Document Title:	**Isomer Differentiation of Novel Psychoactive Substances Using Gas Chromatography Solid Phase Infrared Spectroscopy (GC/IR)**
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1. Executive Summary

Creating designer drugs is an old profession. Consider the following quote taken from *Minutes of the Eleventh Session of the Advisory Committee on Traffic in Opium and Other Dangerous Drugs, 1928*: "The Chairman recalled the well-known fact that, when a narcotic had been declared subject to the provisions of the Conventions, manufacturers hastened to manufacture another narcotic which might escape the consequences of these provisions, at any rate momentarily…" (League of Nations, 1928). Therefore, the practice of modifying controlled substances to create chemical substances uncontrolled by contemporary legislation was practiced as early as 1928.

Throughout the last fifty years many designer drugs have been created; most disappear after a brief period, while a few are still seen many years after their creation. However, by 2009, it had become obvious that, due to a confluence of the internet, drug laws, and international trade, a new era of designer drugs was upon us. At this time a new class of drugs, synthetic cannabinoids, began to be submitted for forensic analysis due to marketing that represented them as "legal highs" (Sedefov, et al., 2009). Synthetic cannabinoids were first synthesized by research laboratories in the 1960s in order to study medicinal applications targeting cannabinoid receptors (Sedefov, et al., 2009). The emergence of synthetic cannabinoids necessitated the need for a new term to replace "designer drugs," which originally referred to clandestinely created substances that were meant to fit the individual needs of users. The meaning of the term "designer drugs" evolved further, coming to represent slight modifications of controlled substances so that they were no longer illegal, but maintained the desired psychoactive effects. Several terms have come and gone (ex. research chemicals, grey-market chemicals, and emerging drugs) that were meant to more inclusively

represent not only designer drugs, but any new chemical introduced into the market of drug abuse. "Novel psychoactive substances" (NPSs) was ultimately adopted by forensic chemists such and will be used here.

The sudden appearance of a bewildering array of synthetic cannabinoids, cathinones, and other NPSs presented three problems to the forensic drug chemist attempting to identify the unknown material: 1) the lack of library reference spectra; 2) the lack of authentic standards or literature; and 3) the analytical specificity required challenge the capability of common techniques. Each of these problems is not uncommon for the forensic chemist to experience, but the relative increased speed at which NPSs enter and exit the drug market make it incredibly difficult to respond on a relevant timescale.

Consider the first problem; the lack of available library reference spectra impedes tentative chemical identifications of an unknown sample. In the past, designer drugs would pop up periodically and their use would spread slowly. Many of the new compounds that became popular as recreational drugs originated in university and pharmaceutical research labs where analytical data was often generated and published. The forensic community, including the Drug Enforcement Agency (DEA), National Institute of Justice (NIJ), and other research labs or U.S. government agencies (ex. Department of Health and Human Services, National Institute of Standards and Technology (NIST), National Institute on Drug Abuse), could then conduct additional research as the popularity of a drug increased. The monographs and publications resulting from the research conducted contained the reference spectra needed by forensic drug chemists to tentatively identify an unknown substance.

However, NPSs now often emerge from clandestine laboratories such that there are no relevant spectra published in digital libraries, accessible books or technical journals when the

NPSs enter the drug abuse market. In response, there are greater efforts to share information such as the construction and frequent updating of commercial or freely-available mass spectral libraries (ex. Mass Spectra of Designer Drugs, the SWGDRUG mass spectral library, and the Cayman Chemical Company mass spectral library). However, there are still significant delays between the emergence of a NPS and its inclusion in such libraries.

The second problem that forensic drug chemists experience is the lack of commercially available reference standards or peer-reviewed analytical data. For many forensic laboratories it is a requirement (promoted by SWGDRUG guidelines) that a standard of the suspected chemical be analyzed and compared to that of the sample in question in order for the substance to be identified. It may additionally be required that published, peer-reviewed spectra of the substance in question be available for comparison. Fortunately, various pharmaceutical companies, chemical suppliers, and academic research labs have begun to synthesize some of the NPS standards. Also, publications such as SWGDRUG Monographs and *Forensic Toxicology* publish validated spectra of NPSs more quickly than in the past. Unfortunately, many smaller state and local laboratories do not have the budget to purchase uncommon reference standards and it is still challenging to obtain reference materials, publications, and spectra in a timely fashion due to the number of new compounds and their relative rate of emergence.

The final challenge that forensic drug chemists face involves determining which analytical assays have sufficient specificity to resolve NPSs sufficiently for identification. Forensic drug chemists rely heavily on gas chromatography/mass spectrometry (GC/MS) that provides low-resolution, electron ionization (EI) mass spectra. Although GC/MS is a highly

discriminating technique, there are some closely related compounds that are difficult to distinguish by this technique. For example 2-fluoromethcathinone, 3-fluoromethcathinone,

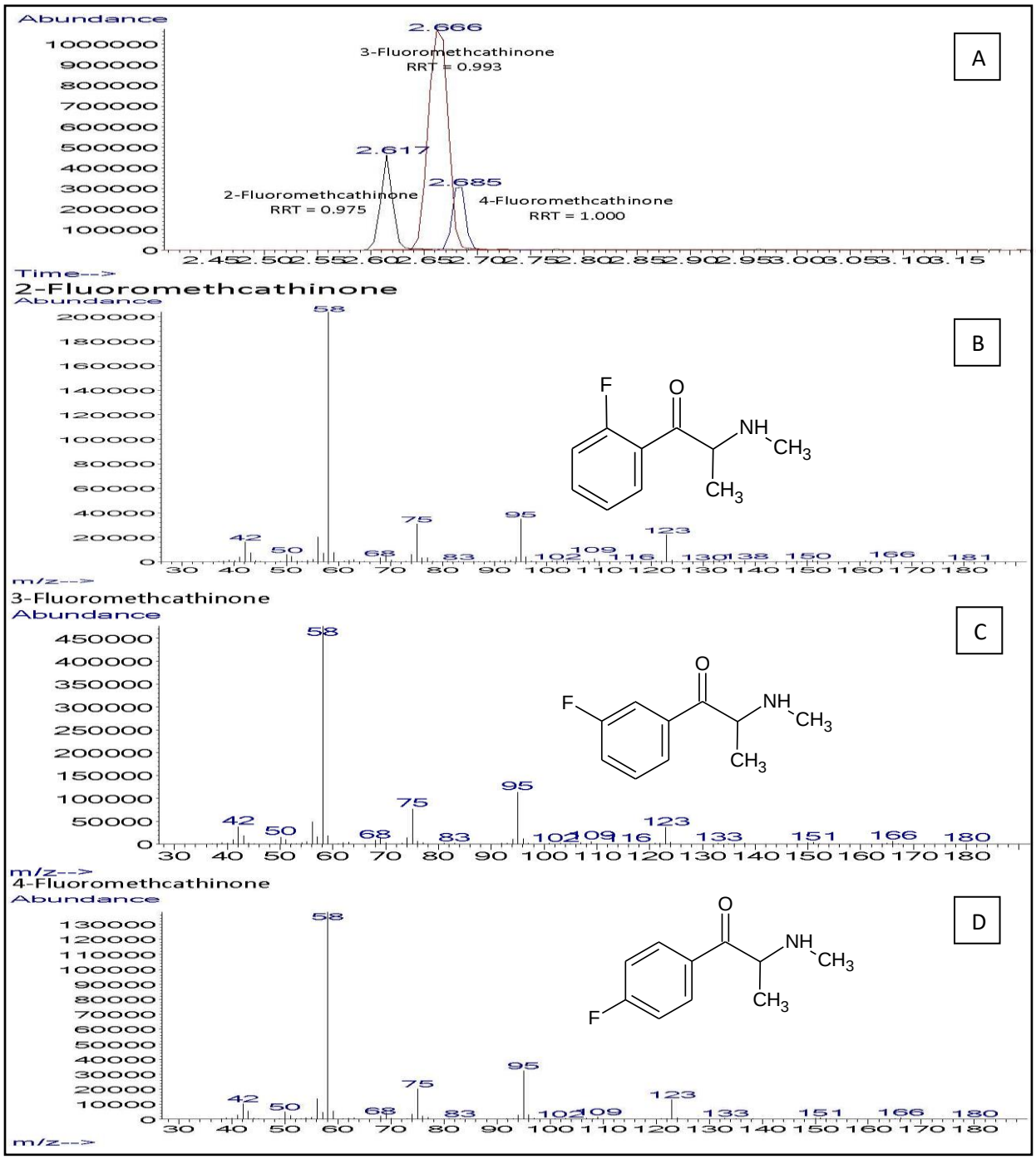

Figure 1: The chromatogram (A) and mass spectra of fluoromethcathinone isomers: 2-fluoromethcathinone (B), 3-fluoromethcathinone (C) and 4-fluoromethcathinone (D).

and 4-fluoromethcathinone are positional isomers that have similar retention times and EI mass spectra (Figure 1) when using common GC/MS conditions. Without more specific methods and reference standards a forensic chemist could not distinguish between the fluoromethcathinone positional isomers with sufficient confidence.

It is well established that it is difficult to differentiate some isomers by GC/MS, and literature is replete with articles demonstrating analytical schemes to differentiate such drugs as methamphetamine, cocaine, MDMA, and heroin from their isomers (Clark, Noggle, Bouhadir, & DeRuiter, 1991; Noggle, Clark, & De Ruiter, 1991; Armstrong, Han, & Han, 1987; Medina, 1989). This is a more significant issue with NPSs due to their large numbers, sudden appearance, and high turnover which do not allow for the execution of comprehensive research. Consequently, non-optimized GC/MS data will only support in limited conclusions.

There are three approaches to NPS isomer identification:

1) A laboratory may choose to issue reports with limited conclusions (ex. "An isomer of fluoromethcathinone was identified…", or "4-fluoromethcathinone or one of its positional isomers was identified…");

2) Positional isomers of NPS's may be synthesized and GC/MS based analytical schemes for them developed; and

3) The NPS may be analyzed using separate, uncorrelated (i.e. orthogonal), highly discriminating techniques in addition to mass spectrometry.

The first approach is reasonable here as the positional isomers of 4-fluoromethcathinone are currently listed as Schedule I controlled substances under United States federal law (Drug Enforcement Administration, Department of Justice, 2017) such that differentiation between each isomer is not necessary. For those occasions where not all

positional isomers are legislated equally, not distinguishing between isomers is a significant limitation.

To distinguish between NPS isomers using the second approach is a time consuming endeavor as each new NPS becomes its own research project, interrupting the normal work flow of a forensic laboratory. Additionally, to accomplish this work using traditional analytical techniques will require highly specific method parameters, sample preparation, analysis of all isomers, highly specific retention time requirements, and the nuanced comparison of ion ratios. Such time and monetary investments for each NPS can be difficult to maintain for even well-funded laboratories.

The last approach involves the application of an orthogonal, analytical technique that provides the discriminating power for NPS isomers with minimal time and research cost. Such an orthogonal technique will complement, and not replace, existing, robust, forensic technologies (ex. GC/MS). A gas chromatograph infrared spectrometer (GC/IR) has the potential to separate complex mixtures into their individual components and acquire their associated Fourier-transform infrared (FTIR) spectra. For example the ortho-, meta-, and para-fluoromethcathinone isomers that were so difficult to differentiate by GC/MS are easily distinguished using their solid-state FTIR spectra (Figure 2). While gas phase IR spectra can also be obtained, the decreased sharpness and resolution of such spectra limit its differentiation power. Unless specifically identified as such, all future iterations of GC/IR used here will be made in reference to the solid-state IR.

A GC/IR with solid phase IR spectra was evaluated for its analytical specificity by differentiating a various NPSs, their isomers, and related compounds. Specifically, a Spectra Analysis DiscovIR™ coupled with an Agilent 7890 gas chromatograph was used for this

evaluation. The GC/IR successfully demonstrated separation repeatability through consistent retention times over 11 weeks for methamphetamine, diazepam, and cholesterol. Similarly, all spectra for methamphetamine, diazepam, and cholesterol consistently matched reference spectra by passing both expert evaluation matching algorithms. The GC/IR also demonstrated inter-laboratory spectra reproducibility despite using independently acquired reference materials, non-uniform sample preparation techniques and different GC/IR acquisition parameters. Over 80 compounds were compared between two laboratories and all spectra fell within the interpretation bounds (3.2 Validation Plan) and were therefore indistinguishable. The GC/IR sensitivity for caffeine was approximately five to ten fold lower than traditional techniques (GC/MS and LC/MS). However, despite higher GC/IR limits of detection (LODs), there is often much more of the target material in evidence than is required to meet instrumental LODs.

Lastly, the GC/IR technique's discriminating power was tested for over 300 compounds including NPS isomers traditionally difficult or impossible to differentiate via GC/MS. The GC/IR successfully produced spectra by which all isomers and homologues were differentiated with the exception of enantiomers and homologues with longer alkyl chains. Enantiomers are a well understood limitation of infrared spectroscopy and are typically differentiated using enantiomer-specific techniques including chiral column separations, derivatization, or polarimetry. Homologues were distinguished by GC/MS, provided that the functionality making up the homologous series is represented within the mass spectra (ex. molecular ion). Overall, the GC/IR provided high discriminating power complimenting traditional forensic chemistry techniques such as GC/MS.

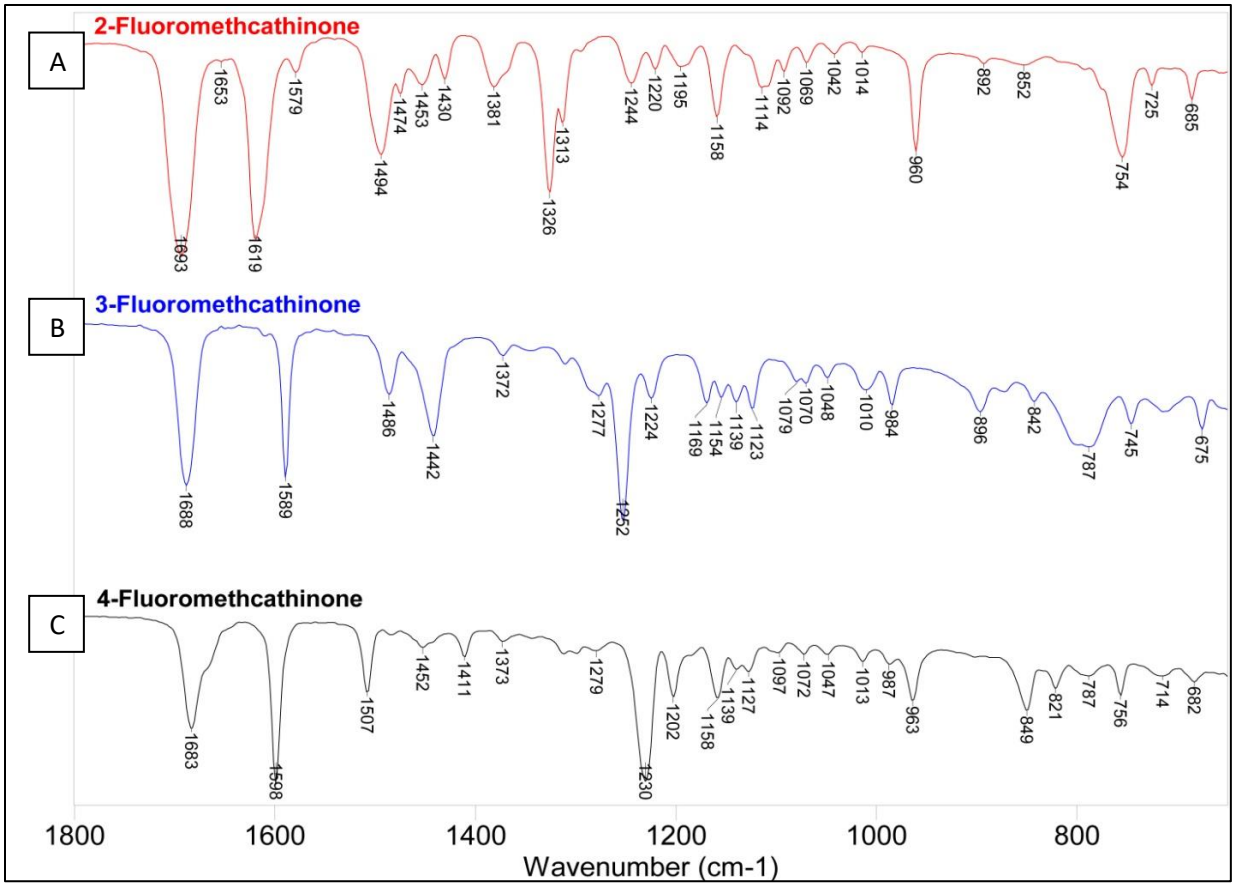

Figure 2: The IR Spectra, expanded for the fingerprint region, of fluoromethcathinone isomers: 2-fluoromethcathinone (A) 3-fluoromethcathinone (B), and 4-fluoromethcathinone (C).

Reference GC/IR spectra for new NPSs can be easily generated and shared with the forensic community. This will not eliminate the need for analytical reference materials for confirmatory comparative analysis. However, only the suspected analyte of interest, as determined when referenced against library spectra, need to be purchased thereby decreasing the financial burden on forensic laboratories.

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2. Introduction

2.1 The Isomer Problem in the Age of Novel Psychoactive Substances

Forensic drug chemists are tasked with analyzing controlled substances and other drugs of abuse. Any analysis scheme must be capable of identifying a substance with a high degree of specificity and confidence such that similar and related substances may be distinguished.

The SWGDRUG and ASTM guidelines for identifying seized drugs categorize analytical techniques by their discriminating power. For instance, Category A techniques have high discriminating power that elucidate chemical structure (ex. Infrared Spectroscopy (IR) and Mass Spectrometry (MS)). While Category B techniques (ex. Gas Chromatography (GC)) have moderate discriminating power (United States Deparment of Justice - Drug Enforcement Administration, 2014).

As a hybrid technique, GC/MS is highly utilized by forensic laboratories to analyze seized drugs due to a multitude of factors: MS discriminating power, mixture deconvolution via GC chromatographic separation, affordability, well established methods for drugs of abuse, robust reproducibility, free and commercial MS libraries, and ease-of-use. Consider the resolving power of the GC alone as it easily separates phentermine, methamphetamine, morphine, and cholesterol in under 11 minutes (Figure 3). Between the phentermine and cholesterol peaks 68 other analytes could be base-line resolved as calculated for the peak capacity (Equation 1). Where p is the conservative estimation of peak capacity between peaks n and m, t_n is the retention time for the nth peak, t_m is the retention time for the mth peak, and w_m is the peak width for peak m.

$$p = 1 + \left(\frac{t_m - t_n}{w_m}\right) \quad [1]$$

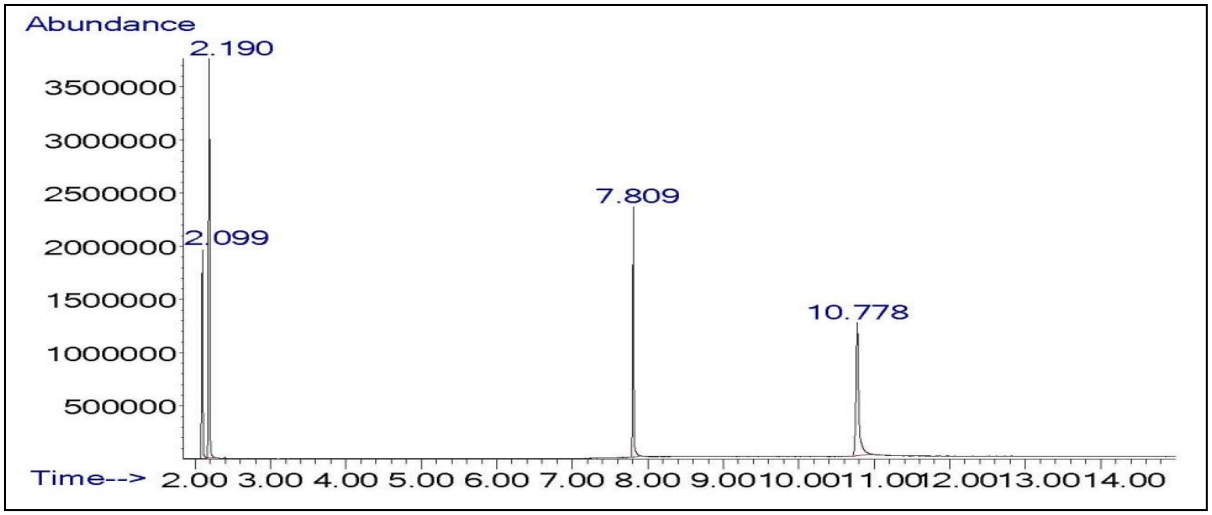

Figure 3: Gas chromatogram of phentermine (2.099 min), methamphetamine (2.19 min), morphine (7.809 min), and cholesterol (10.778 min).

With a clean chromatogram with single non-overlapping peaks, the mass spectra can be safely presumed to represent a single compound. The EI mass spectra for hydrocodone (Figure 4) contains a multitude of peaks representing the molecular ion (*m/z* 299) and many characteristic fragments (ex. *m/z* 242, 270, 185) that cumulatively are highly specific to this compound. If the *m/z* ions 299, 242, and 214 with intensity ranges of 90-100, 45-55, and 25-35 % respectively are searched against the *Wiley 10th Edition NIST 2014 Library* (approximately 700,000 unique compounds) using Agilent's parametric retrieval software, then only hydrocodone is reported as matching all of the criteria.

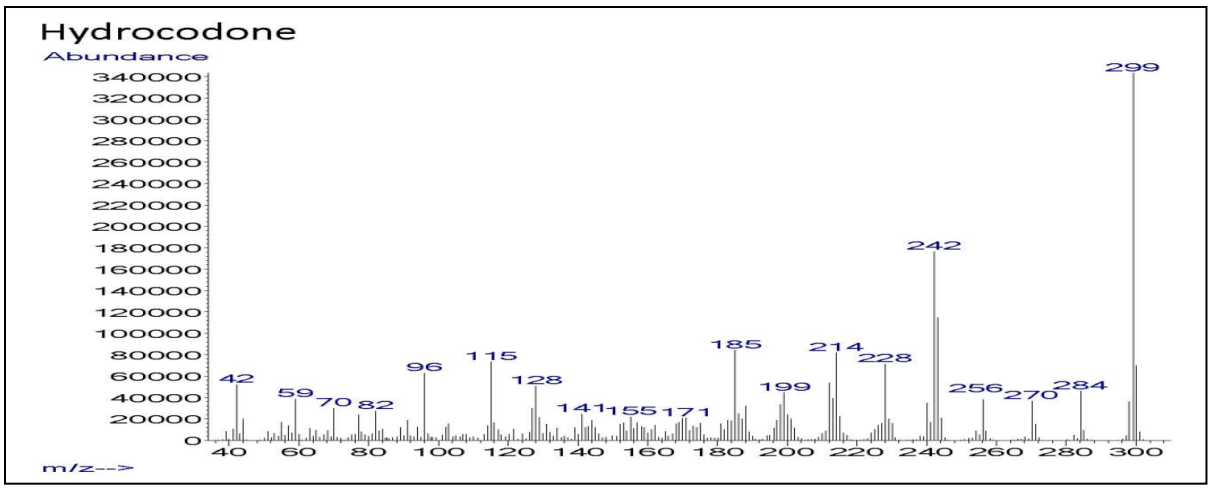

Figure 4: Mass spectrum of hydrocodone.

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The discriminatory power of a mass spectrum is generally dependent on the number, *m/z* value, intensity and uniqueness of its ion peaks. Generally, the more peaks of higher *m/z* value and of greater intensity the greater the discriminating power. Because larger molecular fragments break apart into smaller fragments, the larger *m/z* fragments are less common. Consequently, the probability of an *m/z* ion appearing by chance was calculated to decrease by a factor of two approximately every 130 mass units (McLafferty, Loh, & Stauffer, 1990). Peaks of greater intensity were shown by McLafferty et al. (1990) to be observed less frequently, following a log normal distribution. Lastly, stable ions that are formed from common small-molecule moieties are more frequently detected in a mass spectrum. For example, the *m/z* values 39, 41, and 43 are ten times more likely to be seen than *m/z* 34 (McLafferty, Loh, & Stauffer, 1990).

There are several types of similar compounds for which GC/MS has a greater difficulty differentiating. For instance, the mass spectra often contains little to no information about the relative positioning of functional groups and fragments such that optical, geometric, and positional isomers typically have very similar mass spectra. Additionally, GC retention times and MS spectra are generally correlated such that compounds with a larger molecular weights typically have longer retention times. Consequently, the discriminating power of a GC/MS is limited for a number of specific compound relationships often observed in NPSs.

The mass spectrum of the synthetic cannabinoid JWH-210 (Figure 5) contains both numerous peaks of high intensity and high *m/z* values. However, the 5-ethyl isomer of JWH-210 produces a mass spectra with all of the same high intensity peaks (Figure 6) and is chromatographically unresolved (Figure 7). Consequently, GC/MS does not have the combined discriminating power to differentiate these two isomers.

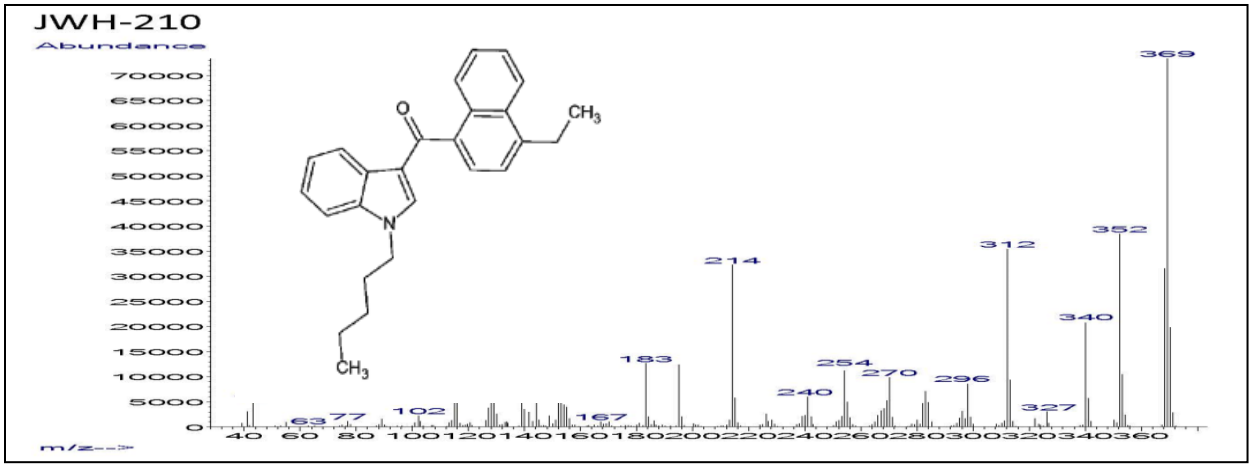

Figure 5: Mass spectrum of JWH-210.

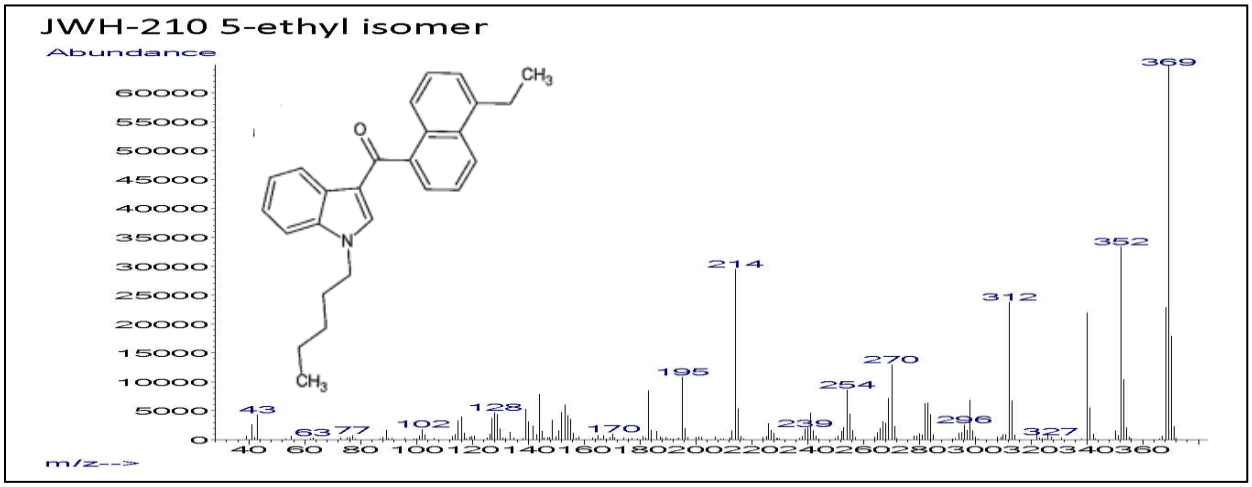

Figure 6: Mass spectrum of JWH-210 5-ethyl isomer.

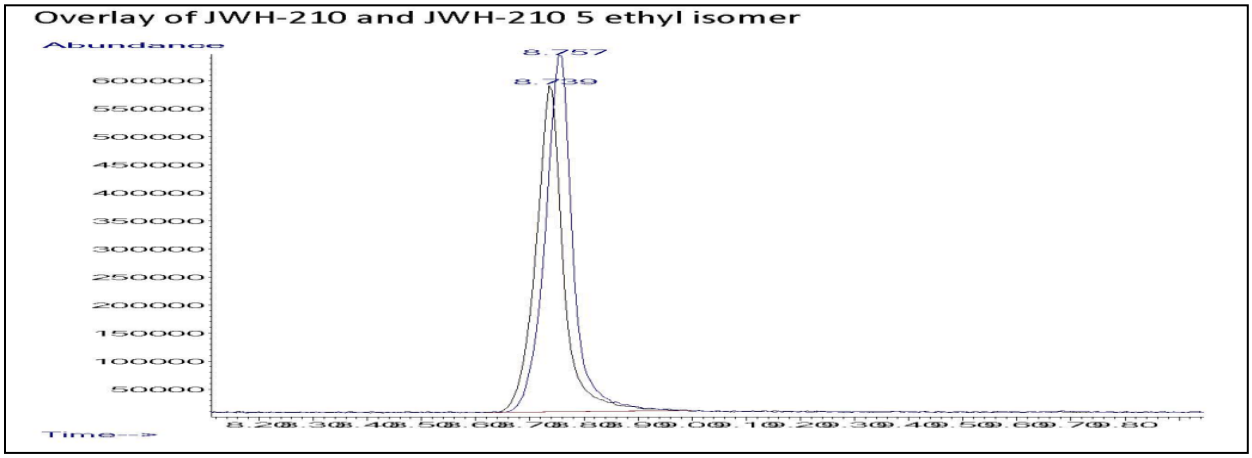

Figure 7: Gas chromatogram overlay of JWH-210 and JWH-210 5-ethyl isomer.

Even information-rich mass spectra have some limitations in their discriminatory power; they cannot discriminate between optical isomers; geometric isomers, including

epimers and diastereomers; constitutional isomers (including positional isomers); and certain isobaric compounds. Many currently encountered NPSs are positional isomers. Positional isomer legal definitions very by the applicable legal authority, but here they are defined as constitutional isomers which have the same molecular formula, core structure, and functional groups. The functional groups may be attached at any position on the core structure, which includes the rearrangement, division or combination of alkyl moieties (Drug Enforcement Administration (DEA), Department of Justice, 2007).

The difficulty with differentiating closely related compounds by GC/MS has been a recognized problem in forensic drug chemistry for many decades (see Appendix E: Drugs of Abuse Isomer Differentiation Bibliography). Since 2009, forensic drug chemists have found themselves in an entirely new environment in which novel psychoactive substances (NPSs) are introduced to the market and evolve at a much faster rate than in the past. Thus new isomers of a currently abused NPSs may be encountered by forensic chemists in the near future. Consider the geometric isomers of cocaine that were synthesized and analyzed in the early 1980's (Allen, Cooper, Kiser, & Cottreli, 1981). Theoretical concerns had arisen in the courts regarding ability of current forensic chemists to differentiate between each cocaine isomer. Consequently, despite a lack of evidence to suggest that such isomers were being sold or consumed, cocaine isomers were synthesized and analytically characterized. Cocaine is a compound derived from a plant, and there would be little economic motivation to complicate its production through the synthesis of its isomers. On the other hand, novel psychoactive substances are synthetic molecules and there may be both legal and economic reasons to synthesize positional isomers. The compounds depicted in Figure 8 are sets of positional isomers of novel psychoactive substances that were advertised for sale on the internet in 2014,

only some of which were controlled at that time. Given the speed and diversity with which NPSs have been encountered by forensic chemists there is significant need for strategies that will allow for their detection with a high degree of specificity.

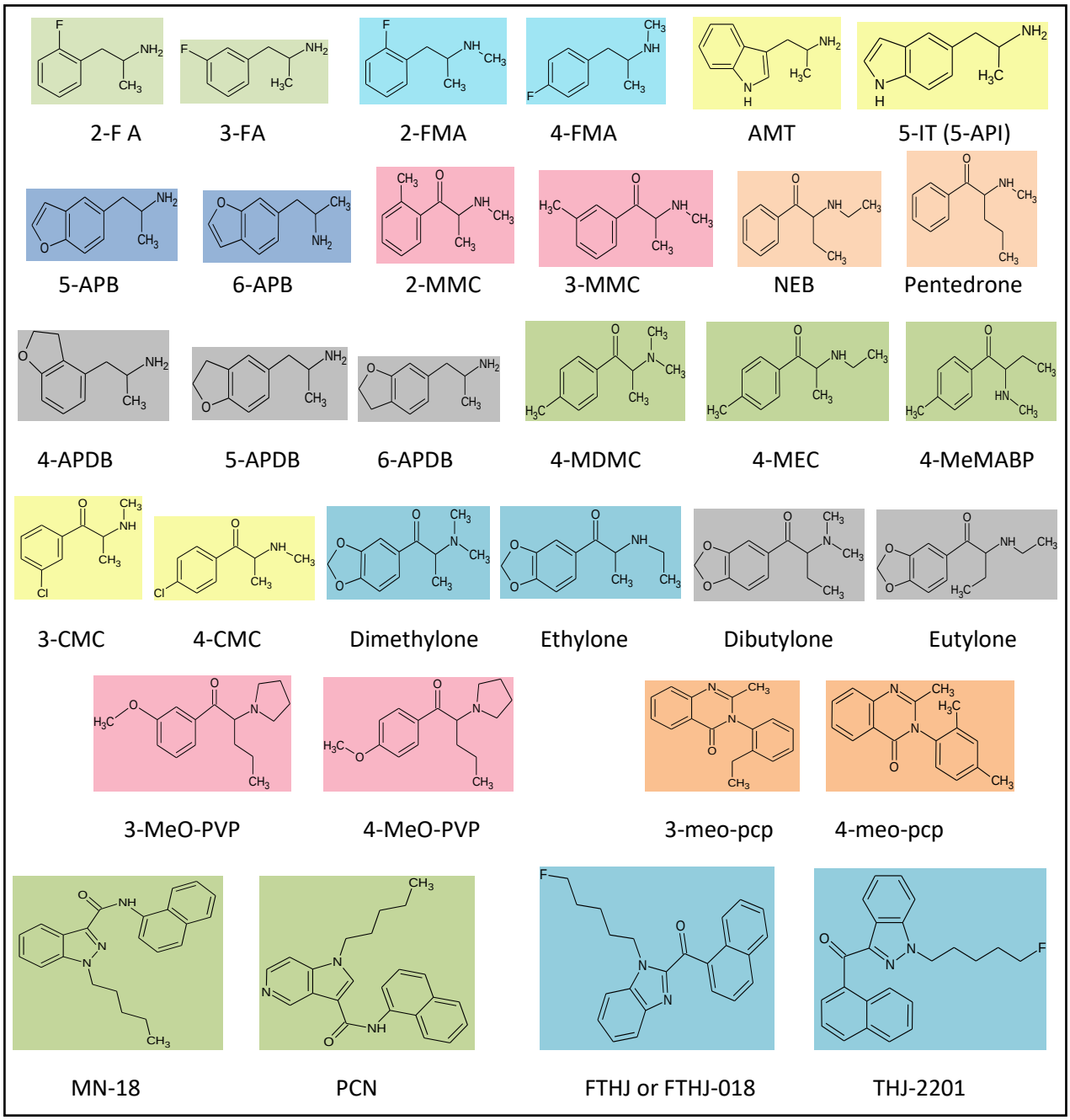

Figure 8: Exemplars of novel psychoactive substances and their isomers.

2.2 Possible Solutions to the Isomer Problem

Presented here are three ways to address the identification challenges of NPSs and their isomers for which reference materials and specific methods are not readily available: 1) report qualitative uncertainty of isomer identification; 2) synthesize isomers and research methods of analysis; or 3) use of an orthogonal technique fundamentally capable of isomer differentiation.

The first proposed solution is to issue a report caveated with qualitative uncertainty regarding the chemical identification of NPSs. For example, instead of reporting the identification of JWH-210, a more measured report identifying "JWH-210 or one of its positional isomers" may be issued. If JWH-210 and all of its positional isomers are controlled in the relevant jurisdiction, then the legal implications may be indistinguishable irrespective of the actual isomer.

The second option is the synthesis and method development and research of NPSs and their isomers. The resulting analytical scheme will be able to differentiate each isomer using traditional techniques. However, as previously discussed, this approach was much more effective in the pre-NPS era when emerging drugs were fewer, infrequent, and sometimes geographically isolated. Additionally, NPSs have relatively short market lifetimes (Figure 9) making the window of opportunity to synthesize reference materials and develop methods very small. The frequency of JWH-018 submitted as evidence diminished from April 2011 to August 2012, corresponded with JWH-018 being controlled in the United States (March 2011). Subsequently, AM2201 was increasingly submitted for forensic analysis temporarily becoming the most popular synthetic cannabinoid. Once controlled AM2201 entered a decline phase similar to JWH-018. The cycle then repeated with XLR11 appearing around June 2012 before all but disappearing by October 2014. Although JWH-018, AM2201, and XLR11 are three of

the most historically popular synthetic cannabinoids, the time between emergence and disappearance was limited to two years.

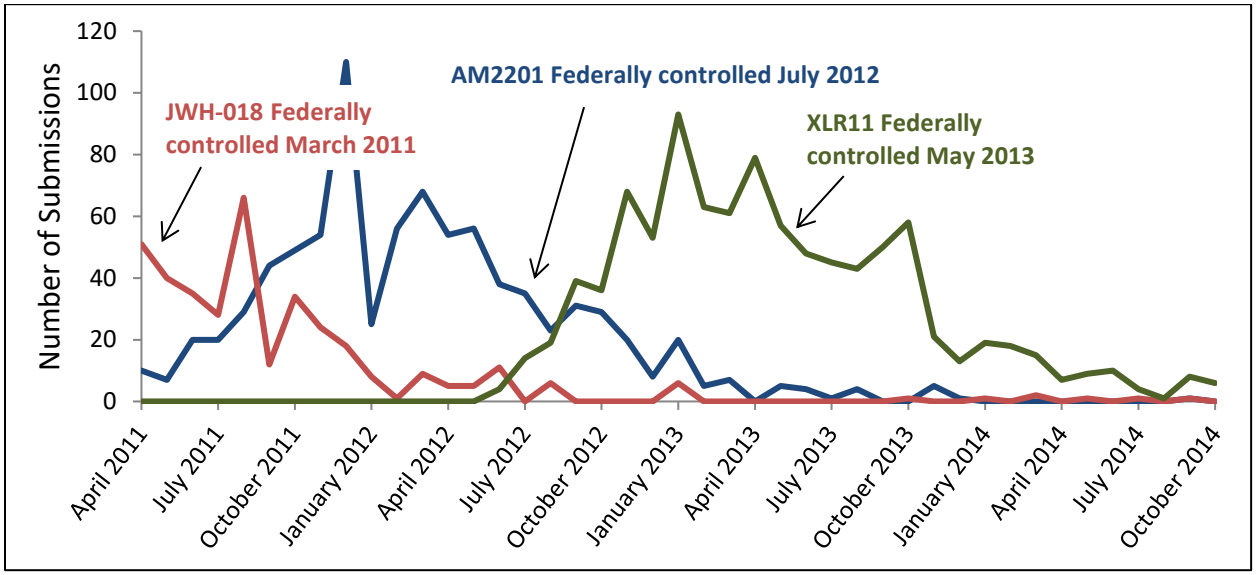

Figure 9: Submission levels for three synthetic cannabinoids (JWH-018, AM2201, and XLR11) between April 2011 and October 2014 at the United States Army Criminal Investigation Laboratory (USACIL).

Fifteen isomers of JWH-018 (Figure 10) were commercially synthesized in order to develop an analytical scheme that could differentiate them and increase reporting conclusion specificity. All 15 of these positional isomers were successfully differentiated from JWH-018 using GC/MS only (data not shown), but by that time JWH-018 was in its decline phase, and had all but disappeared.

Specifically developed GC/MS methods for NPS isomer differentiation typically rely on subtle differences in retention times and/or ion ratios. The poor inter-laboratory precision of such retention times and ion ratios naturally requires that each laboratory analyze all isomeric standards and optimize the GC/MS acquisition to successfully differentiate each isomer. Consequently, digital libraries or published spectra have only limited time or budget savings for forensic chemists.

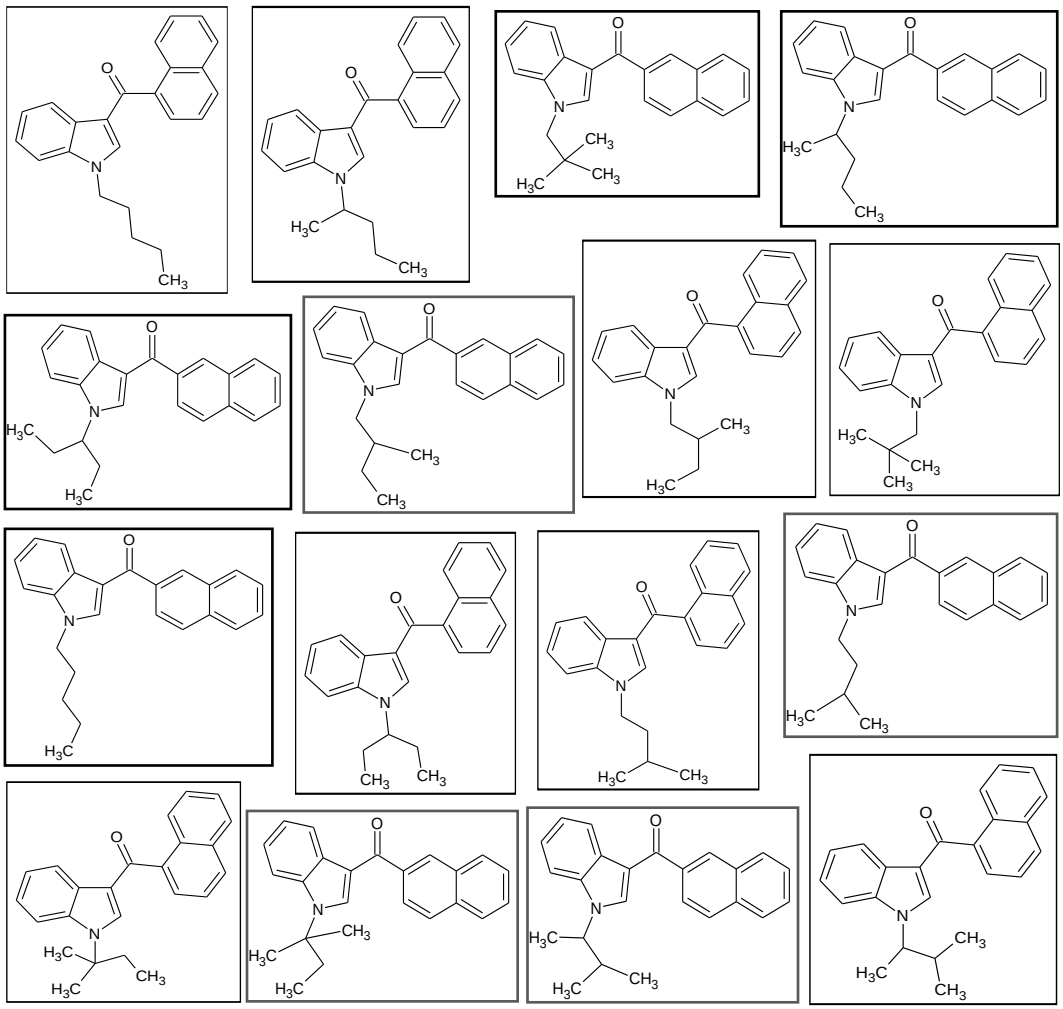

Figure 10: JWH-018 and 15 positional isomers.

The third option is to differentiate NPSs from chemically similar analogues, isomers, etc. by applying an orthogonal technique. To be an effective the technique must be capable of being combined with a technique for the separation of complex mixtures (ex. chromatography). Additionally, it should have high discriminating power to provide data capable of high-confidence, chemical identifications (especially for isomeric compounds) such as a SWGDRUG Category A technique. Lastly, the data should ideally be logically as uncorrelated with mass spectra as possible so as to reduce any redundancy of an additional analysis.

An infrared spectrophotometer coupled to a gas chromatograph meets all of these criteria. While GC/IR instrumentation exist that collect IR spectra of compounds in the solid or gas phase, the solid-state instrumentation typically produce more data rich spectra with greater peak precision and resolution required for isomer differentiation (Figure 11 and 12).

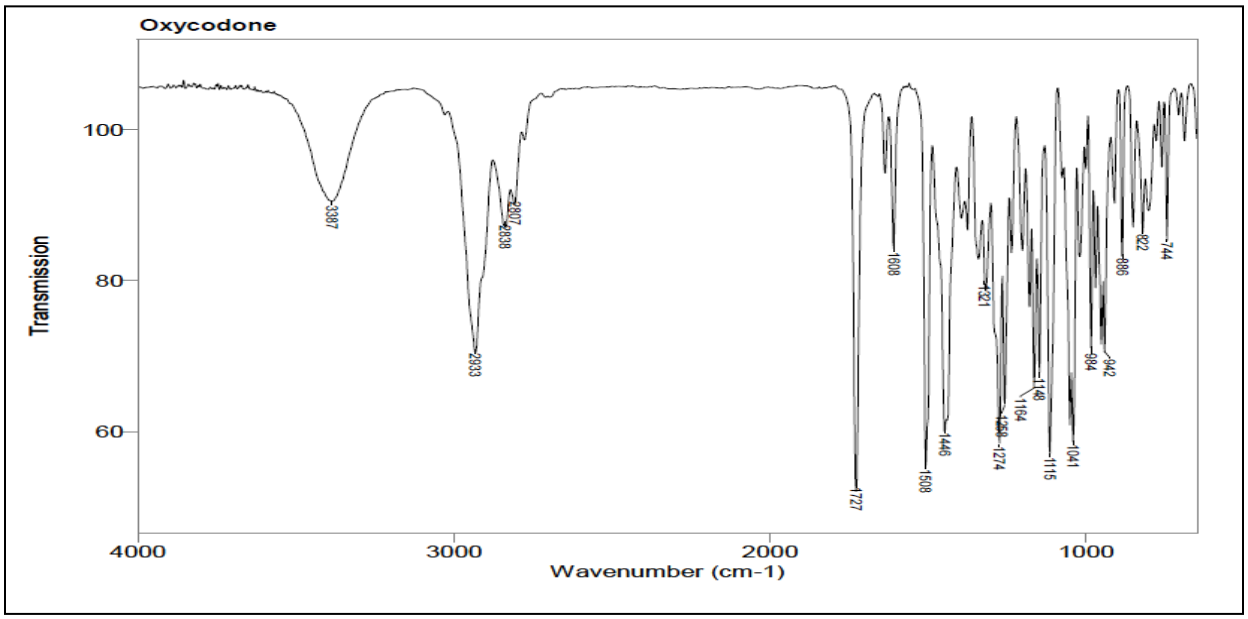

Figure 11: Solid phase GC/IR infrared spectrum of Oxycodone.

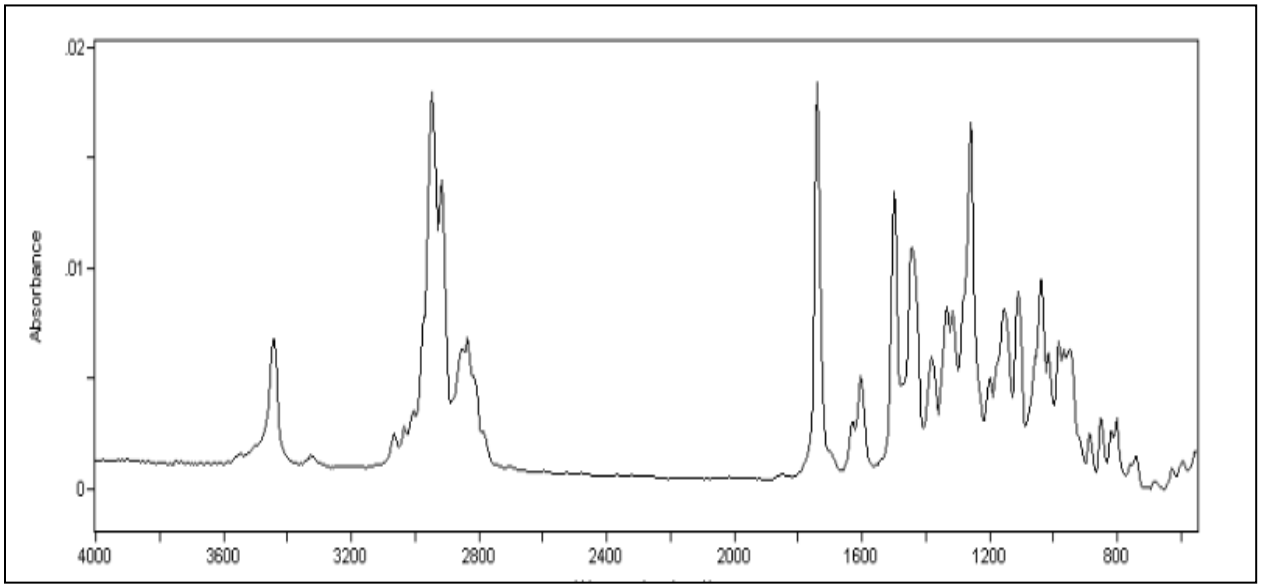

Figure 12: Gas phase GC/IR infrared spectrum of Oxycodone.

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The fingerprint region of the spectra (700 to 1800 cm^{-1}) has much greater resolution when collected by the solid-state IR (Figure 11 and 12). As the fingerprint region affords the greatest discriminating power of IR spectra, the solid-state IR is better suited to differentiate highly similar compounds and future discussion will focus on the solid-state GC/IR. Therefore, unless specifically identified differently, all future iterations of GC/IR used here will be made in reference to the solid-state IR.

2.3 Proposed Work

The objective of this work was to evaluate GC/IR for its ability to discriminate between NPS isomers and other chemically similar compounds relative to GC/MS analyses. The combined the cost and infrastructure requirements of the GC/IR is similar to that of a GC/MS with the exception of the use of approximately 5 L of liquid nitrogen per 8 hrs. of operation. The focus will therefore be on the orthogonality and specificity of the GC/IR with very little further discussion of logistical differences.

The GC/IR utilized was the Spectra Analysis DiscovIR™ infrared spectrophotometer coupled with an Agilent 7890 gas chromatograph. The GC separated compounds were cryogenically cooled using liquid nitrogen and condensed on a rotating disc. Solid-state IR spectra was collected frequently and correlated to the GC retention time, creating an IR-based chromatogram.

Shipman et al. (2013) previously evaluated the Spectra Analysis DiscovIR™ infrared spectrophotometer for forensic drug analyses using pseudoephedrine and cocaine to assess analytical method characterization and optimization (Shipman, Conti, Tighe, & Buel, 2013). The discriminating power of the instrumentation was tested using 35 compounds comprising various positional isomers, homologues, and diastereomers (Shipman, Conti, Tighe, & Buel,

2013). As a result of this initial evaluation, the GC/IR instrumentation demonstrated complimentary data to GC/MS analyses that would further the discriminating power of a forensic laboratory. However, Shipman et al. (2013) failed to demonstrate GC/IR reproducibility.

In order to independently verify that the GC/IR could be used to differentiate isomers of NPS's, the repeatability of retention times, the solid-state IR spectra reproducibility, the limit of detection, and isomer specificity were each evaluated. Isomer specificity was tested using approximately 300 common drugs of abuse and NPSs including positional isomers, homologues, diastereomers, and optical isomers.

Additionally, the solid-state FTIR spectra that was generated for the 300 certified reference materials were combined into a reference library. Such reference libraries could be used to identify the chemical component within an unknown material, enabling the chemist to purchase only the standard needed to meet SWGDRUG analysis guidelines. For this to be appropriate the reference library will be queried to determine to what degree each submission may be distinguished by GC/IR data alone to warrant the purchase of a single certified reference material.

The proposed evaluation was not designed to comment on the specific performance of the instrument models utilized, but rather to comment on the technique and utility of data from a solid-state GC/IR for the forensic chemist. Consequently, no specific evaluation of the cost of ownership and user friendliness were made. Additionally, no discussion was made as to the time or requirements necessary to validate the instrumentation for use within a laboratory due to the specific differences required within individual laboratories.

3. Methods

3.1 Operating Parameters

The GC/IR utilized was the Spectra Analysis DiscovIR™ infrared spectrophotometer coupled with an Agilent 7890 gas chromatograph. The GC operates in the traditional oven based thermal gradient fashion by flash vaporizing solvent based samples and concentrating analytes on the head of the fuzed silica column prior to their temperature dependent separation and elution.

The GC was operated using OpenLAB Chem Station Edition (Agilent Technologies, Rev. C 01.05 [35]) and was outfitted with a 30 m HP-5ms column (0.25 mm outer diameter, 0.25 µm film thickness) and was operated per the method parameters in Table 1. One µL was injected into an inlet heated to 250 °C with a silanized glass liner and a 2:1 split ratio. The column flow rate was 2 mL/min of ultra-pure helium (99.999 %).

Table 1: Agilent 7890 GC method gradients.

Method	Initial Column Temp (°C)	Temp Hold (min)	Temp Ramp (°C/min)	Final Column Temp (°C)	Temp Hold (min)	Total Run Time (min)*
GCIR1	120	1	25	290	1	15
GCIR2	240	1	30	300	11	15

*Total run time accounts for IR acquisition time

The DiscovIR-GC™ Infrared Spectrometer was operated using GRAMS/AI with the operating parameters as listed in Table 2. Deactivated fused silica tubing that was connected to the end of the GC column traveled through a heated transfer line and restrictor and extended into the DiscovIR™ where it hovered perpendicular to the cryogenically cooled infrared-transparent rotating zinc selenide disk. The disk, moving stage, and the restrictor were housed in a vacuum chamber to decrease background water vapor and carbon dioxide and to minimize interactions between the heated and cooled zones. The heated transfer line, restrictor, oven and

the cryogenically cooled (liquid nitrogen) disk were all temperature controlled for method optimized conditions.

Table 2: DiscovIR-GC™ method parameters.

Parameter	GCIR1	GCIR2
Transfer Line	250 °C	290 °C
Oven	250 °C	290 °C
Restrictor	250 °C	290 °C
Disk	-40 °C	-40 °C
Dewar Cap	20 °C	20 °C
Chamber Pressure	~ 4 x 10^{-4} torr	~ 4 x 10^{-4} torr
Resolution	4 cm^{-1}	4 cm^{-1}
Disc Speed	3 mm/min	3 mm/min

The IR was an FTIR with a source close to a black-body emitter in the IR region and a liquid nitrogen cooled mercury/cadmium/telluride (MCT) detector. The spectral resolution could be set from 1 to 128 cm^{-1} and when balancing discriminating power and the impact of resolution on the acquisition time per spectra, a 4 cm^{-1} resolution was applied. The disk moved in a spiral motion by using rotatory and linear motors and the total spiral track length was about 6 meters. As the disk moved, the sample deposits passed through the IR beam and the IR spectra is recorded.

As a point of reference a GC/MS (Agilent 7890A GC and Agilent 7693 MSD) was used to evaluate its limit of detection (LOD) and isomer specificity. One µL was injected into an inlet heated to 250 °C with a splitless silanized glass liner and a 100:1 split ratio. The helium flow rate on a HP-5MS column (30m long, 0.25 mm outer diameter, 0.25 µm film thickness) was 2 mL/min. The MS transfer line (290 °C), source (230 °C), and quadrupole (150 °C) were heated for both GC/MS methods. The MS range was *m/z* 34-550 and an electronic noise threshold of 150 were used to generate mass spectra data.

Table 3: GC/MS method parameters.

Method	Flow Rate (mL/min)	Initial Column Temp (°C)	Temp Hold (min)	Temp Ramp (°C/min)	Final Column Temp (°C)	Temp Hold (min)	Total Run Time (min)
GCMS1	2	120	1	25	290	1	15
GCMS2	2	240	1	30	300	9	14

3.2 Validation Plan Outline

In order to verify differentiation of NPS isomers, the instrument and method were first evaluated for retention time repeatability, spectra reproducibility, and method detection limits.

3.2.1 Retention Time Repeatability

To test gas chromatographic precision, a standard mixture containing methamphetamine, diazepam, and cholesterol was repeatedly injected and the retention times were compared against the average. Specifically one µL (GCIR1 method) of 0.5 mg/mL each of methamphetamine, diazepam, and cholesterol in dichloromethane was analyzed in five consecutive iterations to establish intra-day precision. An additional five analyses were performed on each of eleven more days over the course of seven weeks to establish inter-day precision. A solvent blank consisting only of dichloromethane (99.5 % pure, Macron Fine Chemicals) was run before each sample. Relative retention times of each analyte were expected to vary by no more than two percent (i.e. 0.98 – 1.02) to demonstrate sufficient chromatographic robustness for confidence in the analysis of unknown samples.

3.2.2 Infrared Spectra Reproducibility

The reproducibility of GC/IR spectra was evaluated against spectra spectra acquired by the Canada Border Services Agency (CBSA). The CBSA used a second GC/IR instrument and independently acquired and prepared reference materials to generate the comparison spectra. The presence of peaks (20 % higher than the baseline) within the IR resolution tolerance (±4

cm^{-1}) was used to determine the degree of agreement between spectra for approximately 80 compounds.

3.2.3 Limit of Detection Study: Caffeine

To compare the relative sensitivity of GC/IR to common laboratory instrumentation (GC/MS and LC/MS) varying concentrations of caffeine were analyzed and a direct observation of instrumental limits of detection (LODs). Fresh solutions from 1 to 0.001 mg/mL of caffeine were made and used to probe each instrument's LOD. For a compound to be detected the resulting spectra had to match the library reference spectra using internal software search tools. Additionally, the chromatograms needed to have a limited tailing factor (<1.5). Should the resulting LODs be similar for all three instruments, then a single sample preparation at a given concentration would support analysis on any of these complimentary techniques.

3.2.4 Discriminating Power

Resolving power and isomer differentiation was evaluated by analyzing sets of positional isomers and homologs of NPSs by GC/MS and GC/IR. The detector resolving power was then critically evaluated by considering both the ease of reproducibility and the accuracy of compound identification. Infrared absorbance peaks that were 20 % greater than the baseline were compared between spectra and those that differed by more than four wavenumbers (the instrument's spectral resolution limit) were classified as distinguishable peaks suitable for chemical identification and isomer differentiation. For the resulting mass spectra, ions with relative intensities of at least one percent of the base peak were compared using the maximum permitted tolerances for relative ion intensities developed by the European Union Commission decision (European Communities, 2002). For instance, peaks with

intensities less than ten percent of the base peak can vary up to 50 % for a method used to distinguish ion ratios.

Approximately 300 hundred standards of traditional drugs of abuse and NPSs were analyzed over the course of the study. Two libraries were created consisting of either 63 cathinones or approximately 250 different drugs of abuse. These libraries were exported into PDF format (Appendix B and Appendix C) and they were made available for download (Appendix A, http://forendexforum.southernforensic.org/) and incorporation into the GC/IR reference libraries.

4. Results and Discussion

4.1 Retention Time Repeatability

To test the repeatability of chromatographic retention times of the GC/IR coupled instrumentation, five sequential analysis were made on 12 days over seven weeks to capture both the inter-day and intraday variability. The relative retention times (Table 4) and spectra (Figure 13) of the first five analyses were compared against all subsequent analyses. The resulting chromatograms were determined to be highly repeatable as the retention times for methamphetamine, diazepam, and cholesterol (3.12, 8.69, 12.74 min respectively) did not deviate by more than two percent relative to initial retention time observed (Table 5).

Table 4: Intra-day retention time precision for methamphetamine, diazepam, and cholesterol.

Run	Methamphetamine*	Diazepam*	Cholesterol*
1a	1.00	1.00	1.00
1b	1.00	1.01	1.00
1c	1.00	1.00	1.00
1d	0.98	1.00	1.00
1e	1.00	1.01	1.00

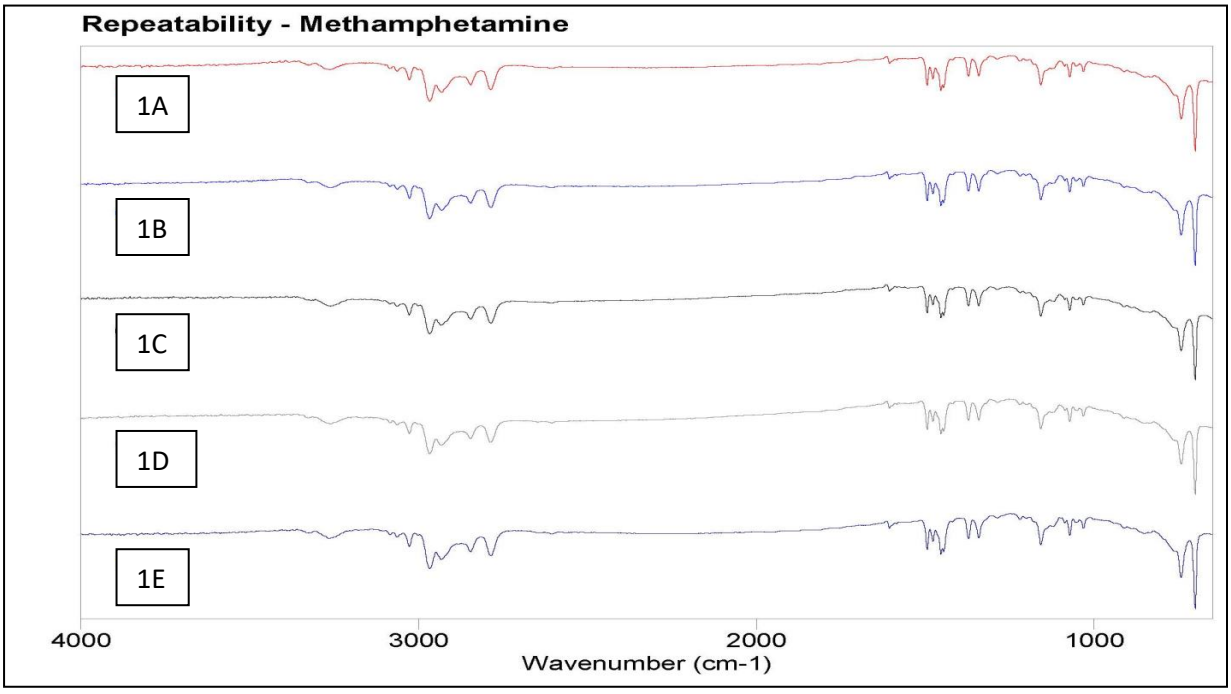
*retention time relative to run 1

Table 5: Inter-day retention time maximum deviation relative to day 1 for methamphetamine, diazepam, and cholesterol.

Day	Methamphetamine	Diazepam	Cholesterol
1	1.00	1.00	1.00
5	0.99	1.00	1.01
12	0.98	1.00	1.00
14	1.00	1.00	1.00
18	0.98	1.00	1.00
19	0.99	1.00	1.00
20	1.00	1.00	1.00
26	0.98	1.00	1.00
27	1.00	1.00	1.00
32	1.00	1.00	1.00
40	1.00	1.00	1.00
47	1.00	1.00	1.00

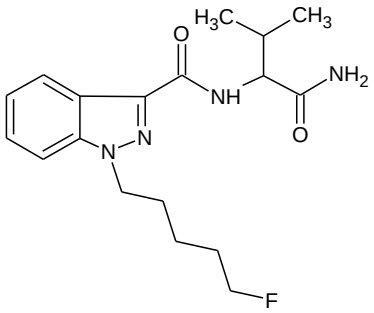
The IR spectral peaks consistently agreed within 4 cm^{-1} (Figure 13) for each compound compared at each time point. Additionally, using the GRAMS software auto report feature, an automated library search for each spectrum returned the proper compound as the most likely target.

Figure 13: GC/IR spectra for methamphetamine from day 1, runs 1a through 1e.

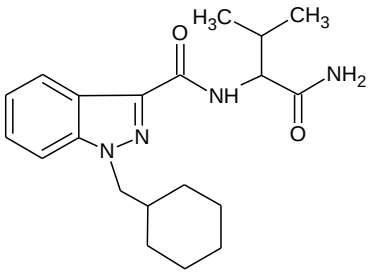


4.2 Infrared Spectra Reproducibility

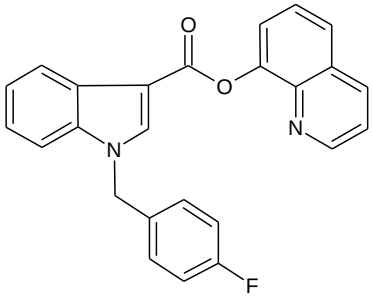
Using methods GCIR1 and GCIR2 the spectra of 84 drugs of abuse were collected and compared to Canada Border Services Agency (CBSA) collected spectra, in order to investigate inter-laboratory reproducibility. The spectra of four compounds (Figure 14) of the 84 drugs of abuse evaluated are presented below as a representative comparison (Figure 15–18). While the CBSA utilized independent reference materials and distinct method parameters (see Appendix D for CBSA method parameters), the GC/IRs still obtained spectra that fell within the bounds of spectra precision.



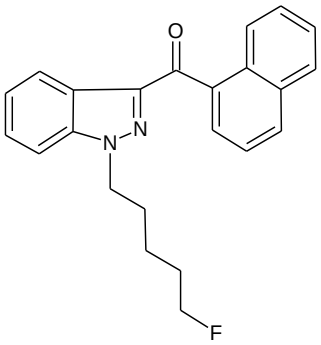
5F-AB-PINACA



AB-CHMINACA



FUB-PB-22



THJ2201

Figure 14: Structures of four representative synthetic cannabinoids used to assess inter-laboratory reproducibility of IR spectra.

Despite the use of different sample solvents, the spectra were reproducible between laboratories. Typically polymorphic effects of recrystallizations out of different solvents

(Yamanobe, Takiyama, H., & Matsuoka, 2002; Khoshkhoo & Anwar, 1993) would have negatively impact the reproducibility of IR spectra. However, because the sample was vaporized, separated chromatographically from the sample solvent, and then cryogenically condensed onto the disc for detection, the resulting spectra are highly reproducible. Consequently, the universal utility of reference library spectra has increased, because it is no longer contingent on the sample solvent. This is important as the salt form of cocaine, for example, produced different IR spectra when acquired on a traditional solid-state attenuated total reflectance FTIR. Whereas the salt form of cocaine does not impact the final spectra when acquired on a solid-state GC/IR.

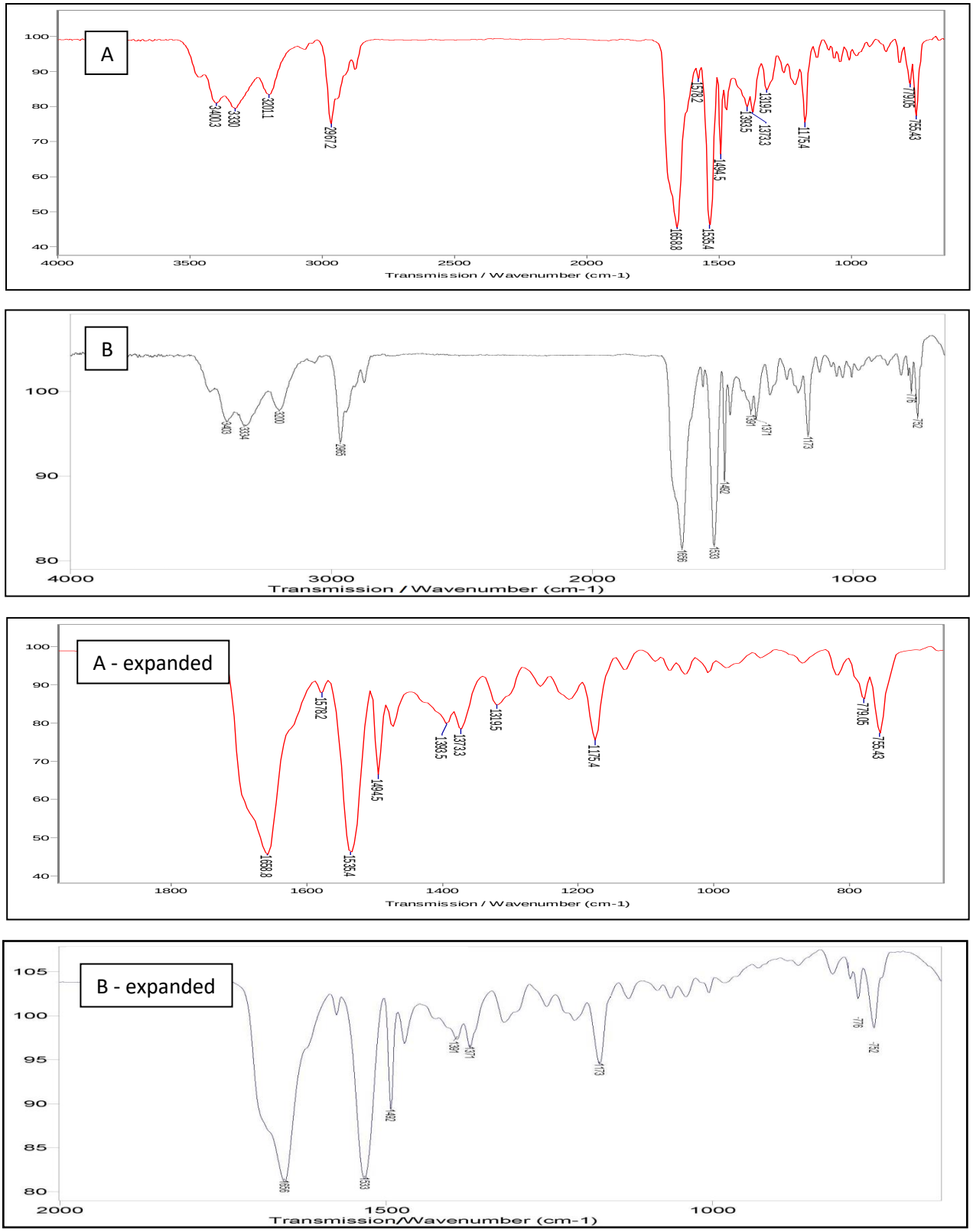


Figure 15: GC/IR Spectra for 5F-AB-PINACA obtained by CBSA at 8 cm^{-1} resolution (A) and the DFSC at 4 cm^{-1} resolution (B) with the fingerprint region expanded for simplified comparisons.

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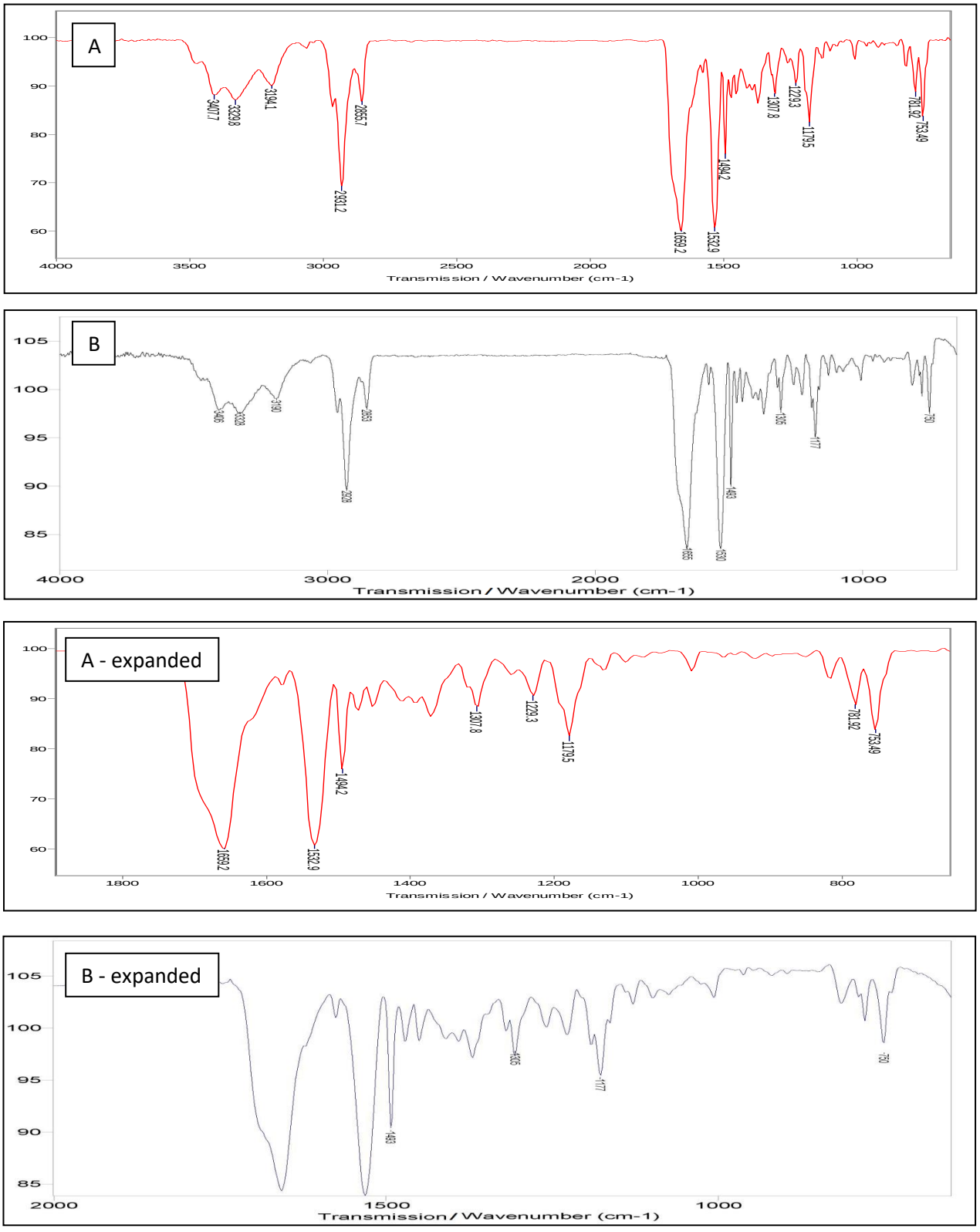


Figure 16: GC/IR Spectra for AB-CHMINACA obtained by CBSA at 8 cm^{-1} resolution (A) and the DFSC at 4 cm^{-1} resolution (B) with the fingerprint region expanded for simplified comparisons.

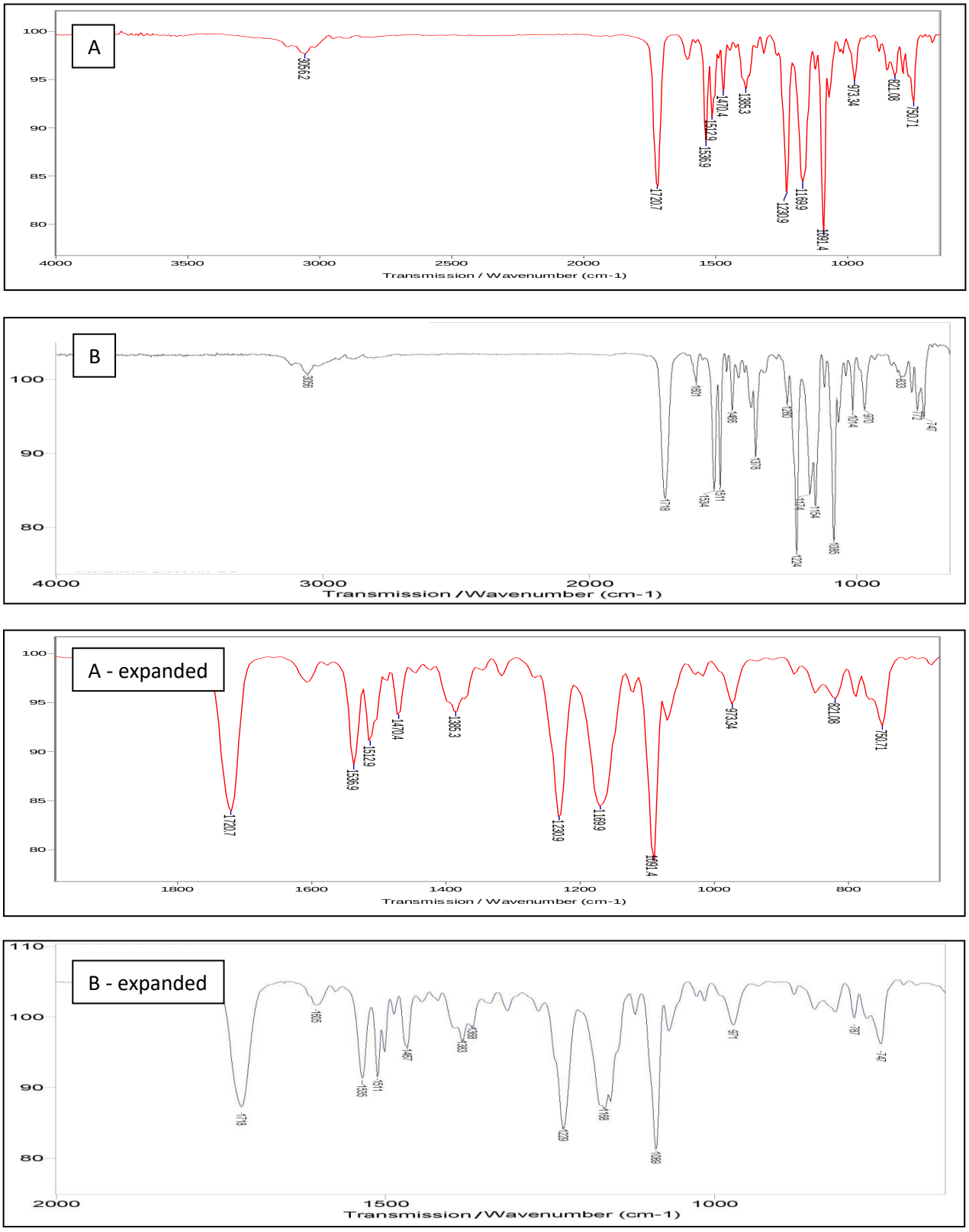
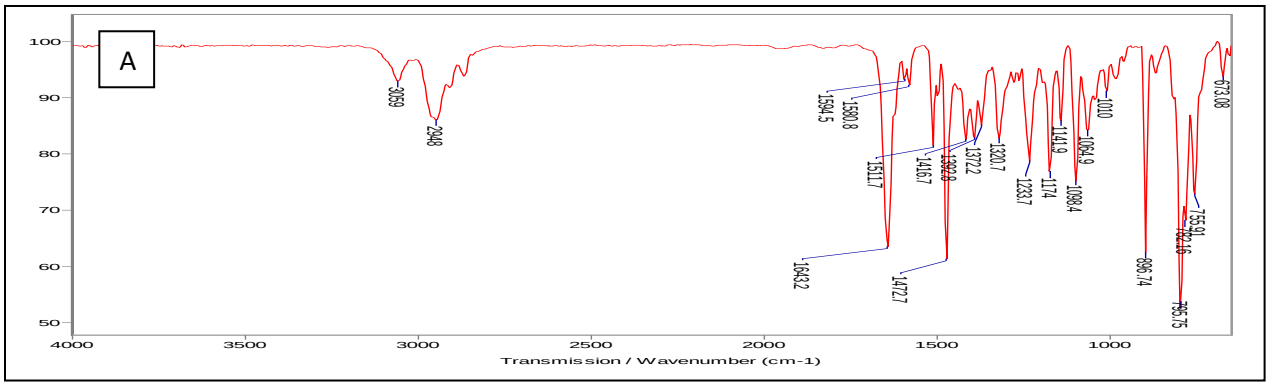
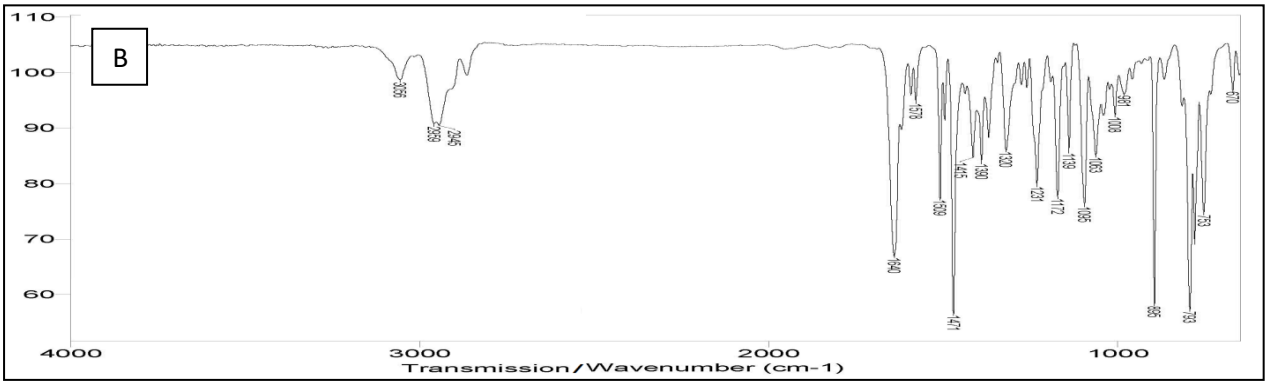
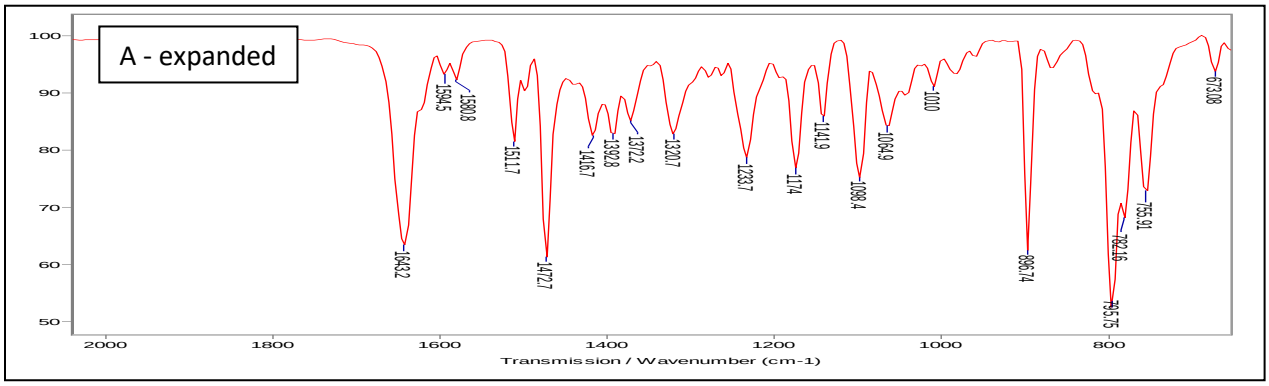


Figure 17: GC/IR Spectra for FUB-PB-22 obtained by CBSA at 8 cm^{-1} resolution (A) and the DFSC at 4 cm^{-1} resolution (B) with the fingerprint region expanded for simplified comparisons.







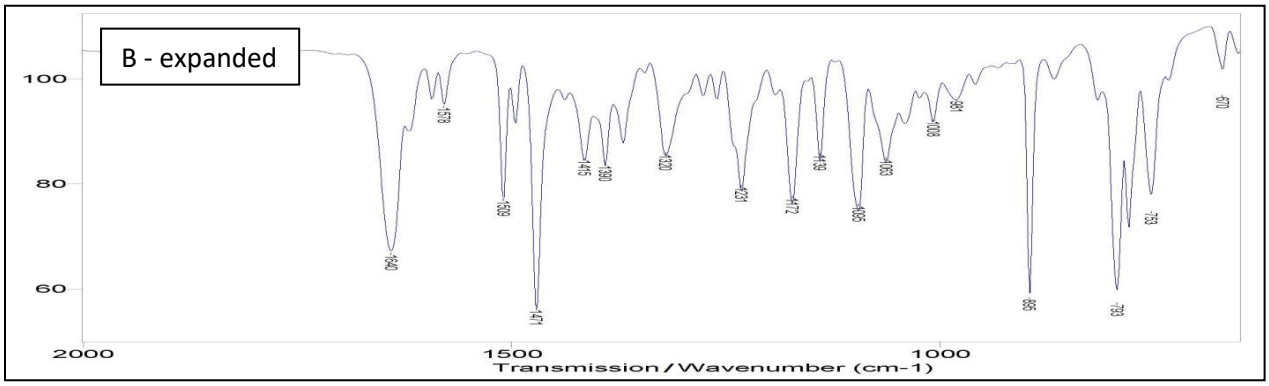


Figure 18: GC/IR Spectra for THJ2201 obtained by CBSA at 8 cm^{-1} resolution (A) and the DFSC at 4 cm^{-1} resolution (B) with the fingerprint region expanded for simplified comparisons.

4.3 Limit of Detection Study: Caffeine

The relative sensitivity of GC/IR to common laboratory instrumentation (GC/MS and LC/MS) was measured using varying concentrations of caffeine to establish instrumental LODs (Table 6). The LC/MS LOD was 10 fold higher when determined using the Total Ion Chromatogram's (TIC) peak. However, by using selective ion monitoring for *m/z* 195, caffeine detected as low as 10 ng on-column and the accompanying mass spectra was ion rich enough to match all of the *m/z* values recorded in reference spectra. In contrast, the GC/MS had a limit of detection of 5 ng on-column and the mass spectra was accurately associated with caffeine in the NIST 2014 EI-MS library. There were enough similarities at 5 ng so that the GRAMS software search function matched caffeine, but the GC/IR LOD was 50 ng on-column because only then did the infrared spectra match all reference spectra peaks.

Table 6: Caffeine instrumental LODs.

Instrument	Method	Split Ratio	LOD (ng)	LOD ratio to GC/MS
LC/MS	LCMS1	N/A	10	2
GC/MS	GCMS1	100:1	5	1
GC/IR	GCIR1	2:1	50	10

At 50 ng on-column of caffeine the Hit Quality Index (HQI), or spectral match, value in GRAMS was <0.5 irrespective of replicate measurements made using differing split ratios. After performing a baseline correction the First Derivative Correlation Algorithm takes the first derivative of both the unknown and library spectra before calculating the HQI. An HQI value of zero represents a perfect match, but a spectrum matched with itself still results in a positive value less than 0.005. A HQI value less than 0.5 is a useful diagnostic threshold when assessing the quality of a searches association, because it was observed to correlate with quality caffeine spectra. However, the HQI on its own may result in false associations as an isomer or other closely related compound can also result in an HQI value of less than 0.5. Consequently it is

important to demonstrate the degree of discriminating power (i.e. specificity) for a set of isomers in order to set appropriate interpretation thresholds.

Despite the relatively higher LOD for the GC/IR, an analysis of seized substances typically has sufficient material to be detected by all three instruments. In the case of trace residue analyses, the LOD plays a more vital role. Aside from traditional techniques to increase the signal strength (larger injection volumes, sample concentration efforts, etc.), the GC/IR is also capable of overlaying multiple deposits of sequential sample injections. This means that a weak sample can be concentrated by depositing consecutive injections onto the GC/IR disk while maintaining chromatographic resolution and generating stronger spectra. Previous work (data not shown) demonstrated a correlation between analyte volatility and the degree to which signal increased using this technique. Testing disk sample retention could be an area of future studies using substances of different molecular weights and volatility as well as adjusting disk temperature.

4.4 Discriminating Power

The analysis of 44 NPS isomers and homologs (chosen as representative of common case-working submissions and to represent analytically challenging classes of chemical structures) was conducted using the GCMS1 and GCIR1 methods. Relative retention times that differed by more than two percent had sufficient chromatographic resolution for the differentiation of analytes. However, retention times are instrument and method specific and must be demonstrated by each laboratory using known reference materials. Likewise, mass-spectral ion ratios are sensitive to instrument and method differences and must be independently demonstrated as reproducible by each laboratory.

4.4.1 Methyl/Ethyl Cathinone Isomers

Positional isomers and homologues differing by the addition and location of alkyl groups (methyl- or ethyl-) produce highly similar spectra. Previous research has demonstrated that the alkyl substituted cathinone isomers can be differentiated via Nuclear Magnetic Resonance (NMR) (Power, et al., 2011; Strano Rossi, et al., 2014), but NMR requires significant sample preparation due to purity requirements. Consequently, spectral differences of GC/MS and GC/IR were evaluated using cathinone isomers with varying alkyl groups (methyl- or ethyl -) at the R2-R6 positions, where R4, R5, and R6 are ortho-, meta- and para-, respectively (Figure 19). The para-substituted cathinone is a recognized substance of abuse and was used to calculate all relative retention times.

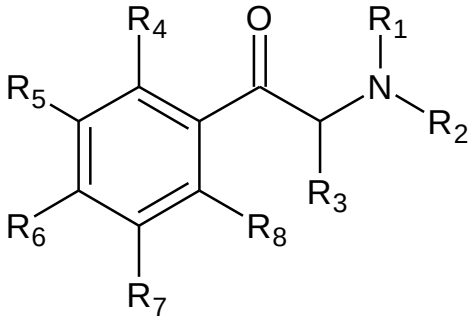


Figure 19: Cathinone positional isomer ring structure.

4.4.1A Methylmethcathinone Isomers

Methylmethcathinone isomers were readily distinguished chromatographically using the GCMS1 method (Figure 20). However, the mass spectra for each positional isomer could not be differentiated due to the similar relative ratios of all major ions (*m/z* 42, 51, 58, 65, 77, 91, 105, and 119).

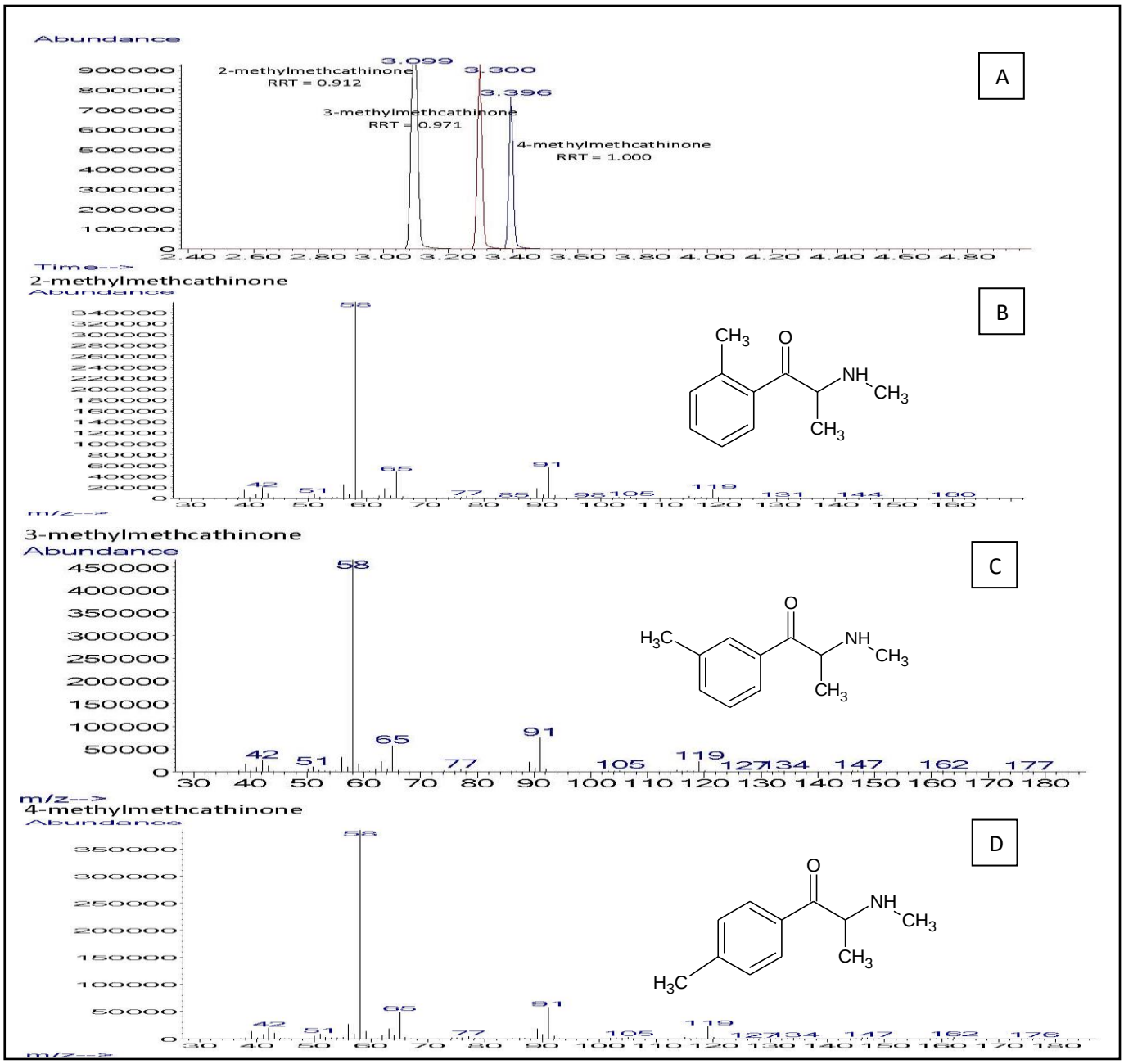


Figure 20: The chromatogram (A) and mass spectra of 2-methylmethcathinone (B), 3-methylmethcathinone (C), and 4-methylmethcathinone (D).

The IR spectra of 2, 3, and 4-methylmethcathinone were readily distinguished (Figure 21). Each positional isomer spectra contained unique peaks (ex. 1291, 1586, and 960 cm^{-1}) in addition to the number of peaks in the fingerprint region that combined are unique to a specific isomer. Consequently, GCIR technology is a valuable technique that was capable of indicating

which methylmethcathinone isomer was contained within a substance. Once the isomer has been indicated via a matching reference spectra the forensic chemist may purchase a single reference standard for comparison instead of demonstrating chromatographic resolution with three separate isomers on a GC/MS.

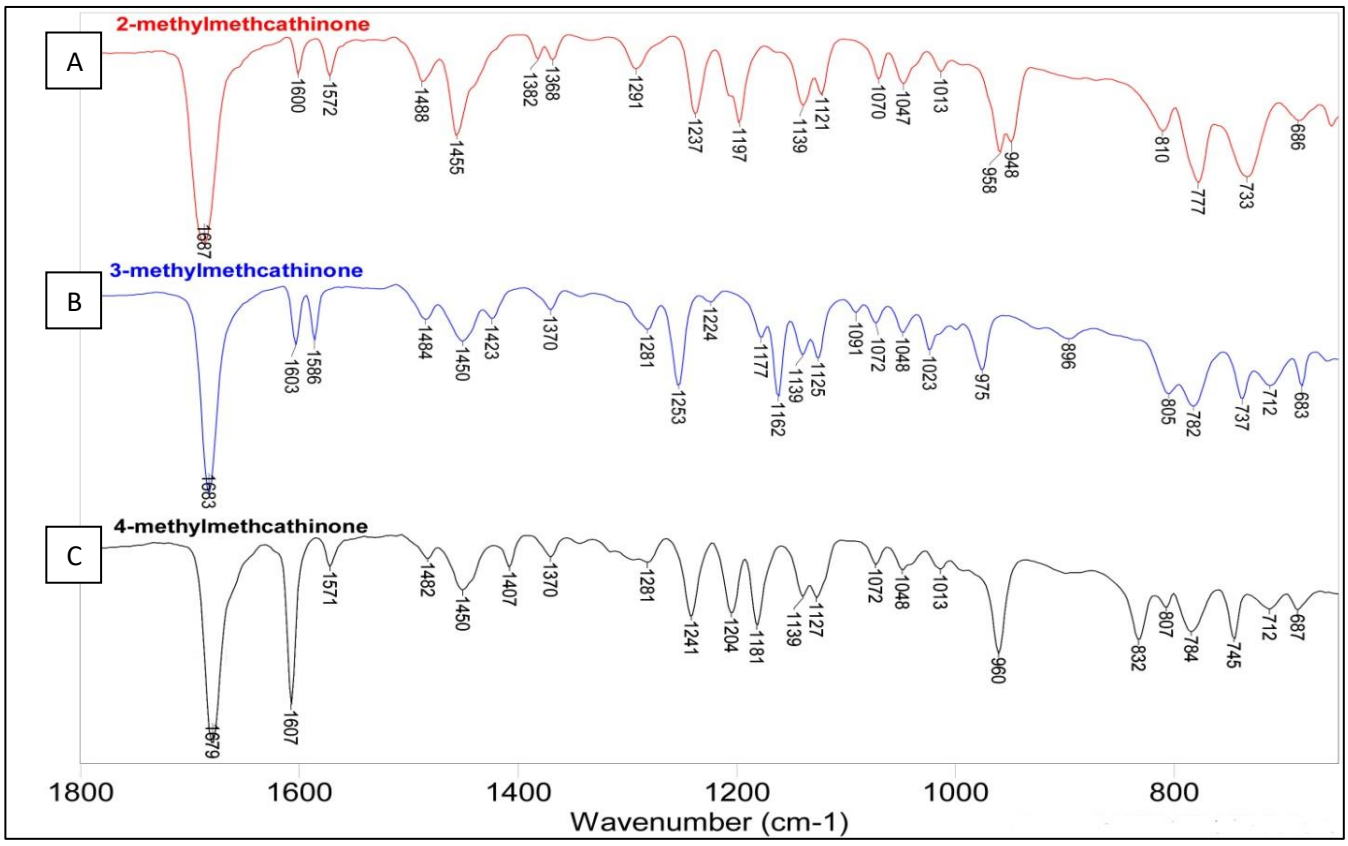


Figure 21: The IR Spectra, expanded for the fingerprint region, of 2-methylmethcathinone (A), 3-methylmethcathinone (B), and 4-methylmethcathinone (C).

4.4.1B Methylethcathinone Isomers

Methylethcathinone isomers consist of a methyl substituent at the ortho-, meta-, and para- positions of ethcathinone. As with the methylmethcathinone isomers, the methylethcathinone isomers could not be distinguished using mass spectrometry, but they can be chromatographically resolved using gas chromatography (Figure 22). Each isomer was differentiated by the combination of infrared peaks observed via GC/IR (Figure 23) and 4-

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methylethcathinone in particular, had a number of unique absorption peaks (ex. 1233, 1213, 1179, 832 cm^{-1}).

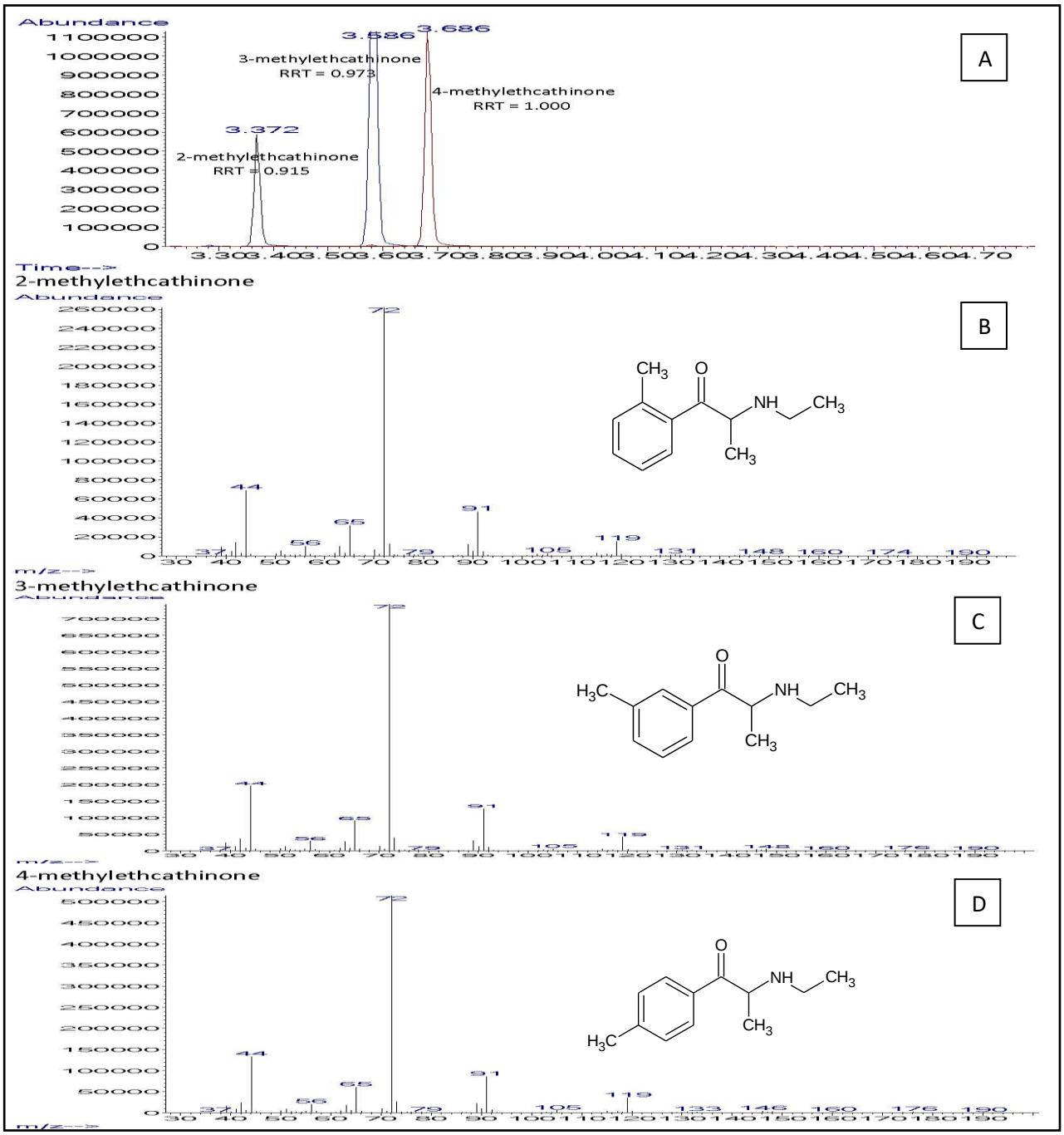

Figure 22: The **c**hromatogram (A) and mass spectra of 2-methylethcathinone (B) 3-methylethcathinone (C), and 4-methylethcathinone (D).

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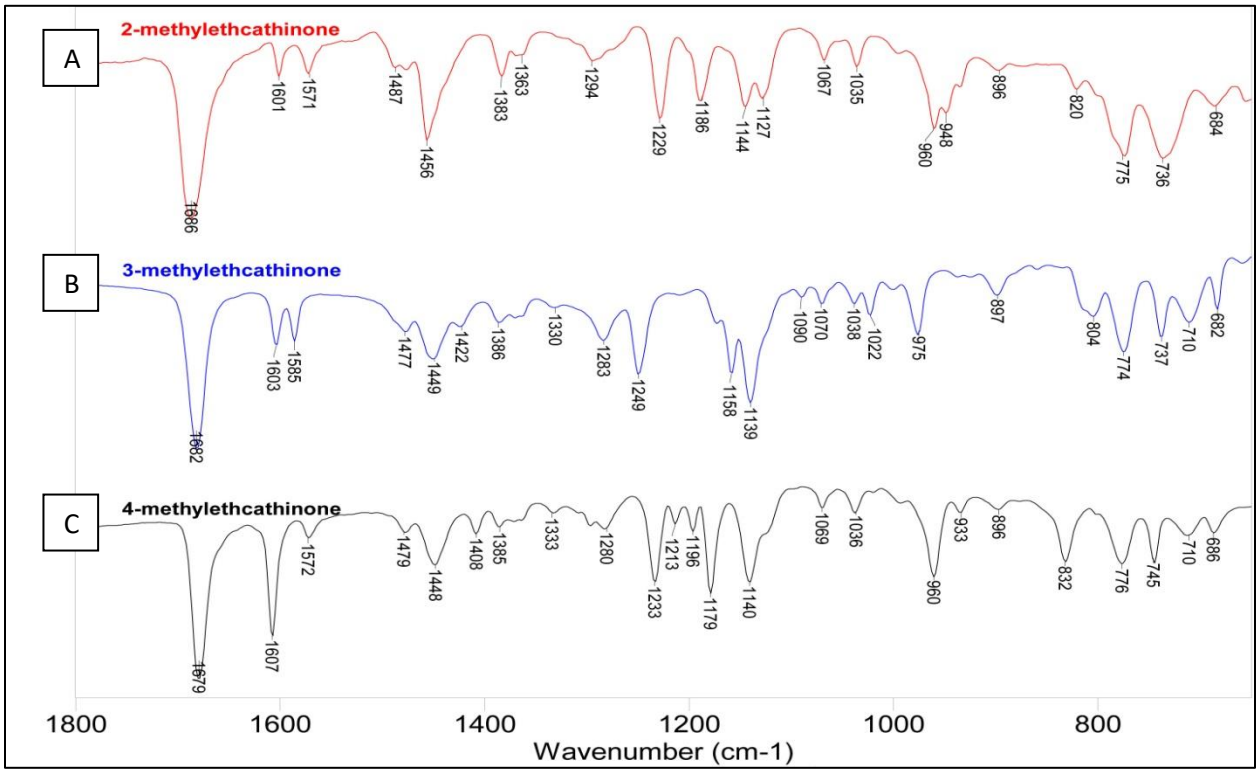

Figure 23: The IR Spectra, expanded for the fingerprint region, of 2-methylethcathinone (A), 3-methylethcathinone (B), and 4-methylethcathinone (C).

4.4.1C Ethylmethcathinone Isomers

The ethylmethcathinone positional isomers consist of an ethyl substituent at the ortho-, meta-, and para- positions of methcathinone. Again, gas chromatography resolved the three positional isomers, but EI-MS did not successfully differentiate them because all significant mass spectral ions (>1 % relative abundance) are observable at similar ratios for all three isomers (Figure 24). The IR spectra, however, differentiated between all three ethylmethcathinone isomers with unique absorption peaks (ex. 1687, 1607, 1161 cm^{-1}) and the combination non-unique peaks (Figure 25).

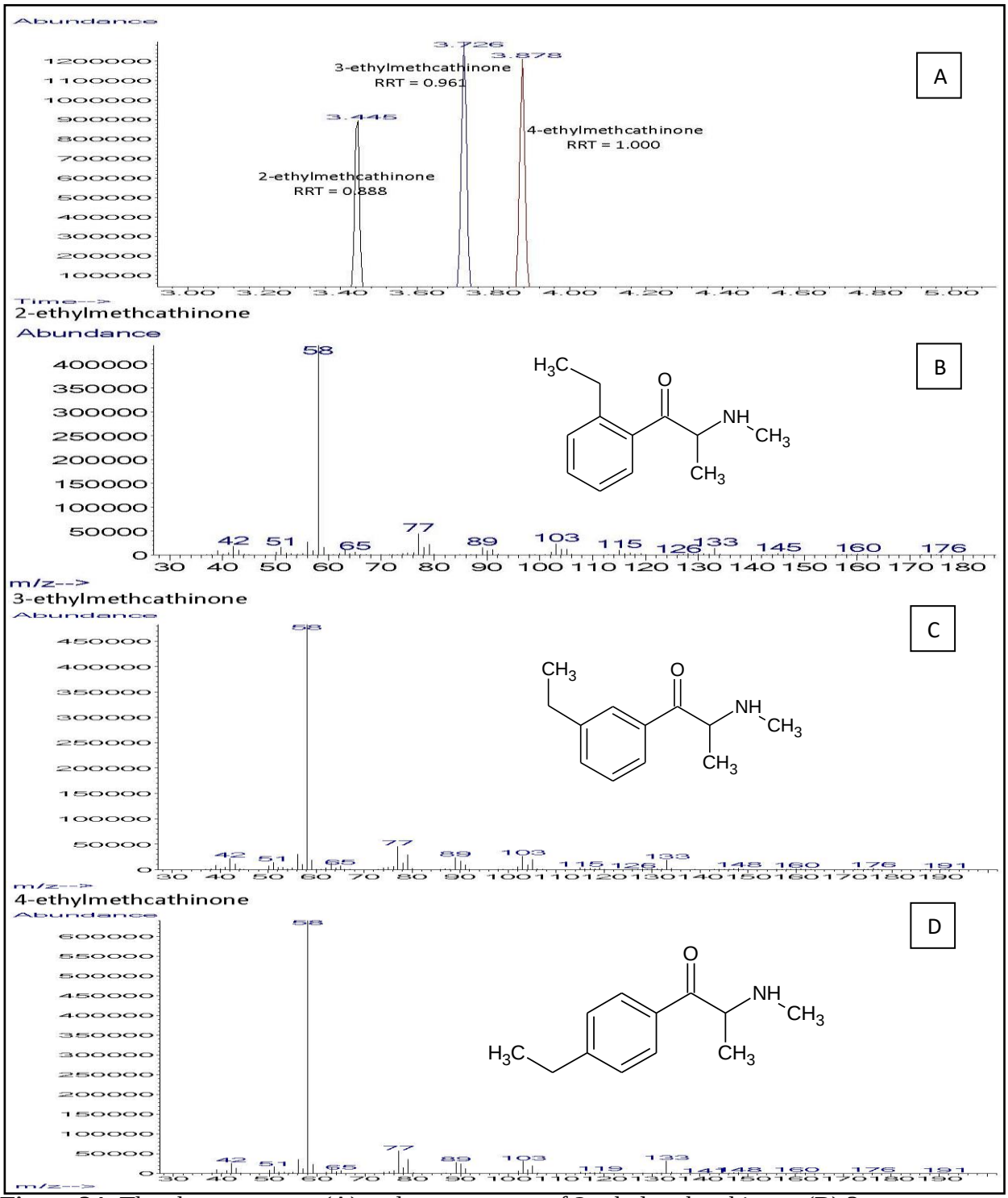

Figure 24: The chromatogram (A) and mass spectra of 2-ethylmethcathinone (B) 3-ethylmethcathinone (C), and 4-ethylmethcathinone (D).

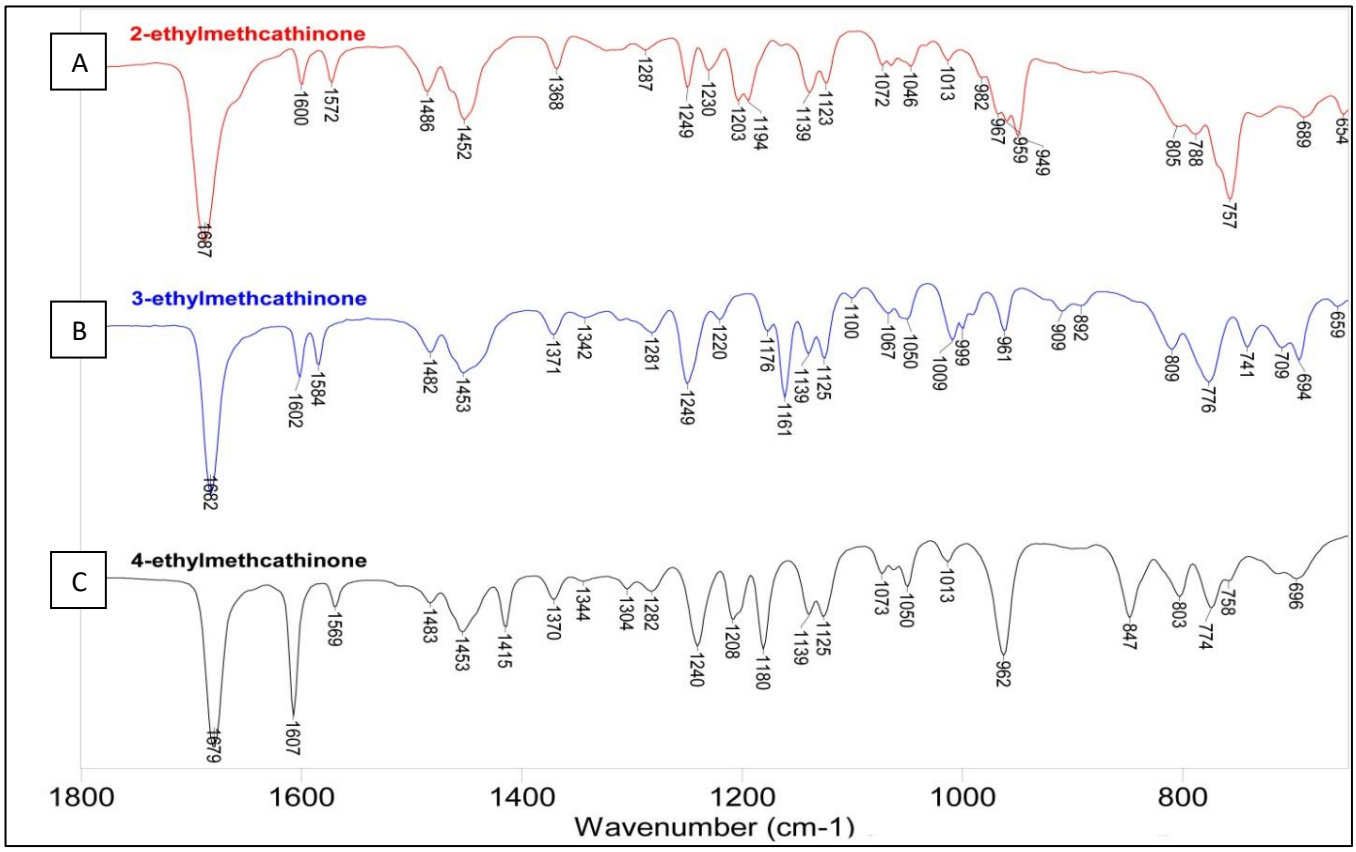

Figure 25: The IR Spectra, expanded for the fingerprint region, of 2-ethylmethcathinone (A), 3-ethylmethcathinone (B), and 4-ethylmethcathinone (C).

4.4.1D Ethylethcathinone Isomers

The ethylethcathinone positional isomers consist of an ethyl substituent at the ortho-, meta-, and para- positions of ethcathinone. As with the previous cathinone positional isomers, the ethylethcathinone positional isomers were successfully chromatographically resolved, but could not be differentiated via EI-MS (Figure 26). The GC/IR spectra for all three isomers contained unique absorption peaks (ex. 1246, 1241, 1231 cm^{-1}) that on their own enable simple isomer differentiation (Figure 27).

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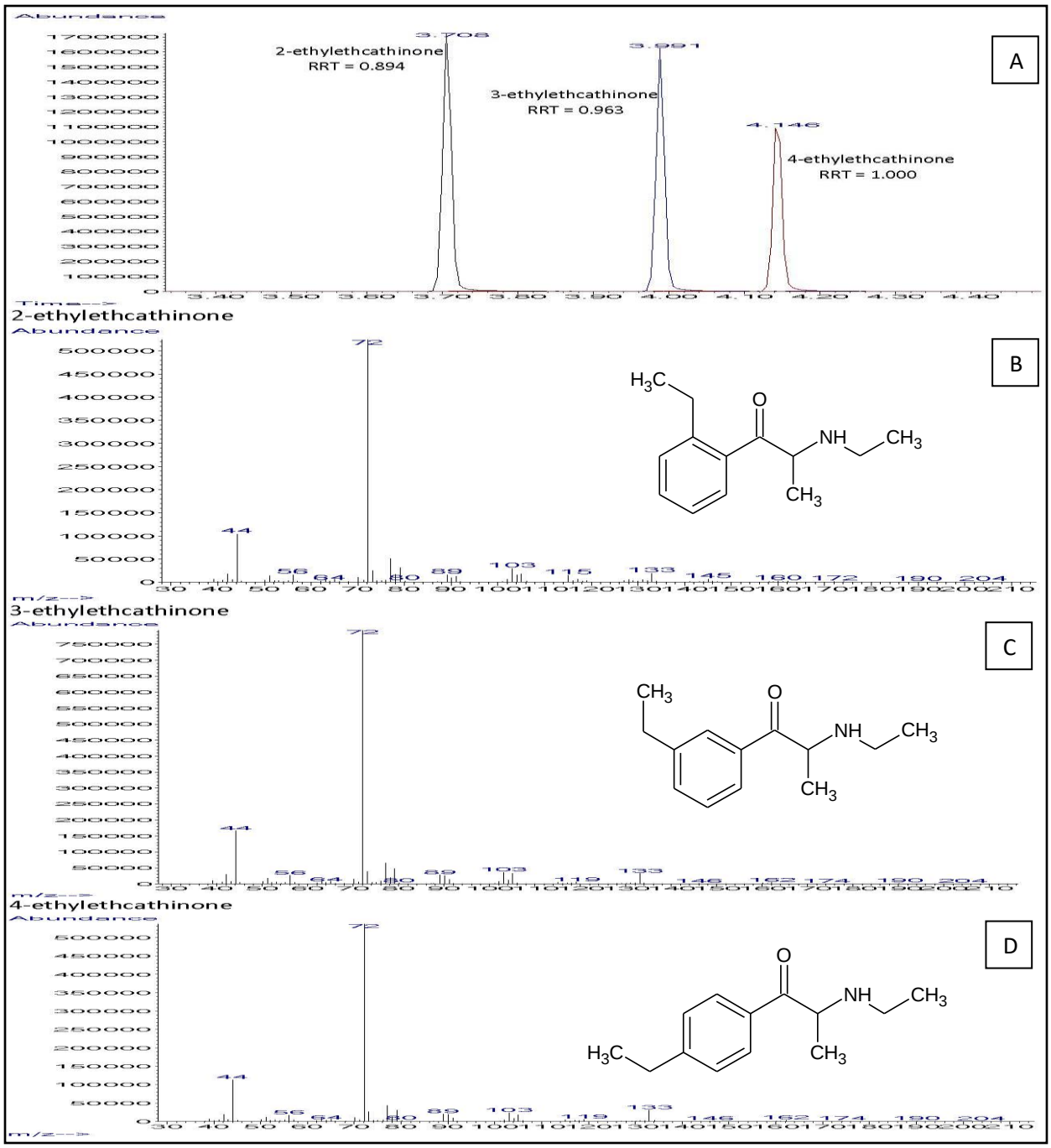

Figure 26: The chromatogram (A) and mass spectra of 2-ethylethcathinone (B), 3-ethylethcathinone (C), and 4-ethylethcathinone (D).

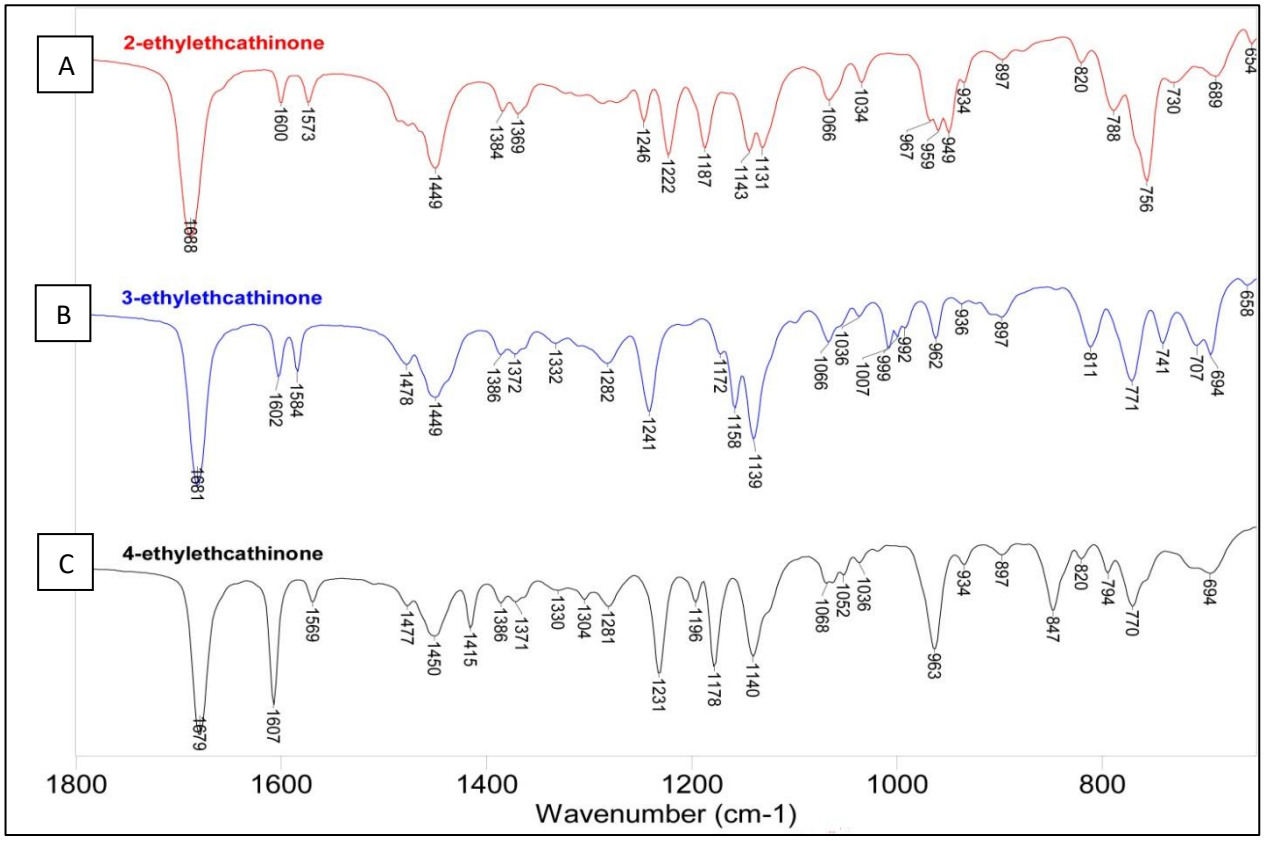

Figure 27: The IR Spectra, expanded for the fingerprint region, of 2-ethylmethcathinone (A), 3-ethylmethcathinone (B), and 4-ethylmethcathinone (C).

In summary, the mass spectra for all of the alkyl-substituted, cathinone, positional isomers were only able to distinguish between methyl- and ethyl-substituted homologues. For example, the methcathinones produced a strong ion at *m/*z 58 ($CH(CH_3)(NHCH_3)+$) consistent with fragmentation between the second and third carbon due to alpha cleavage from the amine, whereas the ethcathinones produced a strong ion at *m/z* 72 ($CH(CH_3)(NHCH_2CH_3)+$) which was also due to alpha cleavage. While all of positional isomers were resolved from one another chromatographically, a laboratory would still need to purchase all three reference materials to demonstrate chromatographic separation if they were to only use GC/MS.

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In contrast the IR spectra for each compound were specific enough in the fingerprint region so as to differentiate each isomer and homologue even though the spectral pattern for homologous compounds were visually similar. For instance, the spectra of 2-methylmethcathinone and 2-methylethcathinone contained distinct features (ex. 1237, 1229 cm^{-1}, the shoulder peak adjacent to 948 cm^{-1}, etc.), but the overall pattern of peaks in the finger print region are difficult to differentiate when first compared (Figure 28). Consequently, the orthogonal analyses of GC/IR and GC/MS, when combined provide strong and simple compound identification for ring substituted alkyl positional isomers and homologues of methcathinone.

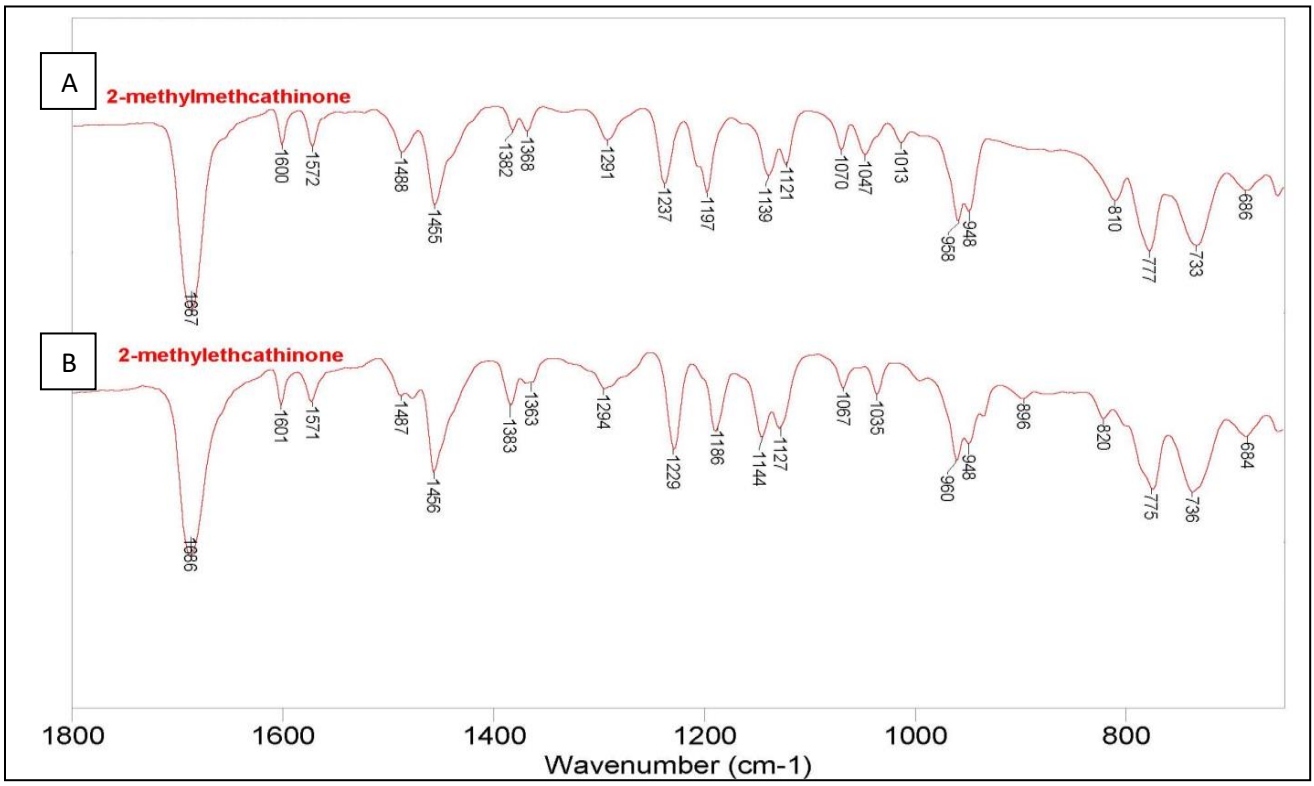

Figure 28: The IR Spectra, expanded for the fingerprint region, of 2-methylmethcathinone (A) and 2-methylethcathinone (B).

4.4.2 Naphthyl Isomers

The drug of abuse naphyrone and its isomers were federally controlled within the United States as of March 2017 (Drug Enforcement Administration, Department of Justice,

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2017). While both naphthrone isomers in this scenario are currently recognized as controlled substances, some isomers have different legal statuses making their specific detection impactful on legal outcomes. Previous research has used NMR and Ion Mobility Spectrometry (IMS) to differentiate the two isomers (Gwak & Almirall, 2015; Brandt, Wootton, De Paoli, & Freeman, 2010). The IMS requires reference materials to differentiate isomers and uses specific methods that do not translate directly to other IMS instruments. The naphthyl isomers

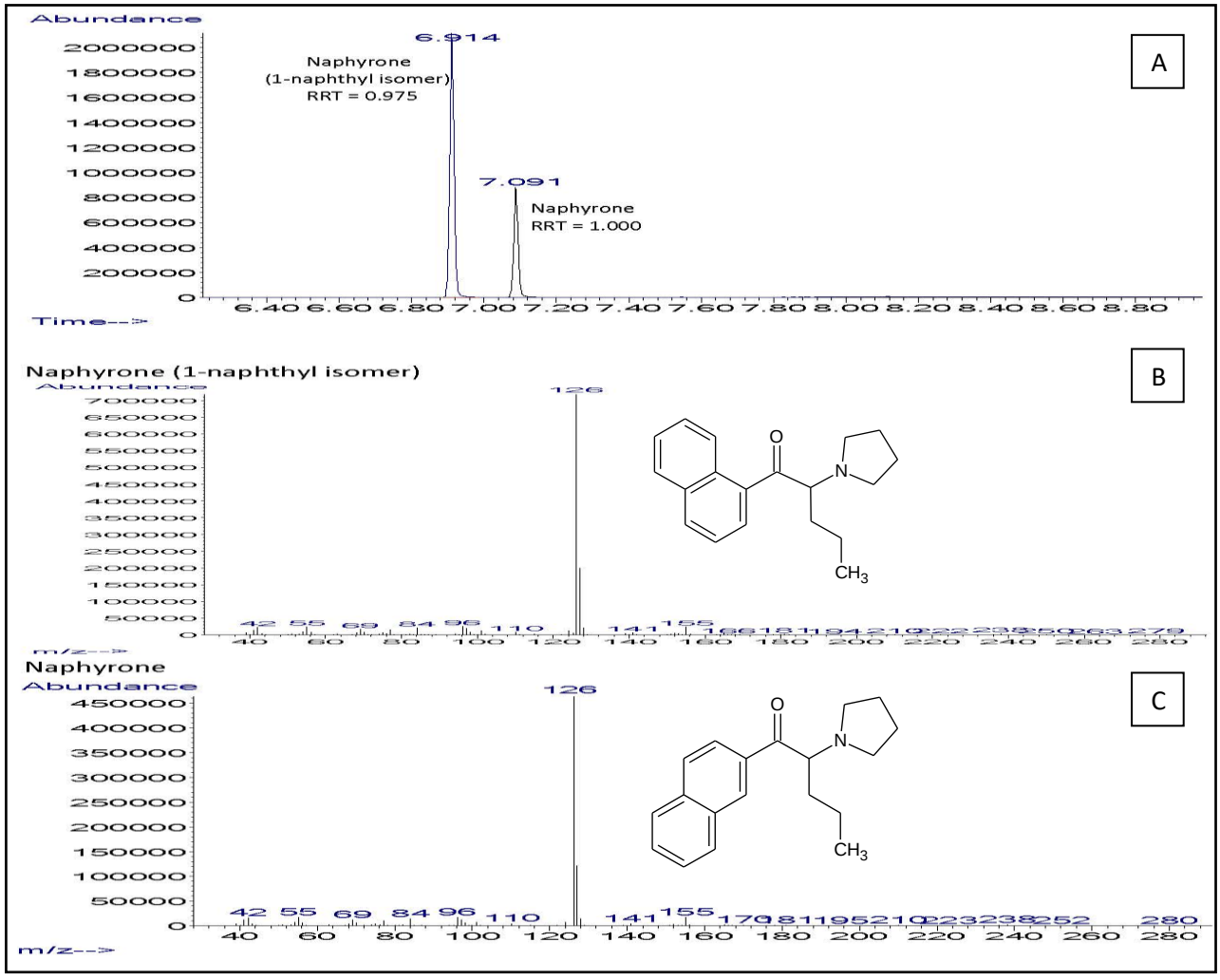

Figure 29: The chromatogram (A) and mass spectra of 1-naphthyl isomer of naphyrone (B) and naphyrone (C).

evaluated in this study had a pyrrolidine group that replaced the cathinone amine and a propyl group at R3 on the basic cathinone backbone (Figure 19). The bicyclic aromatic ring structure,

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naphthalene, can be described in relation to the basic cathinone backbone by the addition of a second aromatic ring at R4-R5 or R5-R6.

Using the GCMS1 method naphyrone and the 1-naphthyl isomer were resolved chromatographically (Figure 29). However, the mass spectra for both isomers were dominated by *m/z* 126 and could not be distinguished using ions of significant abundance. Conversely, the IR spectra for both isomers contained a number of distinct absorbance peaks (ex. 1628, 1598, 1593, 1293, 1288 cm^{-1}) that differentiated the isomers (Figure 30). The peaks at 1677 and 1680 cm^{-1} are characteristic of the strong absorbance peaks expected of aromatically conjugated ketones from 1650 to 1690 cm^{-1} (Silverstein, Webster, Kiemle, & Bryce, 2014).

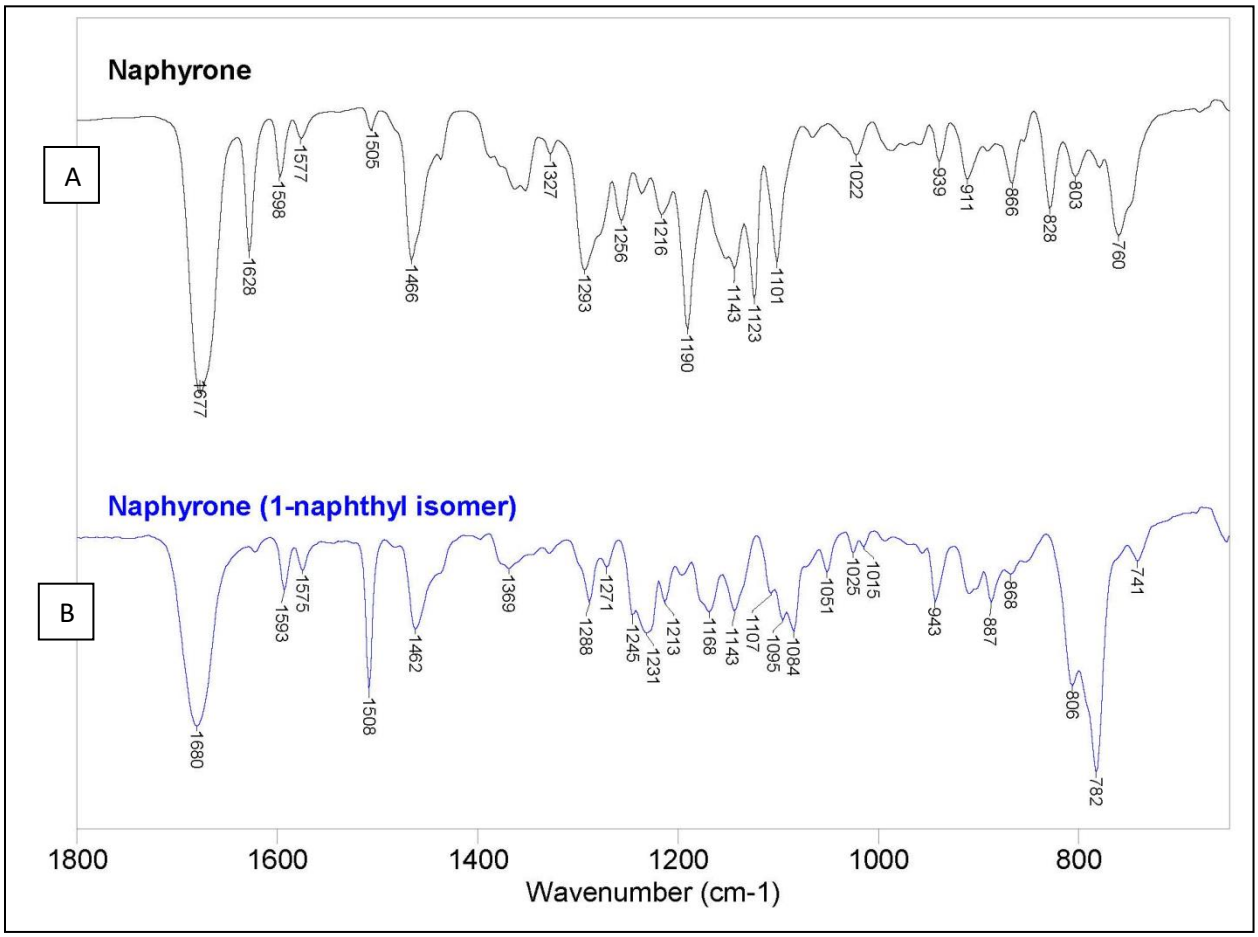

Figure 30: The IR Spectra, expanded for the fingerprint region, of Naphyrone (A) and its 1-naphthyl isomer (B).

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4.4.3 N-pyrrolidine Isomers

The N-pyrrolidine isomers, like naphryone, have a pyrrolidine group replacing the amine of the basic cathinone structure (Figure 19), but instead of a propyl group, there is either a methyl (α-Pyrrolidinopropiophenone) or ethyl (α-Pyrrolidinobutiophenone) group at R3. Additionally, N-pyrrolidine isomers are not bicyclic and consist of a single methyl substitution at the R4/R8, R5/R7, or R6 positions which are ortho-, meta- and para-, respectively (Figure 31). The para- substituted isomers are federally controlled substances within the United States (Drug Enforcement Administration, Department of Justice, 2017) and were therefore used to calculate the relative chromatographic retention times.

4.4.3A α-Pyrrolidinopropiophenone Isomers

As with for other ring substituted positional isomers, the α-Pyrrolidinopropiophenones were resolved chromatographically, but could not be distinguished using EI-MS (Figure 31). The mass spectra for all three α-Pyrrolidinopropiophenone isomers were dominated by *m/z* 98. All ions of significant relative abundance (ex. *m/z* 41, 56, 65, 91, 119) were observed at similar relative ratios in the spectra of each isomer making the EI-MS spectra unsuitable for isomer differentiation. However, the three isomers were easily differentiated from each other using infrared spectroscopy, as shown by absorption peaks at 744, 684 and 834 cm^{-1} (Figure 32) which were unique to 2-methyl-α-pyrrolidinopropiophenone, 3-methyl-α-pyrrolidinopropiophenone, and 4-methyl-α-pyrrolidinopropiophenone respectively.

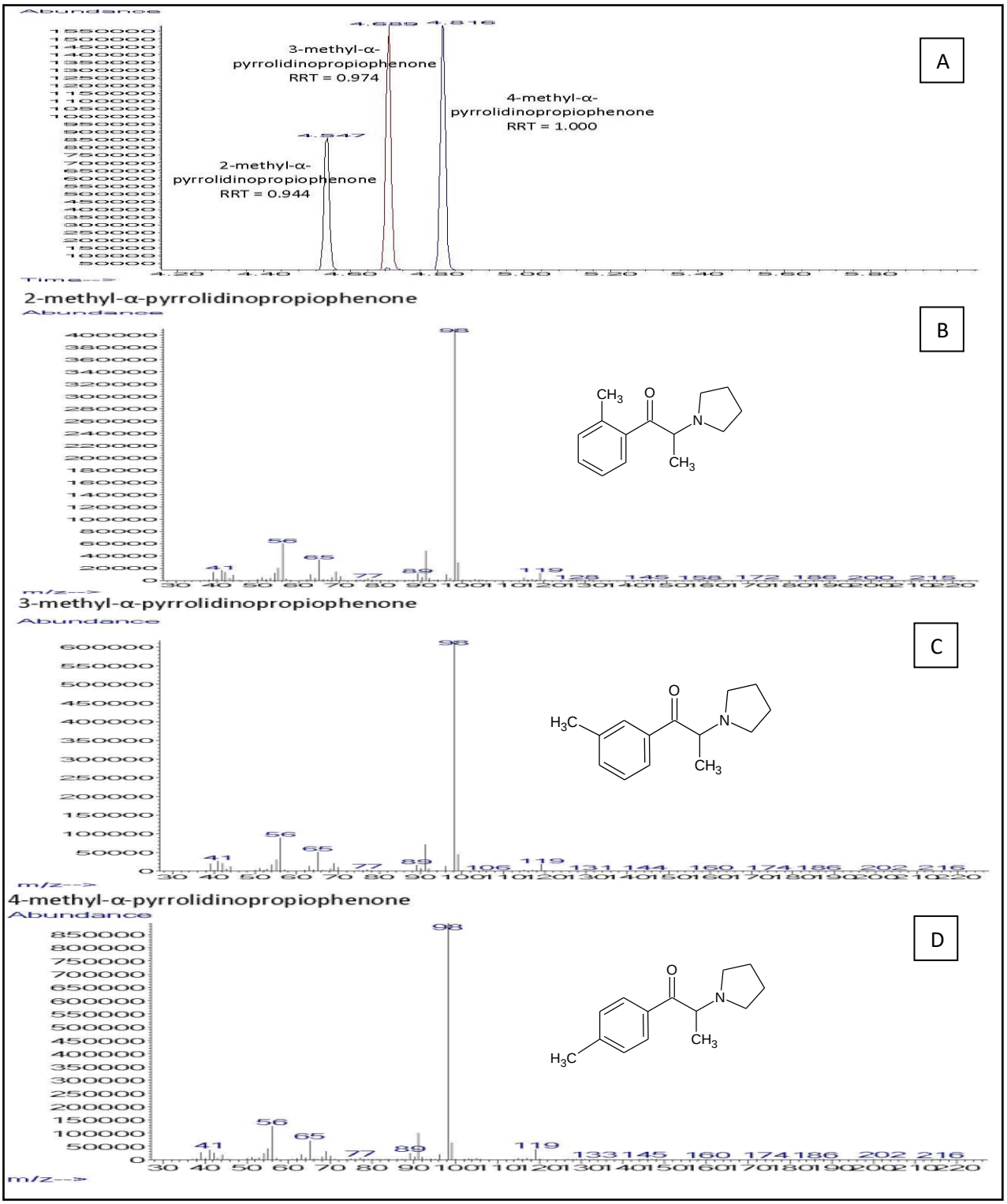

Figure 31: The chromatogram (A) and mass spectra of α-Pyrrolidinopropiophenone substitutions: 2-methyl-α-pyrrolidinopropiophenone (B), 3-methyl-α-pyrrolidinopropiophenone (C), and 4-methyl-α-pyrrolidinopropiophenone (D).

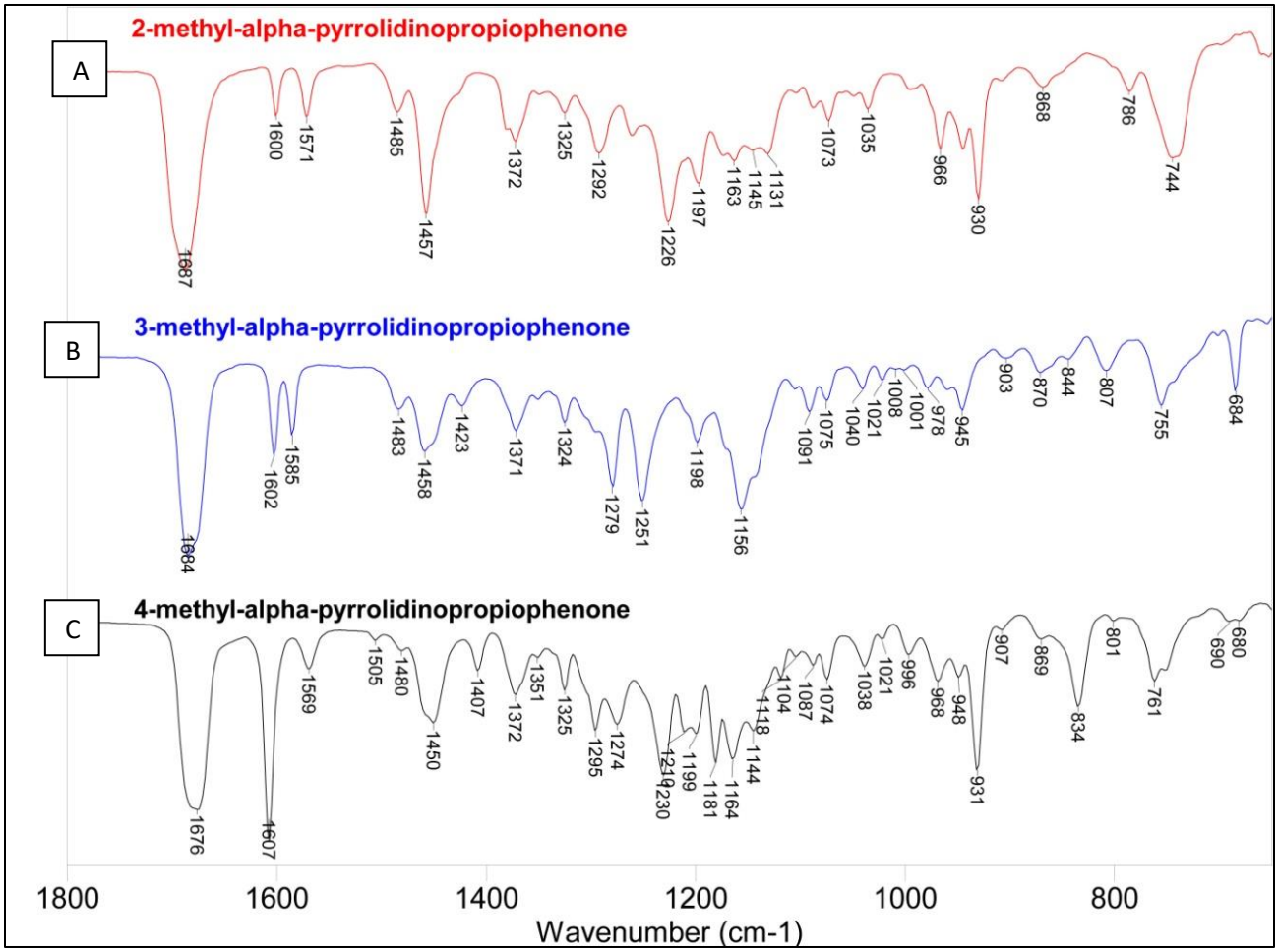

Figure 32: The IR Spectra, expanded for the fingerprint region, of α-Pyrrolidinopropiophenone substitutions: 2-methyl-α-pyrrolidinopropiophenone (A), 3-methyl-α-pyrrolidinopropiophenone (B), and 4-methyl-α-pyrrolidinopropiophenone (C).

4.4.3B α-Pyrrolidinobutiophenone Isomers

The α-Pyrrolidinobutiophenone isomers consisted of a methyl substituent at the ortho-, meta-, and para- positions of α-pyrrolidinobutiophenone. The α-Pyrrolidinobutiophenones were also successfully resolved chromatographically, but could not be distinguished using EI-MS (Figure 33). The mass spectra for all three α-Pyrrolidinobutiophenone isomers were dominated by *m/z* 112 and all ions of significant relative abundance (ex. *m/z* 41, 55, 65, 91, 121) were observed at similar relative ion-ratios. The three isomers were differentiated using

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infrared spectroscopy at 770, 686 and 1607 cm^{-1} (Figure 34) which were unique to the ortho-, meta- and para- substituted α-pyrrolidinobutiophenones respectively.

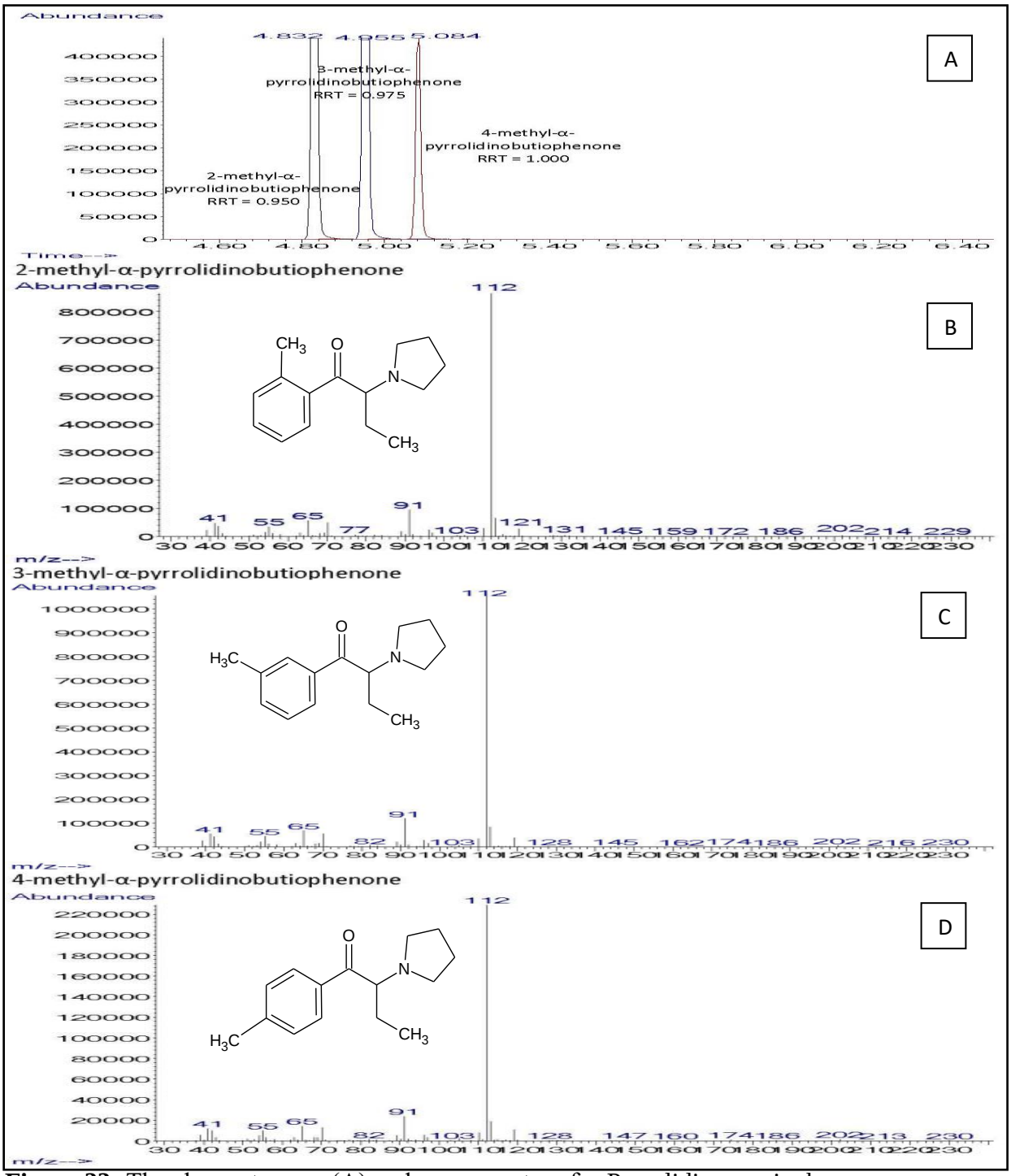

Figure 33: The chromatogram (A) and mass spectra of α-Pyrrolidinopropiophenone substitutions: 2-methyl-α-pyrrolidinobutiophenone (B), 3-methyl-α-pyrrolidinobutiophenone (C), and 4-methyl-α-pyrrolidinobutiophenone (D).

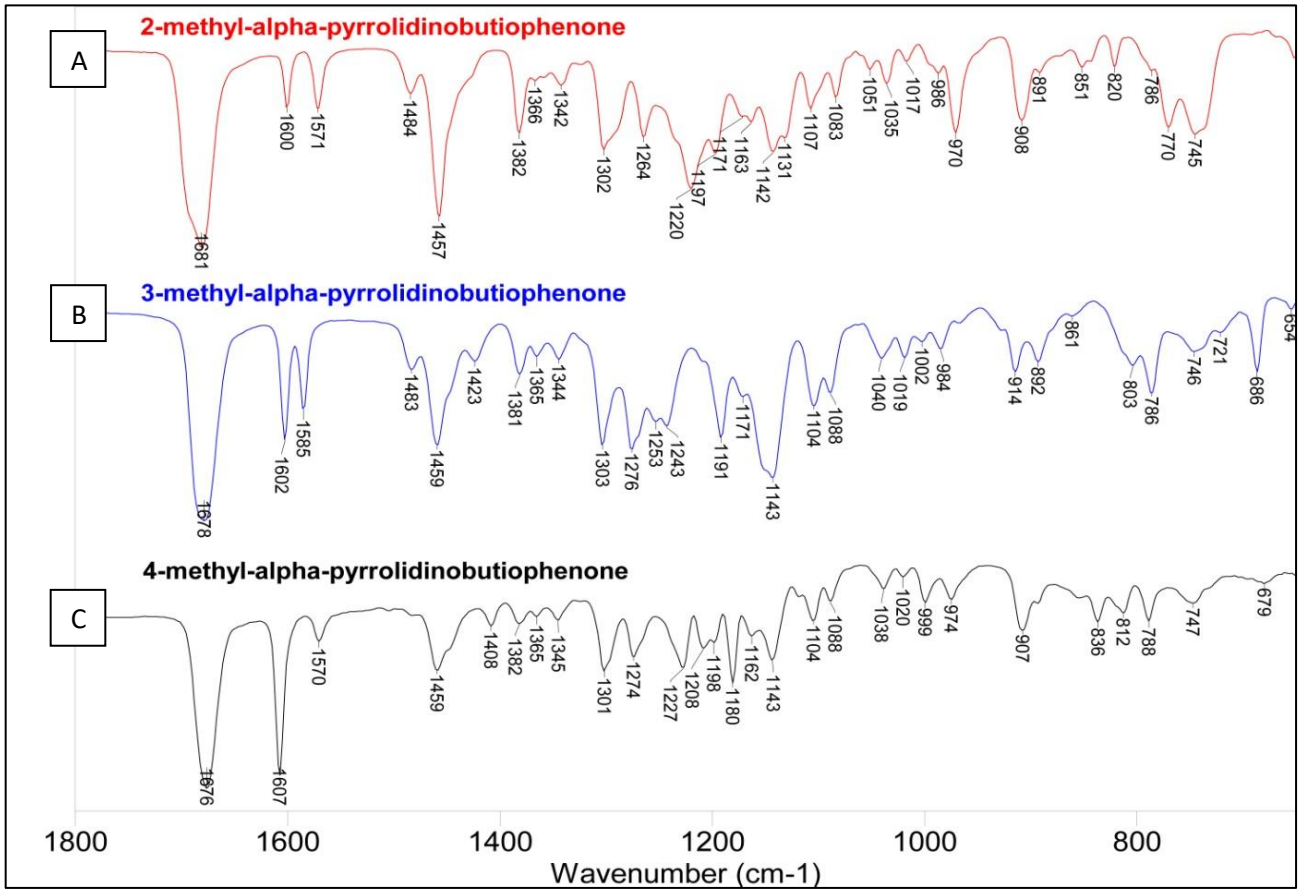

Figure 34: The IR Spectra, expanded for the fingerprint region, of α-Pyrrolidinobutiophenone substitutions: 2-methyl-α-pyrrolidinobutiophenone (A), 3-methyl-α-pyrrolidinobutiophenone (B), and 4-methyl-α-pyrrolidinobutiophenone (C).

In summary, all of the pyrrolidine isomers had similar behavior via GC/MS such that mass spectrometry was only able to differentiate between the α-pyrrolidinopropiophenone and α-pyrrolidinobutiophenone homologues. The base peaks for each homologue were separated by *m/z* 14, consistent with the insertion of CH_2 at R3 for the α-pyrrolidinobutiophenones. While all of positional isomers were resolved from one another chromatographically, a laboratory would still need to purchase all three reference materials to demonstrate chromatographic separation if they were to only use GC/MS.

Although visually similar, the spectra in the fingerprint region was sufficient to differentiate between all isomers and homologues. For instance, the spectra of the homologues

2-methyl-α-pyrrolidinopropiophenone and 2-methyl-α-pyrrolidinobutiophenone contained distinct features (ex. 770, 930, 1017, and 1107 cm^{-1}), but much of the overall pattern of peaks in the finger print region (Figure 35) had similar abundances and vibrational energies (ex. 1600 and 1571 cm^{-1}). Consequently, the orthogonal analyses of GC/IR and GC/MS, when combined provide strong and simple compound identification for pyrrolidine positional isomers and homologues.

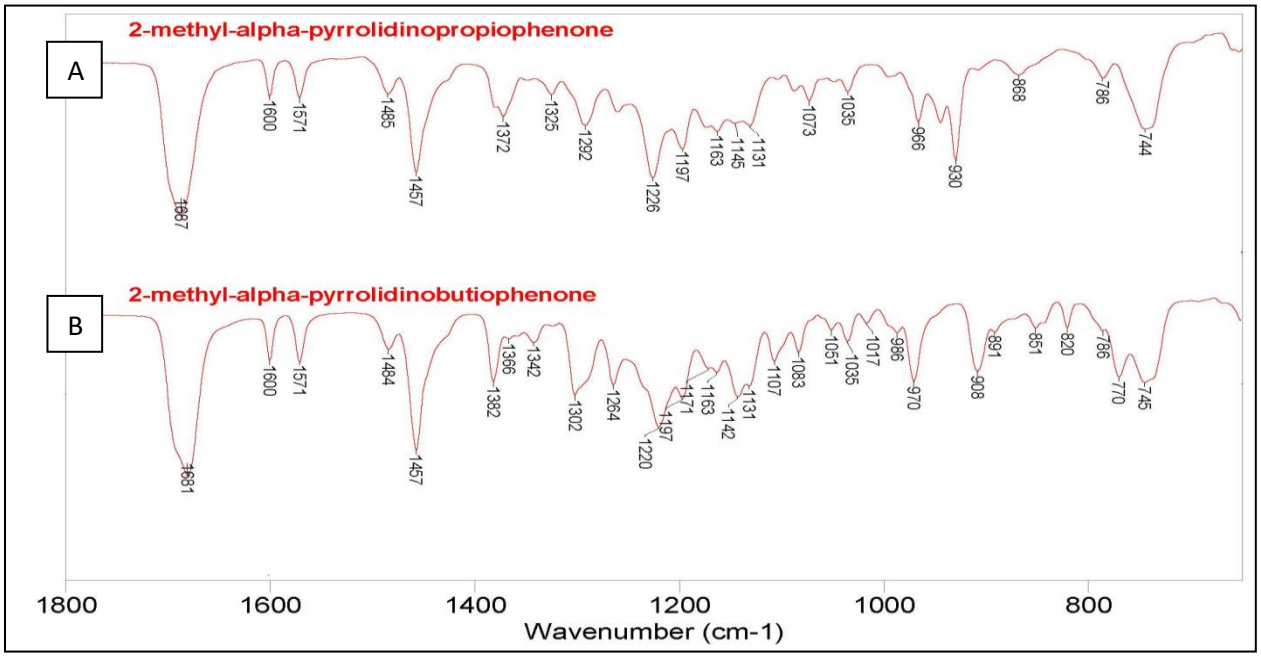

Figure 35: The IR Spectra, expanded for the fingerprint region, of 2-methyl-α-pyrrolidinopropiophenone (A) and 2-methyl-α-pyrrolidinobutiophenone (B).

4.4.4 Alkyl and Ring Methylenedioxy Isomers

The alkyl and ring methylenedioxy isomers have a methylenedioxy functionality attached at either the R6 and R7 positions or the R7 and R8 positions (Figure 19). Additionally, varying alkyl groups (methyl-, ethyl-, propyl-) can be found at the R1-R3 positions. The most commonly observed substances of abuse of the following groupings are butylone, eutylone, pentylone, and methylone (Figure 36). Consequently, these four methylenedioxy compounds were used to calculate relative retention times.

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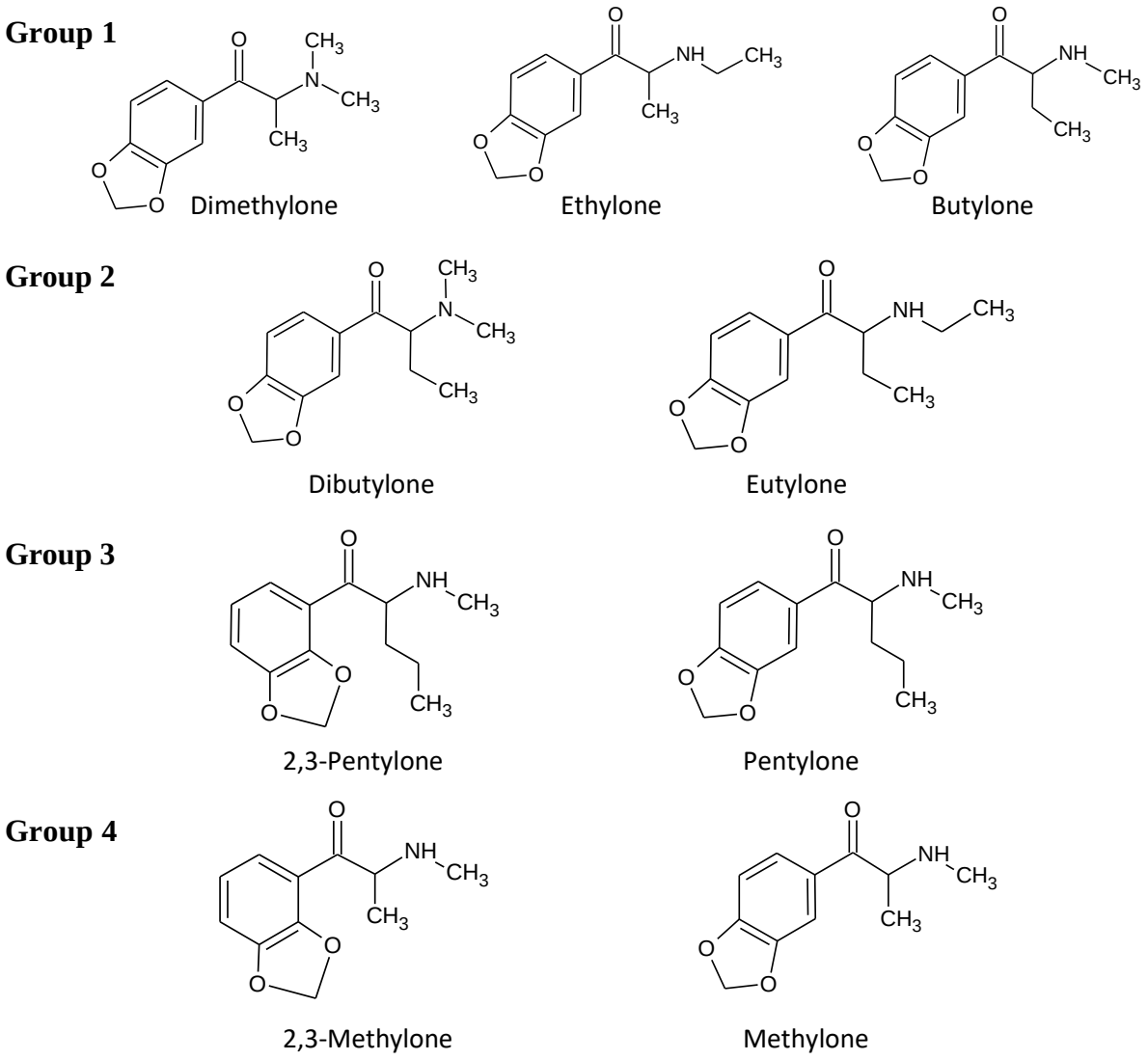

Figure 36: The structures of the methylenedioxy cathinones divided into four groups of isomers.

4.4.4A Methylenedioxy Cathinones: Group 1

While butylone and dimethylone could not be differentiated from ethylone by retention times alone (GCMS1 method) as their relative retention times differed by less than two percent (Figure 37). The mass spectra share all significant ions but the relative abundances of *m/z* 44 and 57 varied by more than 20 % such that they might be used to build an ion ratio based method to differentiate all three isomers. However, as noted previously, ion-ratio based

methods require significant precision and all three analyte standards, such that GC/IR is still an advantageous alternative approach.

While the relative retention times were not significantly resolved to use retention time matching to identify each compound, the peaks displayed baseline separation and therefore any resulting IR spectra can be ascribed to a single component. Additionally, it has been observed (data not shown) that drugs of abuse typically do not come in isomeric mixtures. Using GC/IR the three isomers were differentiated by the resulting spectra (Figure 38). Among the differences noted, the peaks at 1675, 1353, and 834 cm^{-1} were unique to dimethylone, ethylone, and butylone respectively. If however chromatographic resolution were lost and a sample contained a mixture of isomers, the unique absorption peaks for each isomer would enable spectral deconvolution to differentiate group one methylenedioxy cathinones isomers. Many absorbance peaks were common between the spectra as well, of which those strong peaks centered about 1250 cm^{-1} are characteristic of aromatic ether groups as found in the methylenedioxy functionality (Silverstein, Webster, Kiemle, & Bryce, 2014).

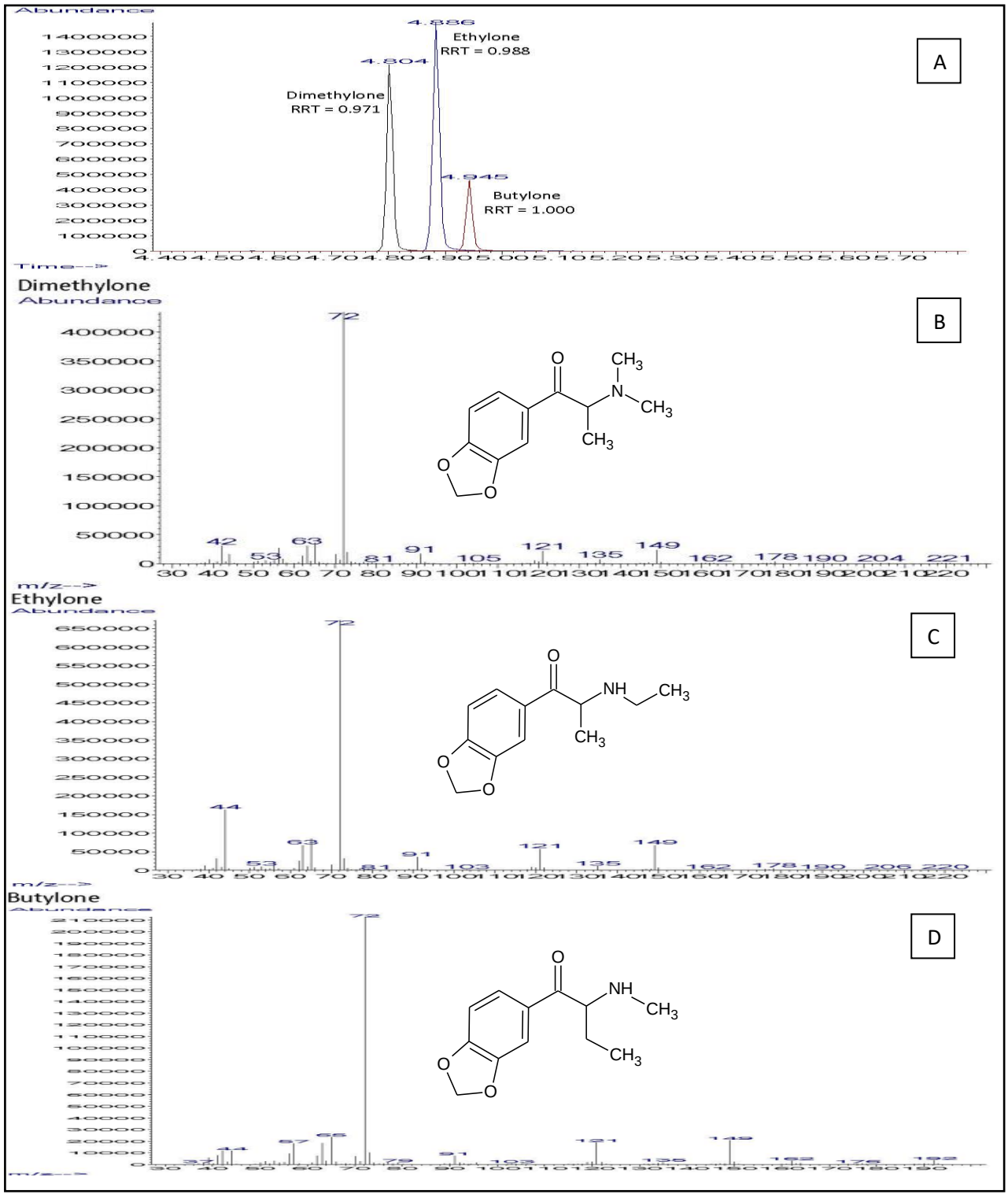

Figure 37: The chromatogram (A) and mass spectra of methylenedioxy cathinone group 1 isomers: dimethylone (B), ethylone (C), and butylone (D).

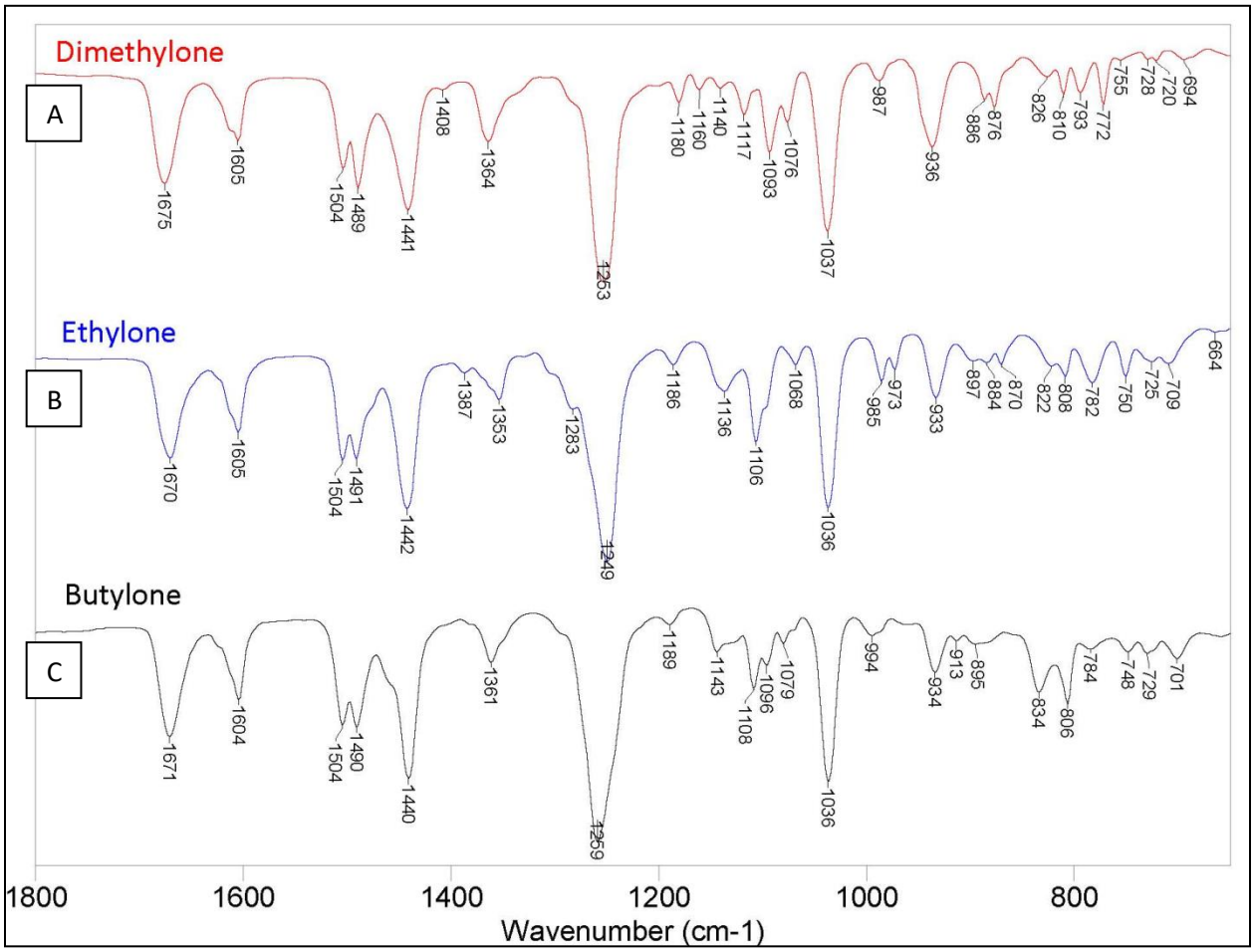

Figure 38: The IR Spectra, expanded for the fingerprint region, of methylenedioxy cathinone group 1 isomers: dimethylone (A), ethylone (B), and butylone (C).

4.4.4B Methylenedioxy Cathinones: Group 2

Just as with group 1 compounds, using the GCMS1 method, dibutylone and eutylone could not be differentiated by retention time alone (despite baseline separation) as their relative retention times differed by less than two percent (Figure 39). While the resulting mass spectra also shared most significant ions, the relative abundances of several ions (ex. *m/z* 65 and 71) varied by more than 20 % such that they might be differentiated by an ion-ratio based method.

Baseline separated group 2 compounds enabled simple, single-component GC/IR spectra interpretation. Using GC/IR, both compounds were differentiated by the resulting infrared spectra (Figure 40). For instance, the peaks at 1361, 1260, 1247, and 1094 cm^{-1} are

characteristic of dibutylone and the peaks at 1354, 1253, and 1106 cm^{-1} are characteristic of eutylone.

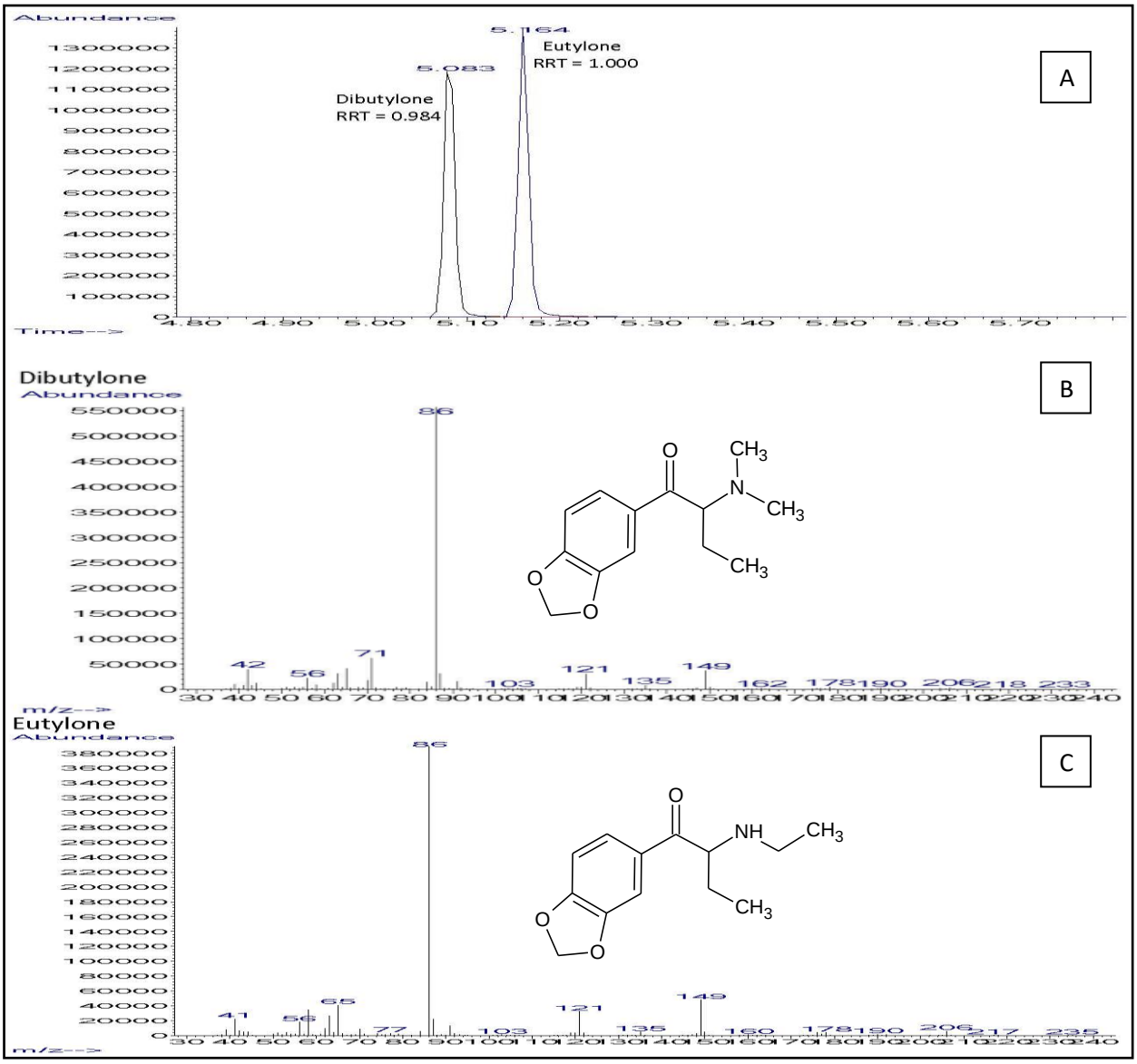

Figure 39: The chromatogram (A) and mass spectra of methylenedioxy cathinone group 2 isomers: dibutylone (B), and eutylone (C).

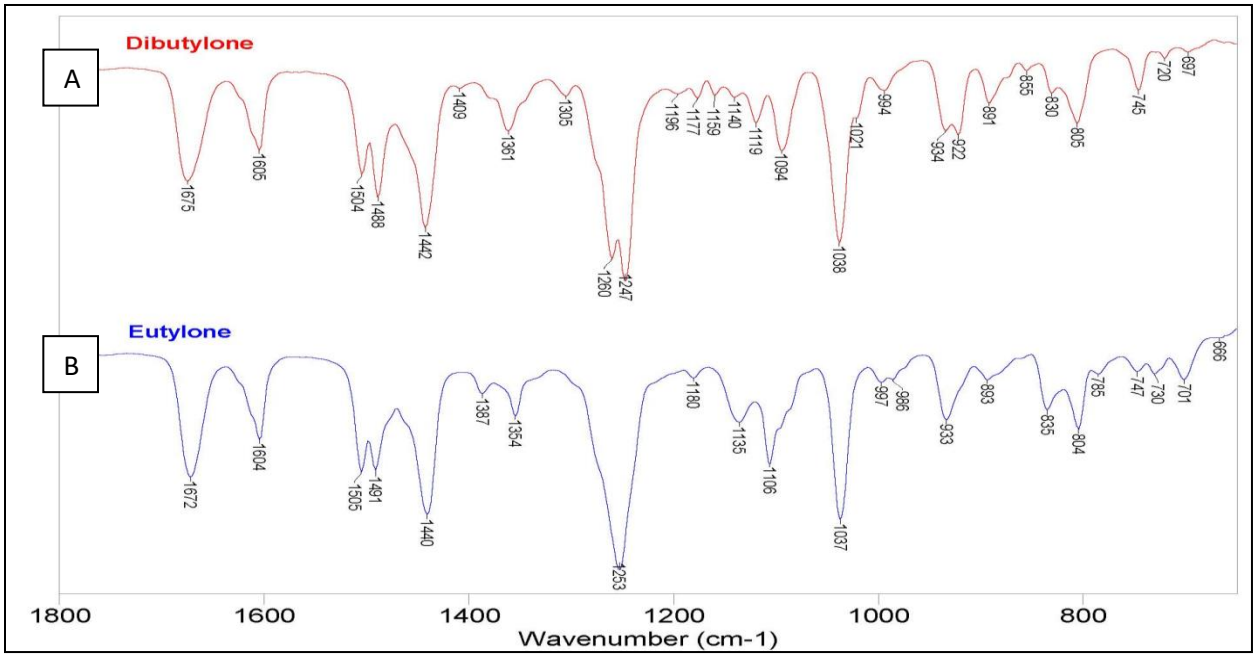

Figure 40: The IR Spectra, expanded for the fingerprint region, of methylenedioxy cathinone group 2 isomers: dibutylone (A), and eutylone (B).

4.4.4C Methylenedioxy Cathinones: Group 3

Pentylone was differentiated from its 2,3-methylenedioxy isomer using gas chromatography (Figure 41), but the mass spectra were equivalent for all significant ions and their relative ratios. However, pentylone was easily distinguished from its 2,3-methylenedioxy isomer using infrared spectroscopy (Figure 42). Among the many differences observed, the peaks at 1256 and 1452 cm^{-1} were unique to pentylone and 2,3 pentylone respectively.

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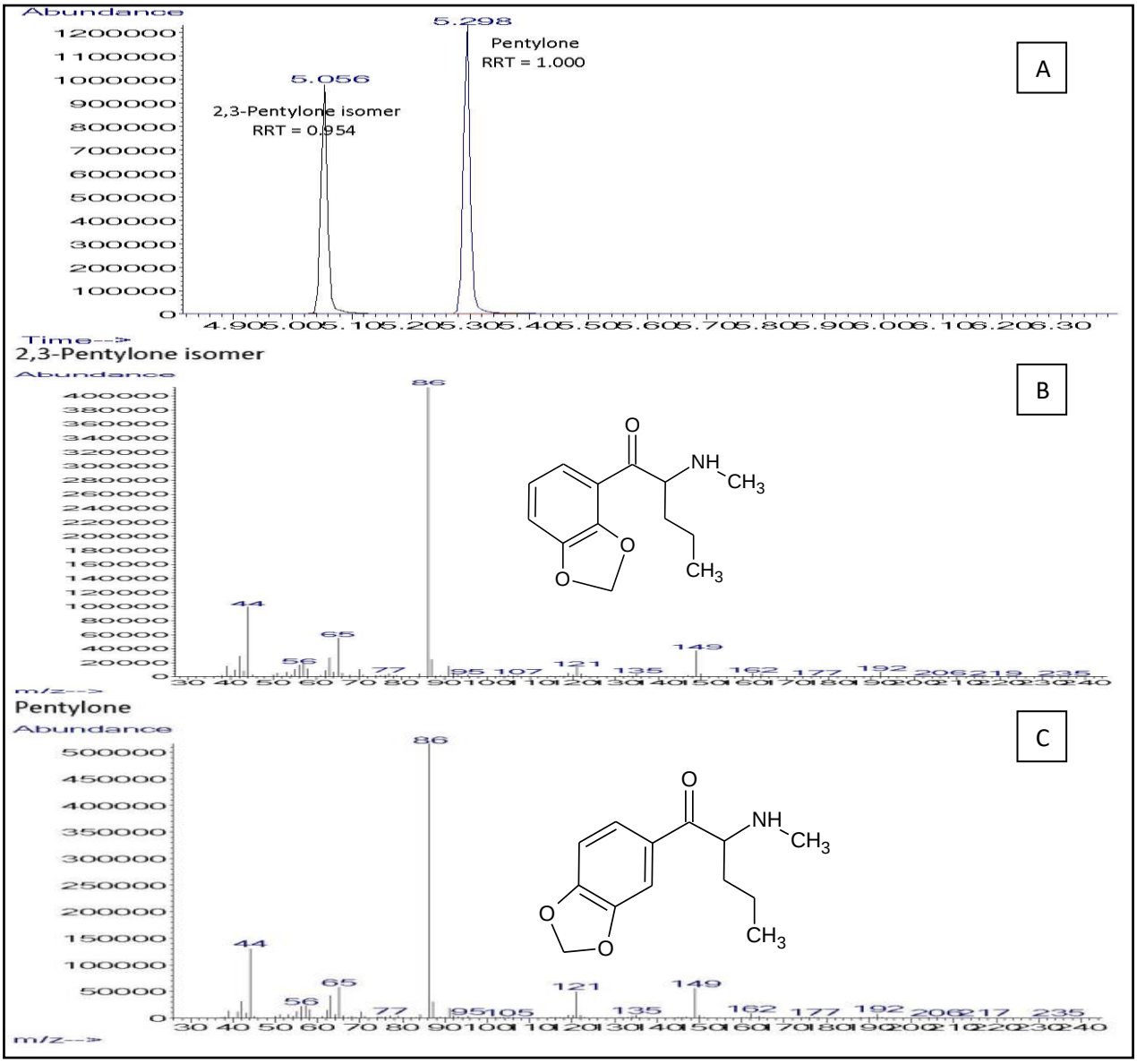

Figure 41: The chromatogram (A) and mass spectra of methylenedioxy cathinone group 3 isomers: 2,3 pentylone (B), and pentylone (C).

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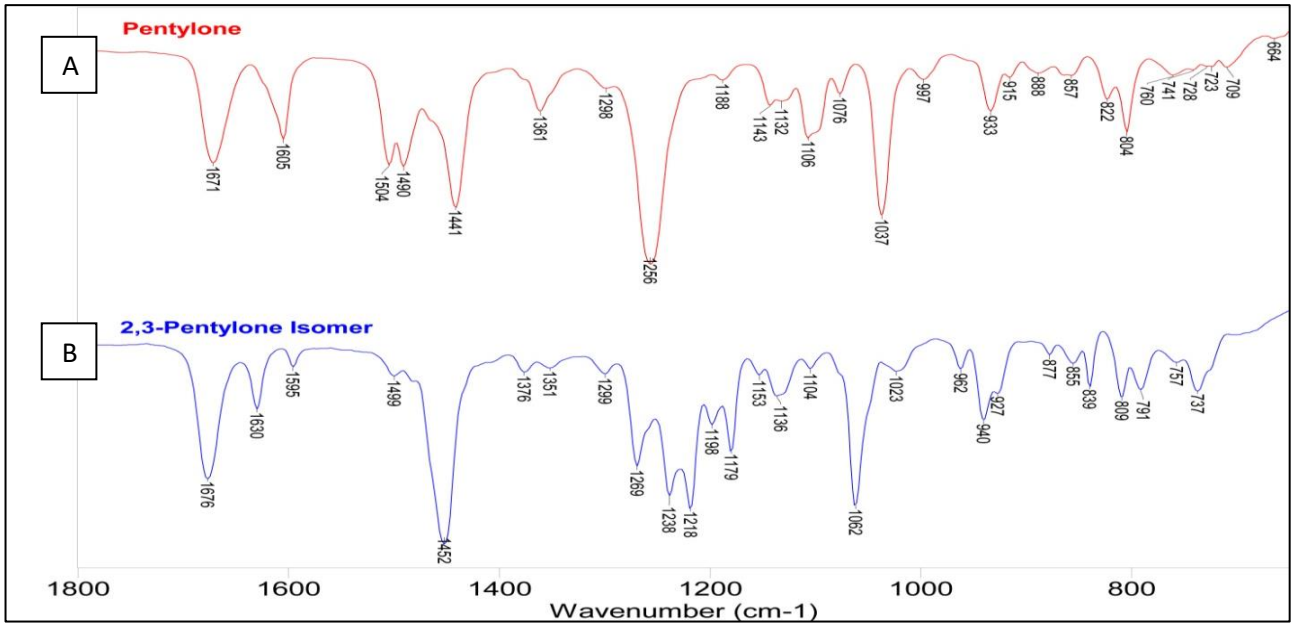

Figure 42: The IR Spectra, expanded for the fingerprint region, of methylenedioxy cathinone group 3 isomers: pentylone (A) and the 2,3-isomer (B).

4.4.4D Methylenedioxy Cathinones: Group 4

Methylone and its 2,3-methylenedioxy isomer were differentiated using gas chromatography, but the accompanying mass spectra could not be distinguished as the relative abundance of all significant ions were mirrored closely in each spectra (Figure 43). However, methylone was easily distinguished from its 2,3-methylenedioxy isomer using infrared spectroscopy (Figure 44). The peaks at 1453 and 1252 cm^{-1} were unique to 2,3 methylone and methylone respectively.

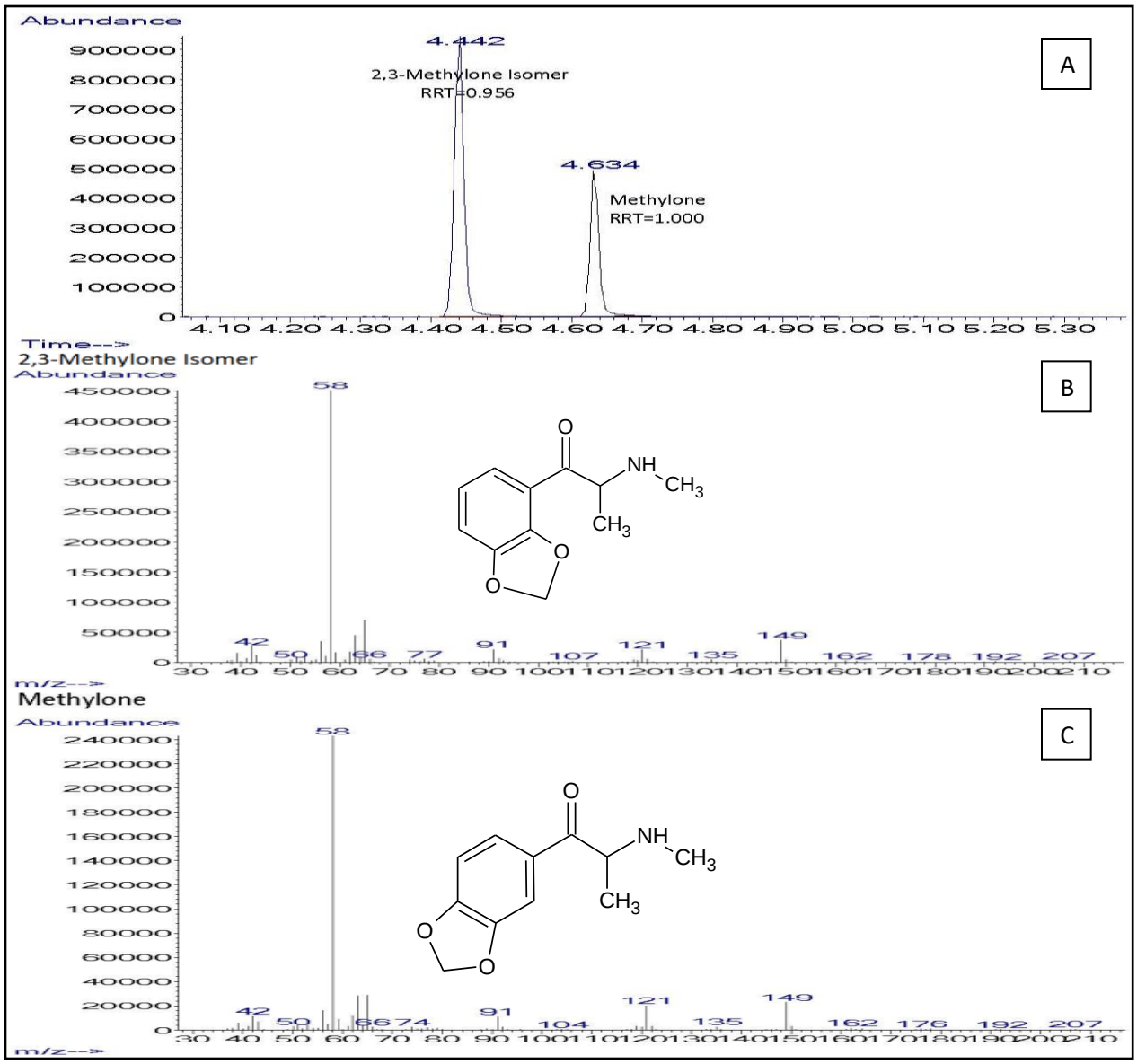

Figure 43: The chromatogram (A) and mass spectra of methylenedioxy cathinone group 4 isomers: 2,3 methylone (B), and methylone (C).

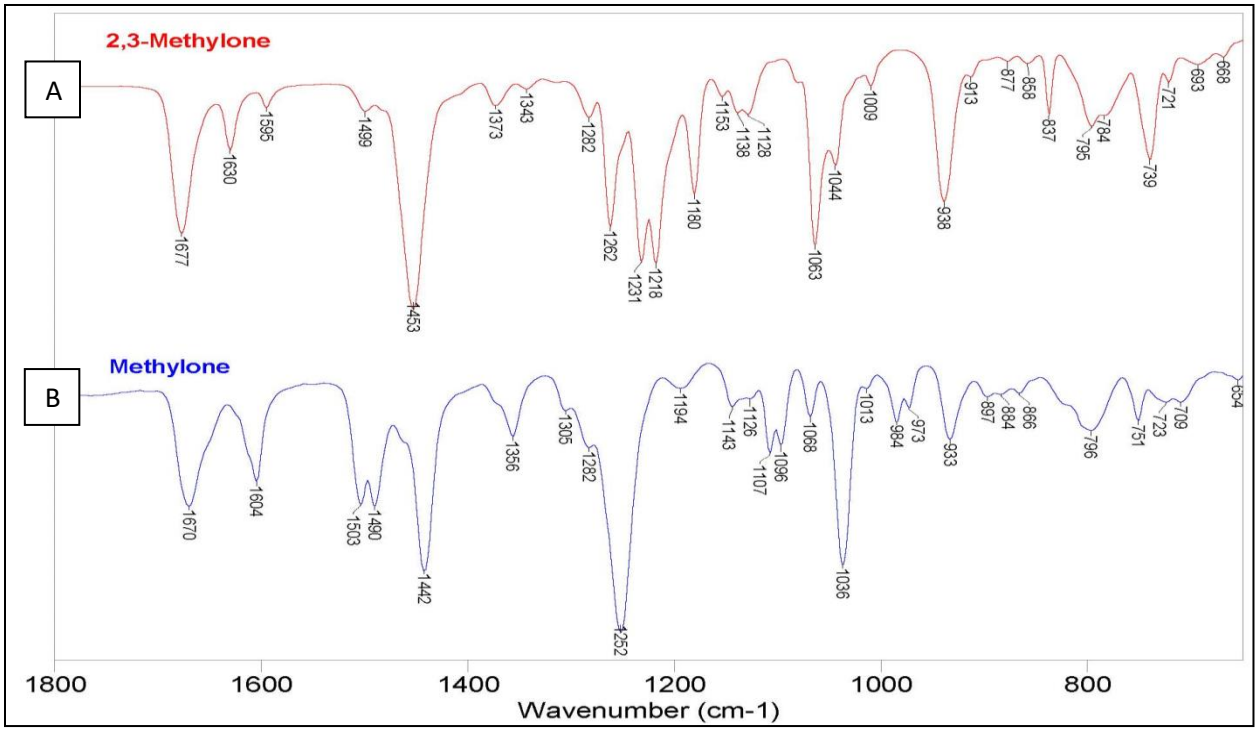

Figure 44: The IR Spectra, expanded for the fingerprint region, of methylenedioxy cathinone group 4 isomers: 2,3 methylone (A) and methylone (B).

4.4.5 Fluorinated Cathinone Isomers

The cathinone fluoro isomers contain a fluorine substituent at the R4-R6 (ortho-, meta- and para-) positions and either a methyl or ethyl group at R2 (Figure 19). The fluoro isomers were of interest given the impact that strong electrophiles like fluorine have on mass spectra and characteristic infrared absorptions (Silverstein, Webster, Kiemle, & Bryce, 2014). Relative retention times were calculated in reference to the para- substituted isomers. The para-substitution of the methyl amine (4-fluoromethcathinone) and its positional isomers are currently listed as Schedule I controlled substances under United States federal law (Drug Enforcement Administration, Department of Justice, 2017).

4.4.5A Fluoromethcathinone Isomers

The isomers in this group consist of a fluorine substituent at the ortho-, meta- and para-positions of fluoromethcathinone (Figure 45). The three fluoromethcathinone positional

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isomers were not resolved chromatographically using the GCMS1 method. The mass spectra were likewise indistinguishable for all significant ions (>1 % relative to base peak, ex. *m/z* 42, 50, 58, 68, 75, 95, and 123). The base peak at *m/z* 58 represents the expected fragmentation

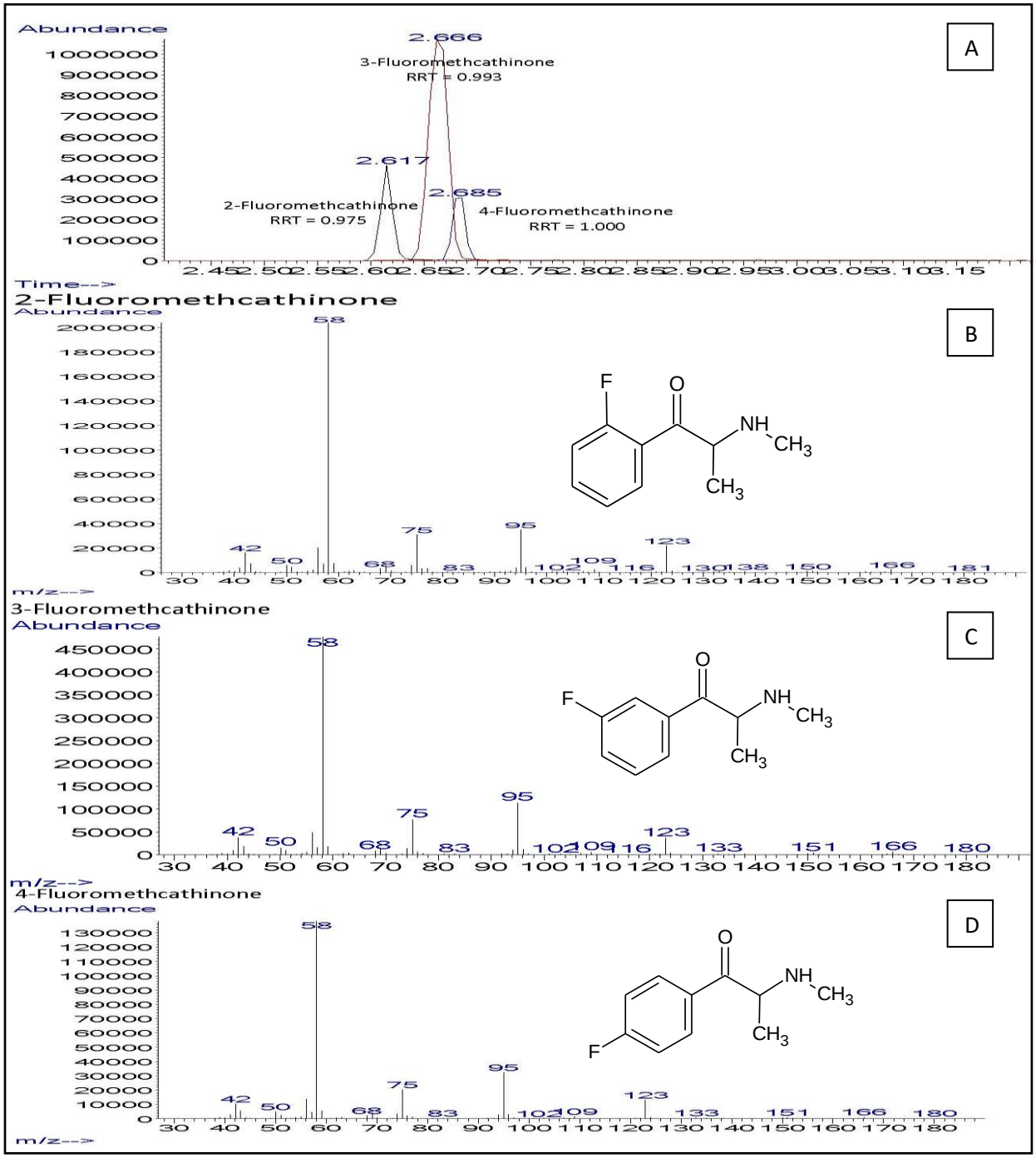

Figure 45: The chromatogram (A) and mass spectra of fluoromethcathinone isomers: 2-fluoromethcathinone (B), 3-fluoromethcathinone (C) and 4-fluoromethcathinone (D).

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between the ketone and the secondary amine ($CH(CH_3)(NHCH_3)+$) due to the stabilizing resonance between the aromatic ring and the alpha ketone.

Using GC/IR the three flouromethcathinone isomers were differentiated by the resulting spectra (Figure 46) which contained a number of unique absorption peaks (ex. 1693, 1688, and 1683 cm^{-1}). The strong 1252 and 1230 cm^{-1} absorptions for 3-fluoromethcathinone and 4-fluoromethcathinone respectively are typical stretches of fluorine bonded to aromatic rings (Silverstein, Webster, Kiemle, & Bryce, 2014). The lack of a similarly strong absorption for 2-fluoromethcathinone is likely due to the proximity of another electron withdrawing group (Silverstein, Webster, Kiemle, & Bryce, 2014).

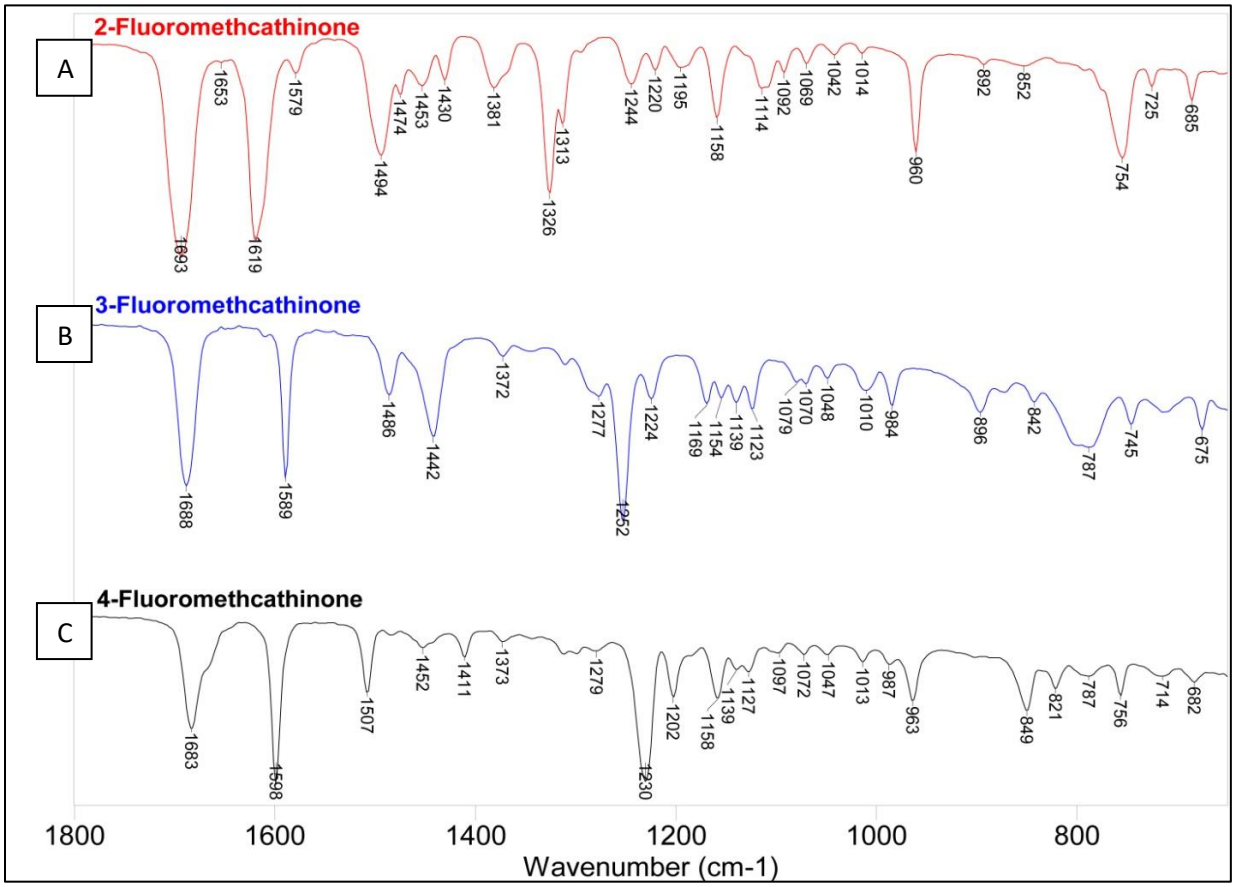

Figure 46: The IR Spectra, expanded for the fingerprint region, of fluoromethcathinone isomers: 2-fluoromethcathinone (A) 3-fluoromethcathinone (B), and 4-fluoromethcathinone (C).

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As the meta- and para- fluoromethcathinone isomers were not chromatographically baseline resolved (Figure 45), the interpretation of unknown sample IR spectra cannot be assumed to result from an individual compound. Nevertheless, unique absorption peaks from single component reference spectra enable future spectral deconvolution of potential isomer mixes.

4.4.5B Fluoroethcathinone Isomers

The fluoroethcathinone isomers consist of a fluorine substituent at the ortho-, meta- and para- positions of fluoroethcathinone (Figure 47). The three fluoroethcathinone positional isomers were not resolved by GC/MS and the mass spectra were indistinguishable for all significant ions (ex. *m/z* 44, 56, 95, 109, and 123). The base peak at *m/z* 72 represents the expected fragmentation between the ketone and the secondary amine ($CH(CH_3)(NHCH_2CH_3)+$) and is *m/z* 14 larger (typical of an additional CH_2) than the base peak for the fluoromethcathinone homologues.

Using GC/IR, the three fluoroethcathinone isomers were differentiated and the resulting spectra (Figure 48) contained a number of unique absorption peaks (ex. 1619, 1589, and 1599 cm^{-1}). The strong 1248 and 1228 cm^{-1} absorptions for 3-fluoroethcathinone and 4-fluoroethcathinone respectively, are typical stretches of fluorine bonded to aromatic rings (Silverstein, Webster, Kiemle, & Bryce, 2014). The lack of a similarly strong absorption for 2-fluoromethcathinone is likely due to the proximity of another electron withdrawing group (Silverstein, Webster, Kiemle, & Bryce, 2014).

As the meta- and para- fluoromethcathinone isomers were not chromatographically baseline resolved (Figure 47) the interpretation of an unknown sample's IR spectra cannot be assumed to result from an individual compound. Nevertheless, despite poor chromatographic

resolution, observing a lack of overlapping peaks unique to different fluoromethcathinone isomers would indicate isomeric purity.

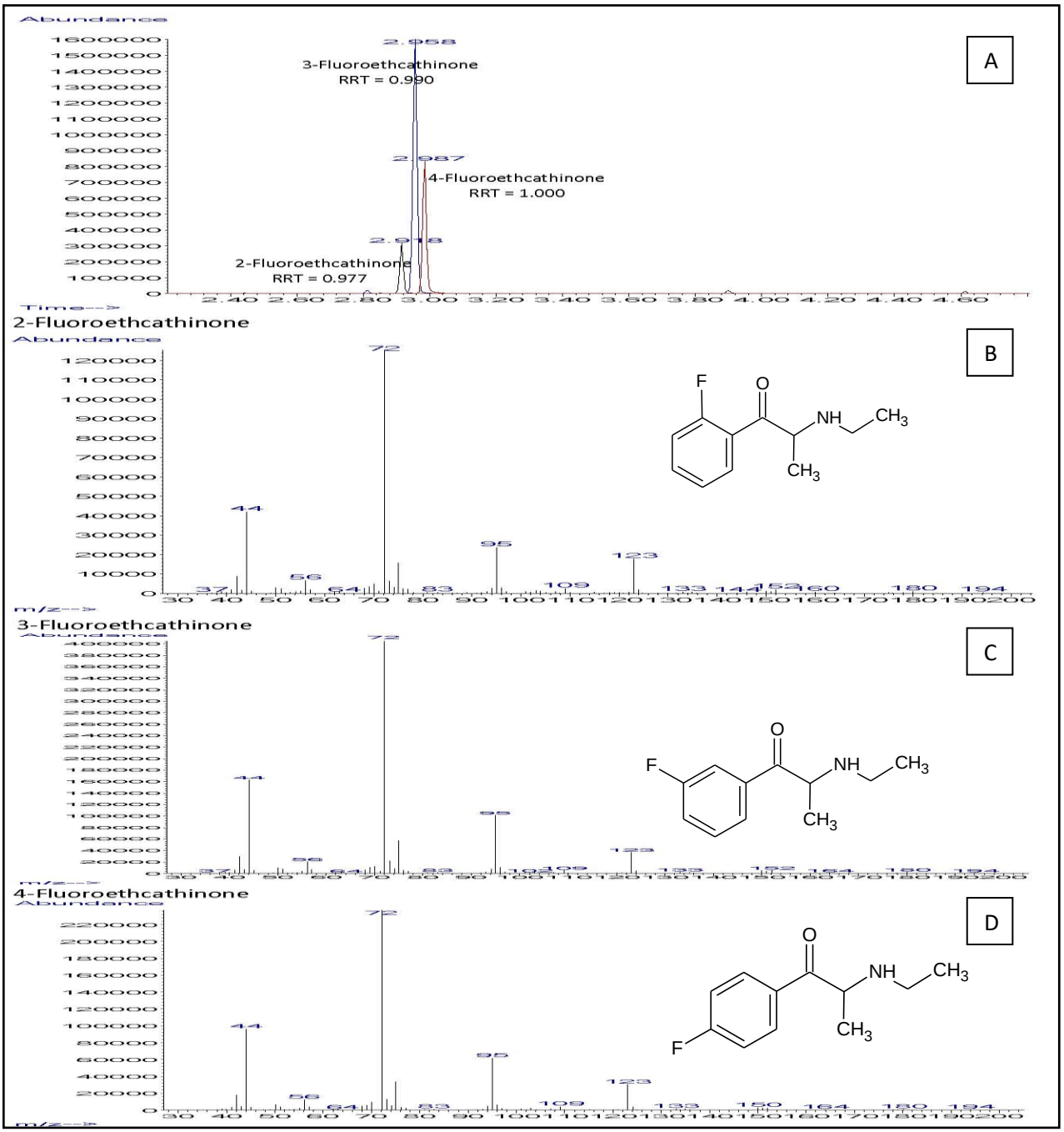

Figure 47: The chromatogram (A) and mass spectra of fluoroethcathinone isomers: 2-fluoroethcathinone (B), 3-fluoroethcathinone (C) and 4-fluoroethcathinone (D).

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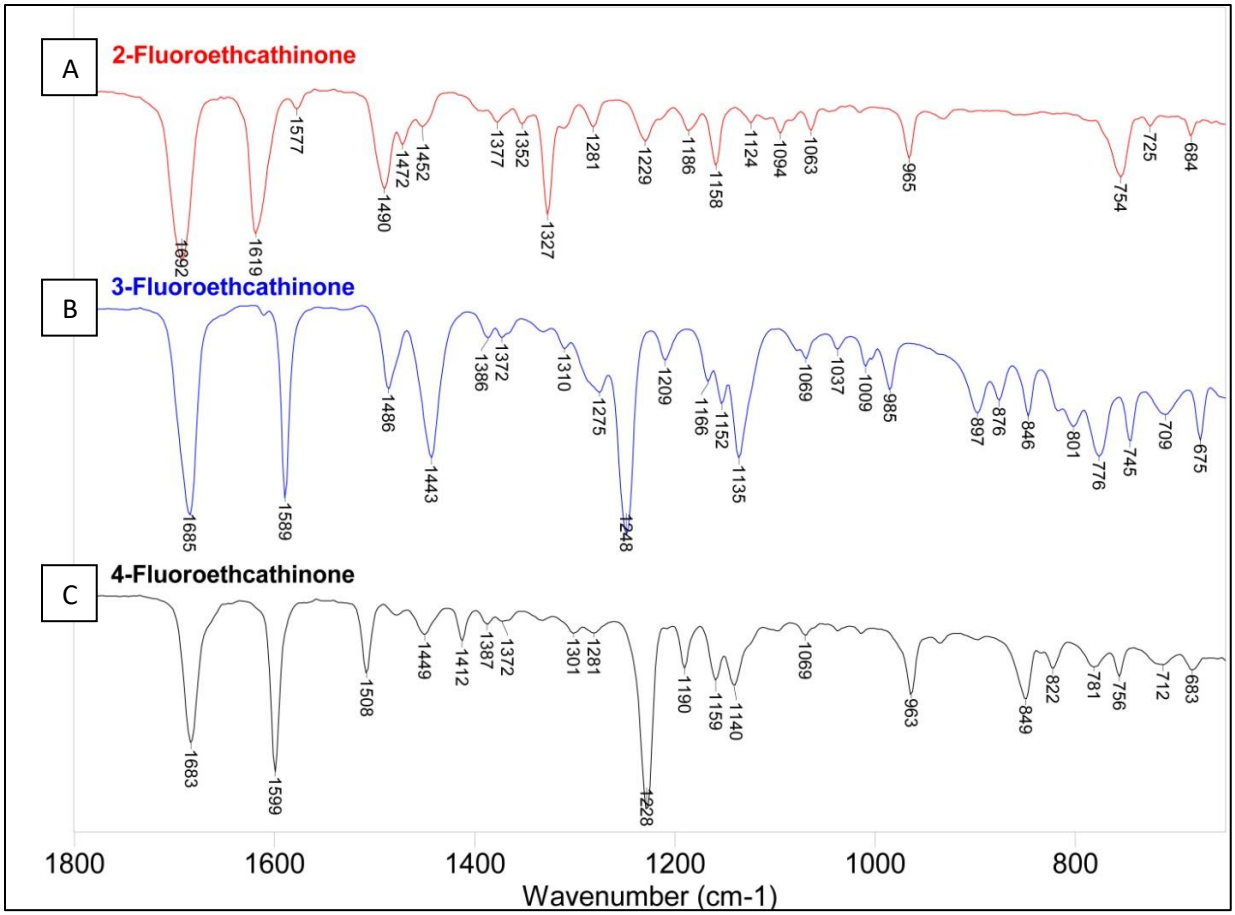

Figure 48: The IR Spectra, expanded for the fingerprint region, of fluoroethcathinone isomers: 2-fluoroethcathinone (A) 3-fluoroethcathinone (B), and 4-fluoroethcathinone (C).

In summary, all of the fluorinated cathinone isomers had similar behavior via GC/MS such that only the homologues of fluoromethcathinone and fluoroethcathinone could be resolved. The base peaks for each homologue were separated by *m/z* 14 consistent with the addition of CH_2 at R2 for the ethyl substituted on the amine (fluoroethcathinone). Due to the observed lack of chromatographic resolution between fluorinated isomers GC/MS provides only minimal discriminating power.

Though the IR spectra exhibited strong similarities due to the compounds' similar structures, the IR spectra in the fingerprint region were specific enough to differentiate each fluorinated cathinone isomer and homologue studied. The spectra of the homologues 2-fluoromethcathinone and 2-fluoroethcathinone (Figure 49) contained few distinct features (ex.

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1381 and 965 cm^{-1}). Therefore, the combined discriminating power of GC/IR and GC/MS provided a clear differentiation of fluorinated cathinone homologues and isomers.

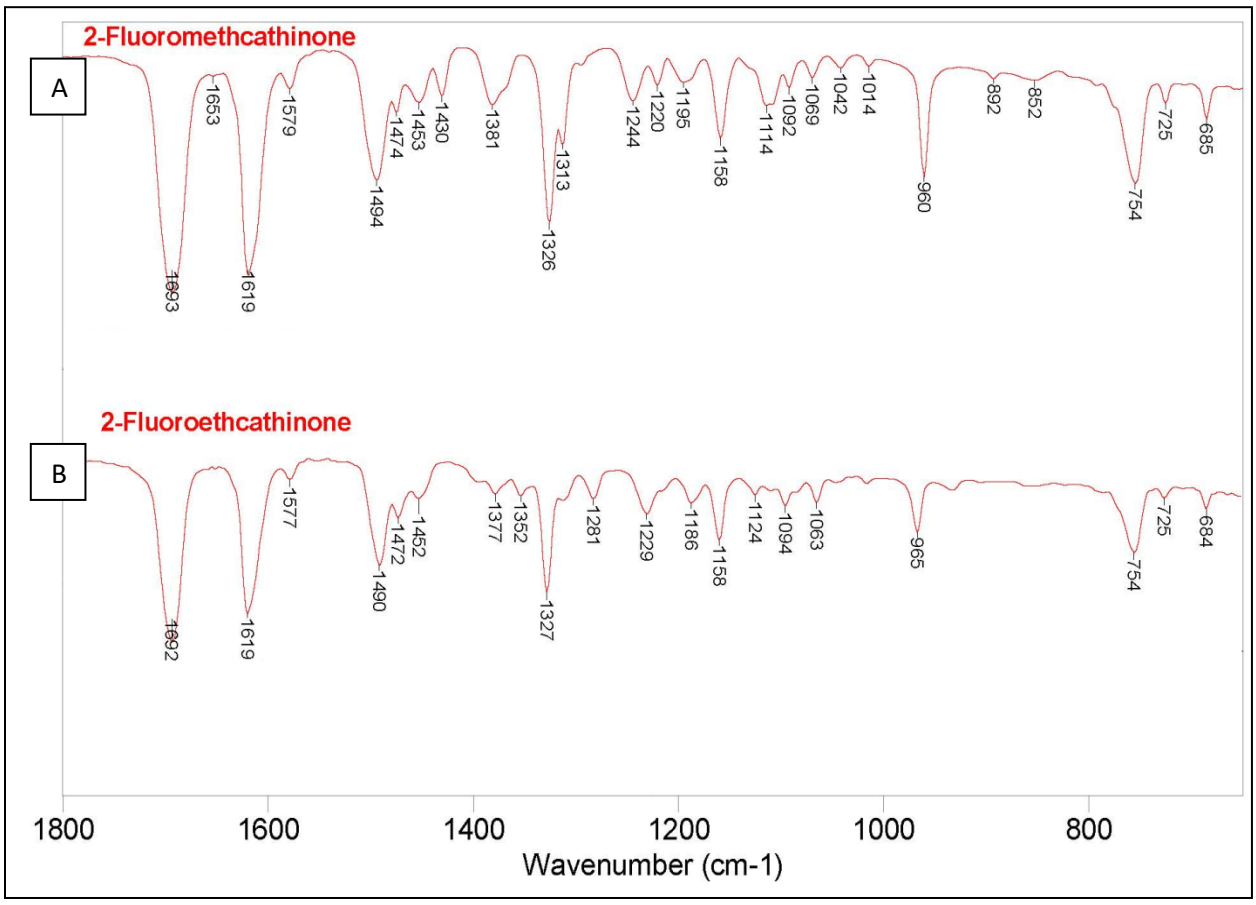

Figure 49: The IR Spectra, expanded for the fingerprint region, of 2-fluoromethcathinone (A) and 2-fluoroethcathinone (B).

4.4.6 Methoxy Substituted Cathinone Isomers

The methoxy substituted cathinone isomers consisted of a methoxy group bonded to the basic cathinone structure at the R4-R6 positions (Figure 19). These isomers are of interest due to the strong electron donating nature of the methoxy group which can impact the mass spectral ions and IR absorption peaks.

The three methoxymethcathinone positional isomers were sufficiently resolved chromatographically using the GCMS1 method (Figure 50). Only *m/z* 107 had a relative abundance that varied sufficiently to differentiate 2-methoxymethcathinone from the other two

isomers via mass spectrometry. The *m/z* 107peak, observed in the meta and para methoxy isomer mass spectra, may represent the fragmentation and loss of the electron withdrawing group (i.e. ketone containing branch off of the aromatic ring). The resulting hydrogen-poor anisole ion ($+C_6H_4(OCH_3)$) would be stabilized by the electron donating methoxy group expected in the mass spectra of aromatic ethers (Silverstein, Webster, Kiemle, & Bryce, 2014).

All three of the methoxymethcathinone isomers were differentiated by GC/IR spectra (Figure 51) which contained a number of unique absorption peaks (ex. 1668, 1683, and 1673 cm^{-1}). The C-O-C asymmetrical stretching typical of aryl alkyl ethers may be represented by the absorption peaks at 1247, 1261, and 1241 cm^{-1} for the ortho-, meta- and para- substituted methoxymethcathinones respectively (Silverstein, Webster, Kiemle, & Bryce, 2014). Such differences between the IR spectra make GC/IR a valuable orthogonal technique to differentiate isomers where mass spectrometry fails to do so as is the case here for 3-methoxymethcathinone and 4-methoxymethcathinone.

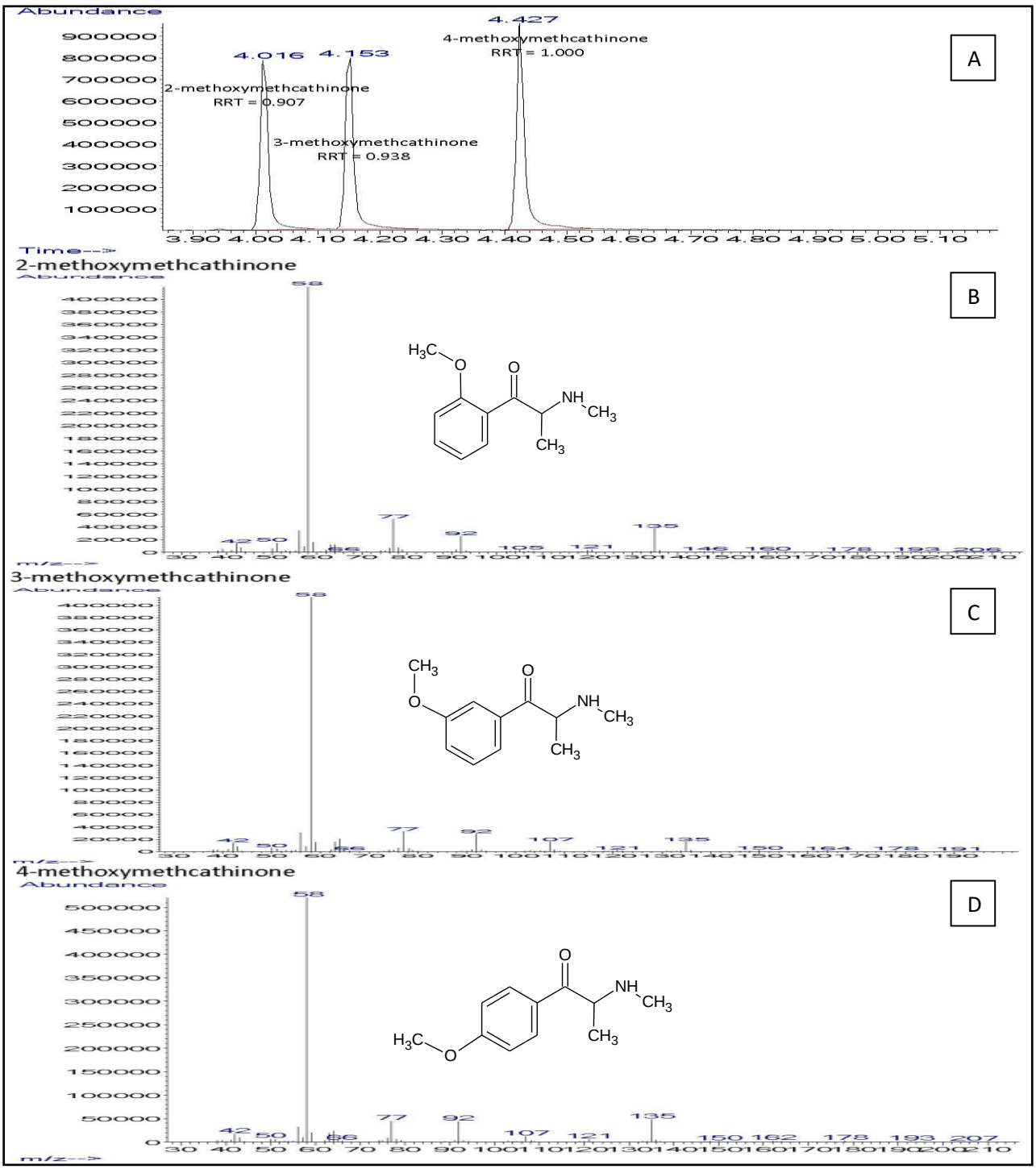

Figure 50: The chromatogram (A) and mass spectra of methoxymethcathinone isomers: 2- methoxymethcathinone (B), 3-methoxymethcathinone (C) and 4-methoxymethcathinone (D).

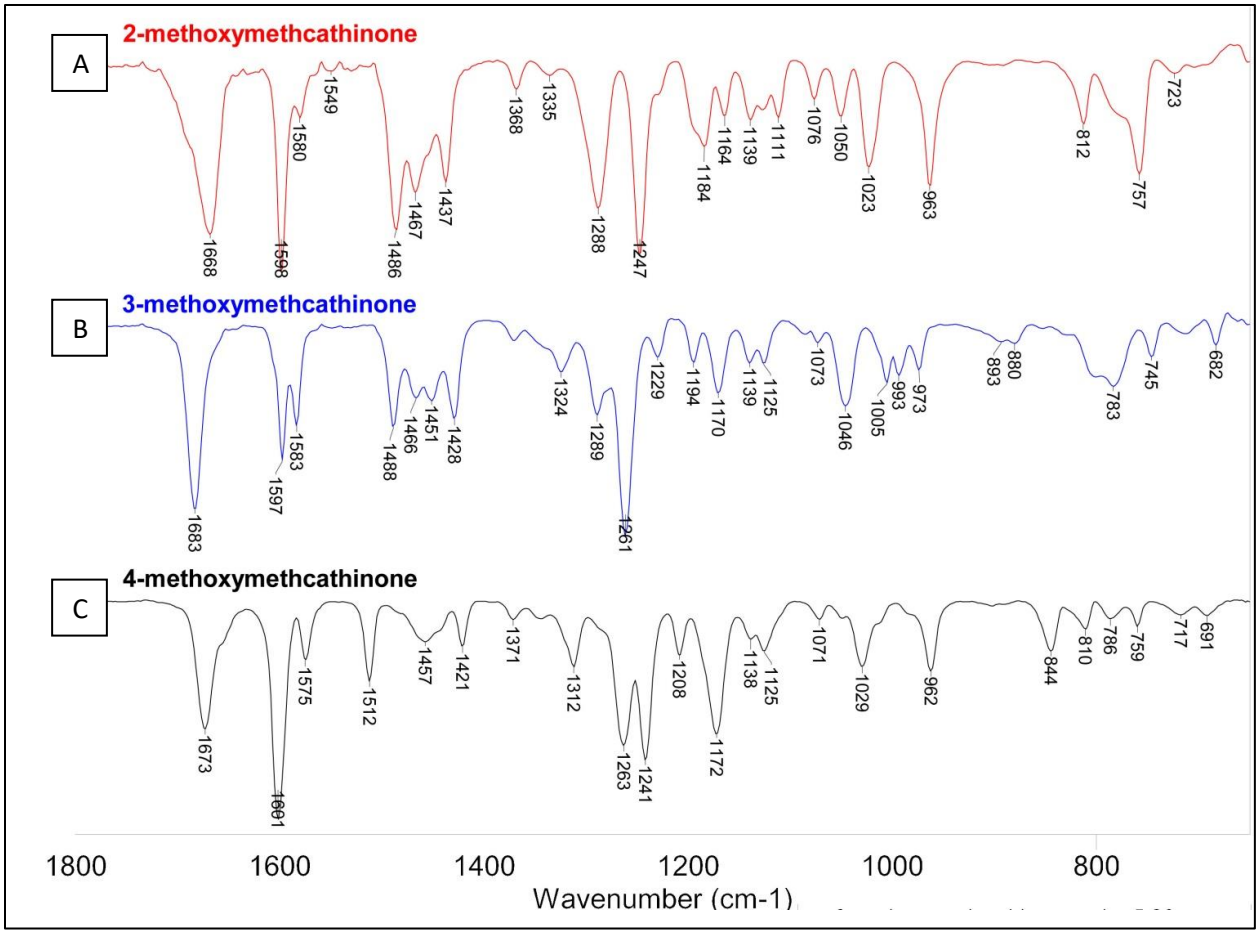

Figure 51: The IR Spectra, expanded for the fingerprint region, of methoxymethcathinone isomers: 2-methoxymethcathinone (A) 3-methoxymethcathinone (B), and 4-methoxymethcathinone (C).

4.4.7 Synthetic Cannabinoid Homologues

Some drugs of abuse, such as the synthetic cannabinoids 1-pentyl-3-(1-naphthoyl)indole (JWH-018) and 1-Hexyl-3-(naphthoyl)indole (JWH-019), differ structurally by only a single CH_2 in longer alkyl group (Figure 52). It was expected that the vibrational energies of such homologues would not be significantly shifted by the difference of a single CH_2 extension. Therefore, these homologues tested the limitations of infrared spectroscopy's discriminating power.

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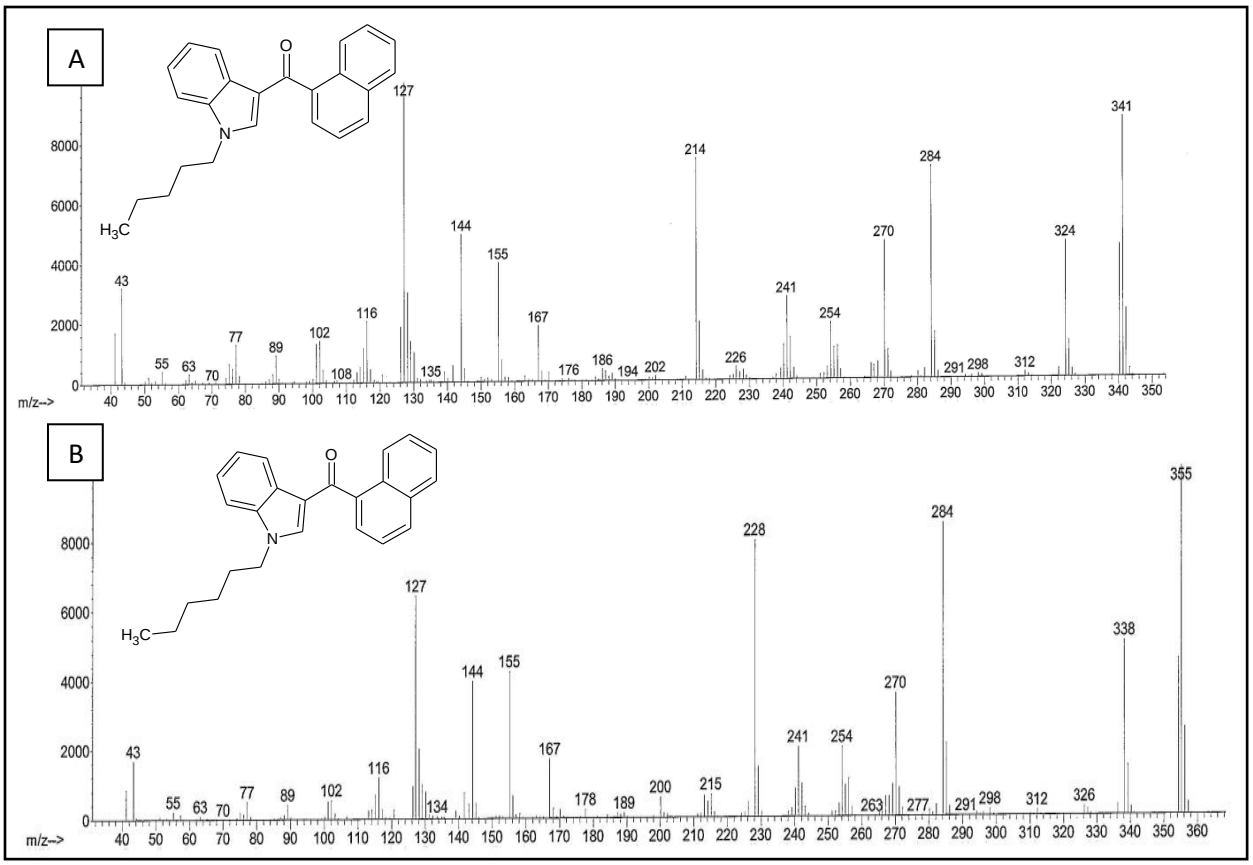

Figure 52: Mass spectra of JWH-018 and JWH-019.

The homologues JWH-018 (6.79 min) and JWH-019 (7.59 min) were sufficiently resolved chromatographically using the GCMS2 method. Additionally, the mass spectra contained multiple unique ions (ex. *m/z* 214 vs. 228) separated by *m/z* 14 which is characteristic of the difference of a single alkyl chain (Figure 52). Thus, GC/MS had sufficient discriminating power for JWH-018 and JWH-019. On the other hand, the resulting GC/IR spectra (Figure 53) contained no significantly abundant absorption peaks that differed by more than four wavenumbers. Therefore, the resulting spectra may be considered to have diminished discriminating power to differentiate these two homologues. While there were some minor differences between the JWH-018 and JWH-019 spectra (ex. minor peaks below 1012 and above 1234 cm^{-1}), these may be difficult to be recognized in low quality spectra.

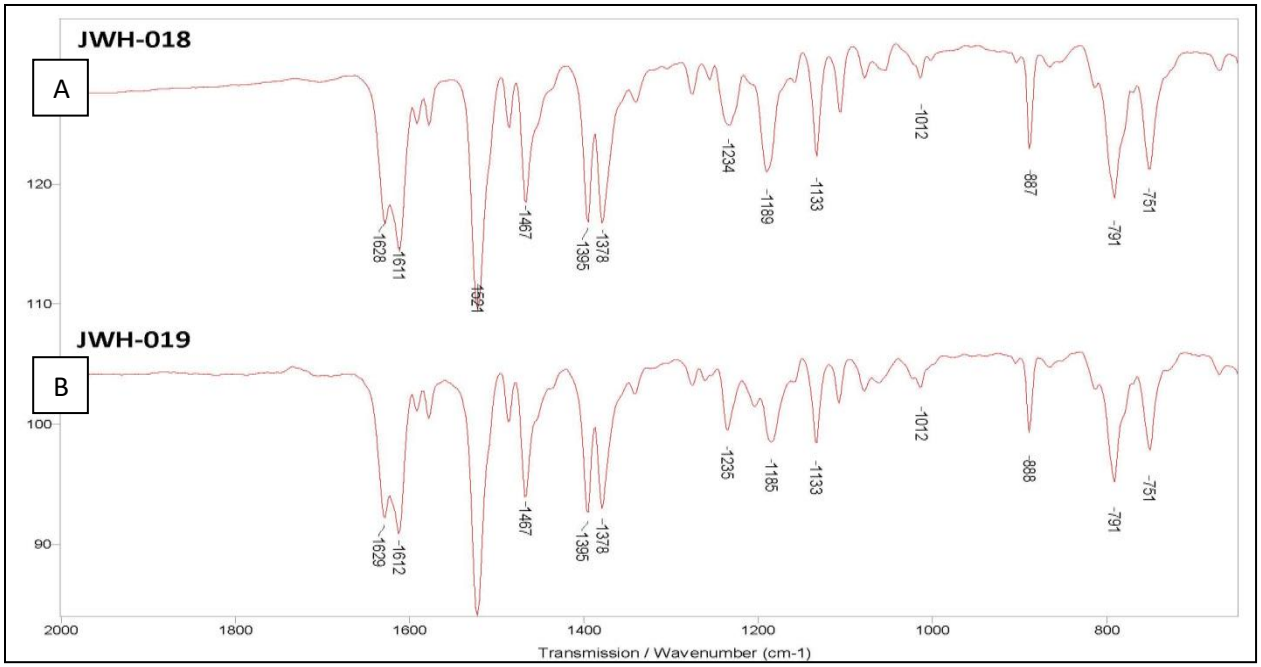

Figure 53: The IR Spectra, expanded for the fingerprint region, of JWH-018 (A) and JWH-019 (B).

4.4.8 Ephedrine Diastereomers and Enantiomers

Spectra were obtained for 1R-2S-ephedrine, 1S-2S-pseudoephedrine, 1R-2S-N-methyl-ephedrine and 1S-2R-N-methyl-ephedrine to evaluate GC/IR discriminating power for diastereomers and optical isomers (enantiomers). The identification of an optical isomer can have investigative value, for instance some synthetic pathways will preferentially produce D-methamphetamine. Of compounds containing multiple chiral centers only enantiomers were expected to produce indistinguishable FTIR spectra (Silverstein, Webster, Kiemle, & Bryce, 2014).

Ephedrine and pseudoephedrine were not adequately resolved chromatographically using the GCMS1 method (Figure 54). Additionally, the mass spectra for the two diastereomers could not be distinguished as all ions of significant abundance (ex. *m/z* 42, 51, 58, 77, 105, 117, 146) were found at similar relative ratios in both mass spectra. The IR spectra, while containing numerous peaks representing similar vibrational energies, were differentiated for

pseudoephedrine and ephedrine (Figure 55) using a number of unique absorption peaks (ex. 1339, 1353, 1160, 1139, 983, 759, and 740 cm^{-1}). The absorption peak differences between the spectra result from various atoms moving in unison in a periodic motion, different from functional group frequencies, that is almost impossible to predict from theoretical calculations (Drug Enforcement Administration (DEA), Department of Justice, 2007).

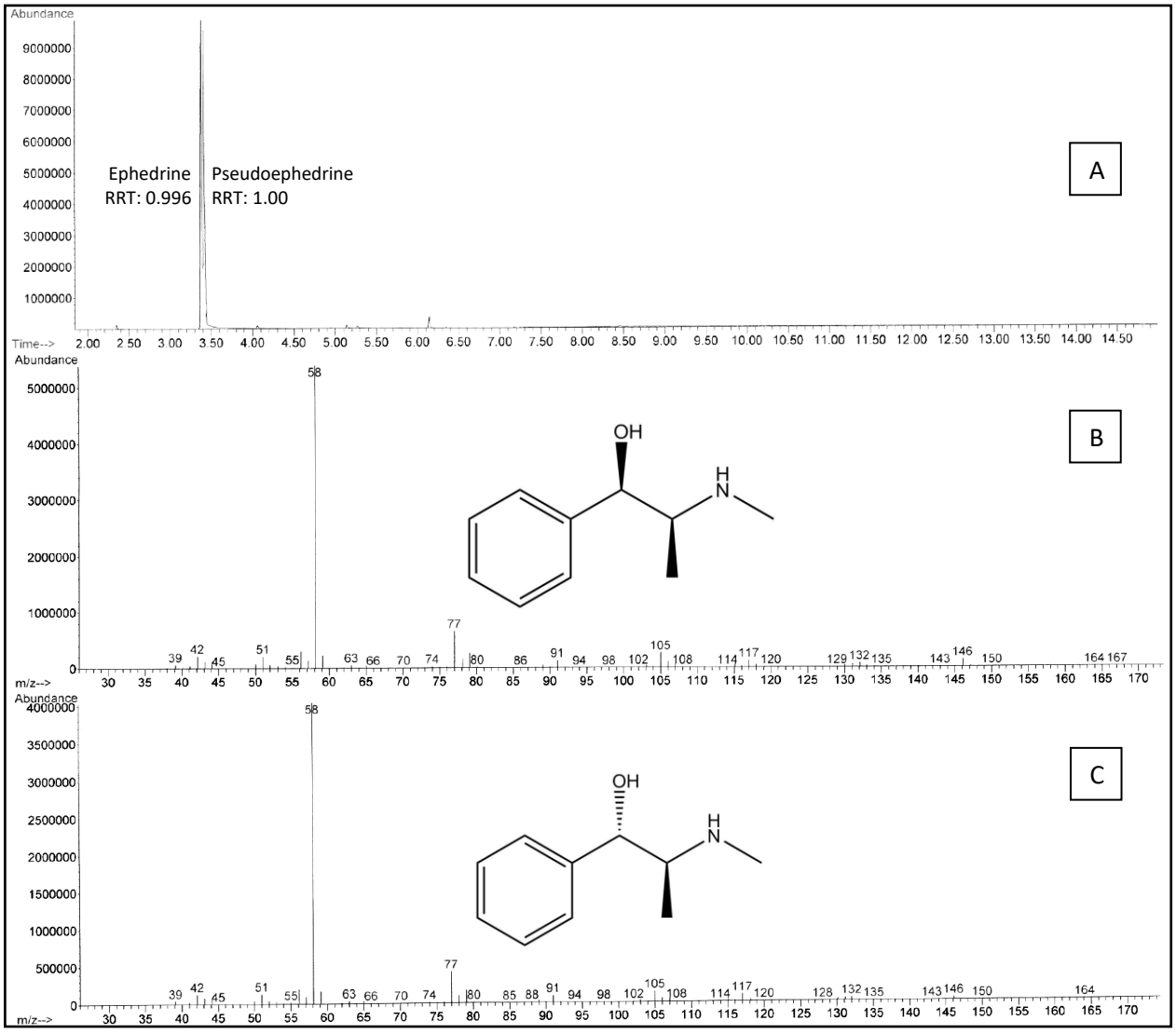

Figure 54: The chromatogram (A) and mass spectra of 1R-2S-(l)-ephedrine (B) and 1S-2S-(d)-pseudoephedrine (C).

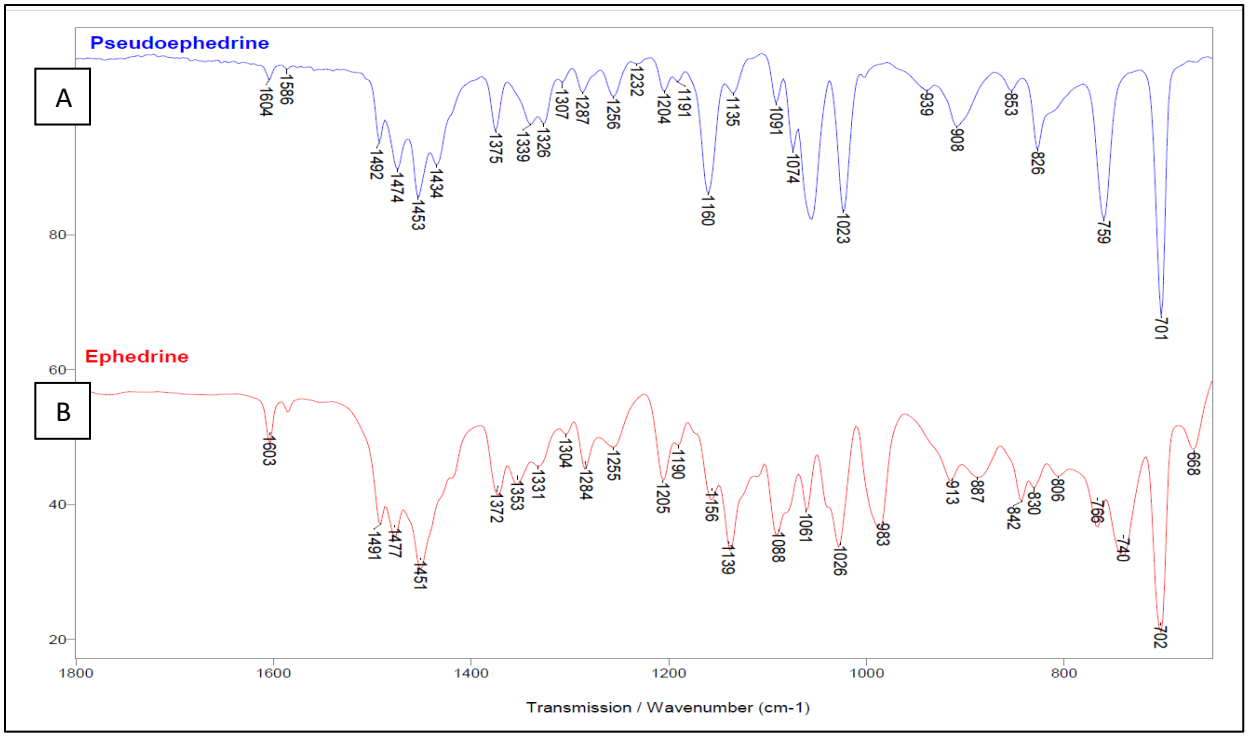

Figure 55: The IR Spectra, expanded for the fingerprint region, of 1S-2S-(d)-pseudoephedrine (A) and 1R-2S-(l)-ephedrine (B).

The enantiomers 1R-2S-(l)-N-methyl-ephedrine and 1S-2R-(d)-N-methyl-ephedrine were not resolved chromatographically (Figure 56) and the mass spectra contained all of the same ions at similar relative abundances such that the enantiomers could not be differentiated using GC/MS. The greatest difference between the IR spectra for 1R-2S-(l)N-methyl-ephedrine and 1S-2R-(d)-N-methyl-ephedrine (Figure 57) was two wavenumbers for a single absorption peak. Two wavenumbers was not significant enough to differentiate the enantiomers via GC/IR. To differentiate 1R-2S-(l)-N-methyl-ephedrine and 1S-2R-(d)-N-methyl-ephedrine enantiomers, techniques such as chiral chromatographic columns and polarimetry are typically applied.

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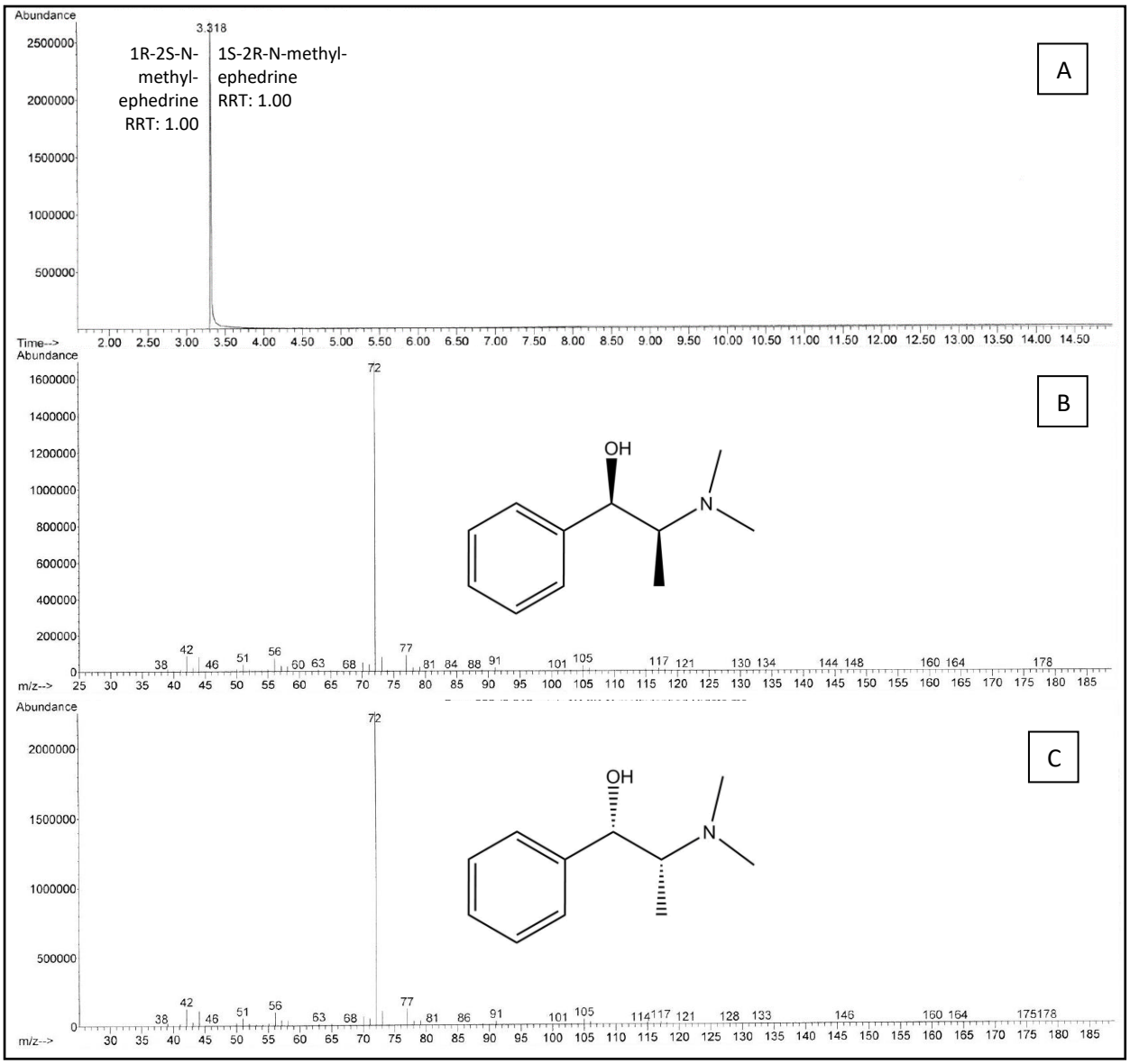

Figure 56: The chromatogram (A) and mass spectra of 1R-2S-(l)-N-methyl-ephedrine (B) and 1S-2R-(d)-N-methyl-ephedrine (C).

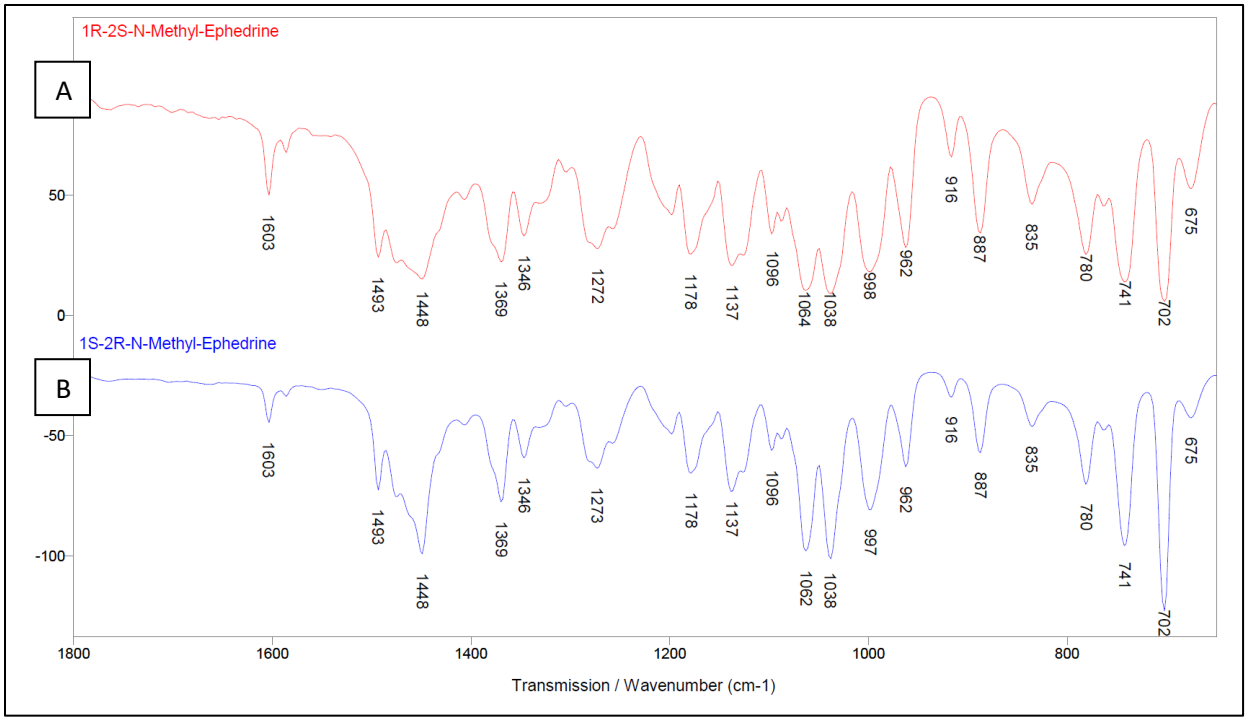

Figure 57: The IR Spectra, expanded for the fingerprint region, of 1R-2S-(l)-N-methyl-ephedrine (A) and 1S-2R-(d)-N-methyl-ephedrine (B).

5. Conclusion

The Spectra Analysis DiscovIR-GC™ successfully separated the individual components of mixtures and generated high resolution, reproducible infrared spectra. The GC/IR spectra generated met the structurally elucidating requirement for Category A SWGDRUG tests, and chromatographic separation met Category B SWGDRUG test requirements. Of the approximately 300 compounds for which library quality data was acquired only enantiomers and homologous compounds containing long alkyl chains were indistinguishable via their GC/IR spectra. Therefore, the GC/IR successfully differentiated almost all compounds representative of typical case-work, sample submissions (99 %) including multiple varieties of positional isomers, homologues, and diastereomers. Individual synthetic cannabinoids and cathinones were difficult to differentiate from some of their positional isomers using GC/MS. The GC/MS did however provided spectra with more readily

apparent differences between compounds in a homologous series. Therefore, the two techniques combined (GC/MS and GC/IR) represent an analytical scheme with high discriminating power for NPSs.

Reference libraries for the compounds of interest were successfully created and made available to forensic chemists. The IR spectra were shown to be reproducible across laboratories such that reference library data can be shared among laboratories to identify unknown chemical constituents. Once identified, the isomeric standard can be purchased for direct comparison to an unknown sample without the need to purchase all isomers to demonstrate chromatographic separation on a GC/MS. Therefore, the use of the GC/IR will save the forensic laboratory in both time and money.

Future efforts may explore the discriminating power of chromatographically coupled infrared ion spectroscopy for forensic analytes, the continued expansion of library reference spectra and the verification of the discriminating power of GC/IR for NPSs as they evolve.

6. Acknowledgements

1. Jeremy Triplett, the drug chemistry supervisor at the Kentucky State Police Central Forensic Laboratory in Frankfort, provided the cathinone standards used in the validation studies.

2. Cathy Copeland and Philippe St-Amour, Canada Border Services Agency, provided the spectra used in the comparison section of the validation study, and provided comments and suggestions.

3. Josh Yohannan, lab manager for the Allegheny County Office of the Medical Examiner provided comments and suggestions.

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8. Appendices

Appendix A: Directions for downloading and installing the libraries on the DiscovIR™

Step 1. Saving the library files

1. Log in to **Forendex Forum**. If you are not a member, you will need to register first. http://forendexforum.southernforensic.org/
2. Click on the "**Designer Drugs**" forum
    - Click on the topic called "**Commercially Available Designer Drug Excel Spreadsheet**"
    - Scroll down until you see the two comments labeled "**Test**" and "**Test: GC-IR library**". These contain links to Dropbox™ to download the library files for the Cathinones library and the USACIL drug library, respectively.
3. Save all five library files to the same location/path on the computer's hard drive.
4. The files should have the same name, excluding the extensions (Ex. Cathinones.idx and Cathinones.ipl).

Step 2. Adding the Library

1. Open **GRAMS**.
2. On the **Toolbar**, click **Launch Spectral ID** (fingerprint icon).
3. Under the **Tools** menu, click **Library Manager.**
4. Click **Add**
    - Access the location/path of the stored library files.
    - Open the .lib file for the library you wish to add. **Note:** This should be the only file type displayed. If not, use the drop down menu to display only .lib files.
5. The library selected should now appear in the "Library List".
    - Click **Done.**

Step 3. Including the library in the Spectrum Search results

1. Under the **Tools** menu, click **Choose Libraries**.
2. Check the box next the desired library.
    - Select **OK**.
    - **Note:** You can also uncheck boxes to exclude libraries from Spectrum Search Results.

Removing a library

1. Under the **Tools** menu, click **Library Manager.**
2. Click the correct library.
3. Click **Remove.**
4. Choose **Yes** to confirm.
5. Select **Done** to exit the **Library Manager.**

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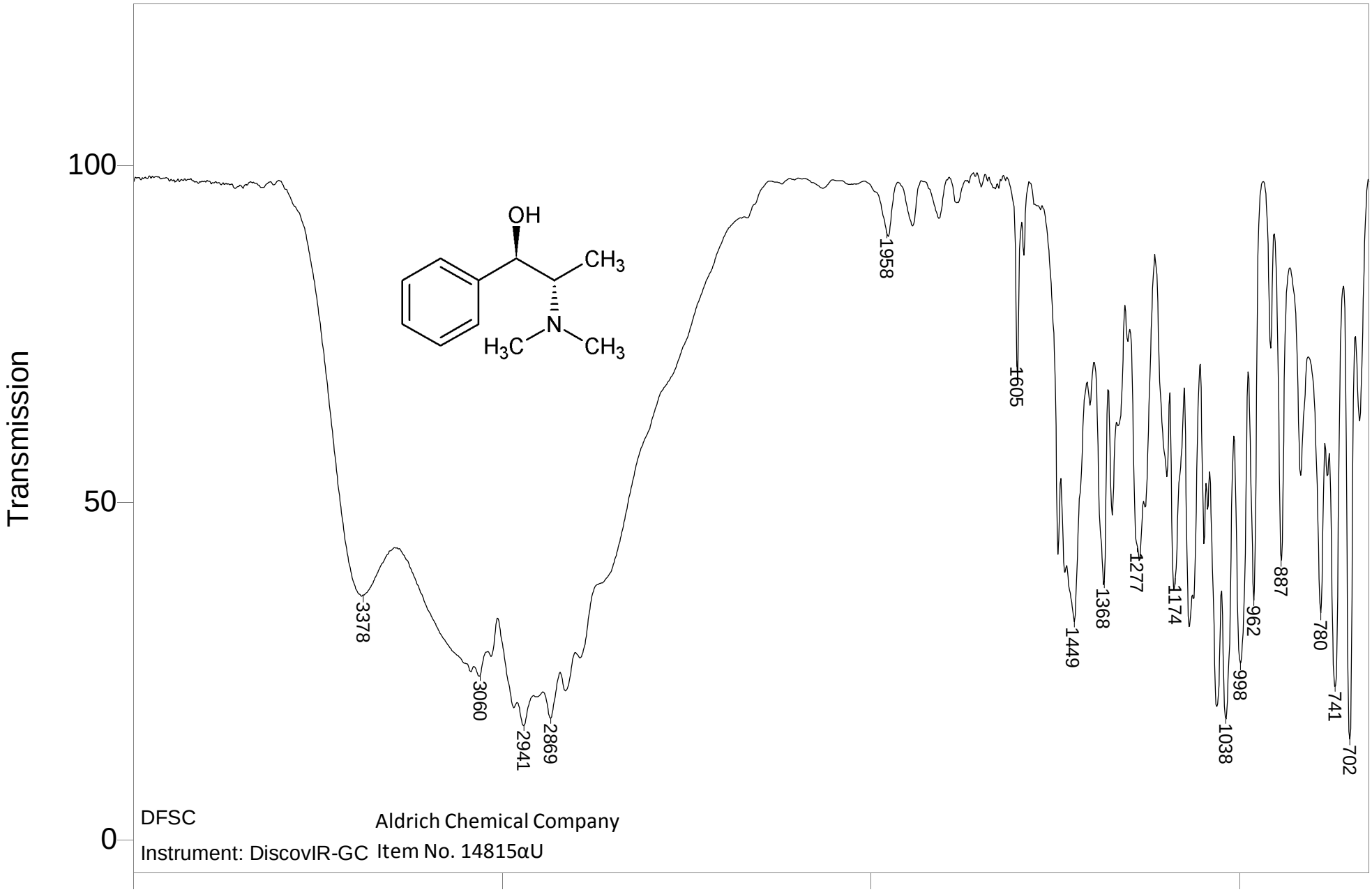
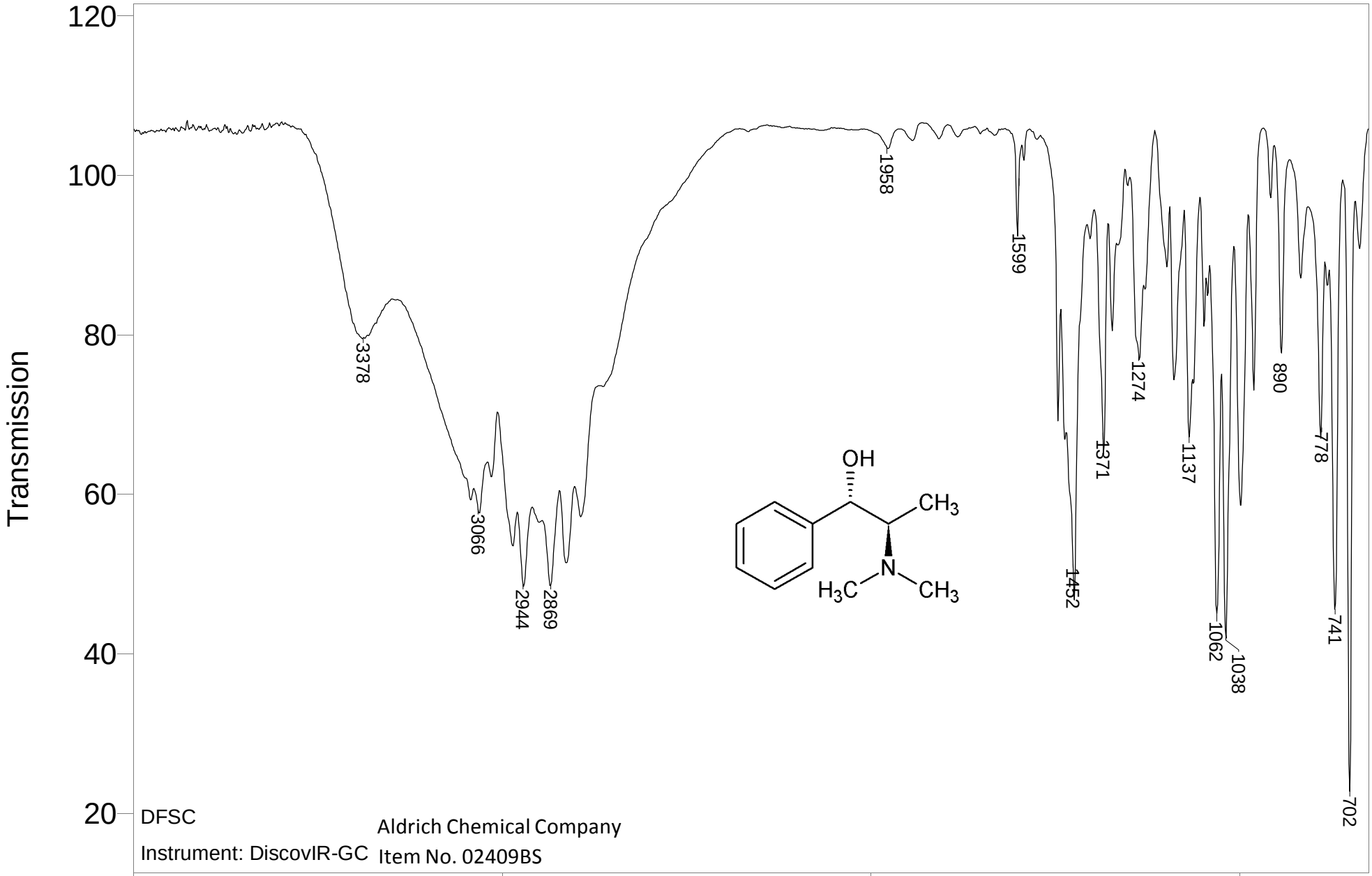
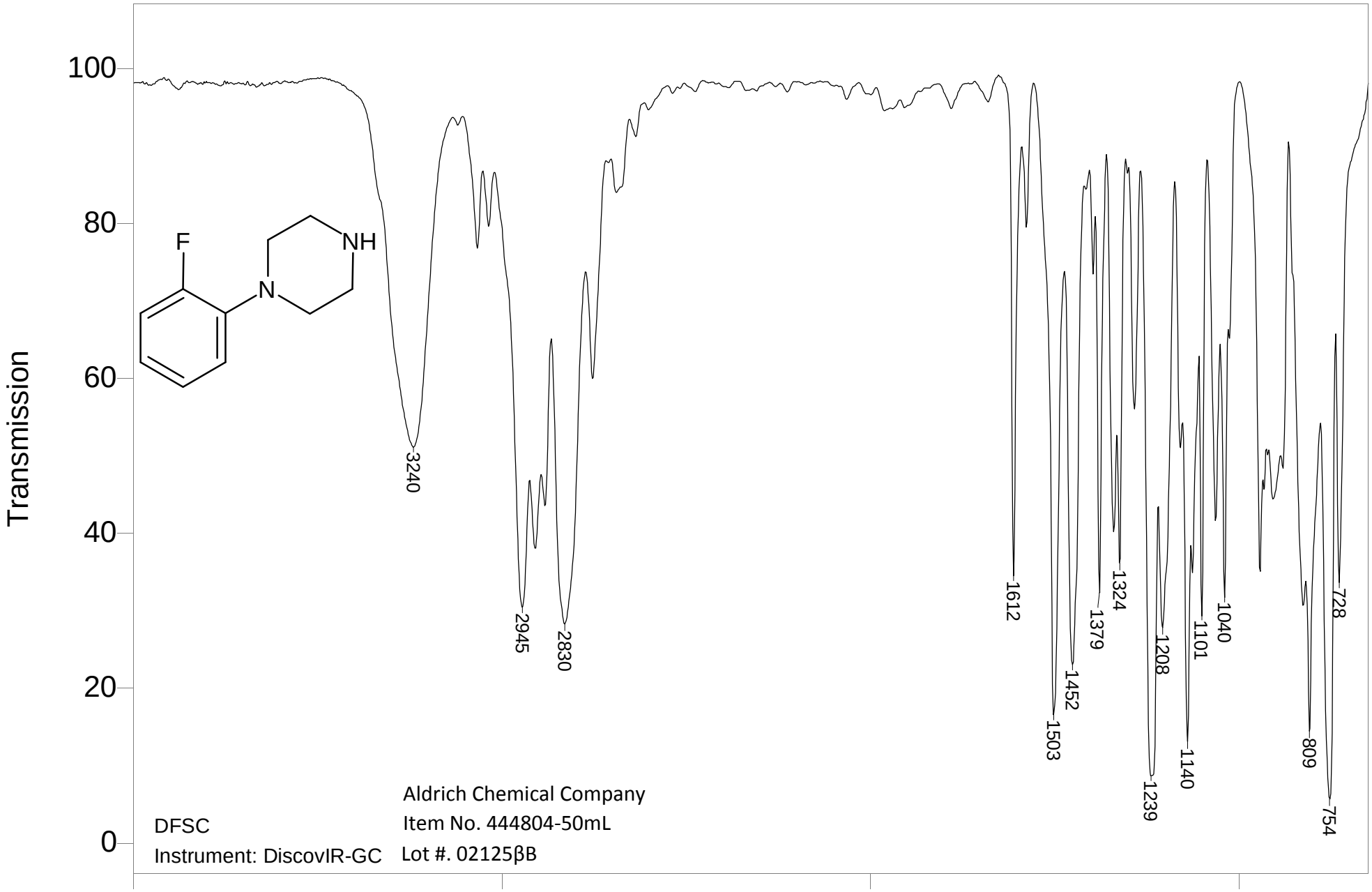
Appendix B: GC/IR Spectra Library: USACIL Drugs

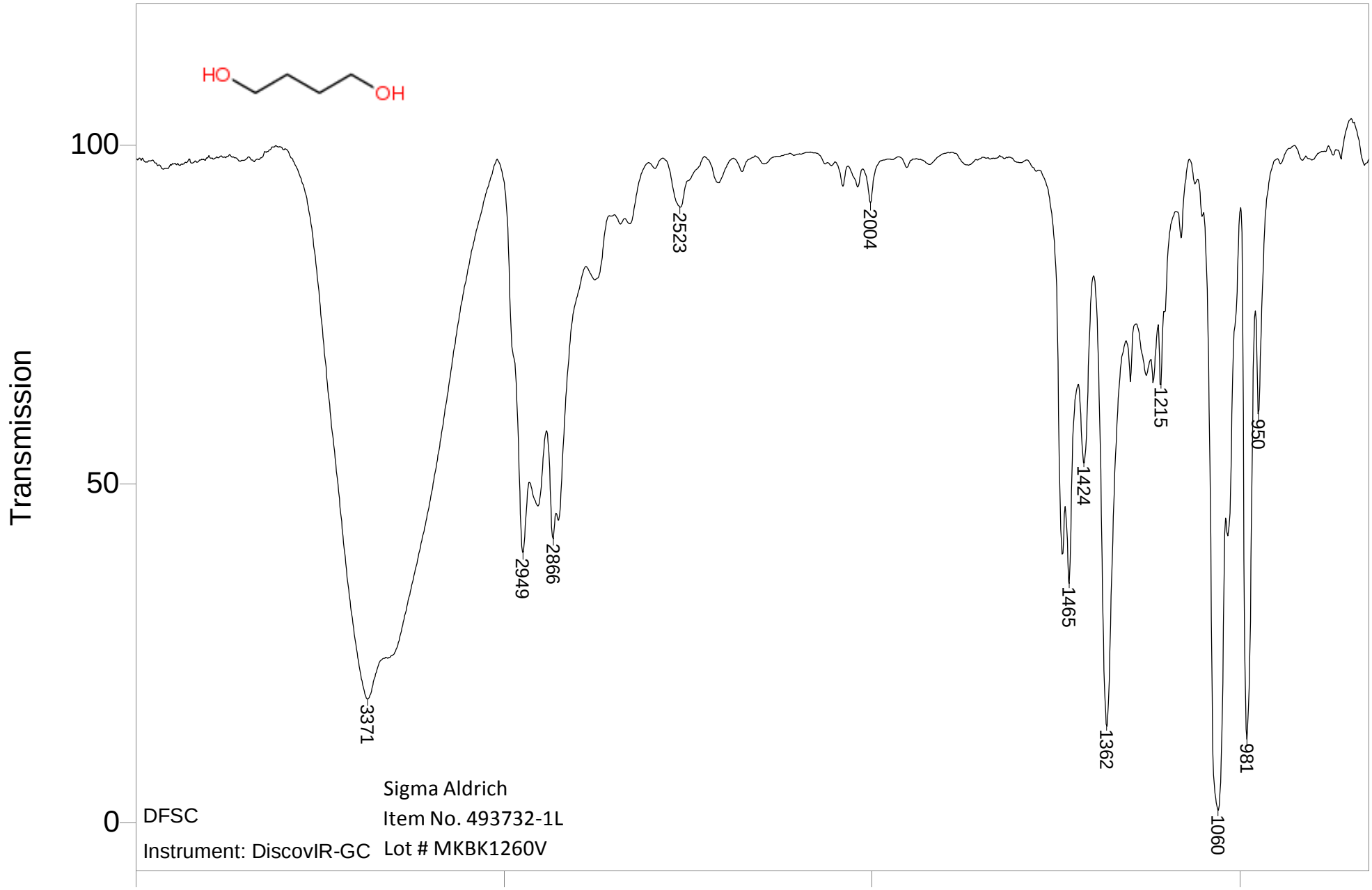
The GC/IR Spectra Library contains 250 non-repeating solid-state infrared absorption spectra of NPSs and related compounds as acquired on the DiscovIR-GC™. Several chemical structures (those with colored heteroatoms), as found associated with IR spectra, were copied from the Forendex database maintained by the Southern Association of Forensic Scientists (Southern Association of Forensic Scientists, 2017). The product information of the standard material used to acquire reference spectra, typically used for traceability assurance, was unavailable for the following compounds: alprazolam, clonazepam, methylphenidate, cocaine, methenolone acetate, tamoxifen, propofol, and the UR-144N-(2-chloropentyl) analog.

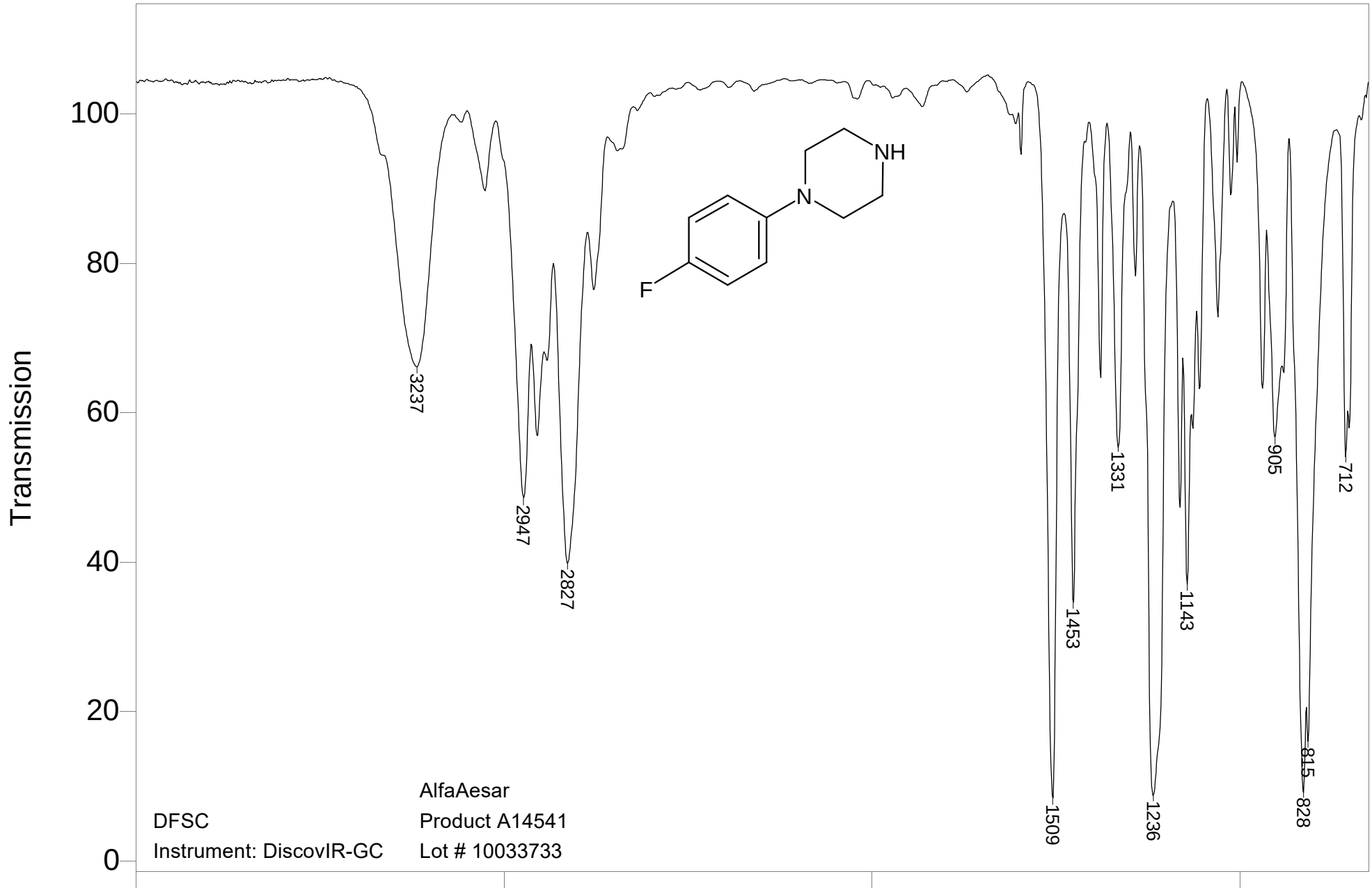


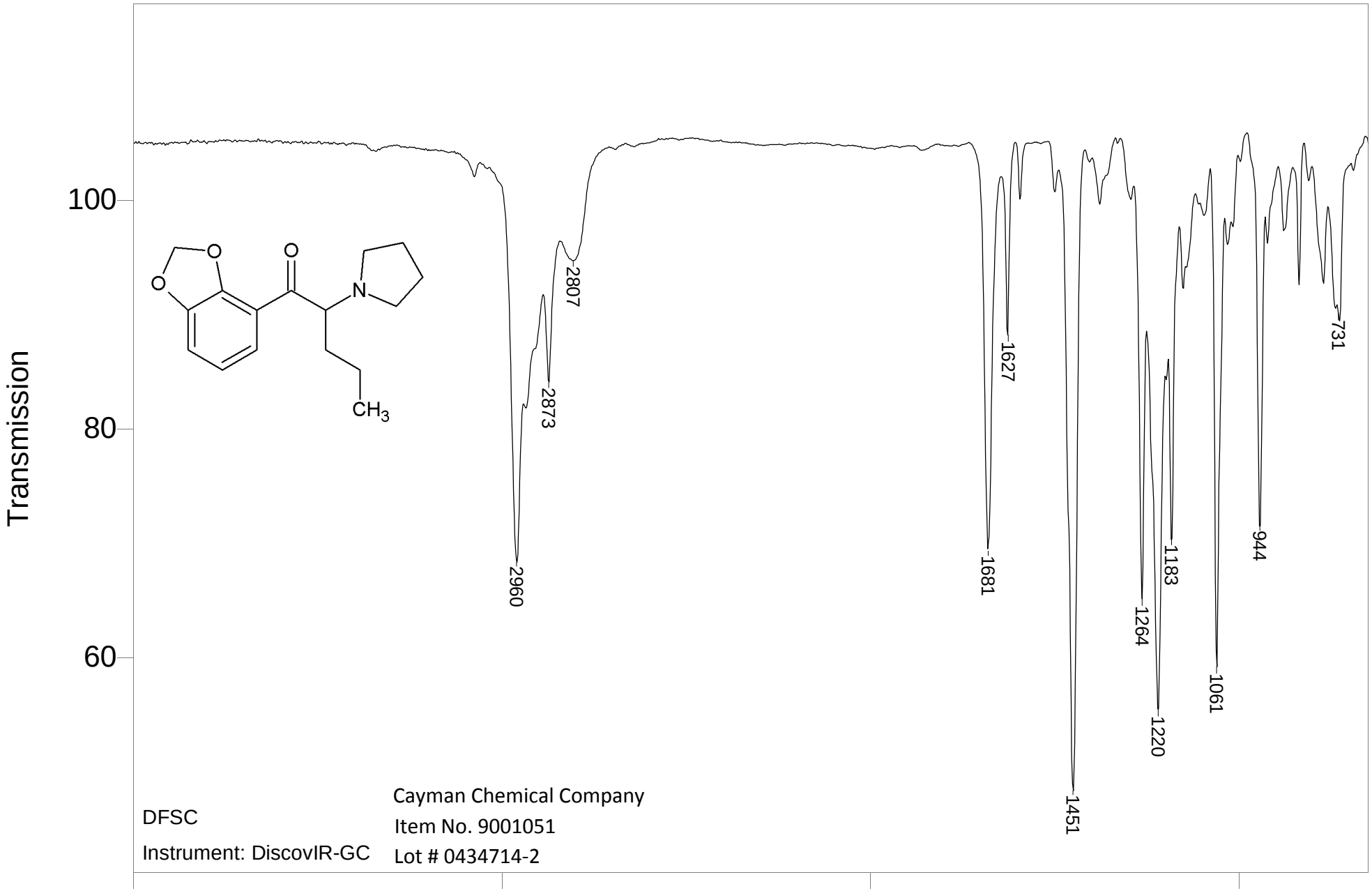
(1S,2R)-N-Methyl-Ephedrine

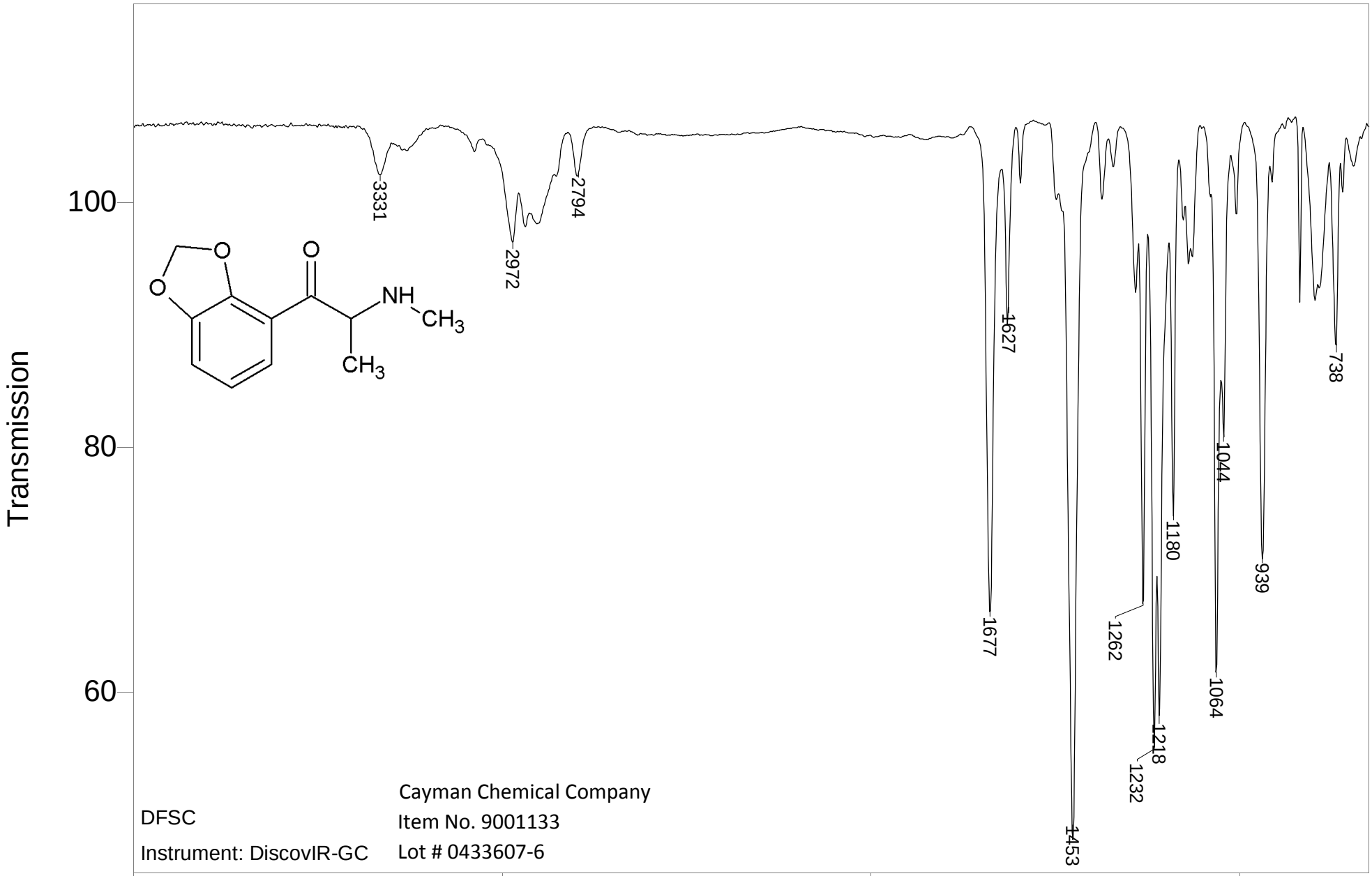




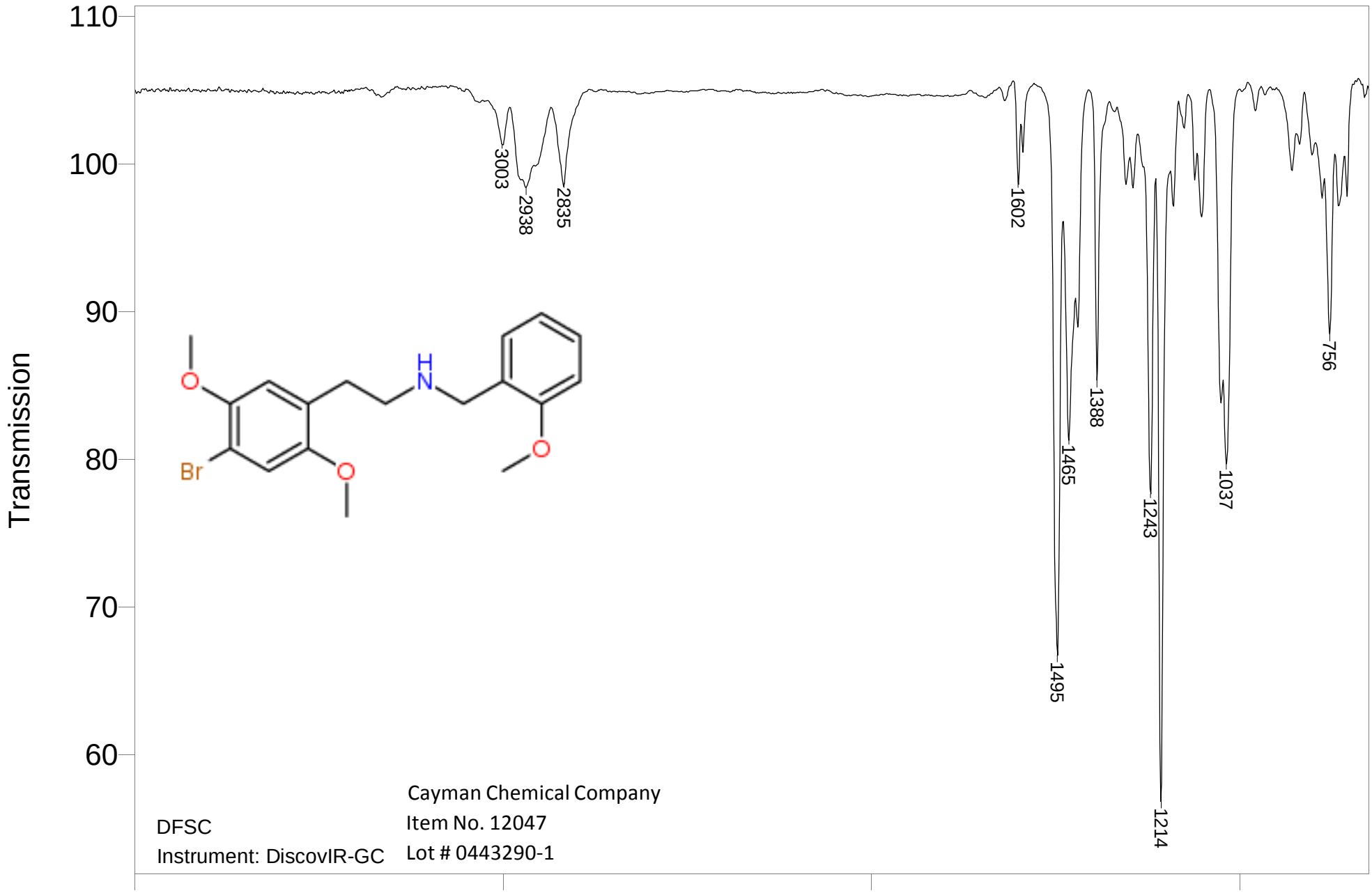


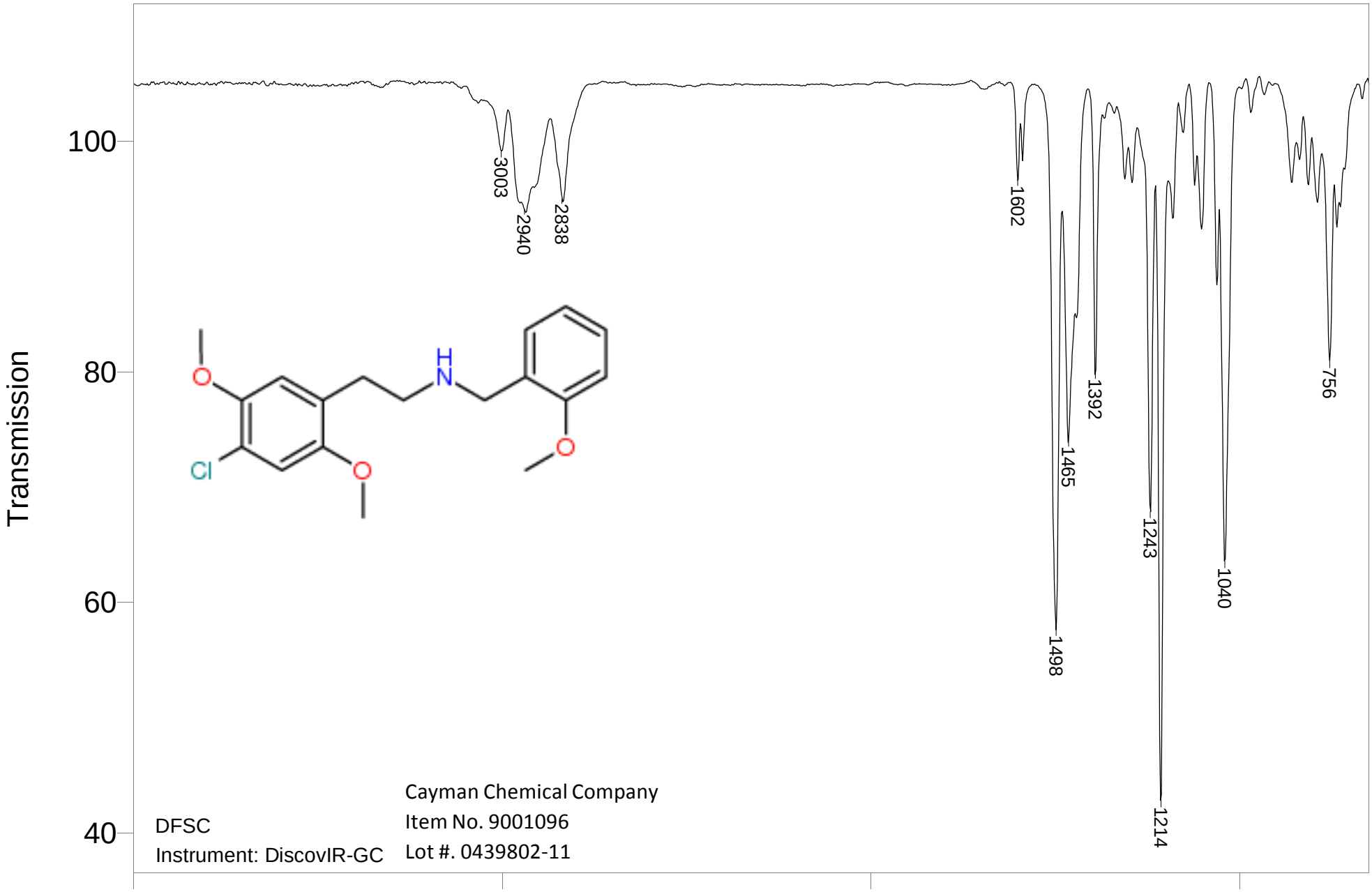


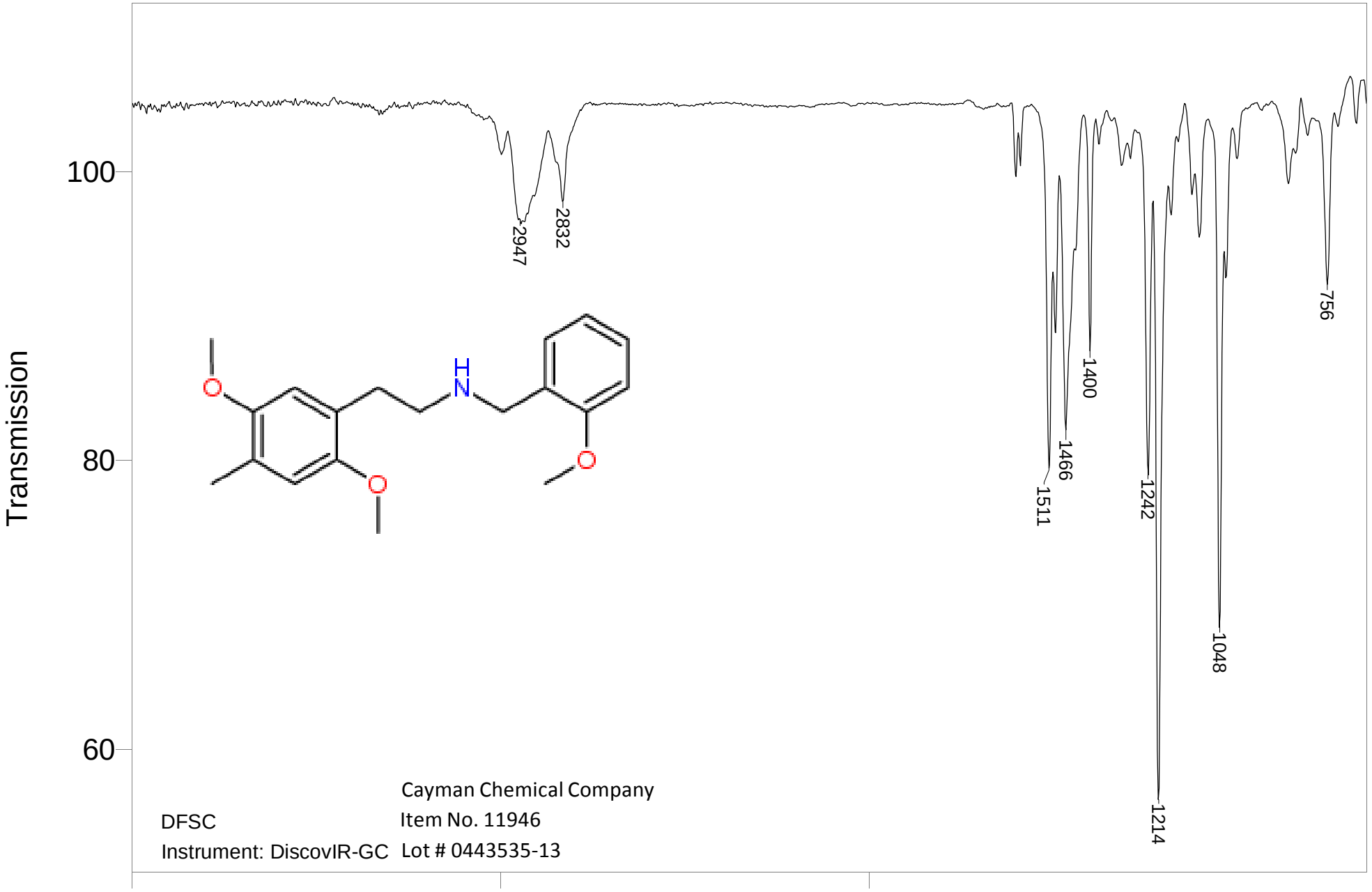


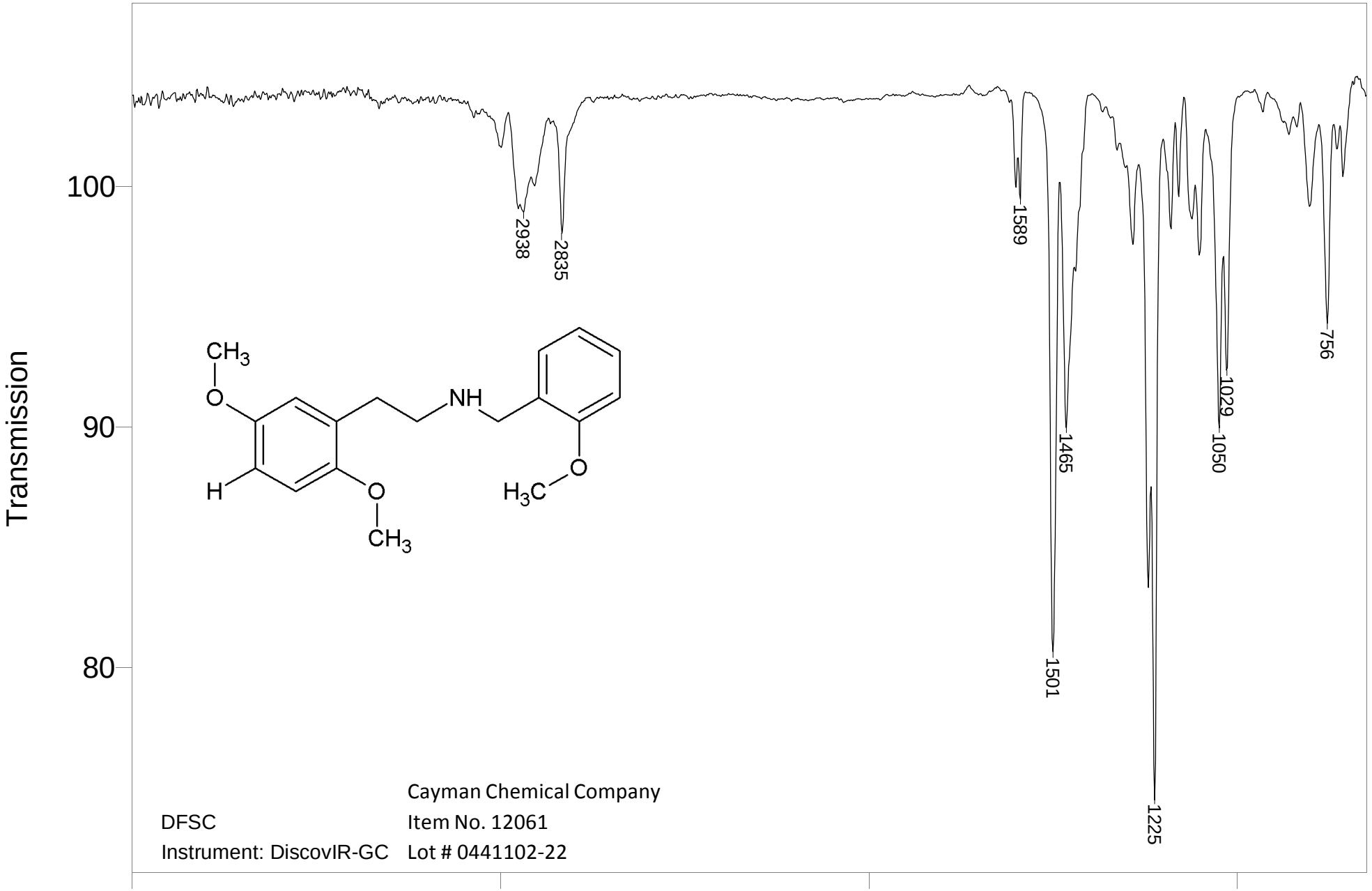


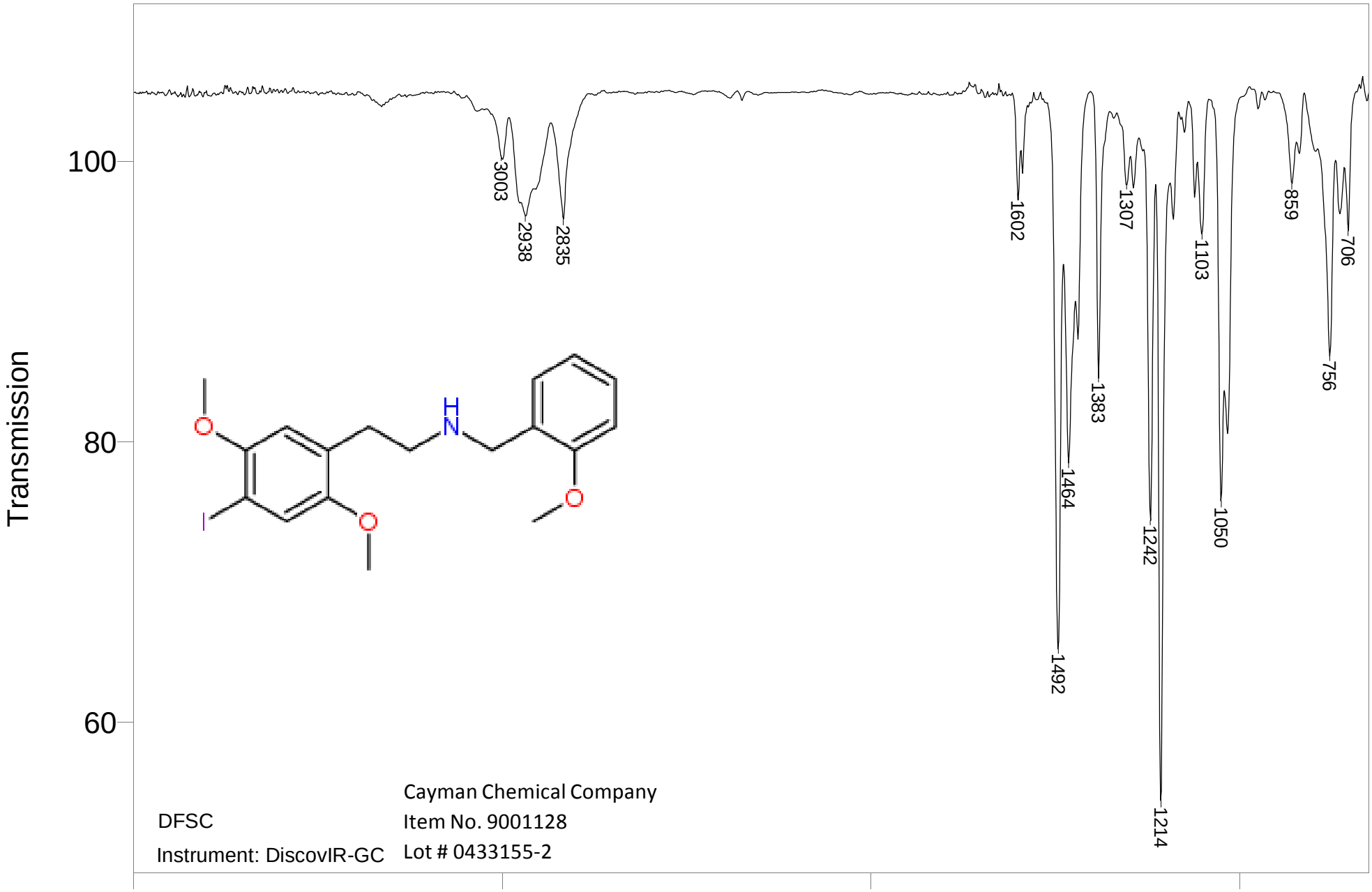
25B-NBOMe

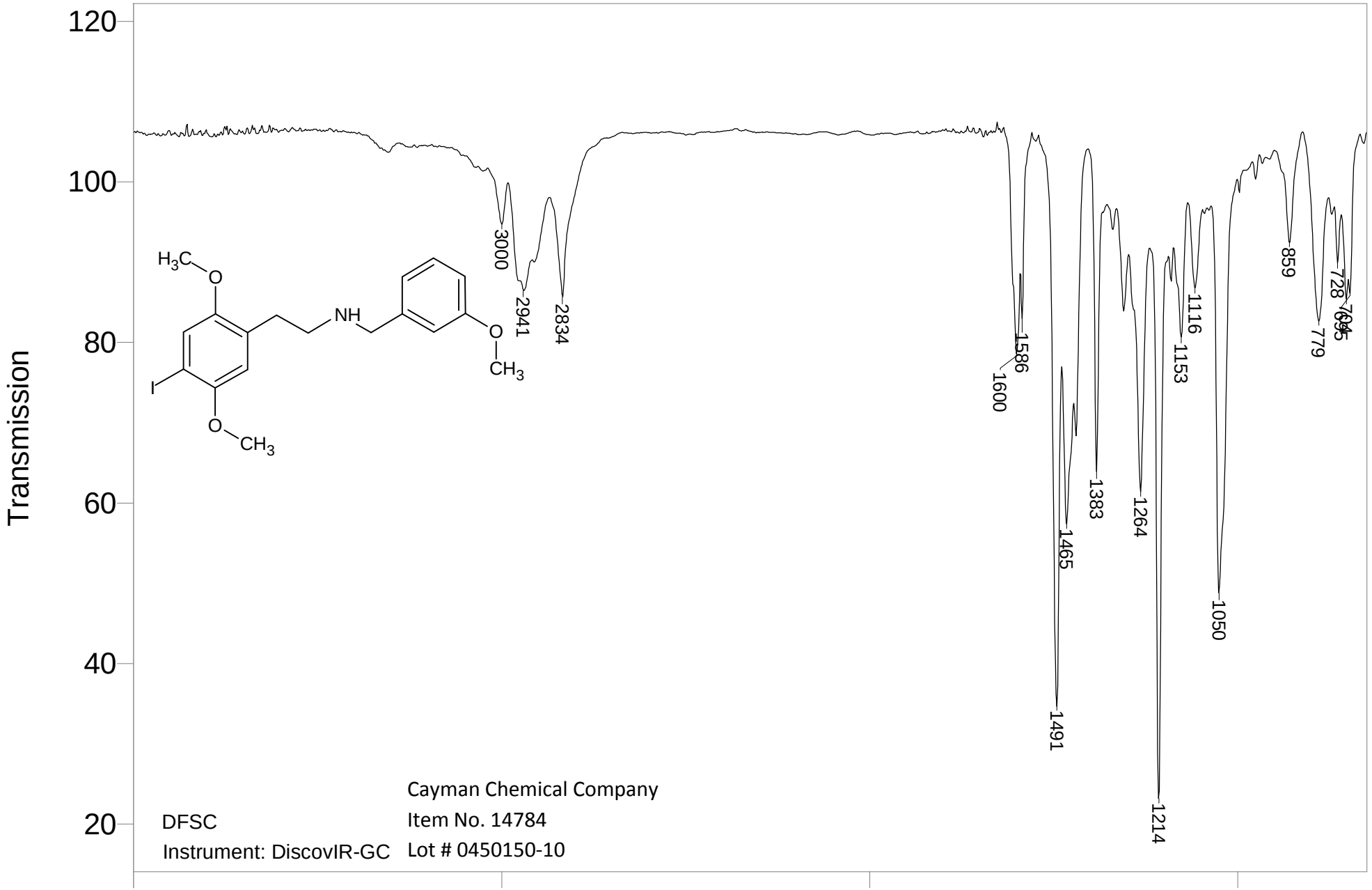


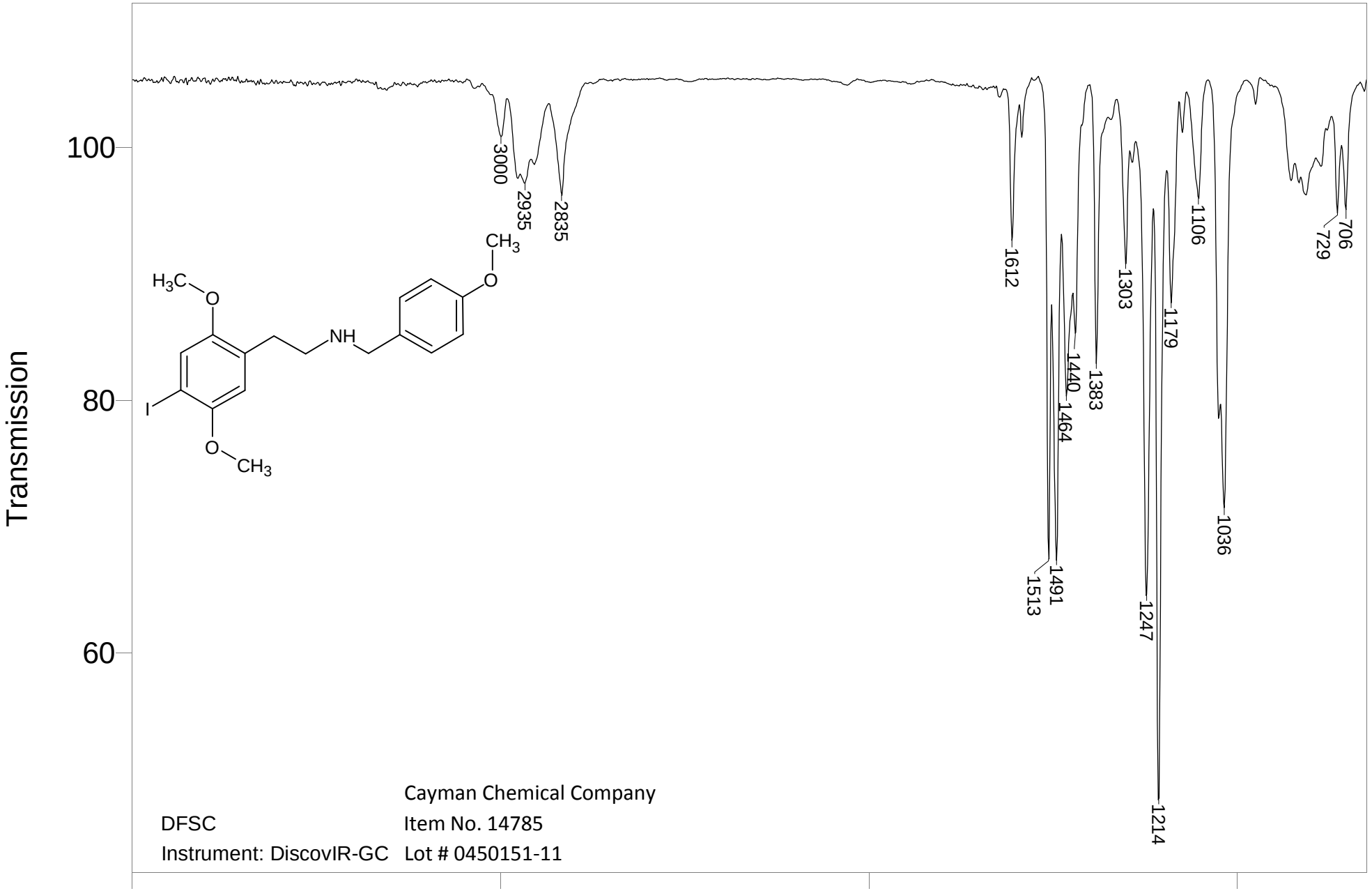


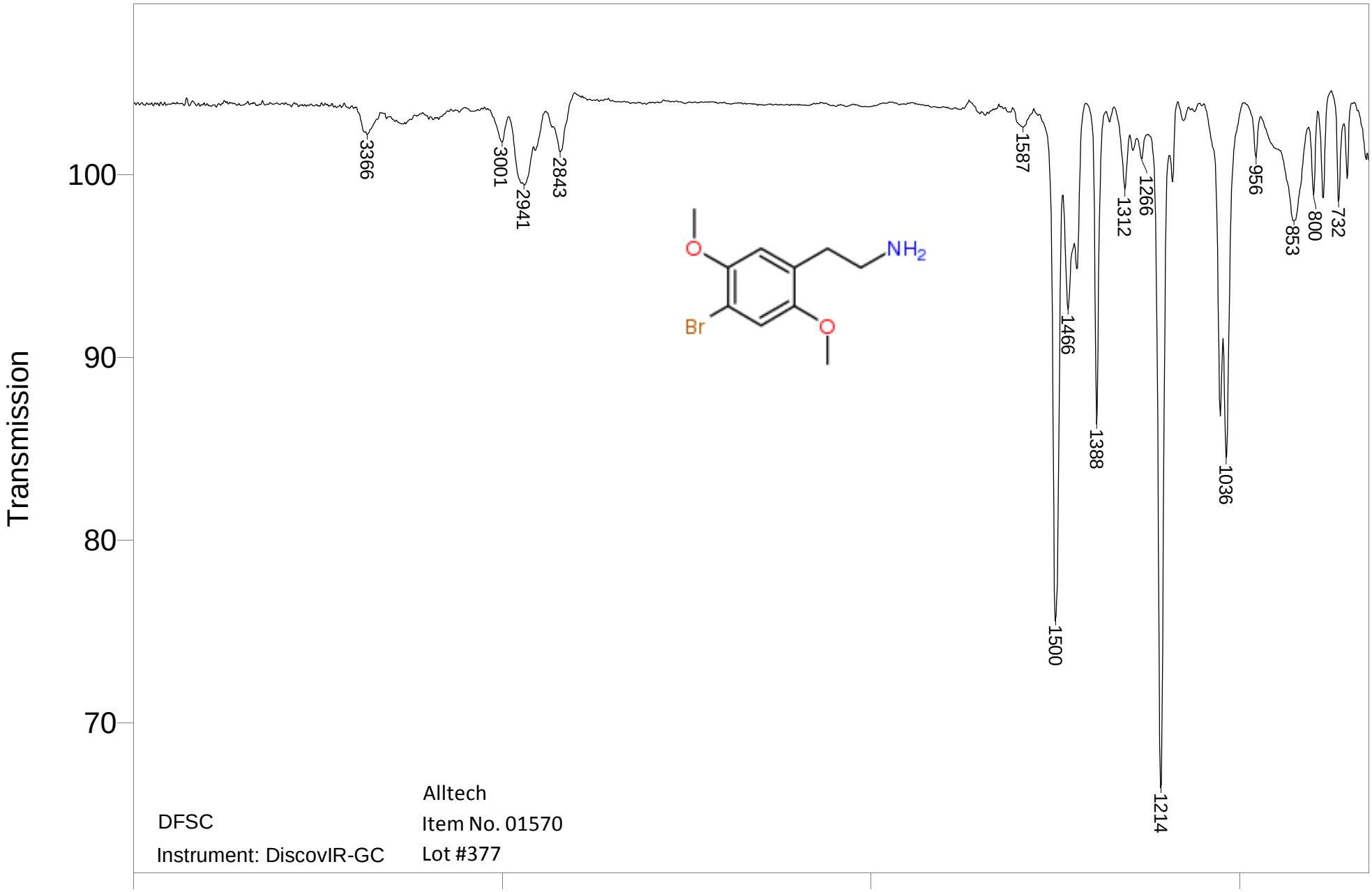


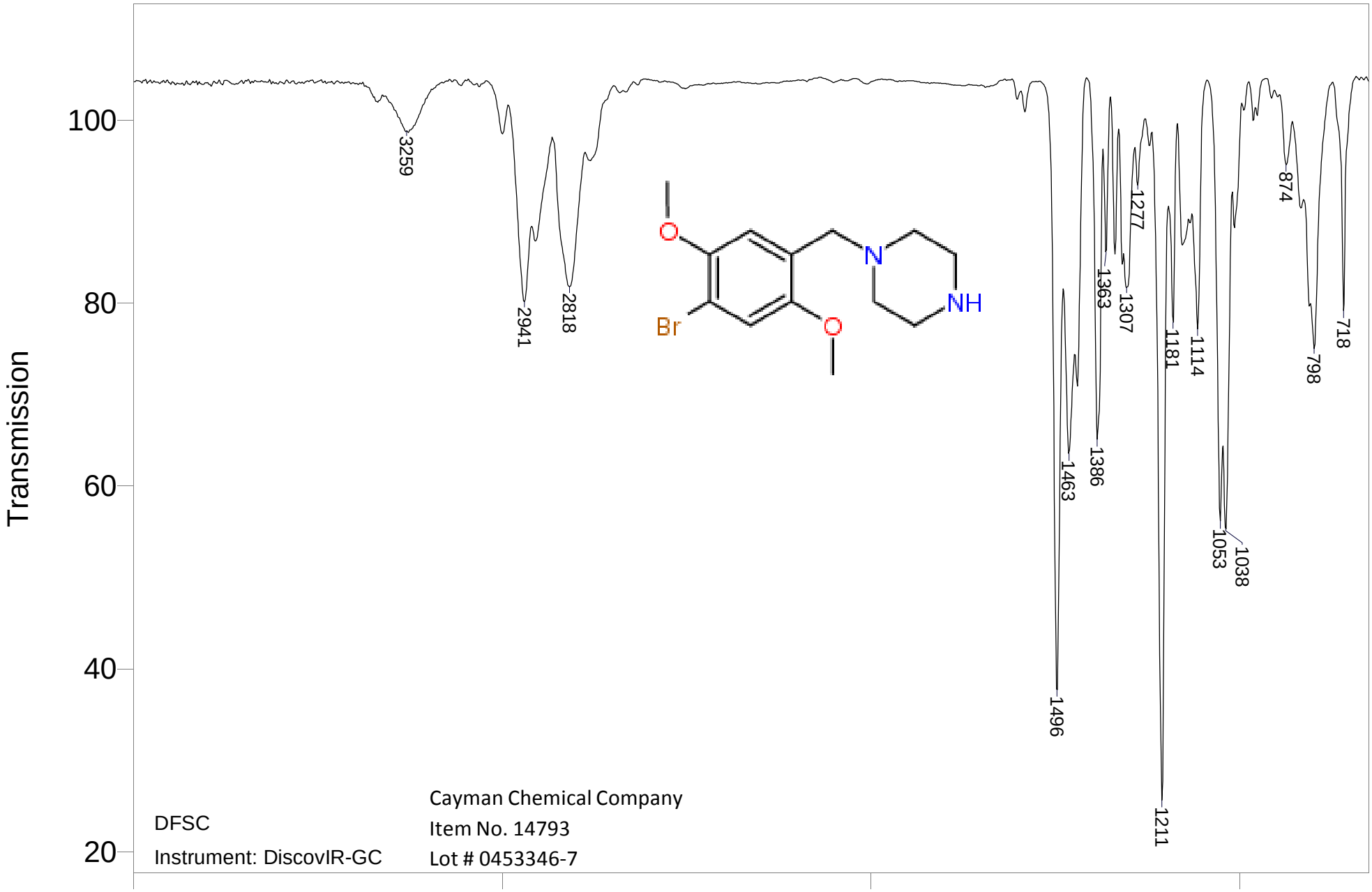


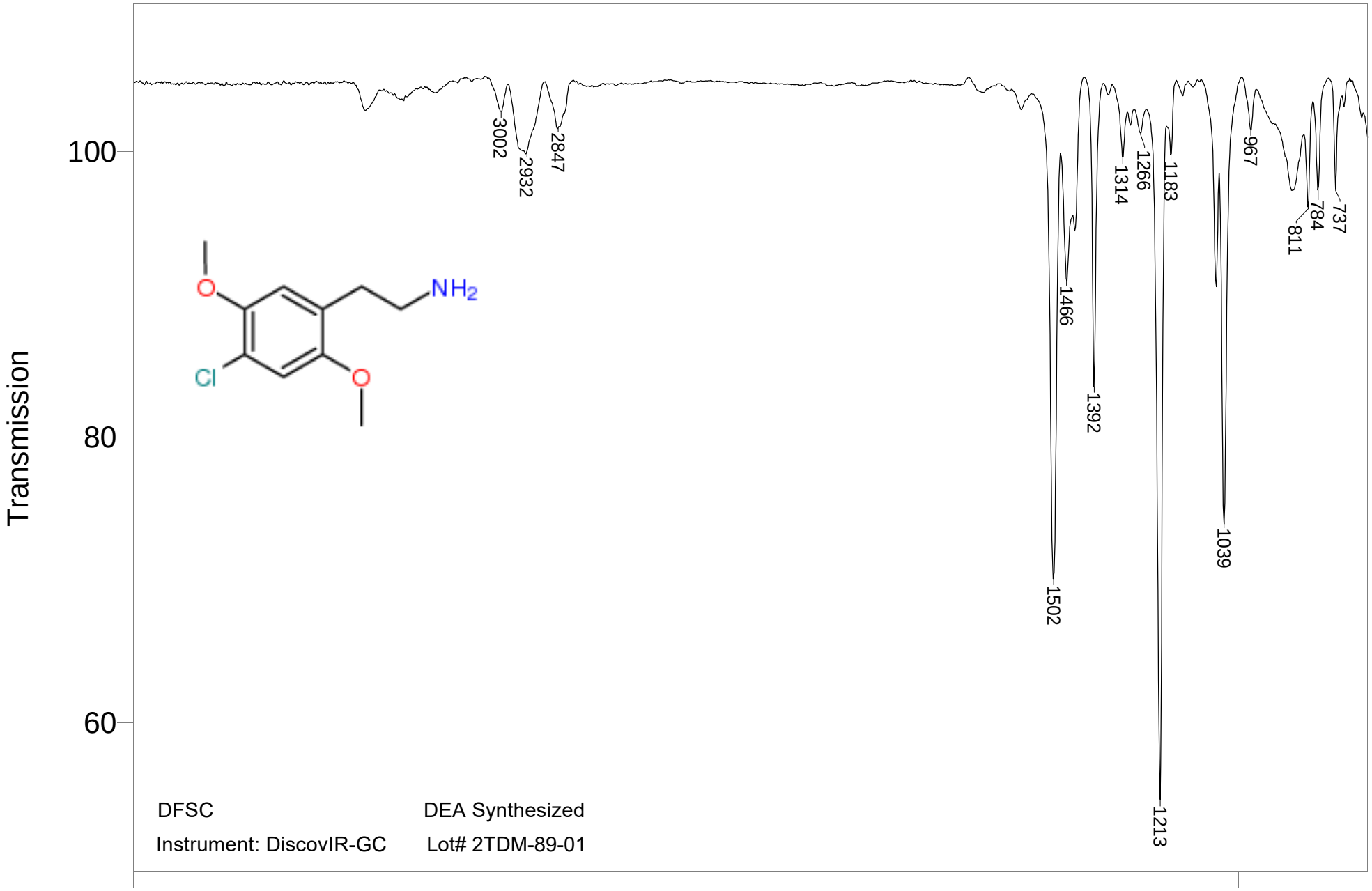


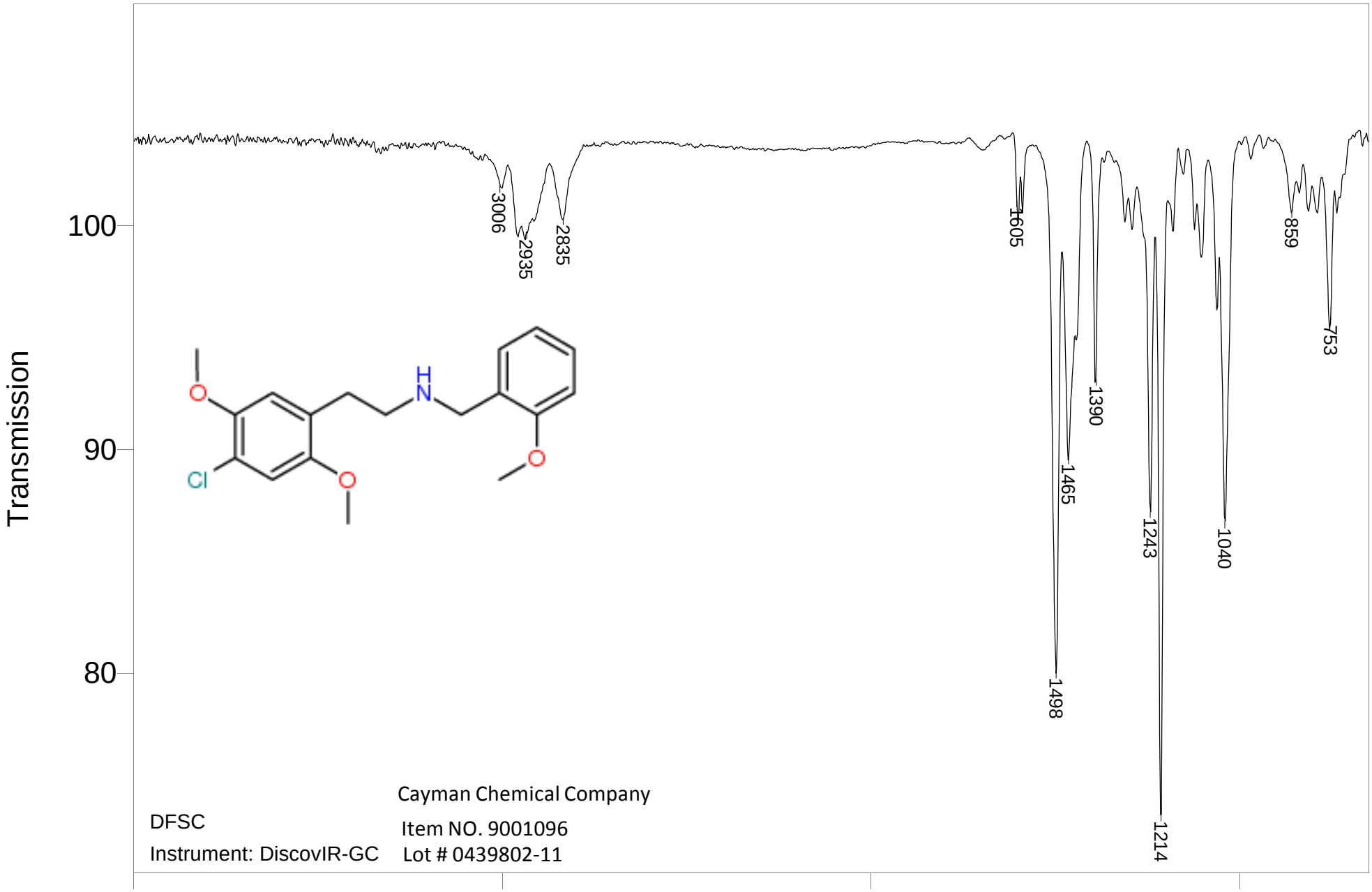


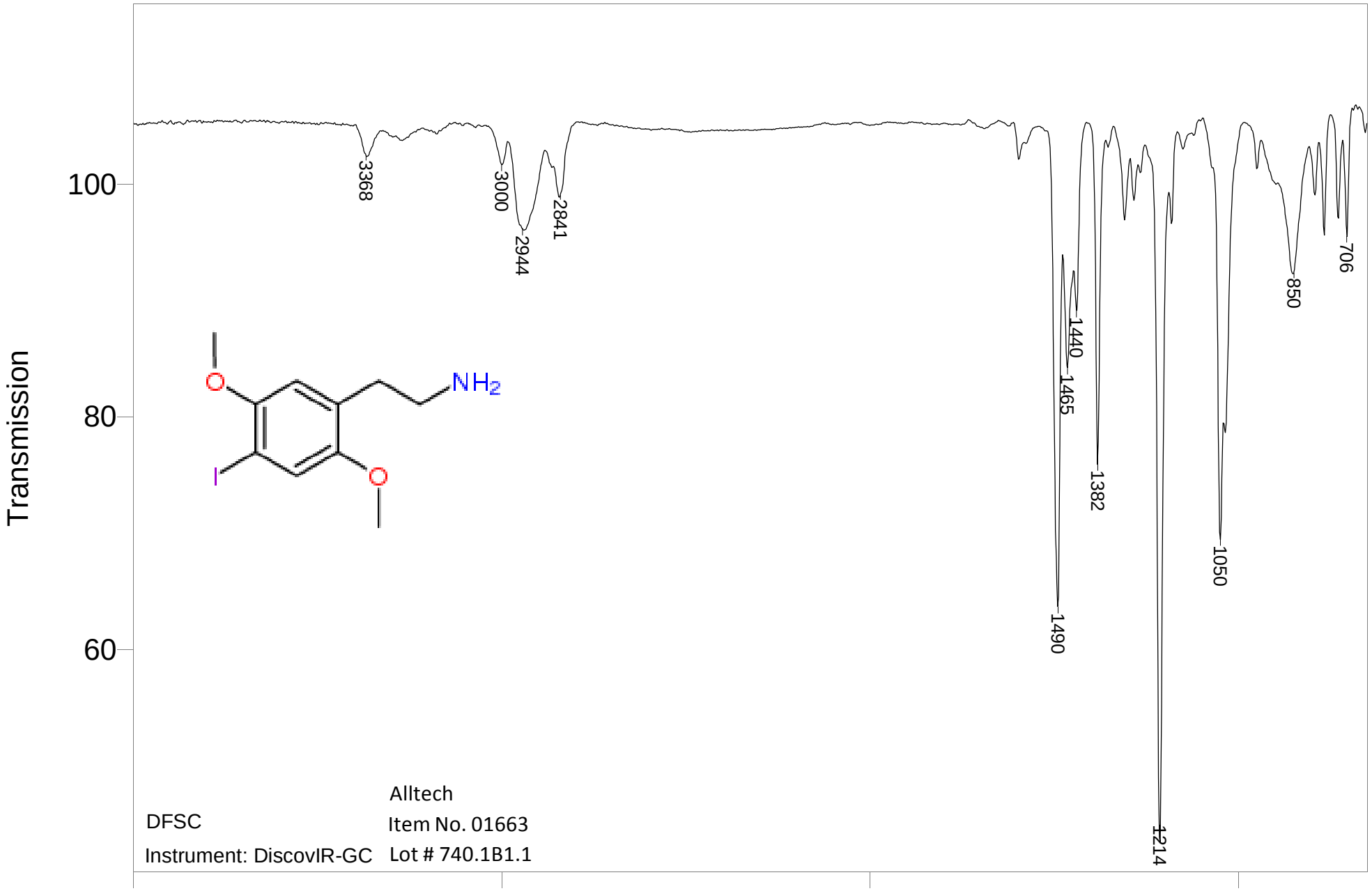


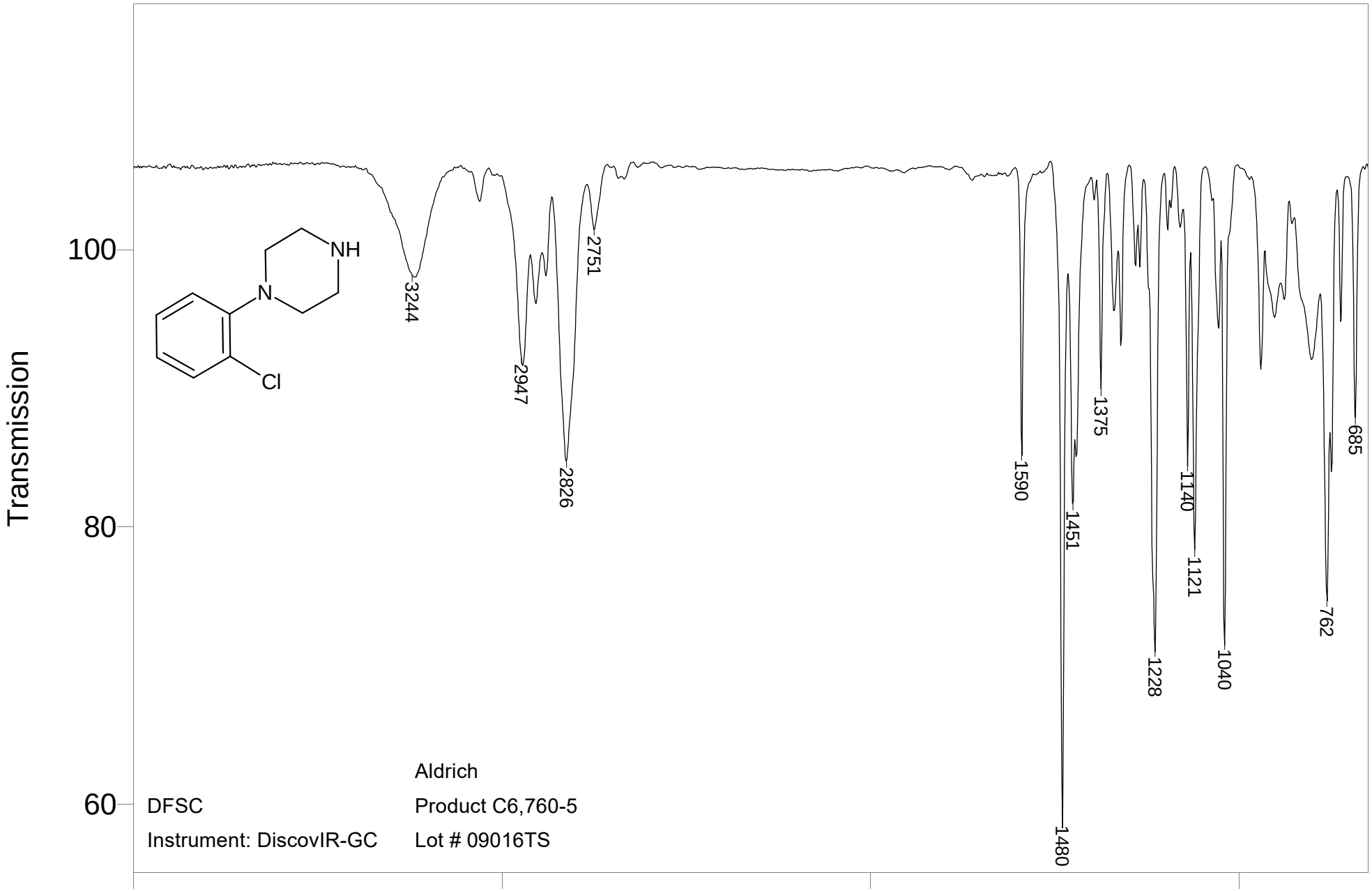




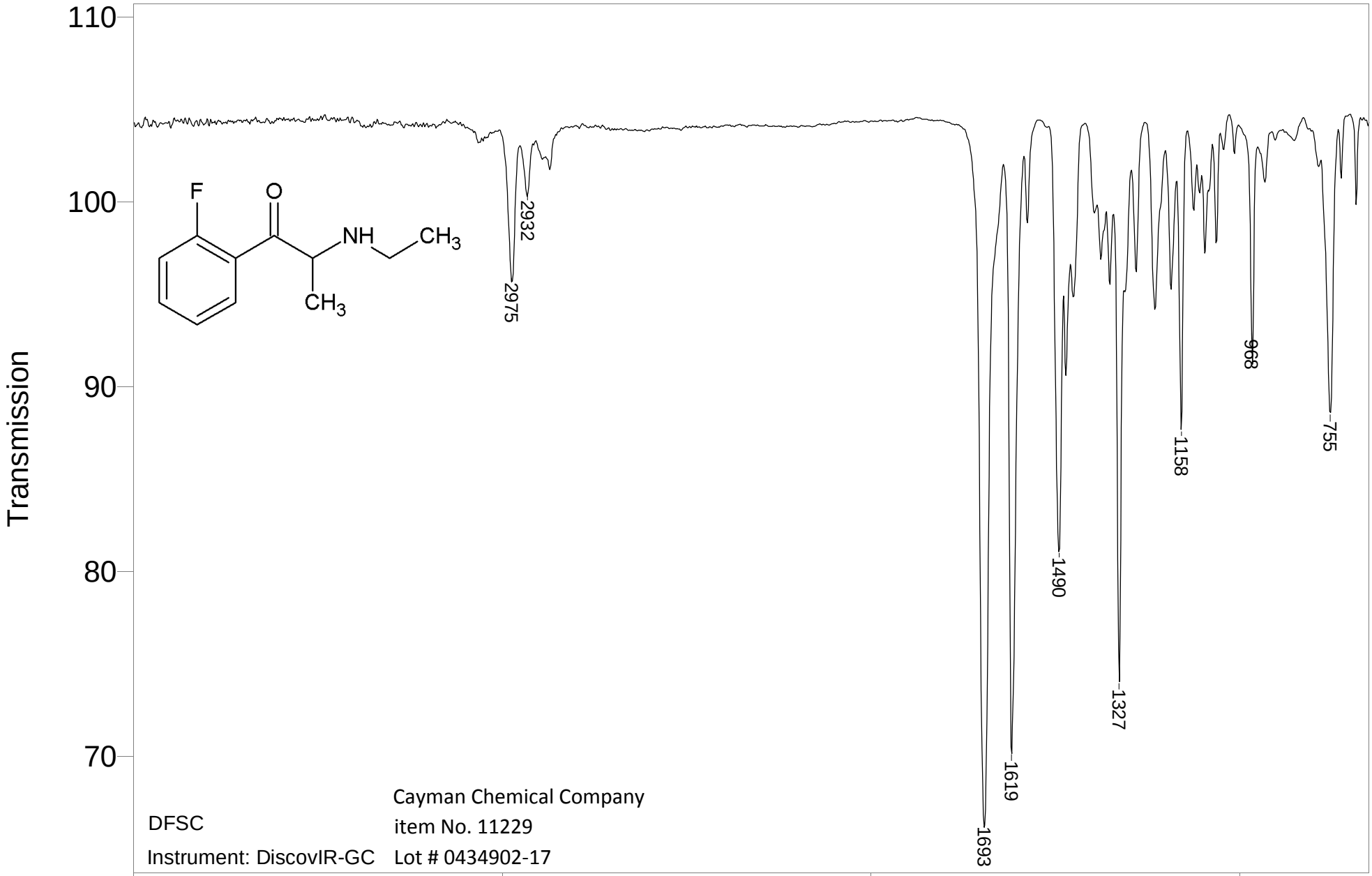


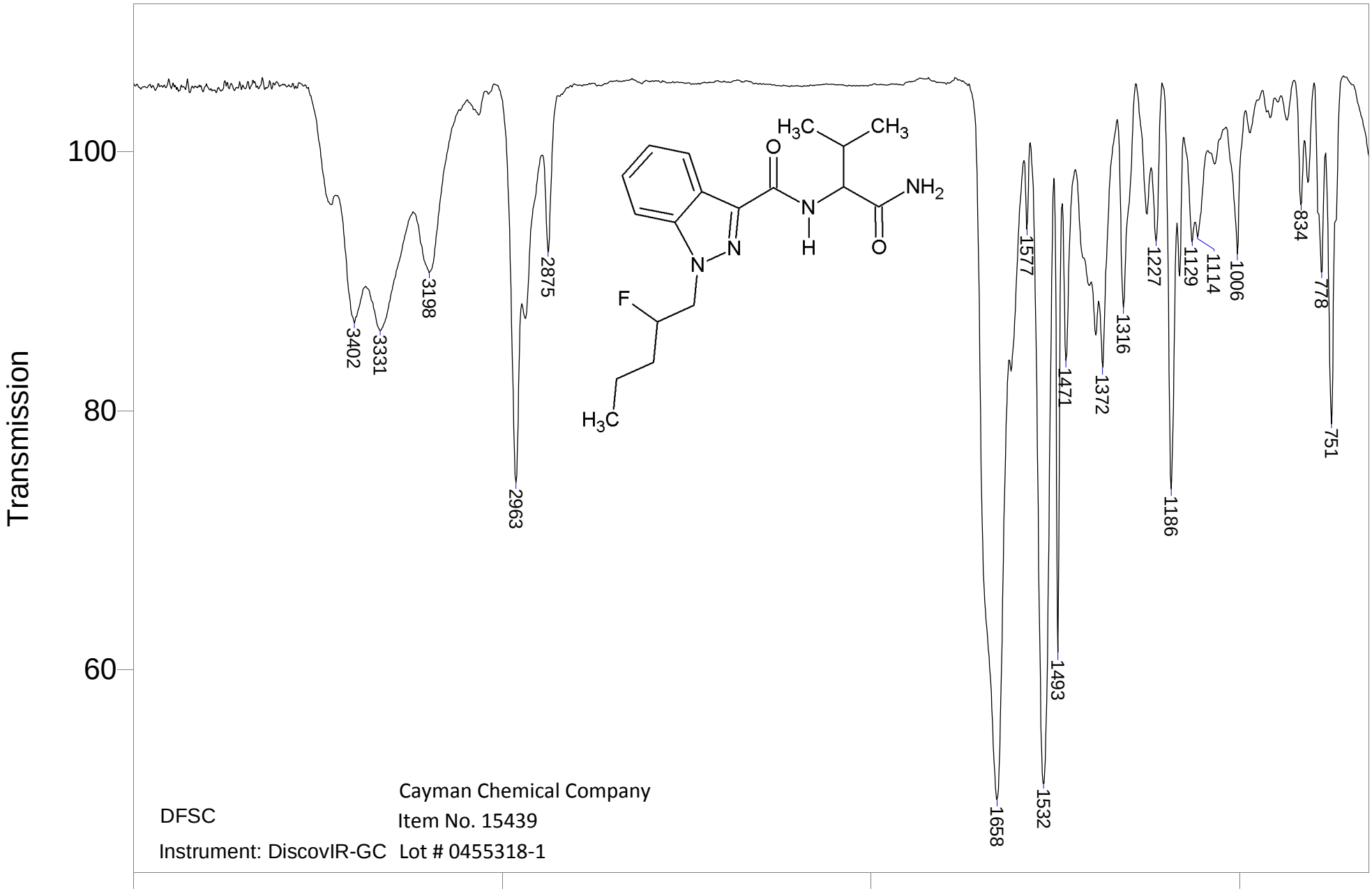


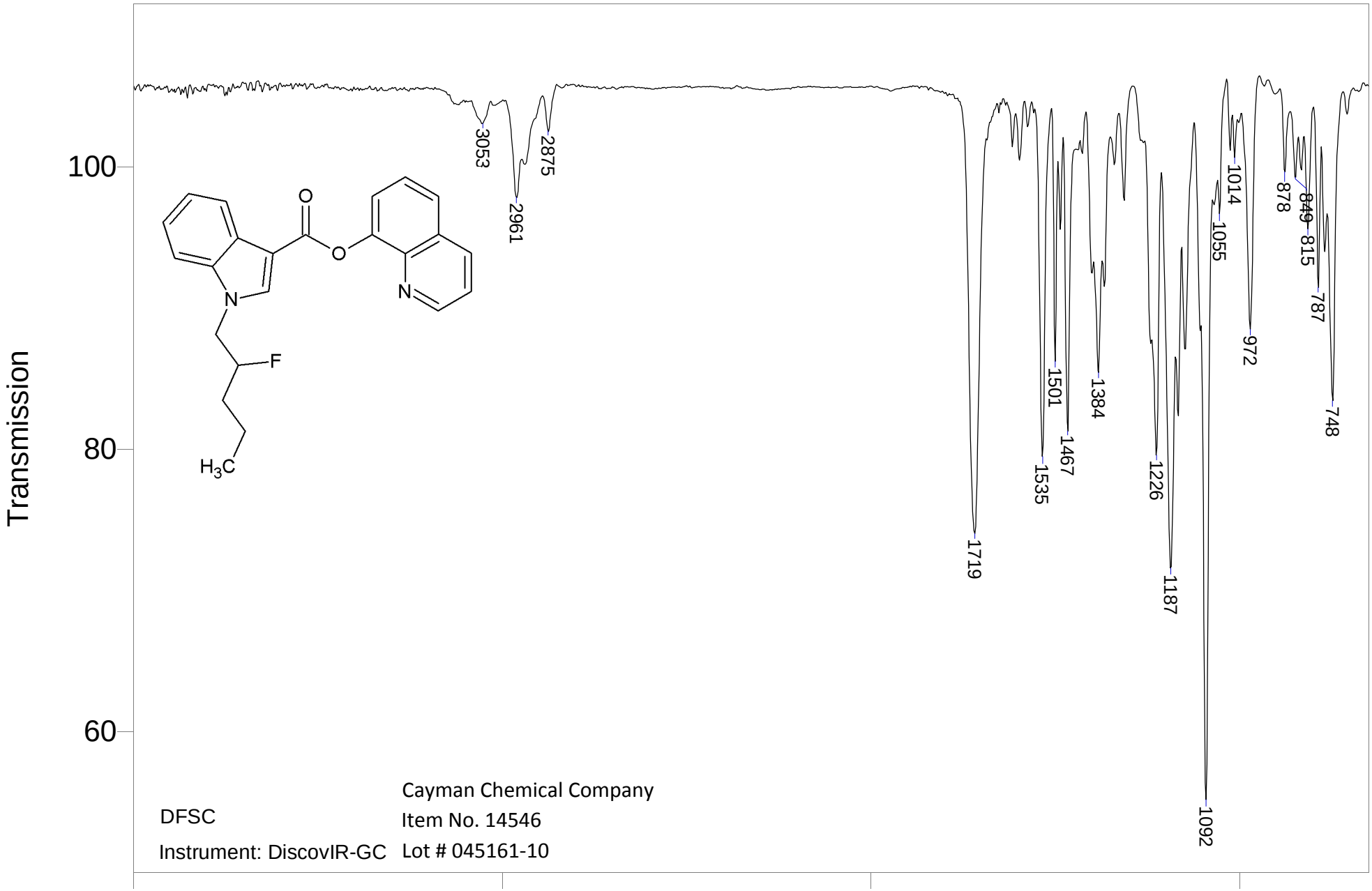




2-Fluoroethcathinone







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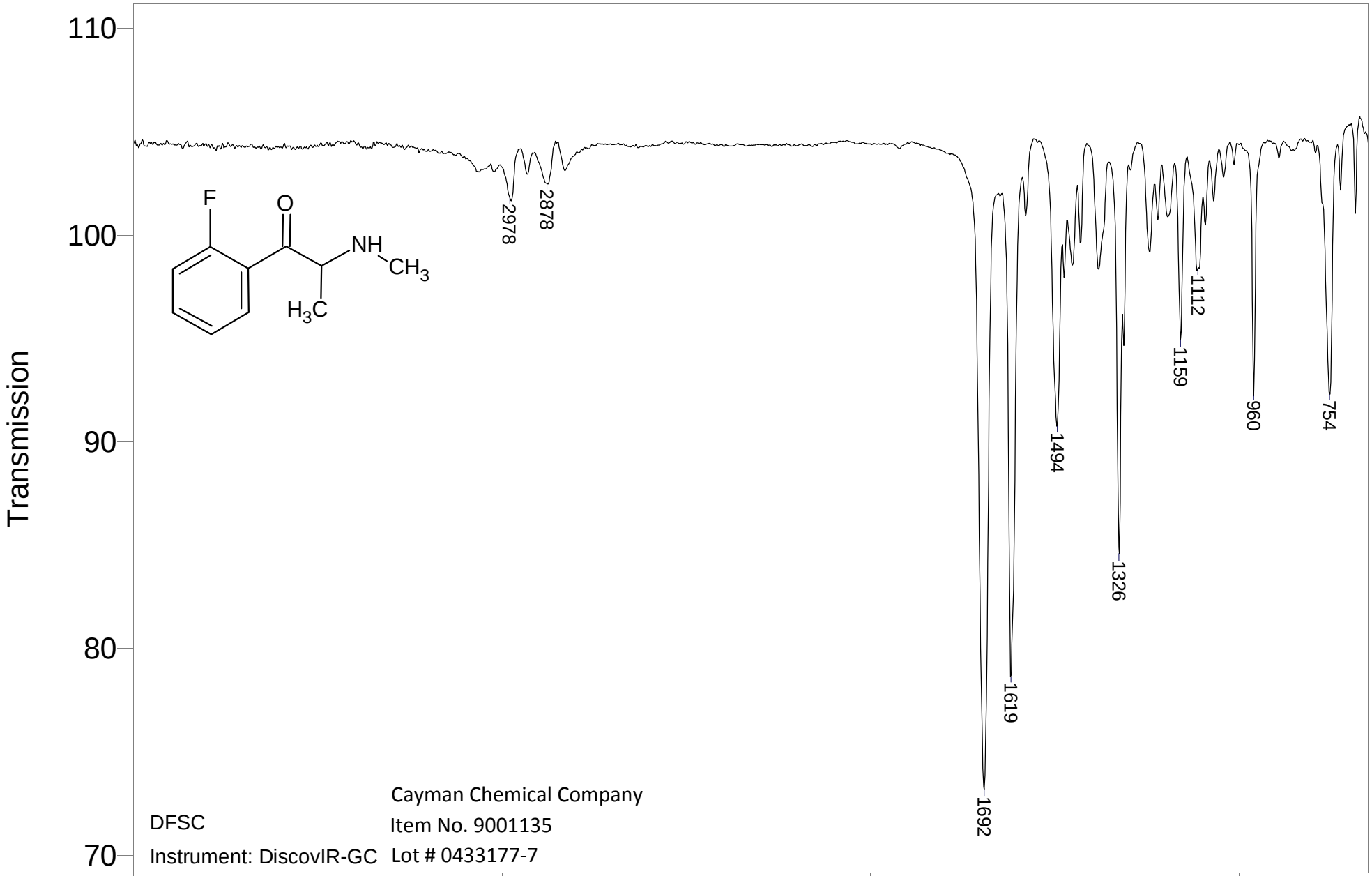

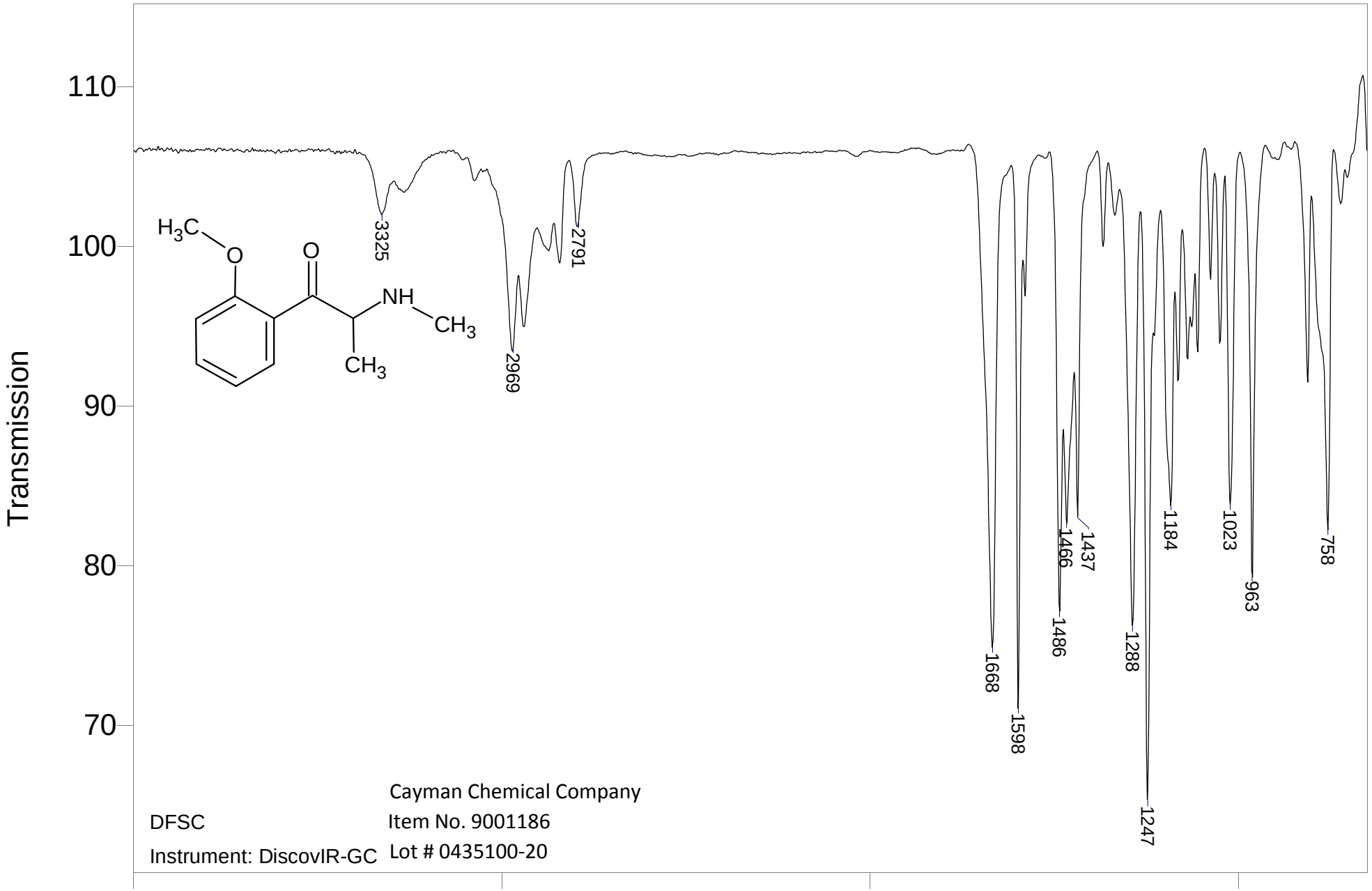

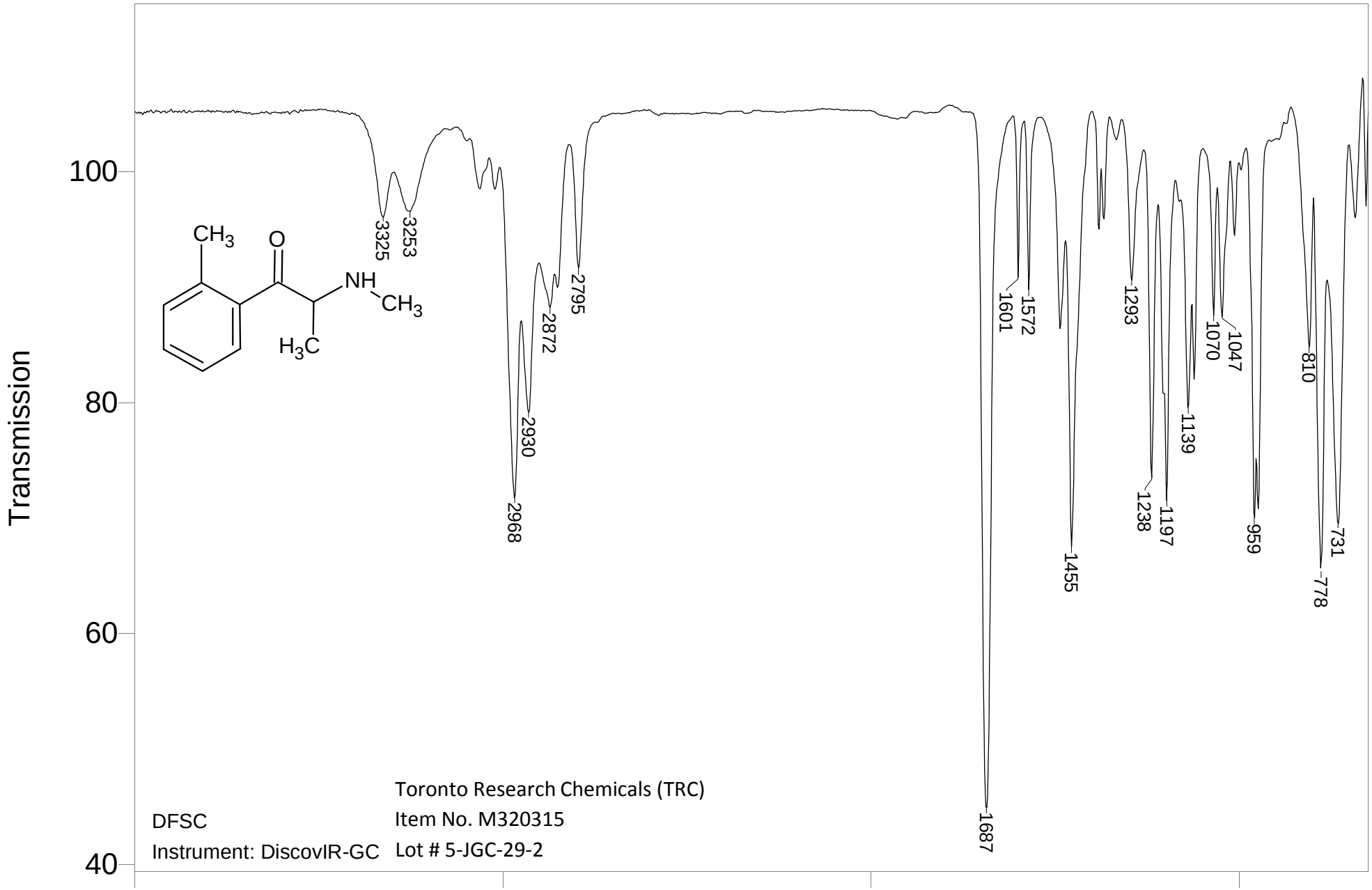

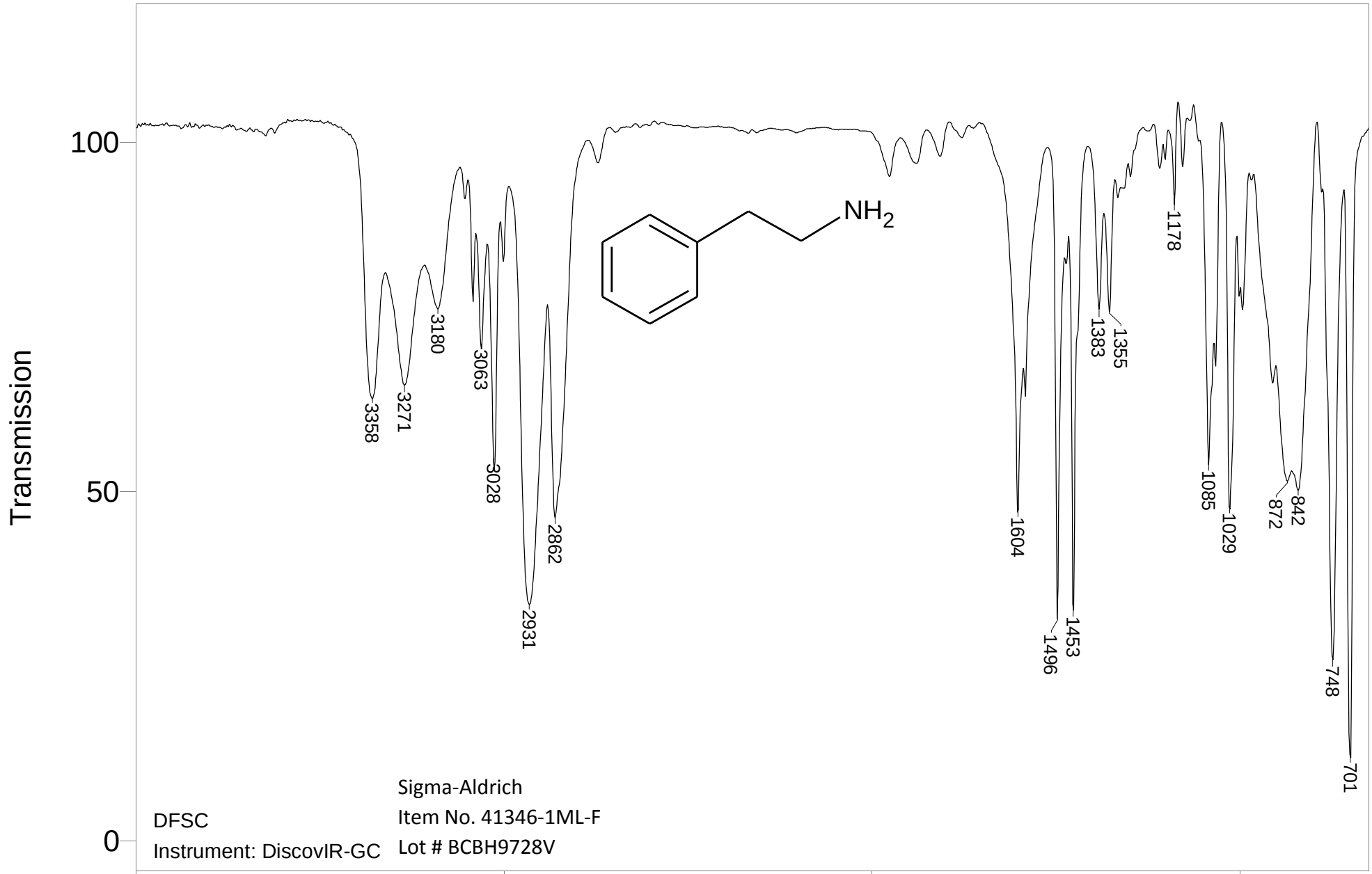

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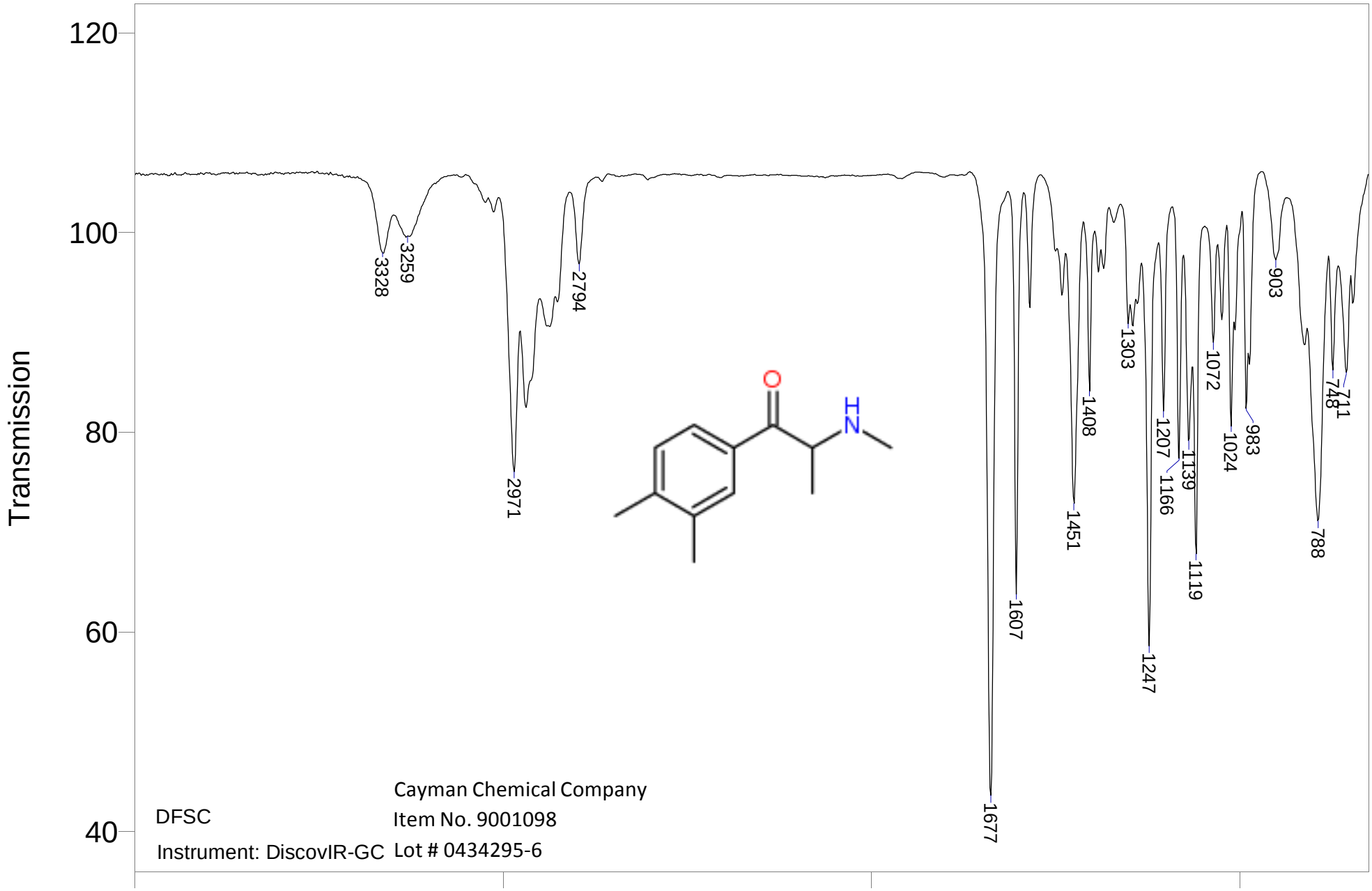
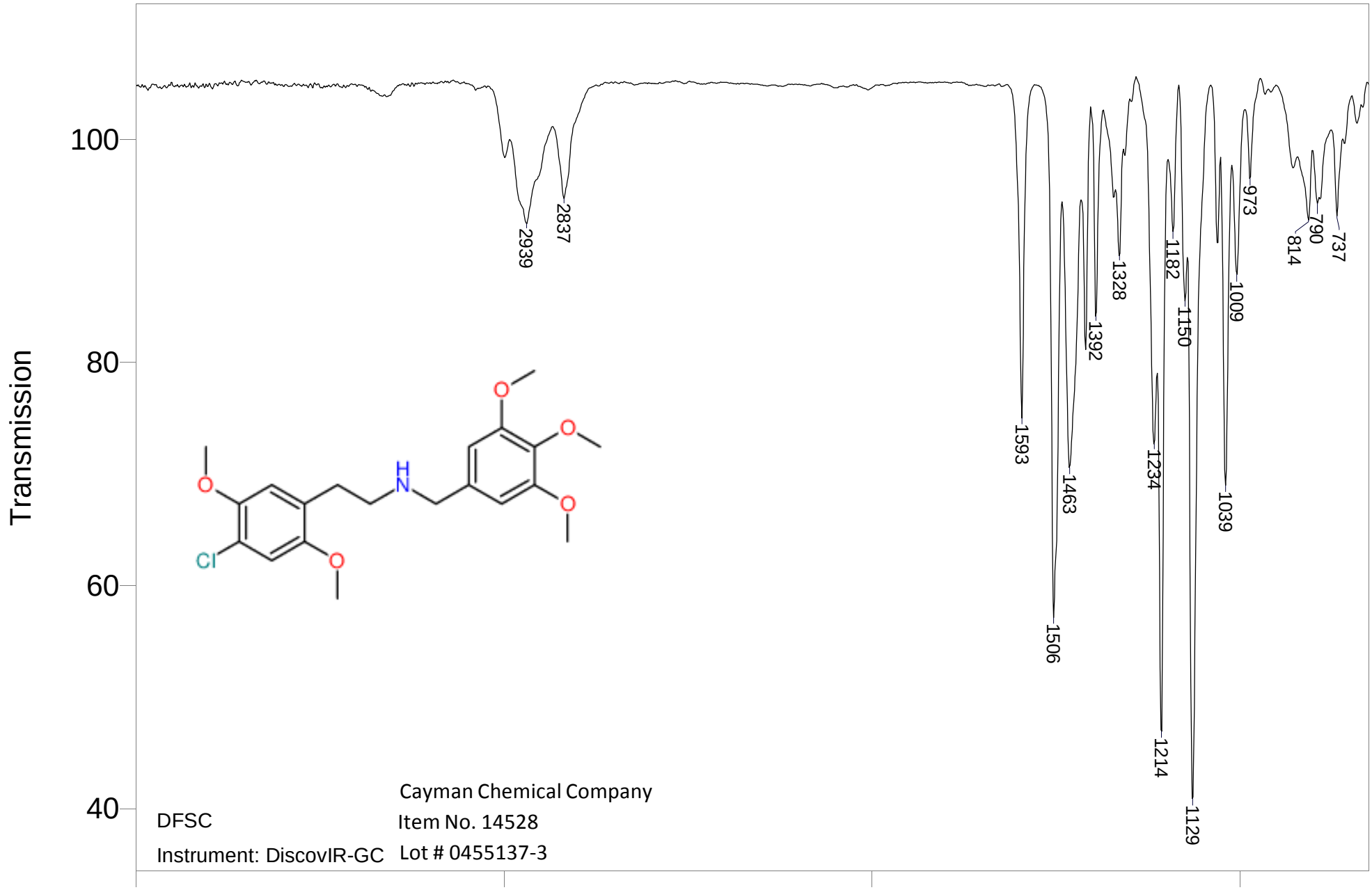
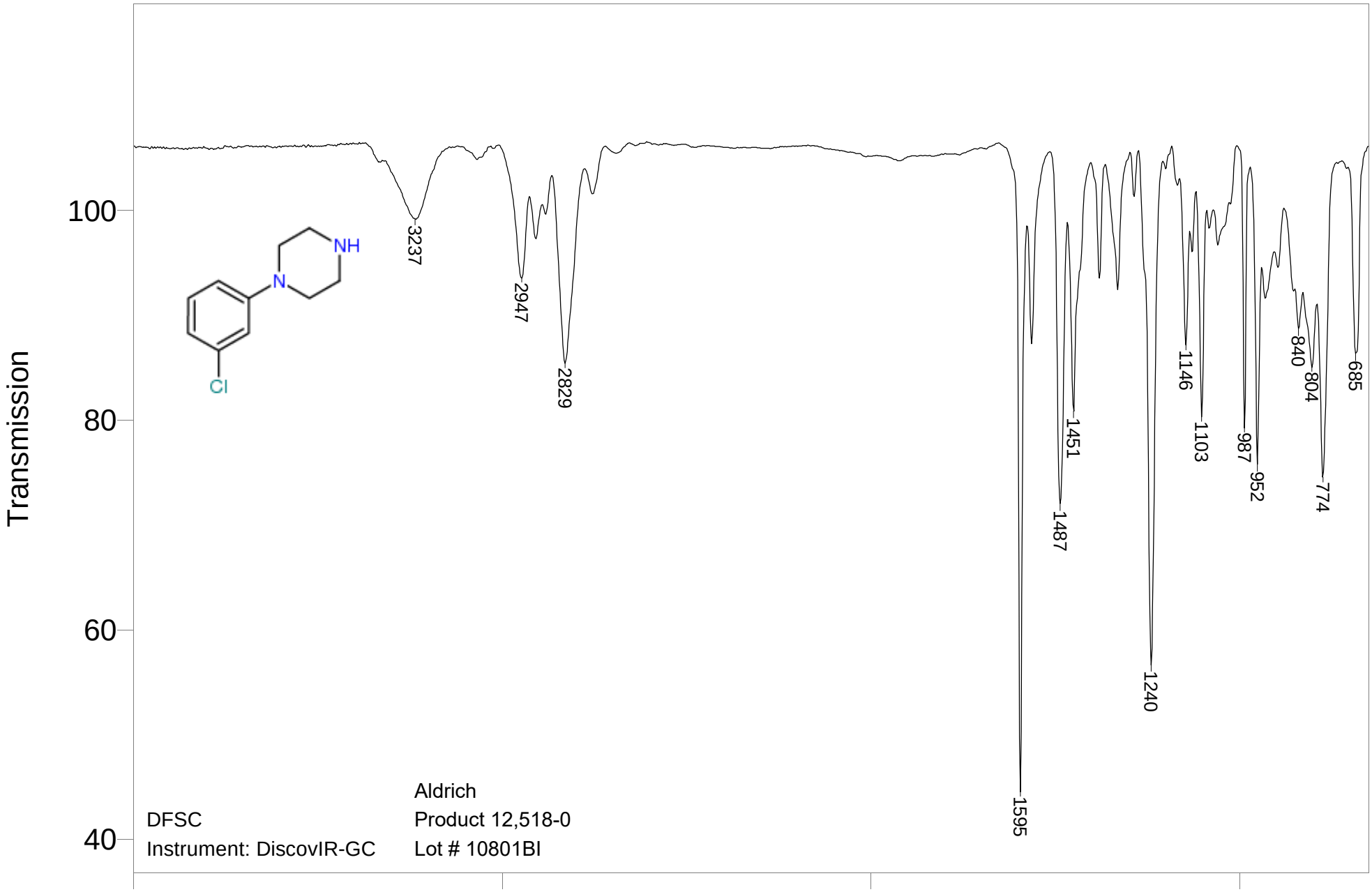
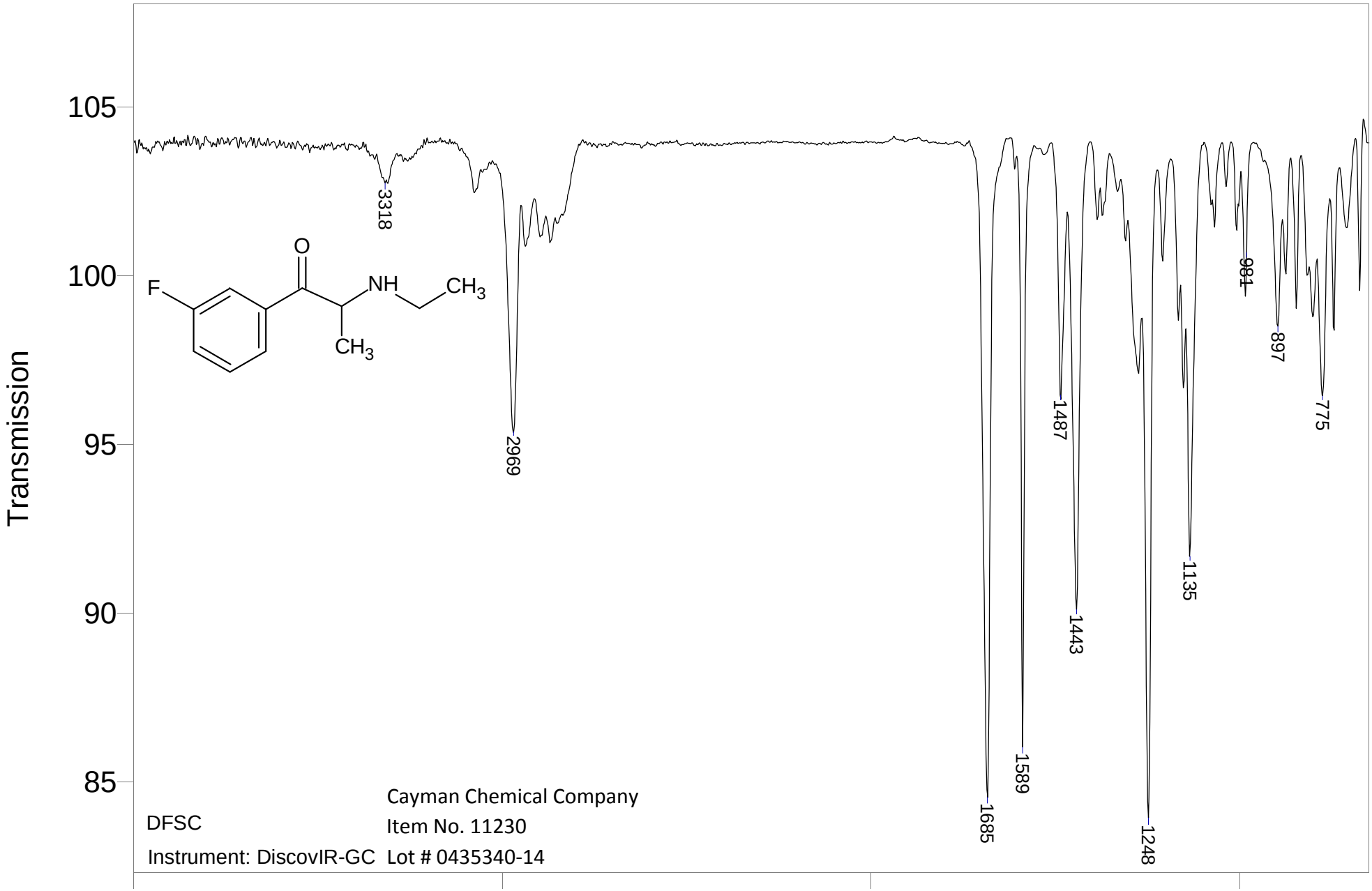
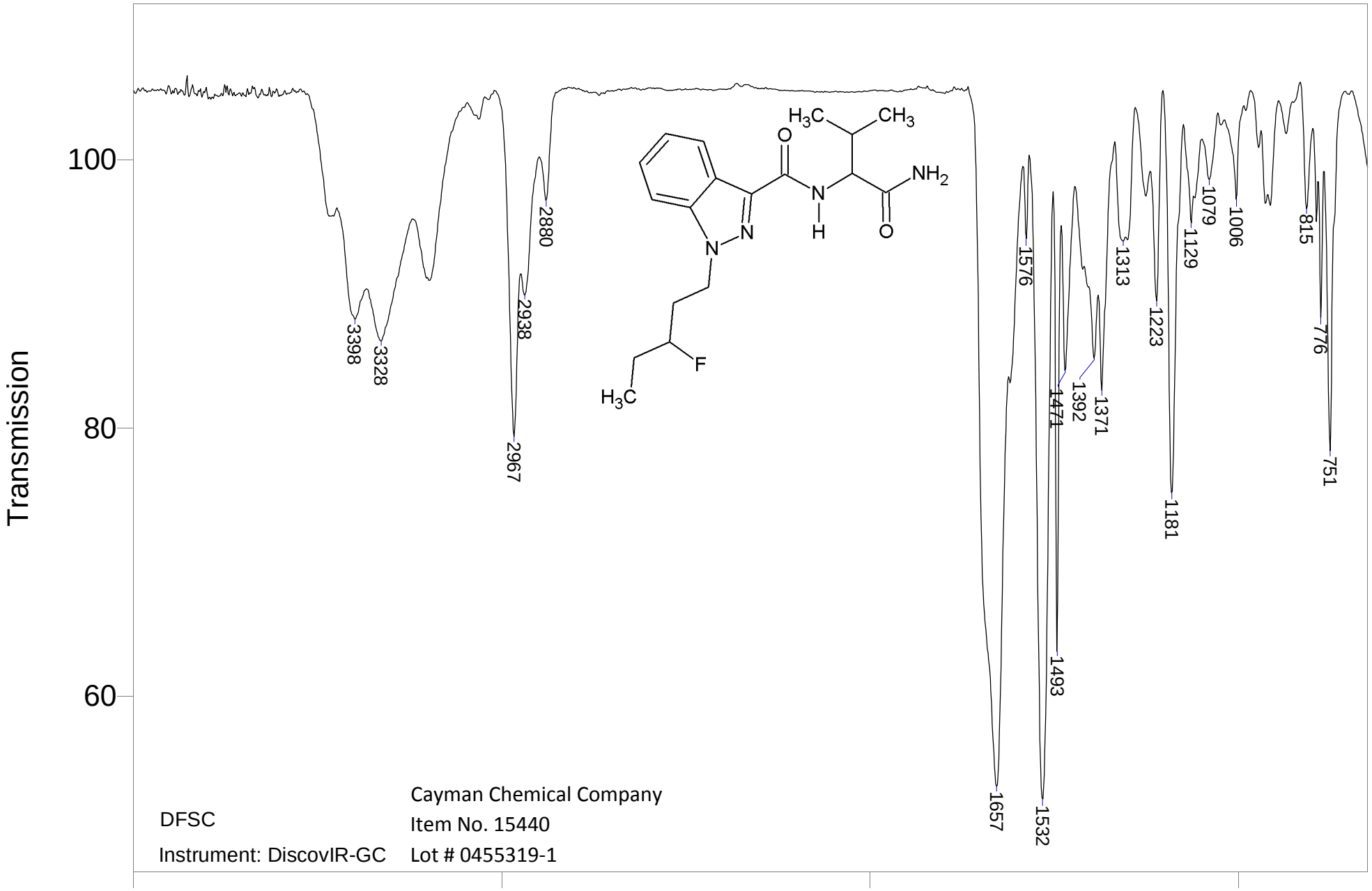
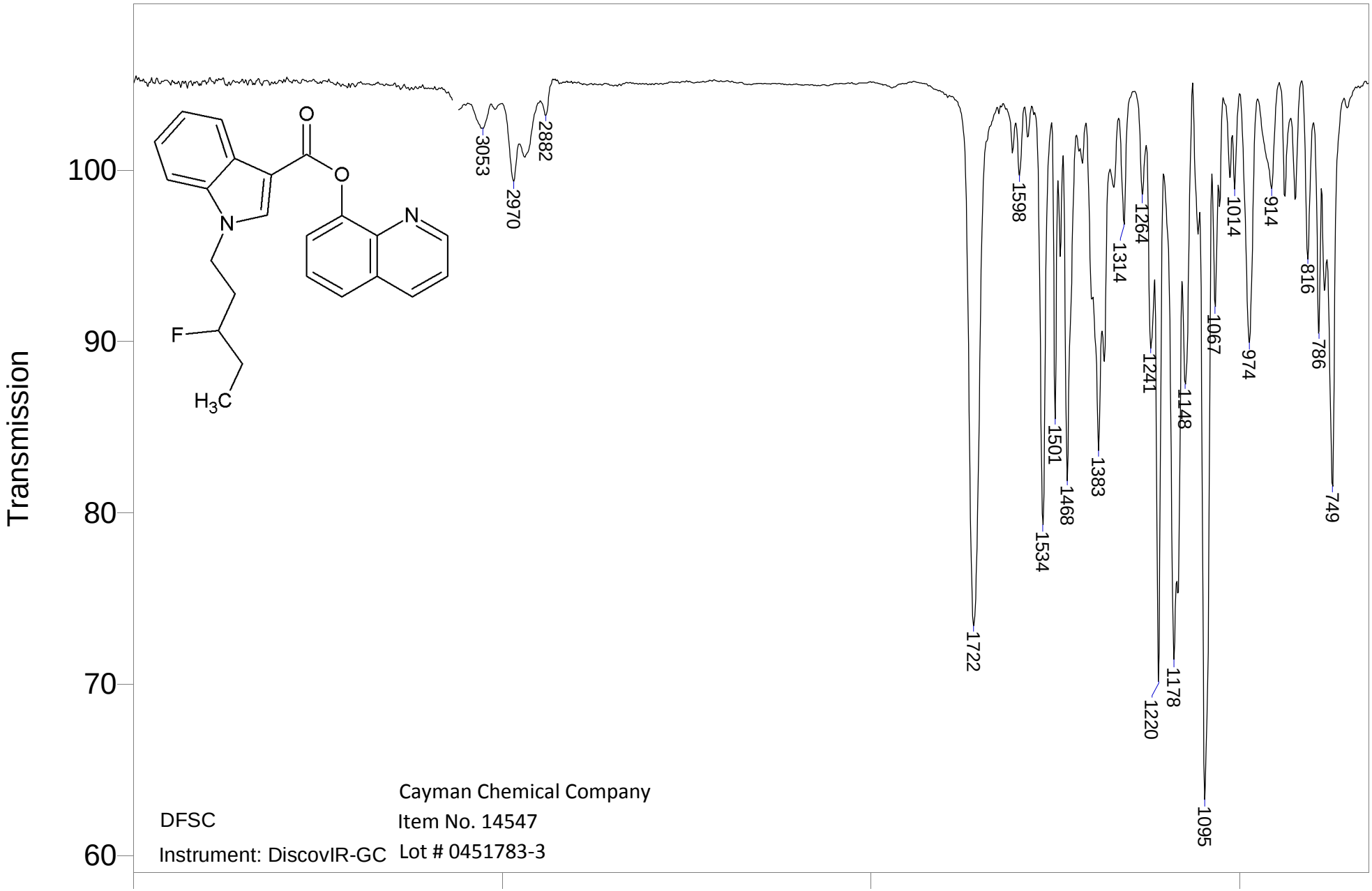


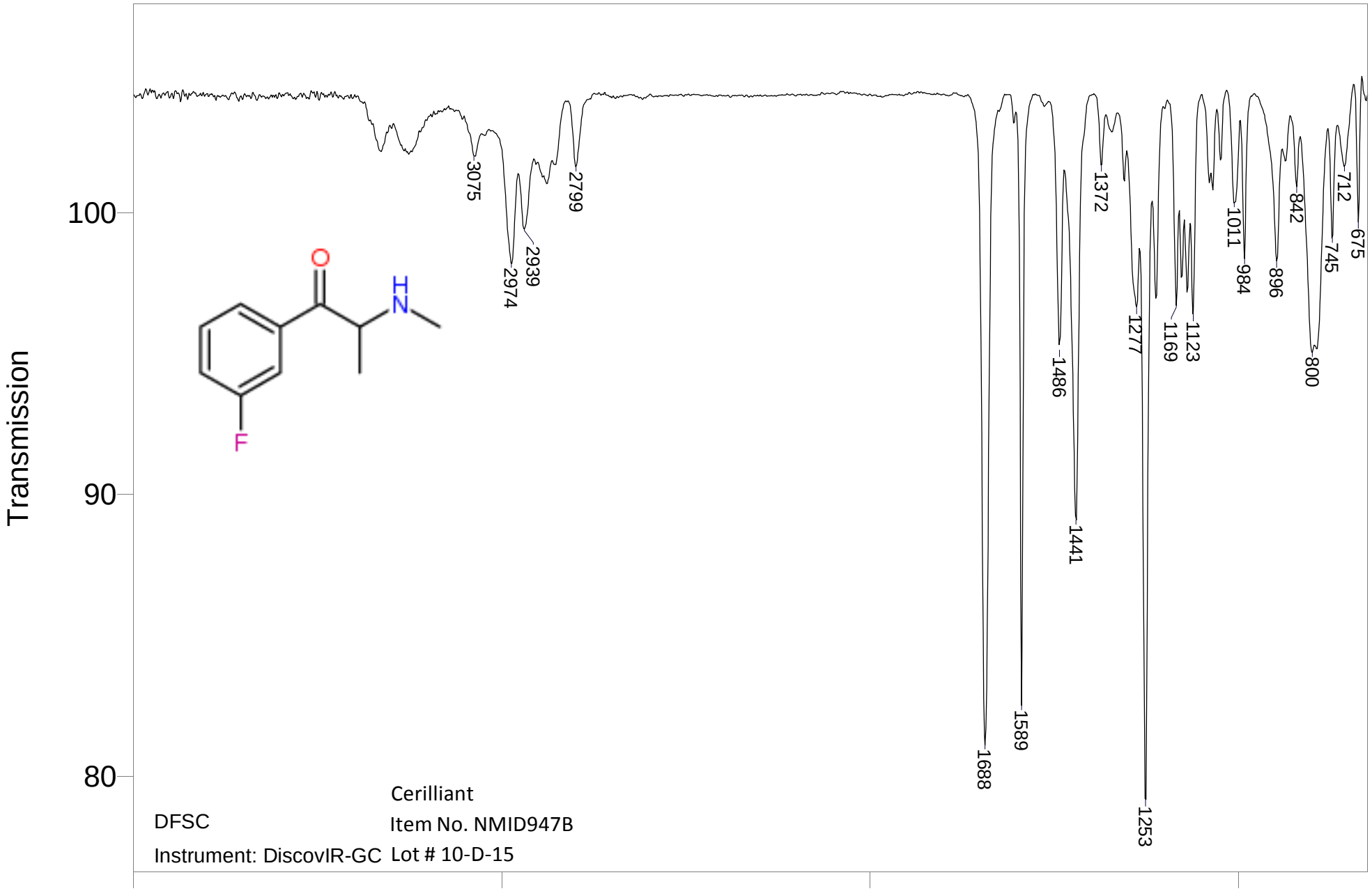


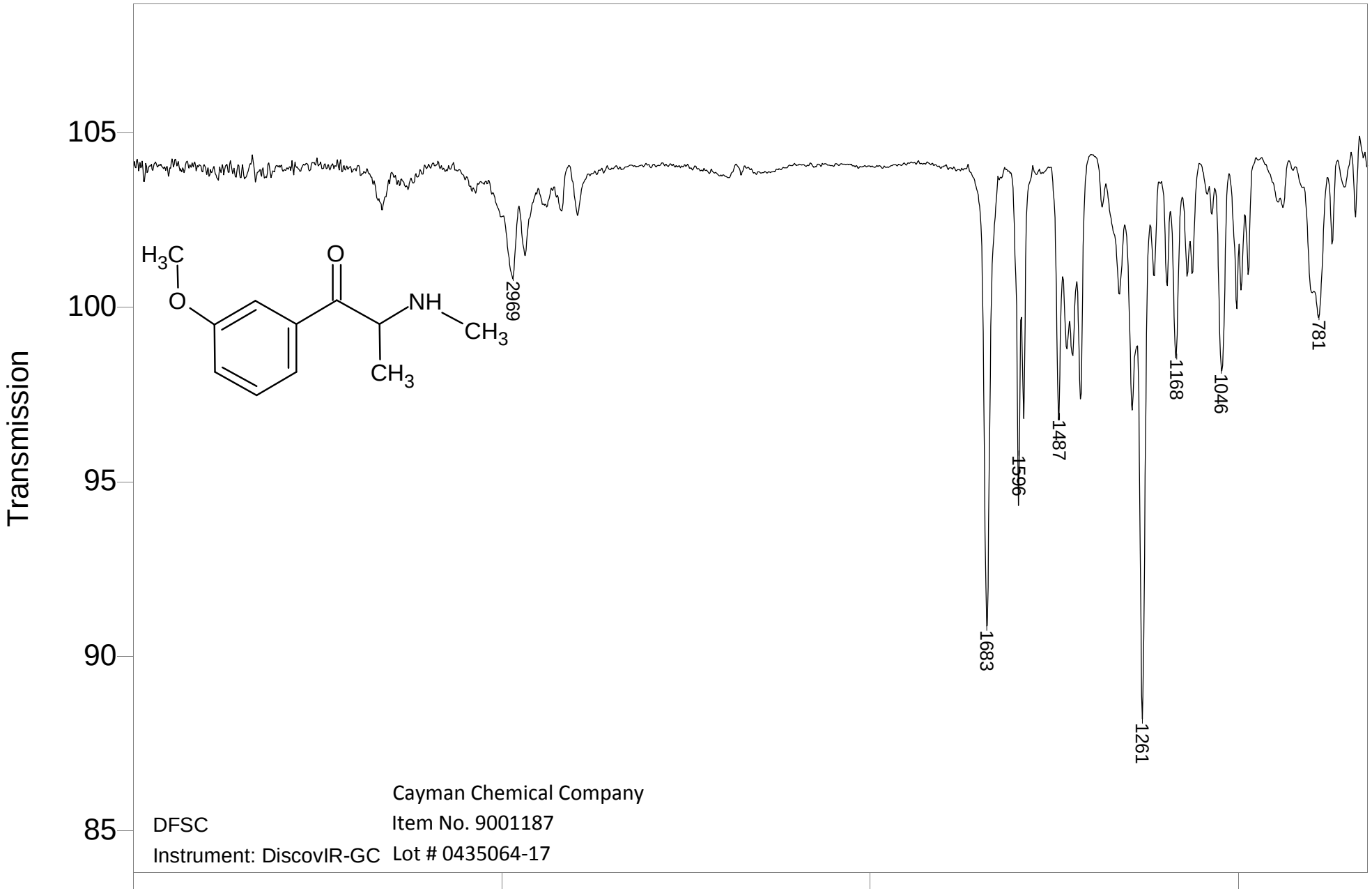


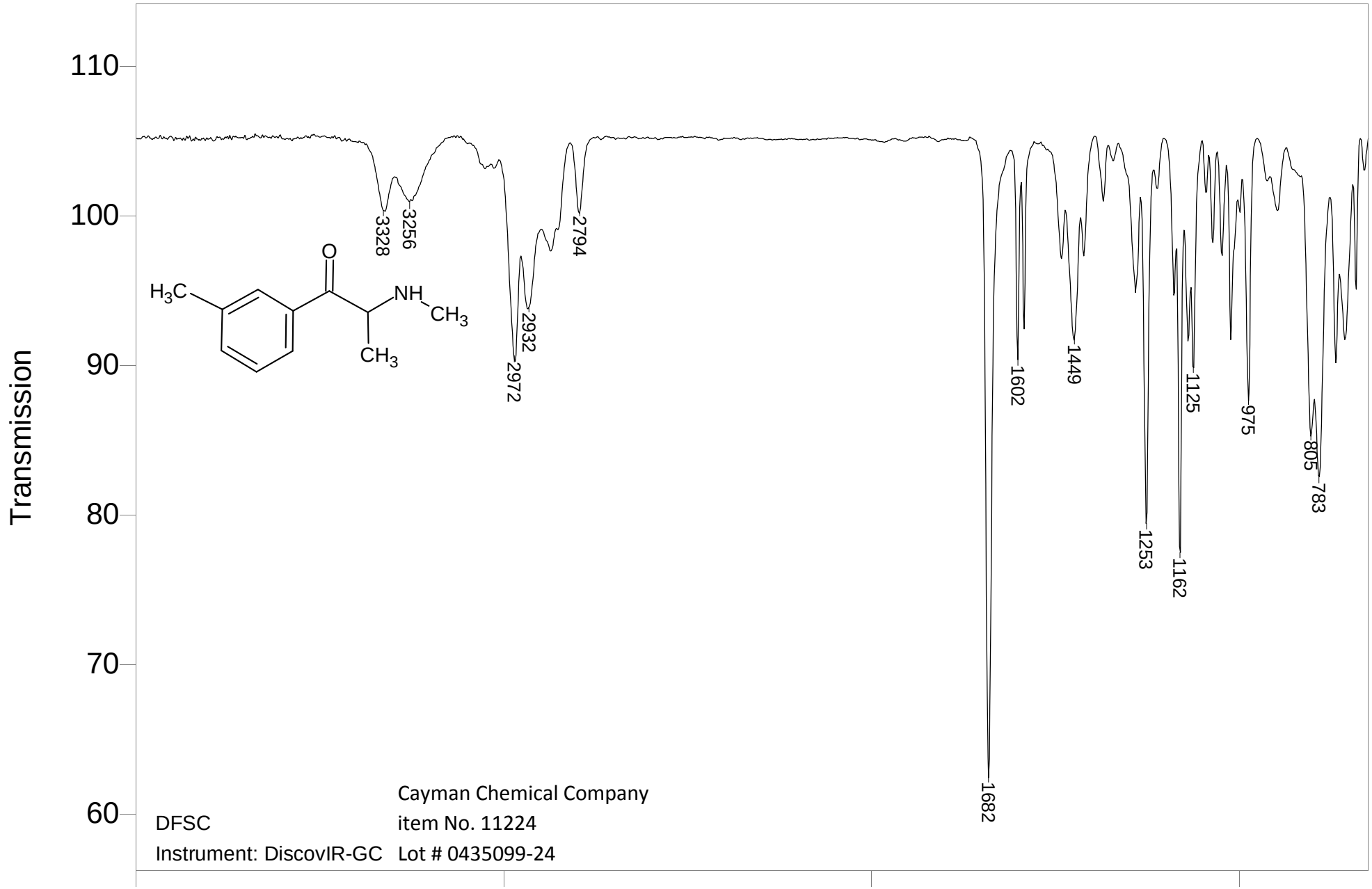


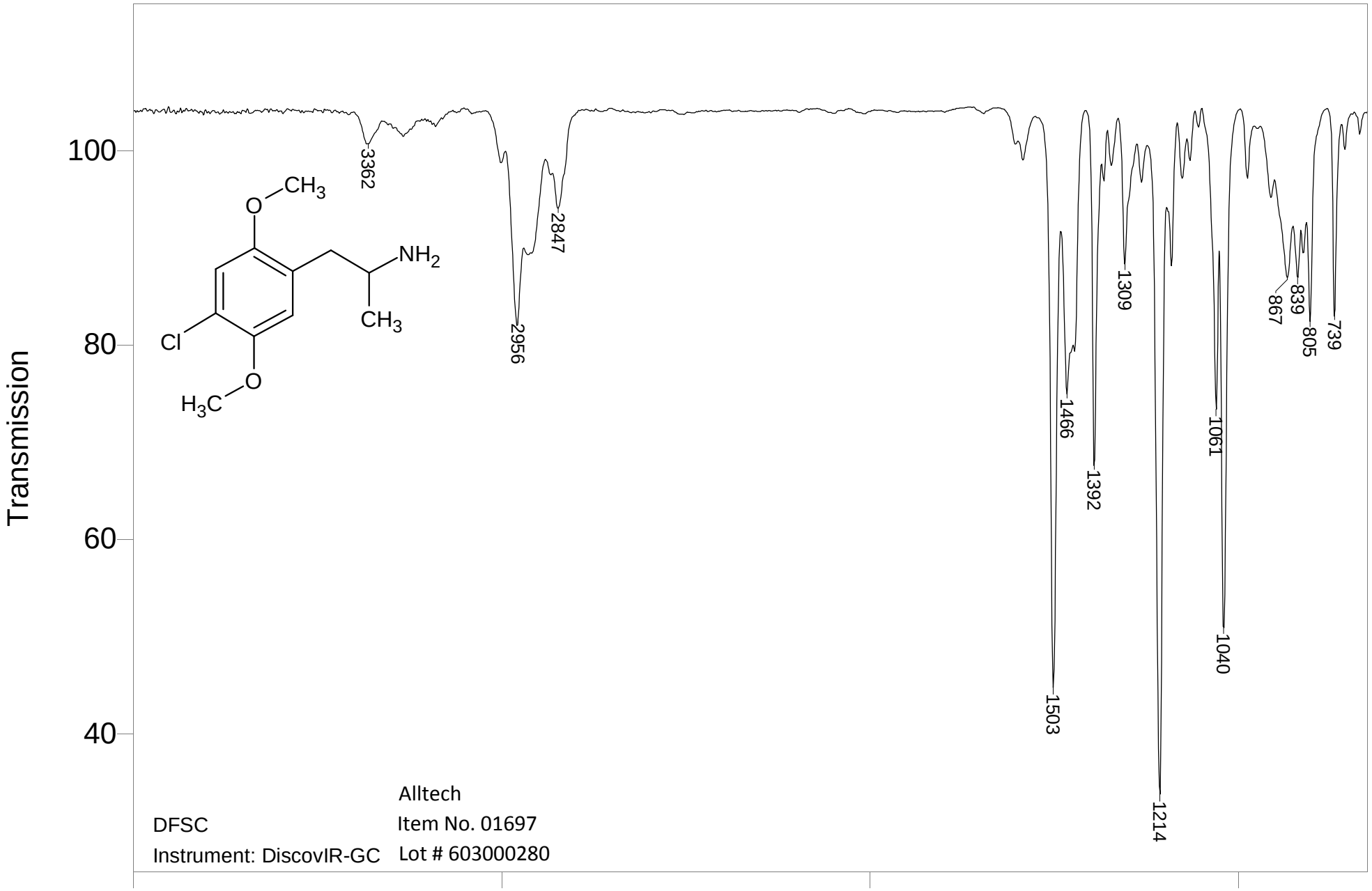


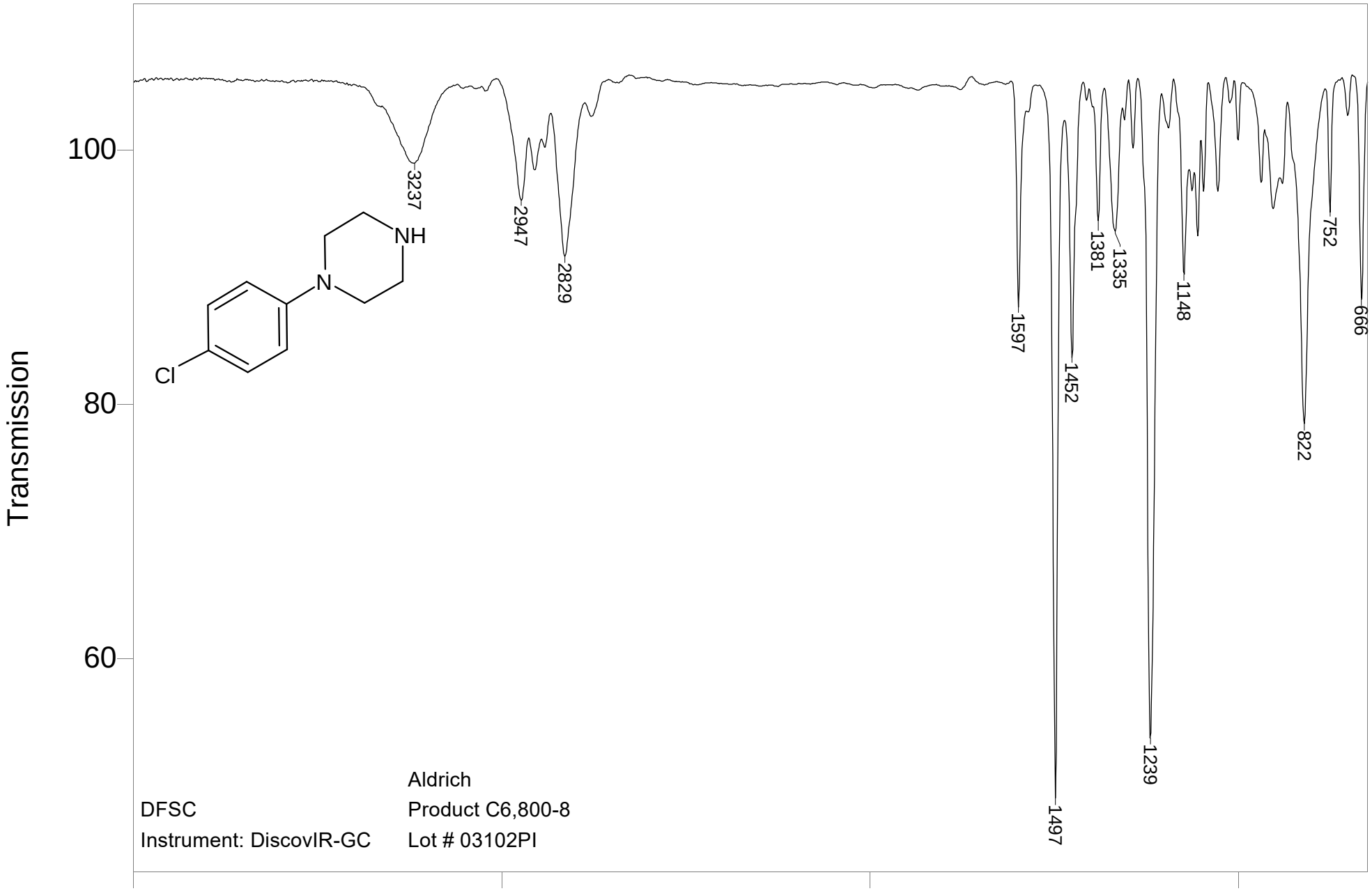


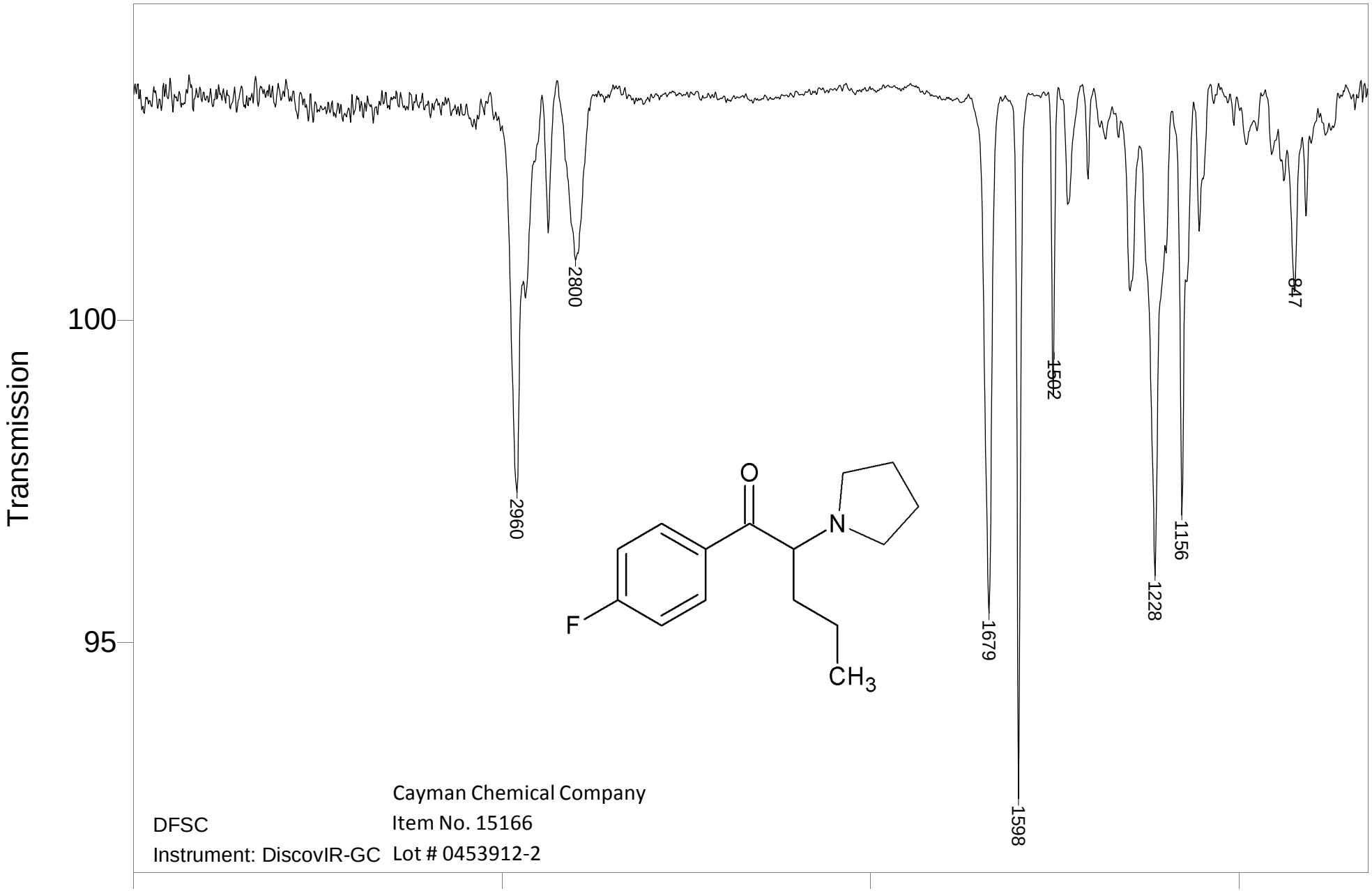


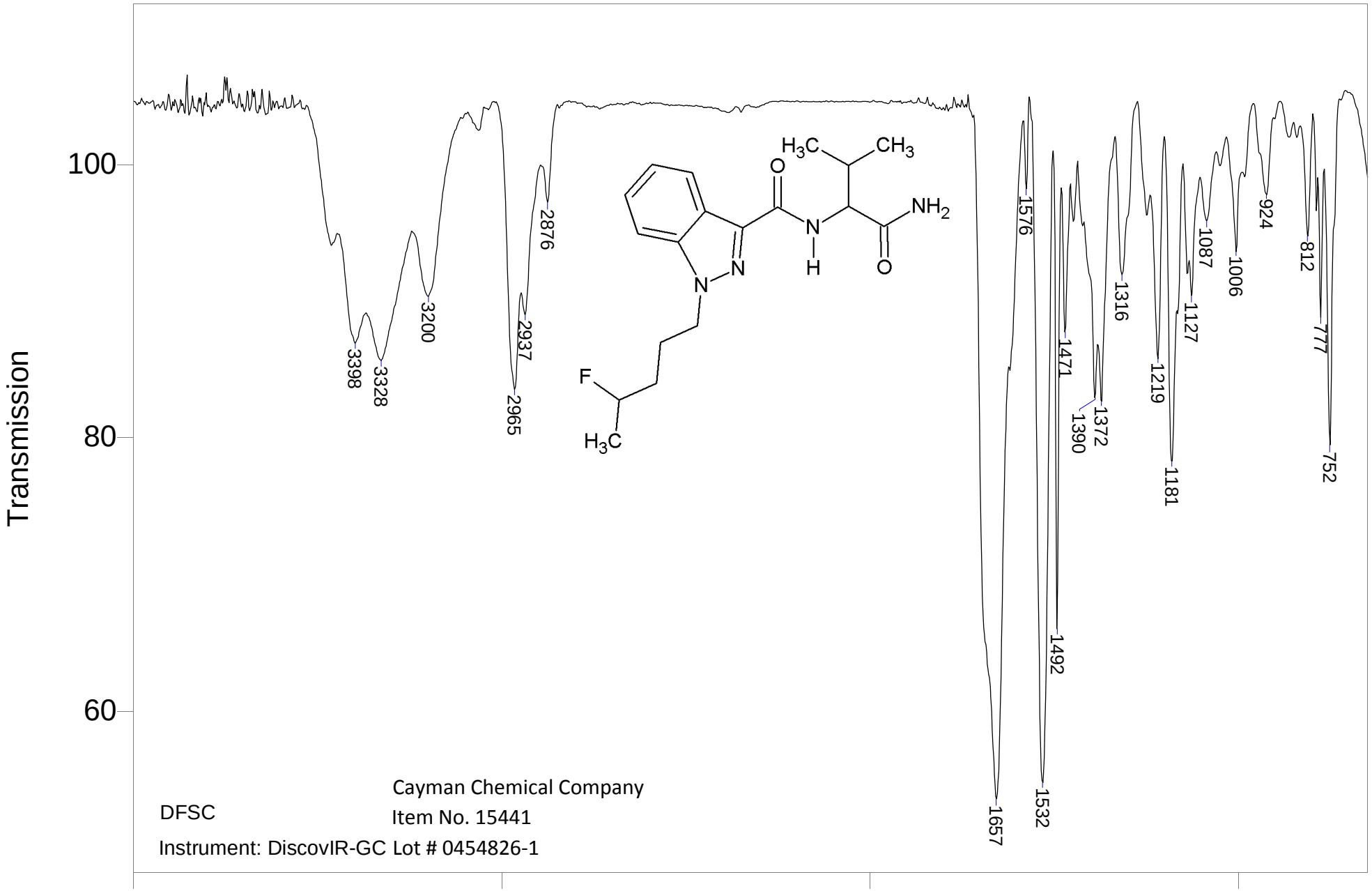


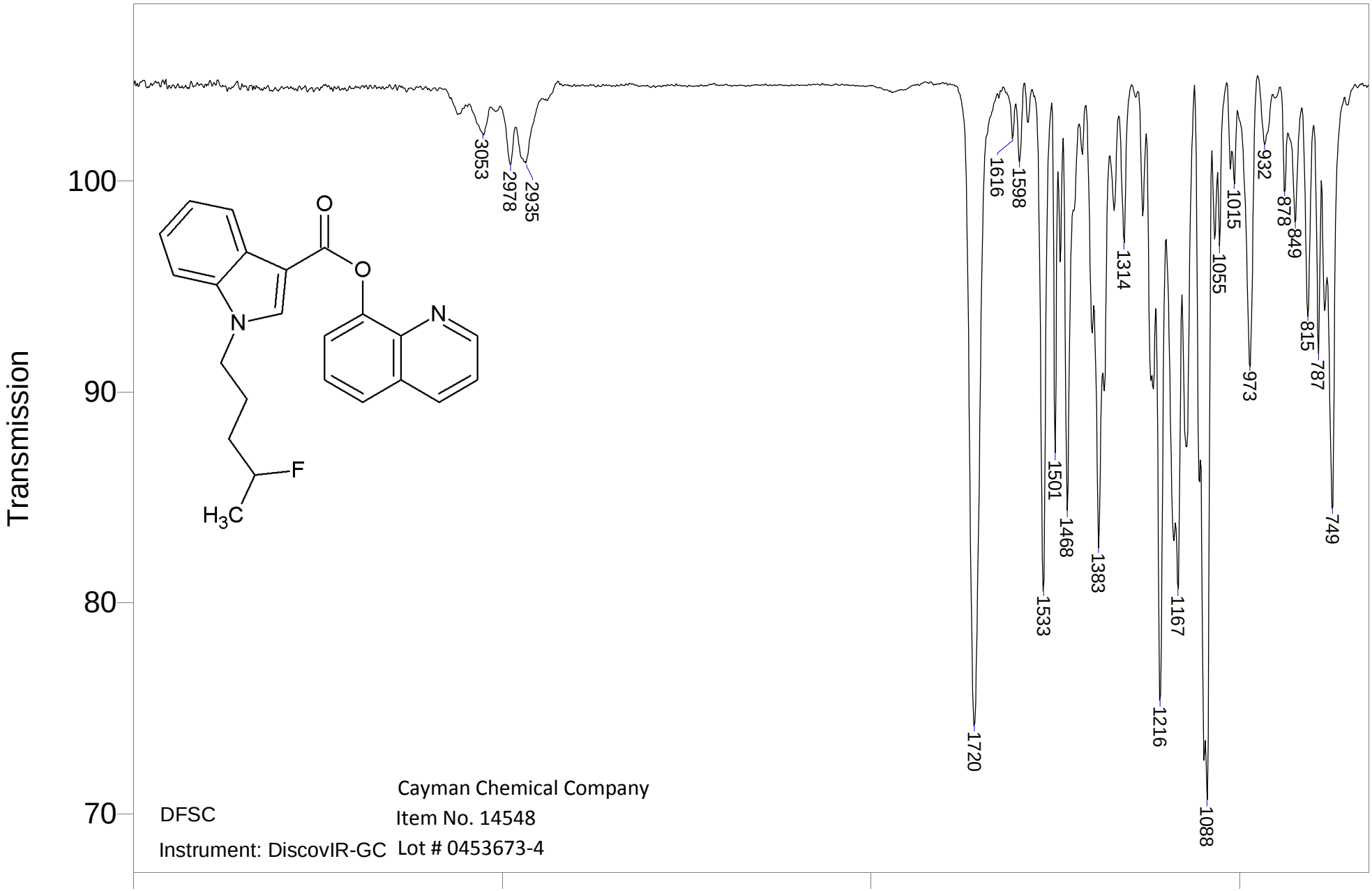


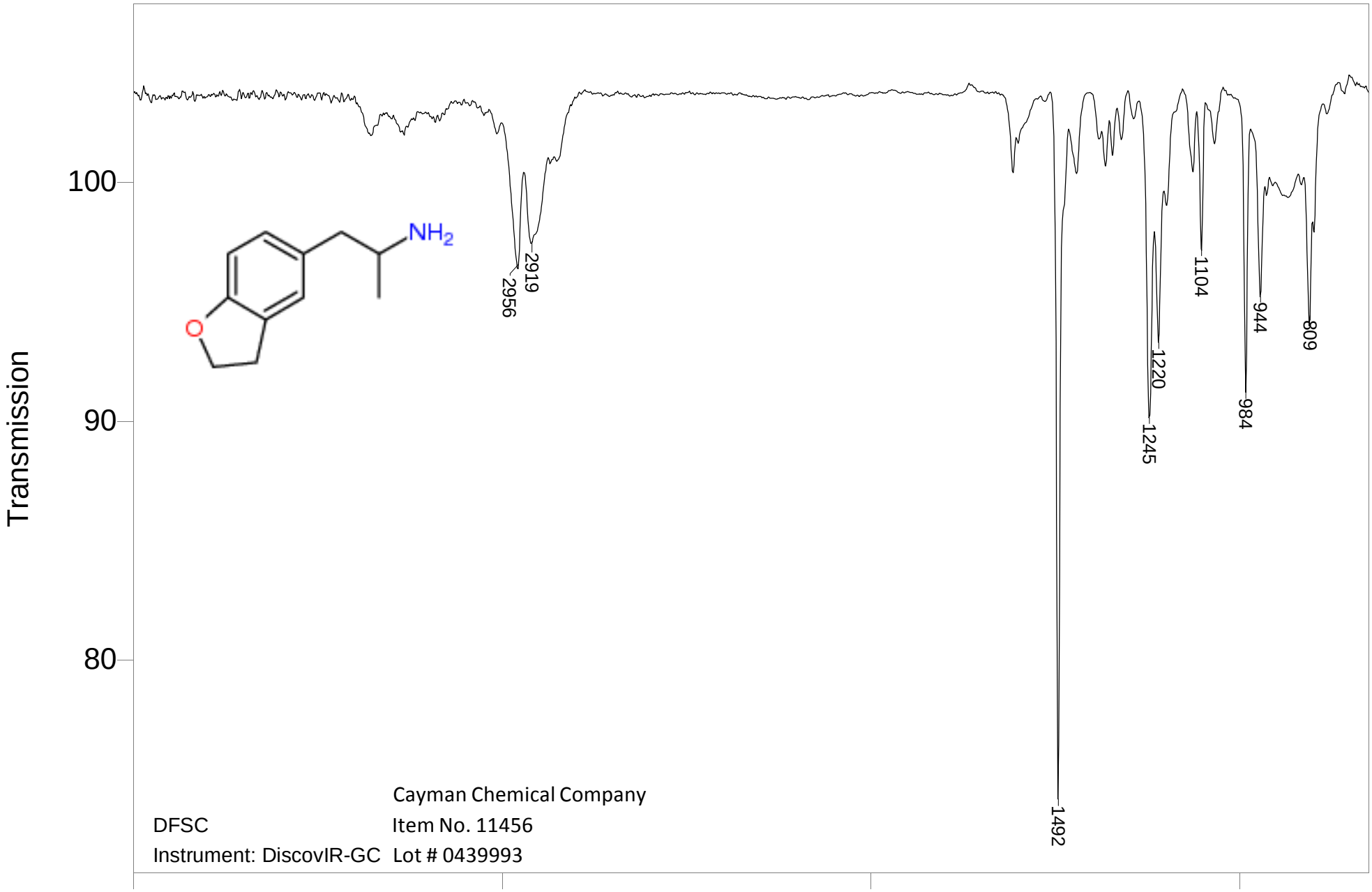


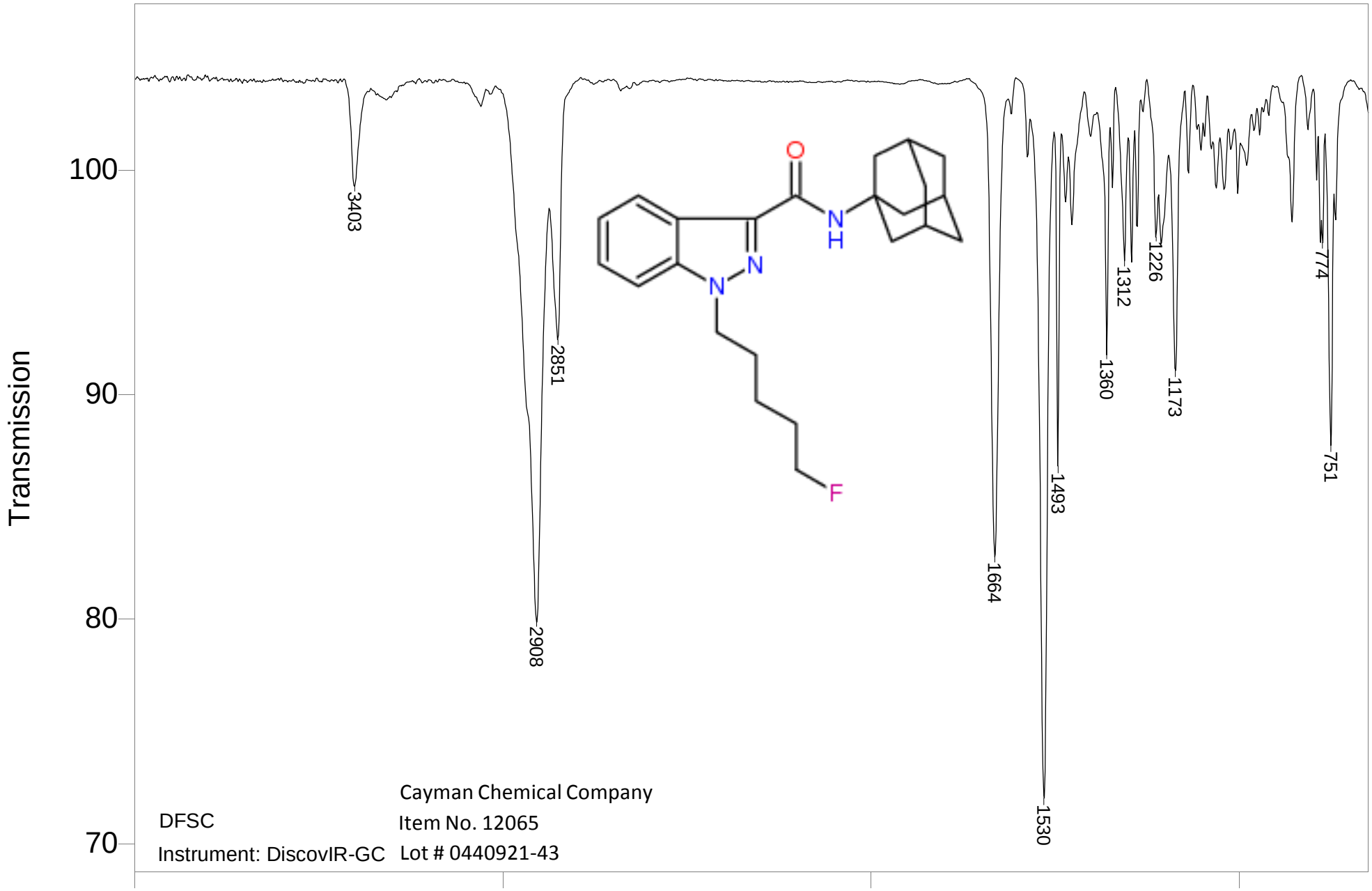


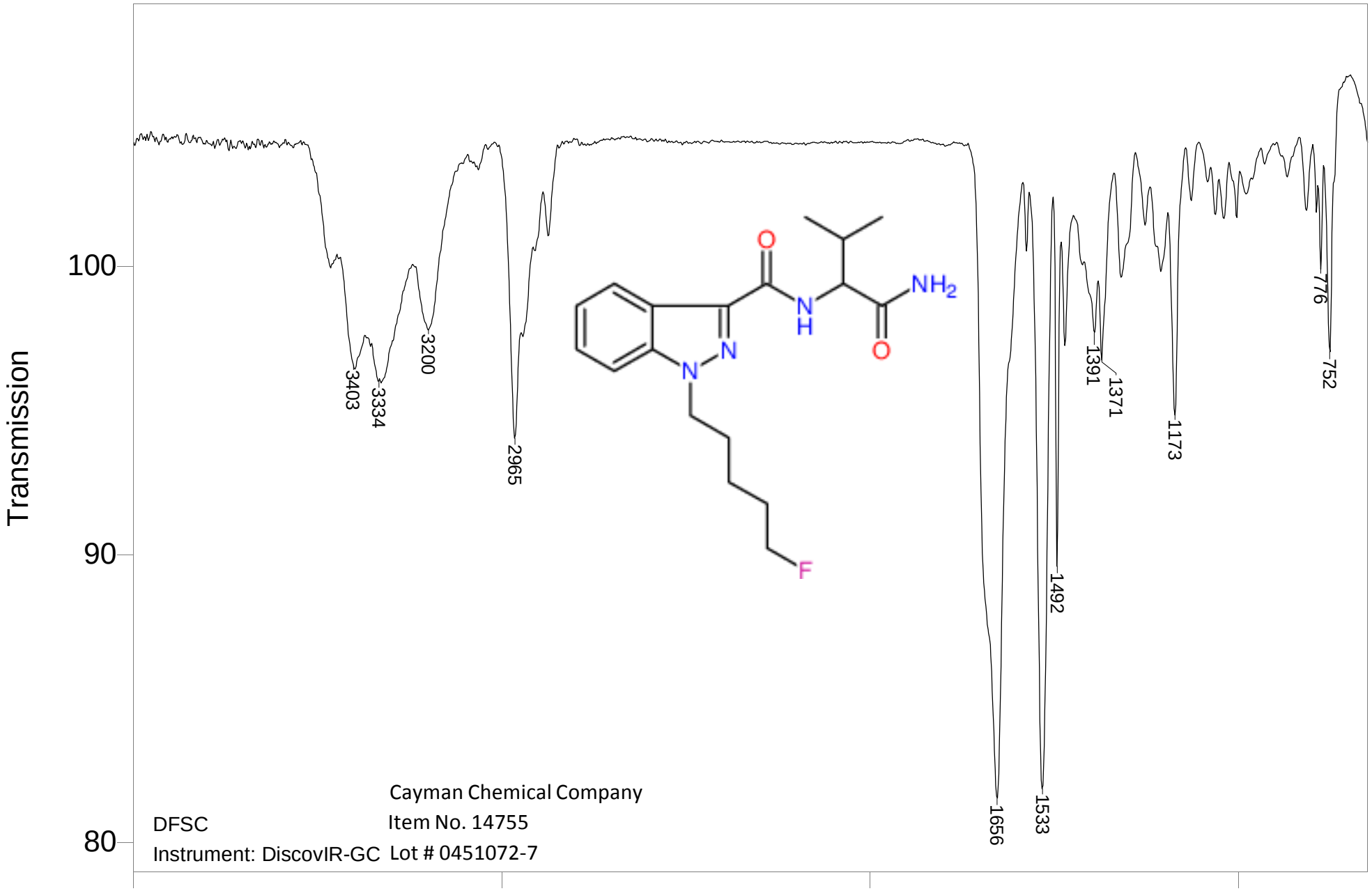


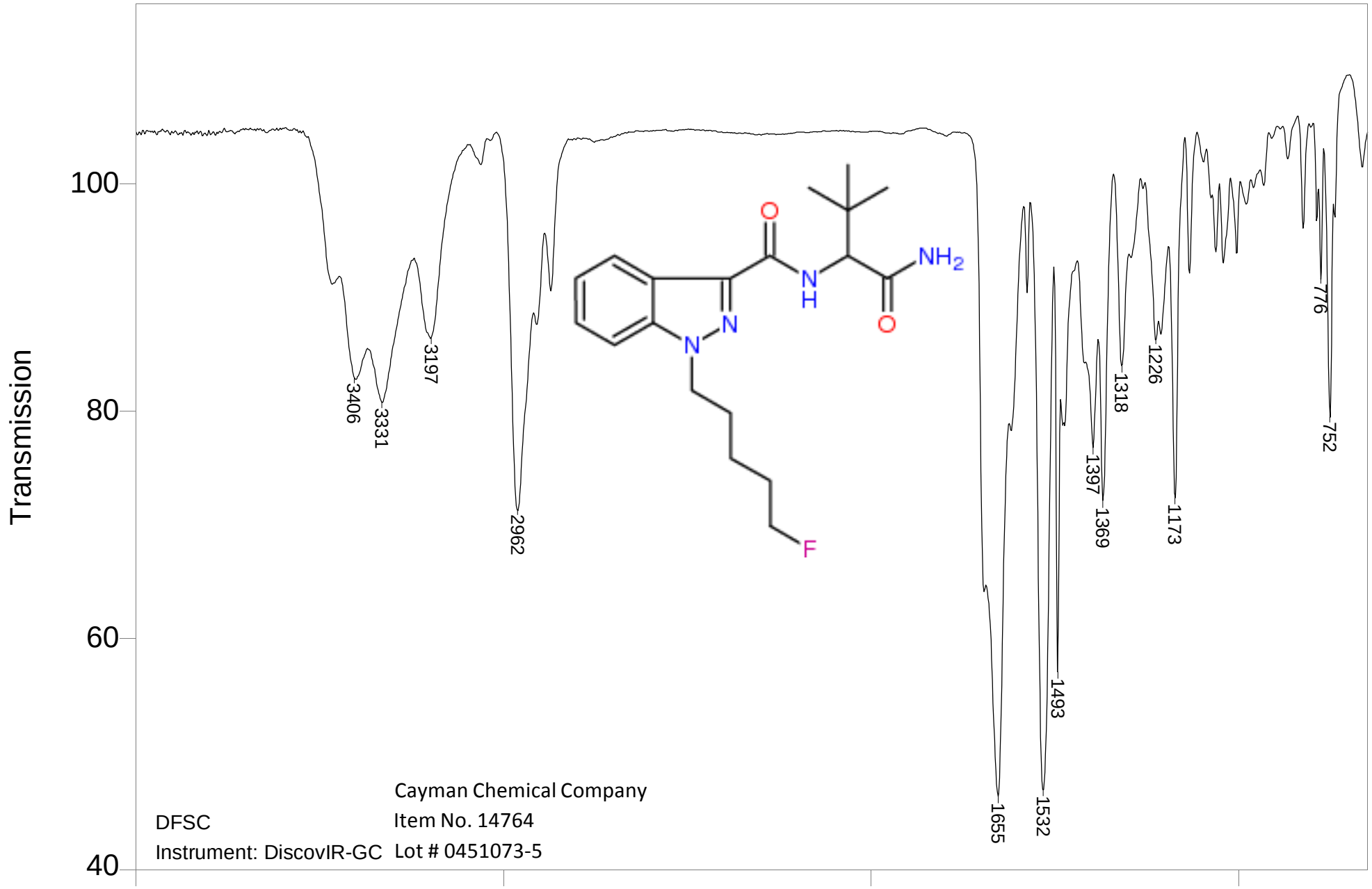


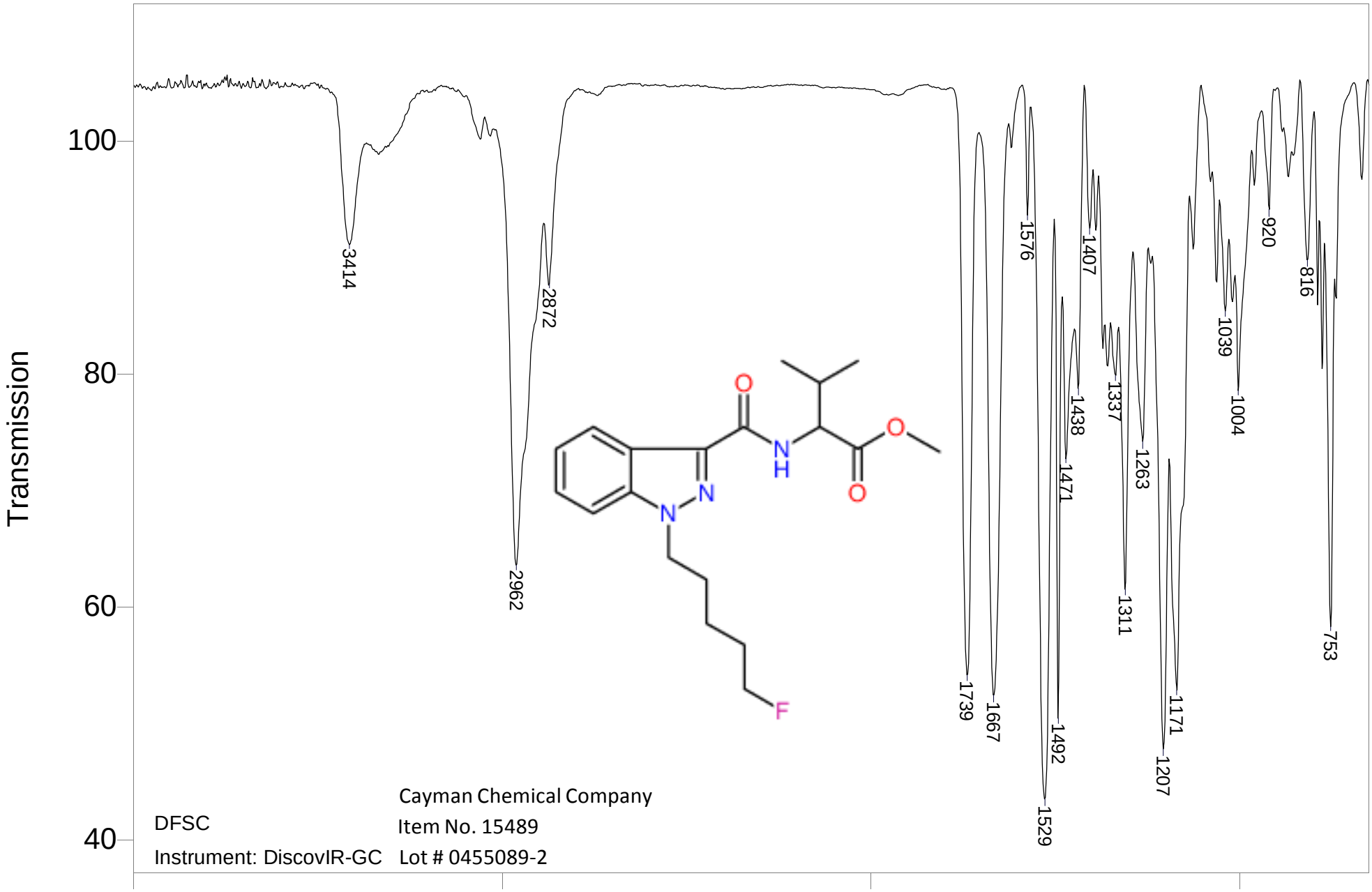


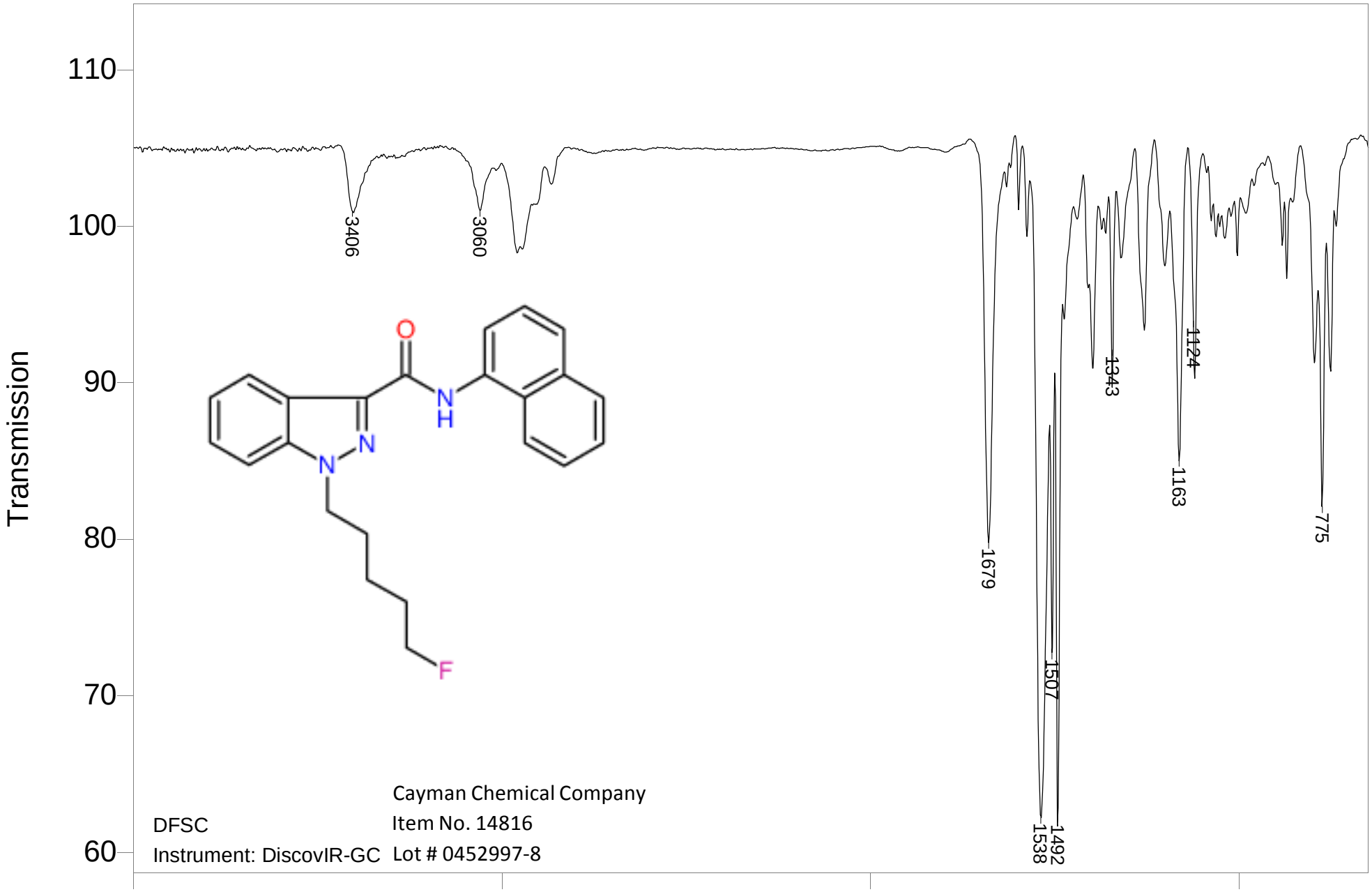


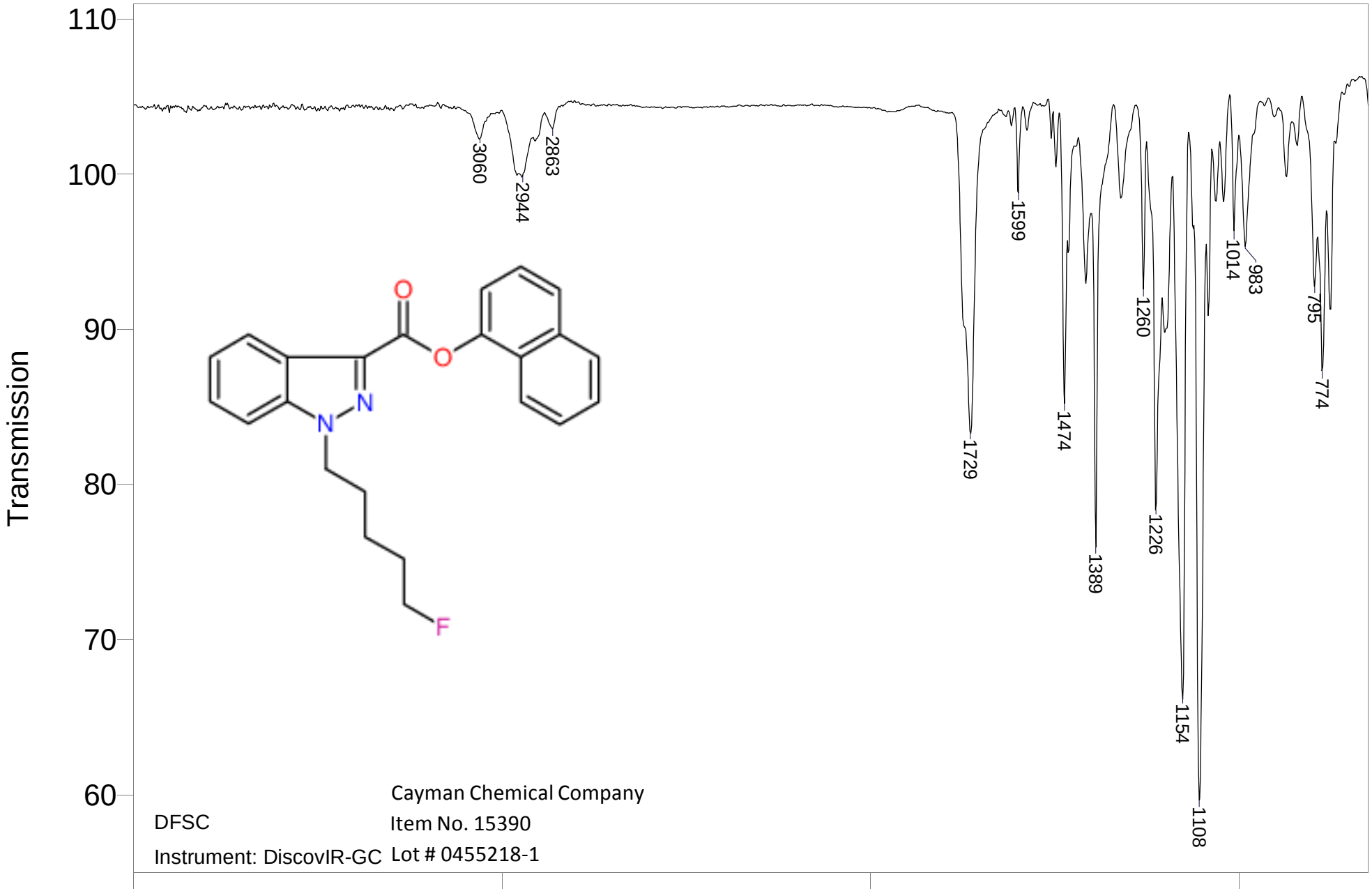


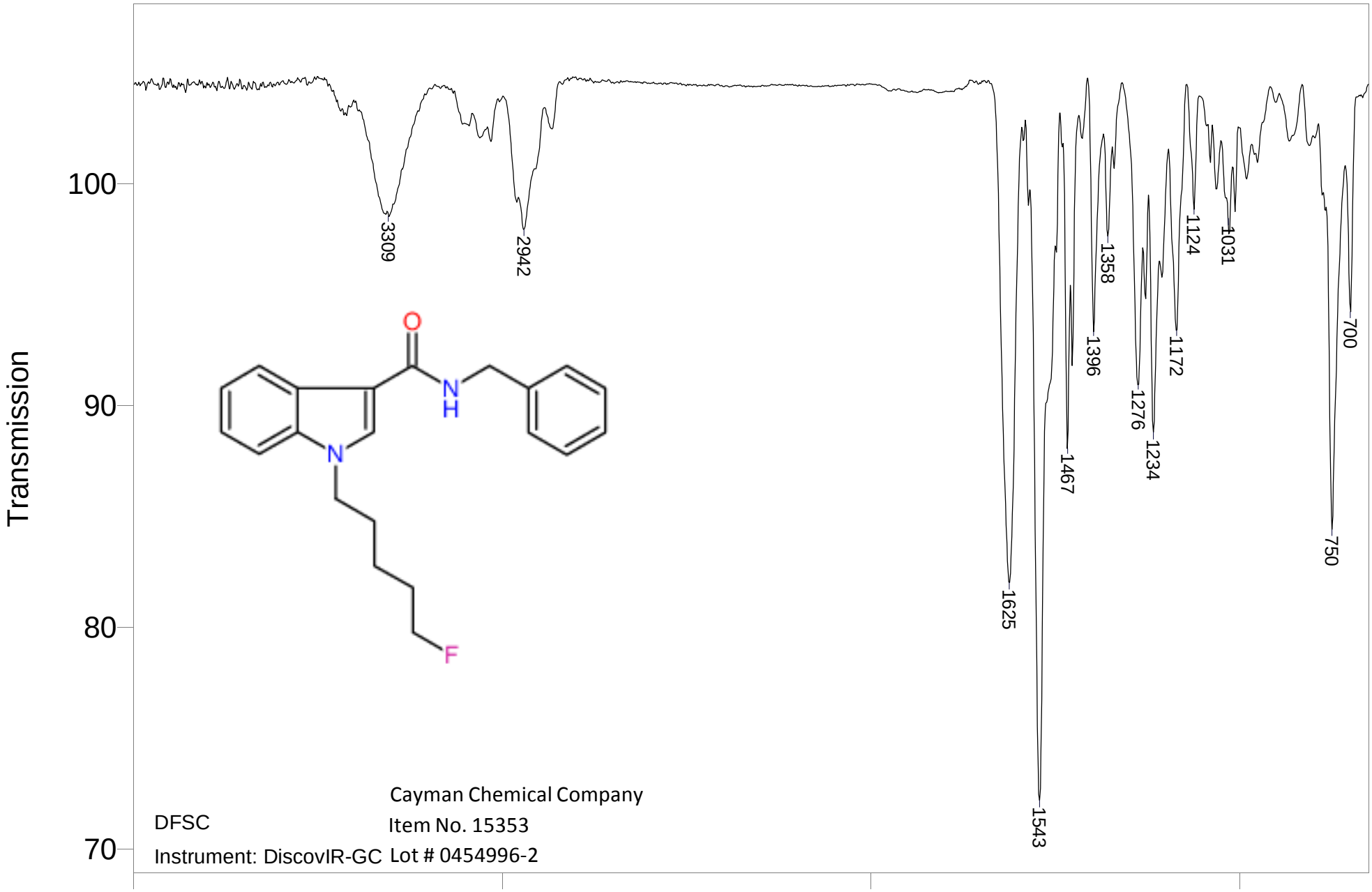


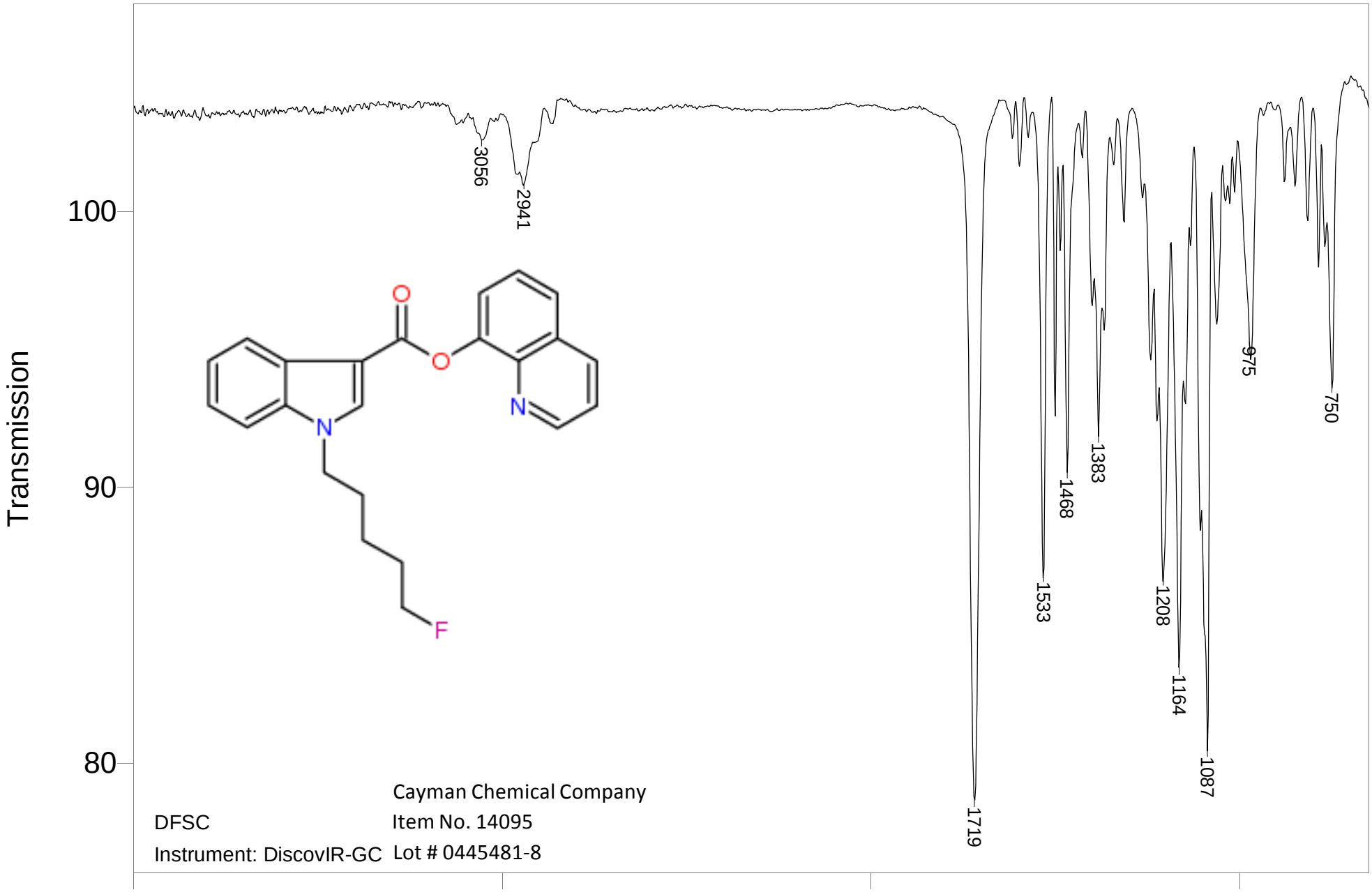


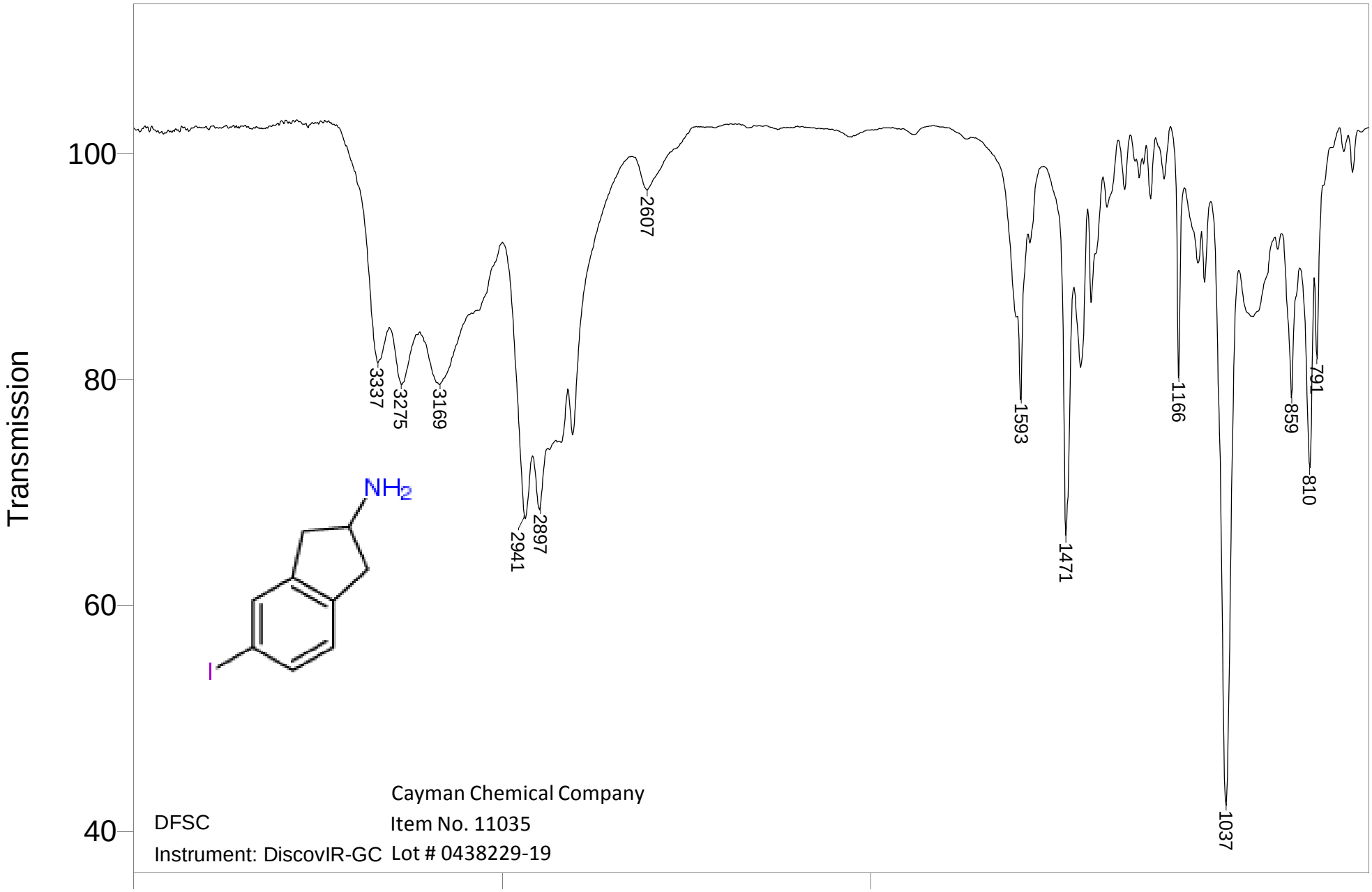


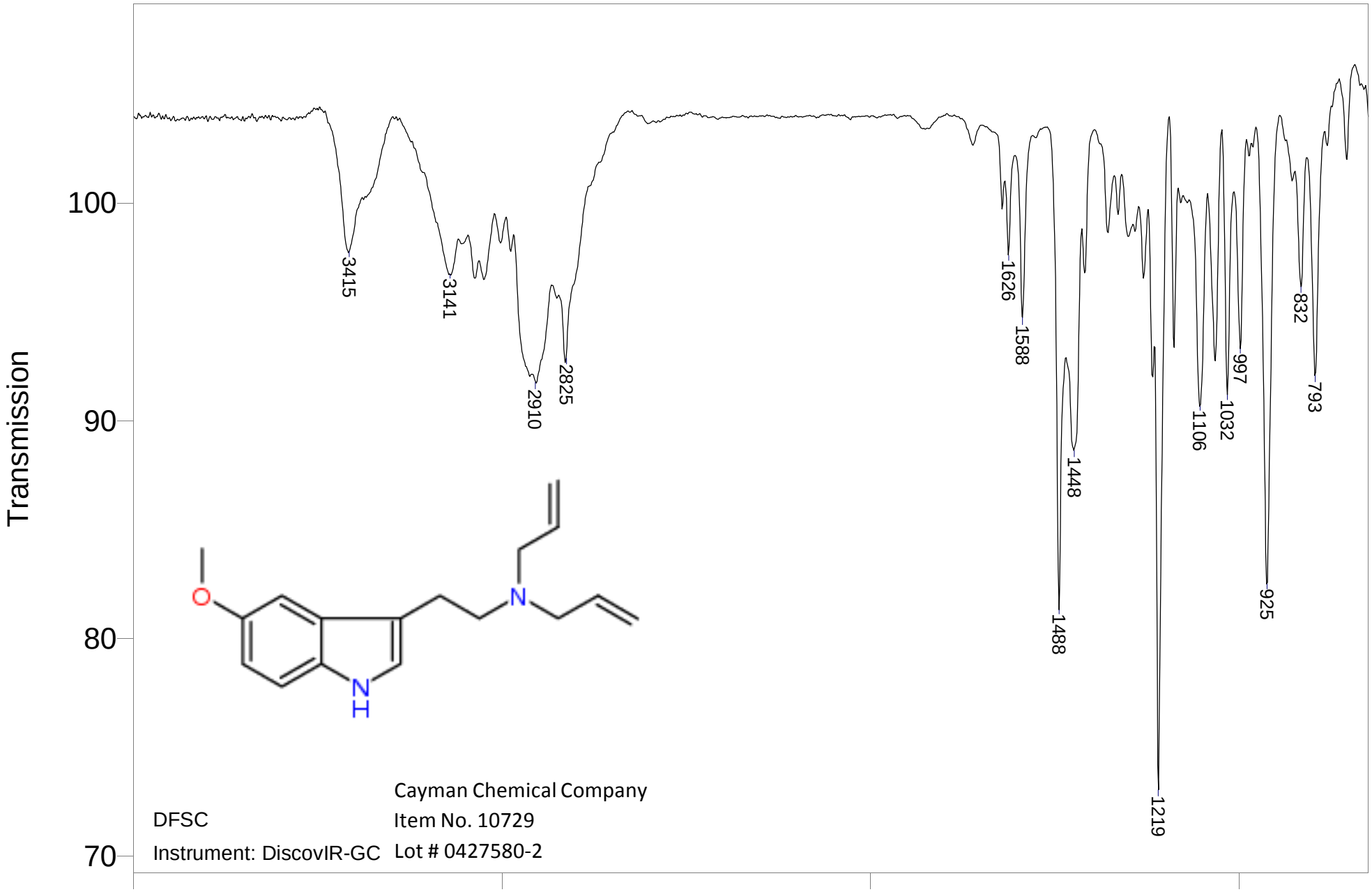


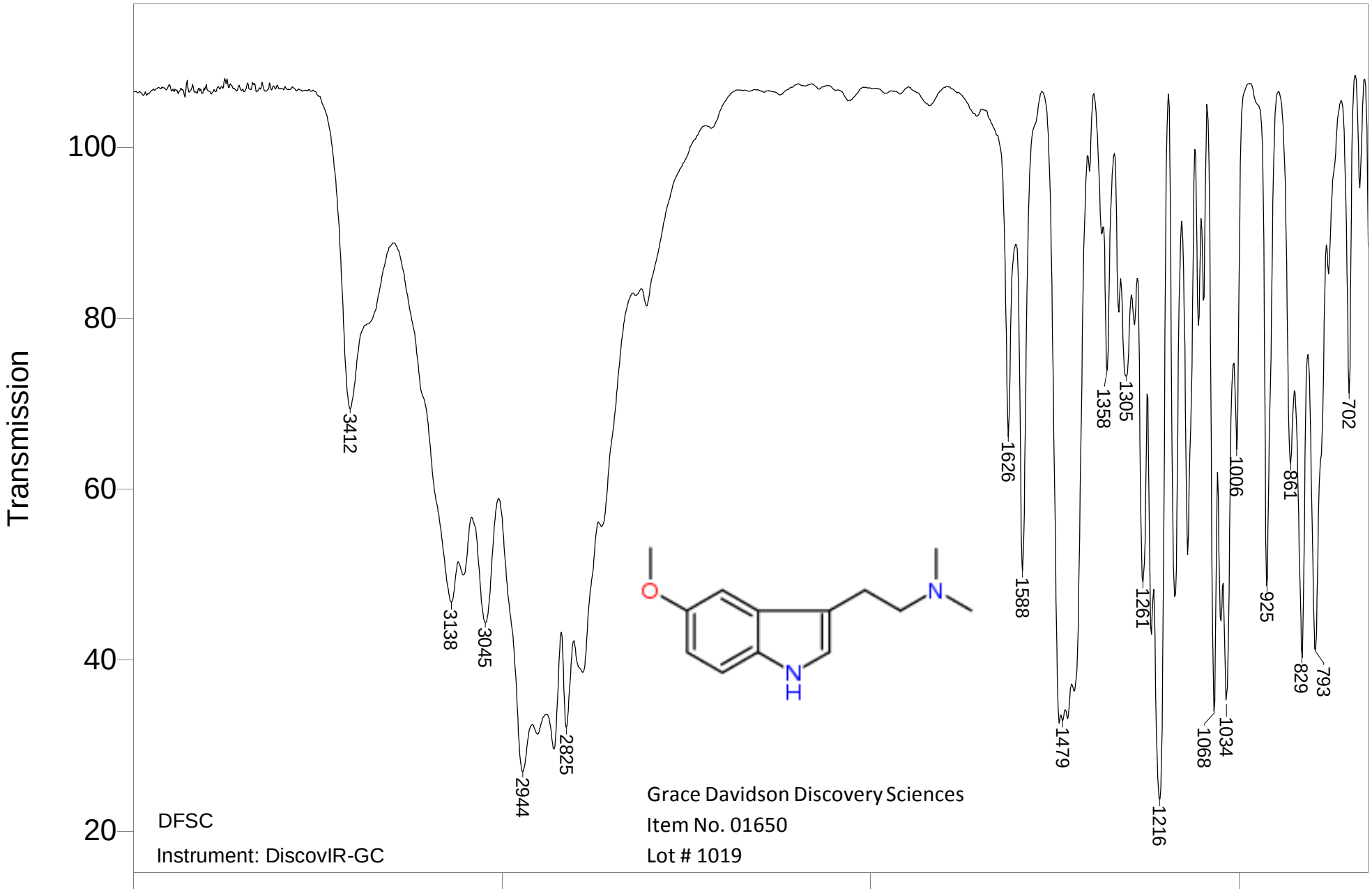


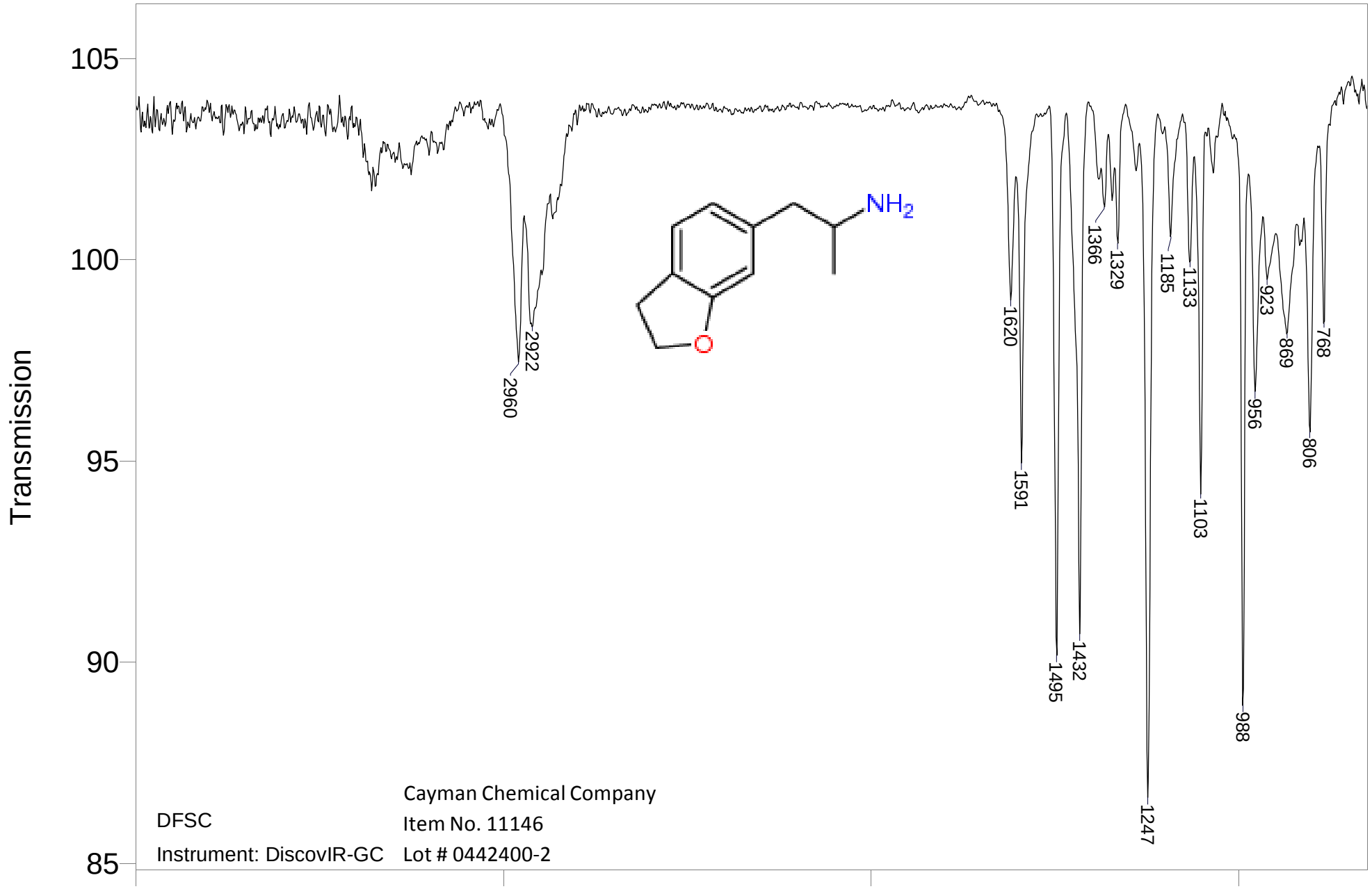


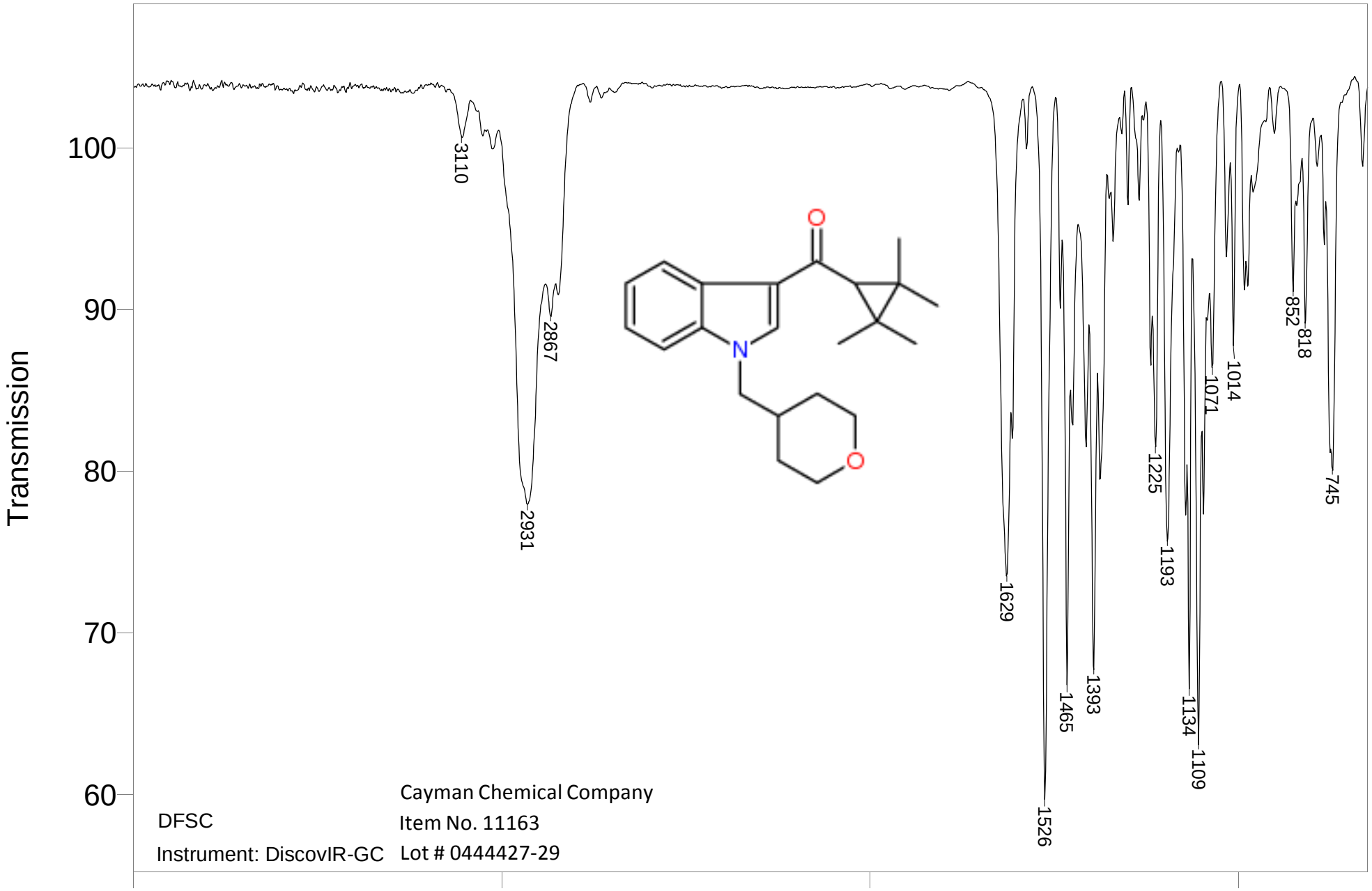


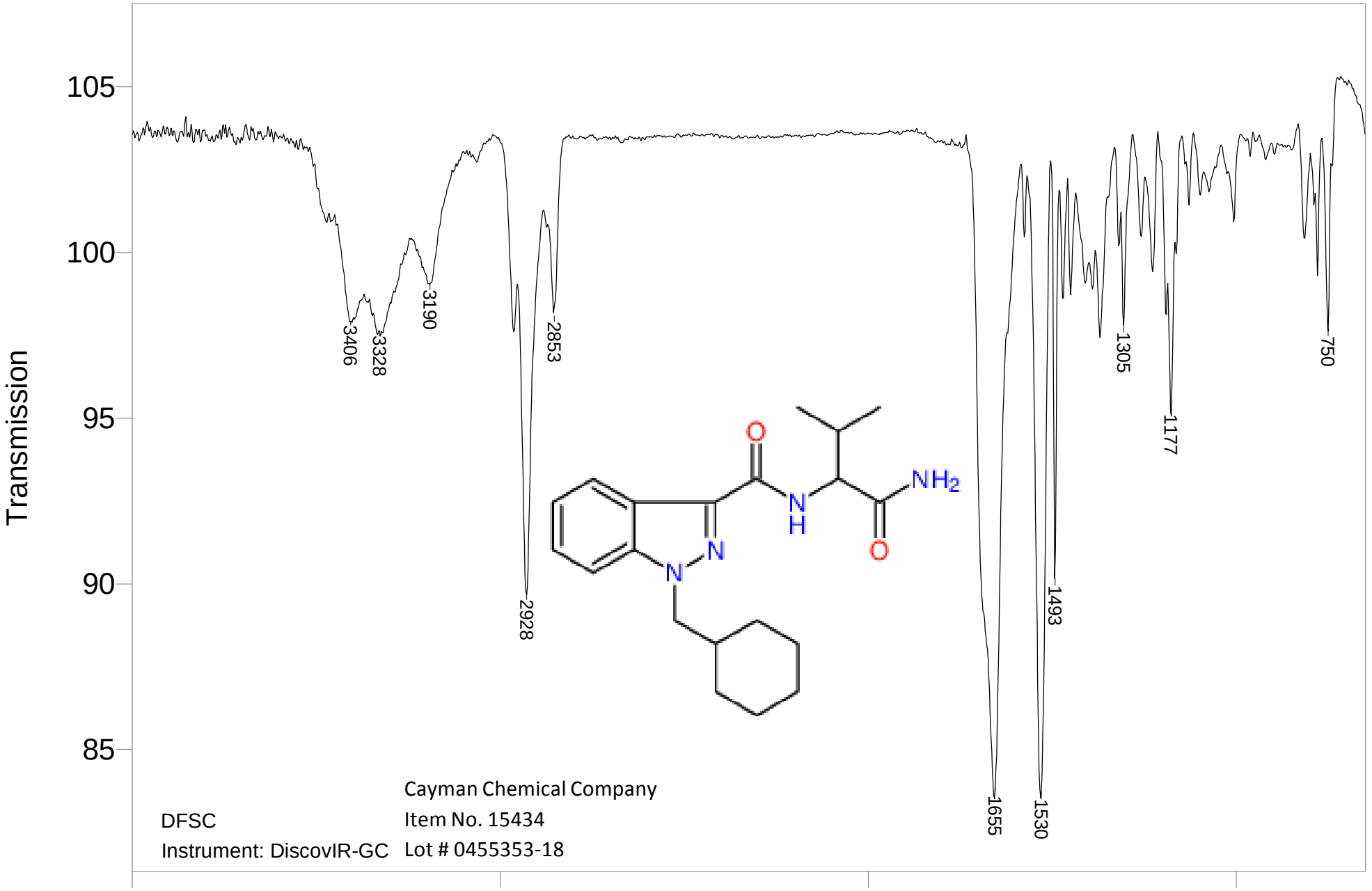




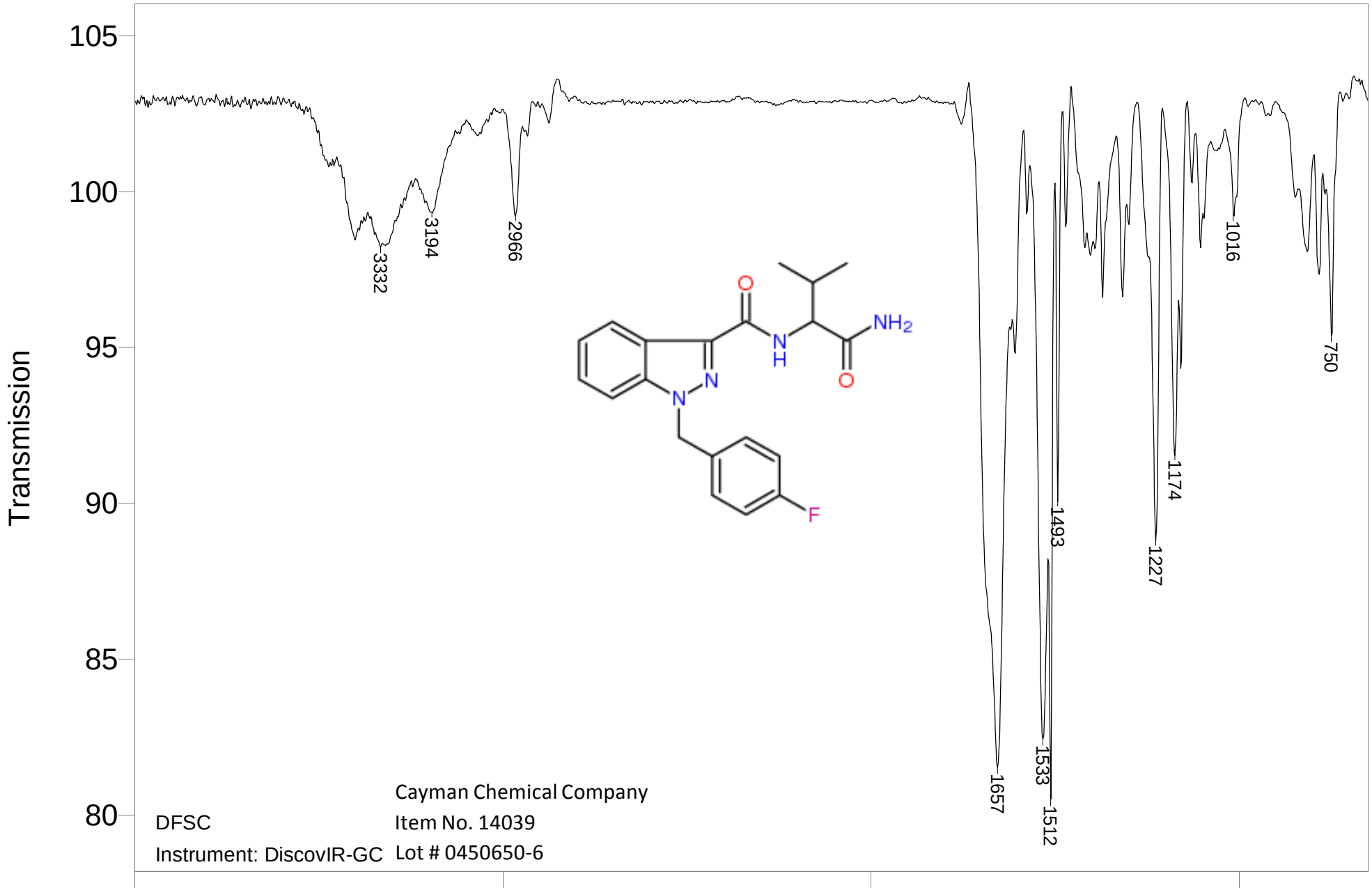


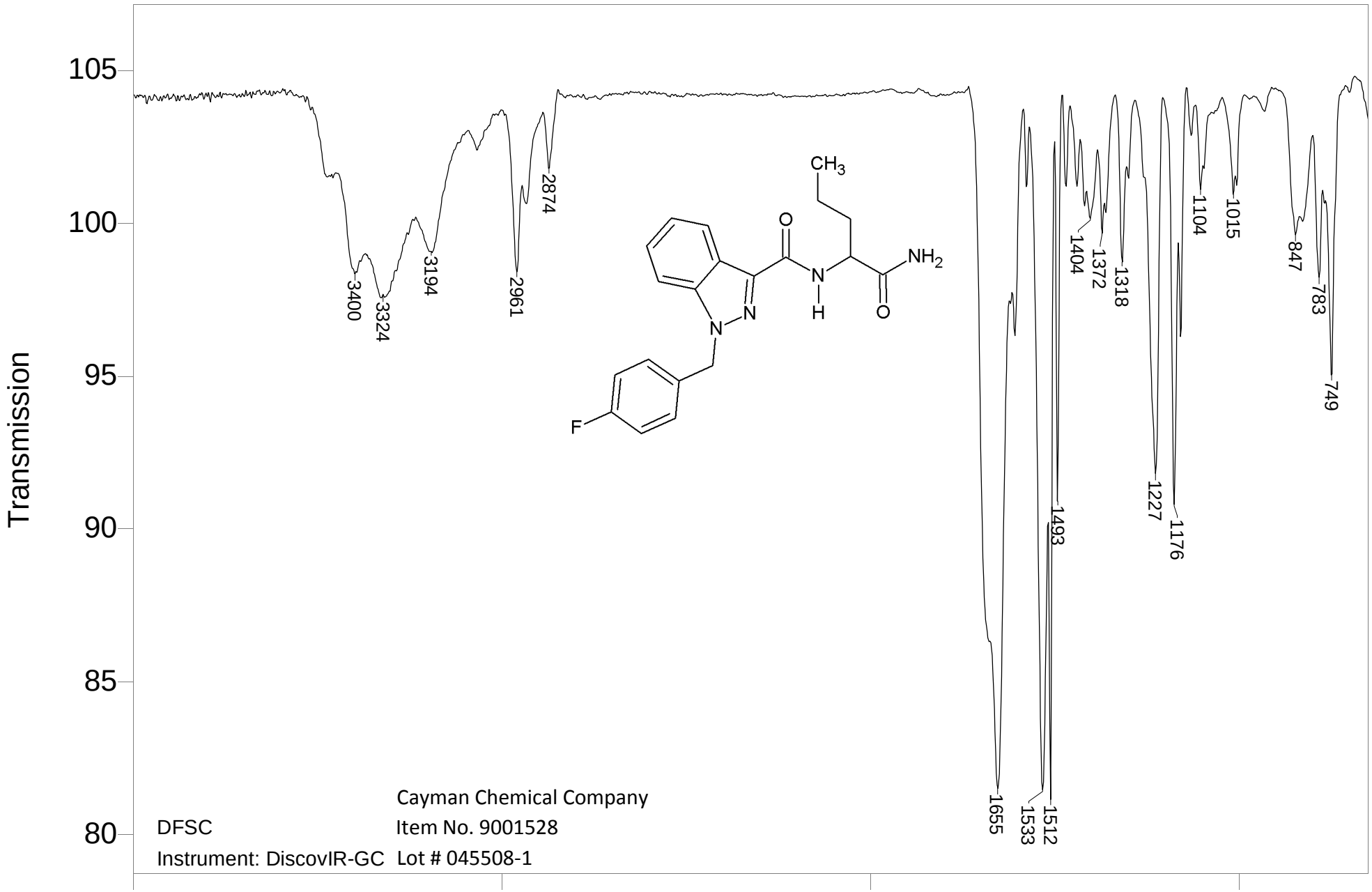


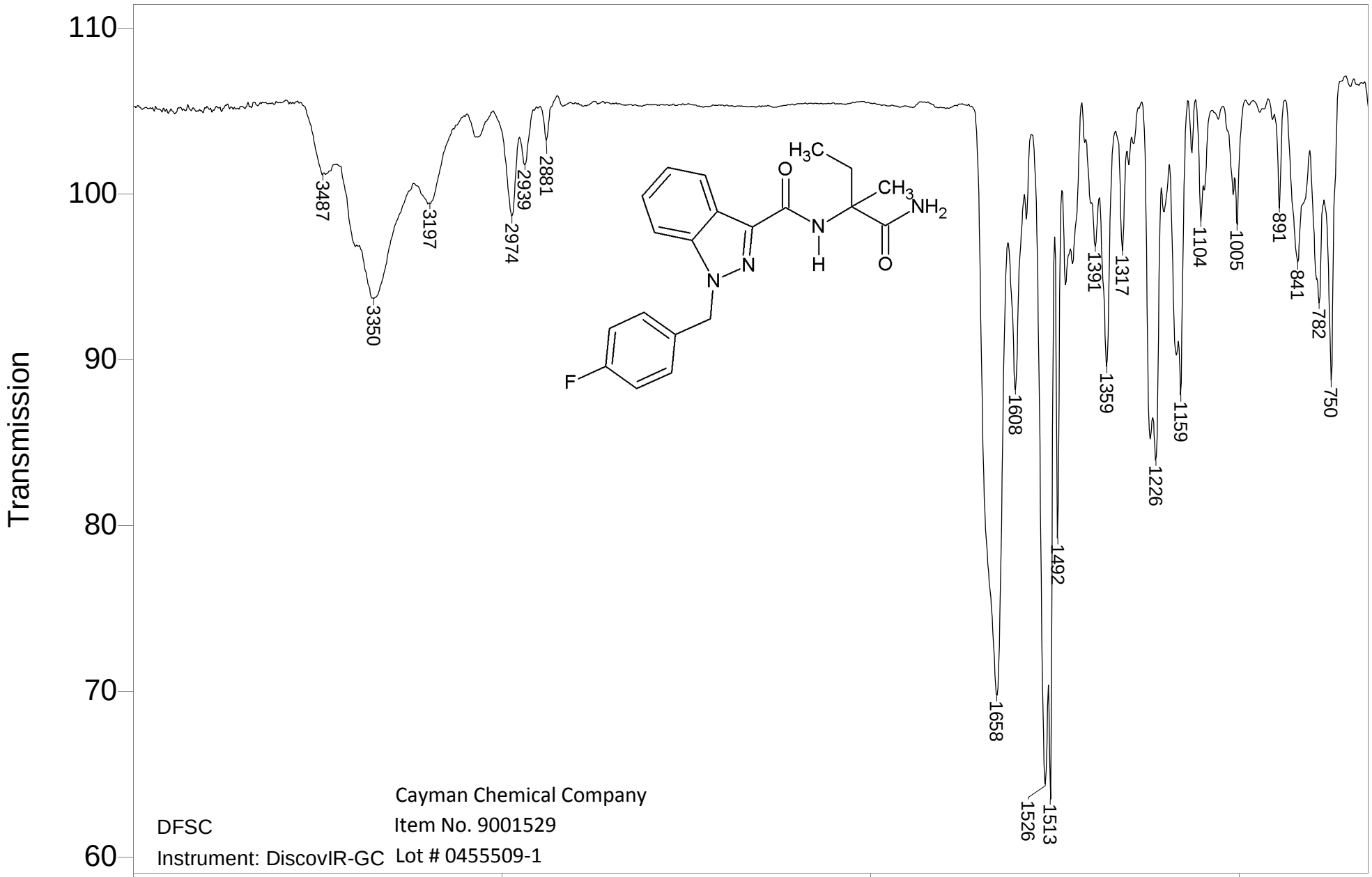


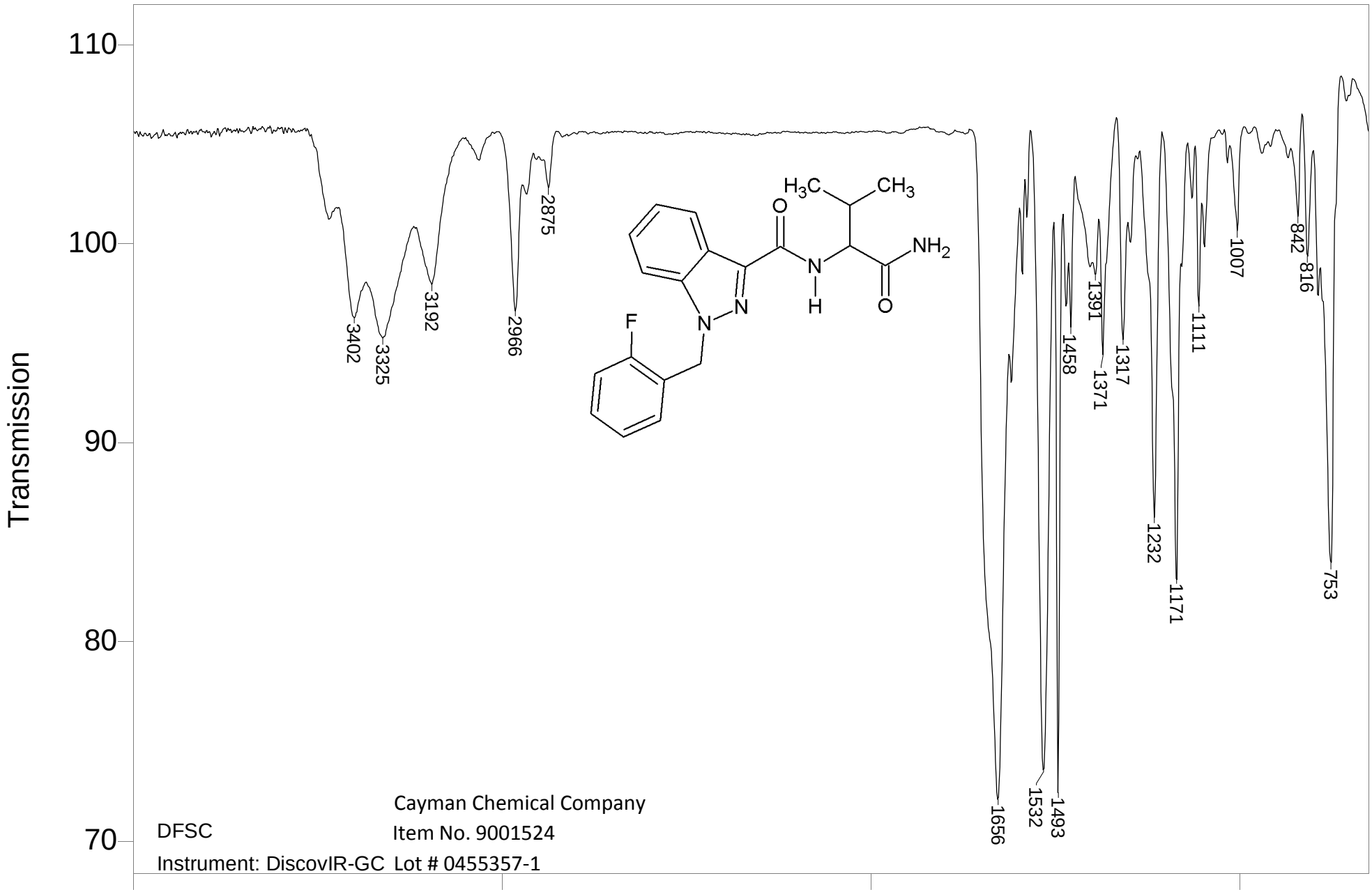


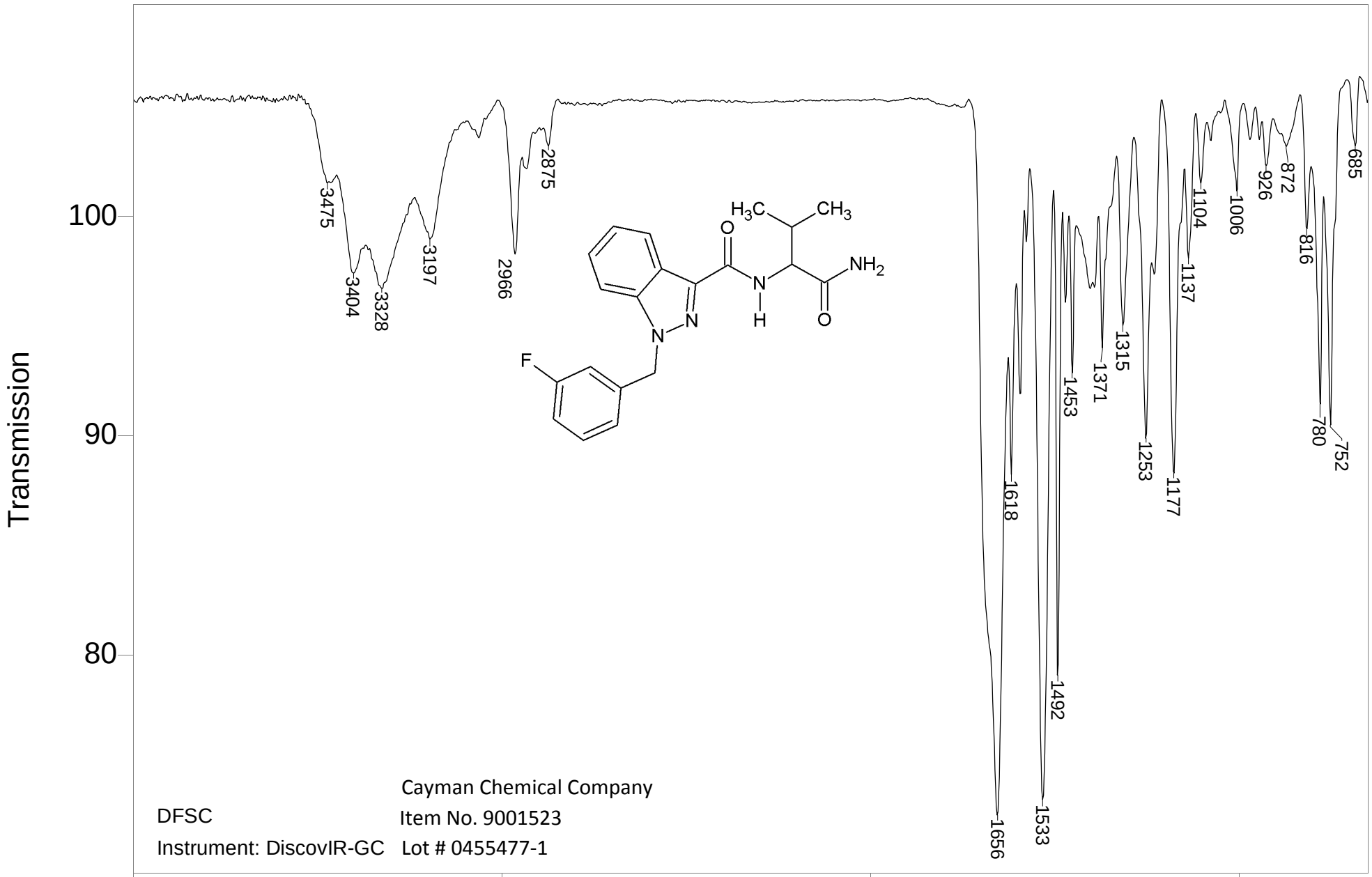
AB-FUBINACA

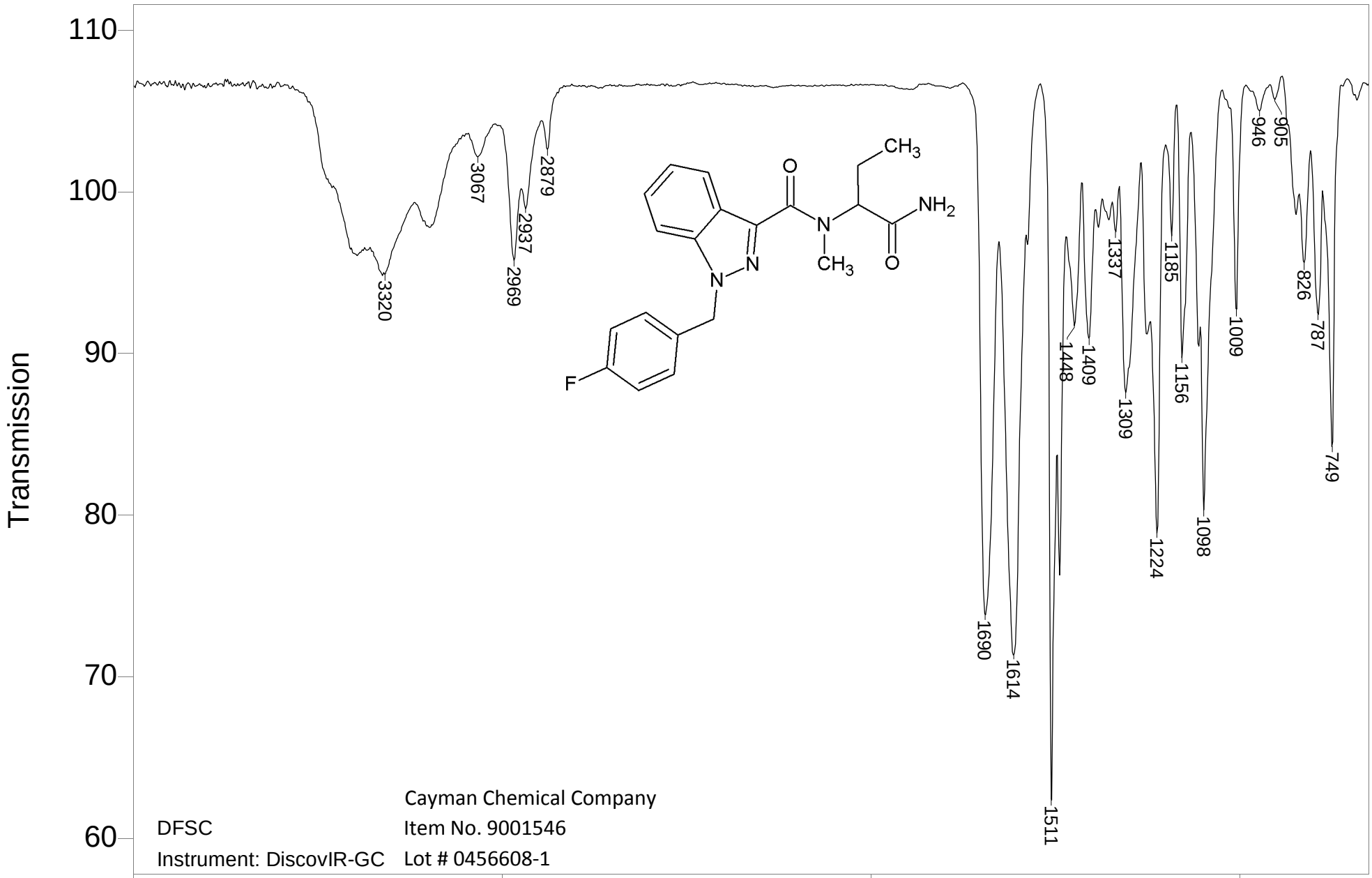


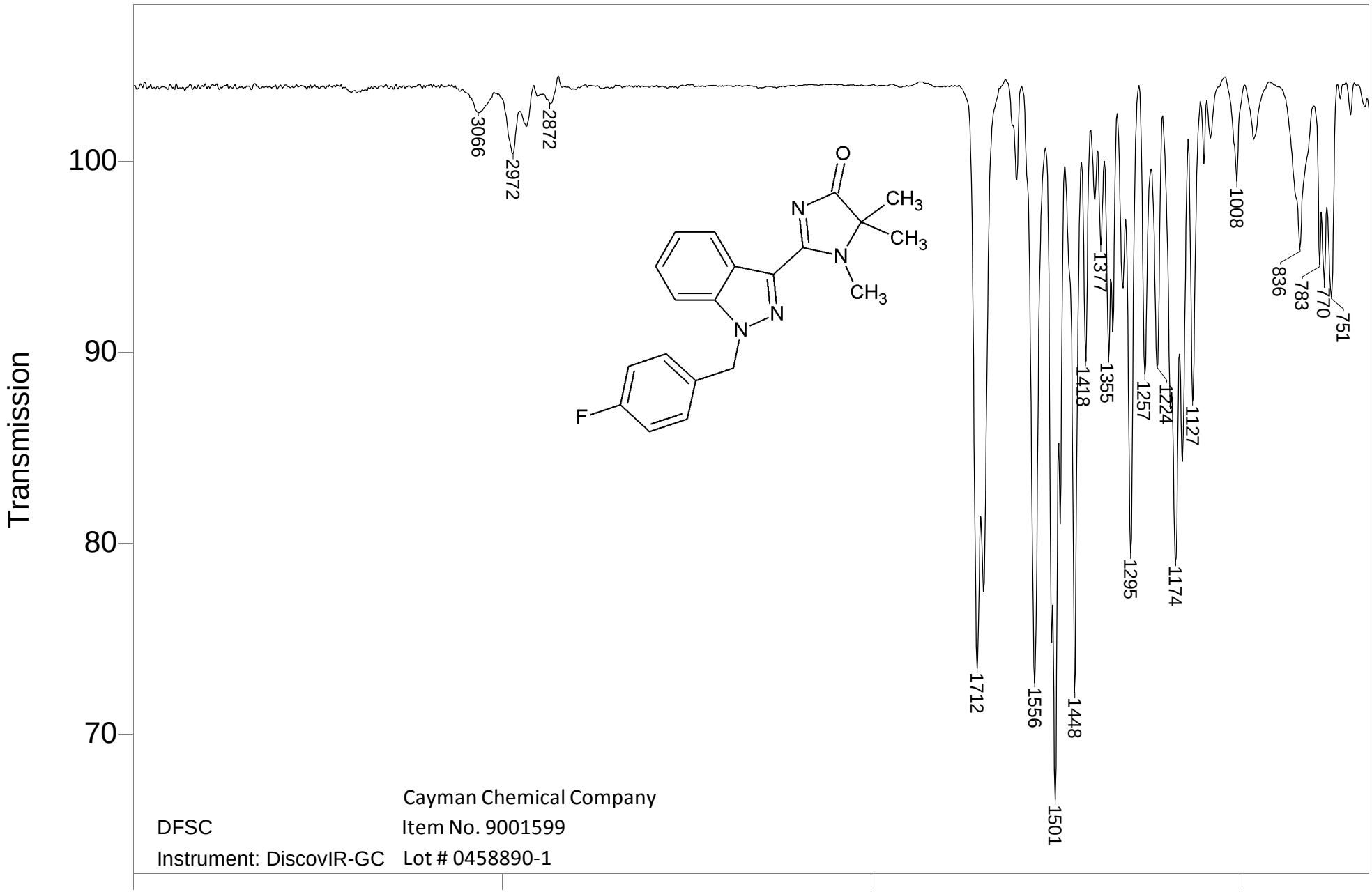


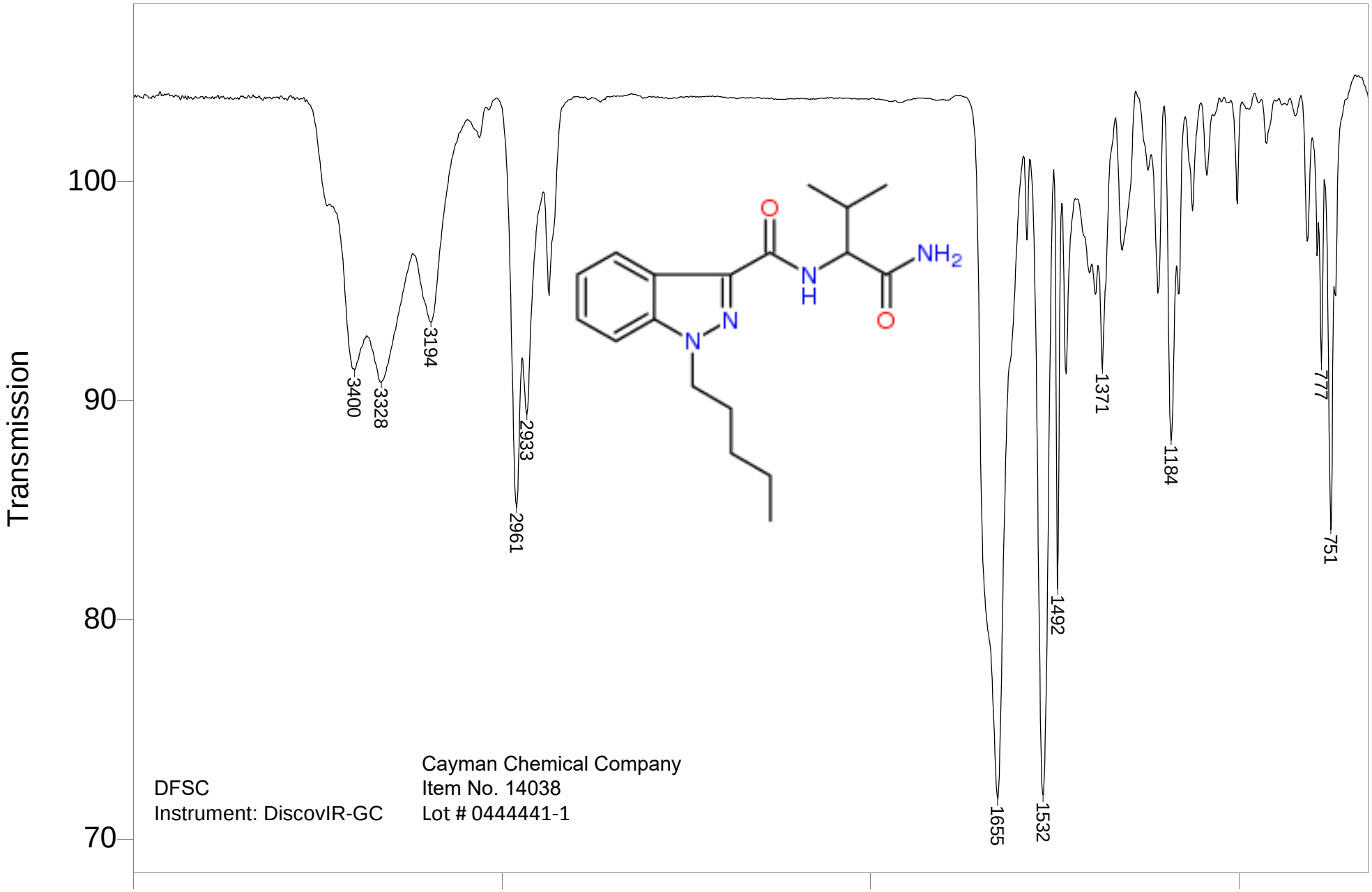


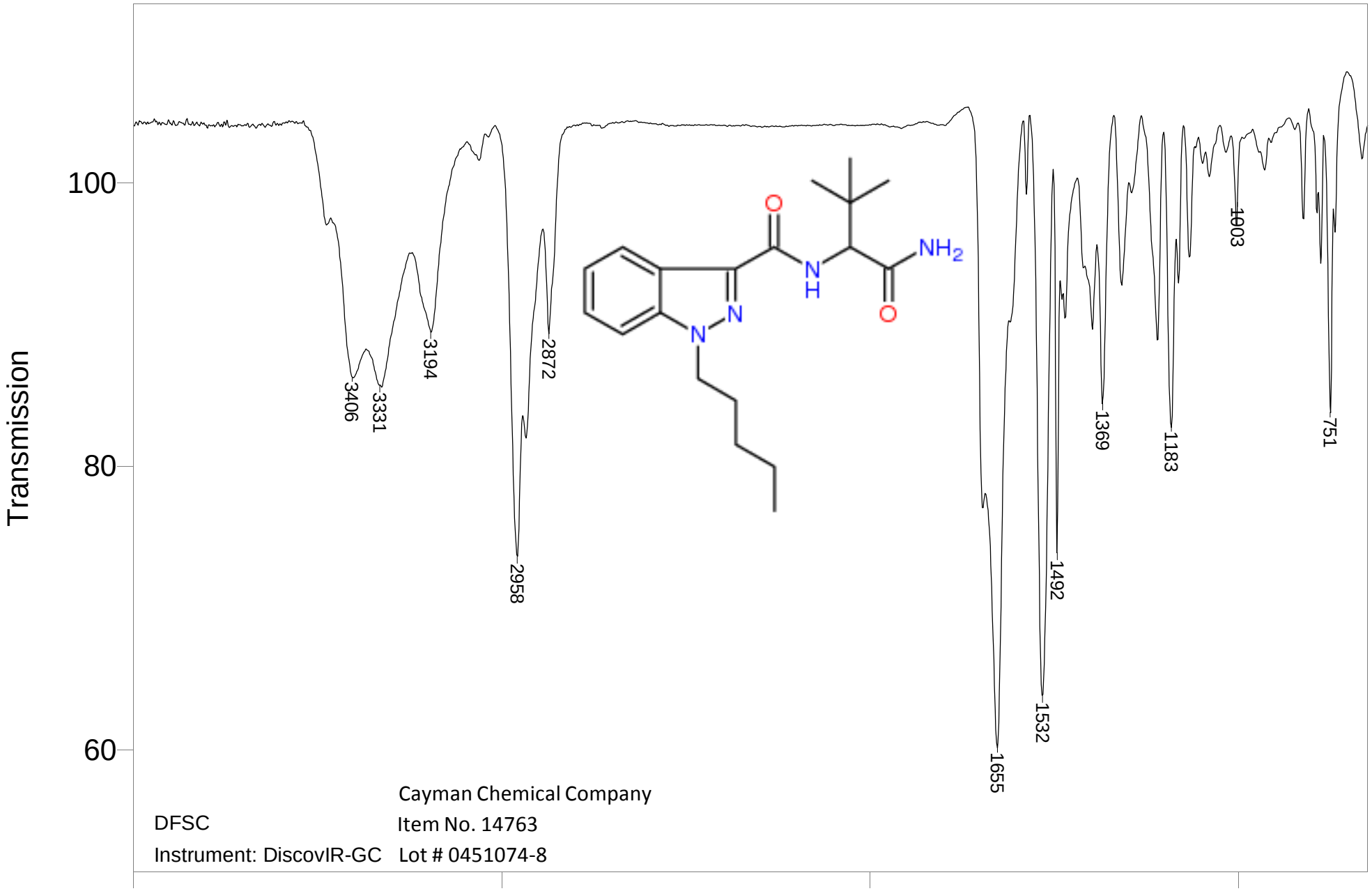


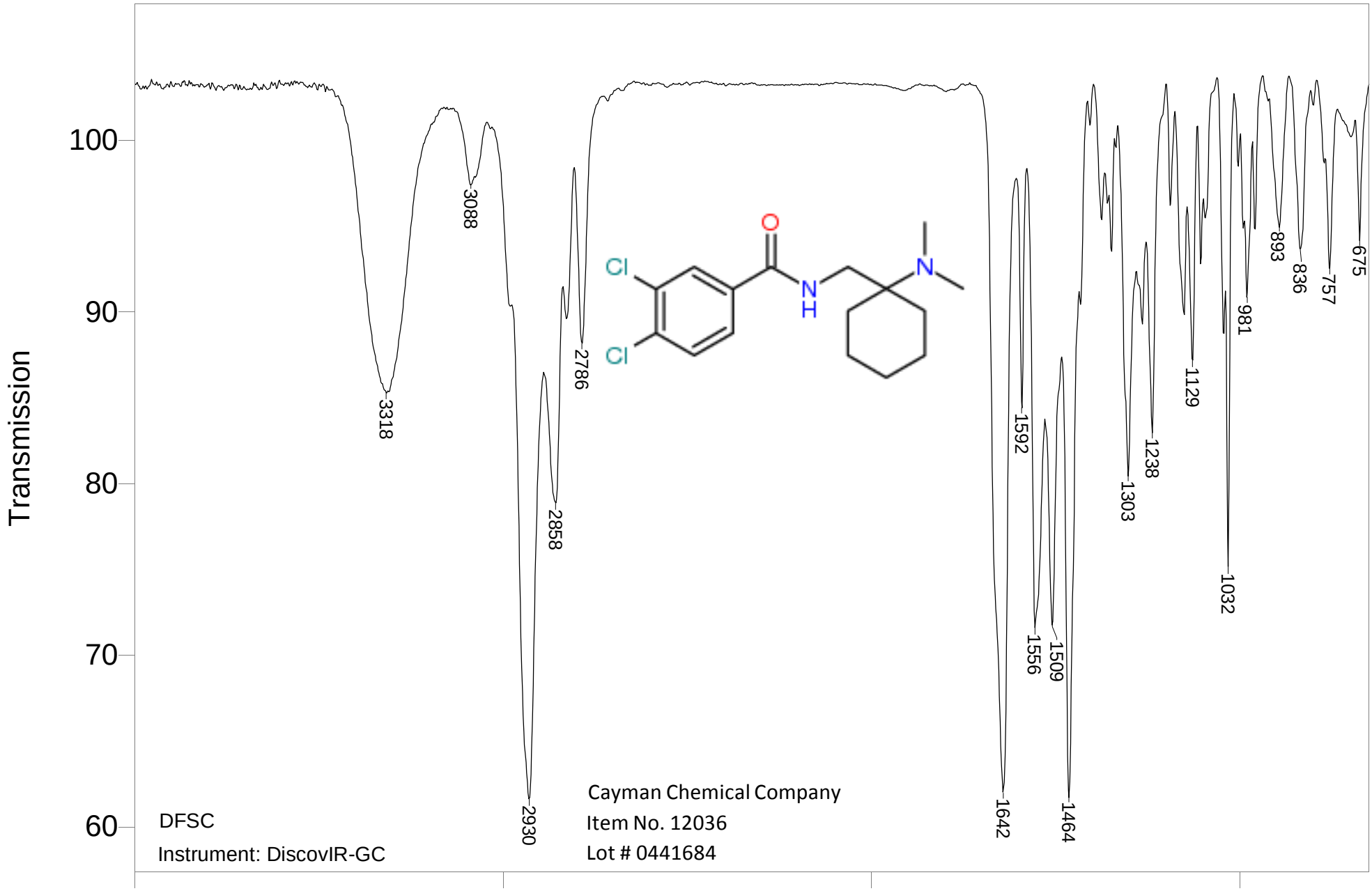


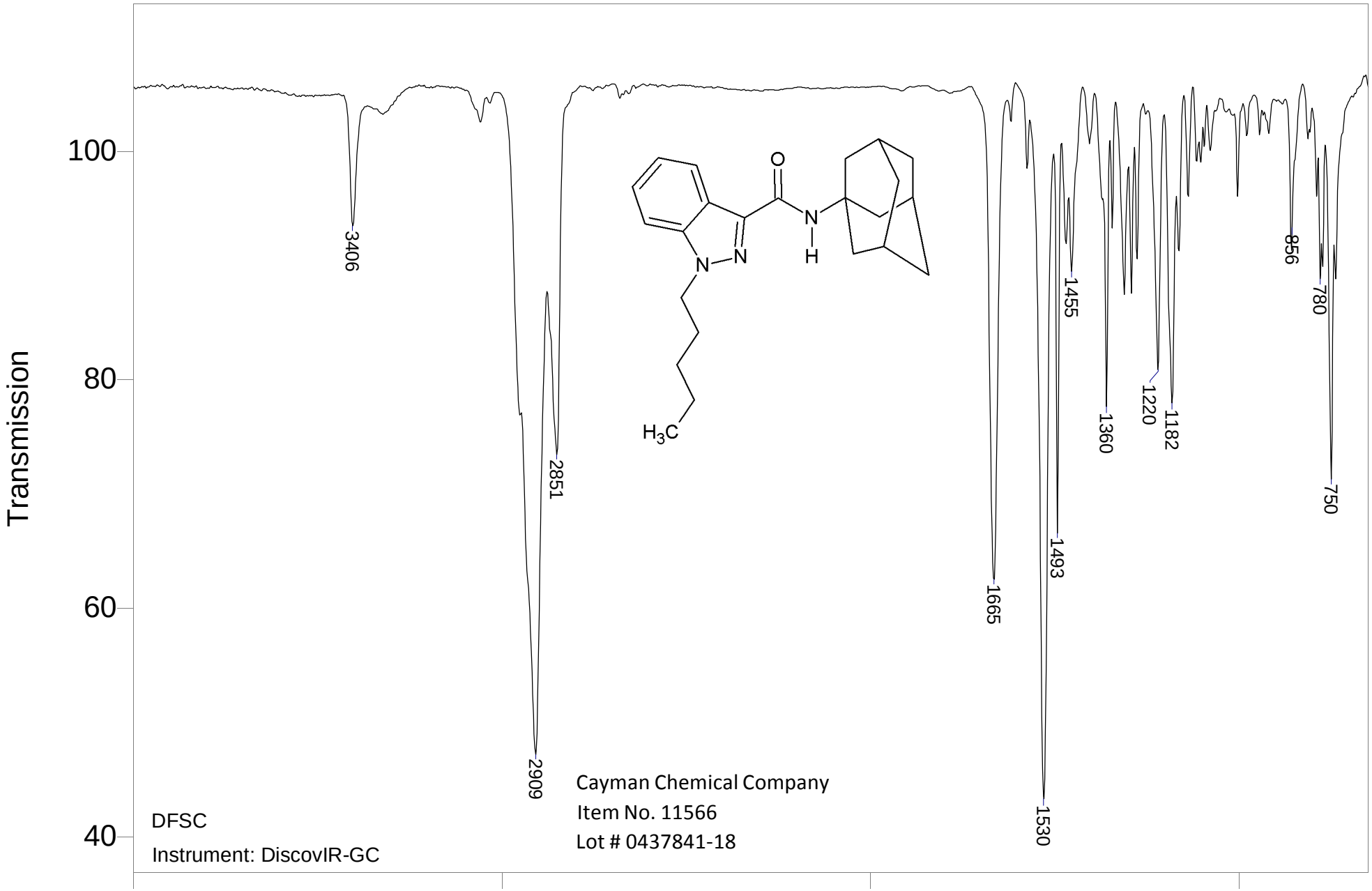




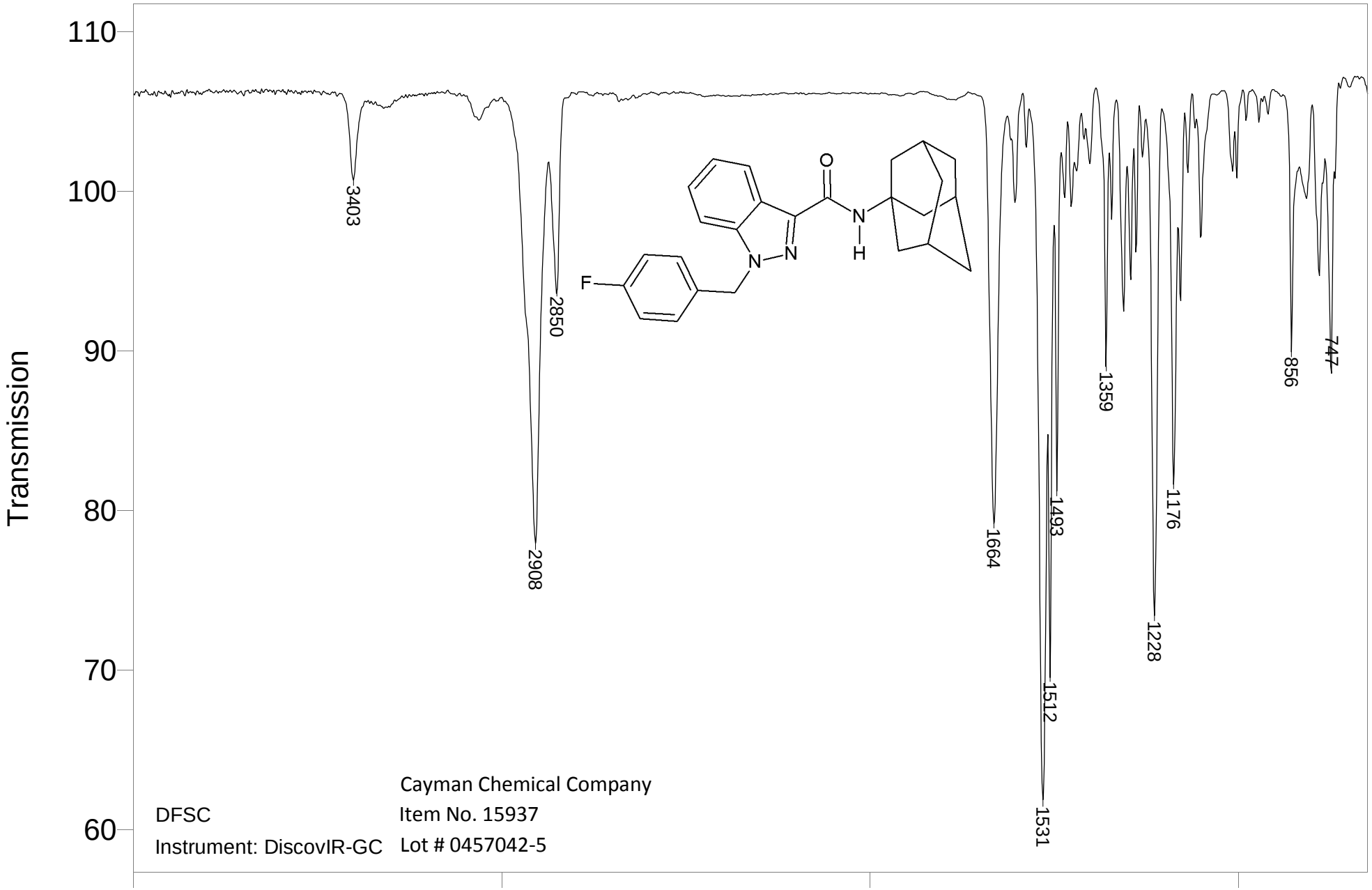


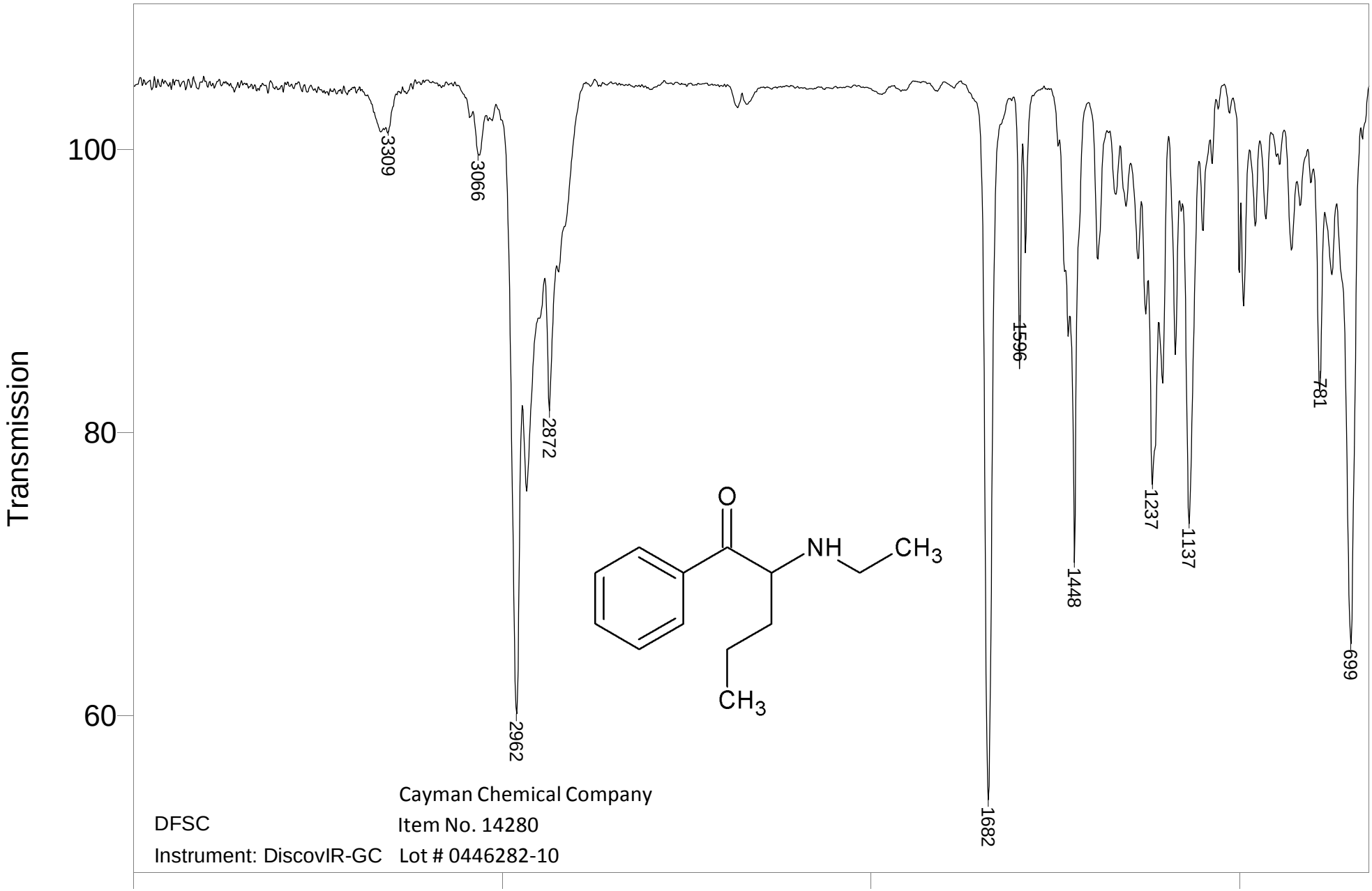


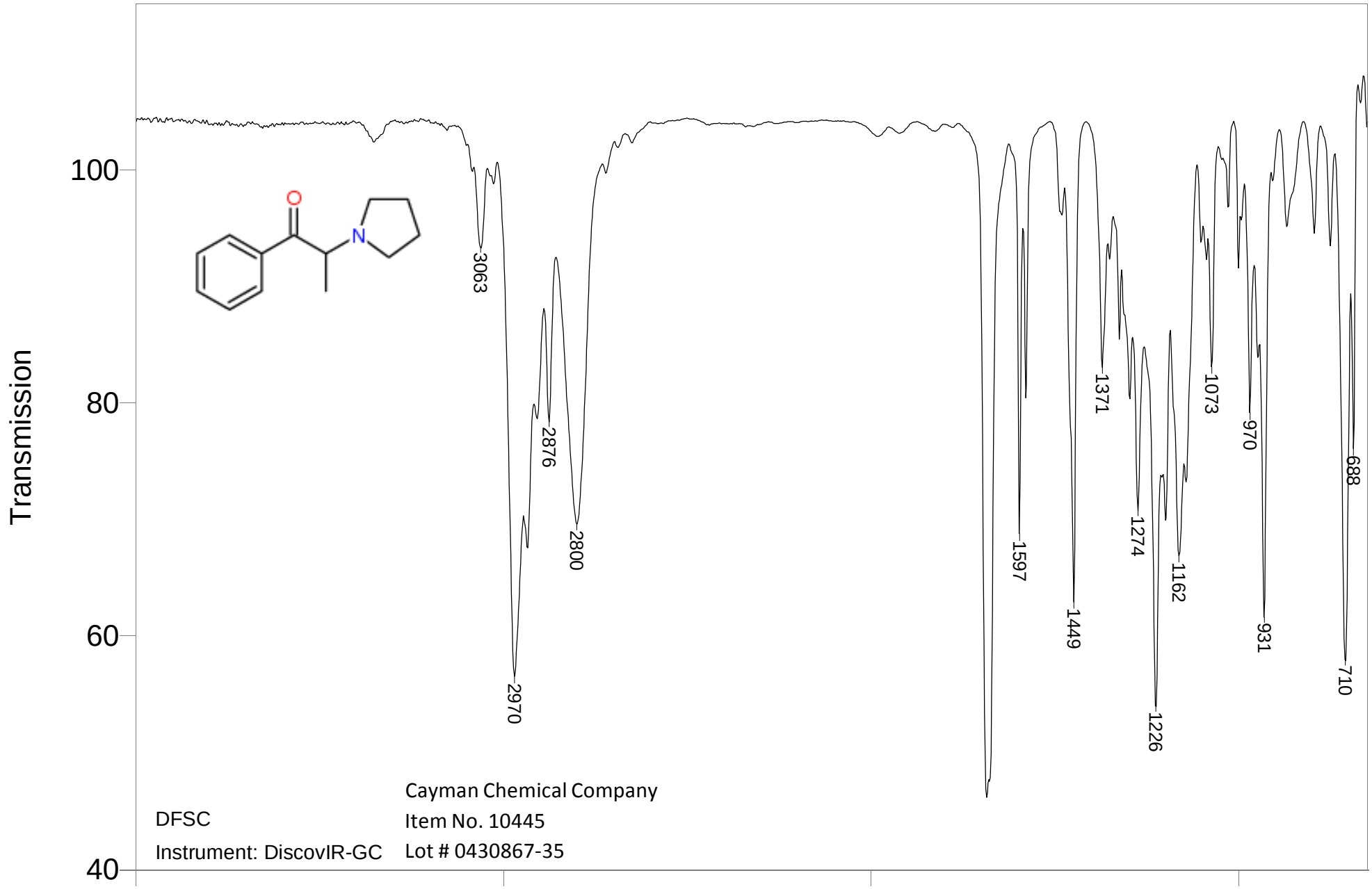


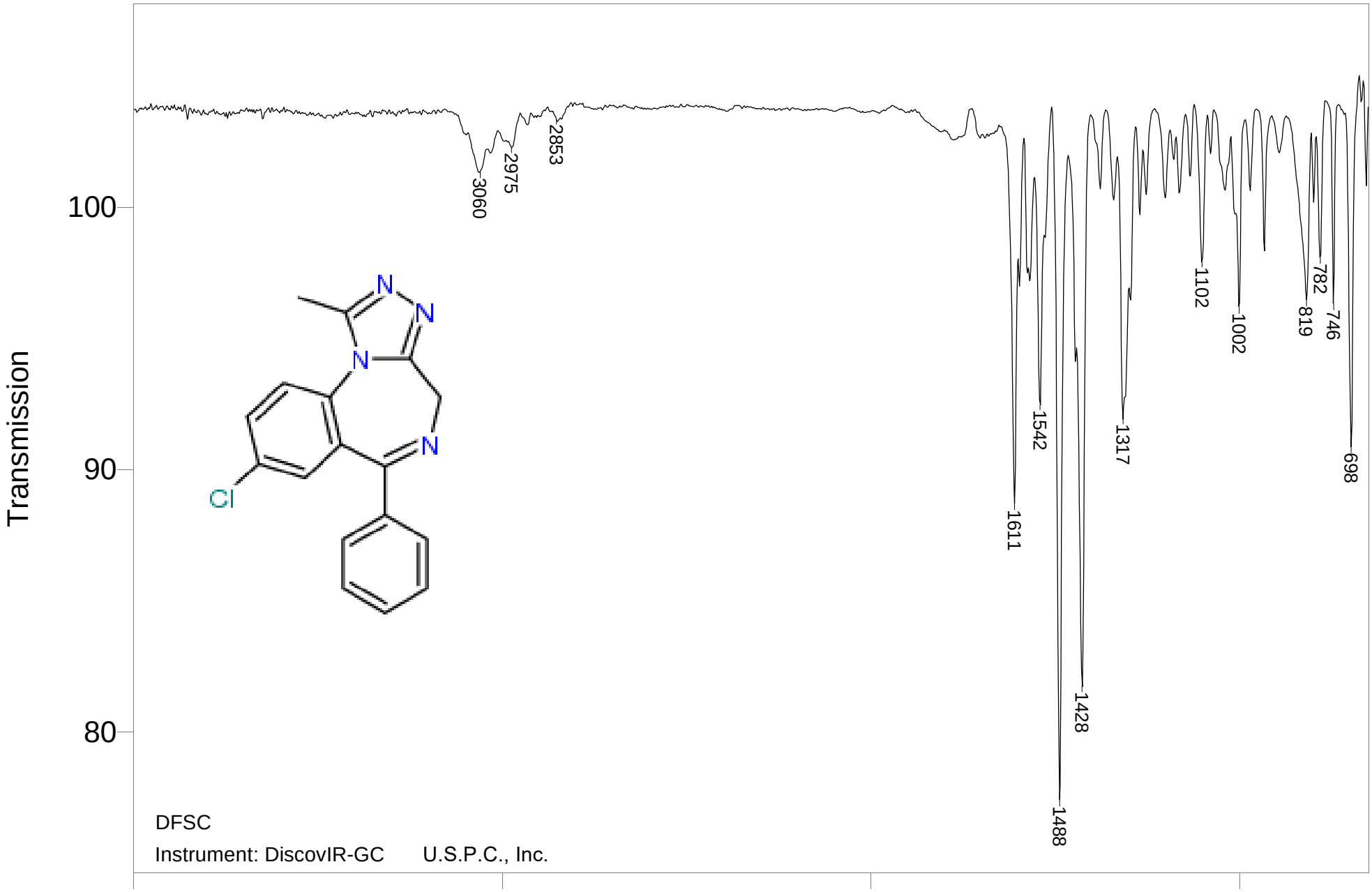


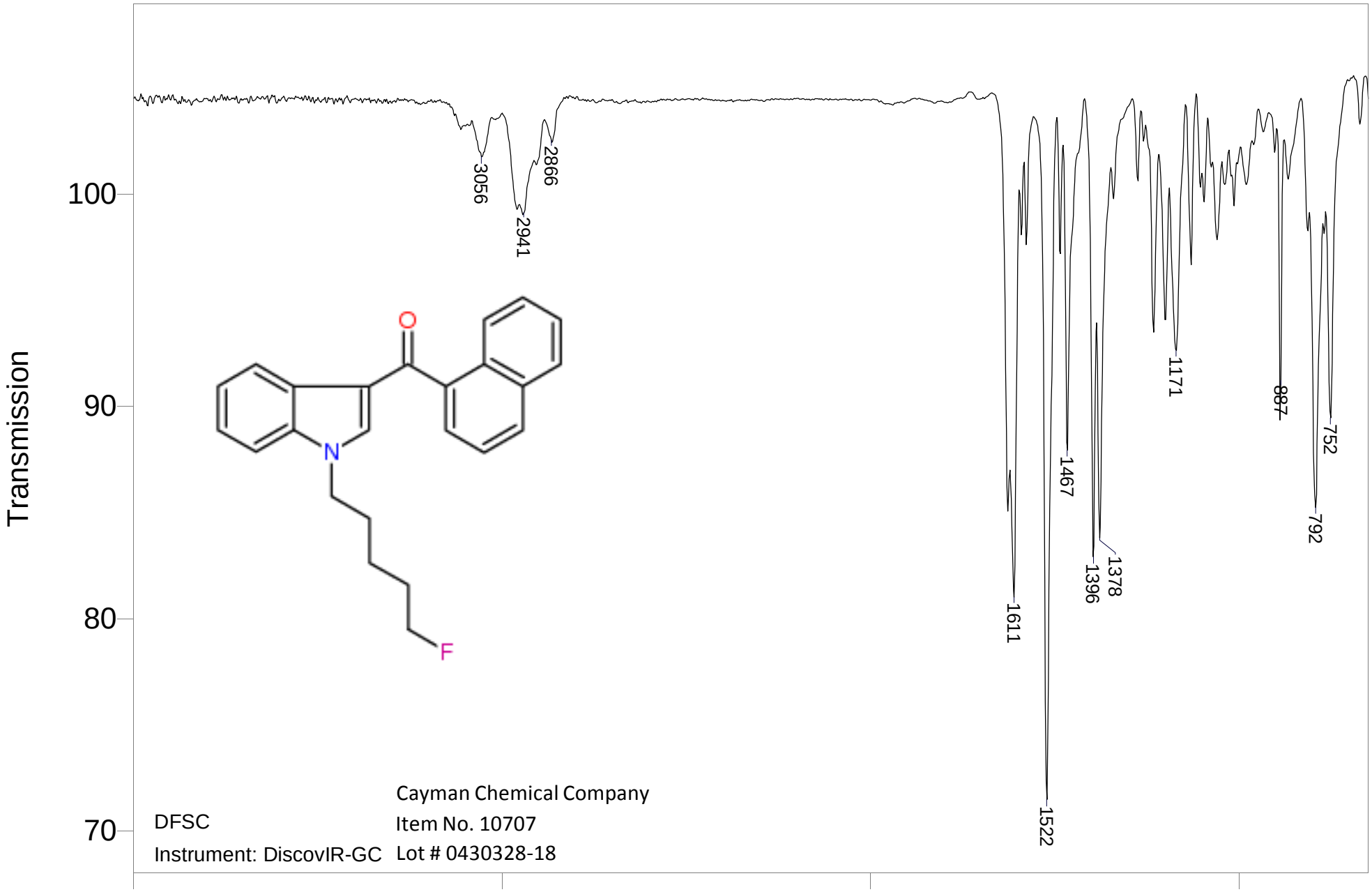
AKB48 N-(4-fluorobenzyl) analog

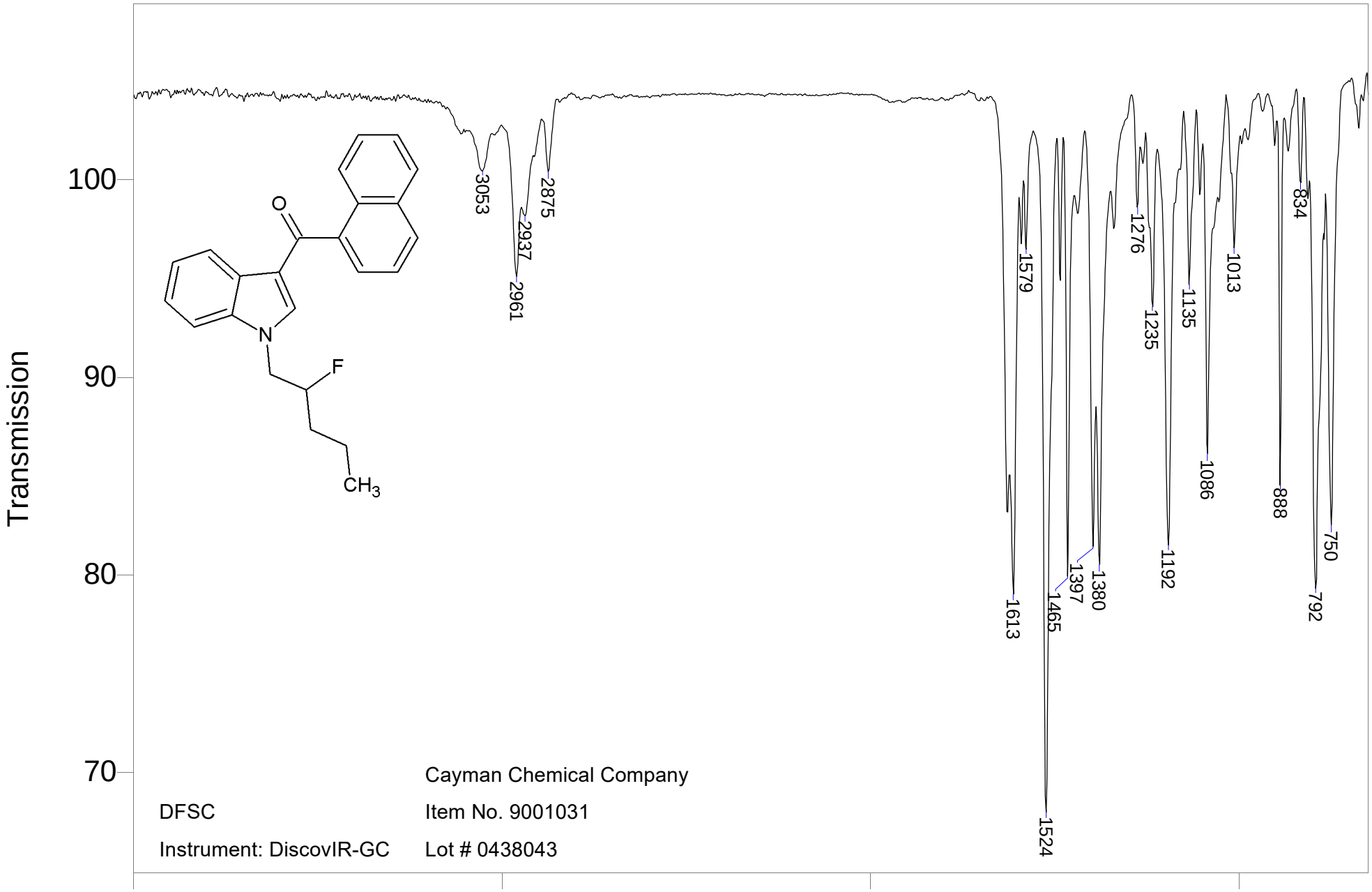


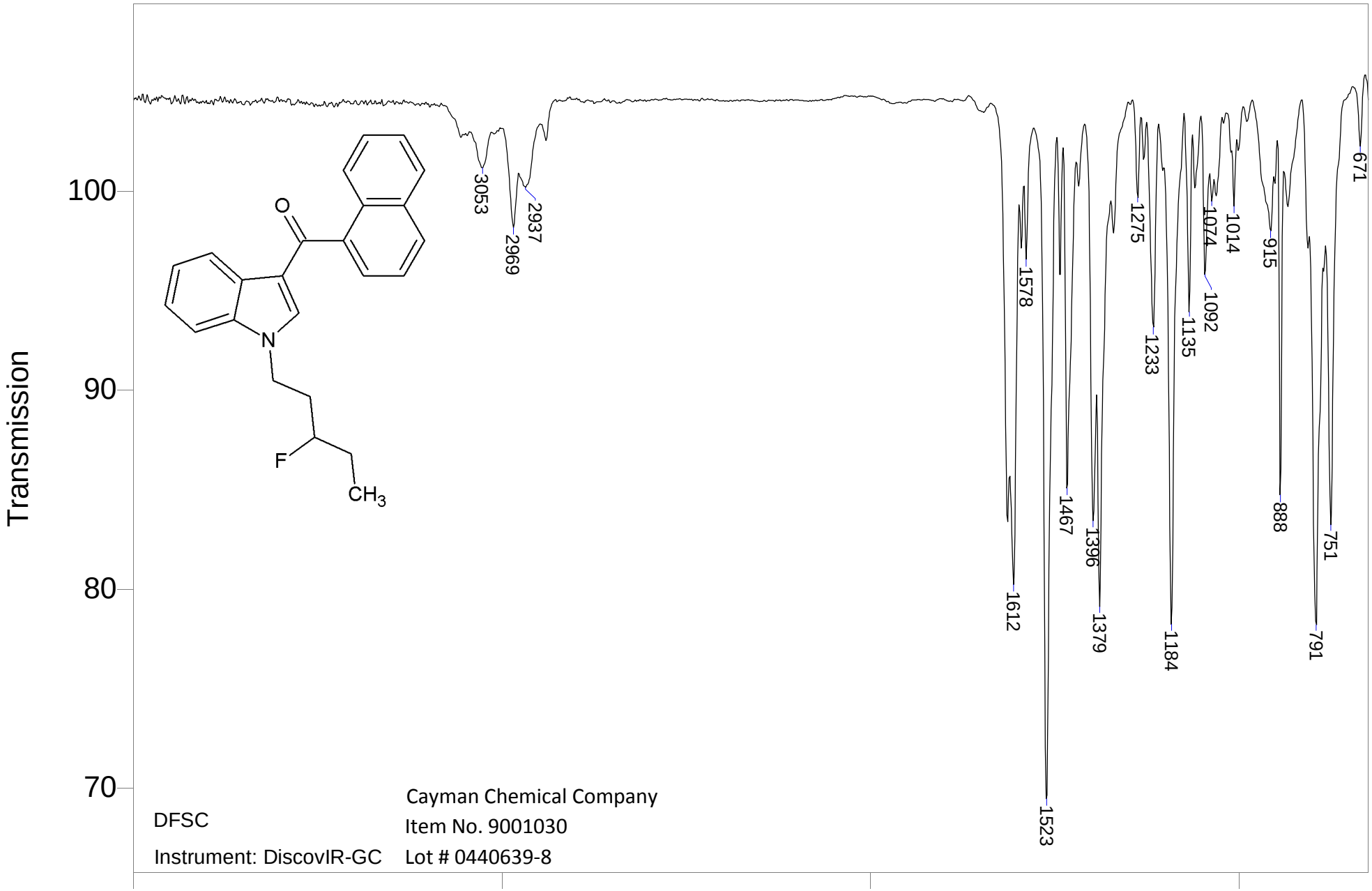


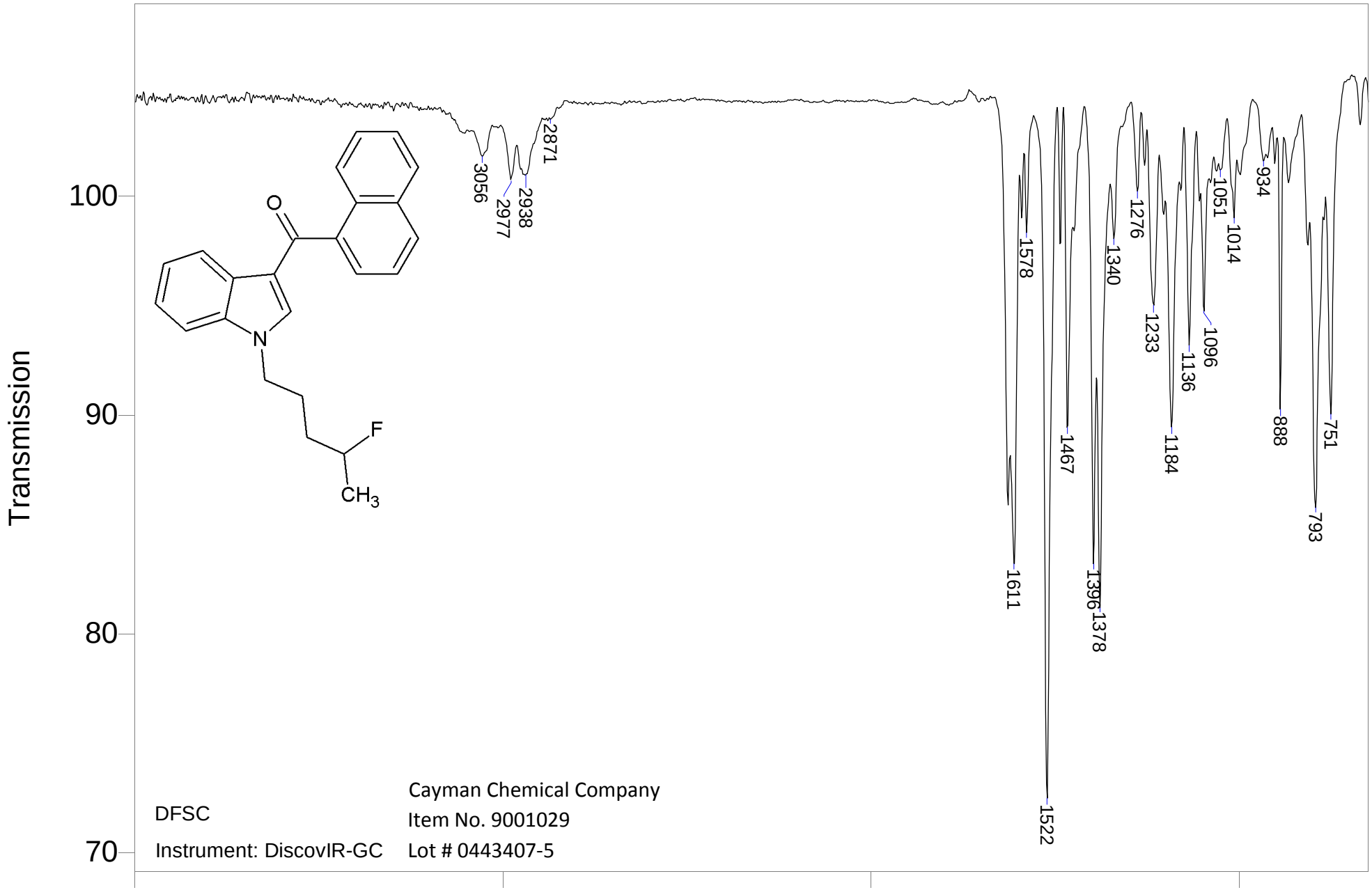


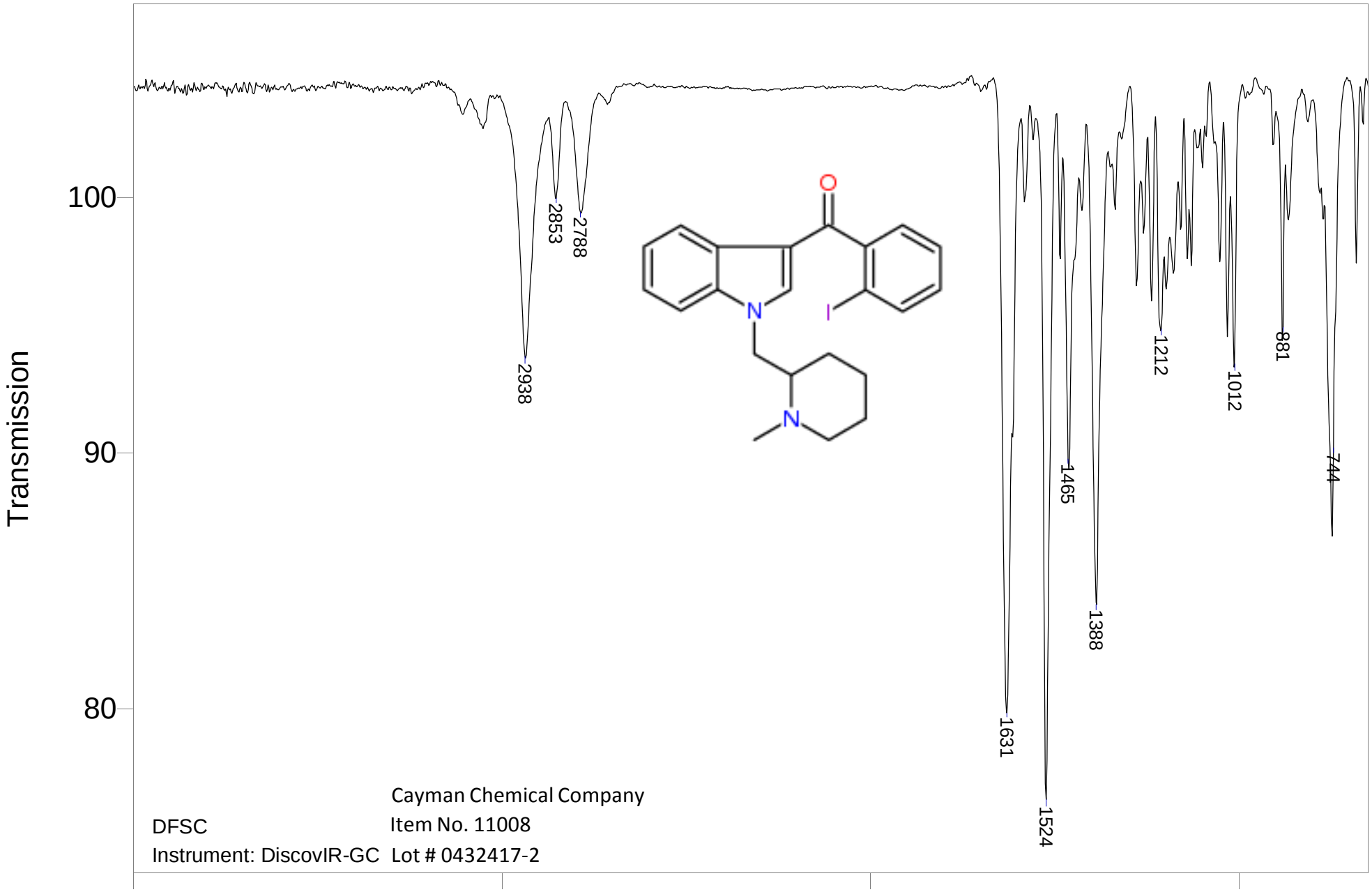


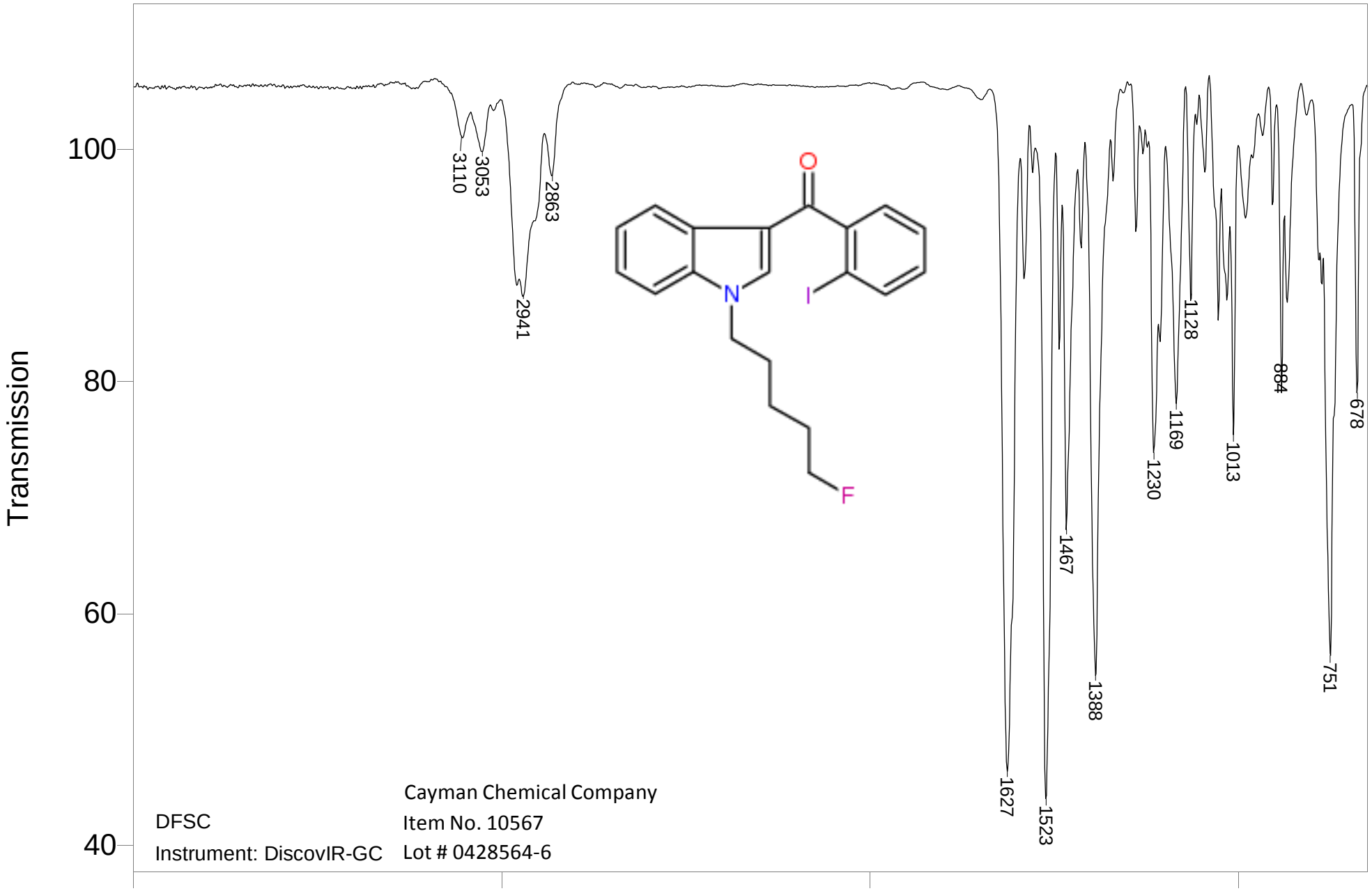


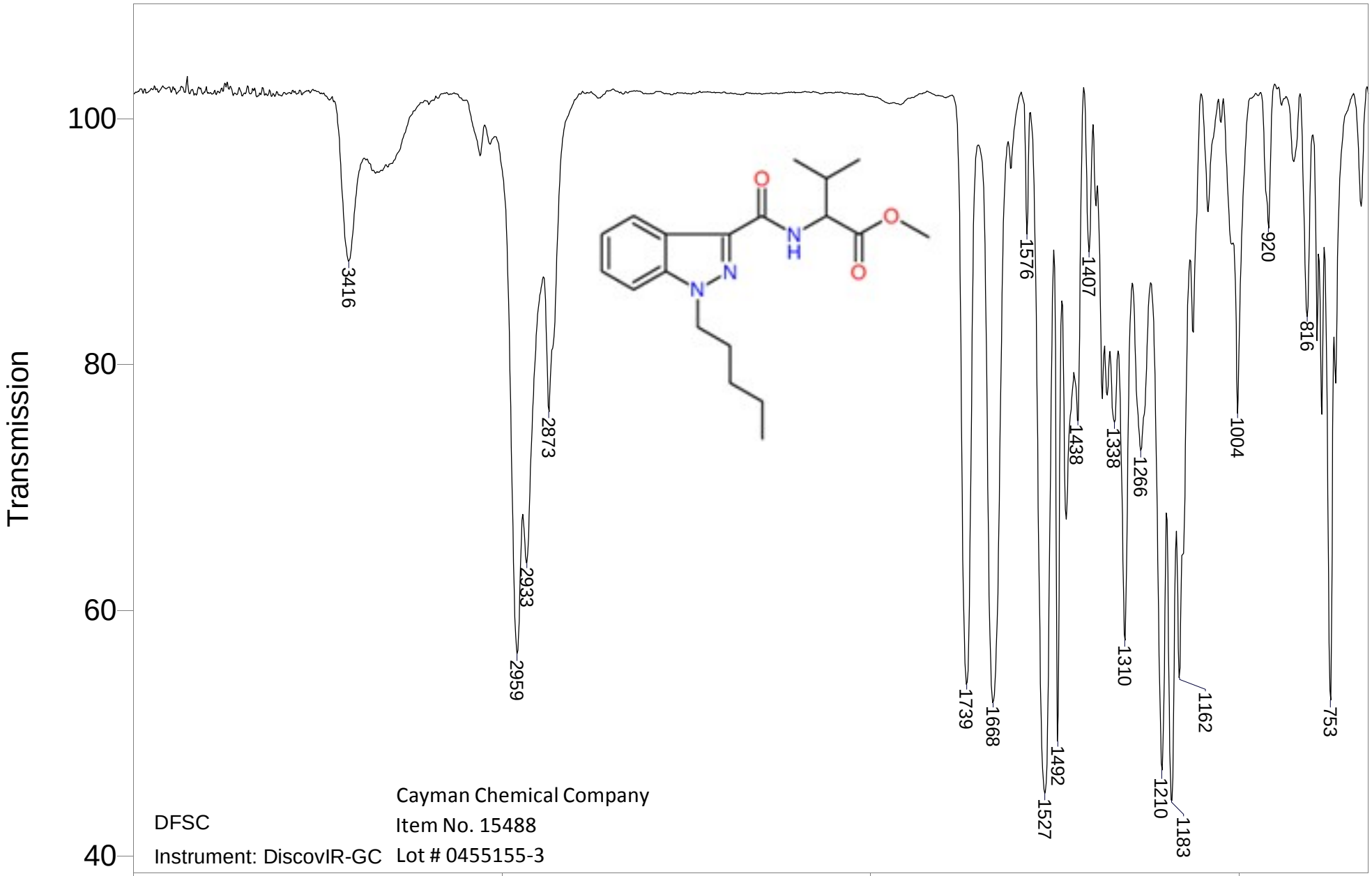




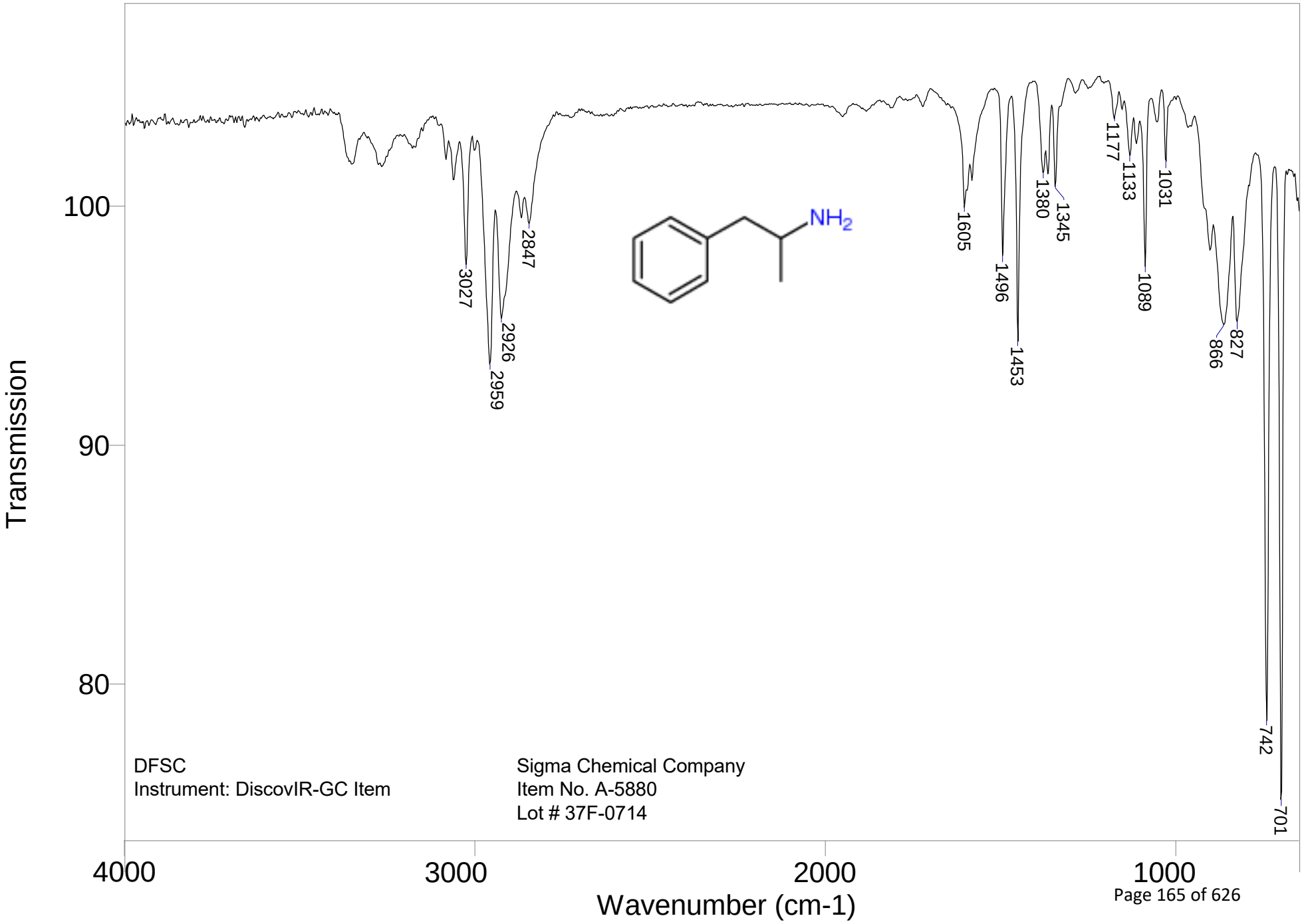


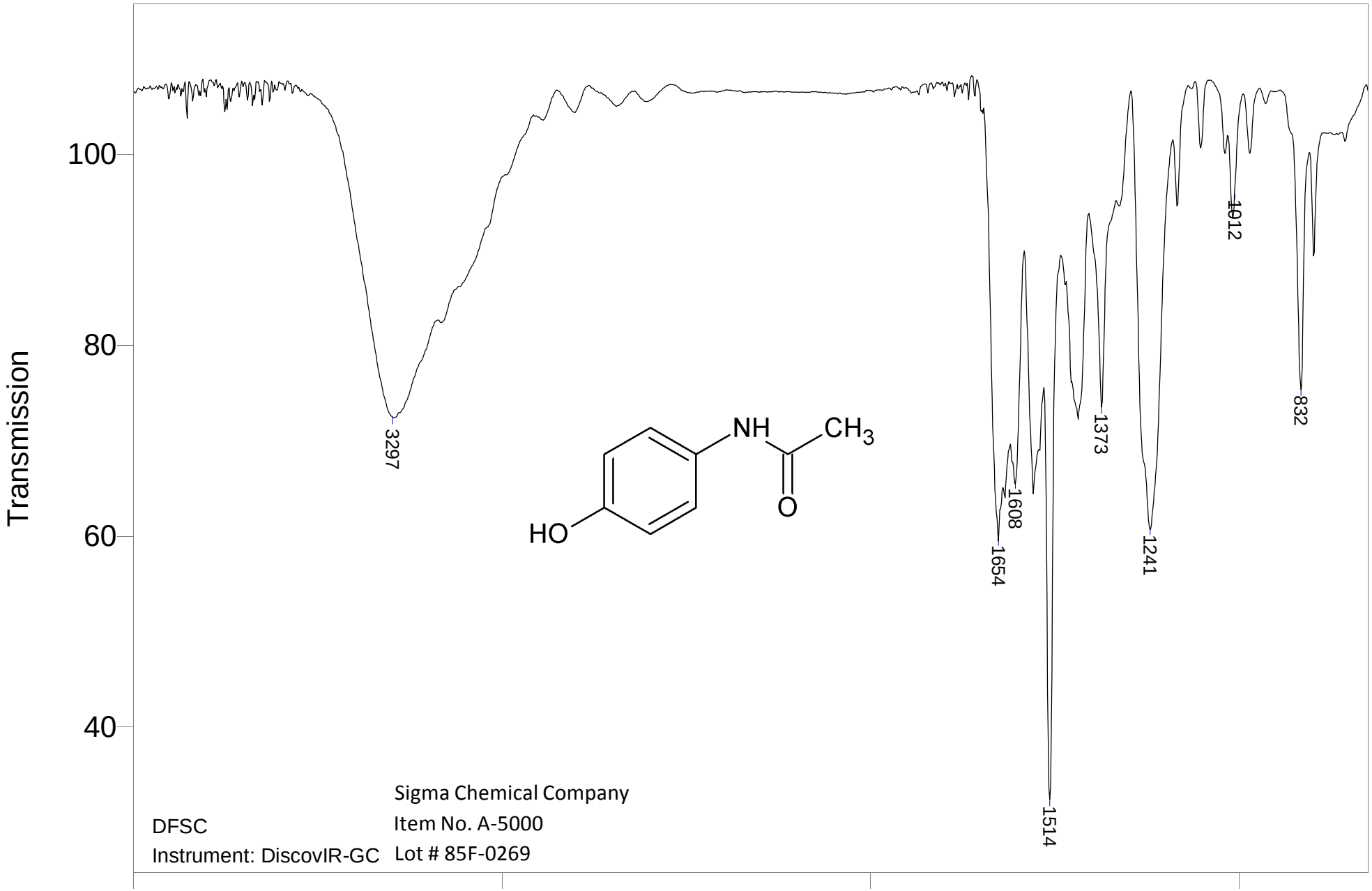




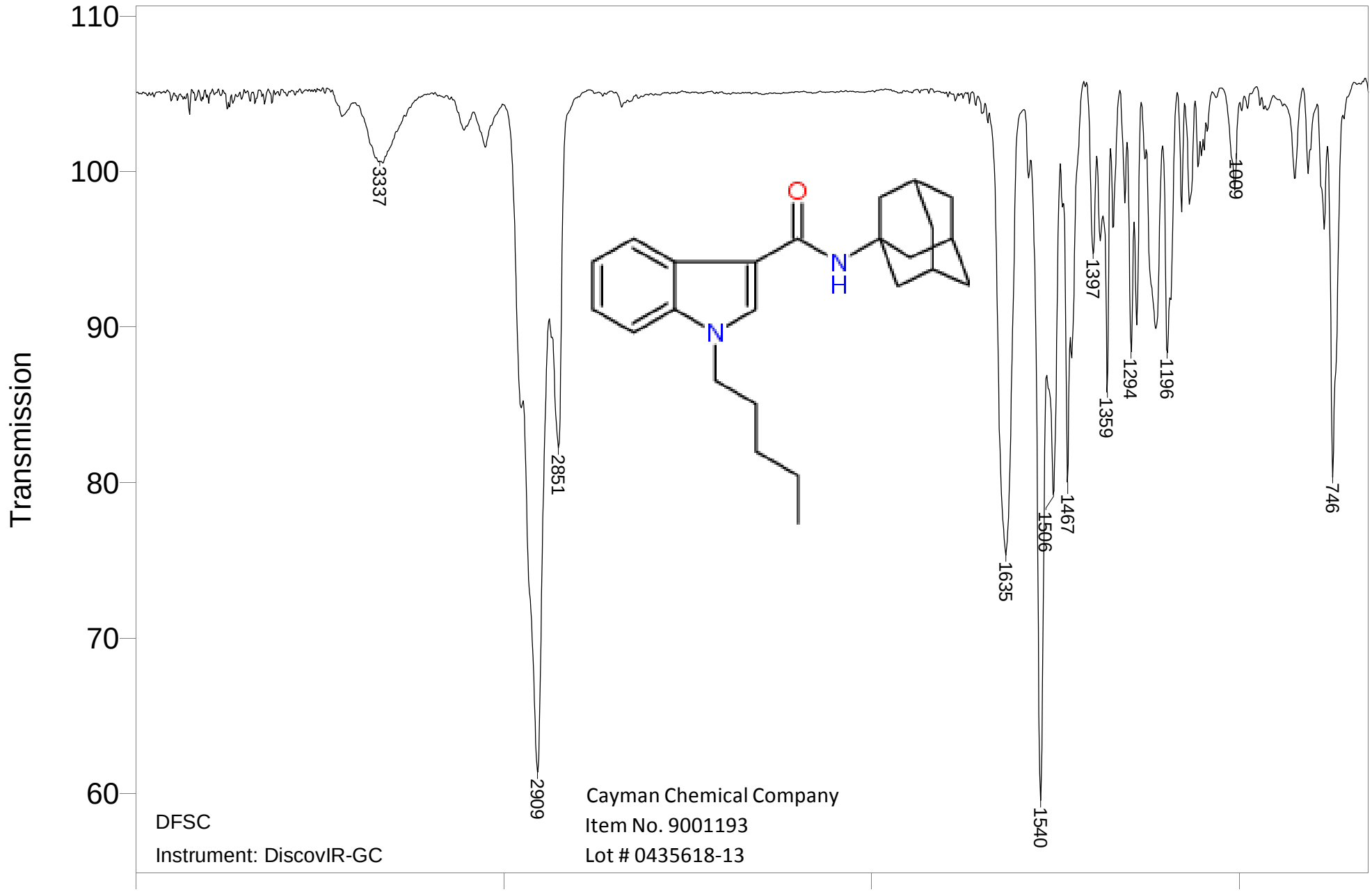


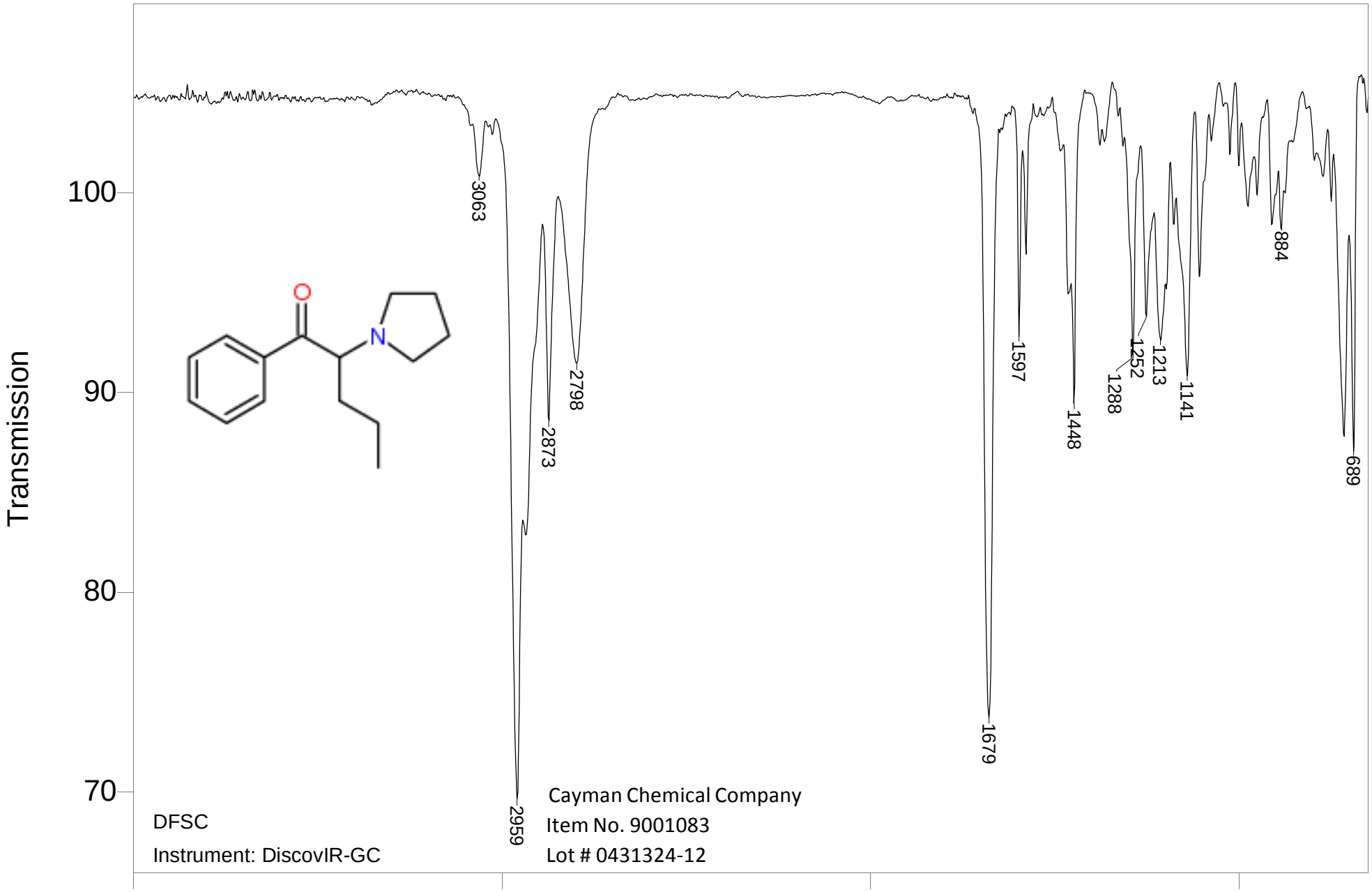
Amphetamine

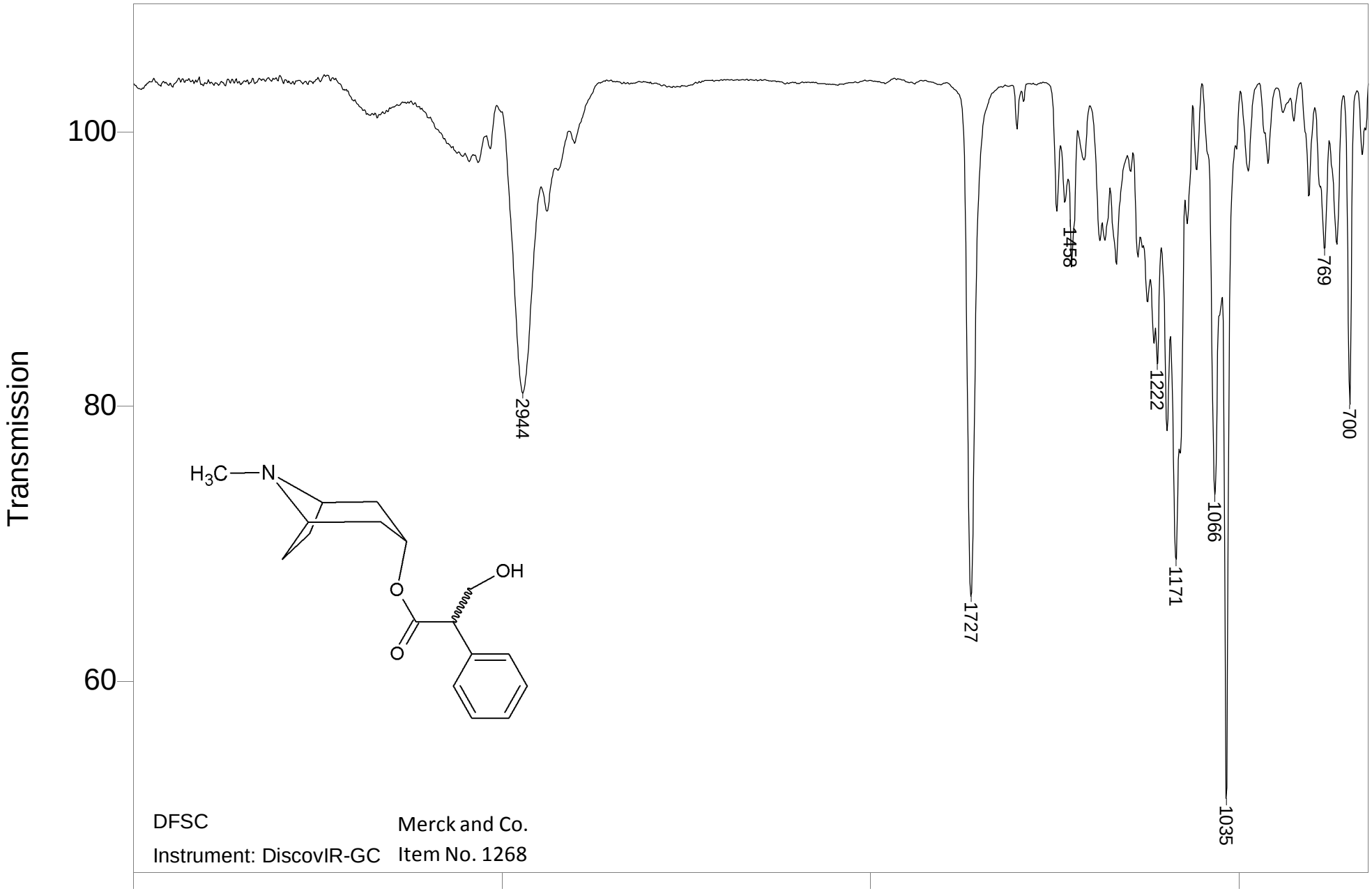


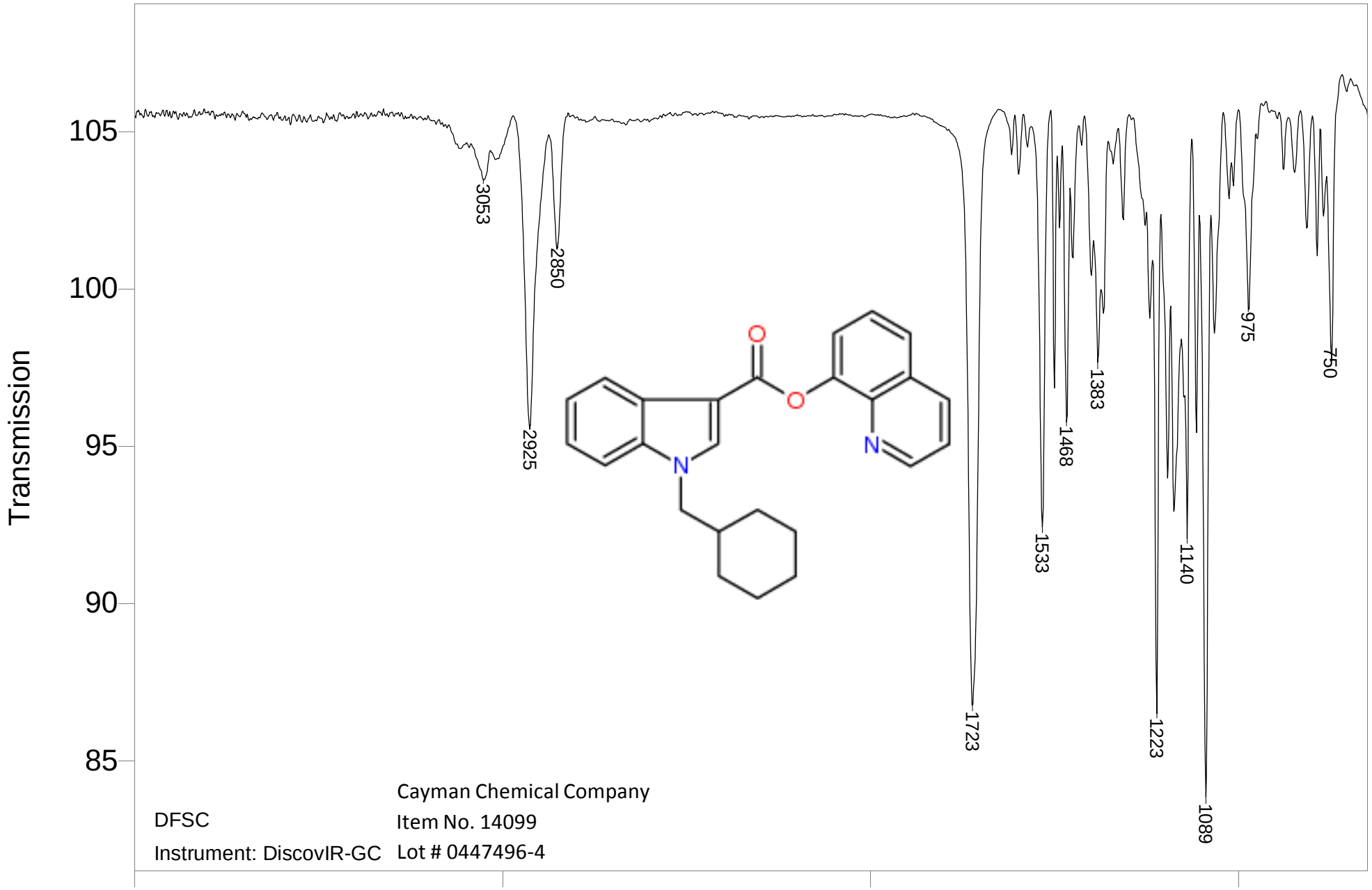


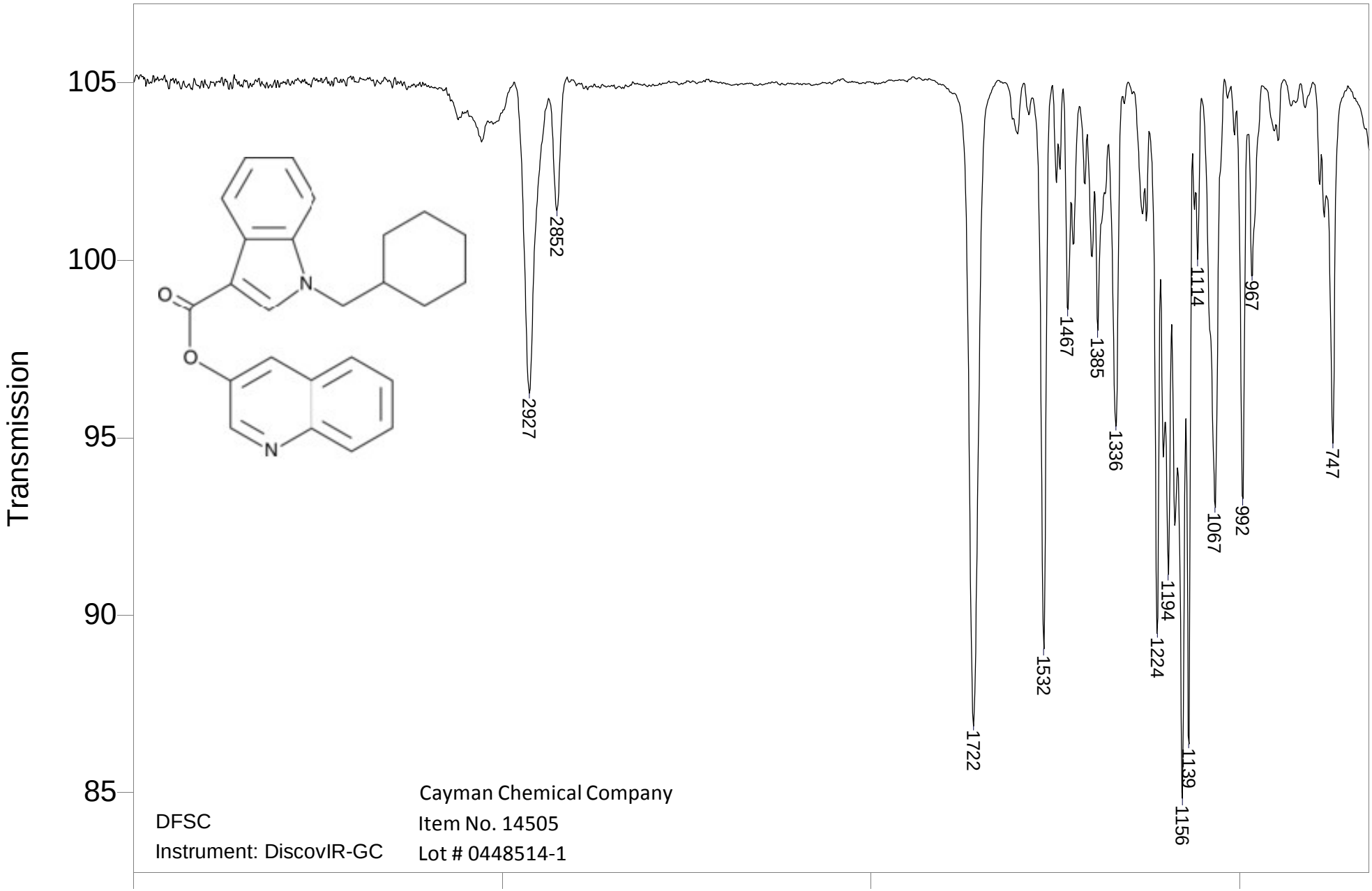
APICA **JWH-018 adamantyl carboxamide**

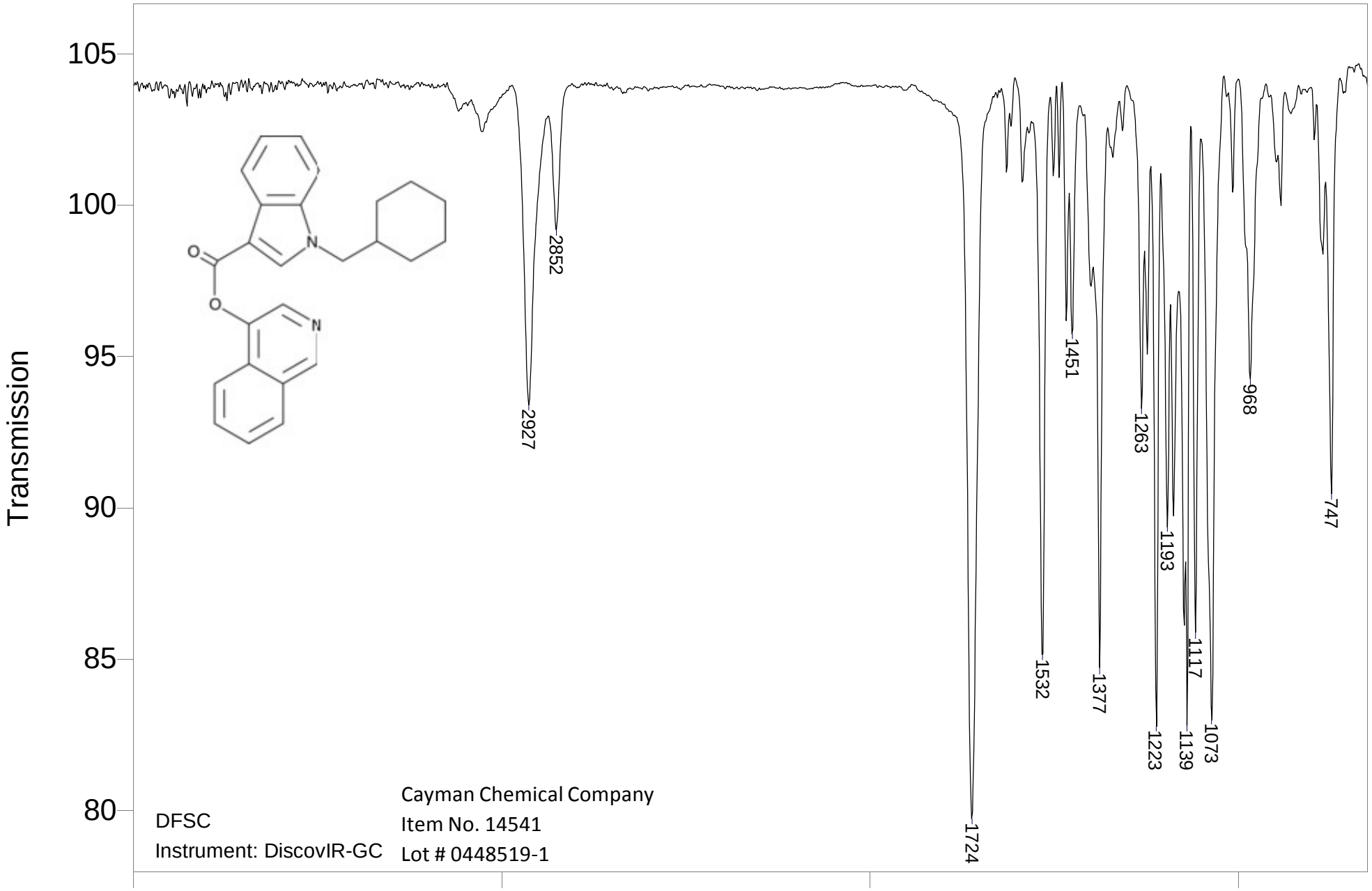


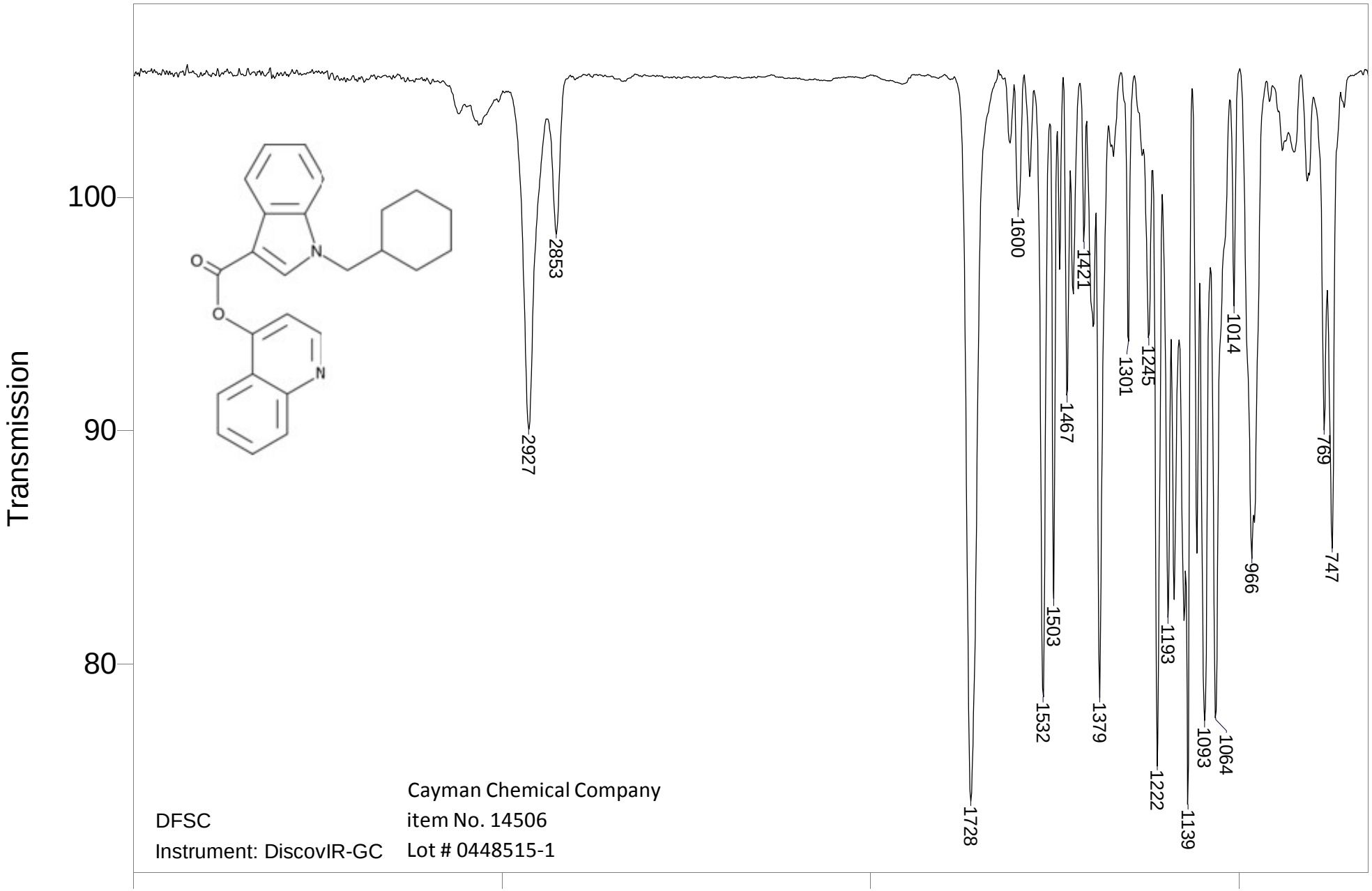




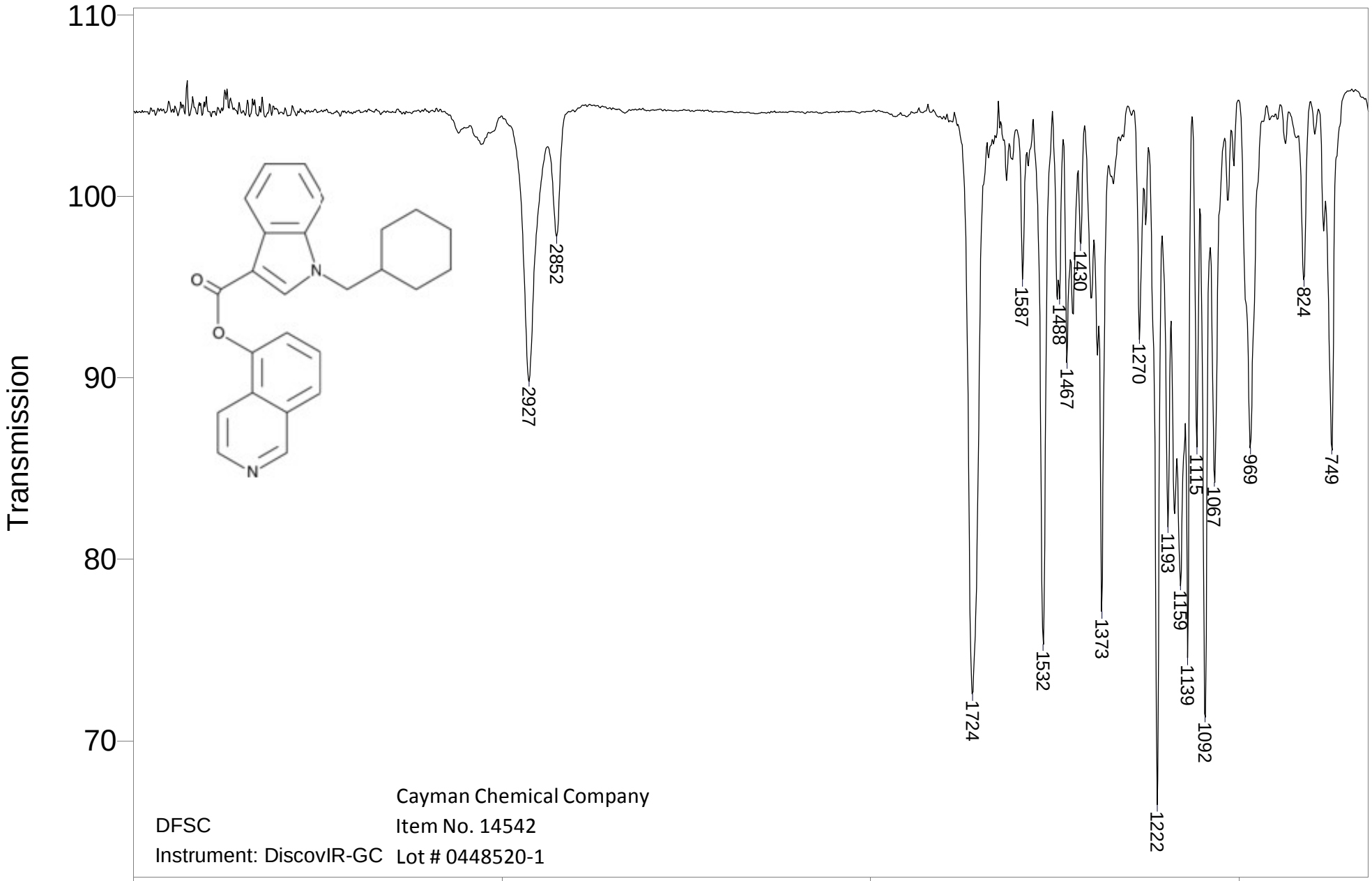


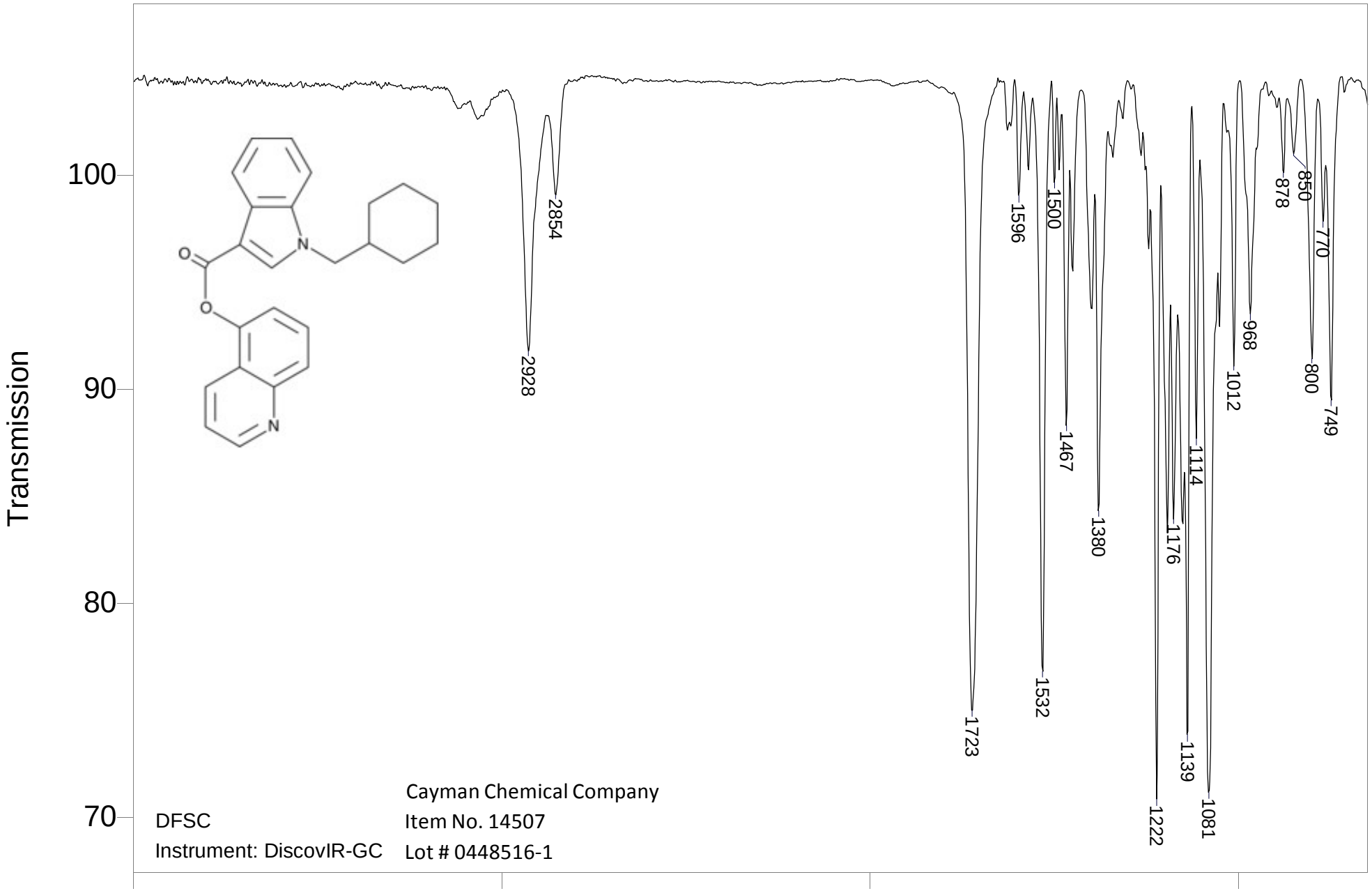


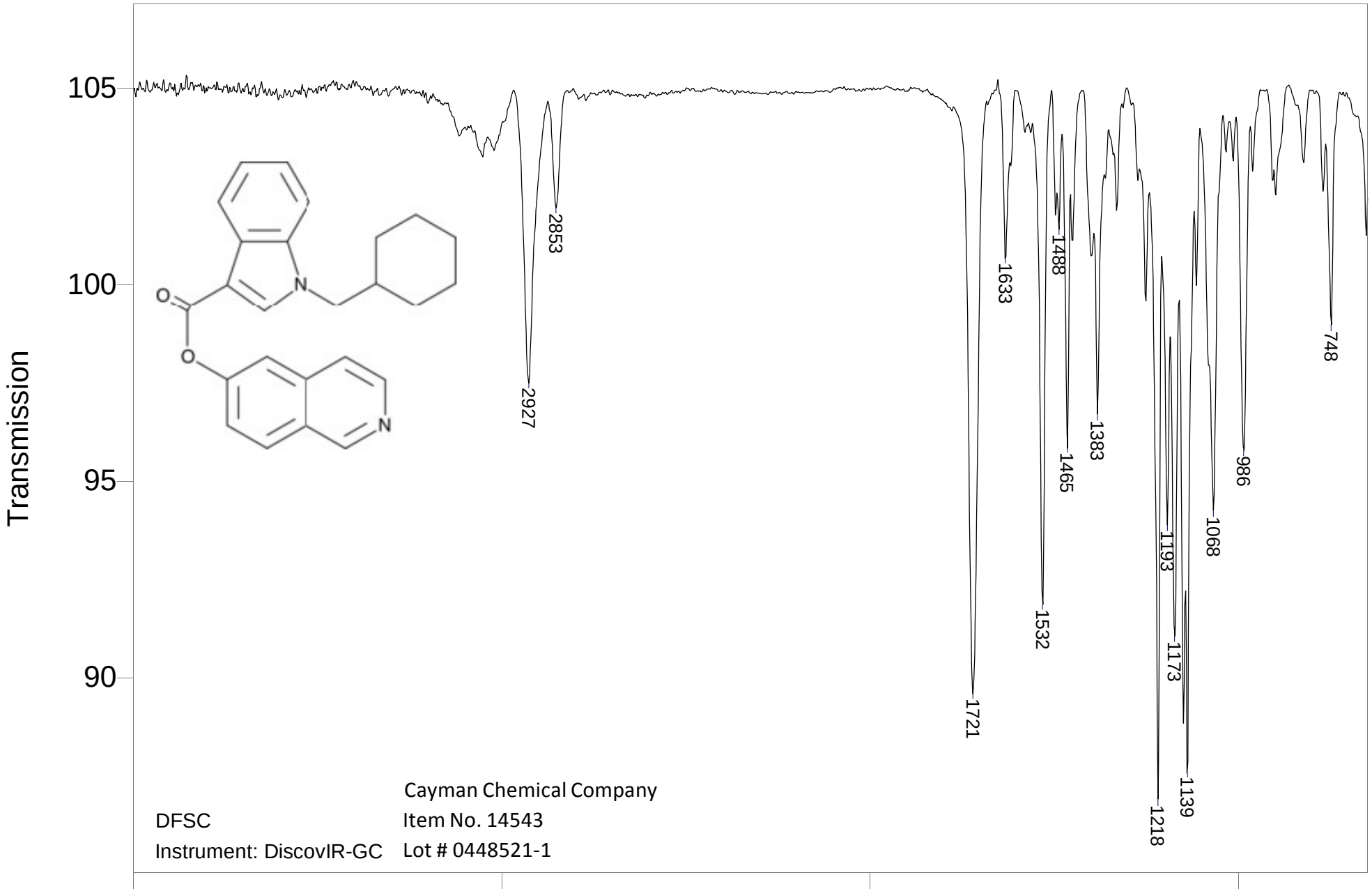


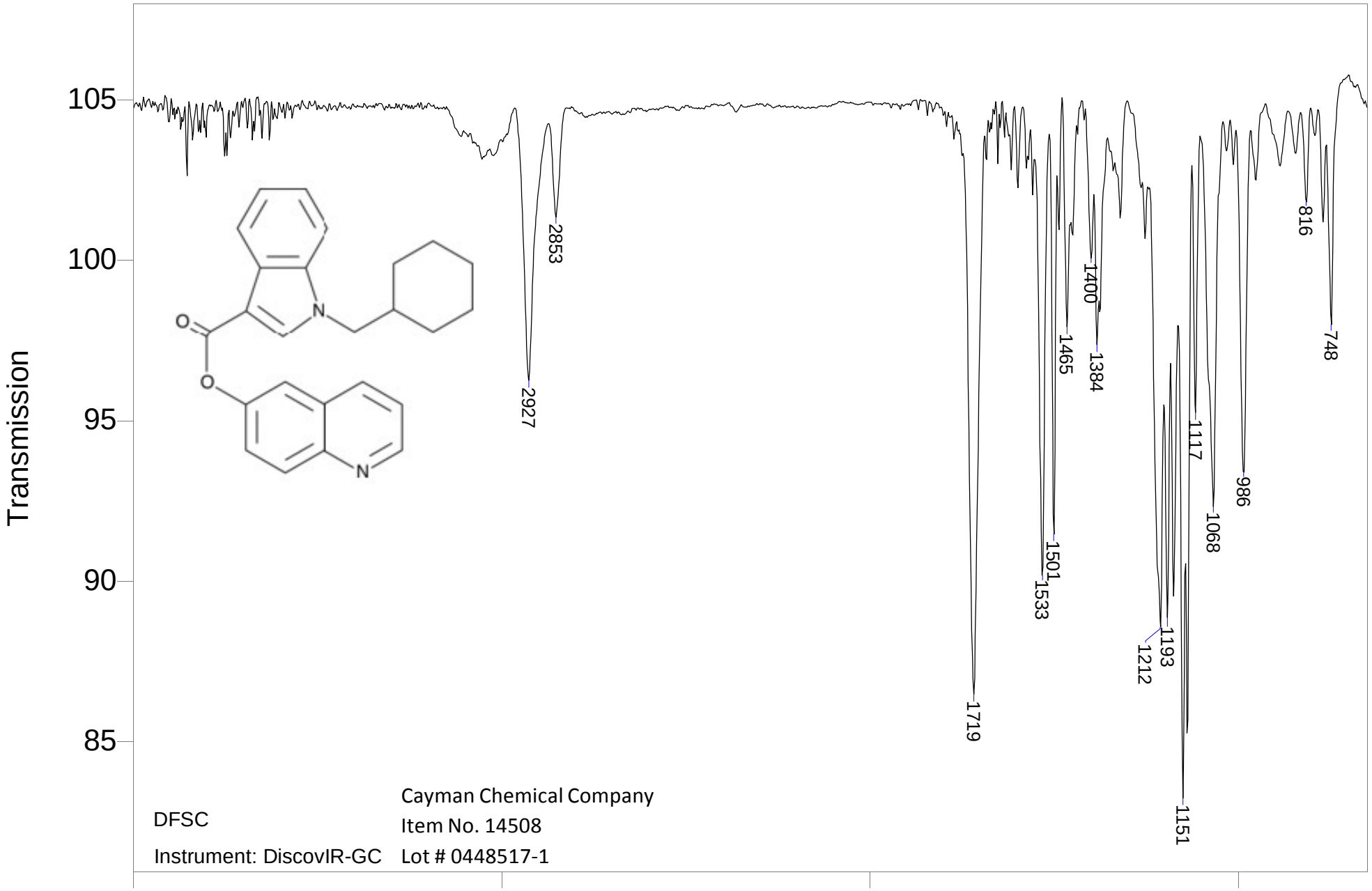


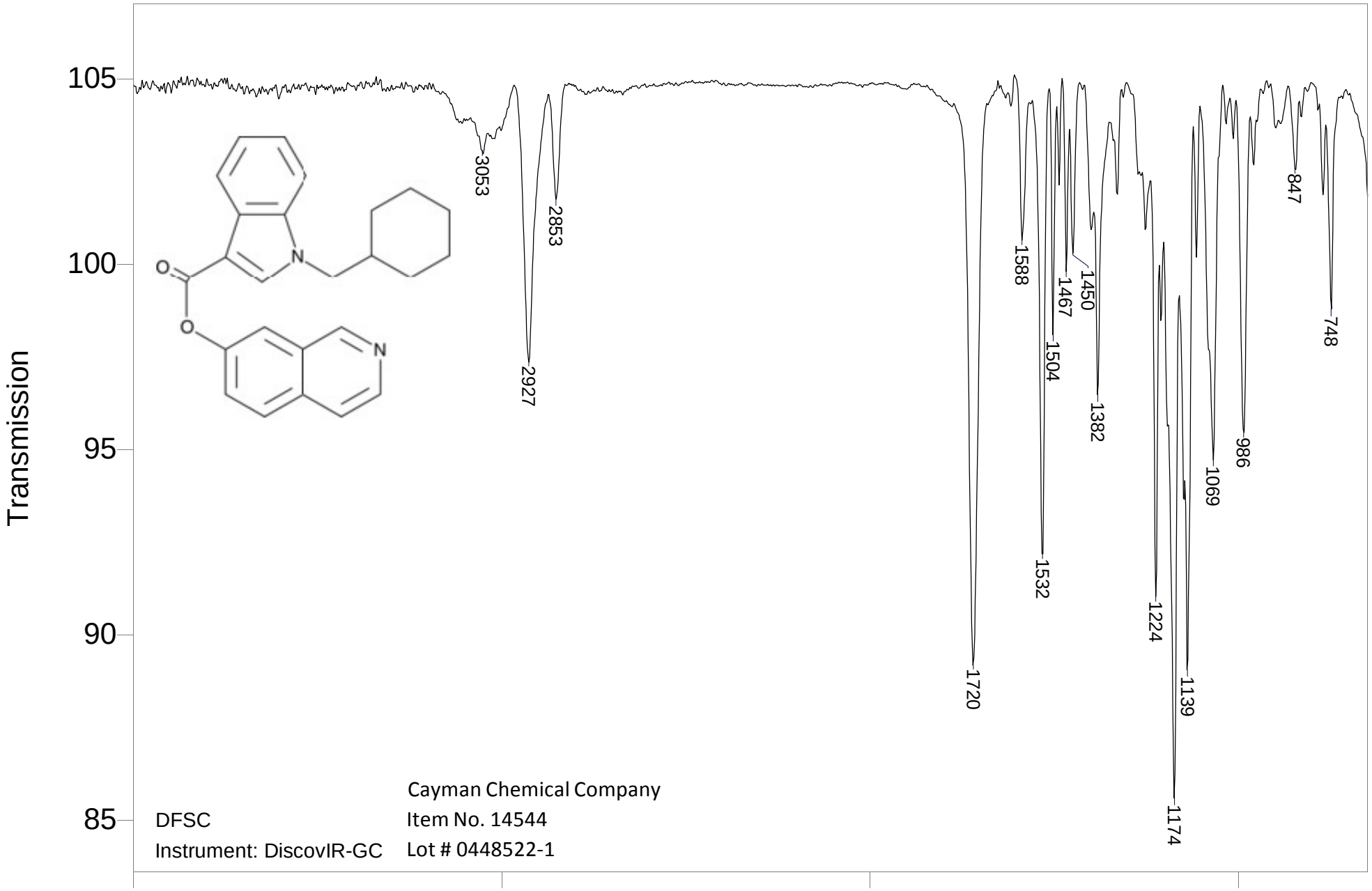
BB-22 5-hydroxyisoquinoline isomer

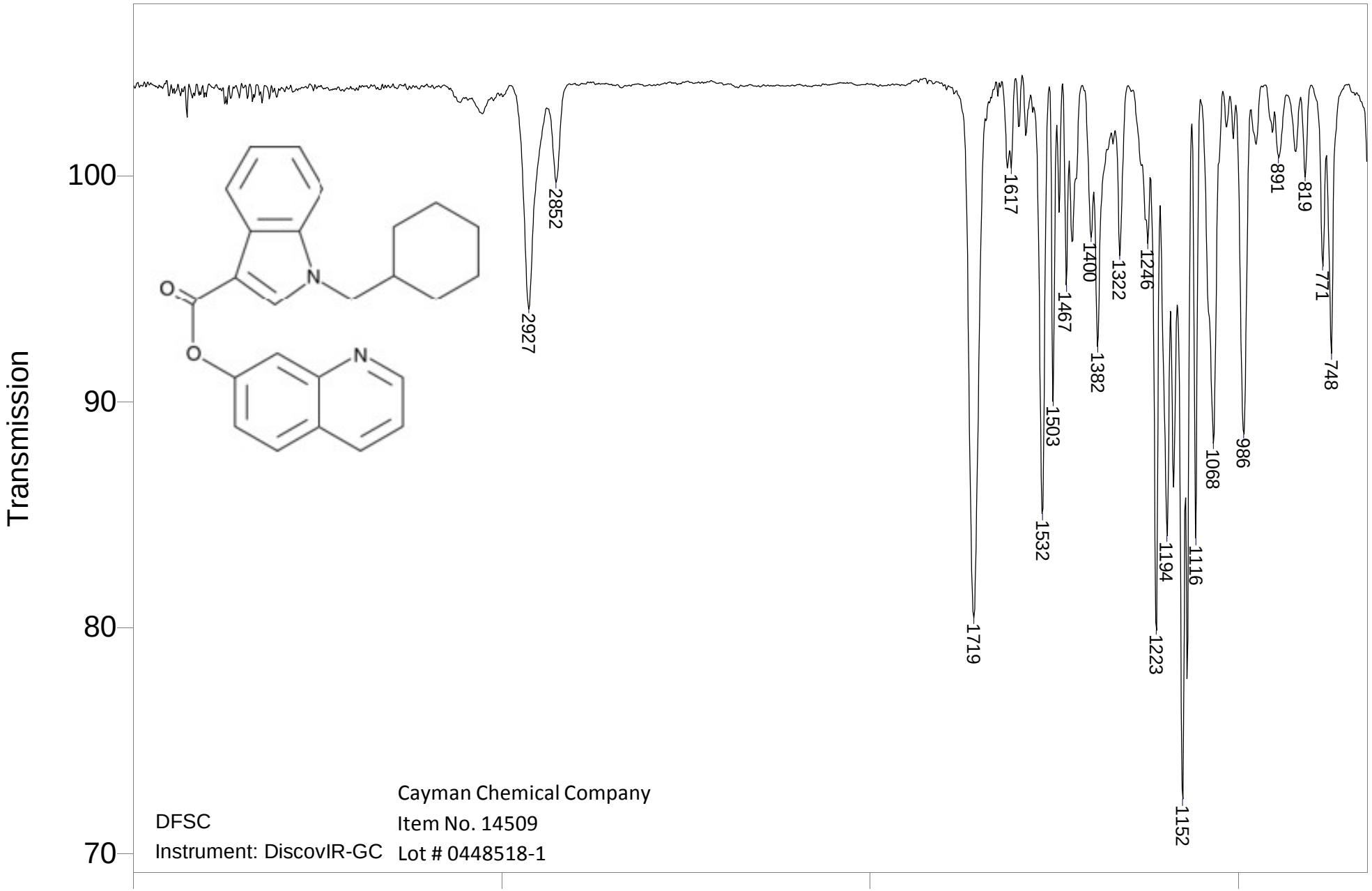


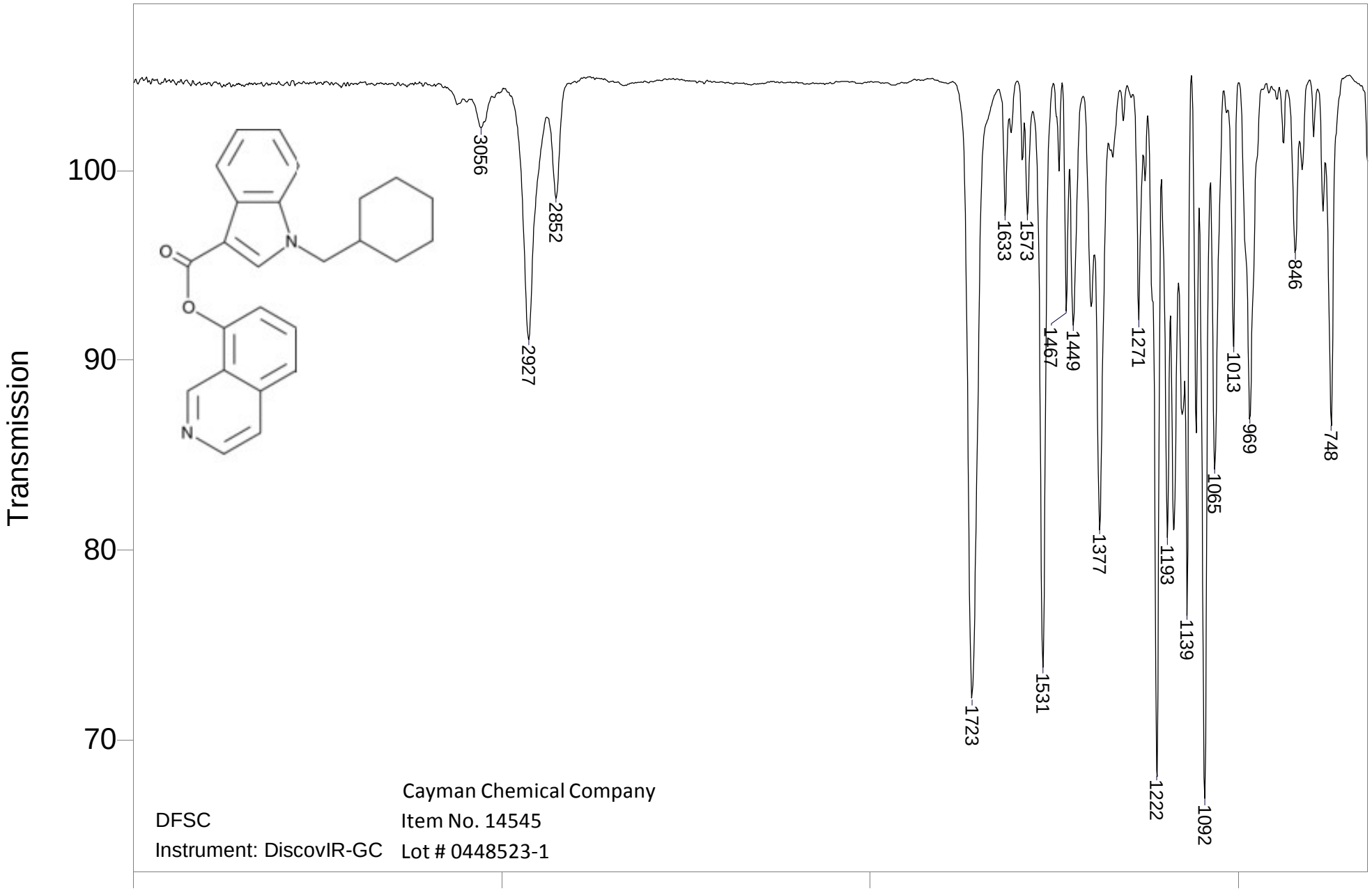


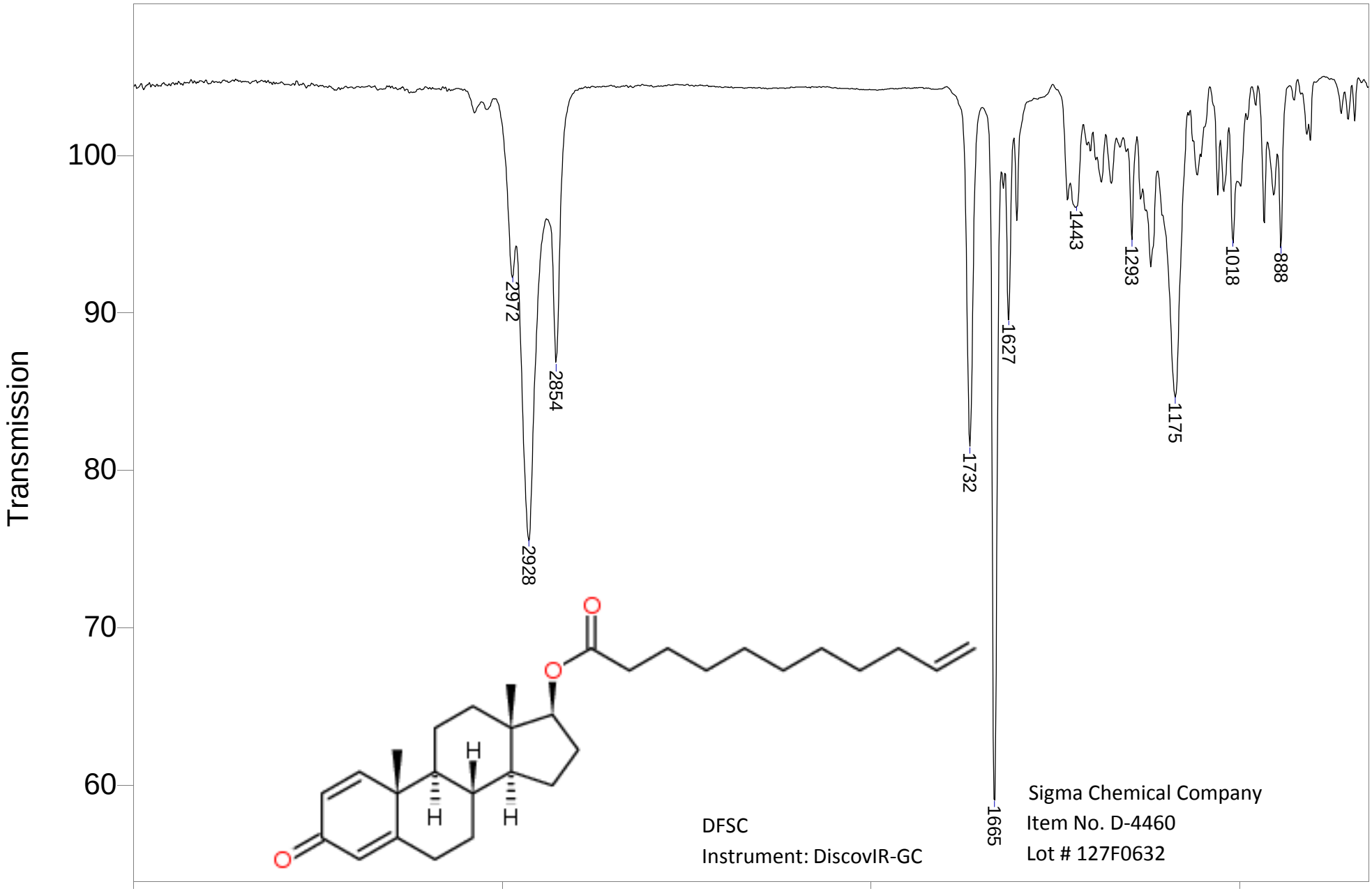












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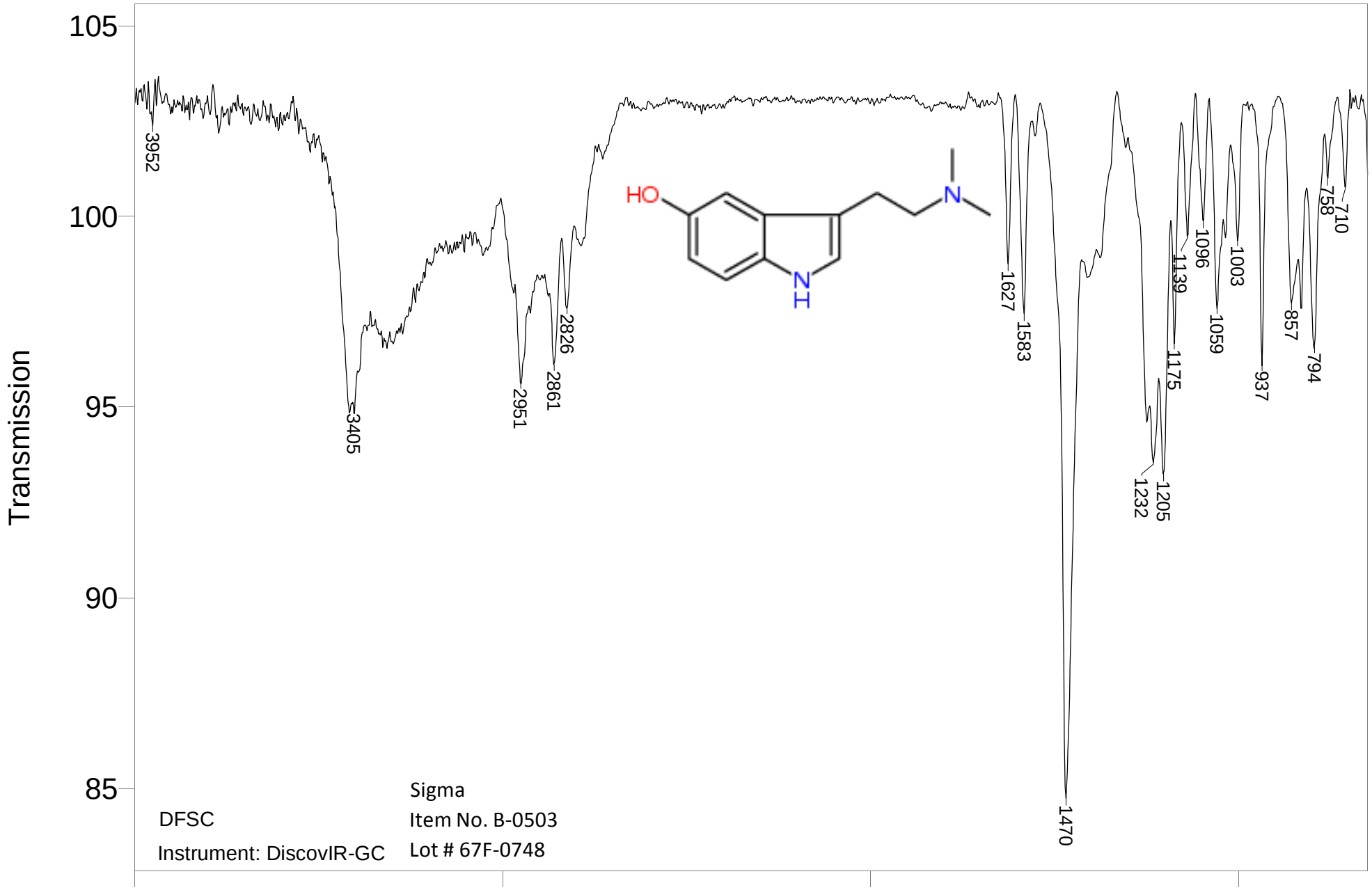
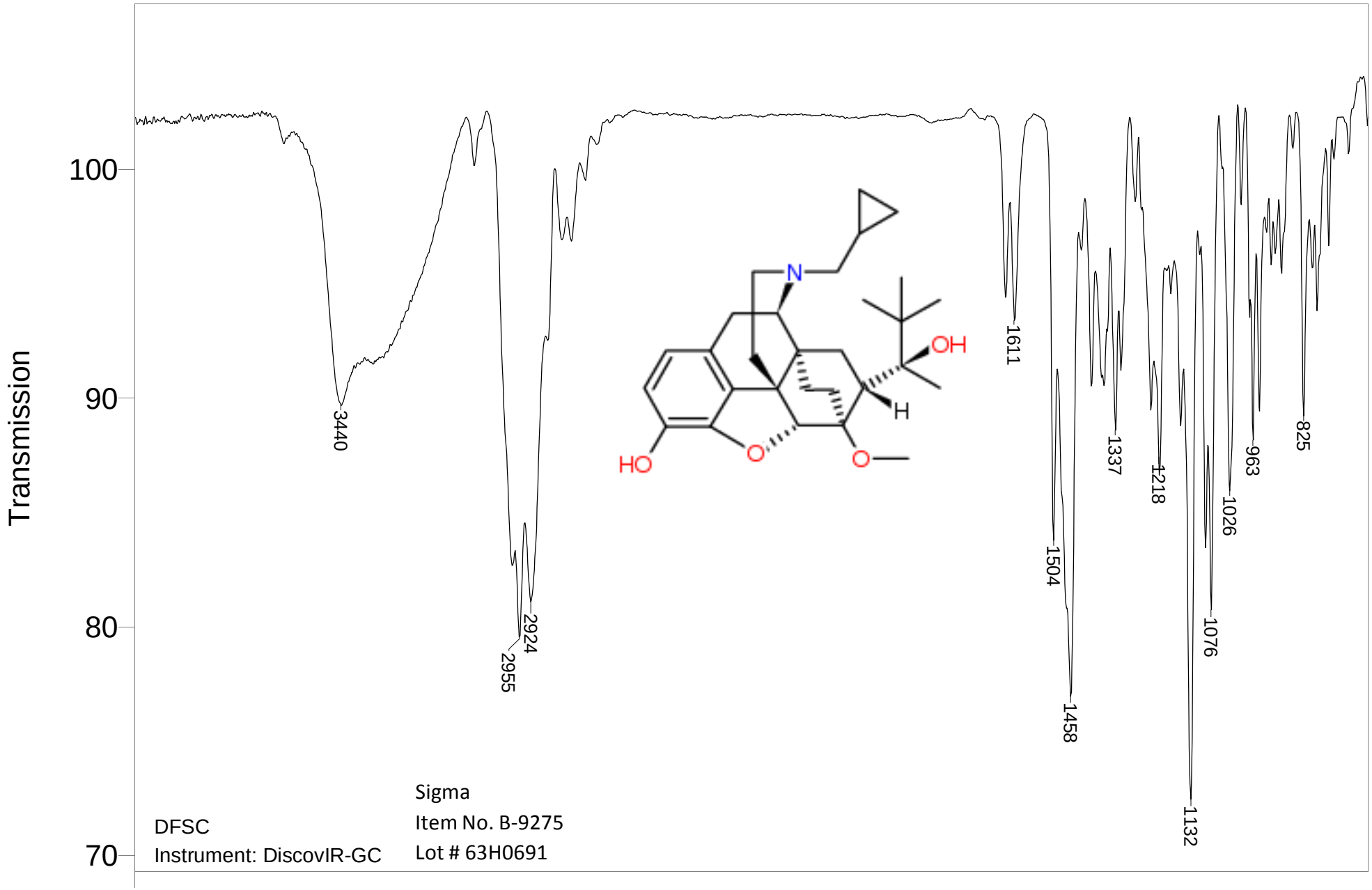
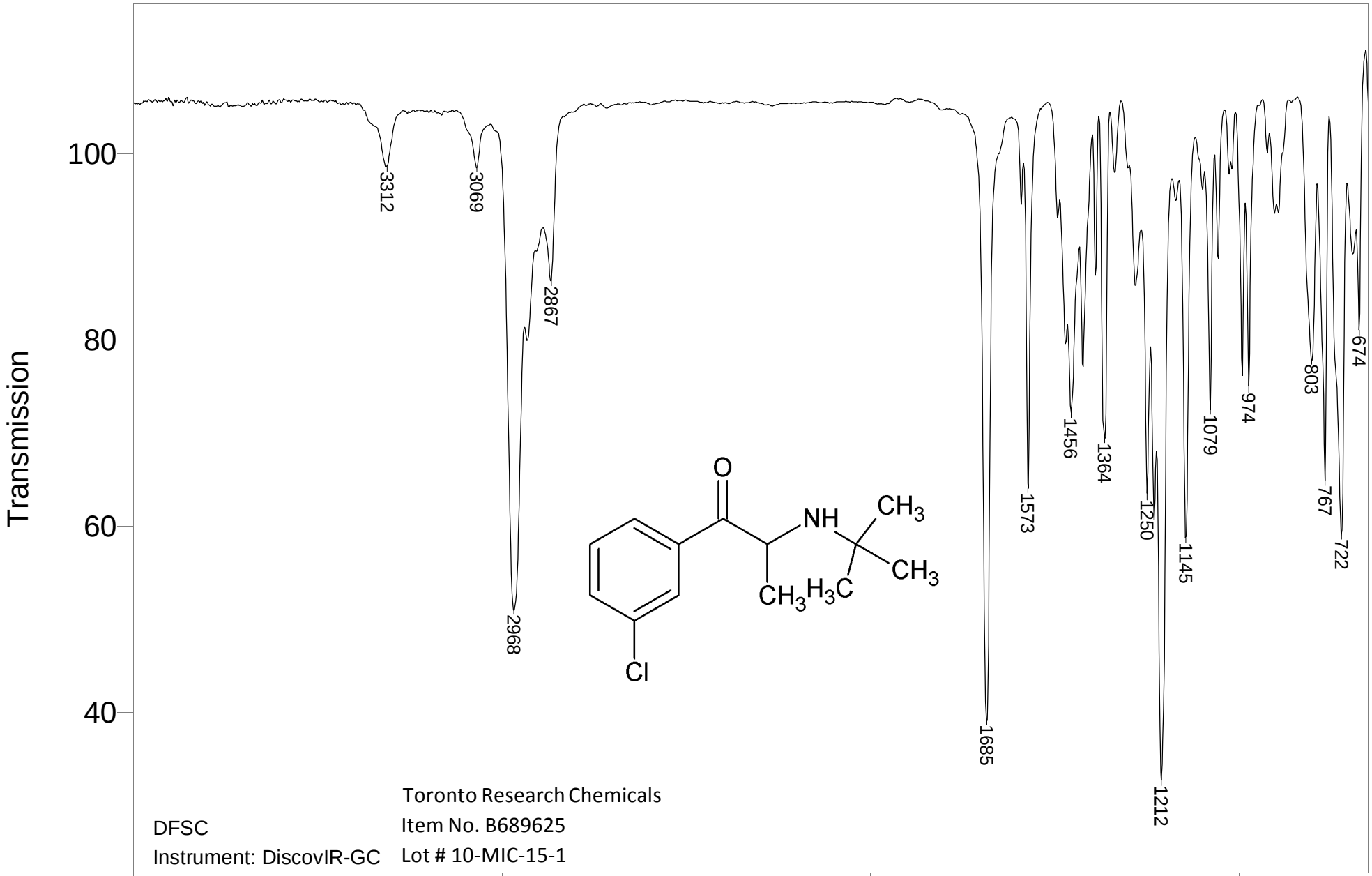
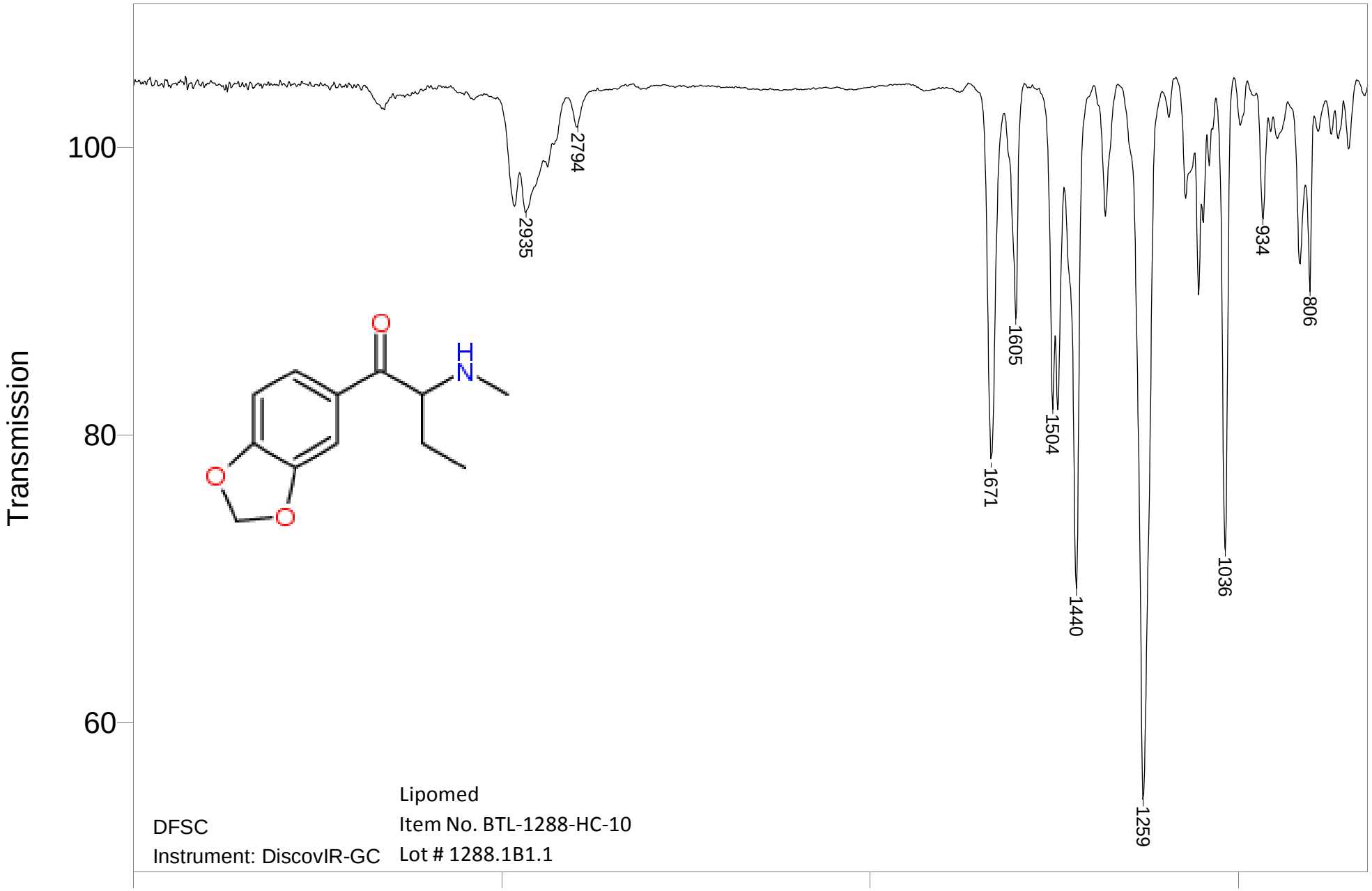
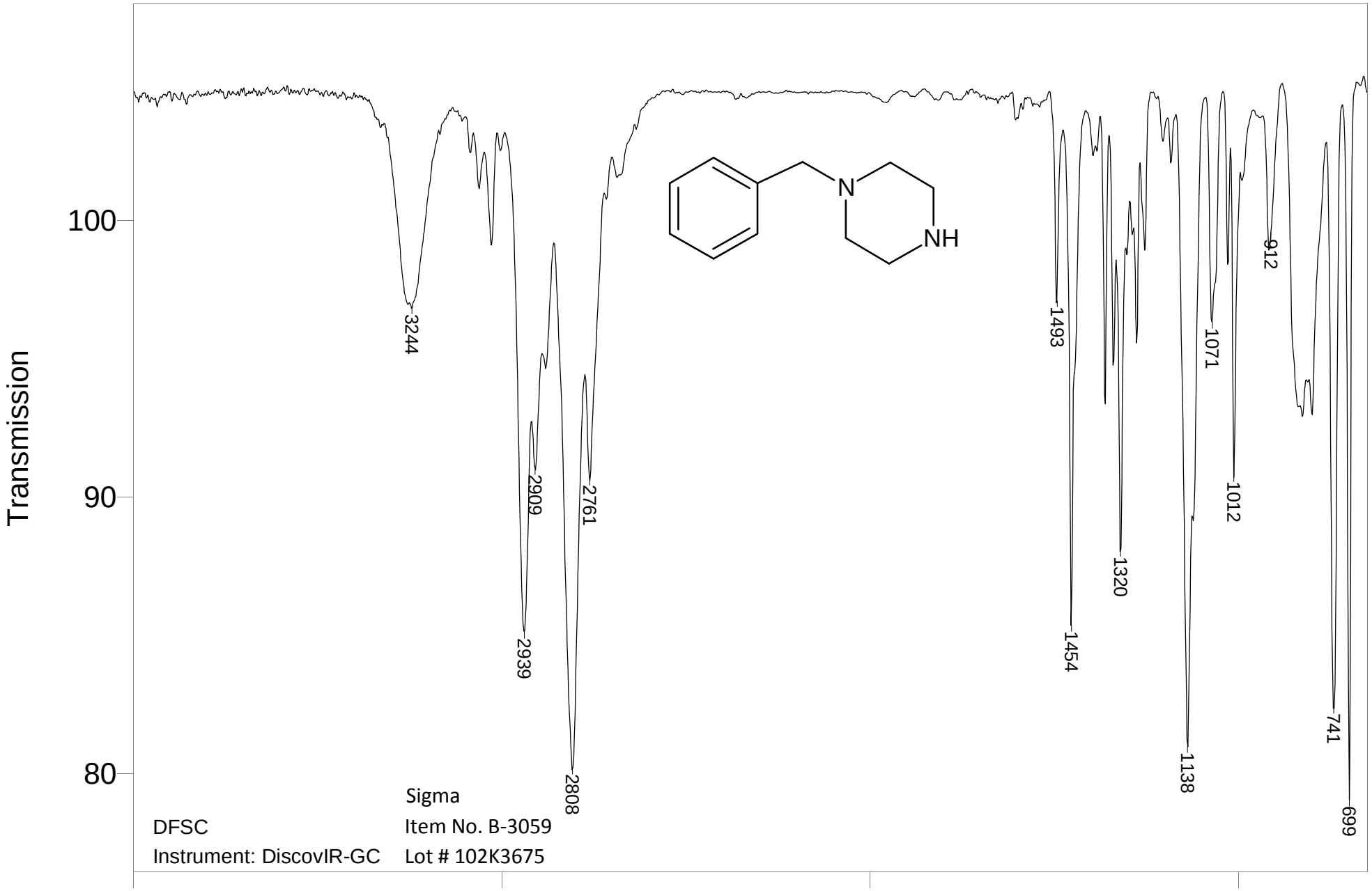
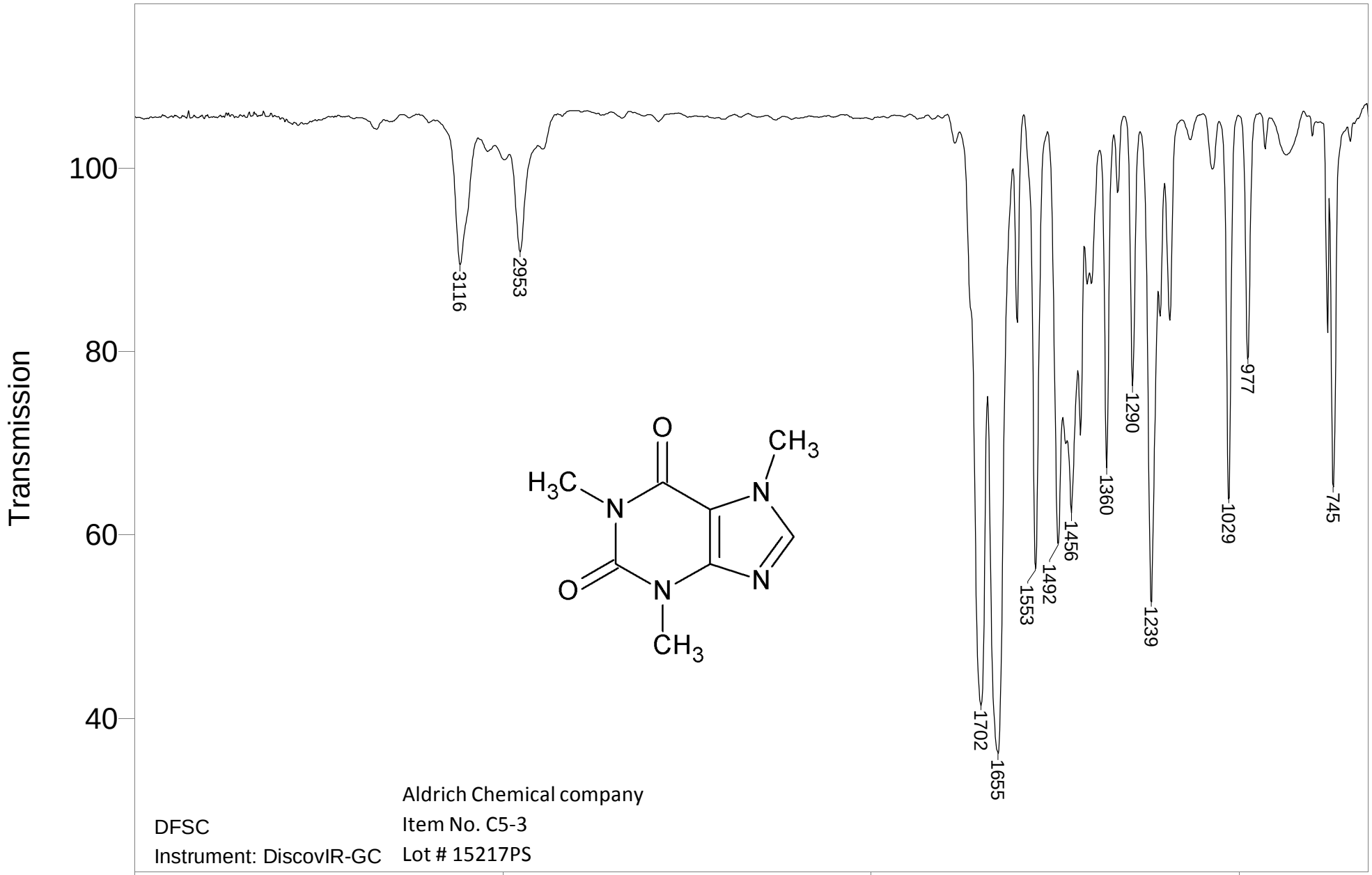




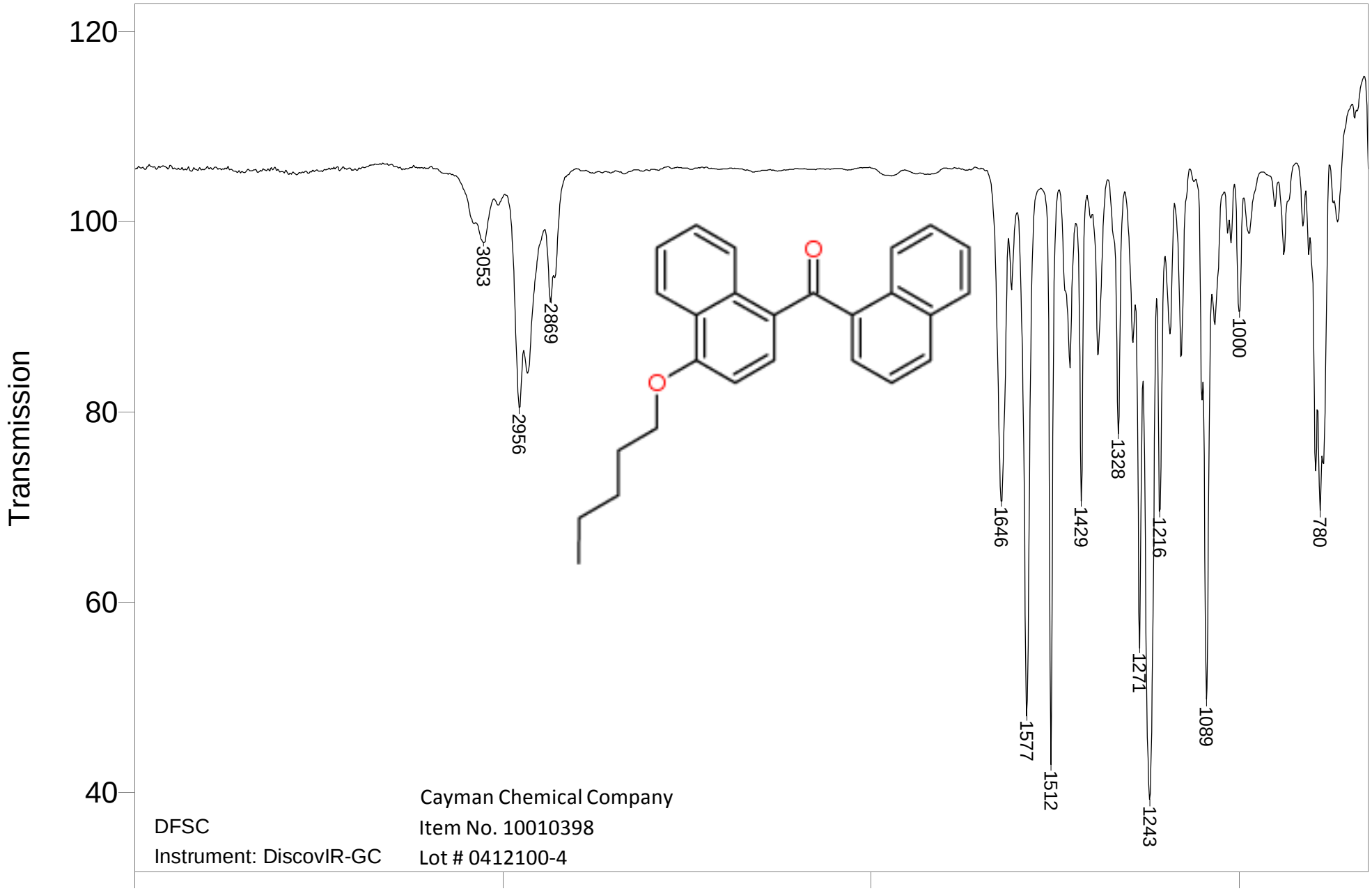


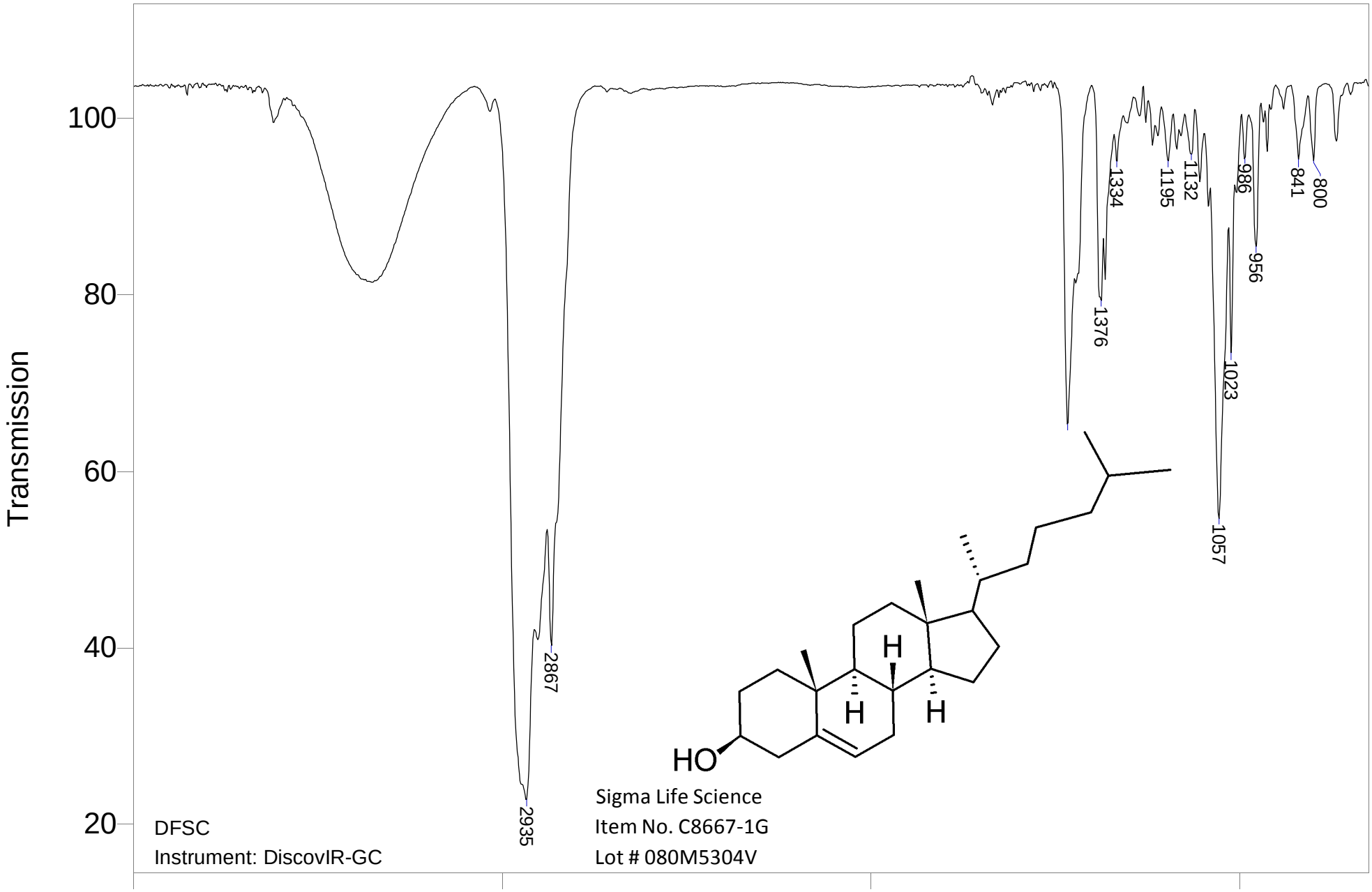


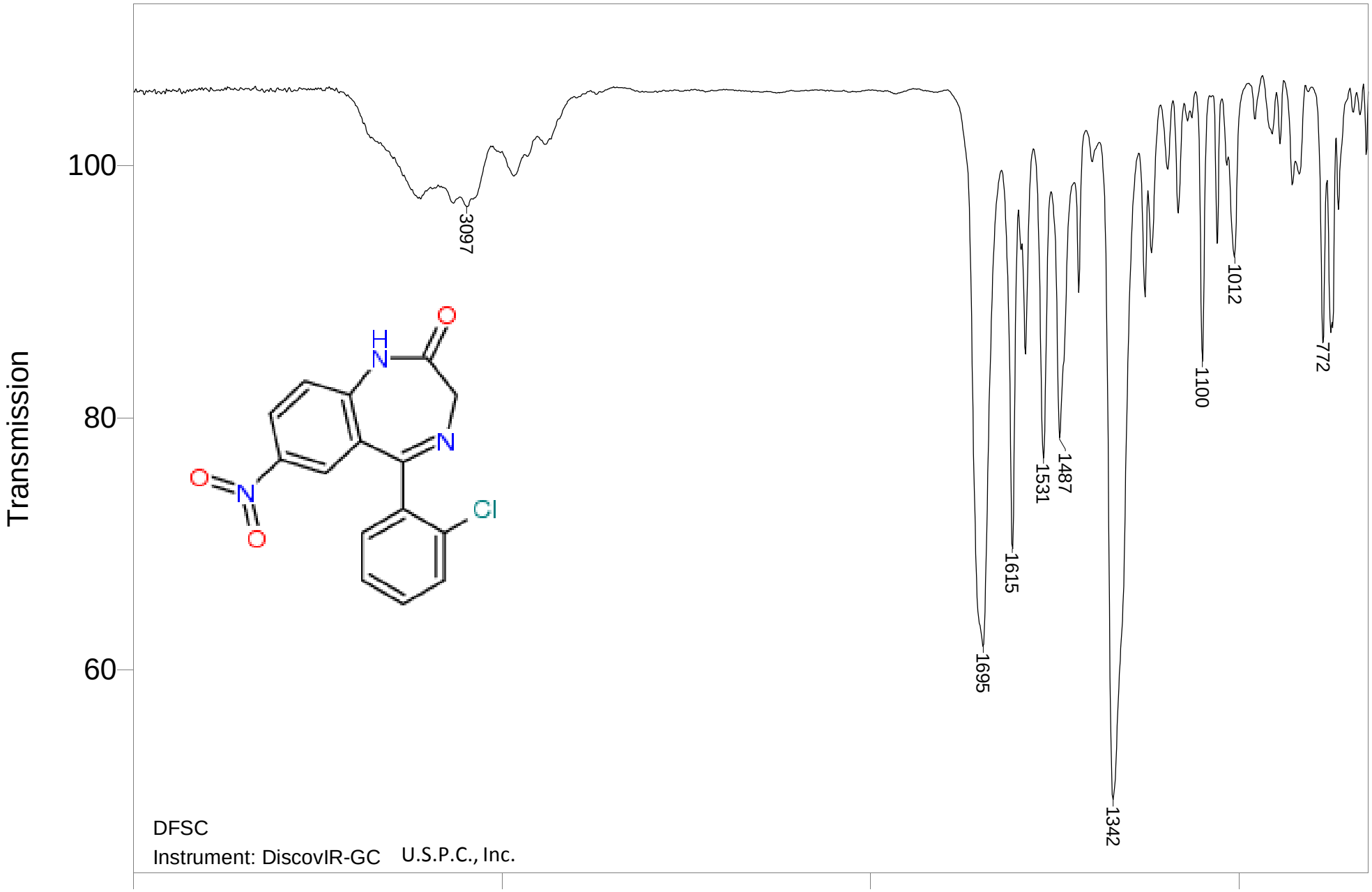


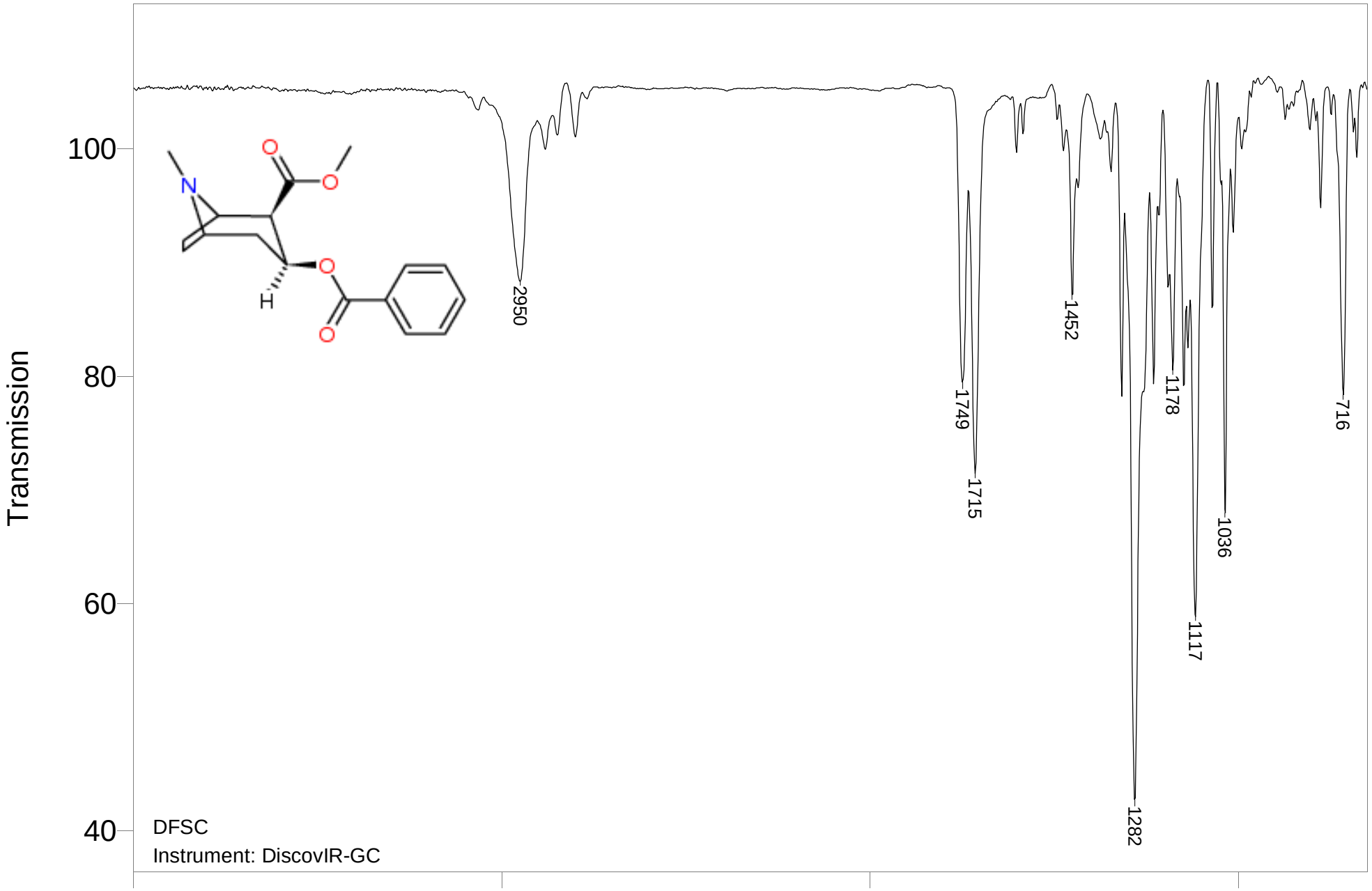


CB-13

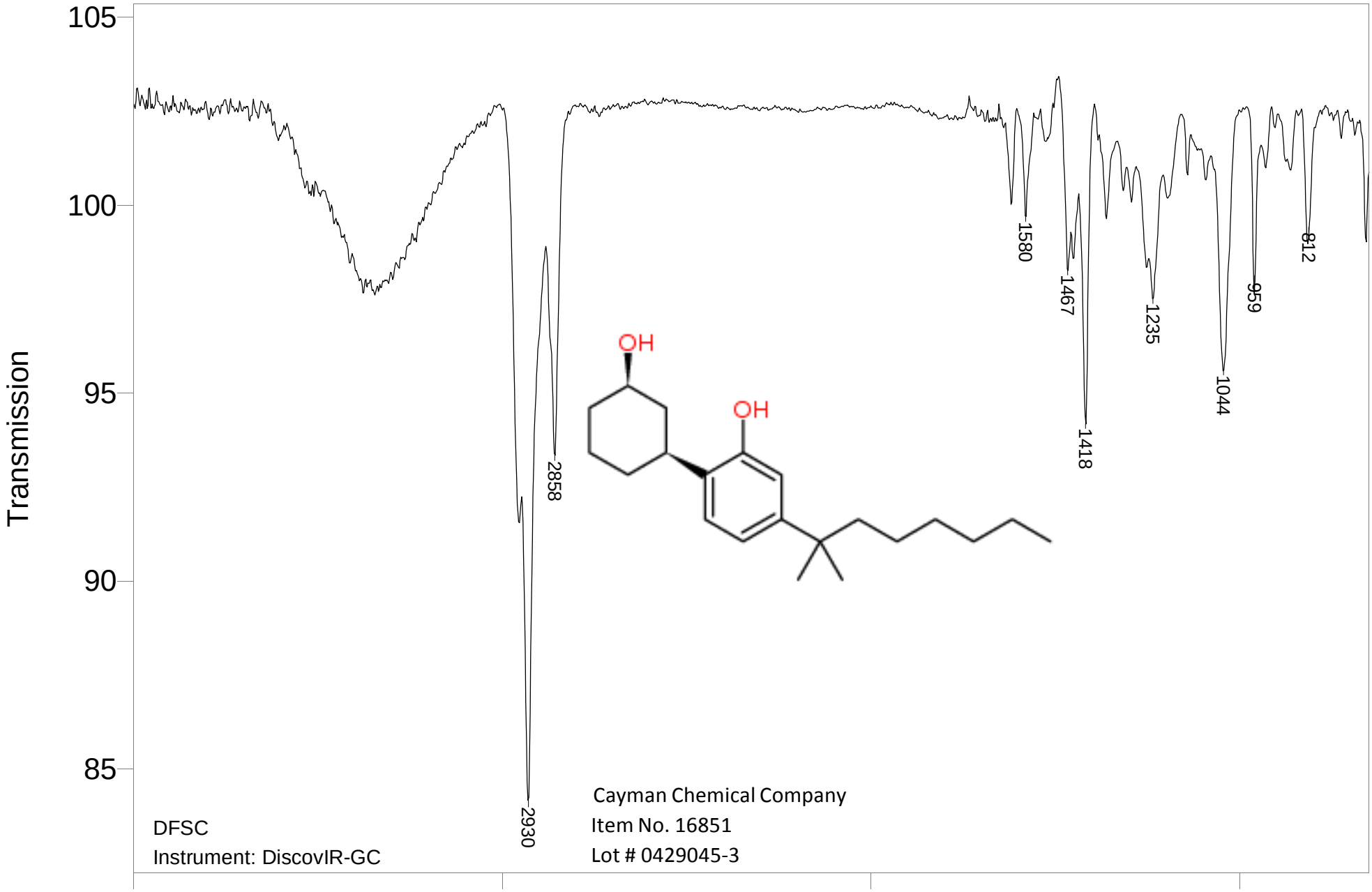


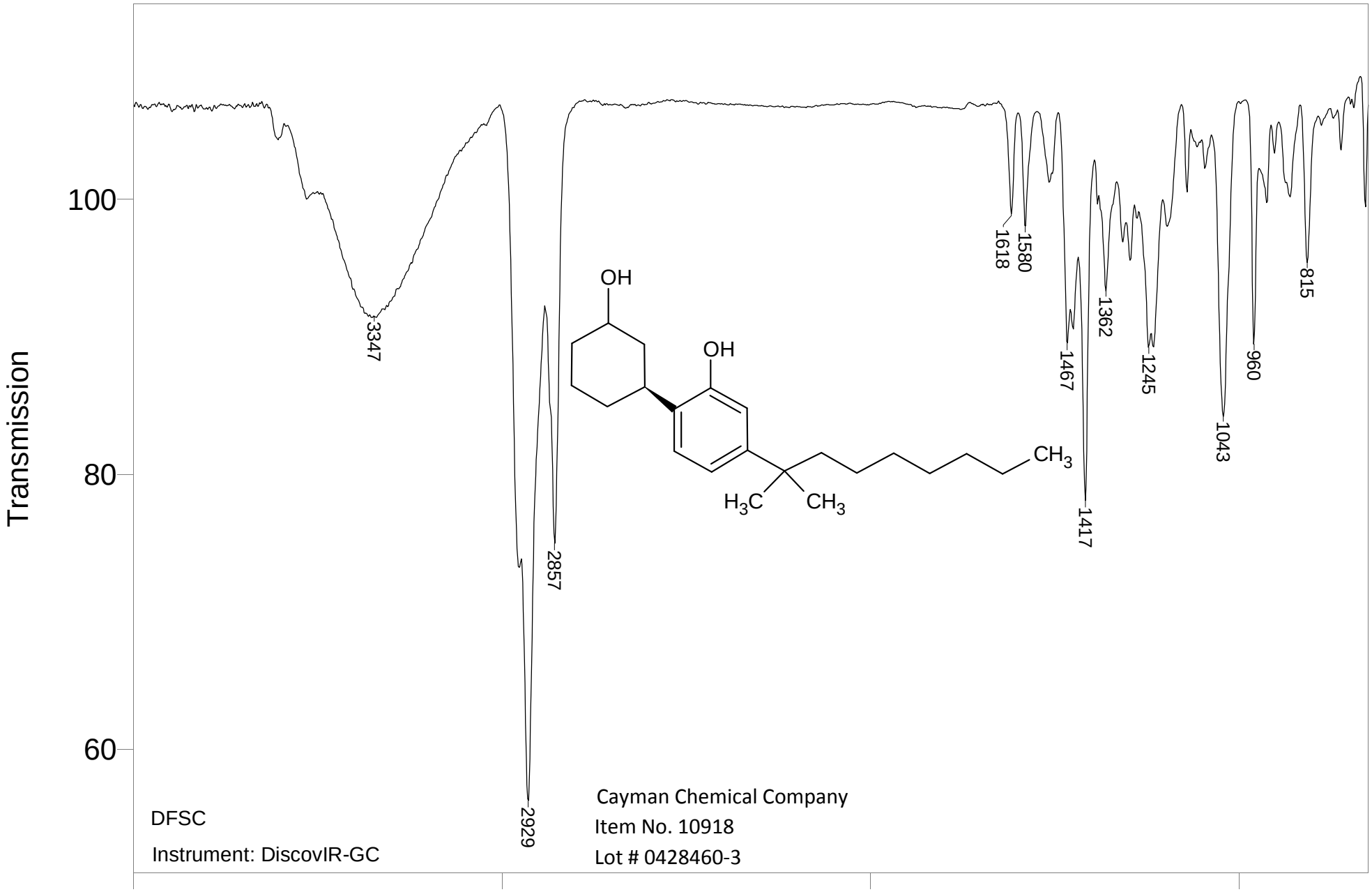


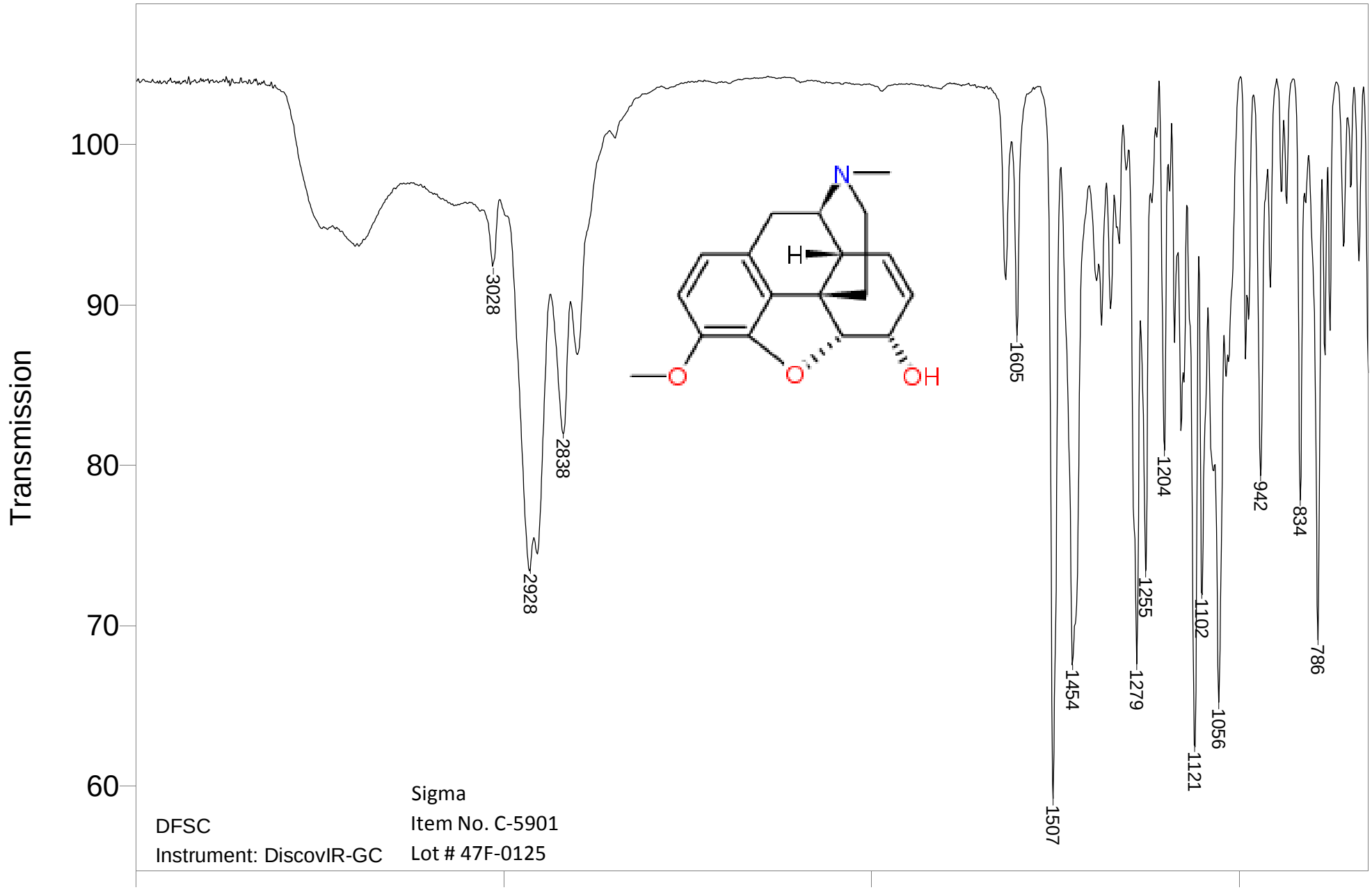


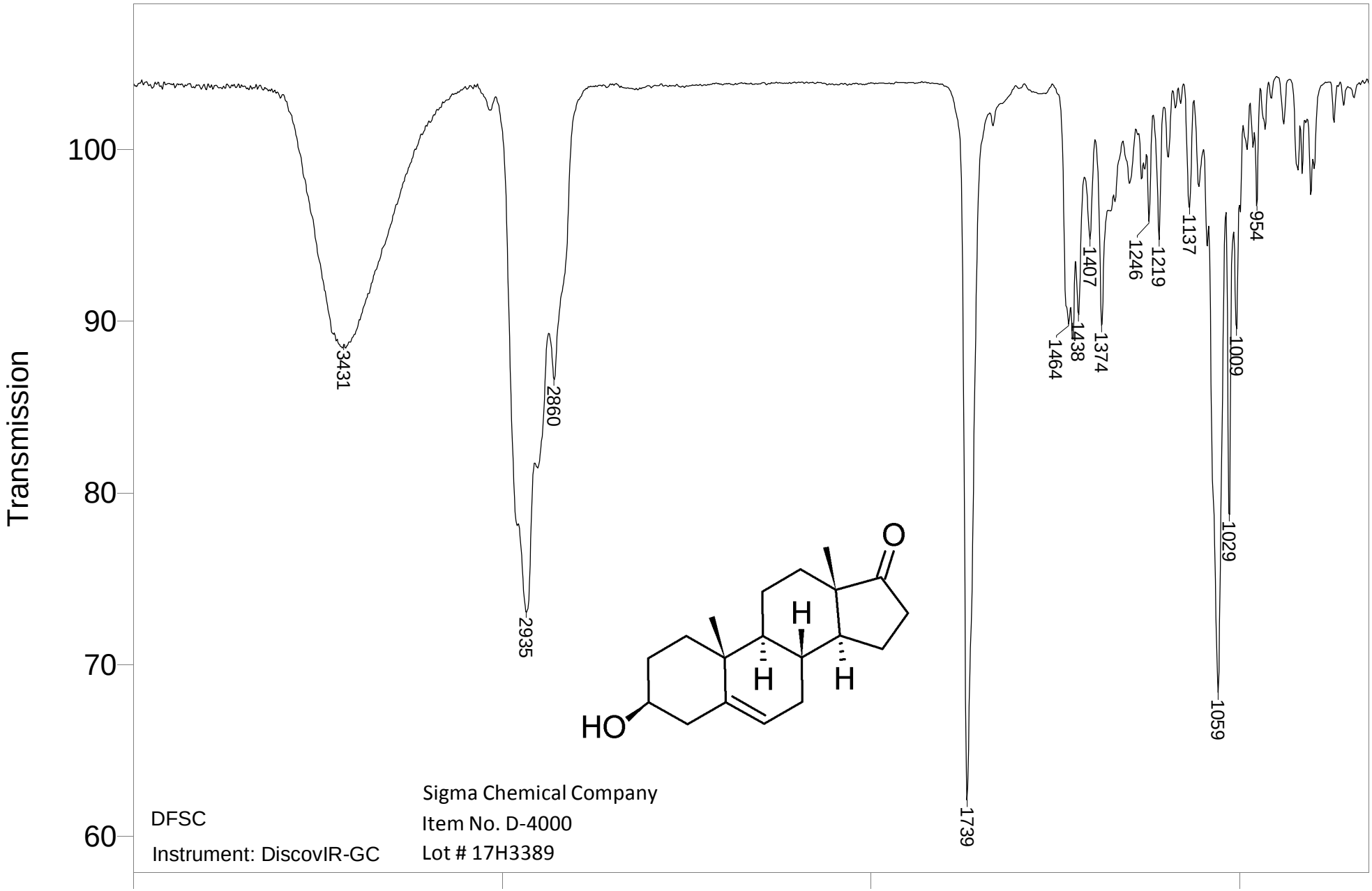


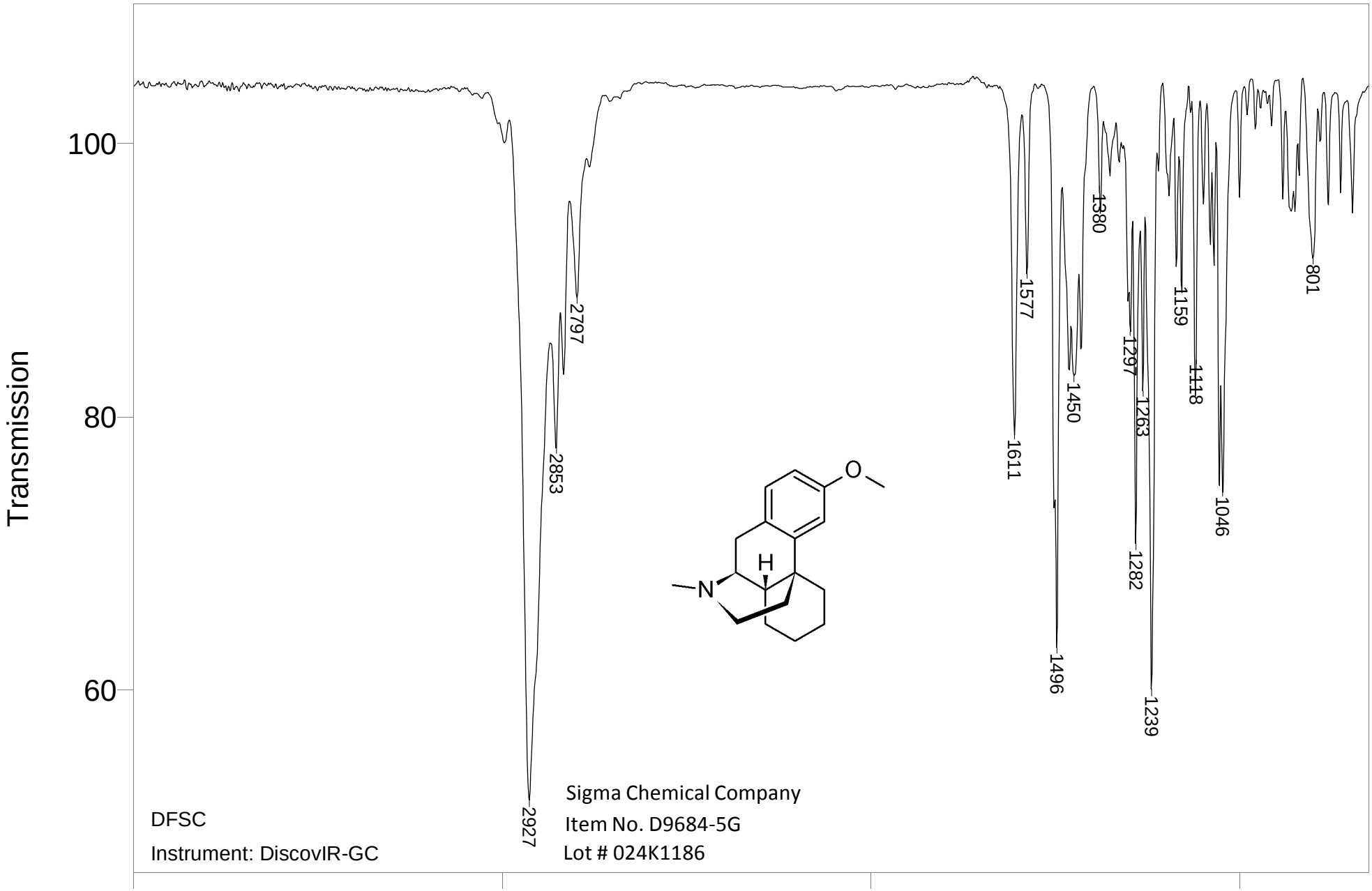
CP-47, 497

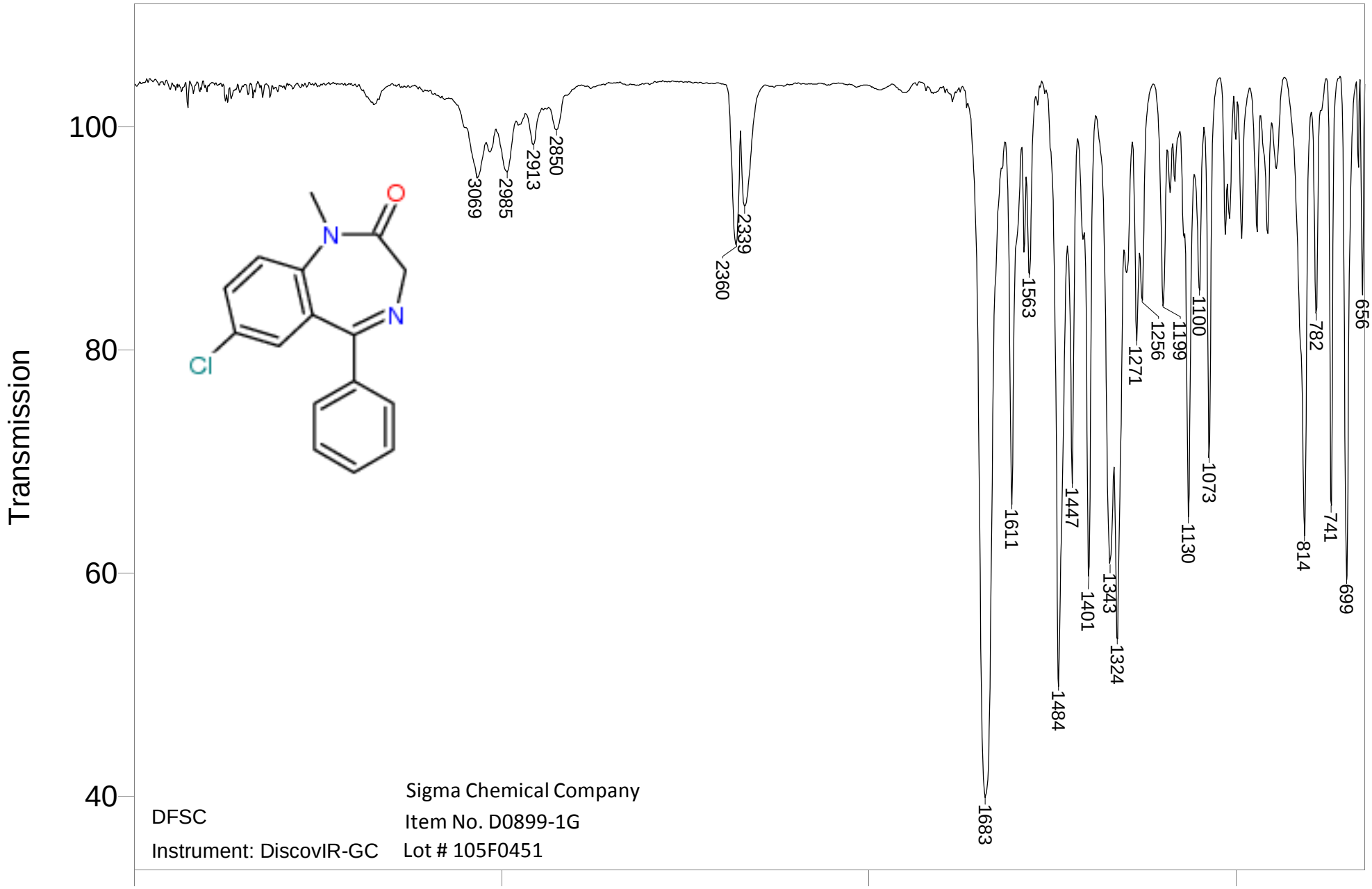


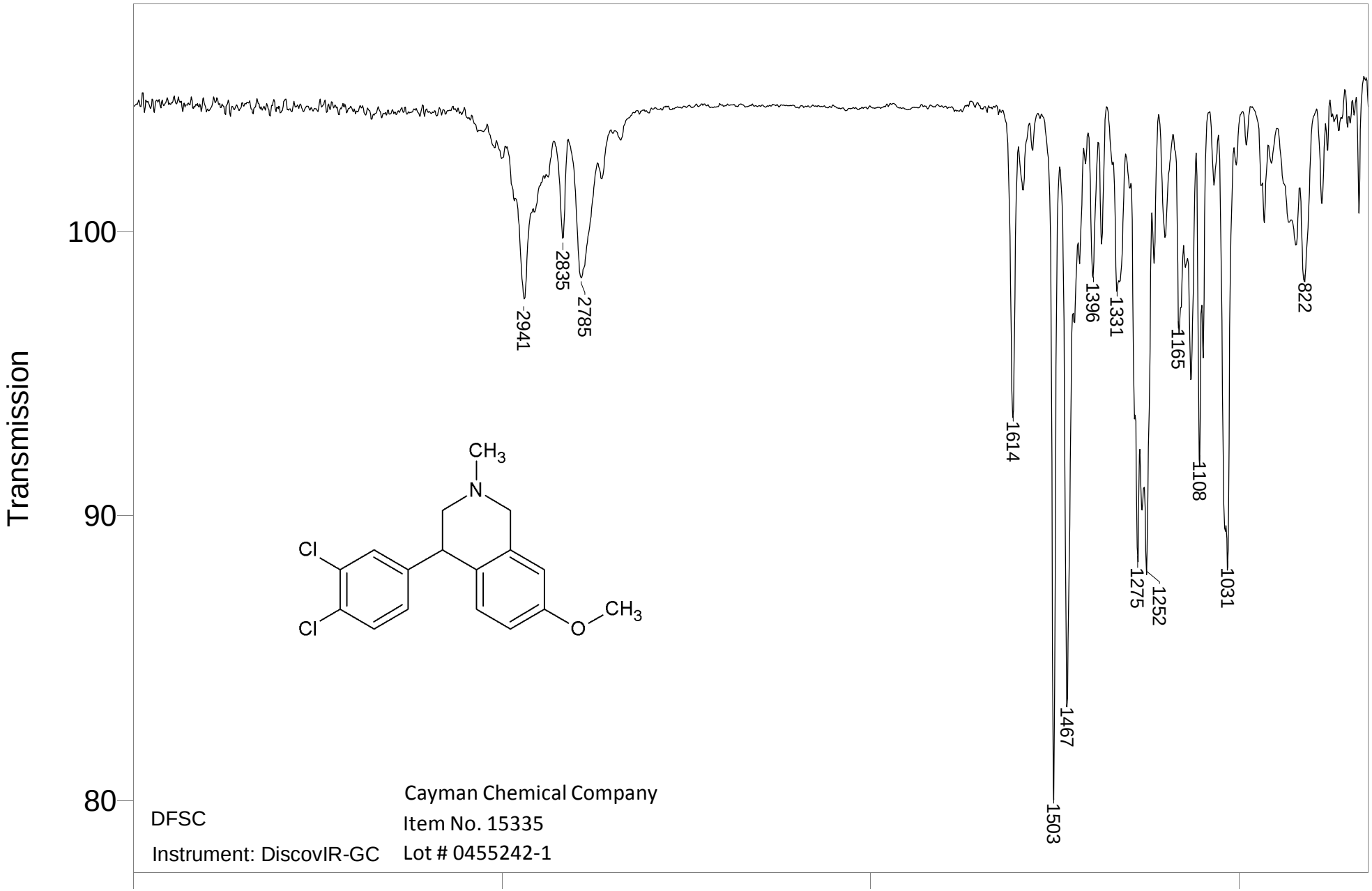


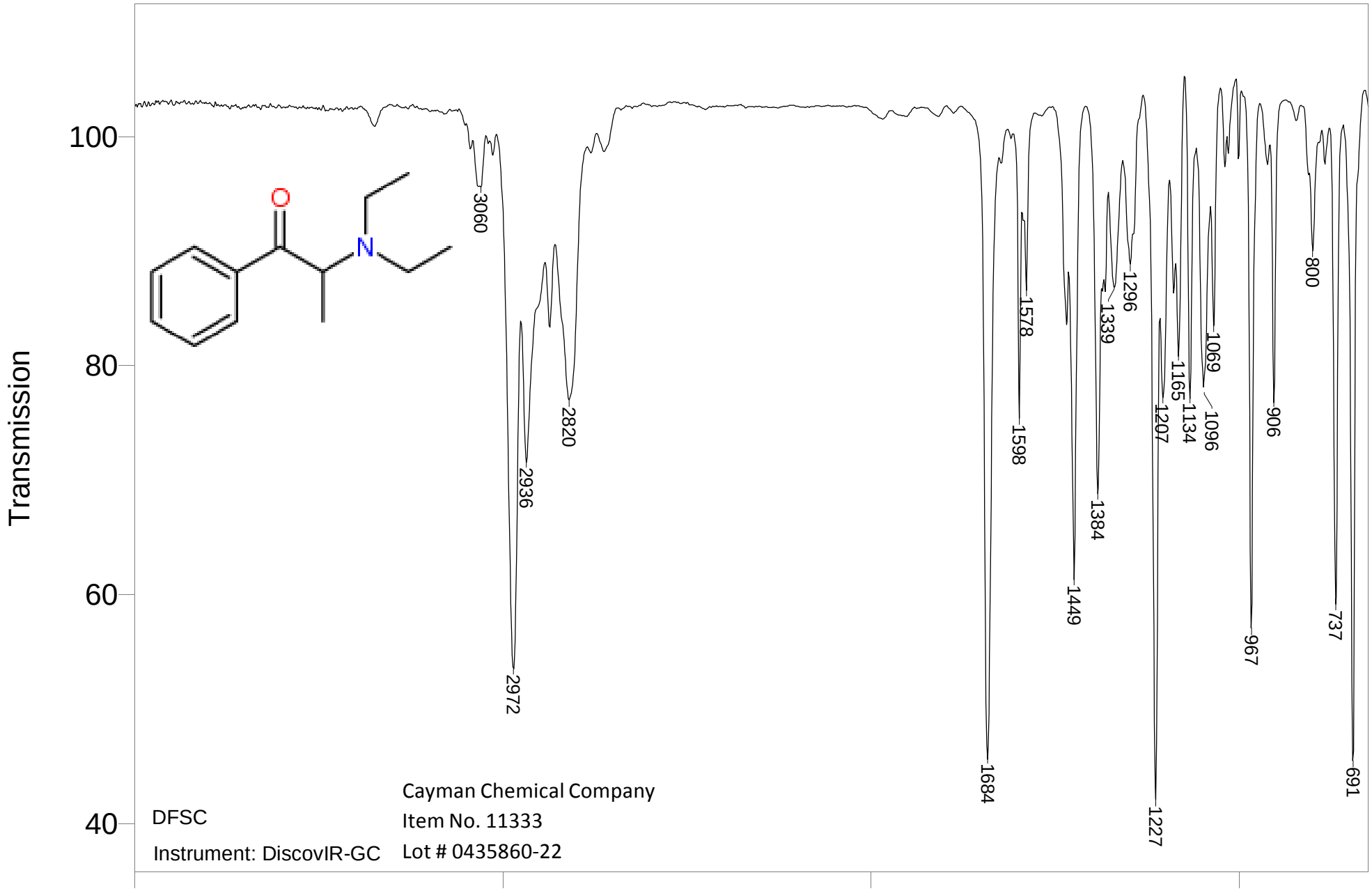












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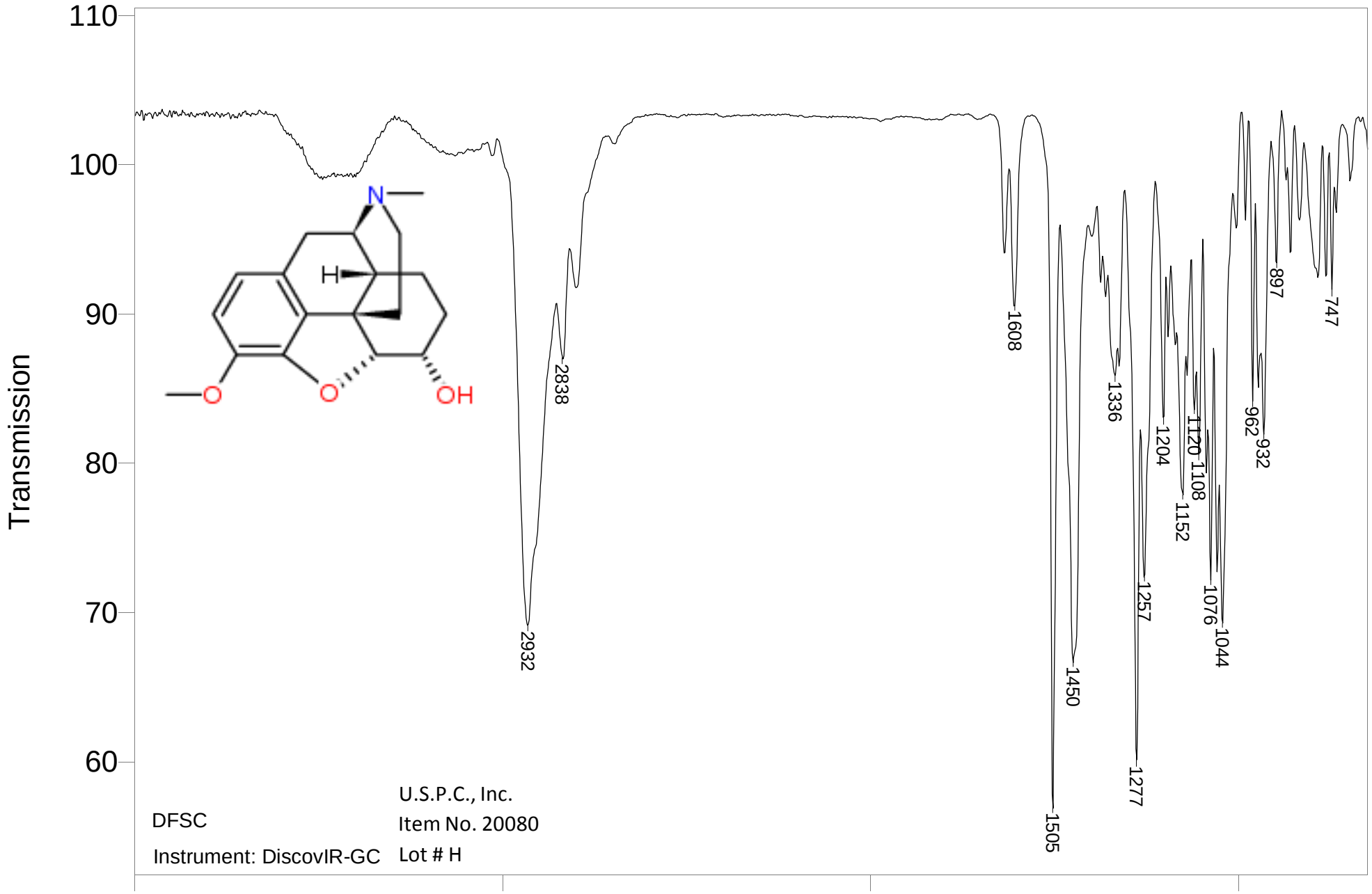

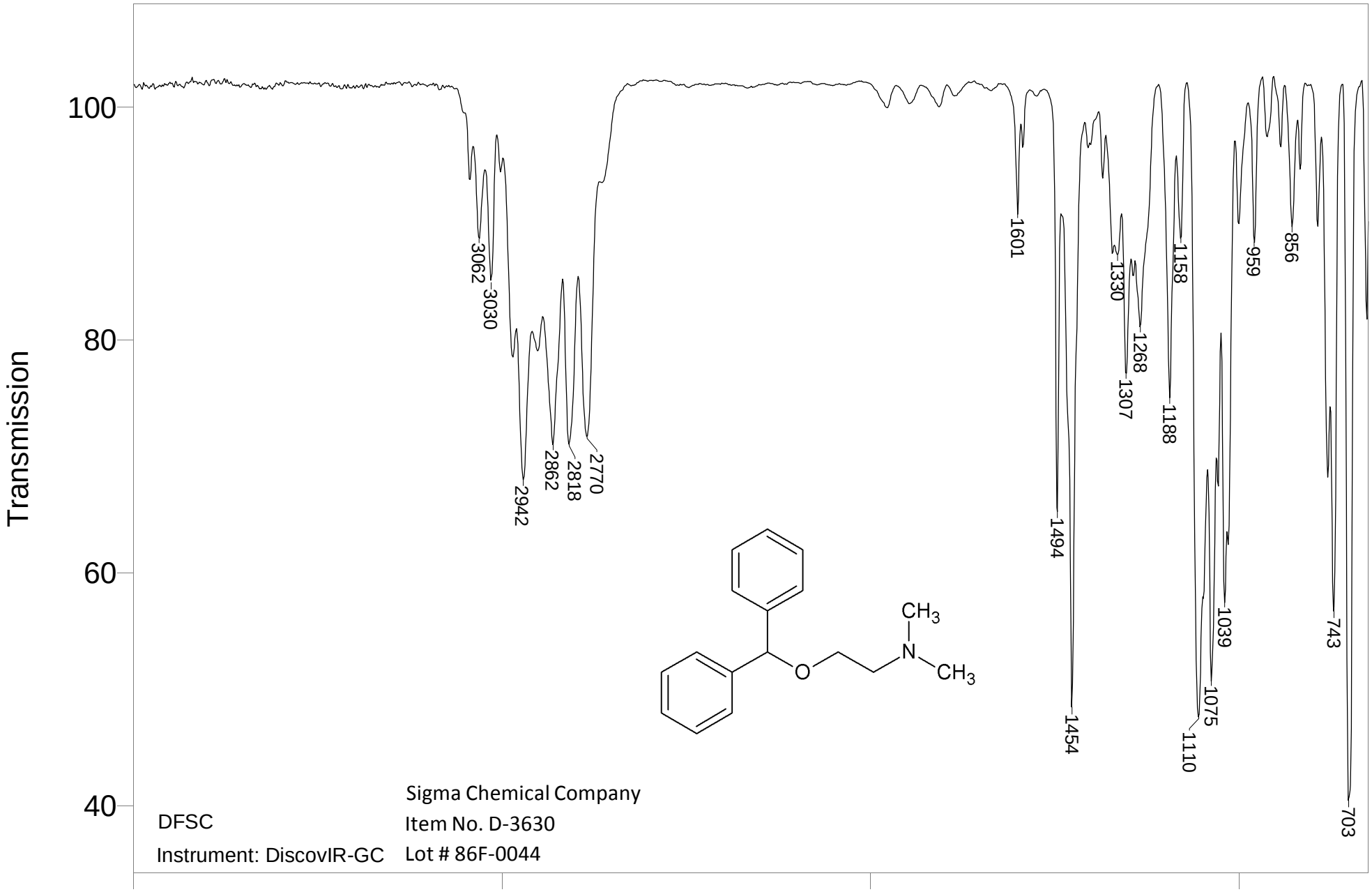

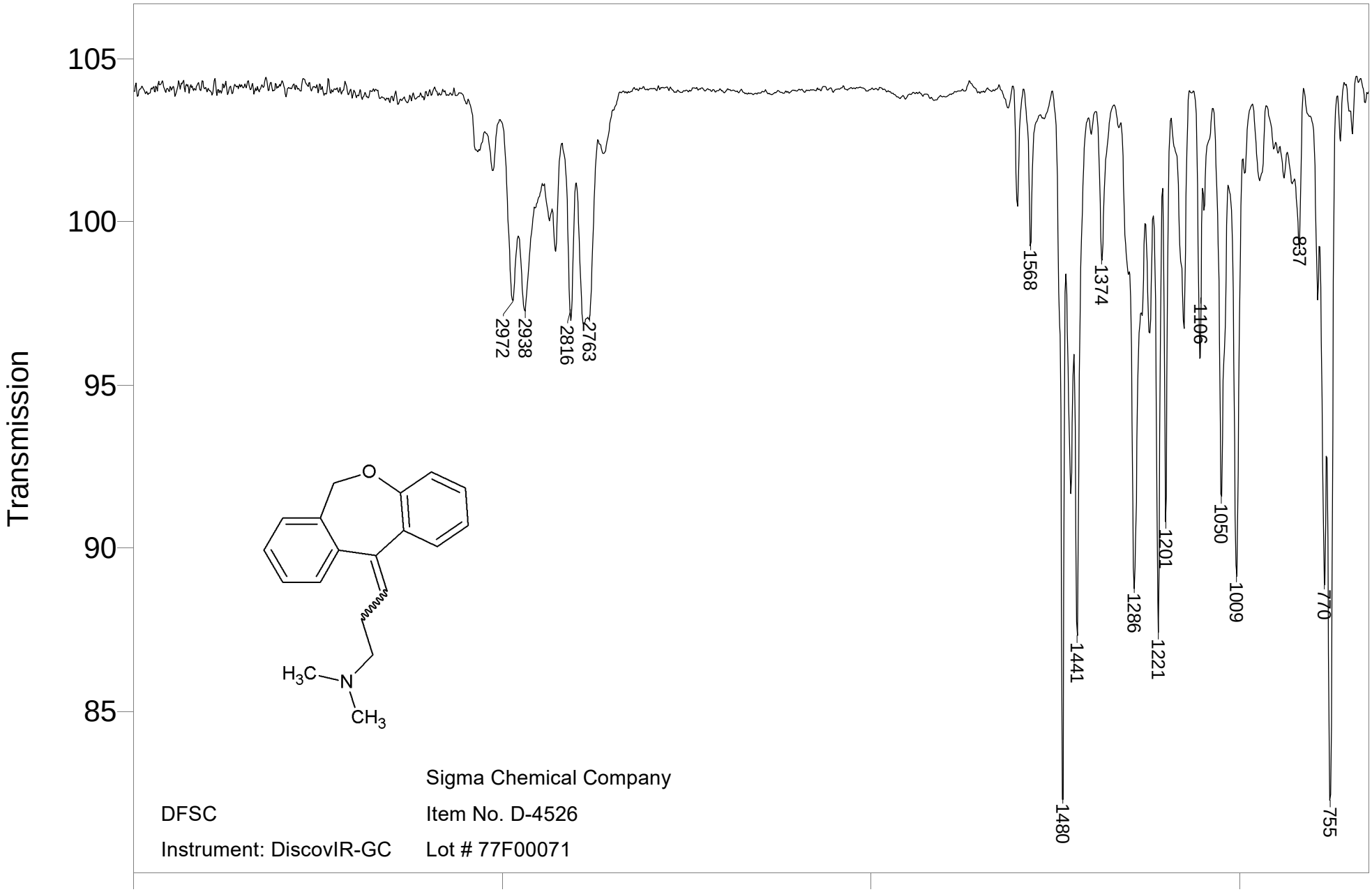

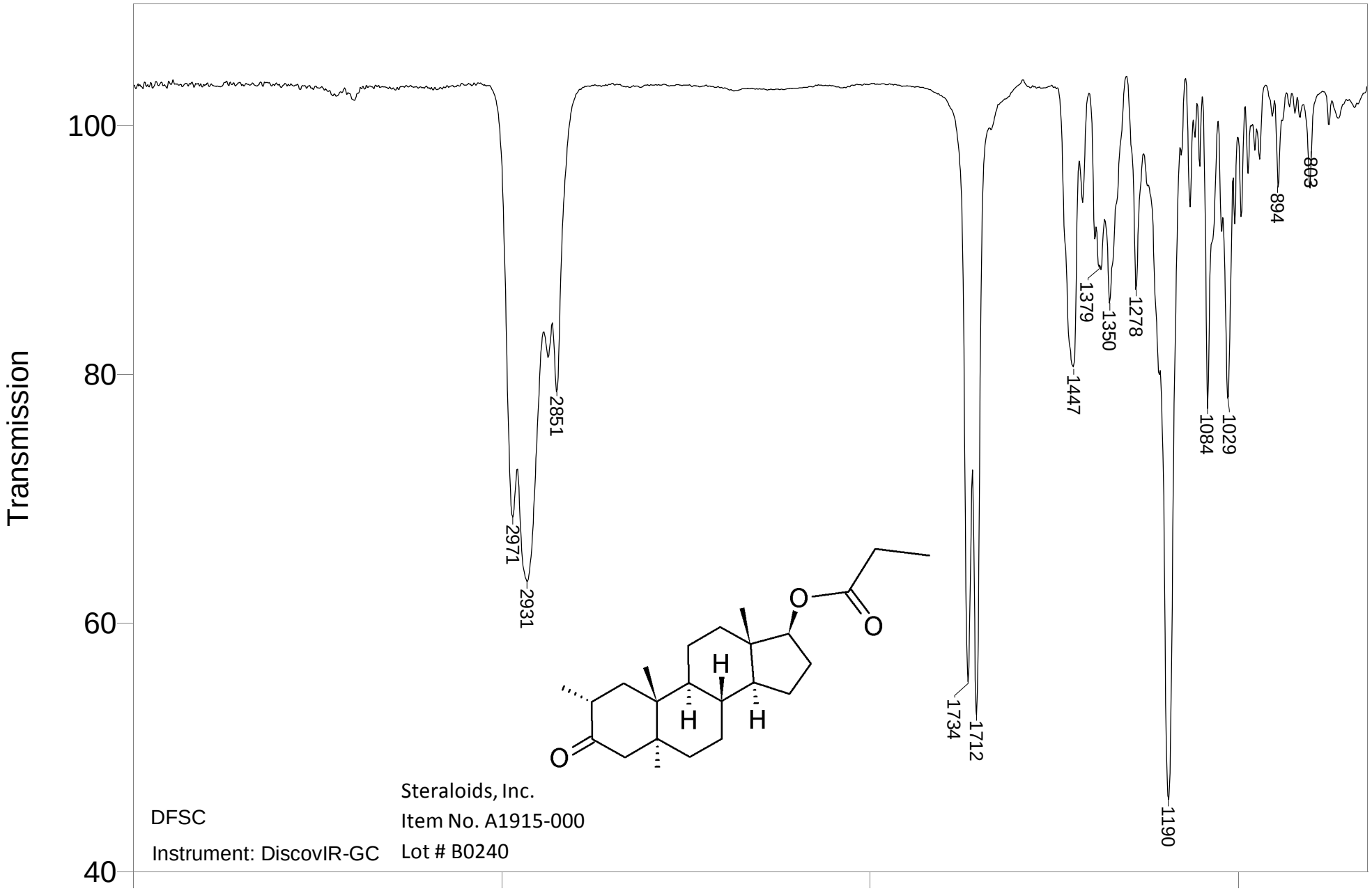

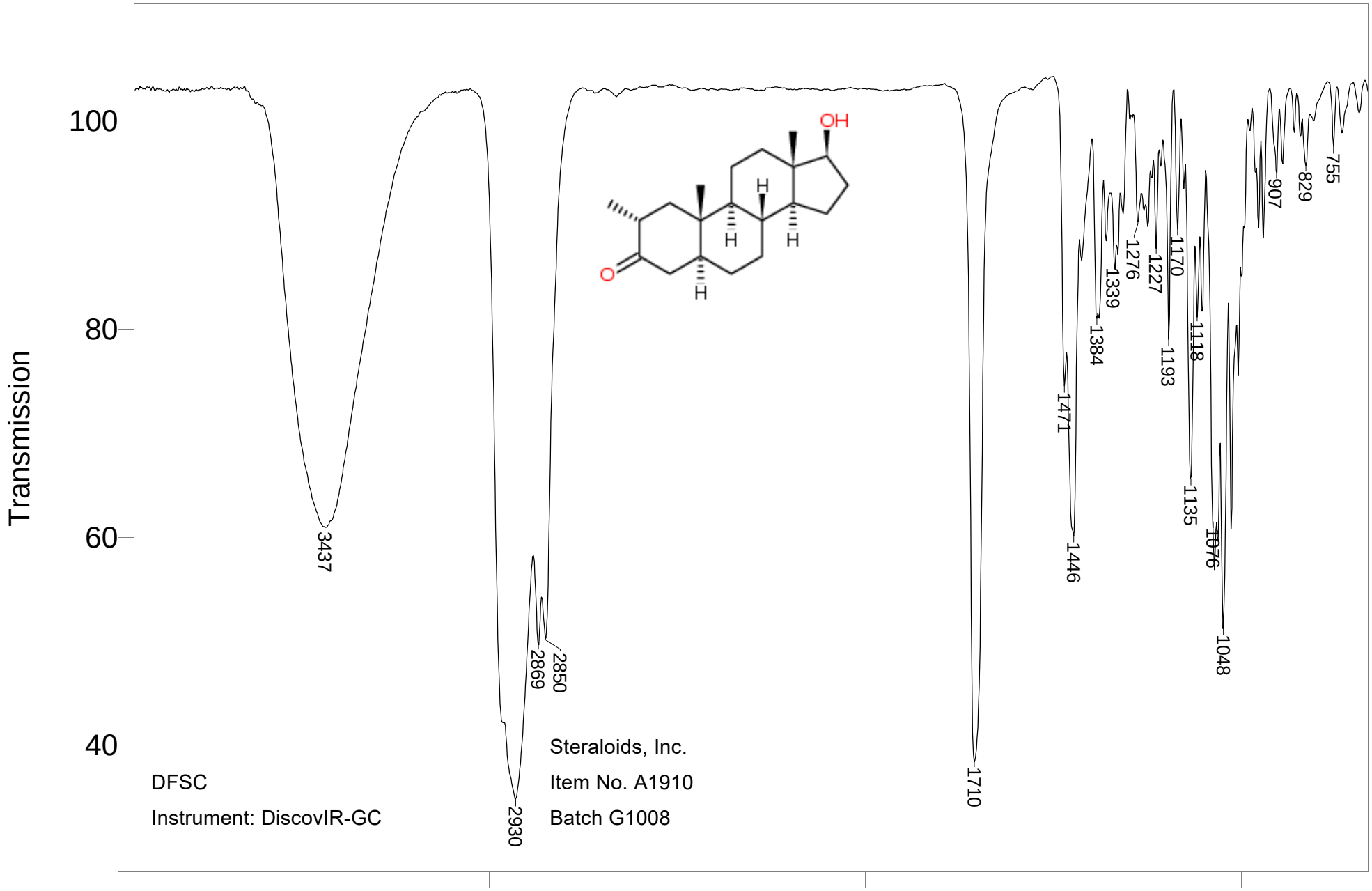

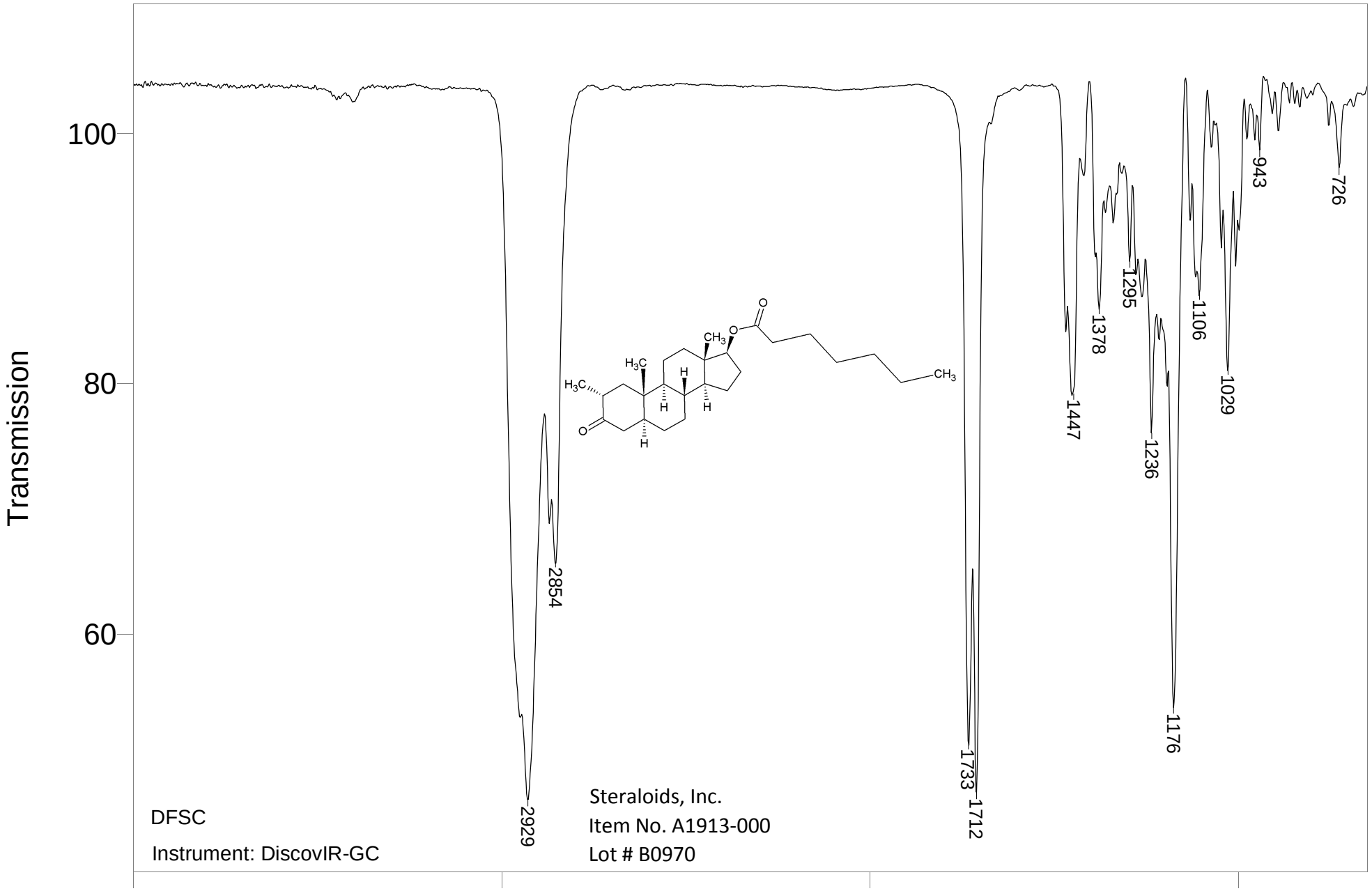

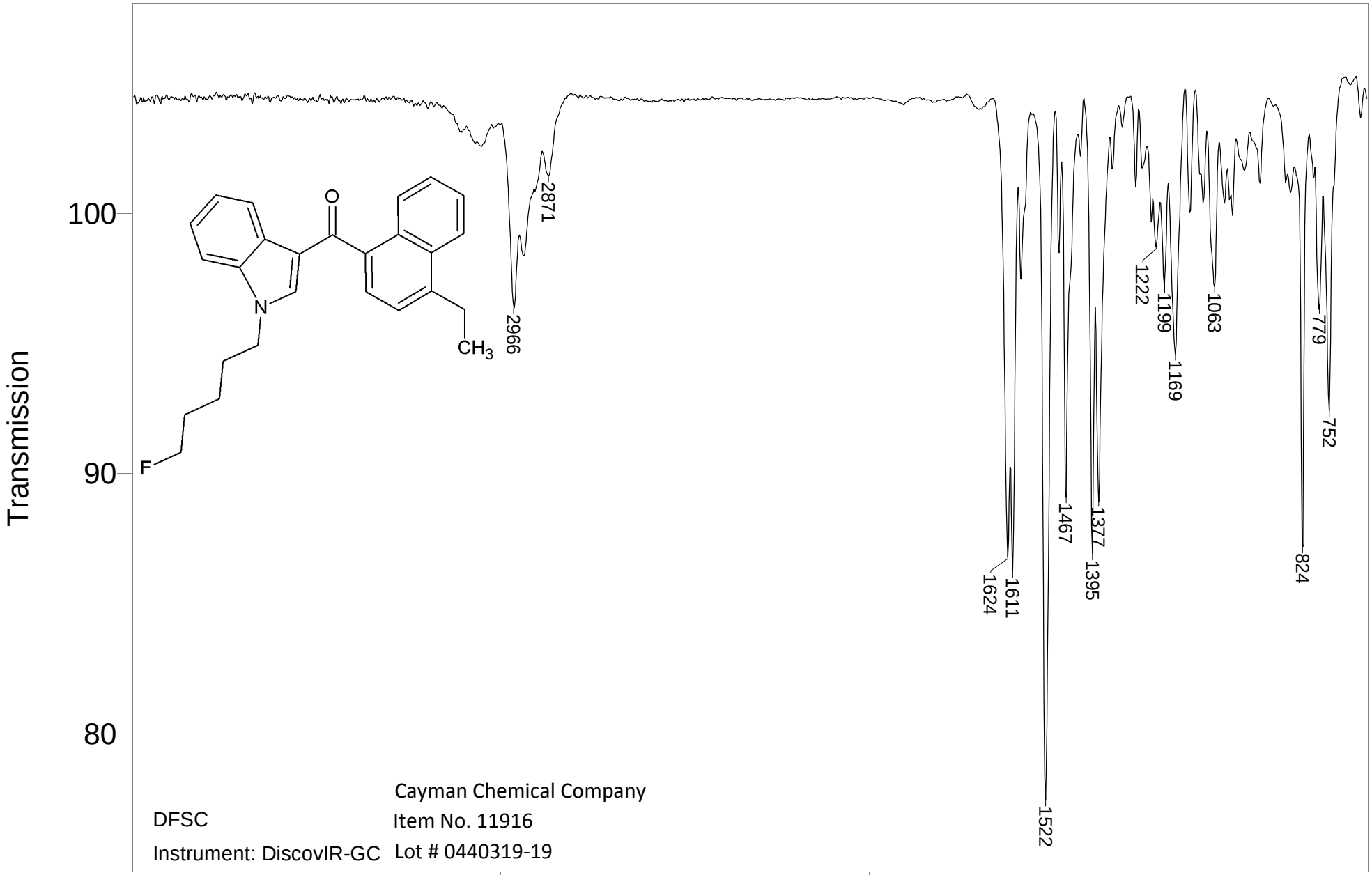

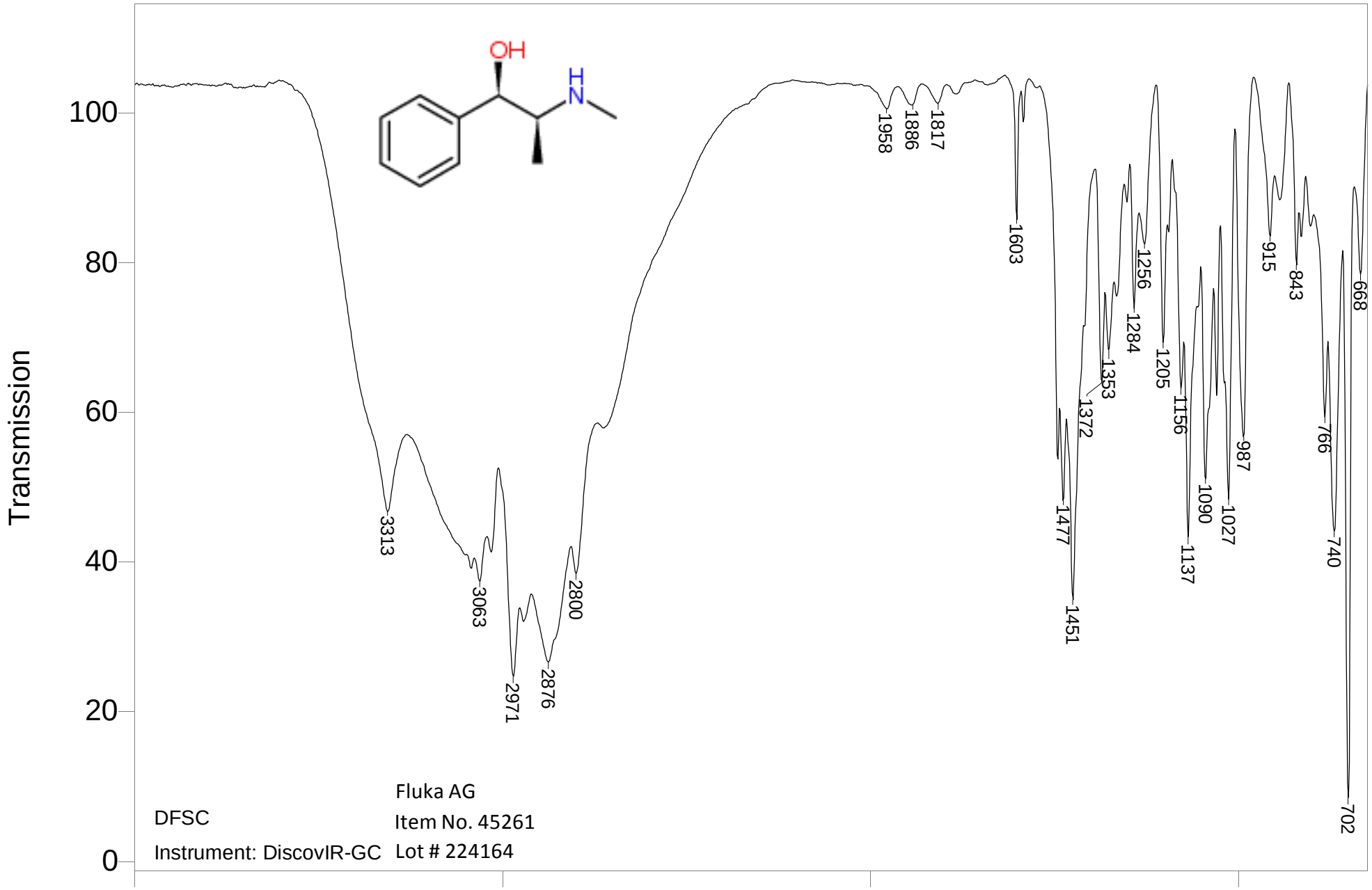

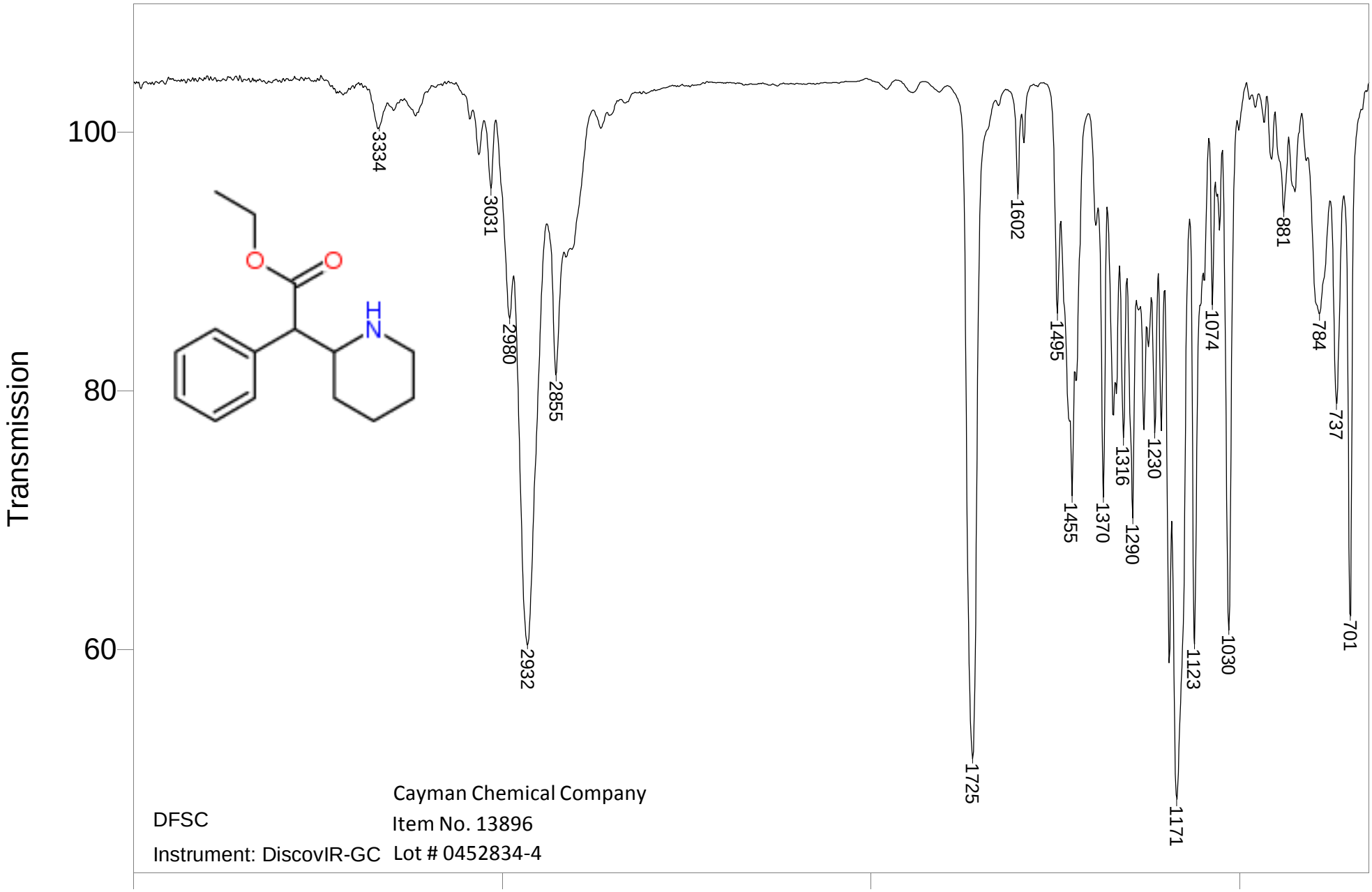

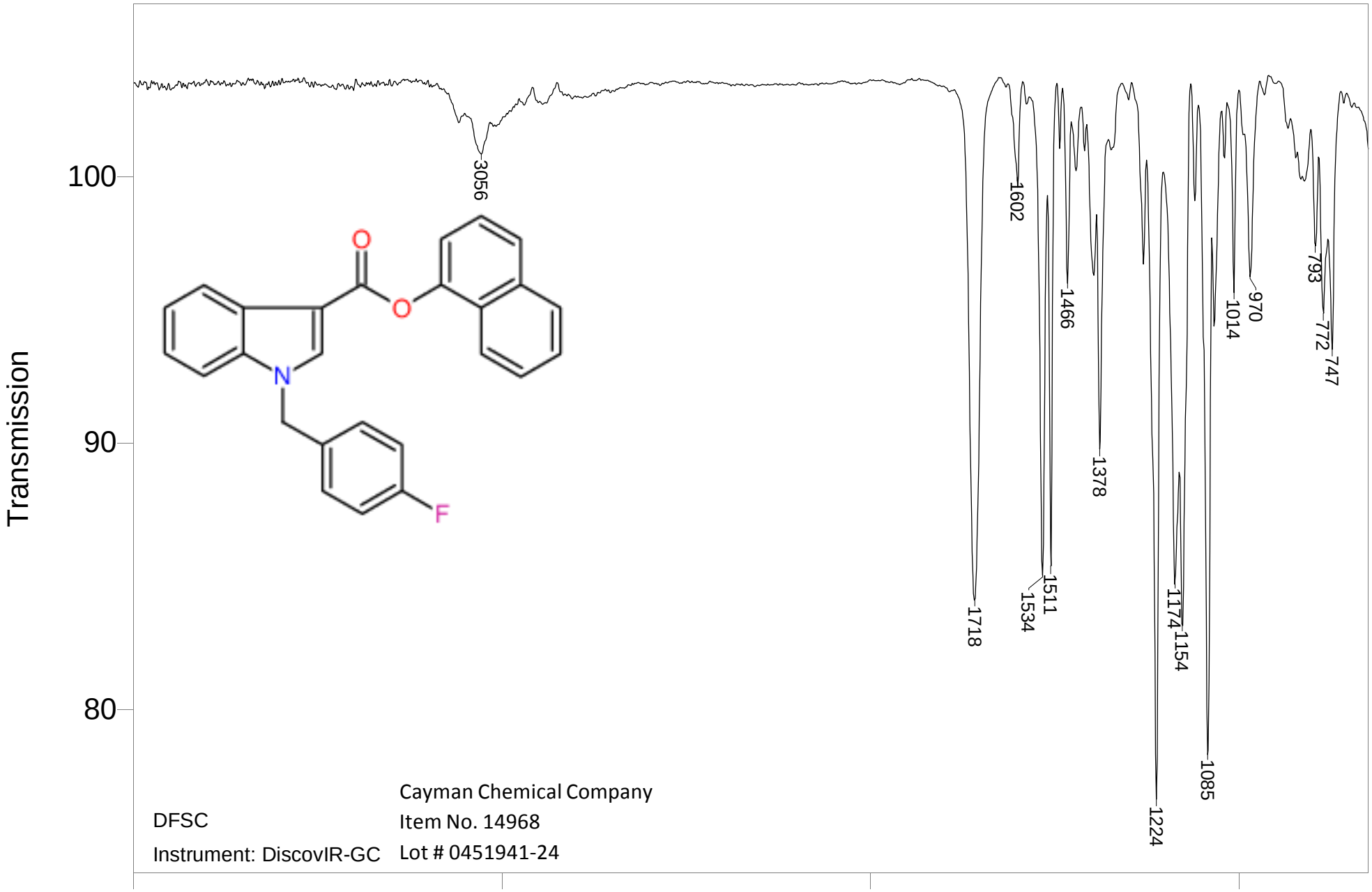

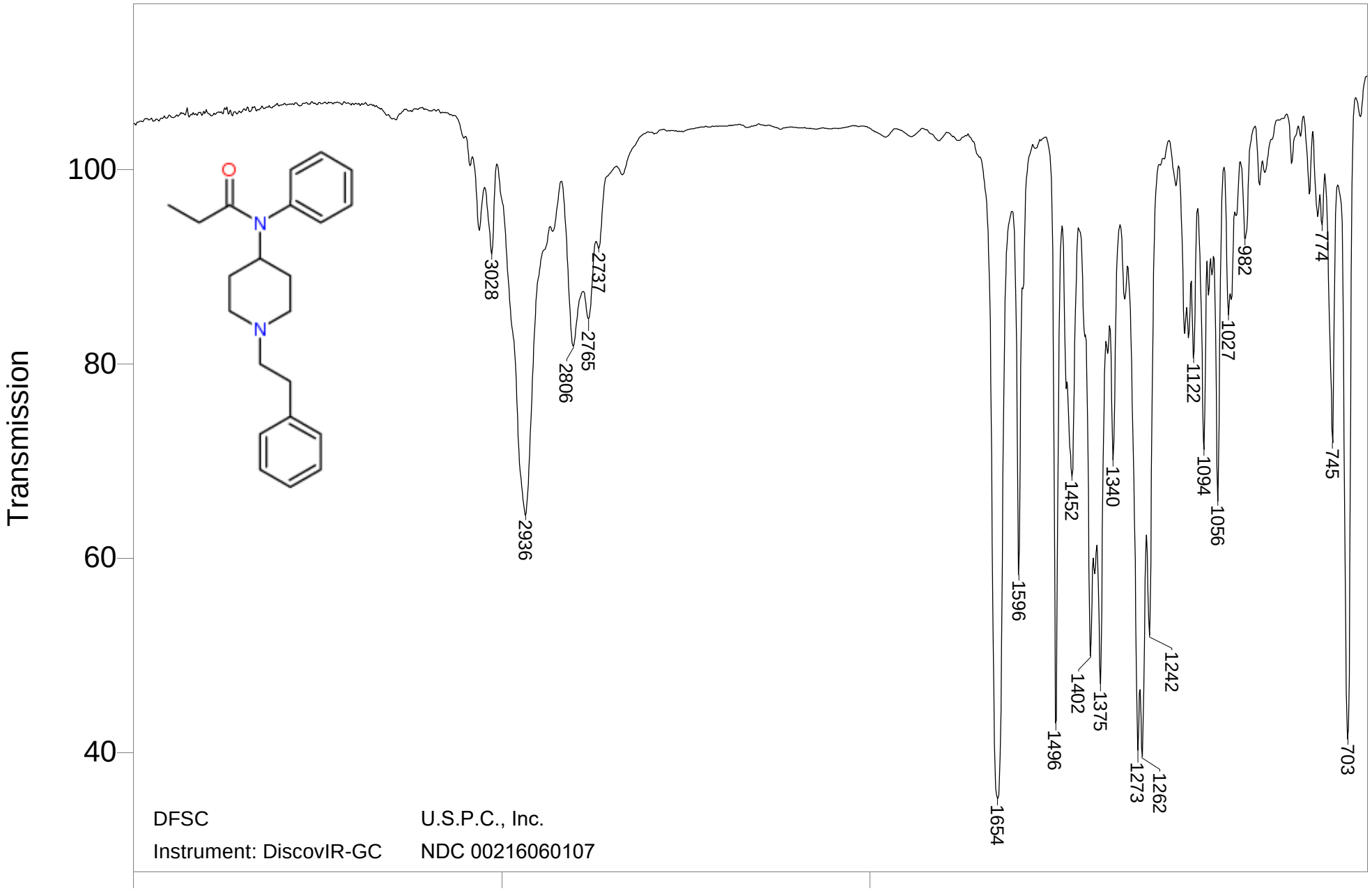

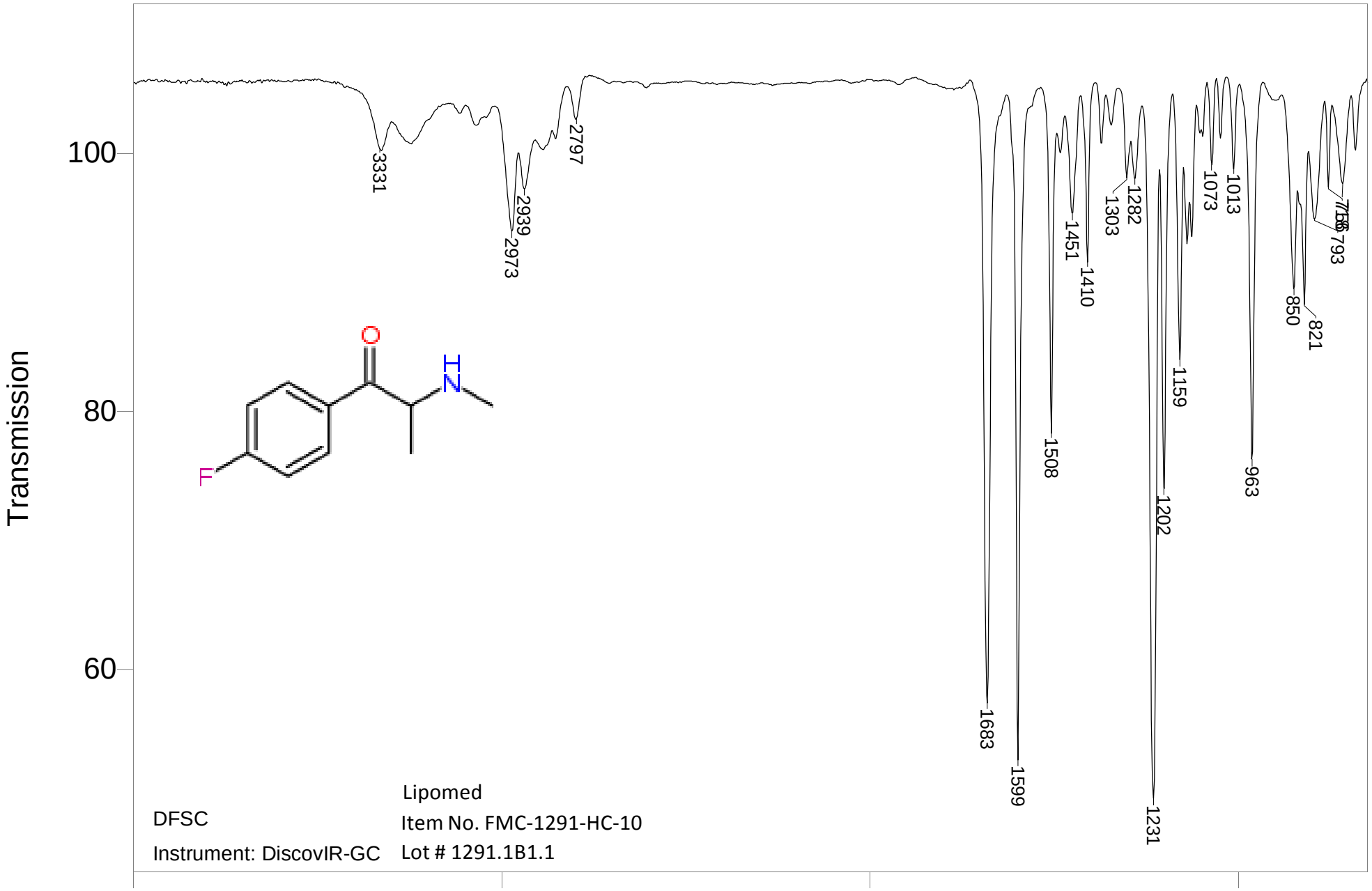

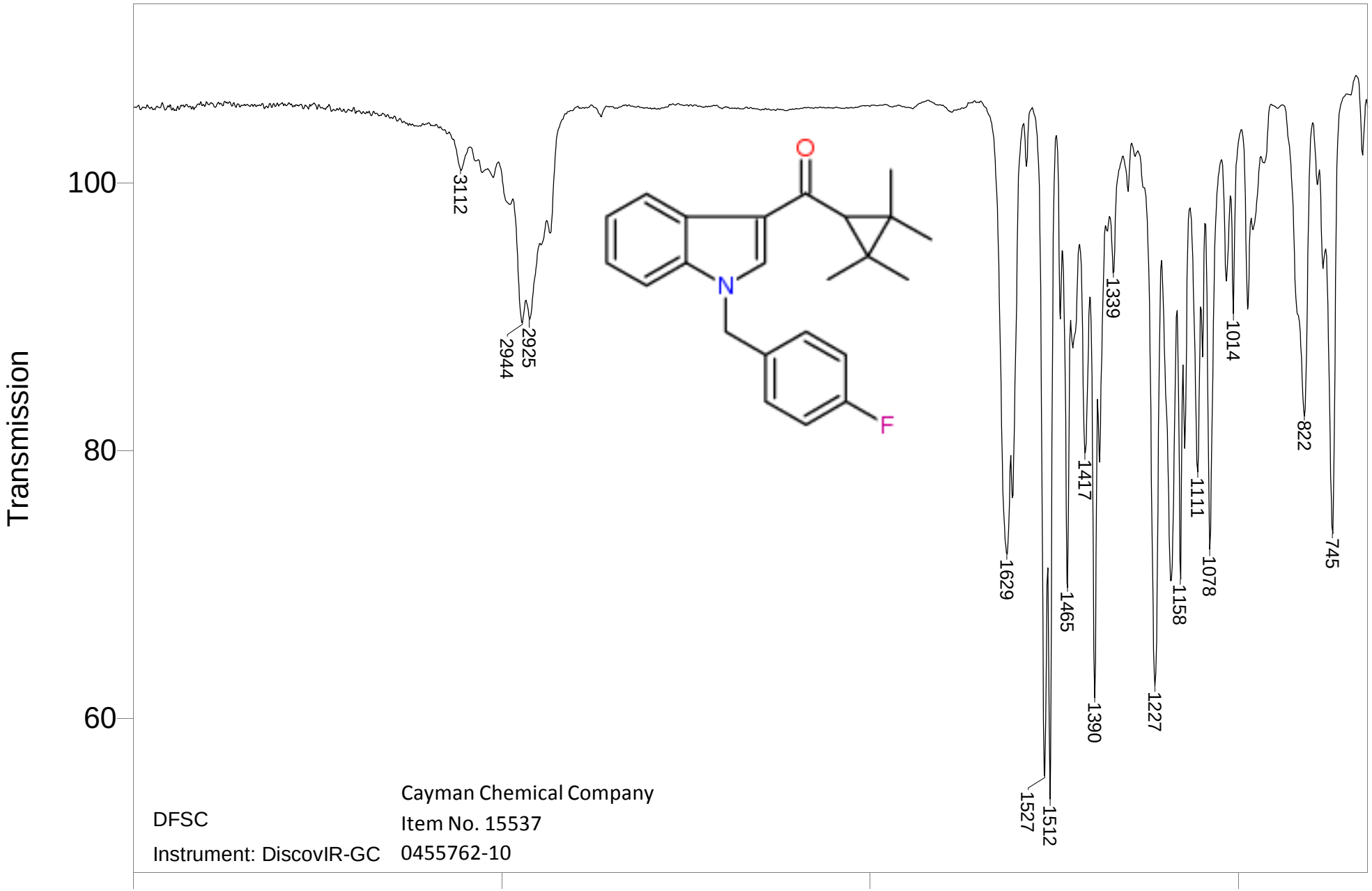

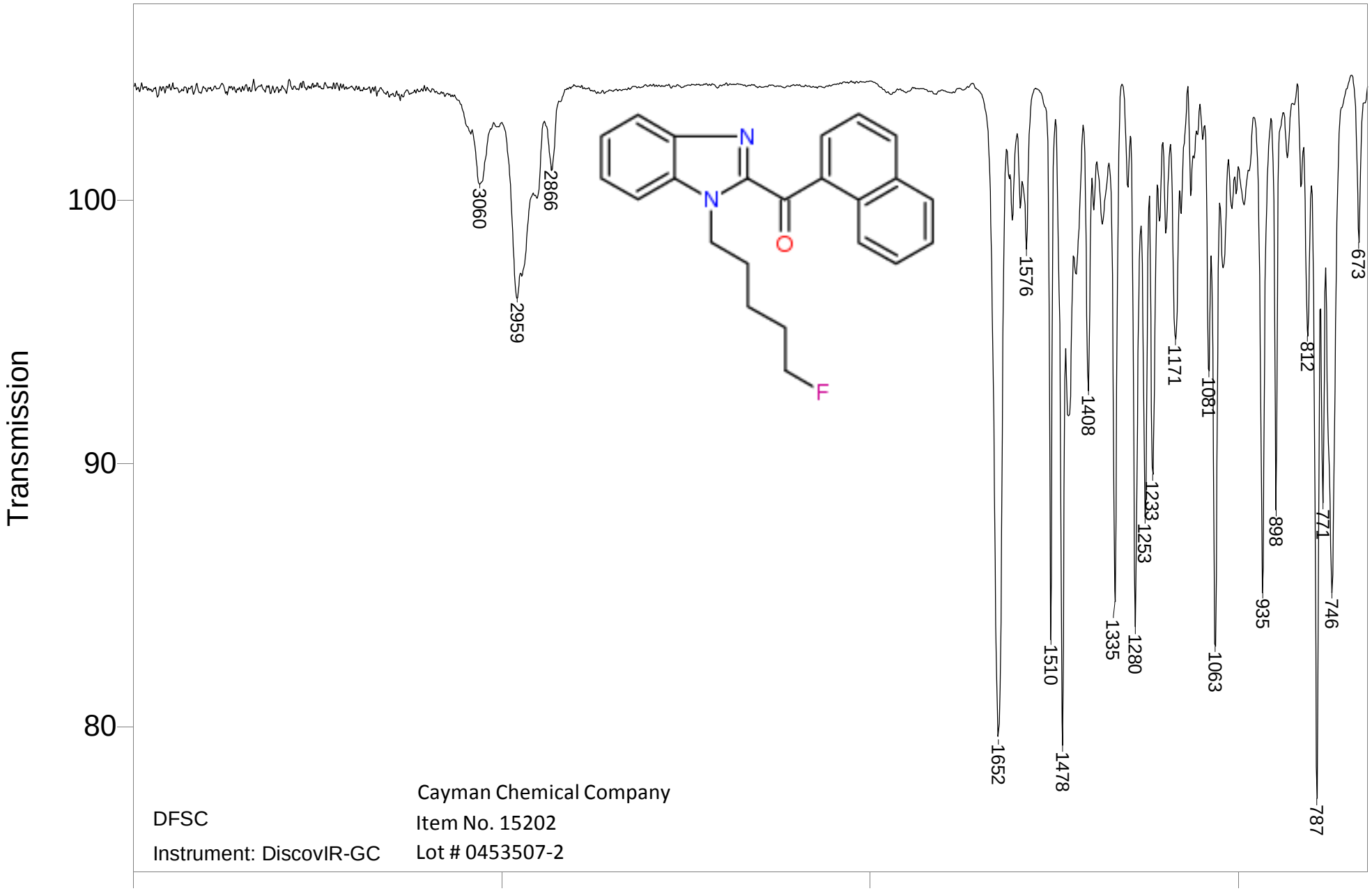

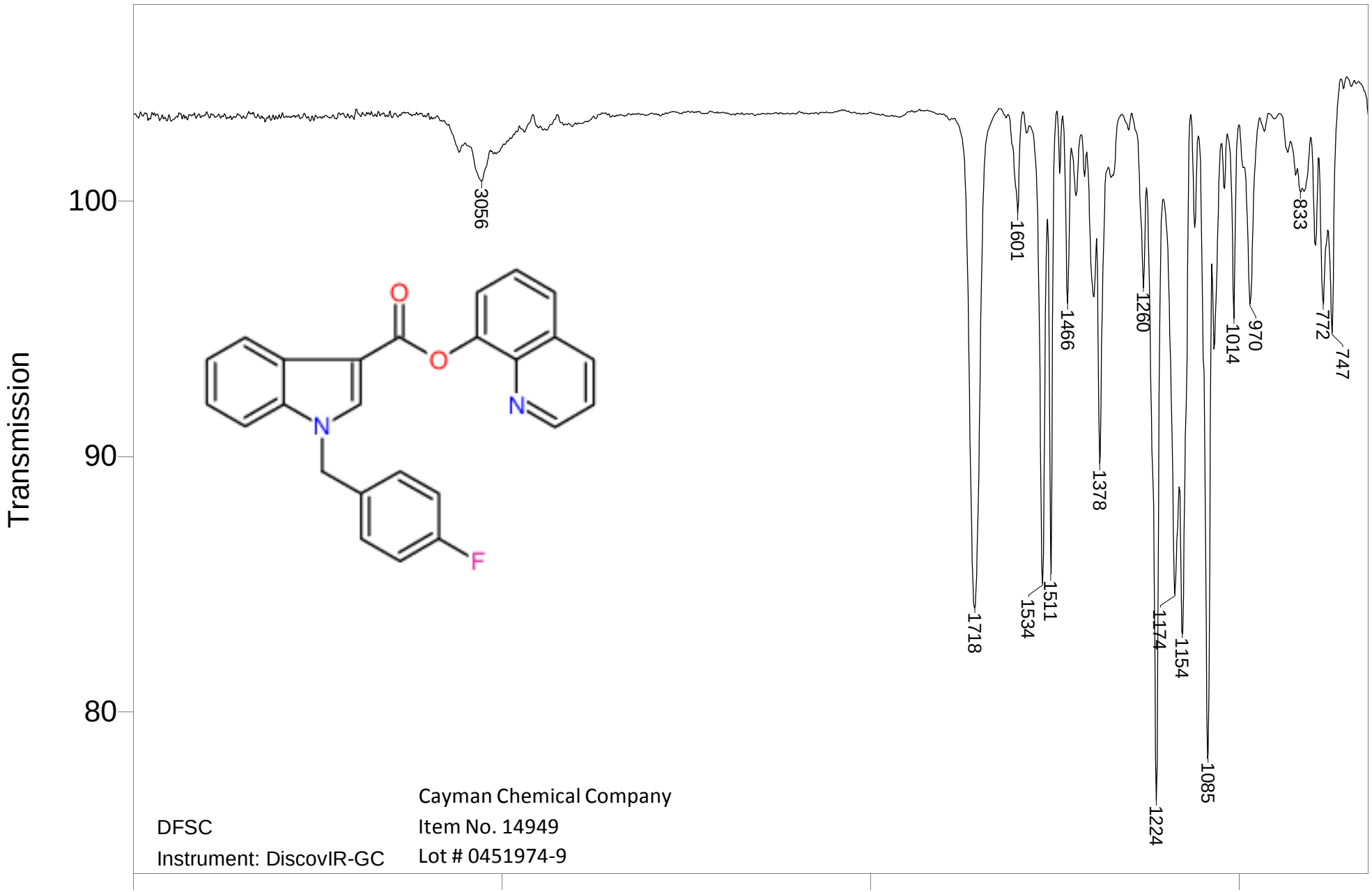

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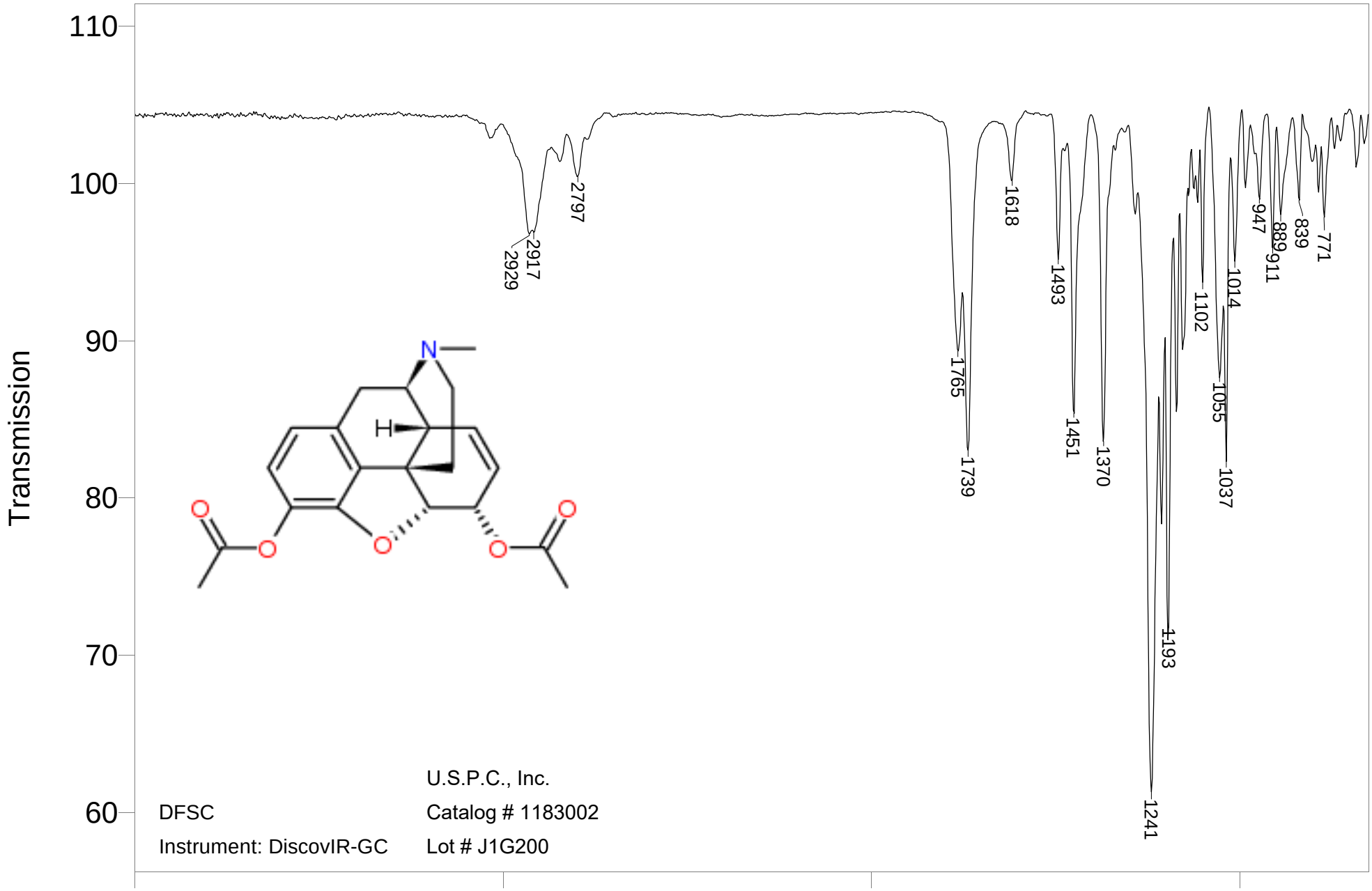

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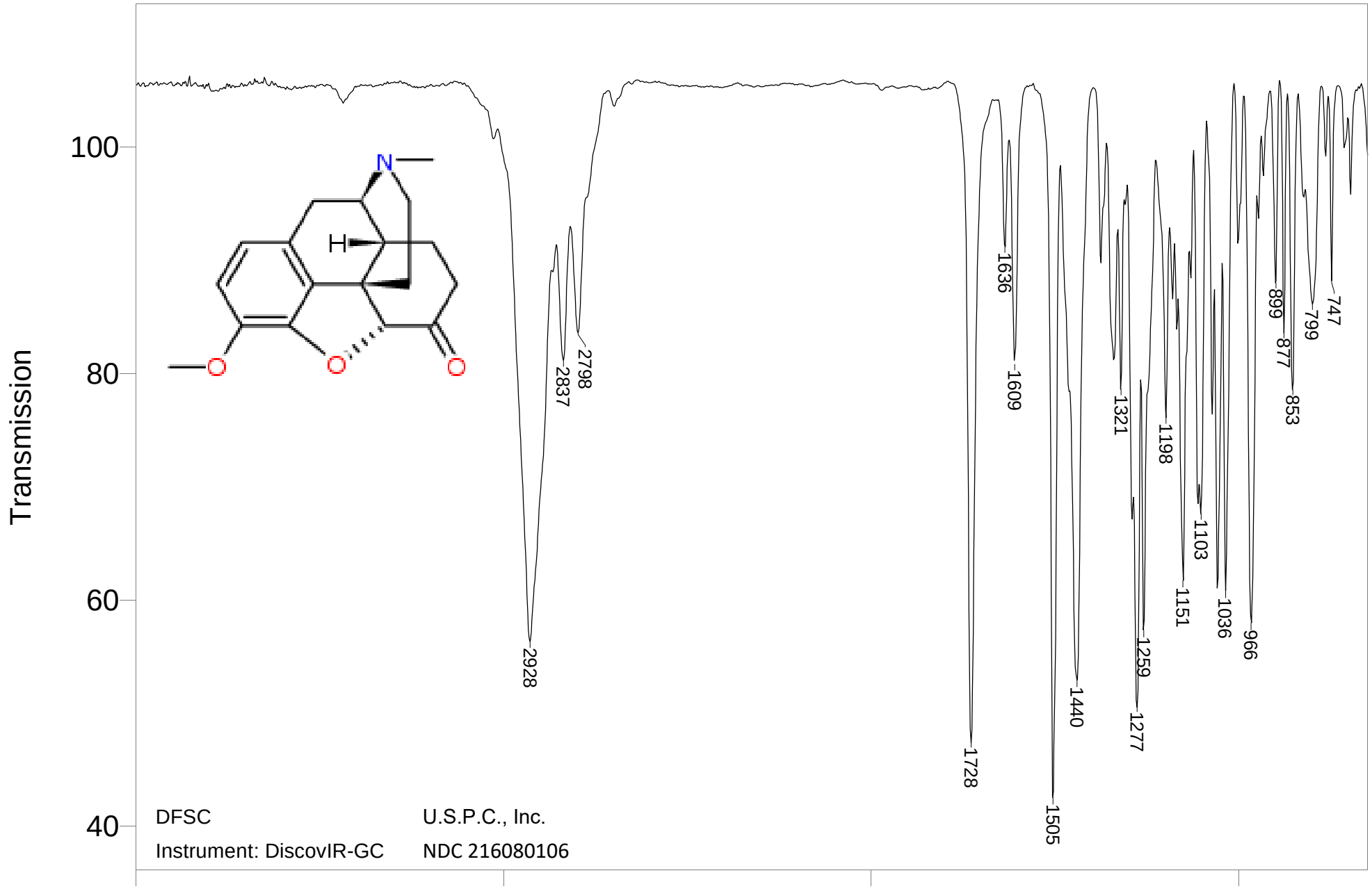
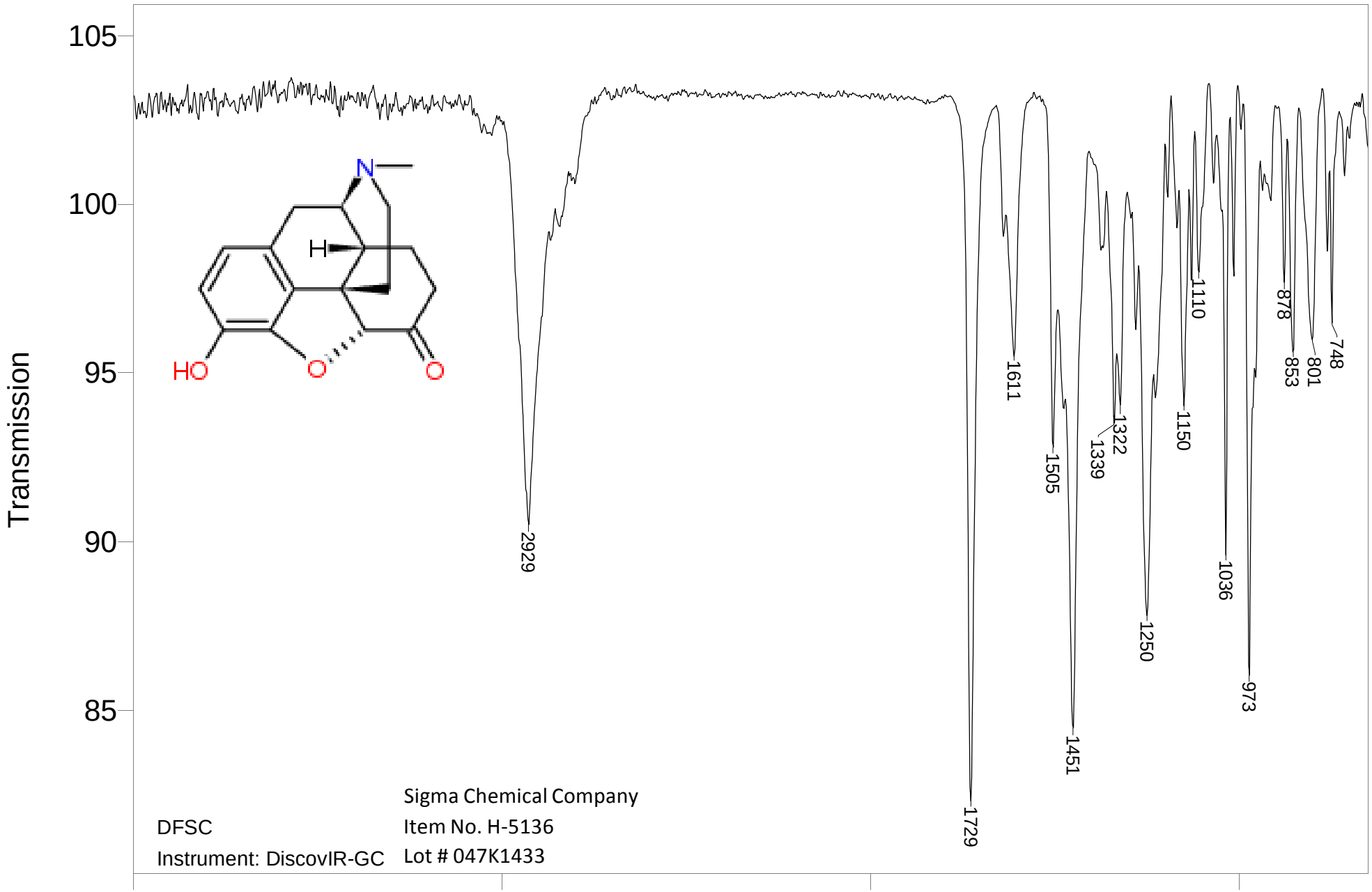
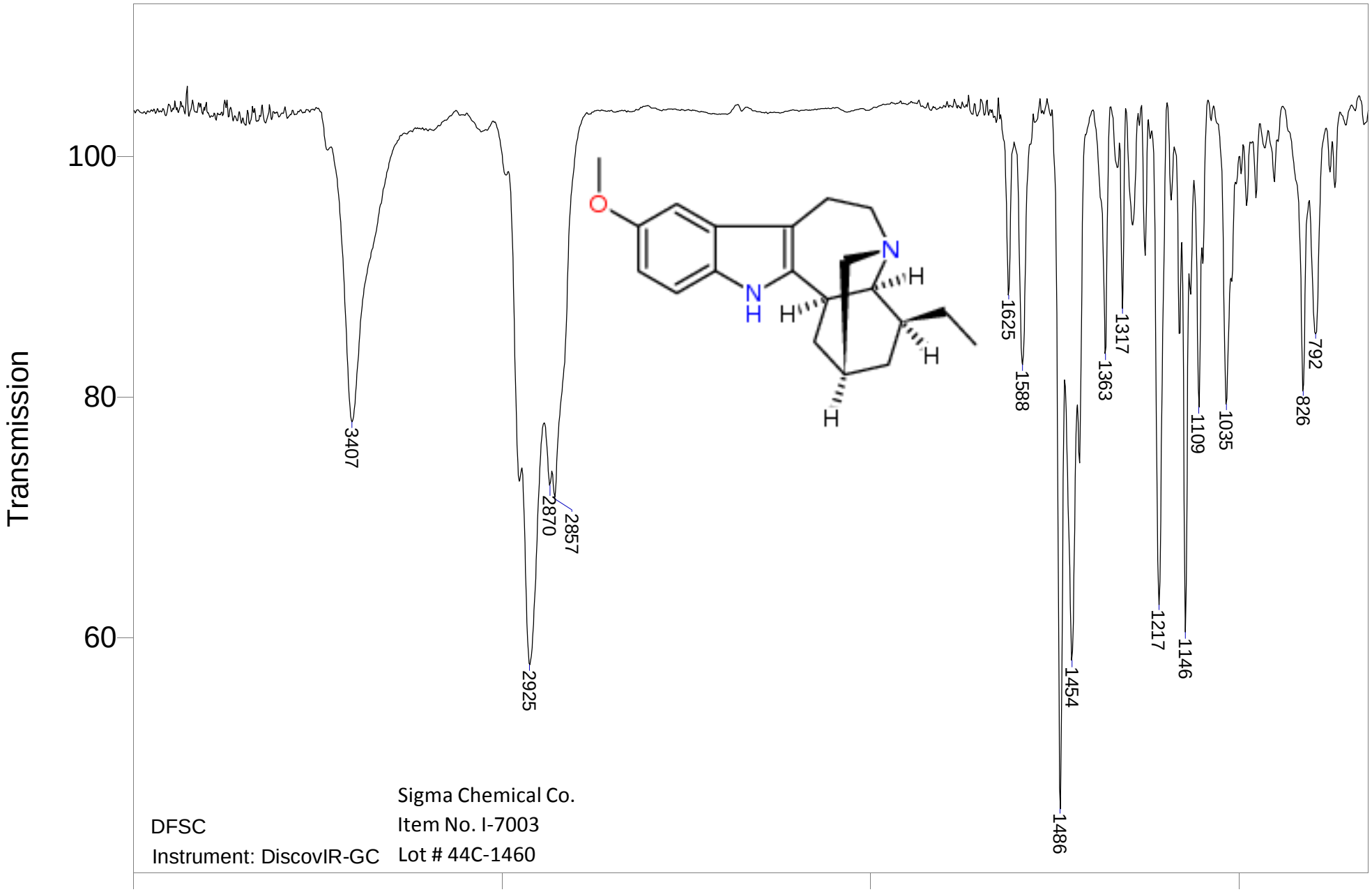
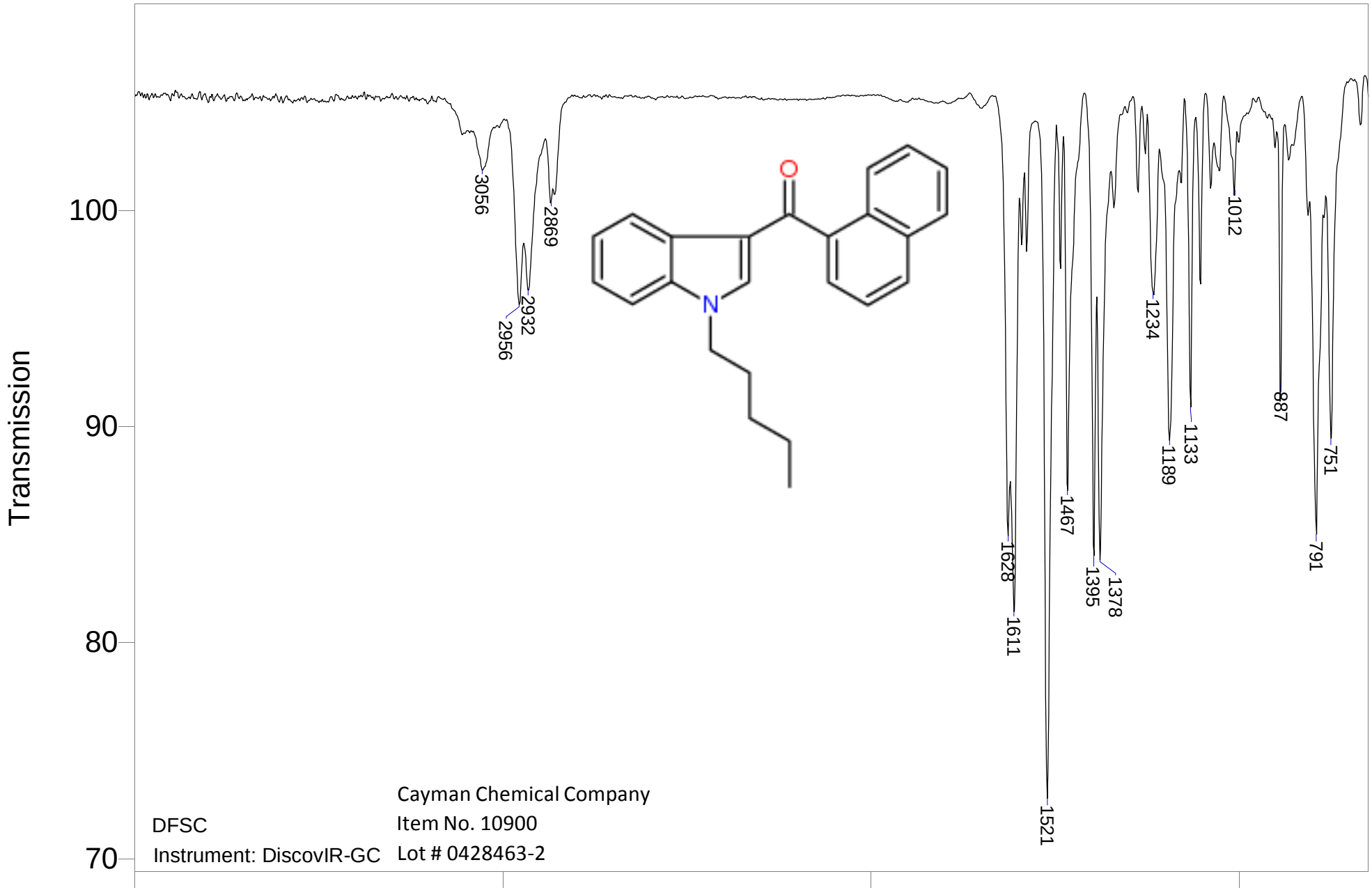
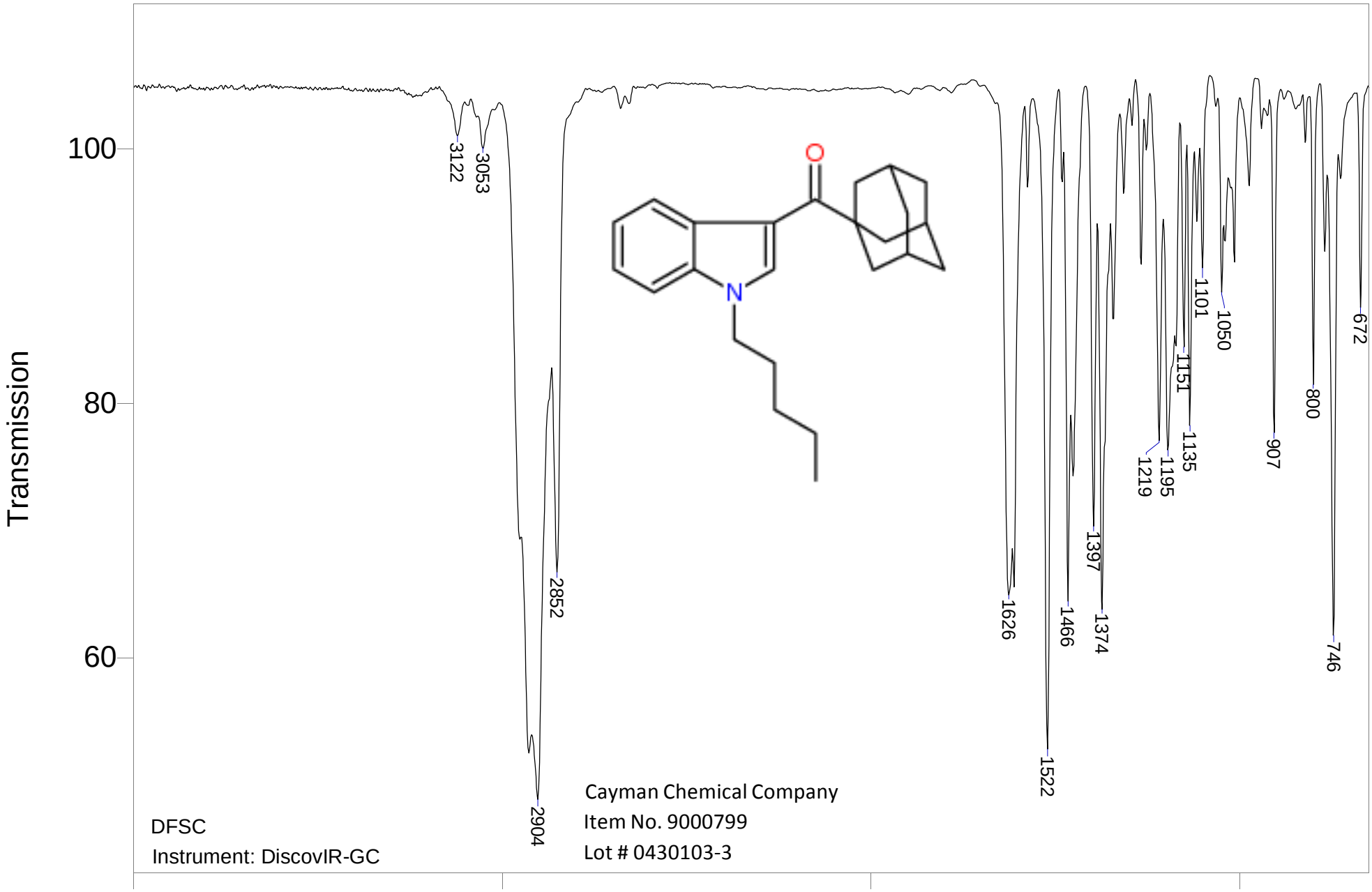


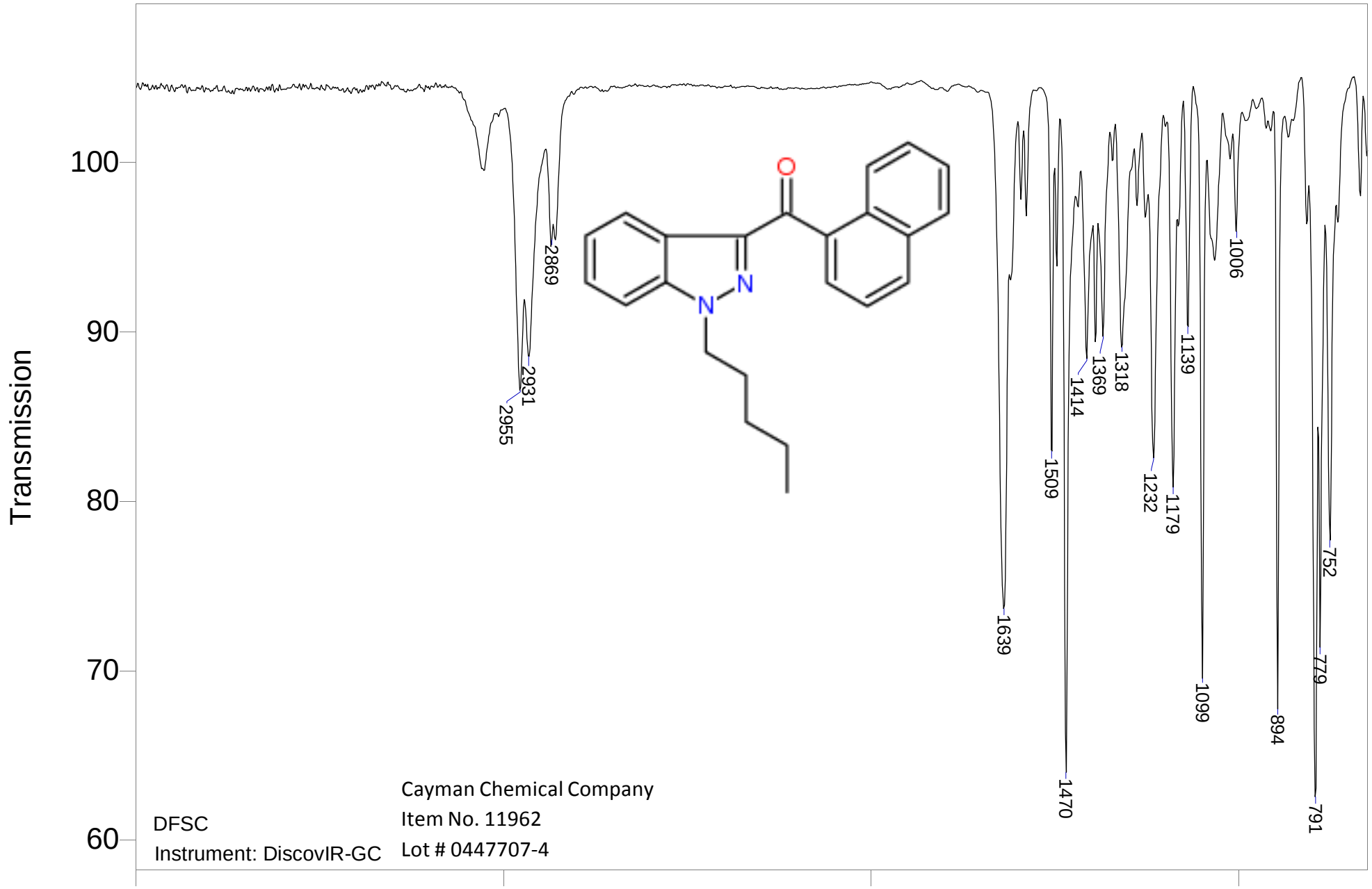
Hydromorphone

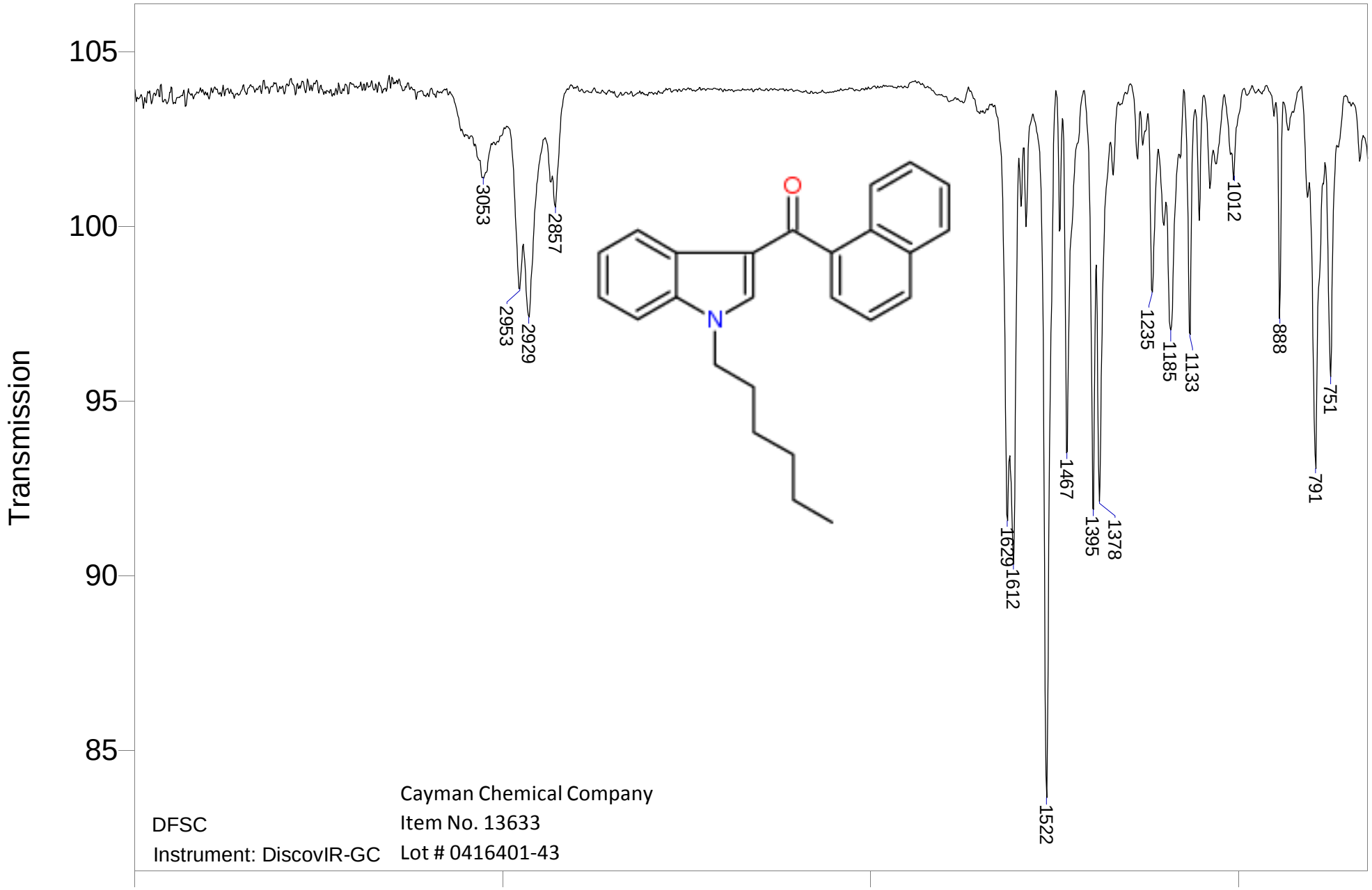


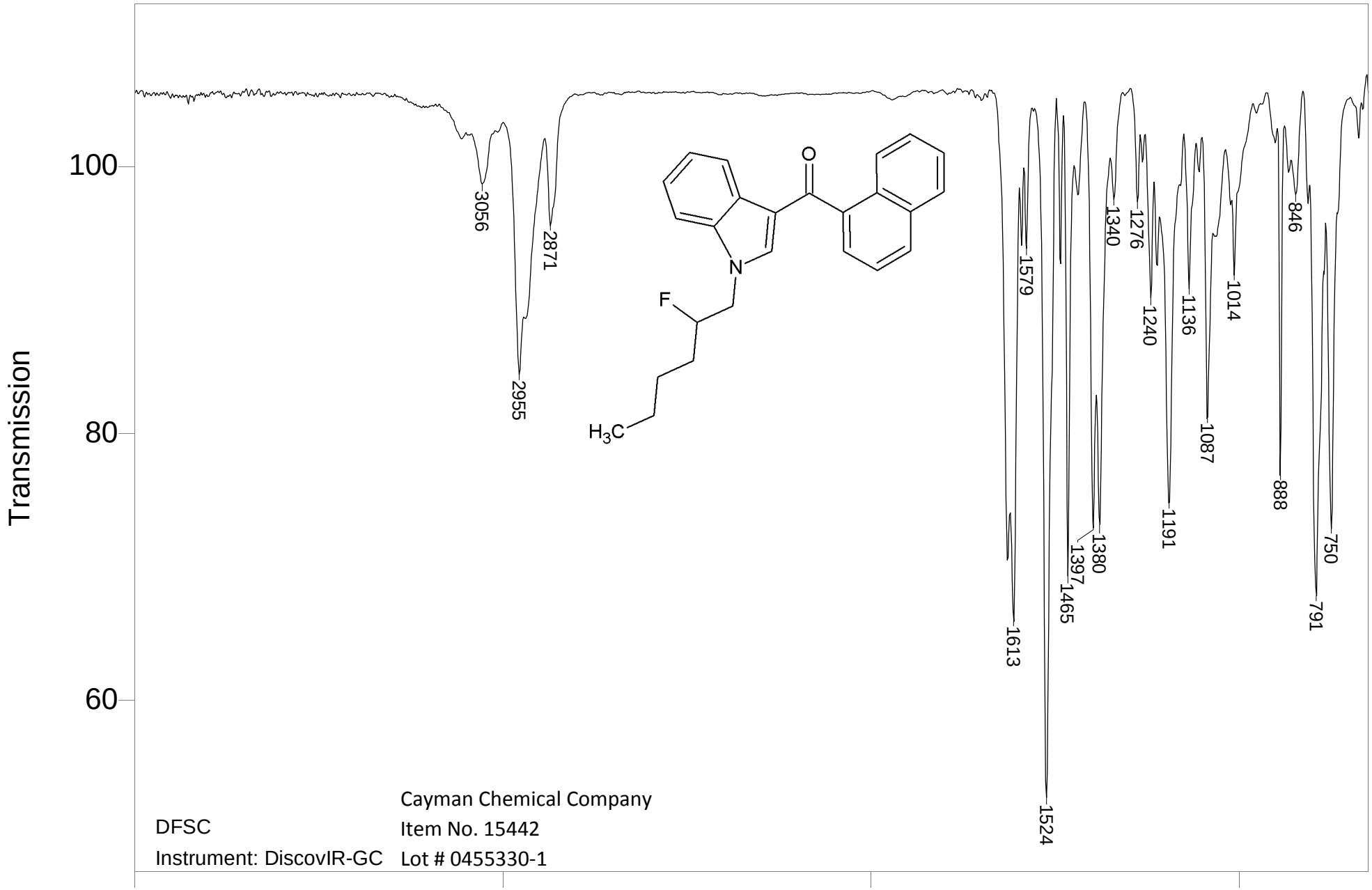


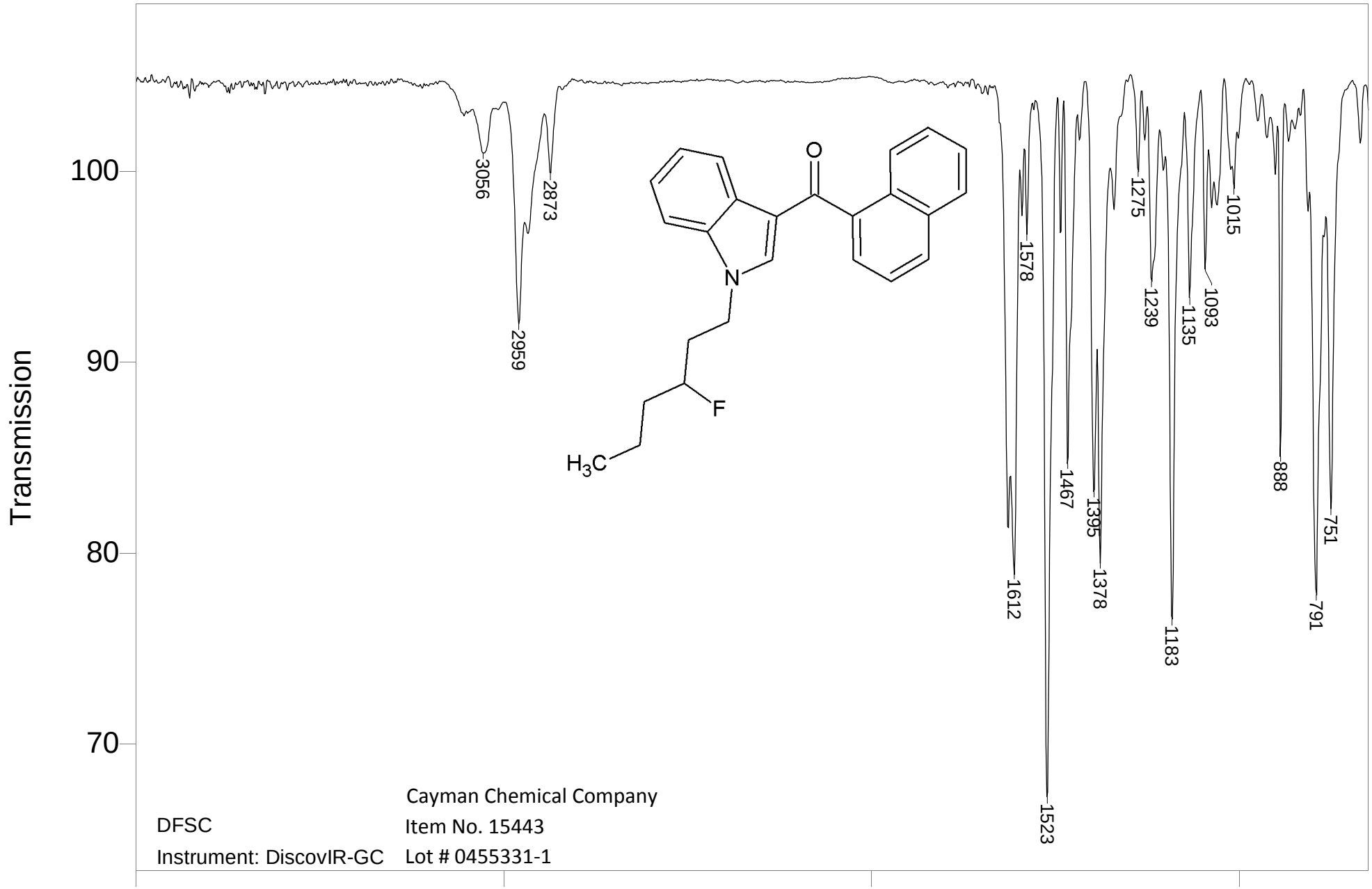


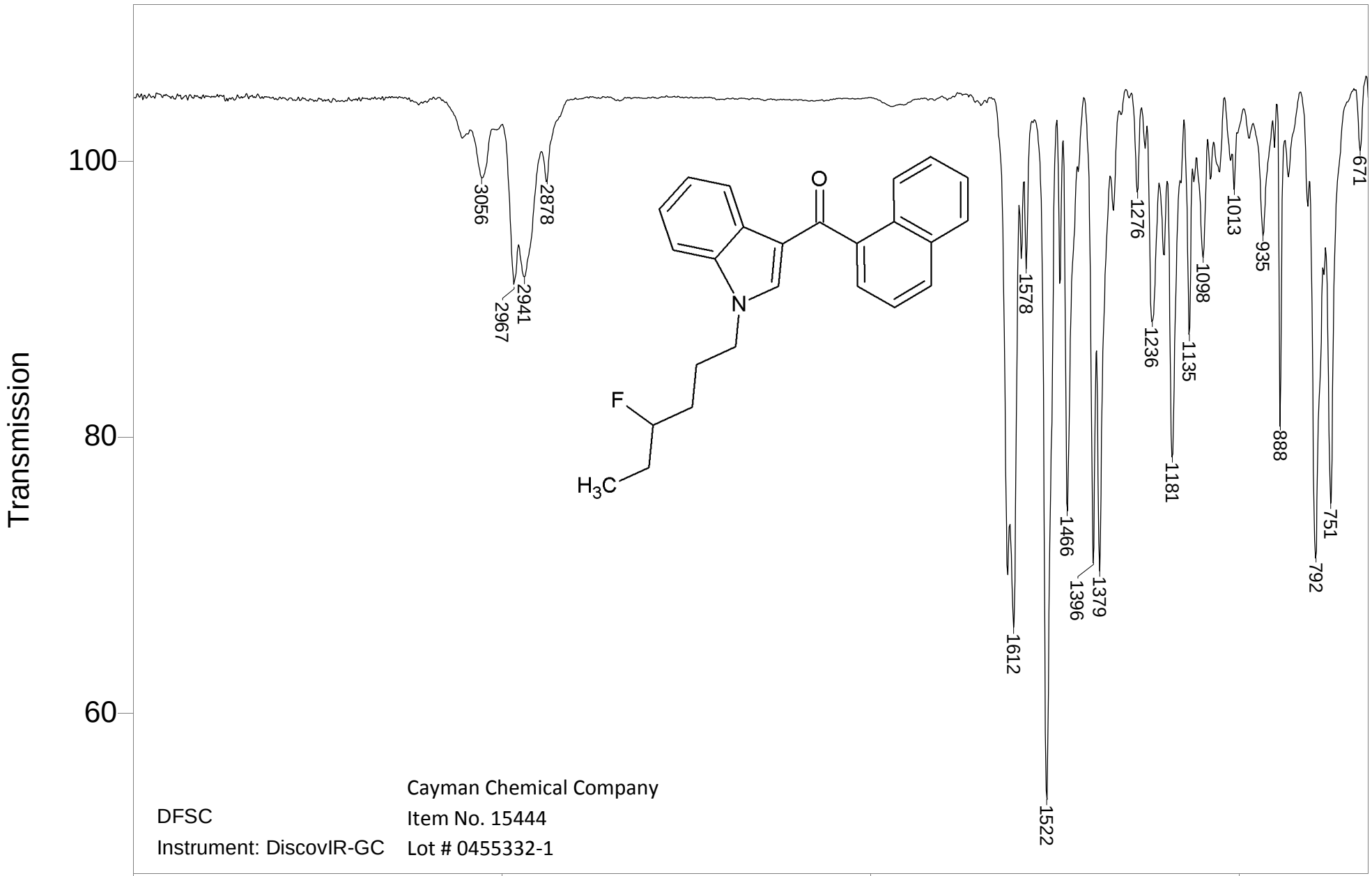


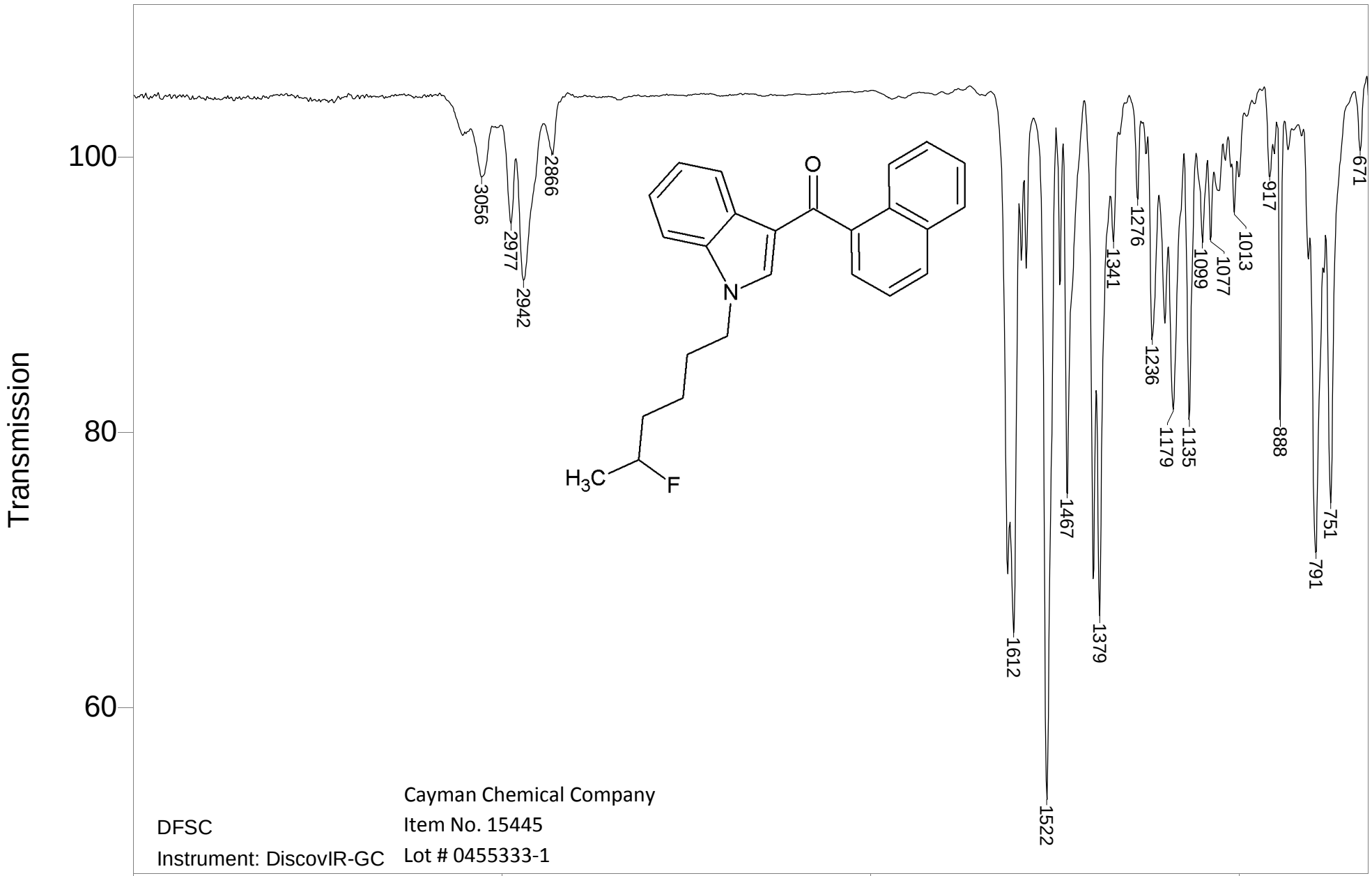


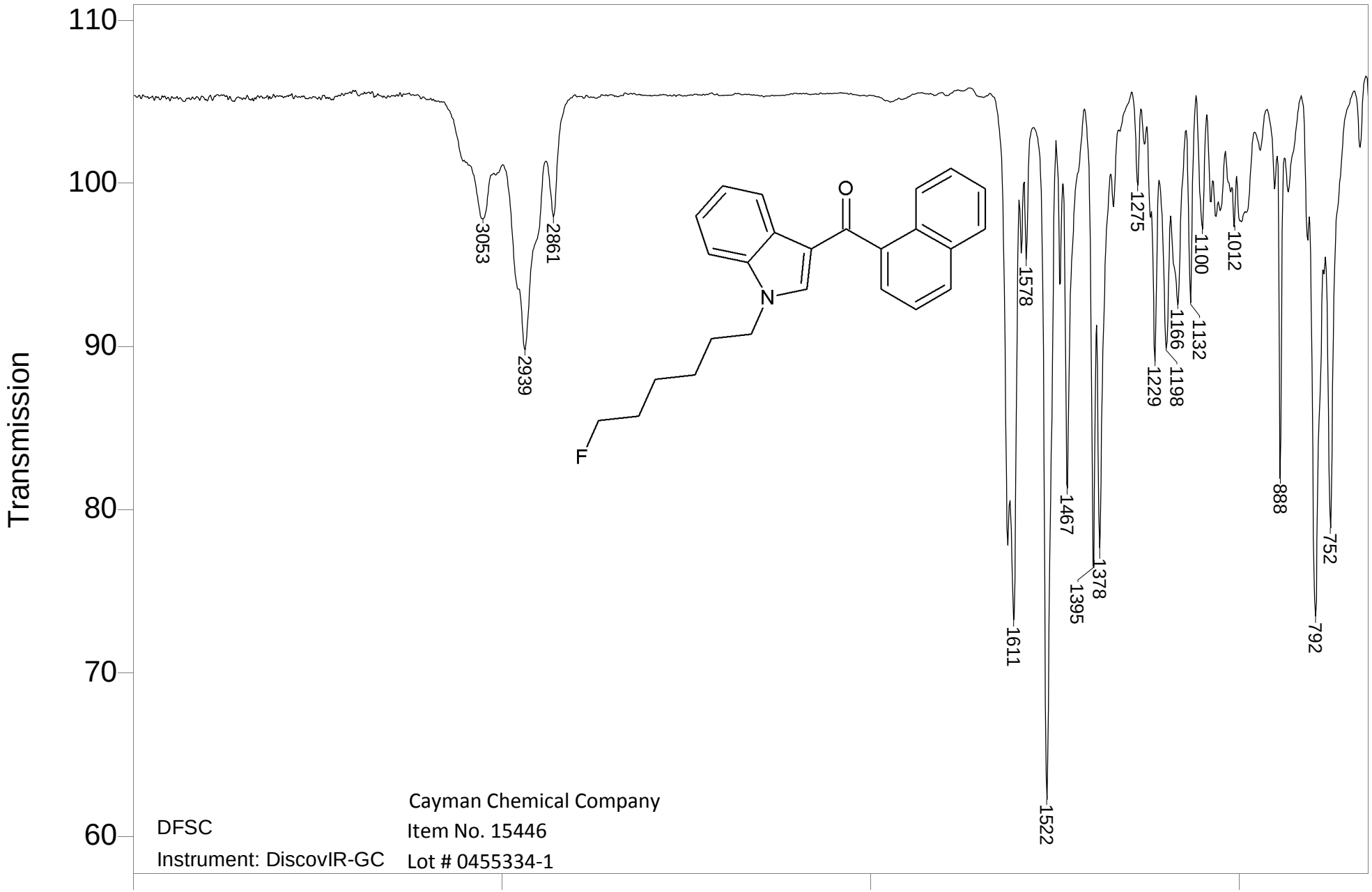


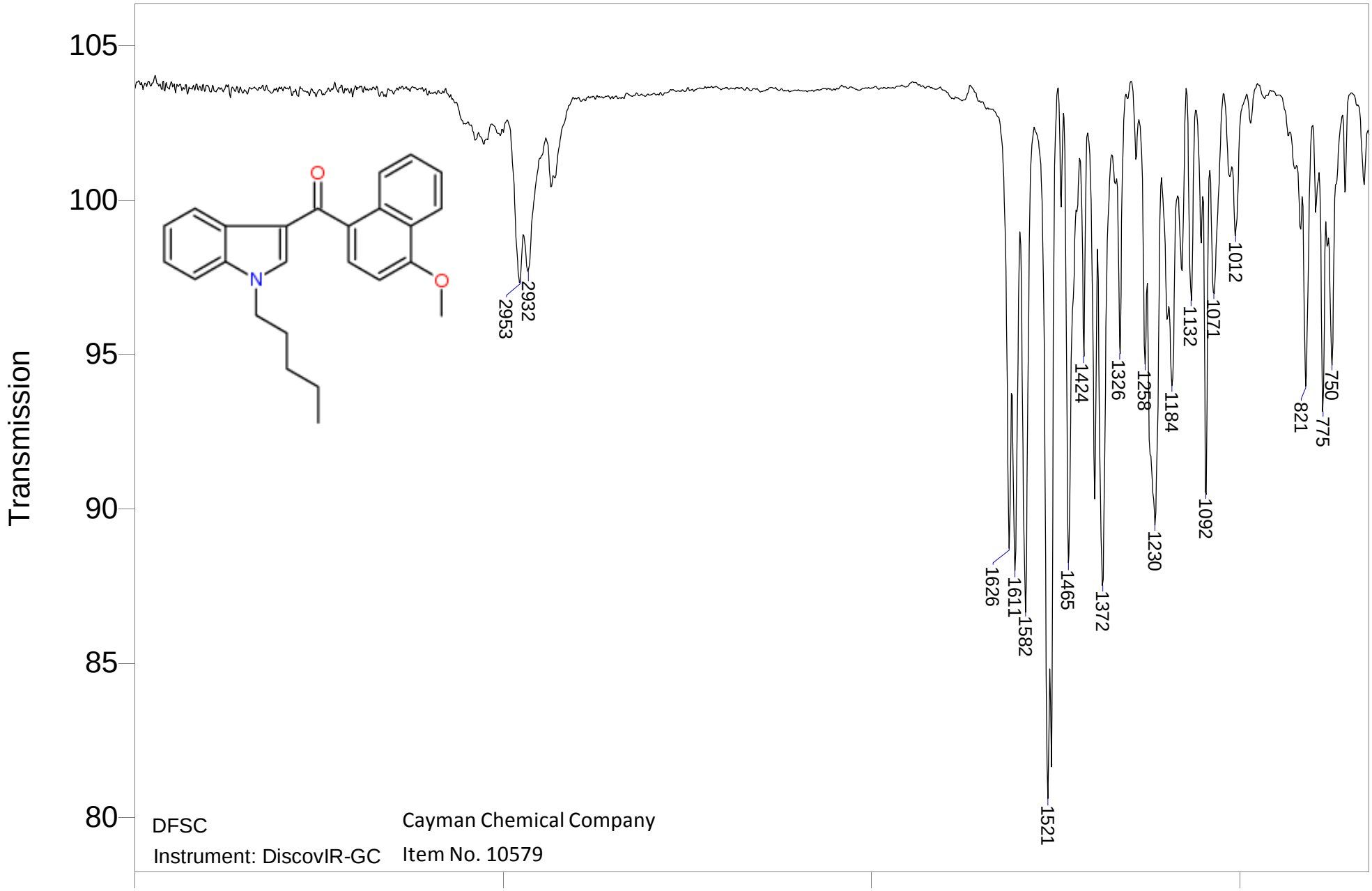


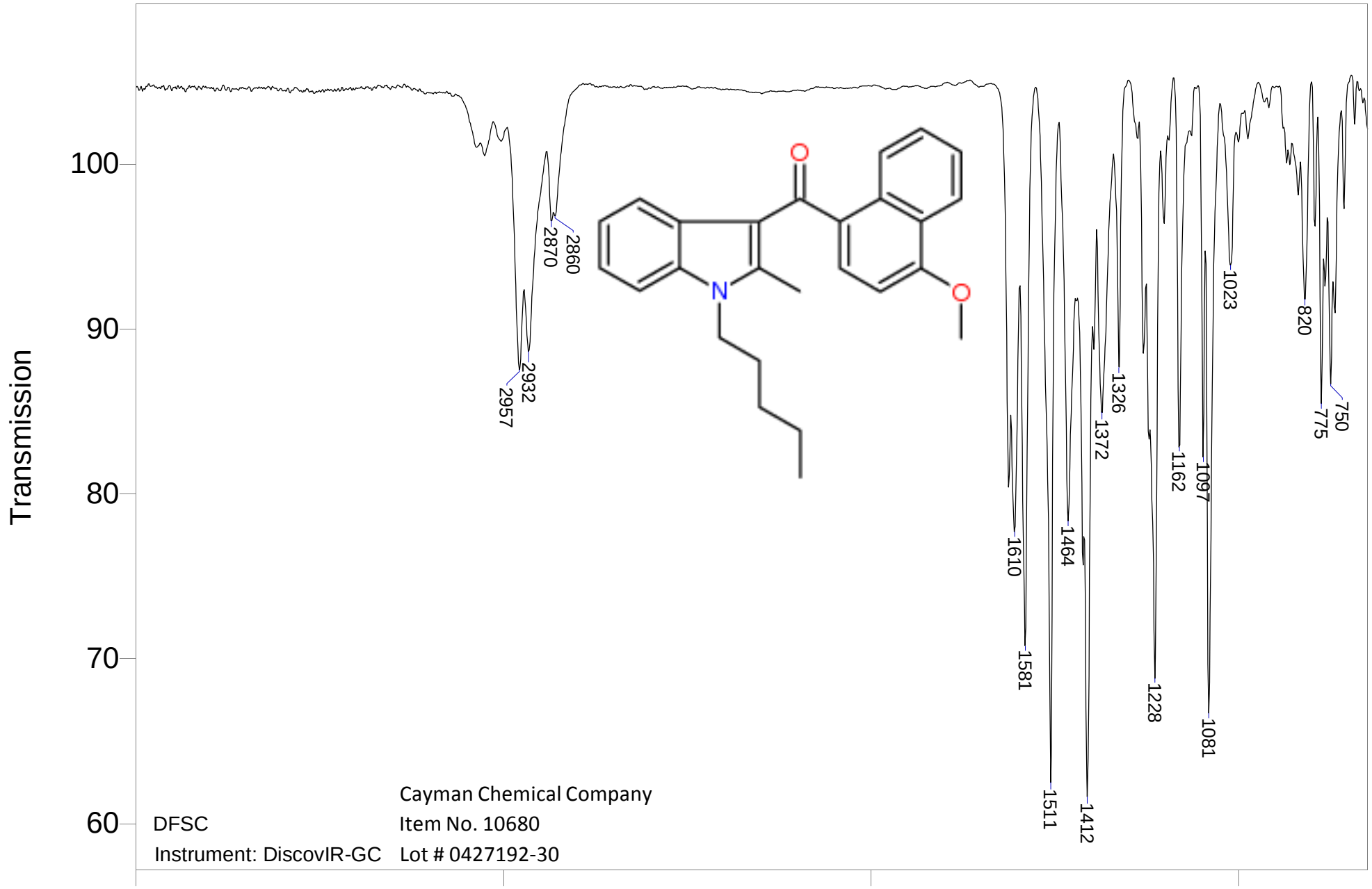


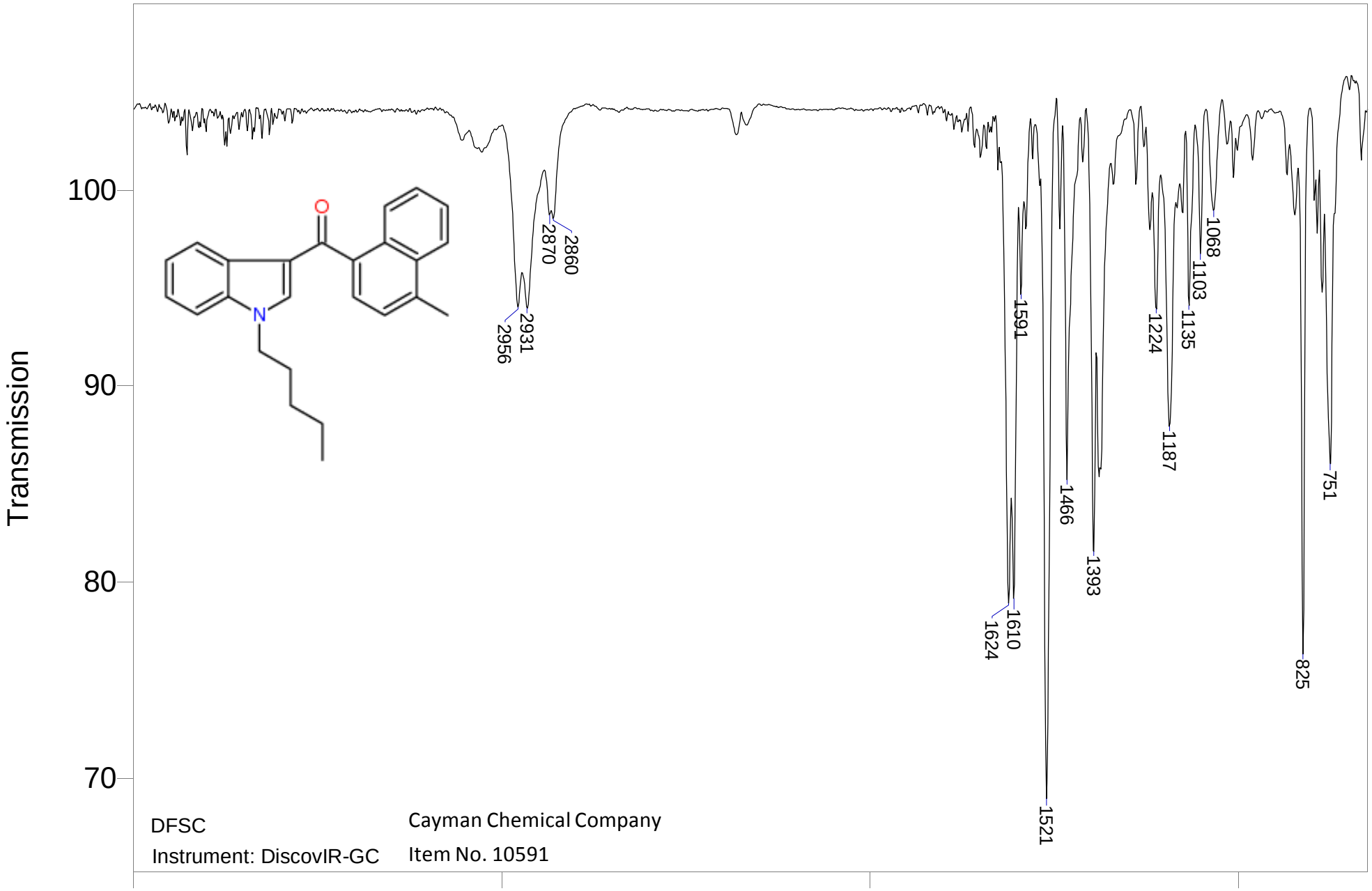


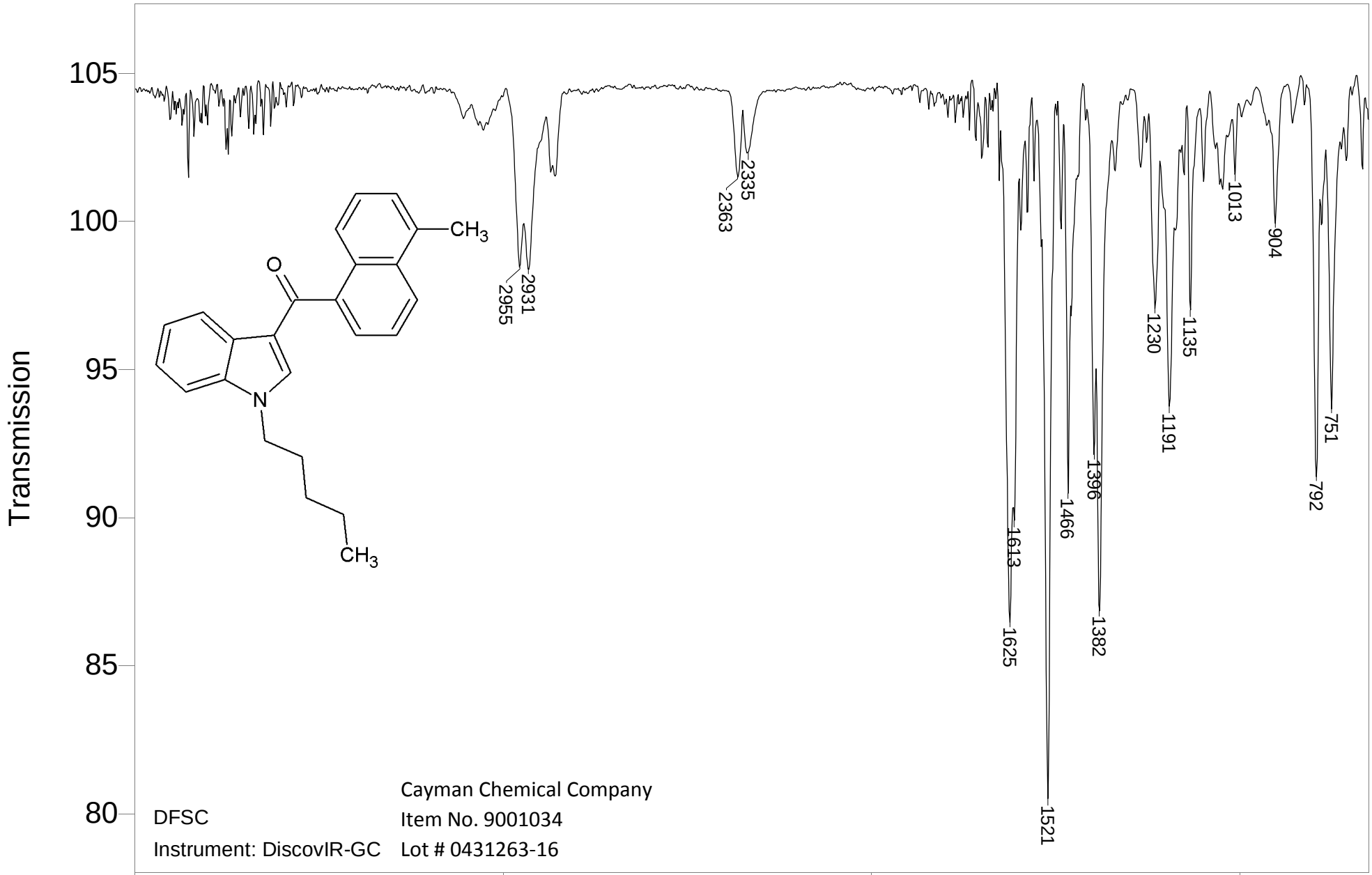


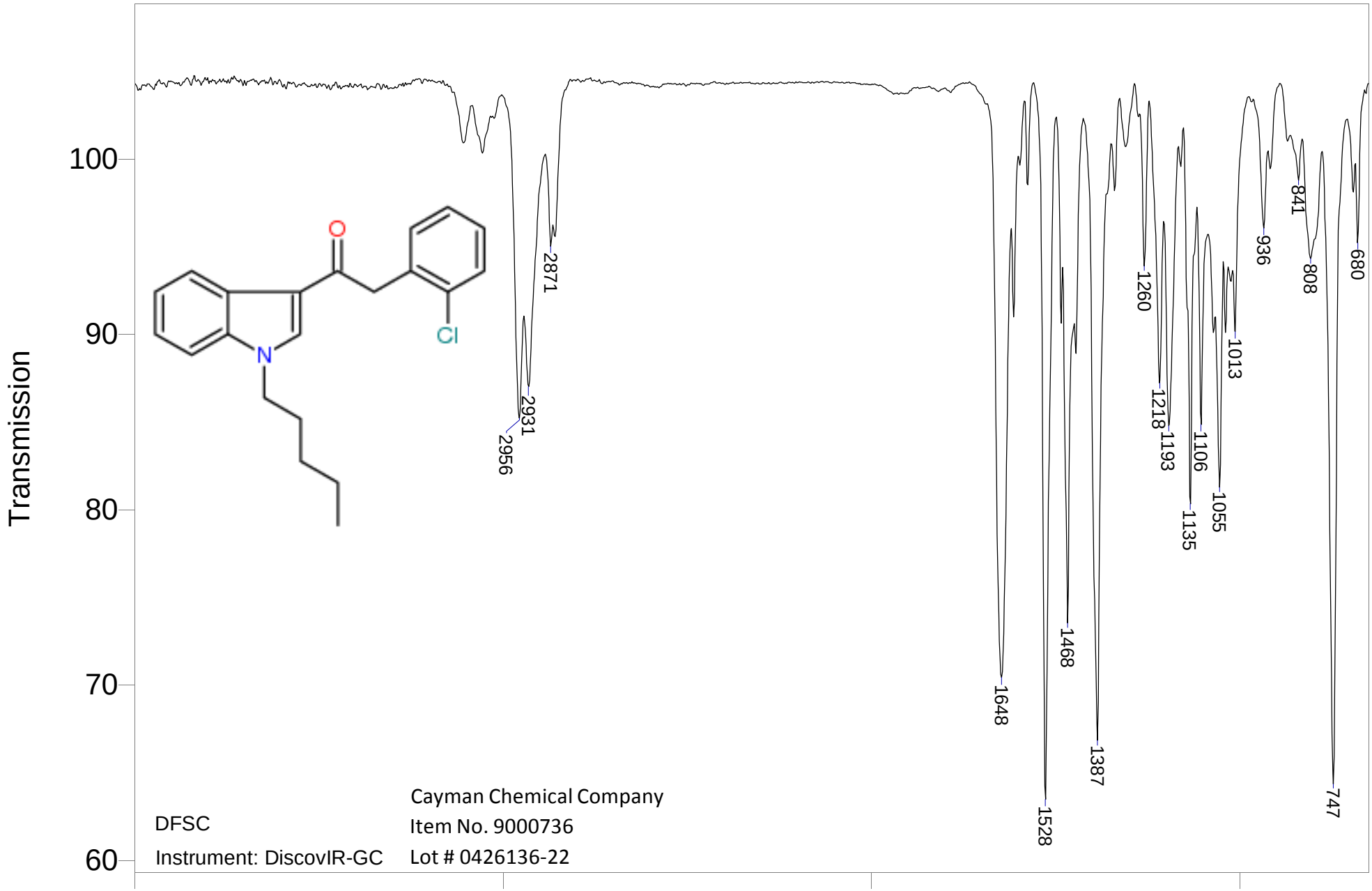


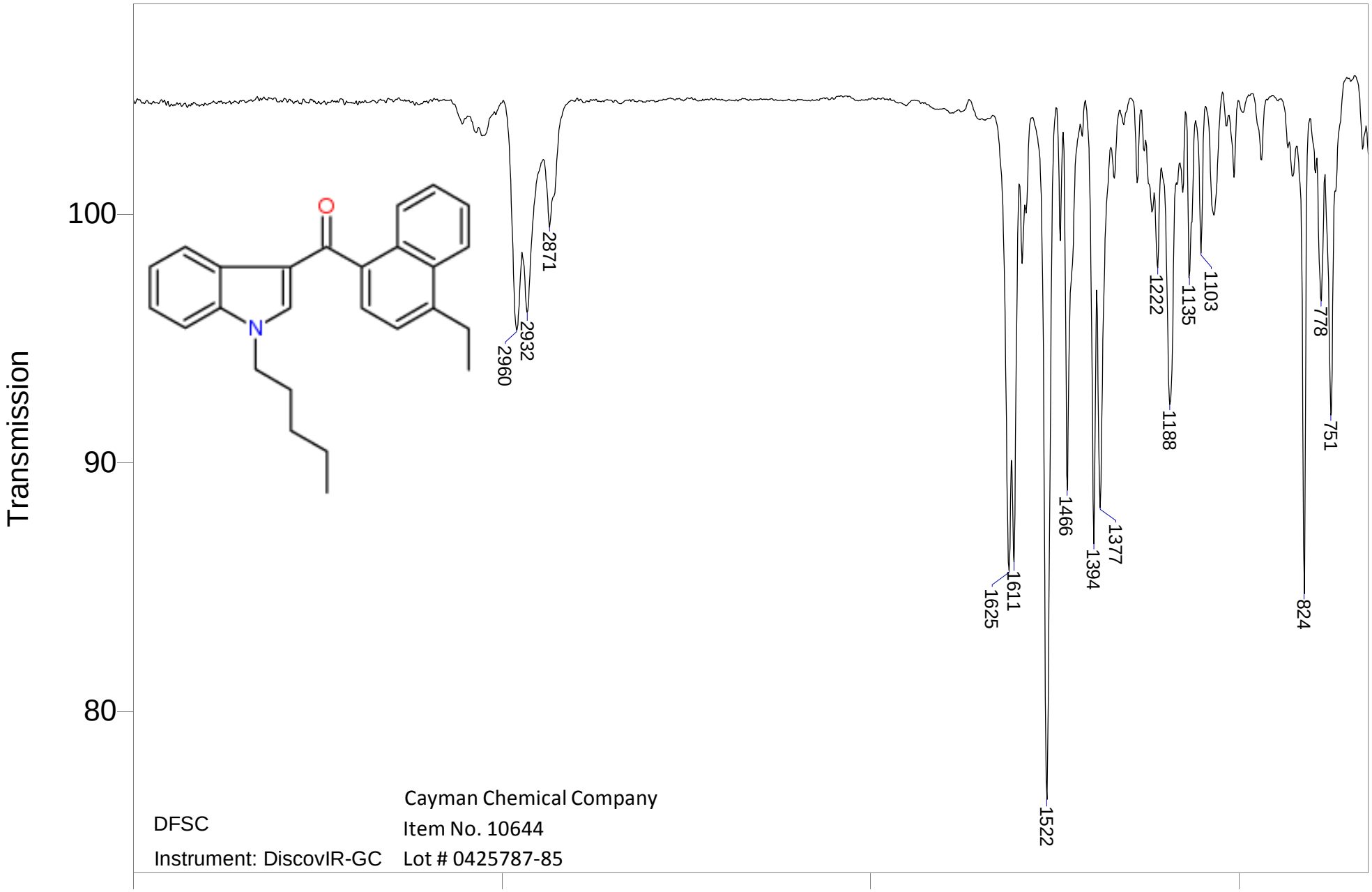




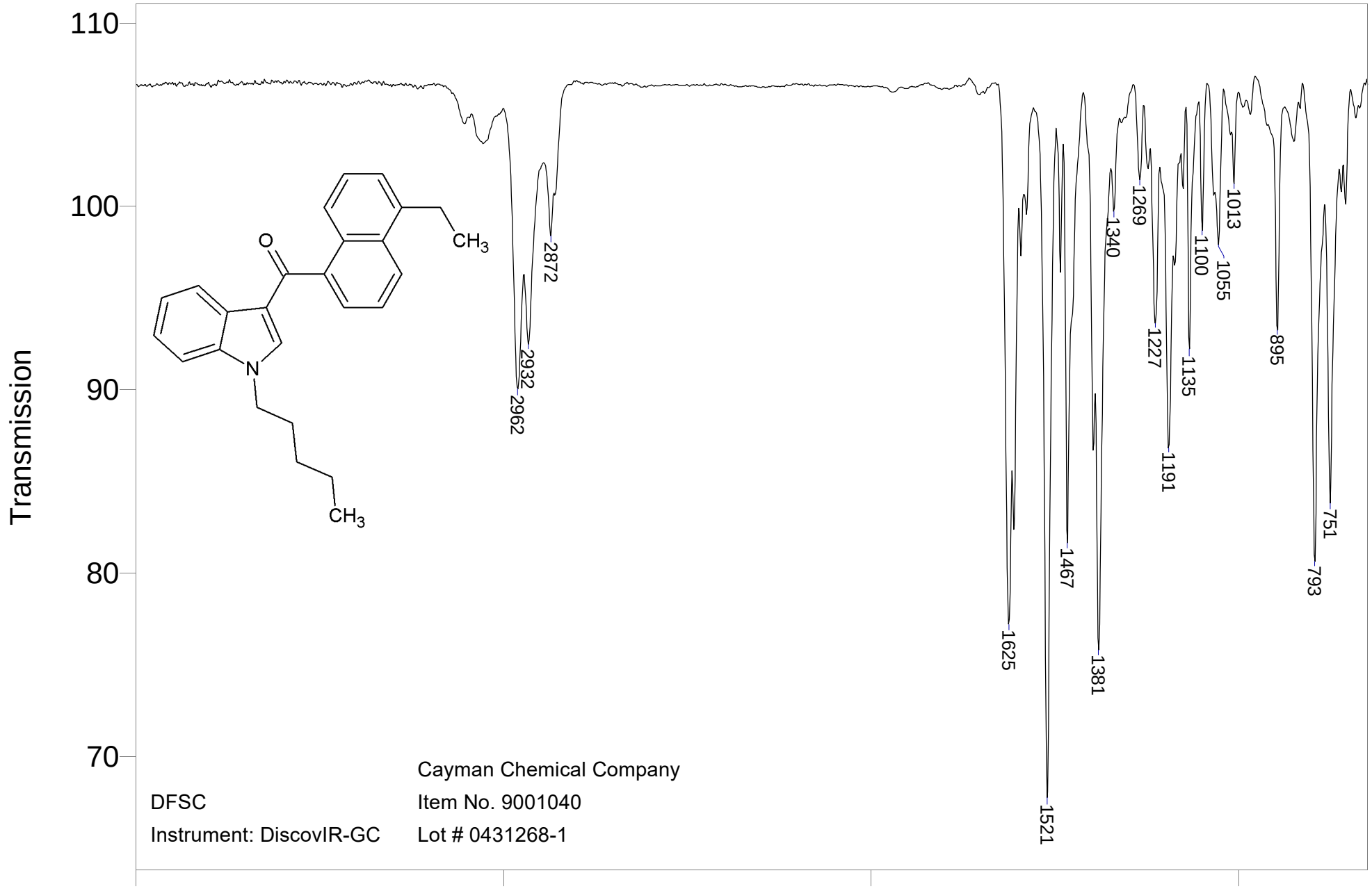


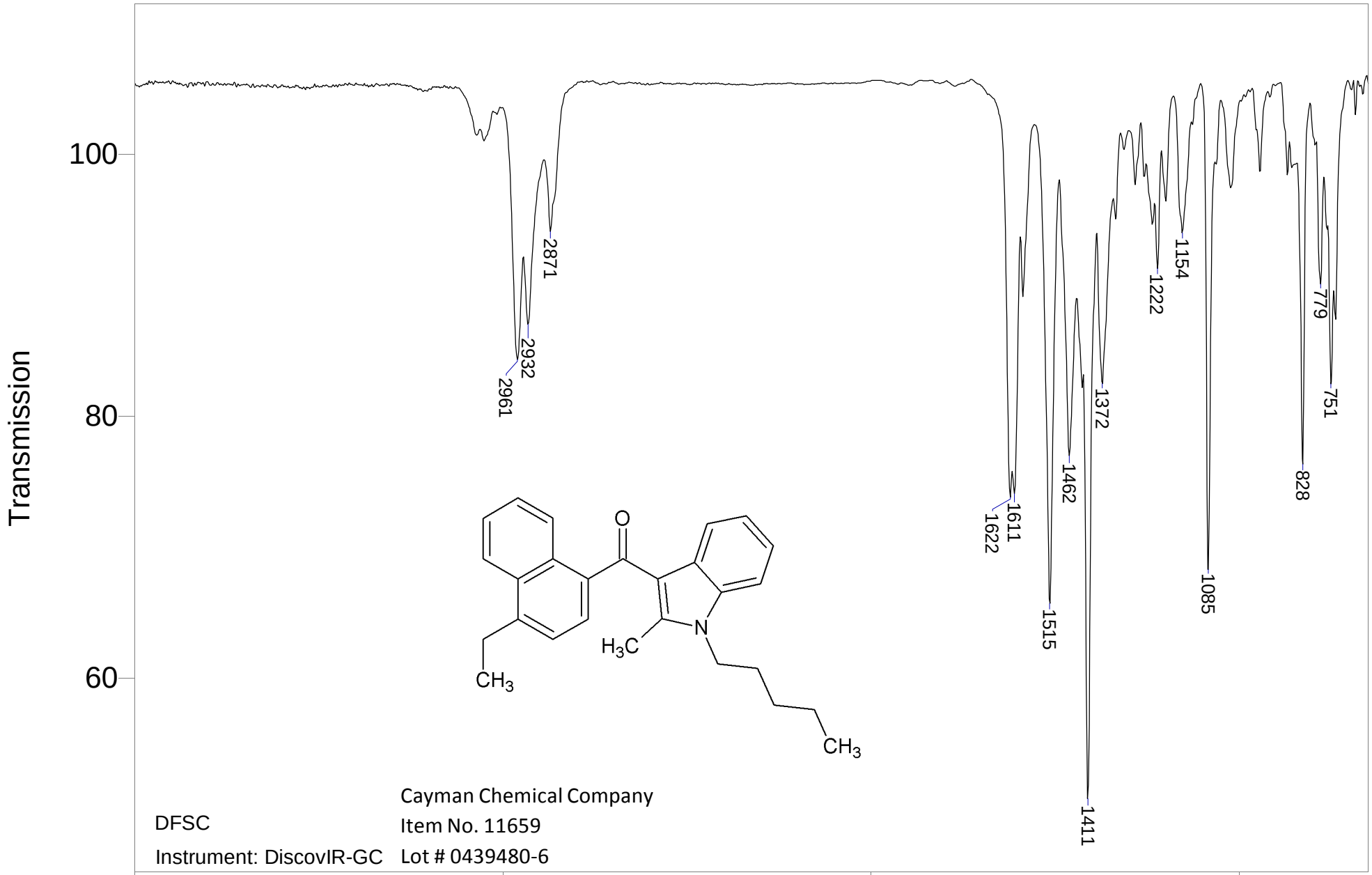


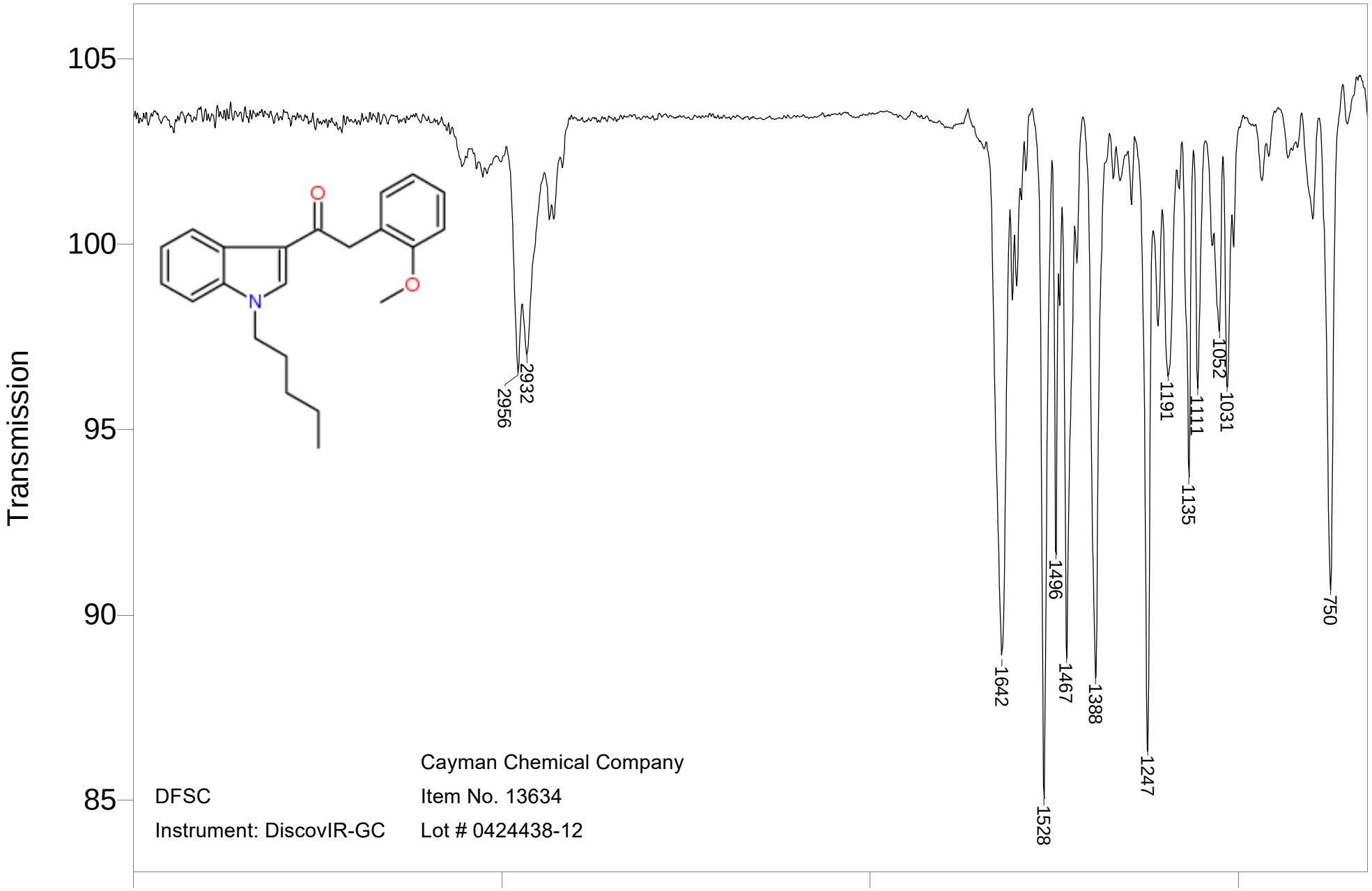


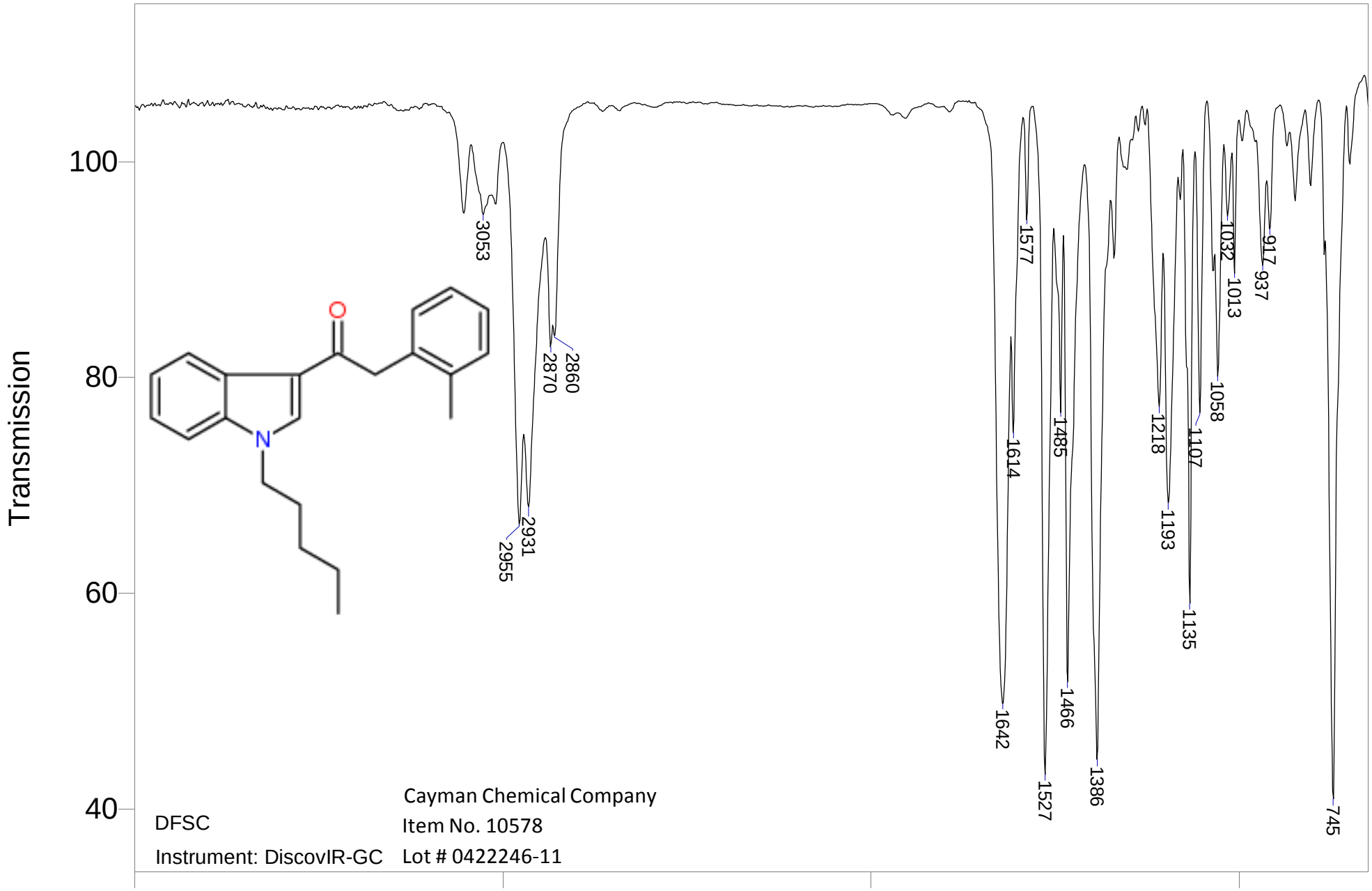


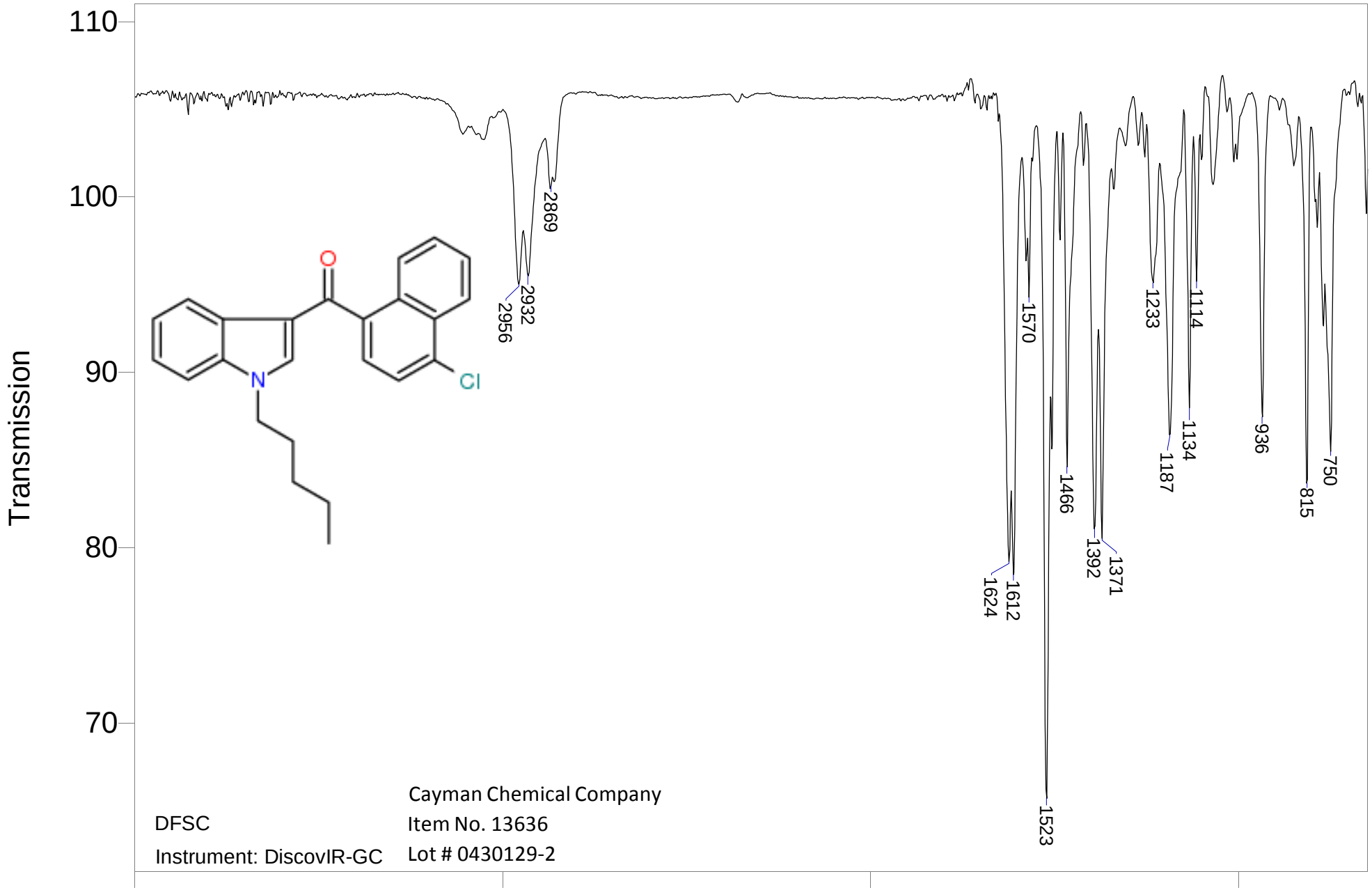
JWH-210 5-ethylnaphthyl isomer



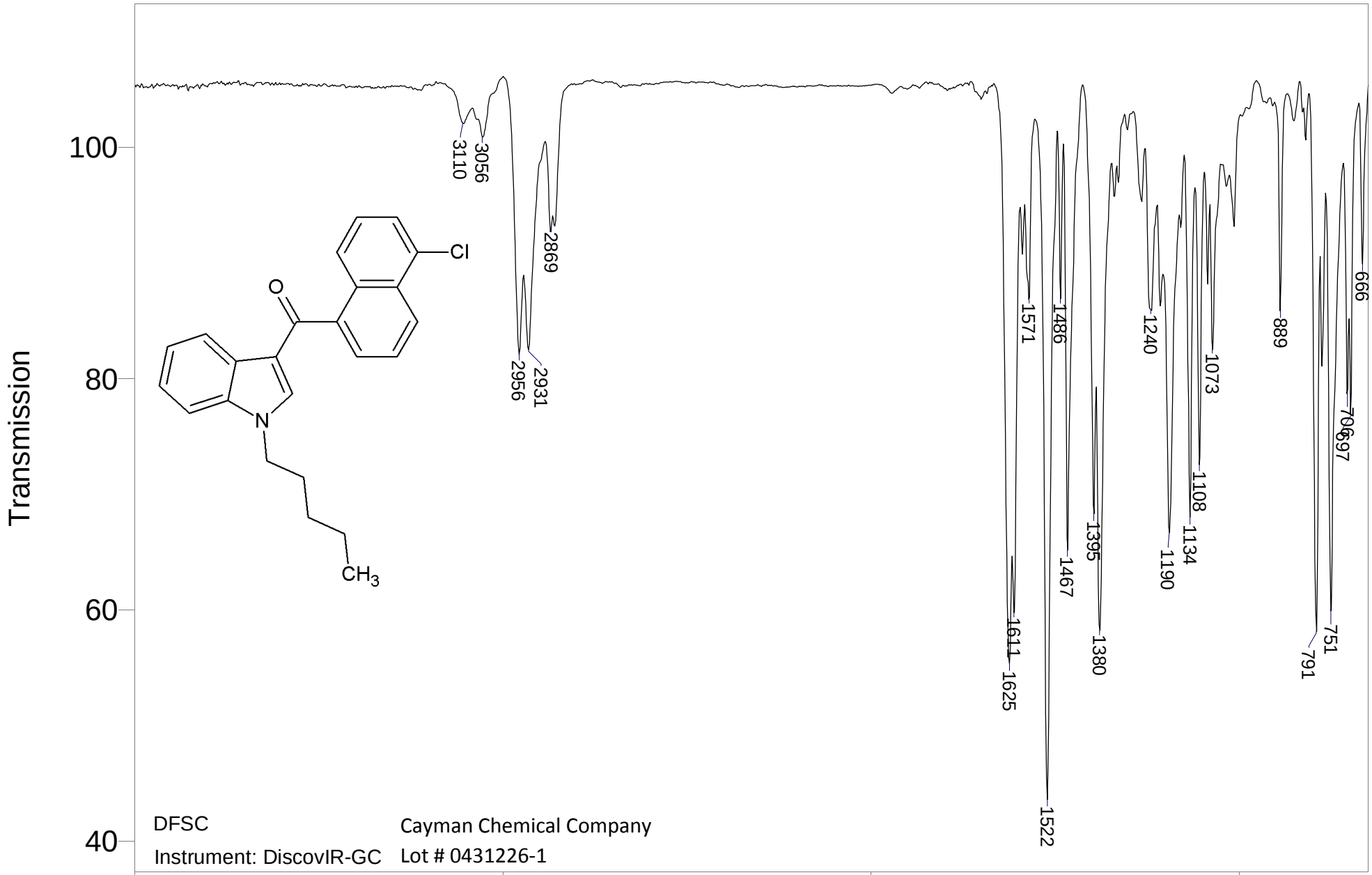




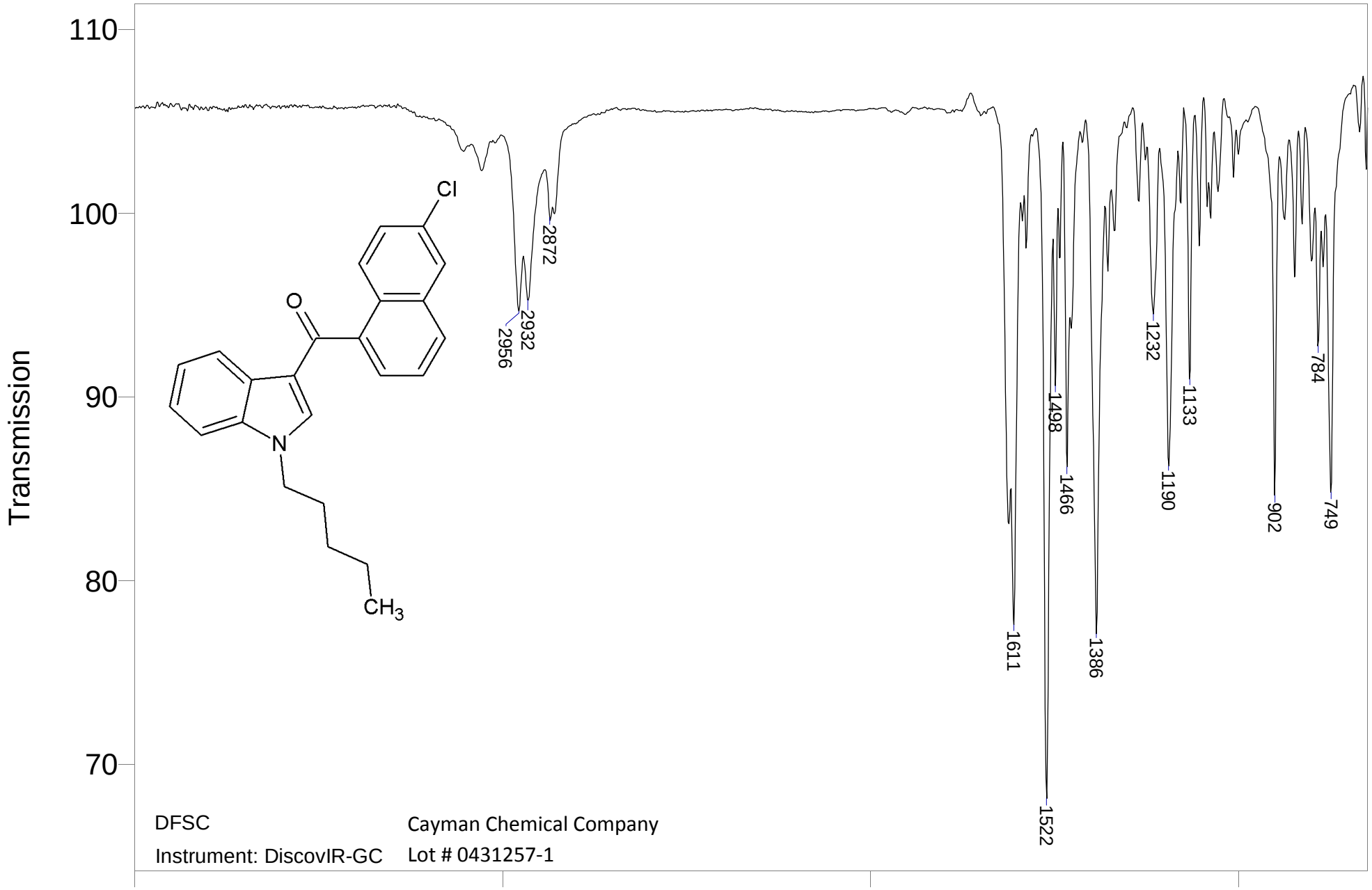


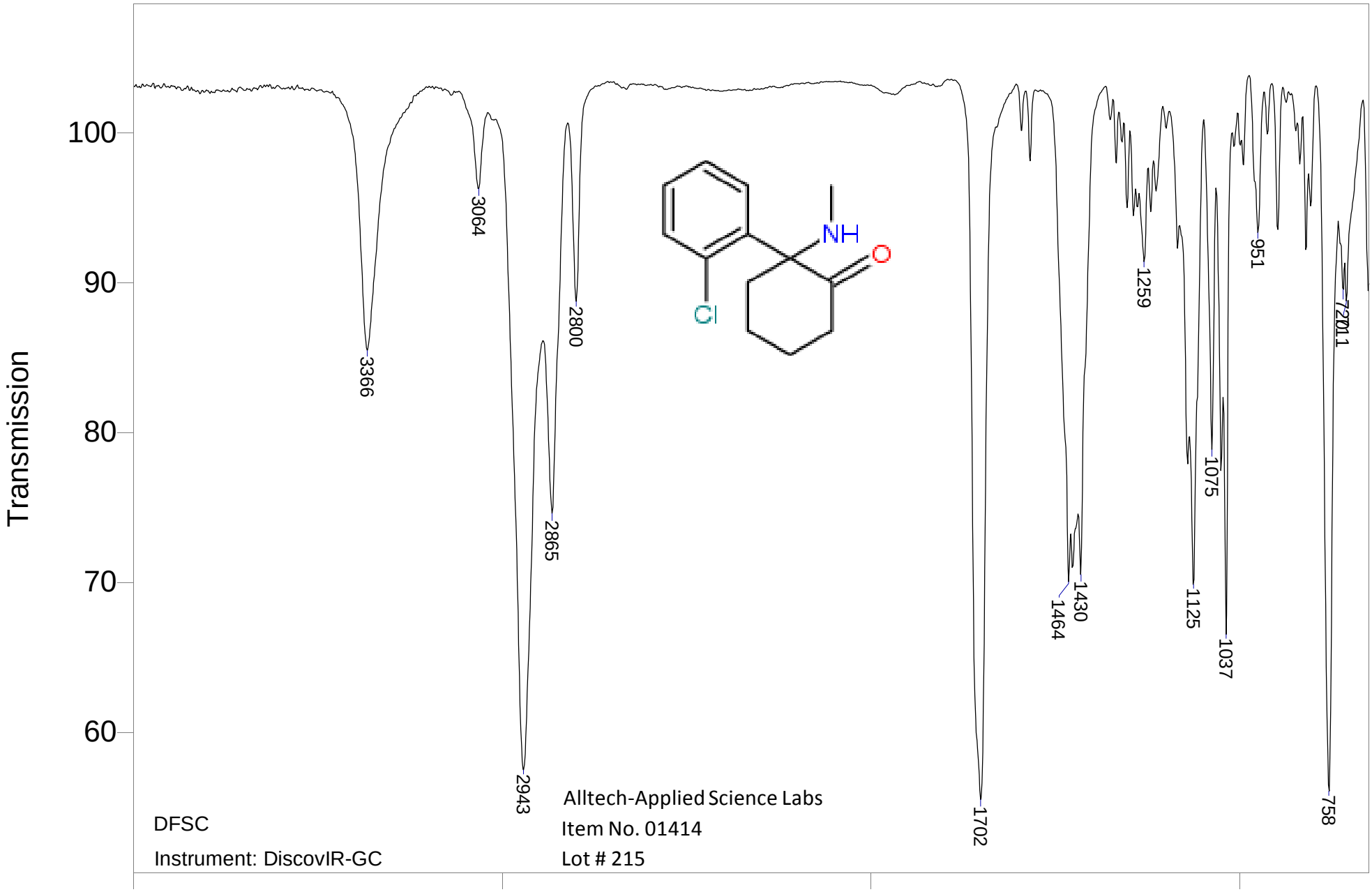


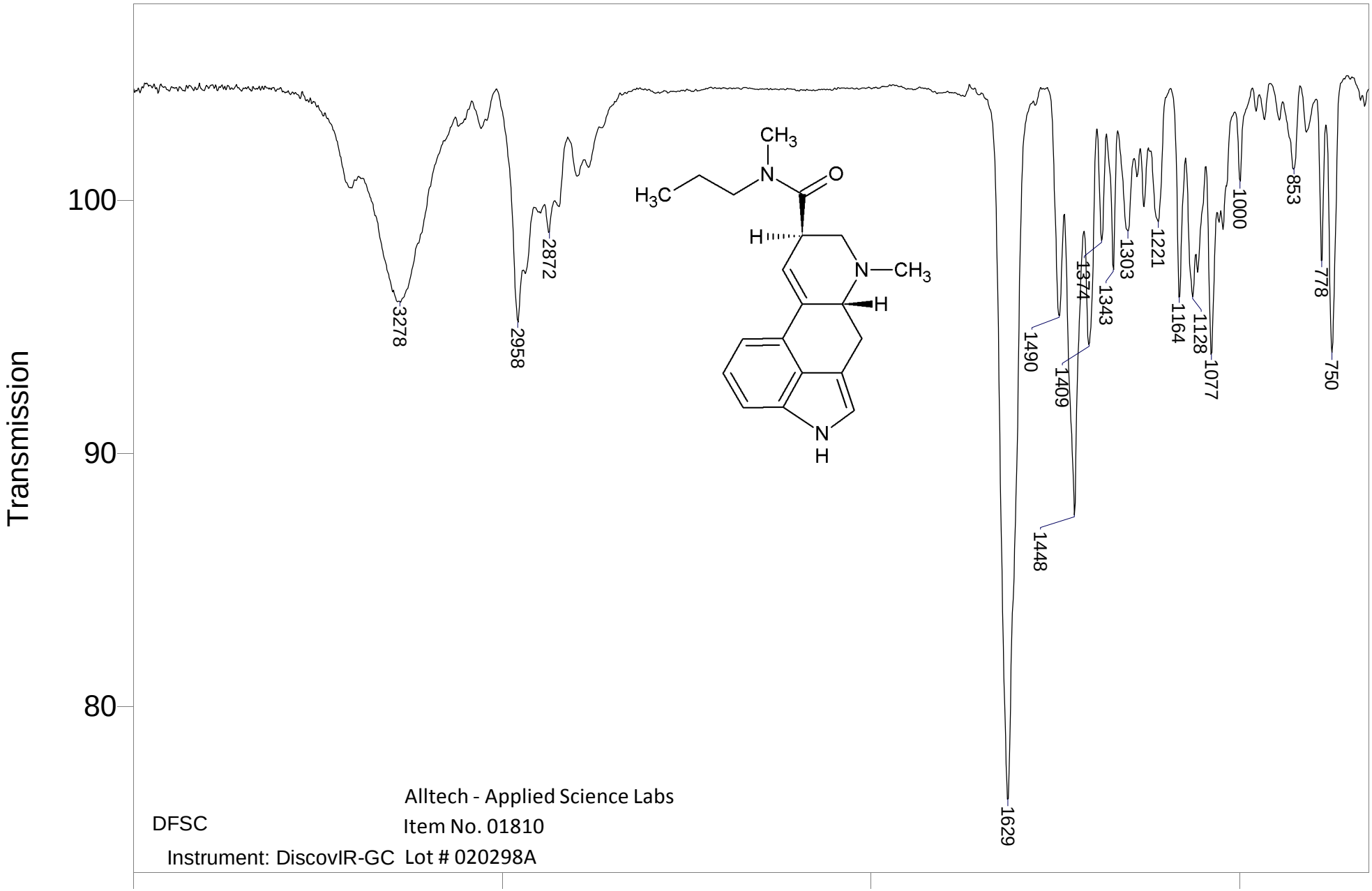
JWH-398 5 Isomer

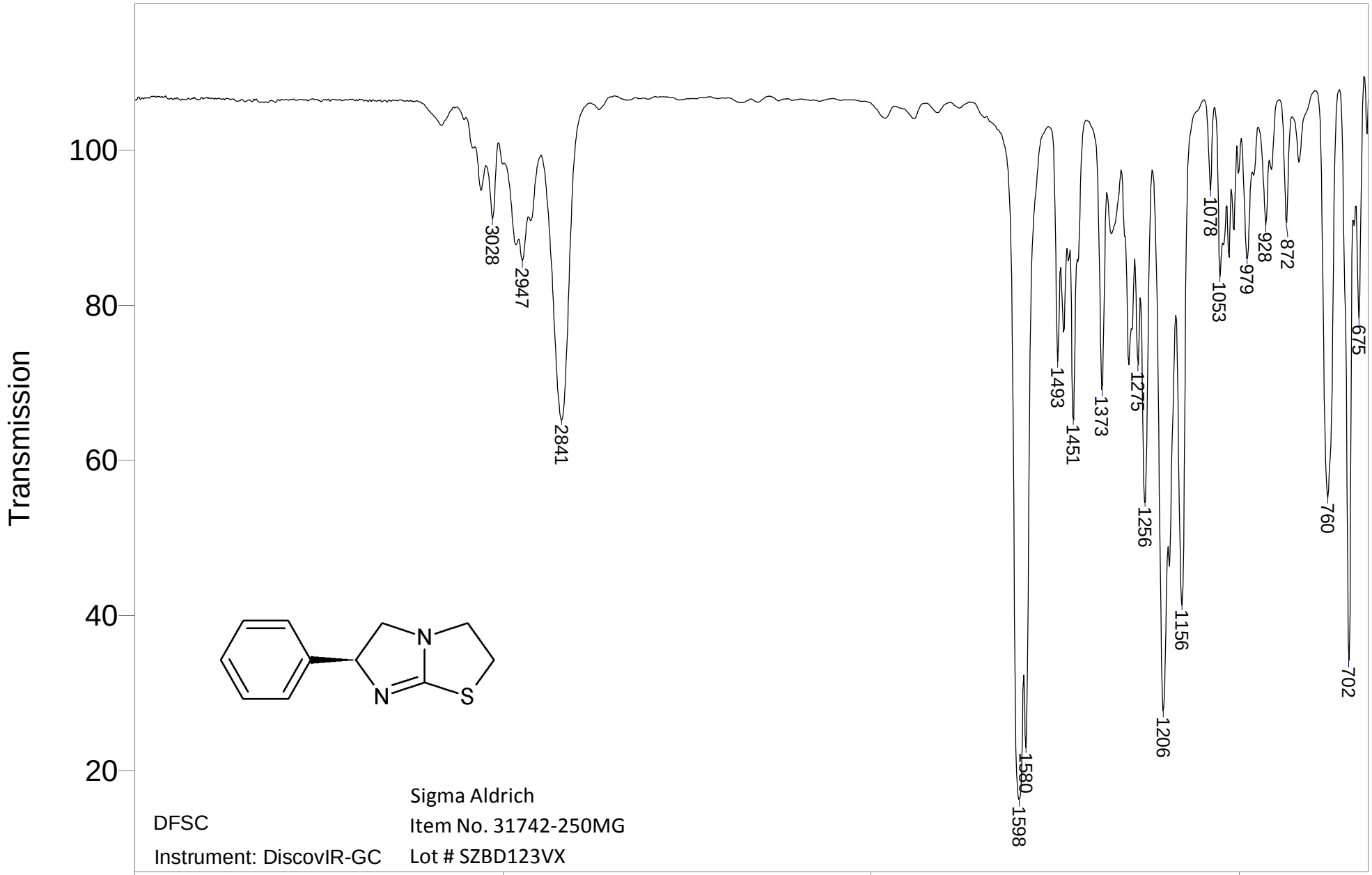


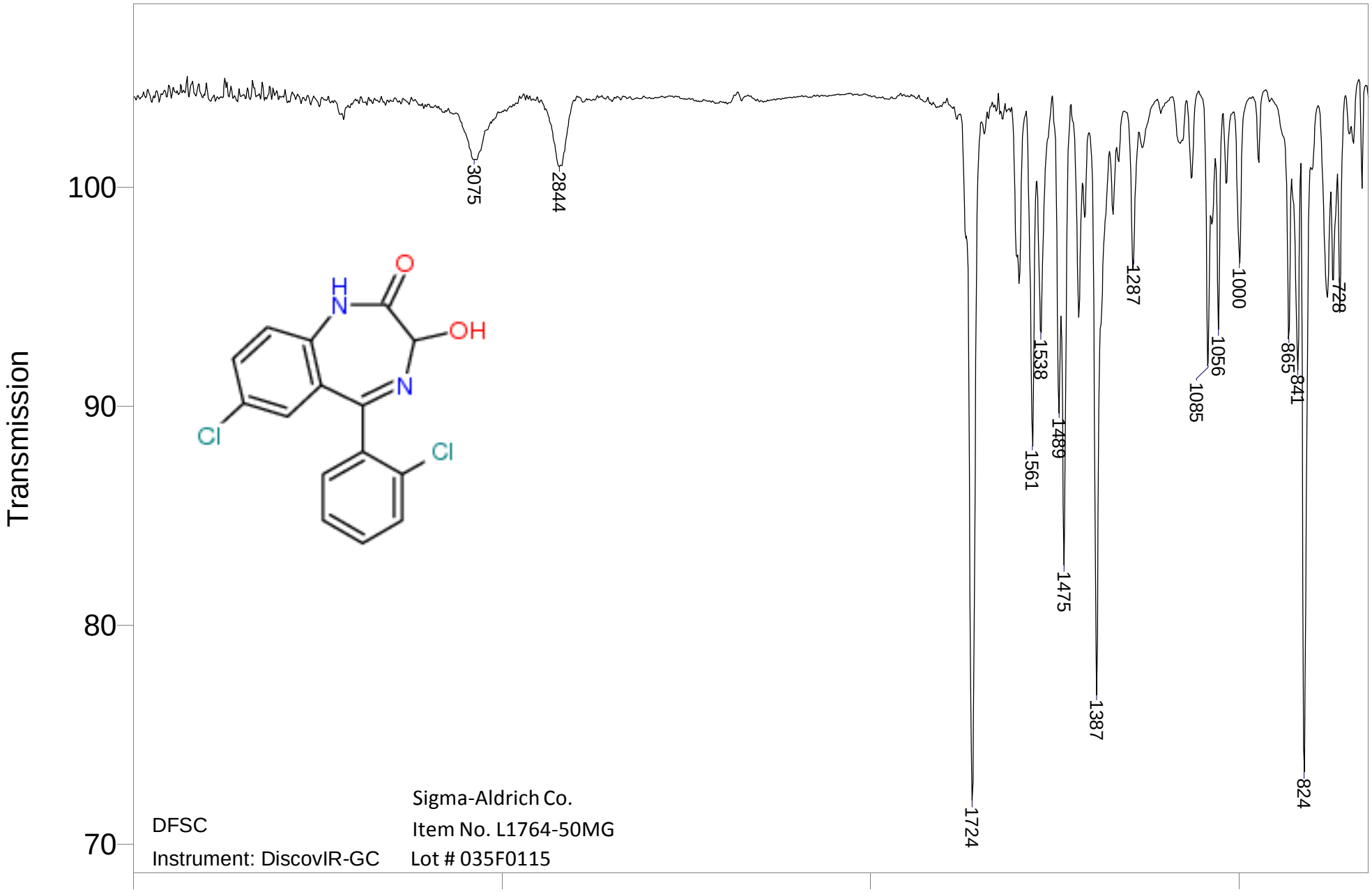
JWH-398 6 isomer

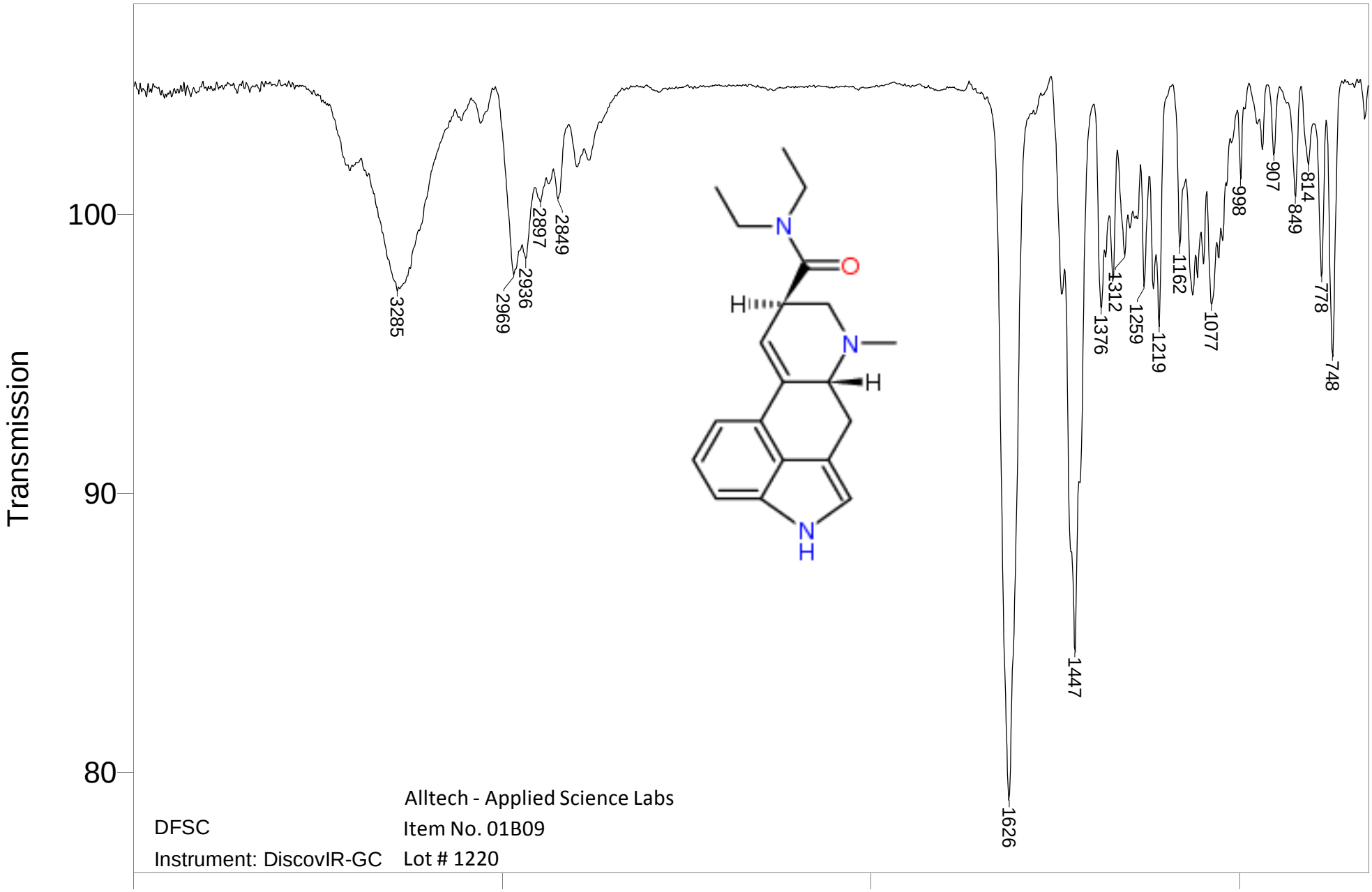


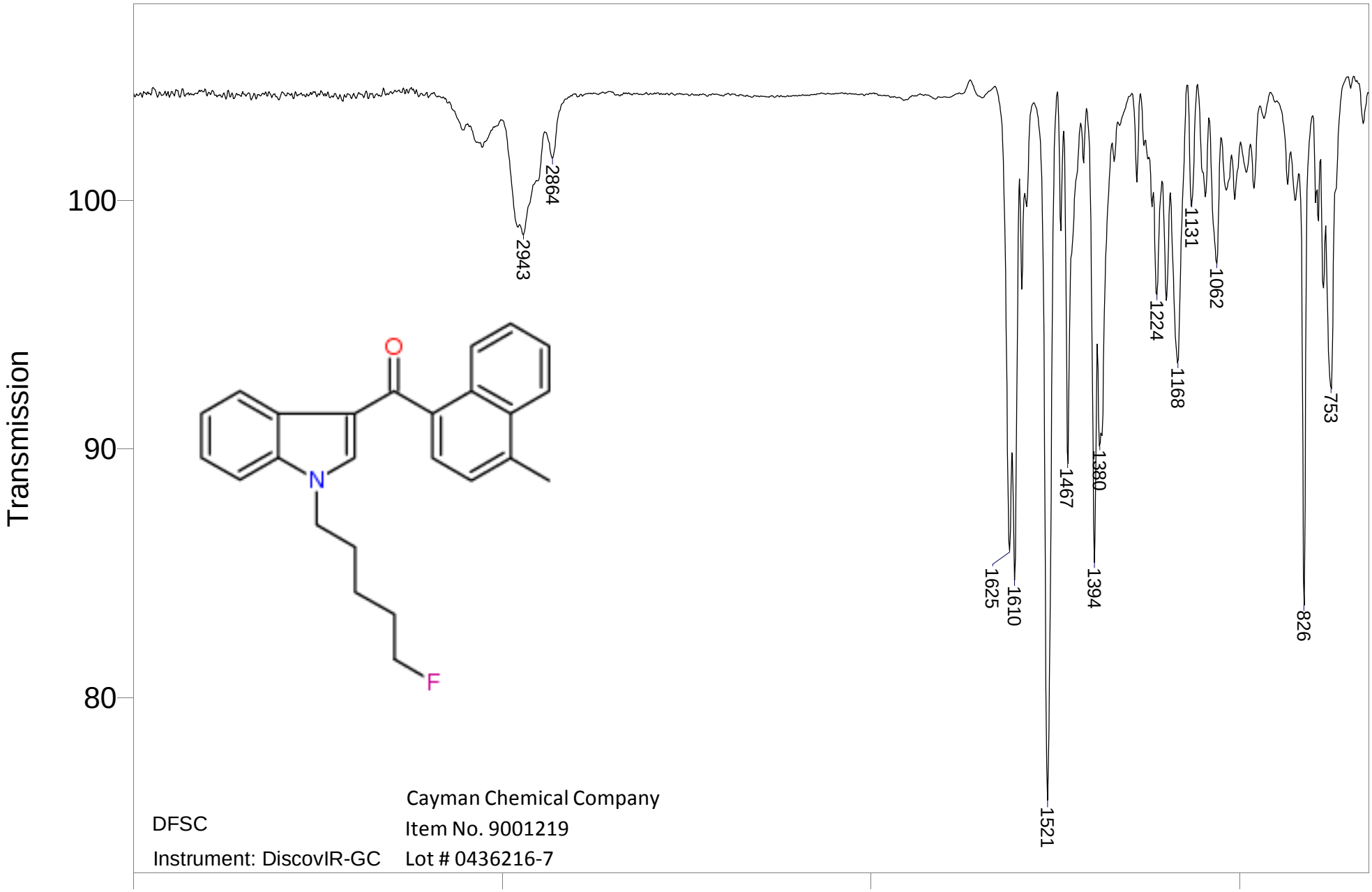


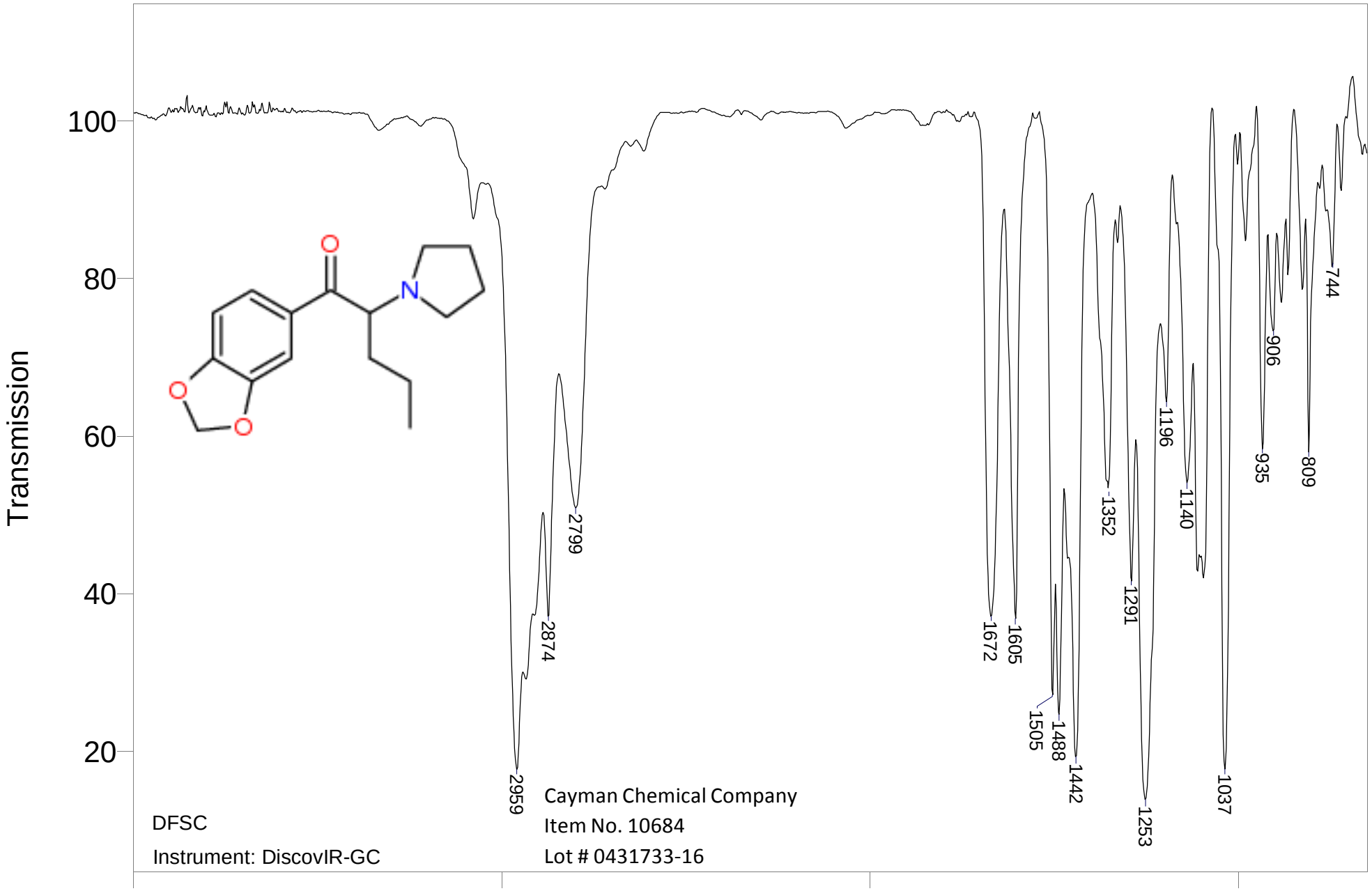


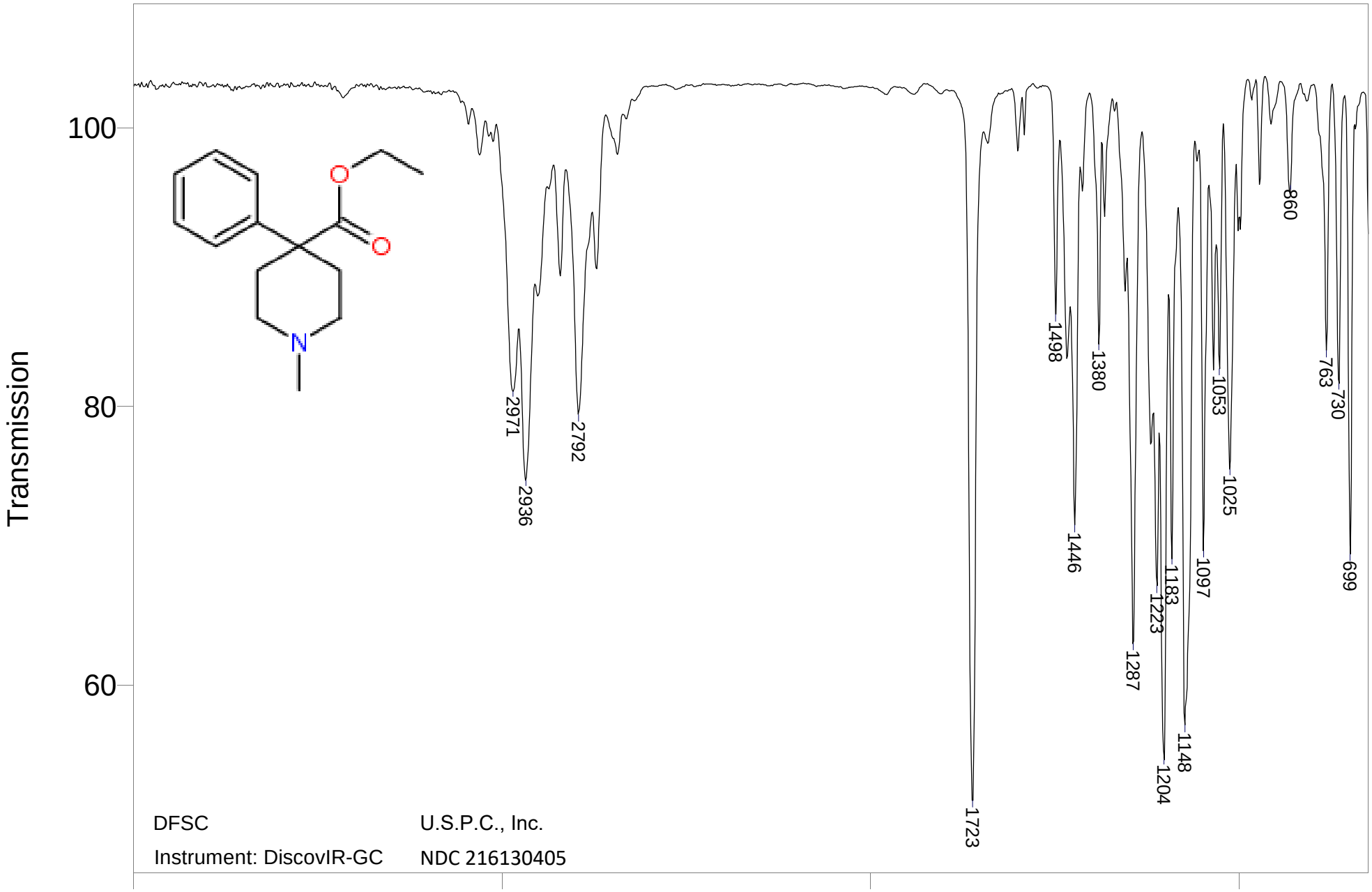


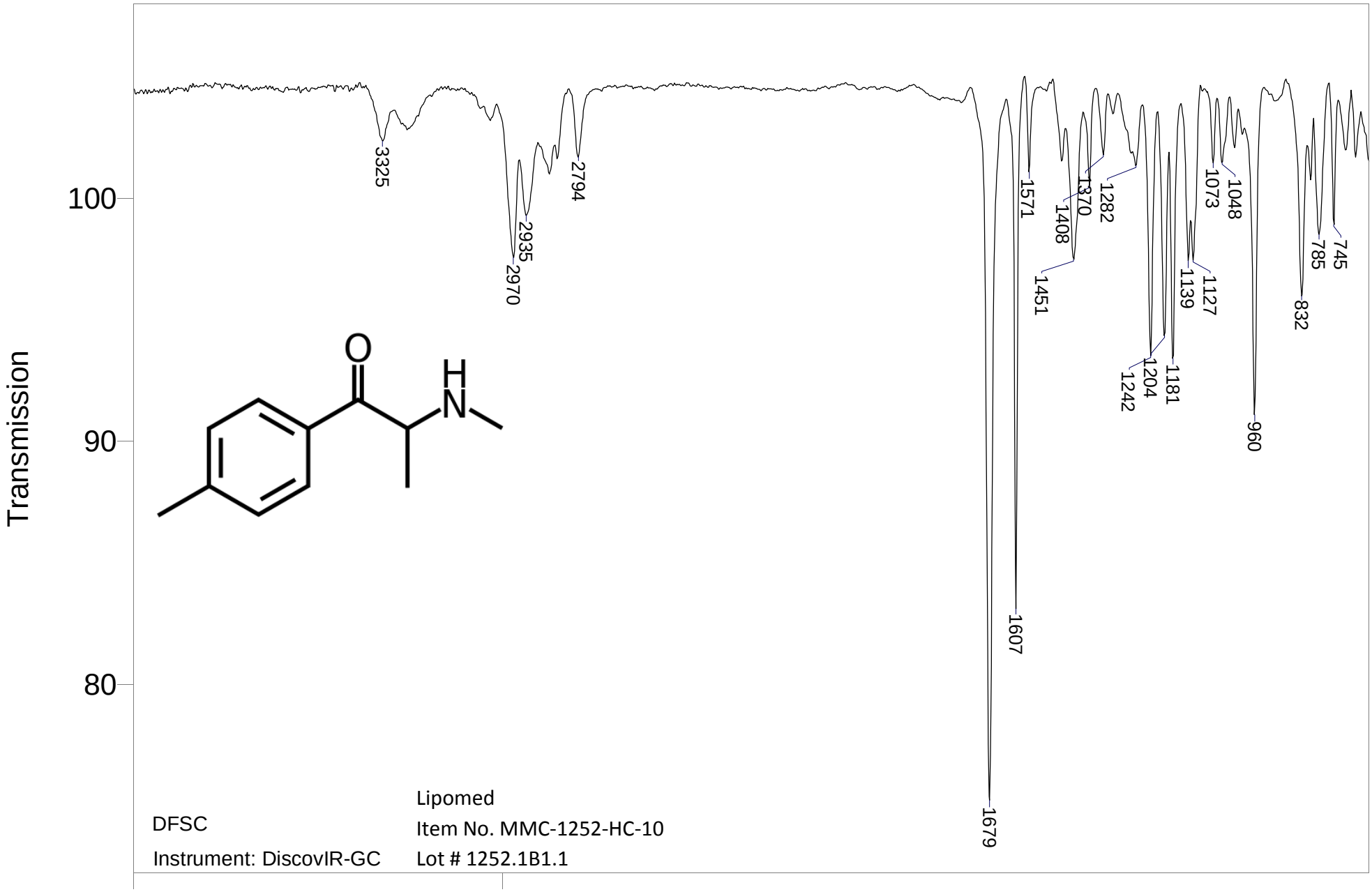


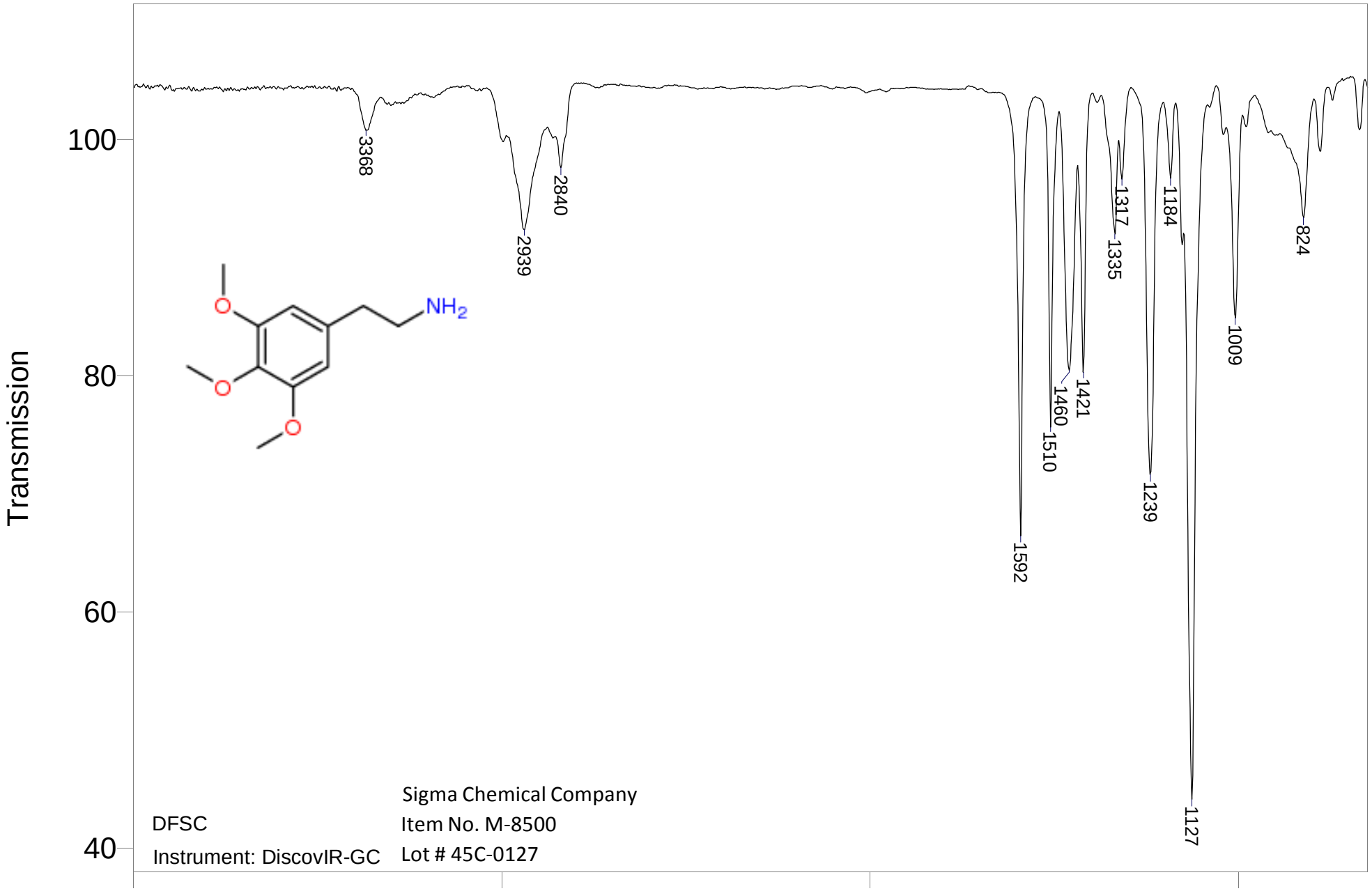


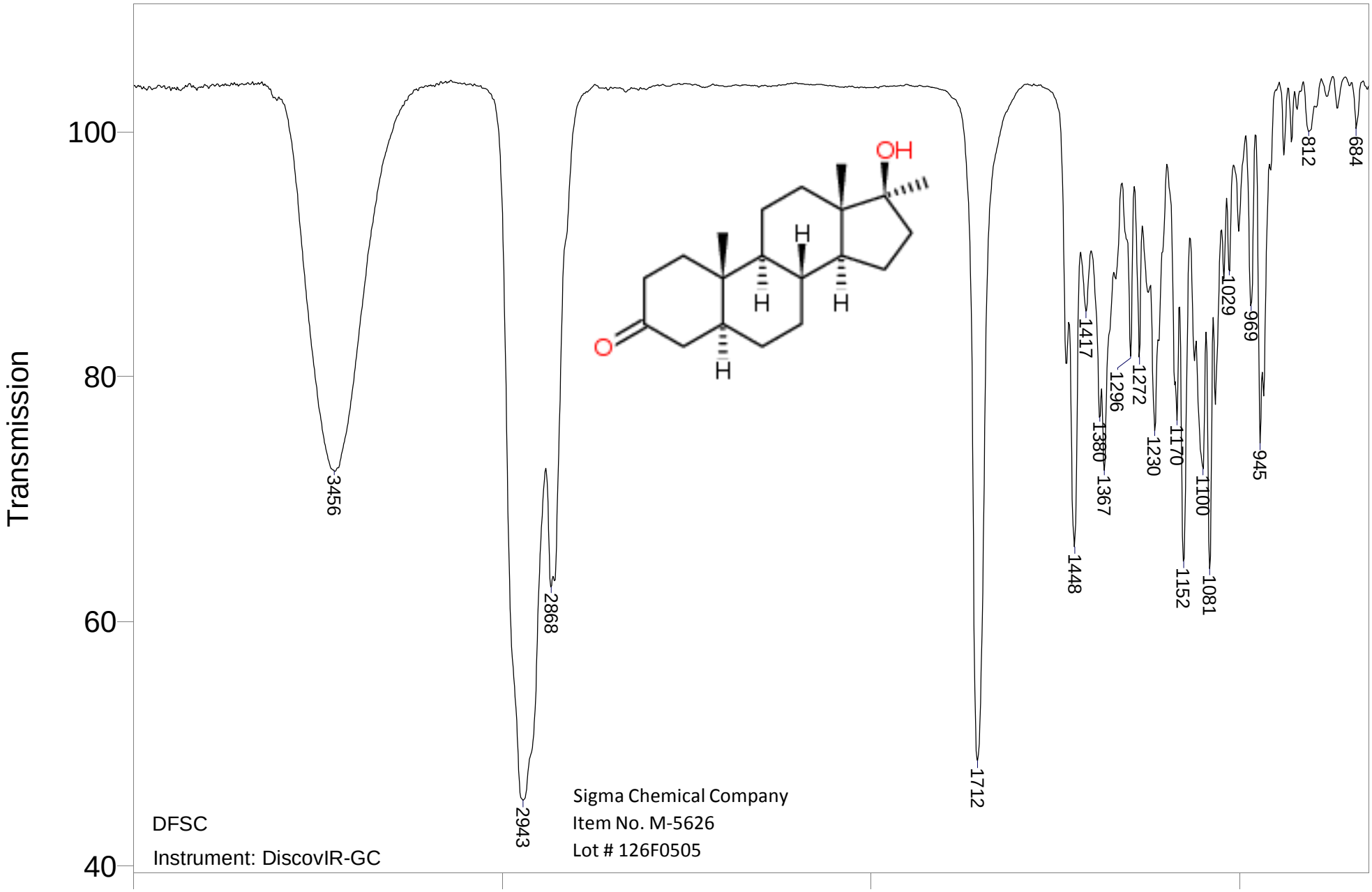


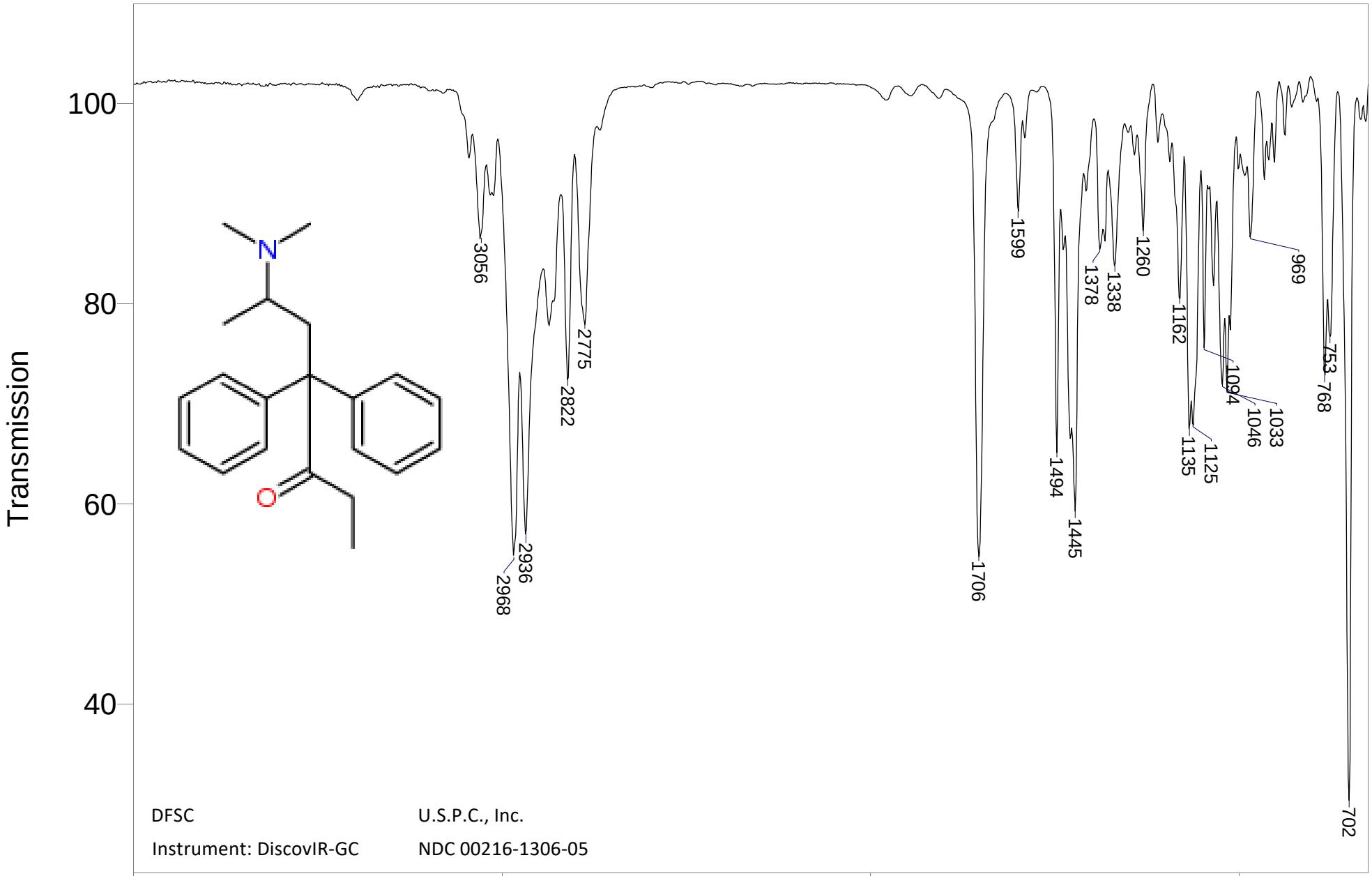




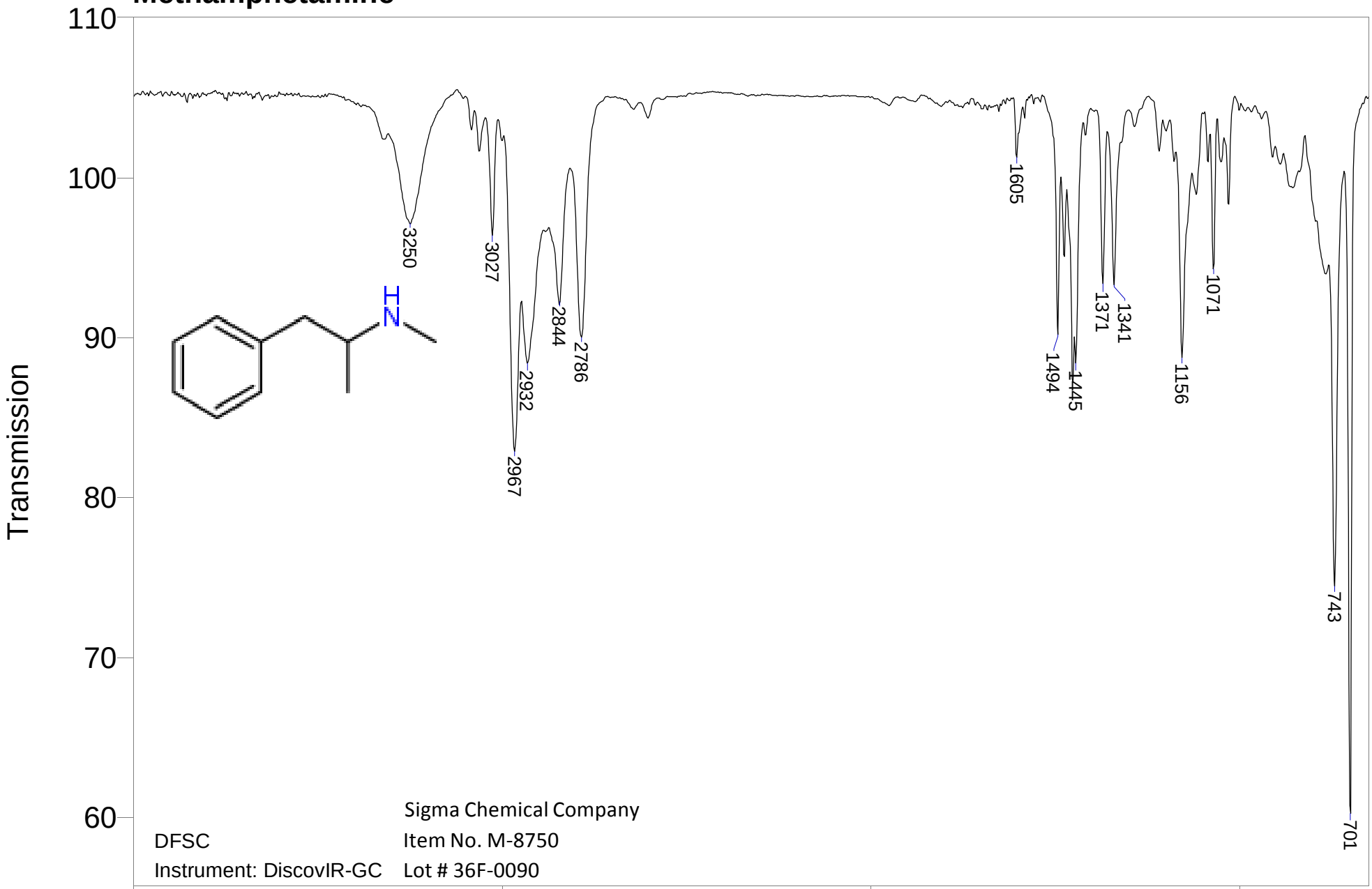


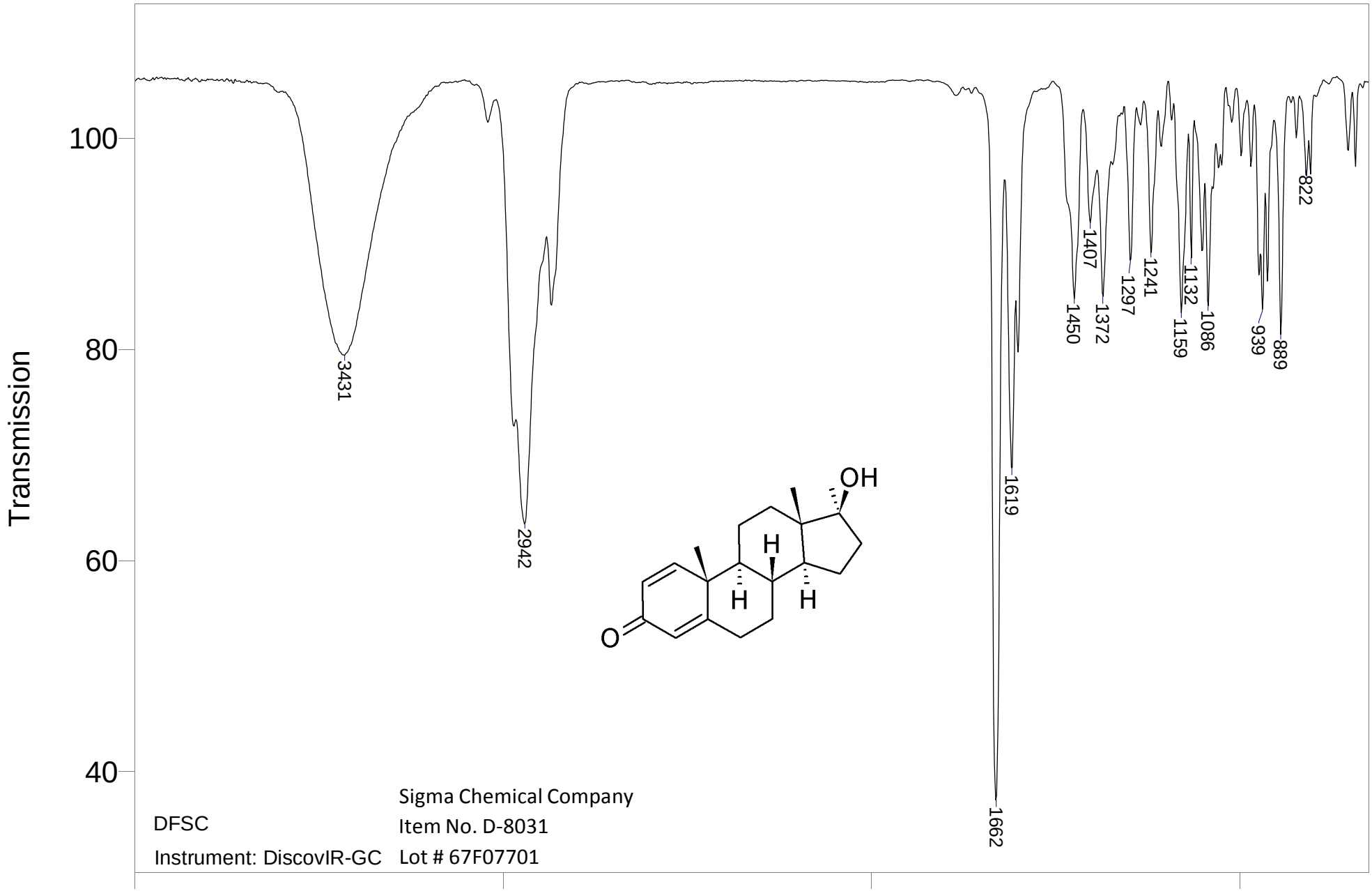


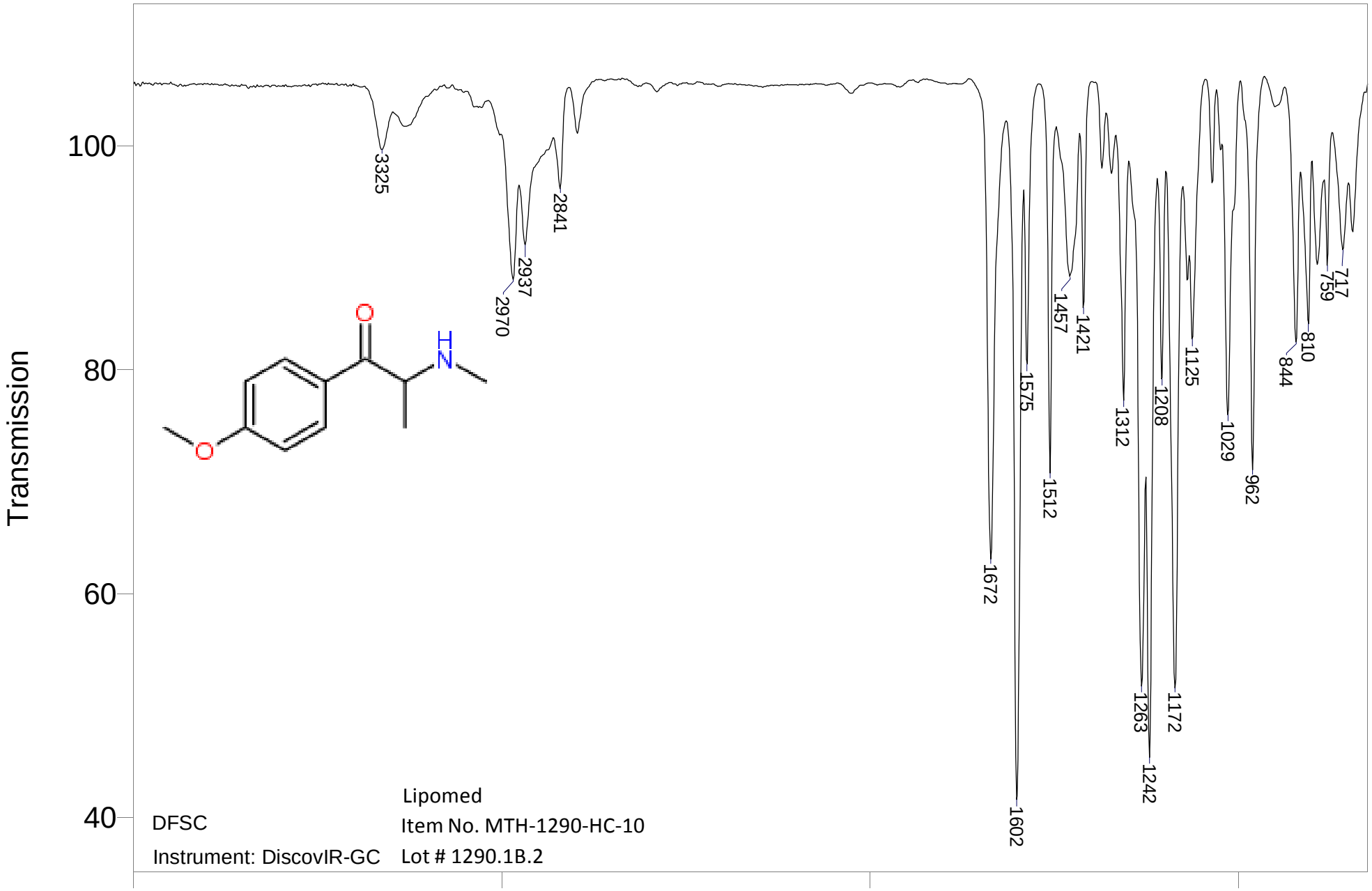


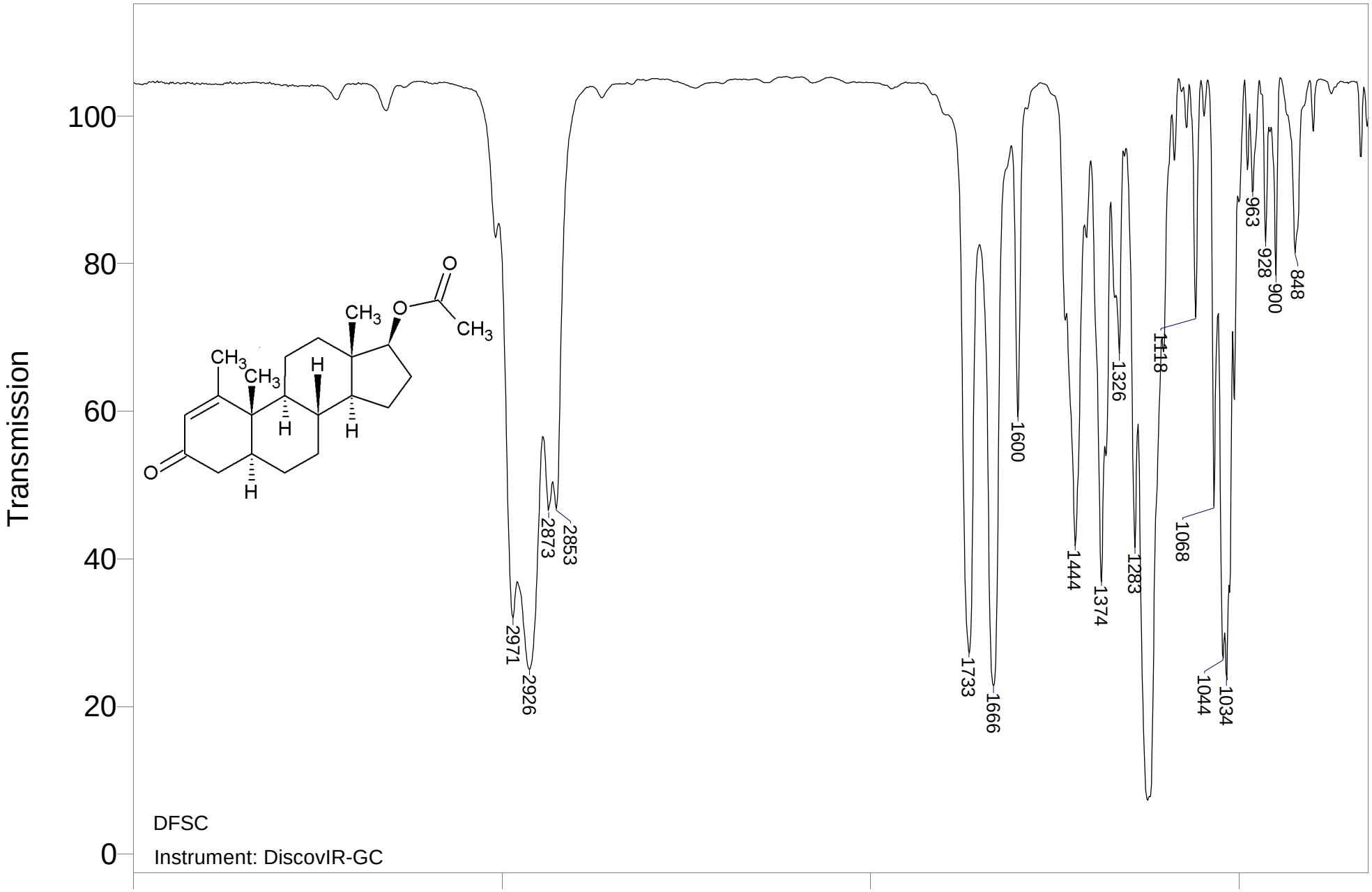


Methamphetamine

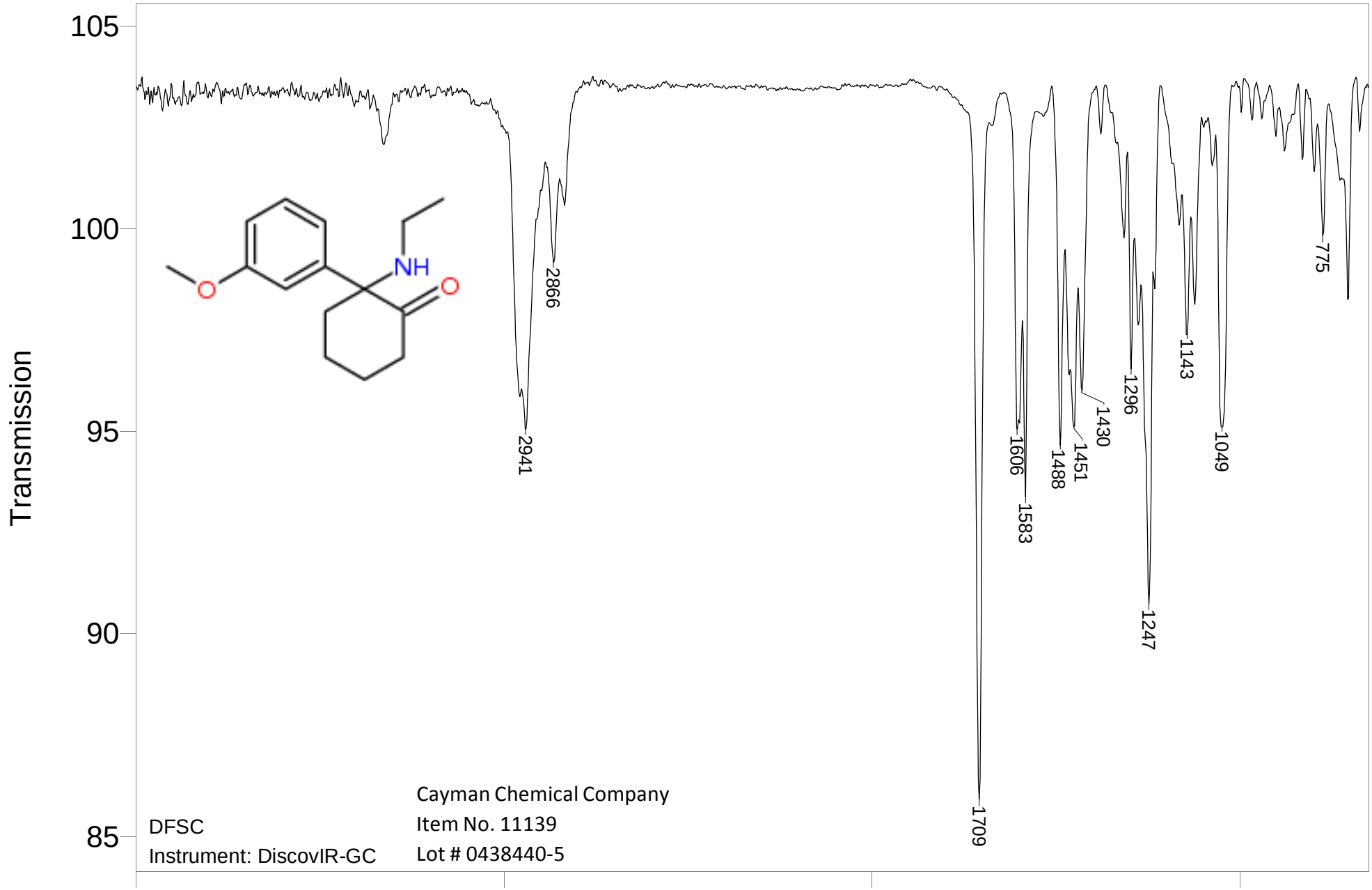


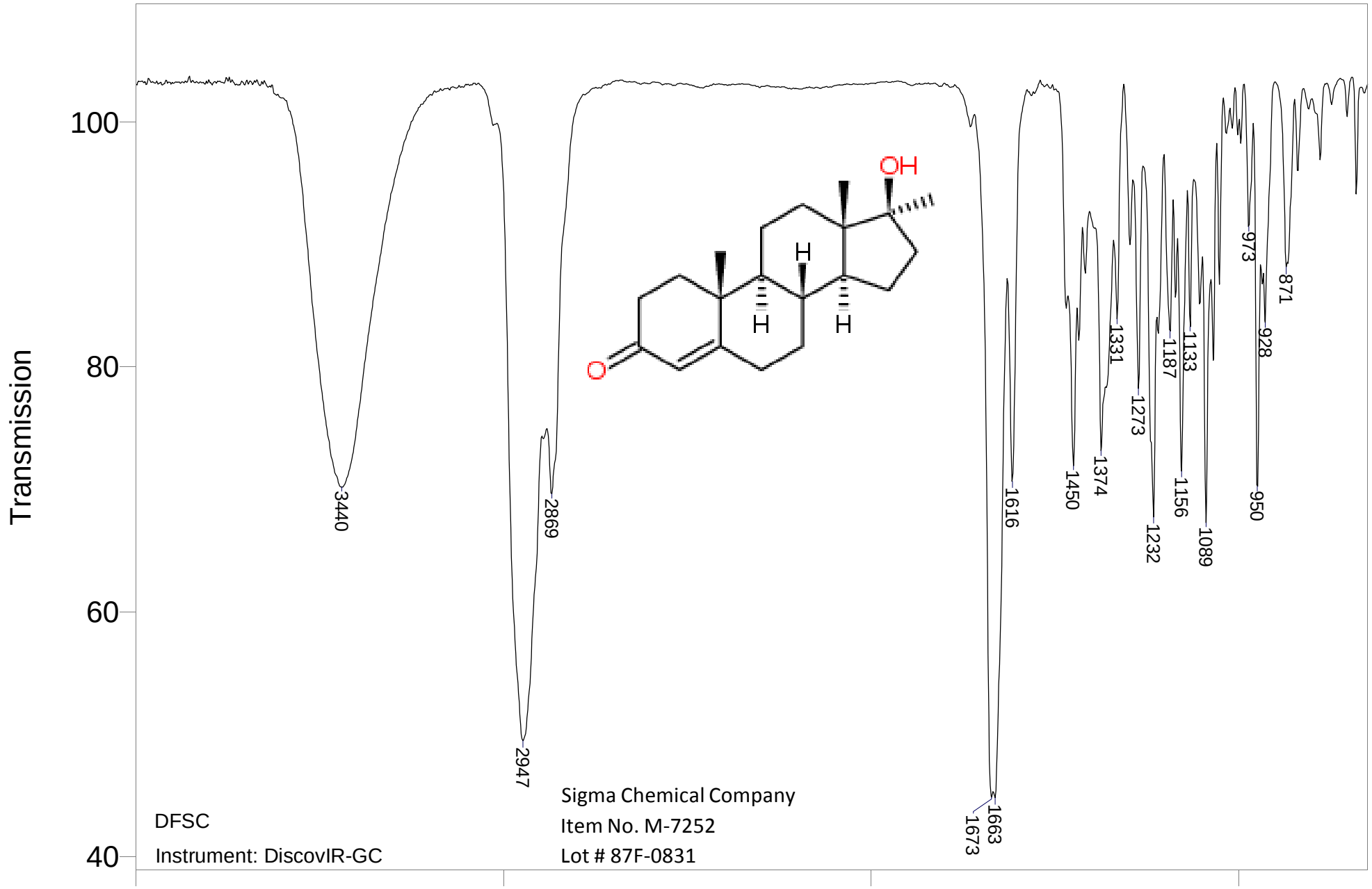


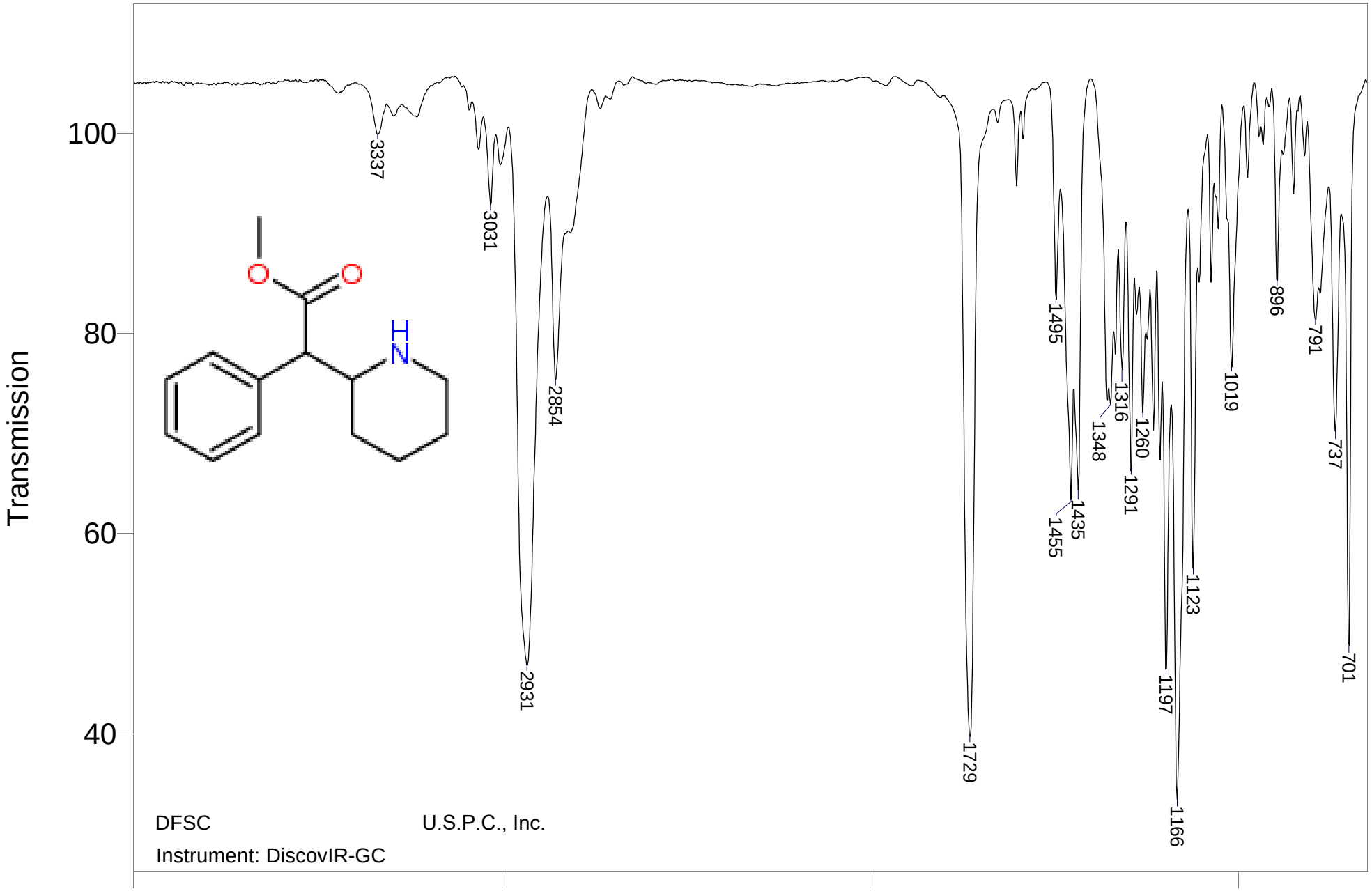


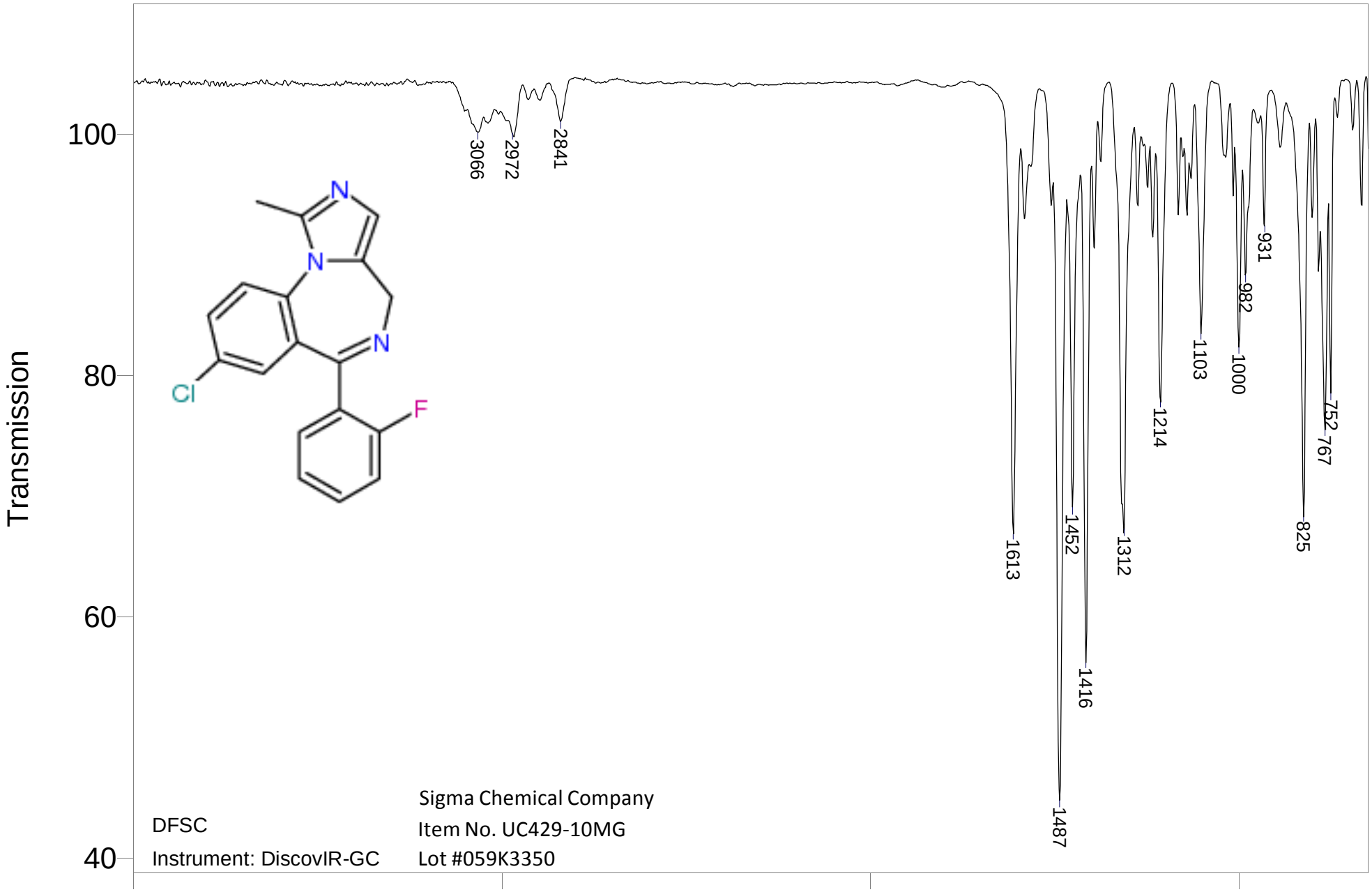


Methoxetamine

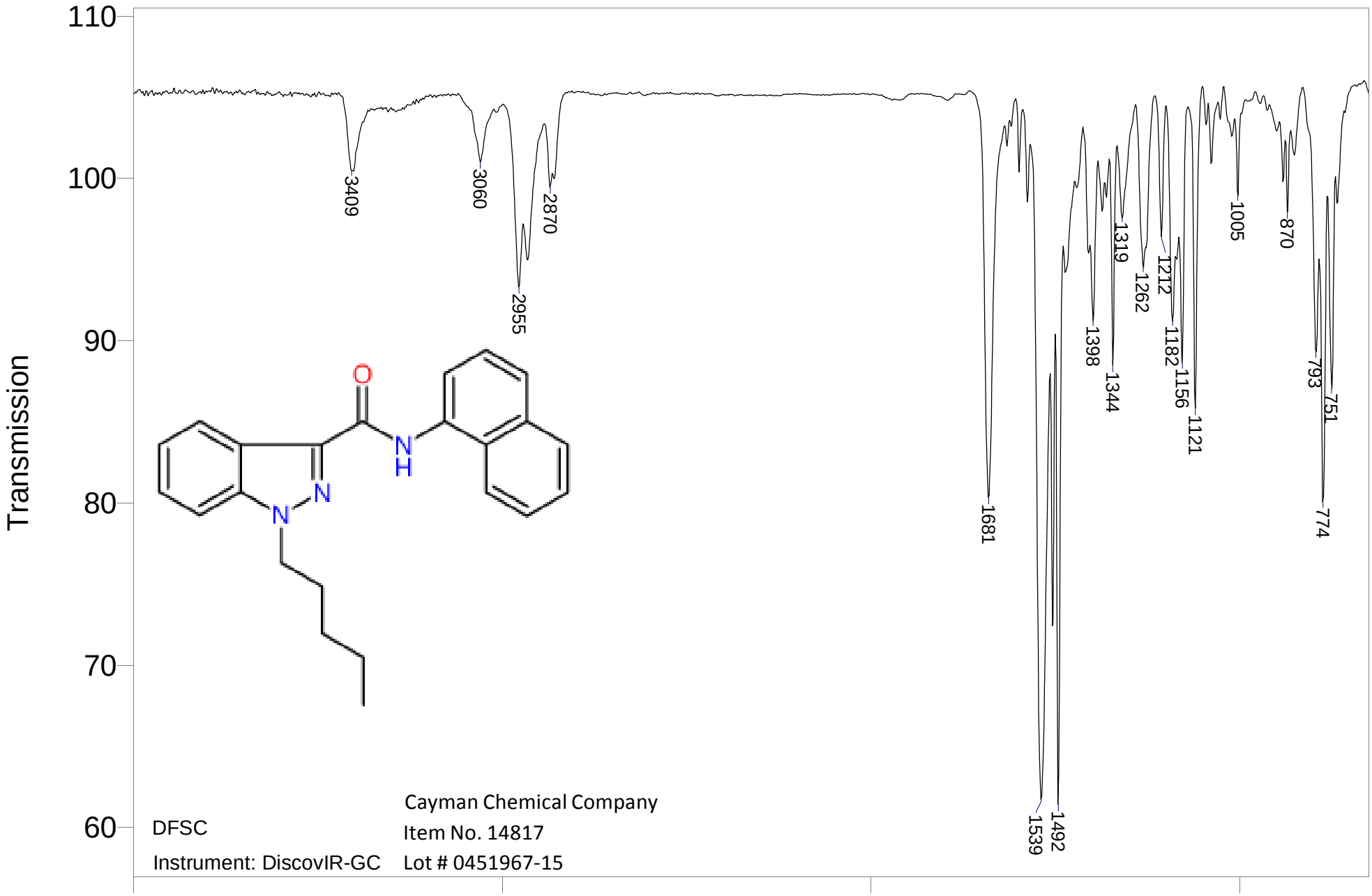


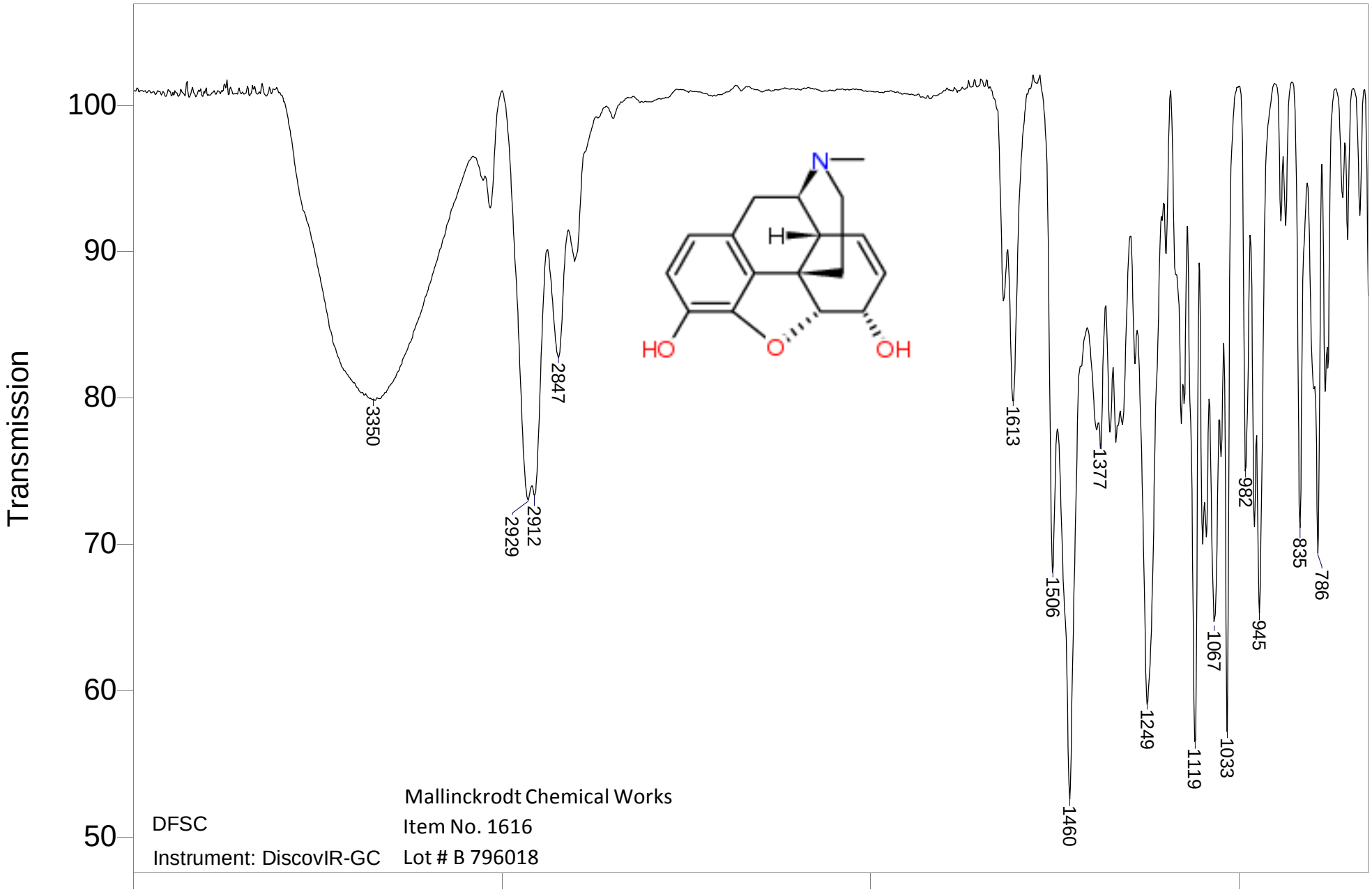


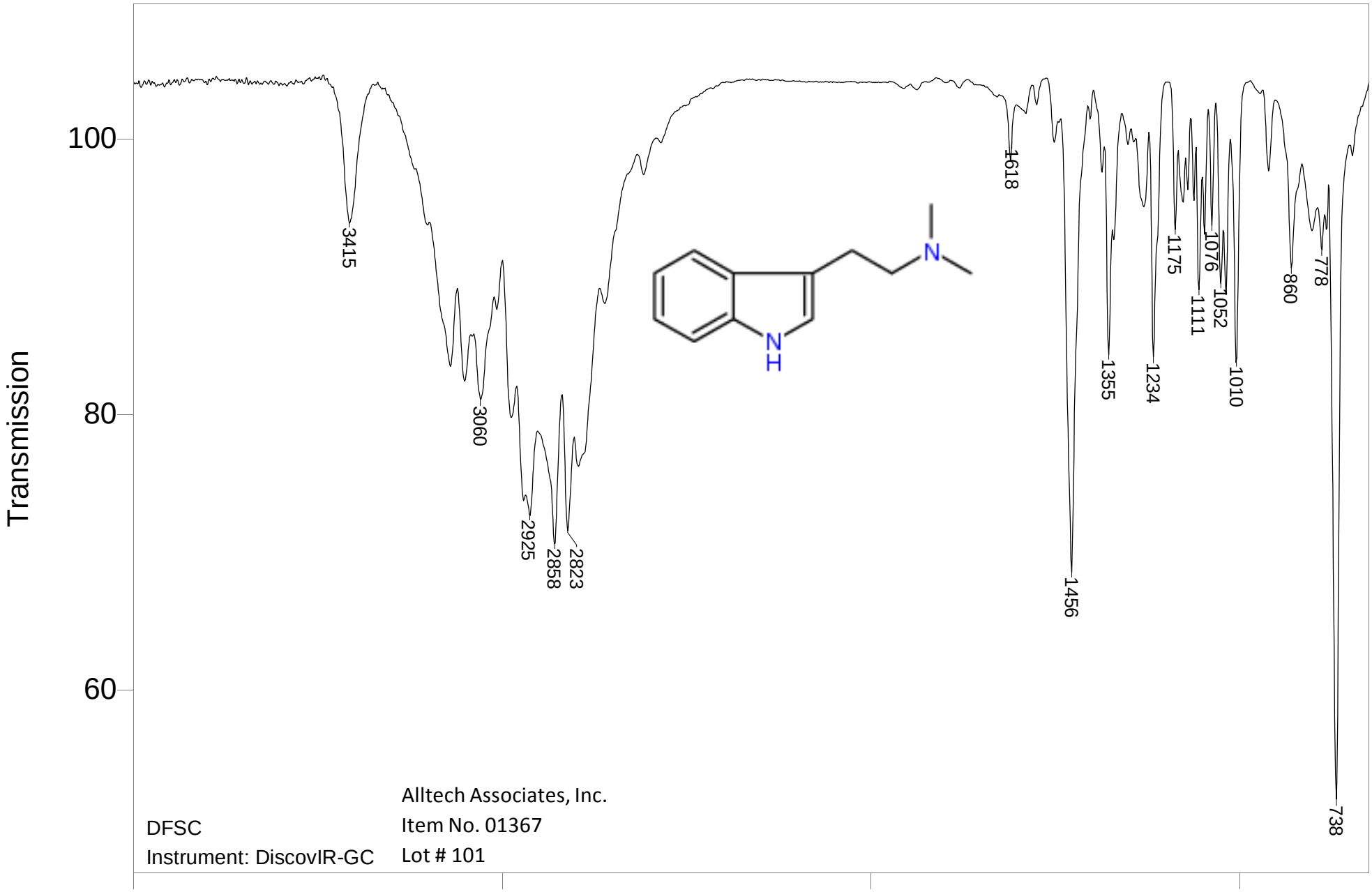


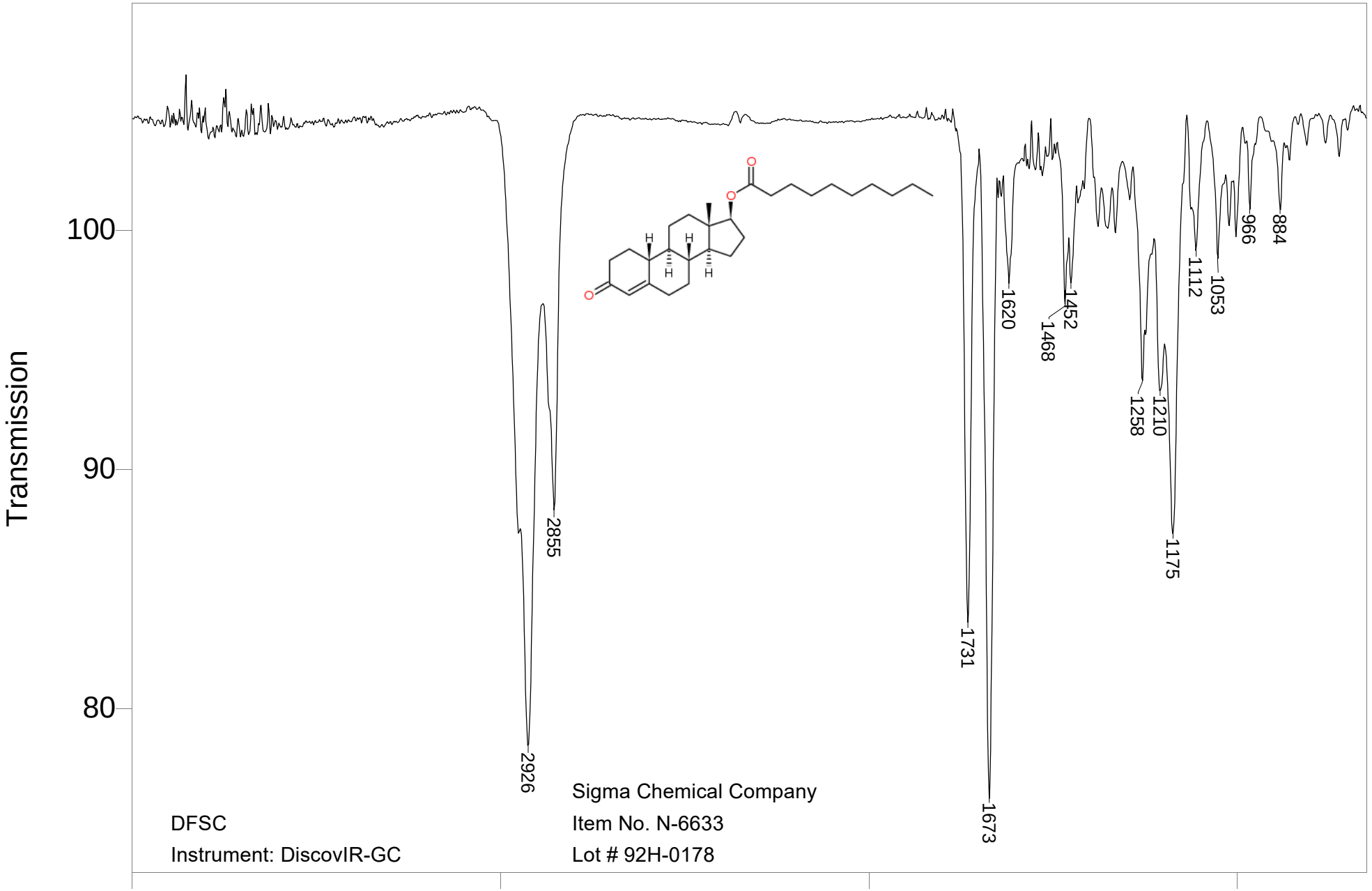


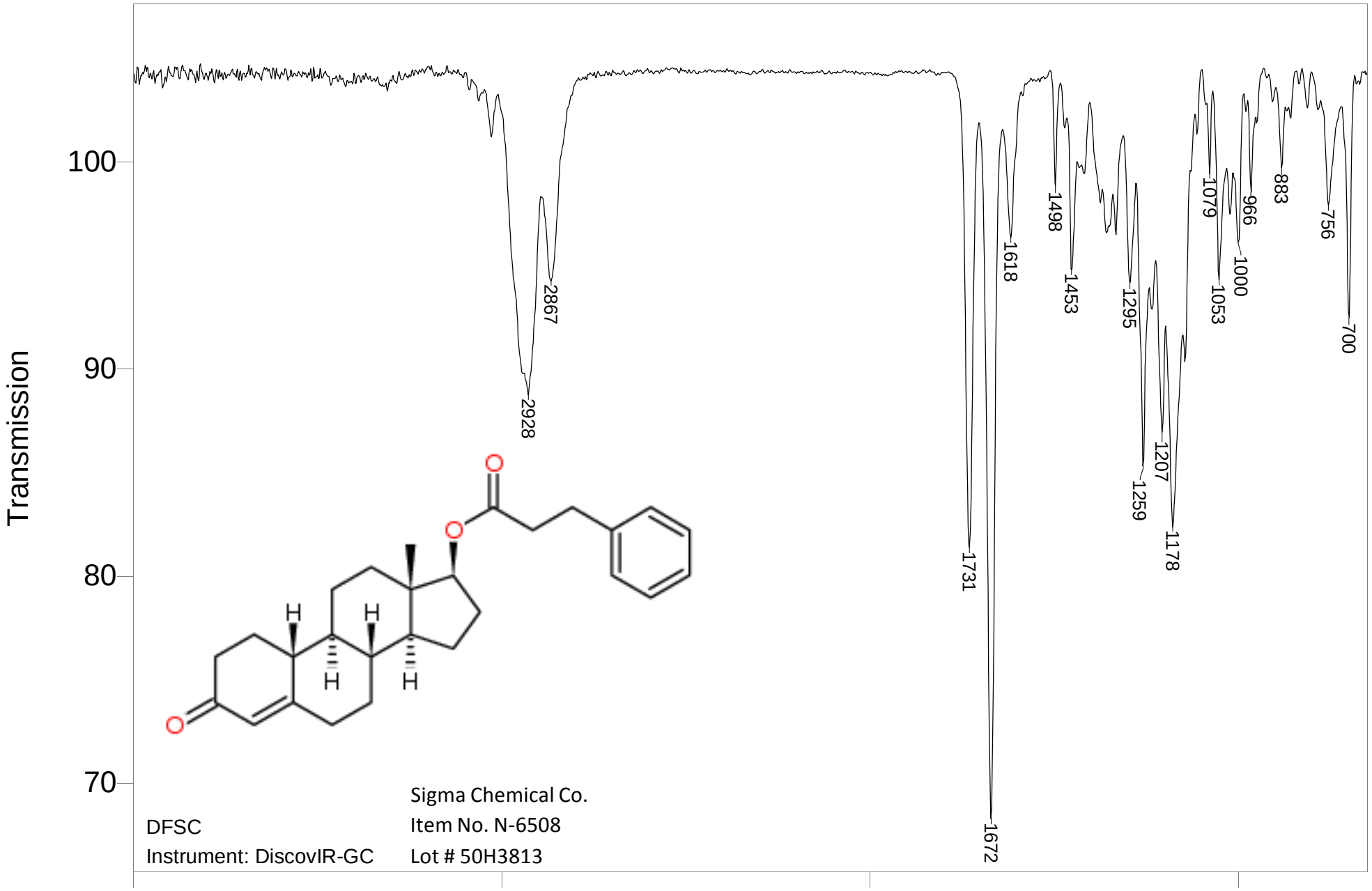
MN-18











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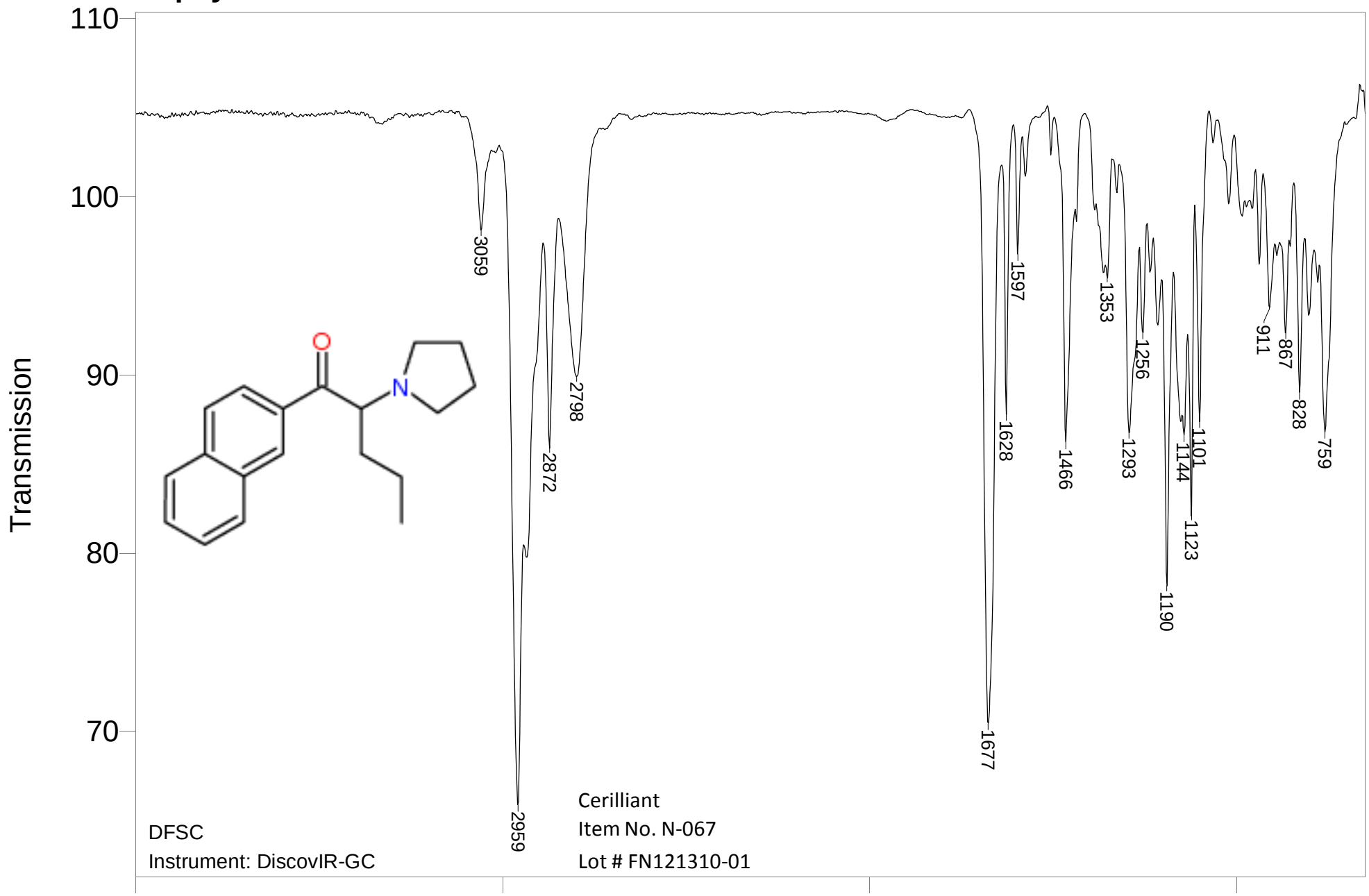
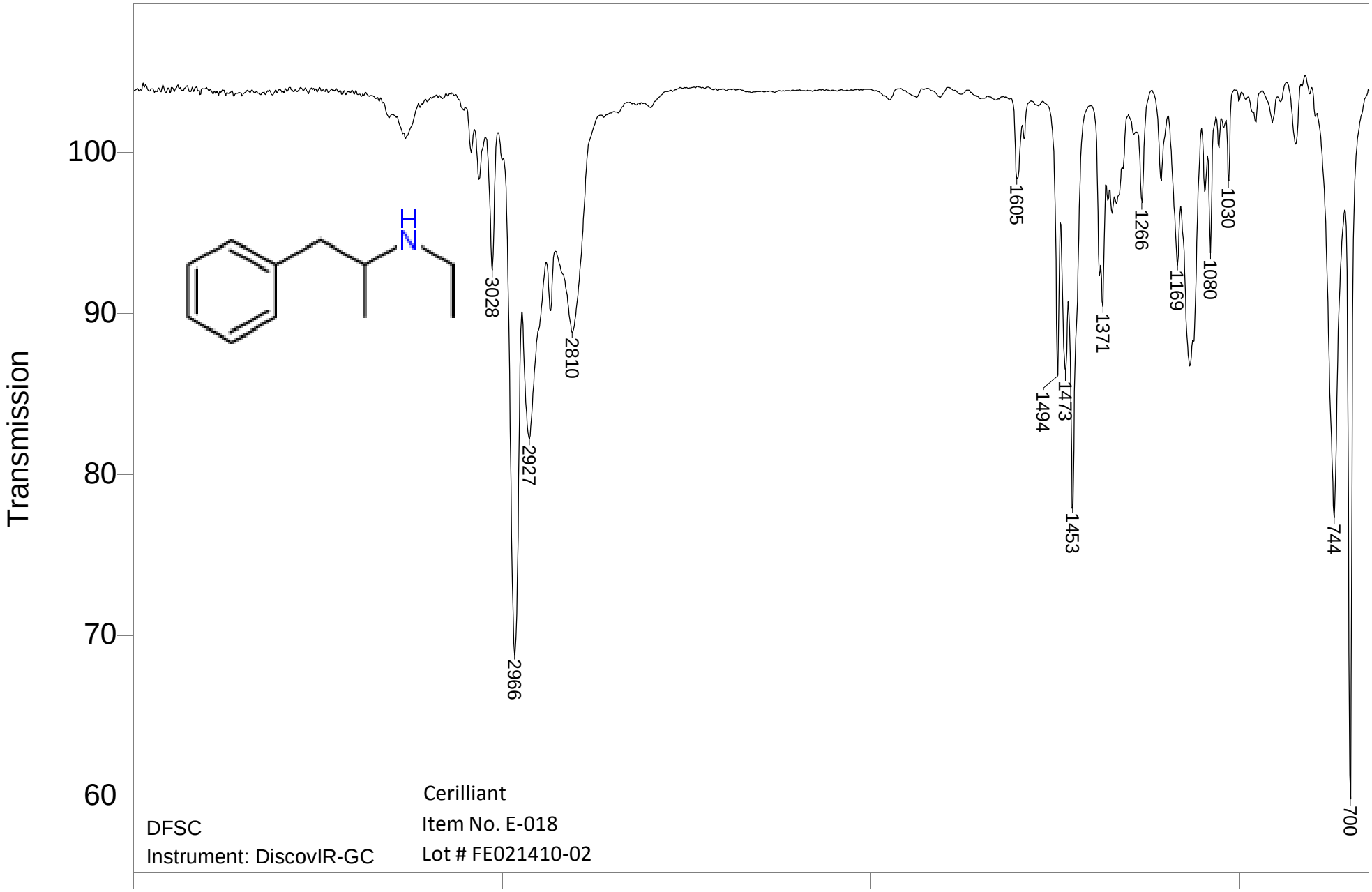
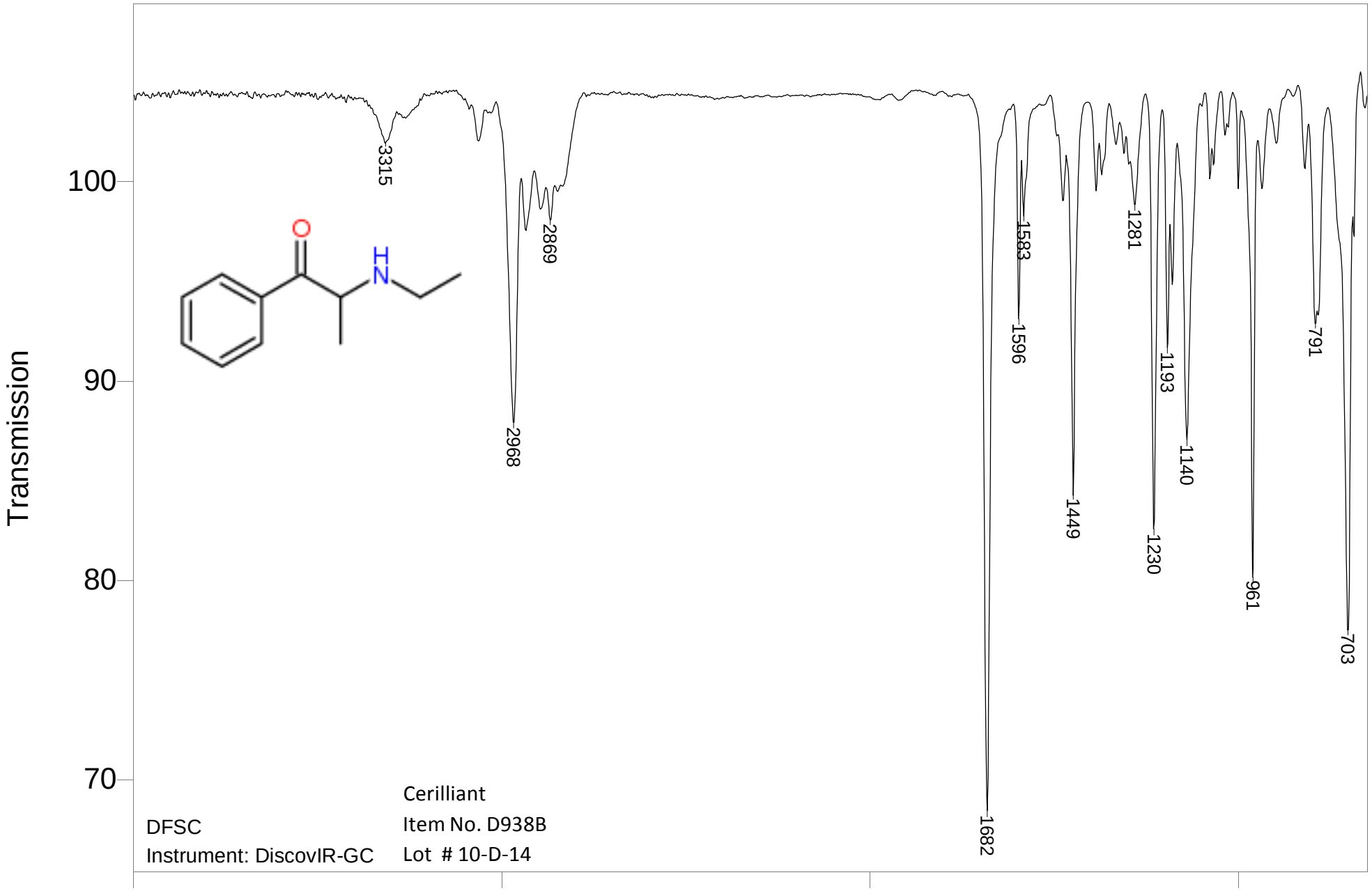
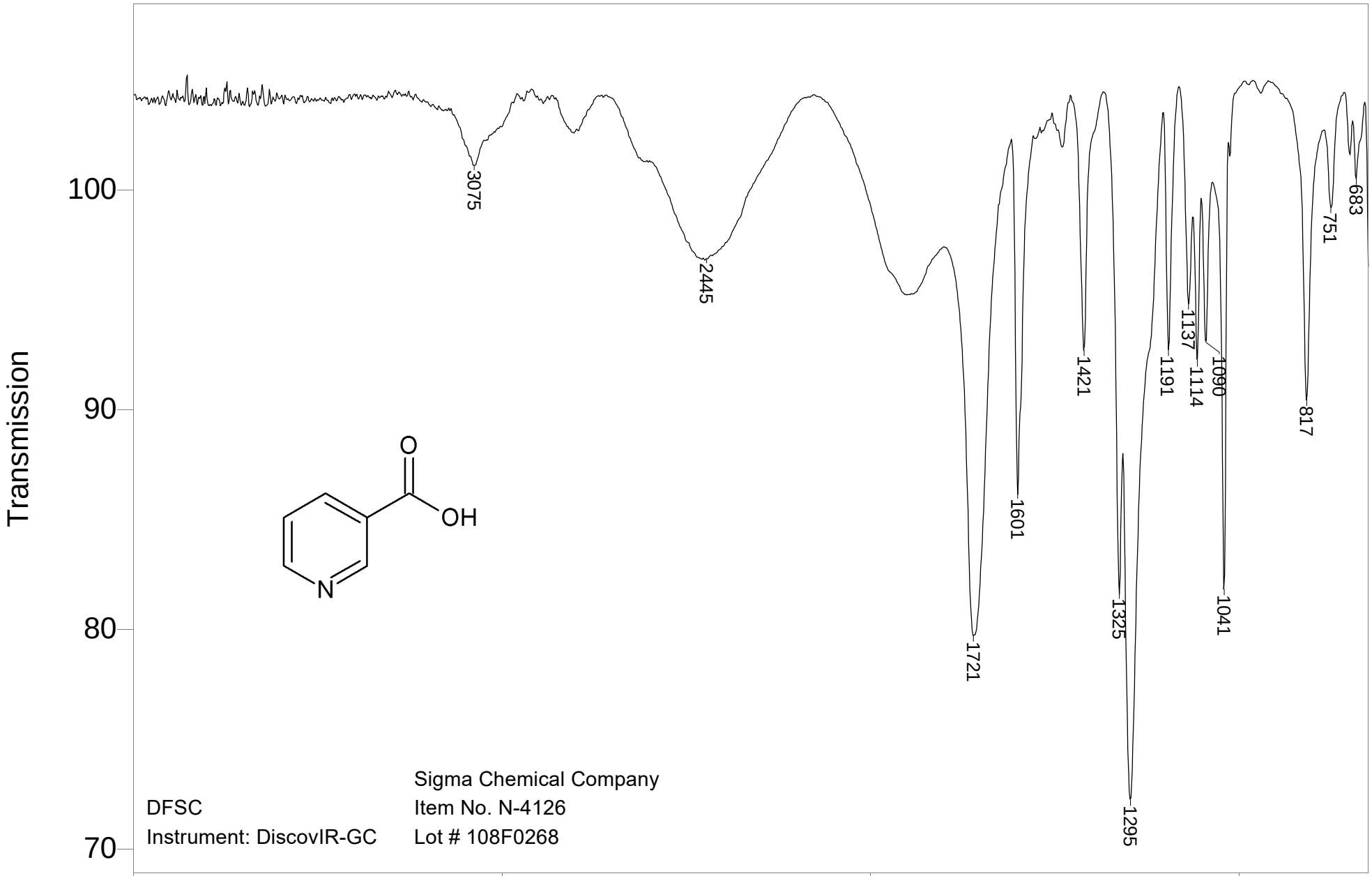
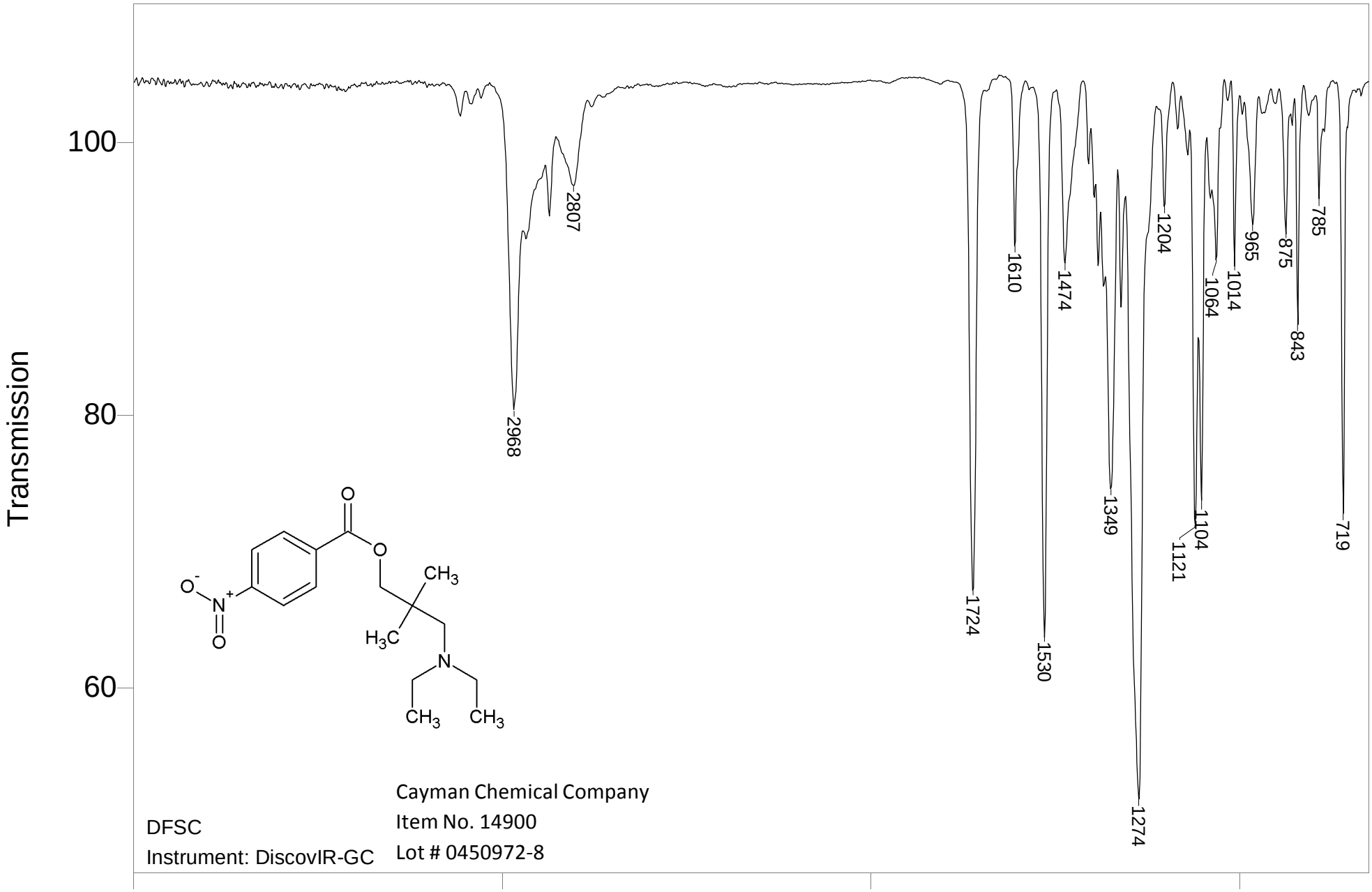
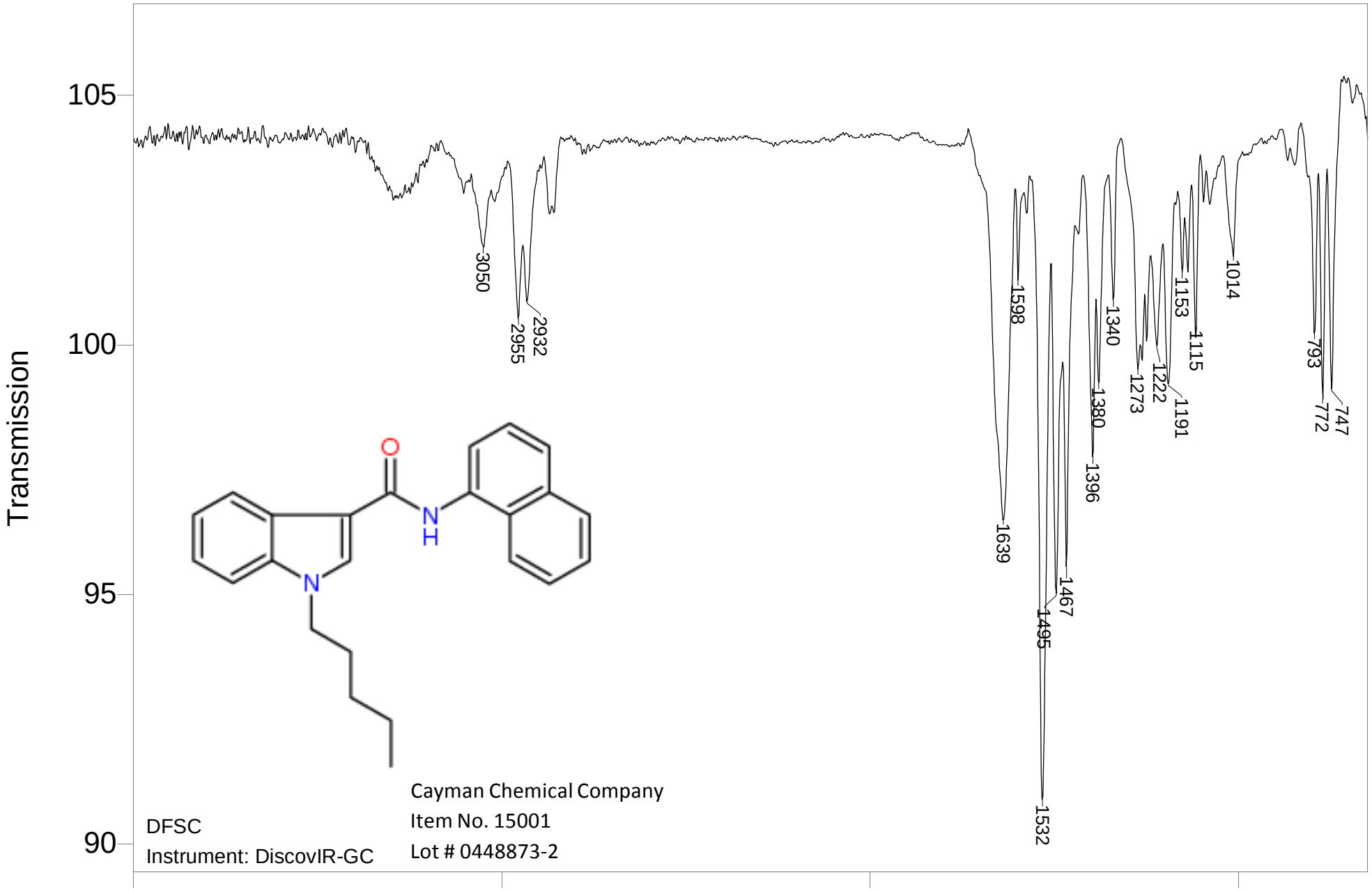


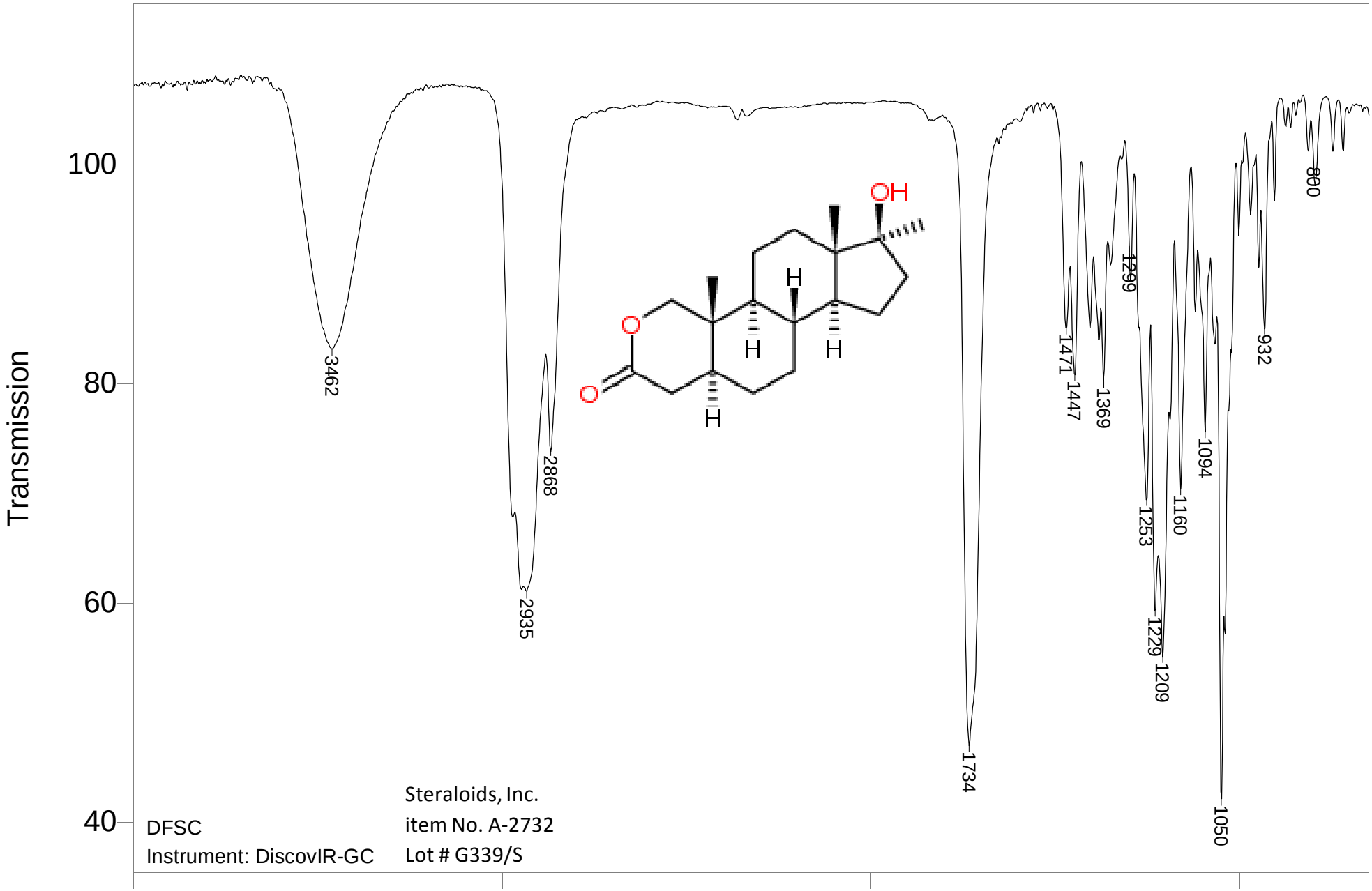


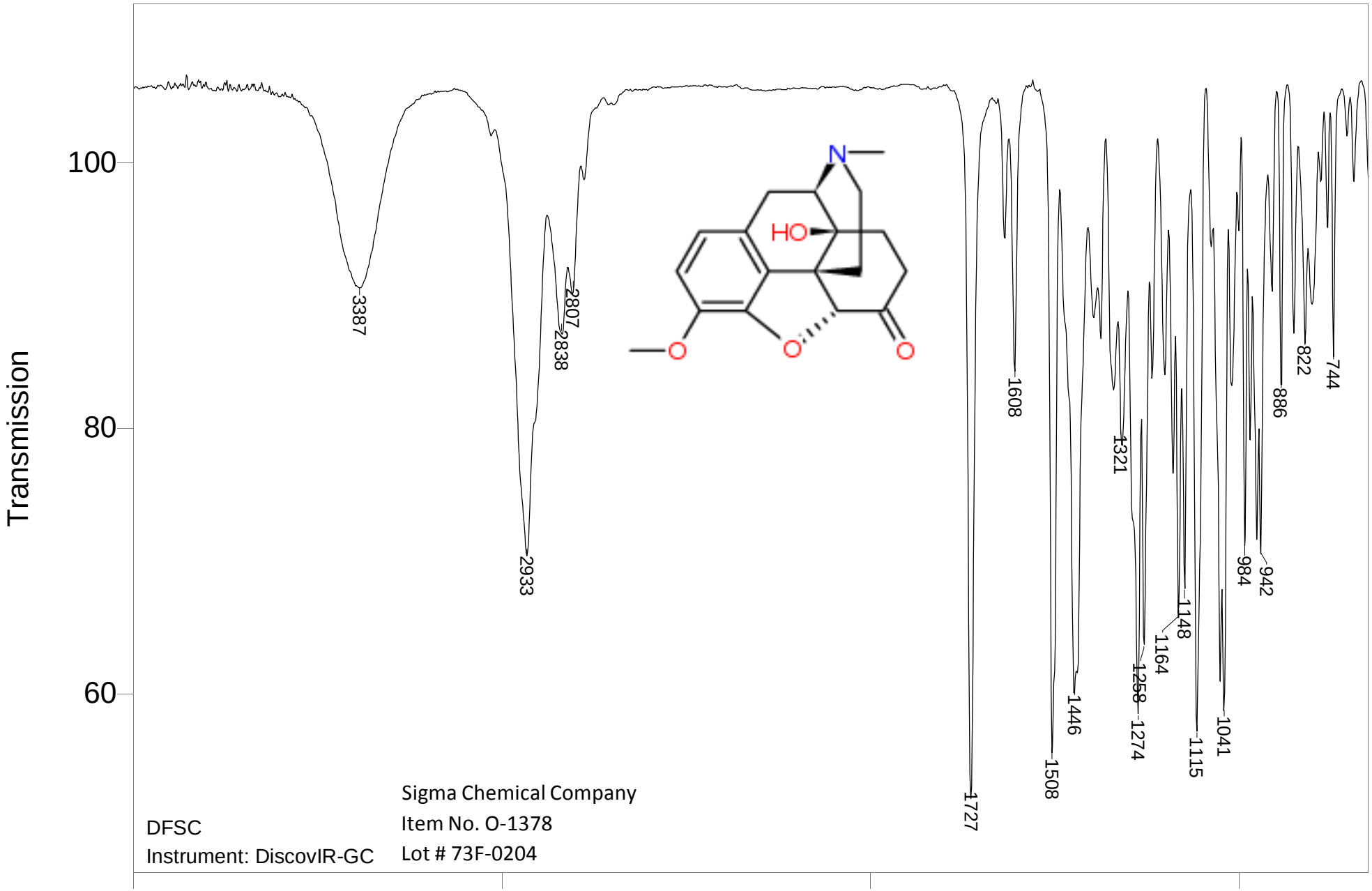


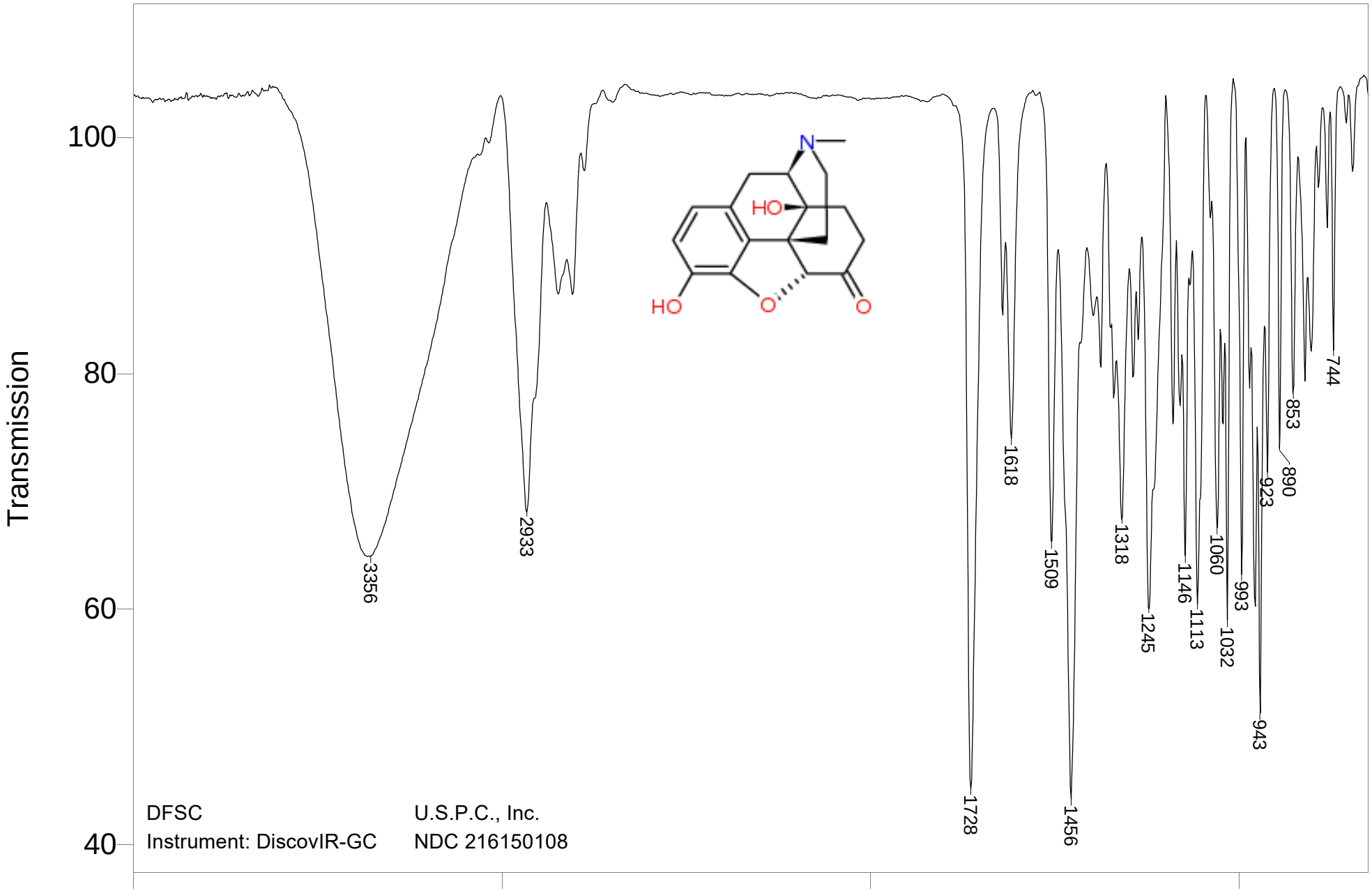


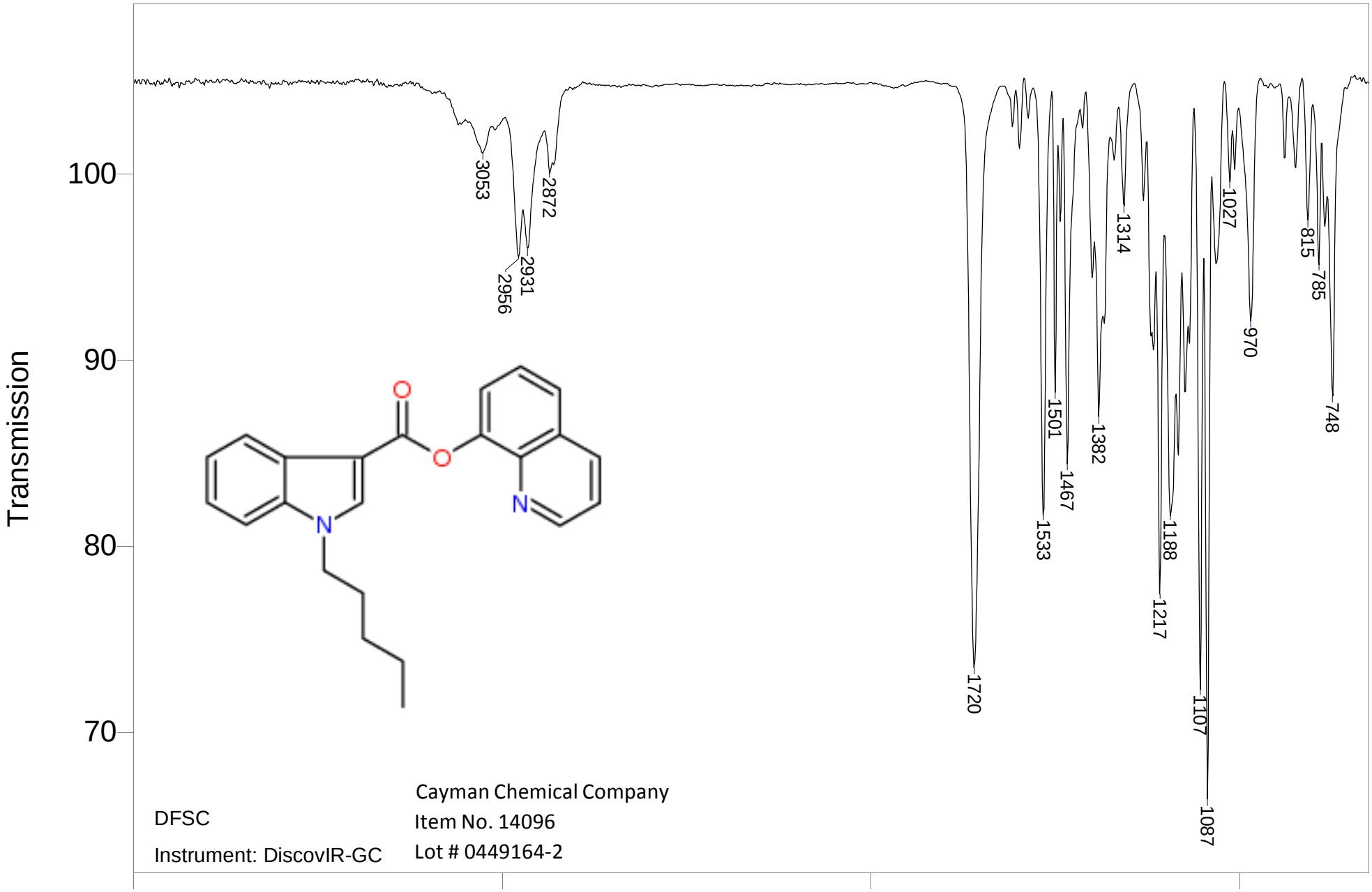


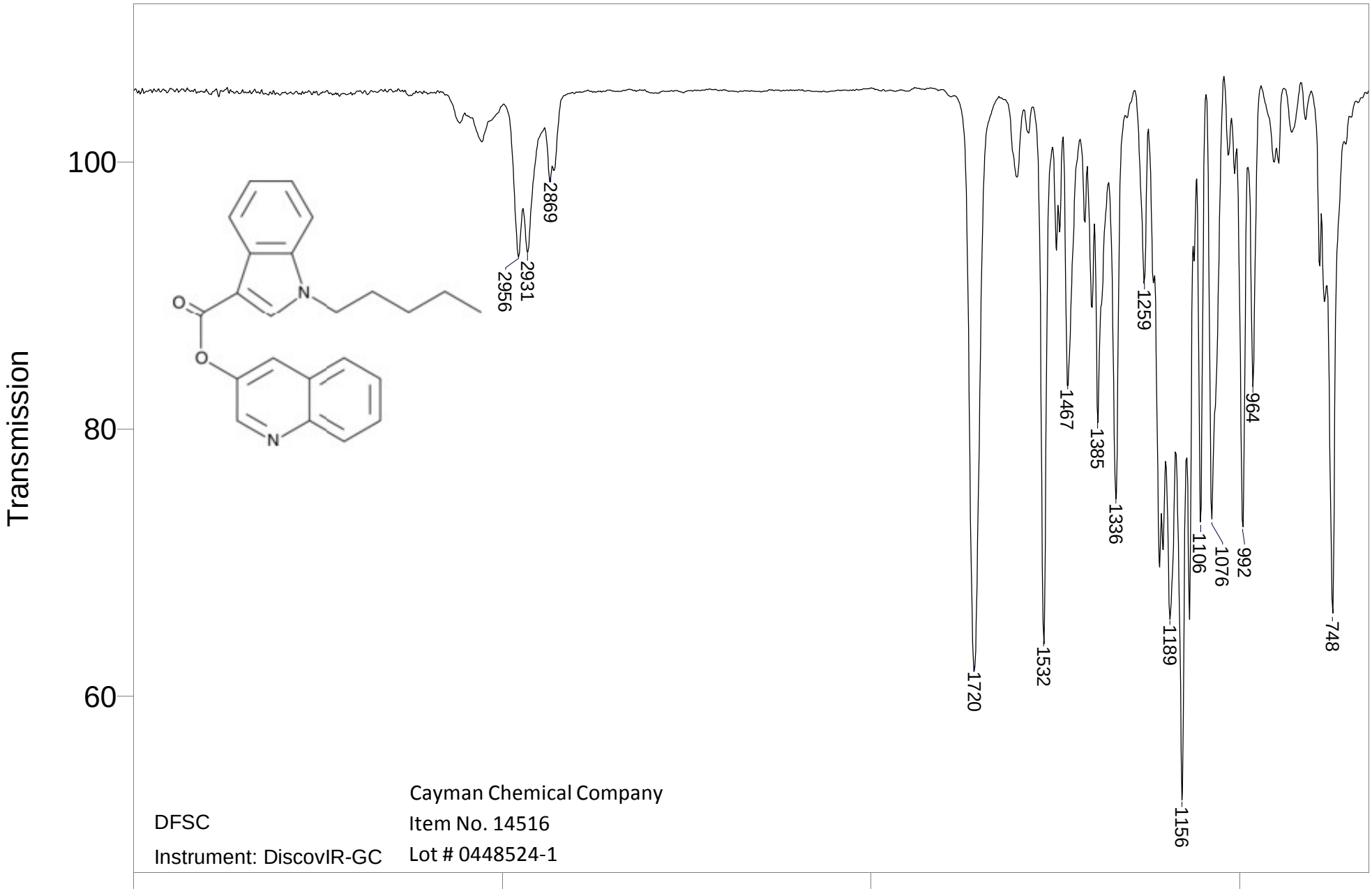


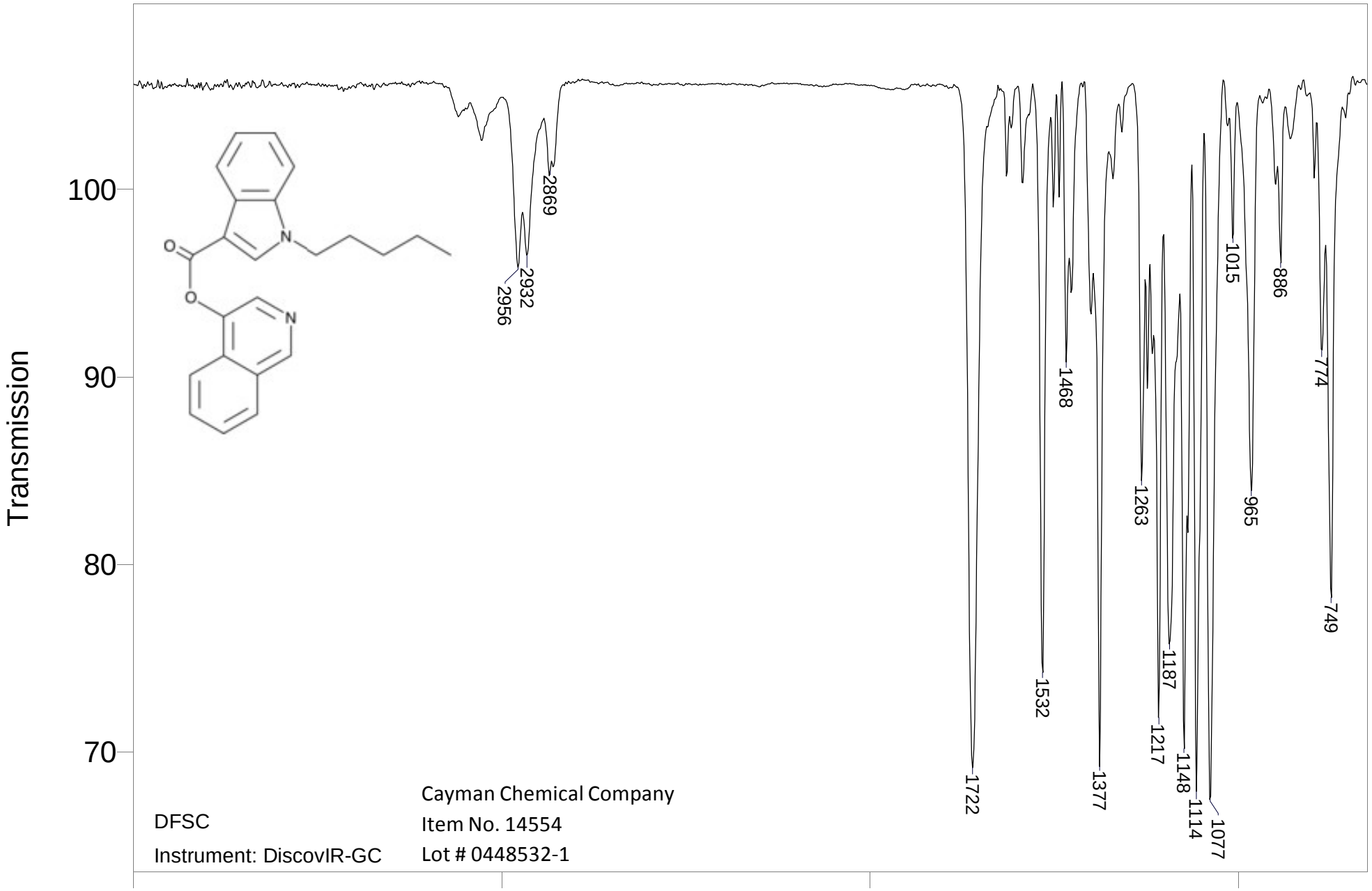




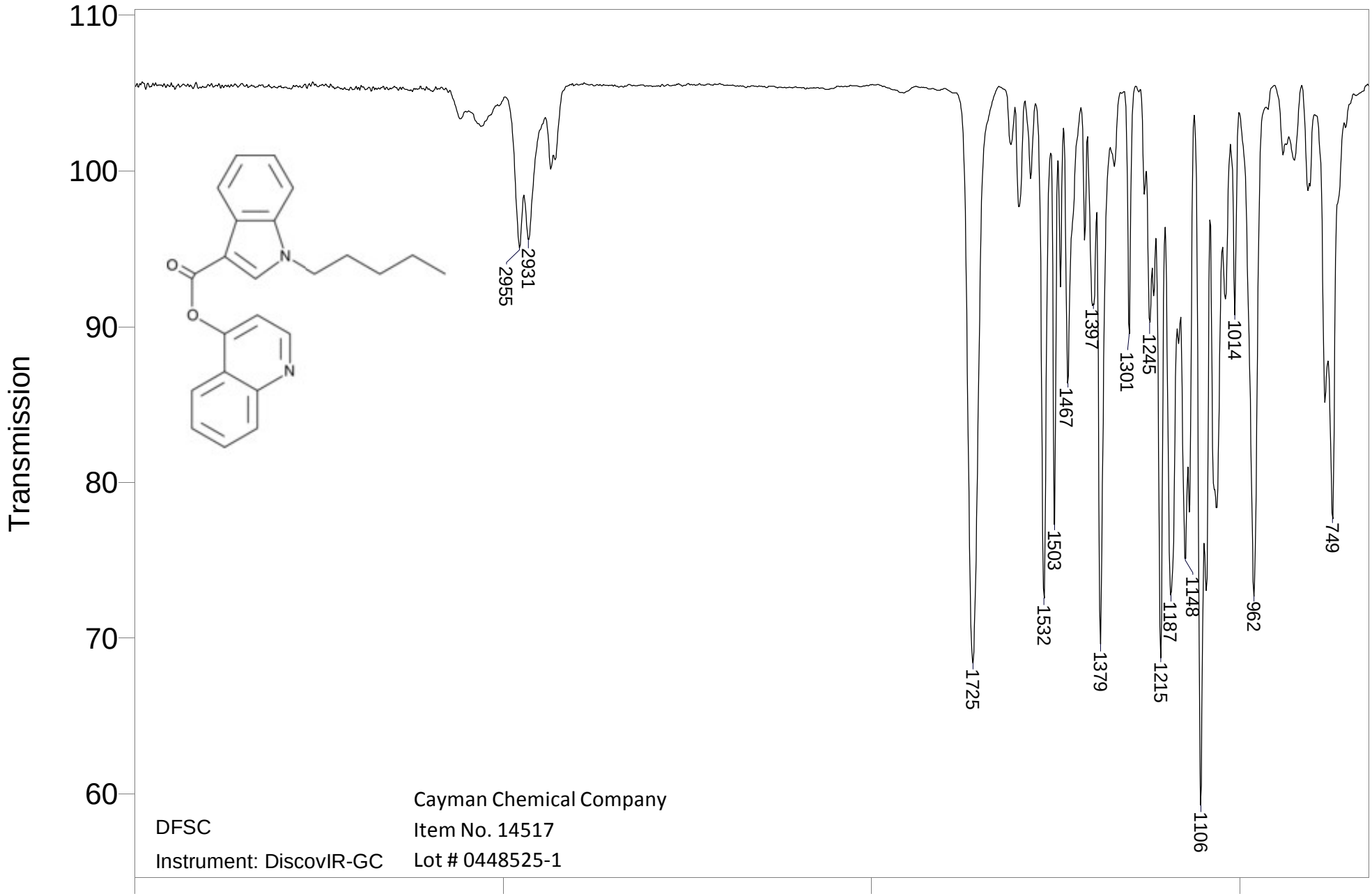


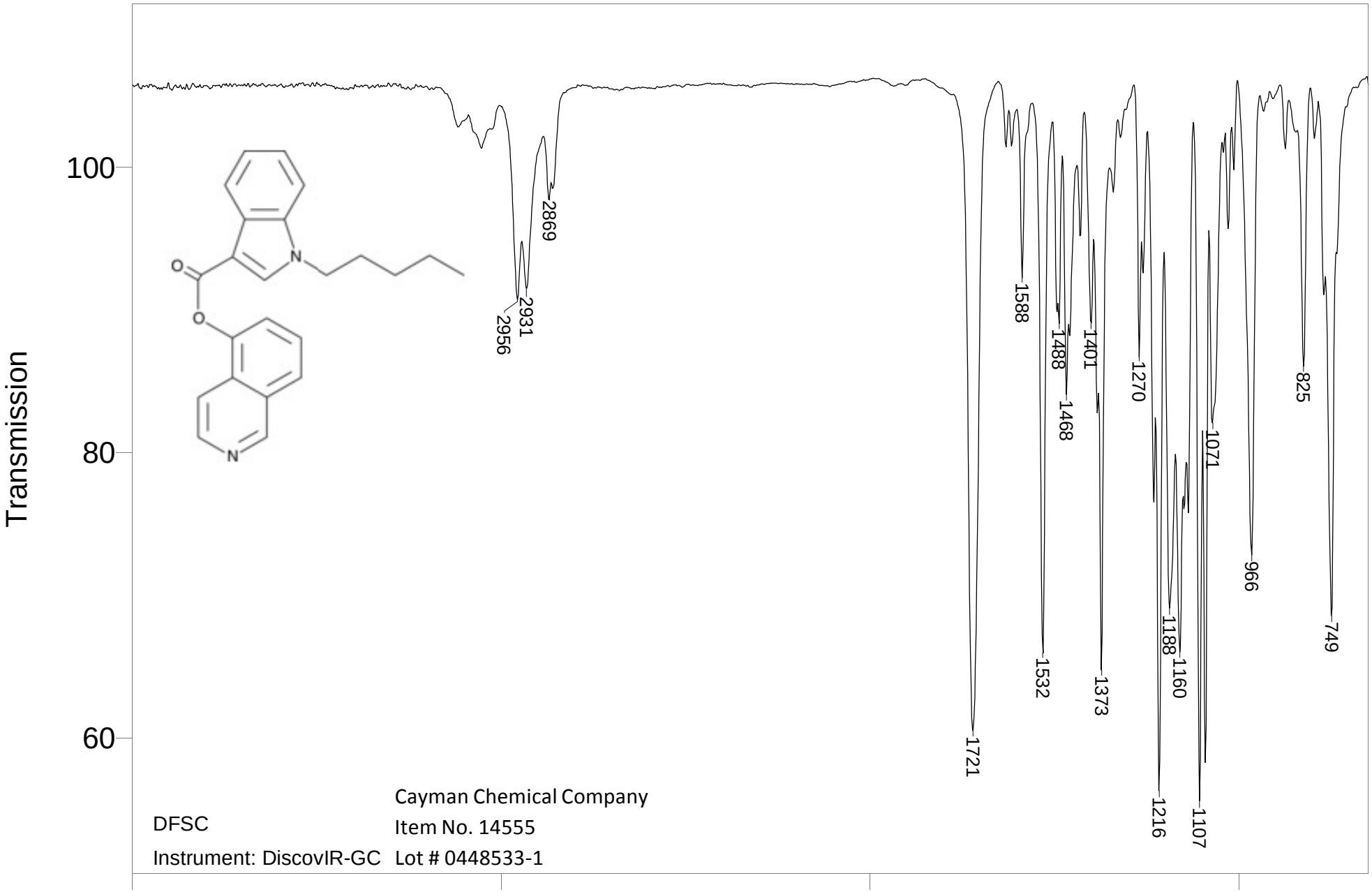


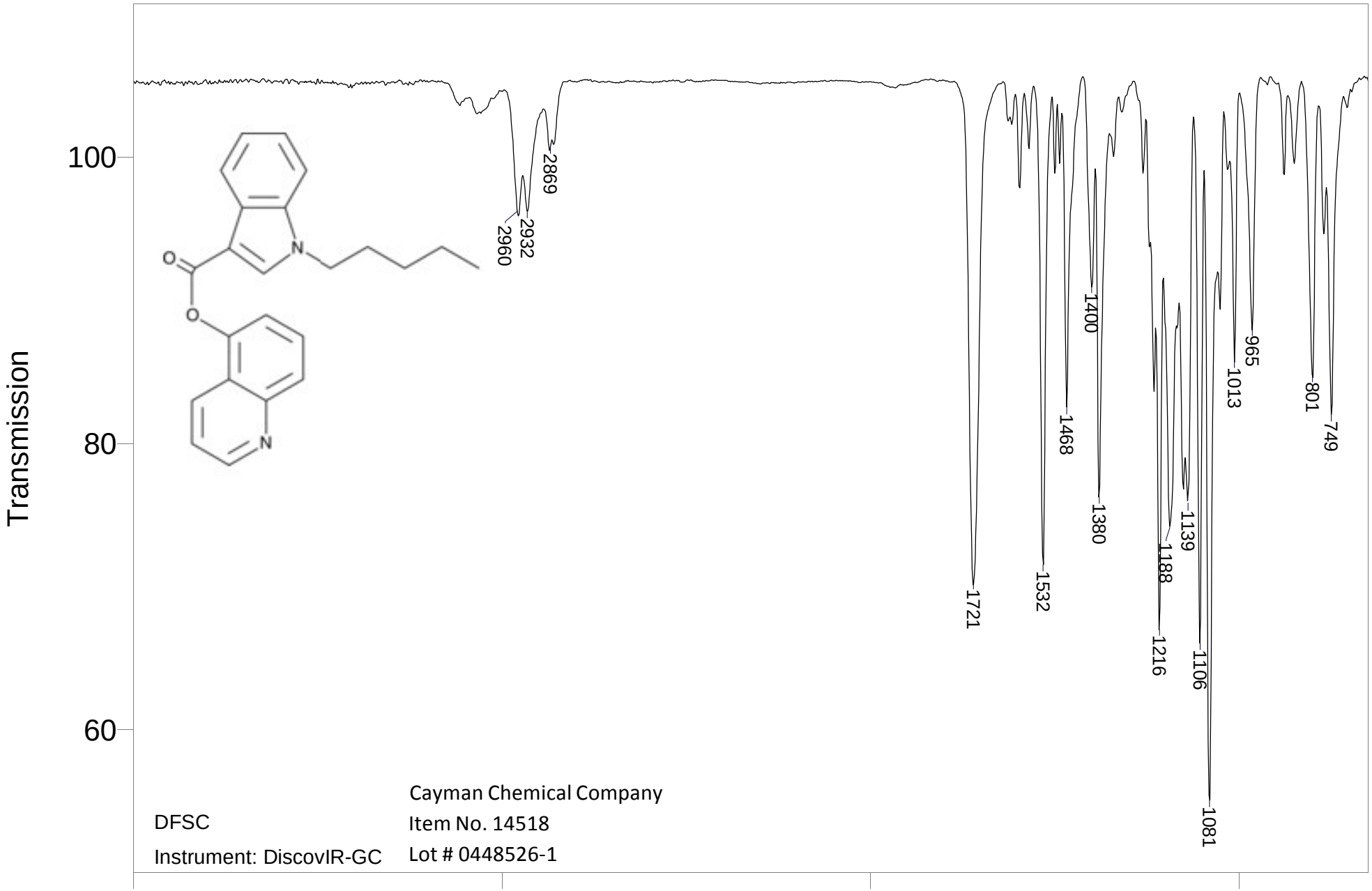




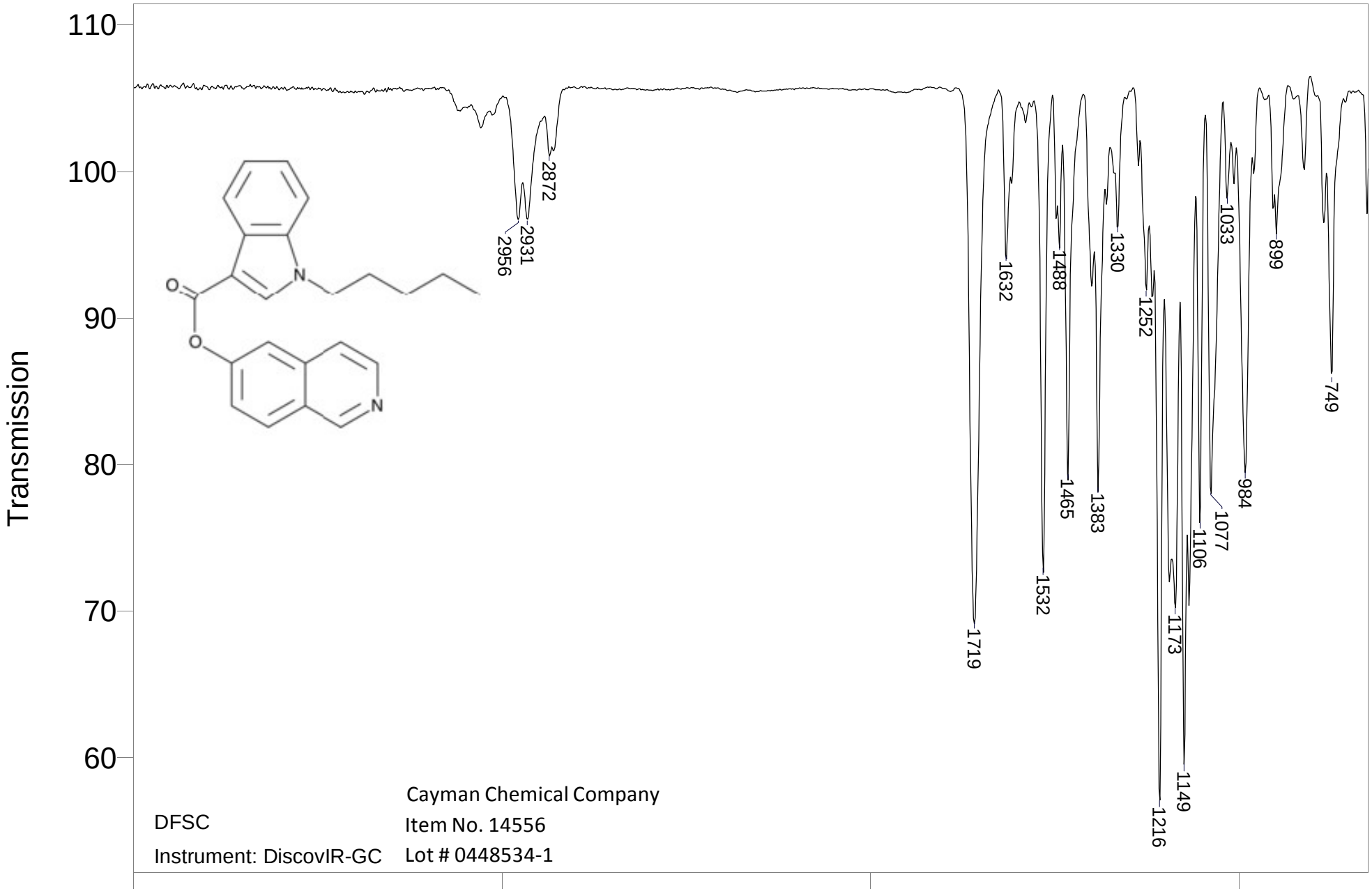
PB-22 4-hydroxyquinoline isomer

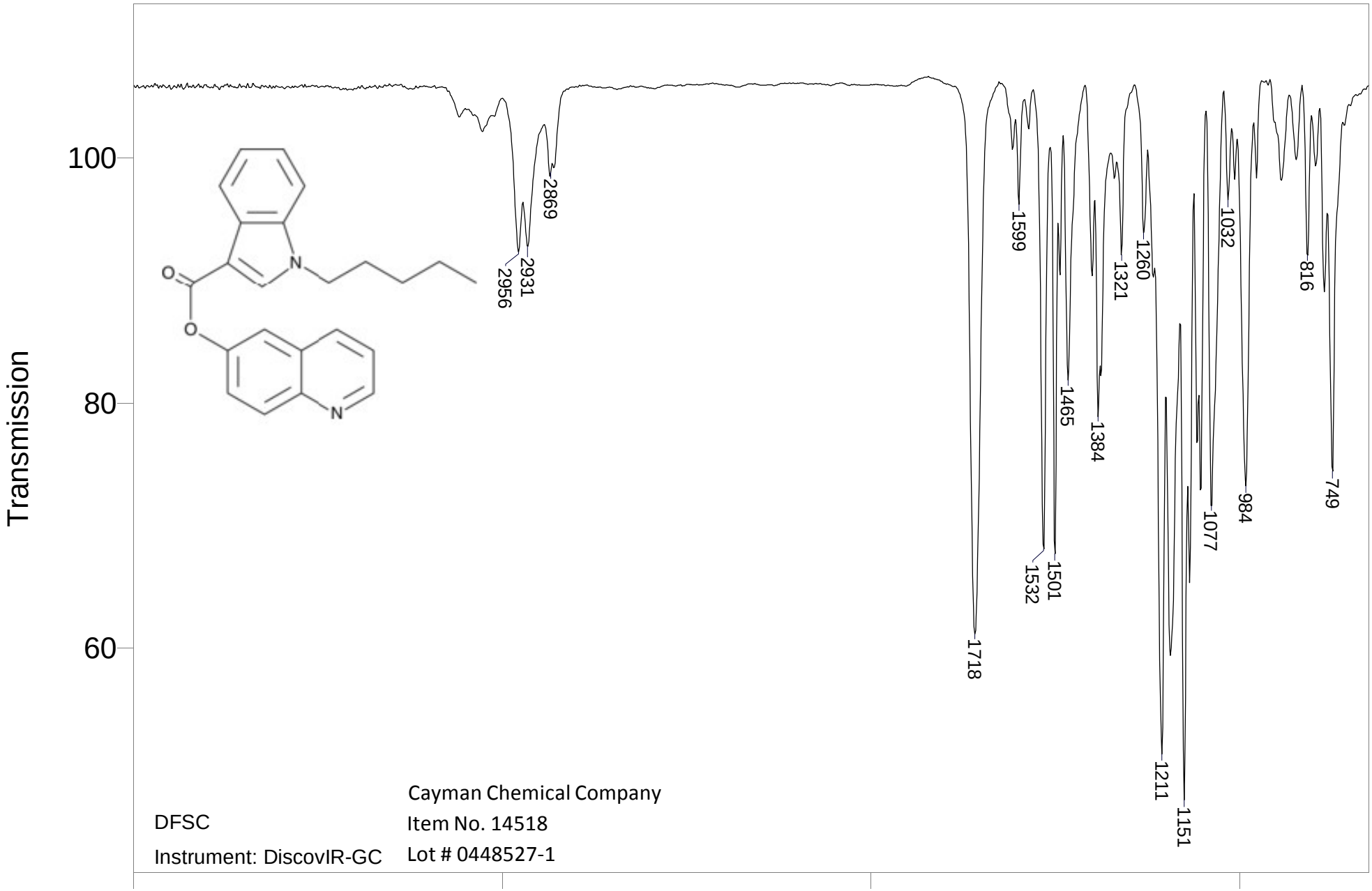


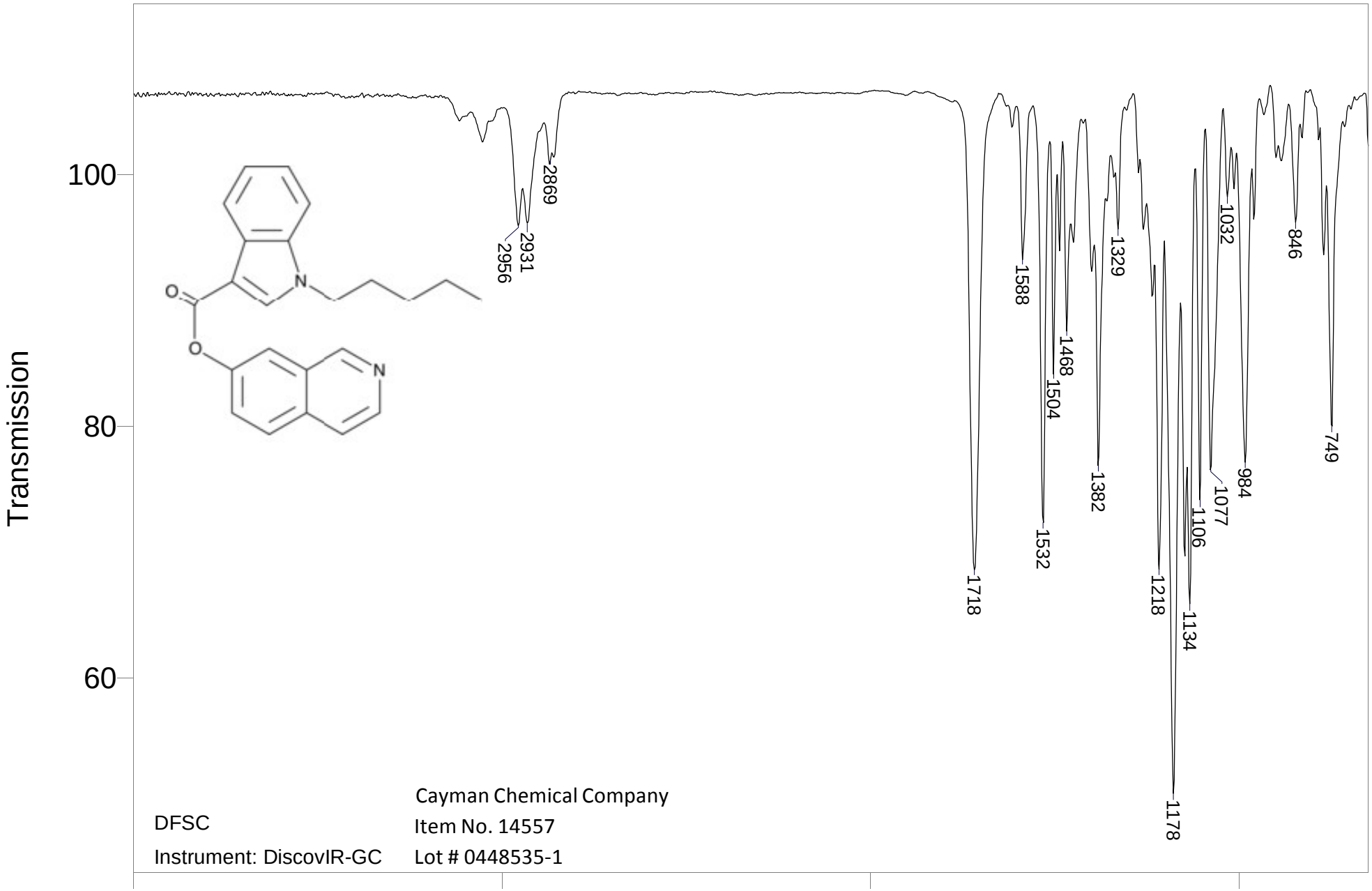


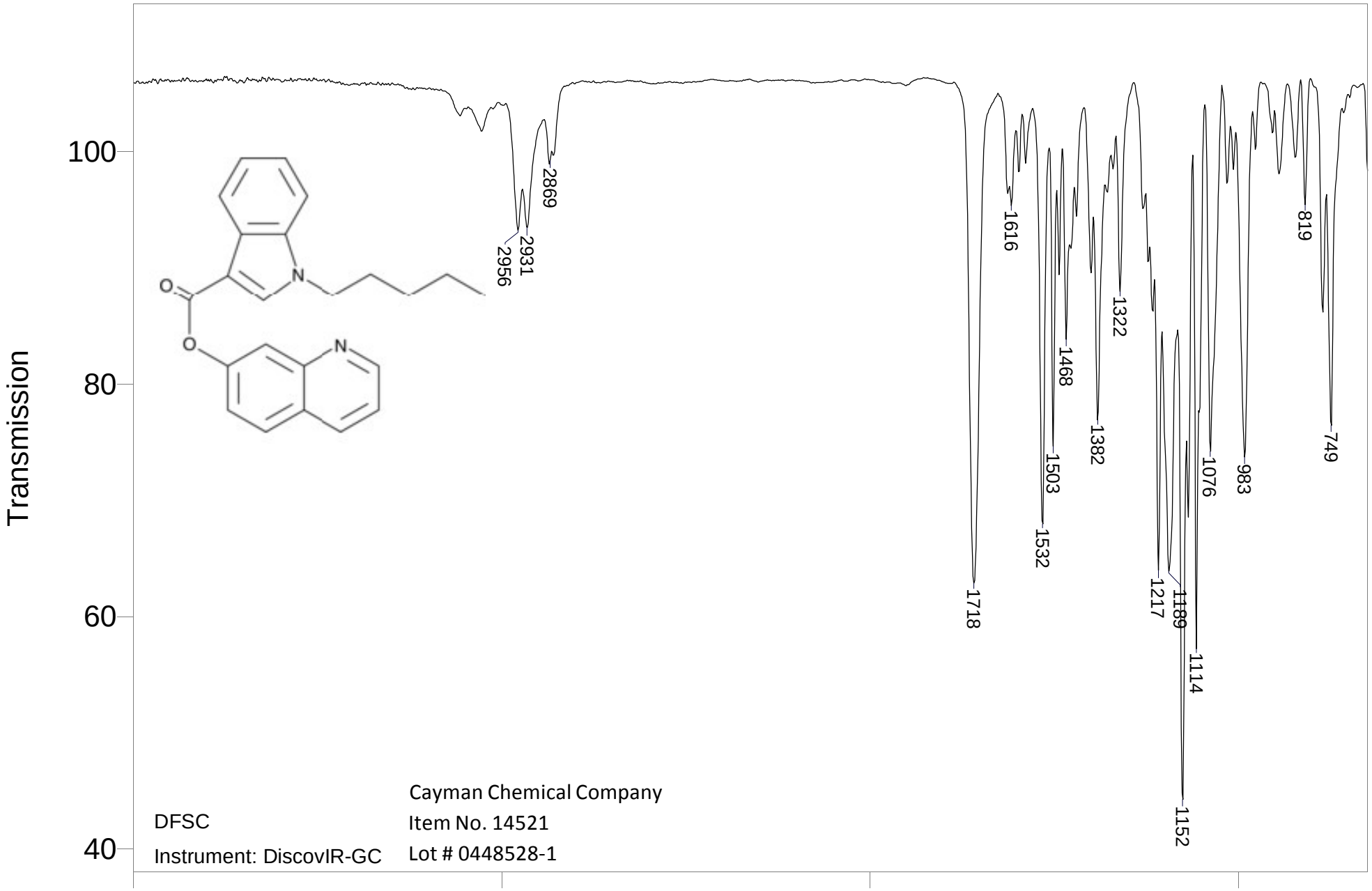


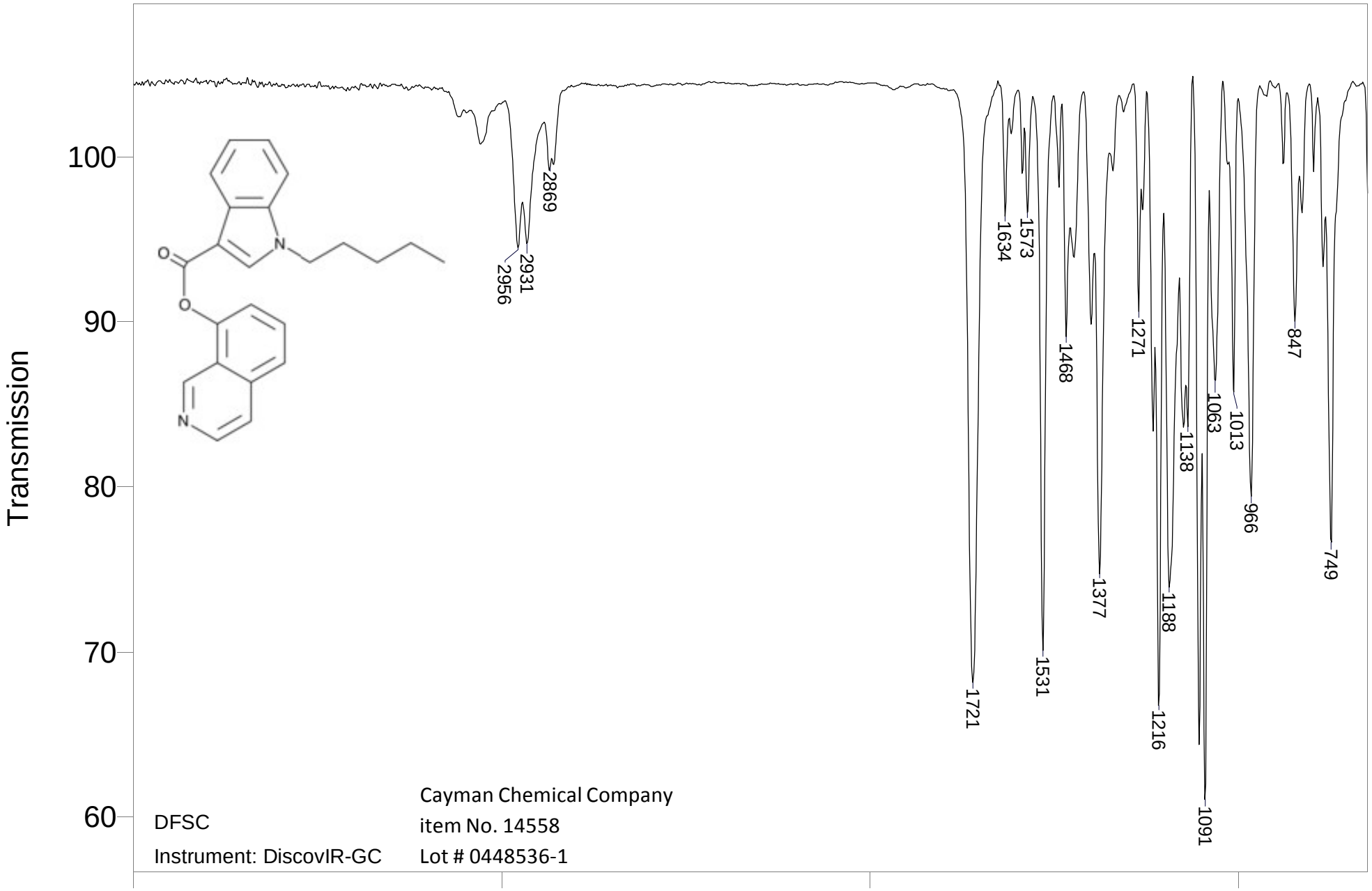
PB-22 6-hydroxyisoquinoline isomer

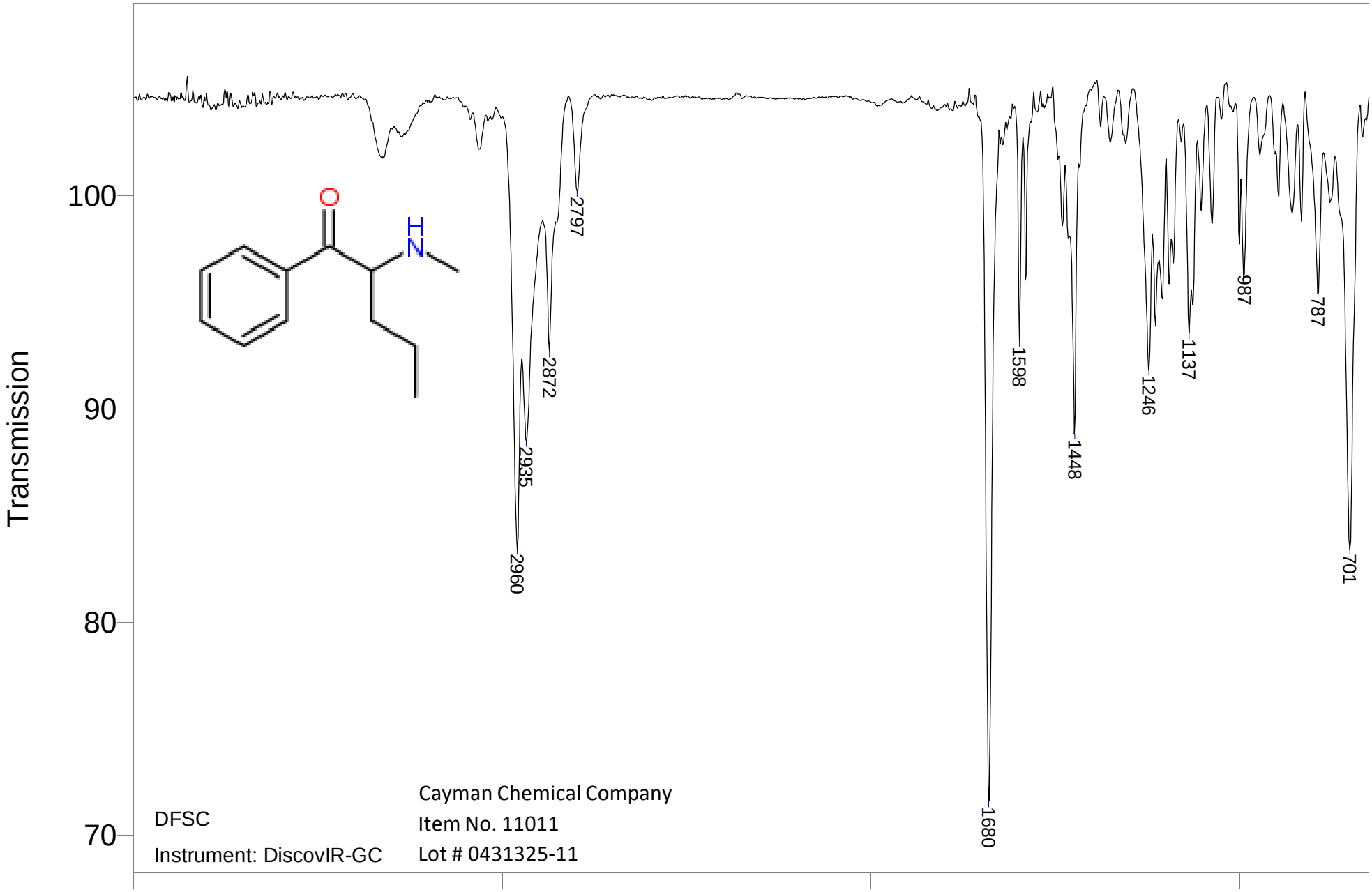


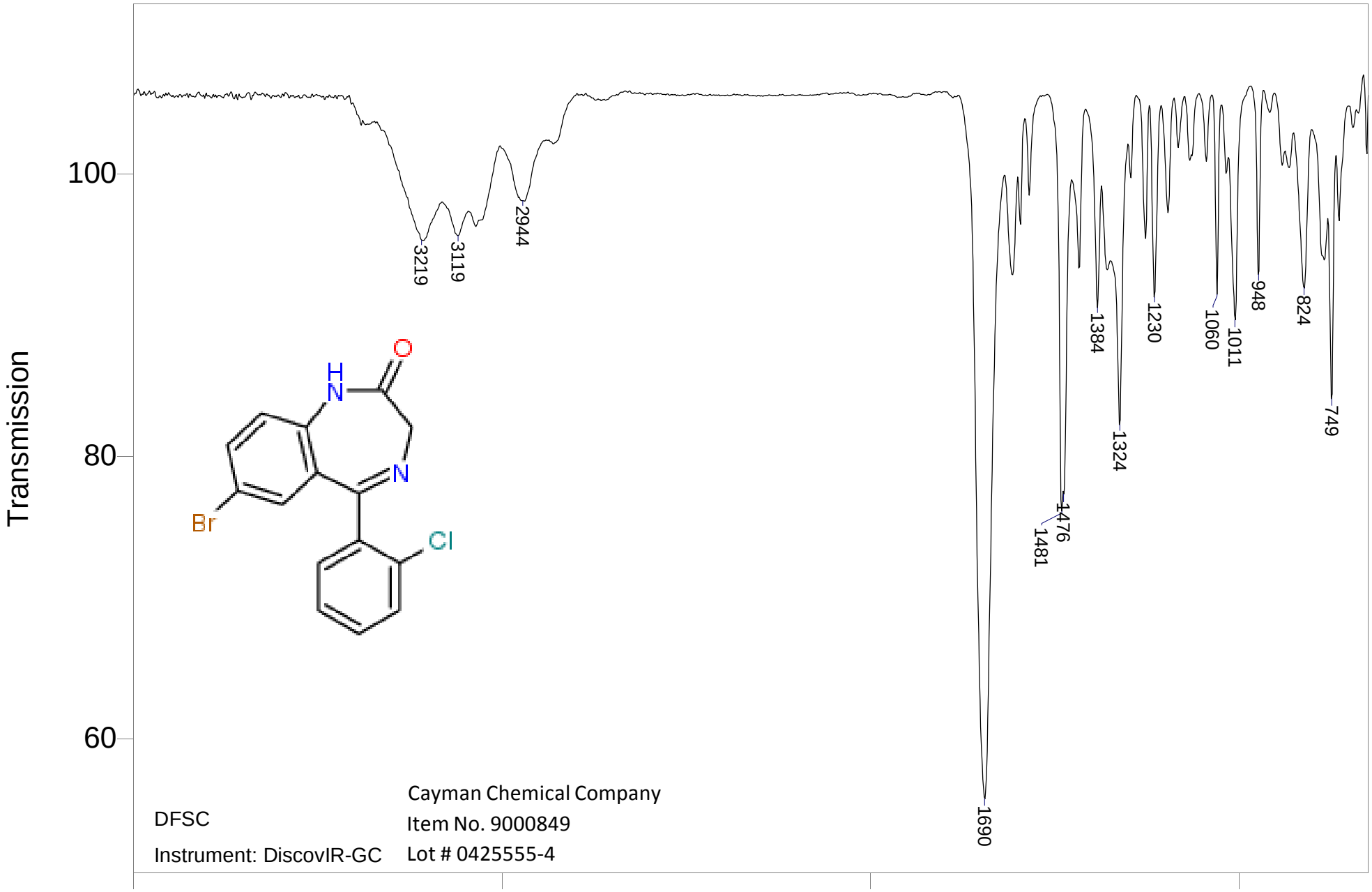


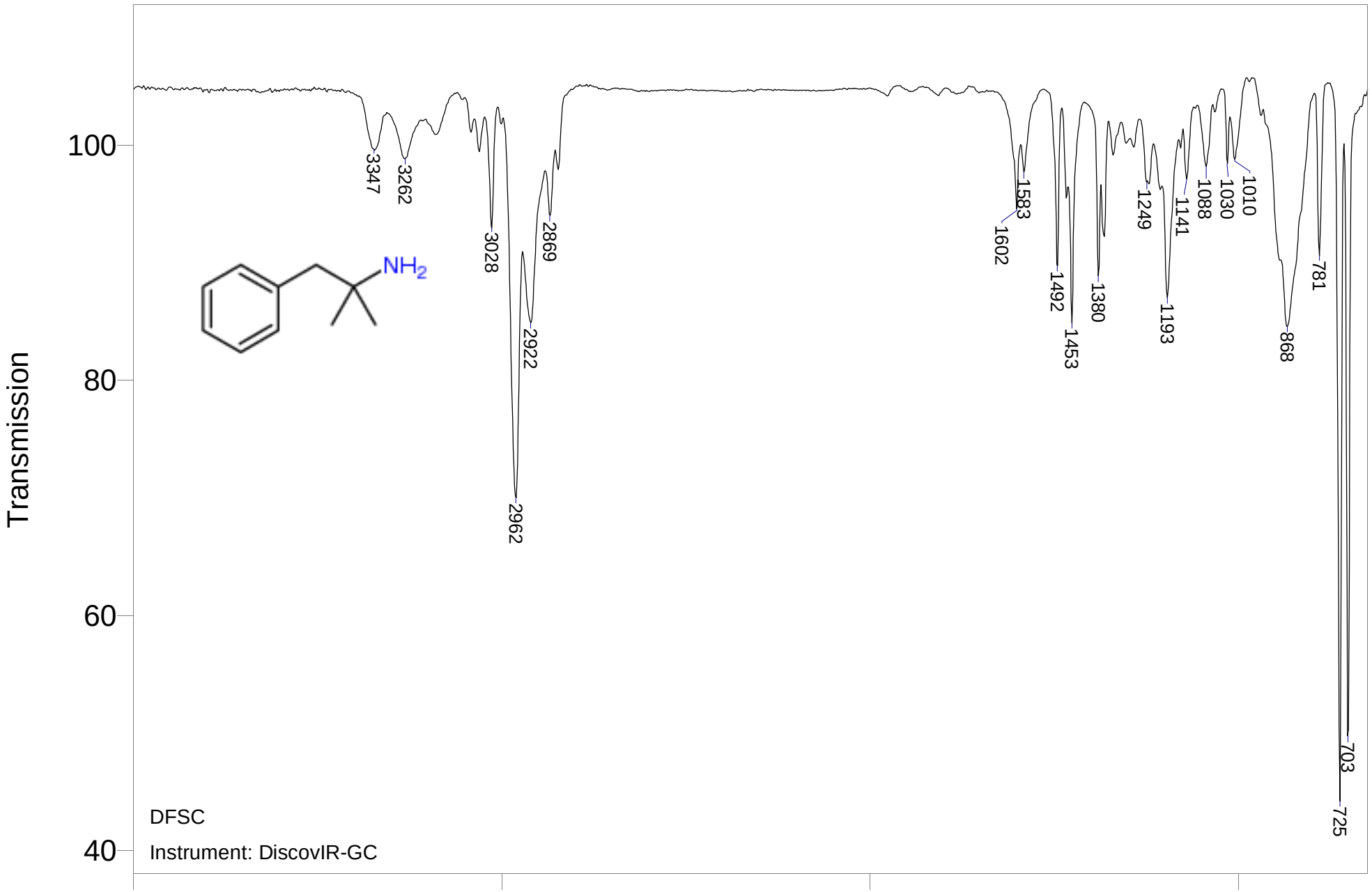


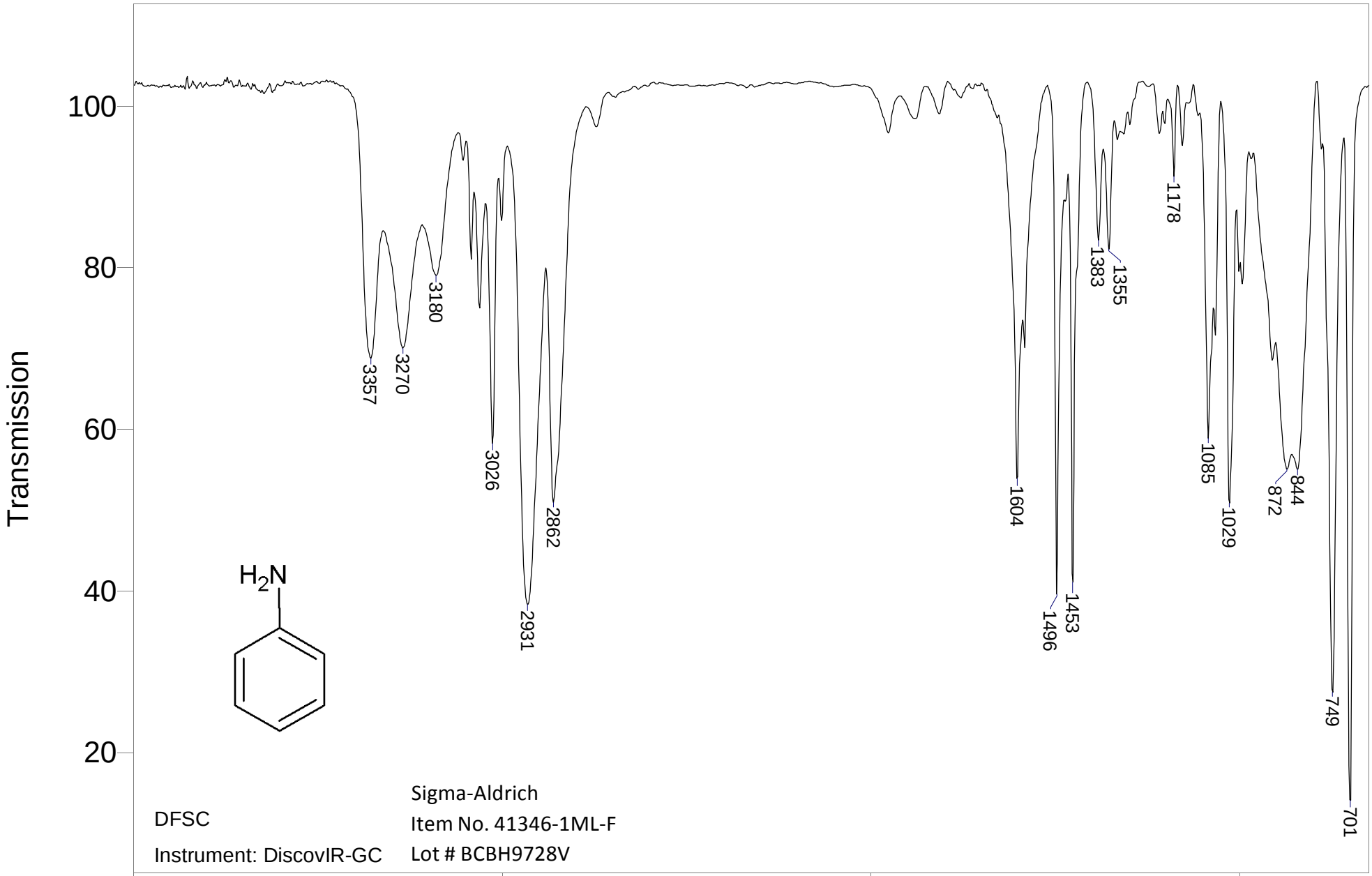


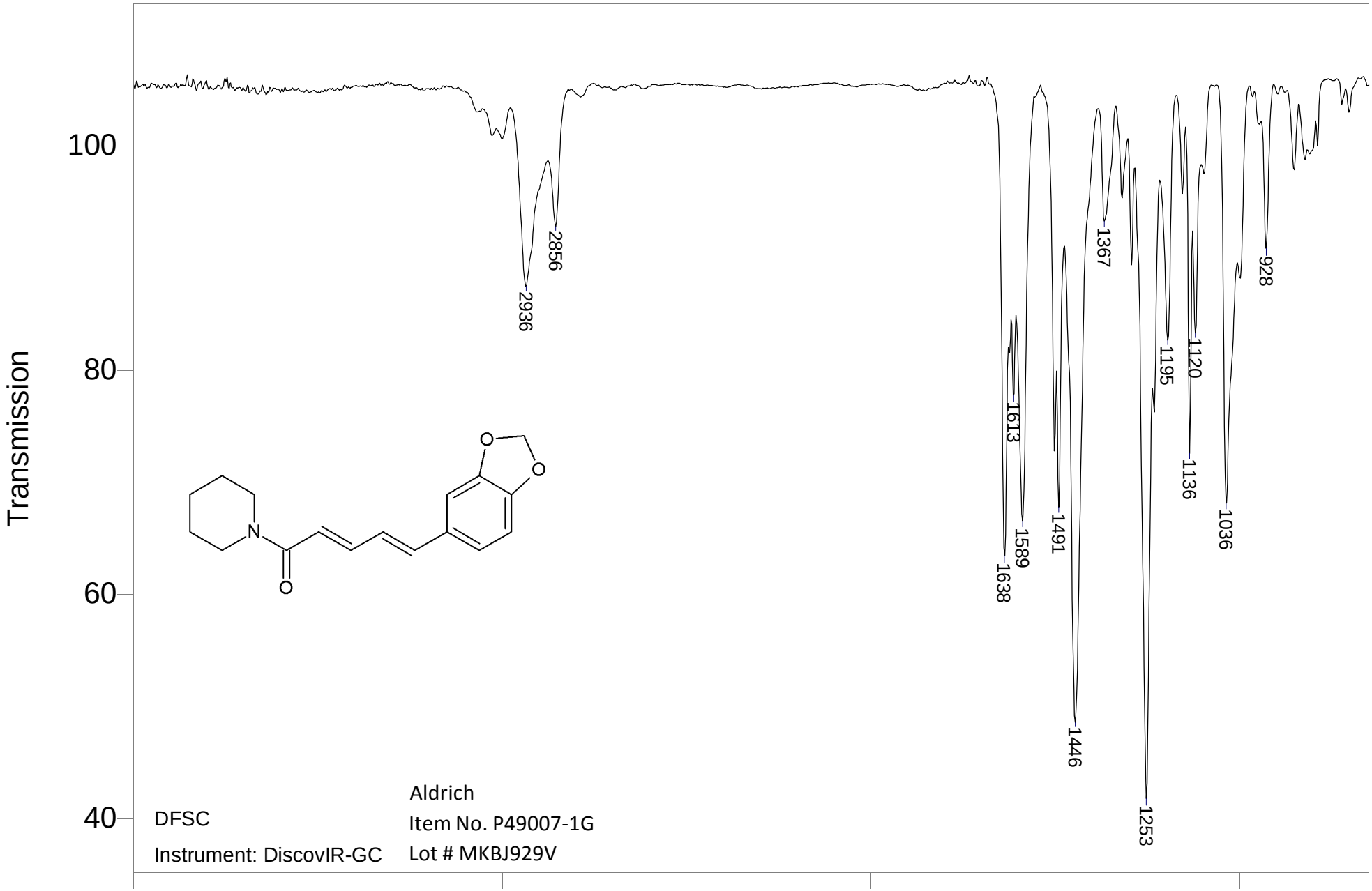


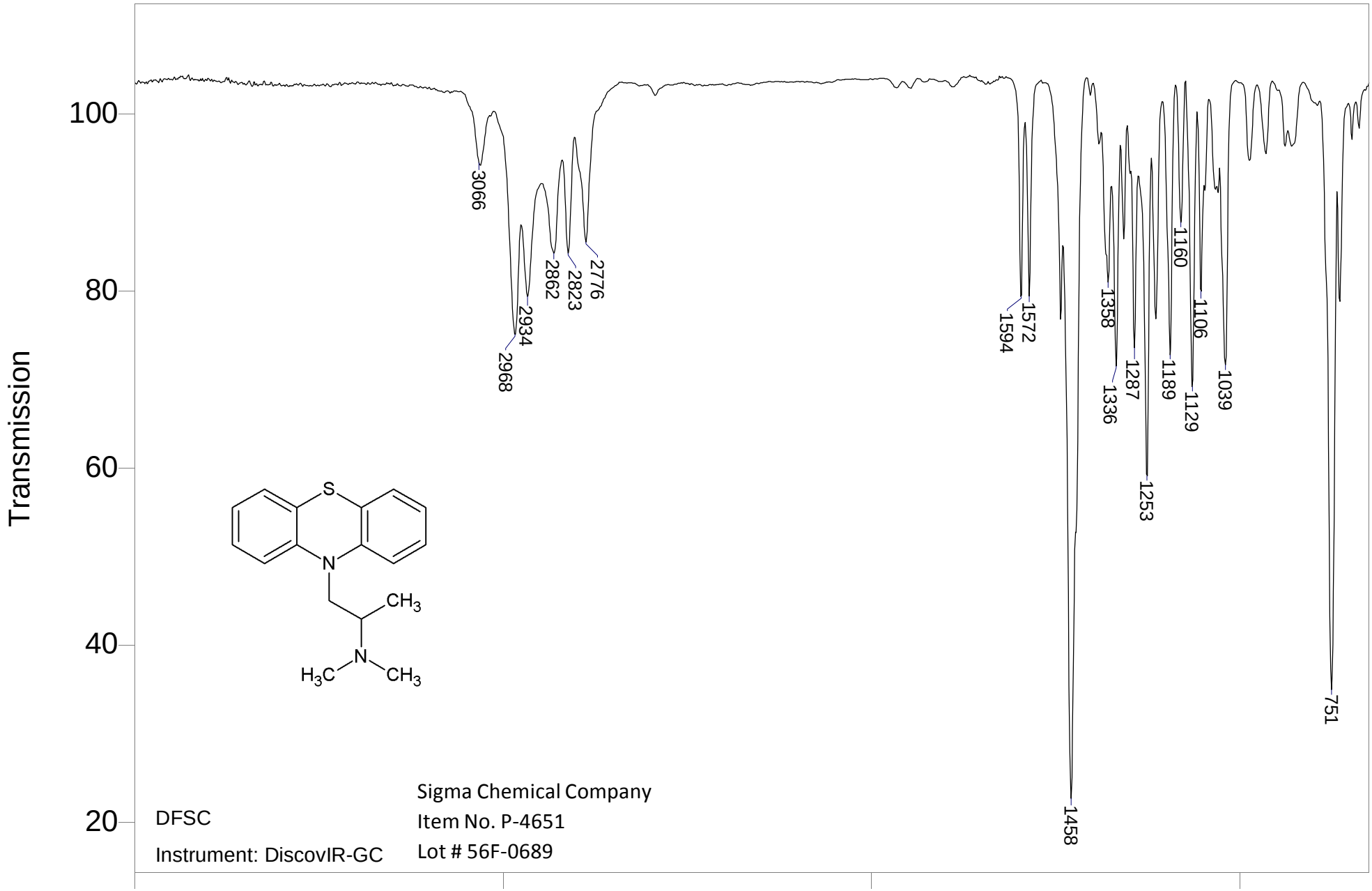


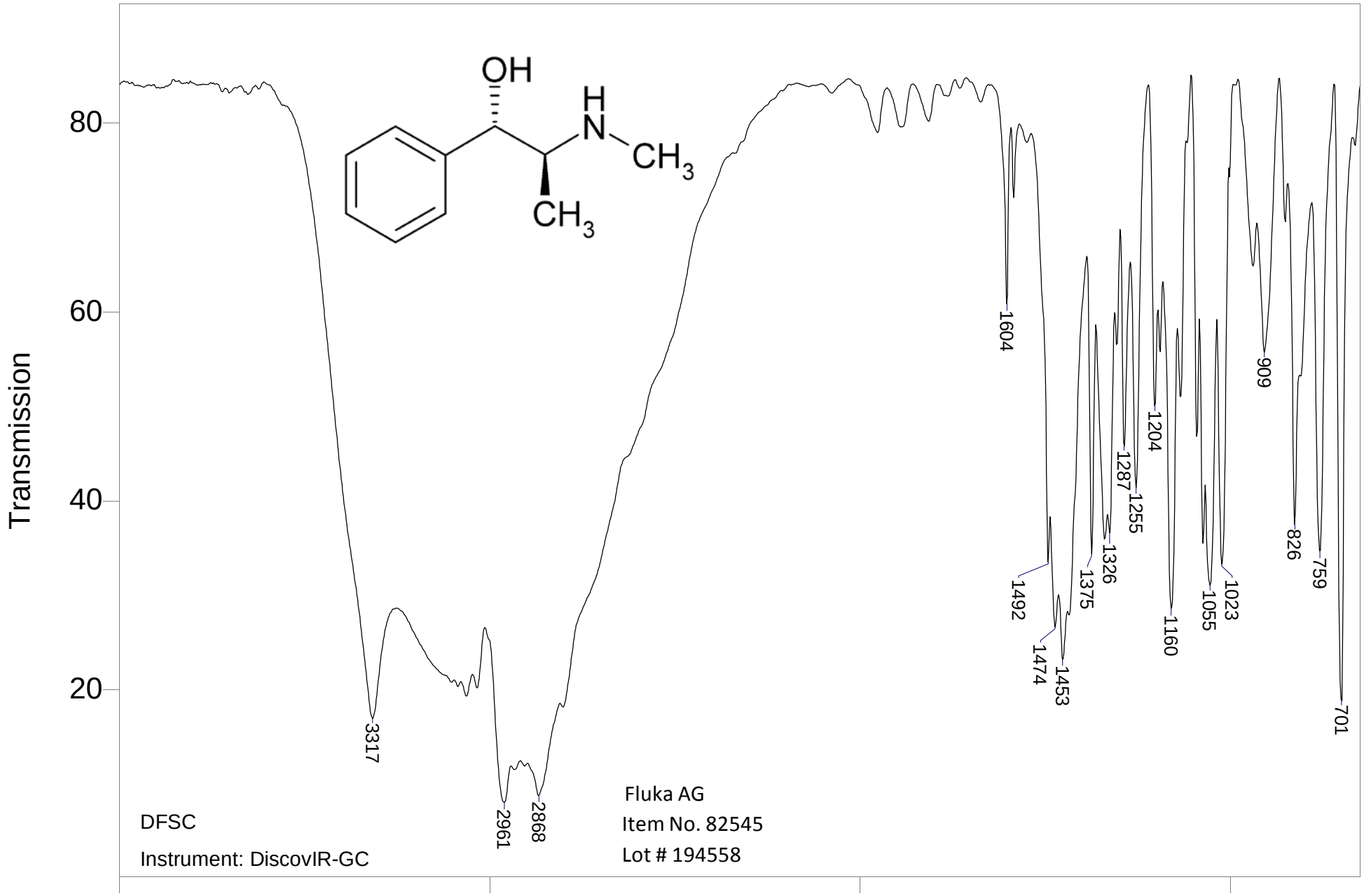


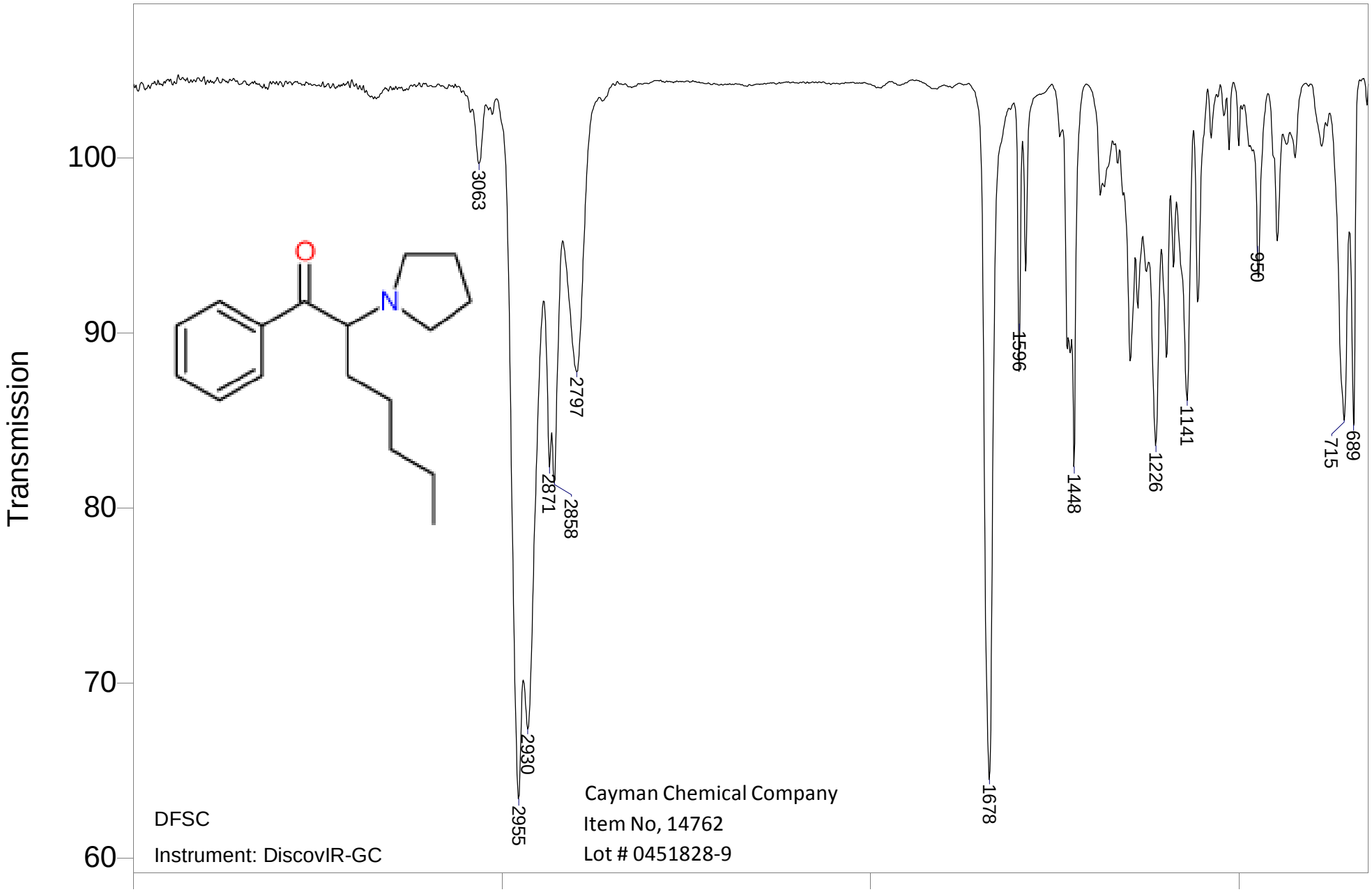


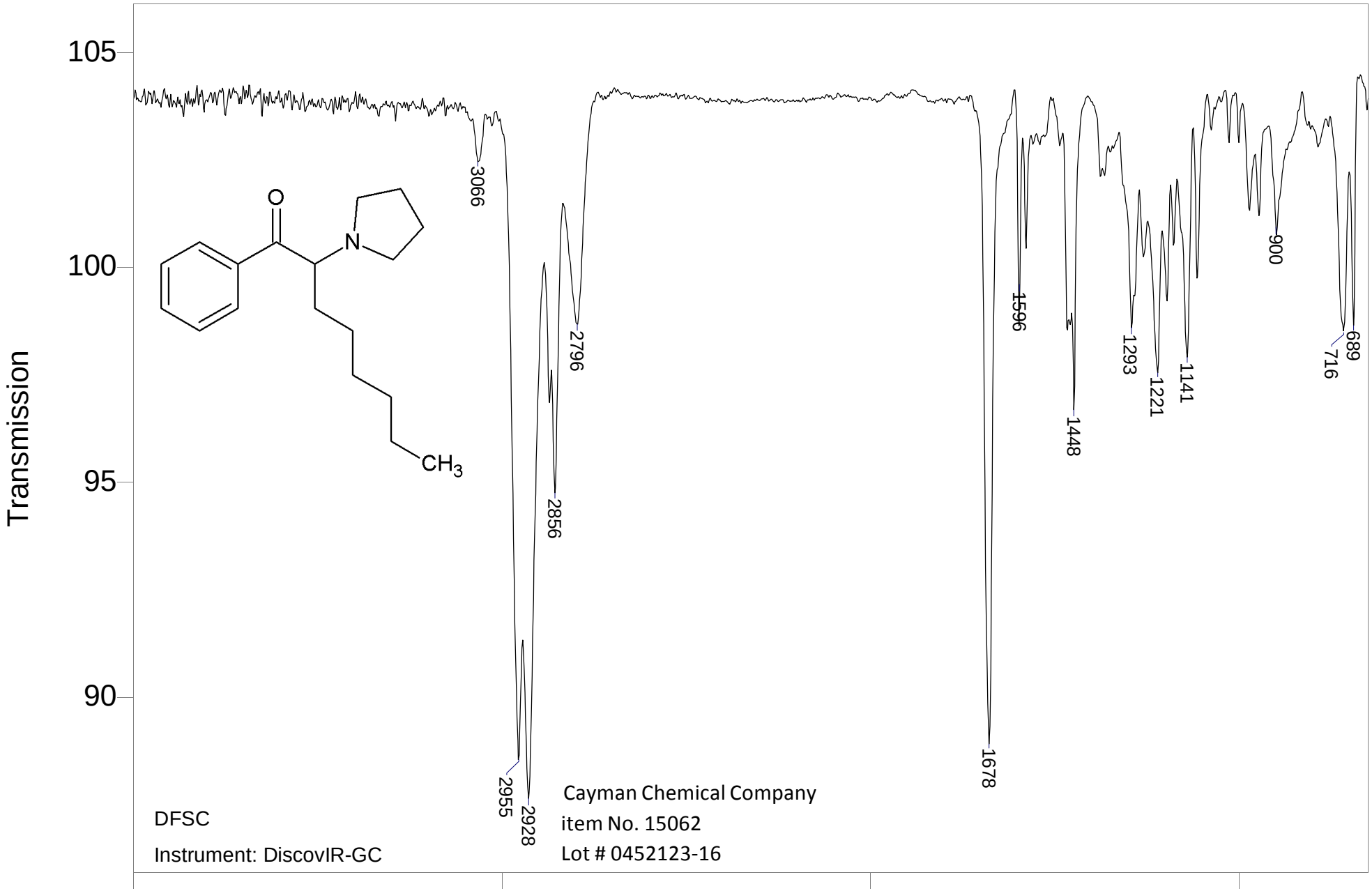


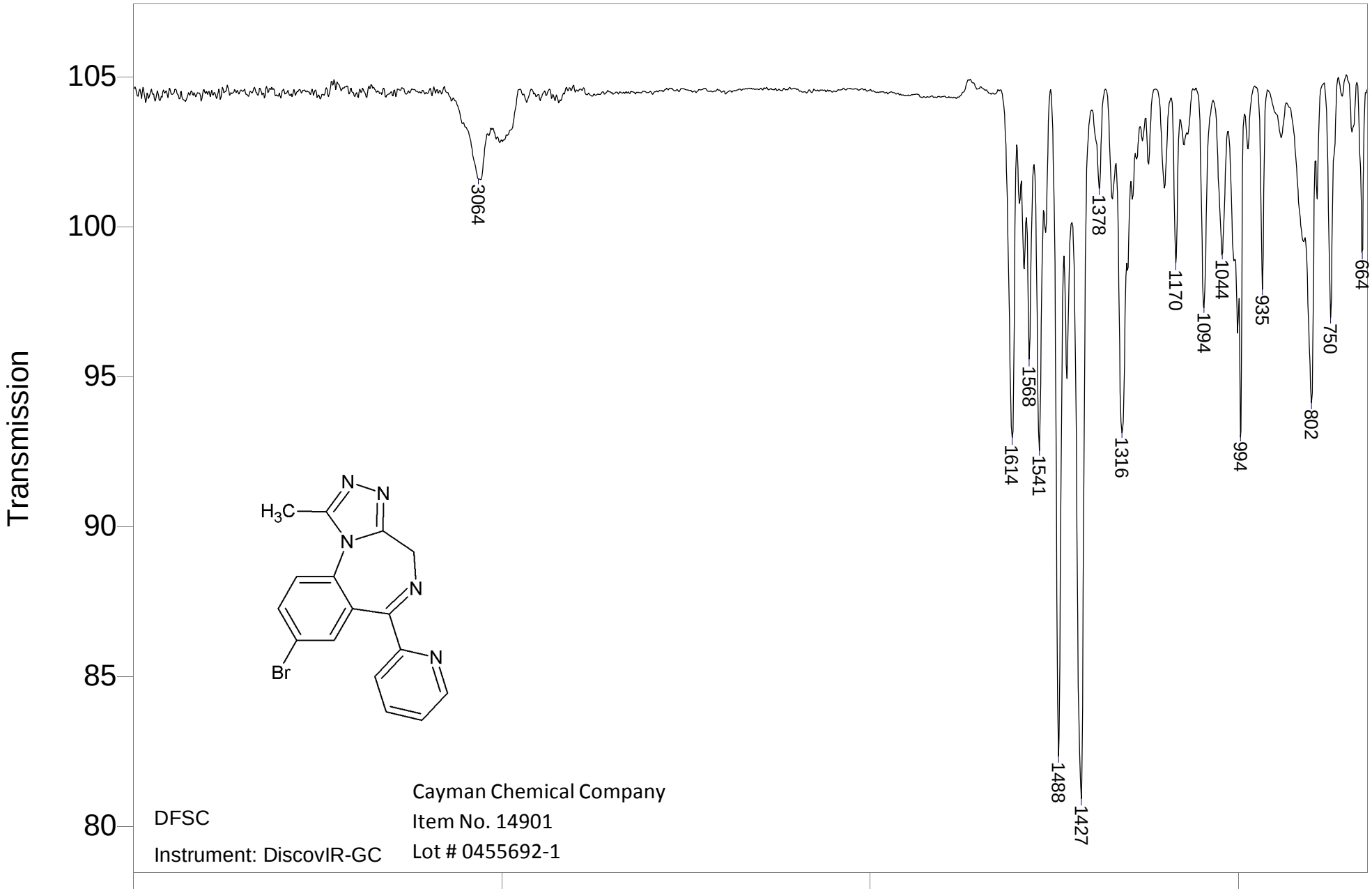


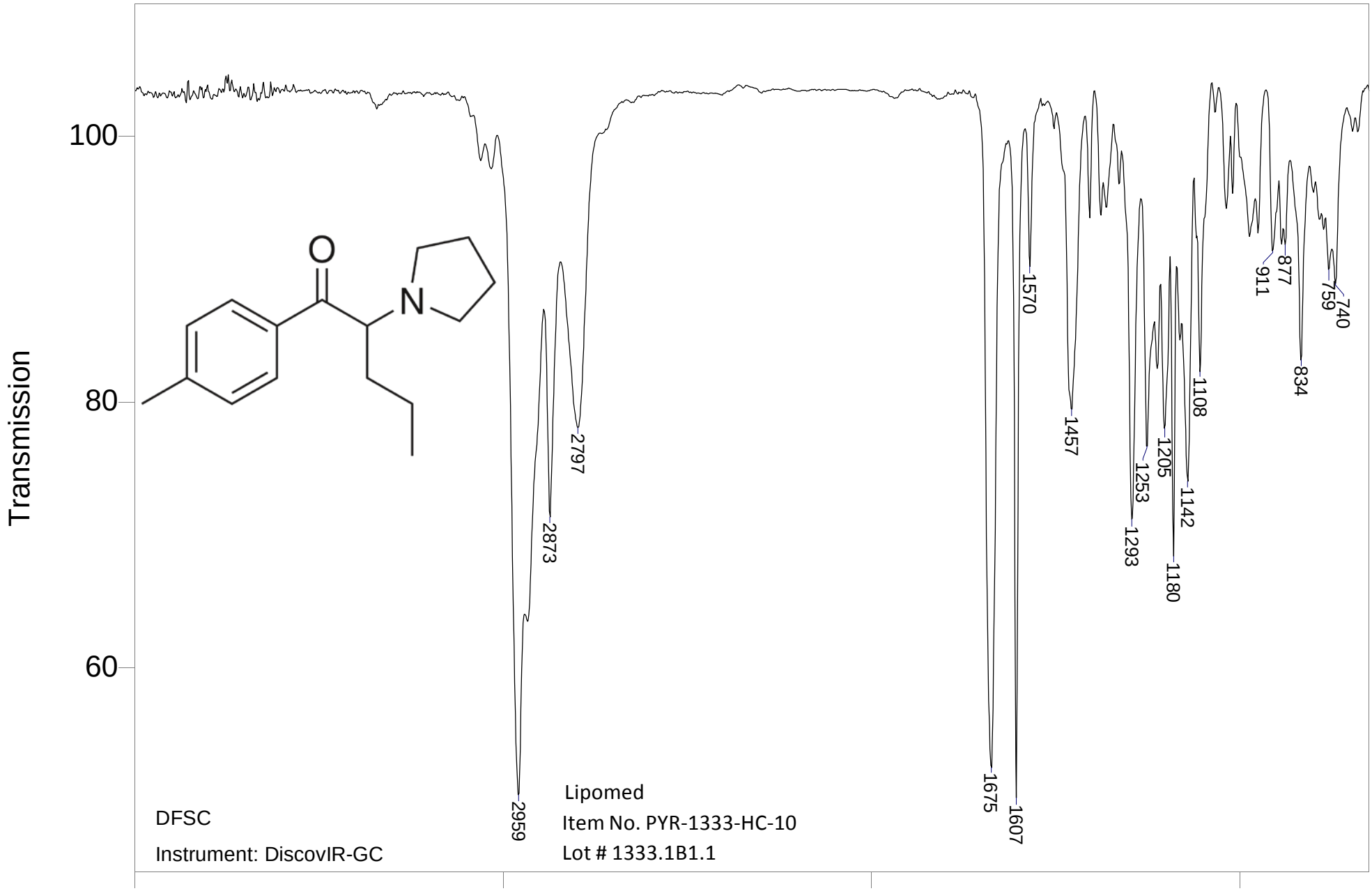




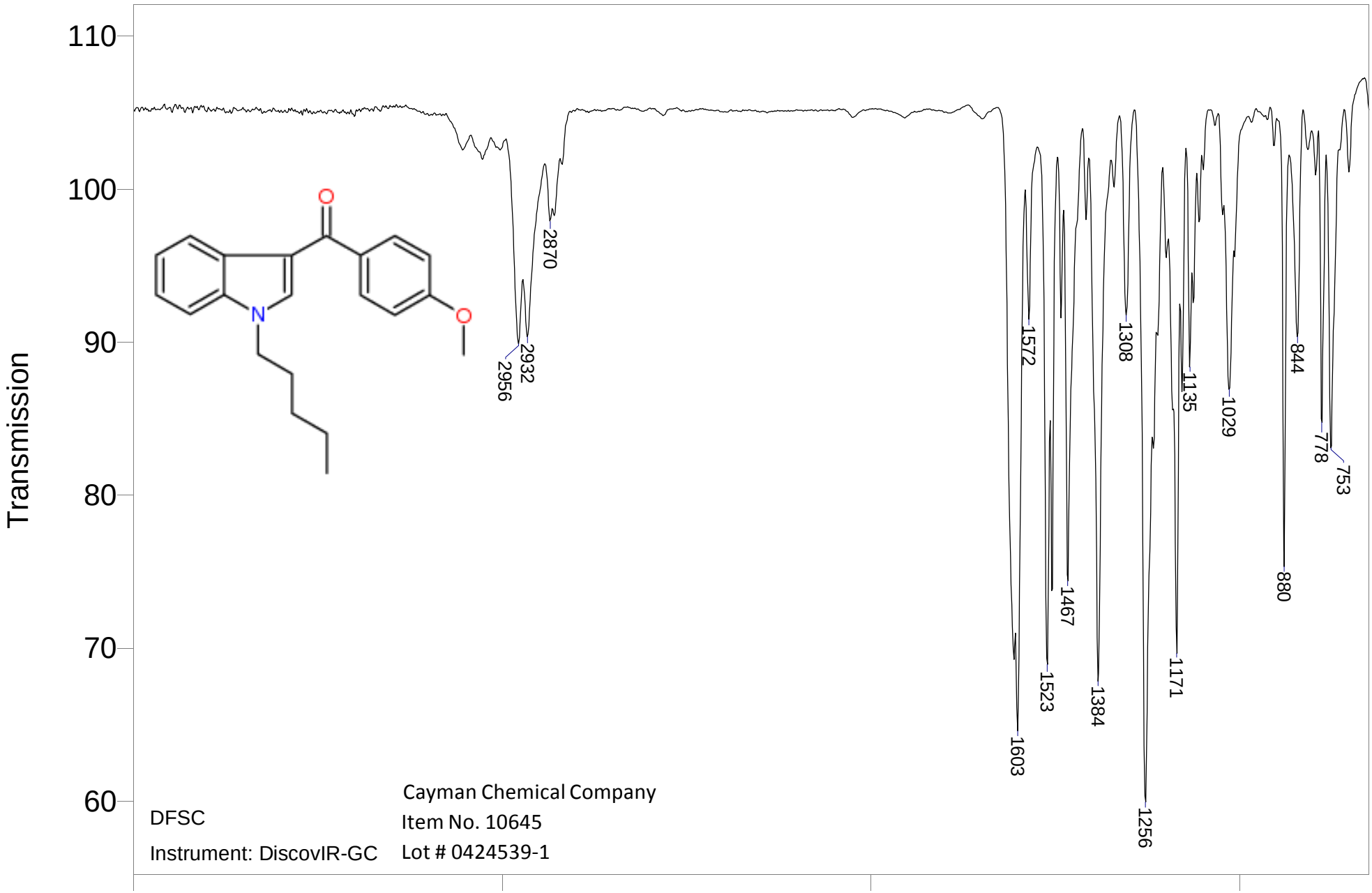


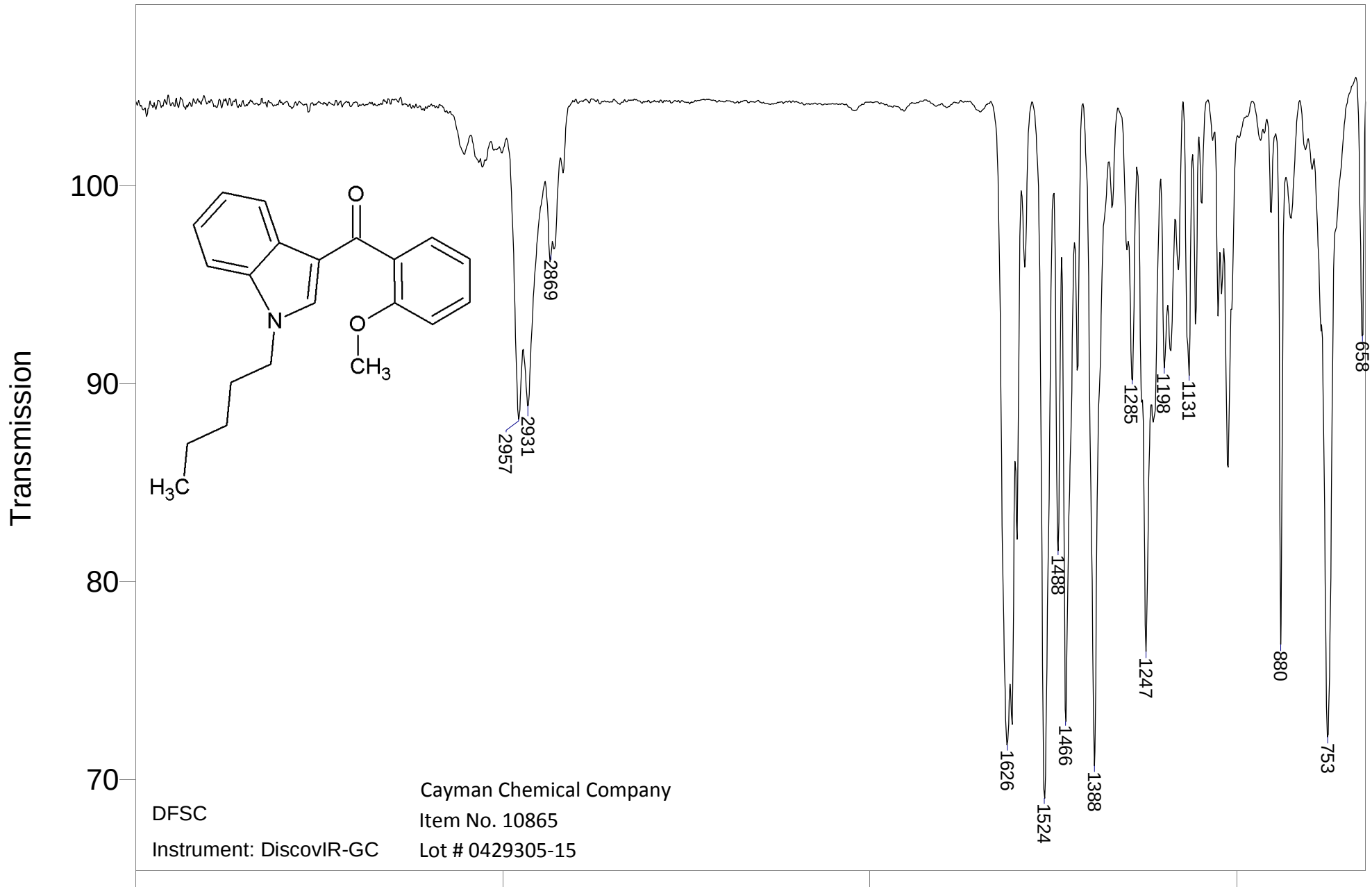


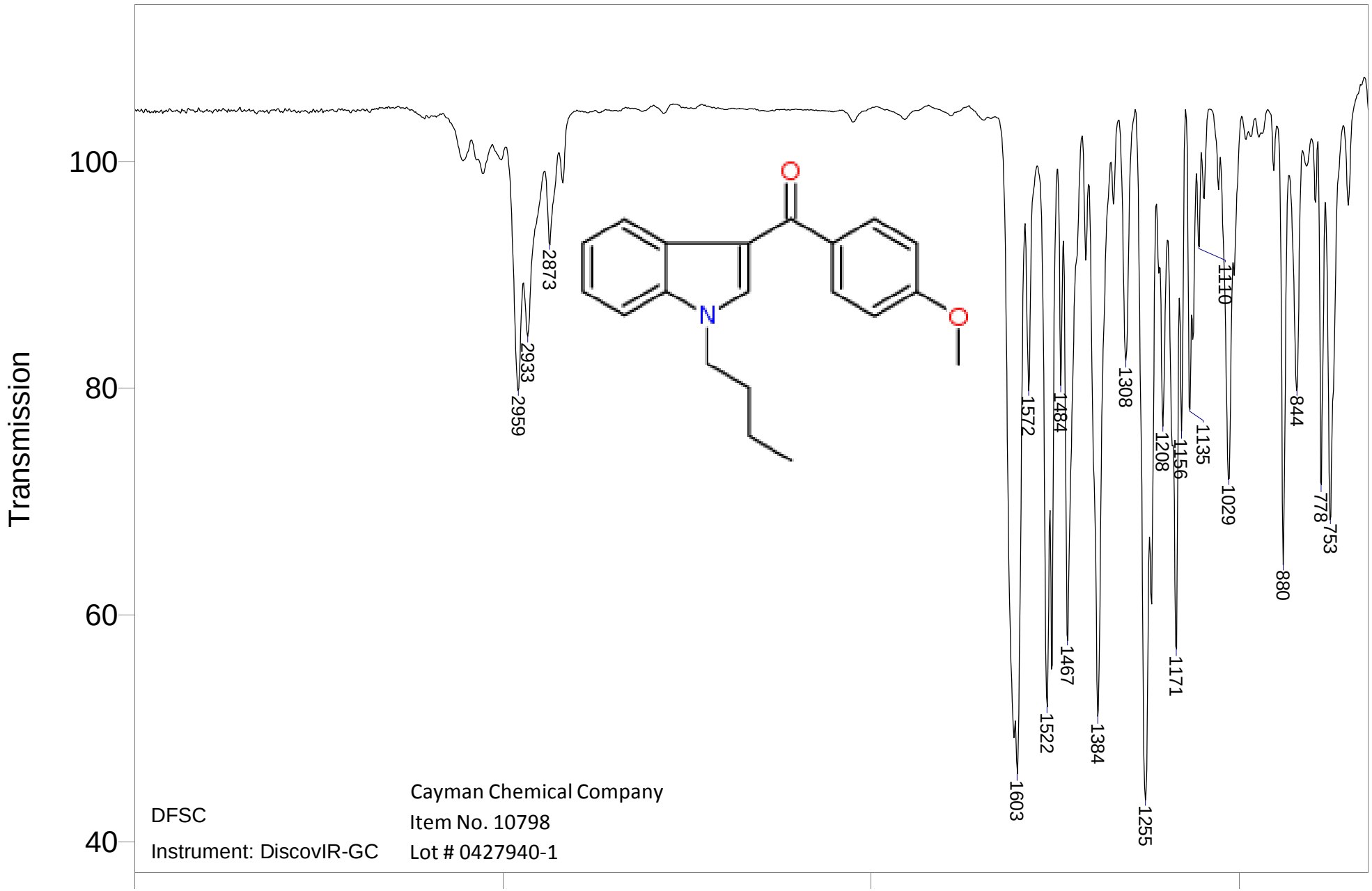




RCS-4







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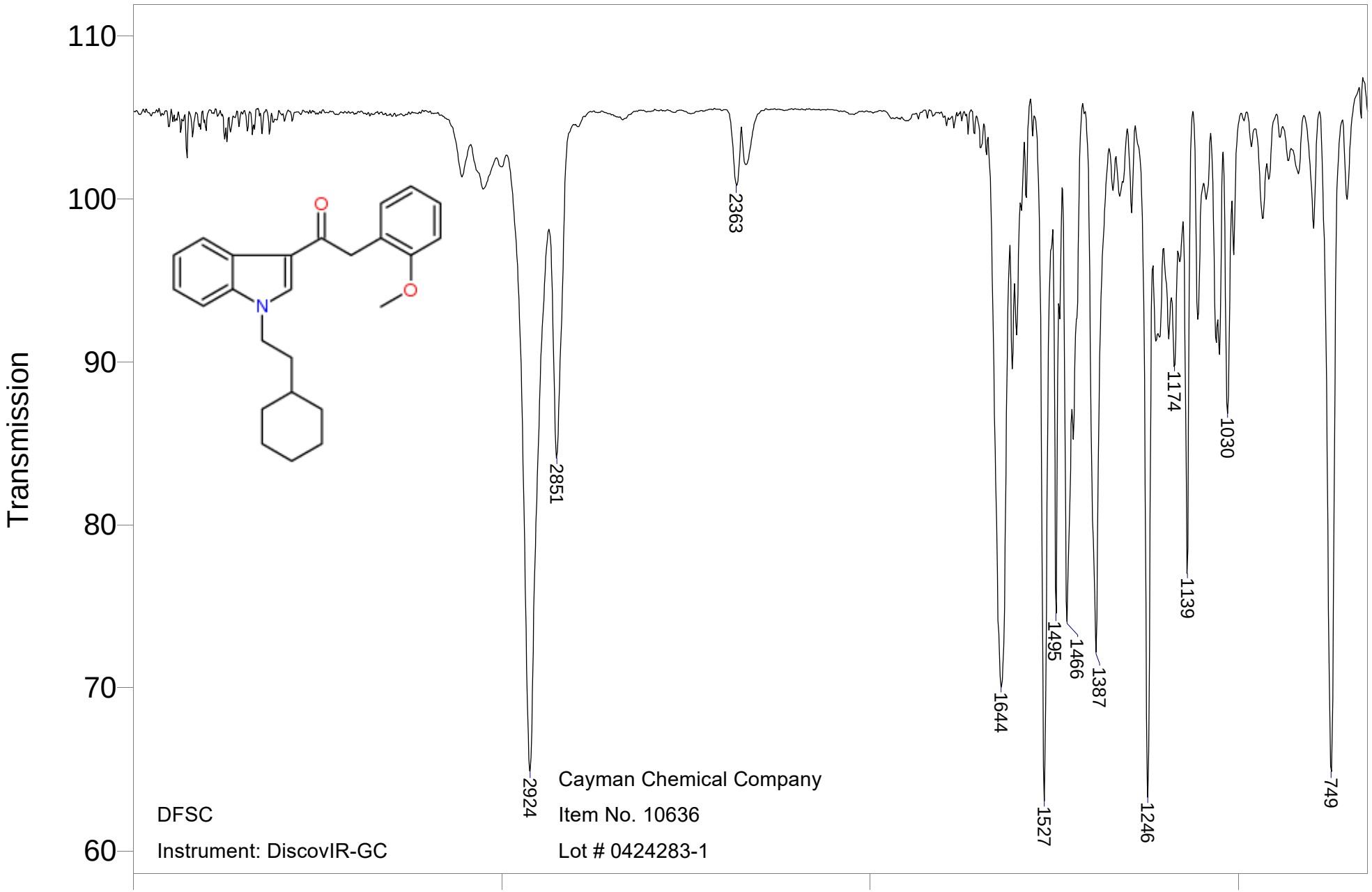
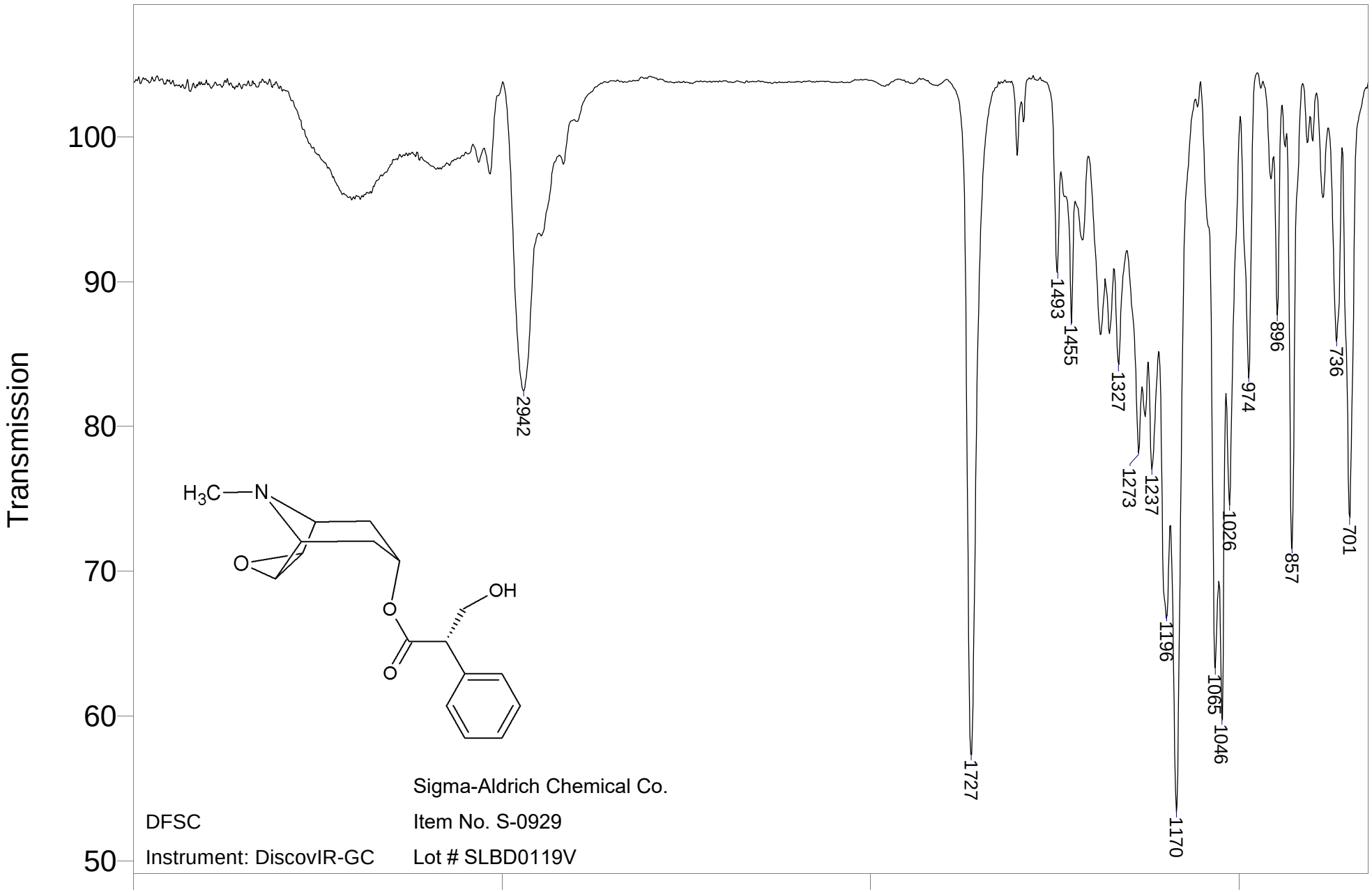
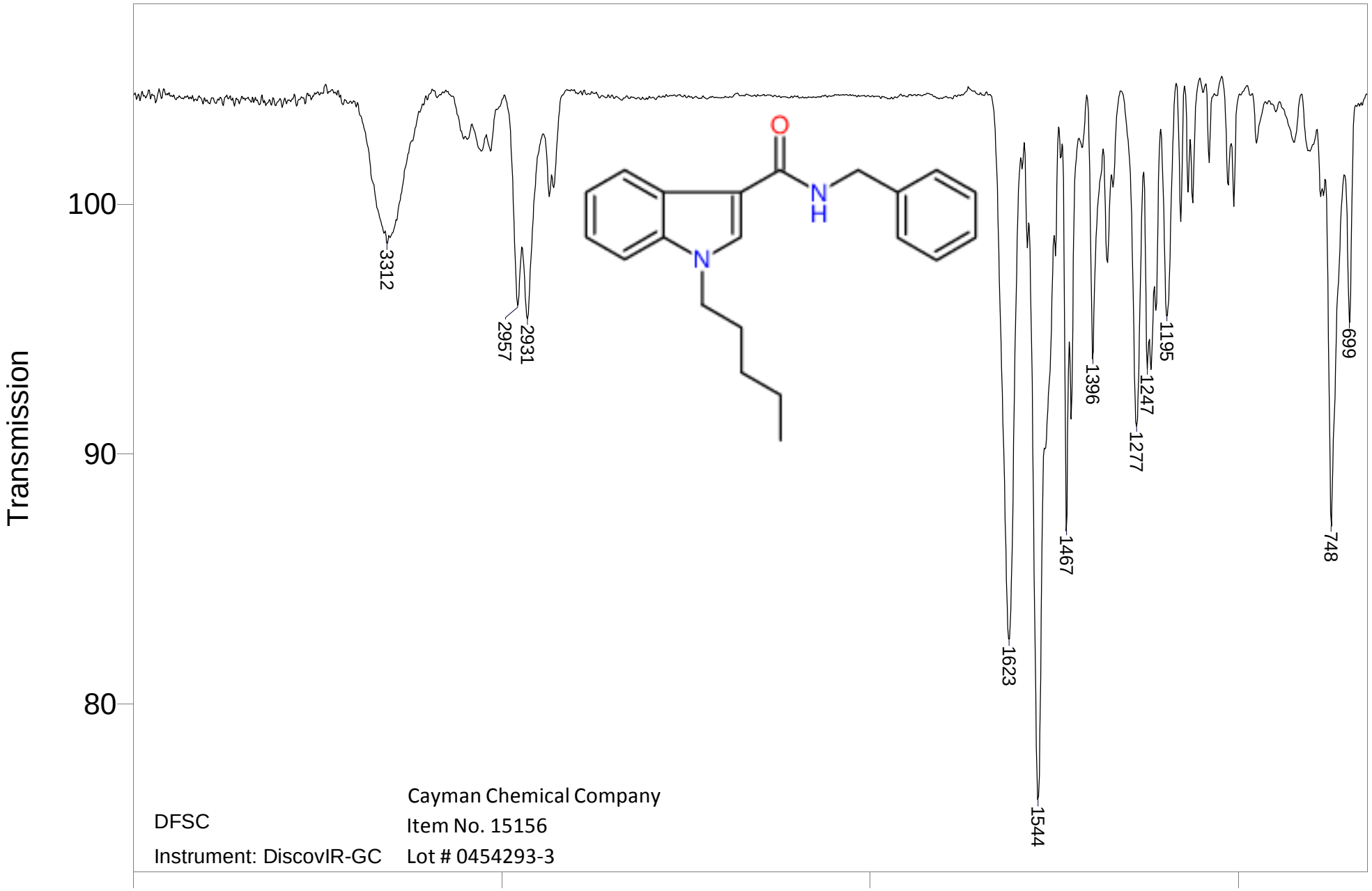
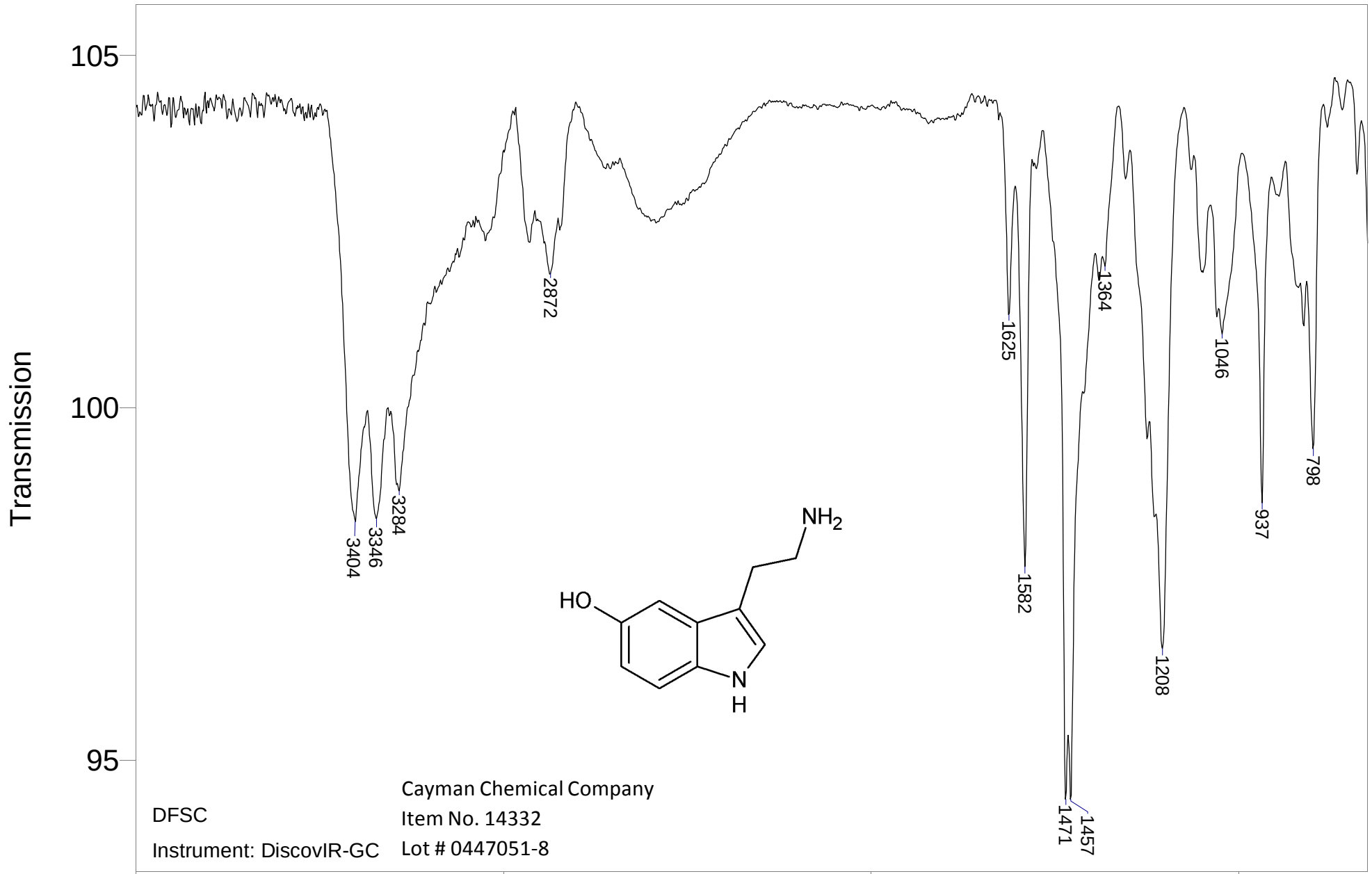
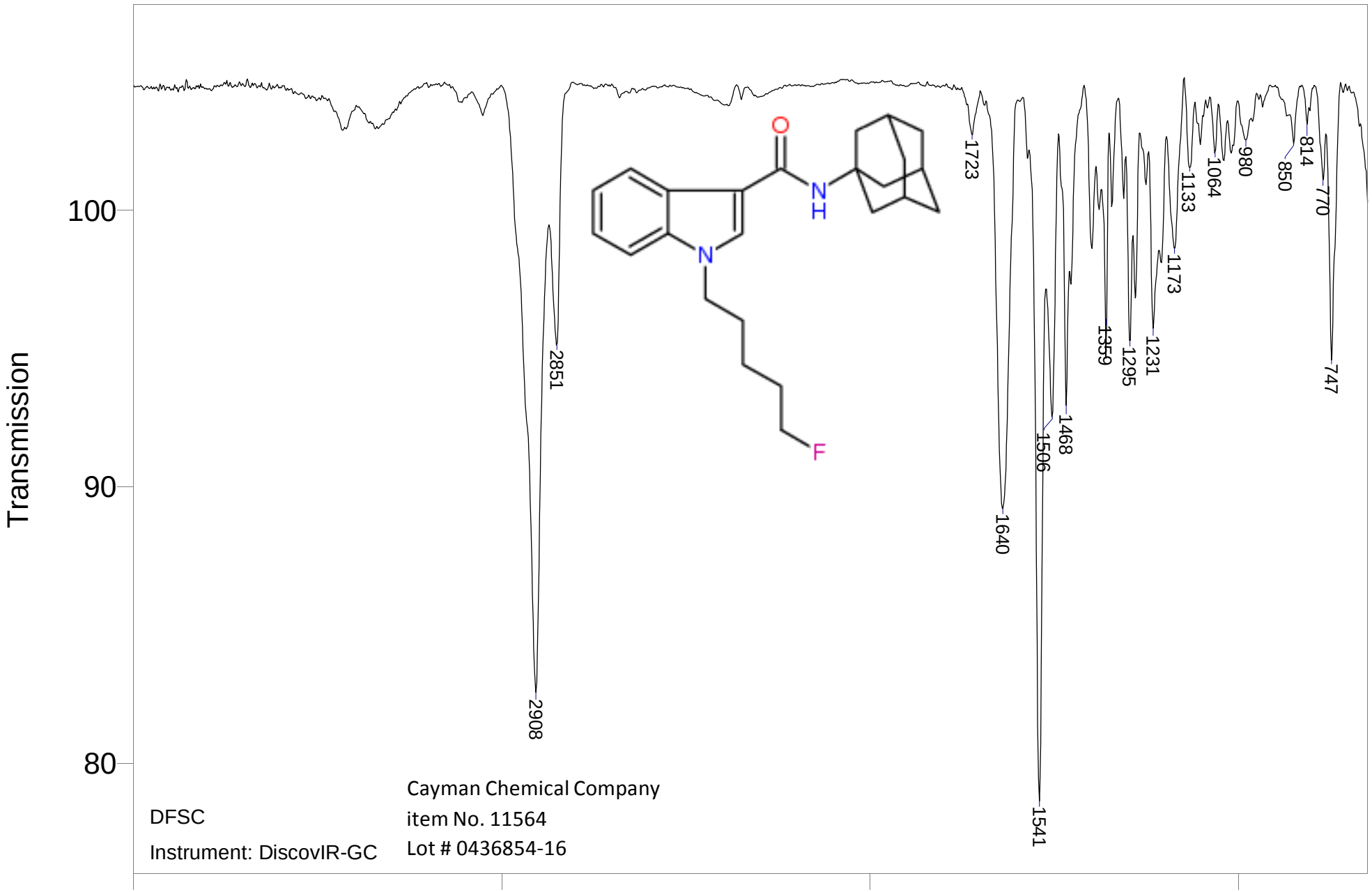
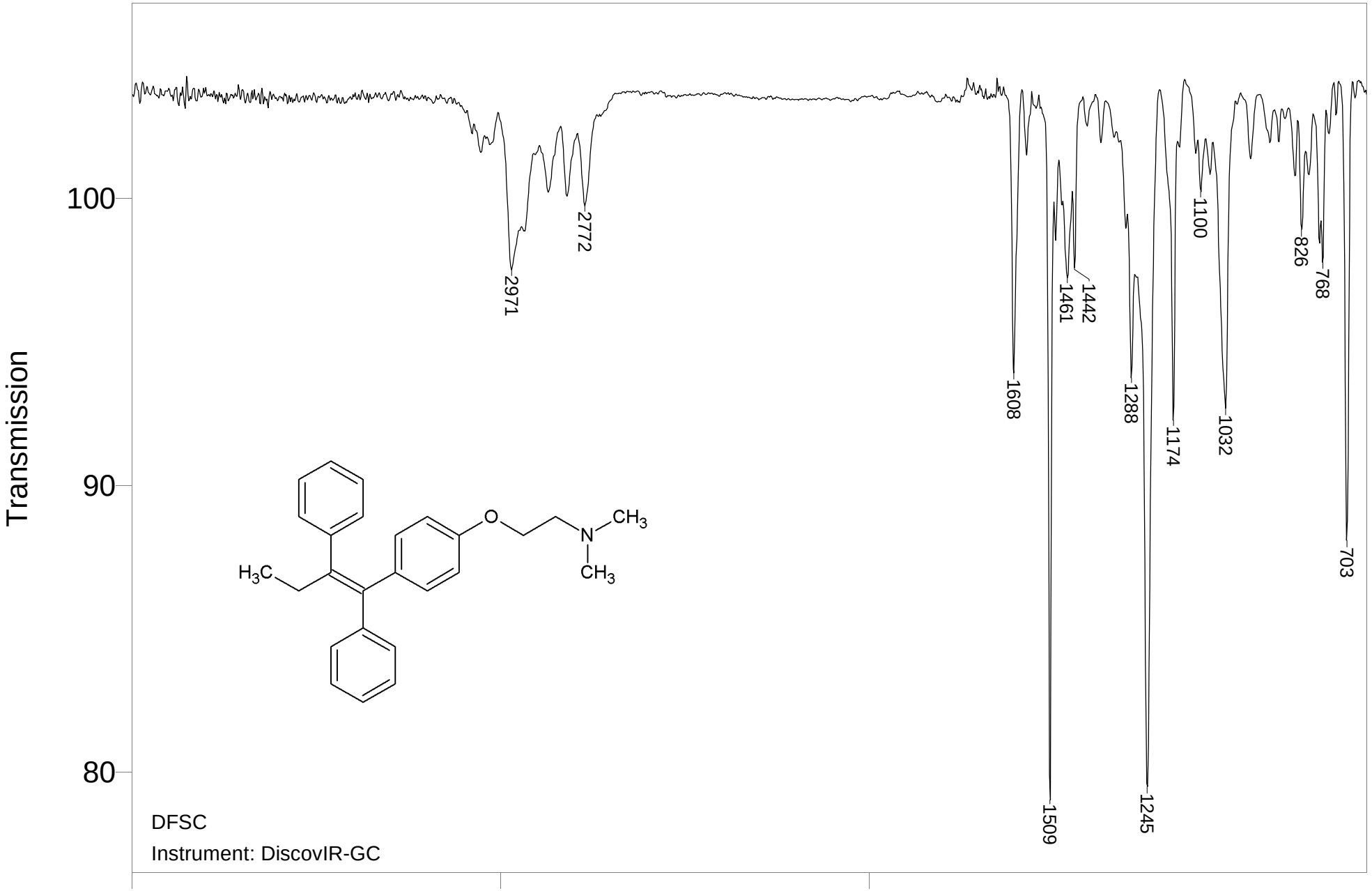


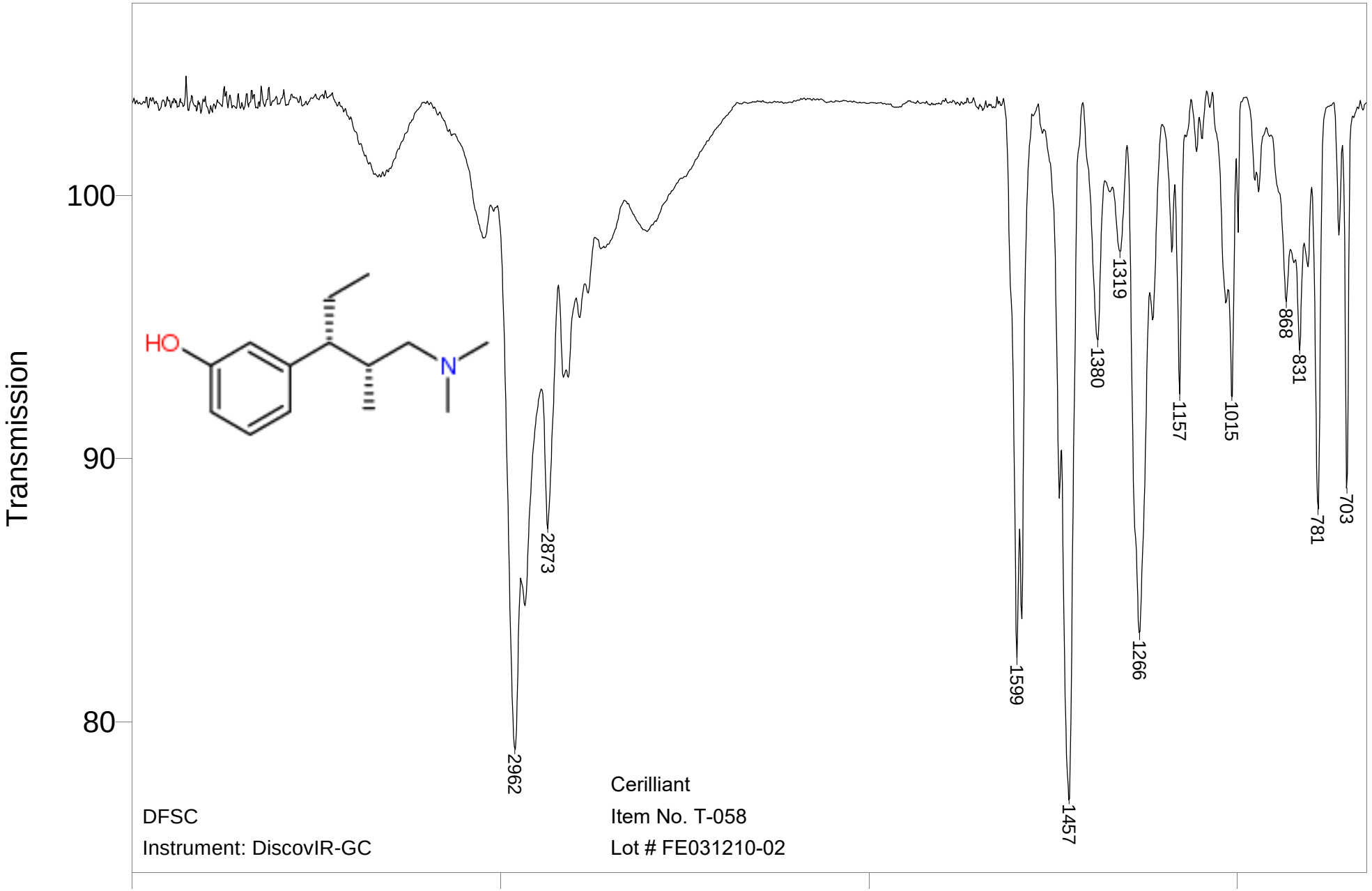


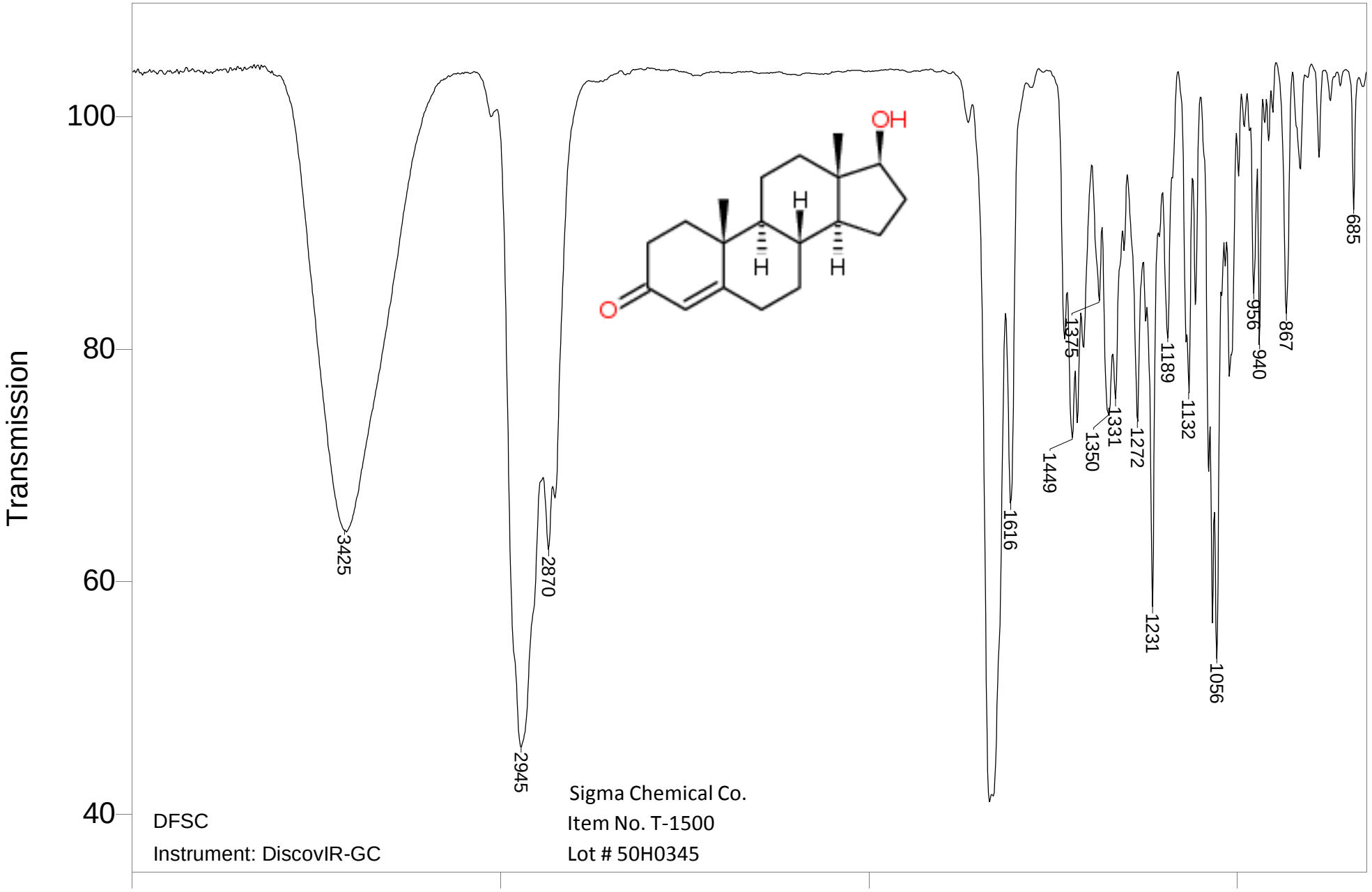


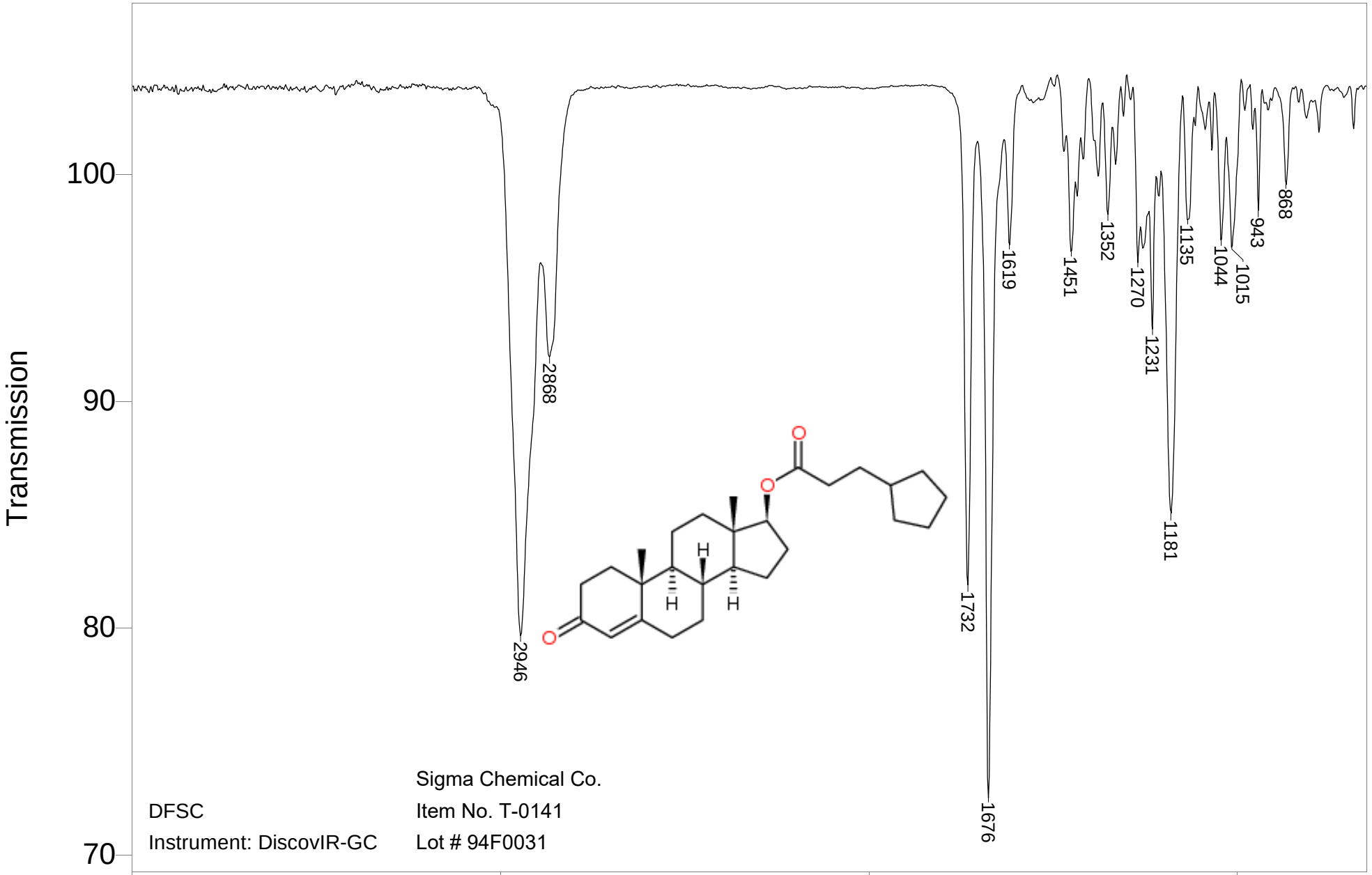


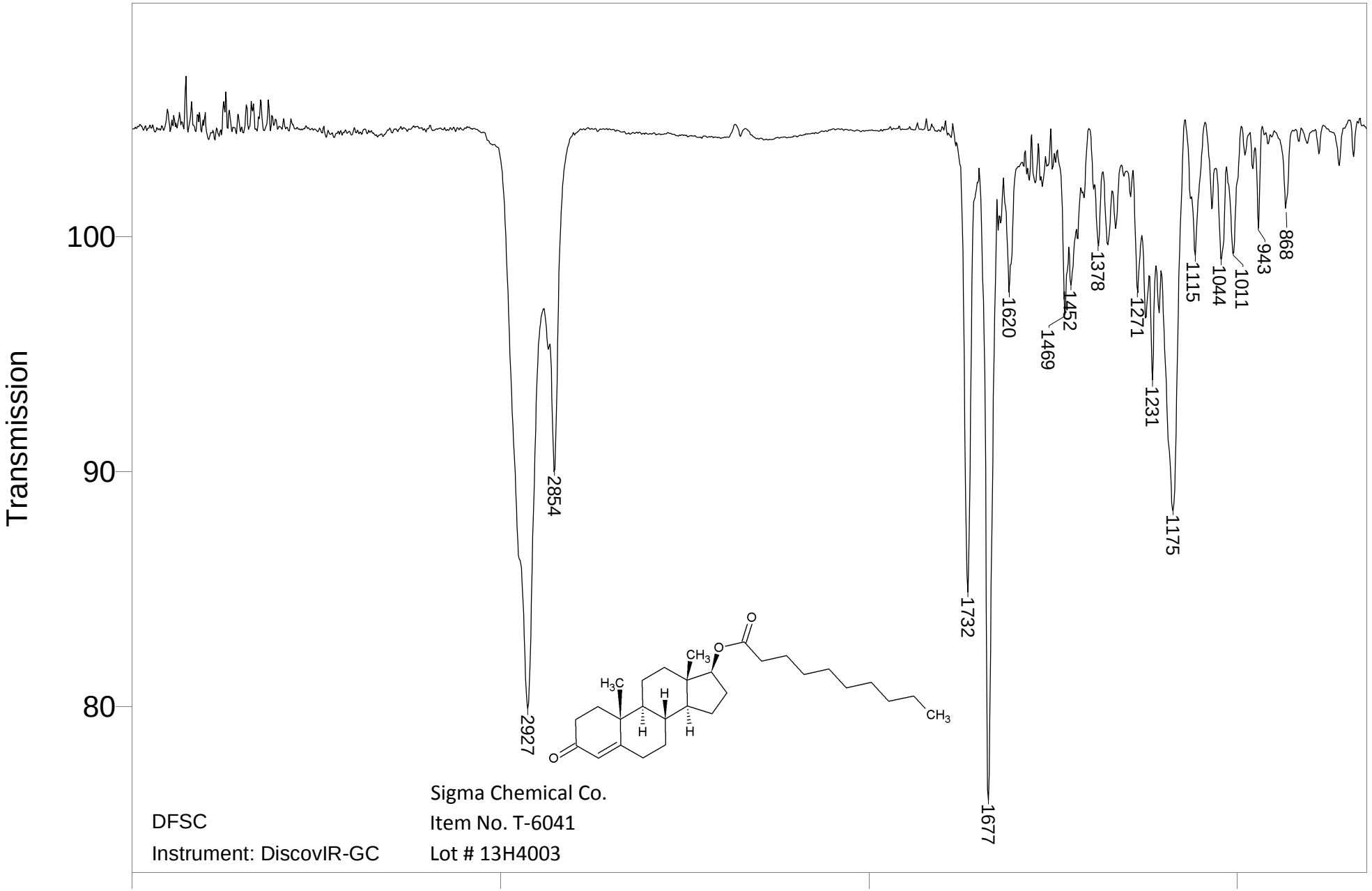




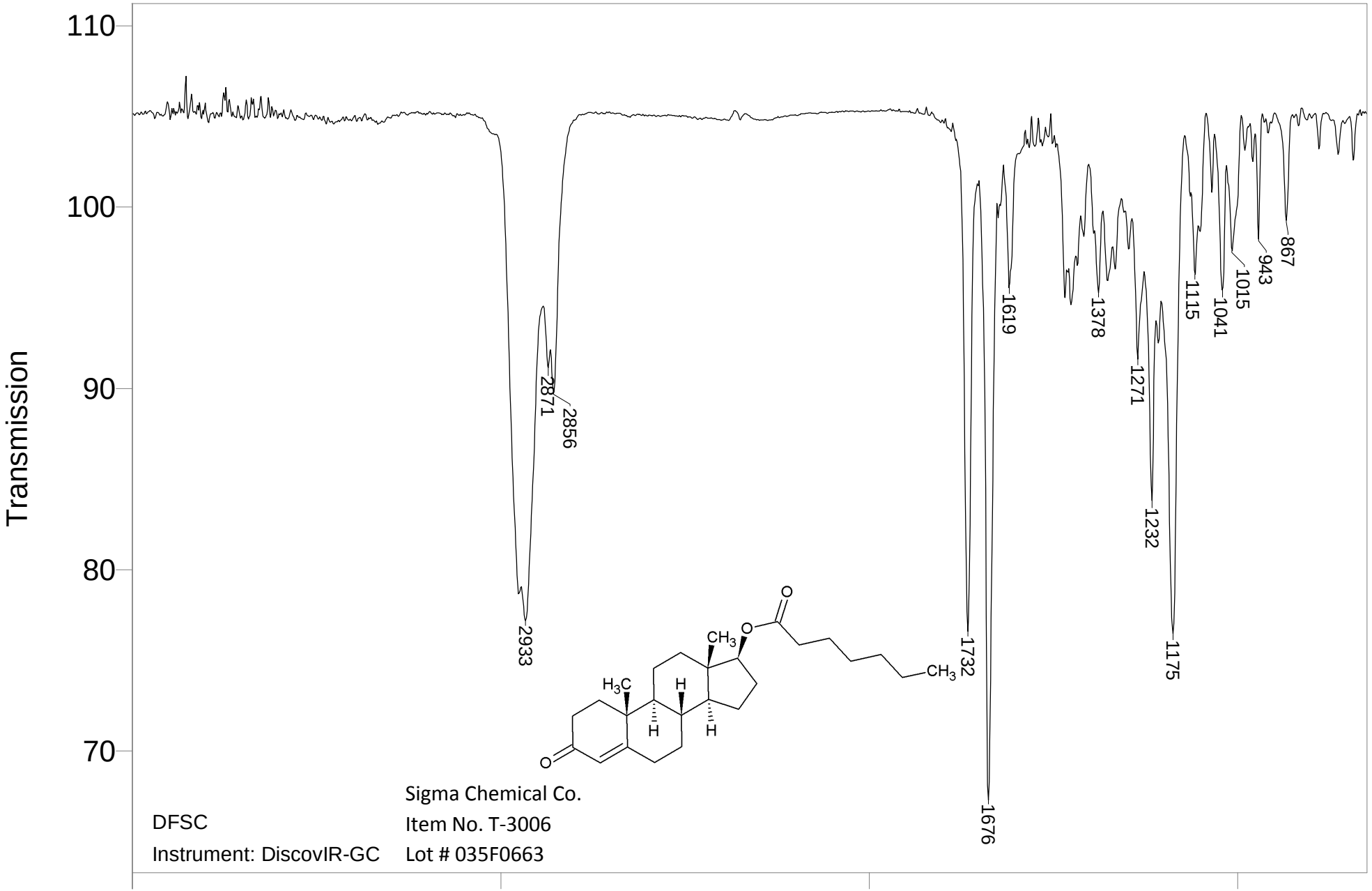




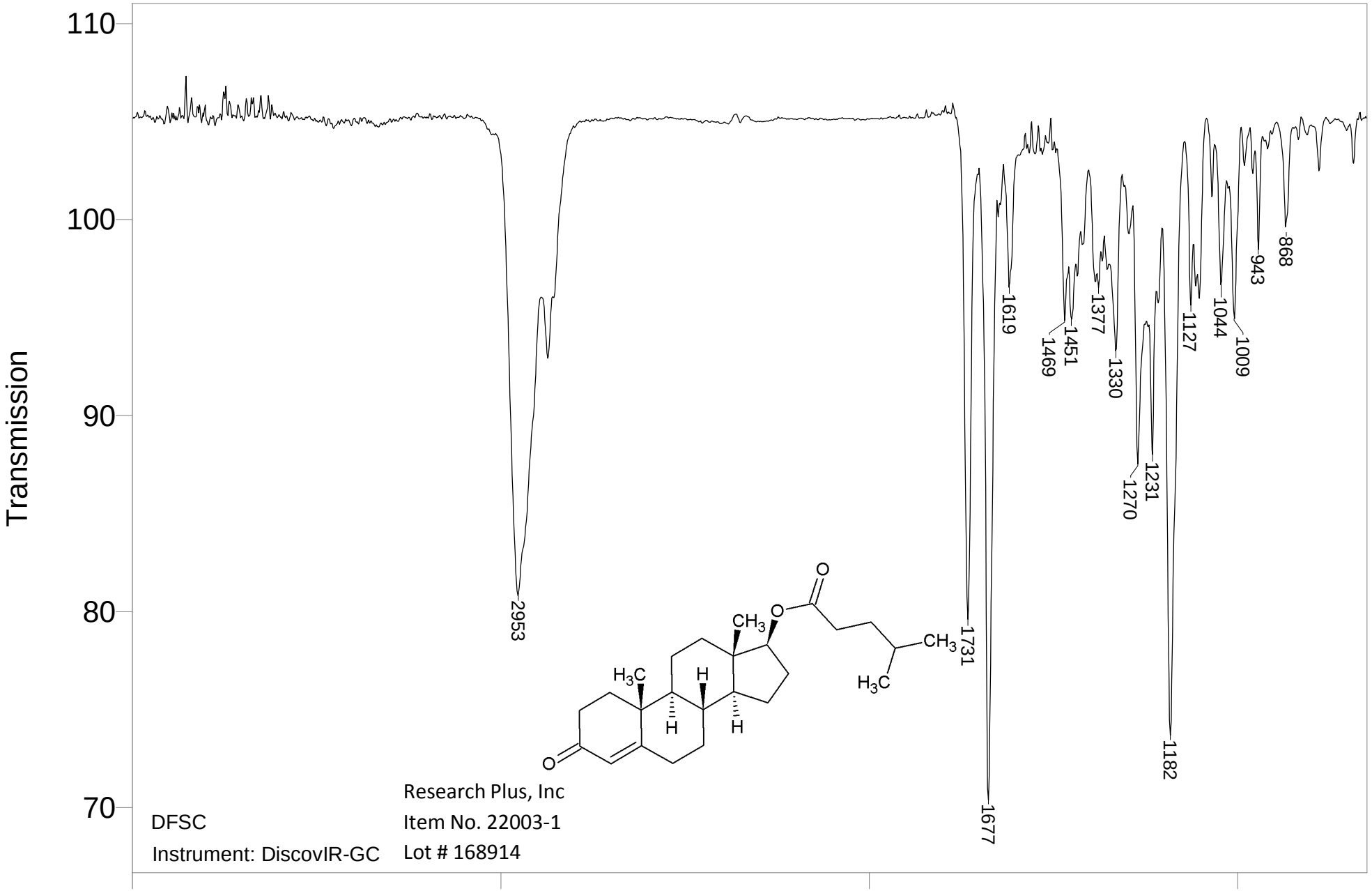




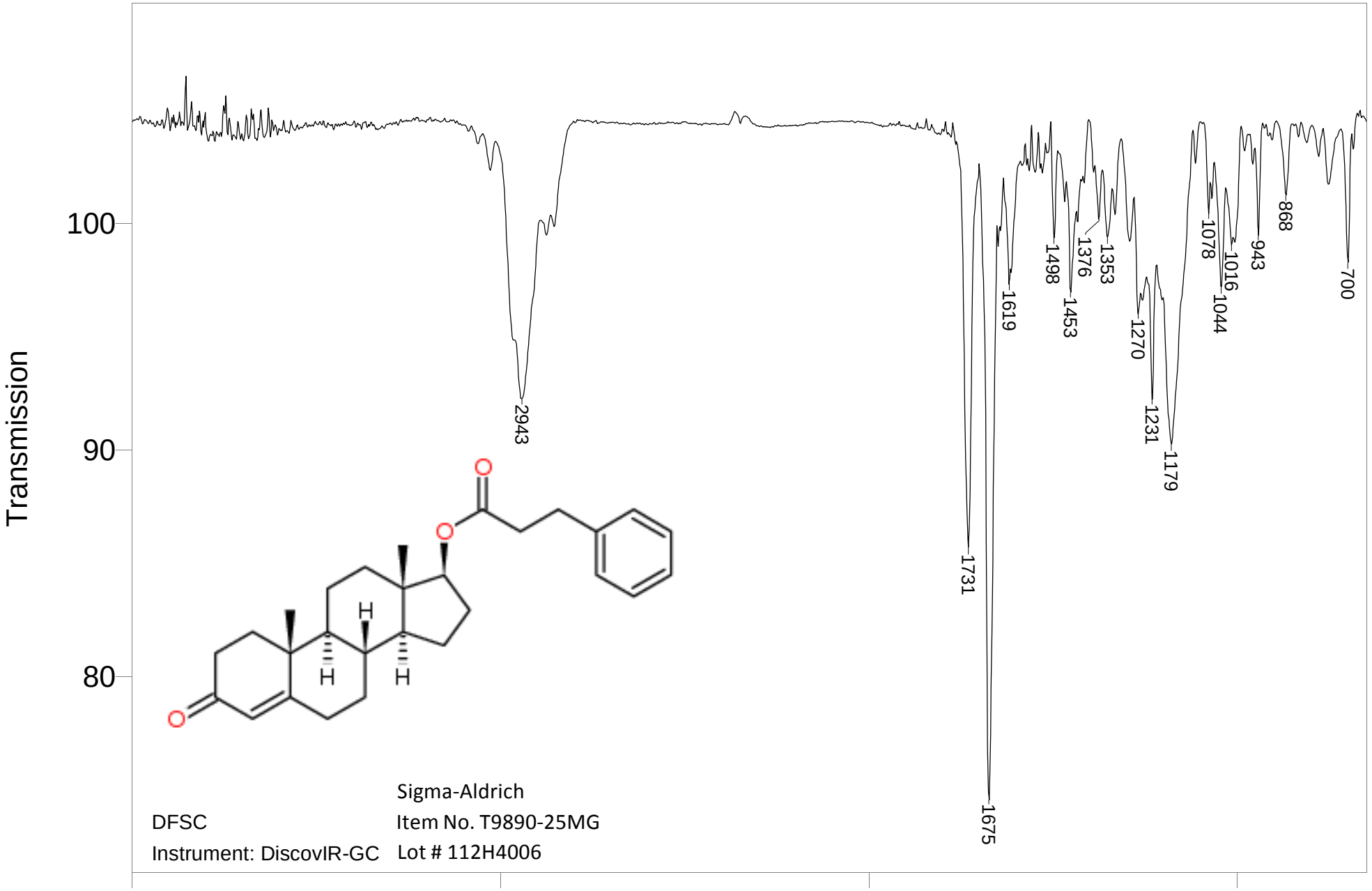
Testosterone Enanthate



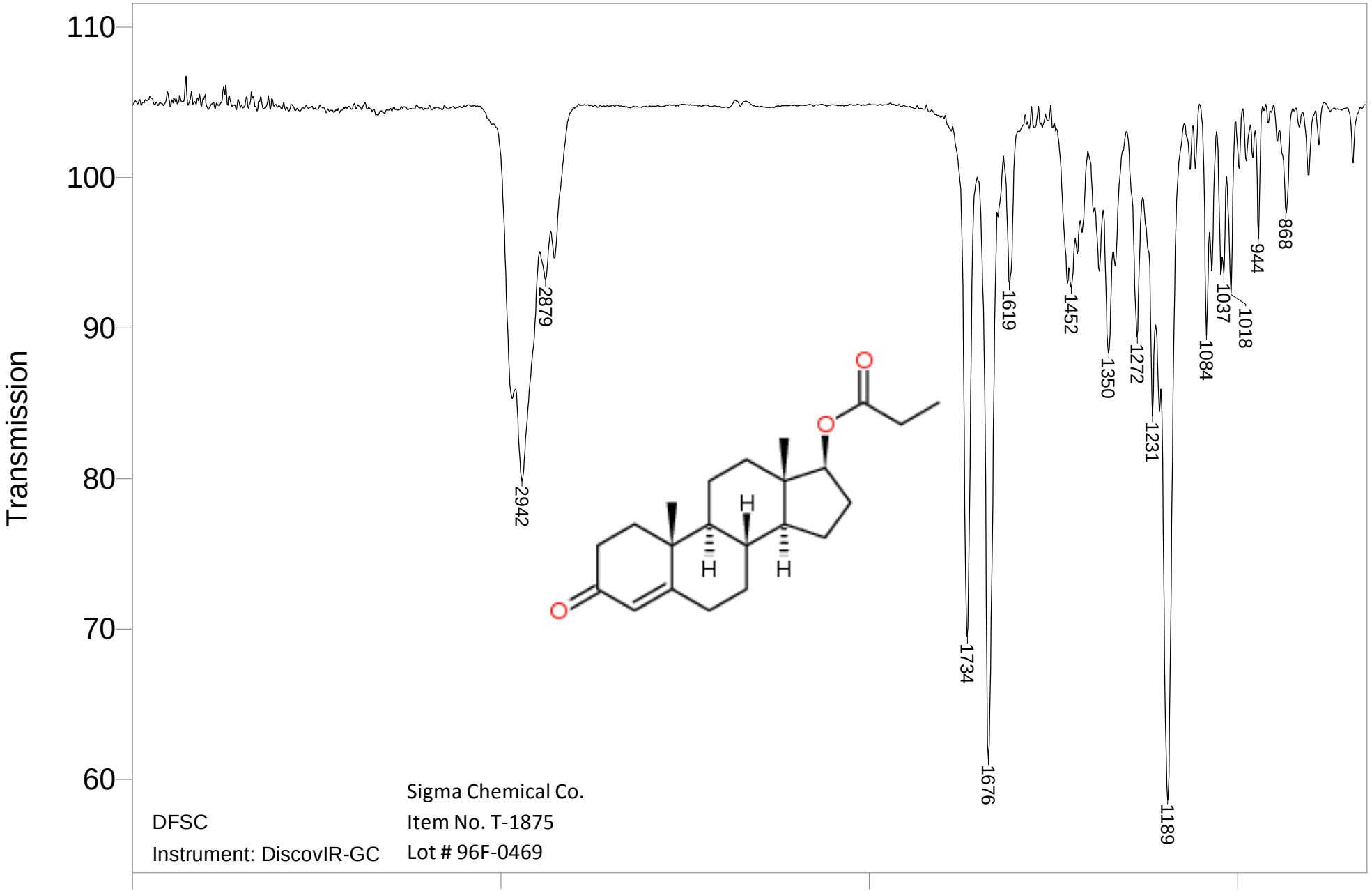
Testosterone Isocaproate



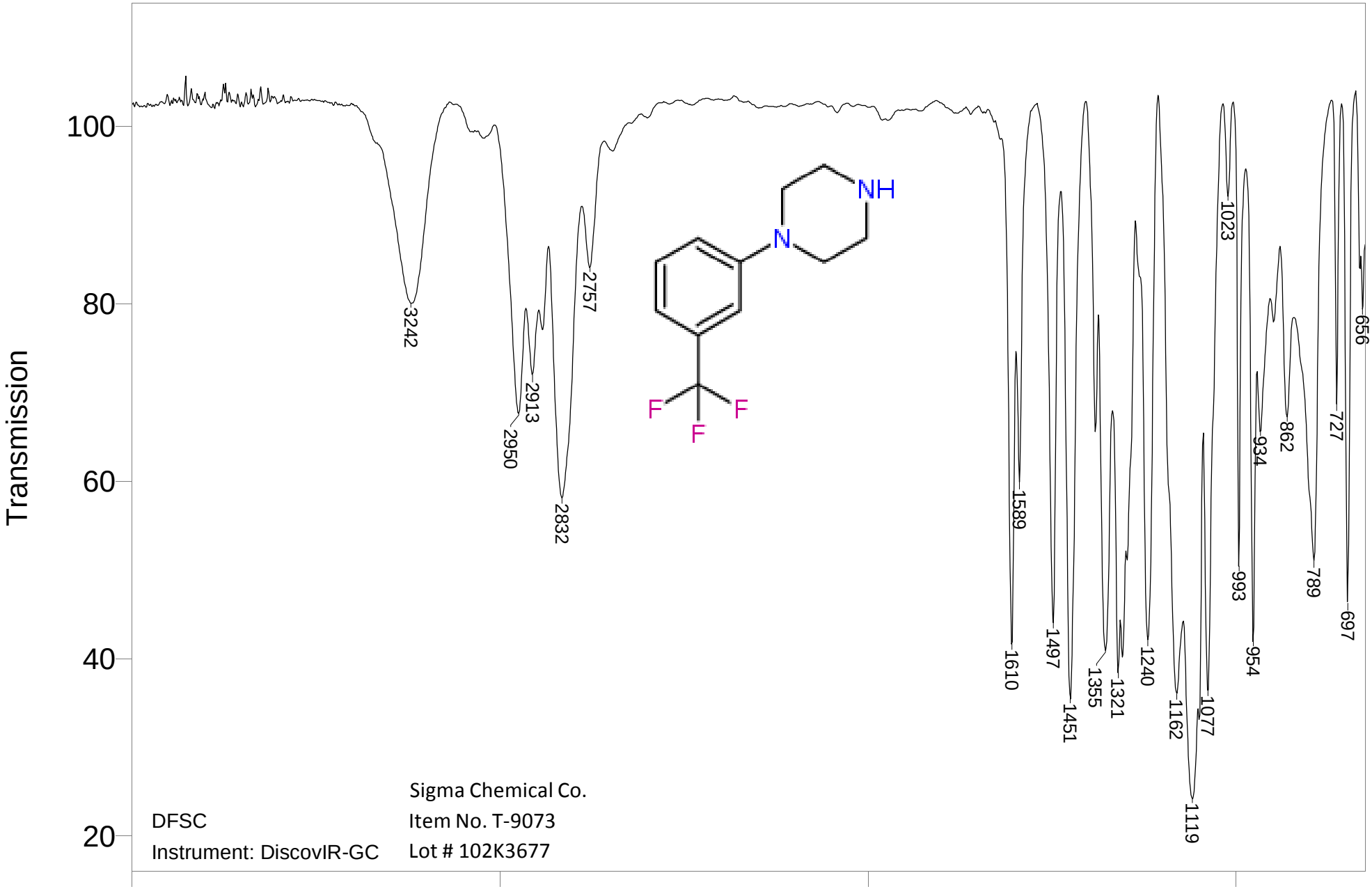
Testosterone Phenylpropionate

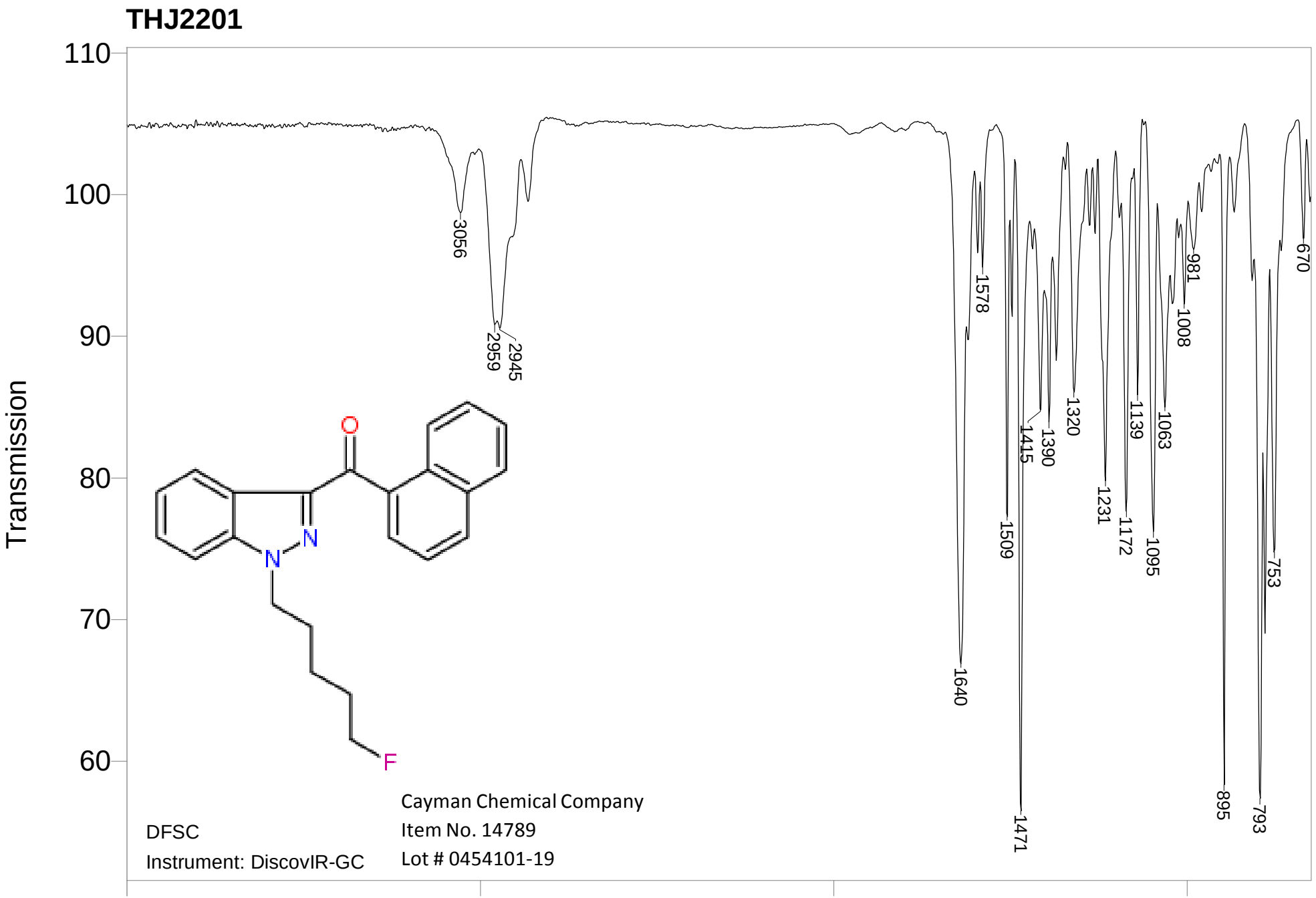


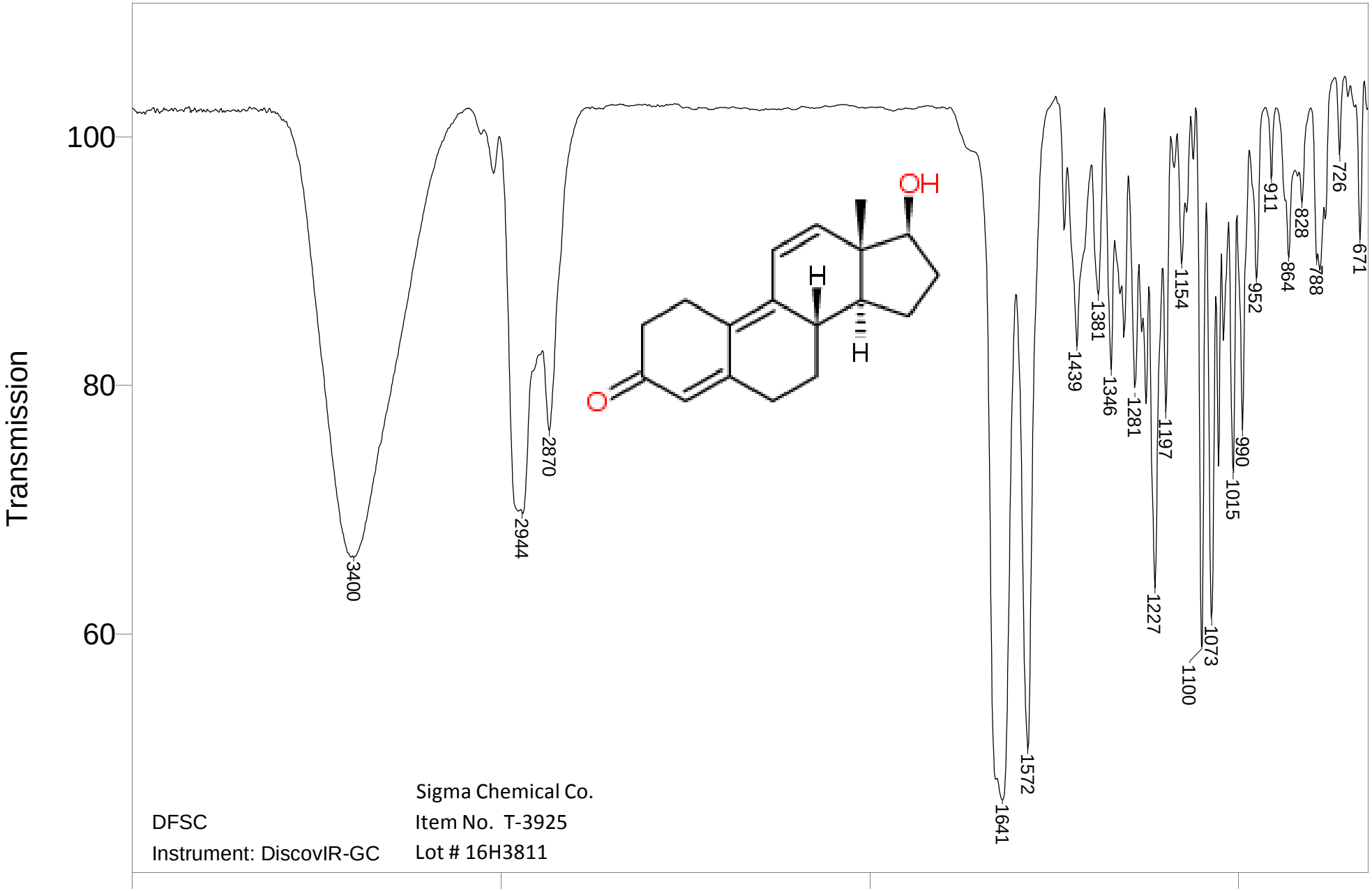
Testosterone Propionate

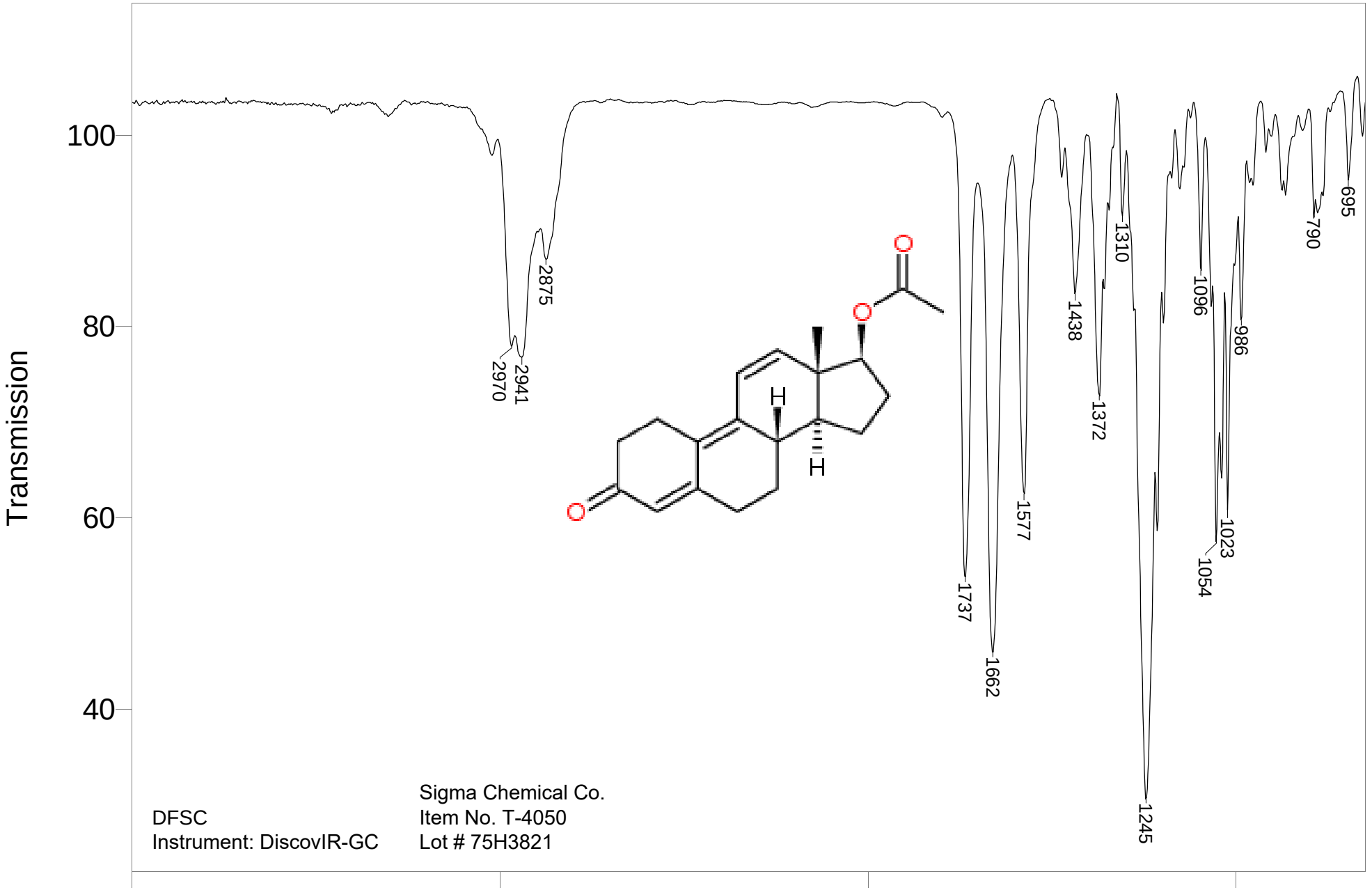


TFMPP 1(a,a,a-trifluoro-m-tolyl)-piperazine

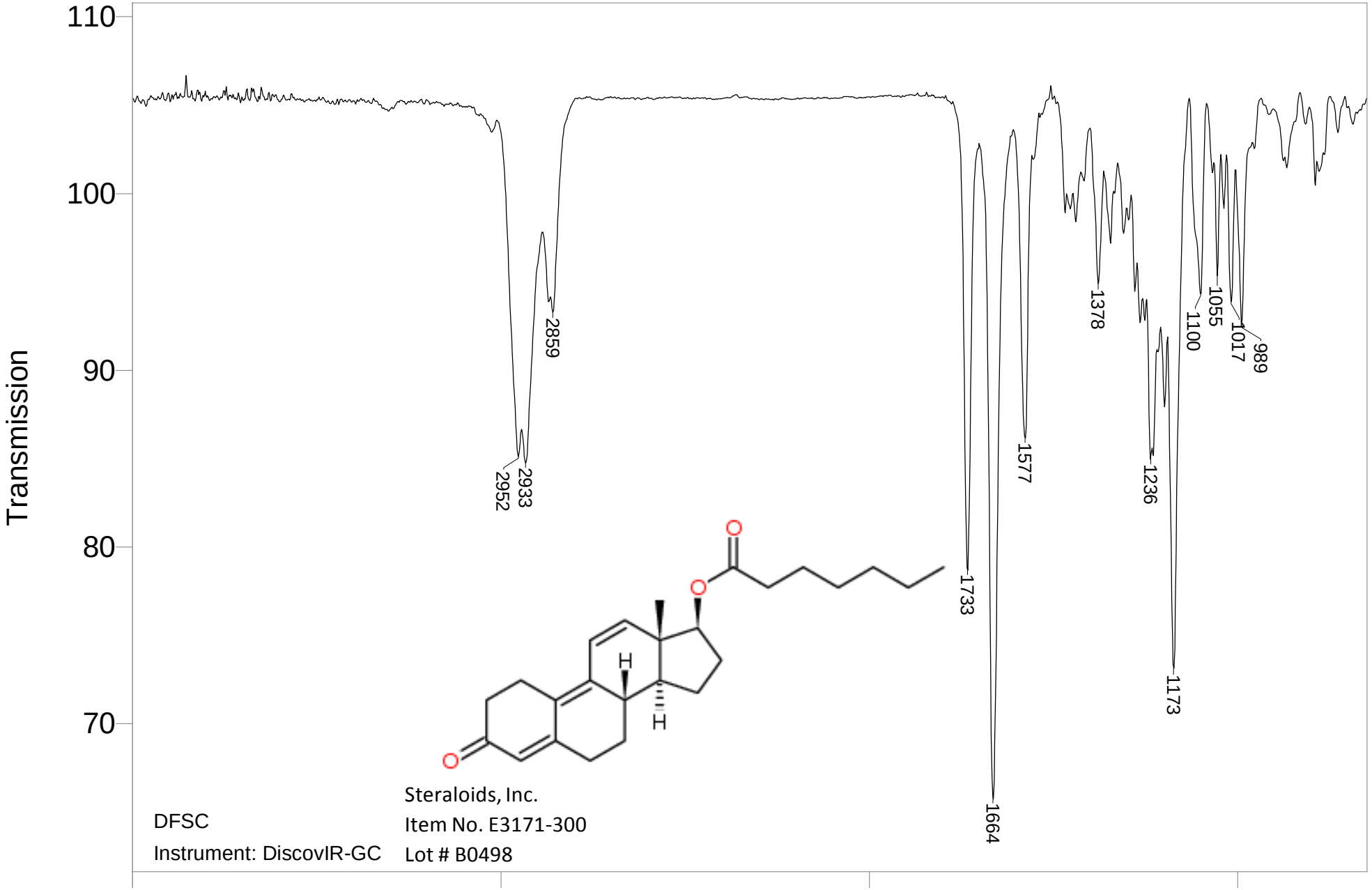


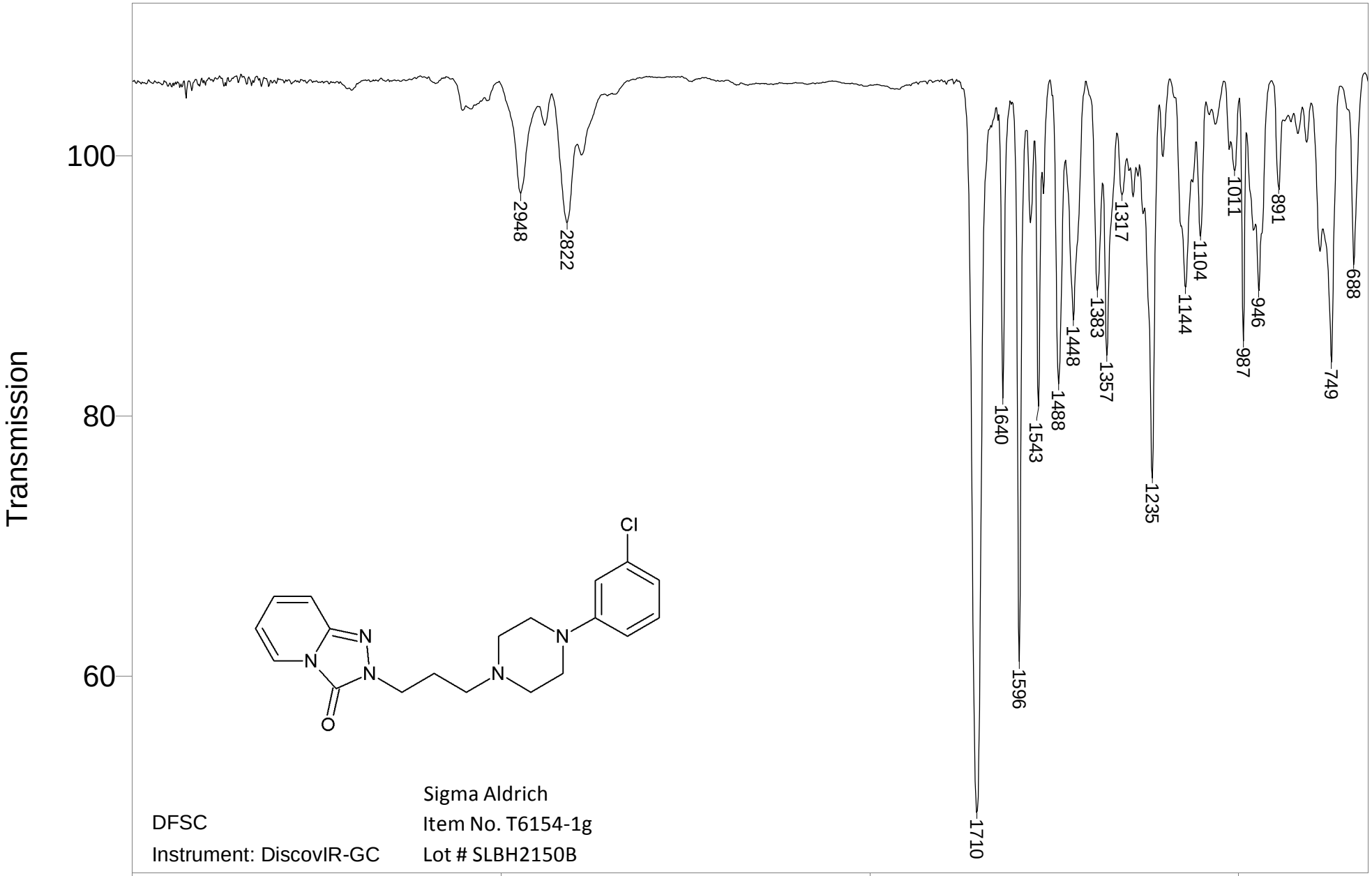


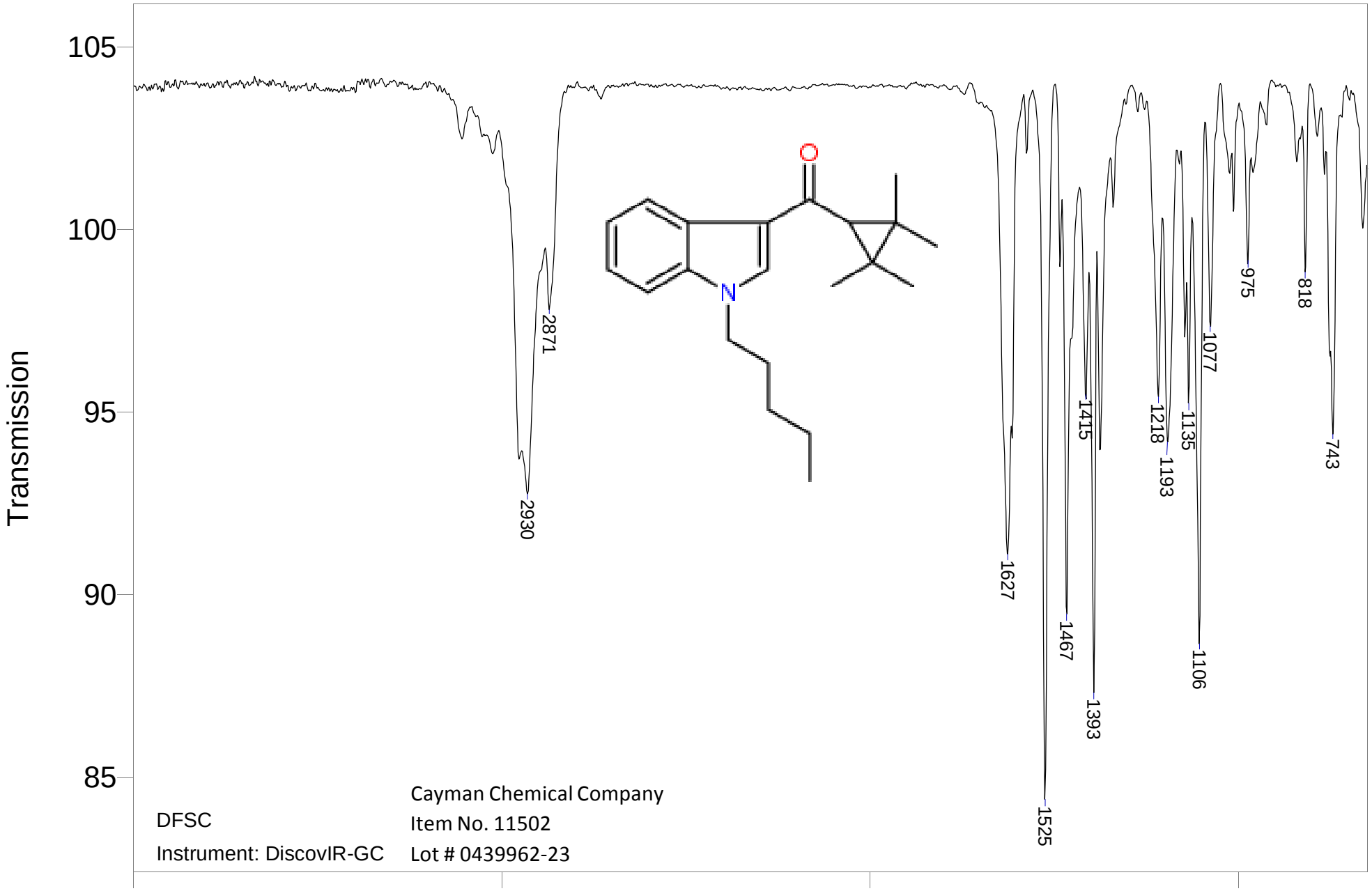


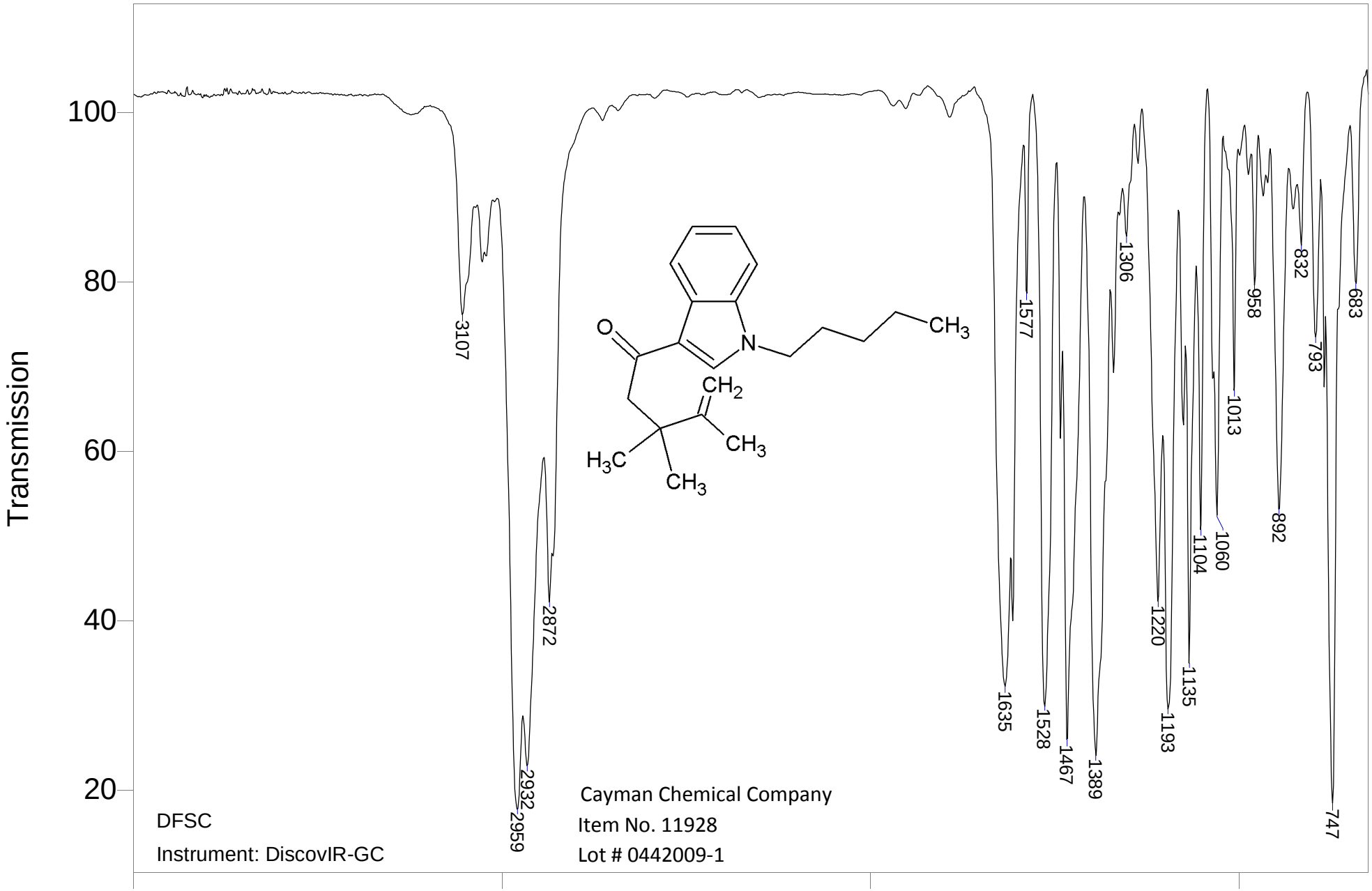


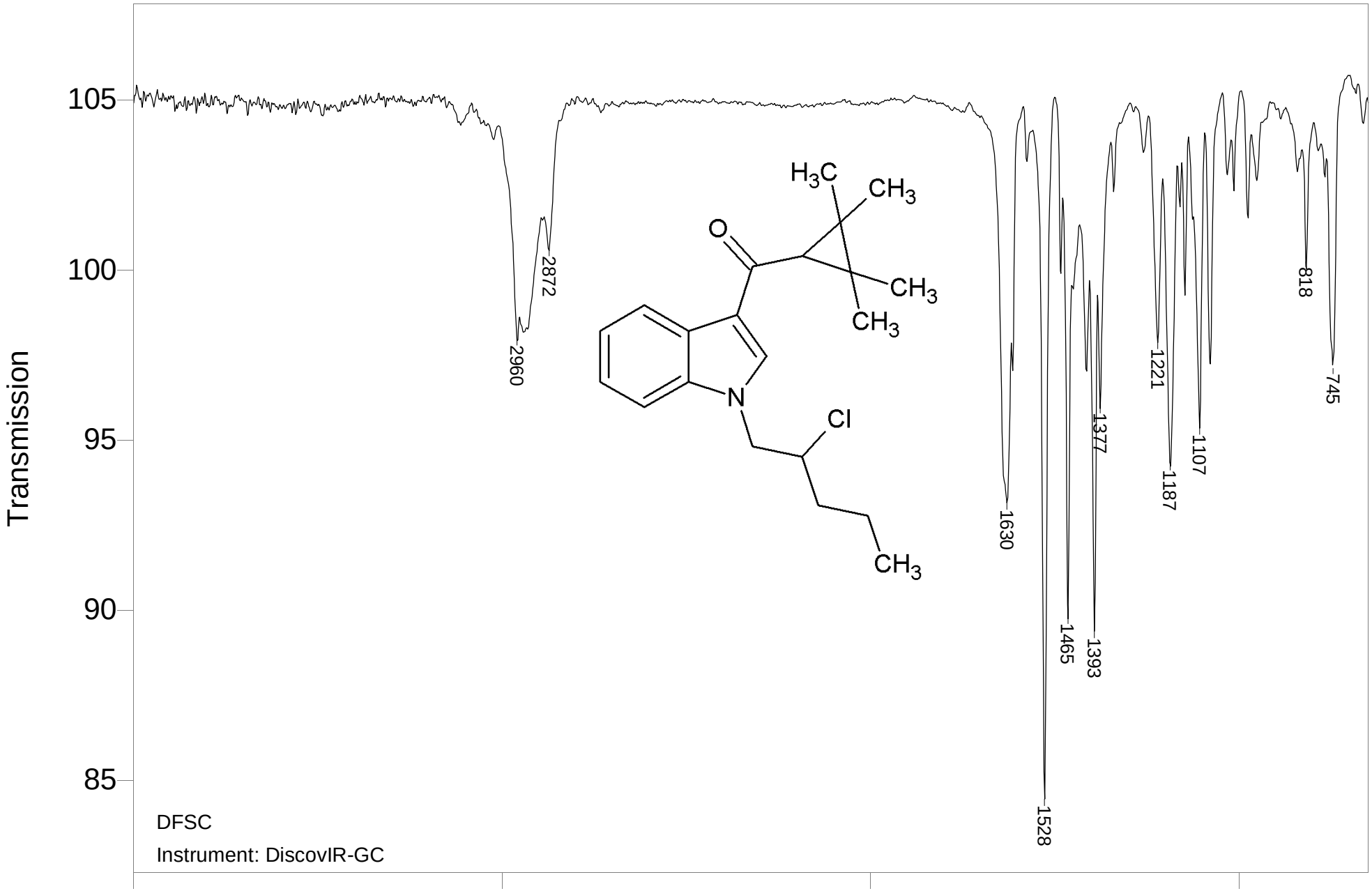
Trenbolone Enanthate

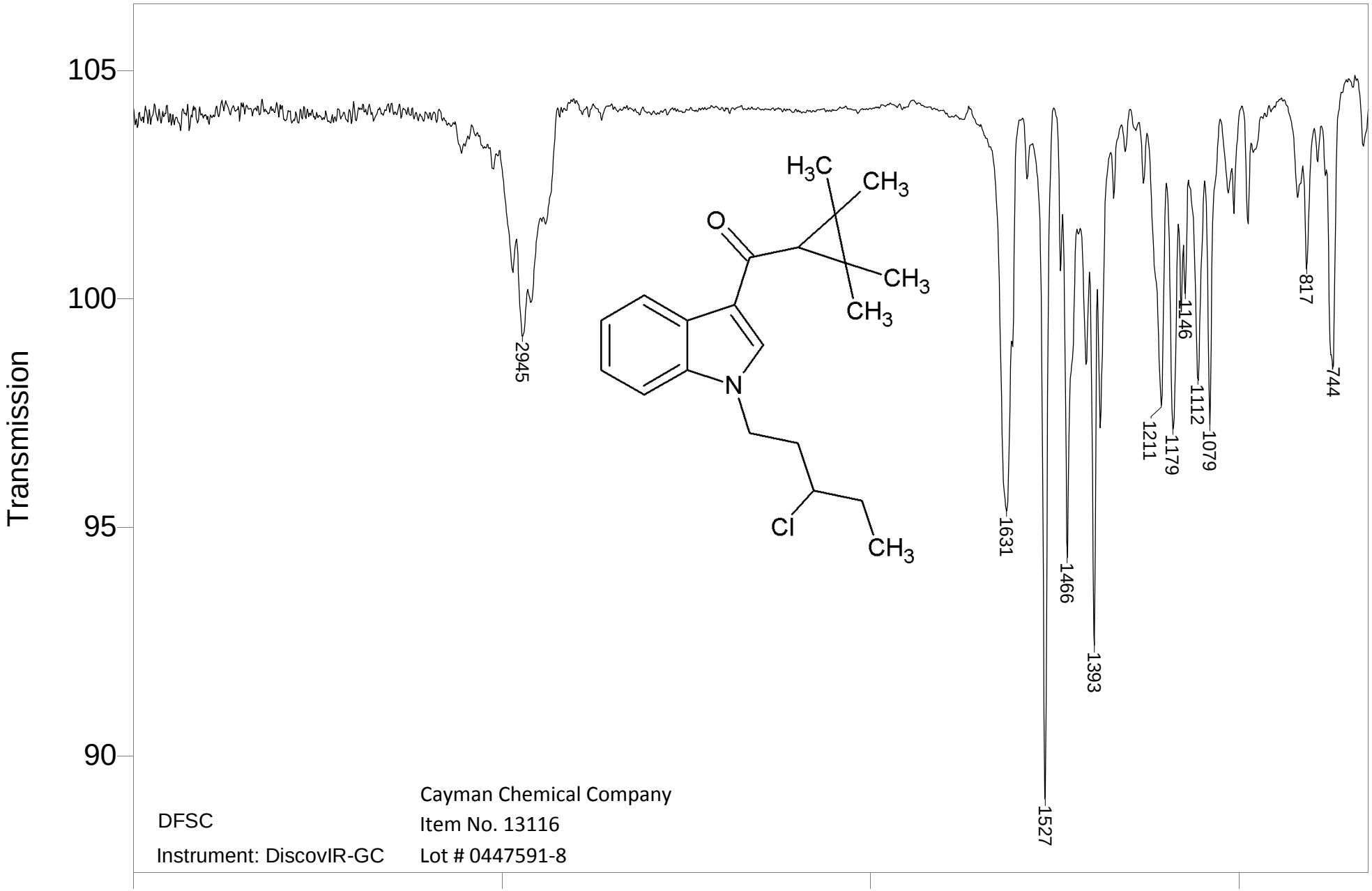


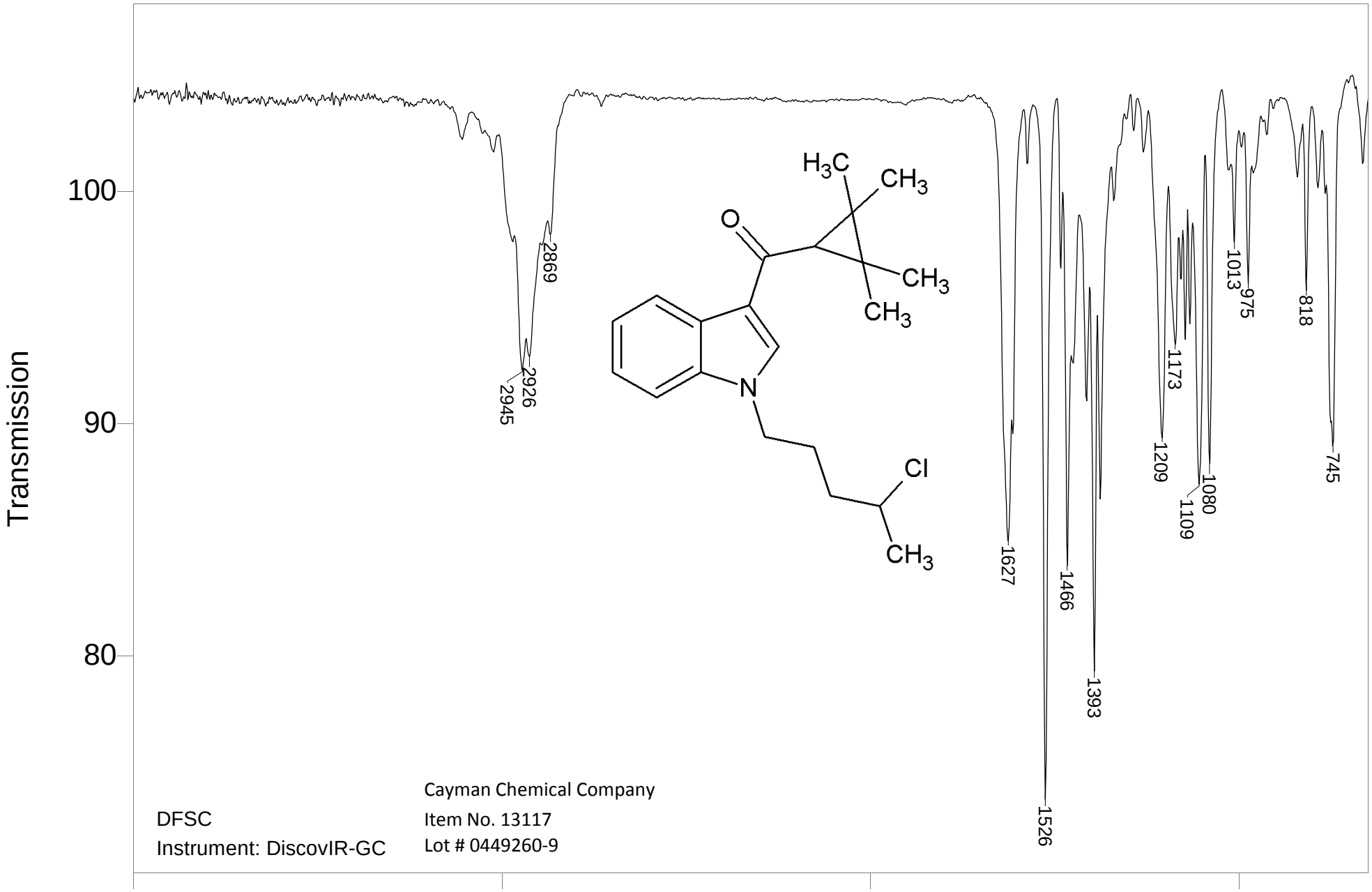


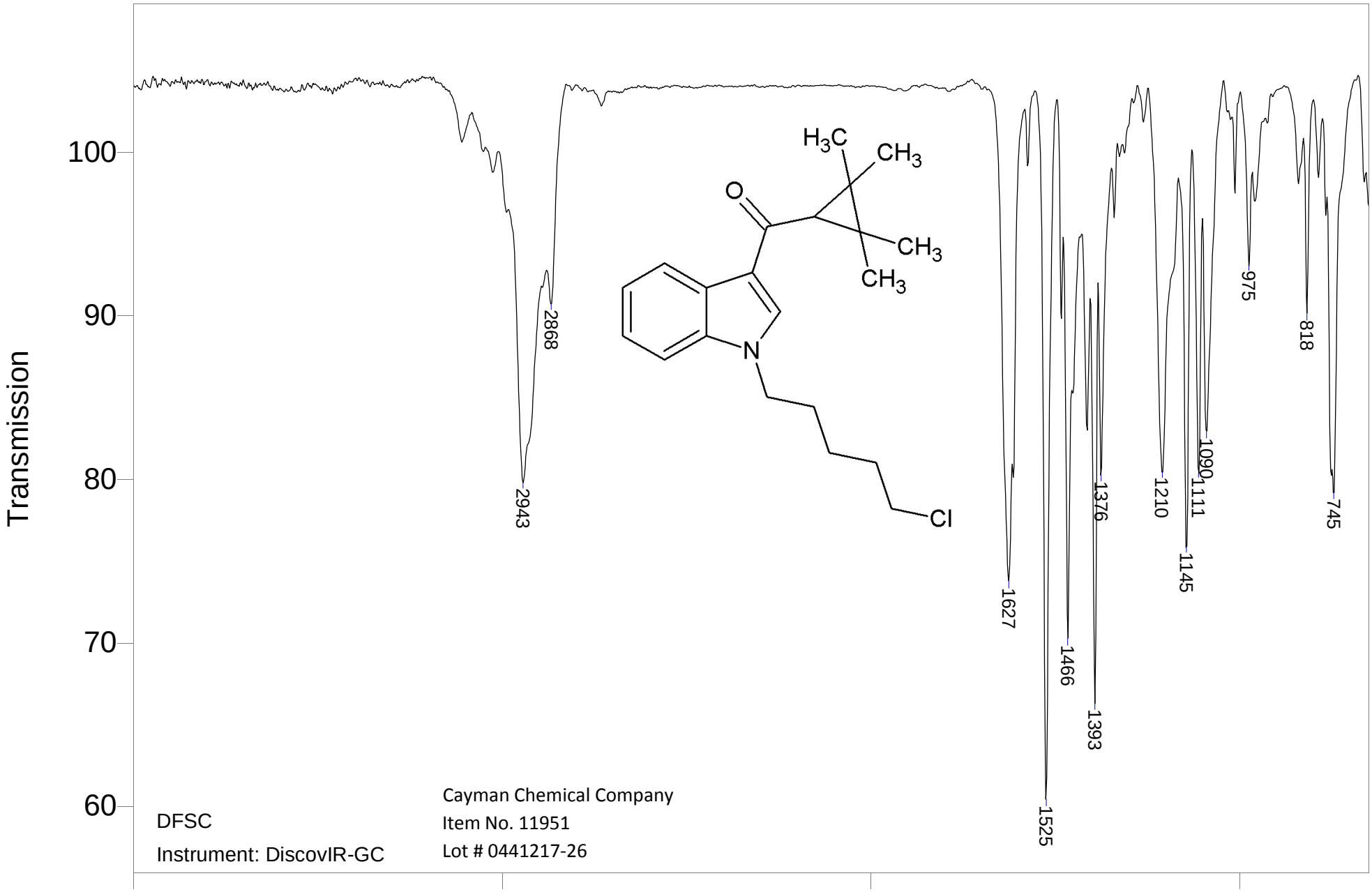


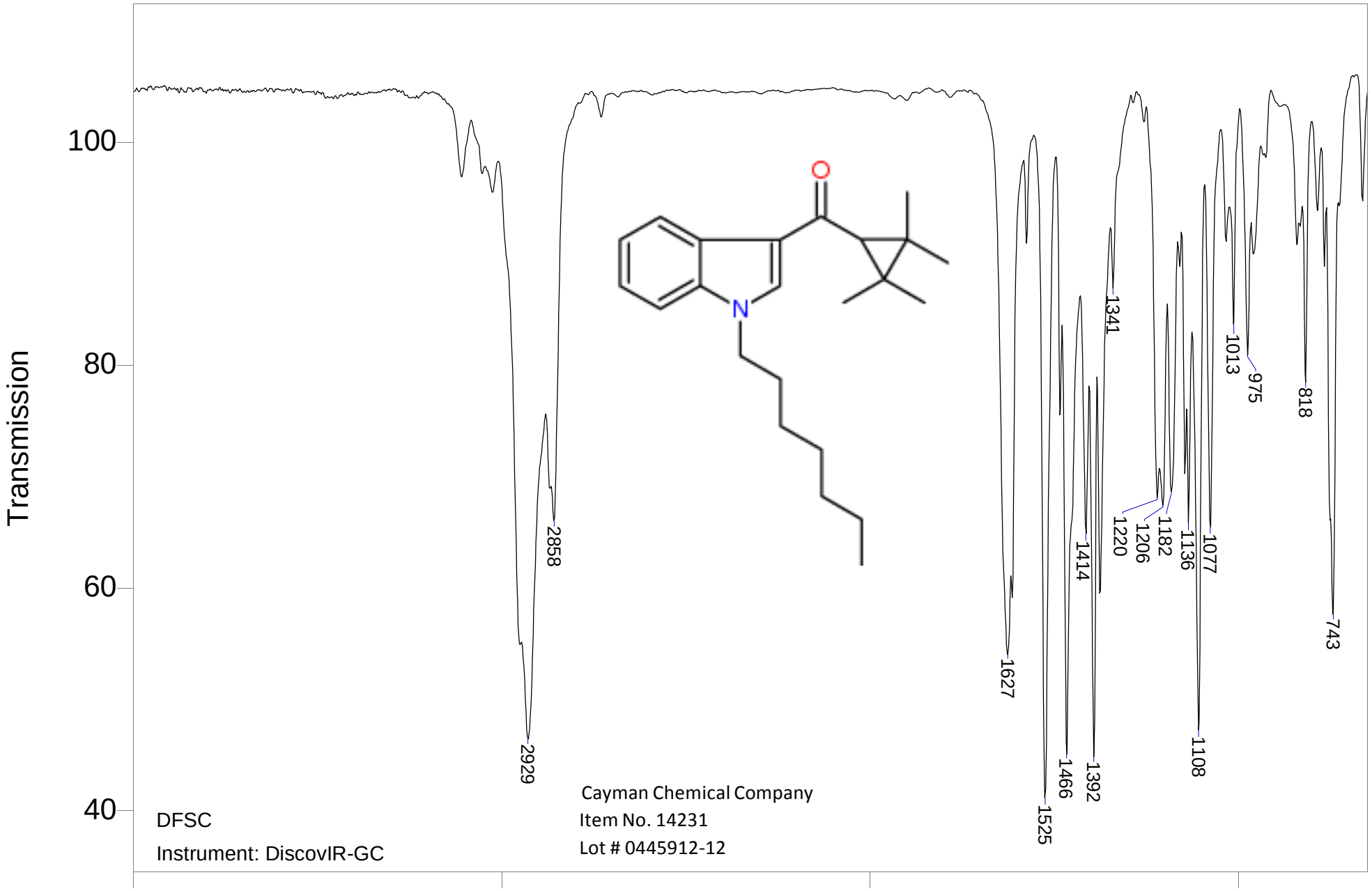


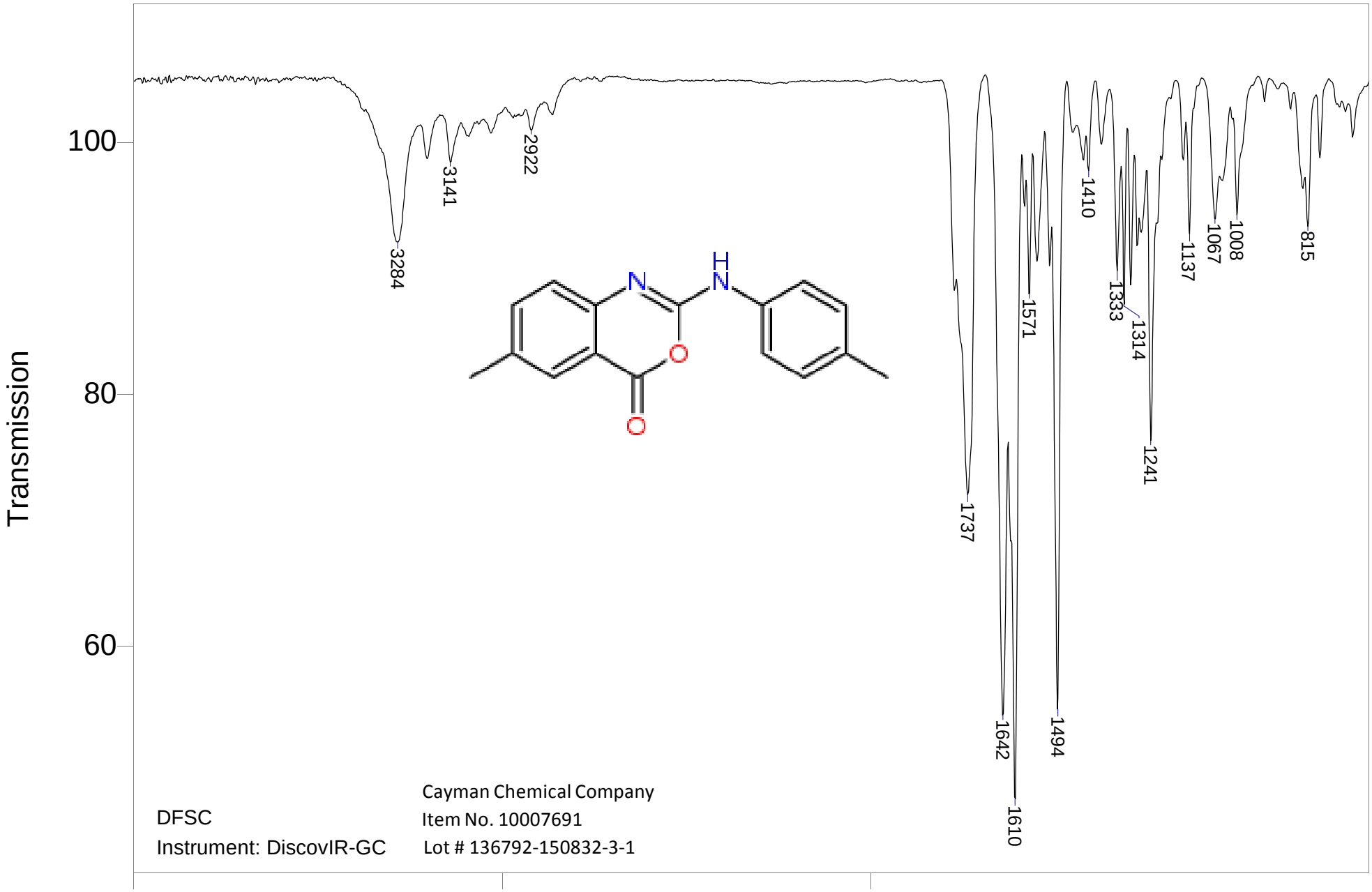


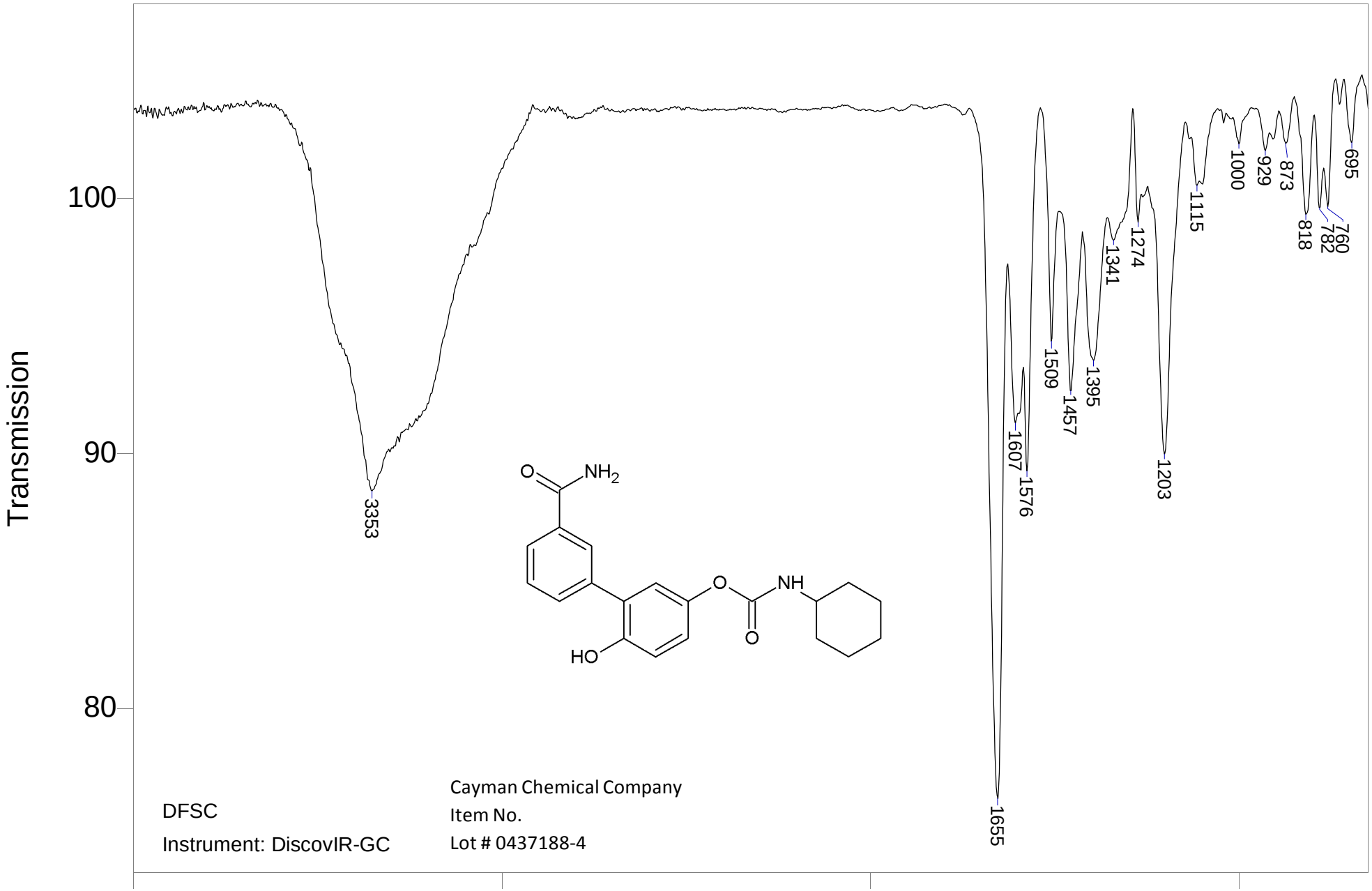


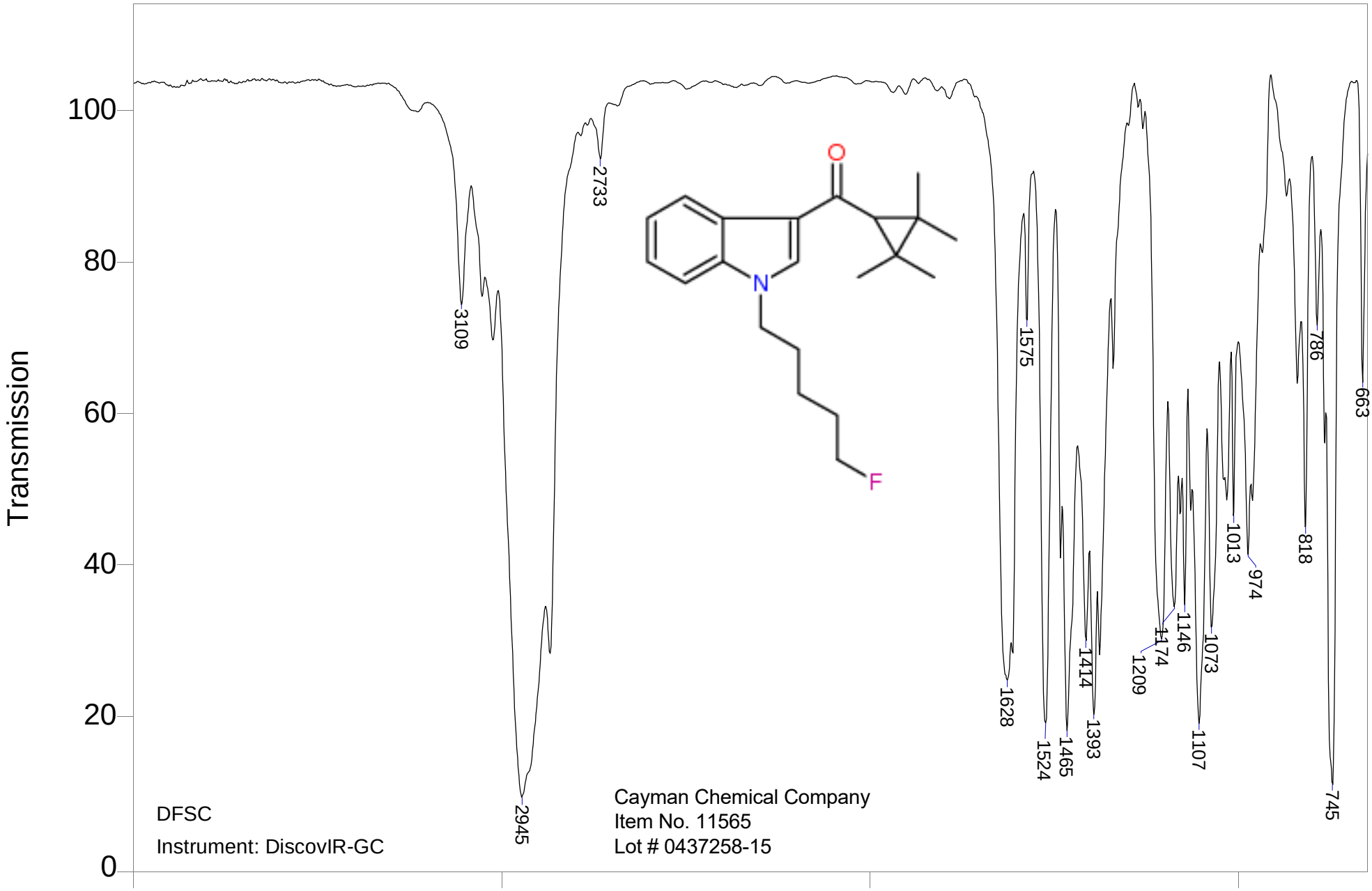


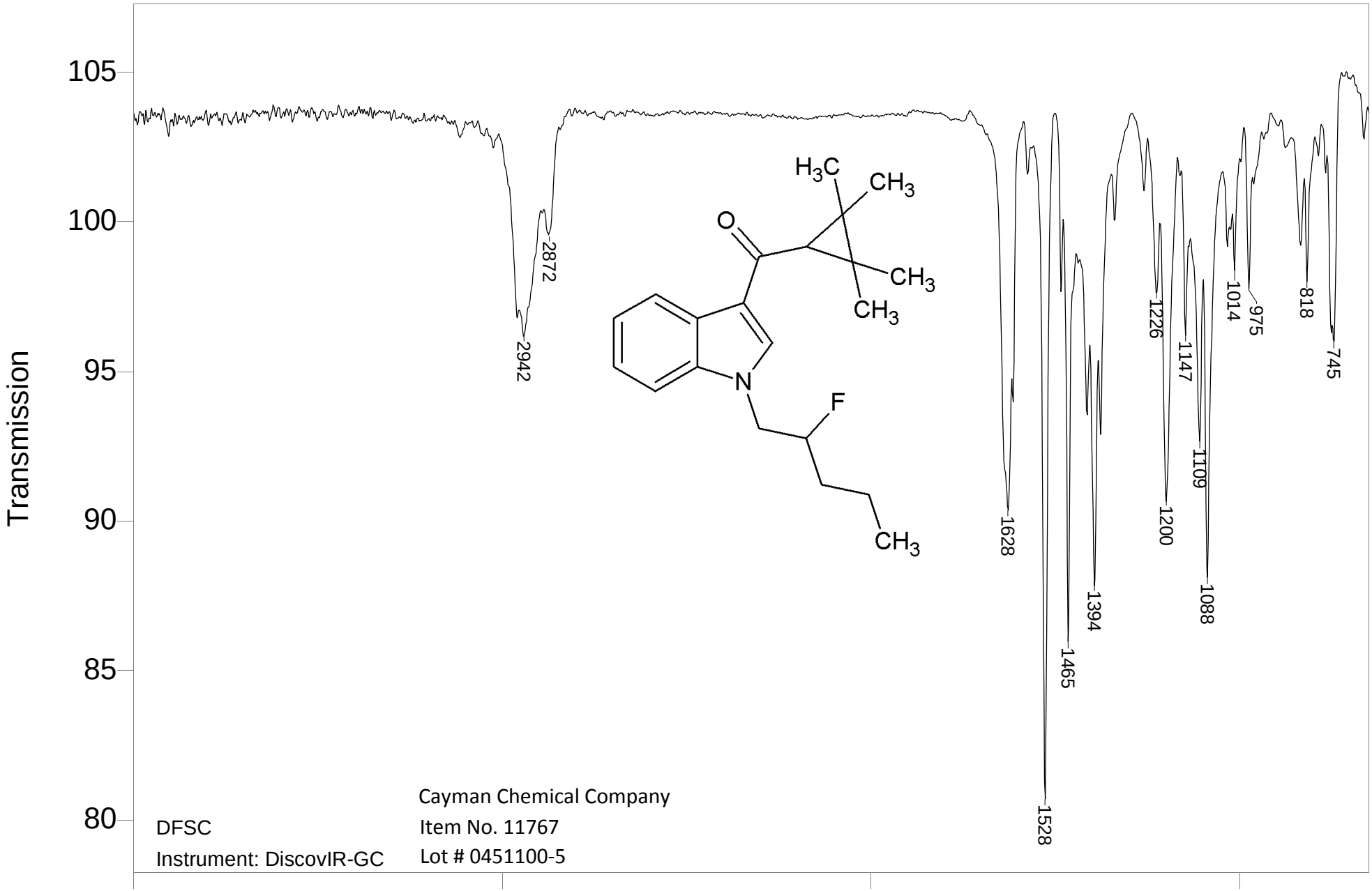


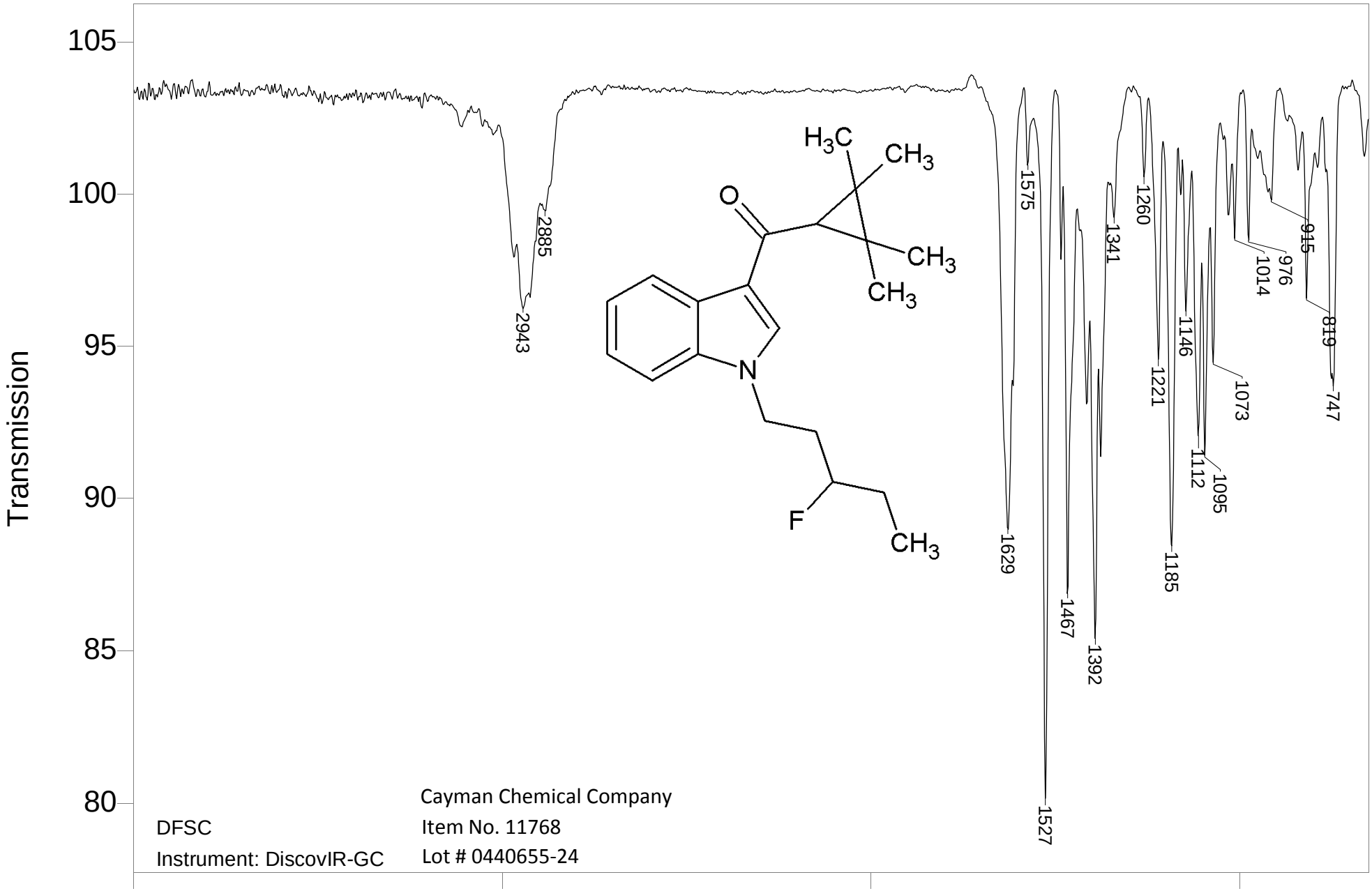


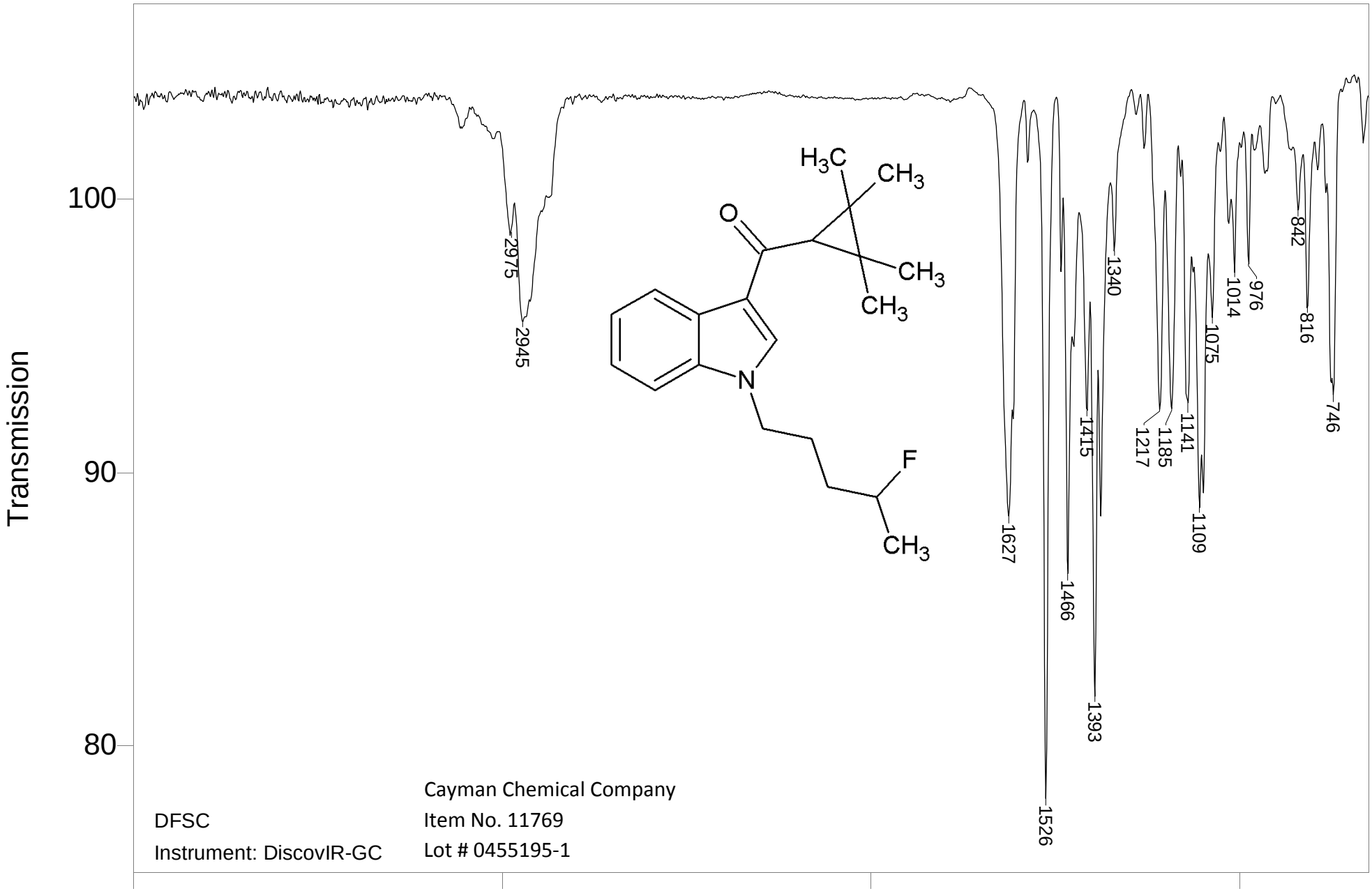


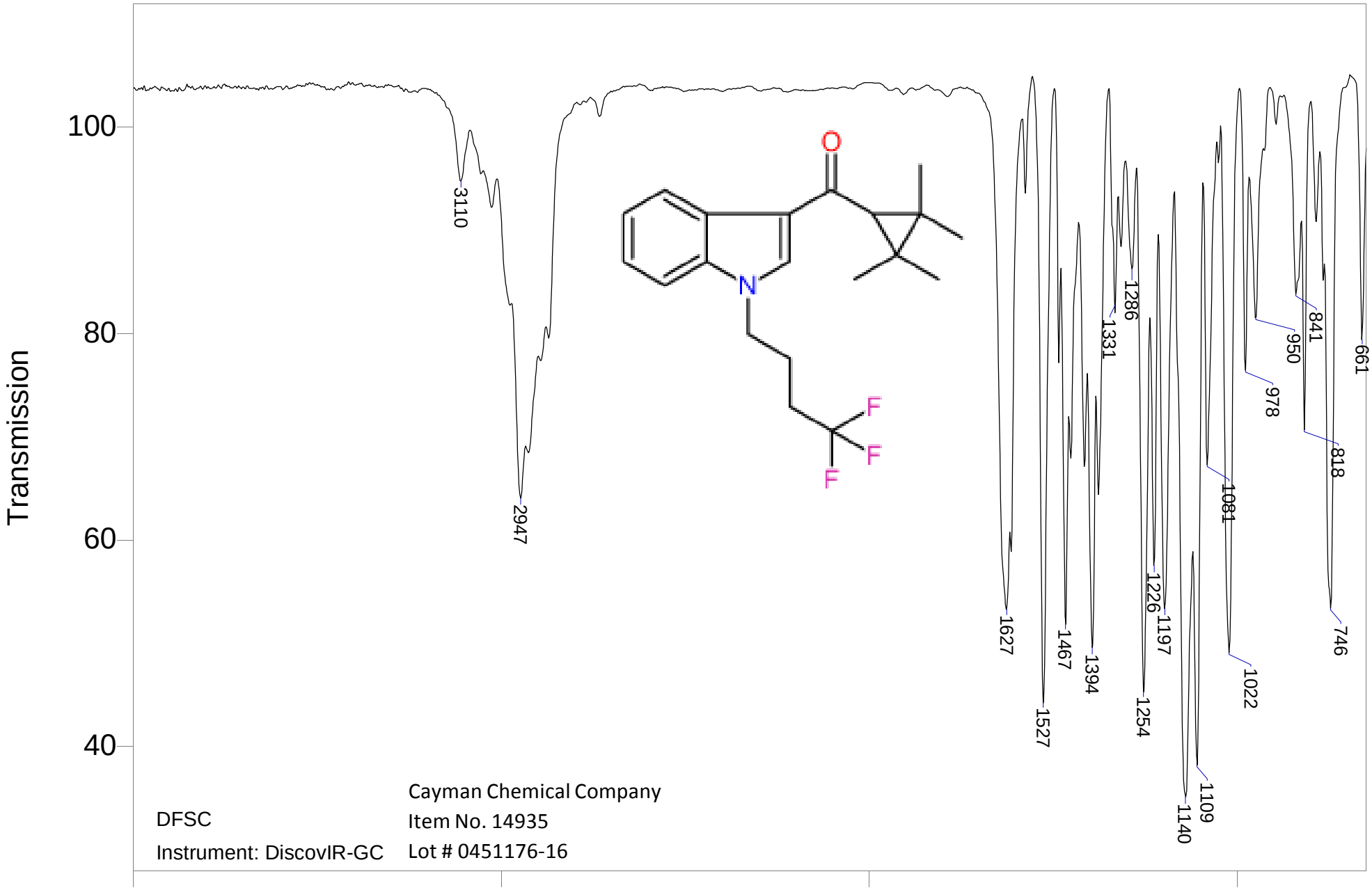


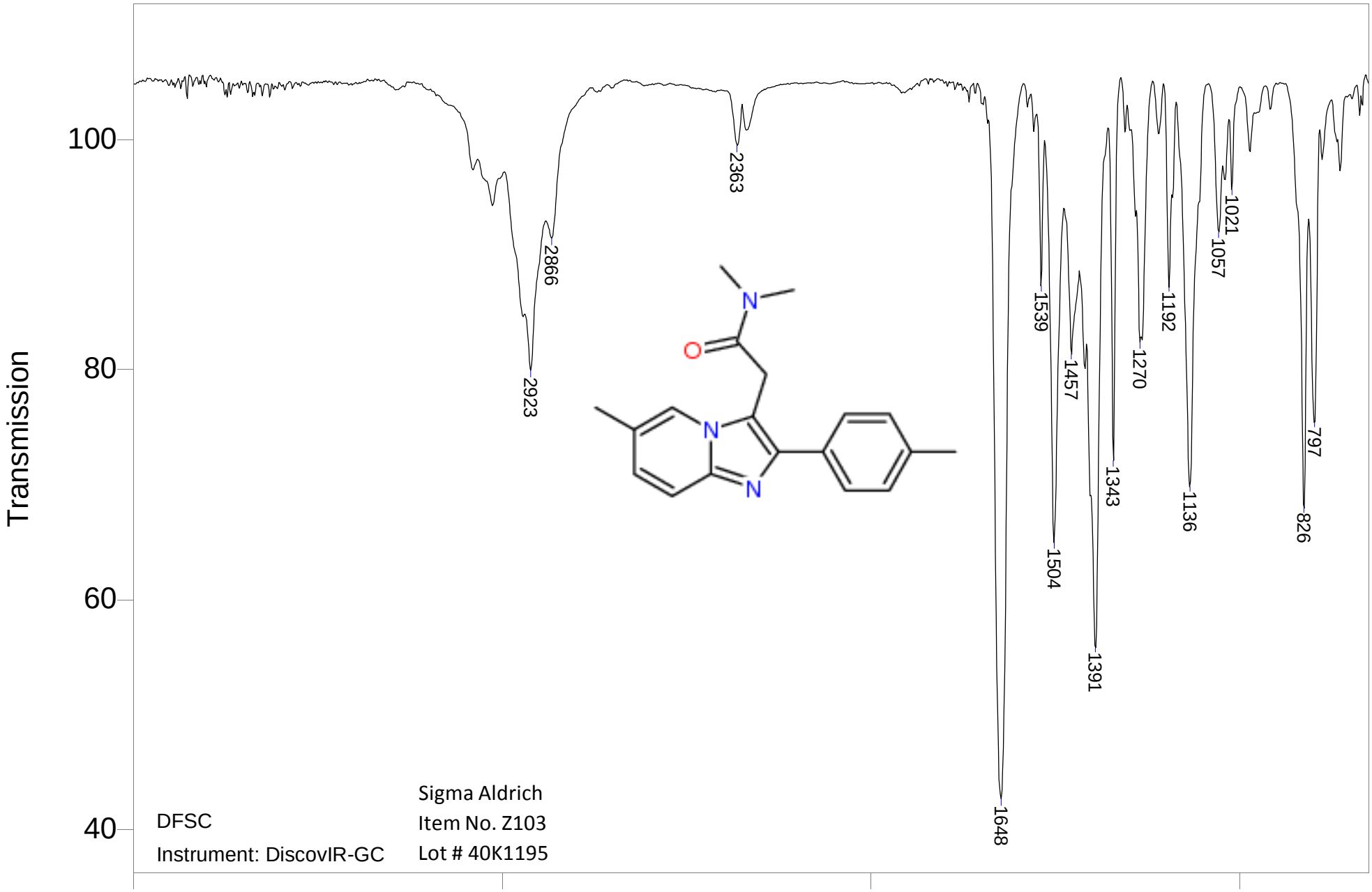


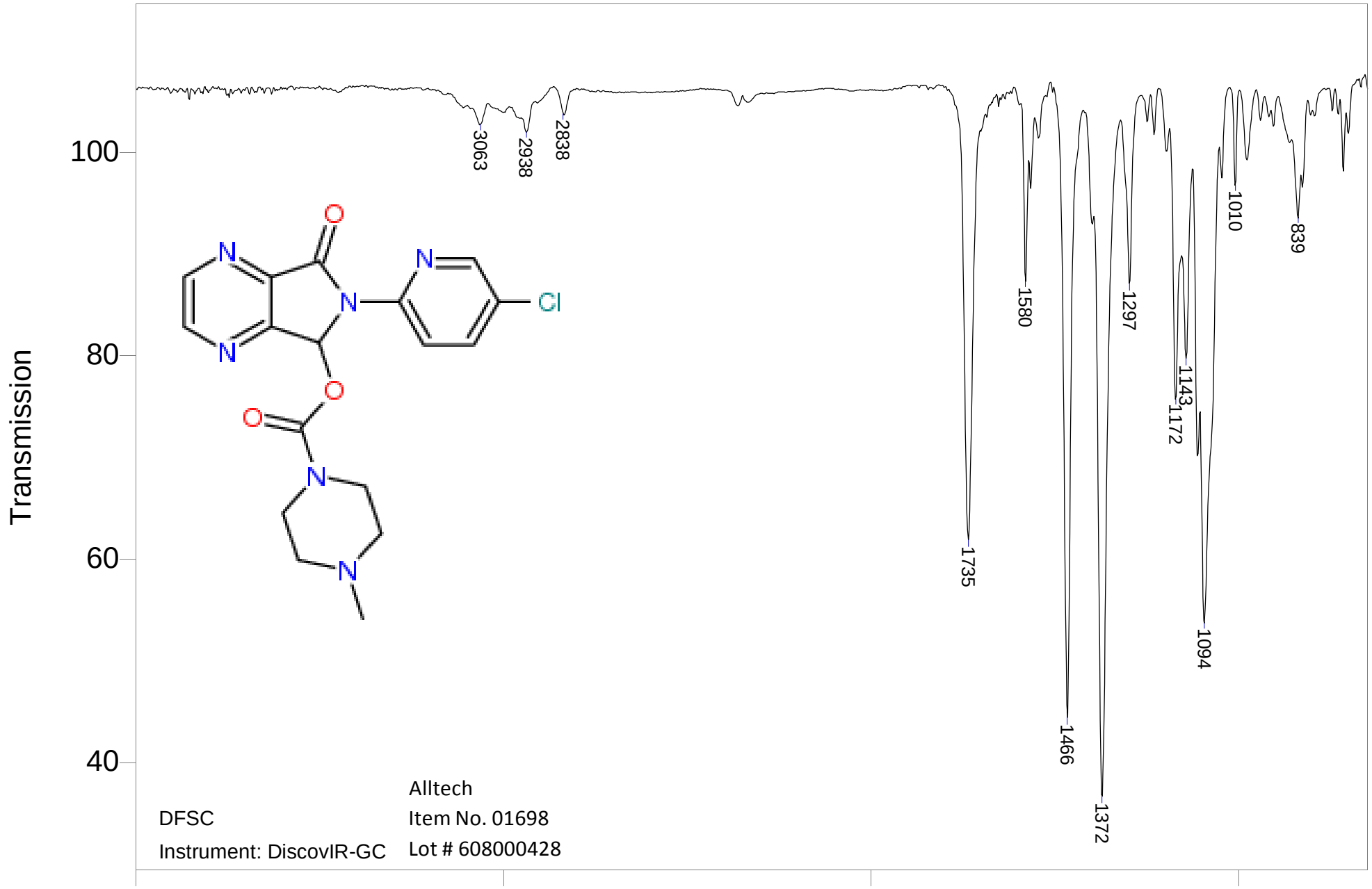








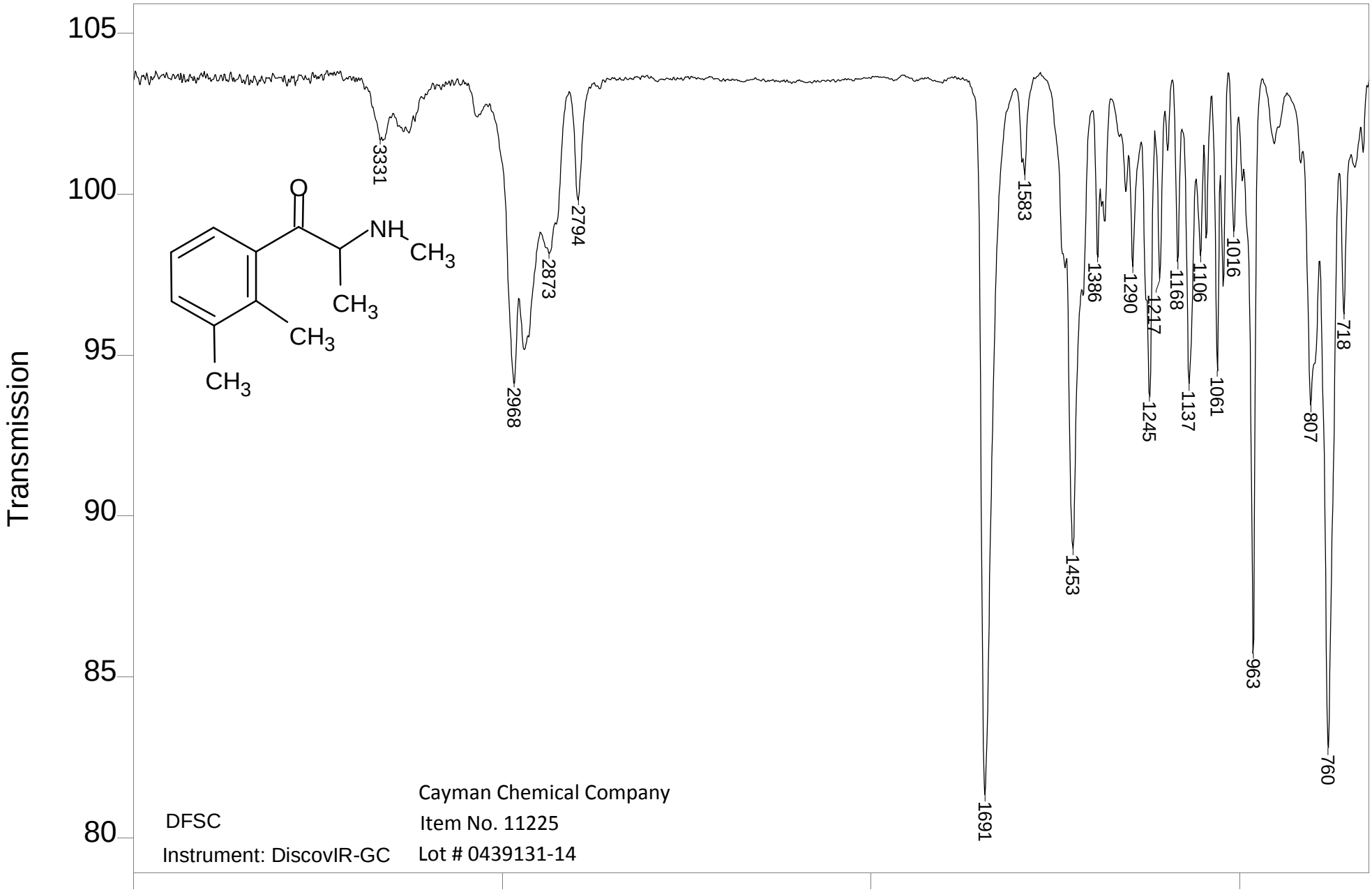


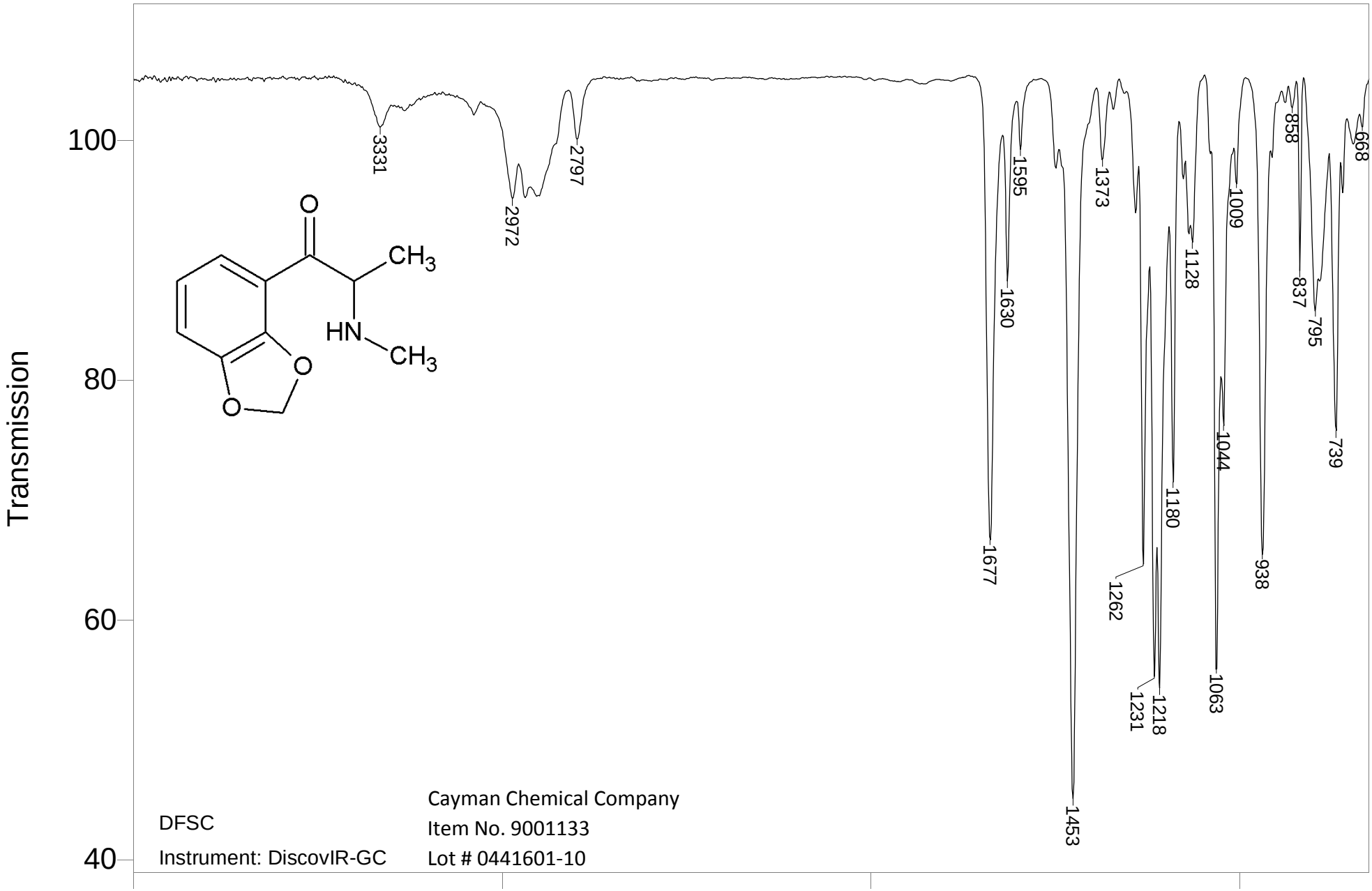


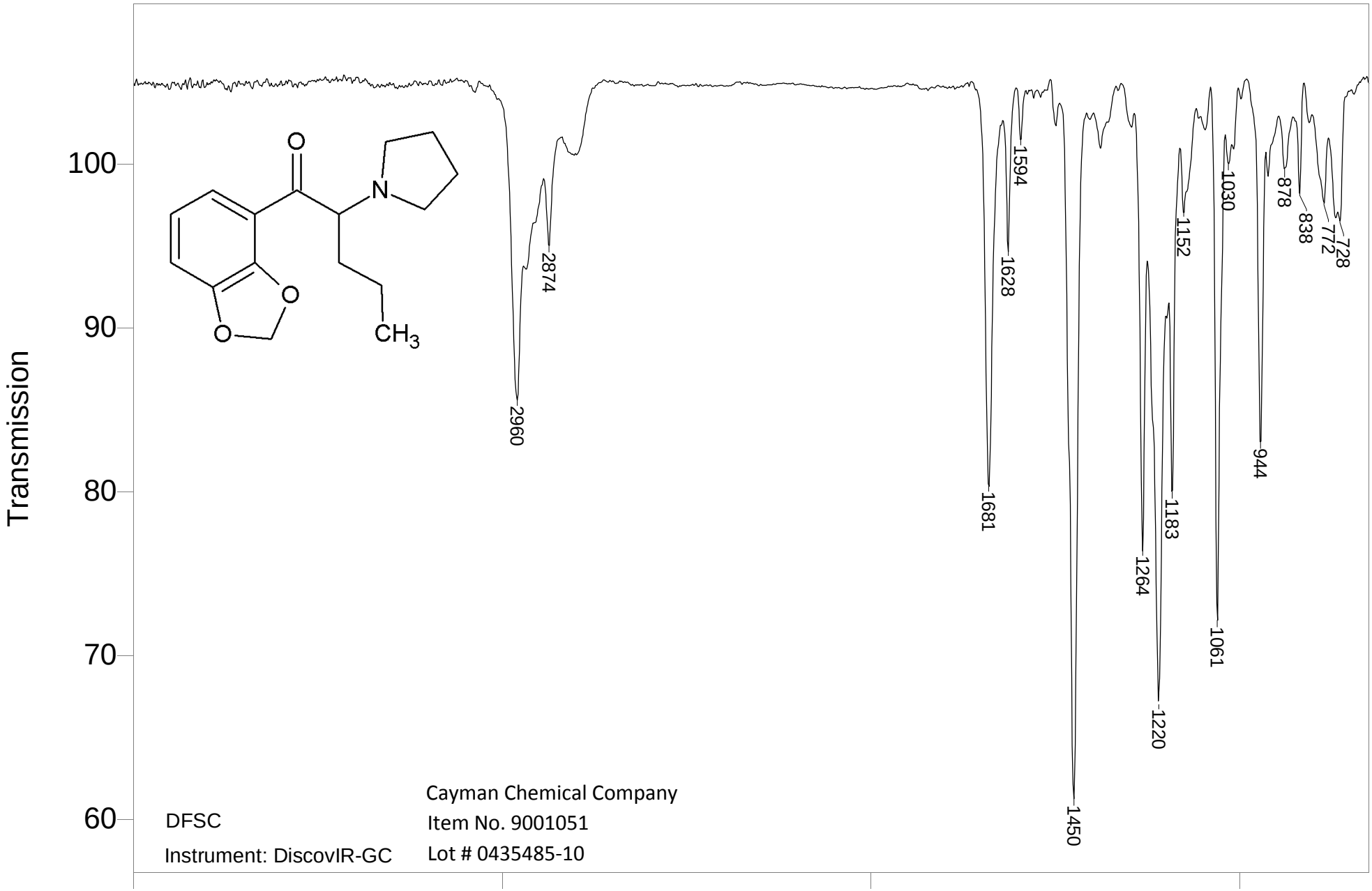
Appendix C: GC/IR Spectra Library: Cathinones

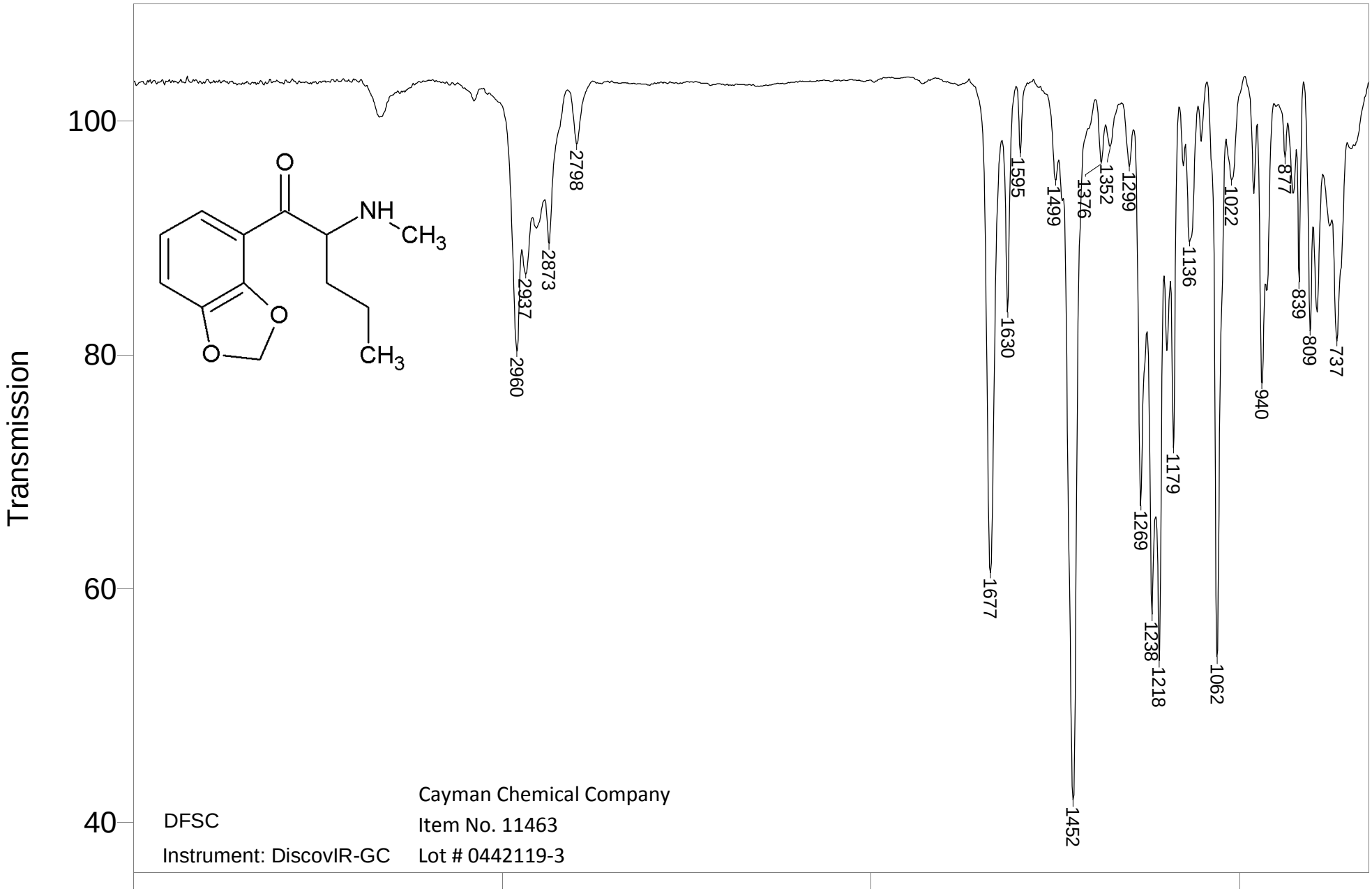
The GC/IR Spectra Library contains 64 solid-state infrared absorption spectra of cathinone related compounds as acquired on the DiscovIR-GC™. Several spectra contained here are also contained within the USACIL Drugs library (Appendix B). Several chemical structures (those with colored heteroatoms), as found associated with IR spectra, were copied from the Forendex database maintained by the Southern Association of Forensic Scientists (Southern Association of Forensic Scientists, 2017).

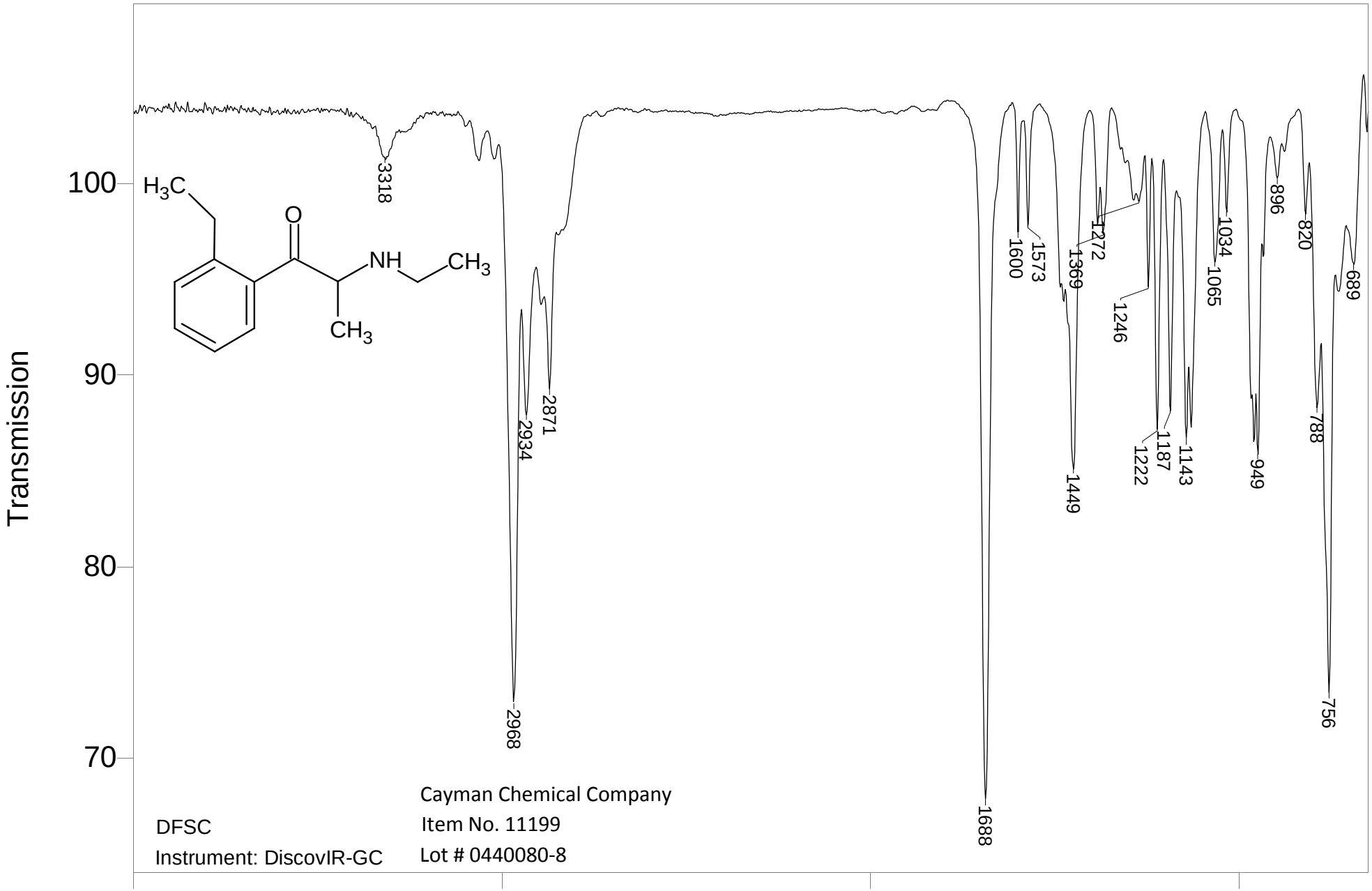
2,3-dimethylmethcathinone

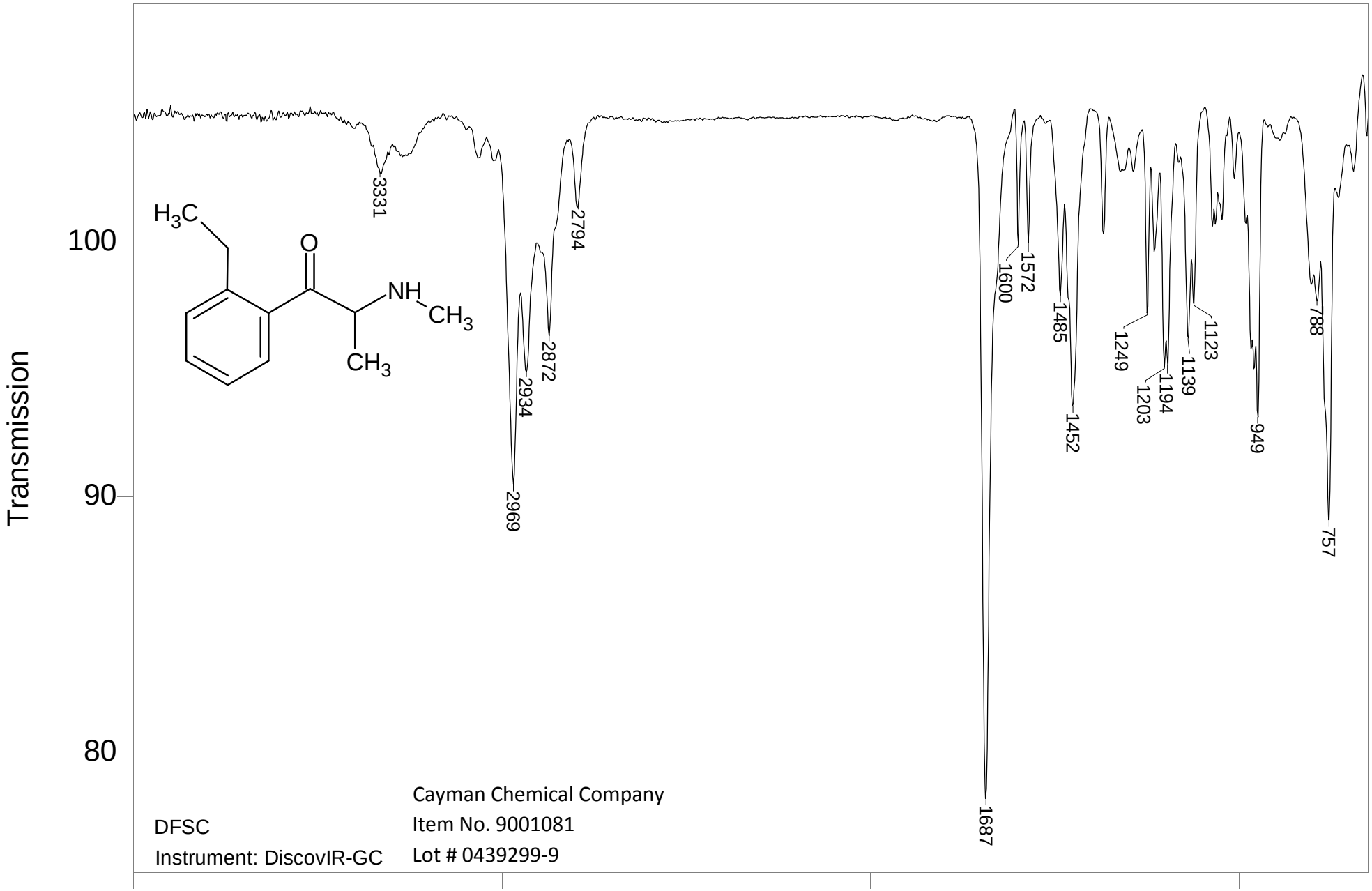




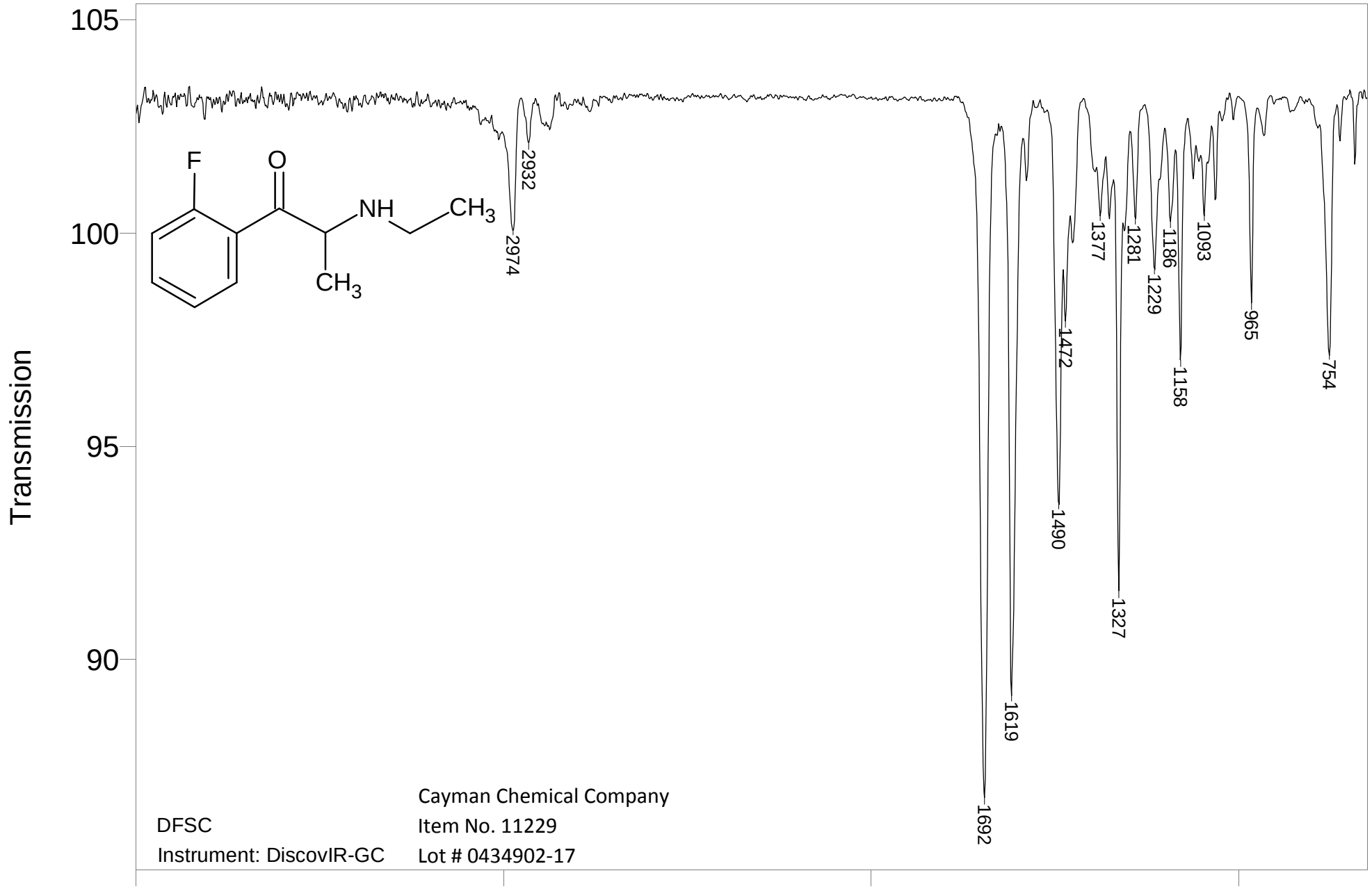


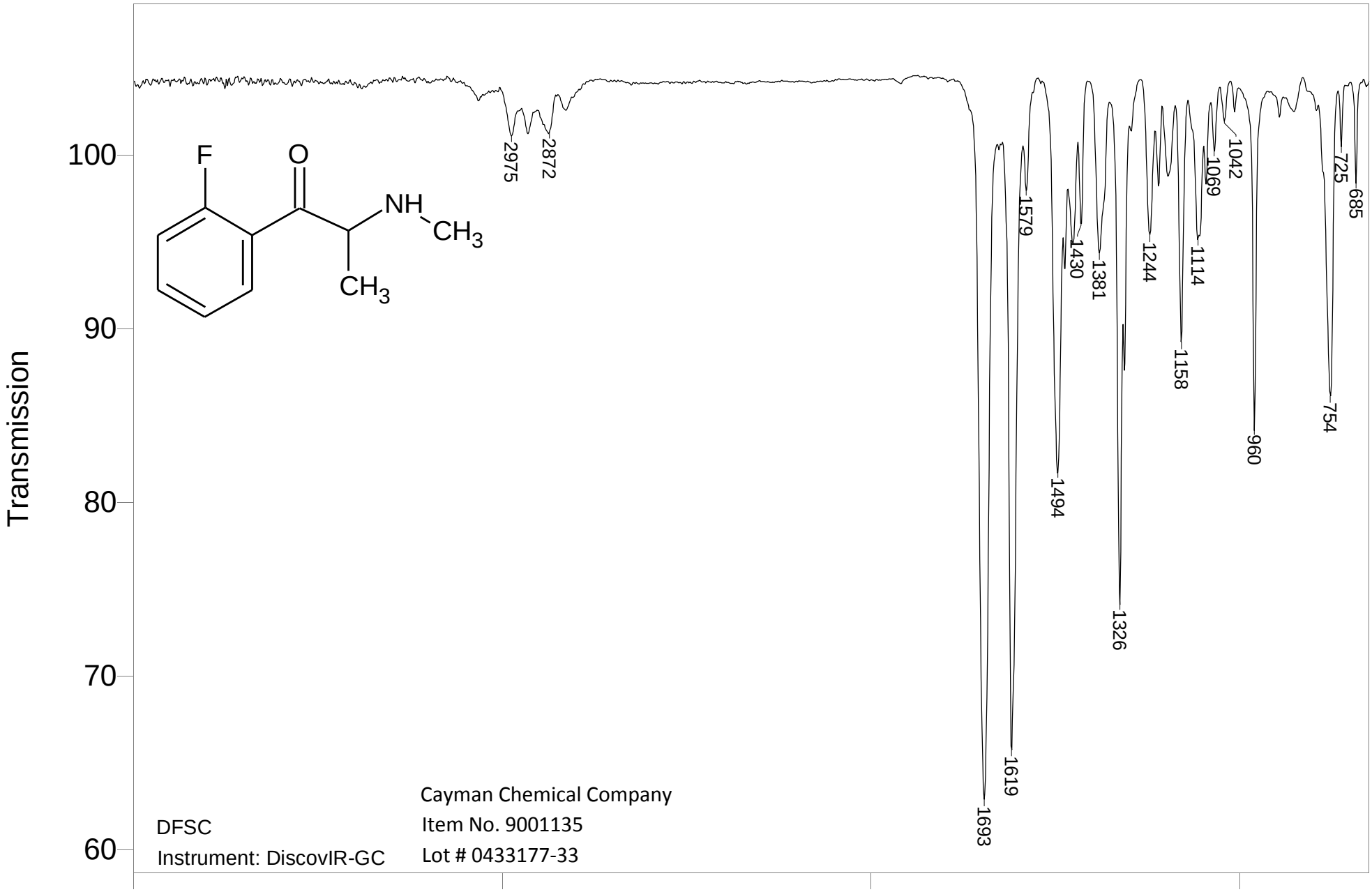


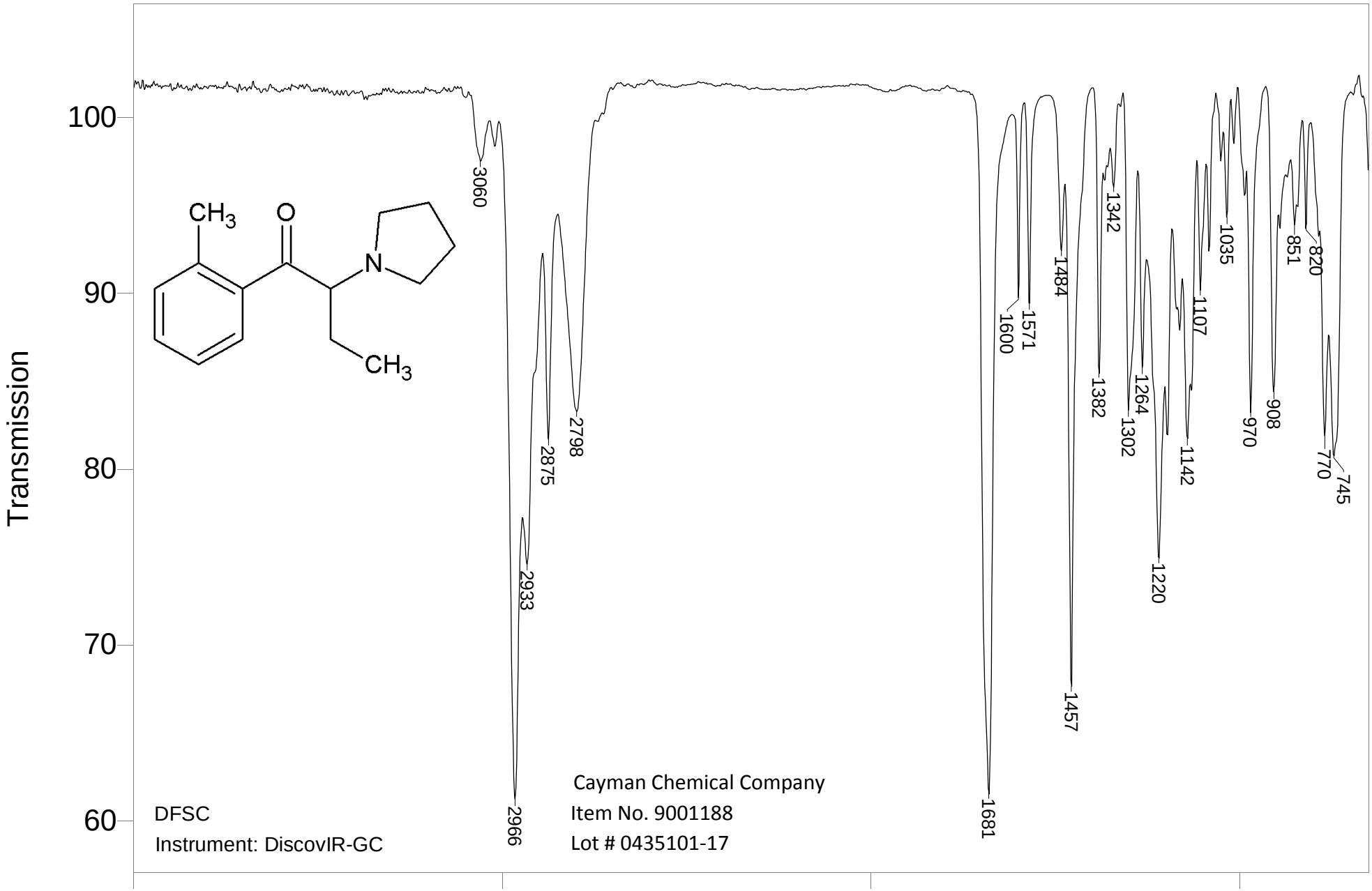


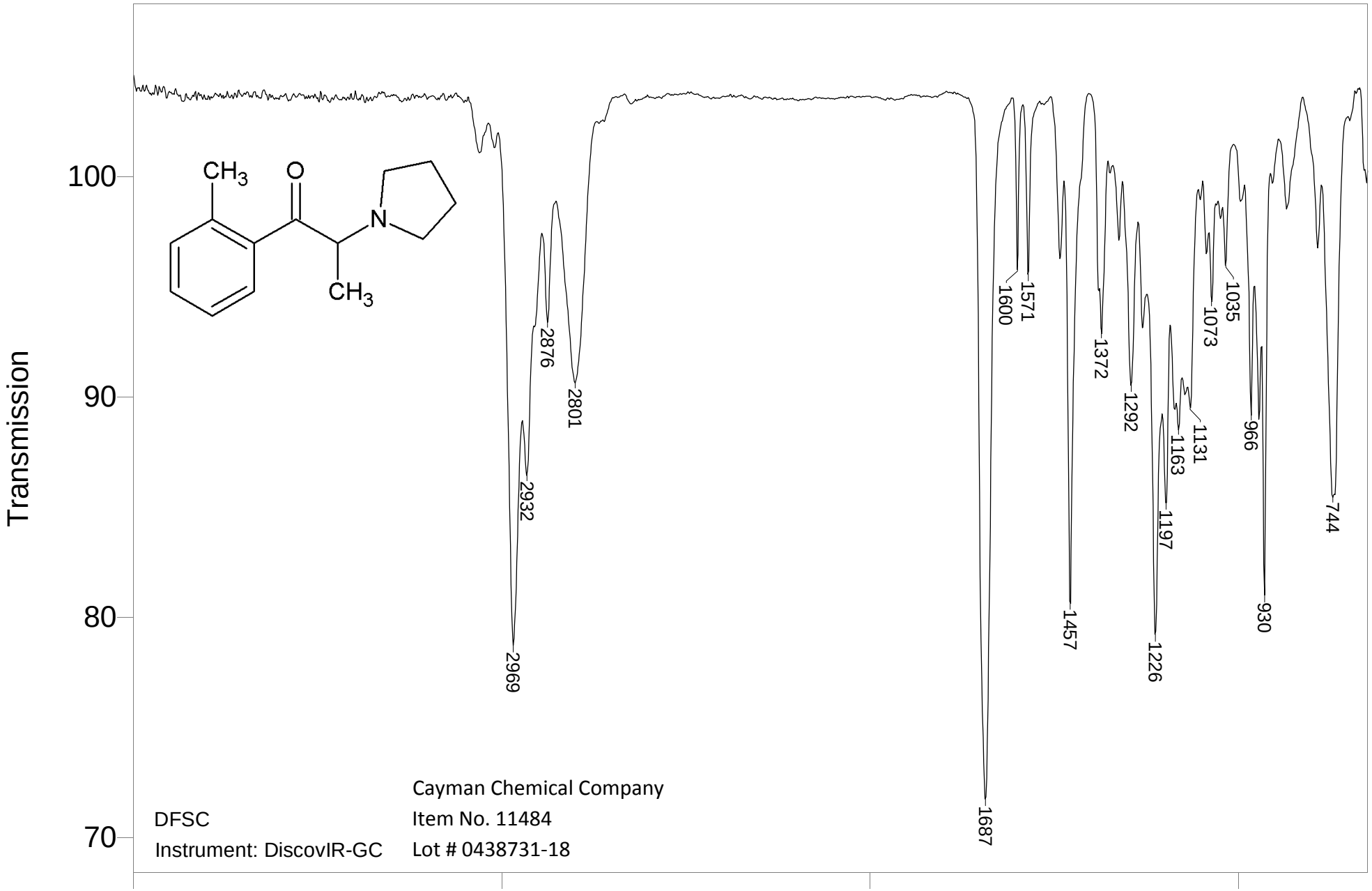


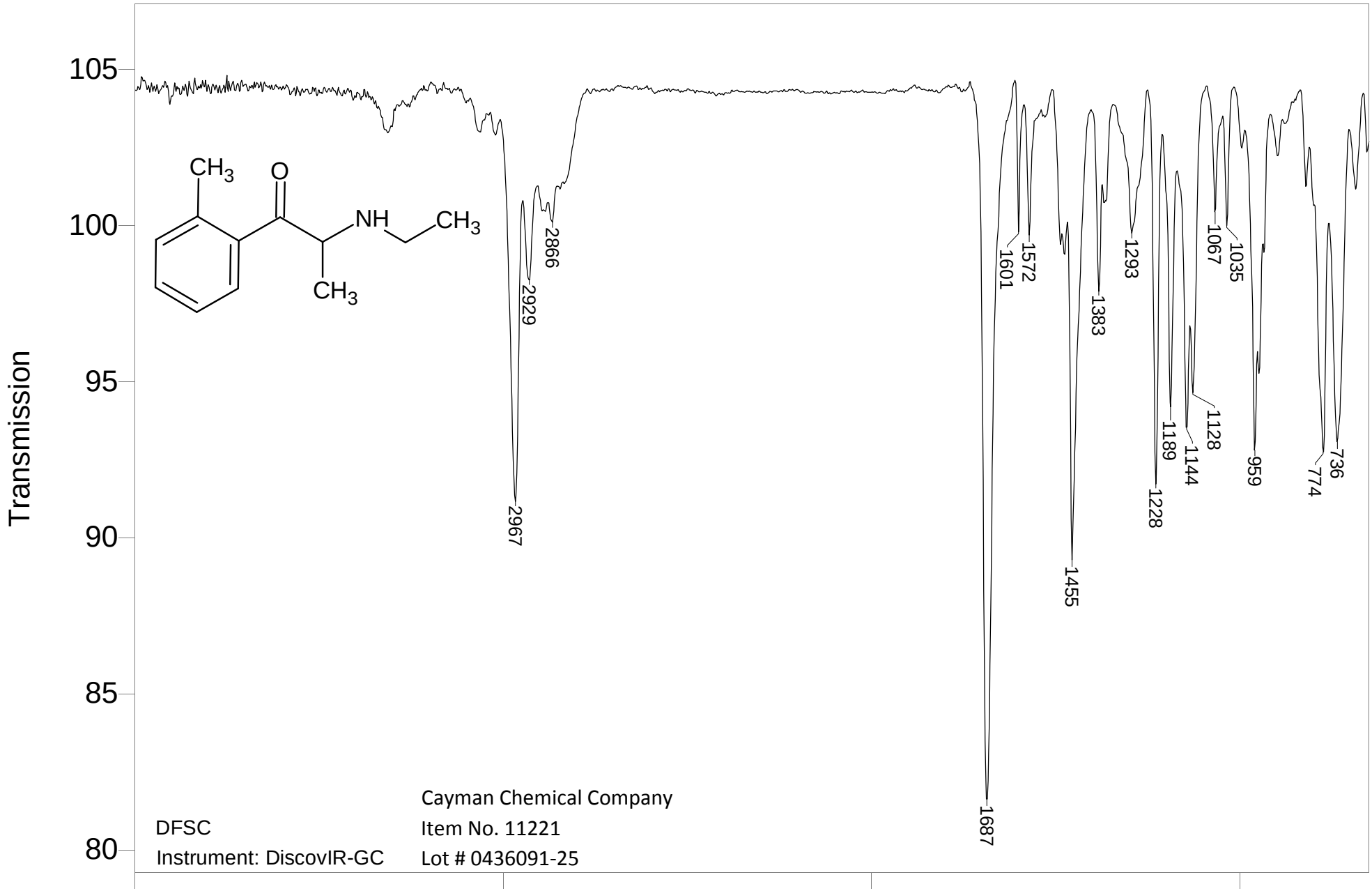
2-fluoroethcathinone

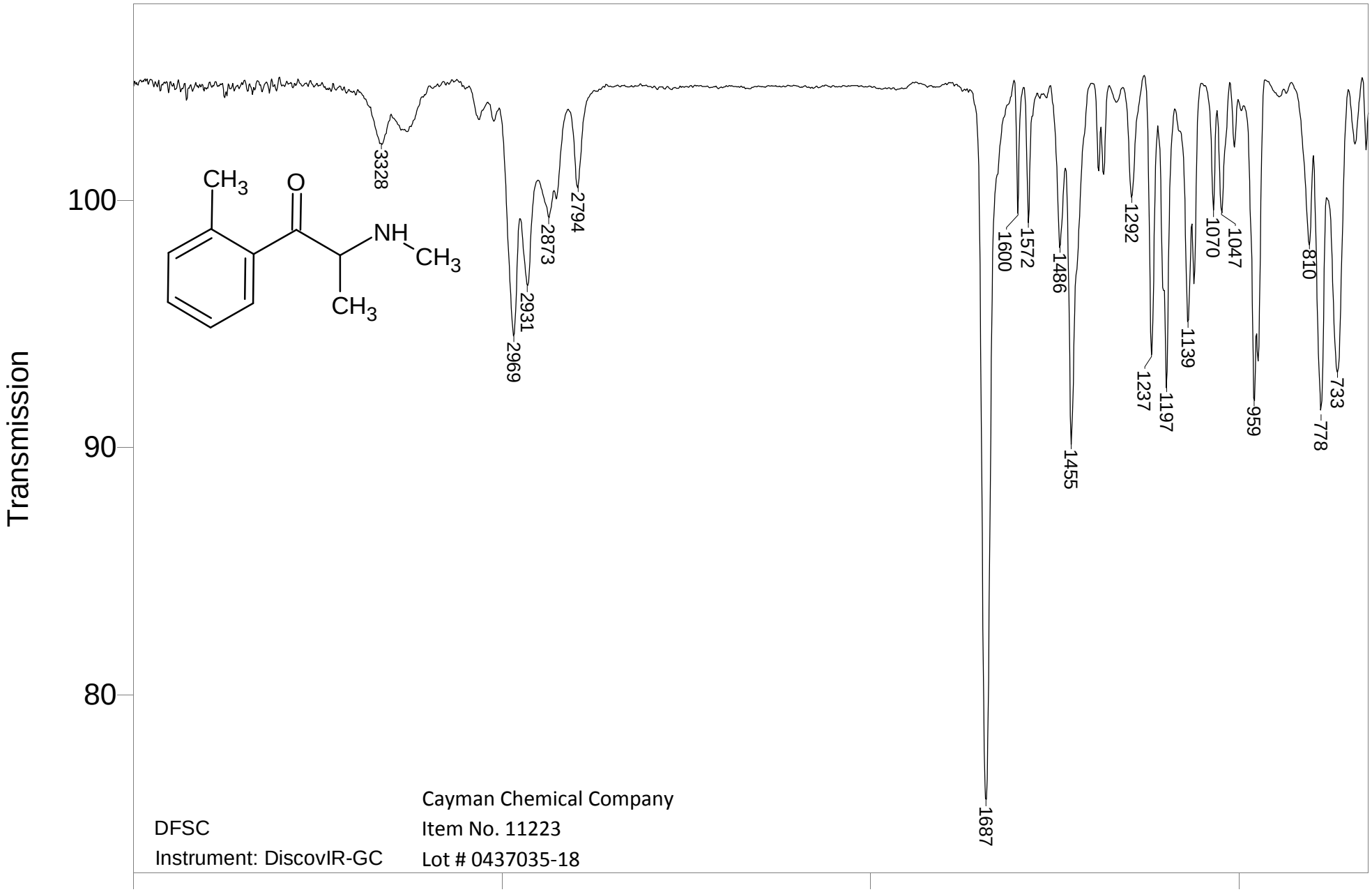


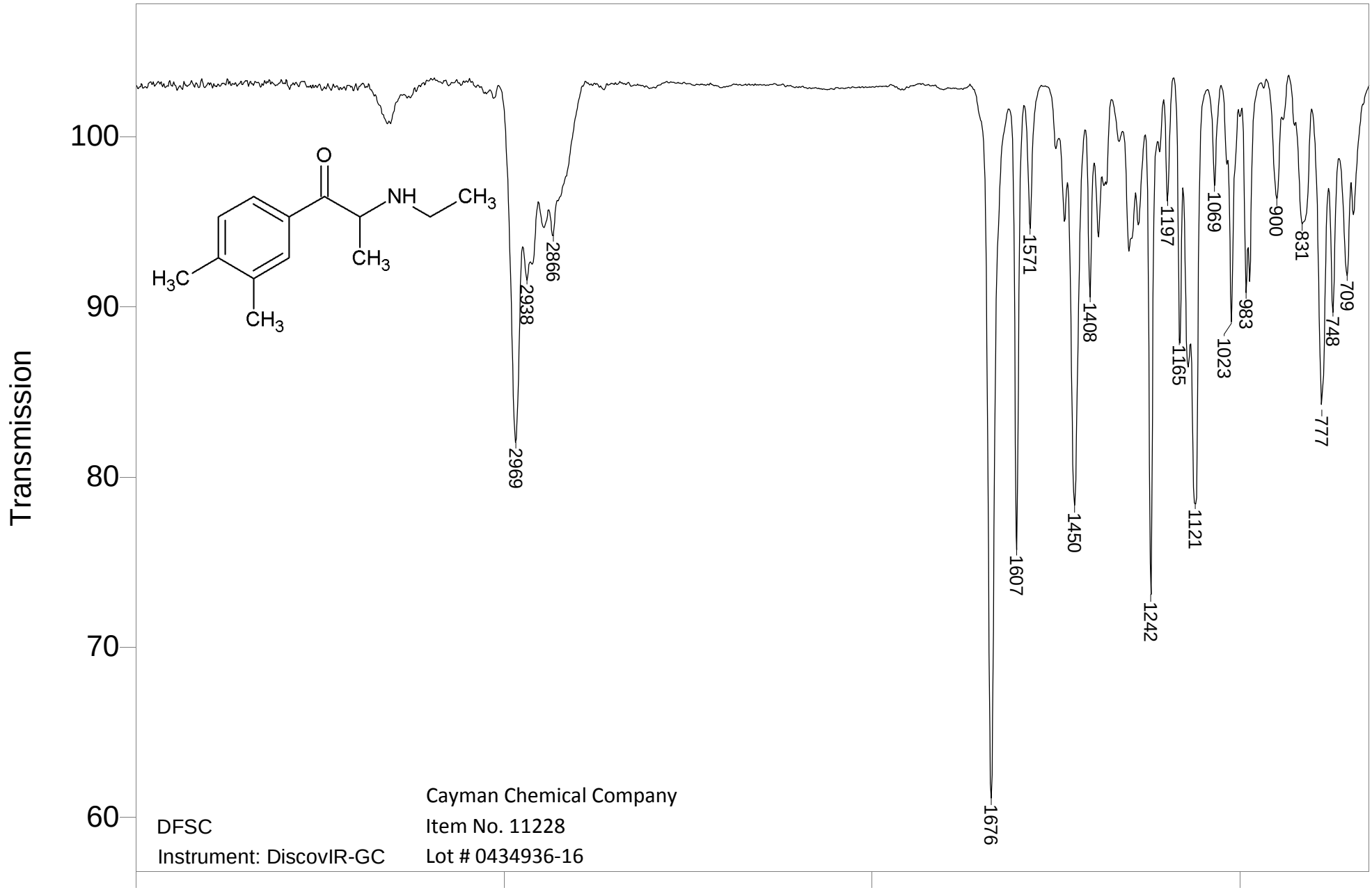


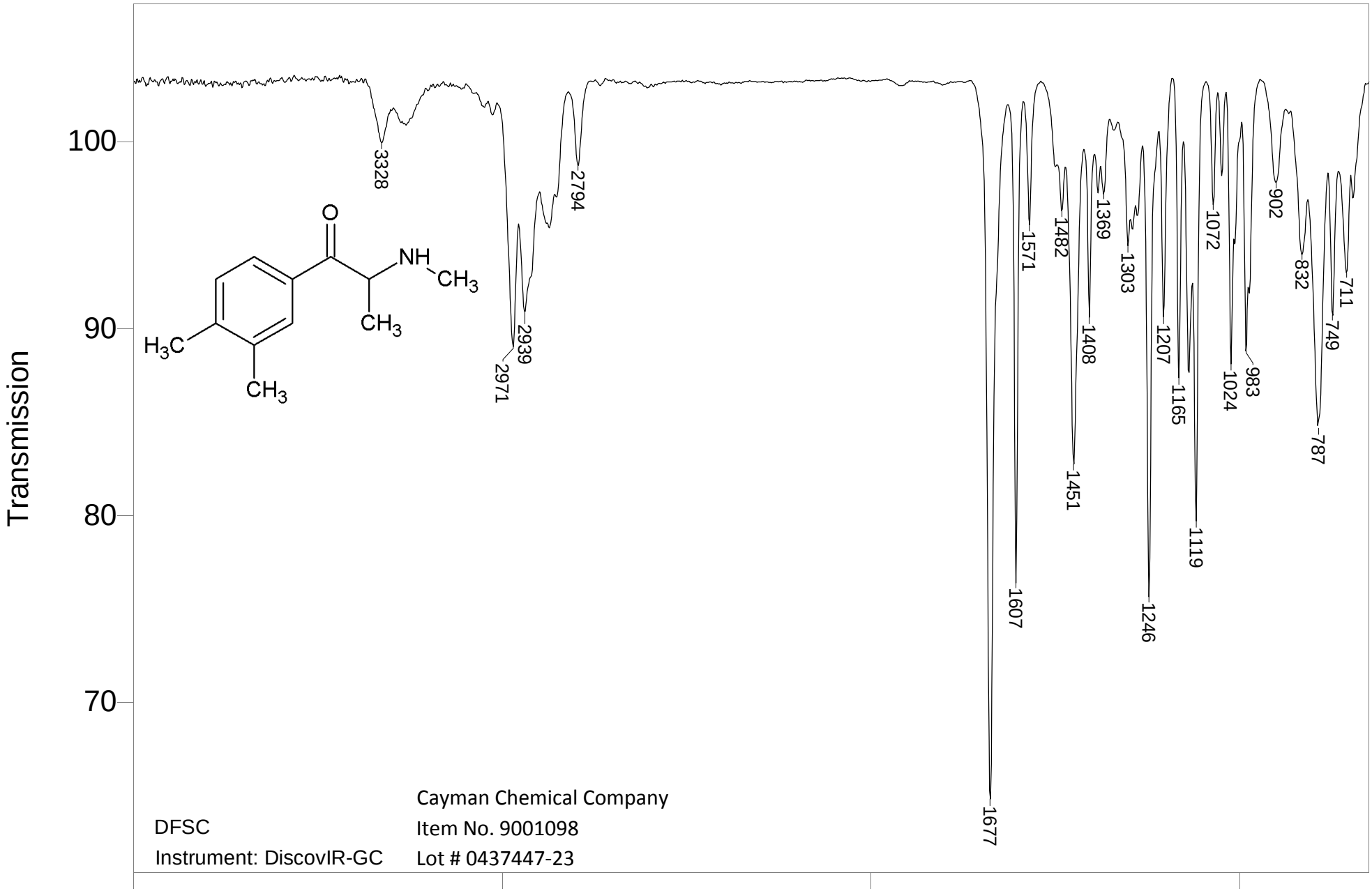


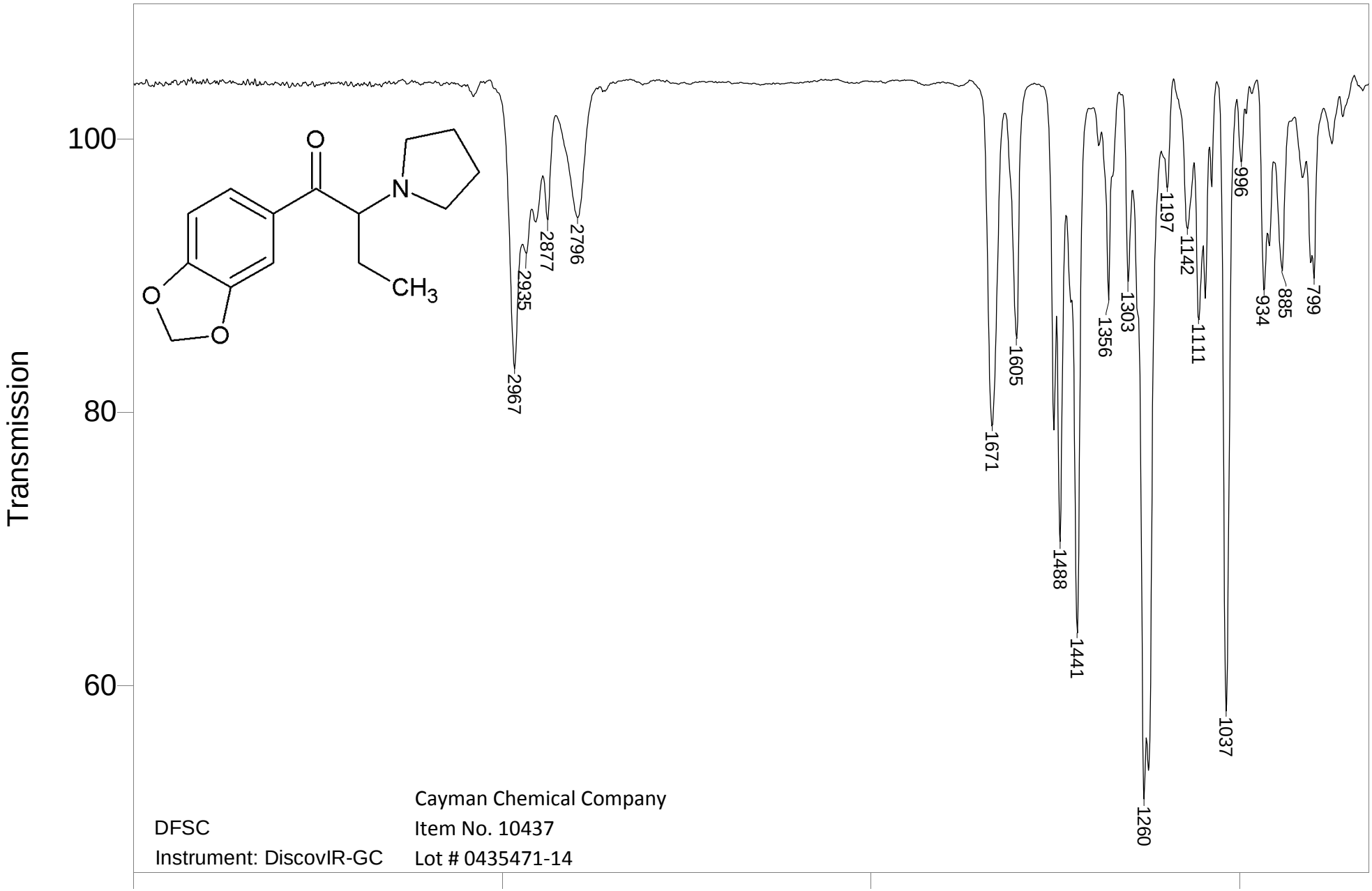


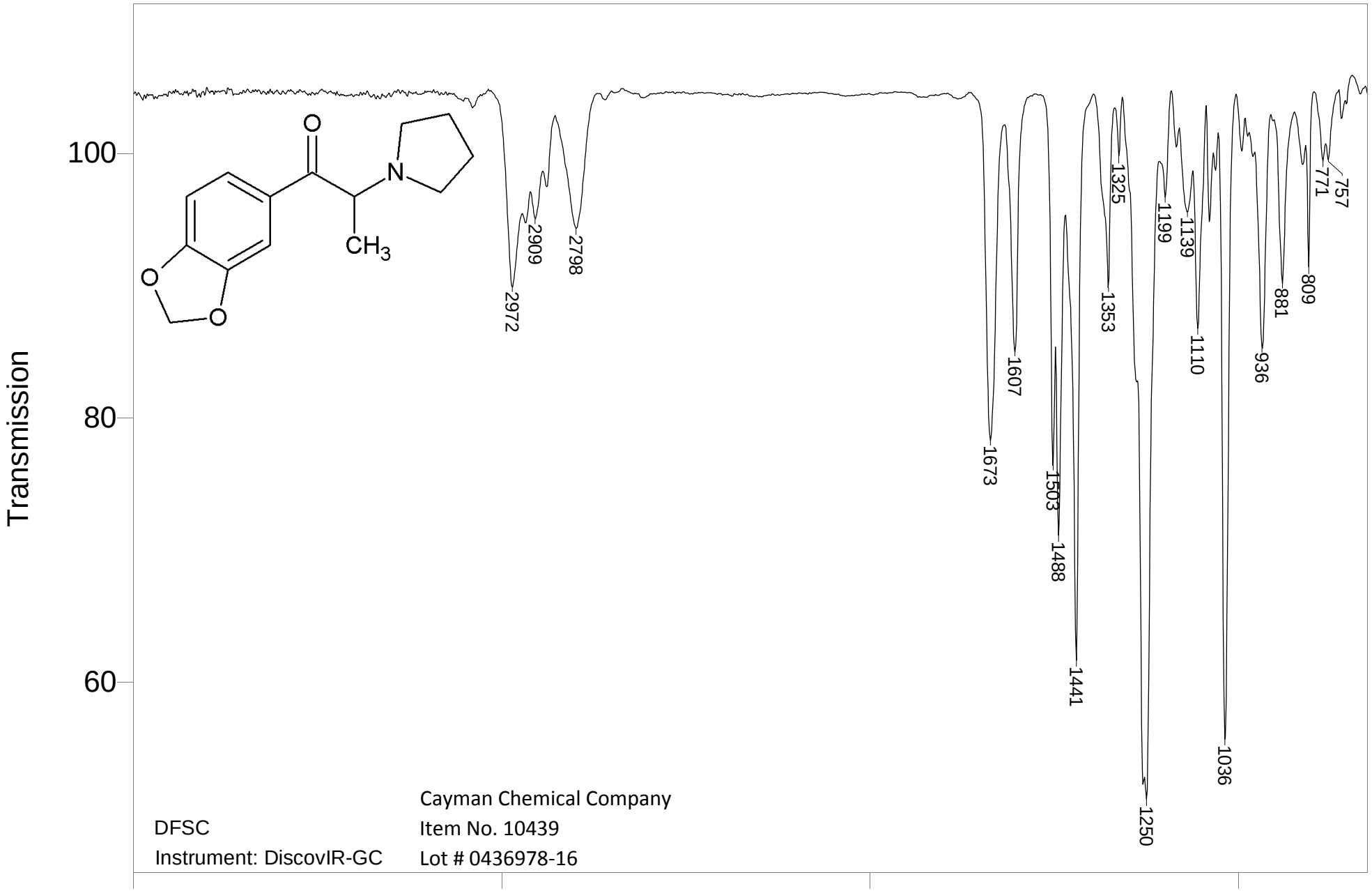


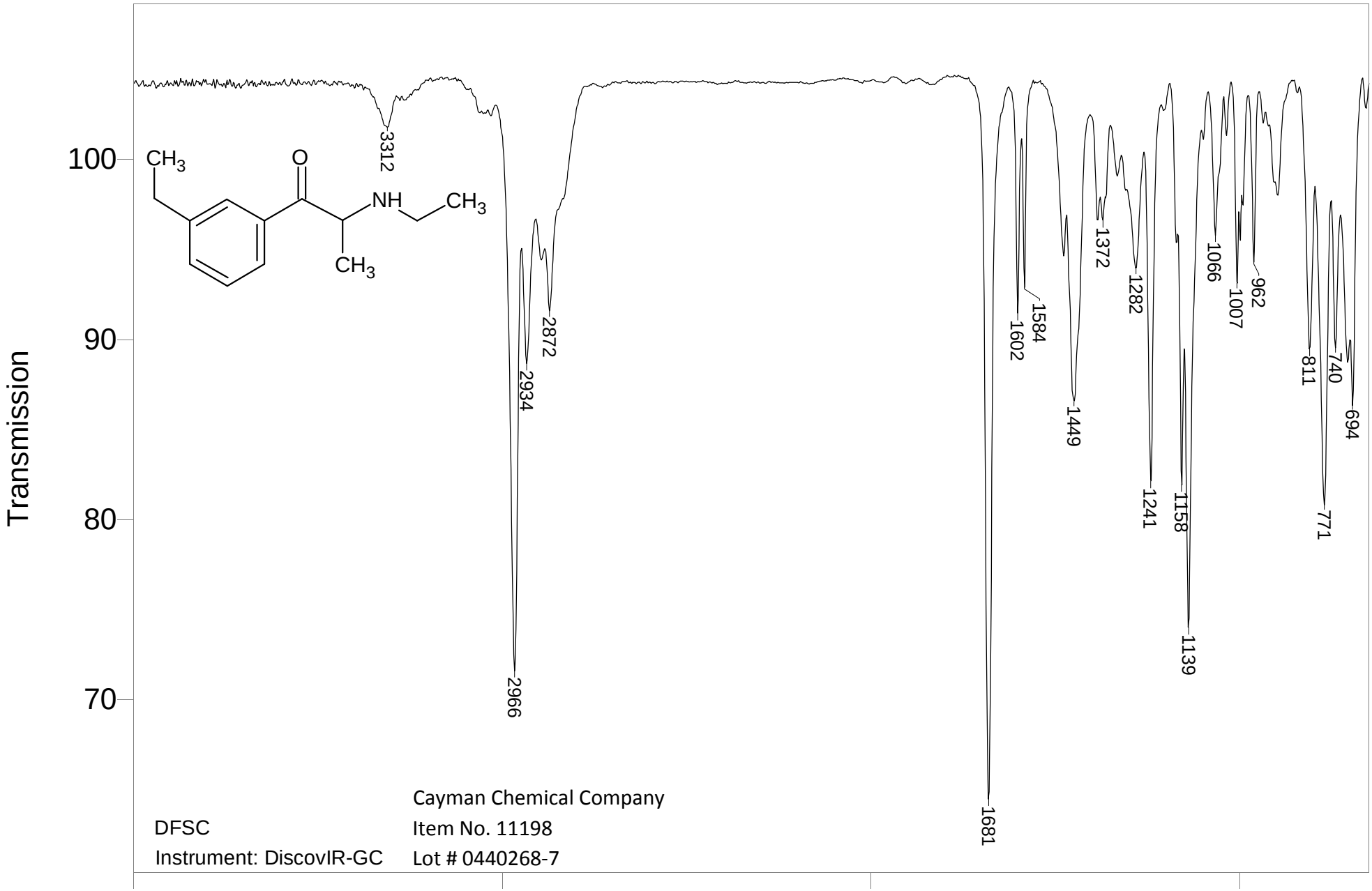


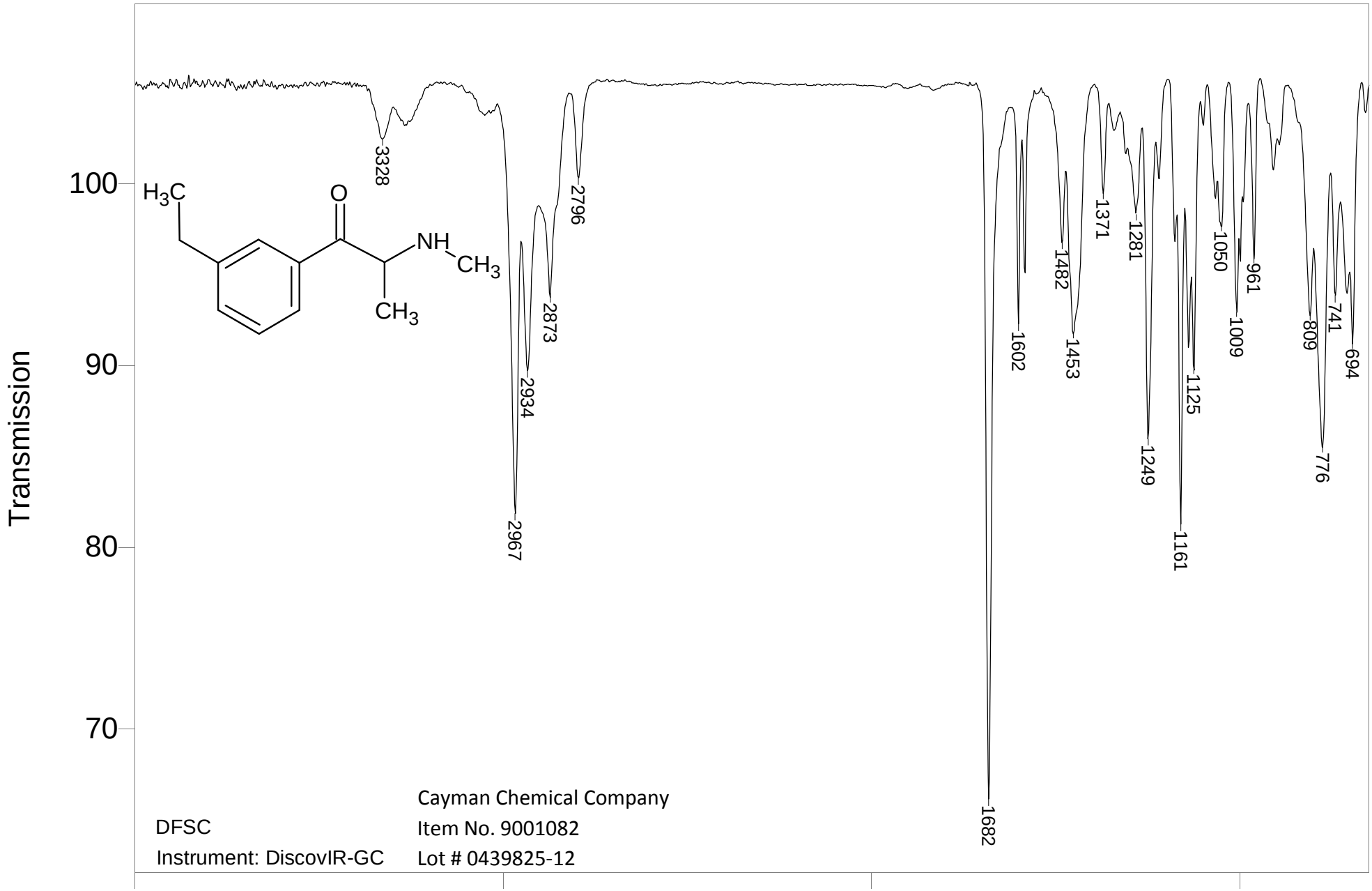


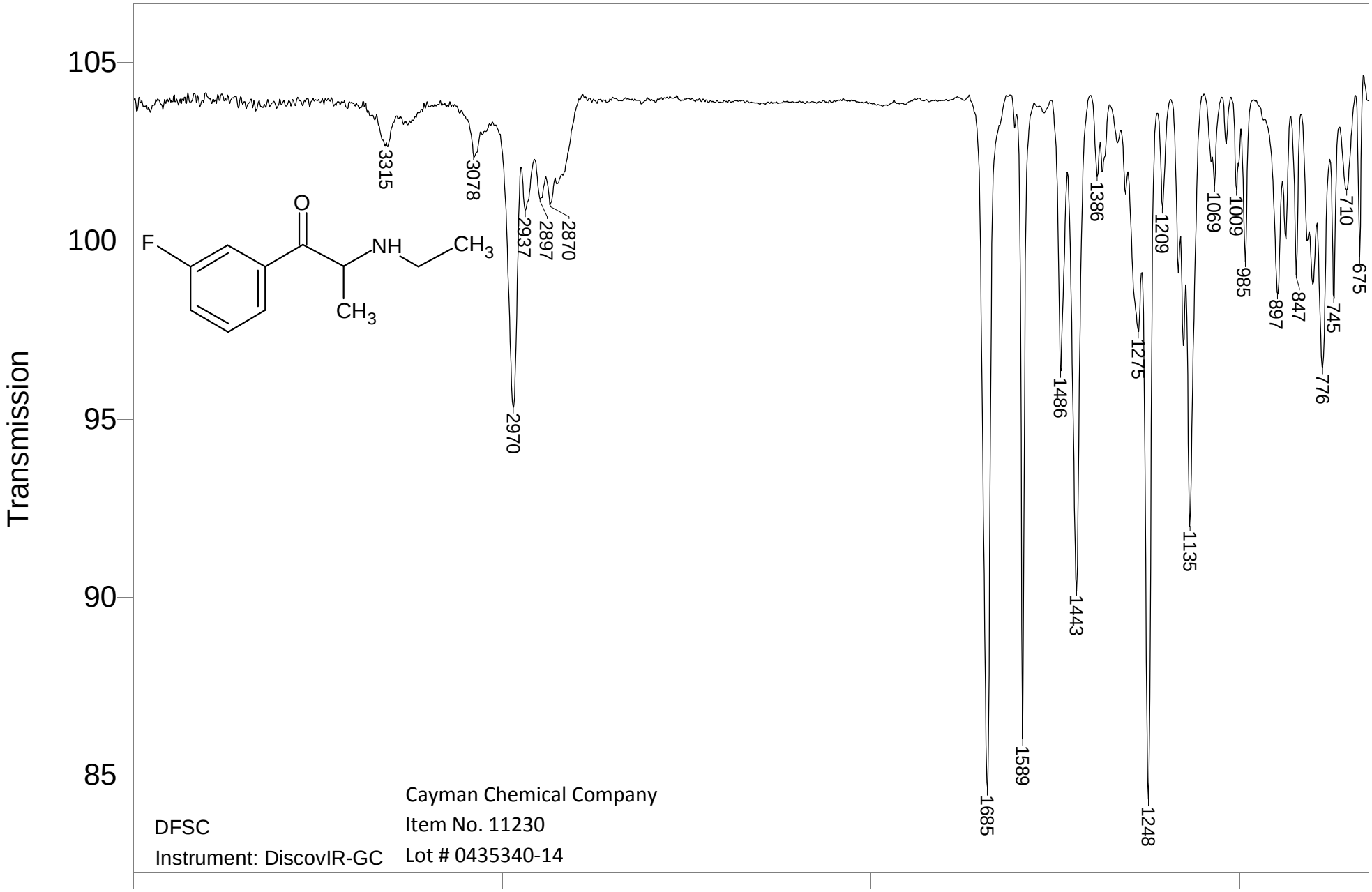


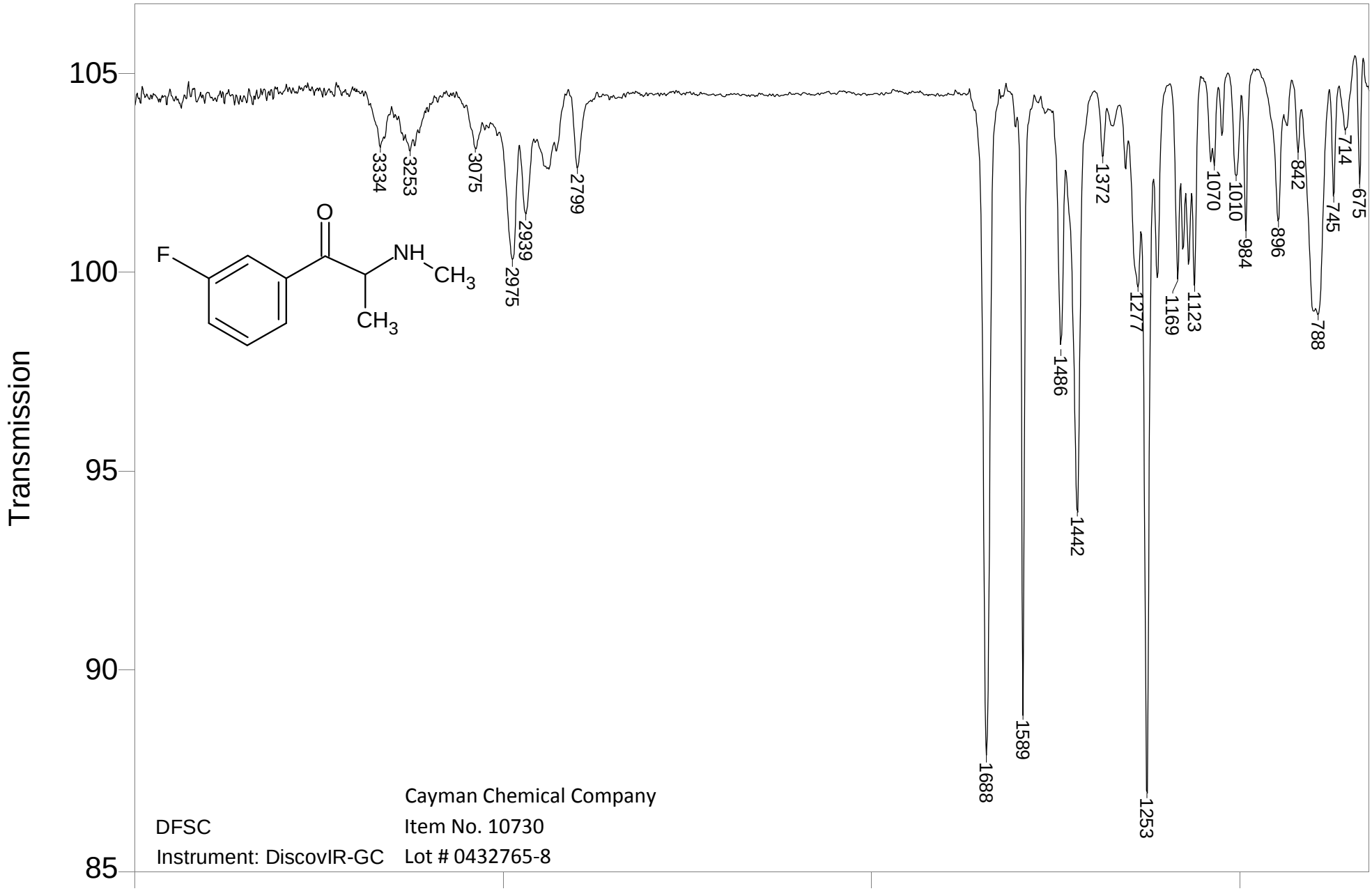


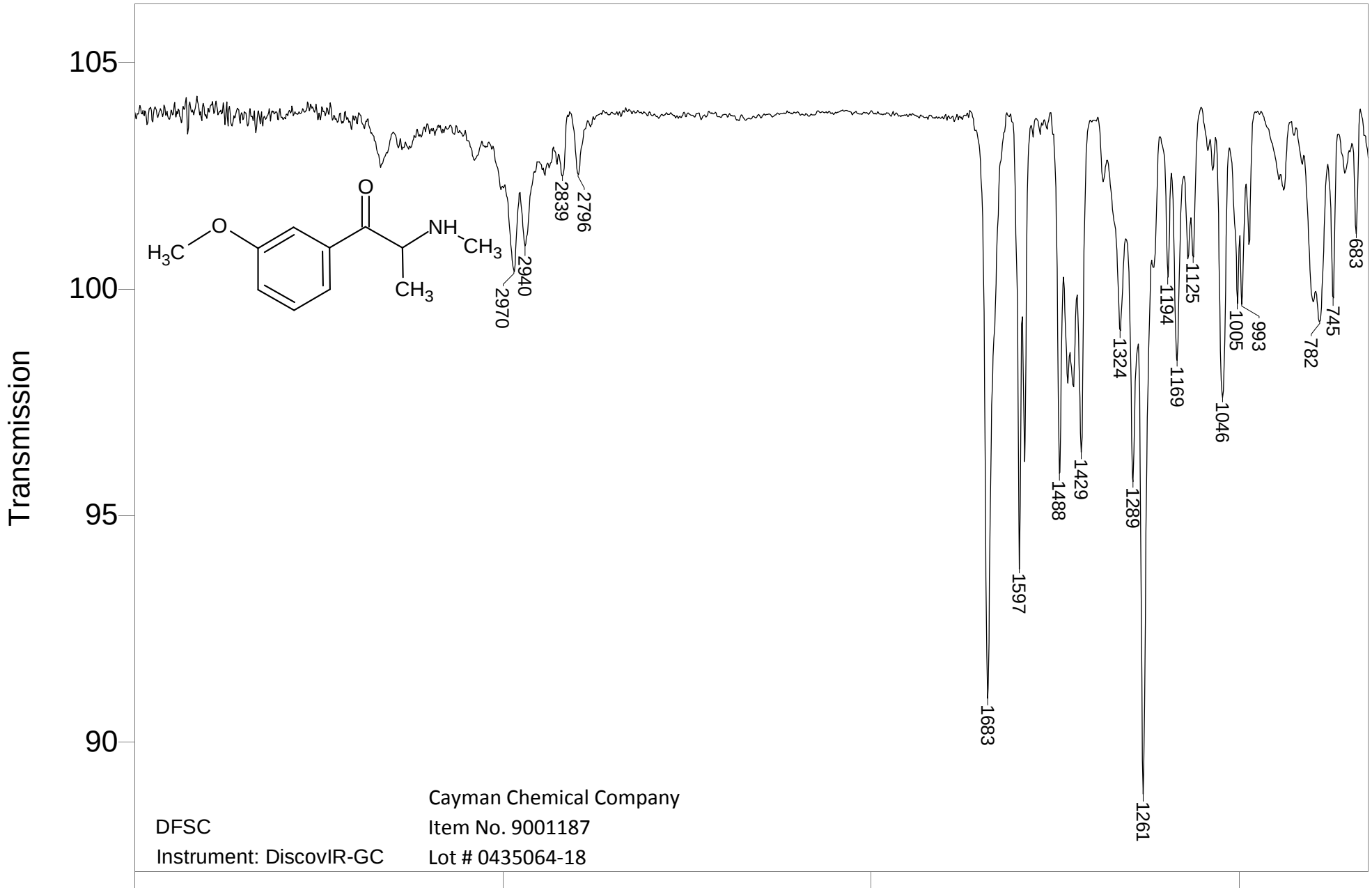


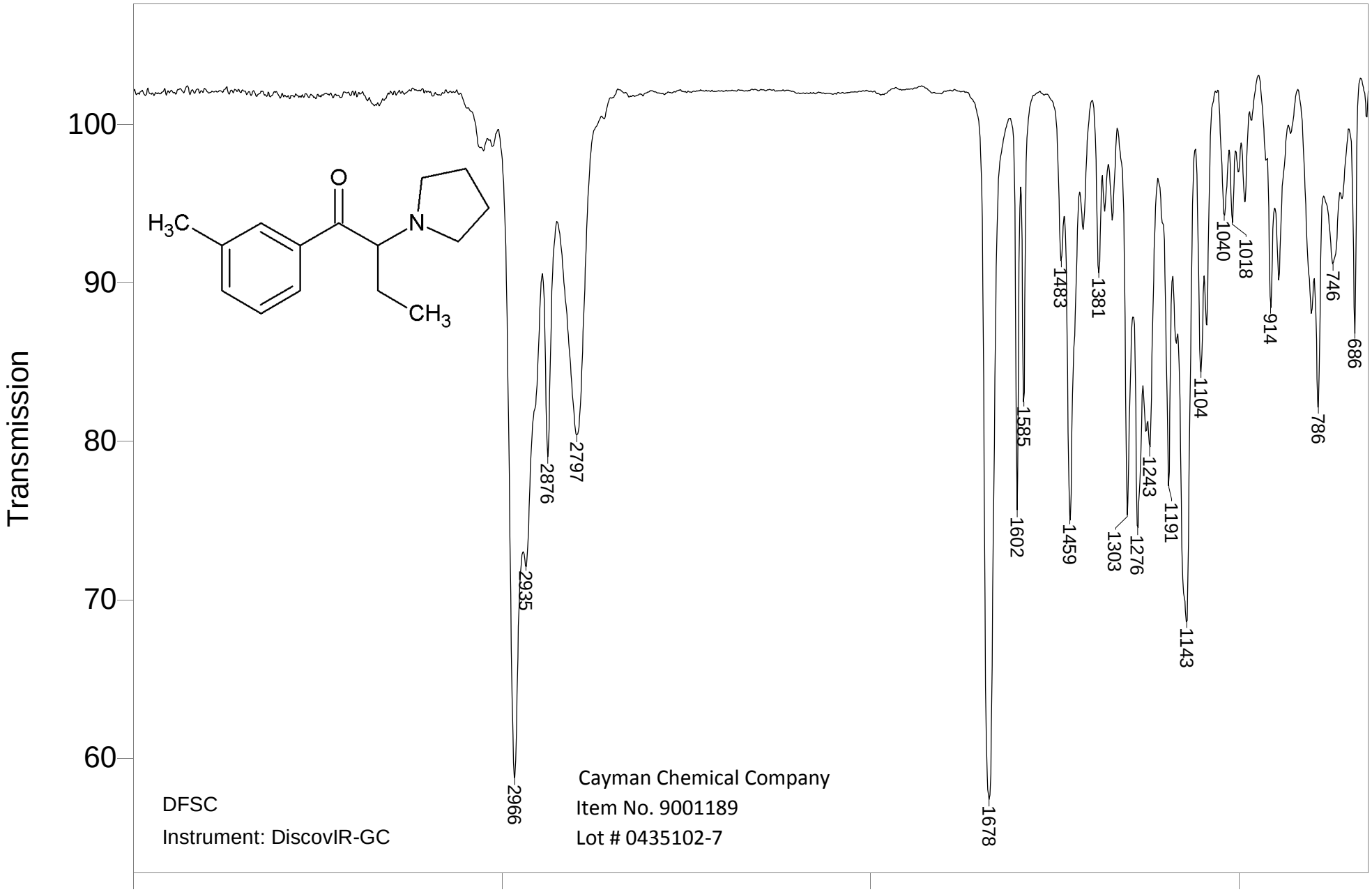


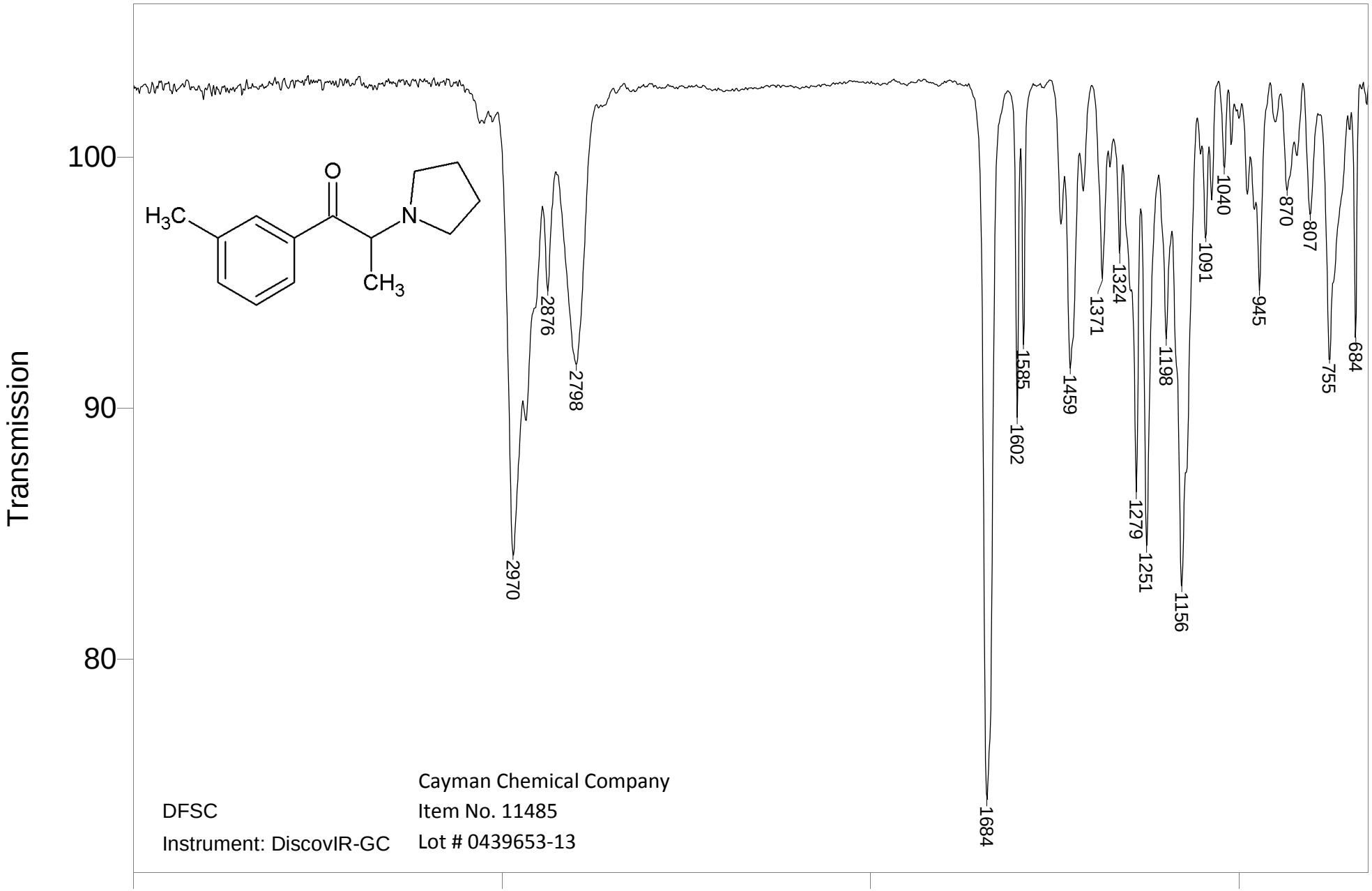


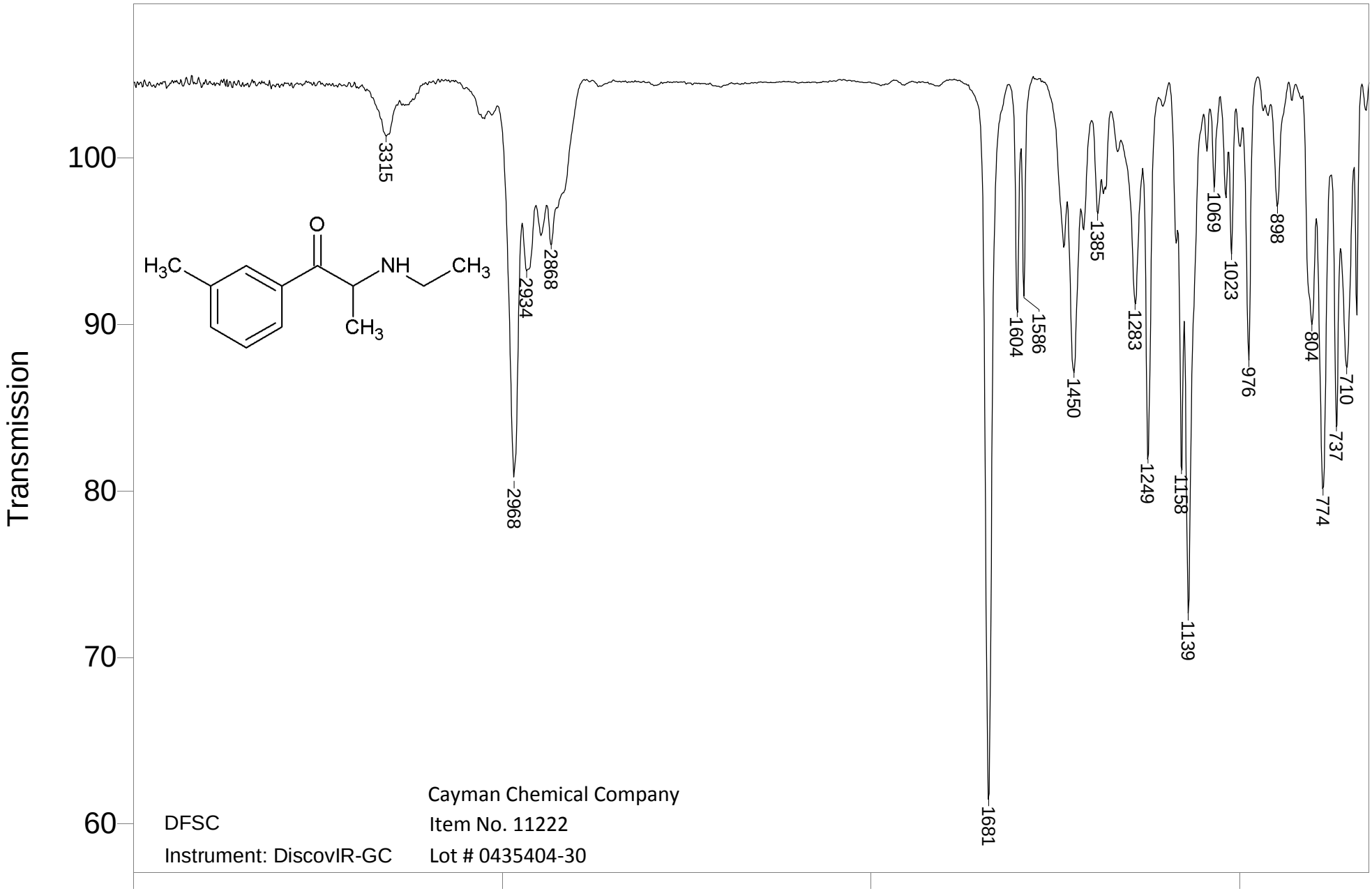


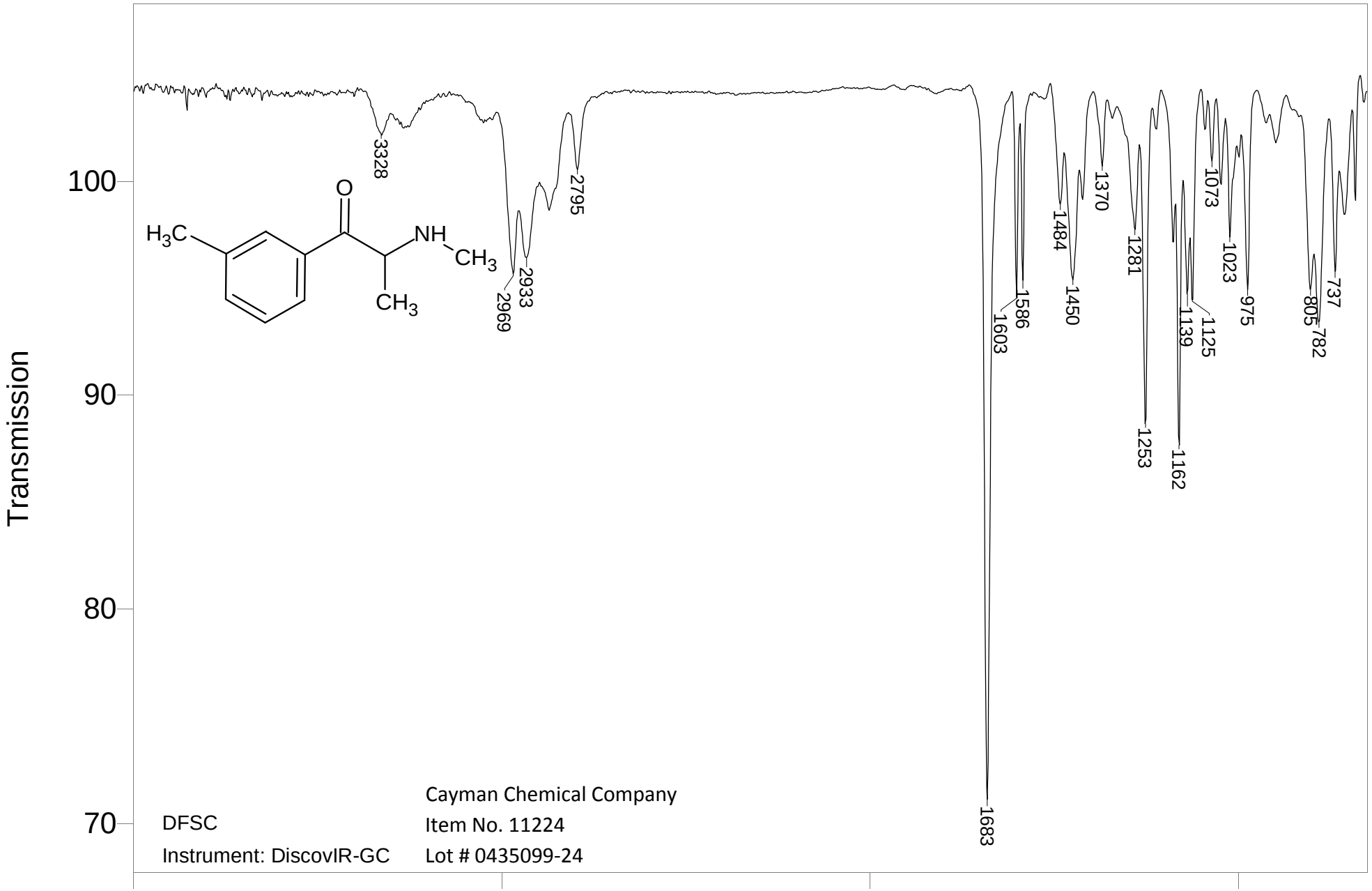


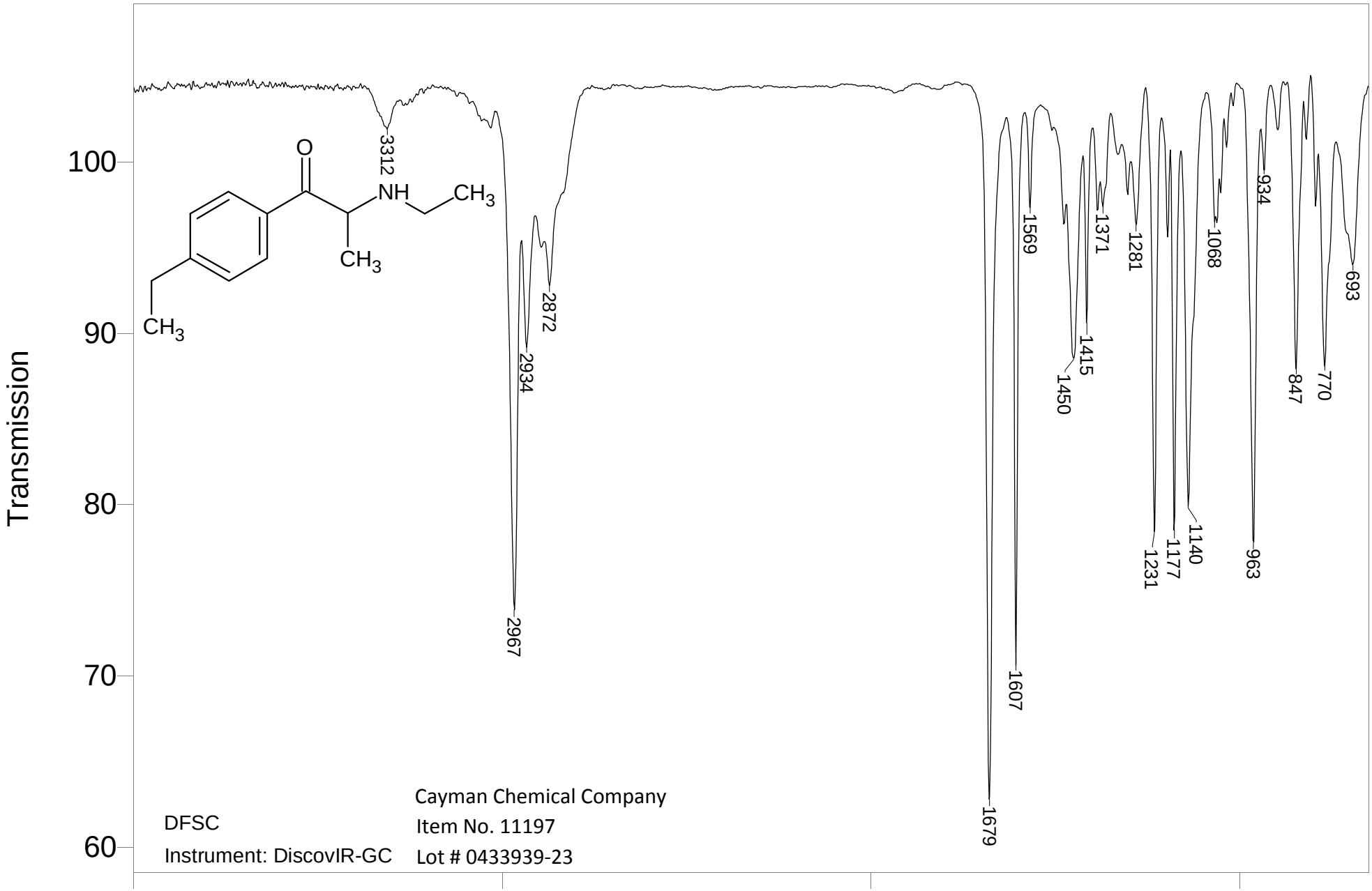


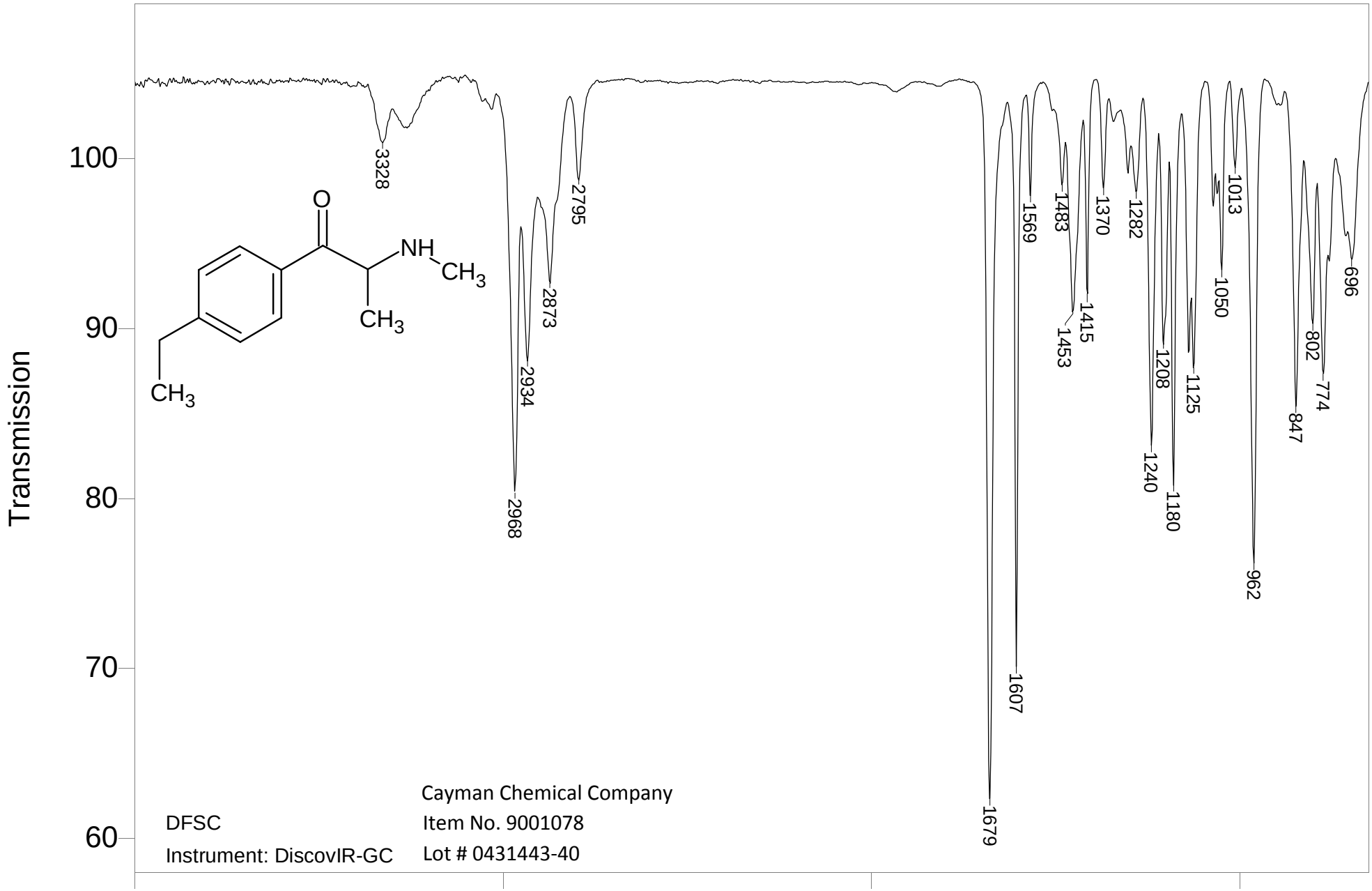


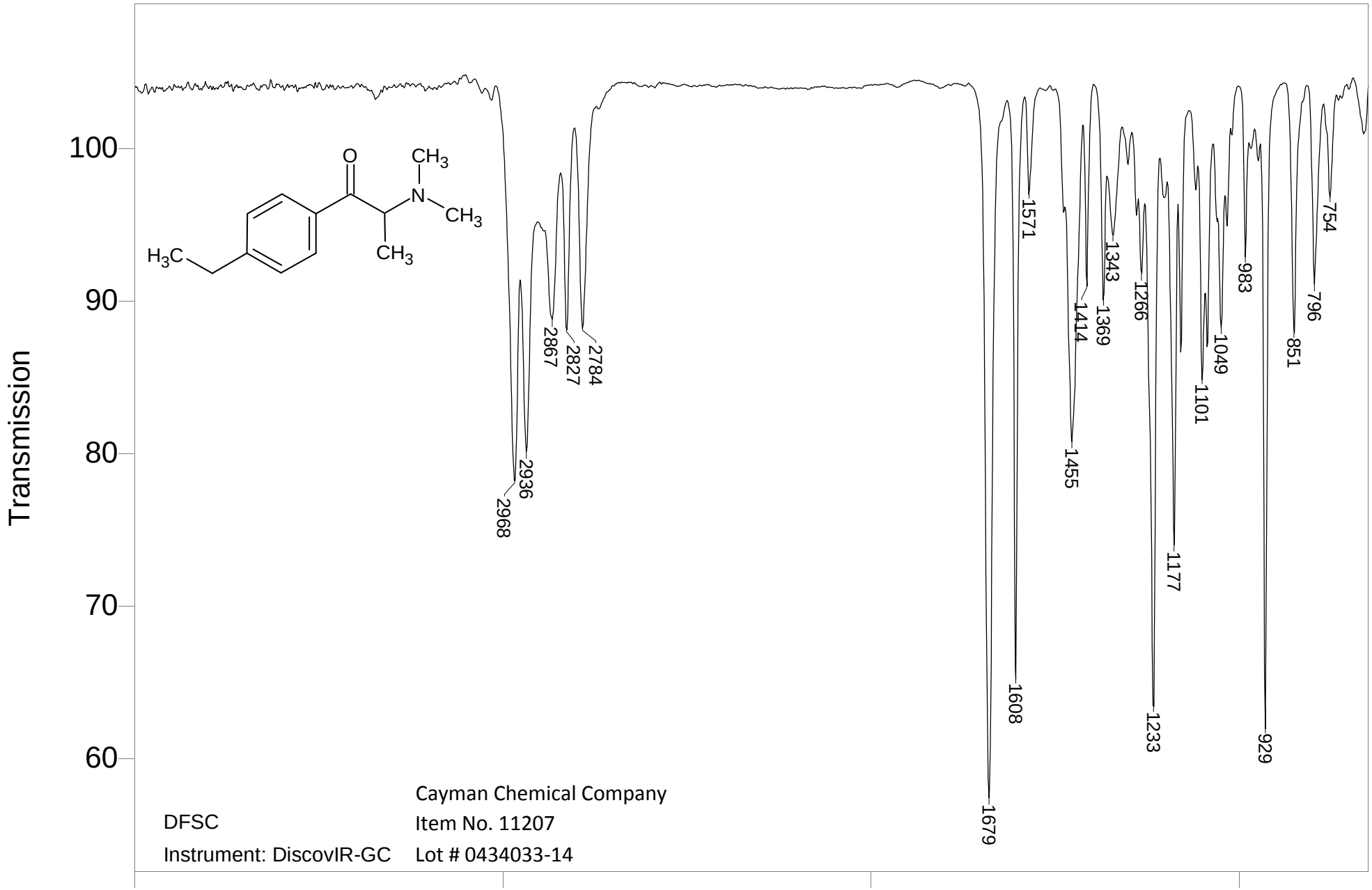




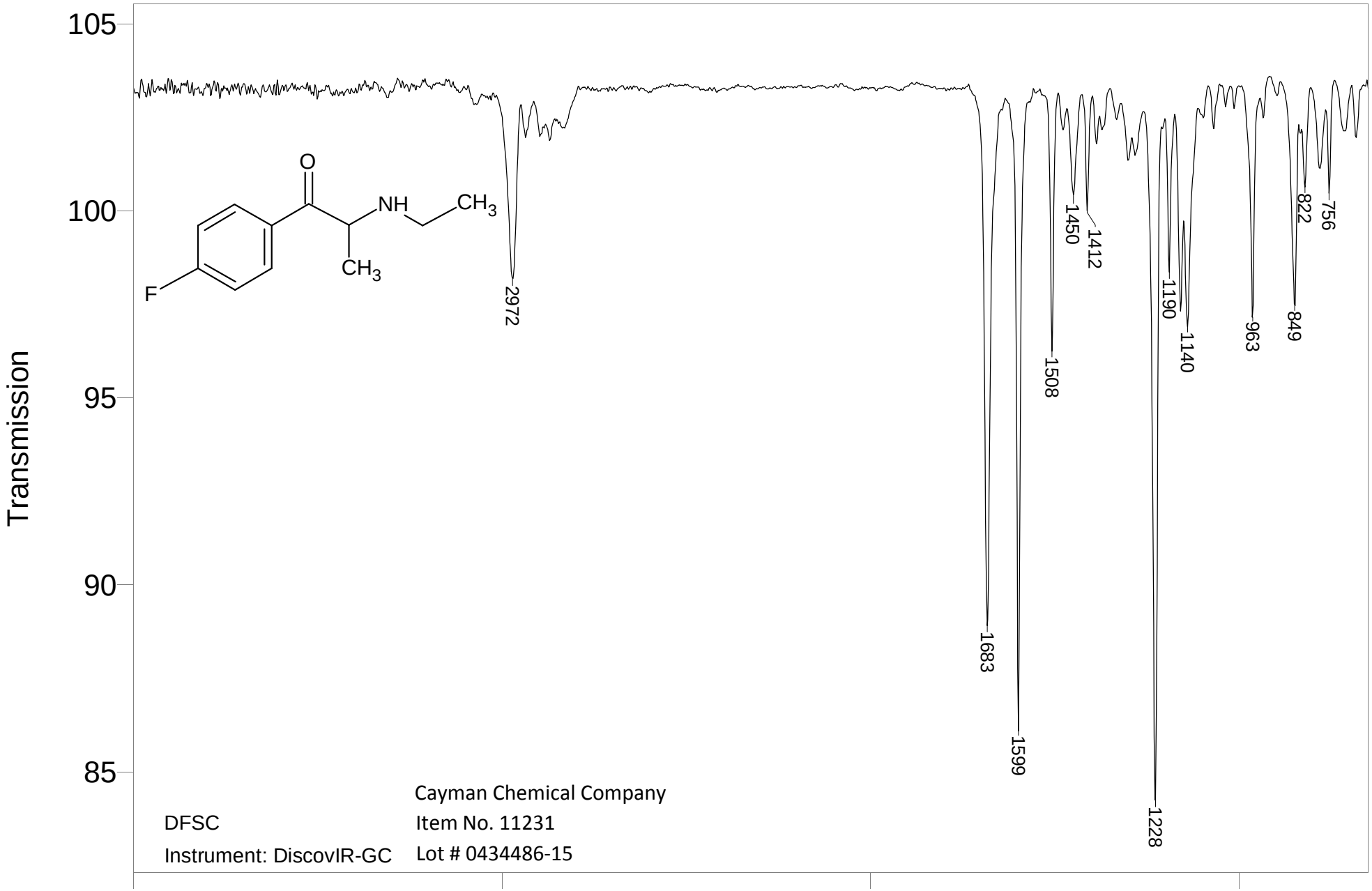








4-fluoroethcathinone



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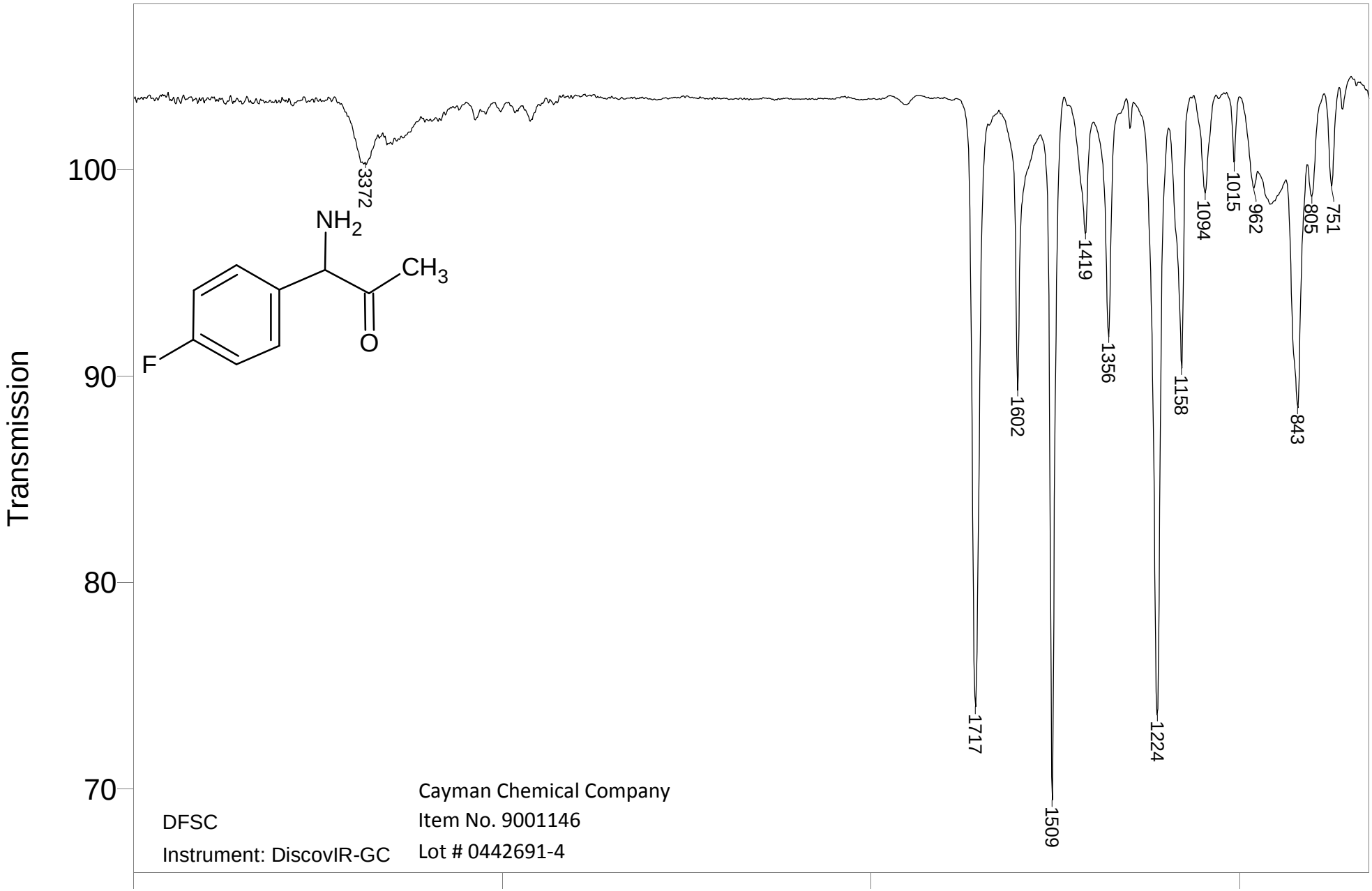

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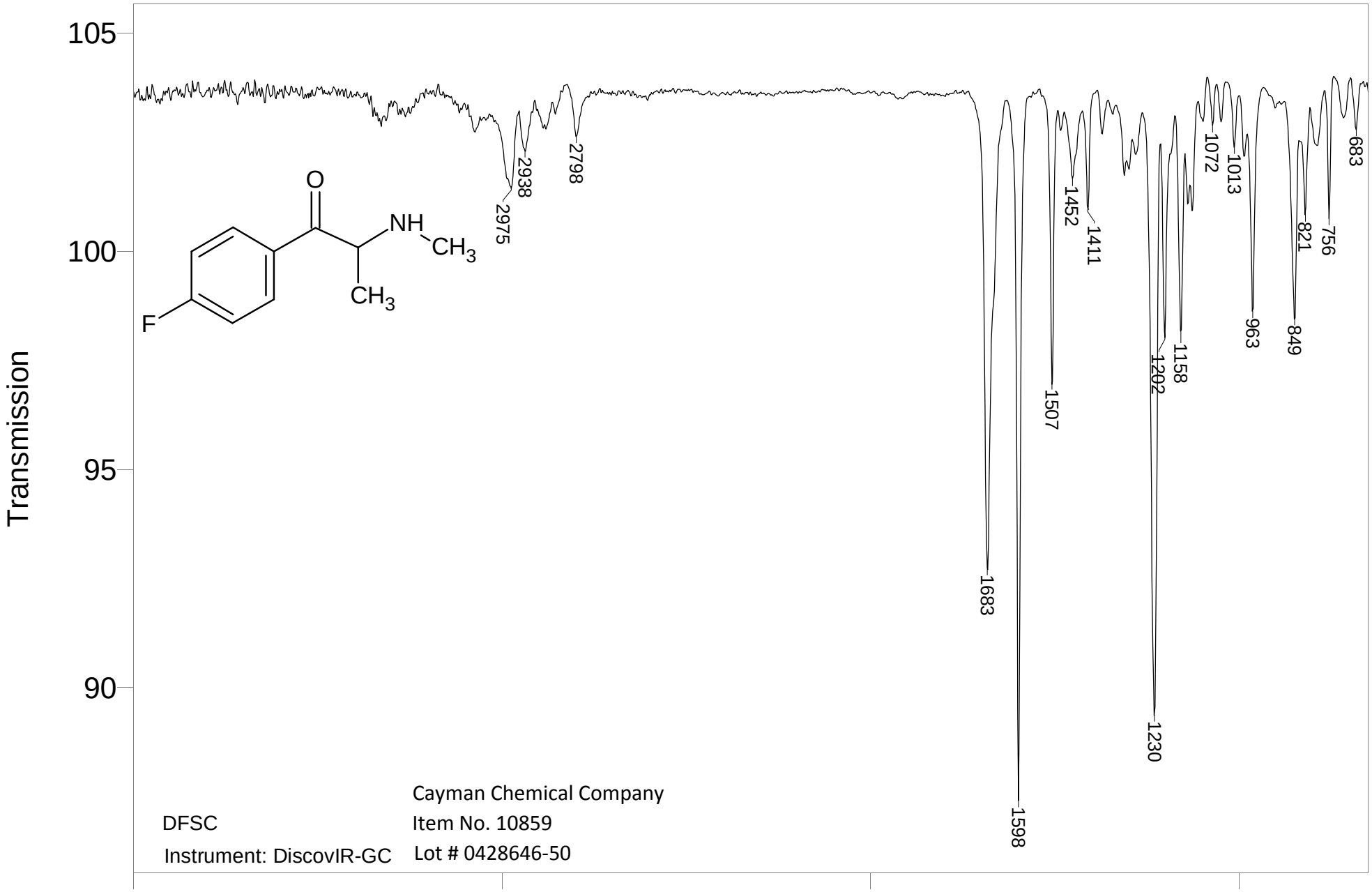
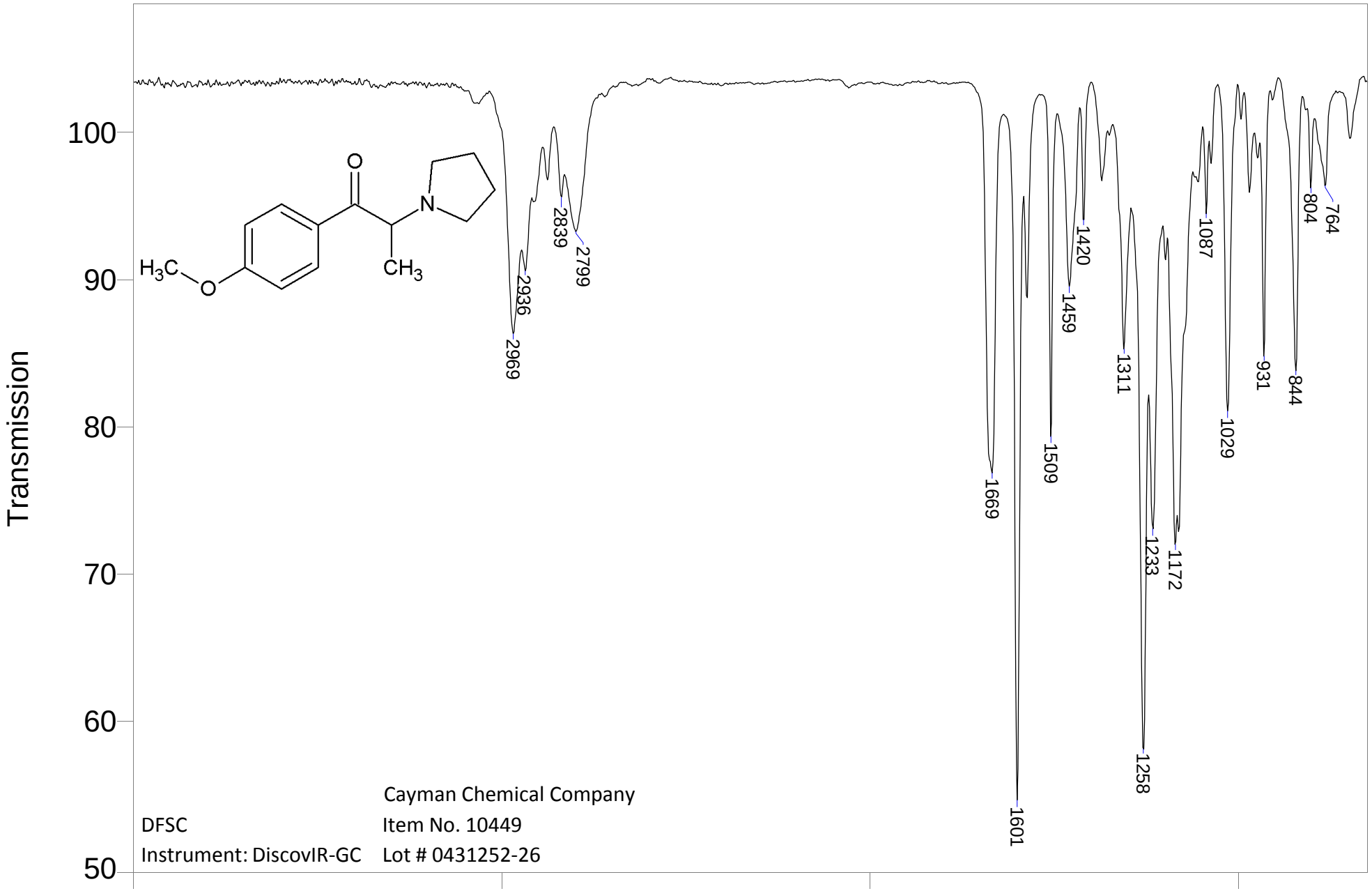
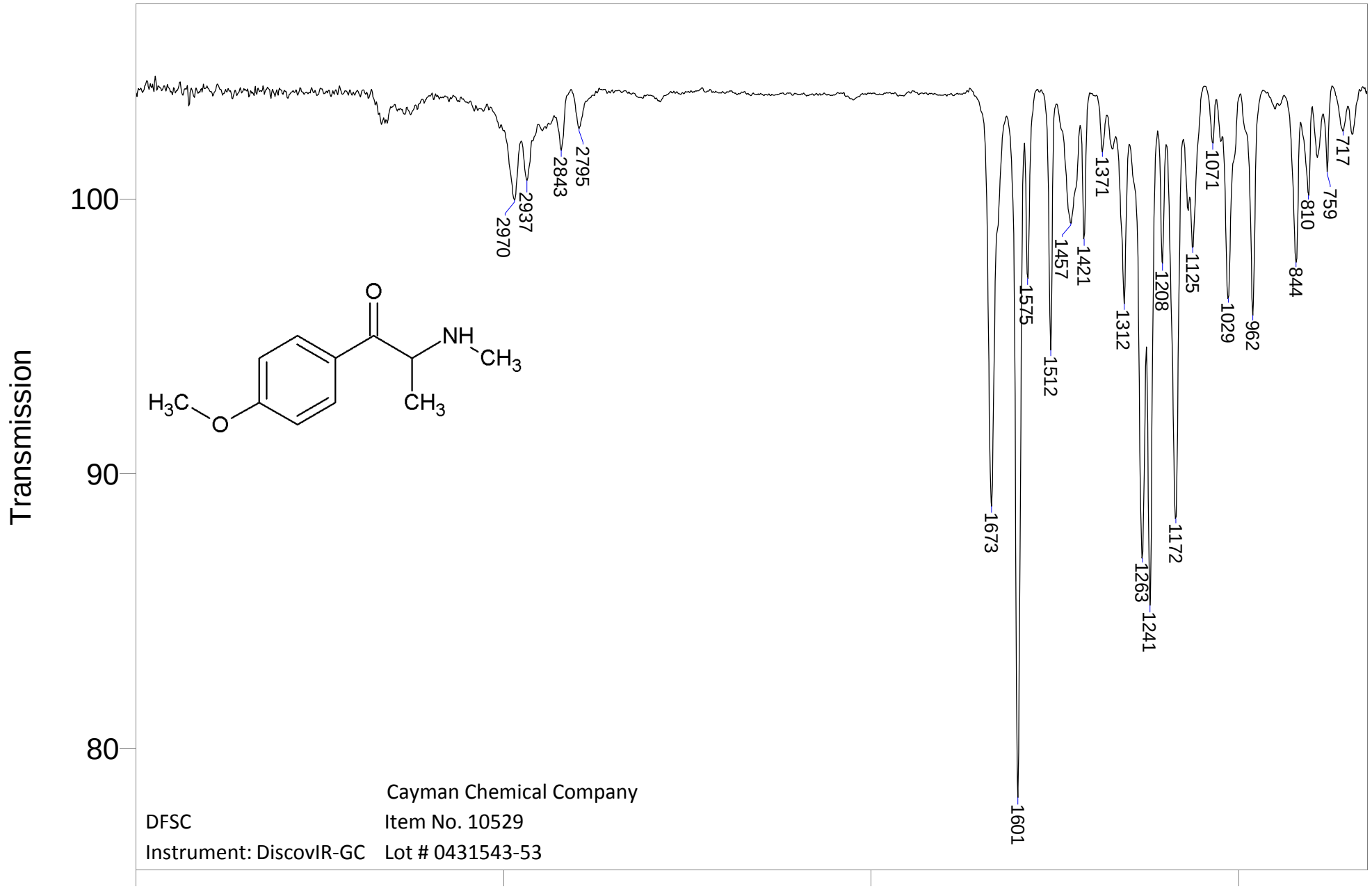
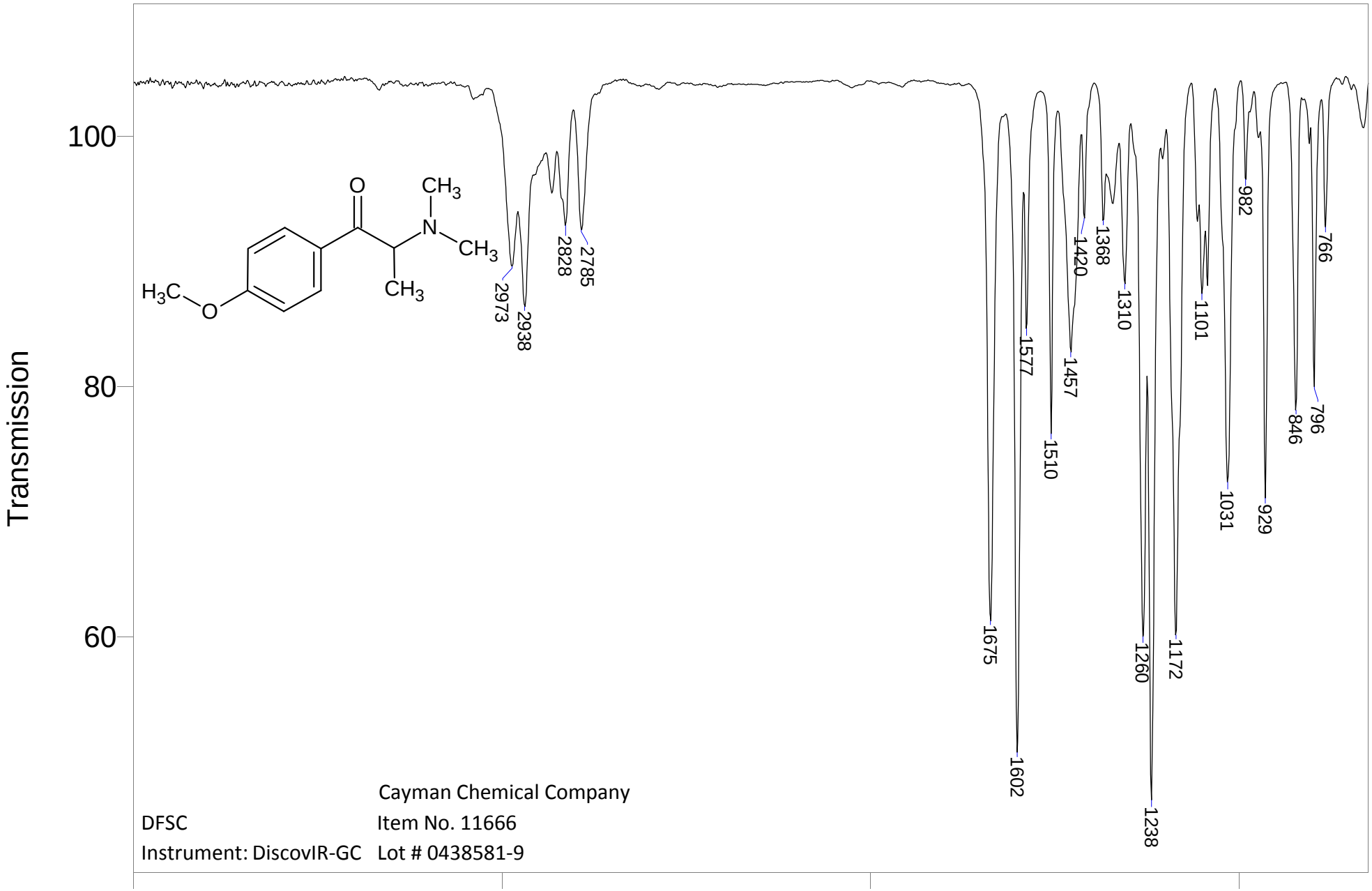
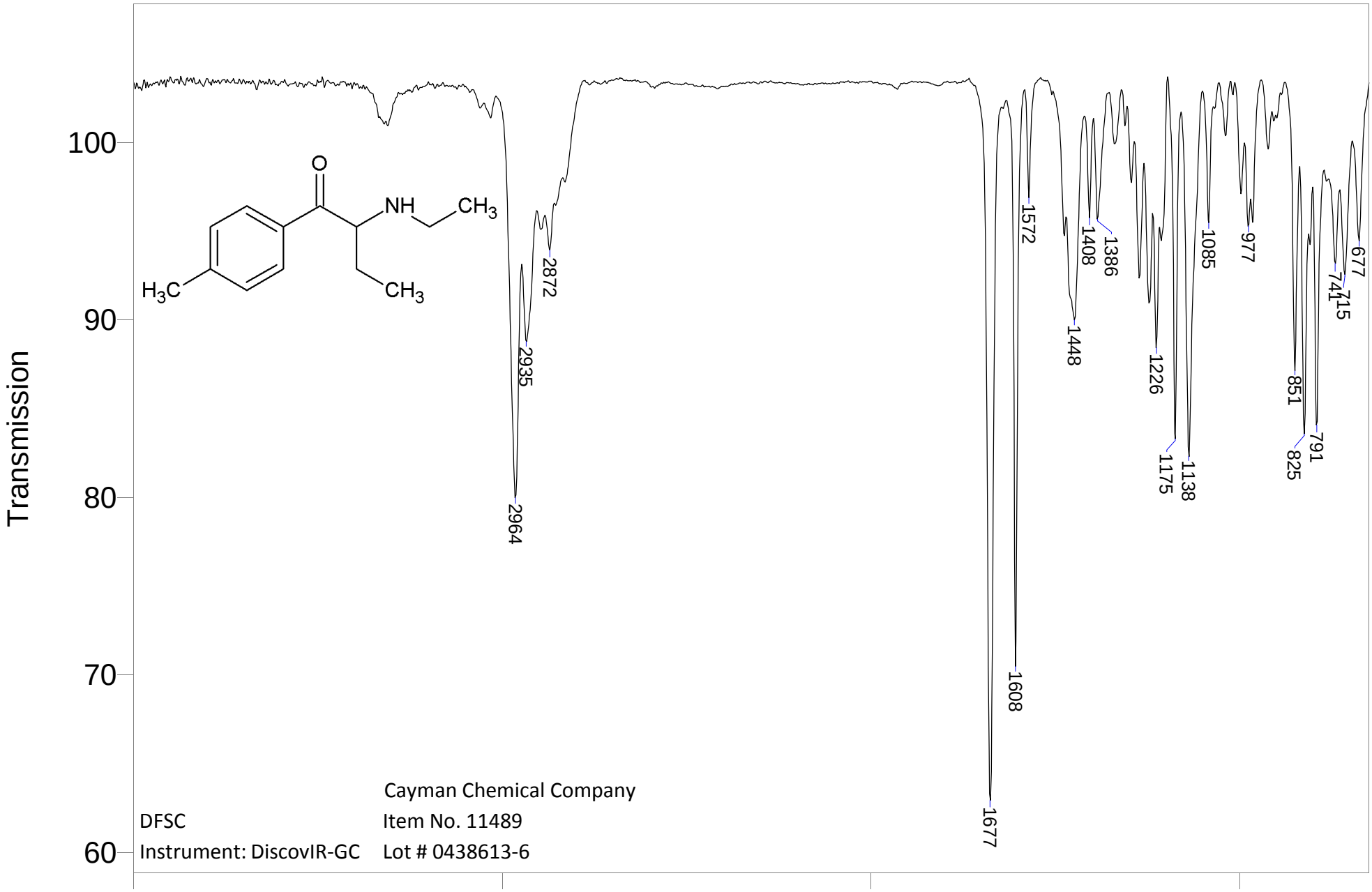
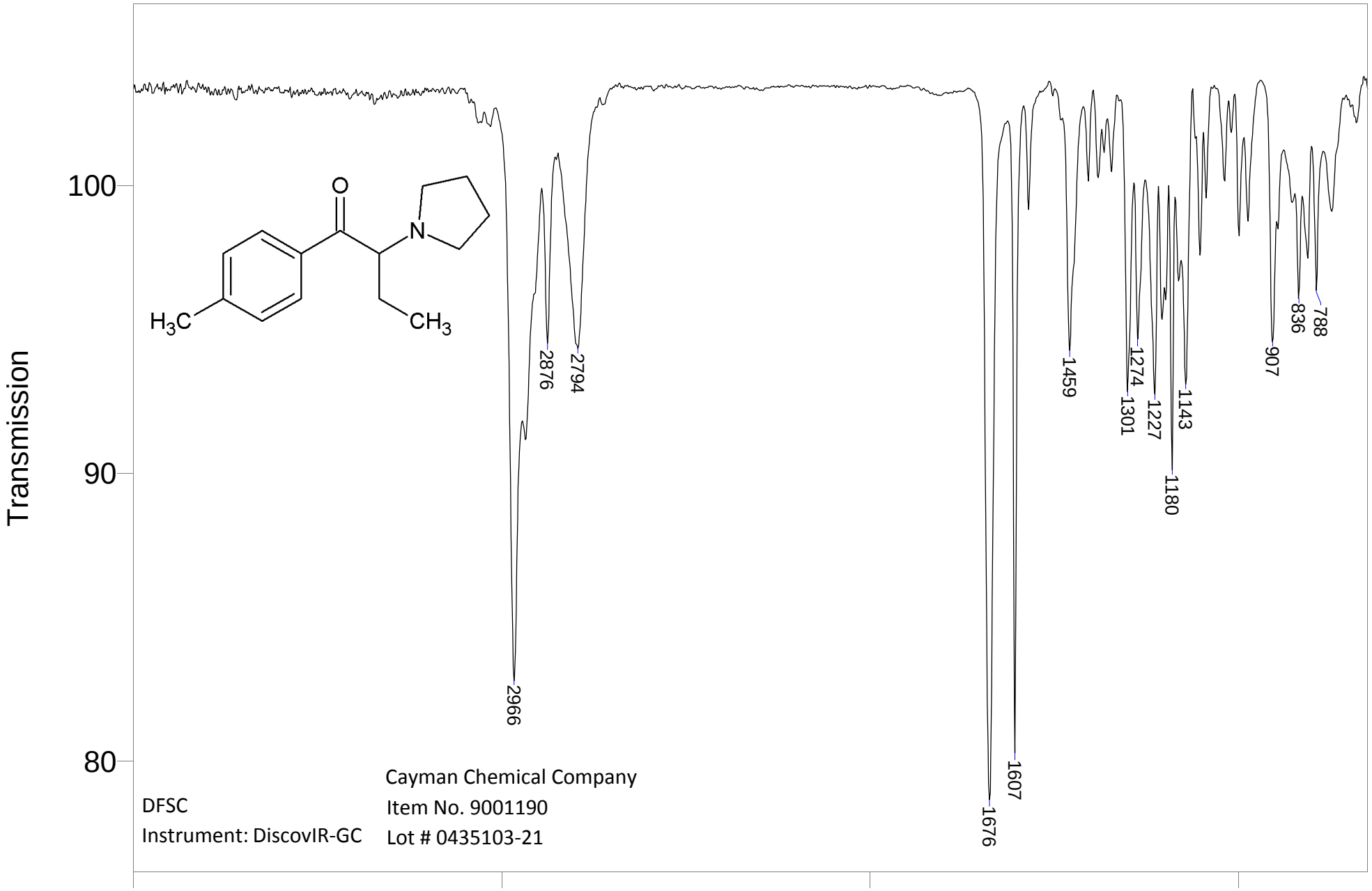


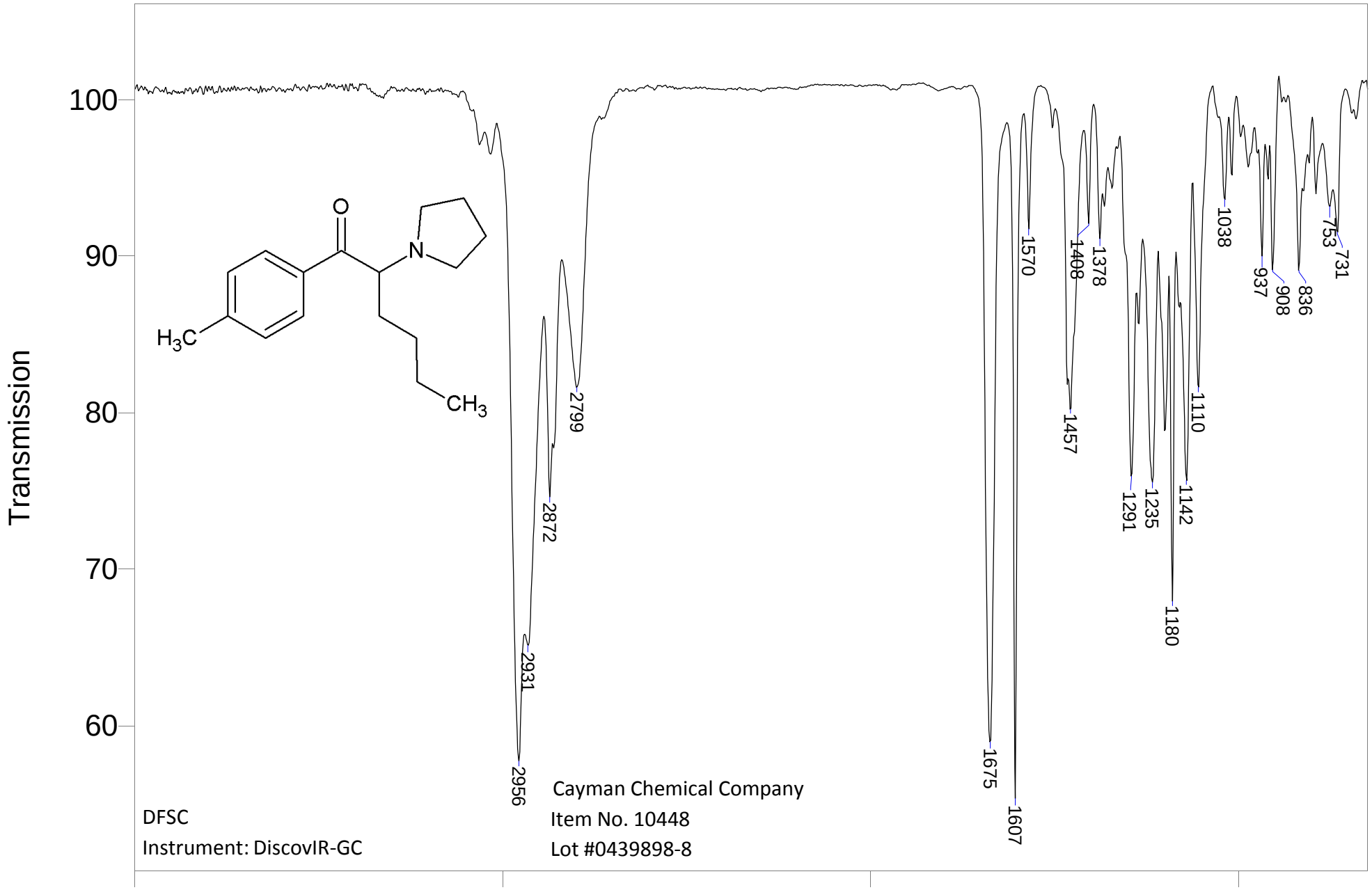


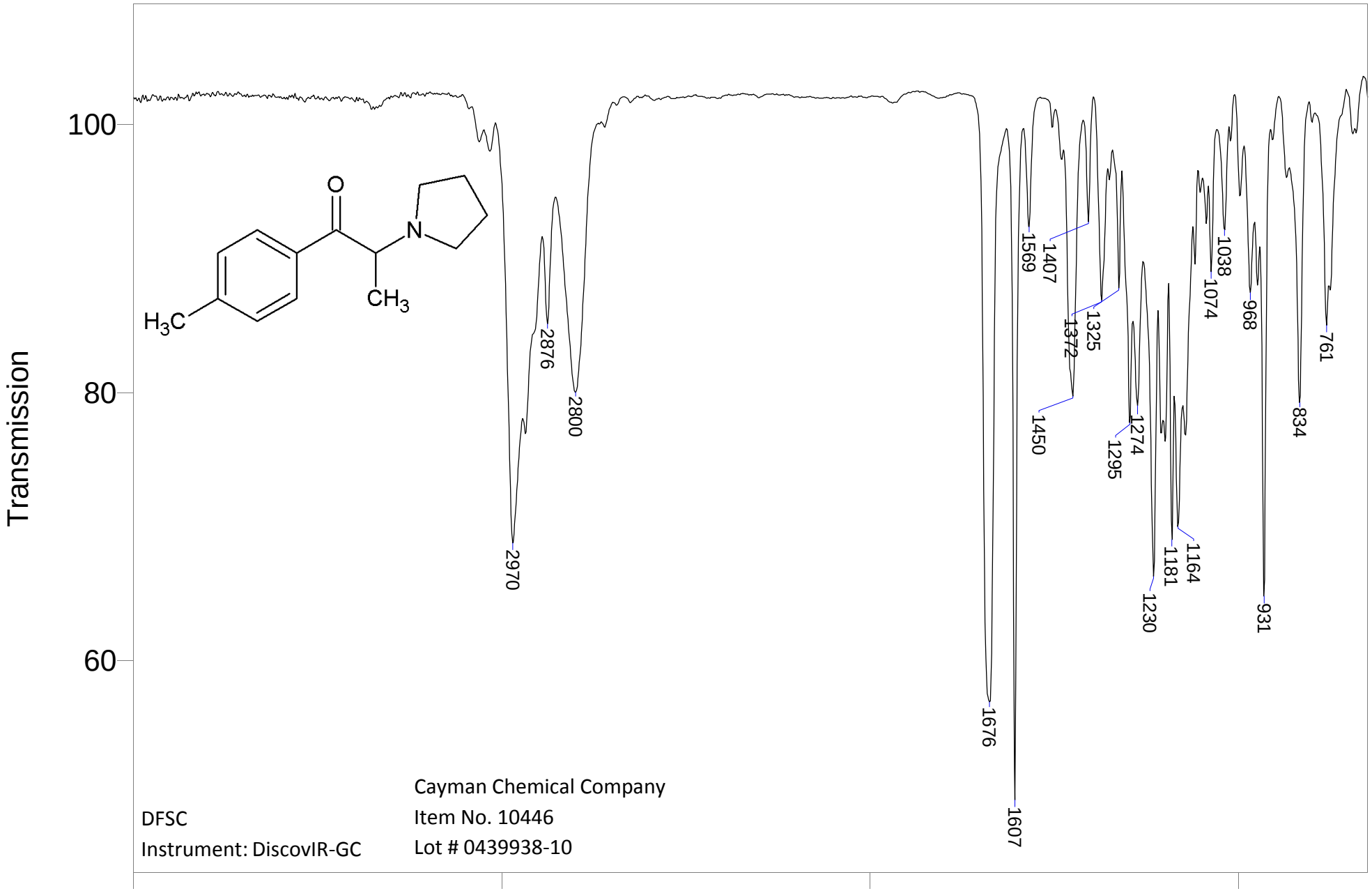


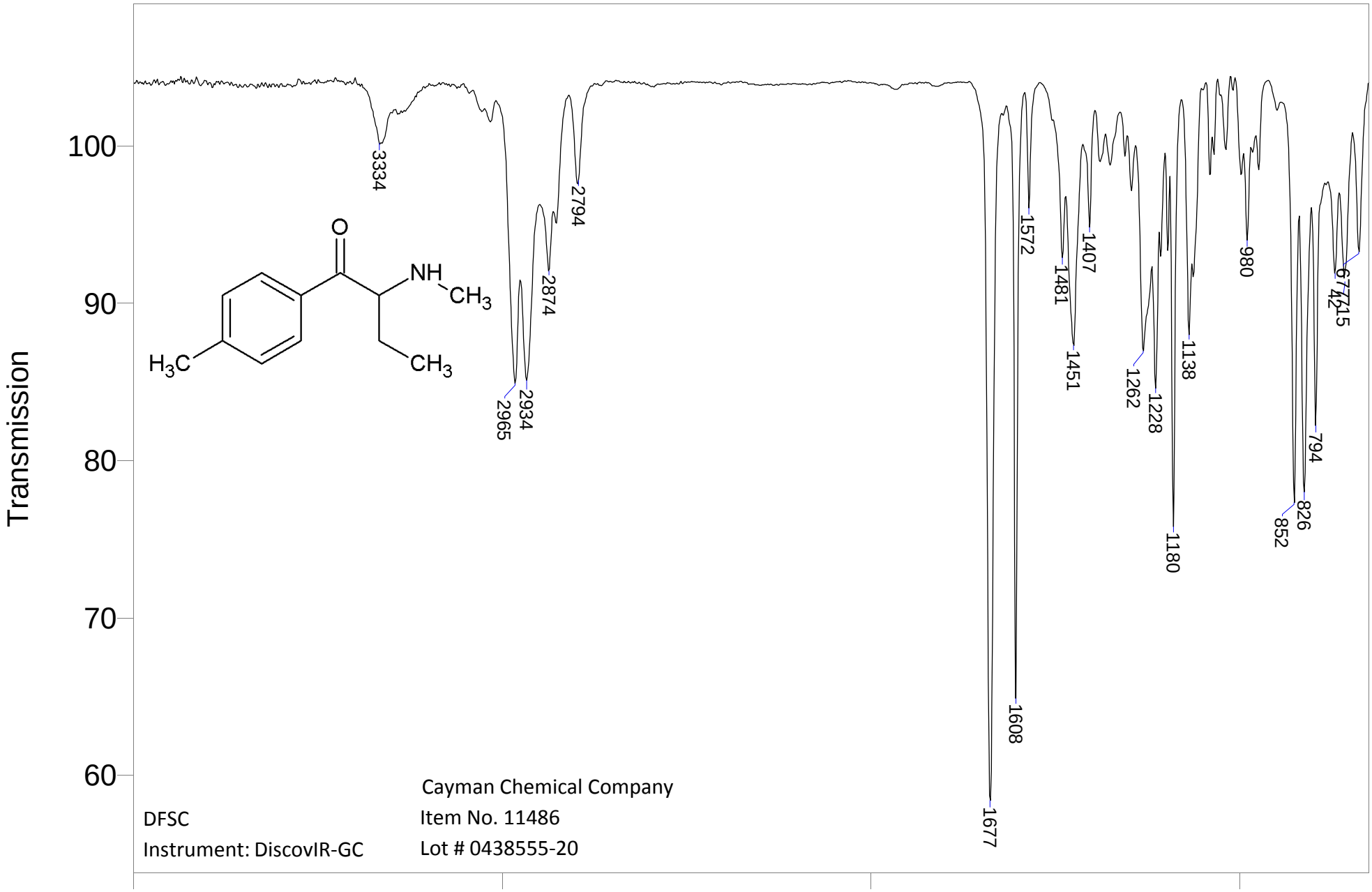


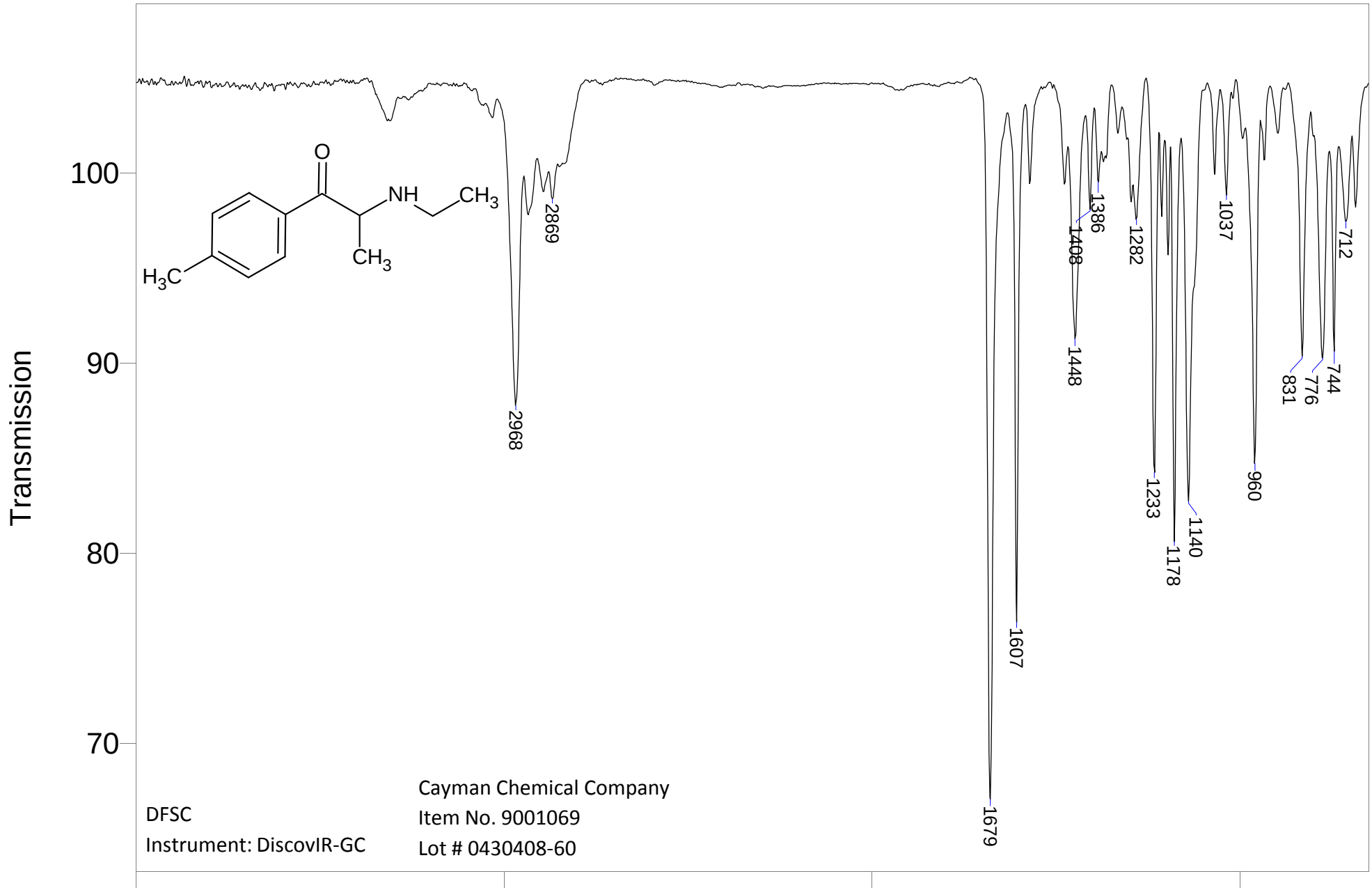


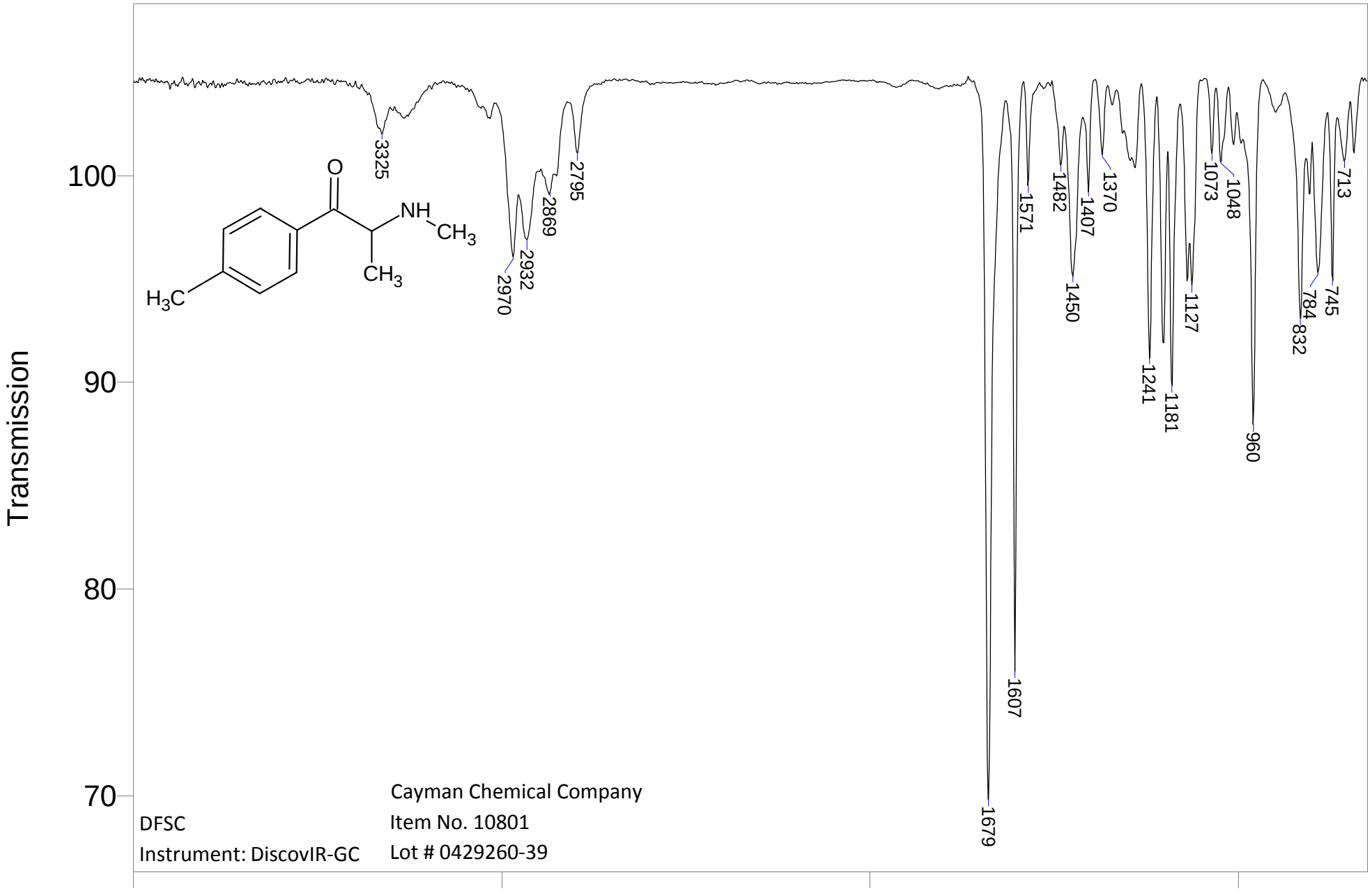


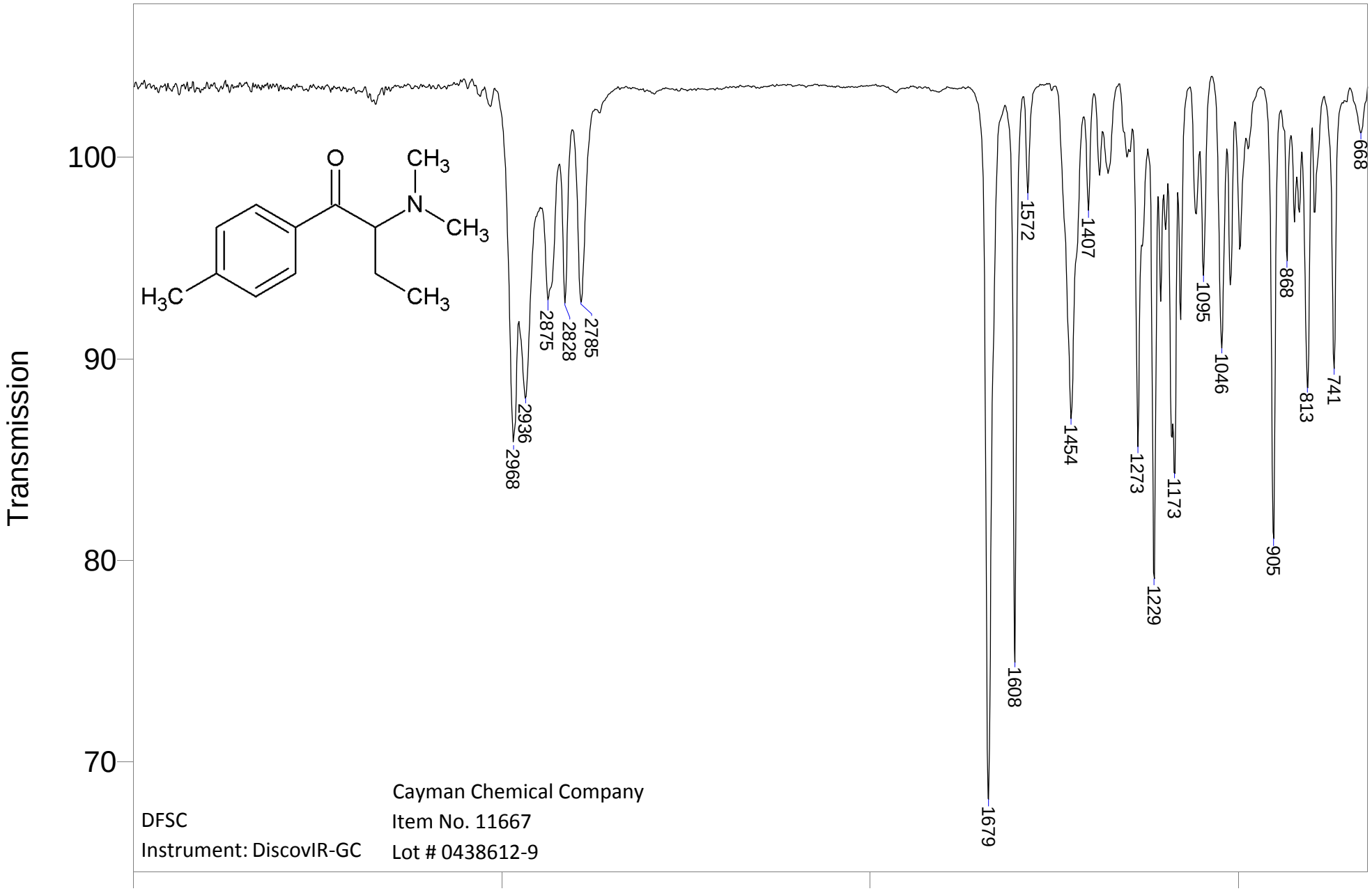


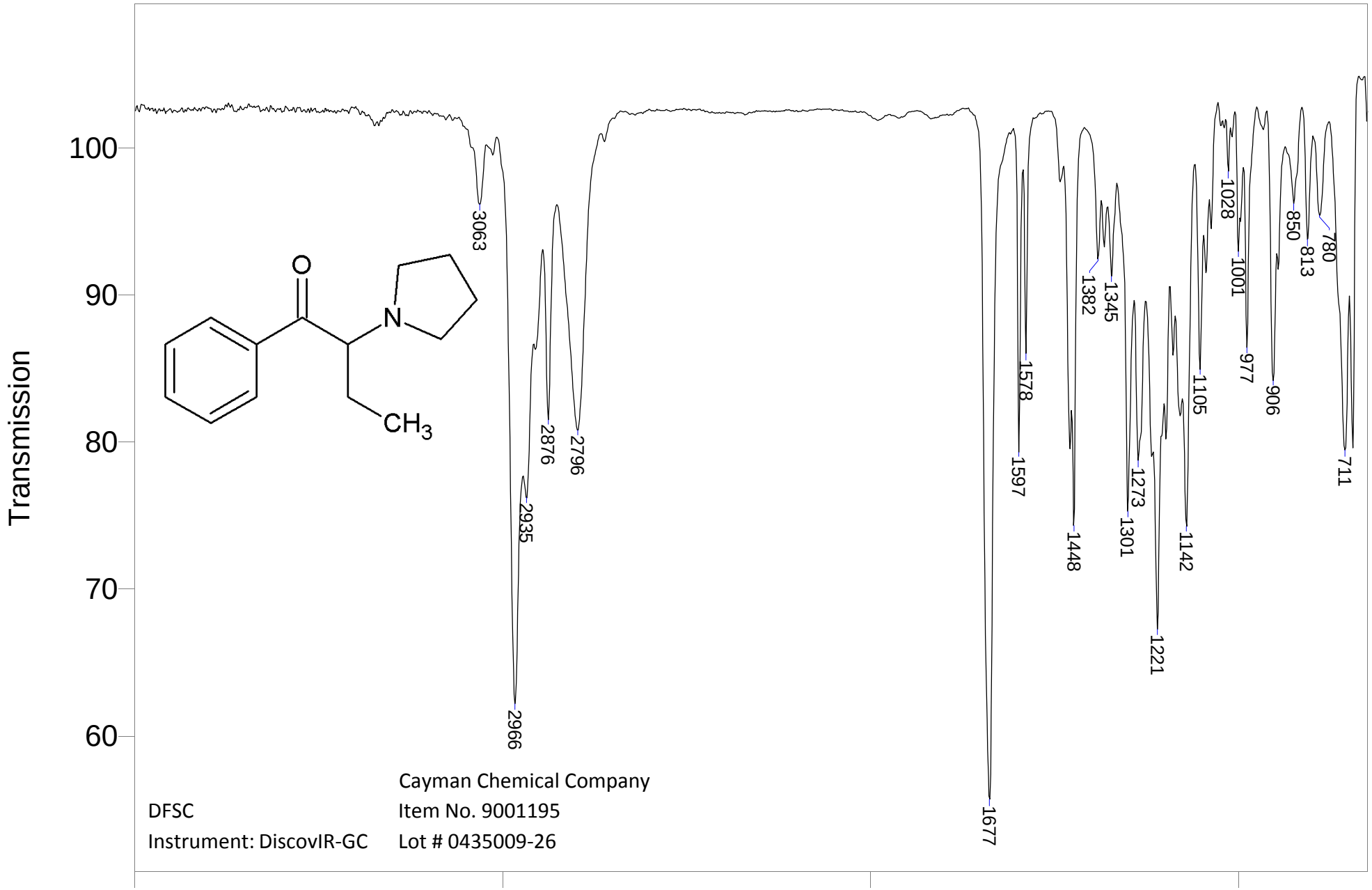


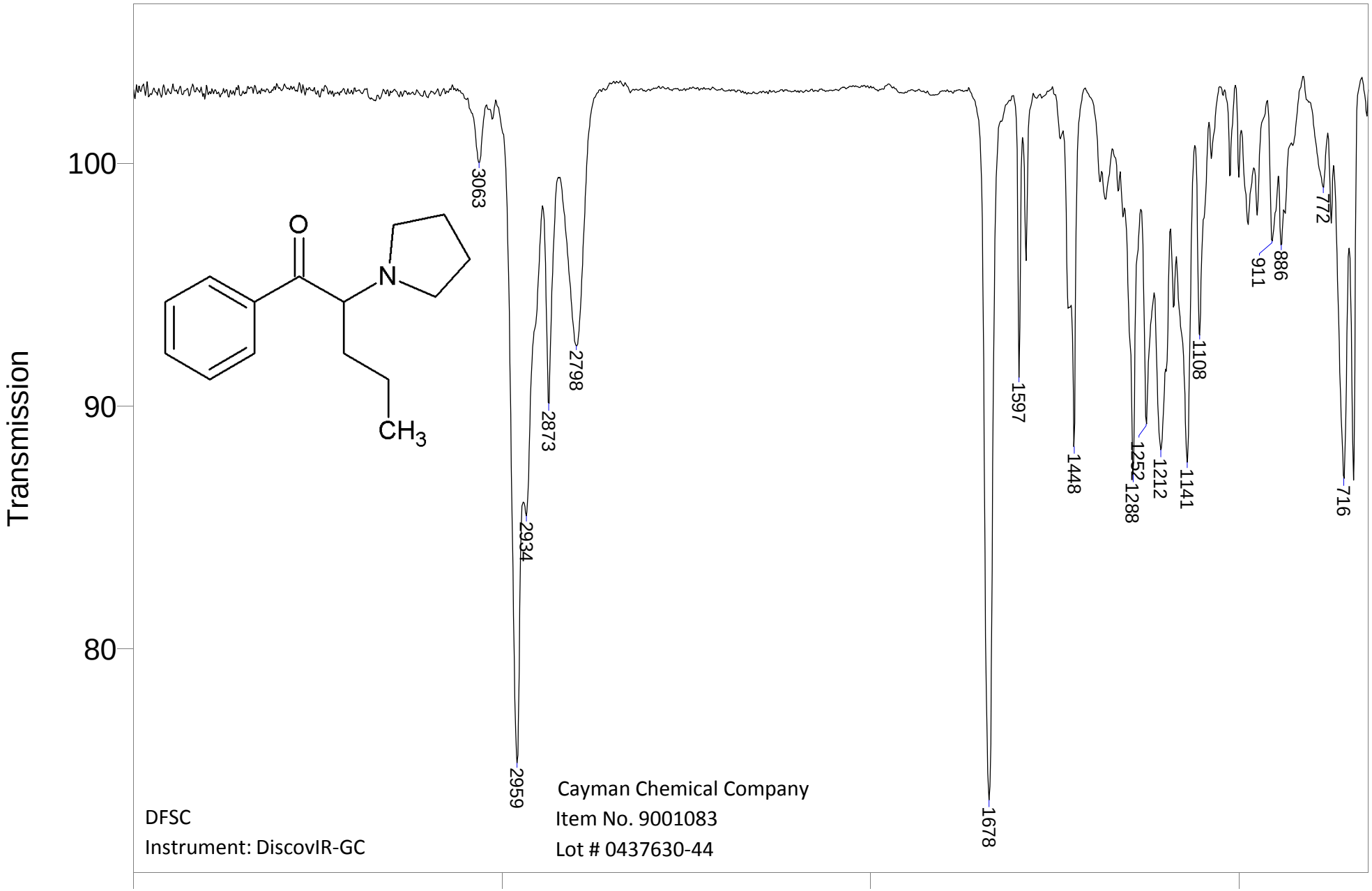


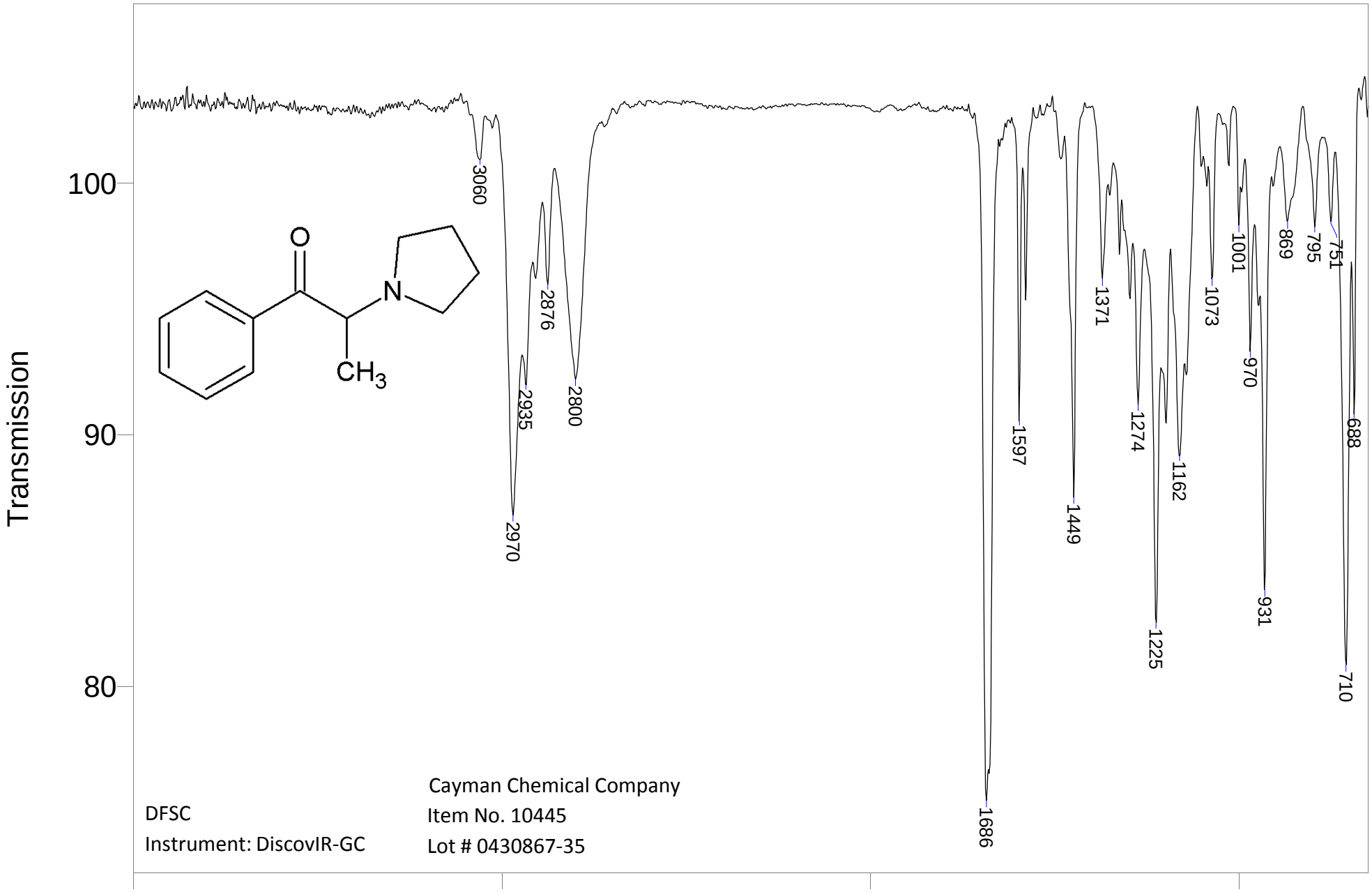


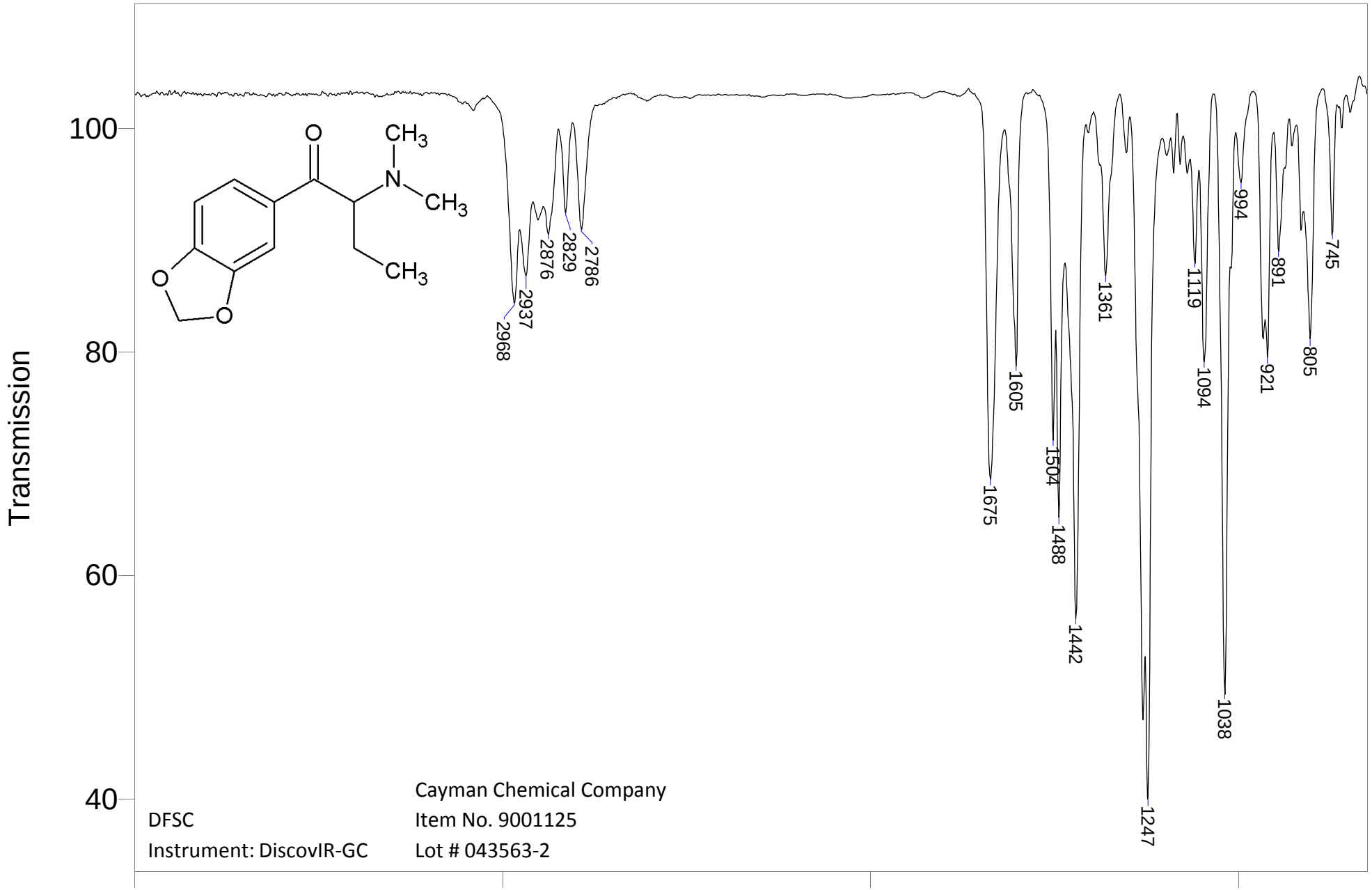


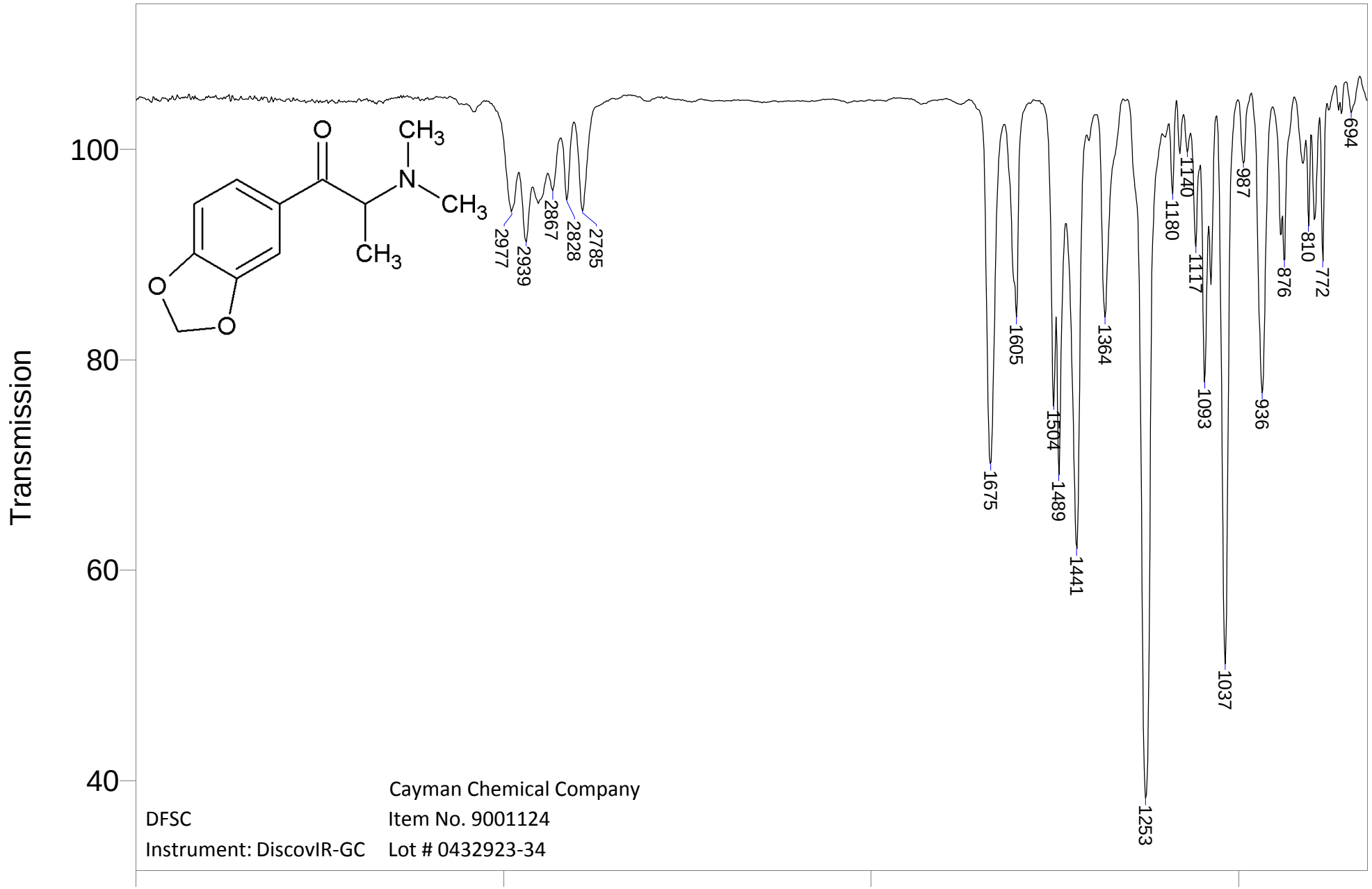


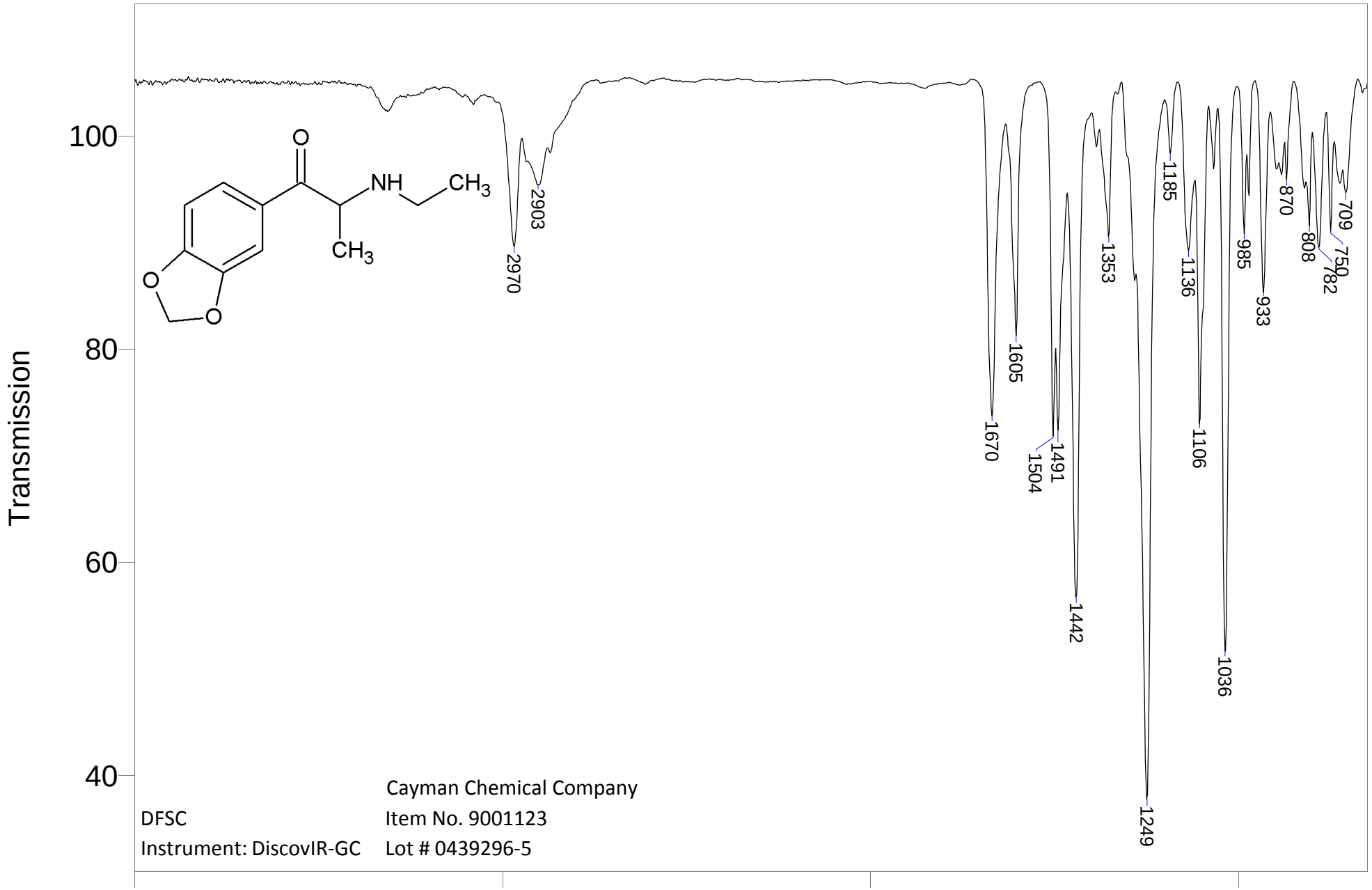


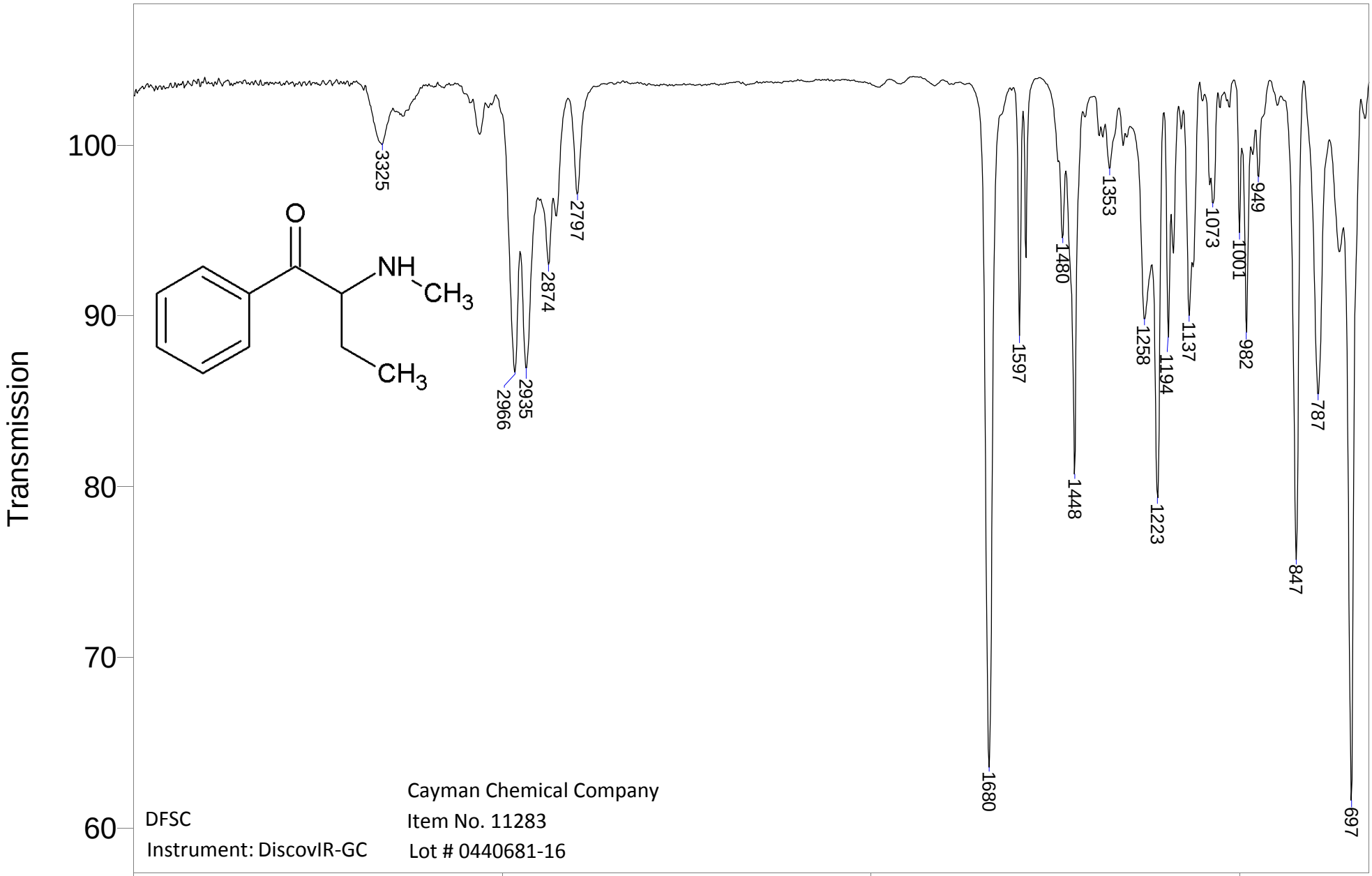


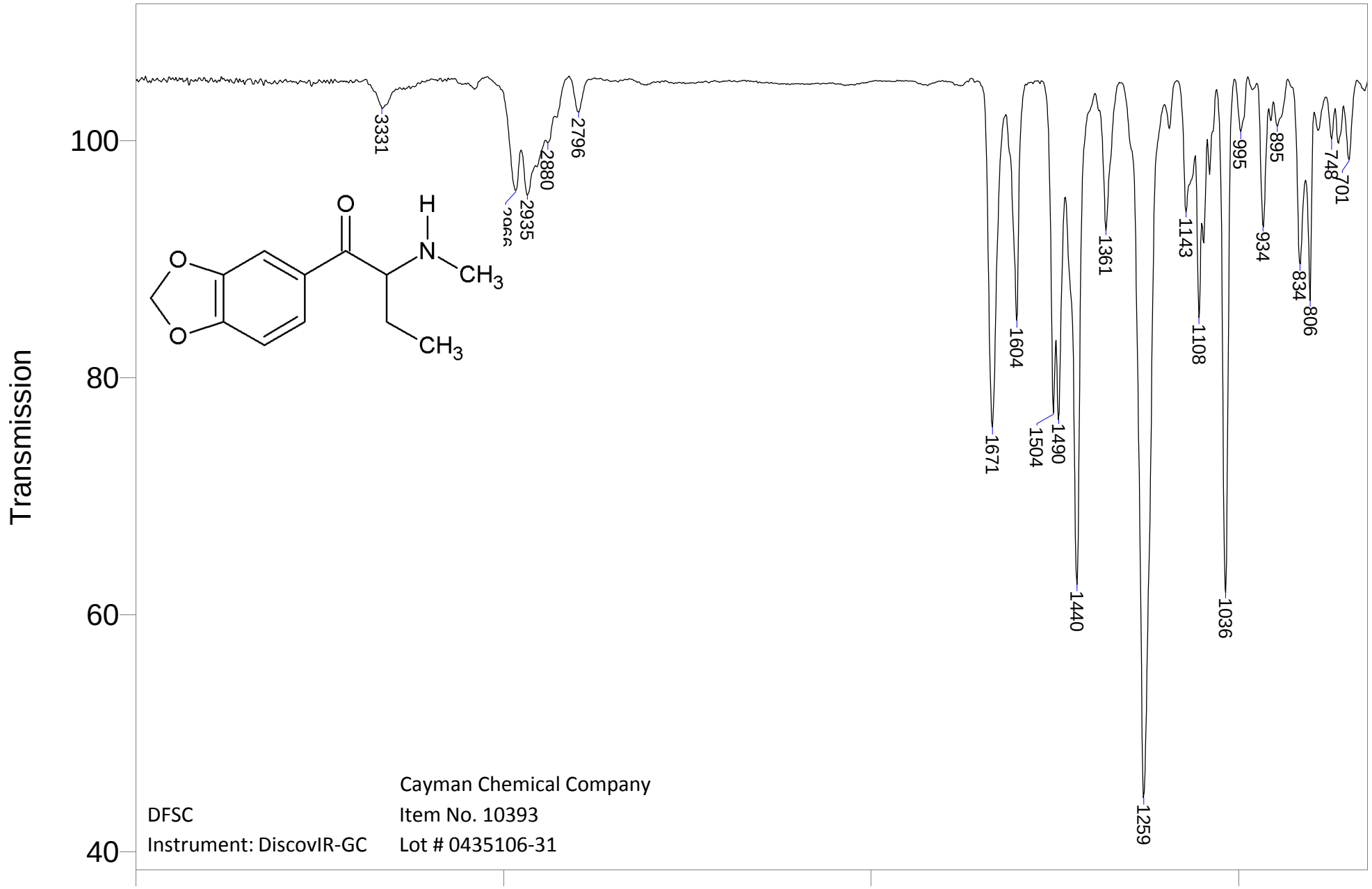


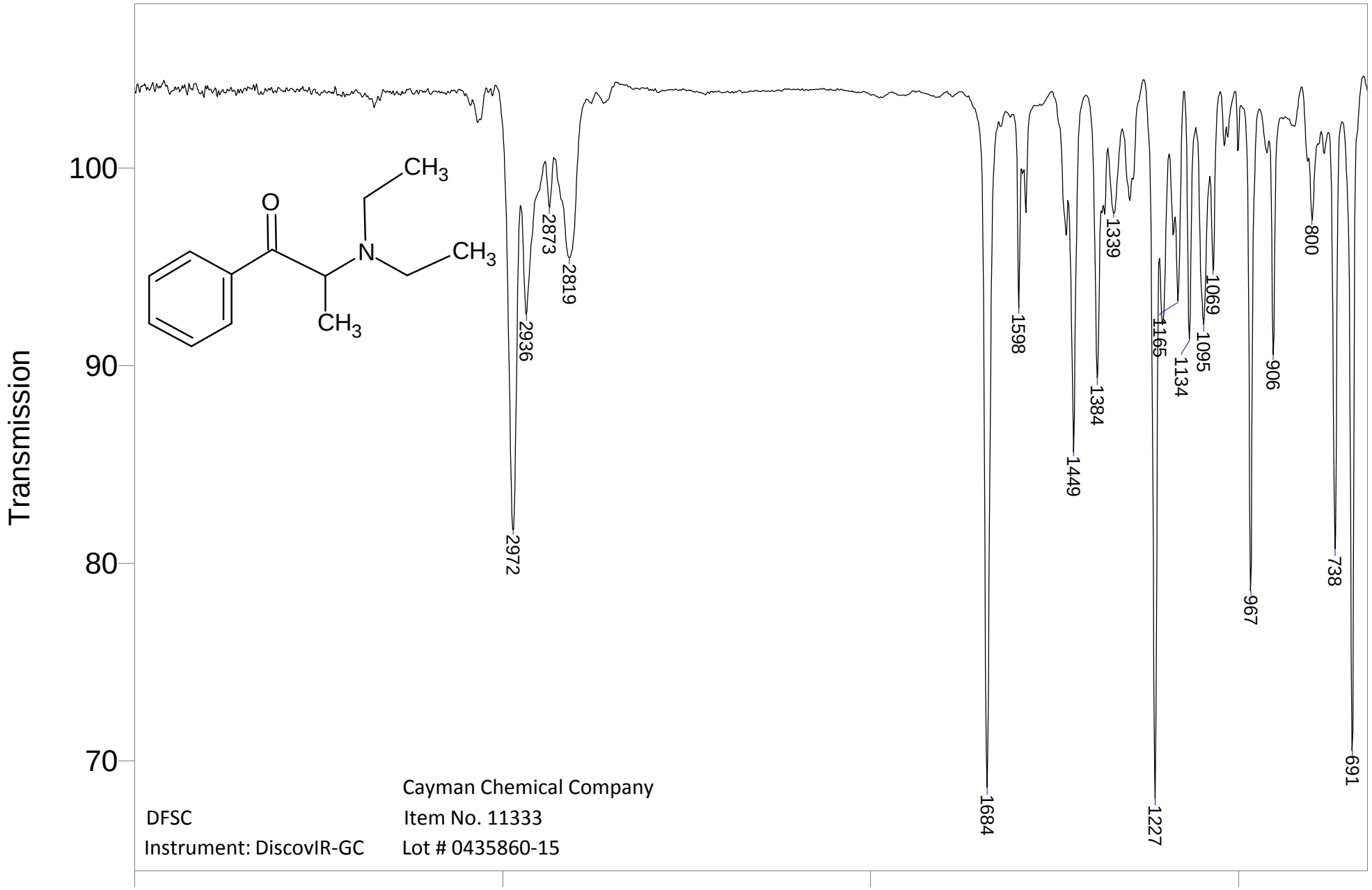


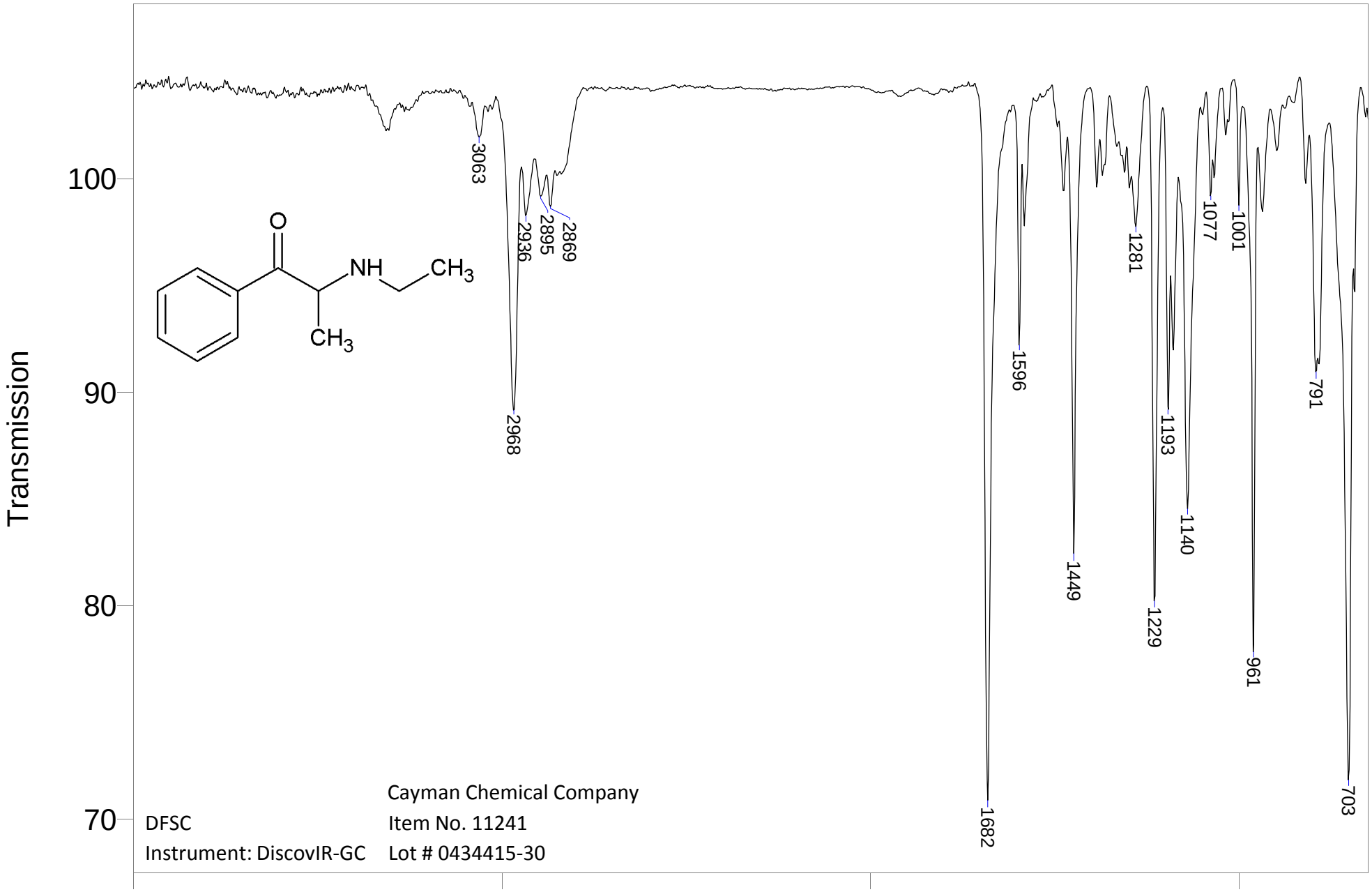


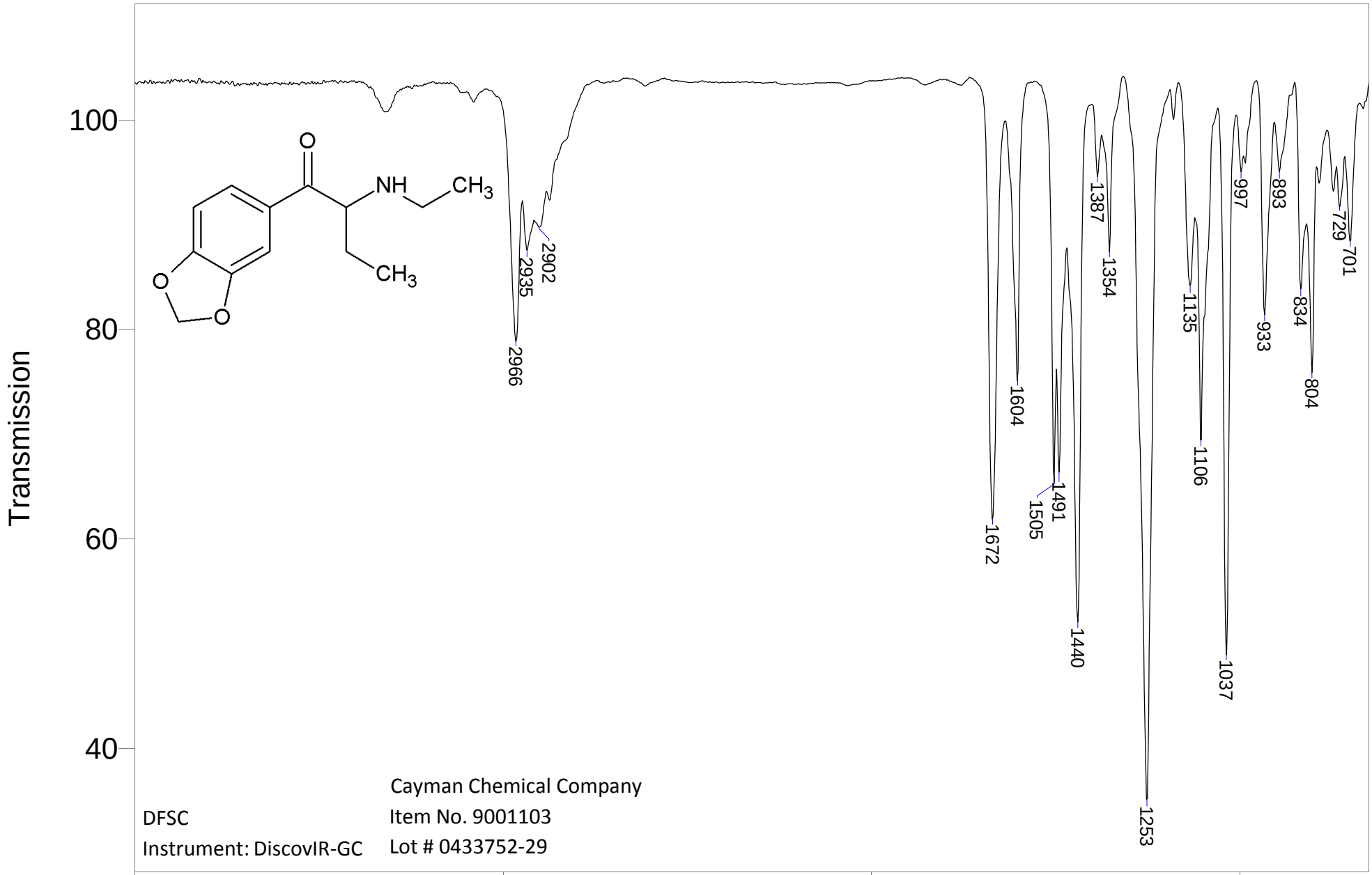


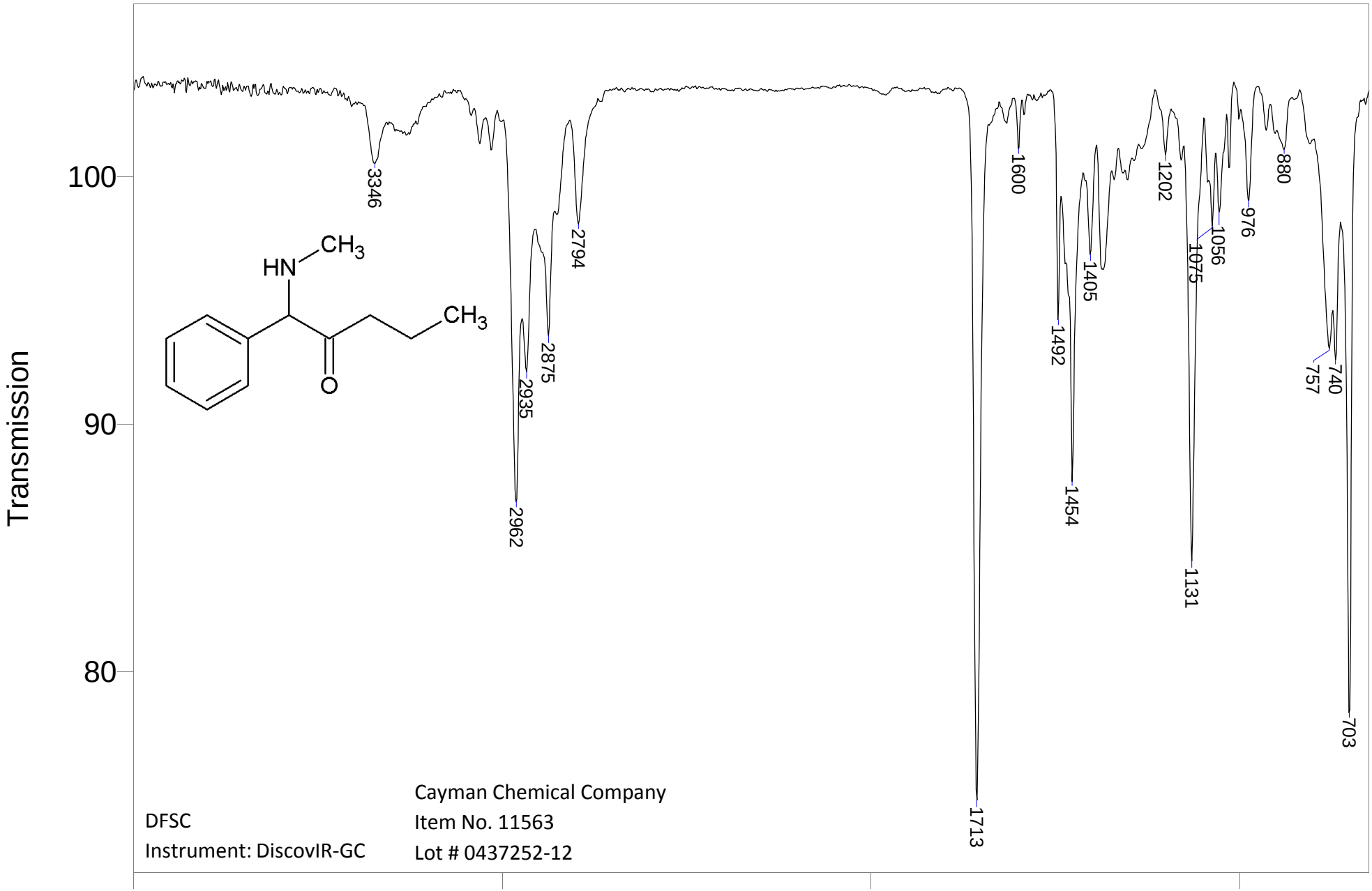


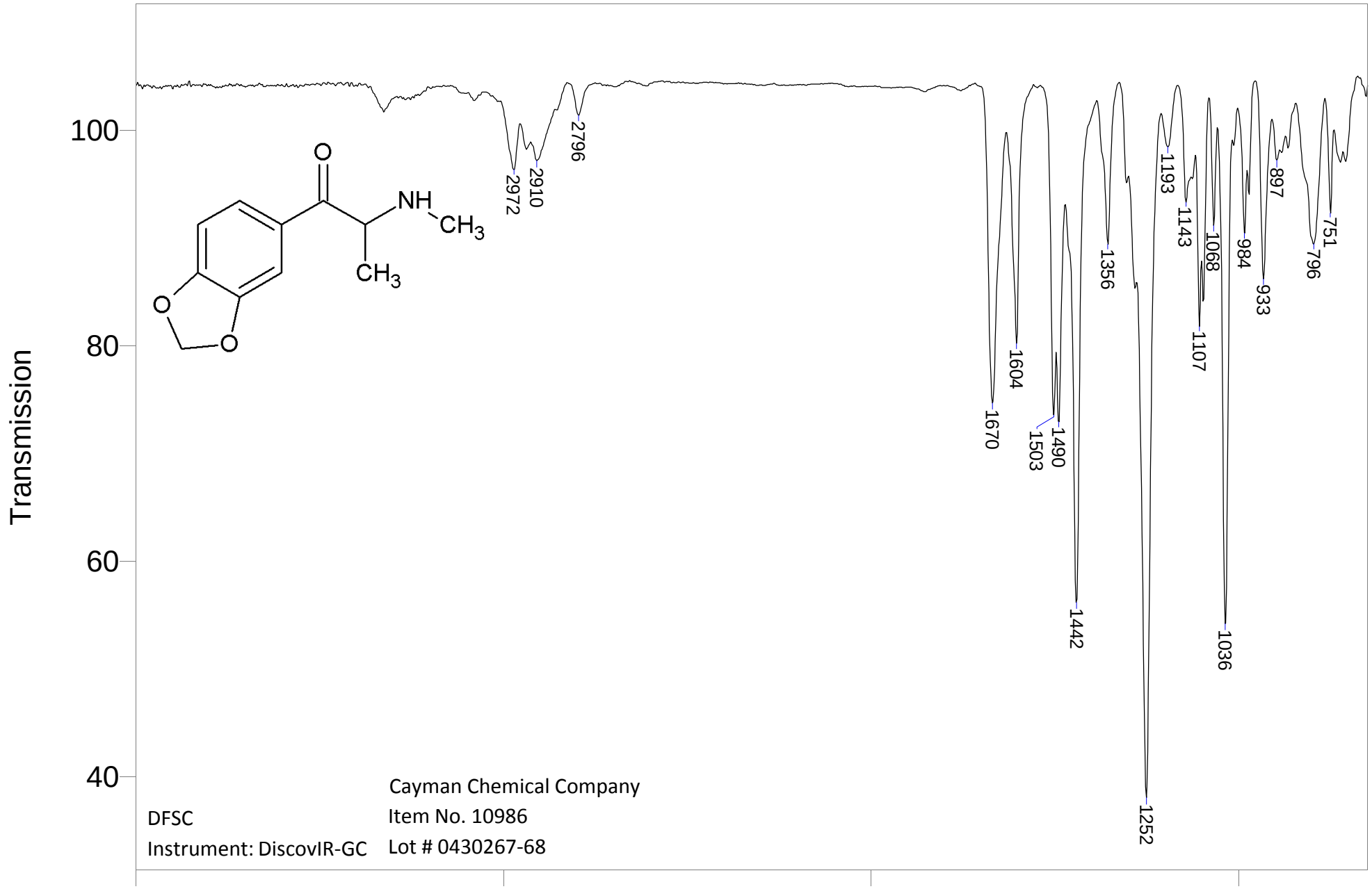


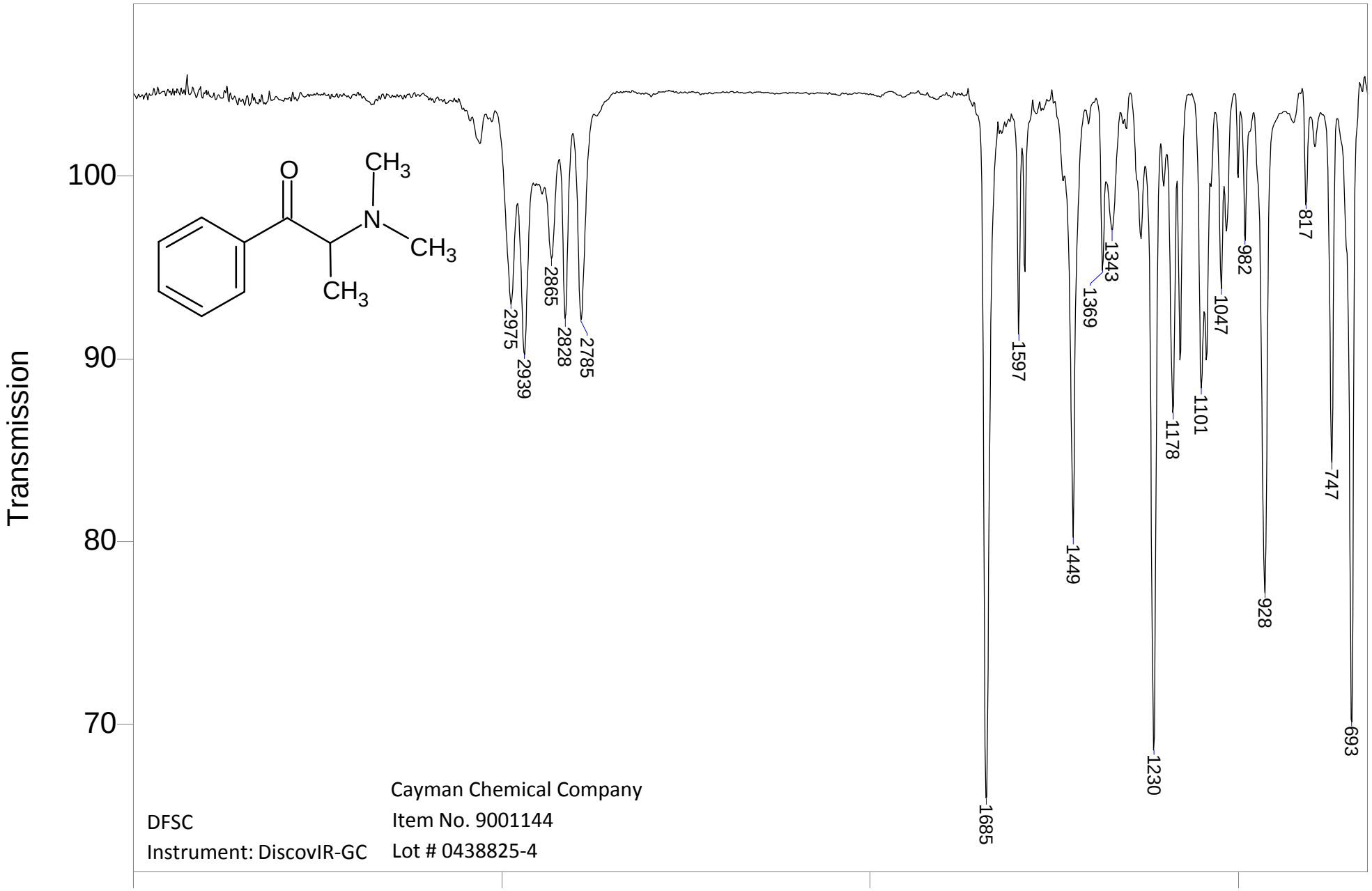




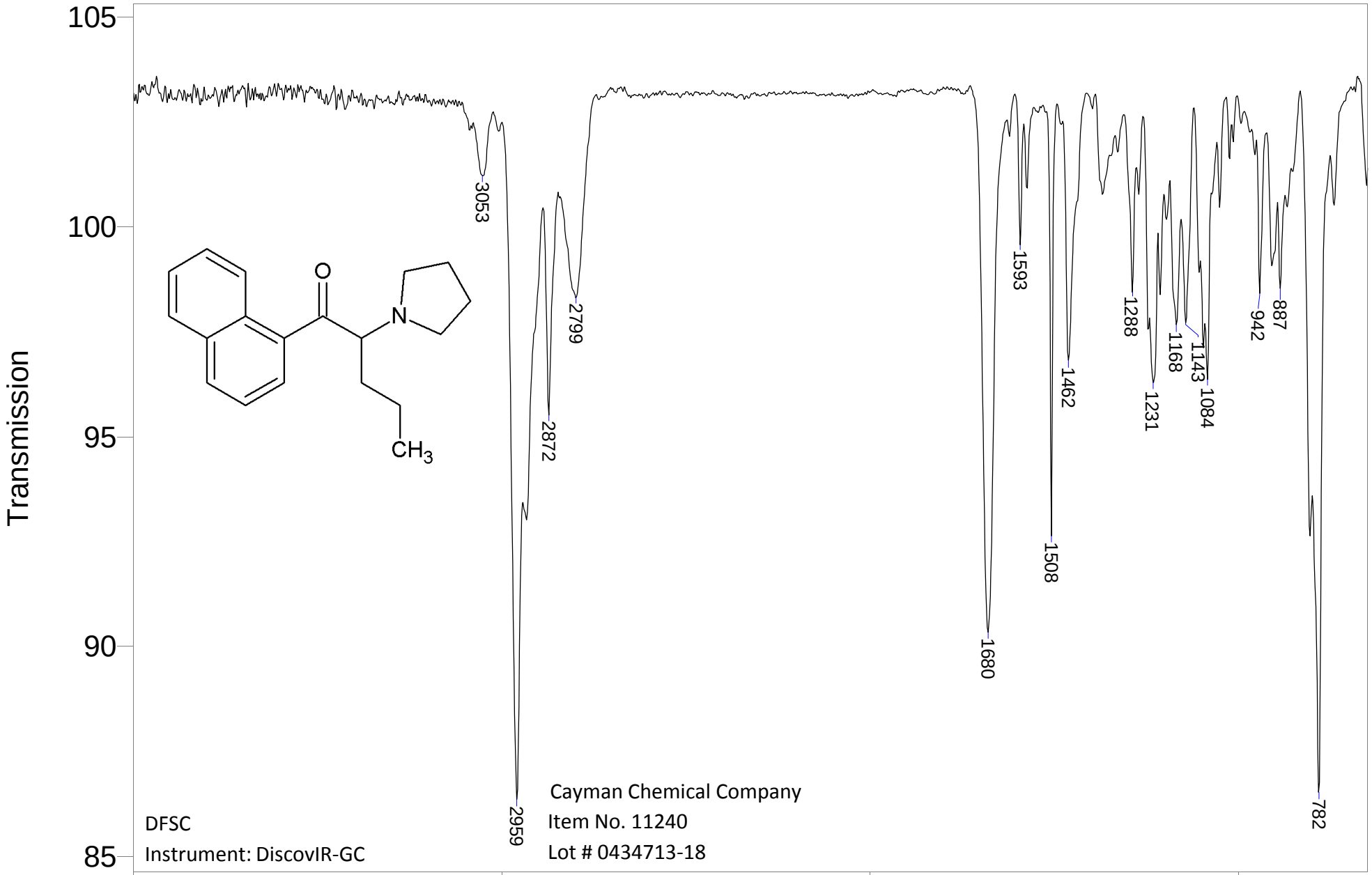


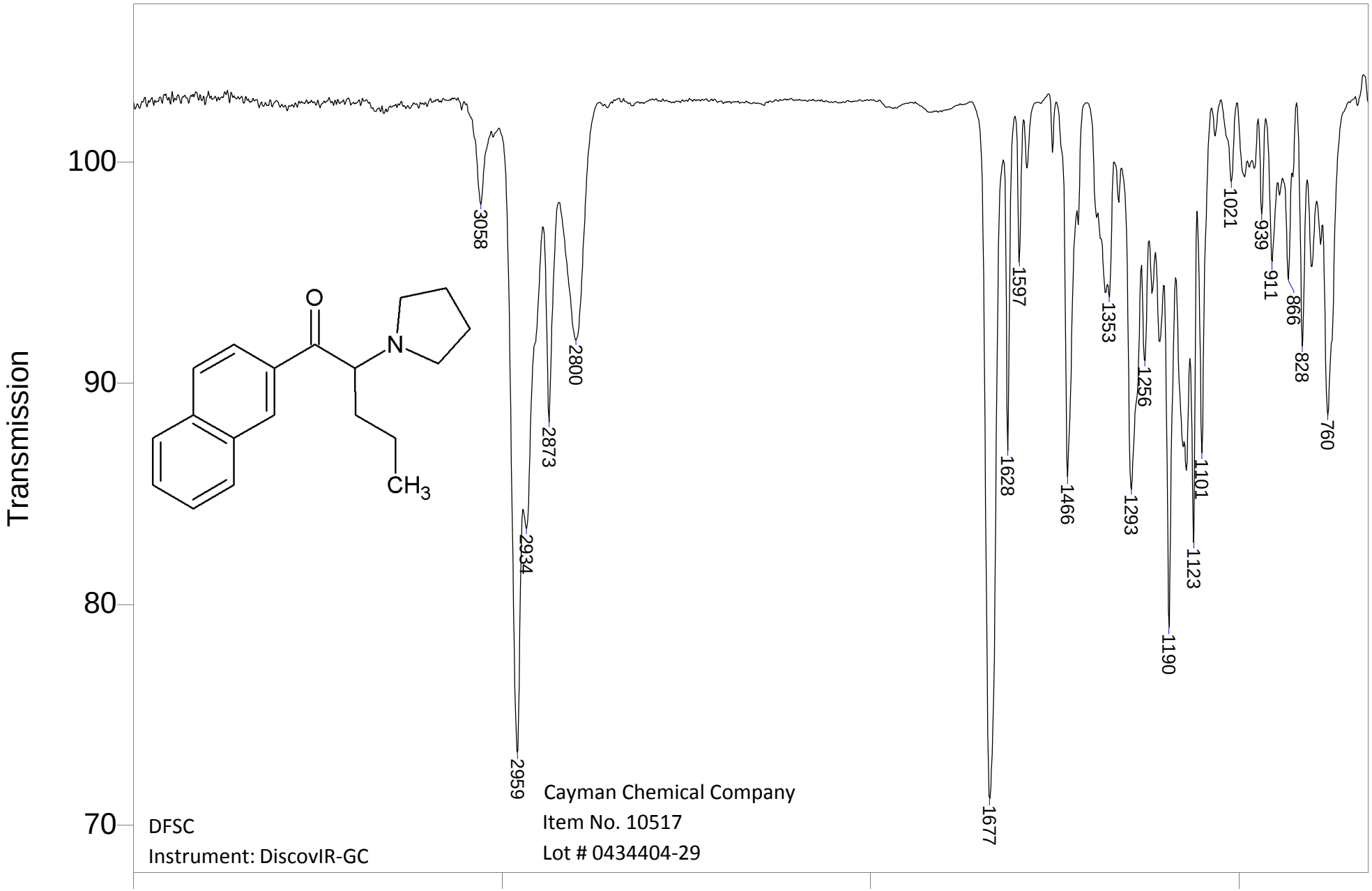


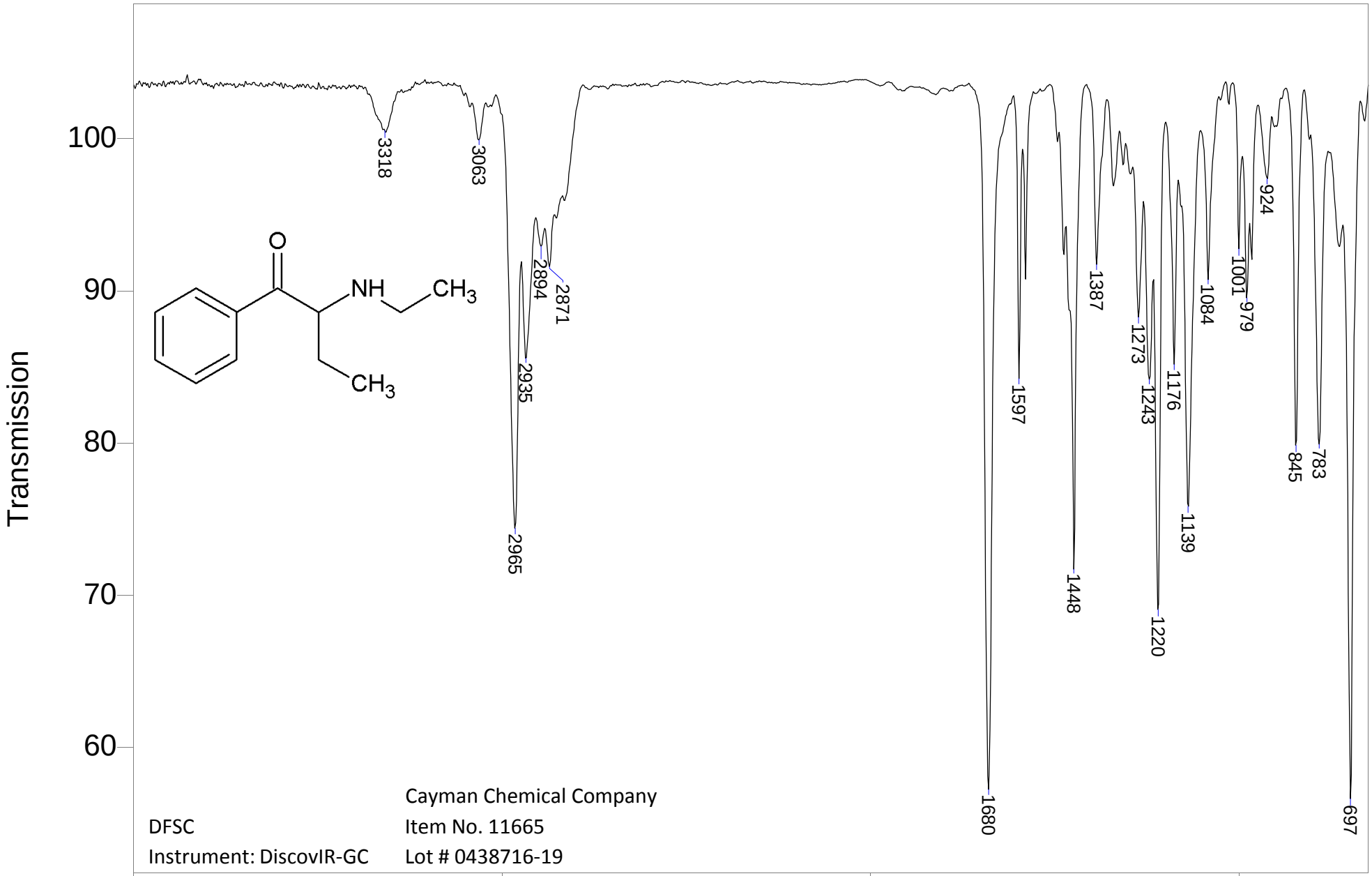


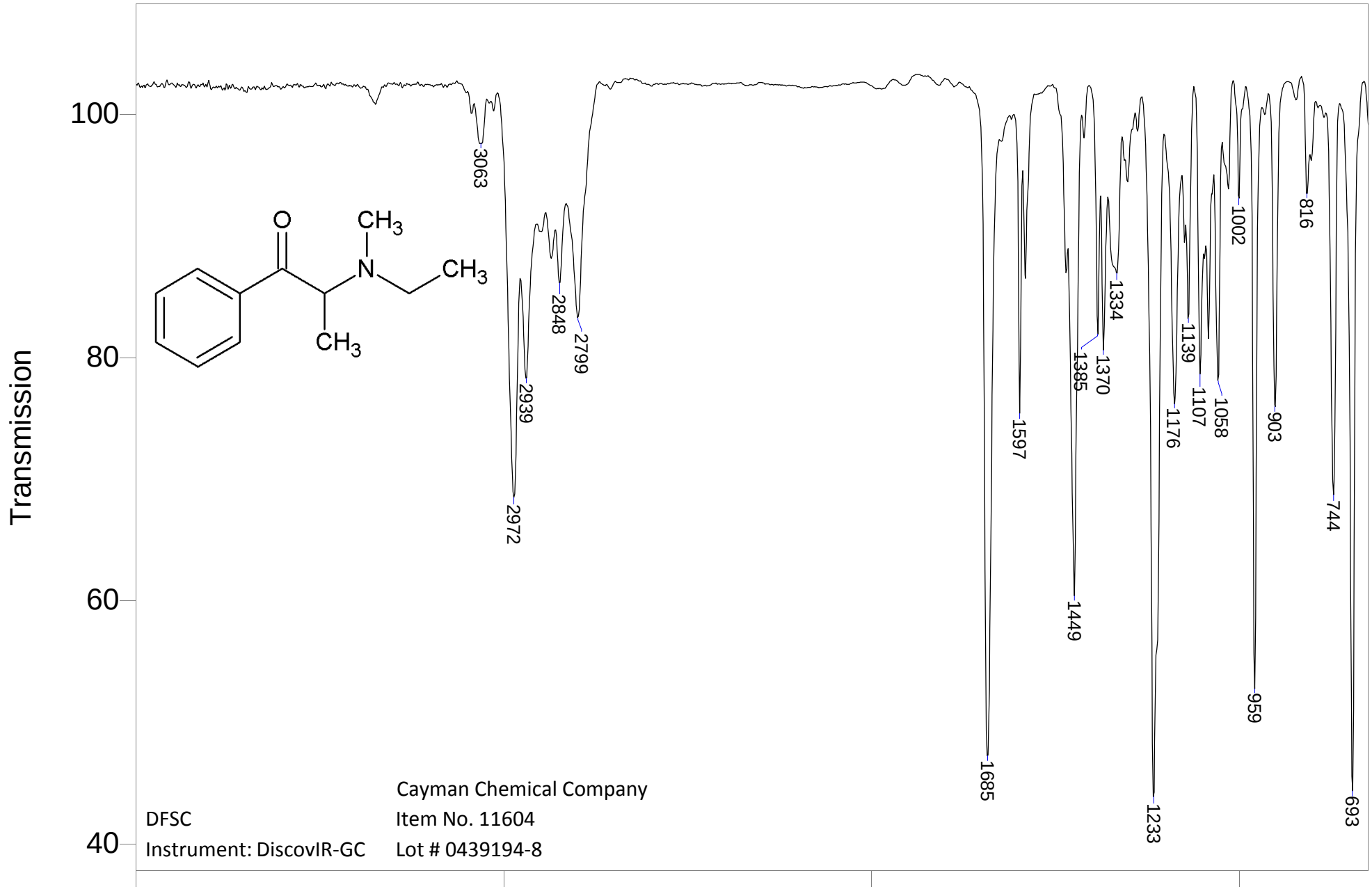


Naphyrone (1-naphthyl isomer)

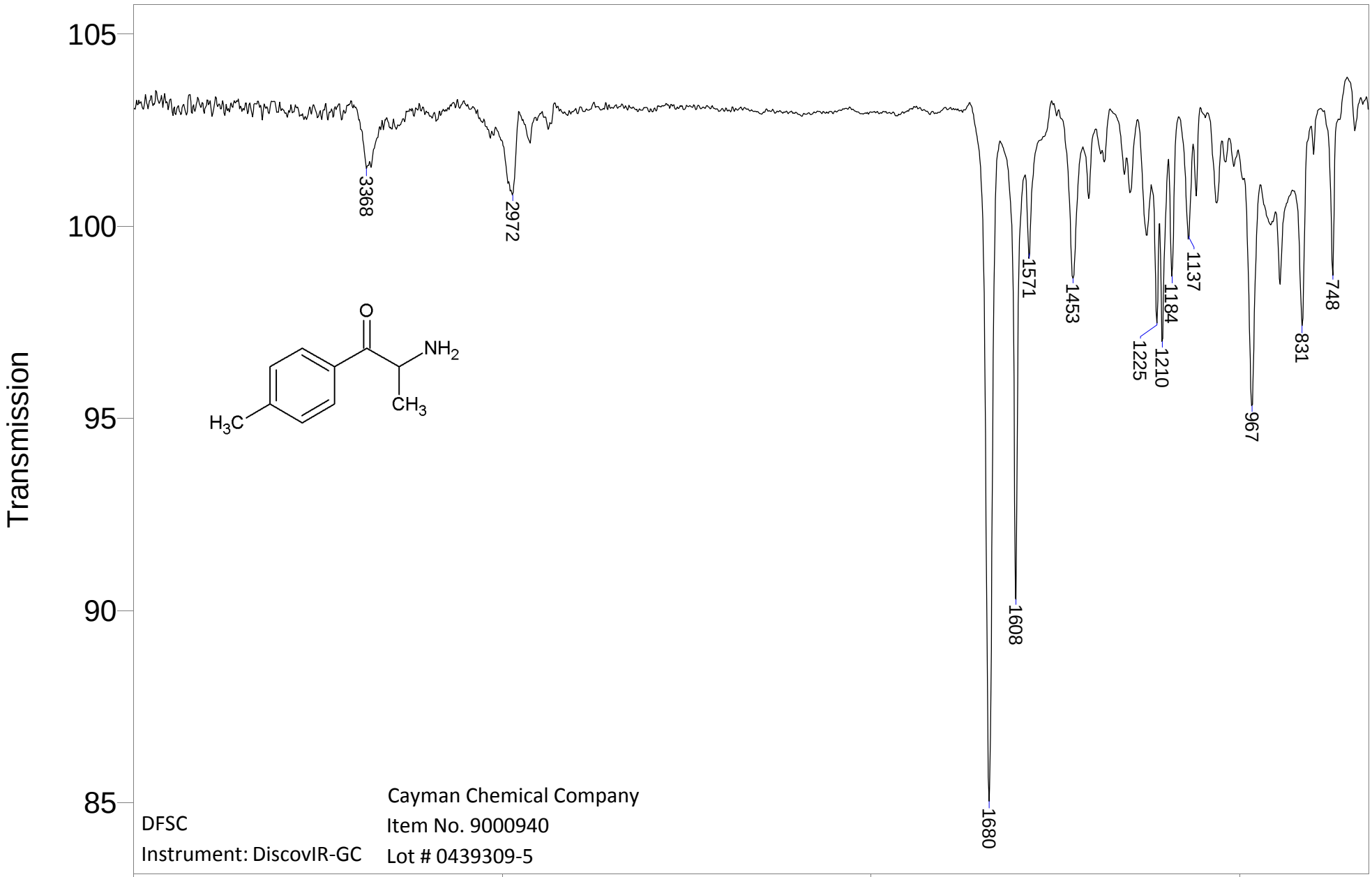


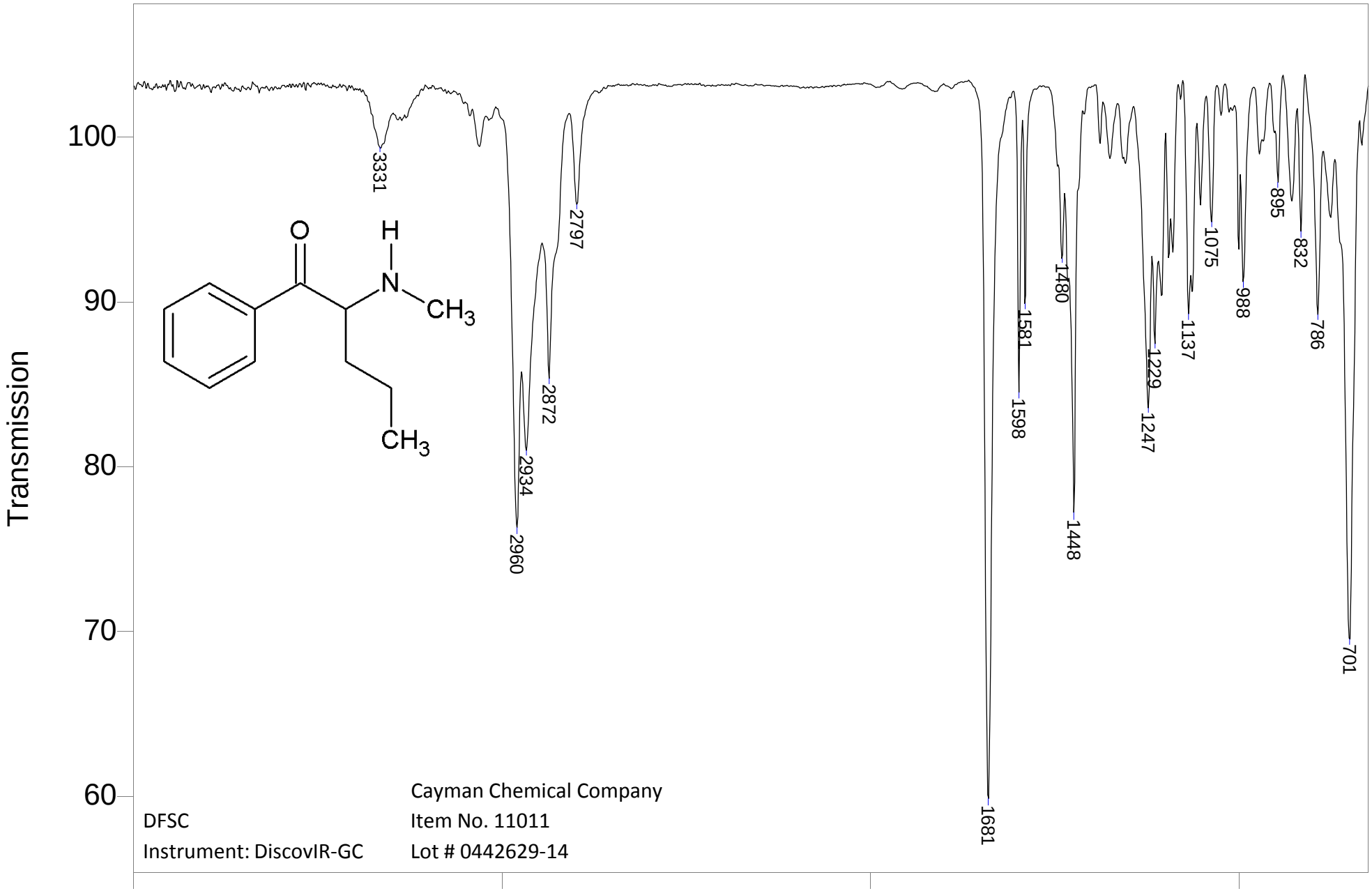


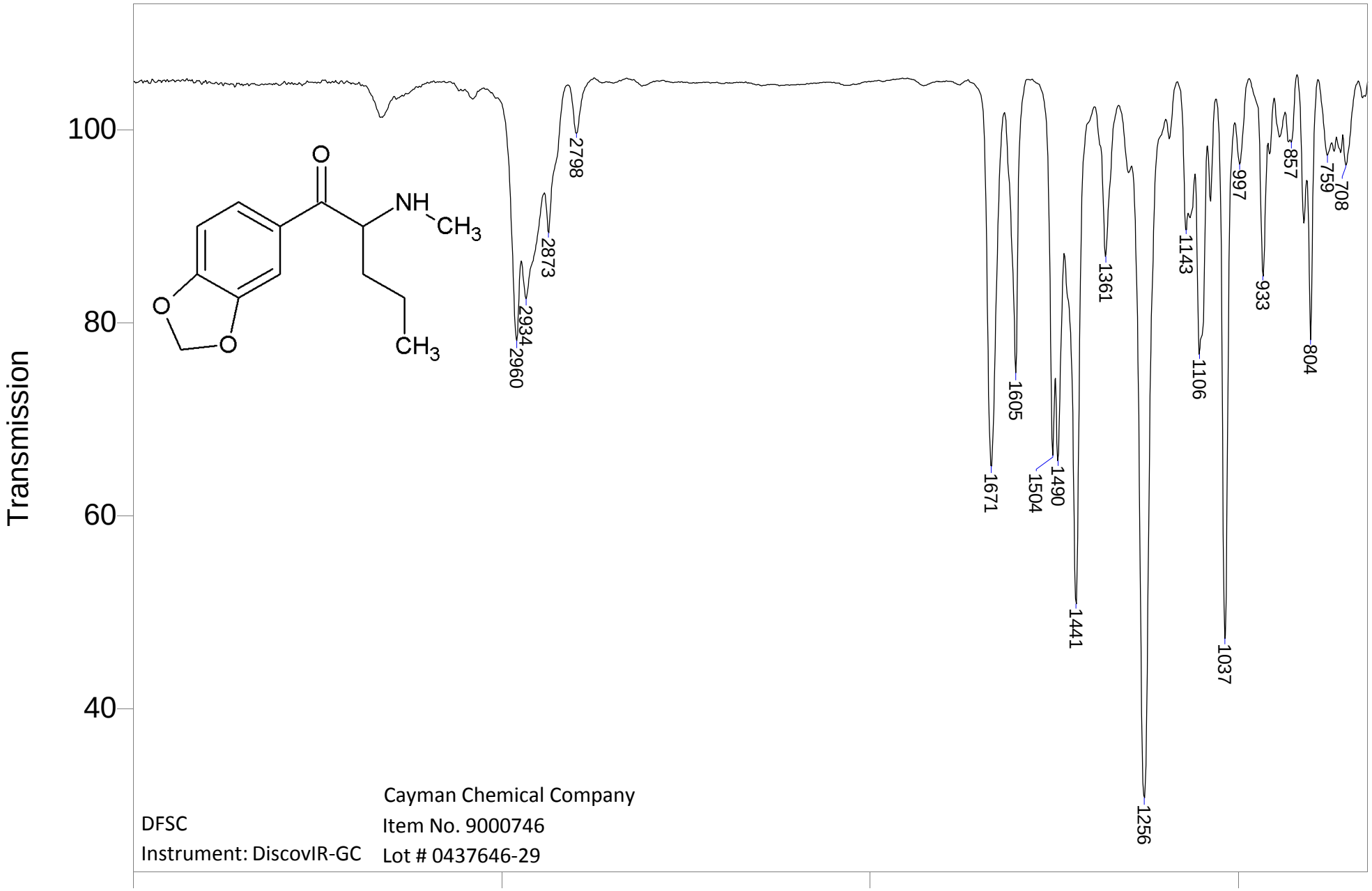


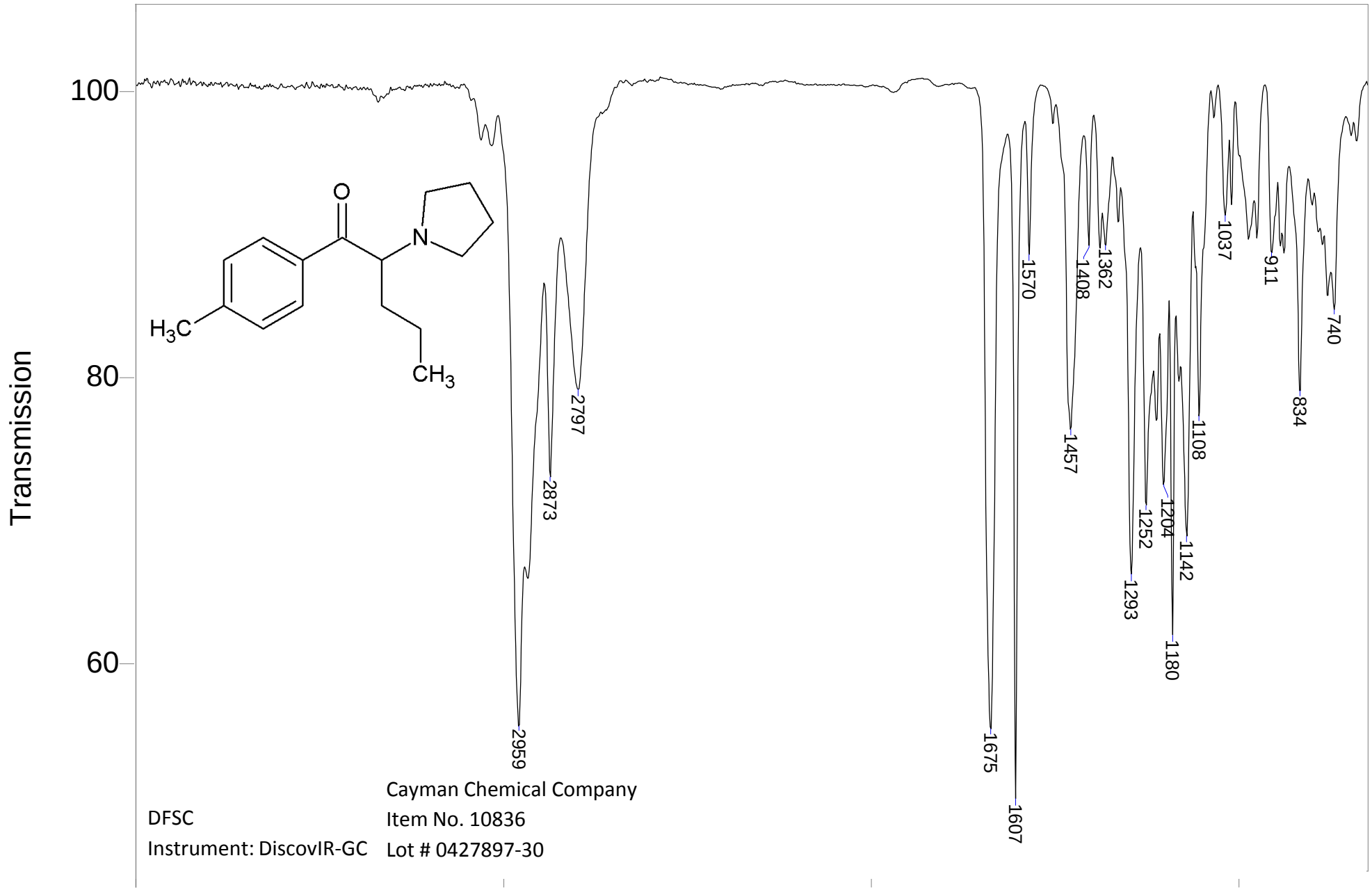


Nor-mephedrone









Appendix D: Inter-laboratory reproducibility: Spectra Comparison

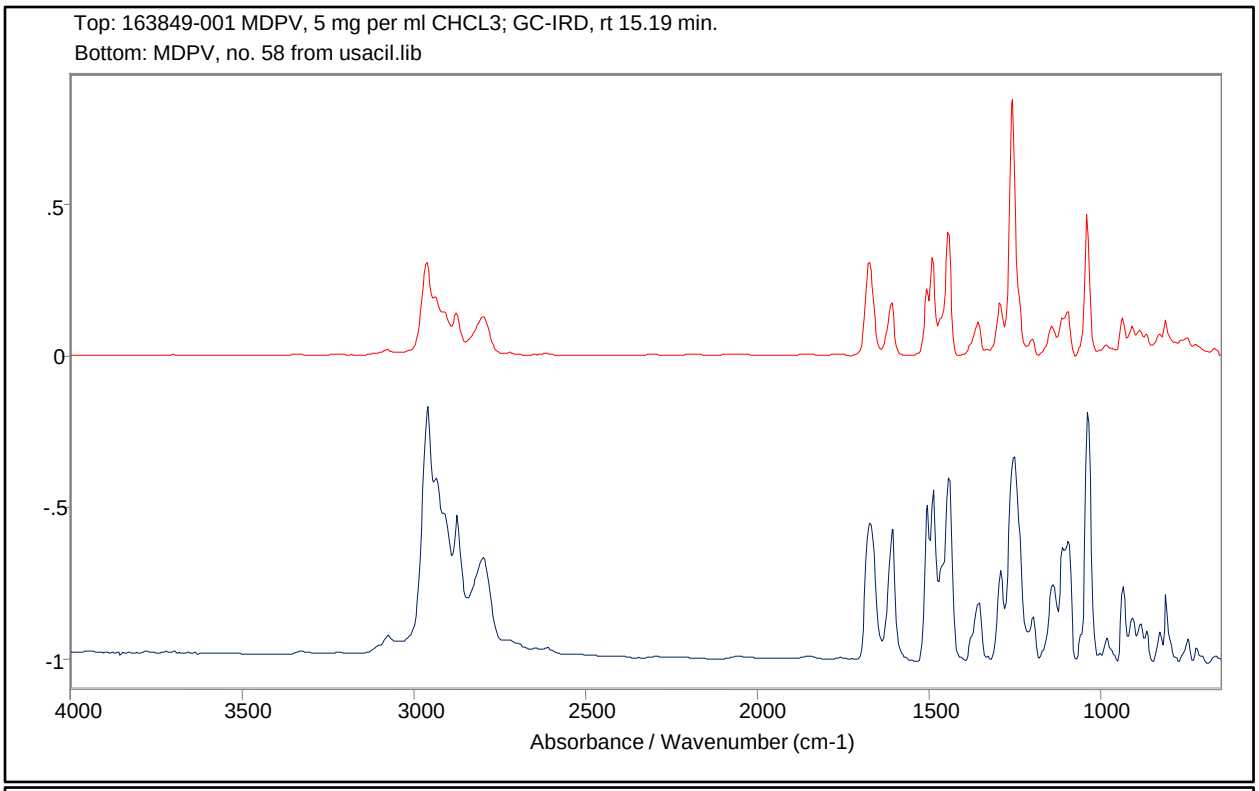
High purity standards of NPSs (n=84) were analyzed independently by the Defense Forensic Science Center (DFSC) and the Canada Border Services Agency (CBSA). Comparisons are shown below with the Canadian data (red) on top and the library match in from the DFSC library spectral data (blue) reported in Appendix B and C (usacil.lib or cathinones.lib respectively).

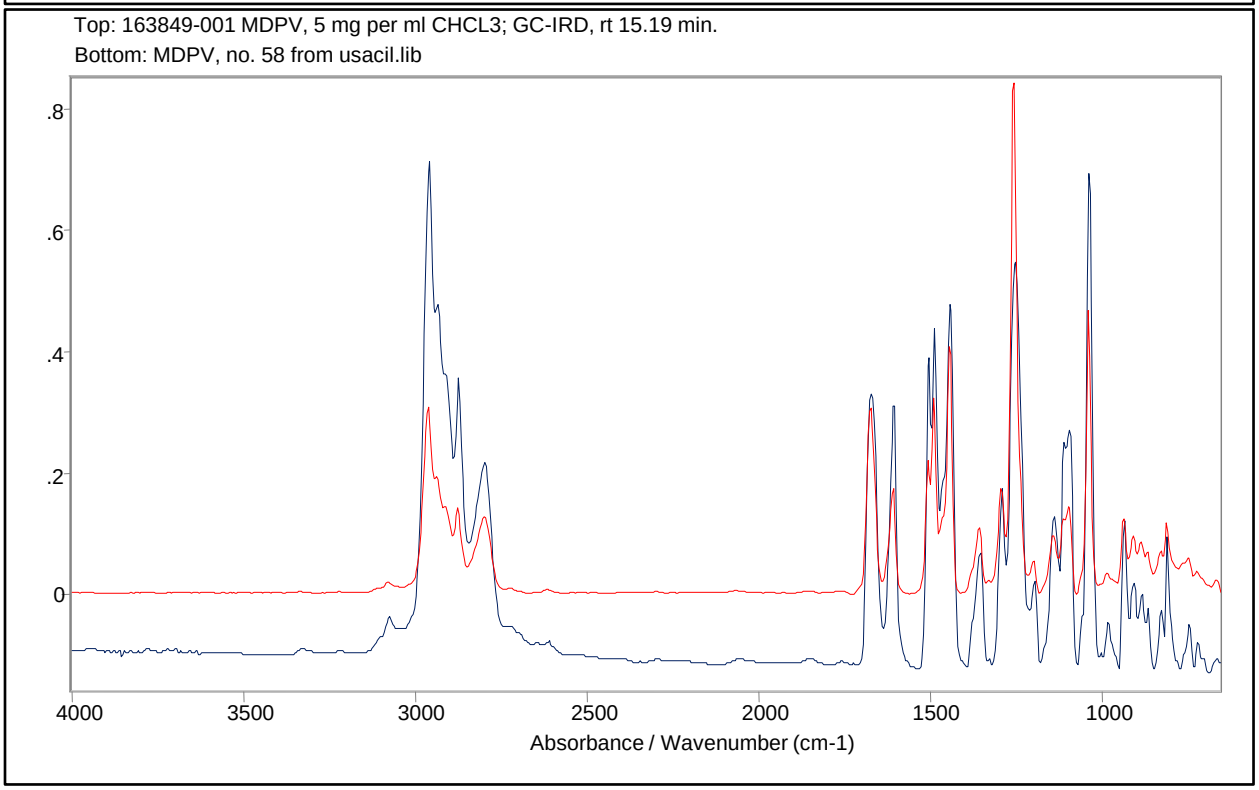
The CBSA ran at 8 cm^{-1} resolution, -40 °C on the IR disk (3mm/min rotation speed), a restrictor temperature of 300 °C, an oven temperature of 300 °C, and a transfer line temperature of 300 °C (Appendix Table D-1). One µL of sample was injected at a 10:1 split ratio. The GC inlet was heated to 250 °C while the oven was ramped from 100 °C to 300 °C over 20 minutes and held there for five minutes before re-equilibrating the oven over a half minute to prepare for the next sample. The column experience a constant flow of helium (1ml/min).

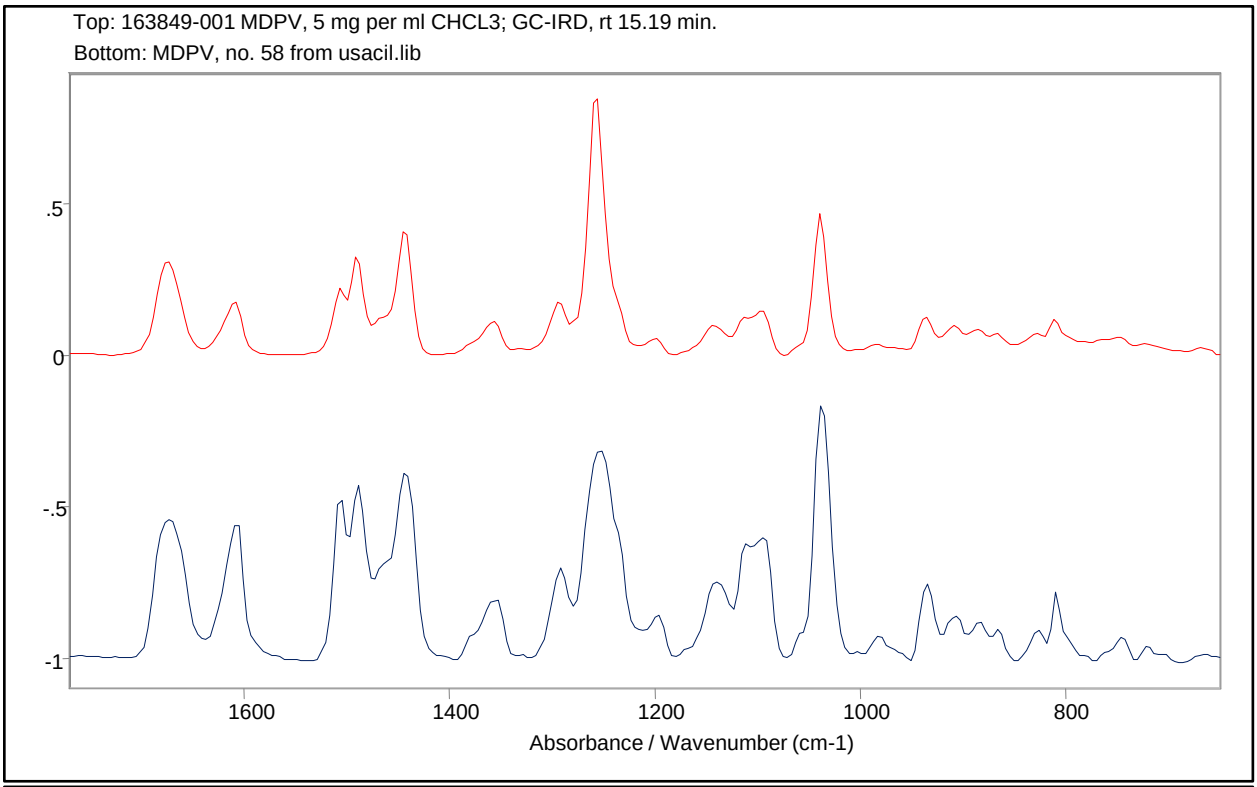
Generally the data generated by the two laboratories compare very well to each other. There were some differences in the relative intensities of the absorption peaks in some spectra (ex. MDPV and 1-(4-fluorophenyl)piperazine). However, despite the different relative absorption intensities, both spectra contained all peaks of significance at comparable wavenumbers and therefore should not lead to any chemical misidentification. The different relative absorptions may have resulted from an effect of the sample concentration differences.

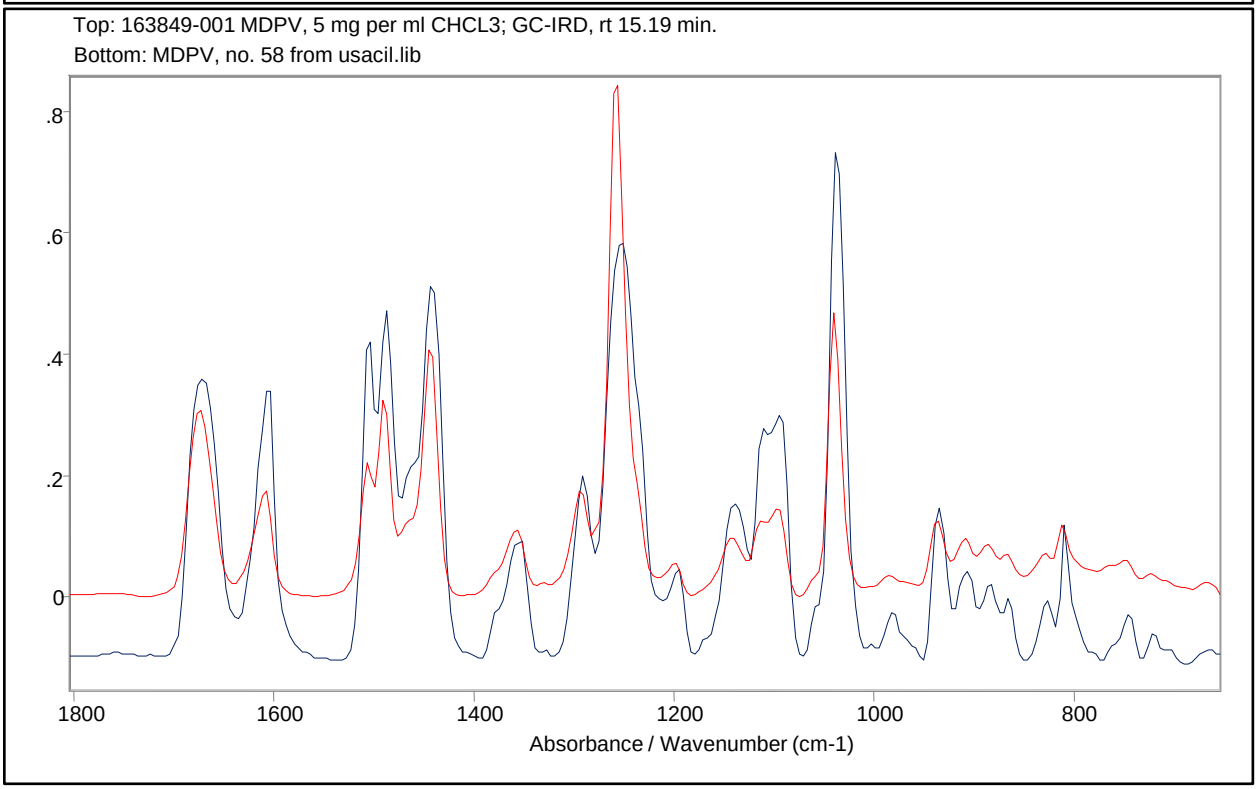
Appendix Table D-1: Method parameters for reproducibility comparison between CBSA and DFSC.

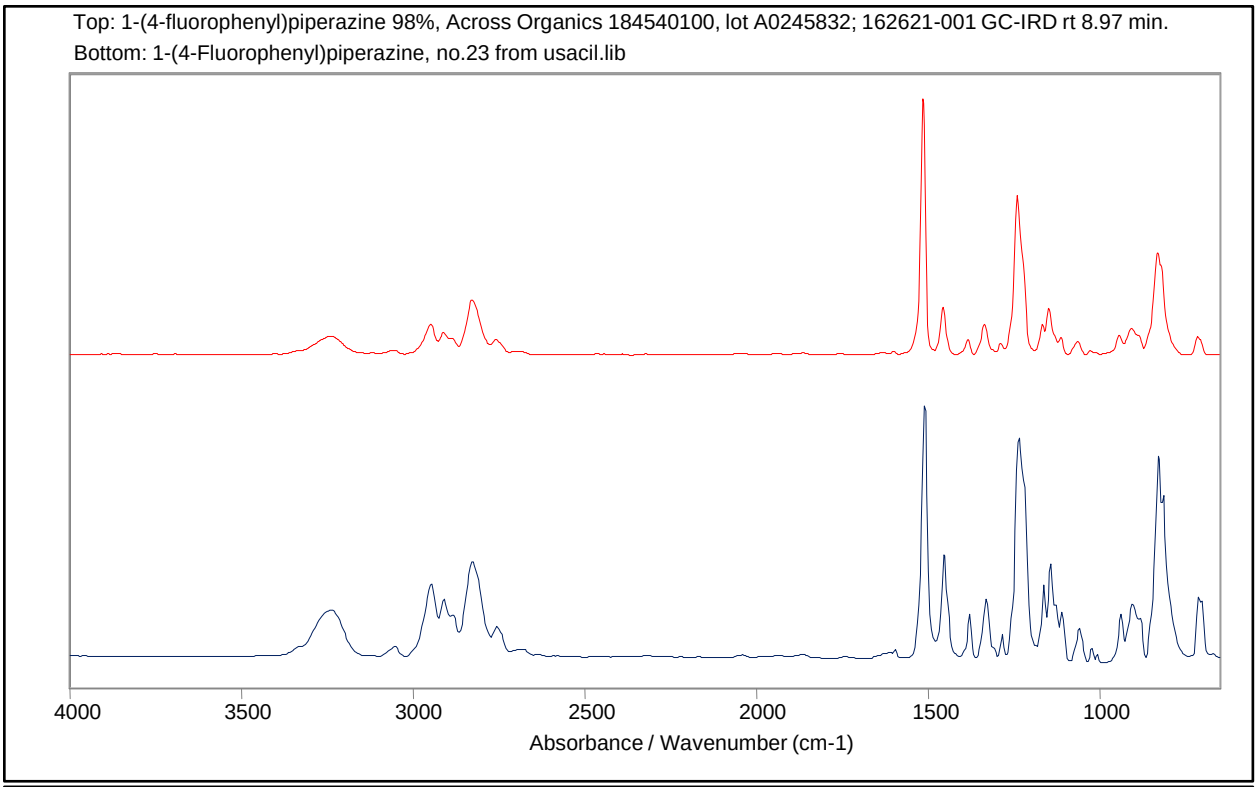
		CBSA	DFSC (GCIR1 method)
Infrared Detector	Spectral Resolution (cm^{-1})	8	4
	Disk (°C)	-40	-40
	Restrictor (°C)	300	250
	Transfer Line (°C)		250
	Disk Speed (mm/min)	3	3
Gas Chromatograph	Inlet (°C)	250	250
	Split ratio	10:1	2:1
	Oven (°C)	100	120
	Oven Program, Temperature Gradient	10 °C/min to 300 °C, hold 5 min	25 °C/min to 290 °C, hold 1 min
	Column Flow (ml/min)	1	2

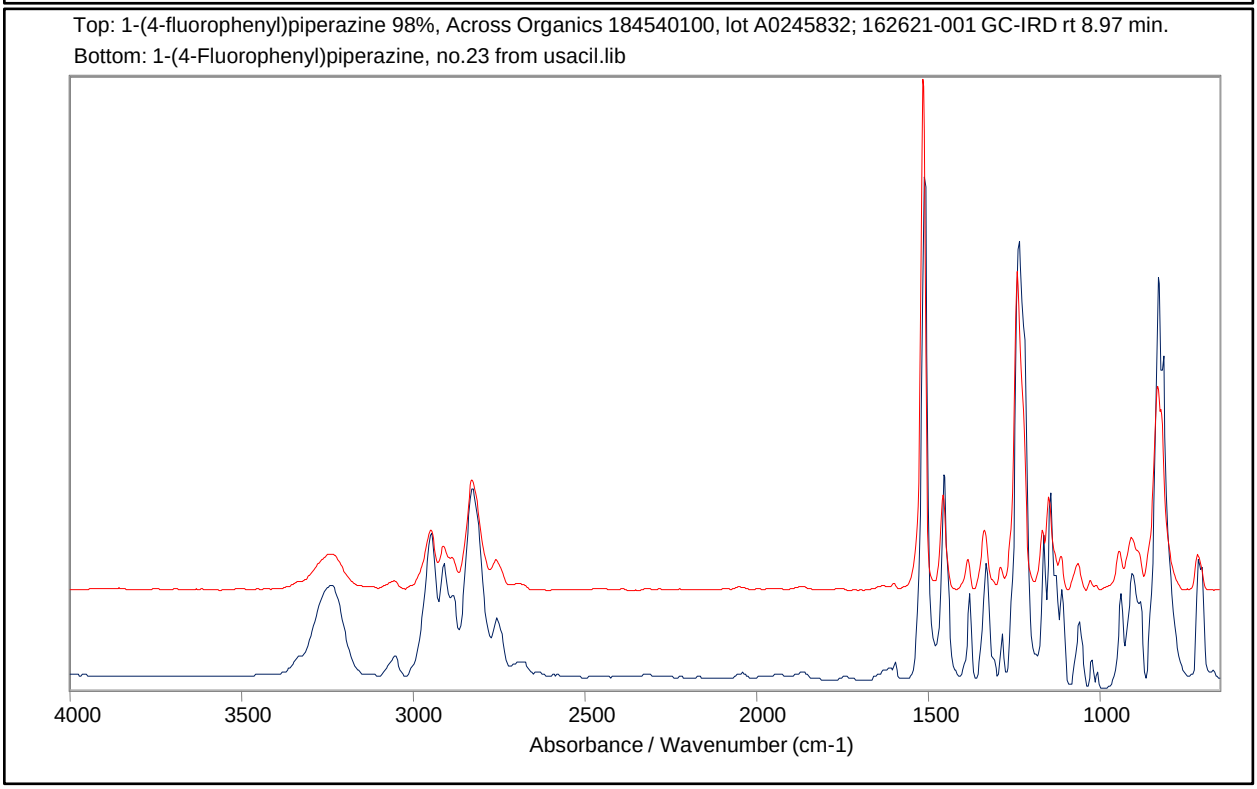




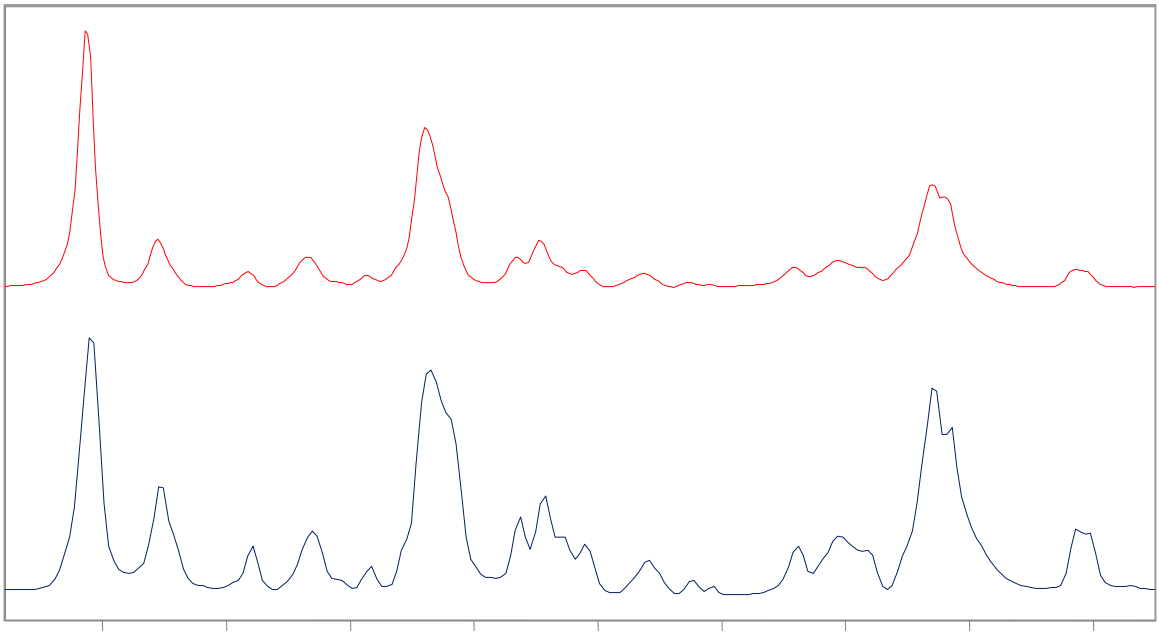




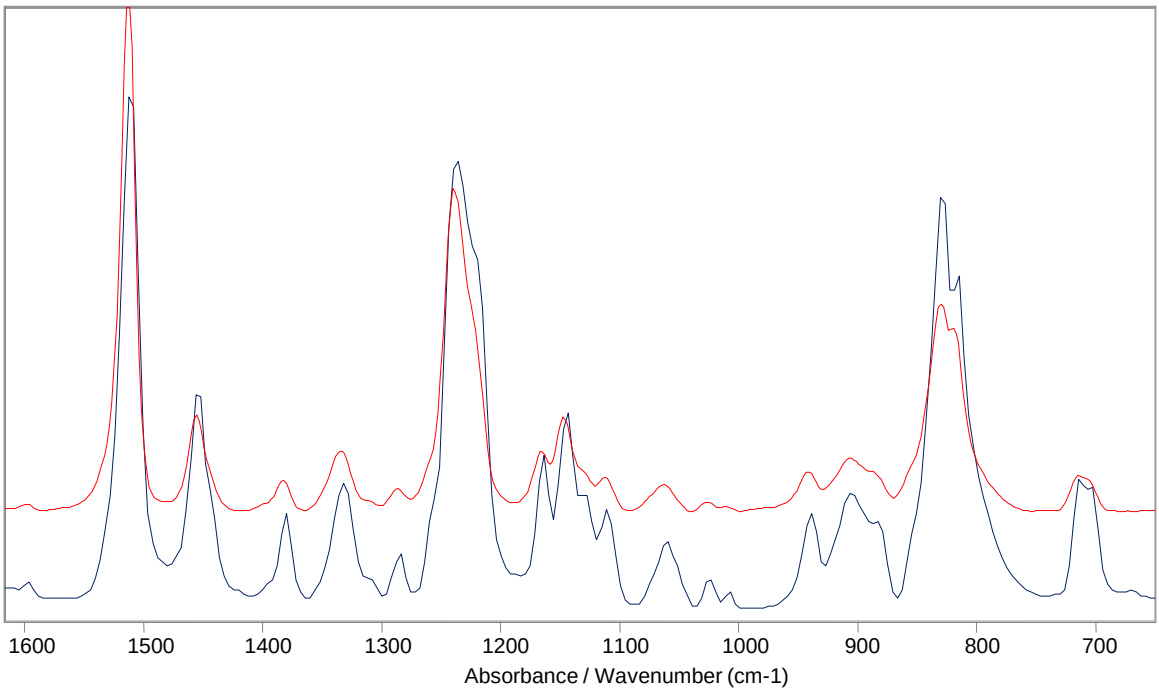


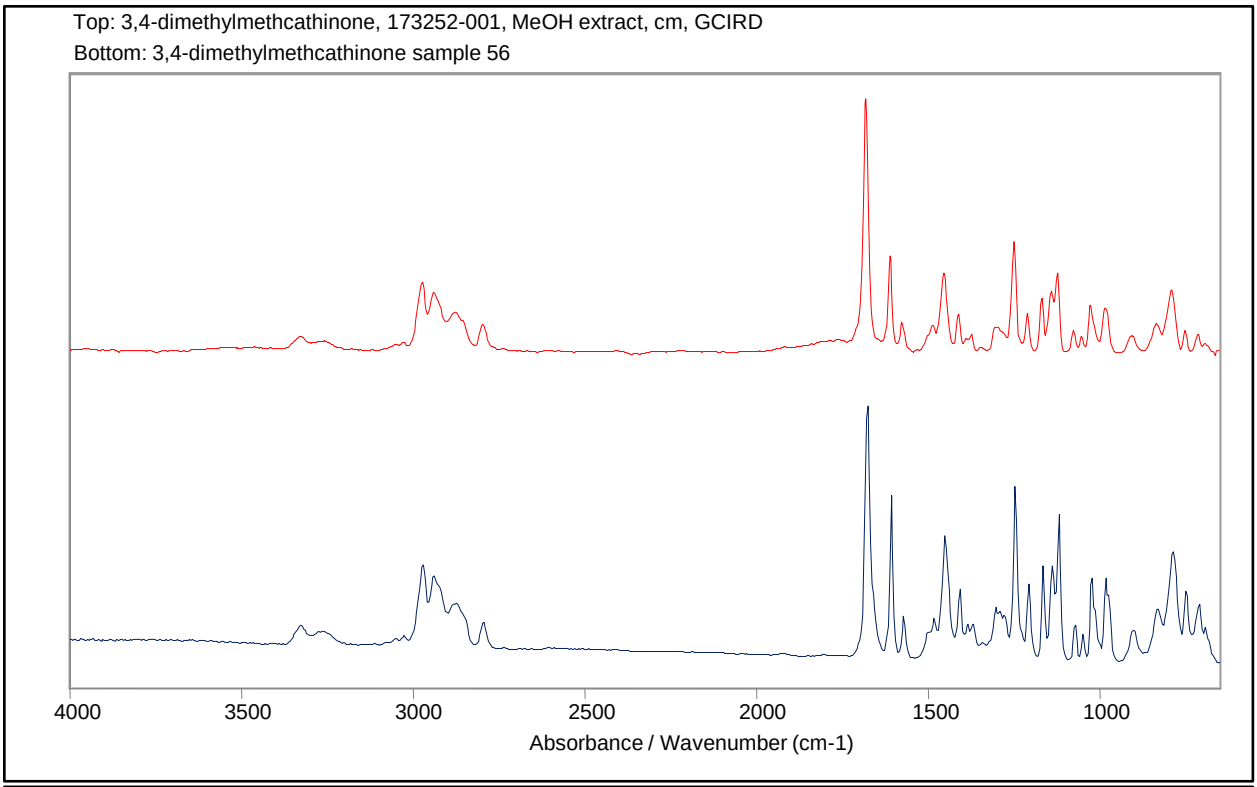


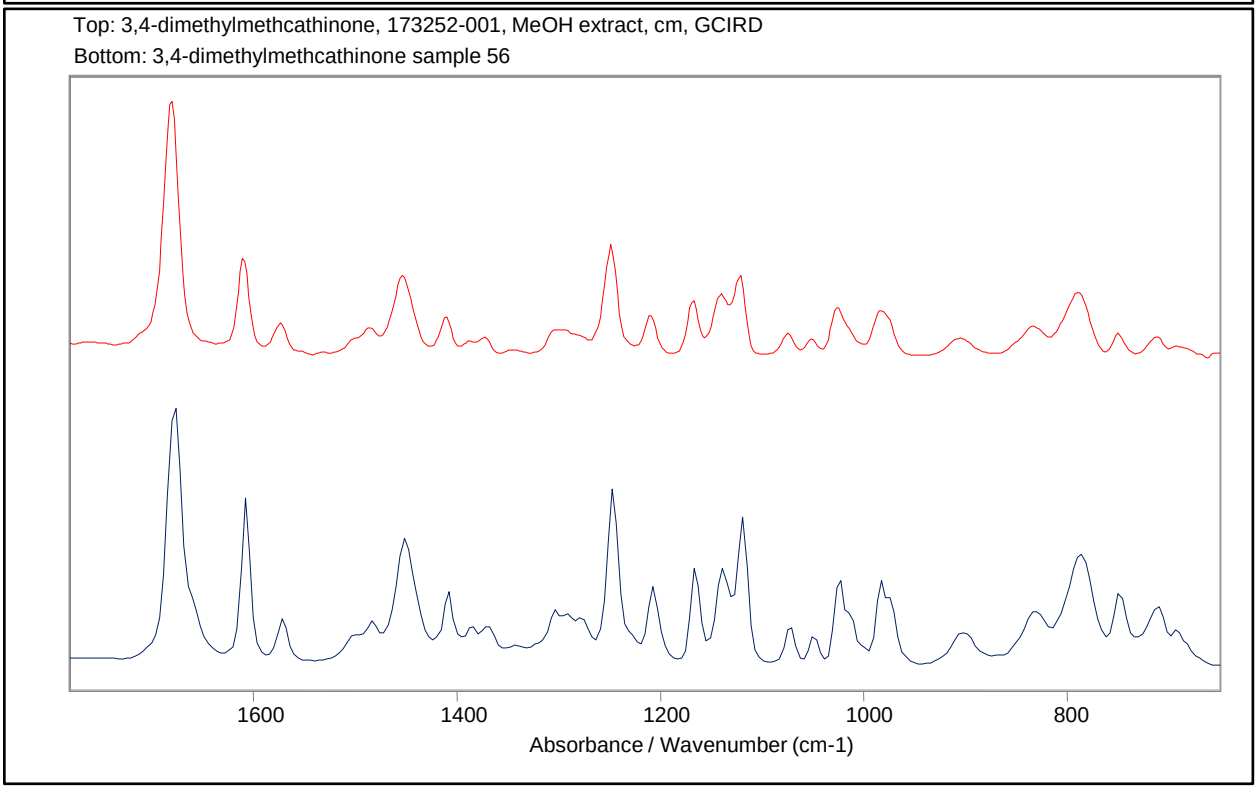
Top: 1-(4-fluorophenyl)piperazine 98%, Across Organics 184540100, lot A0245832; 162621-001 GC-IRD rt 8.97 min.
Bottom: 1-(4-Fluorophenyl)piperazine, no.23 from usacil.lib

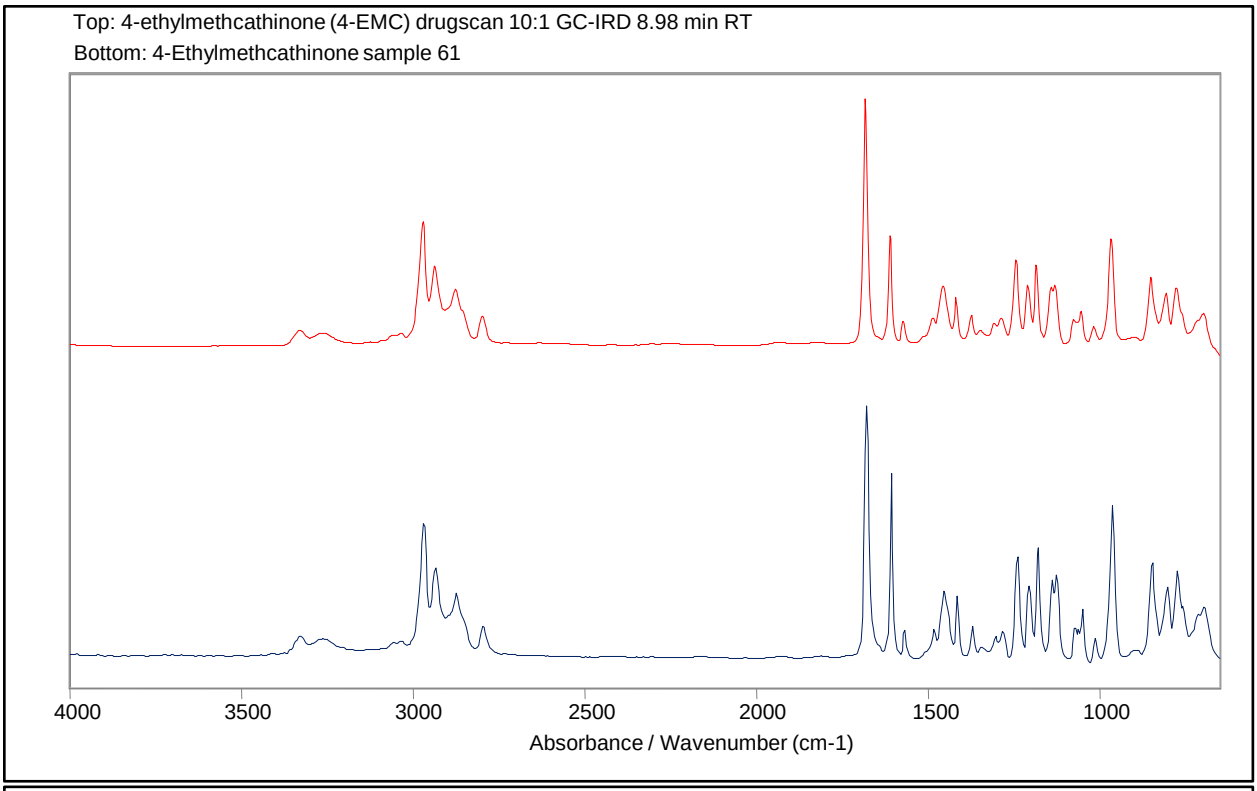


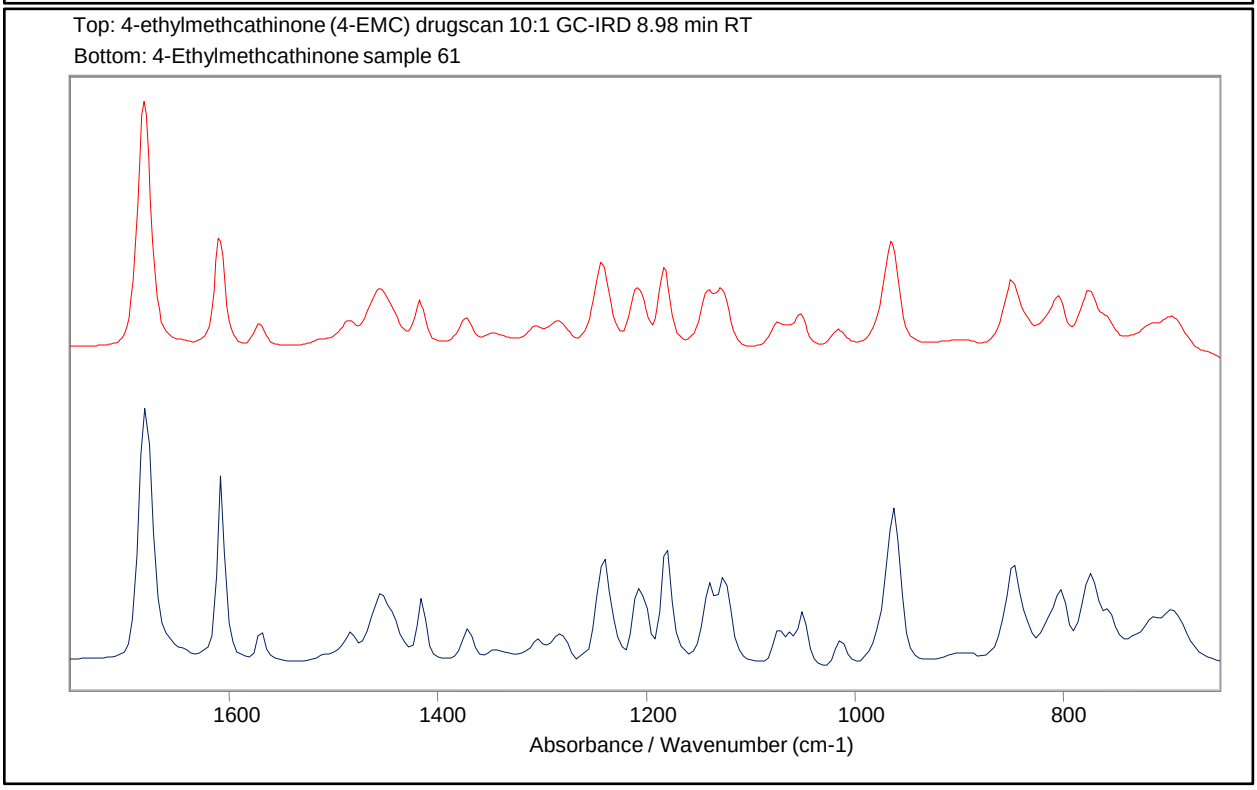
Top: 1-(4-fluorophenyl)piperazine 98%, Across Organics 184540100, lot A0245832; 162621-001 GC-IRD rt 8.97 min.
Bottom: 1-(4-Fluorophenyl)piperazine, no.23 from usacil.lib

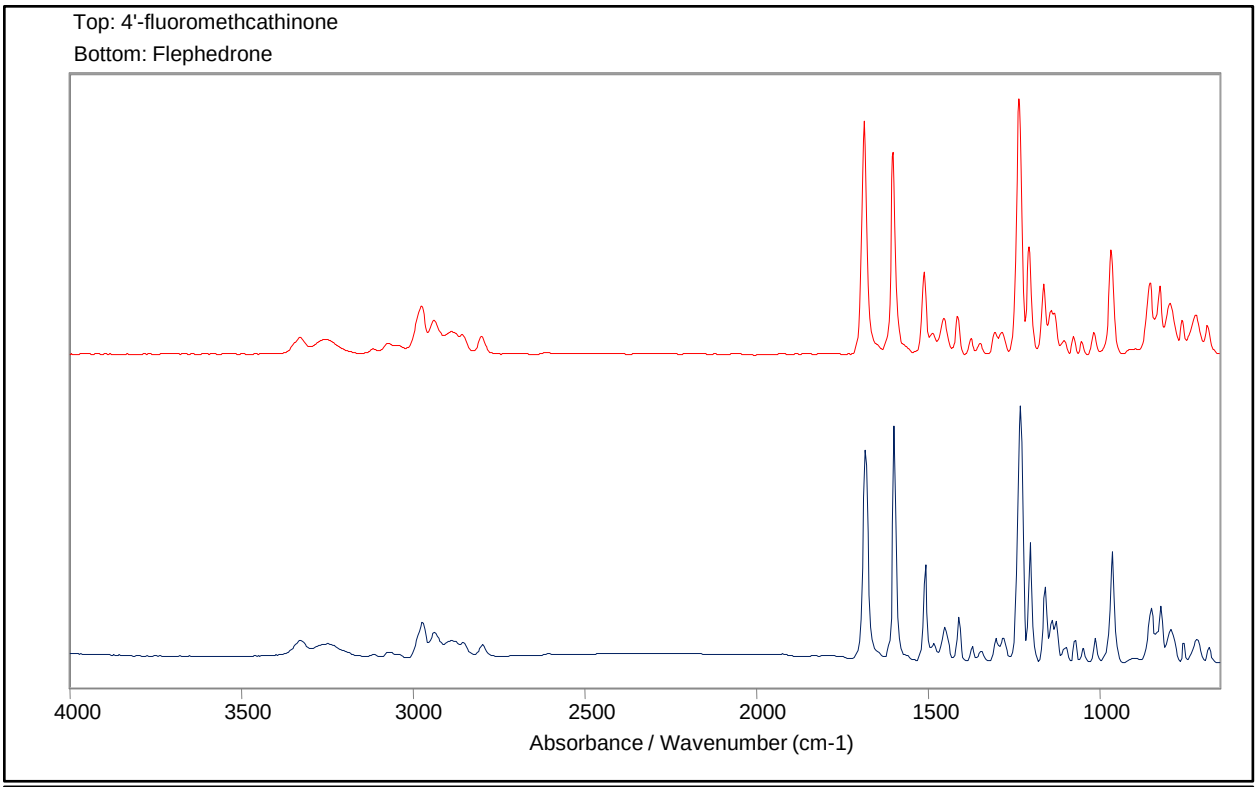


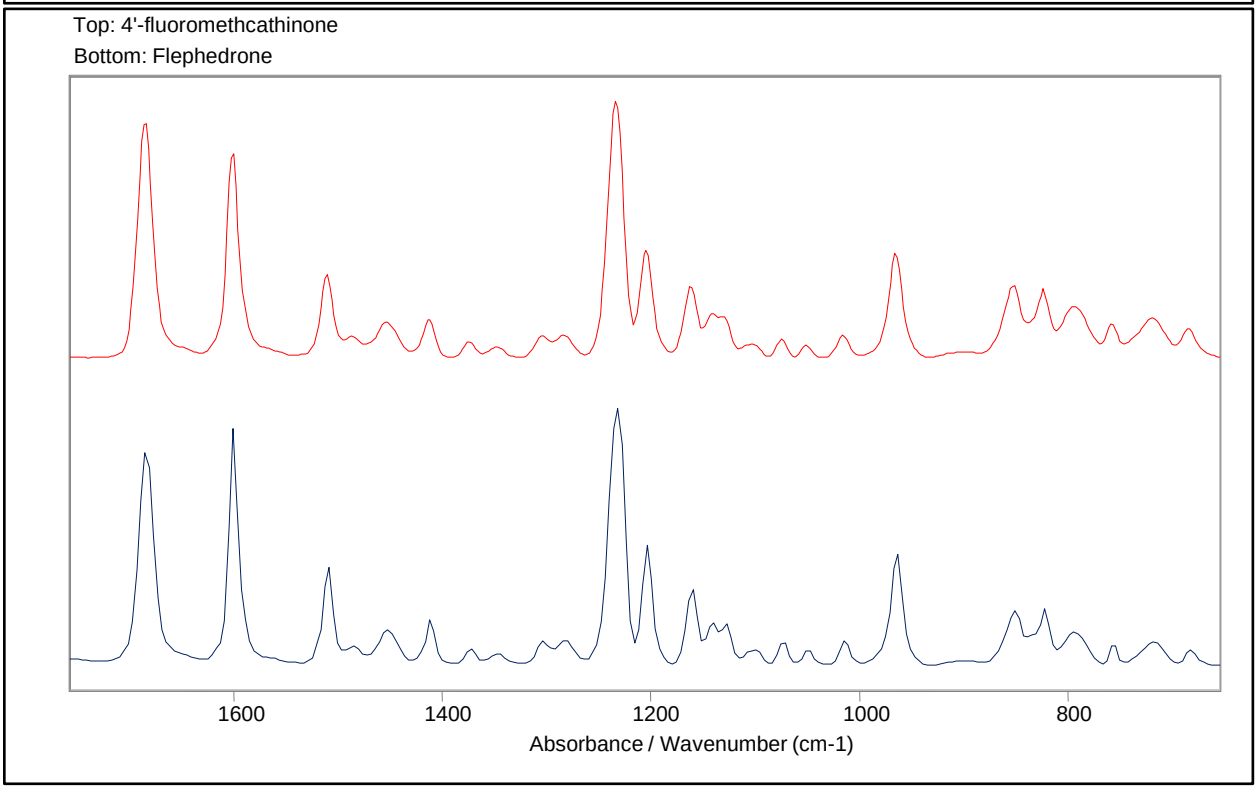


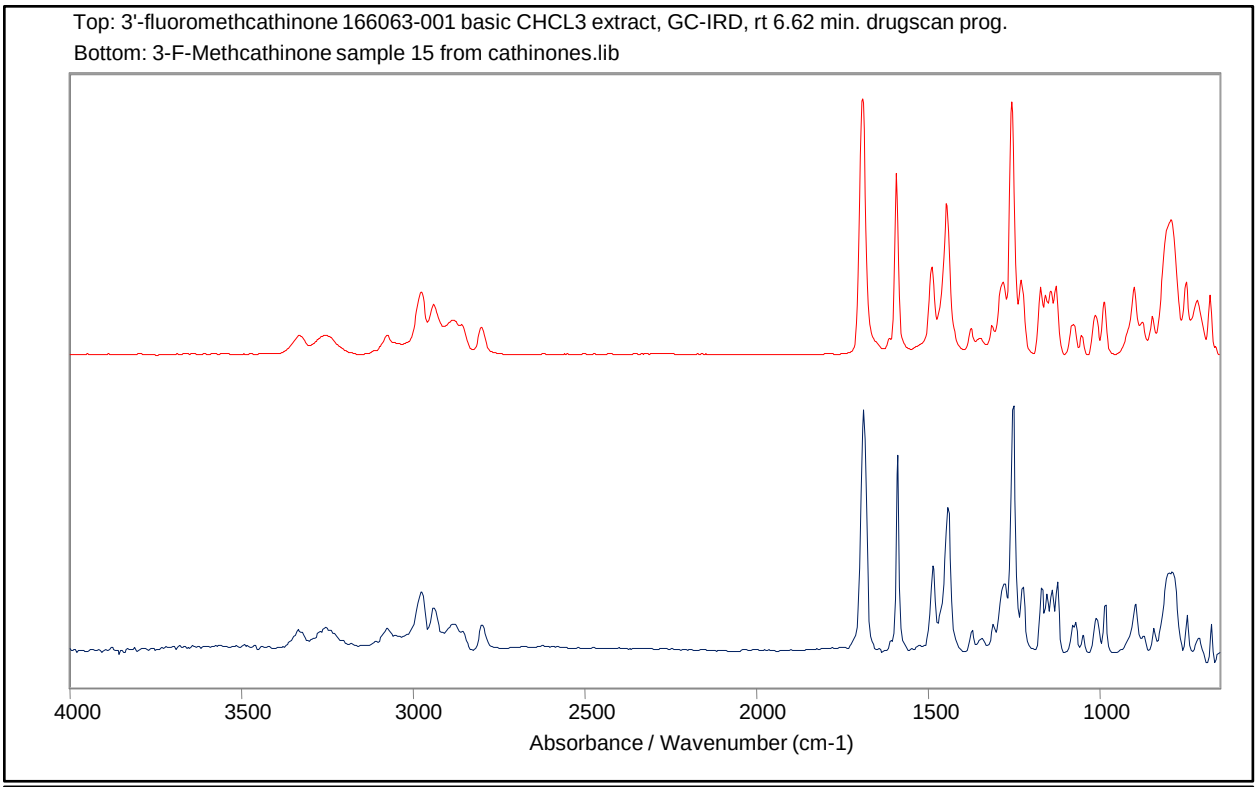


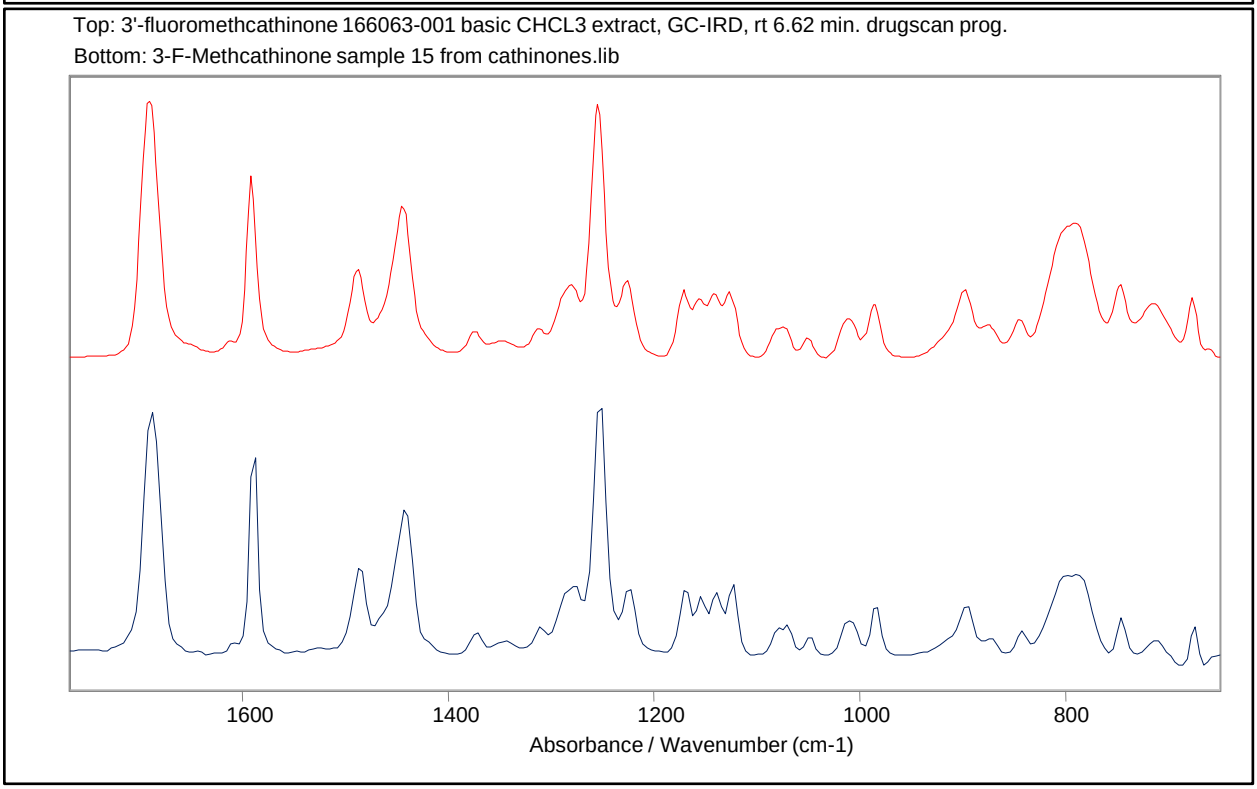


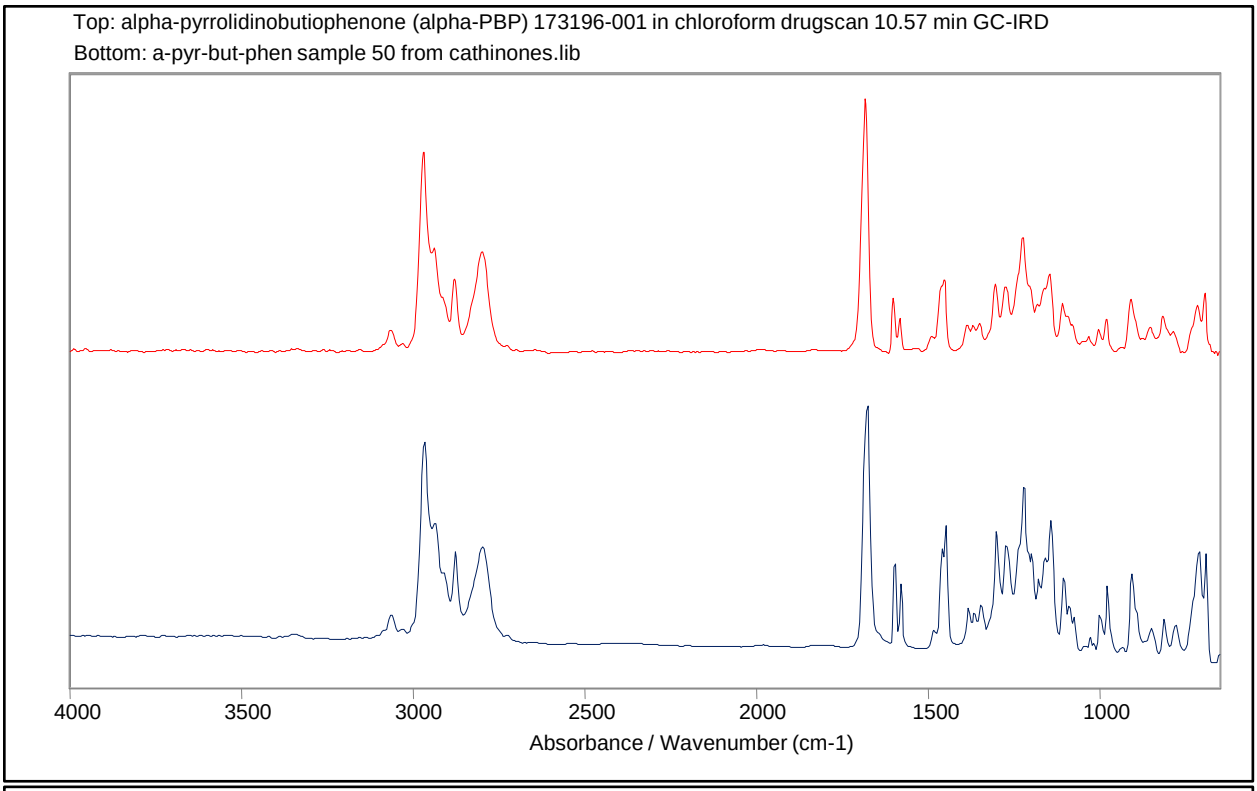


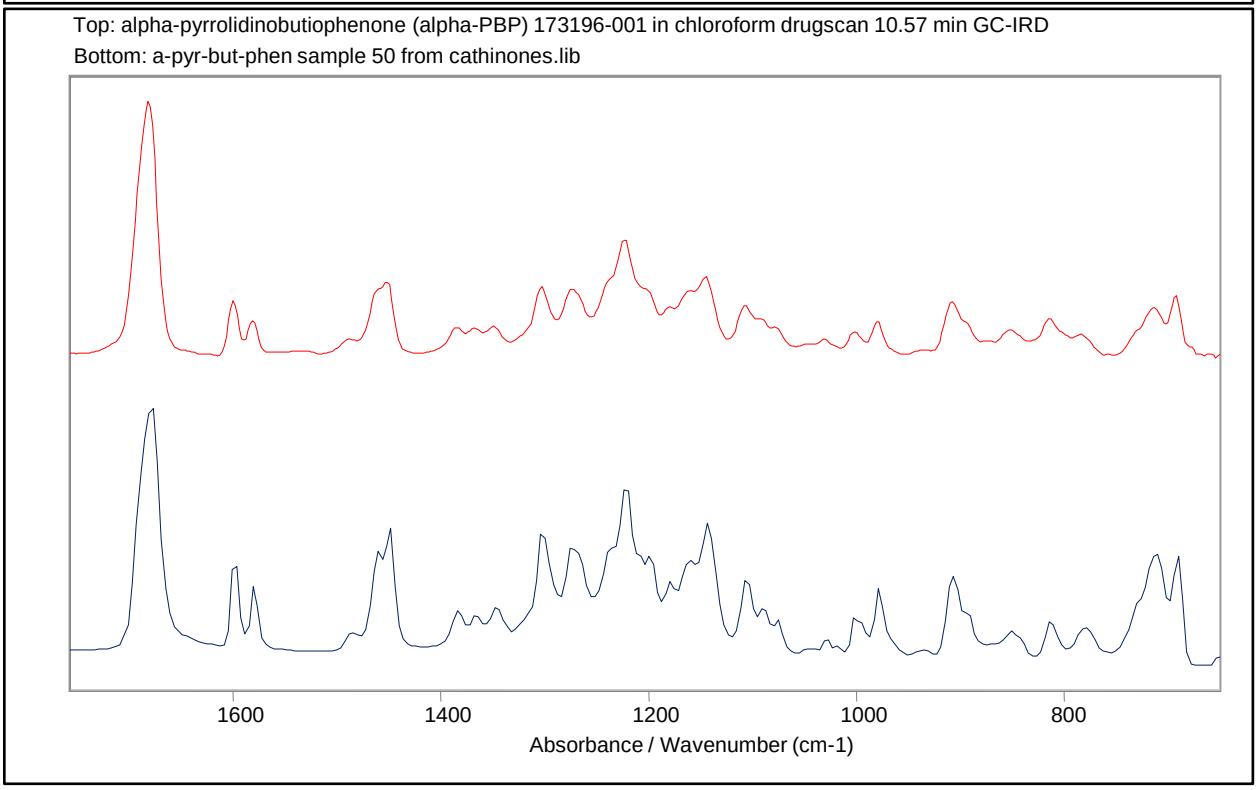


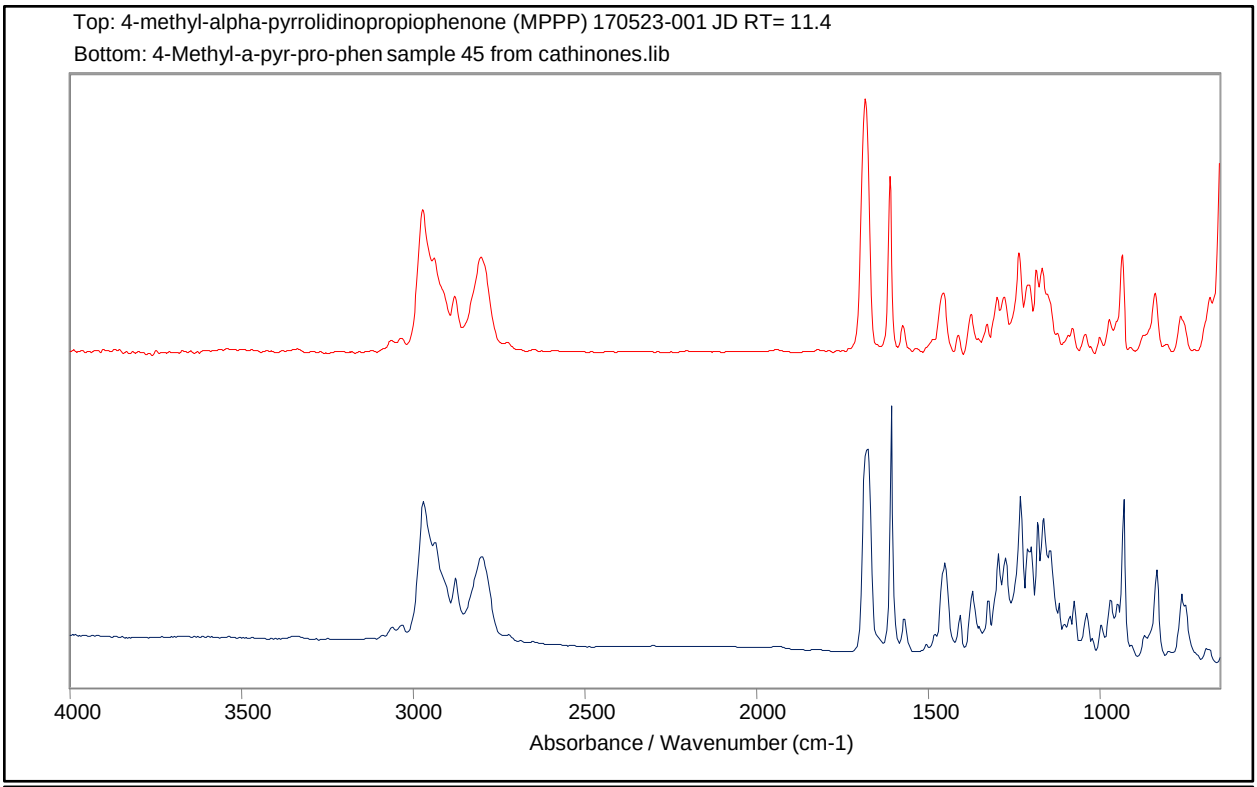


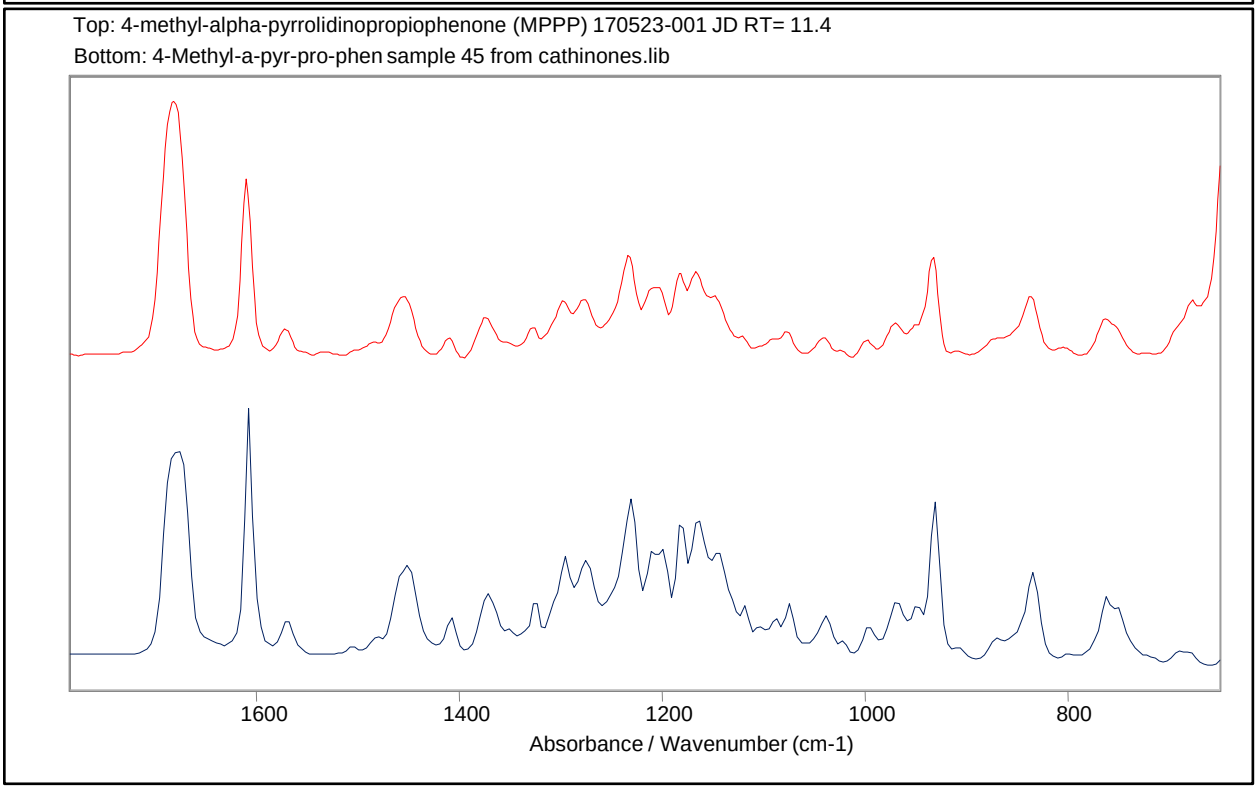


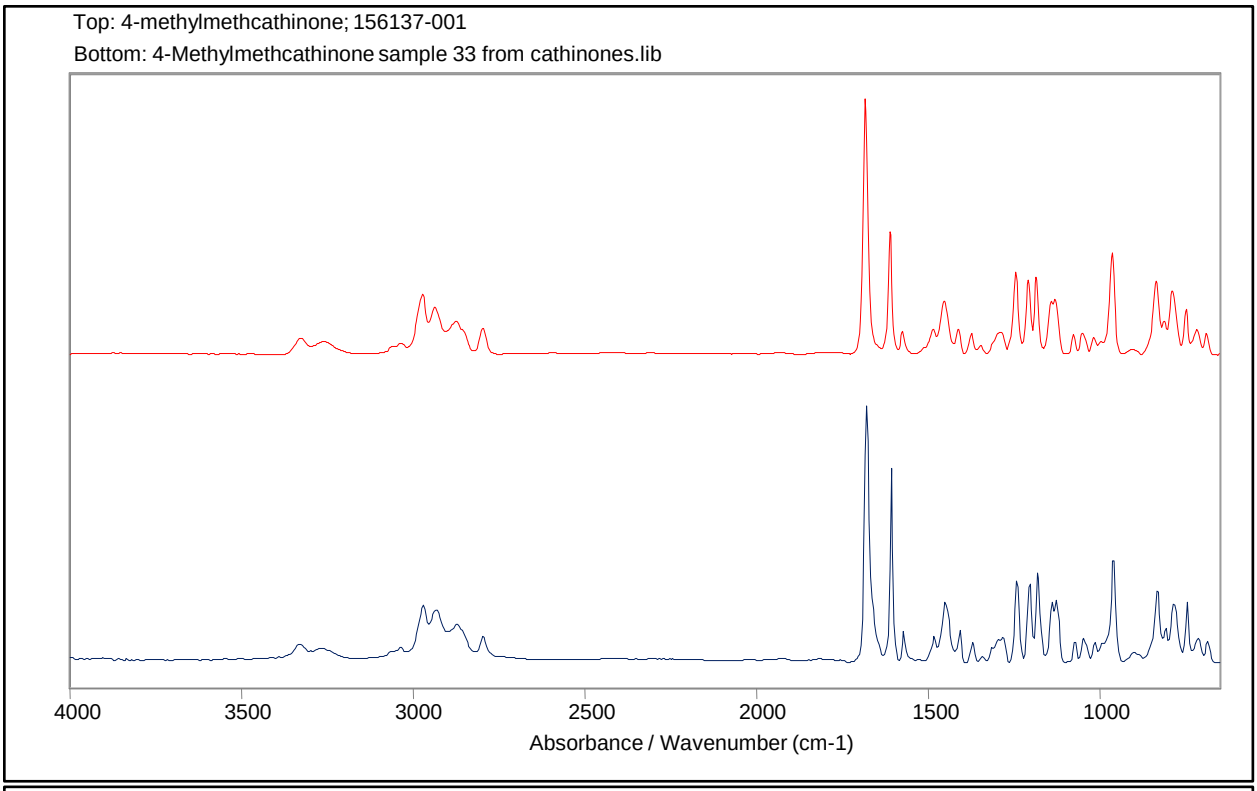


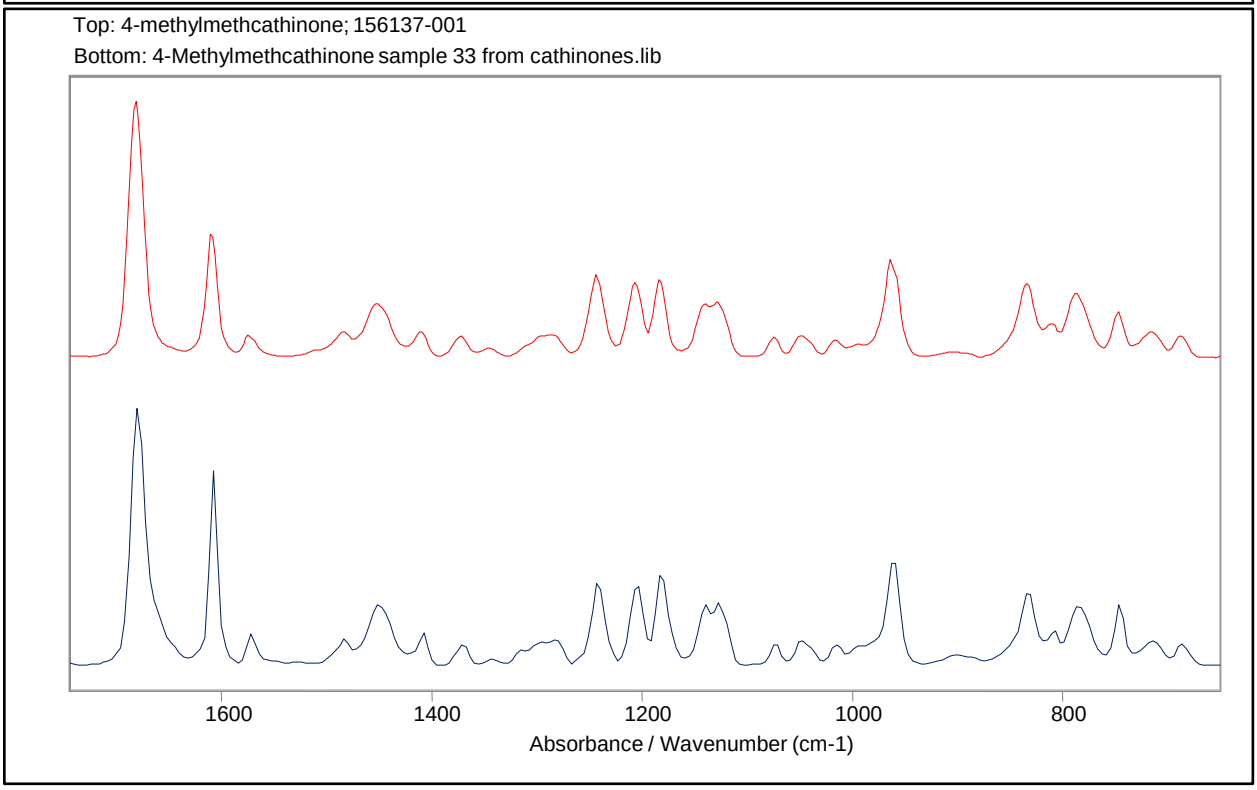


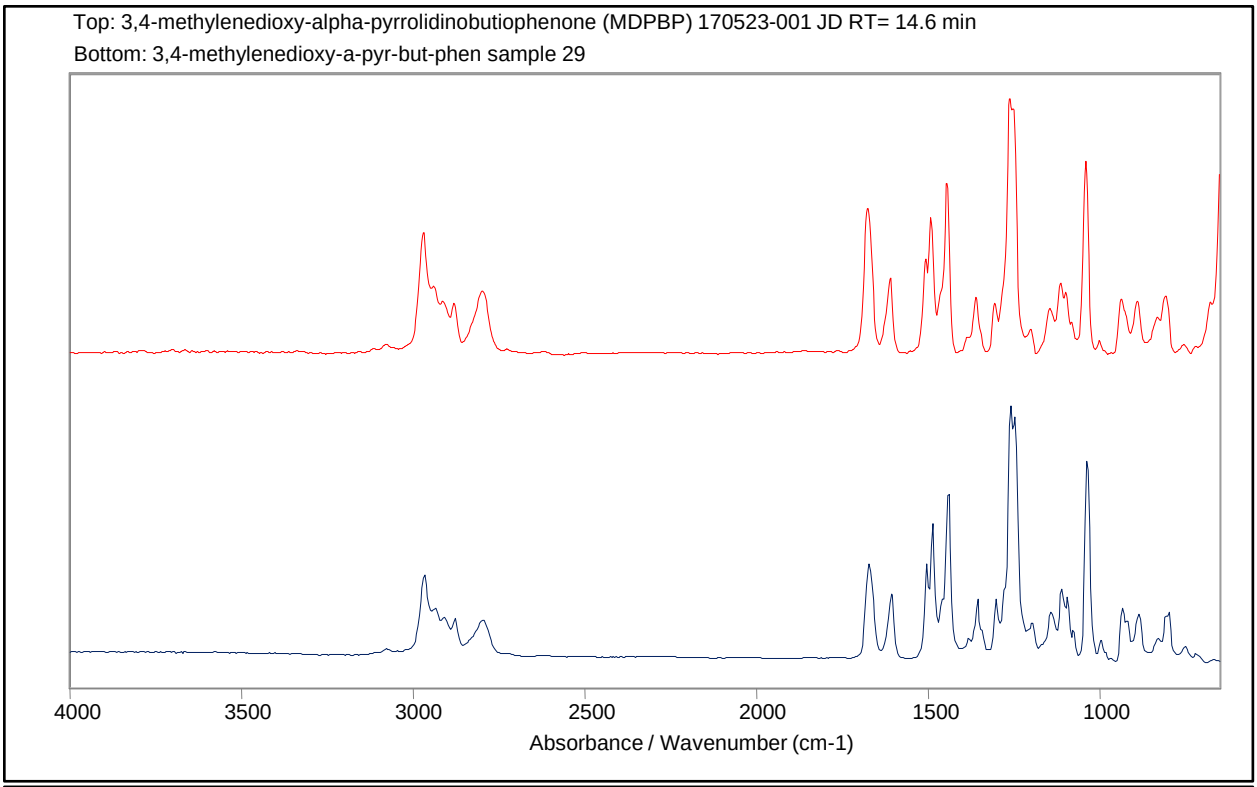


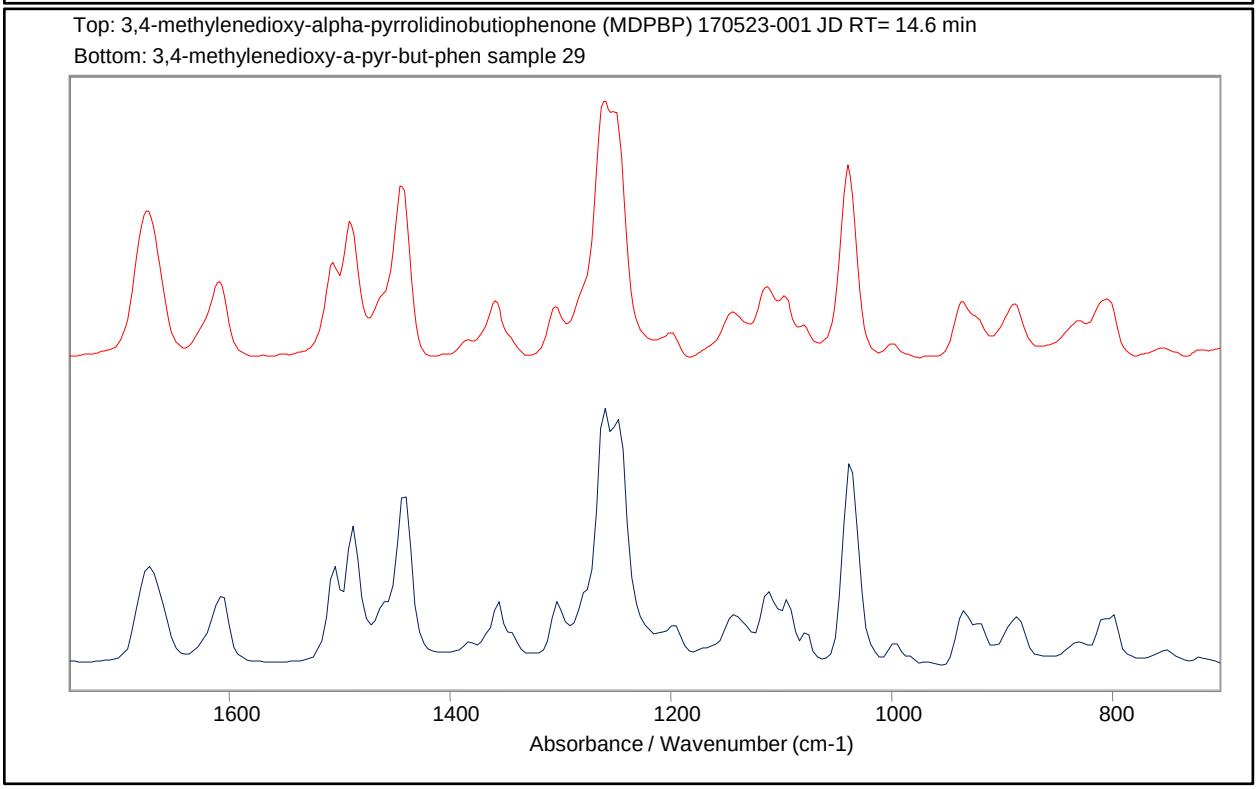


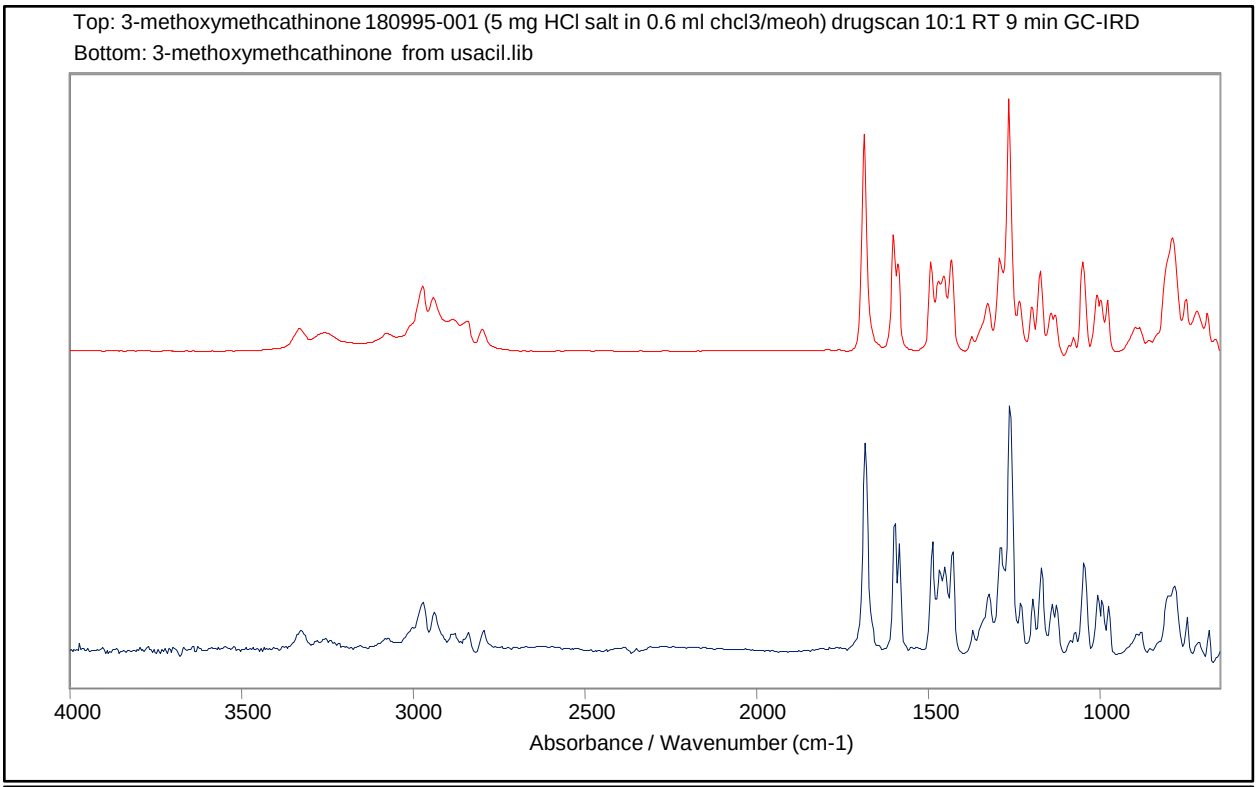


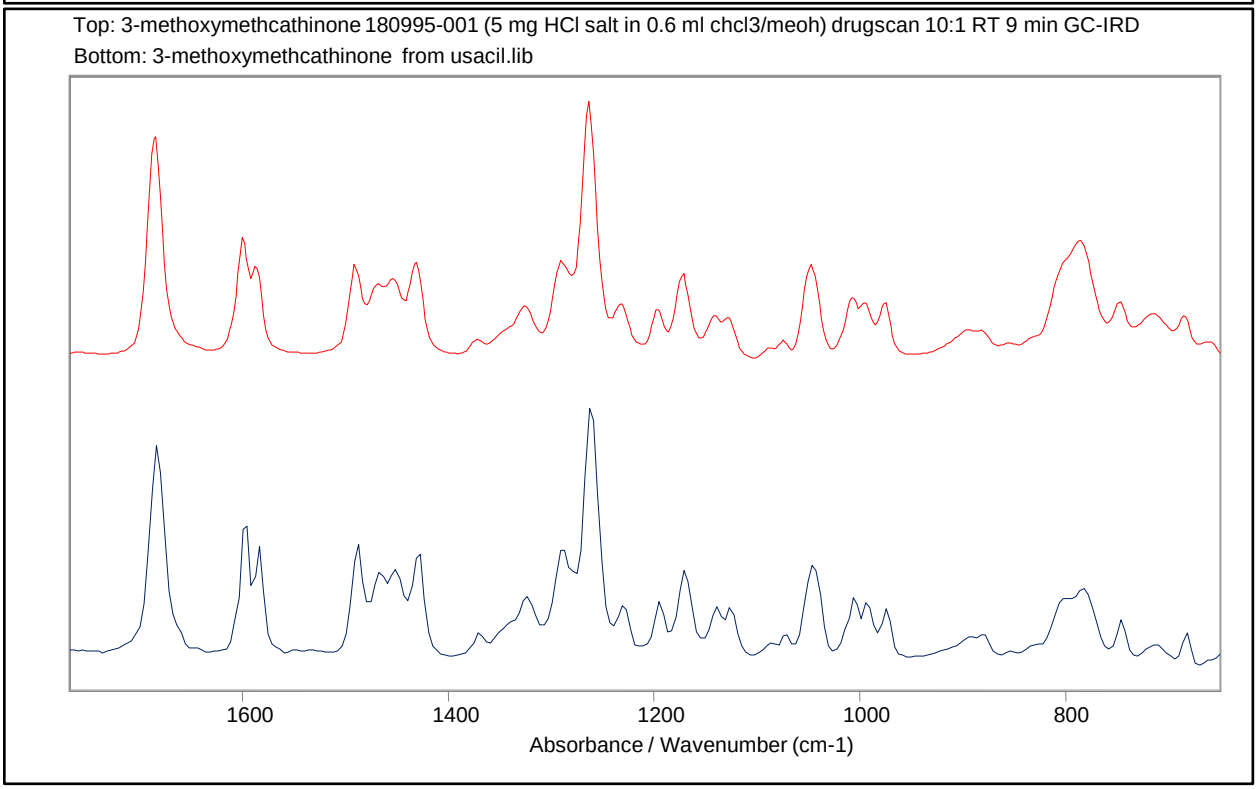


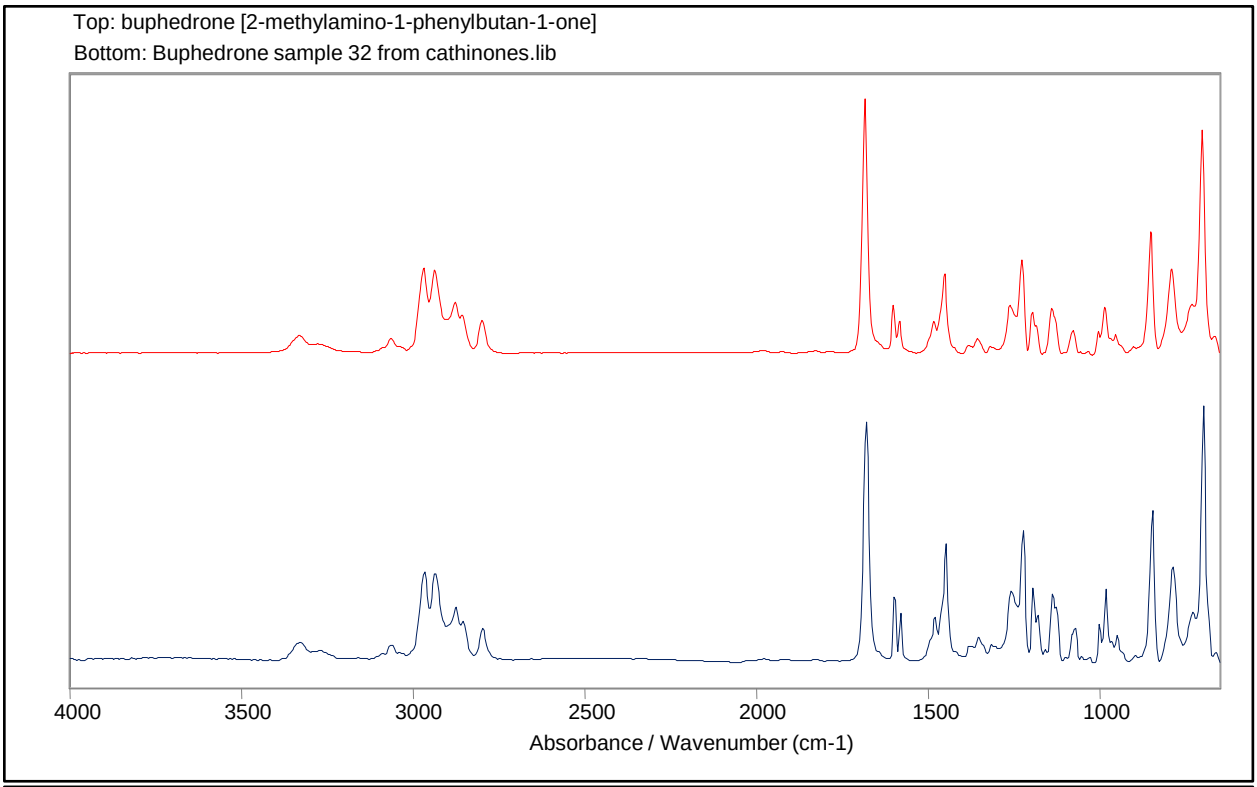


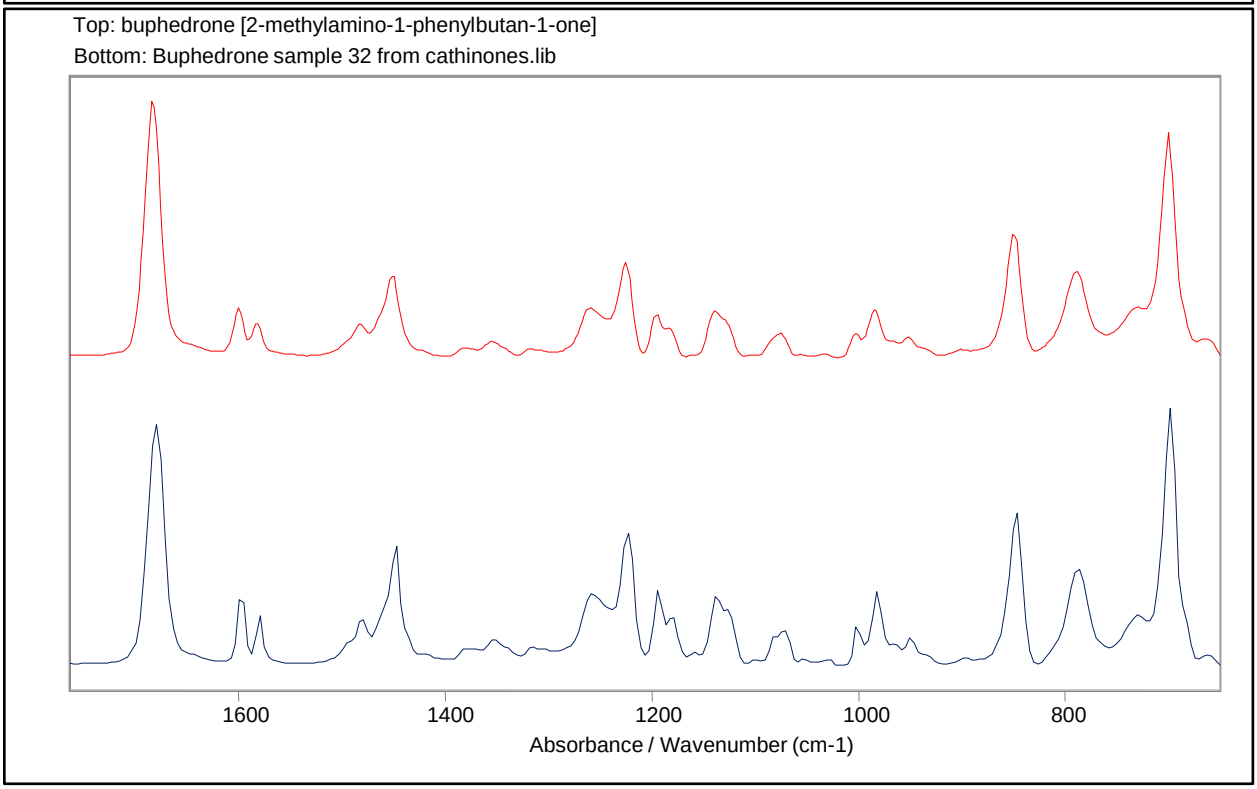


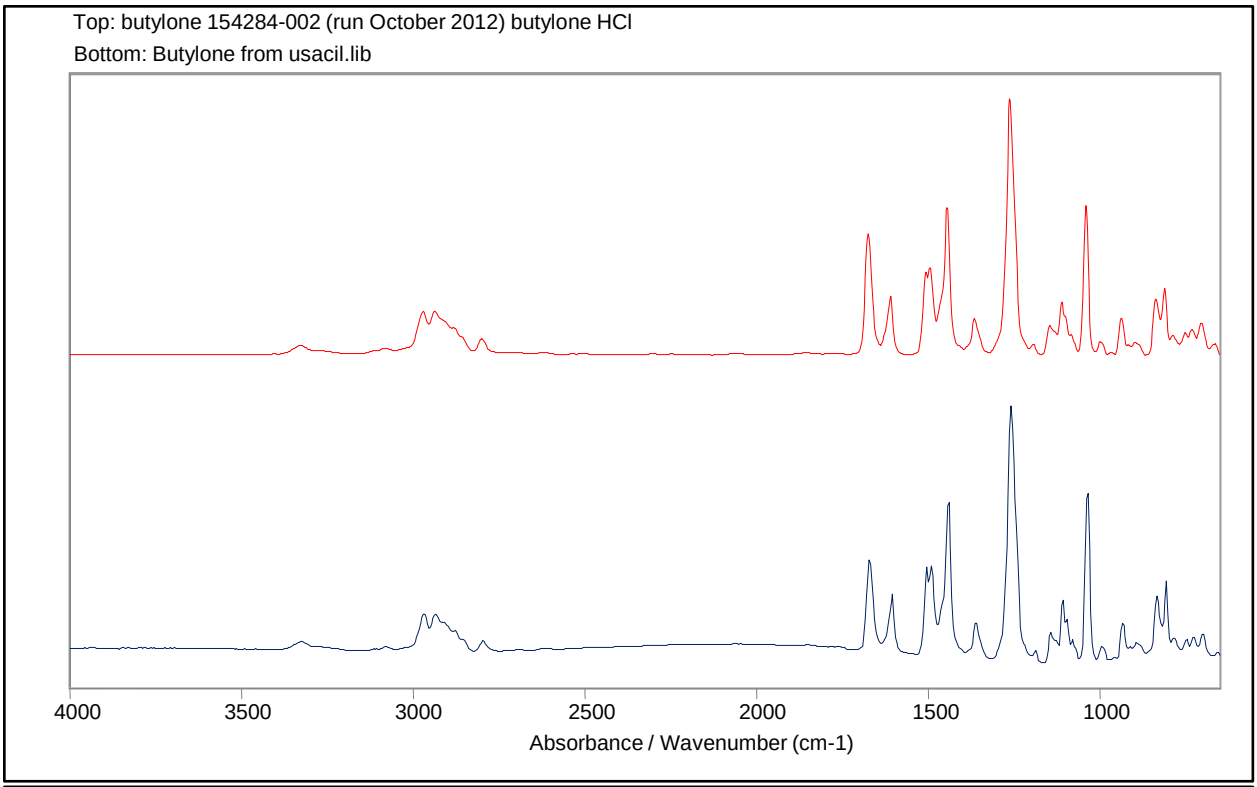


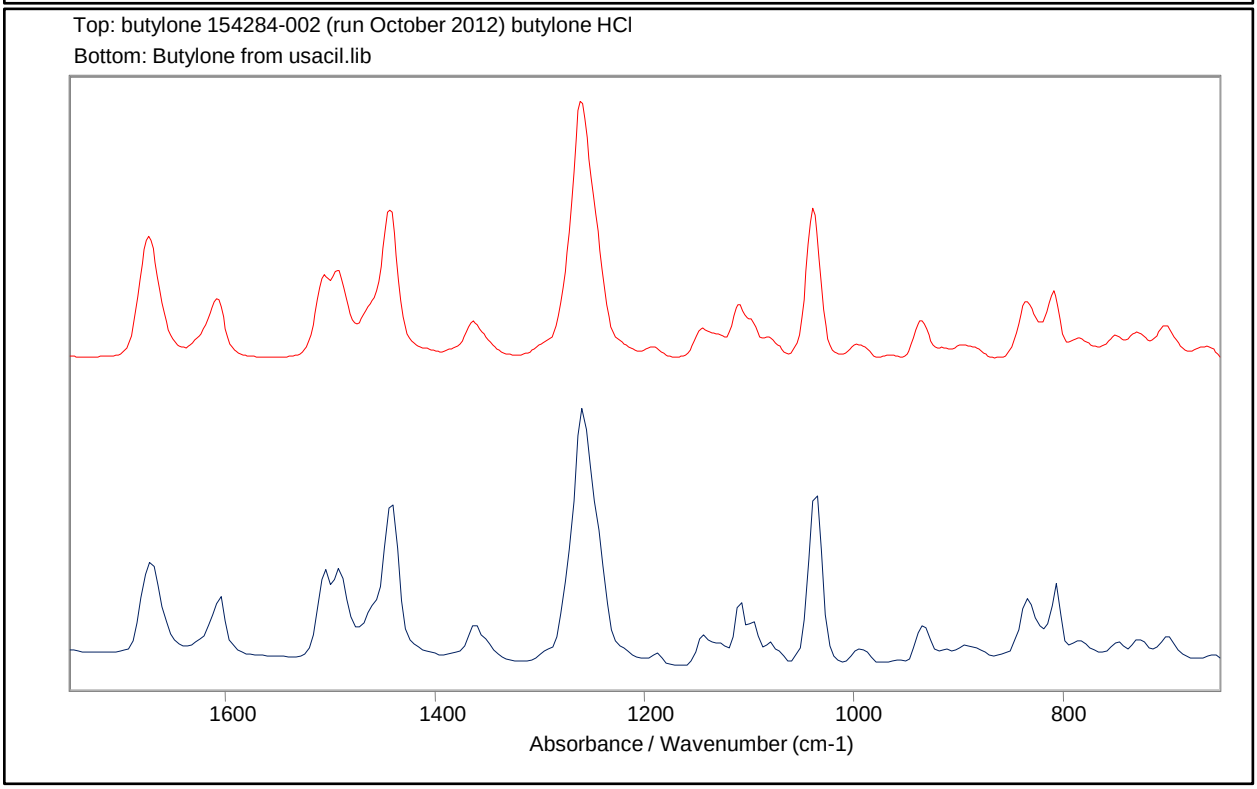


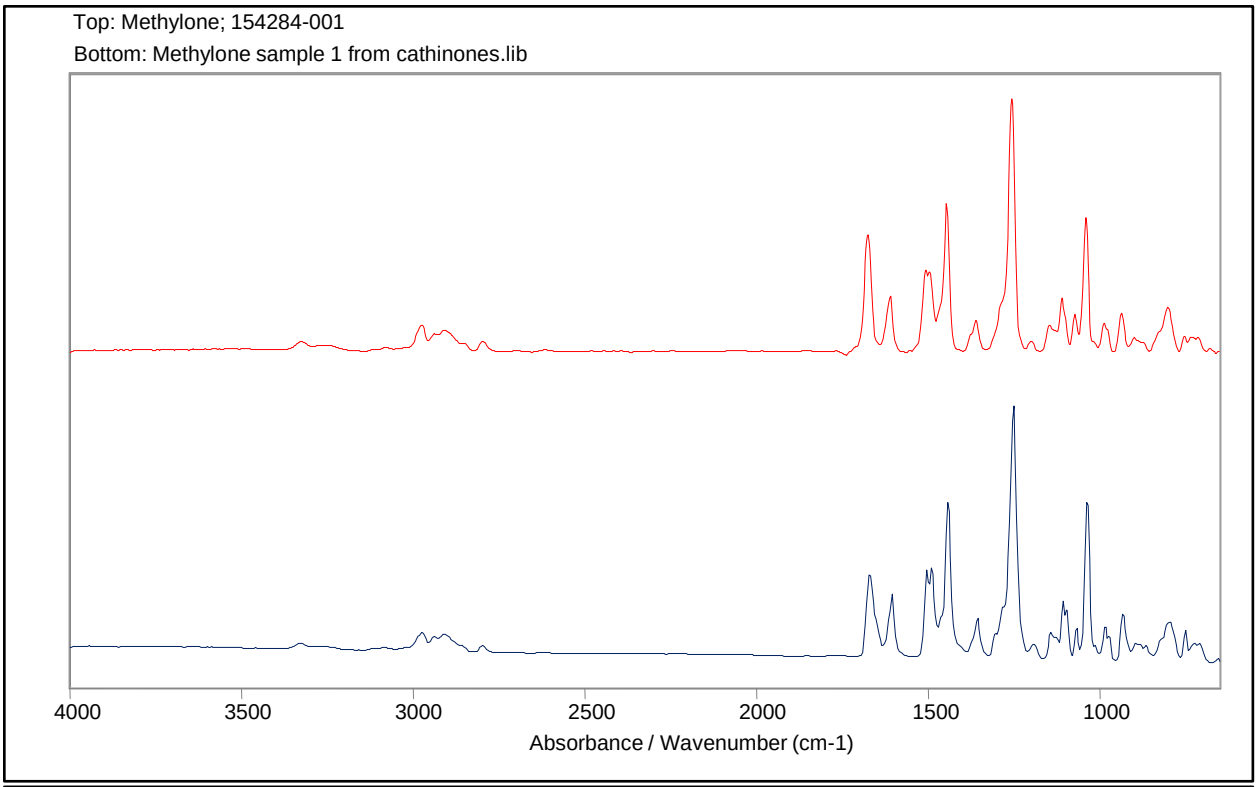


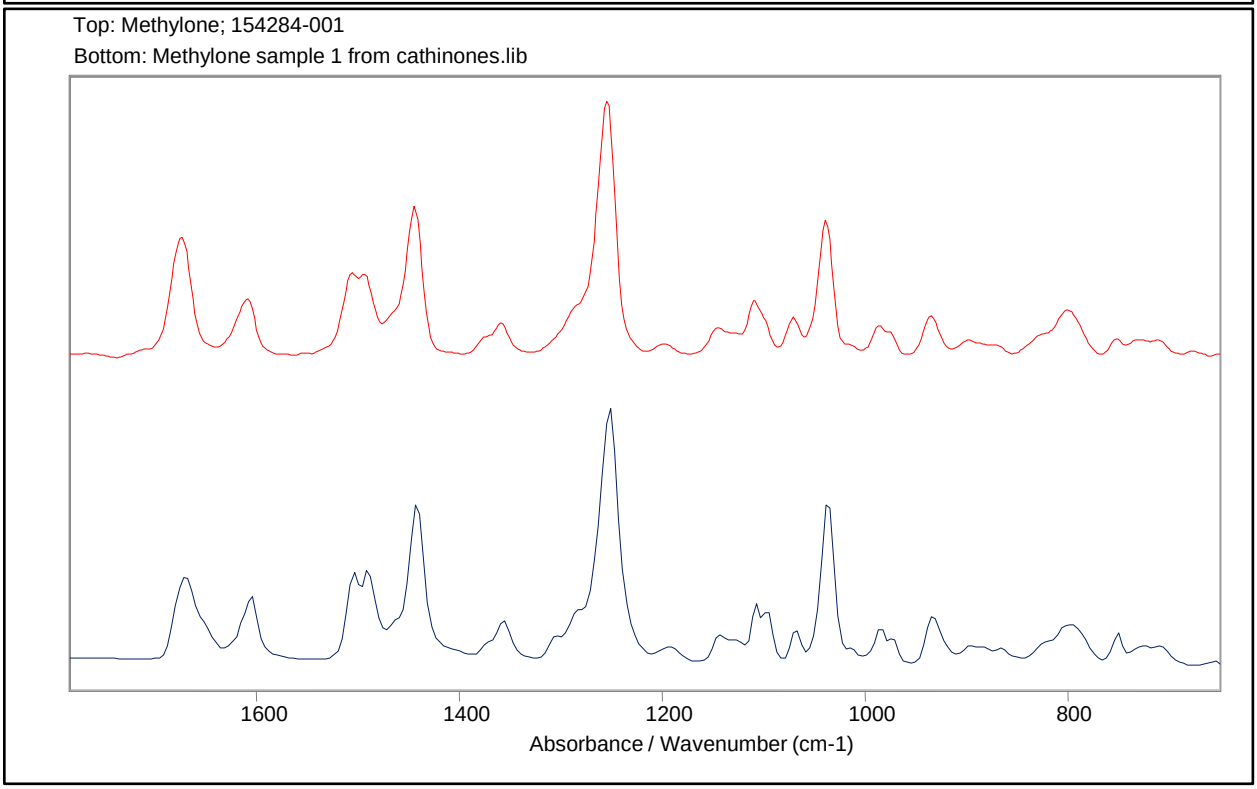


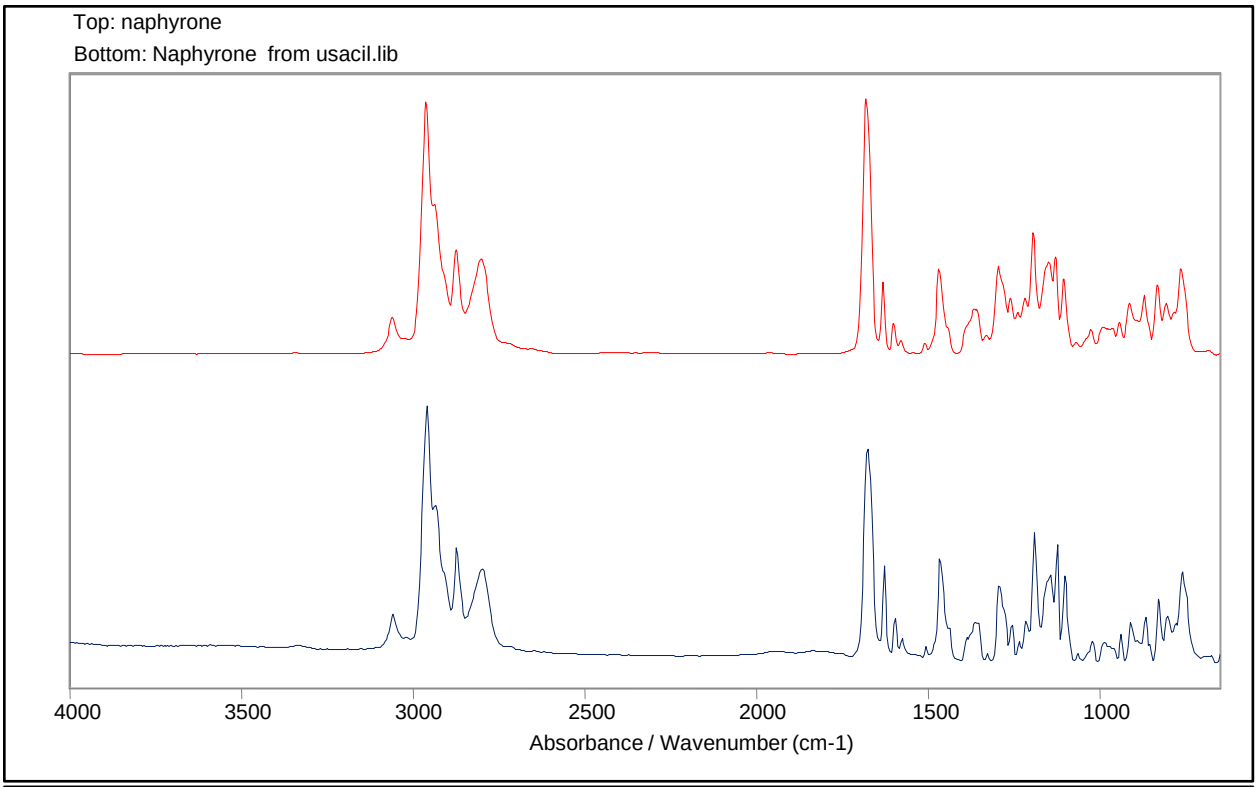


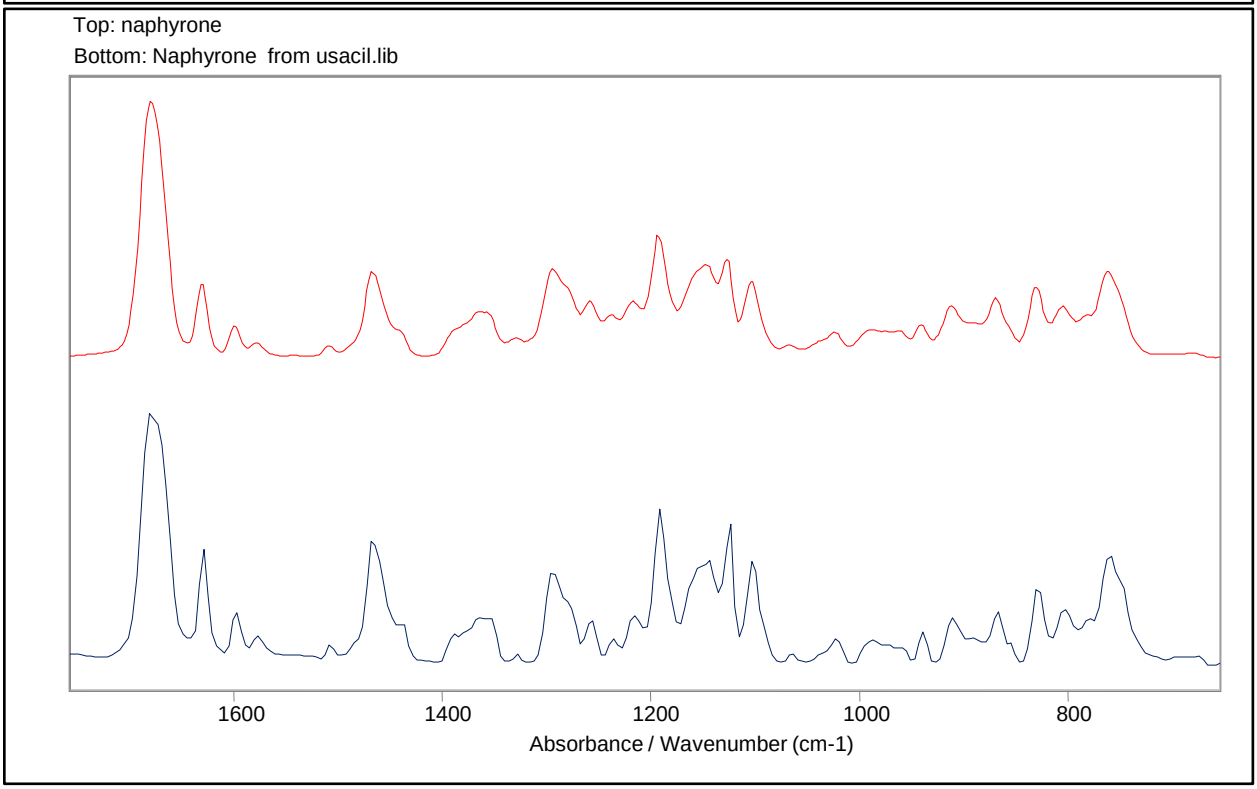


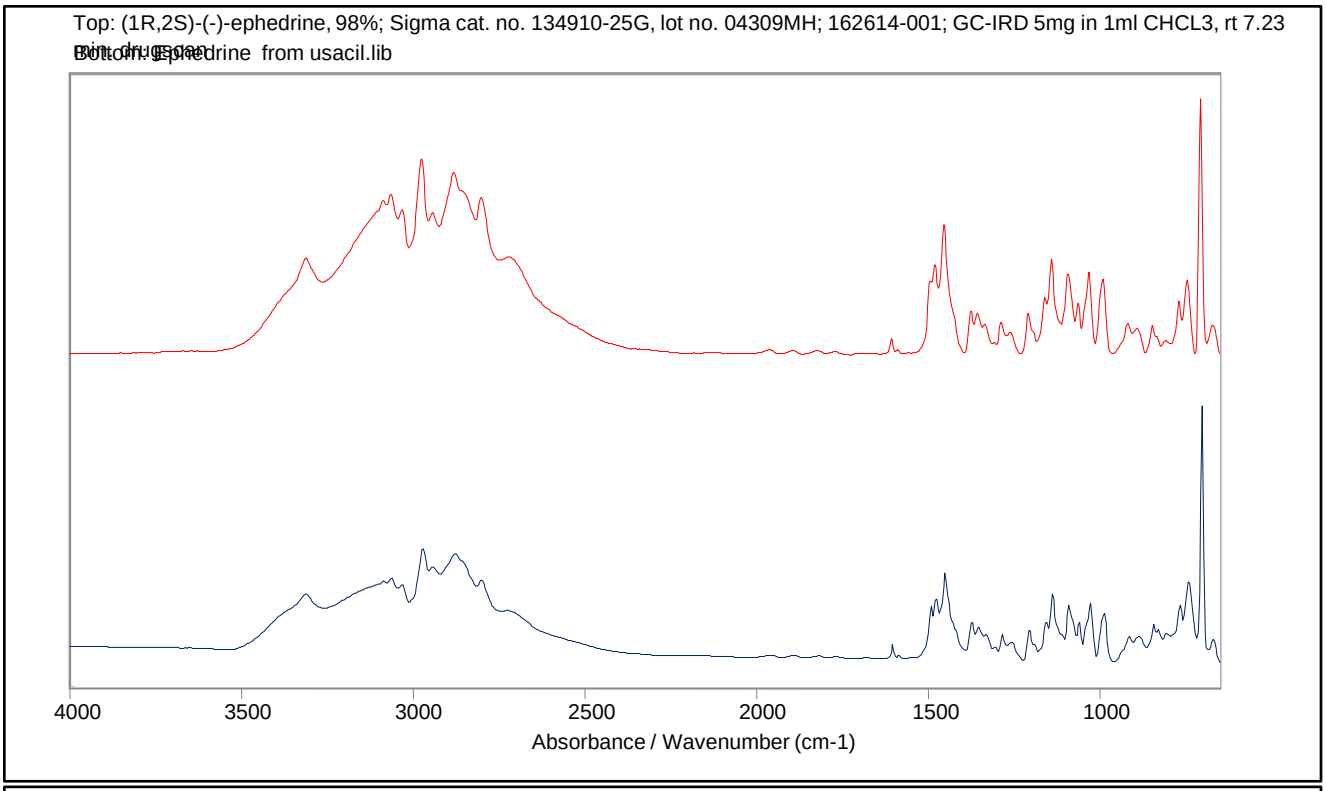


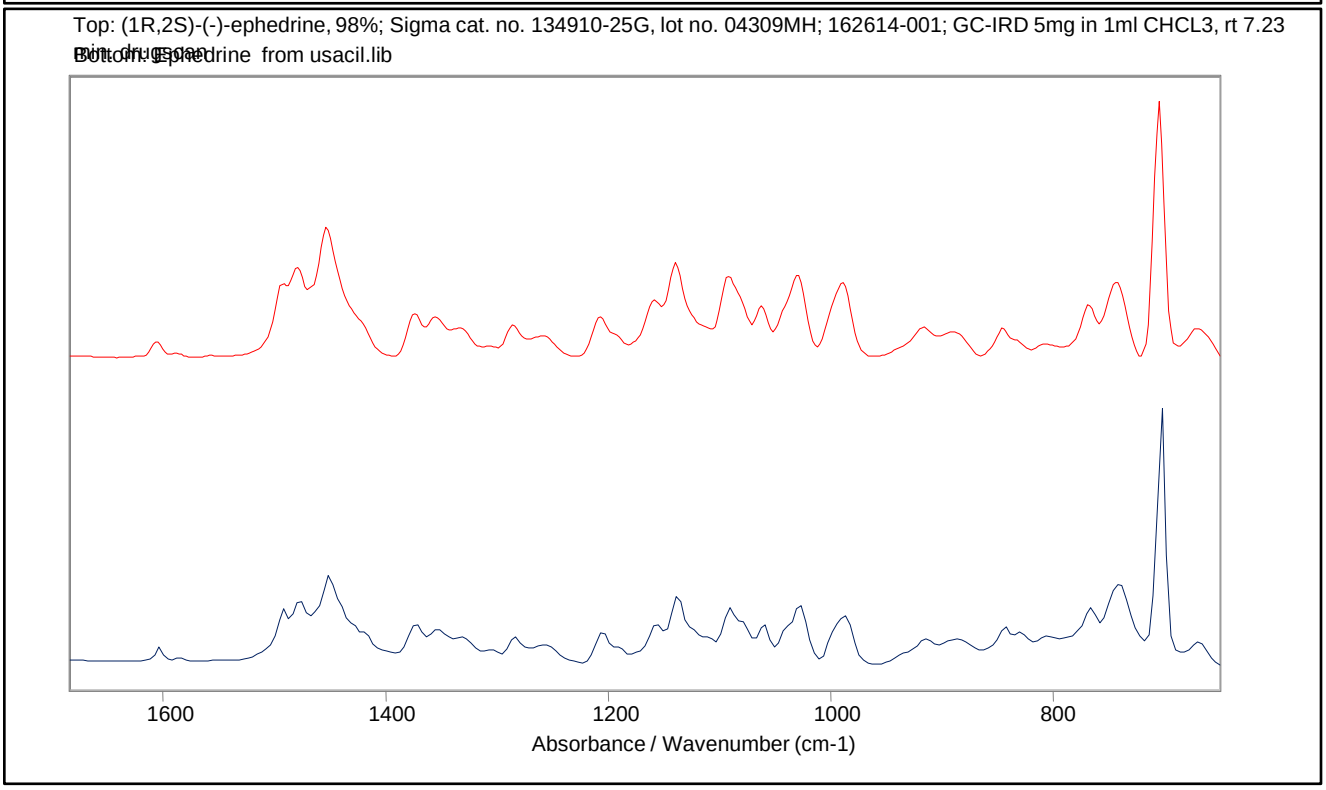


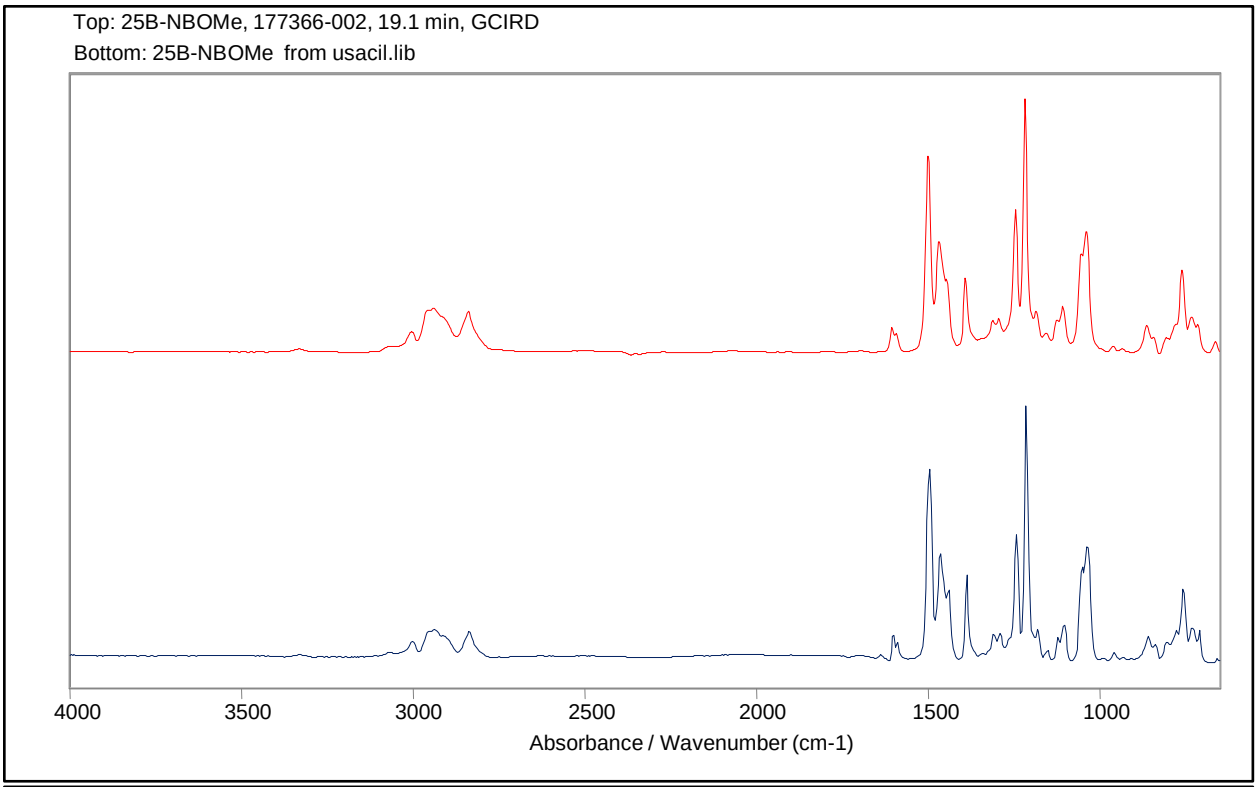


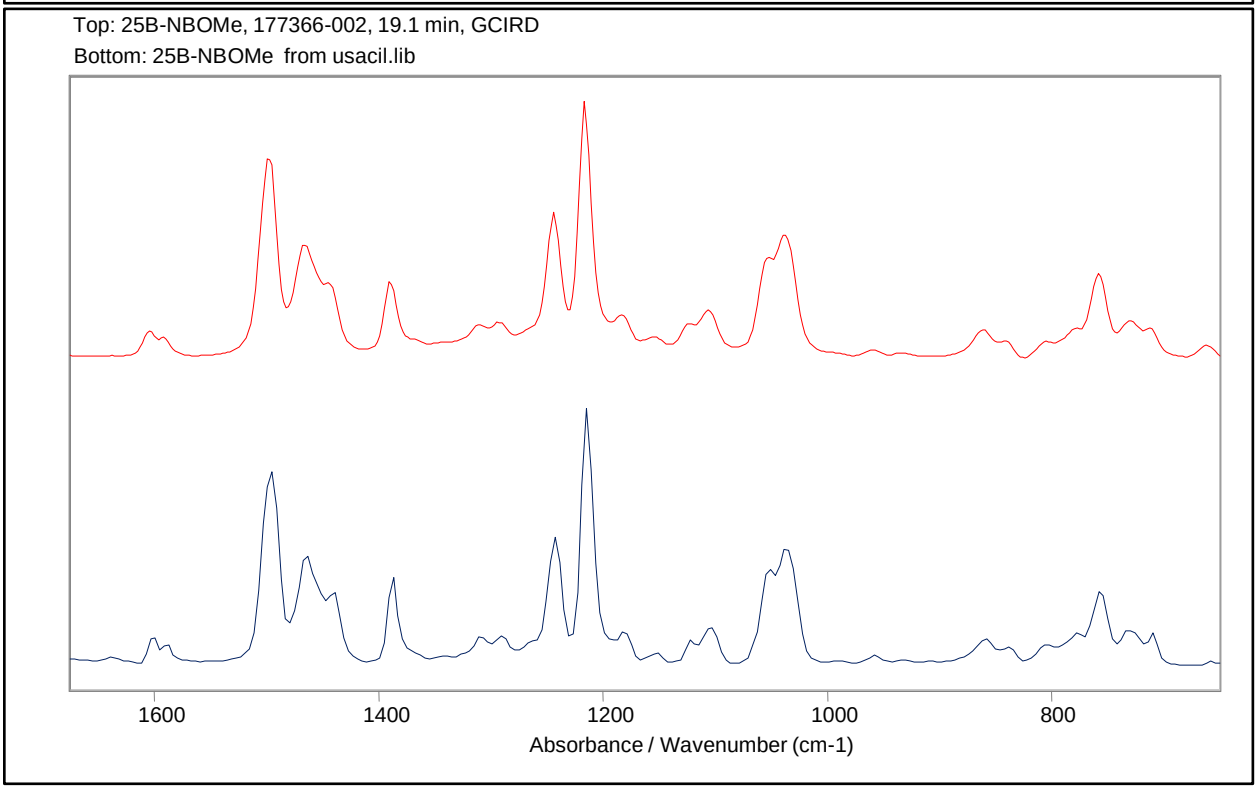


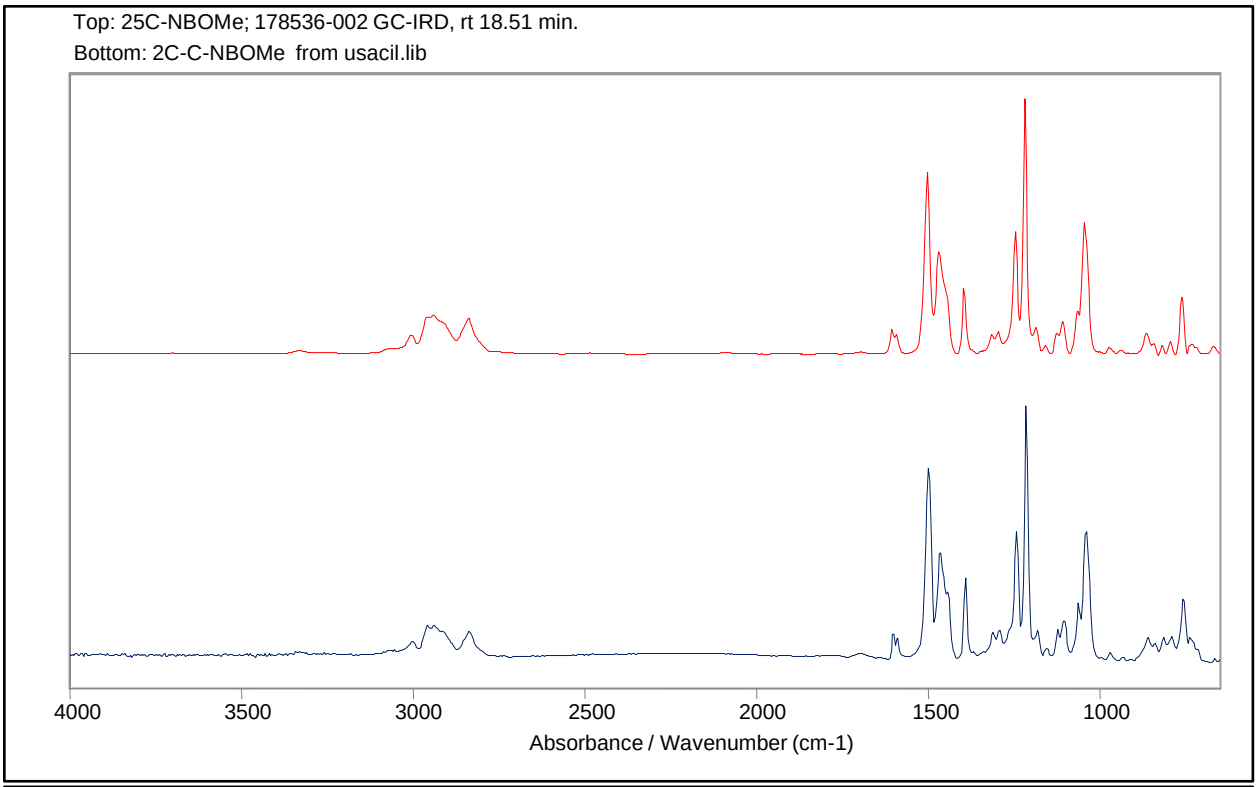


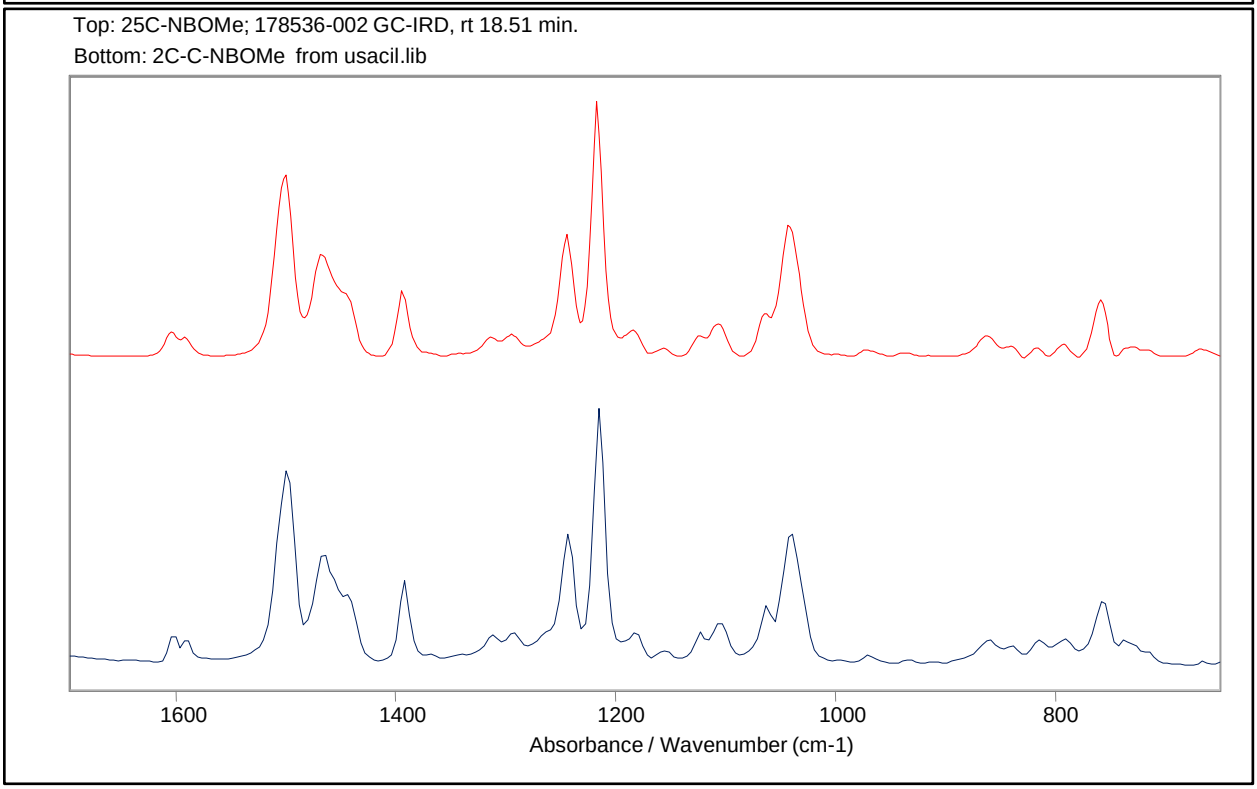


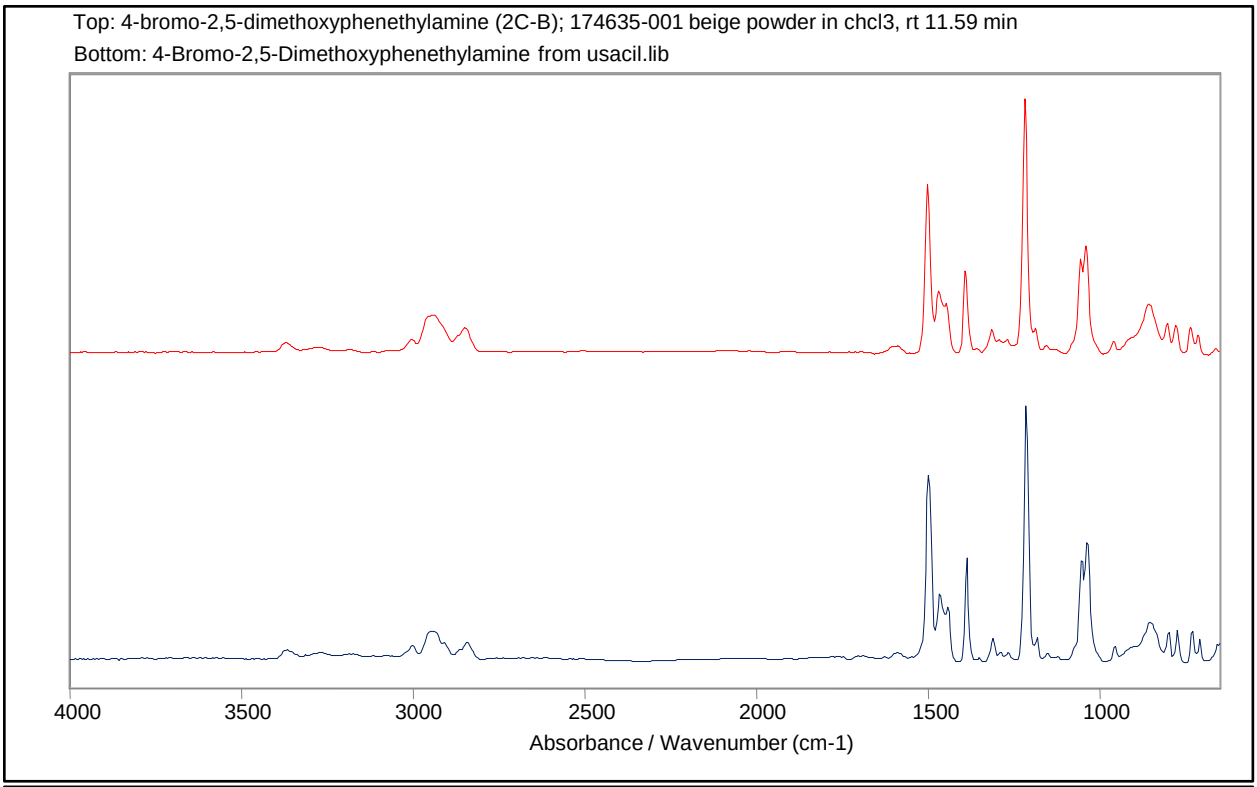


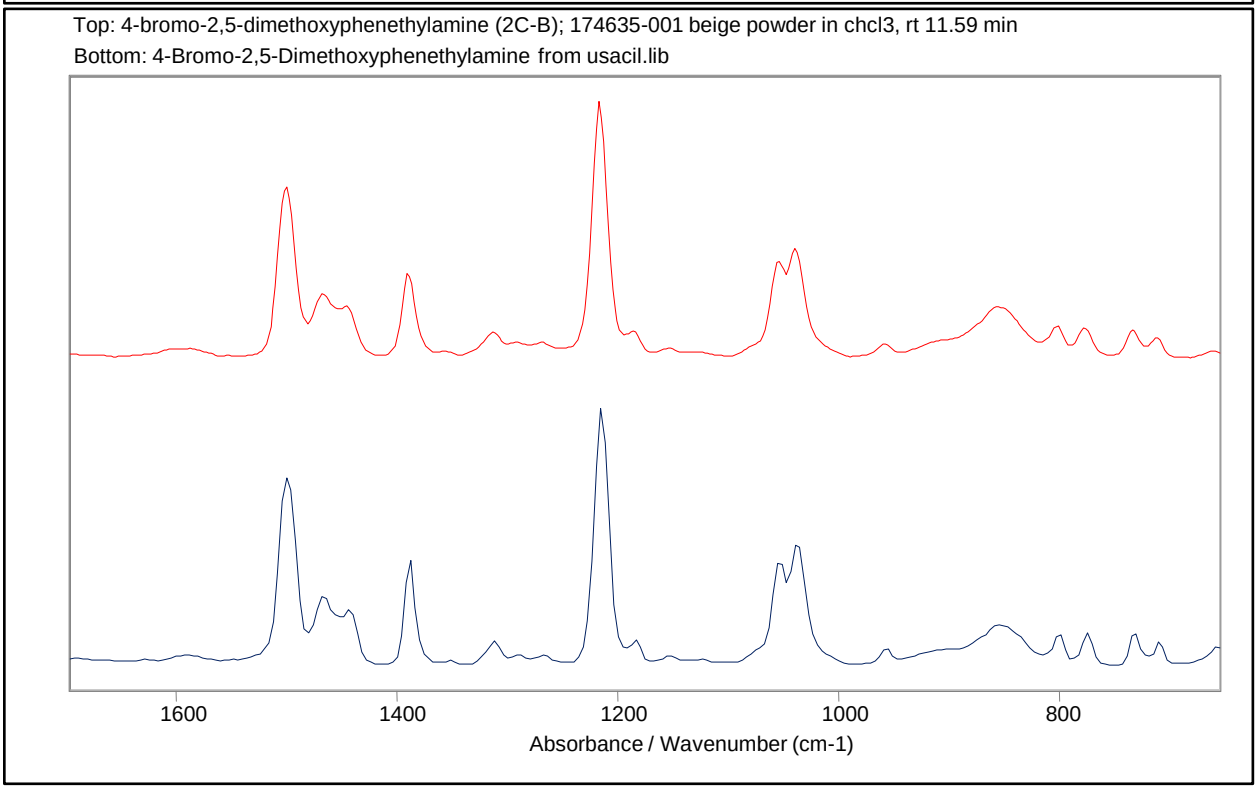


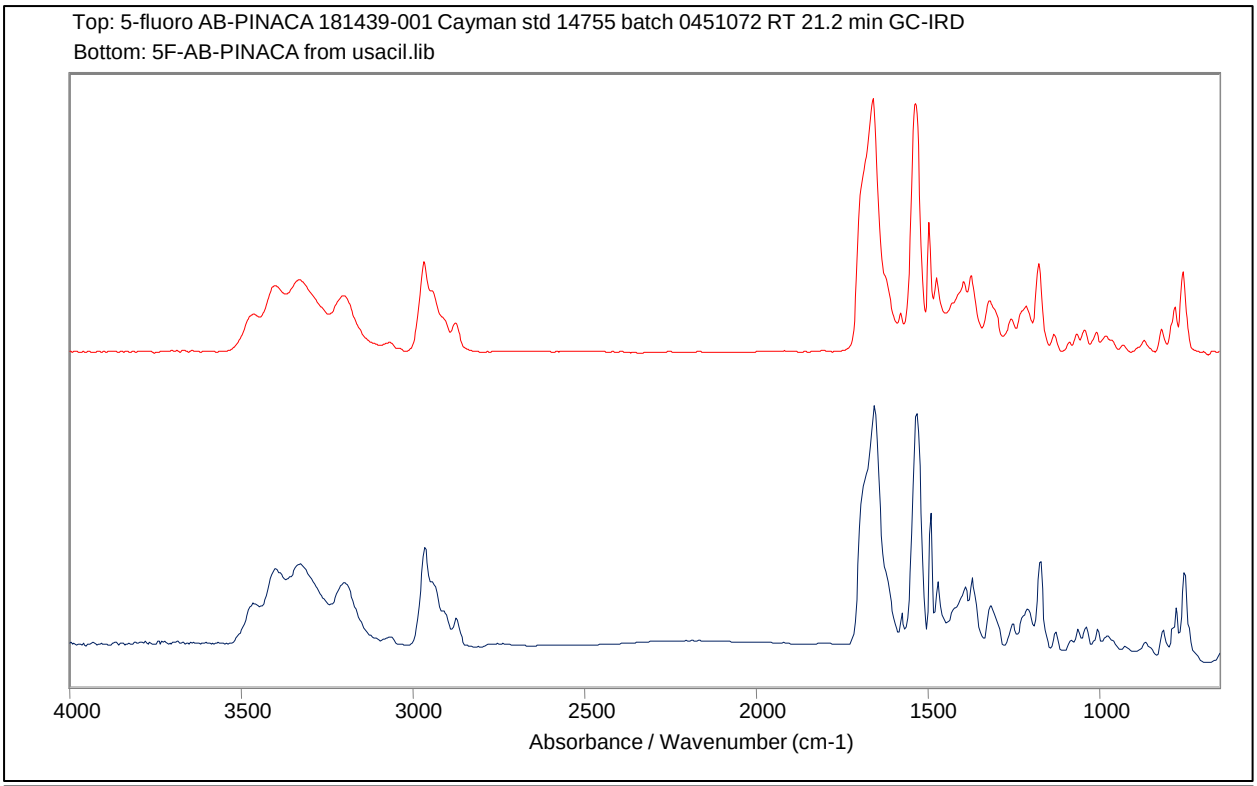


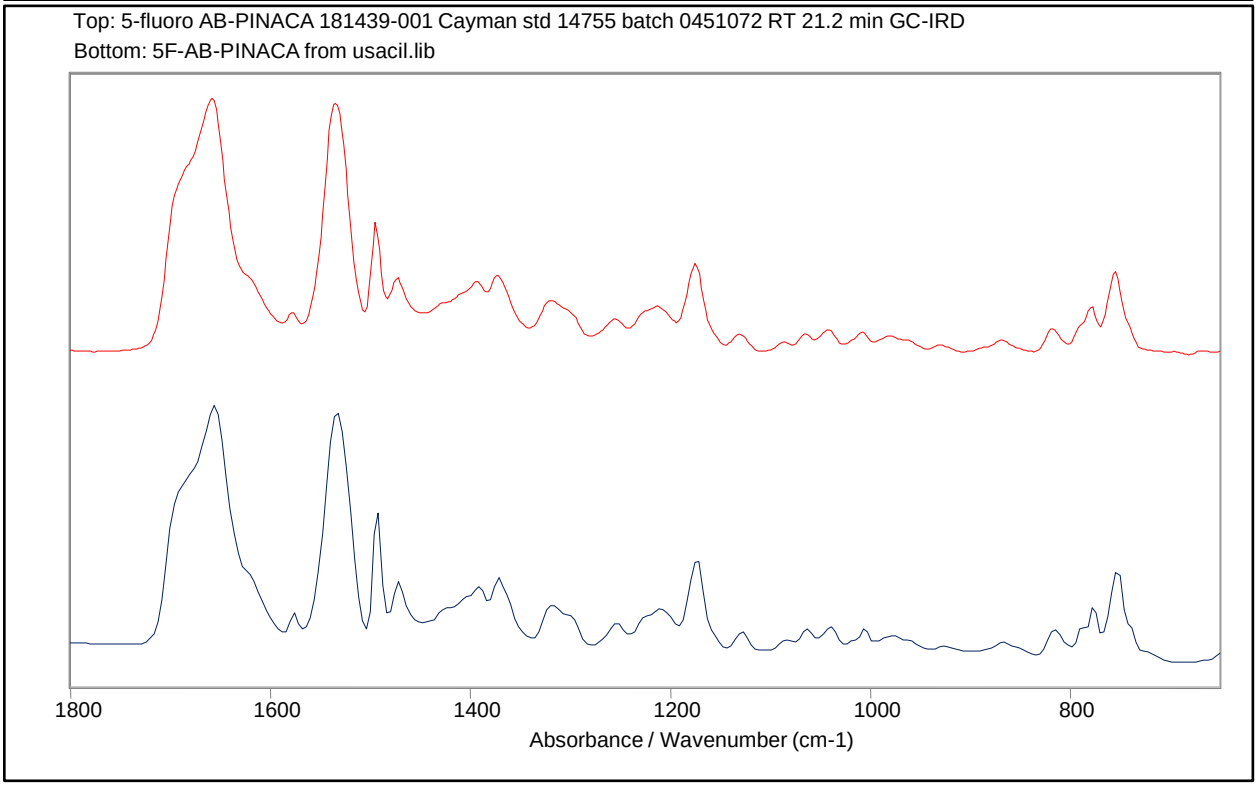


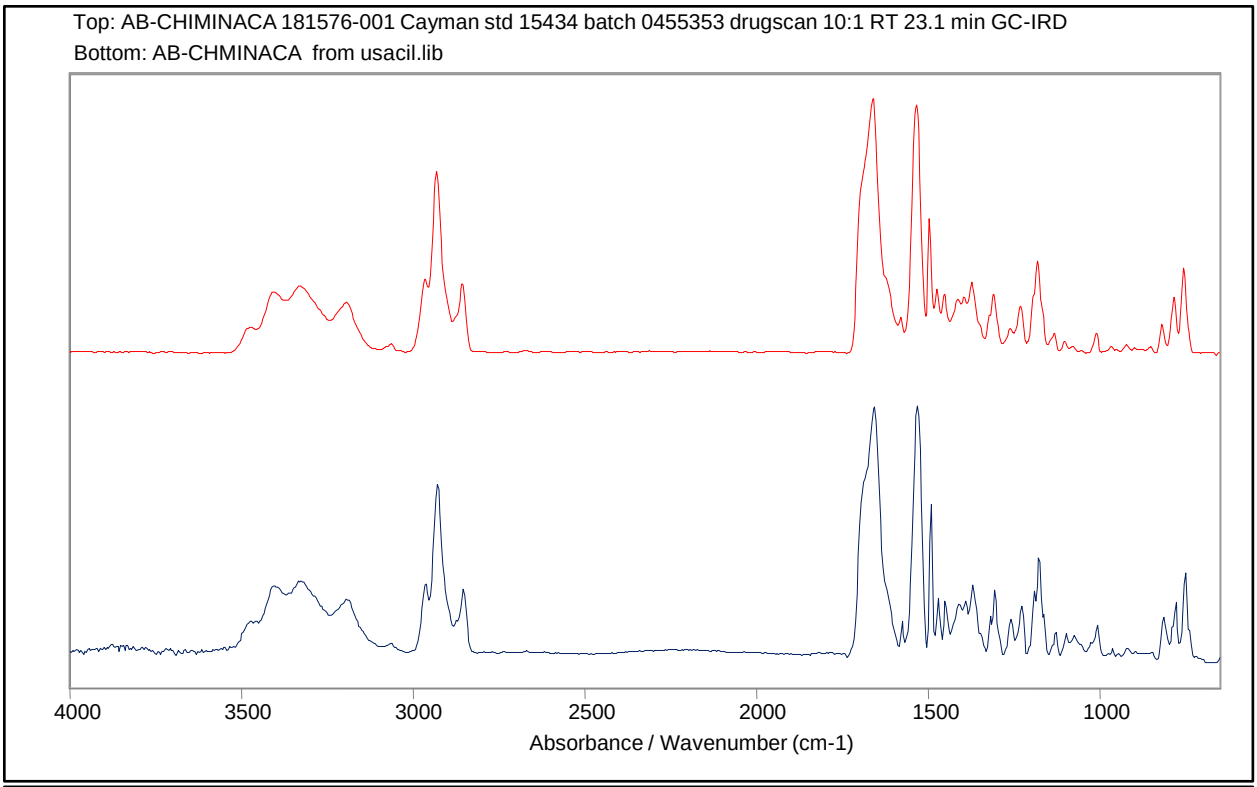


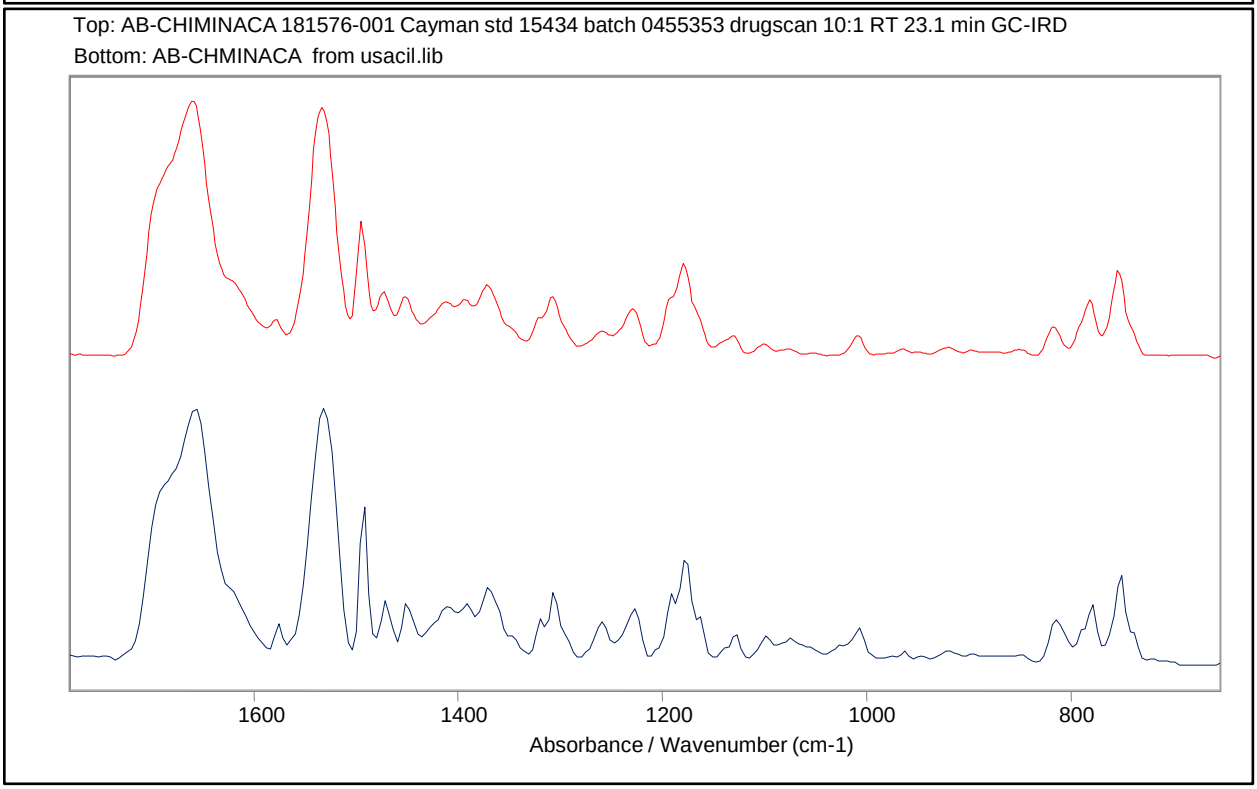


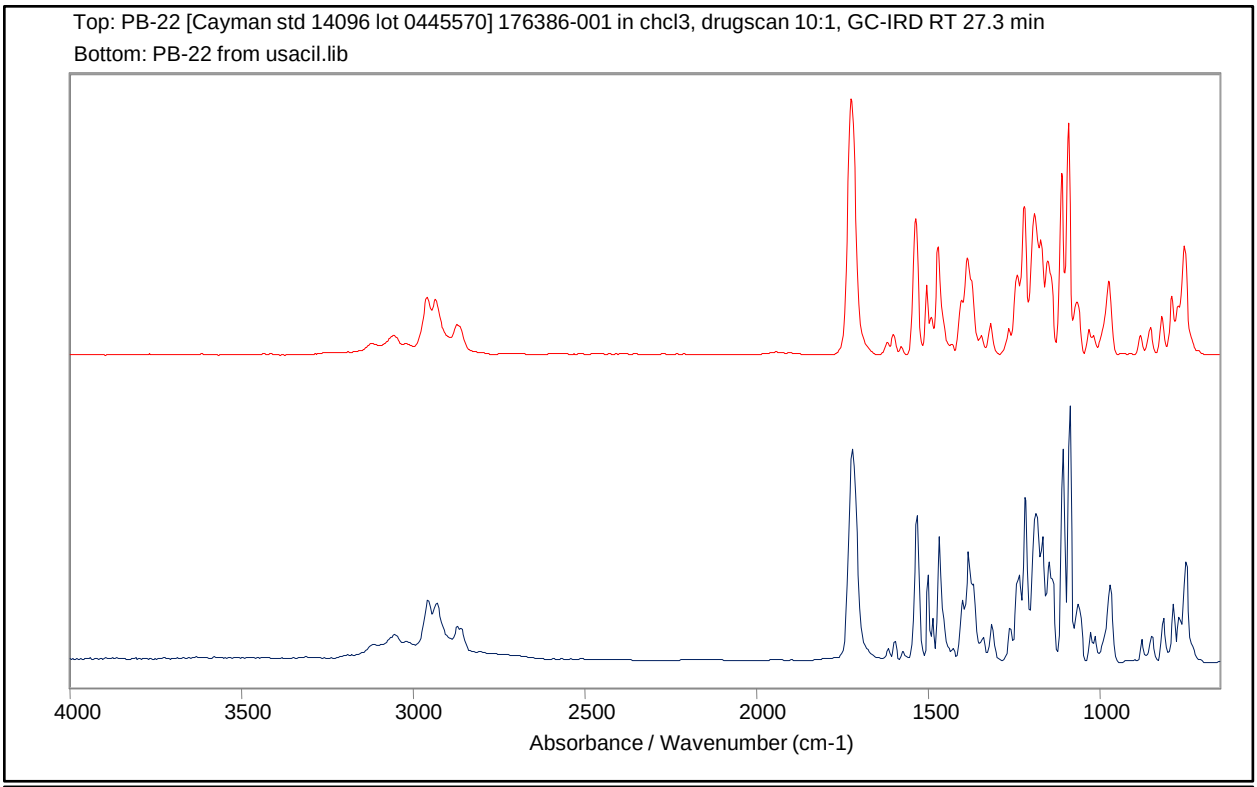


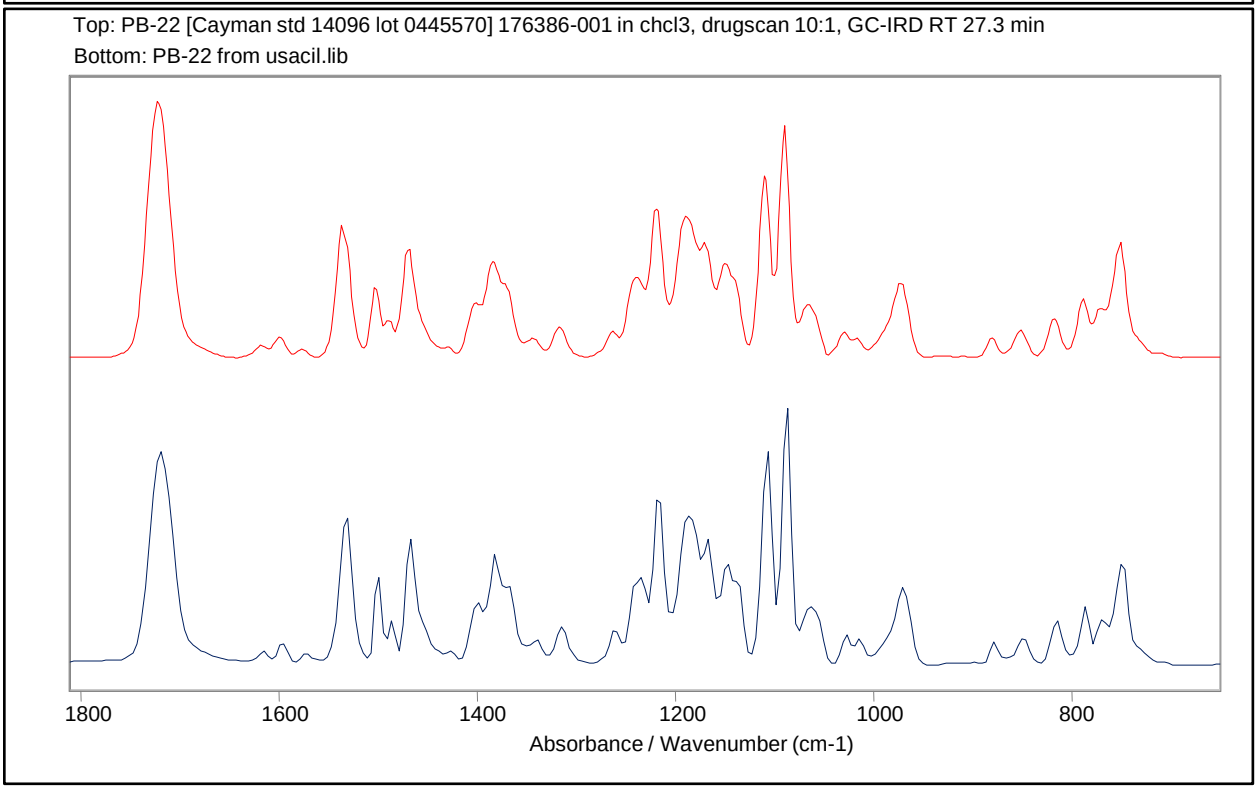


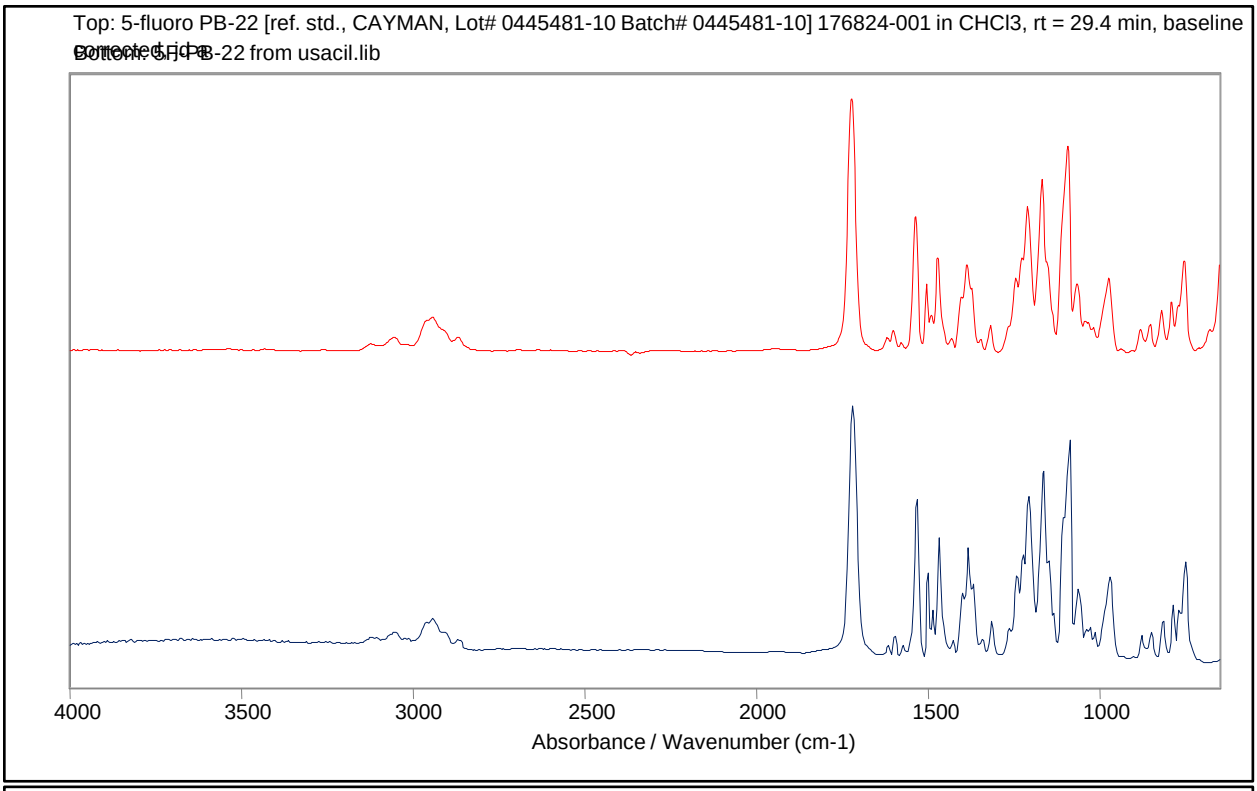


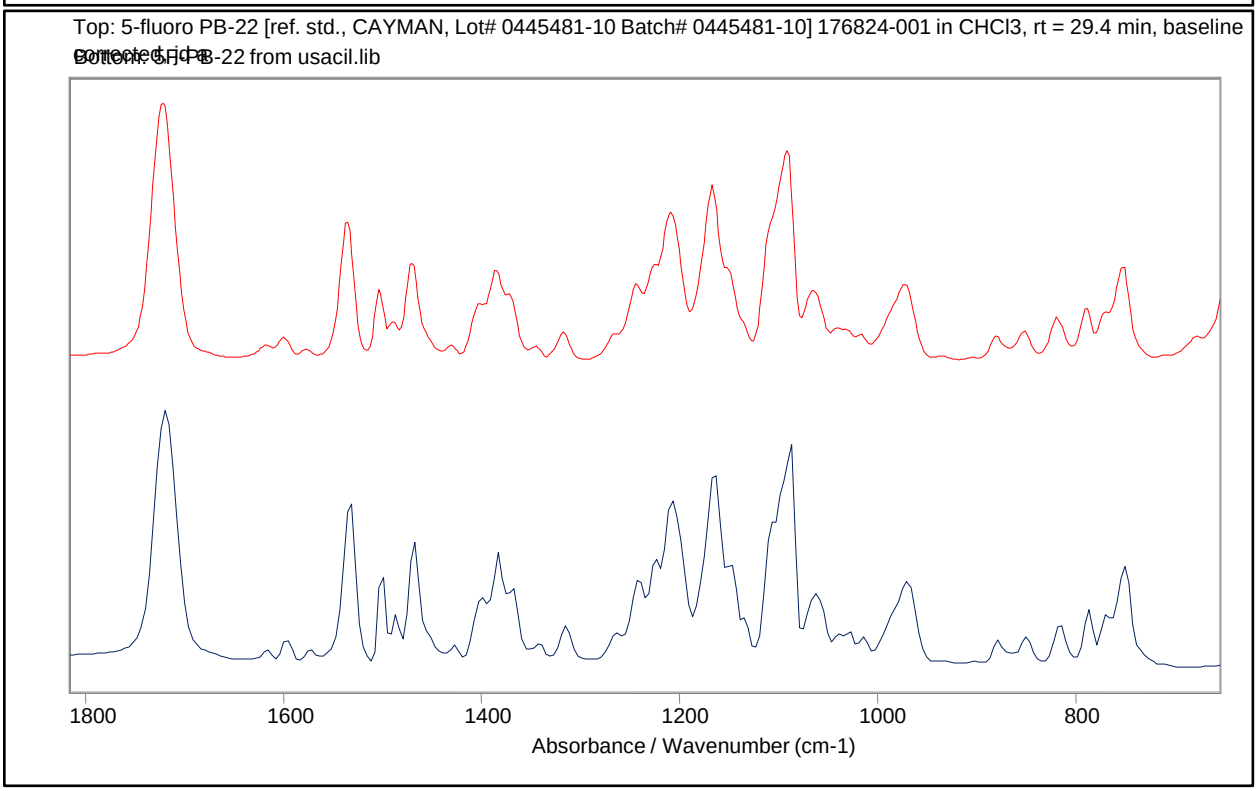


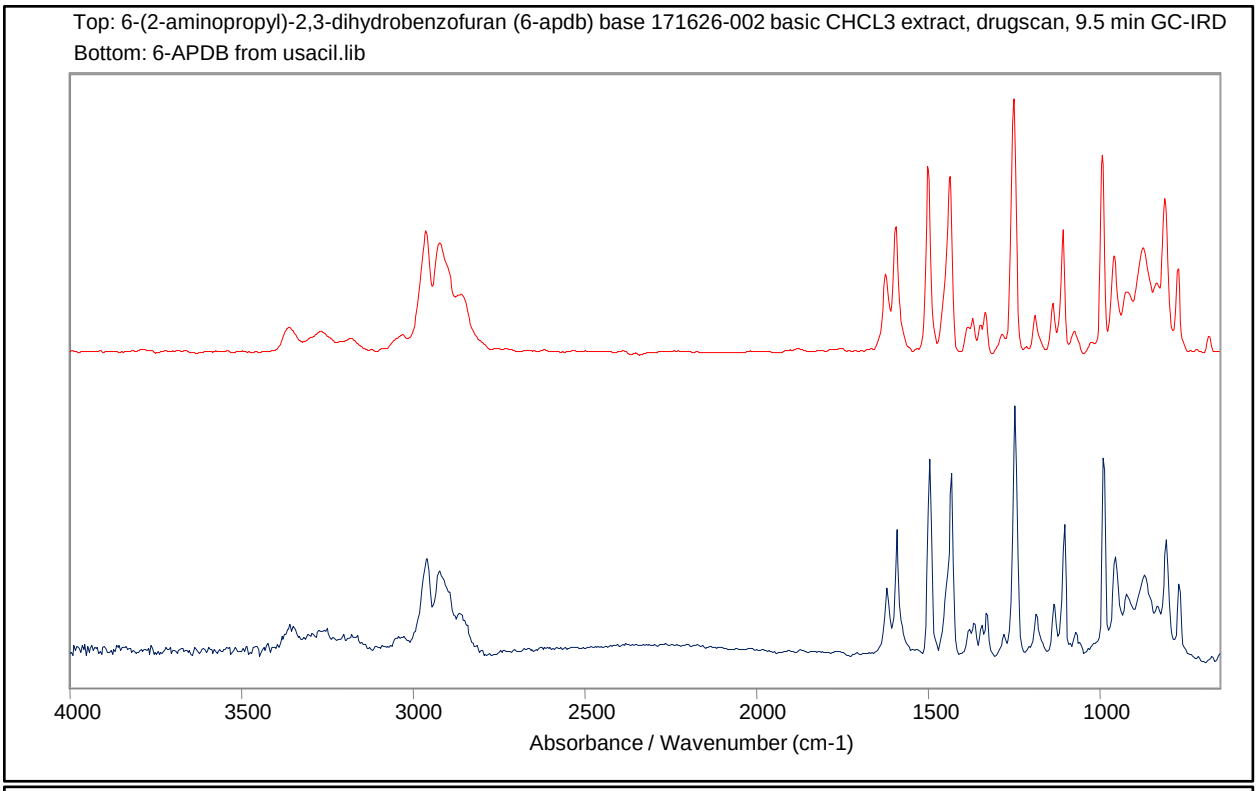


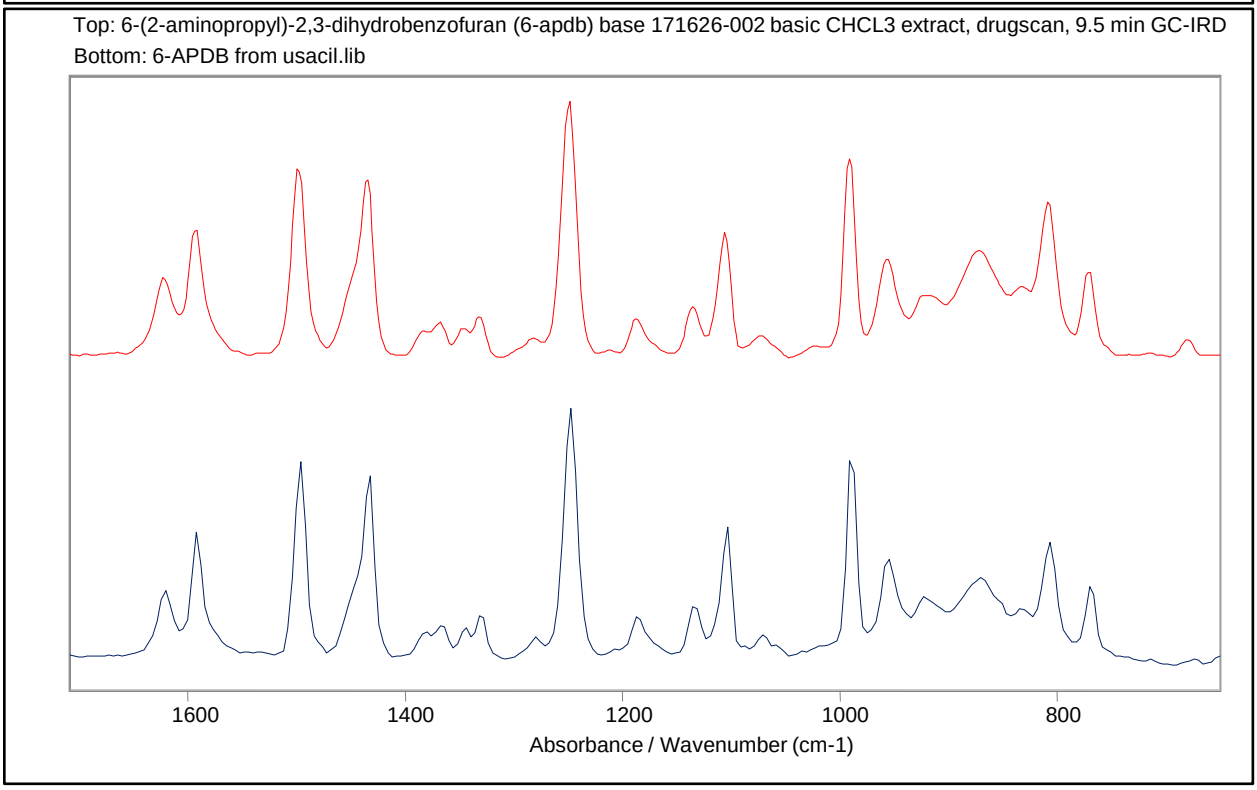


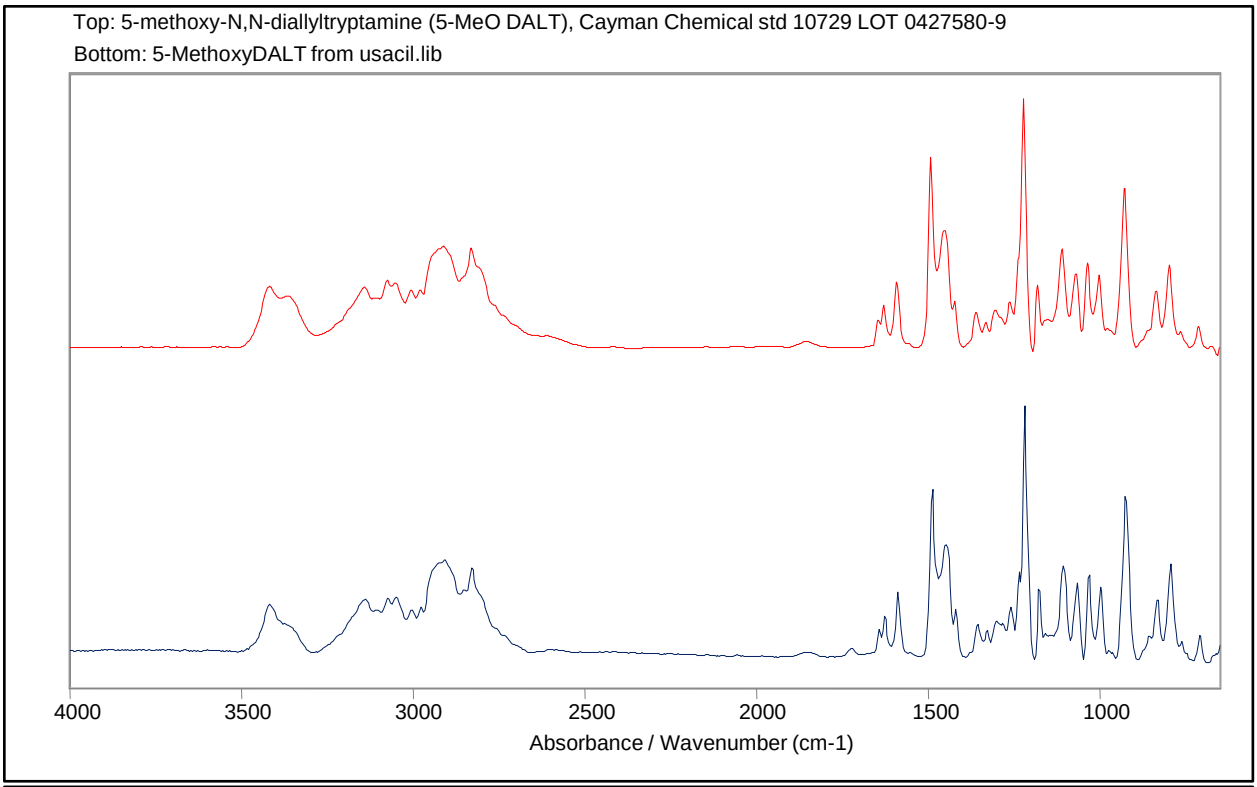


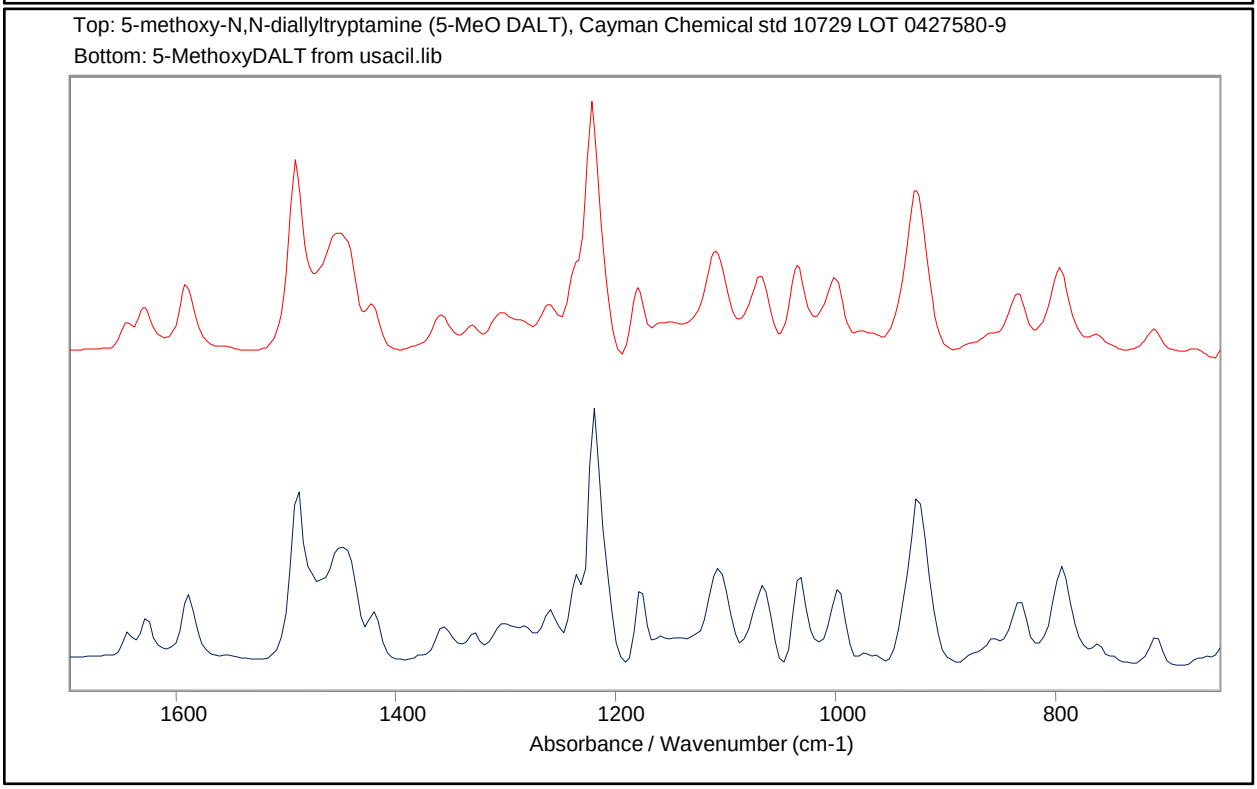


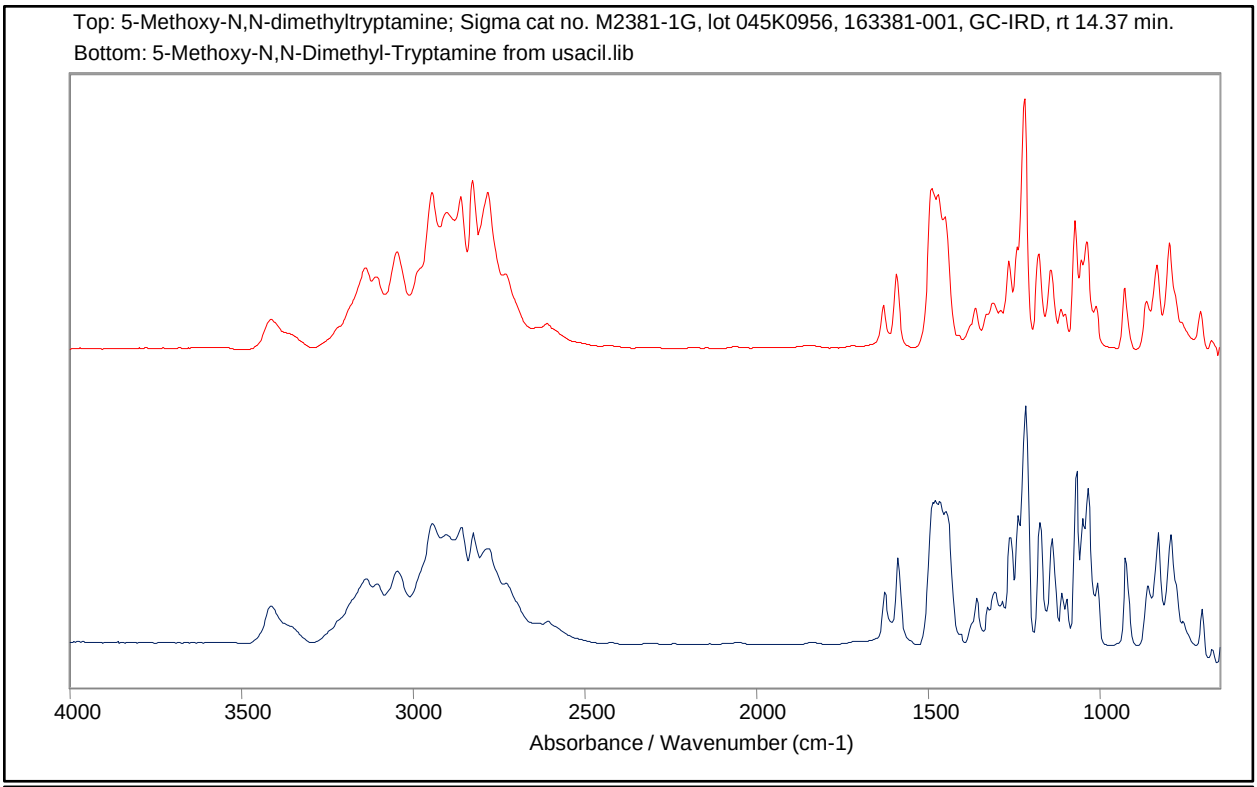


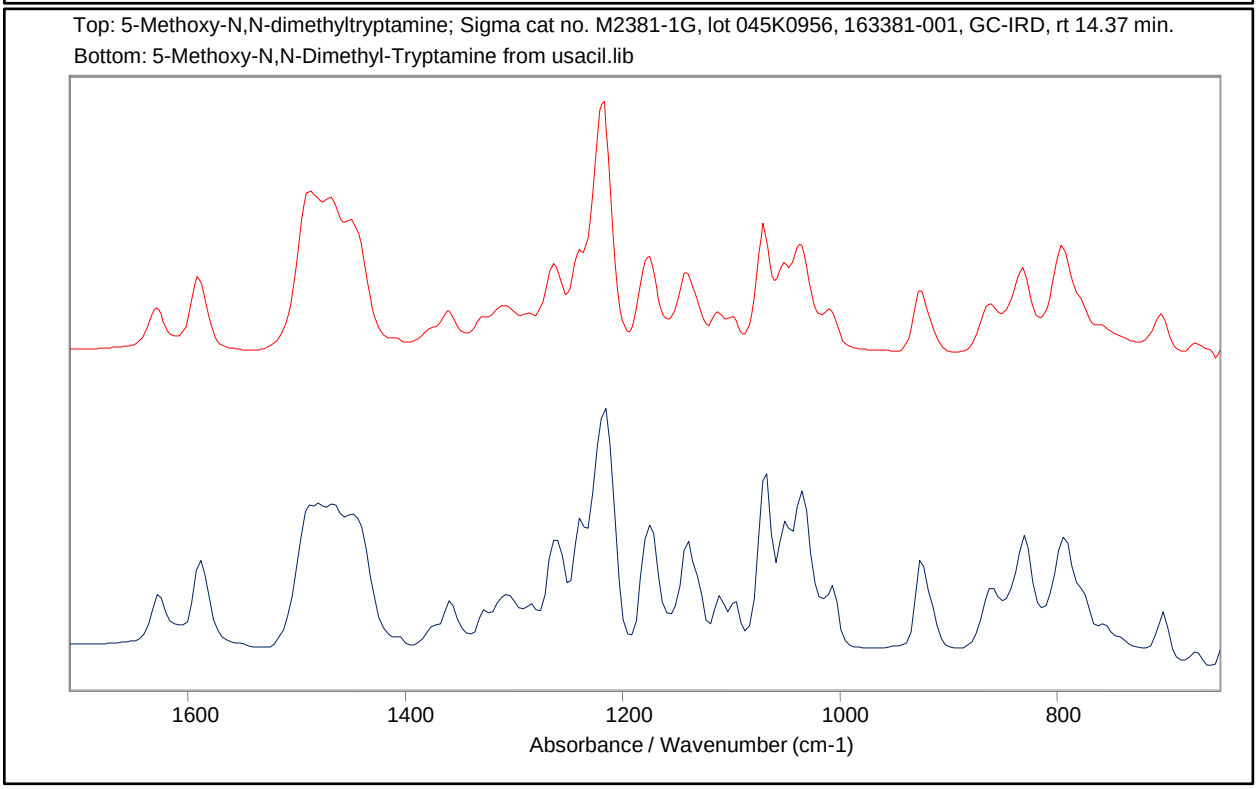


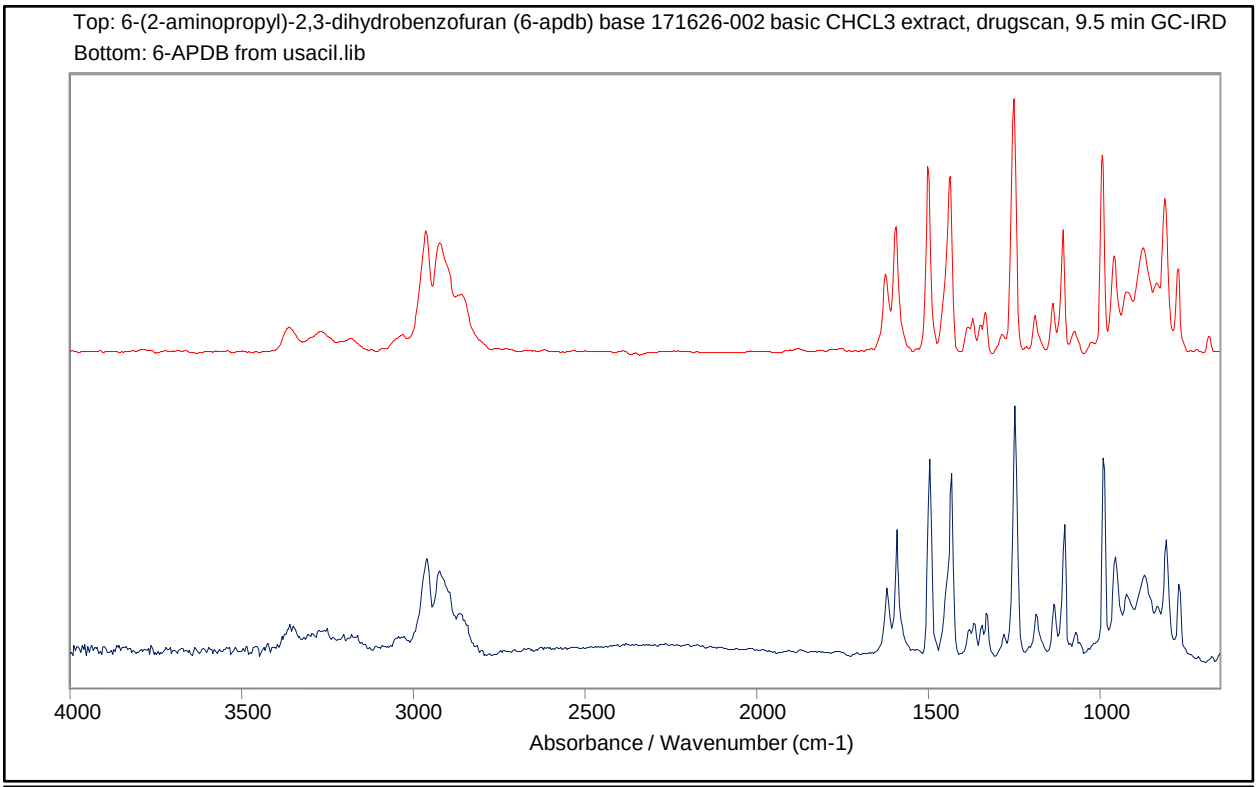


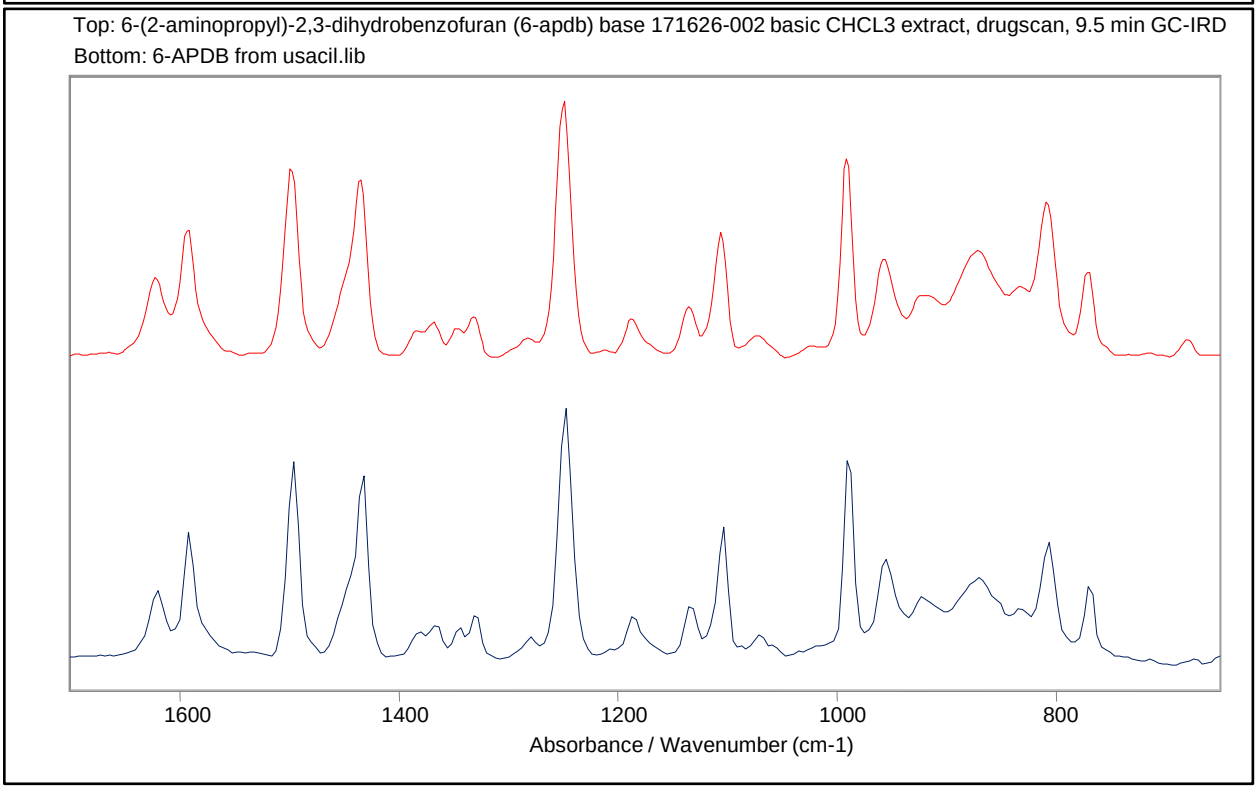


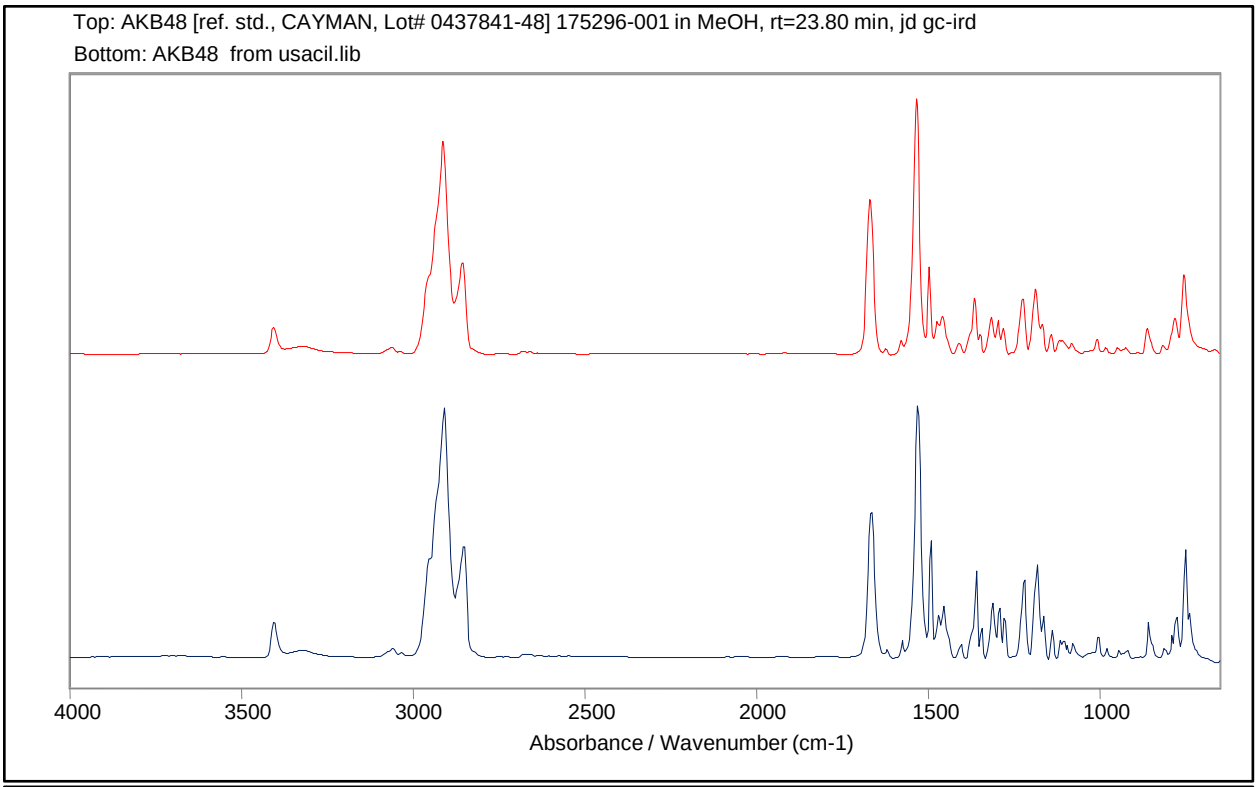


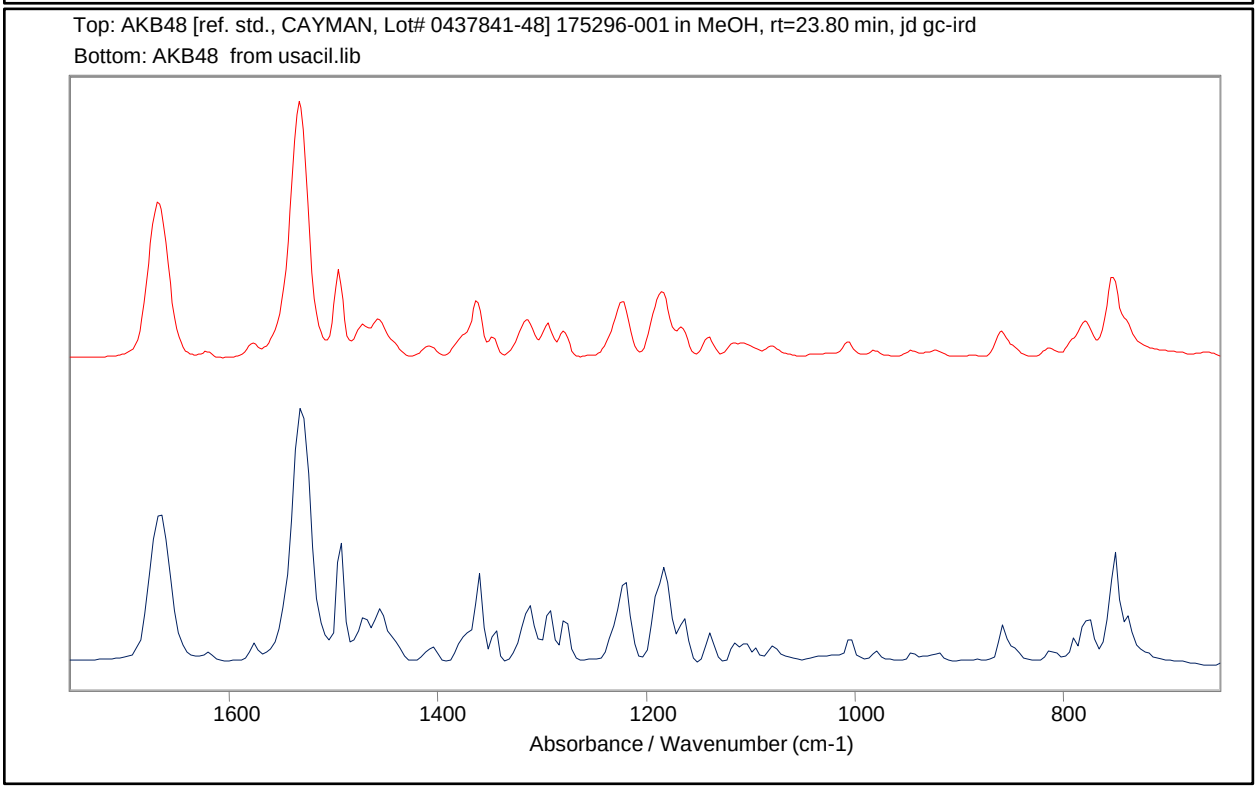


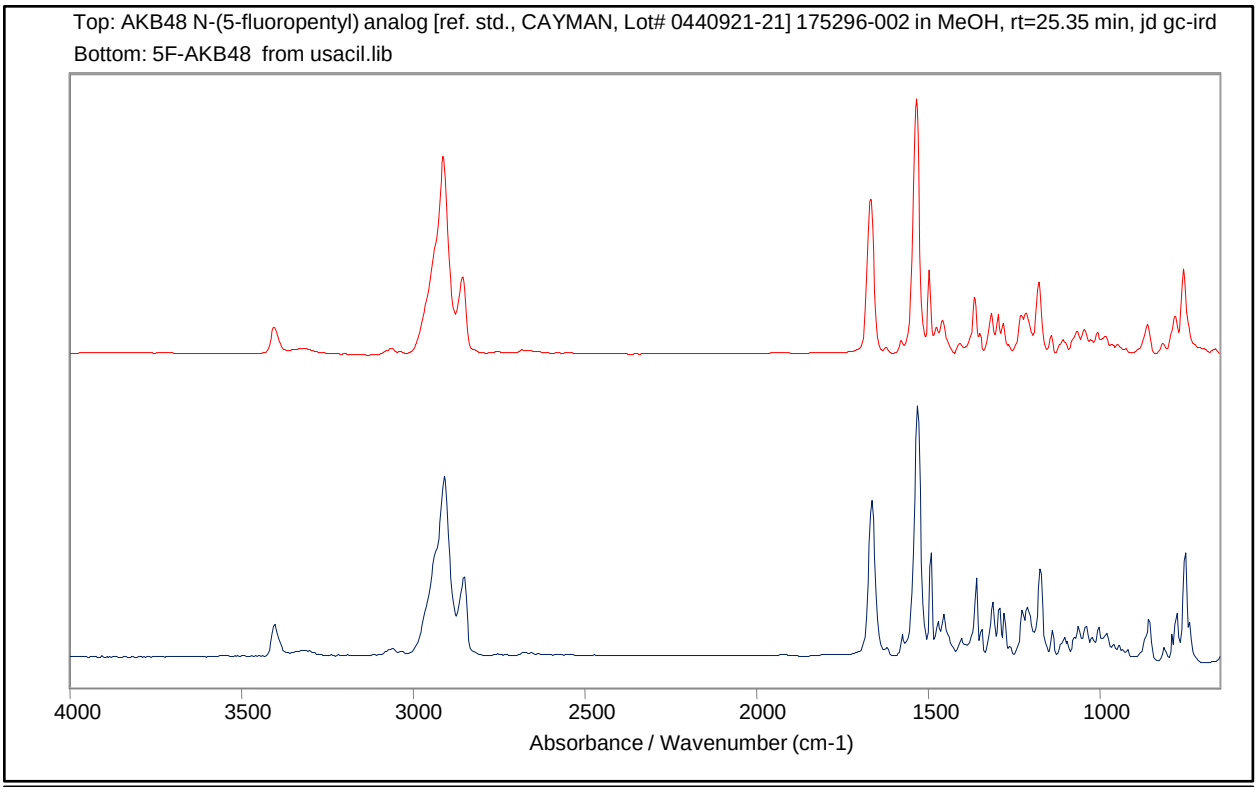


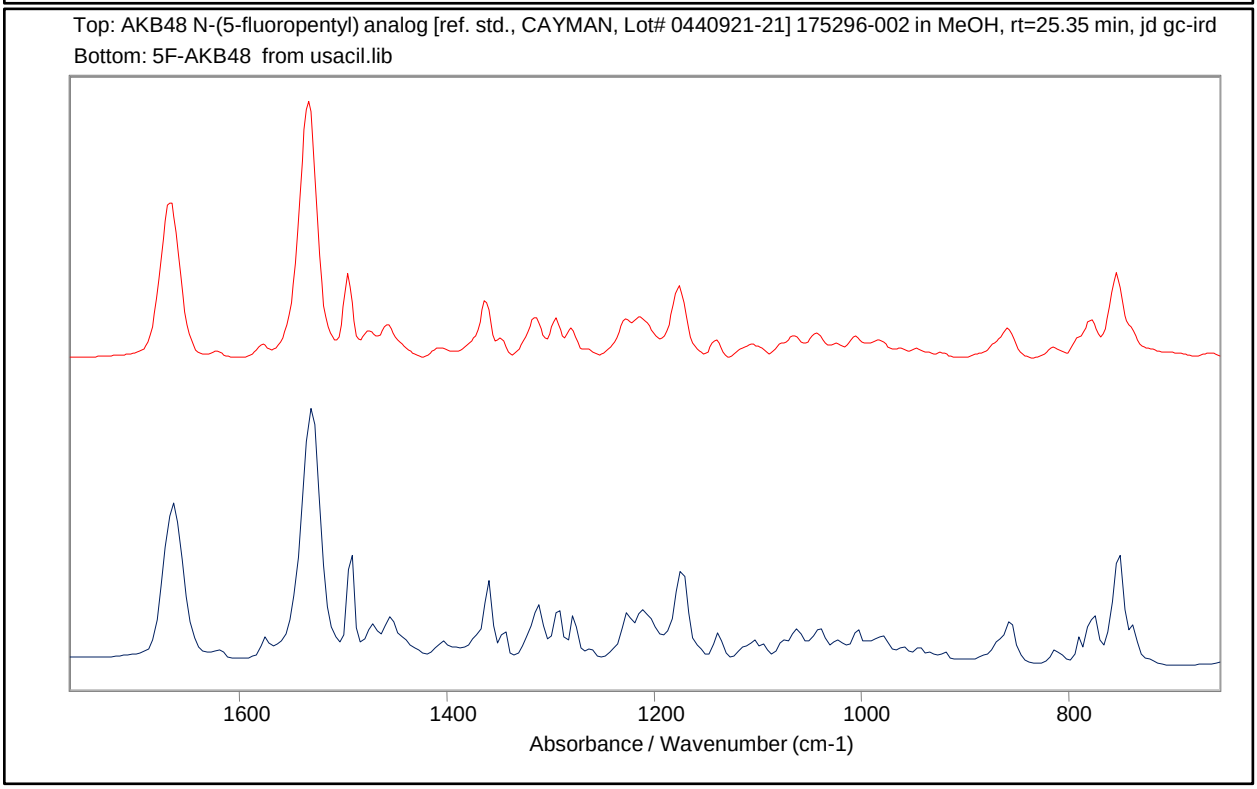


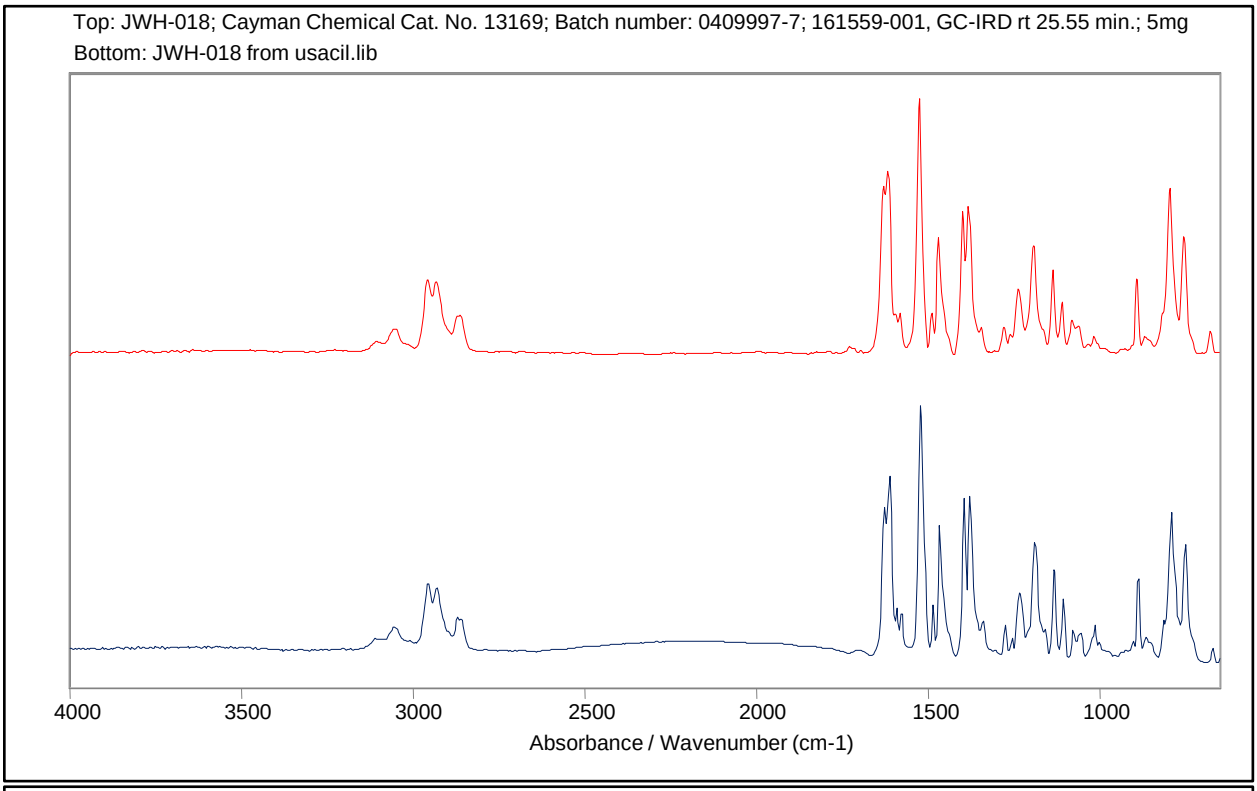


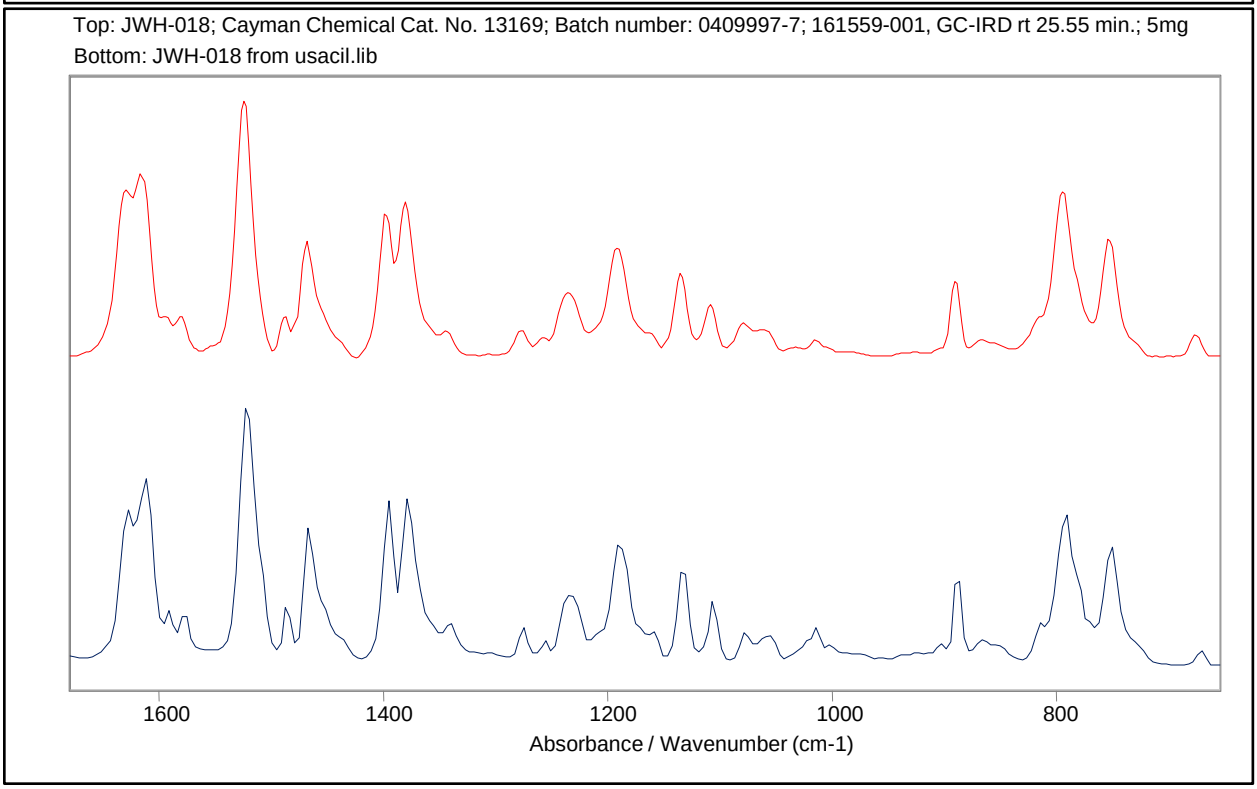


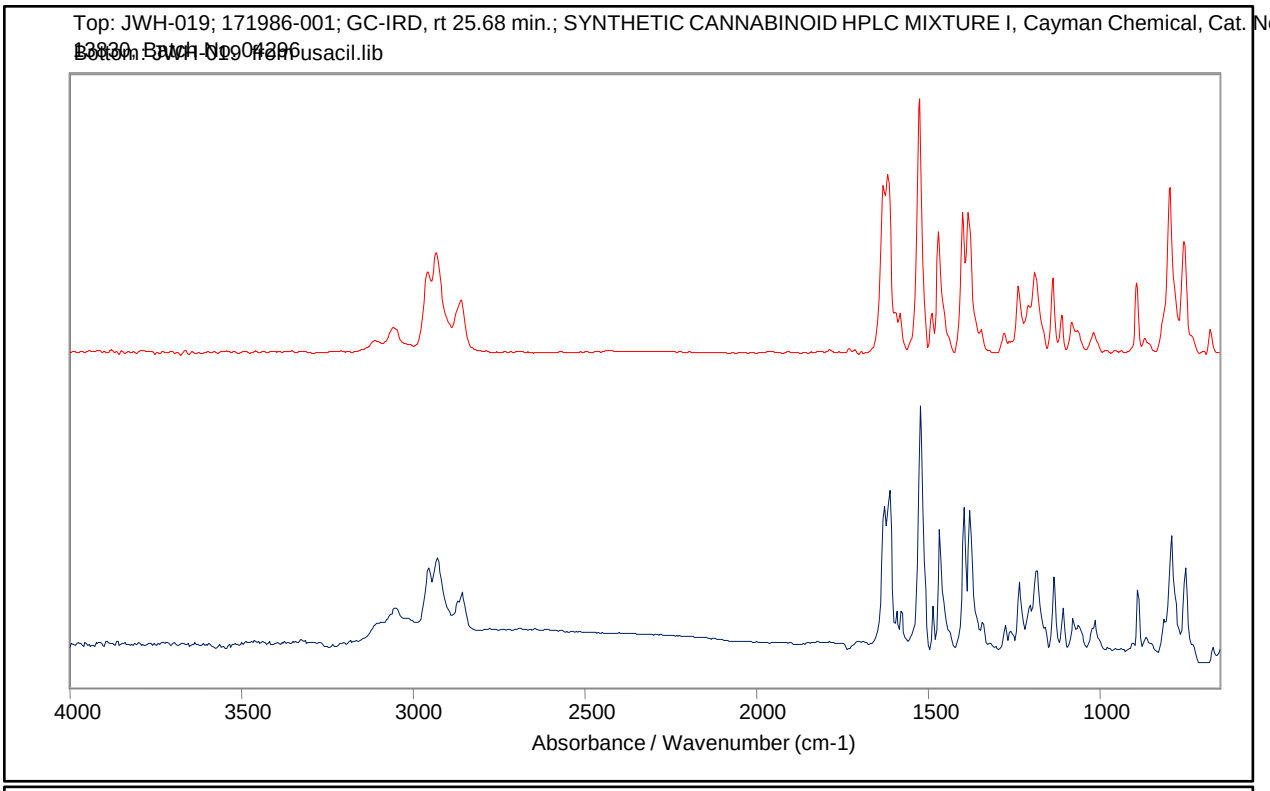


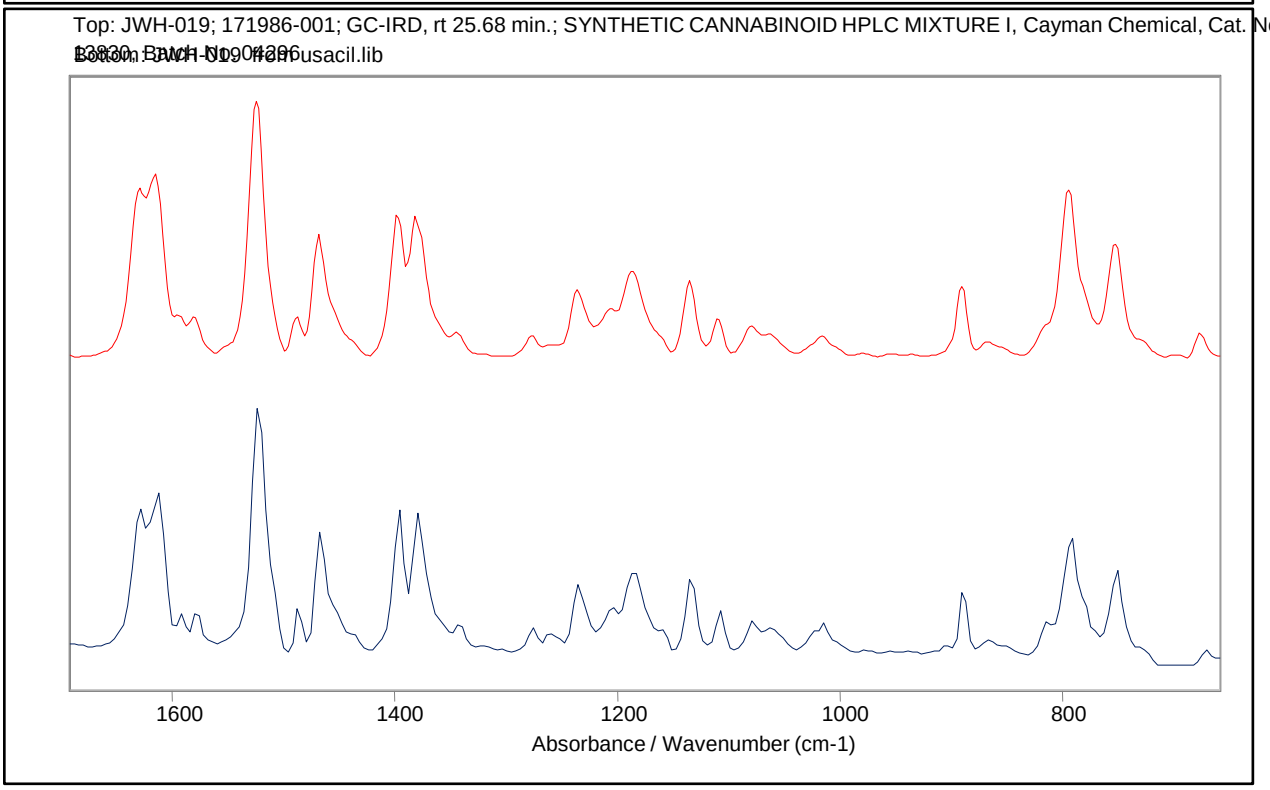


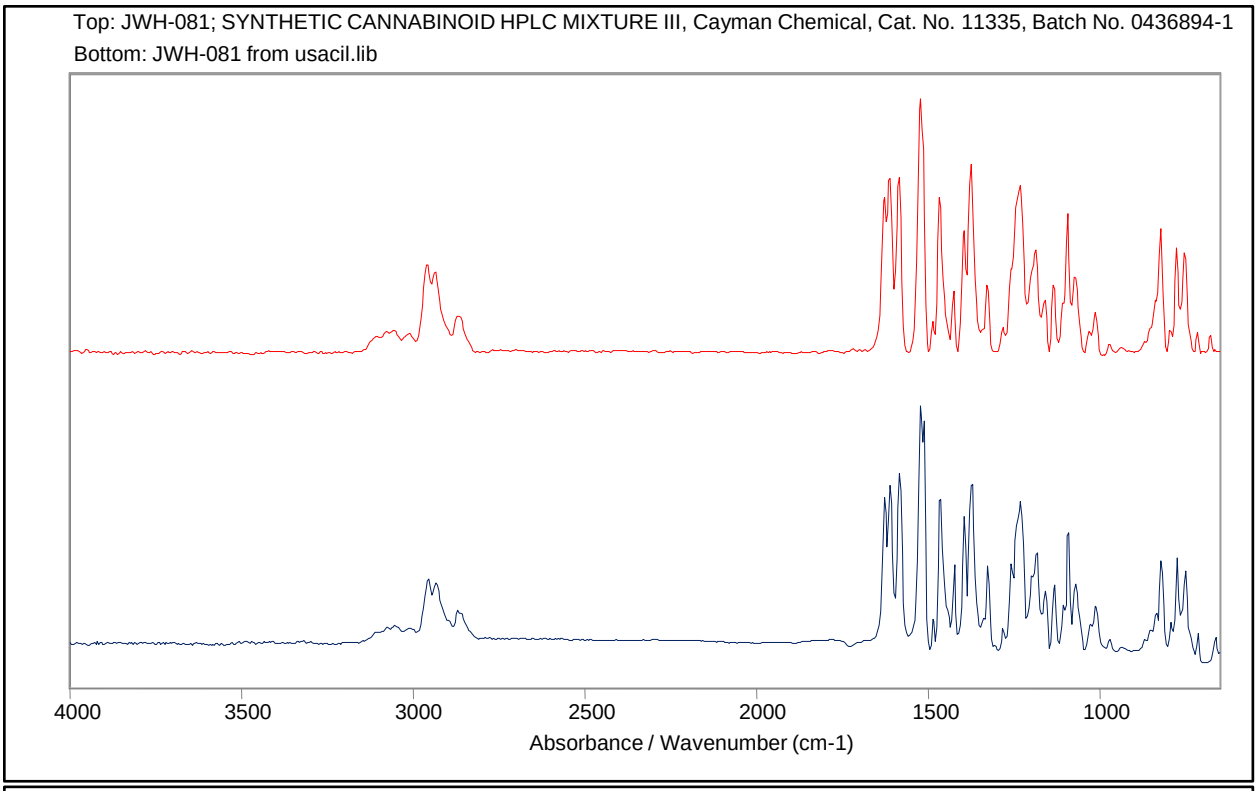


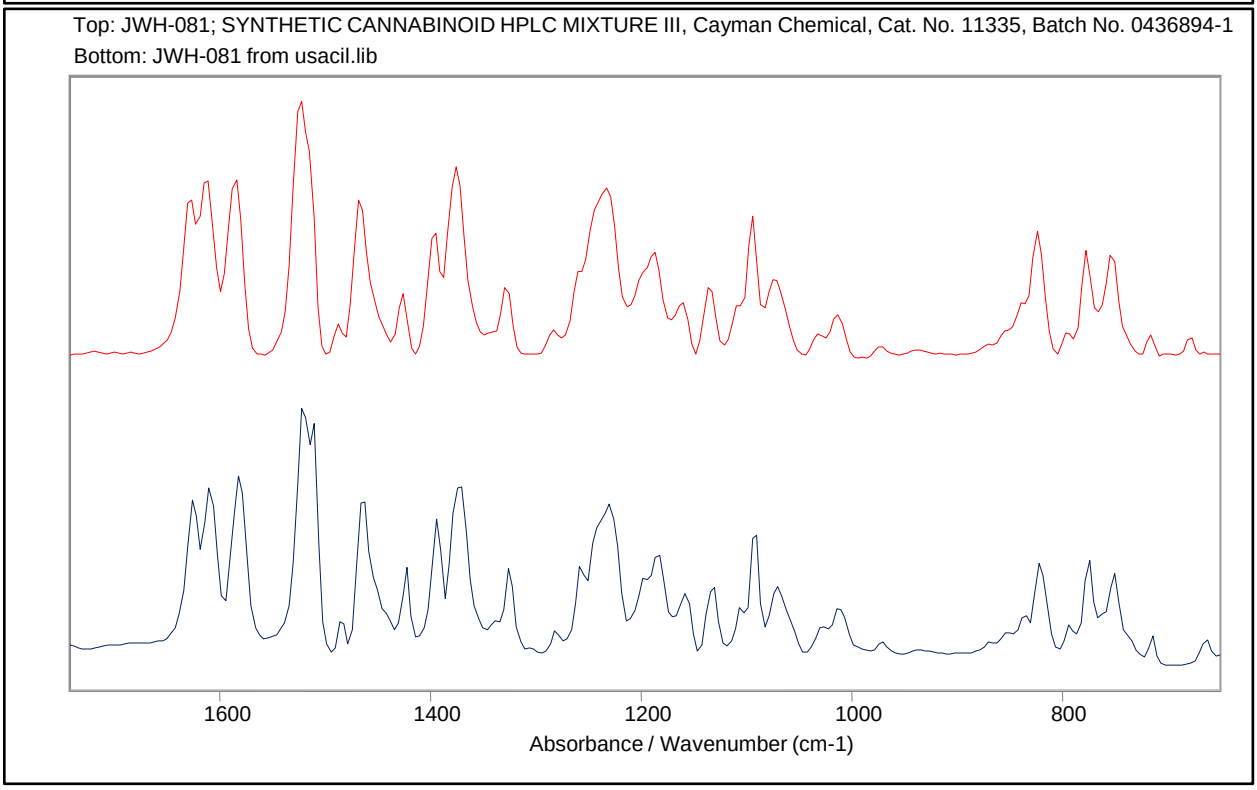


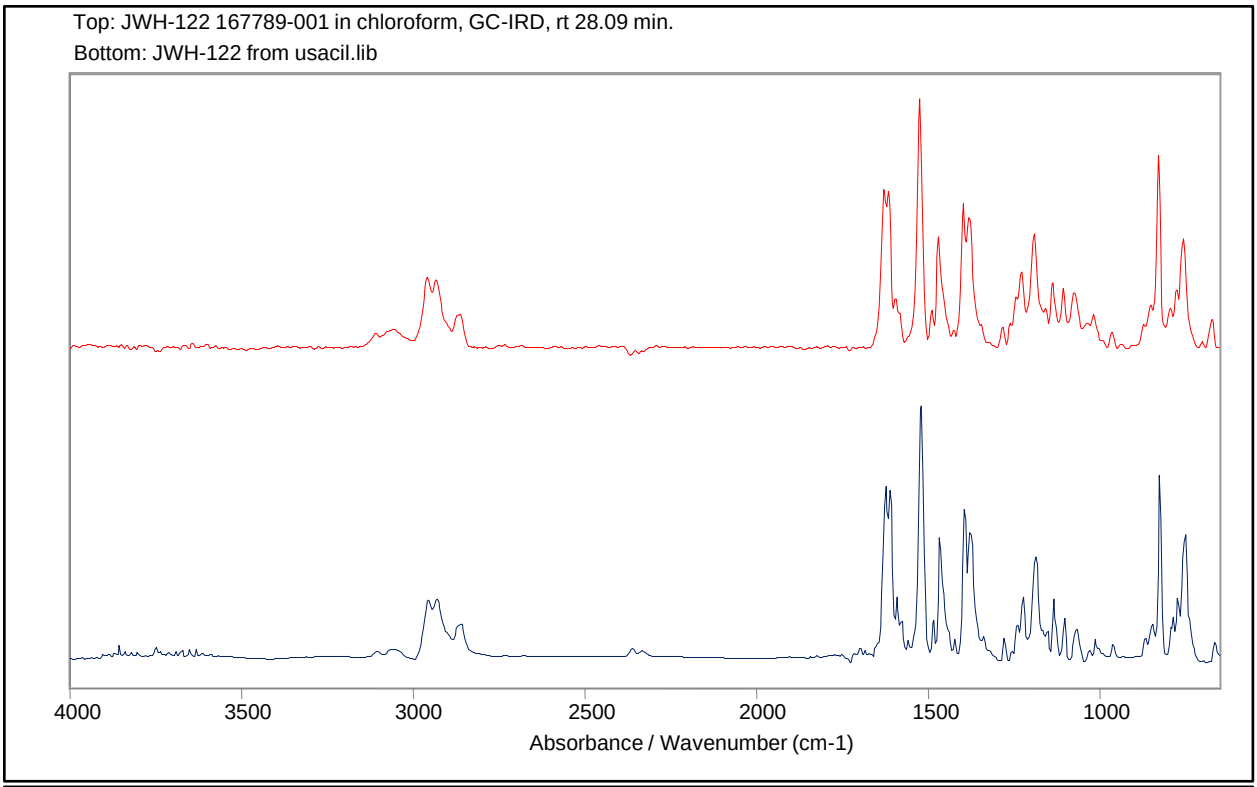


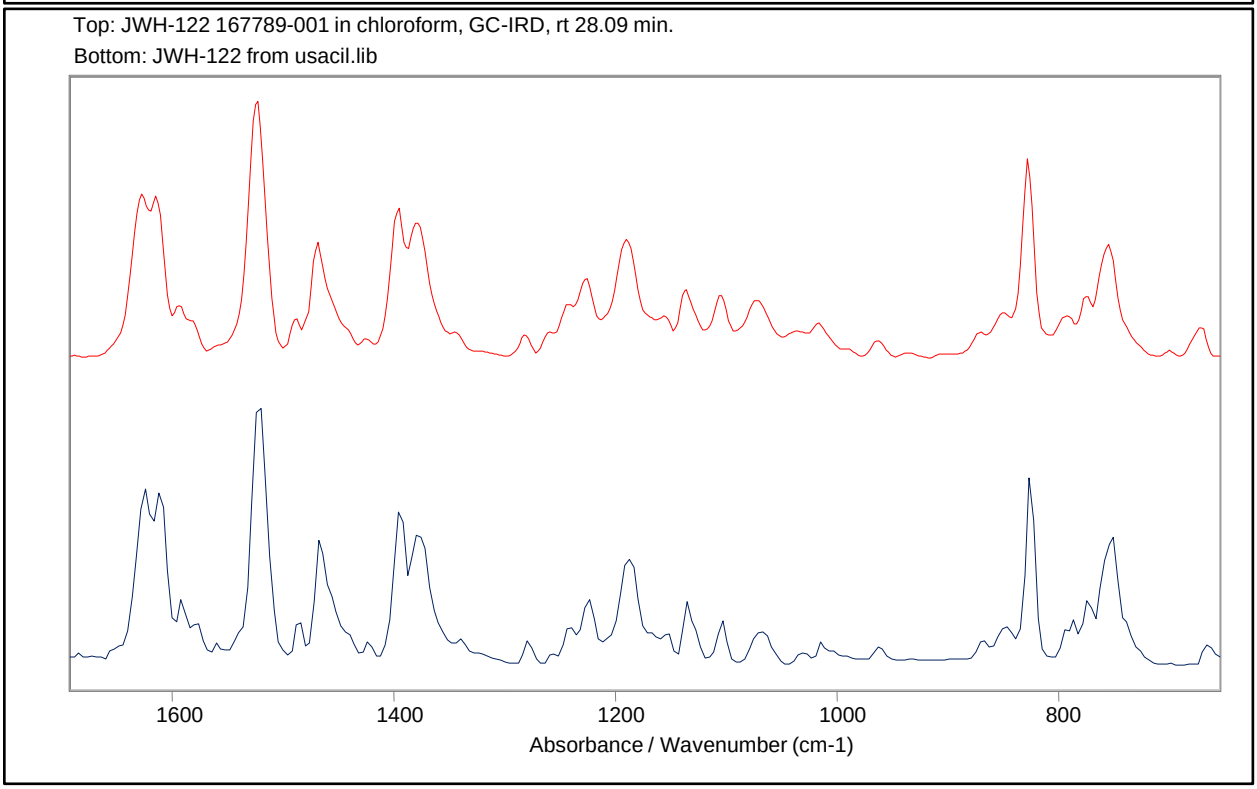


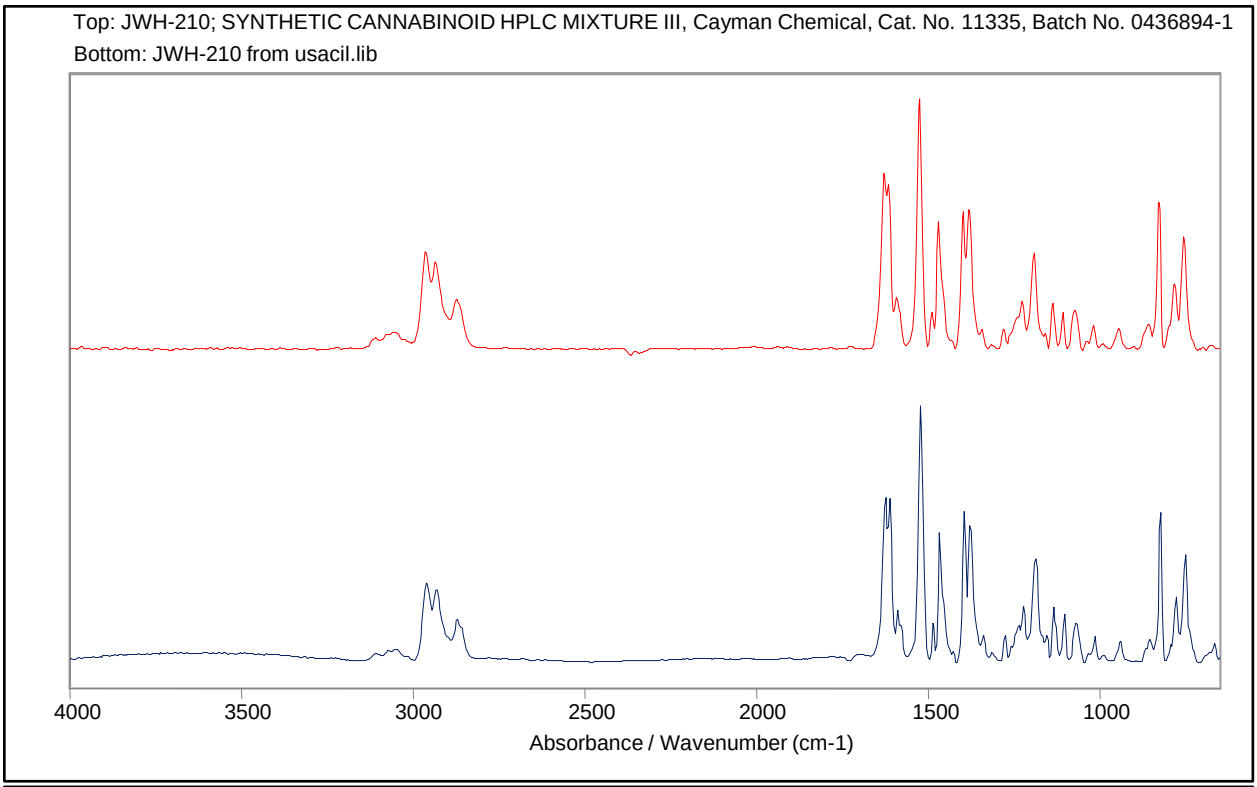


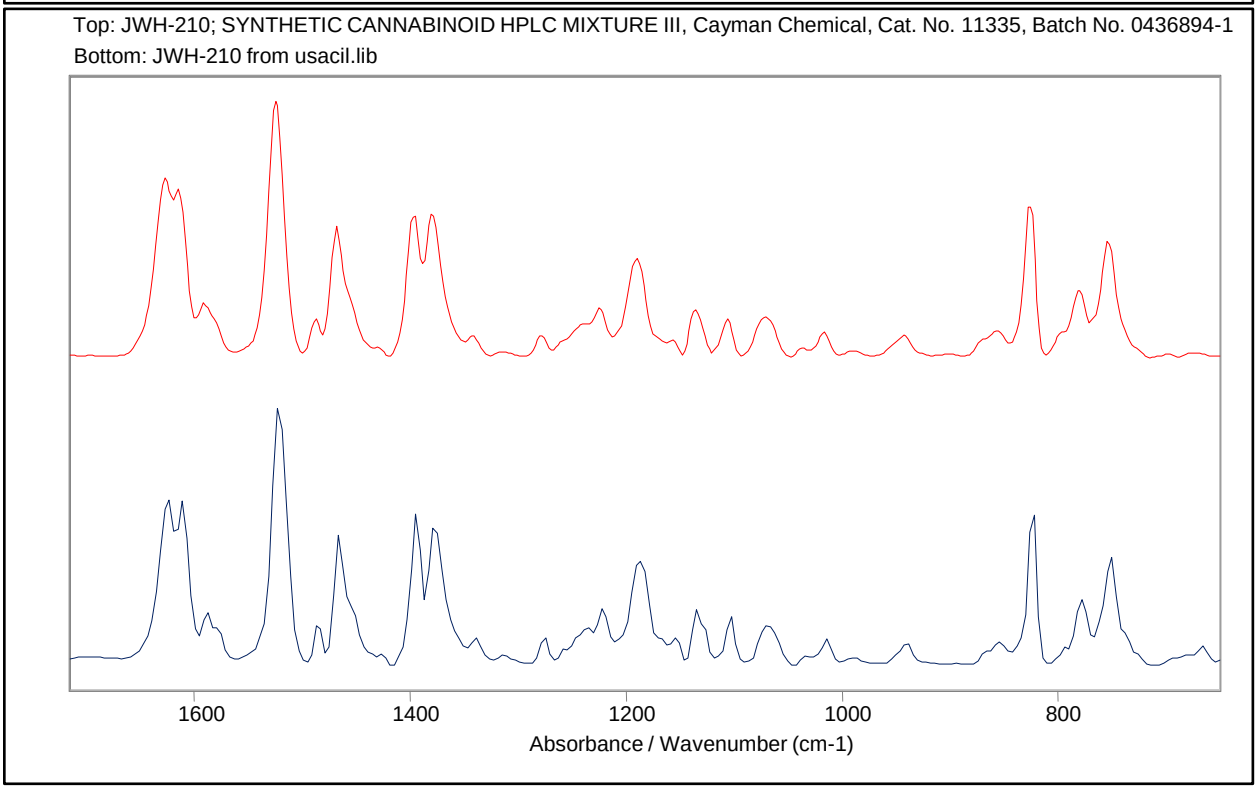


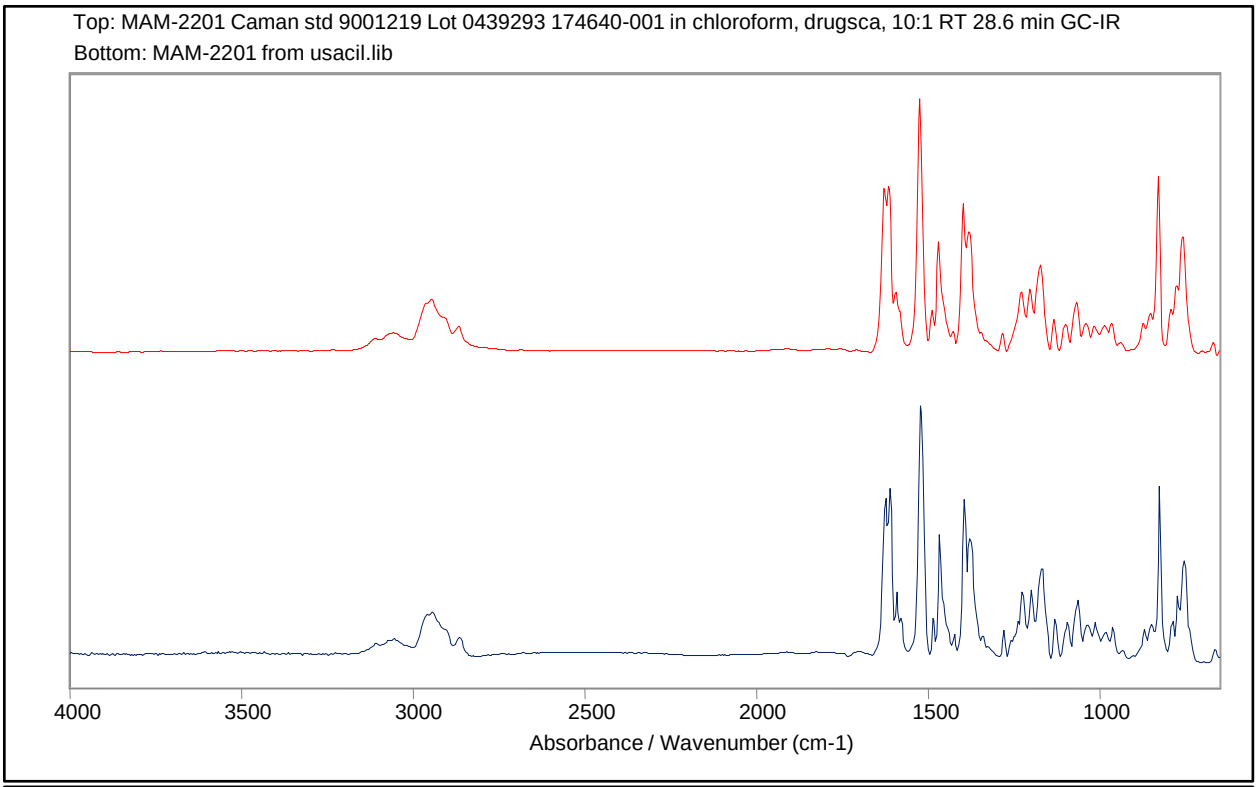


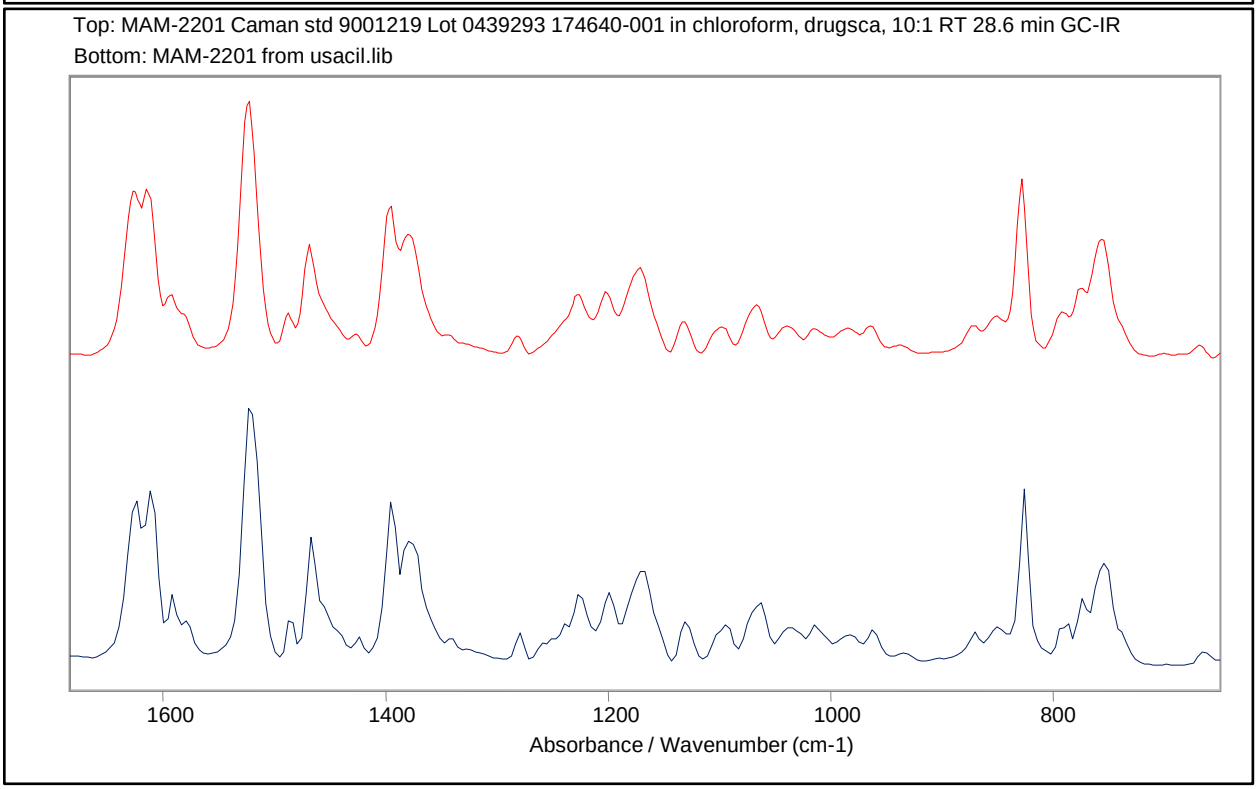


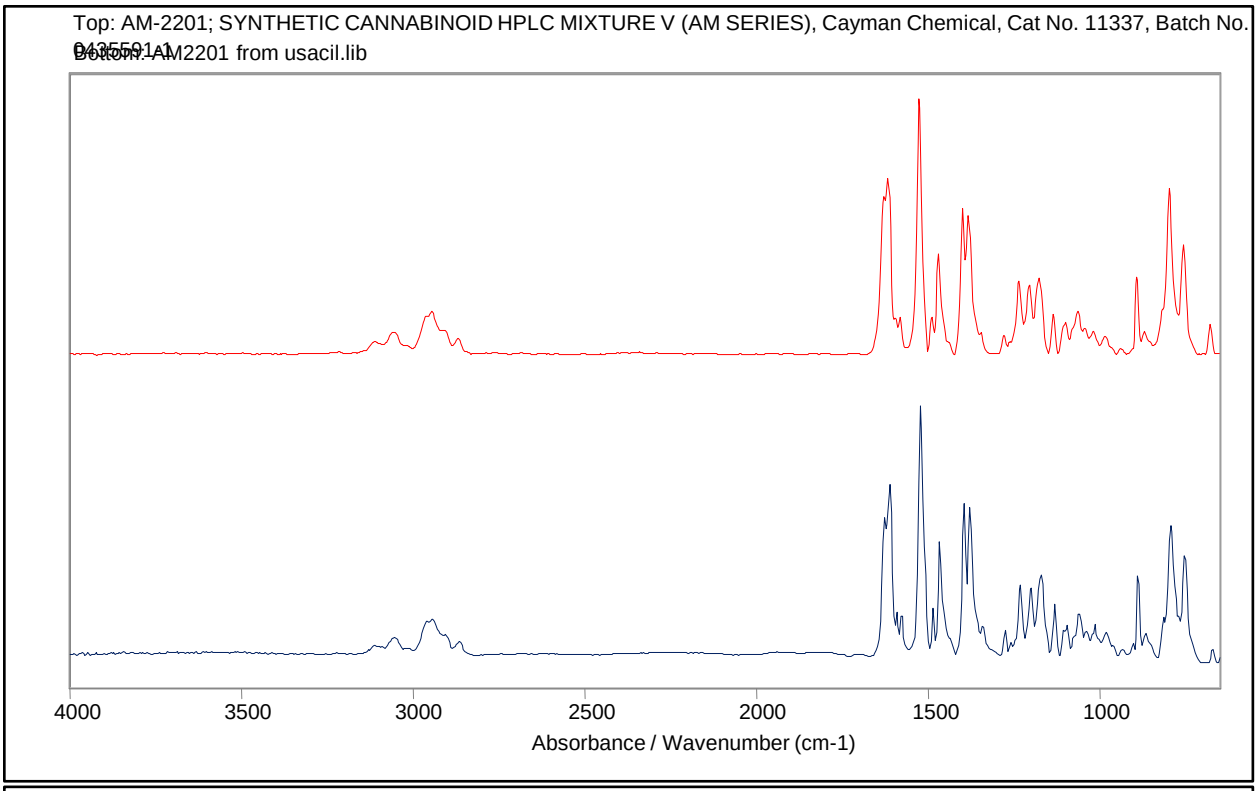


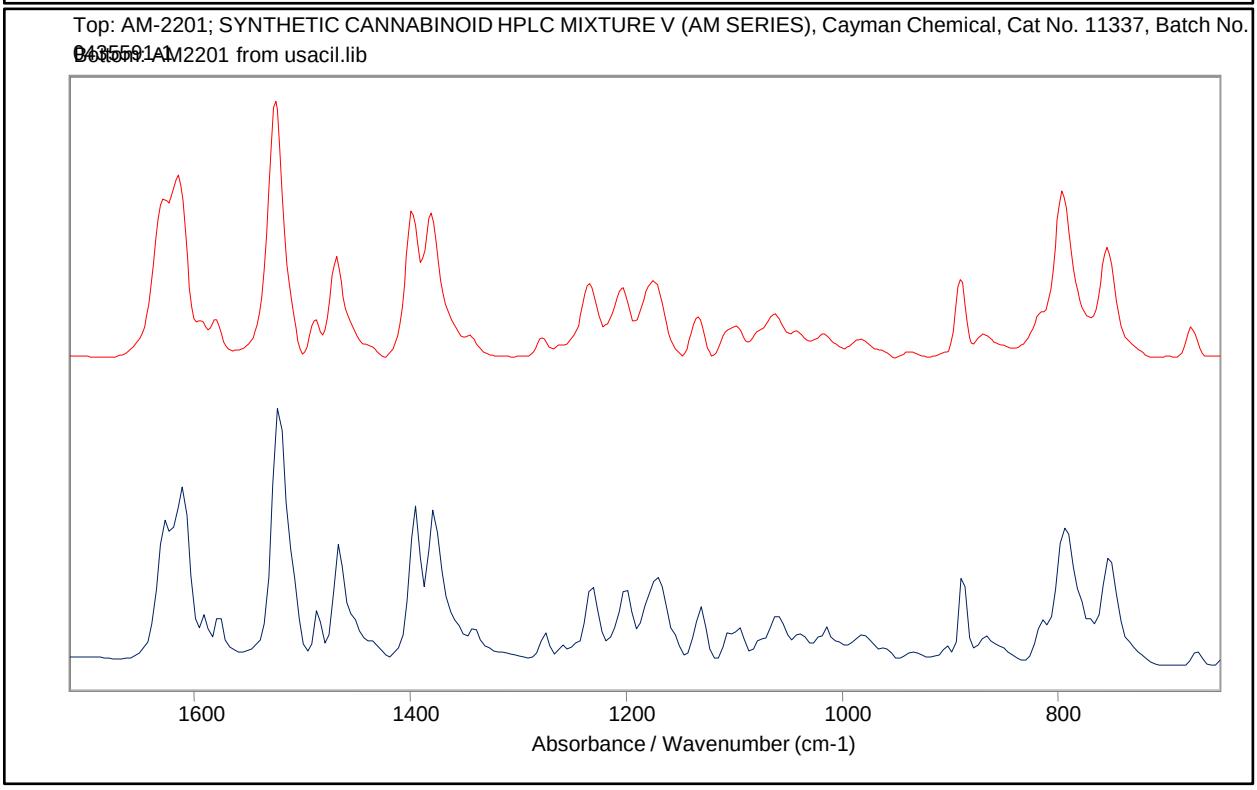


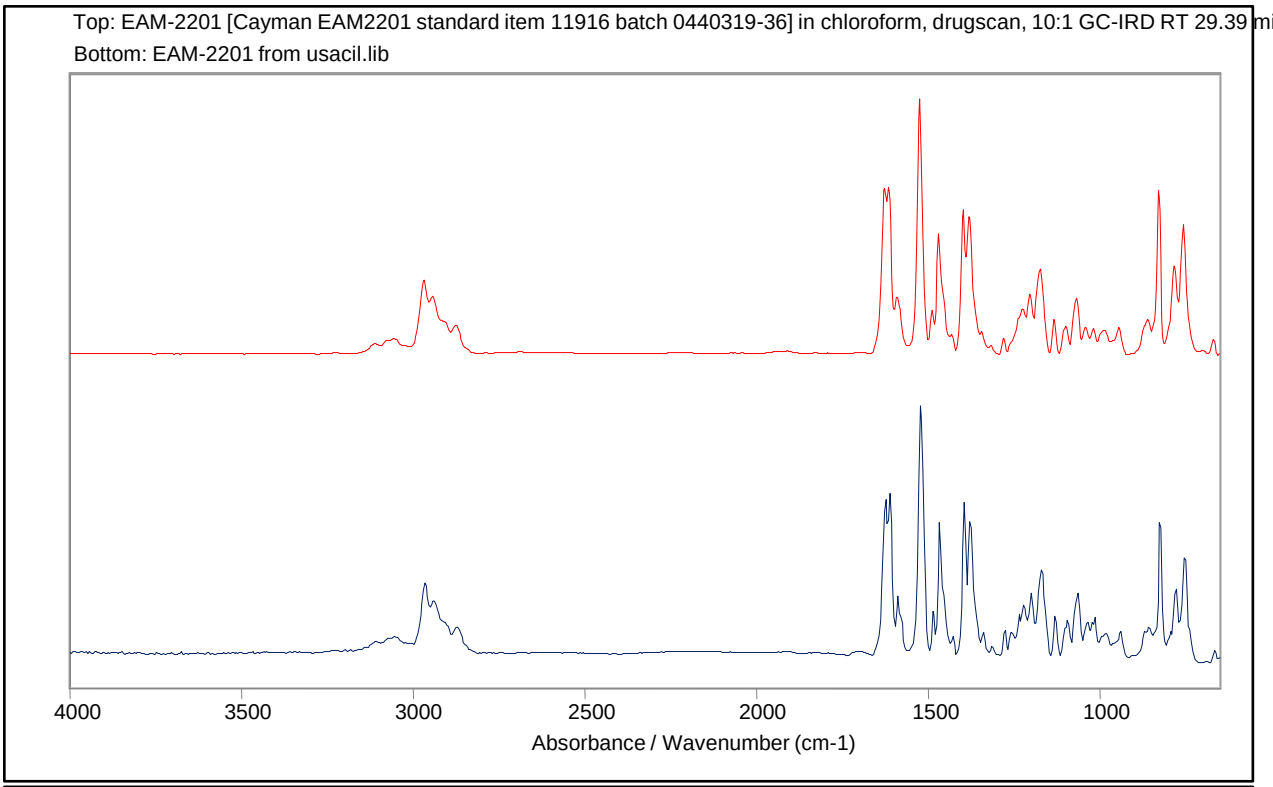


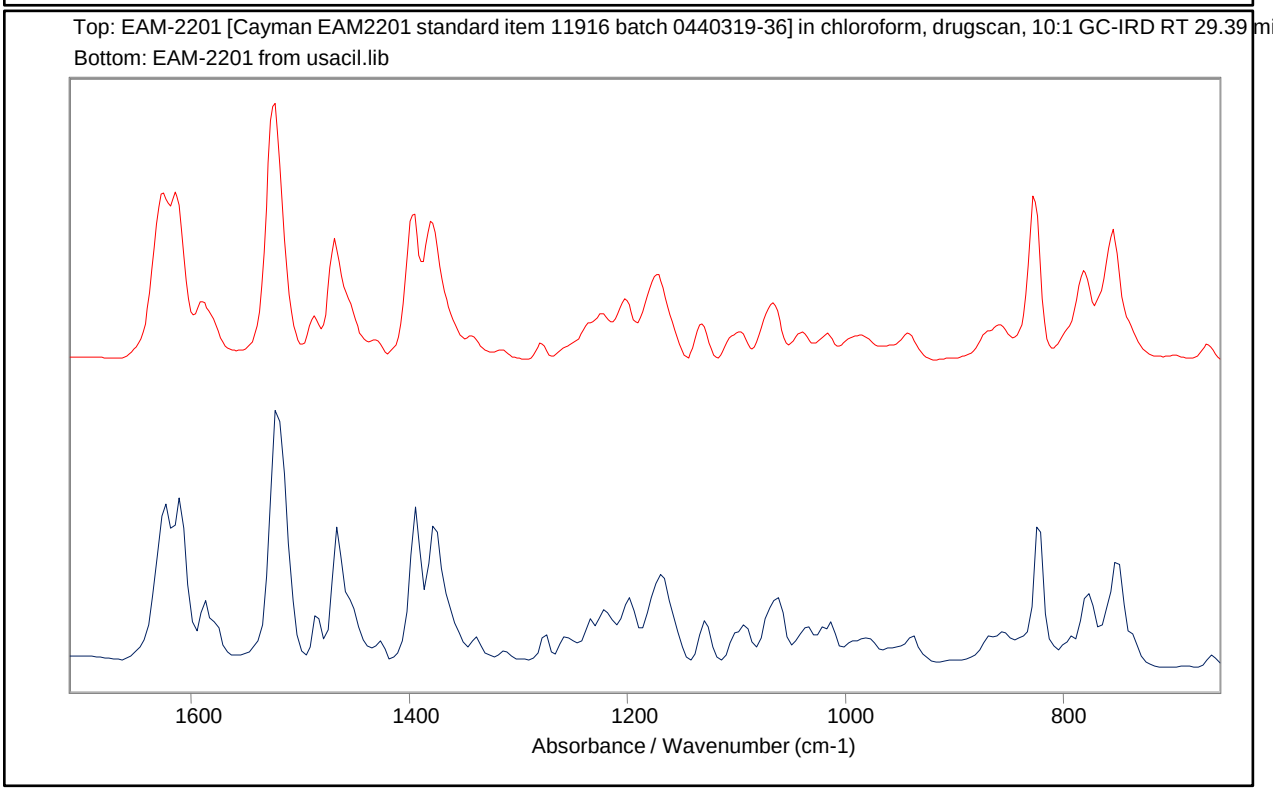


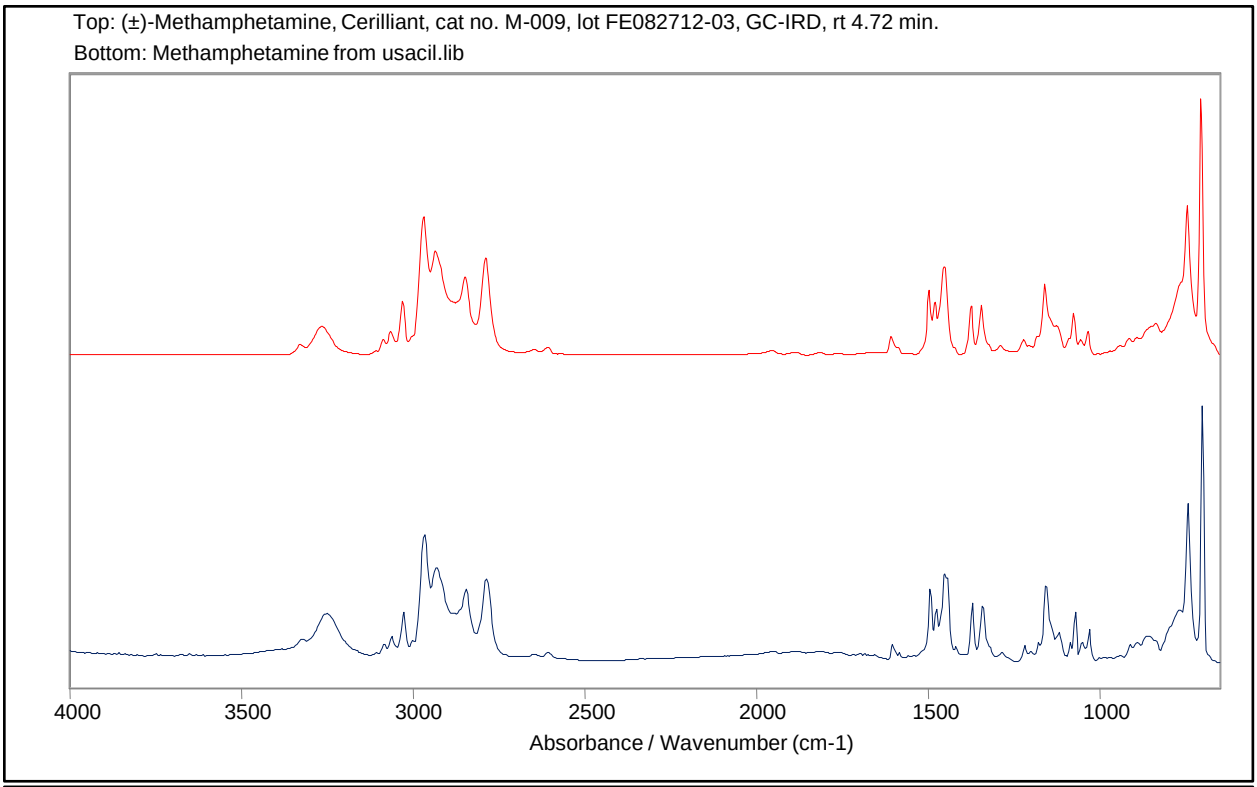


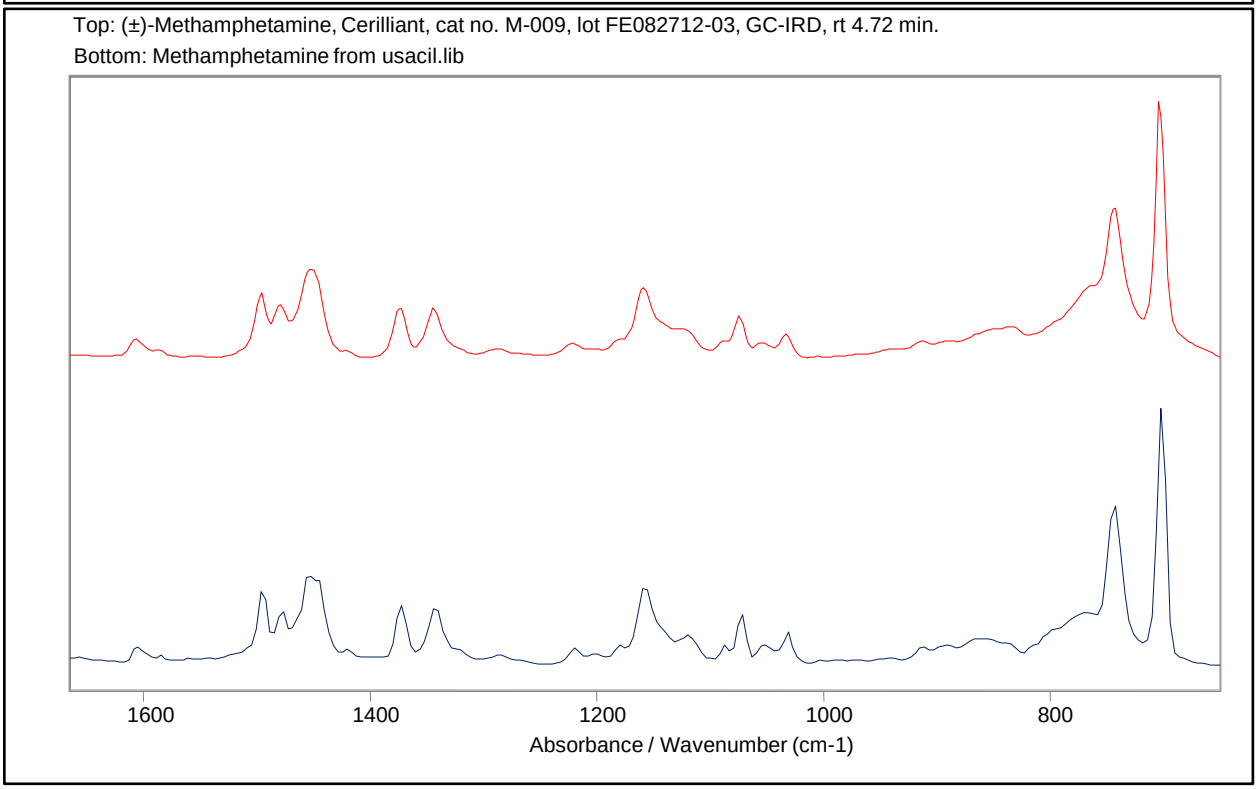


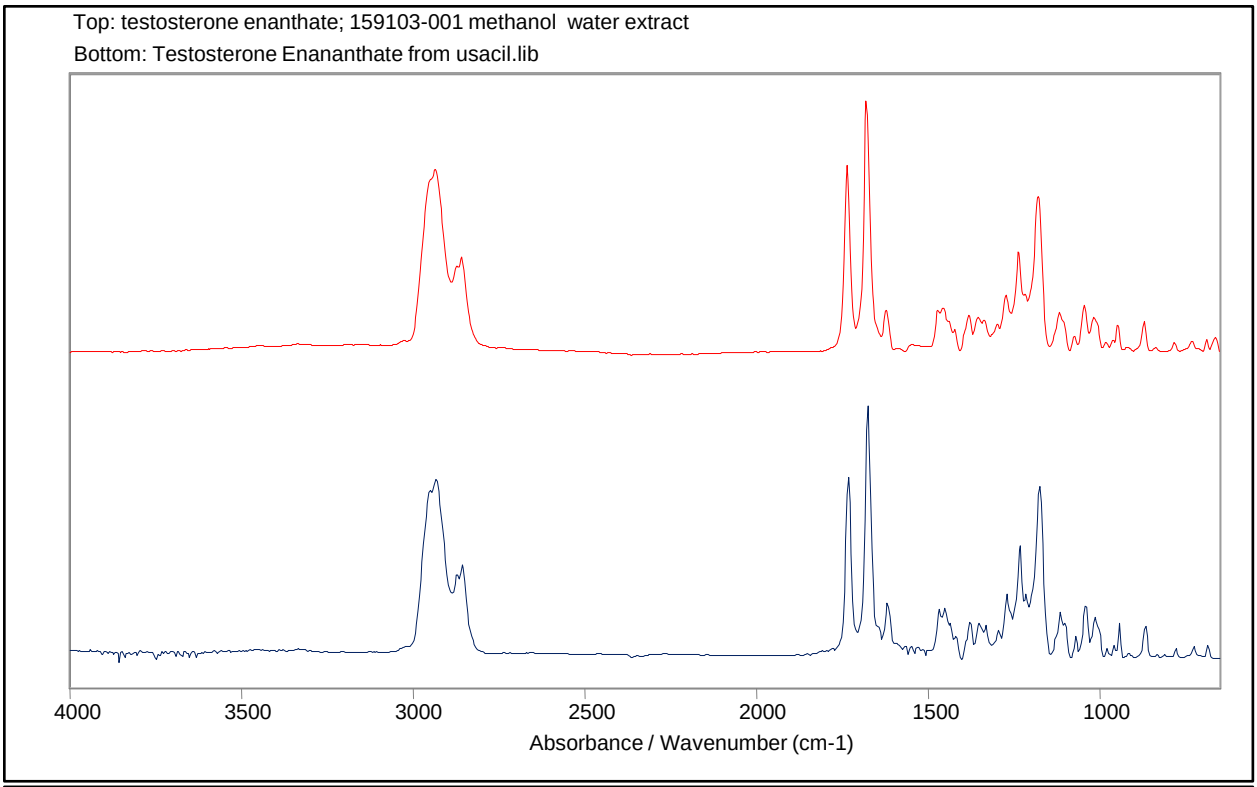


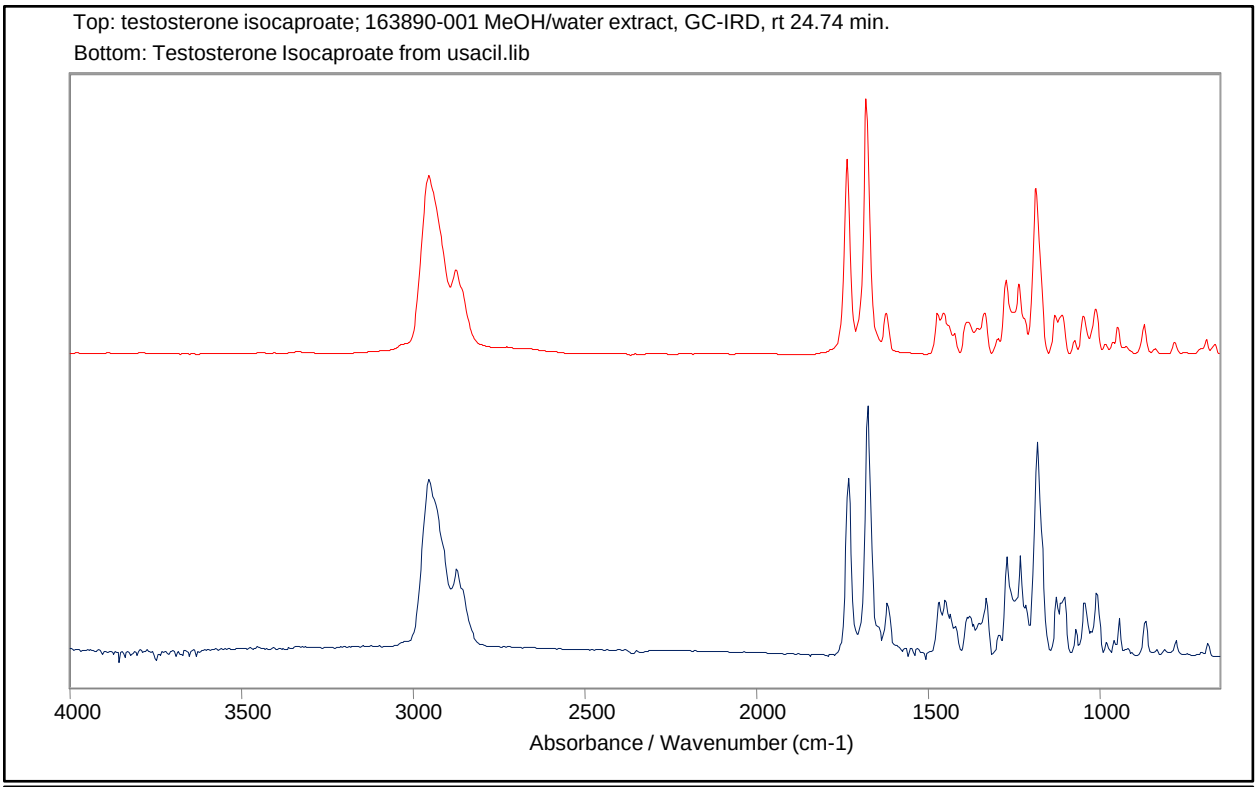


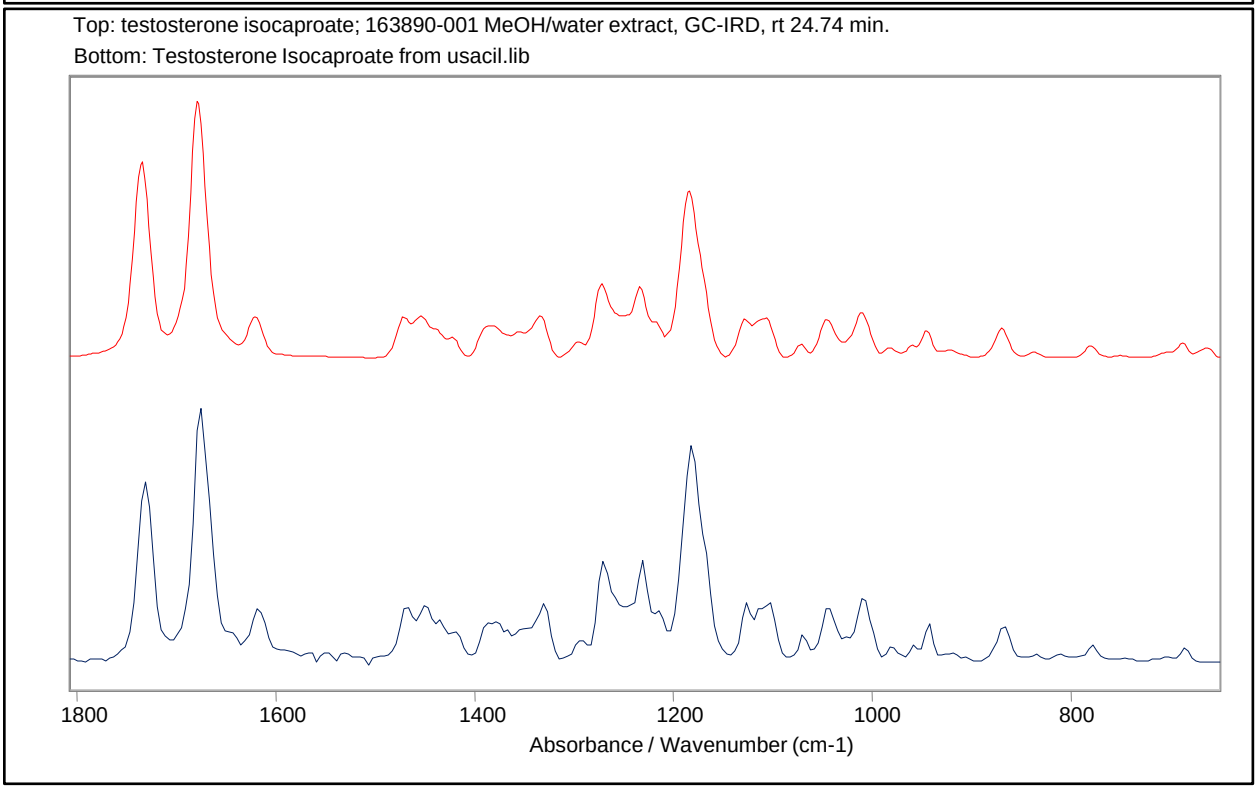


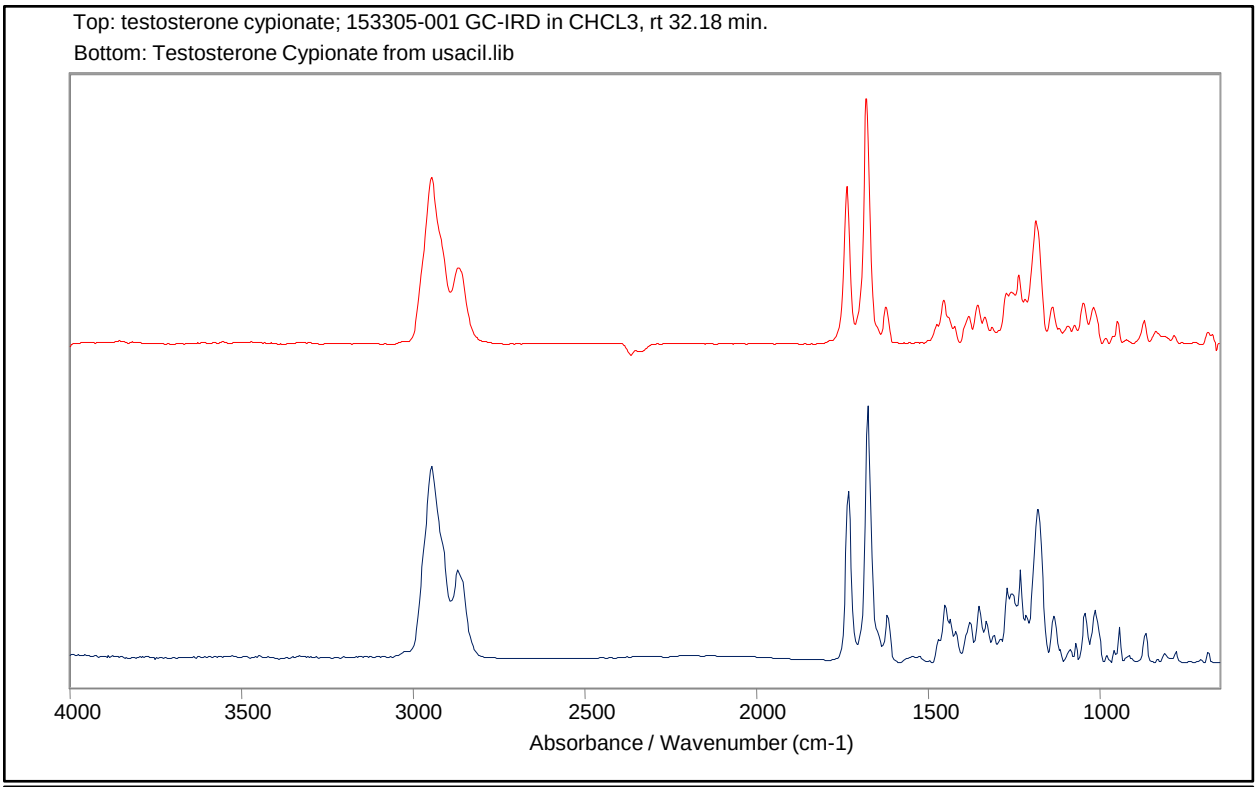


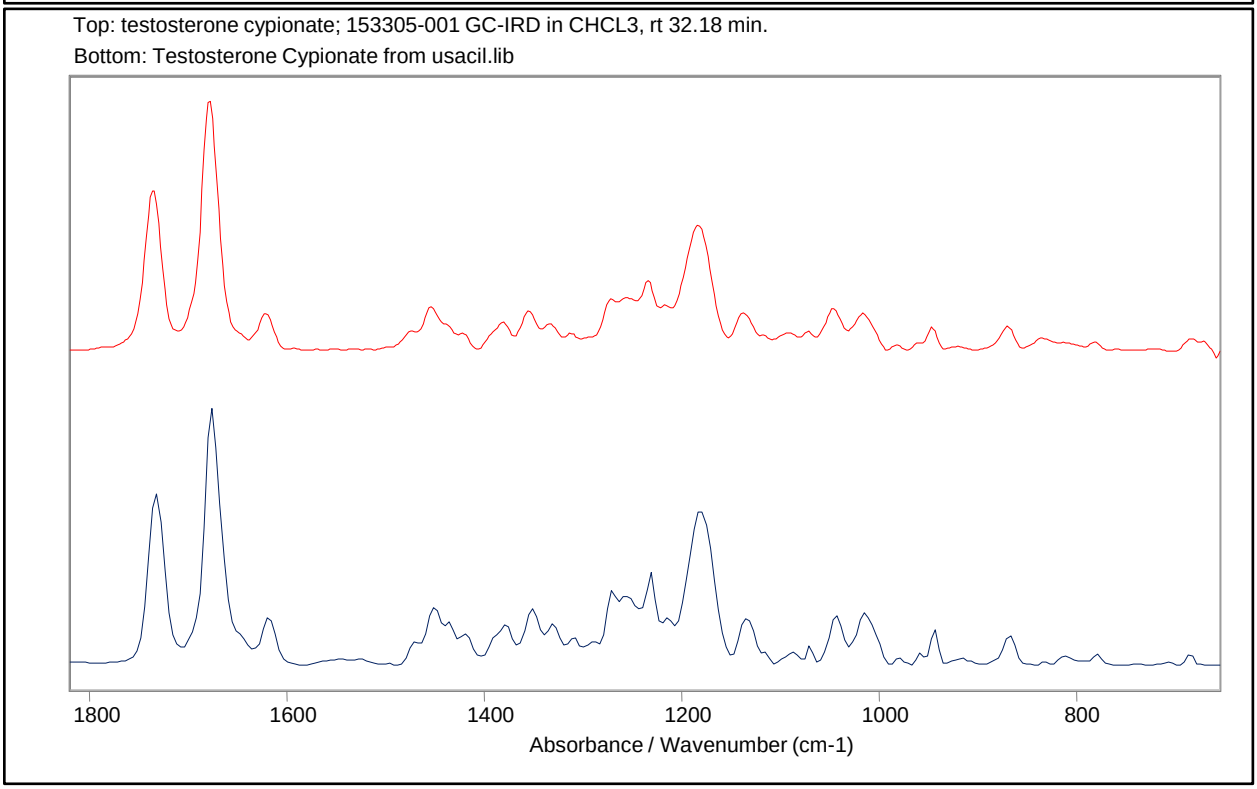


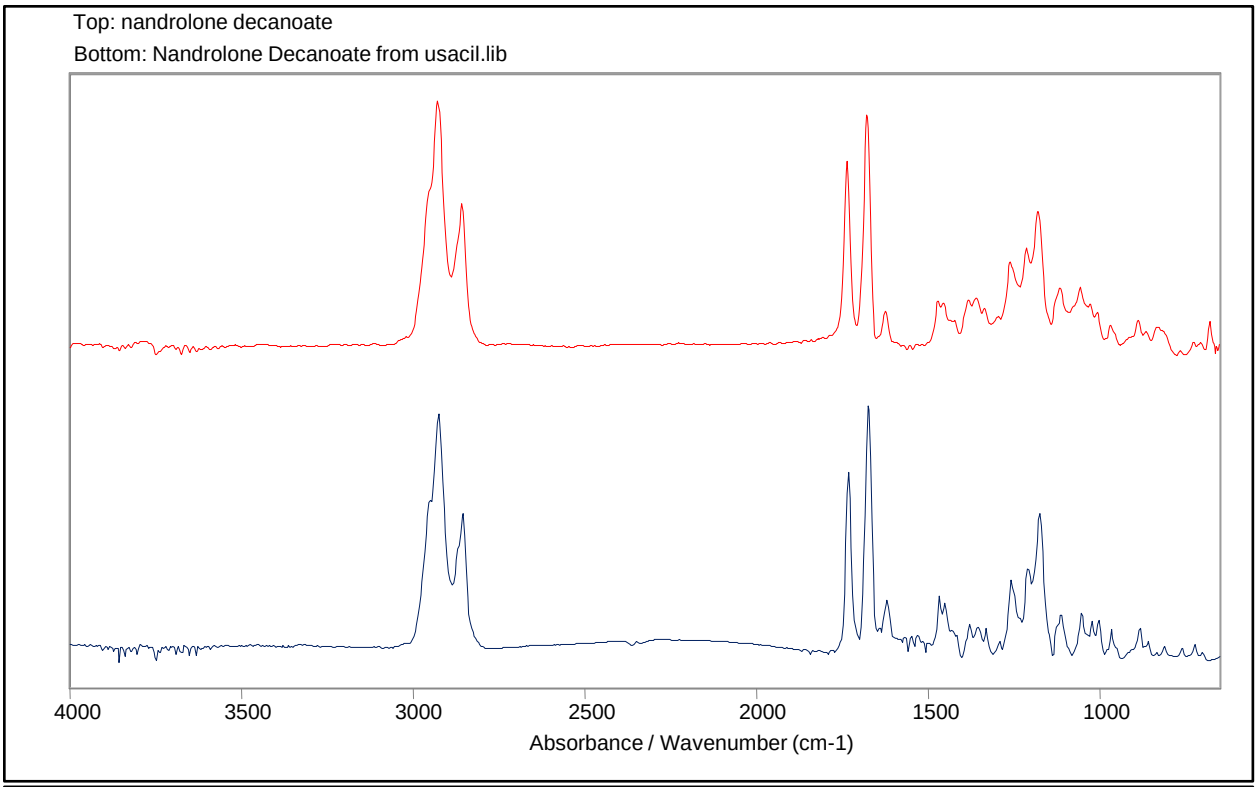


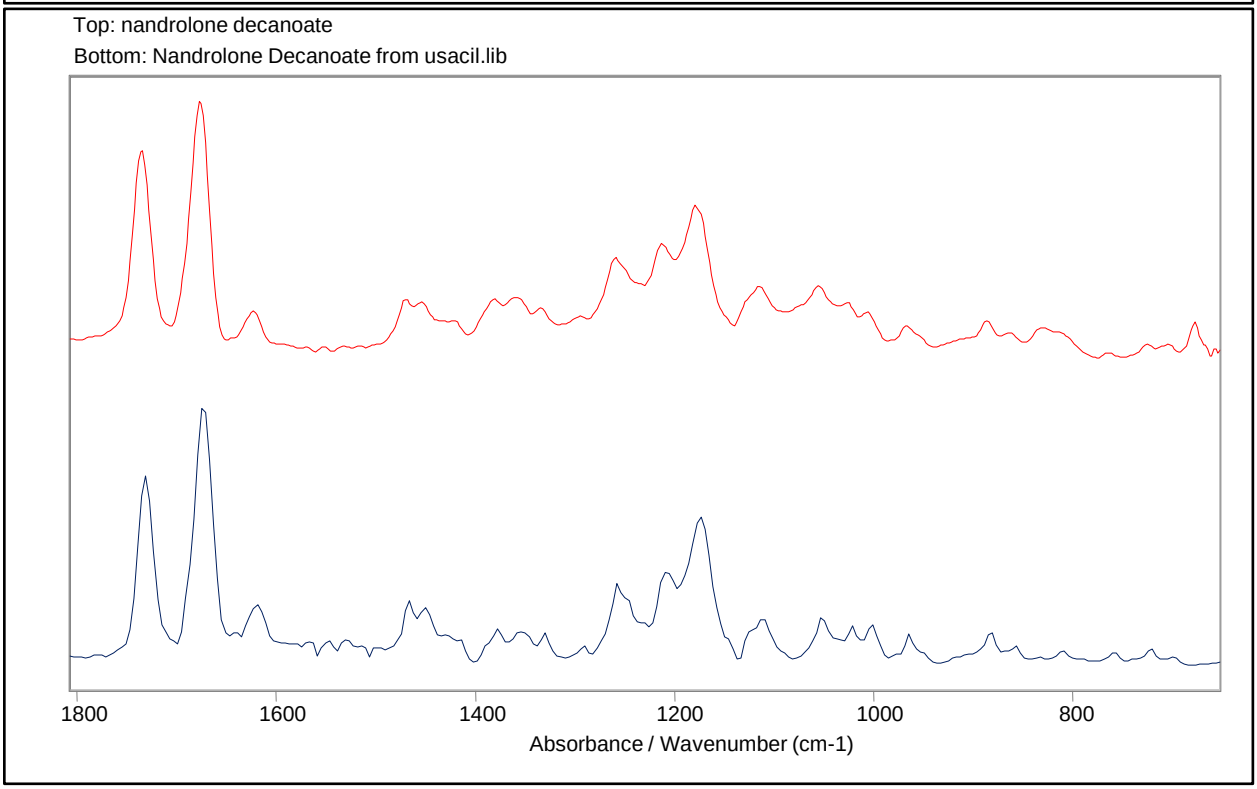


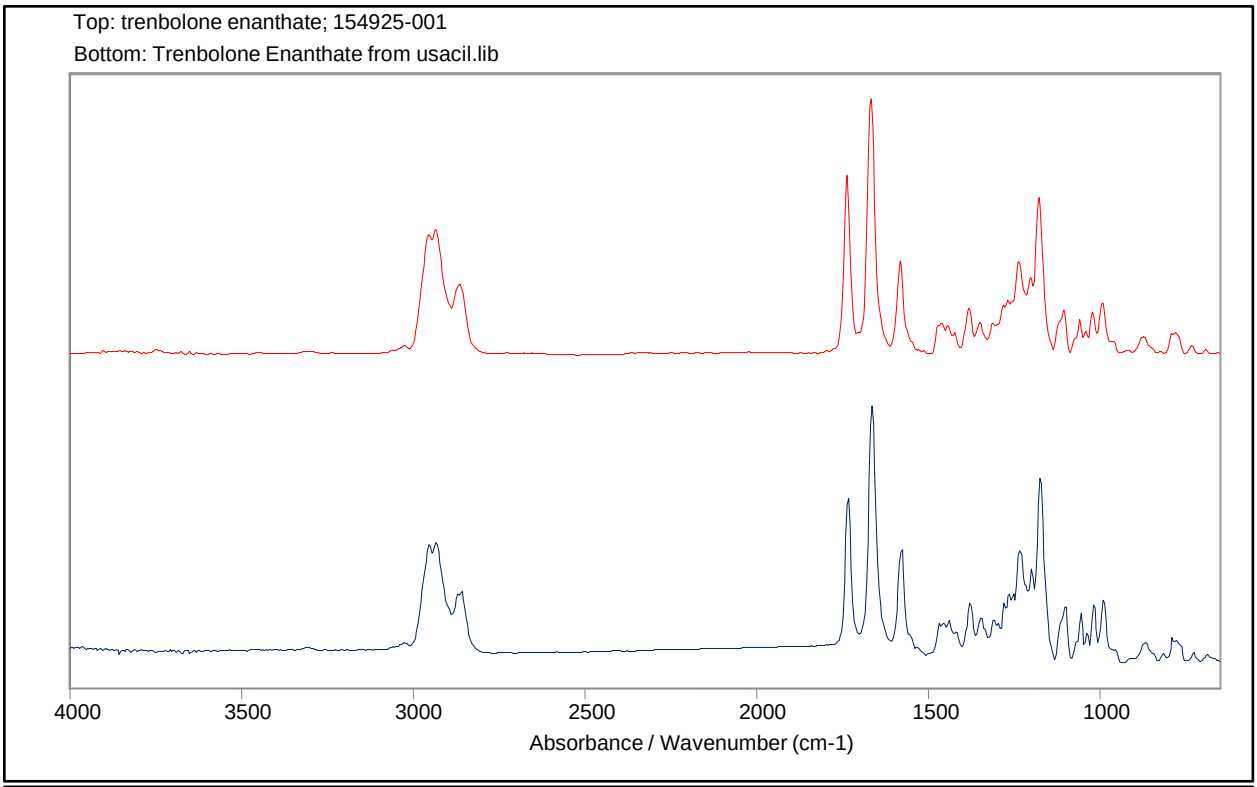


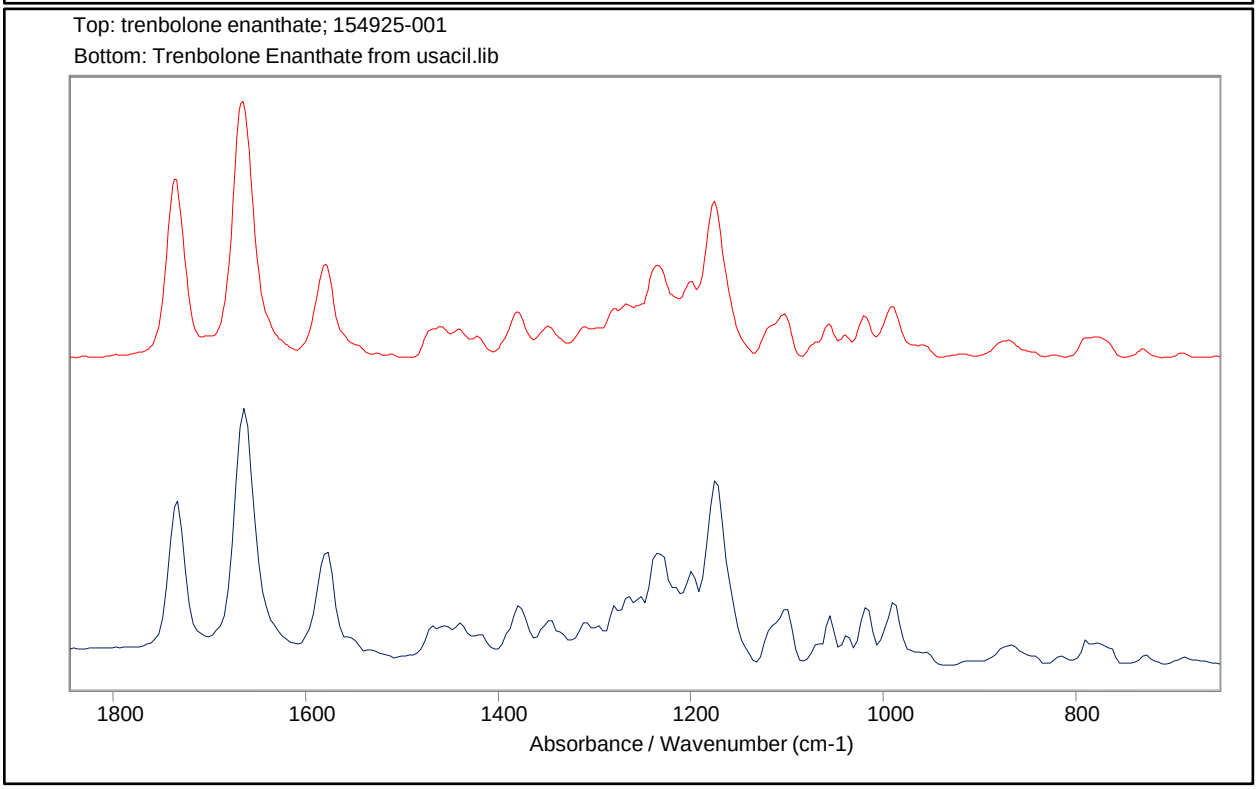


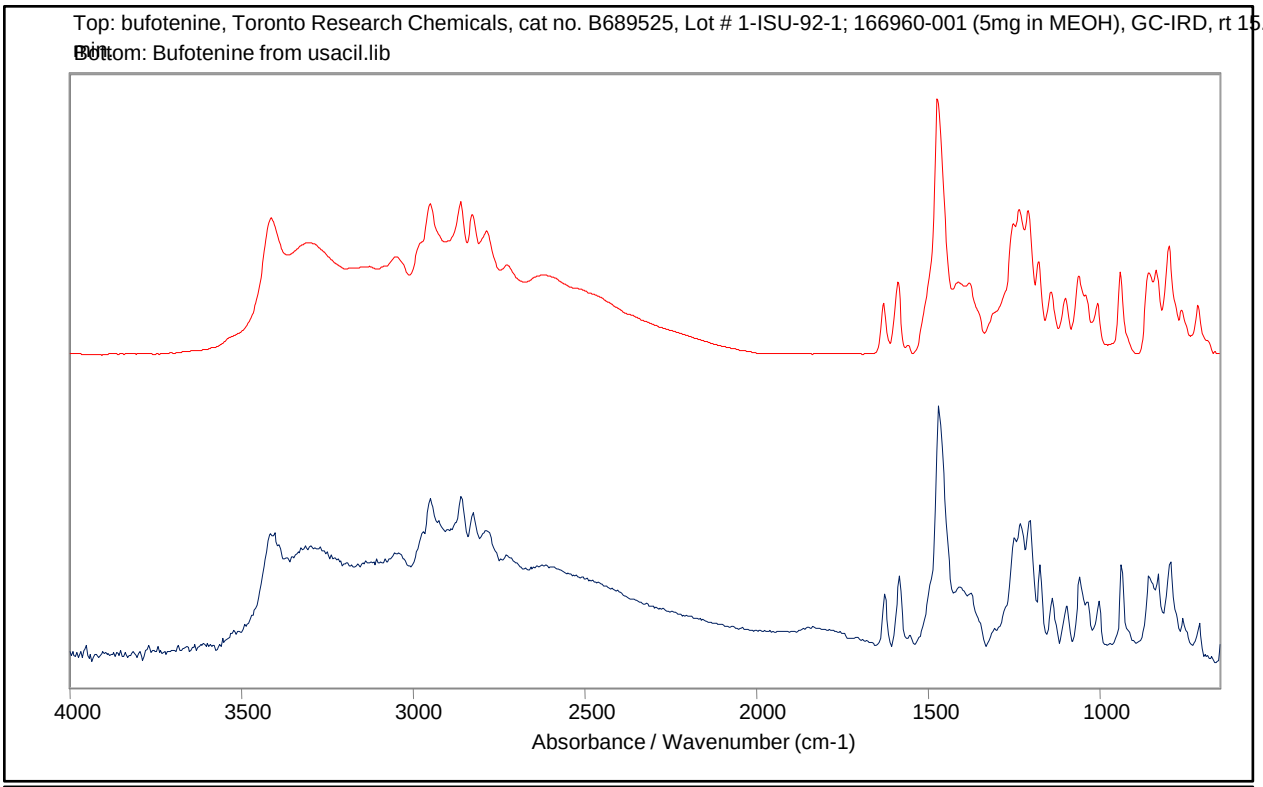


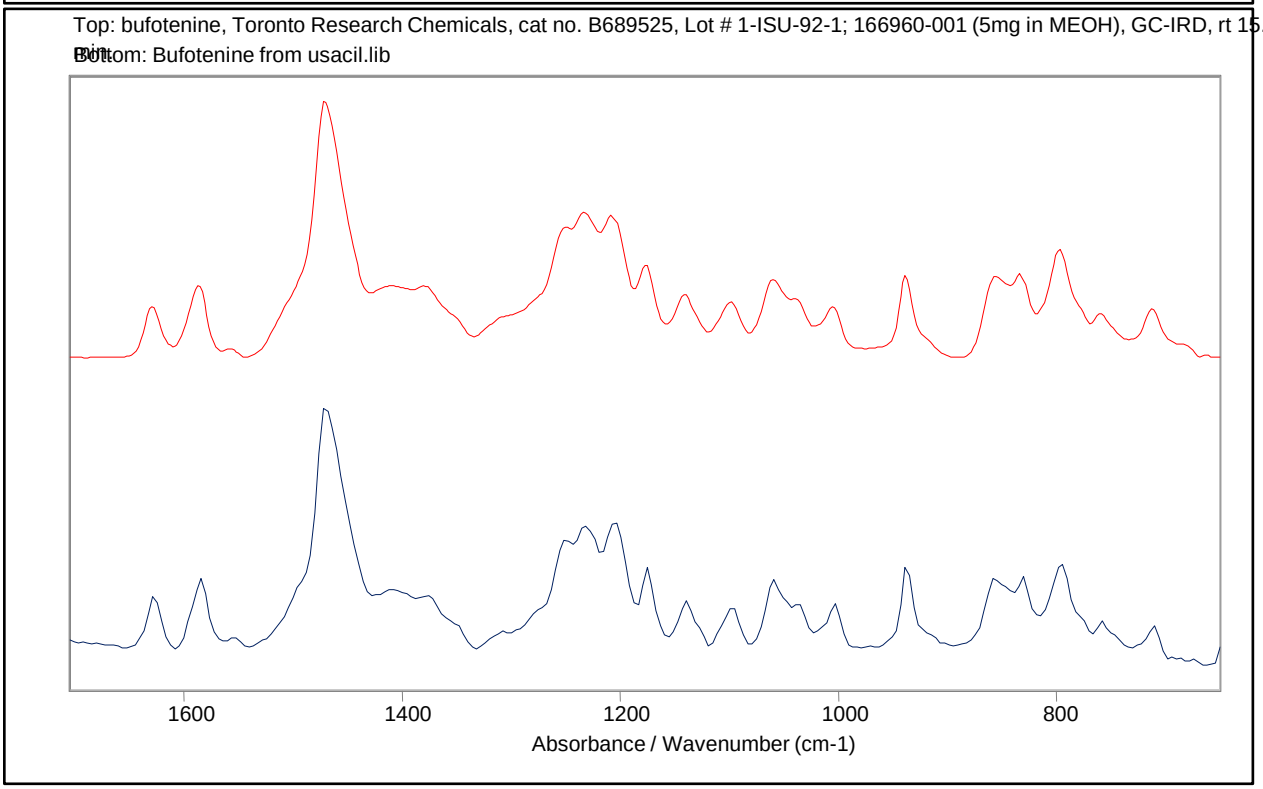


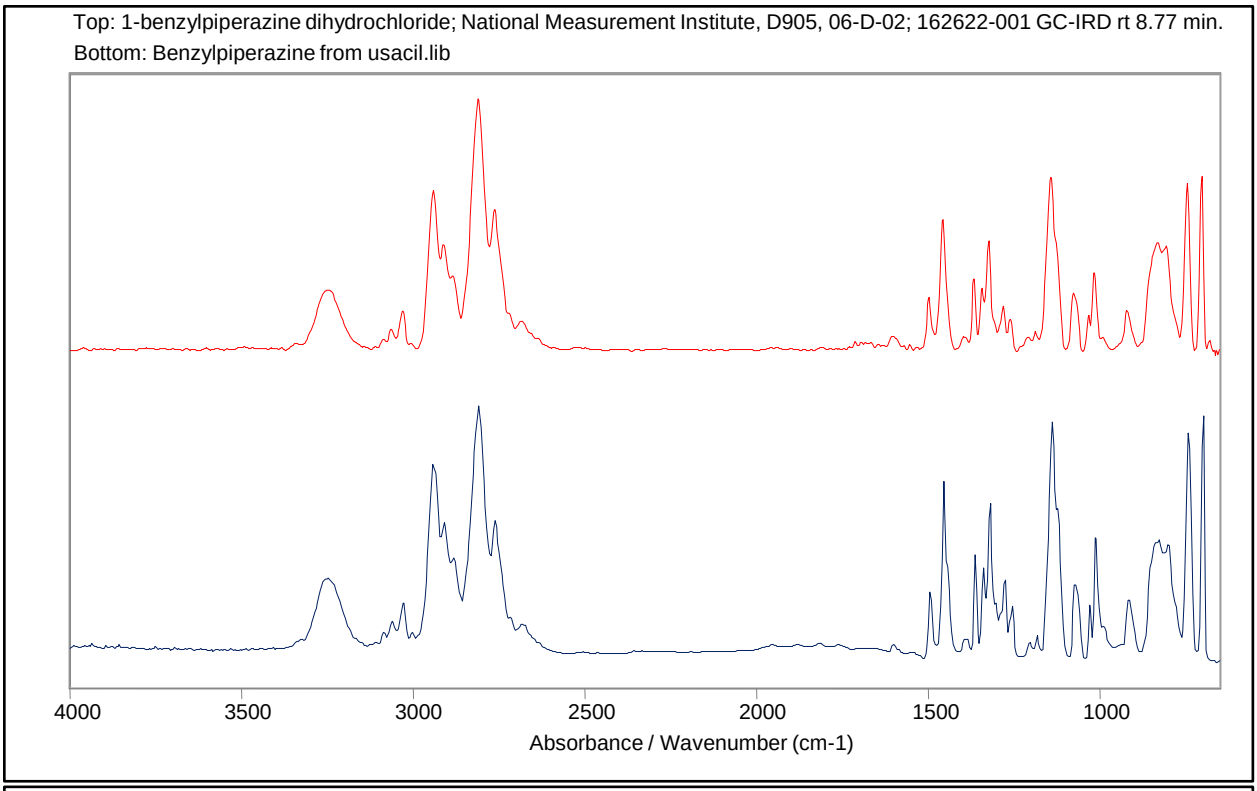


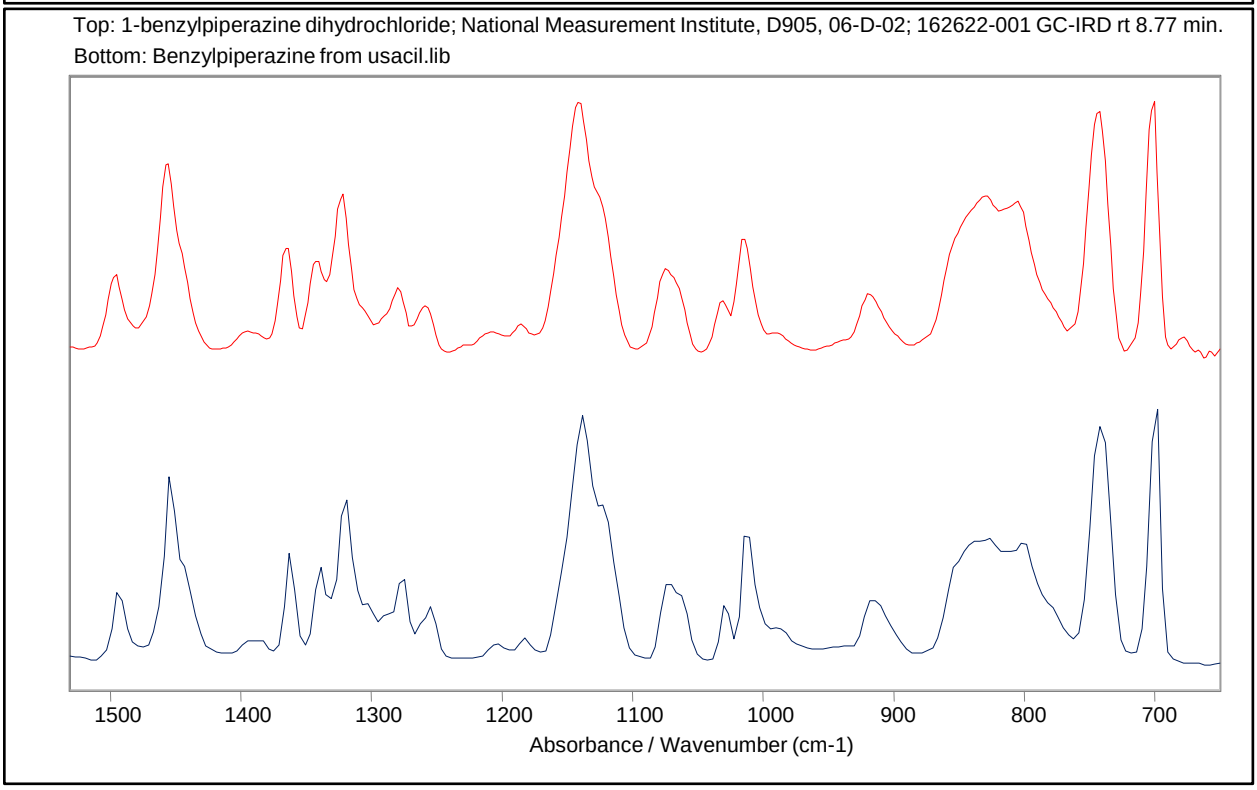


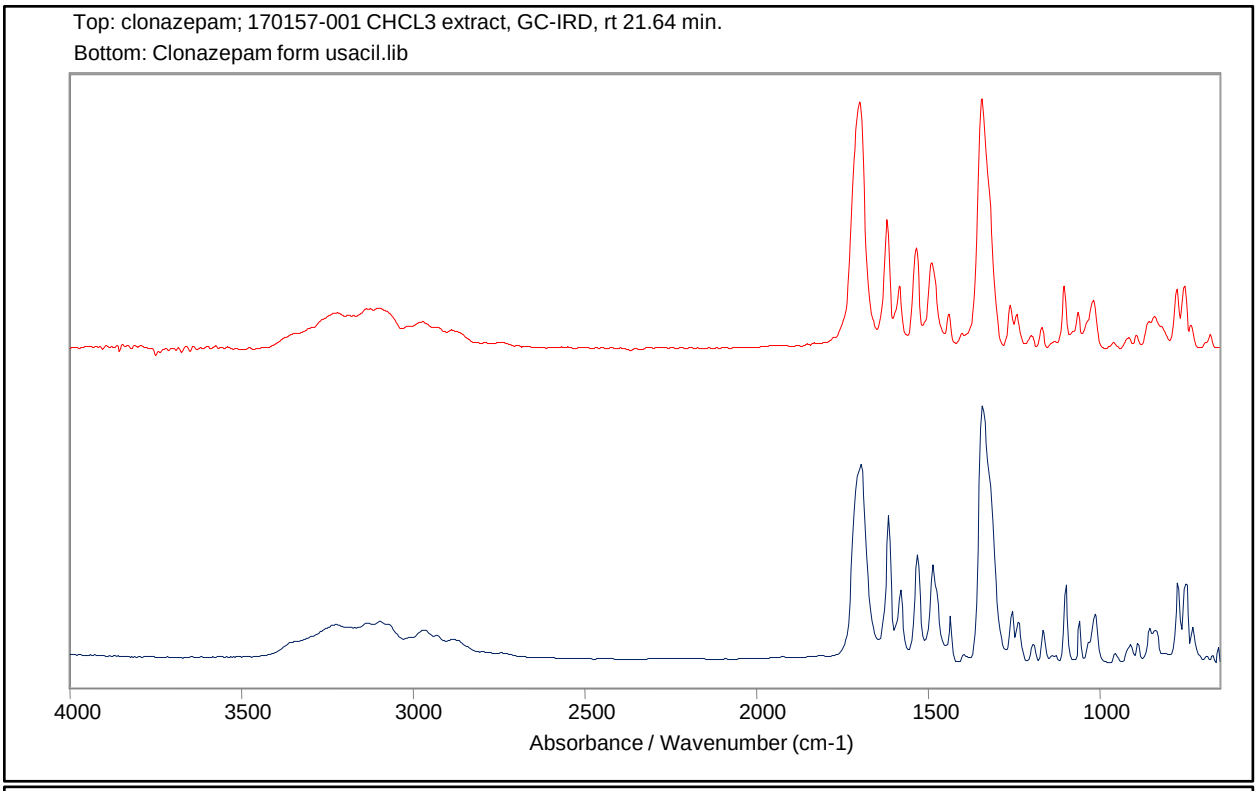


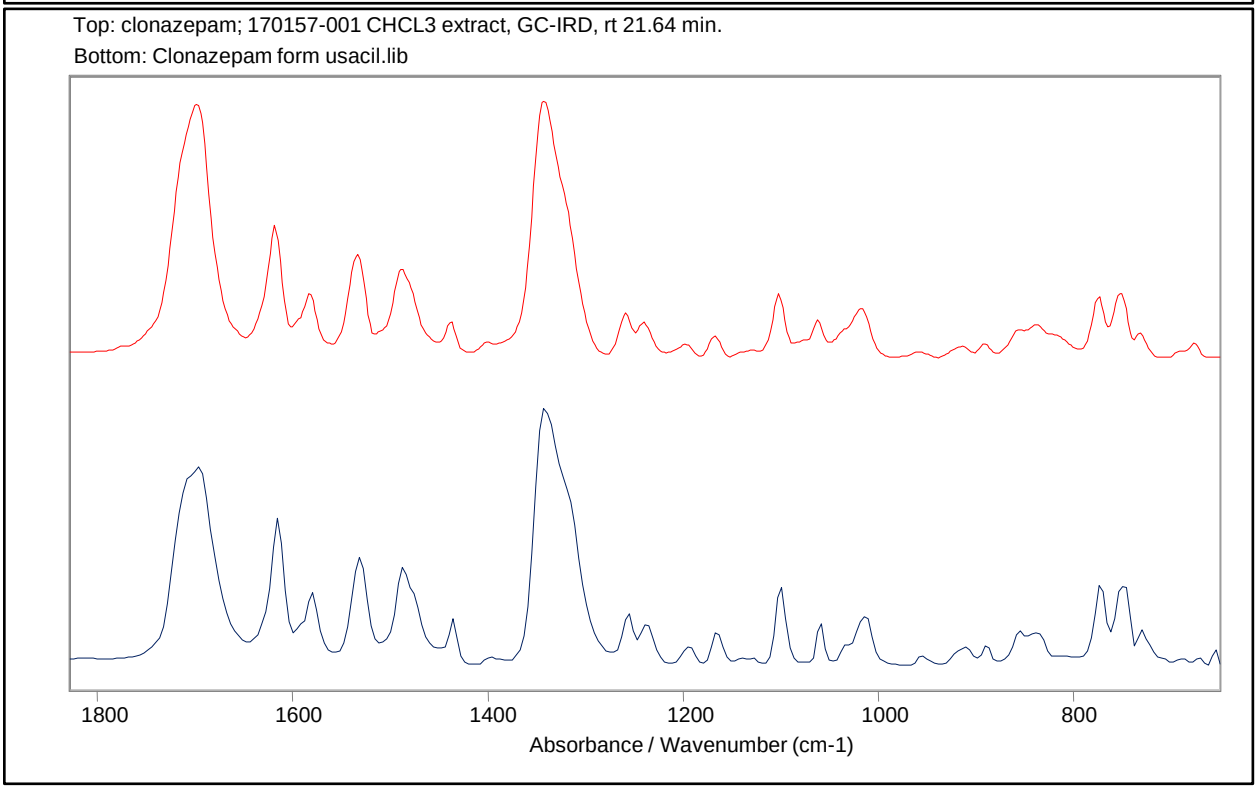


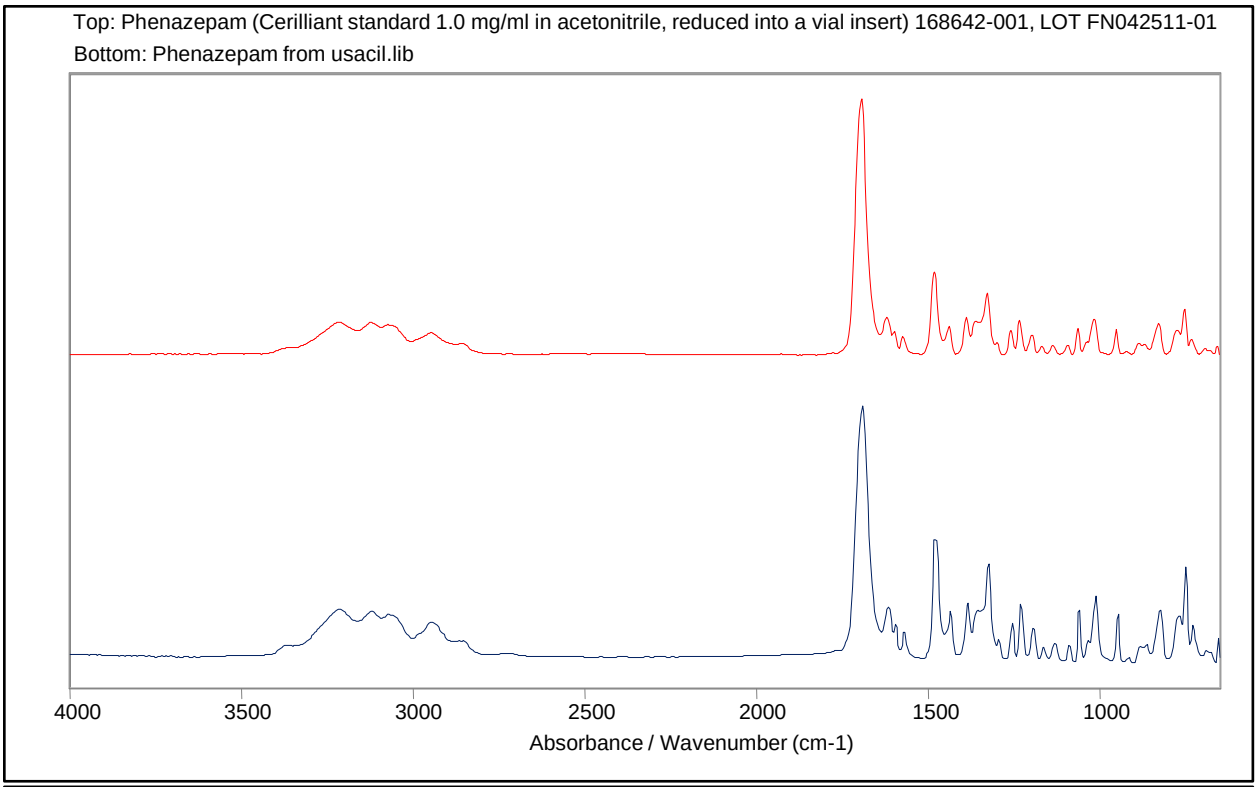


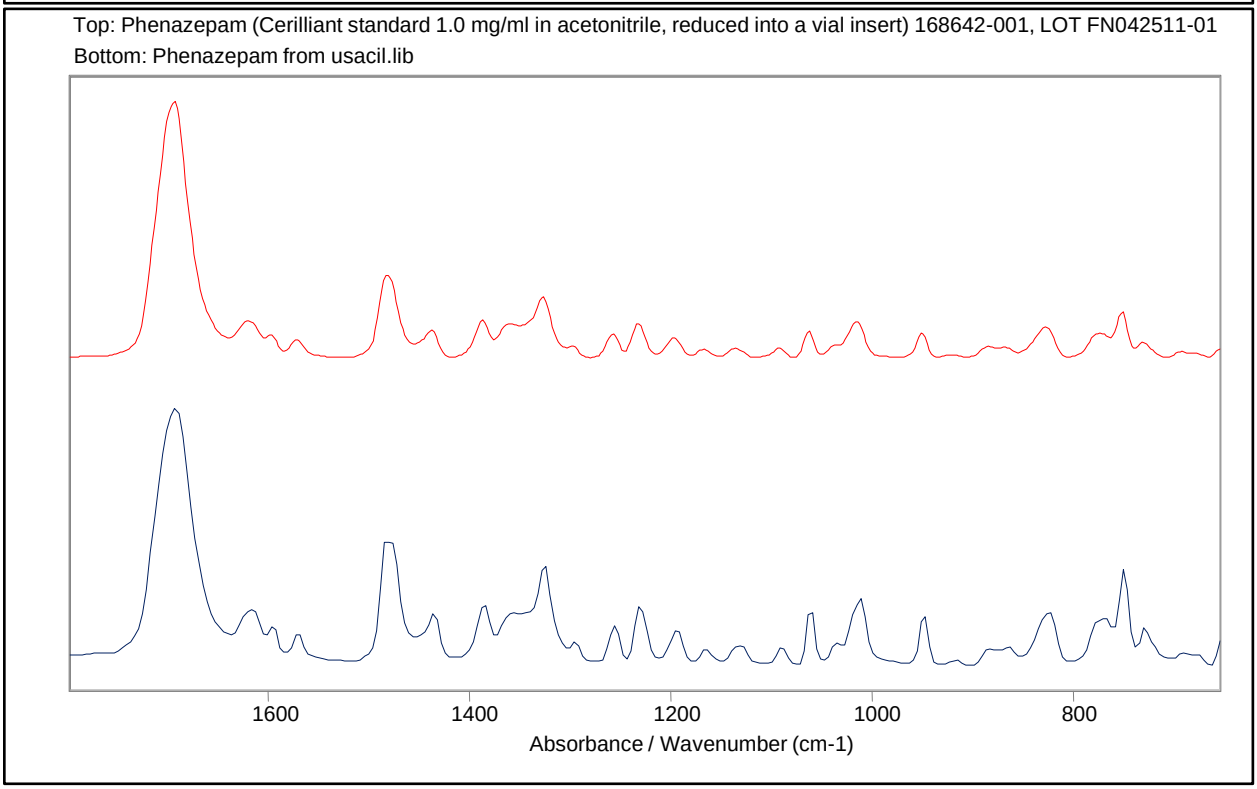


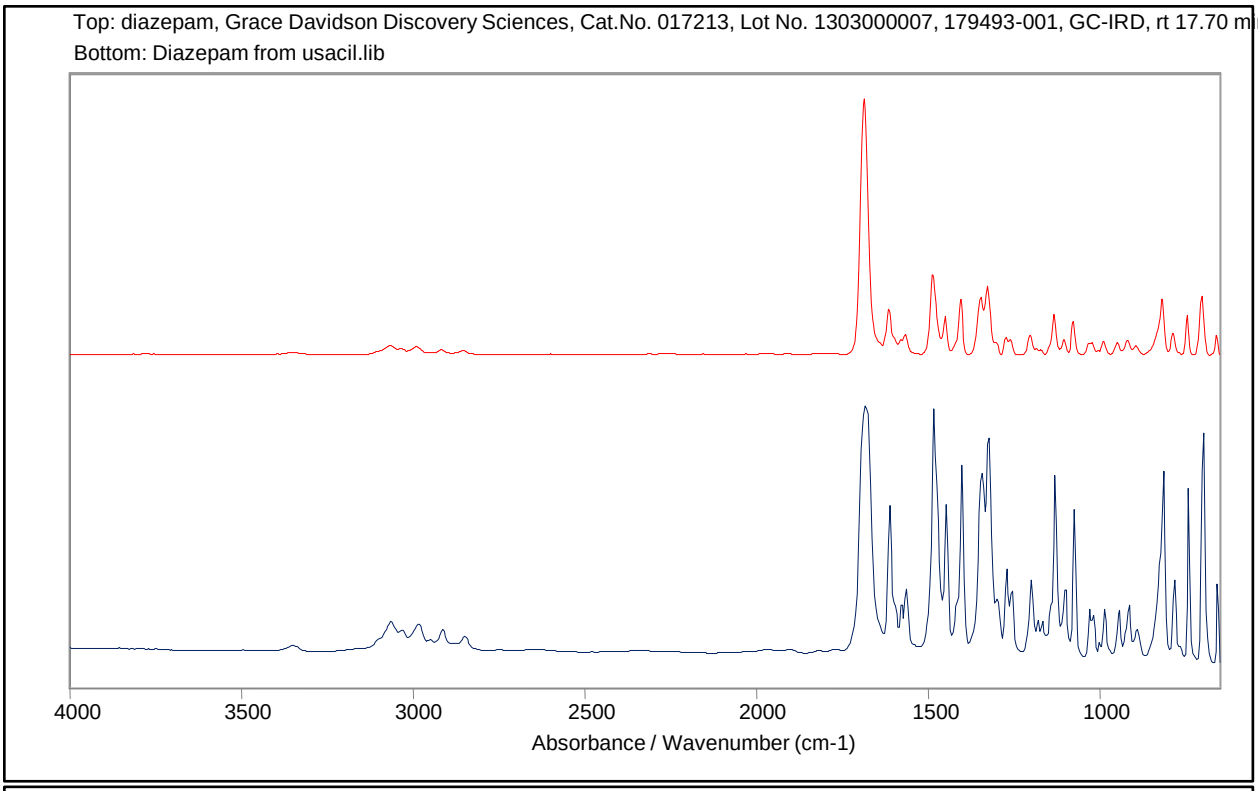


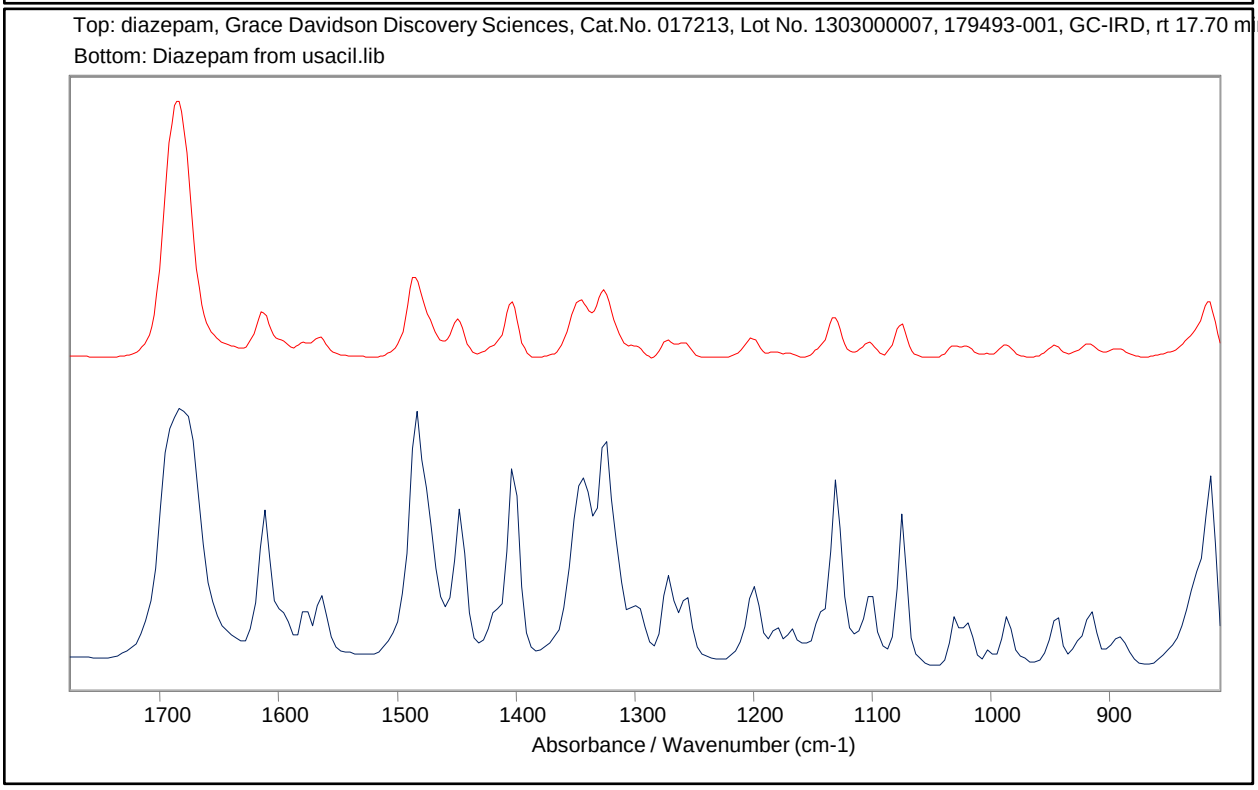


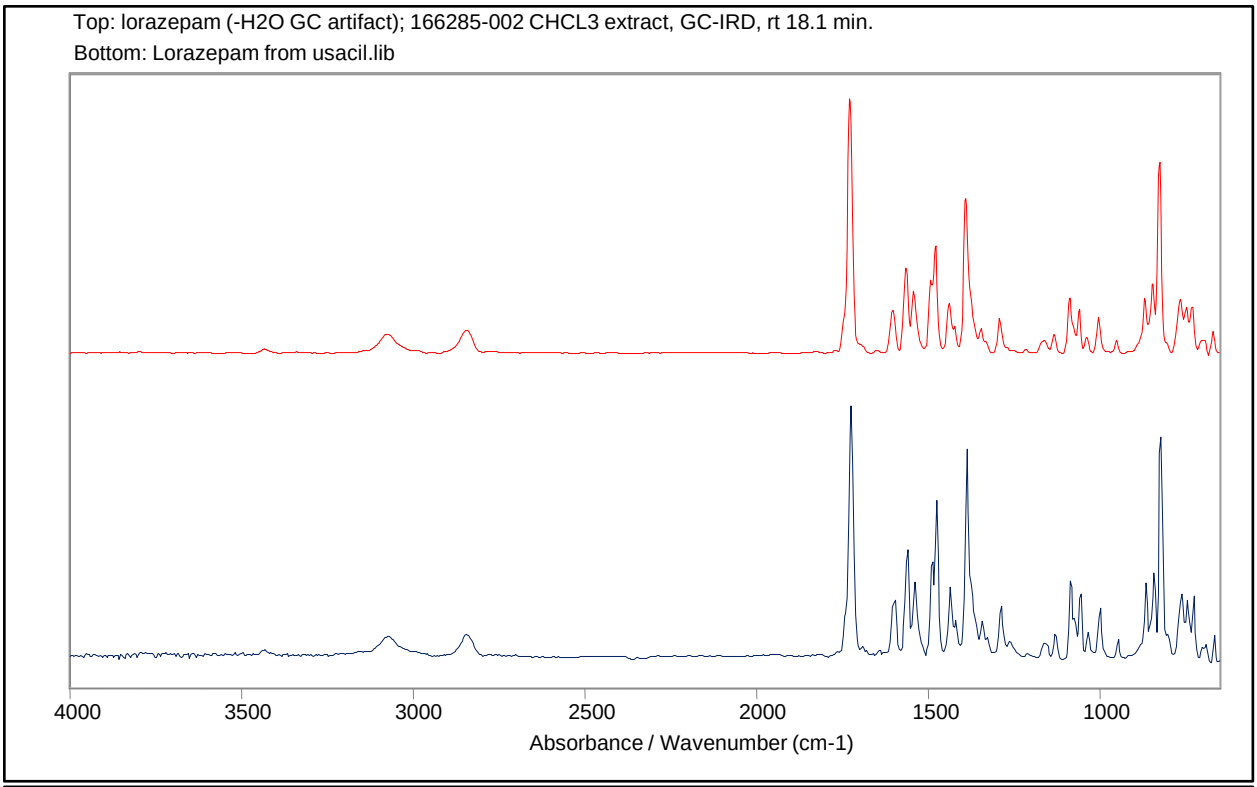


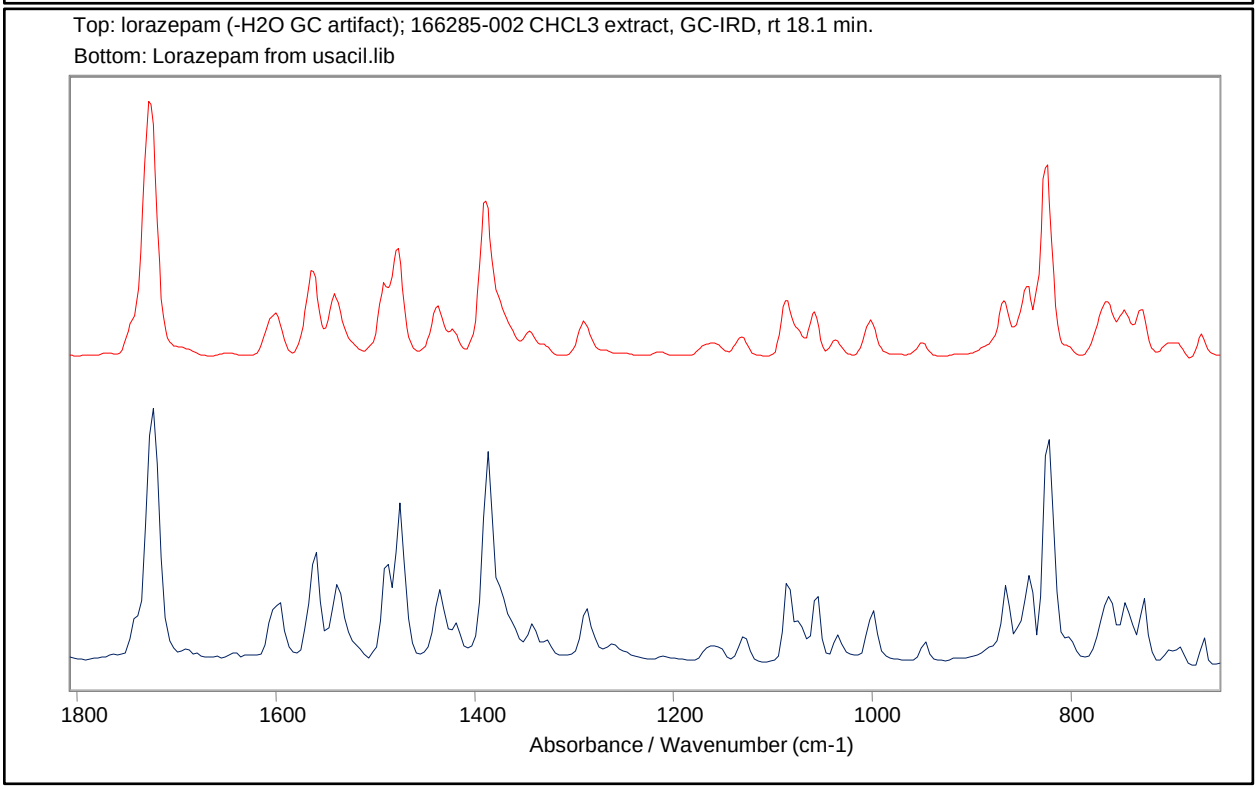


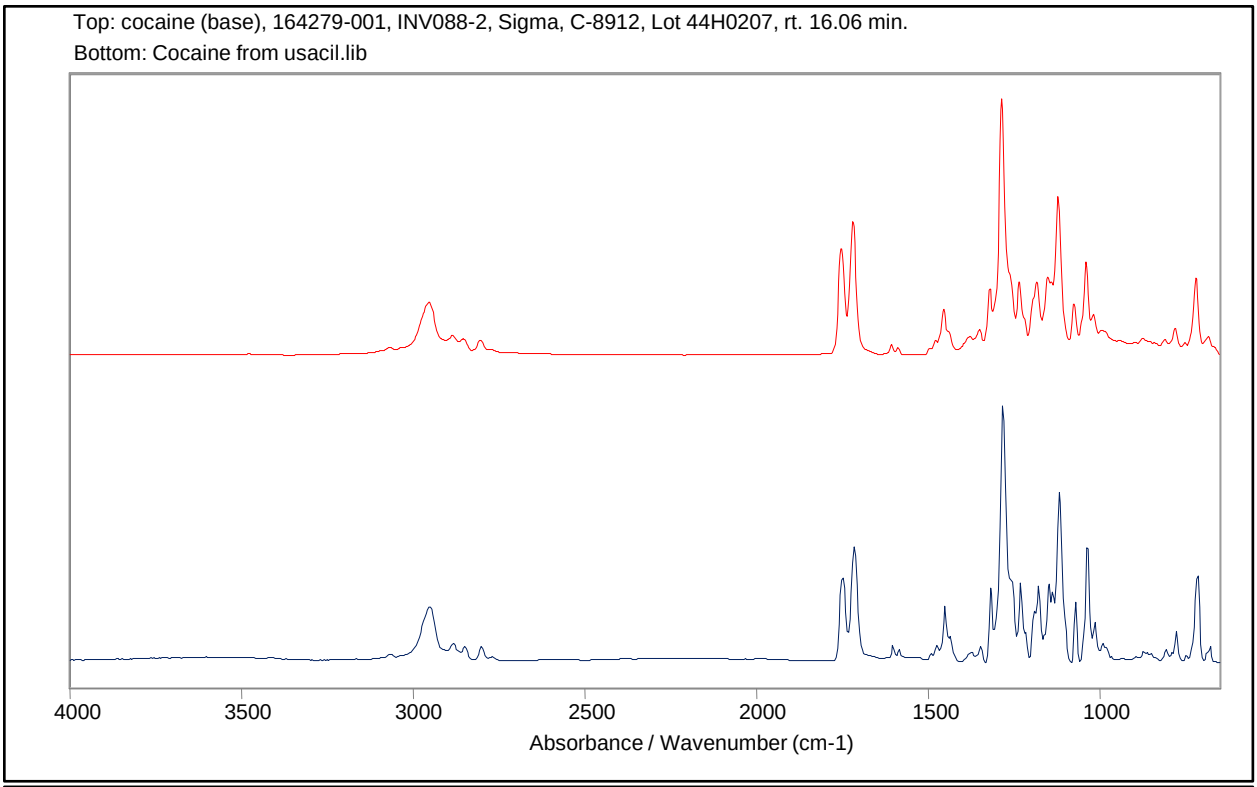


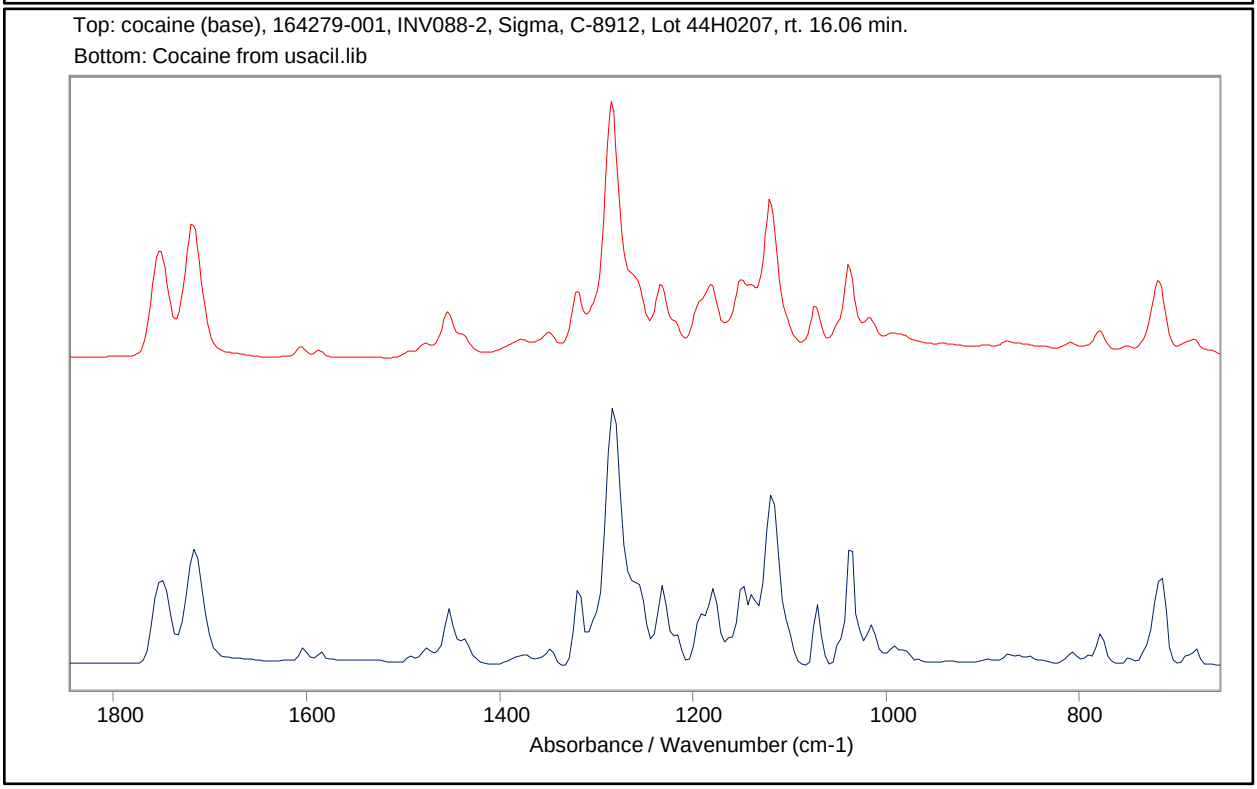


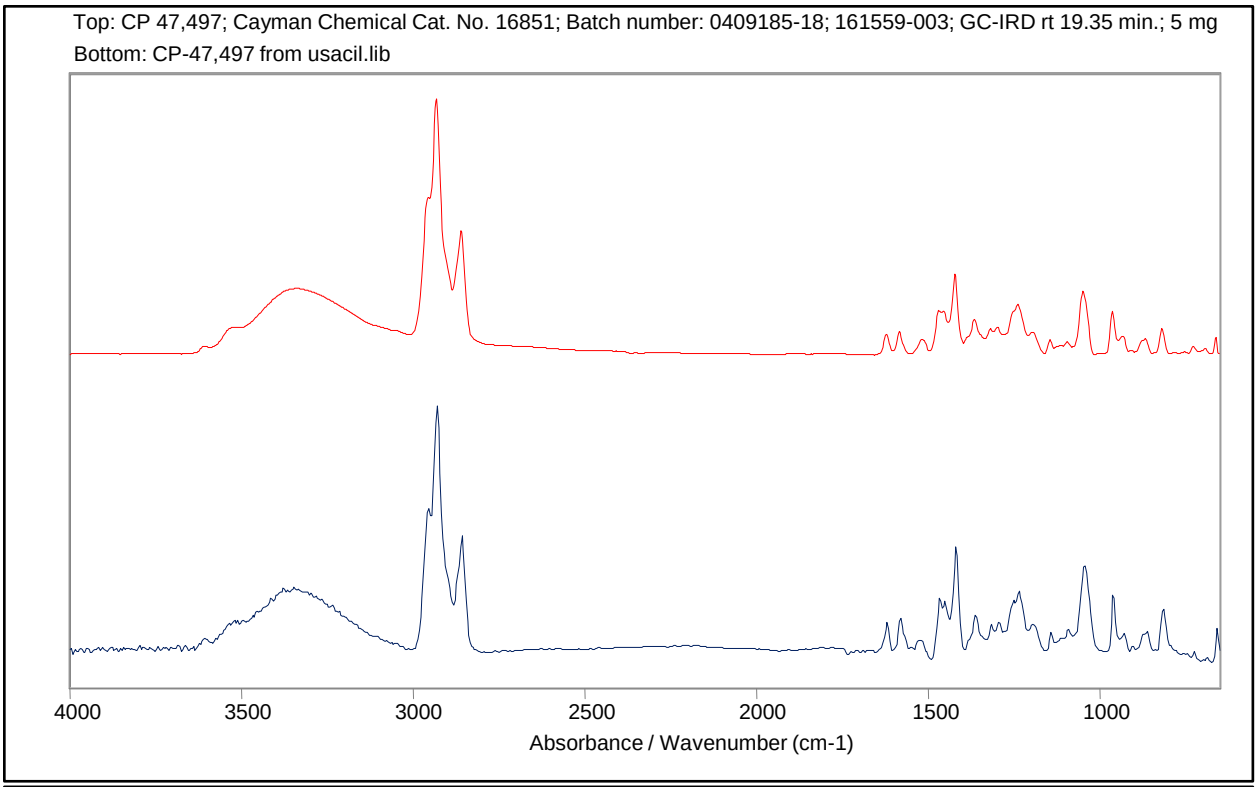


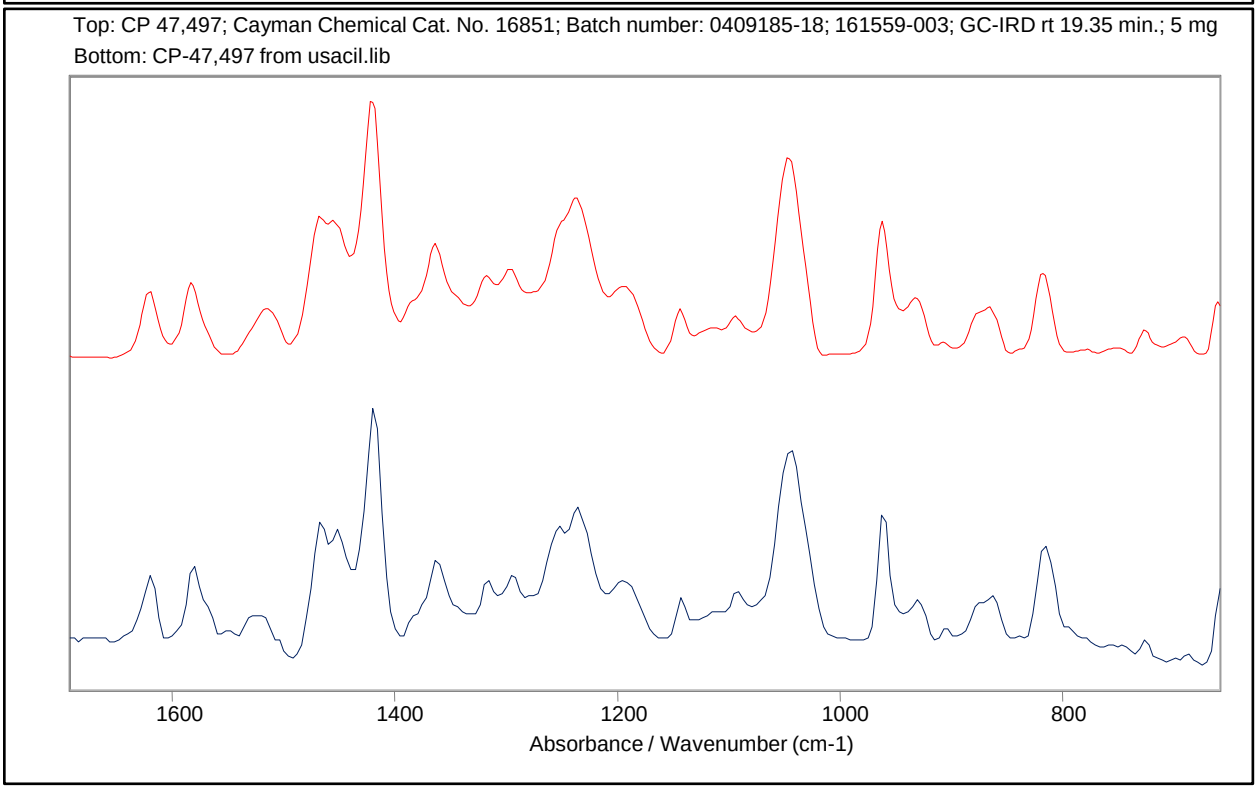


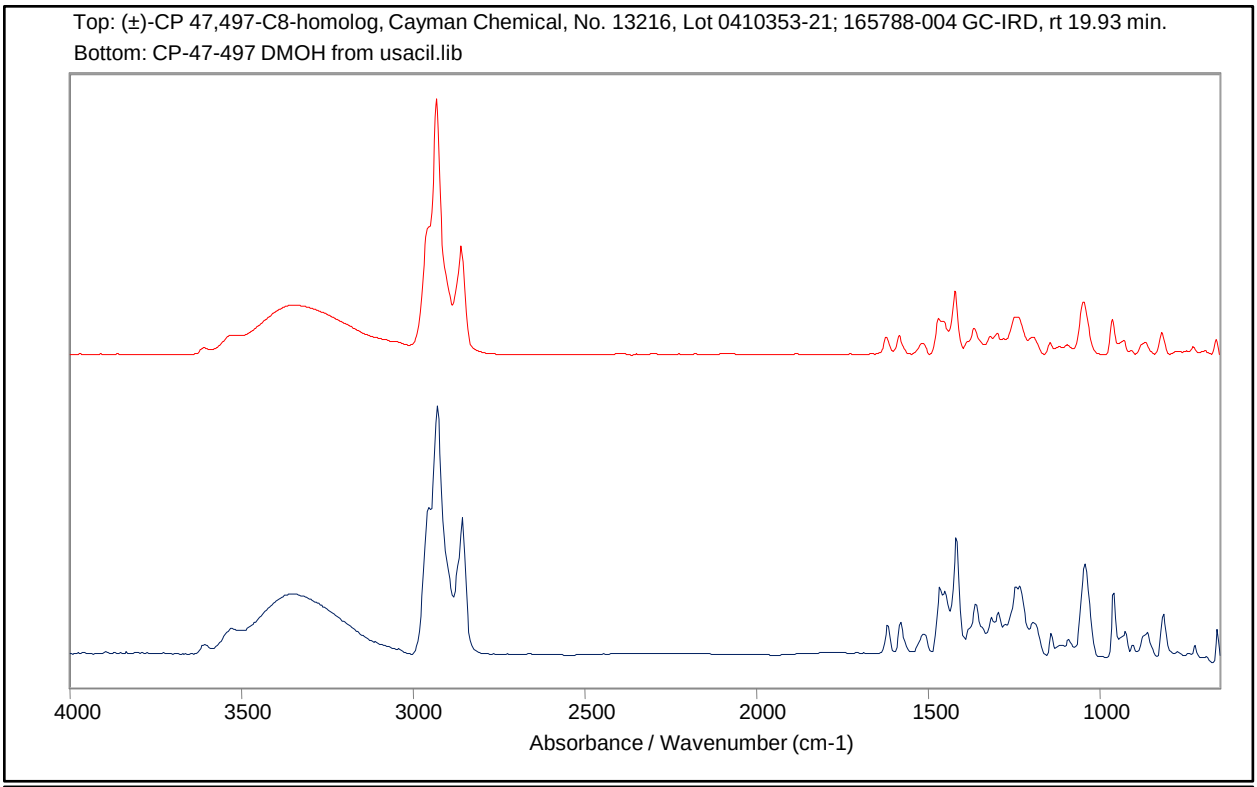


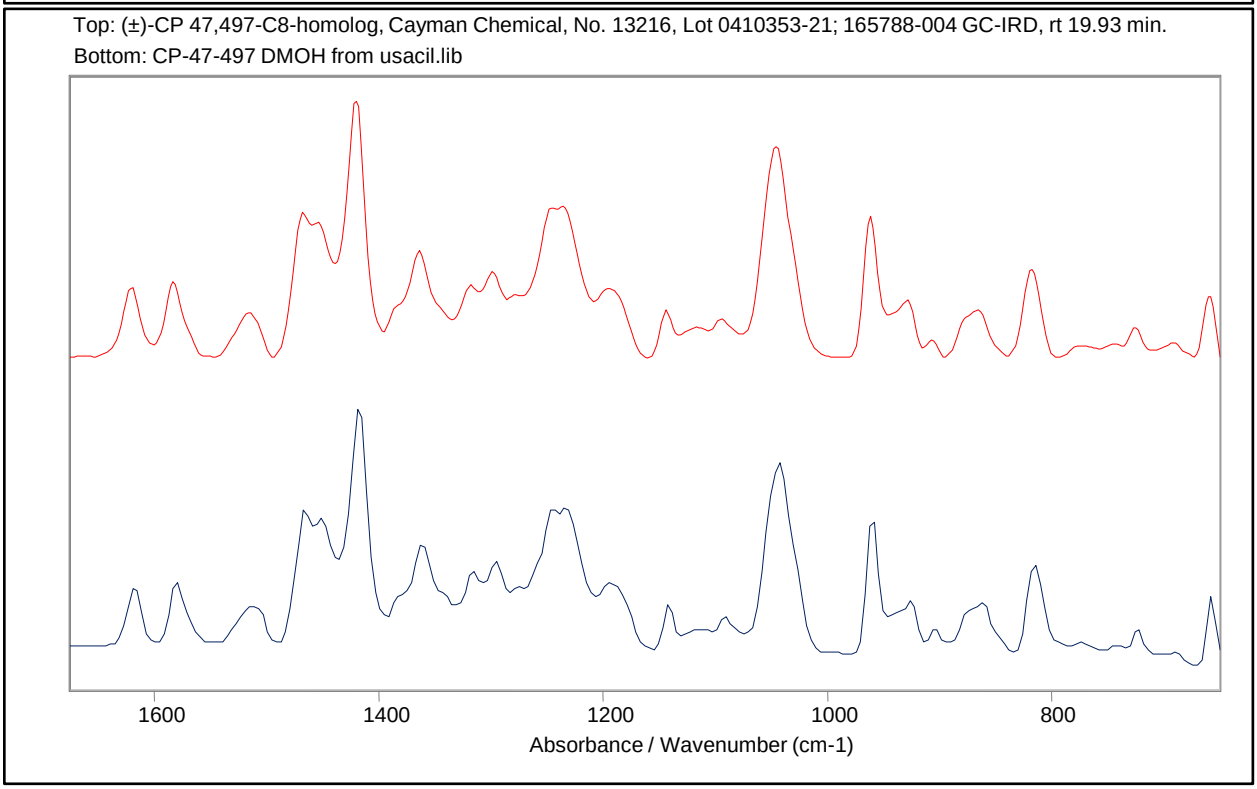


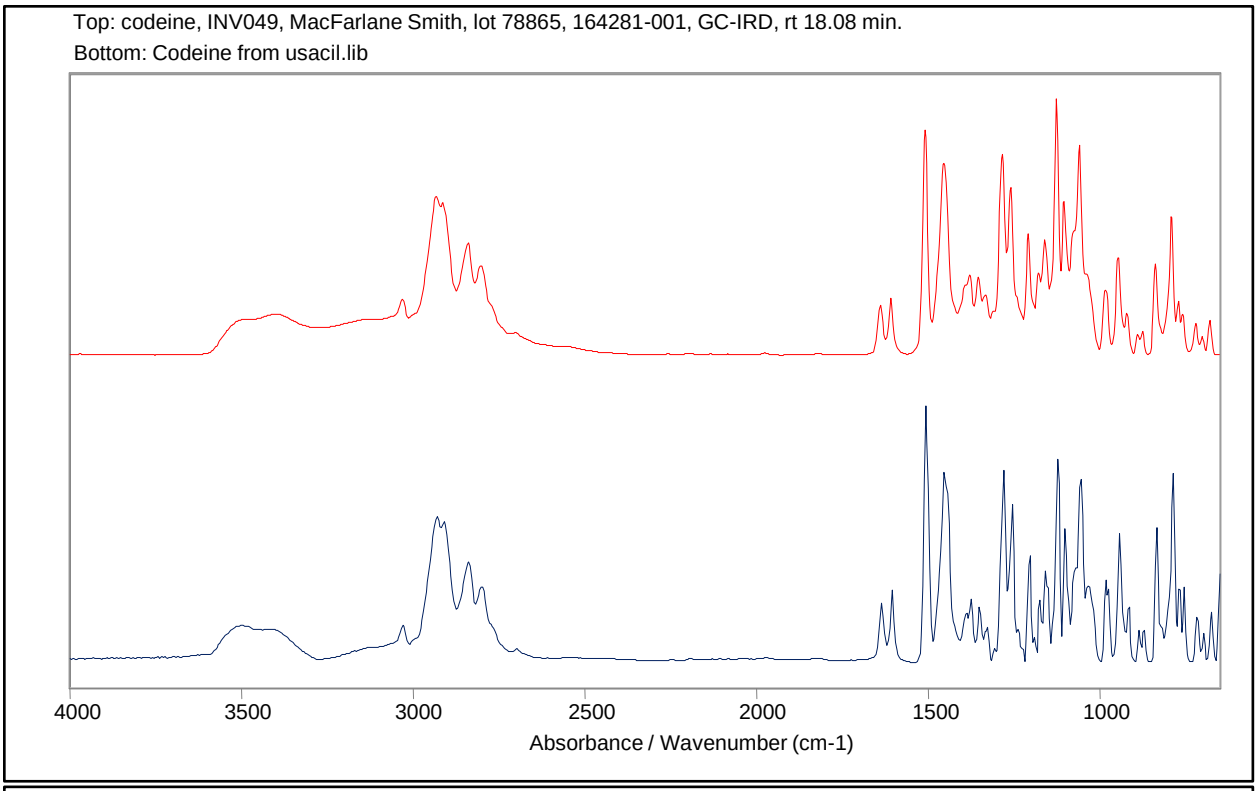


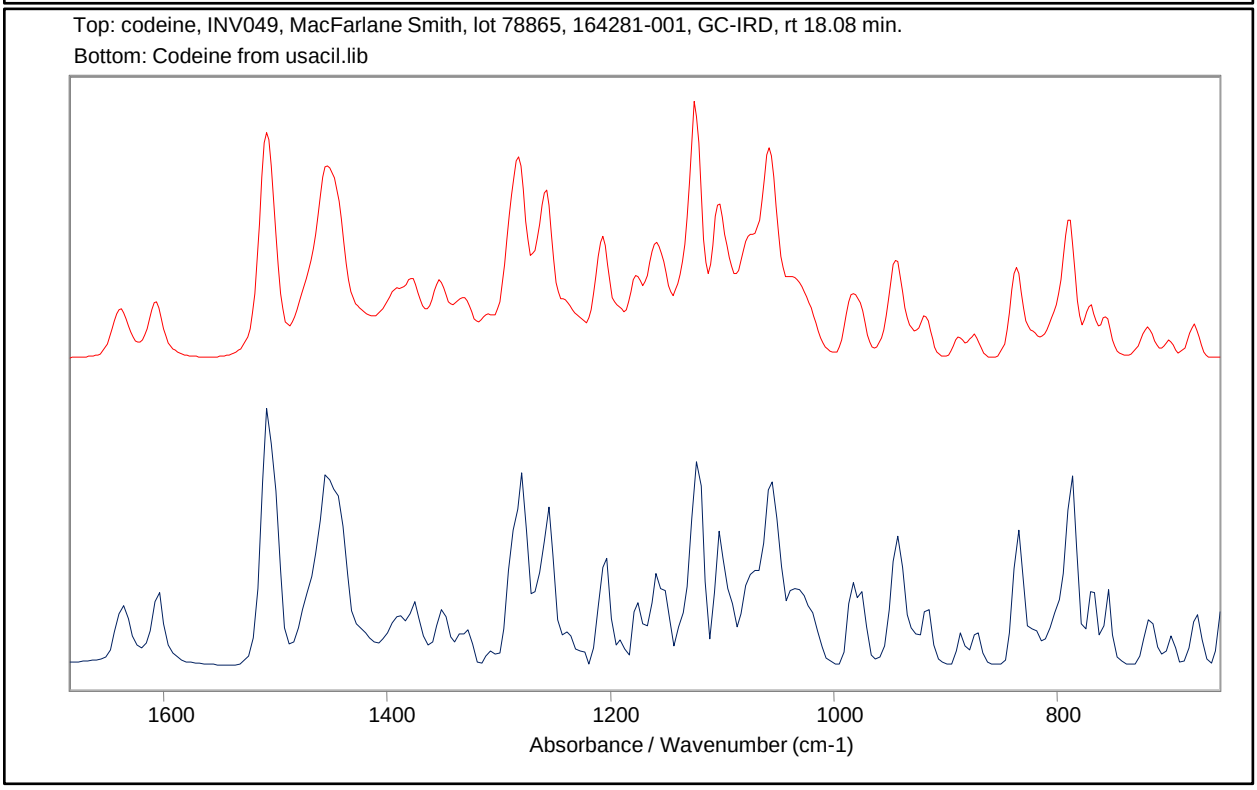


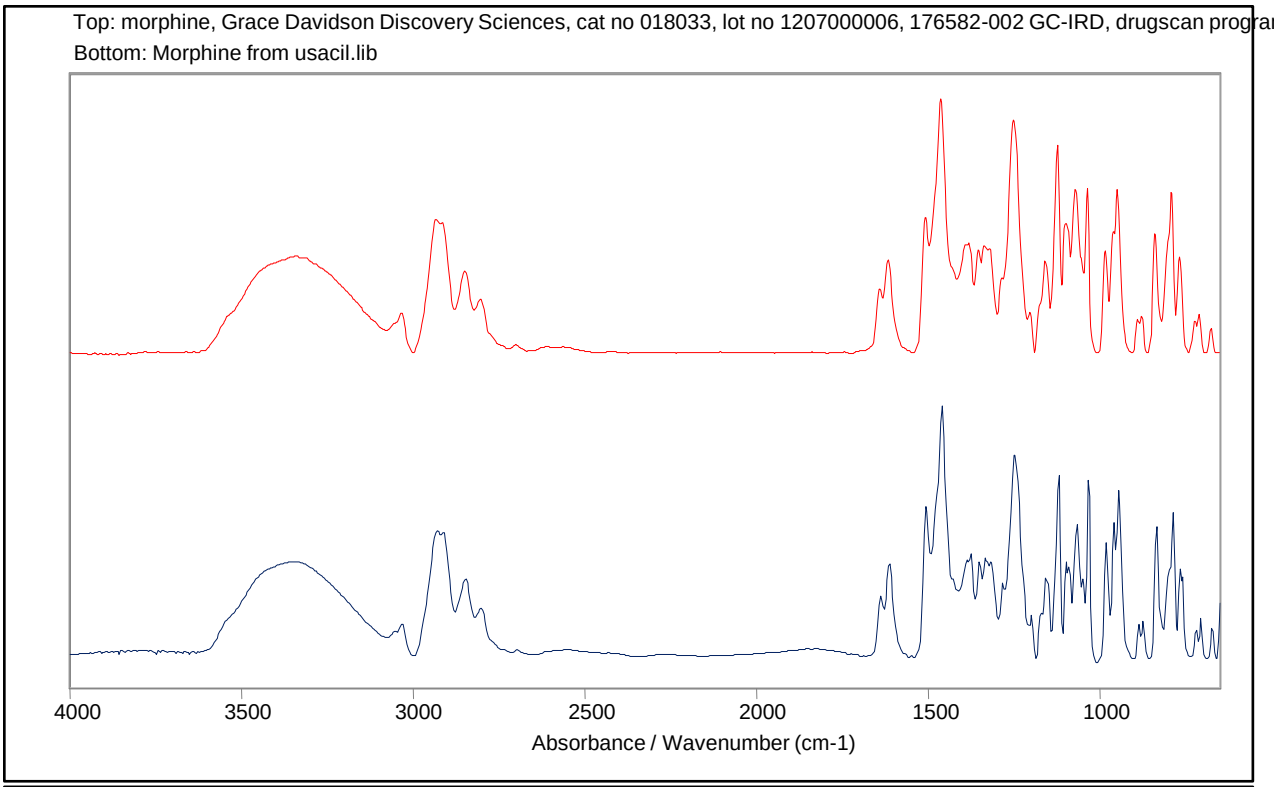


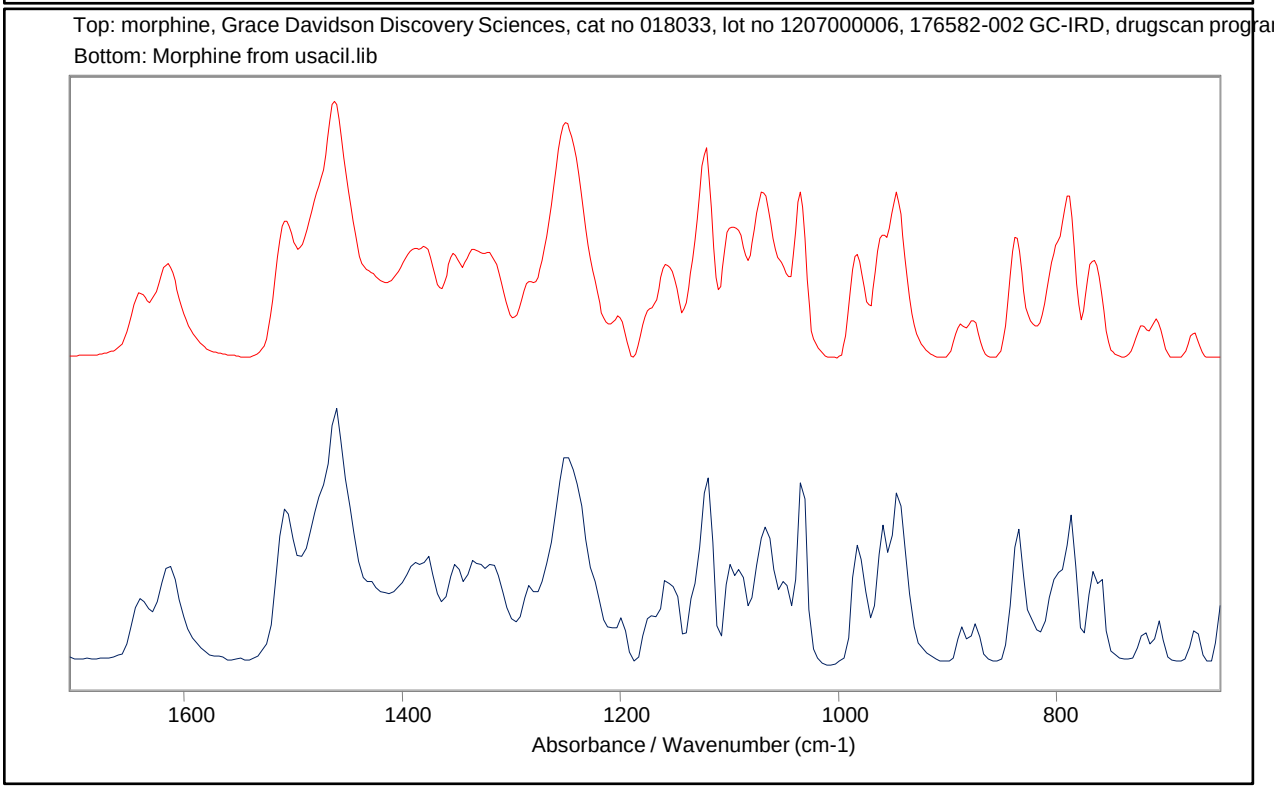


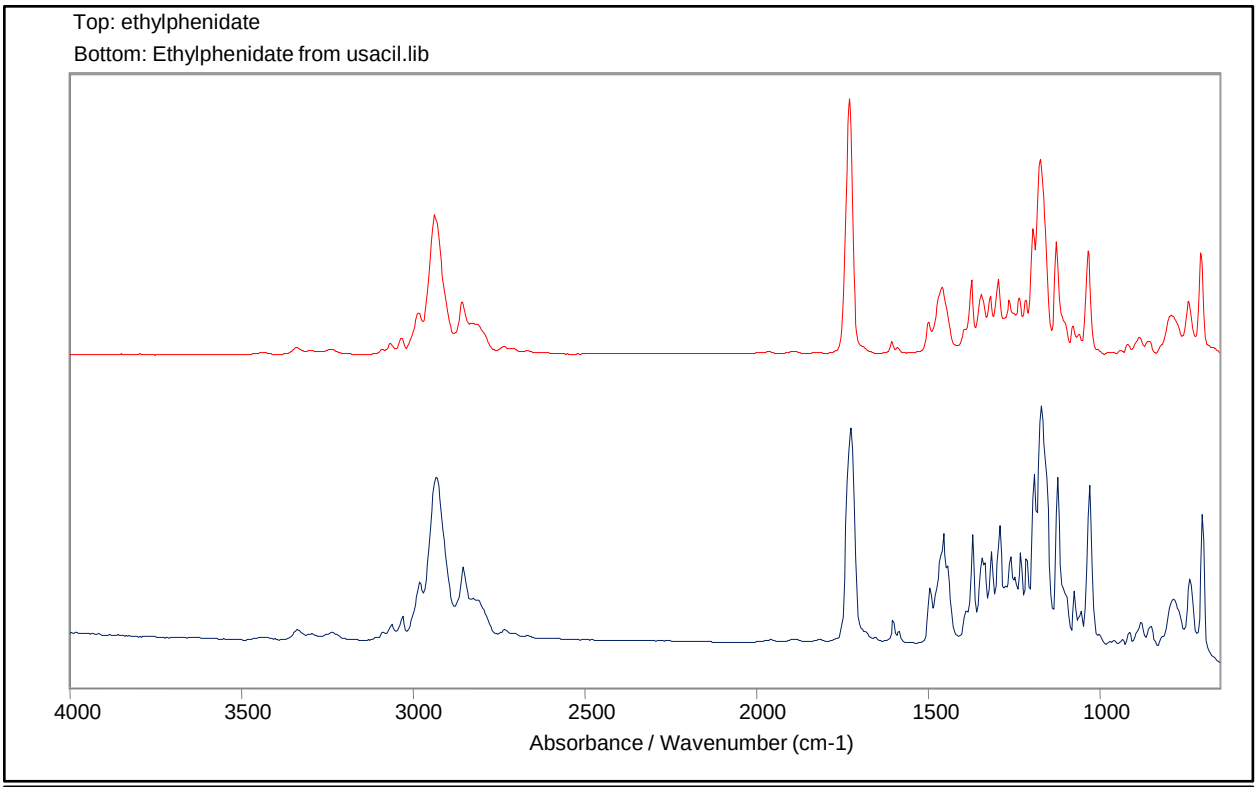


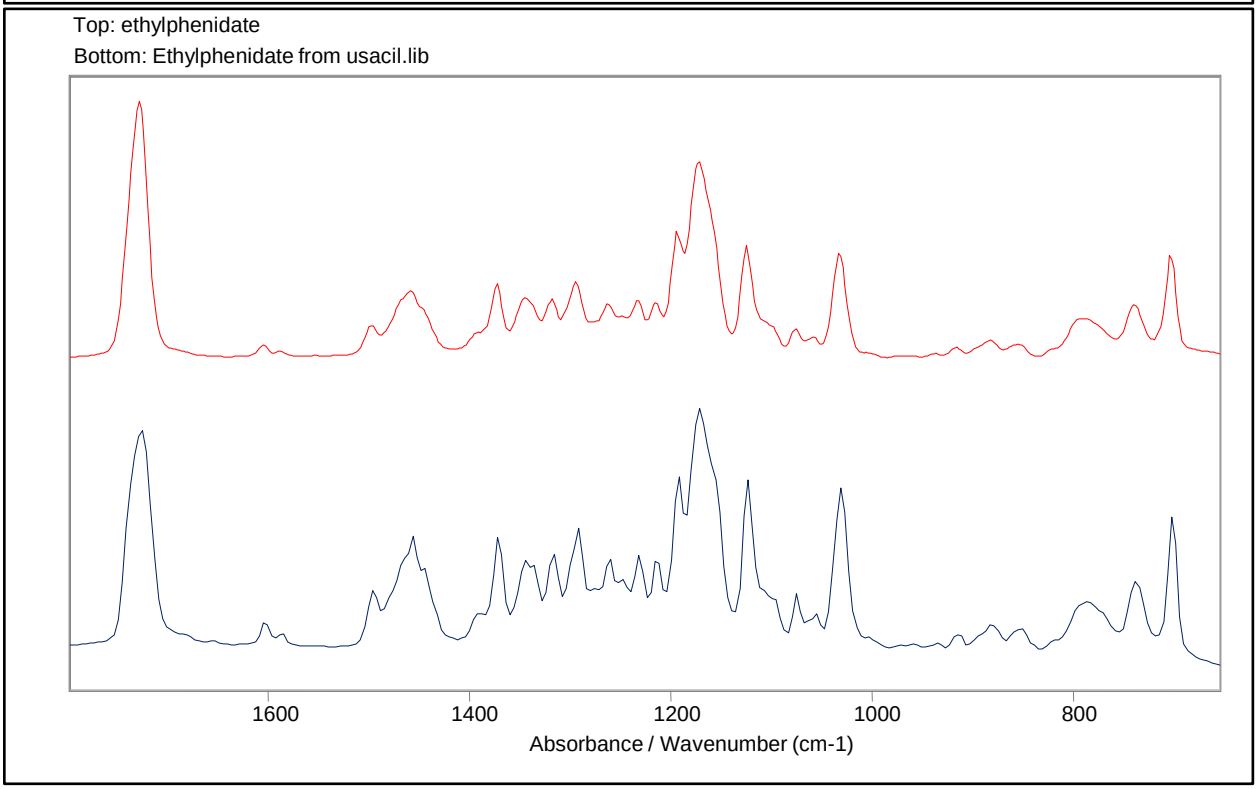


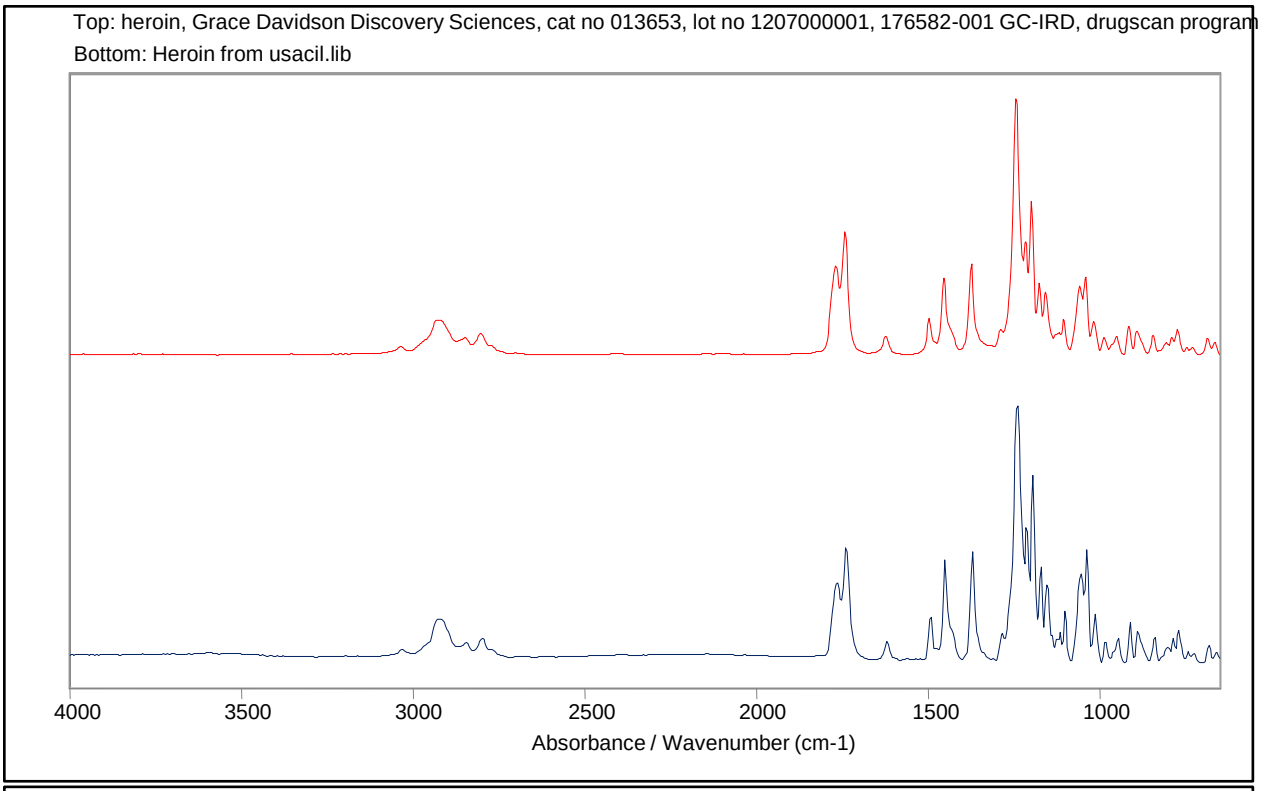


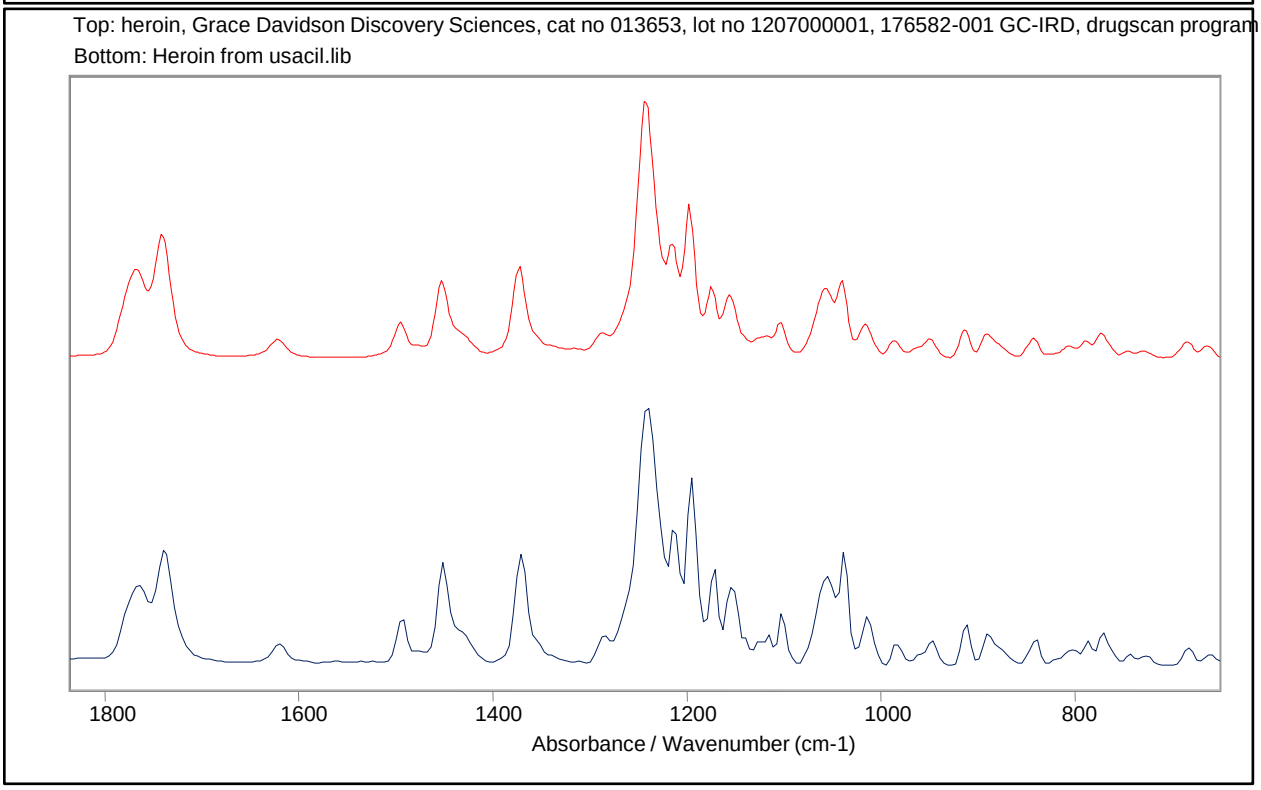


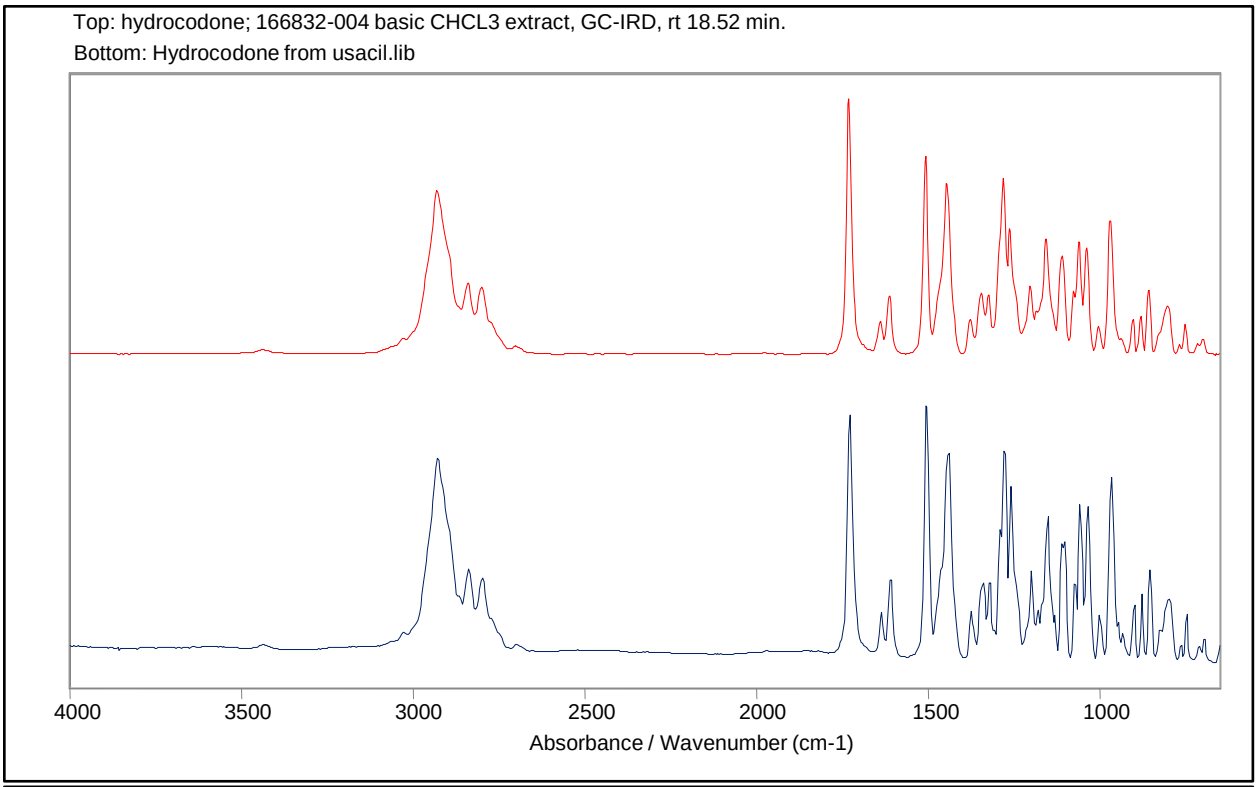


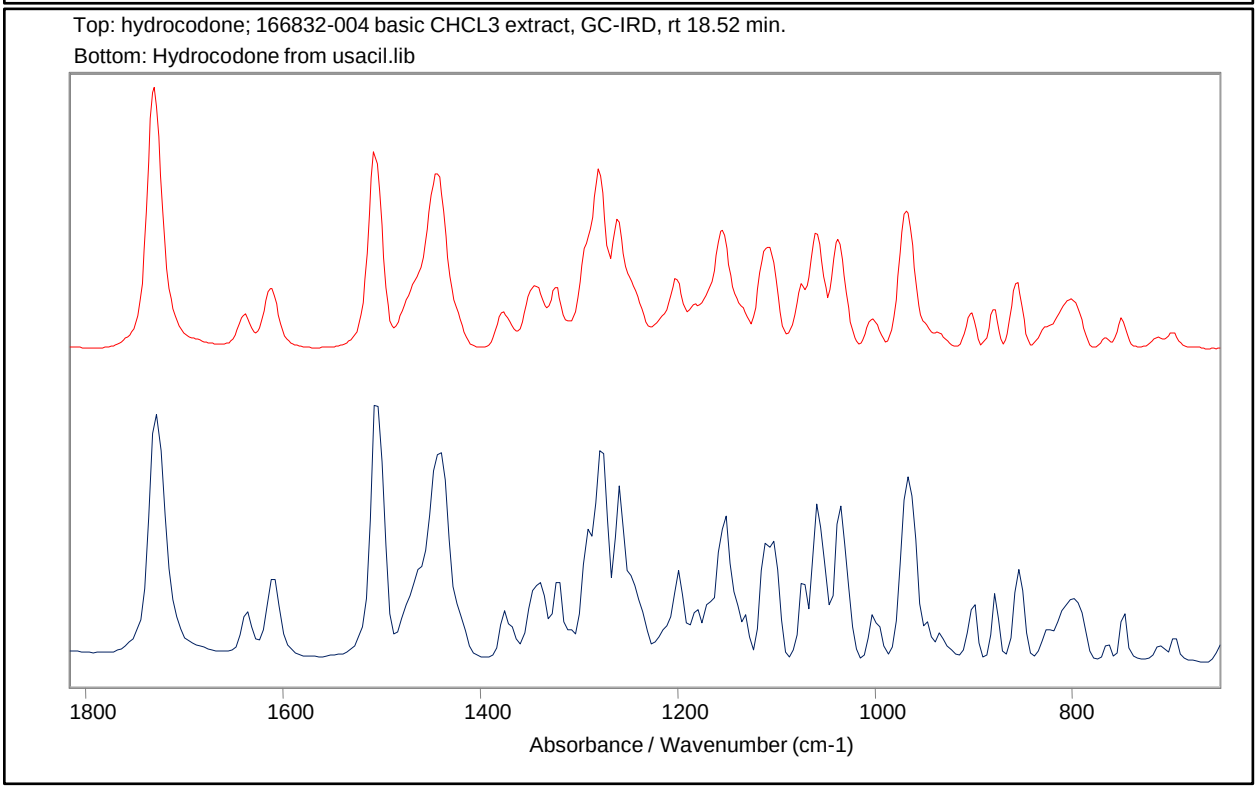


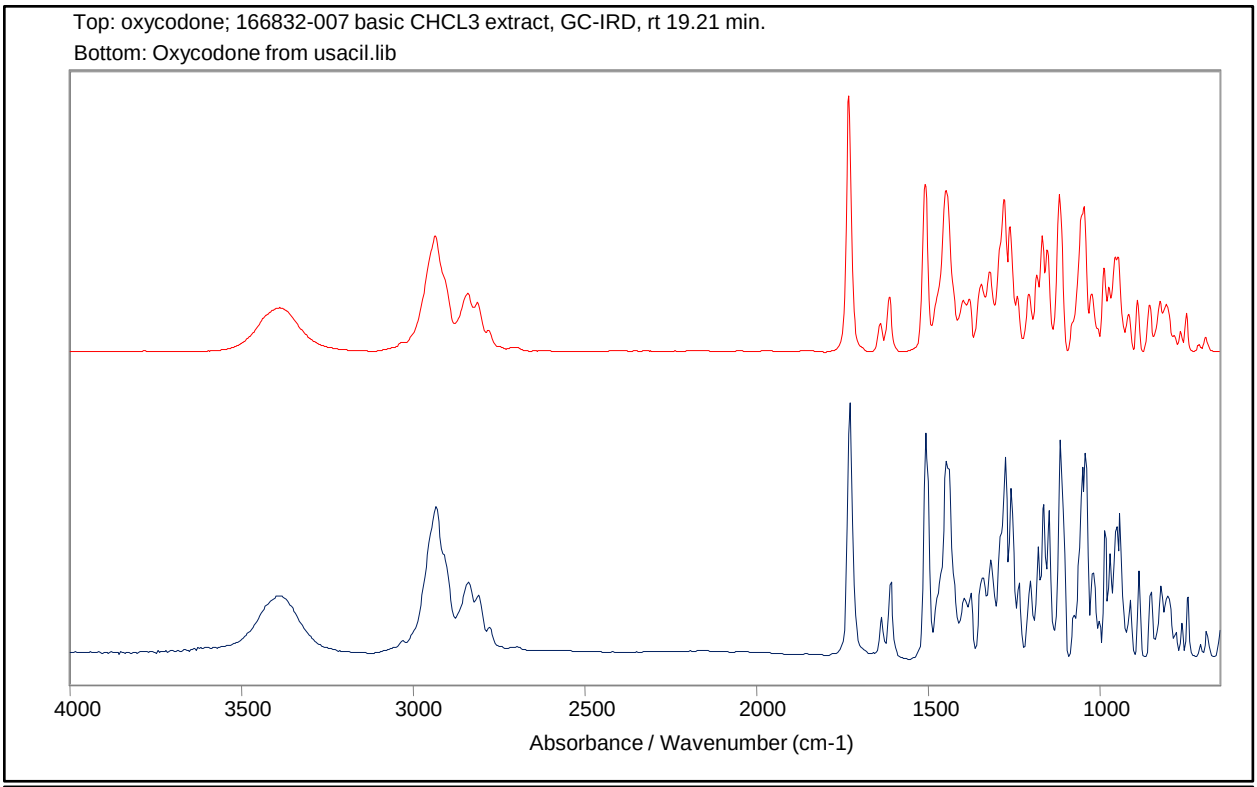


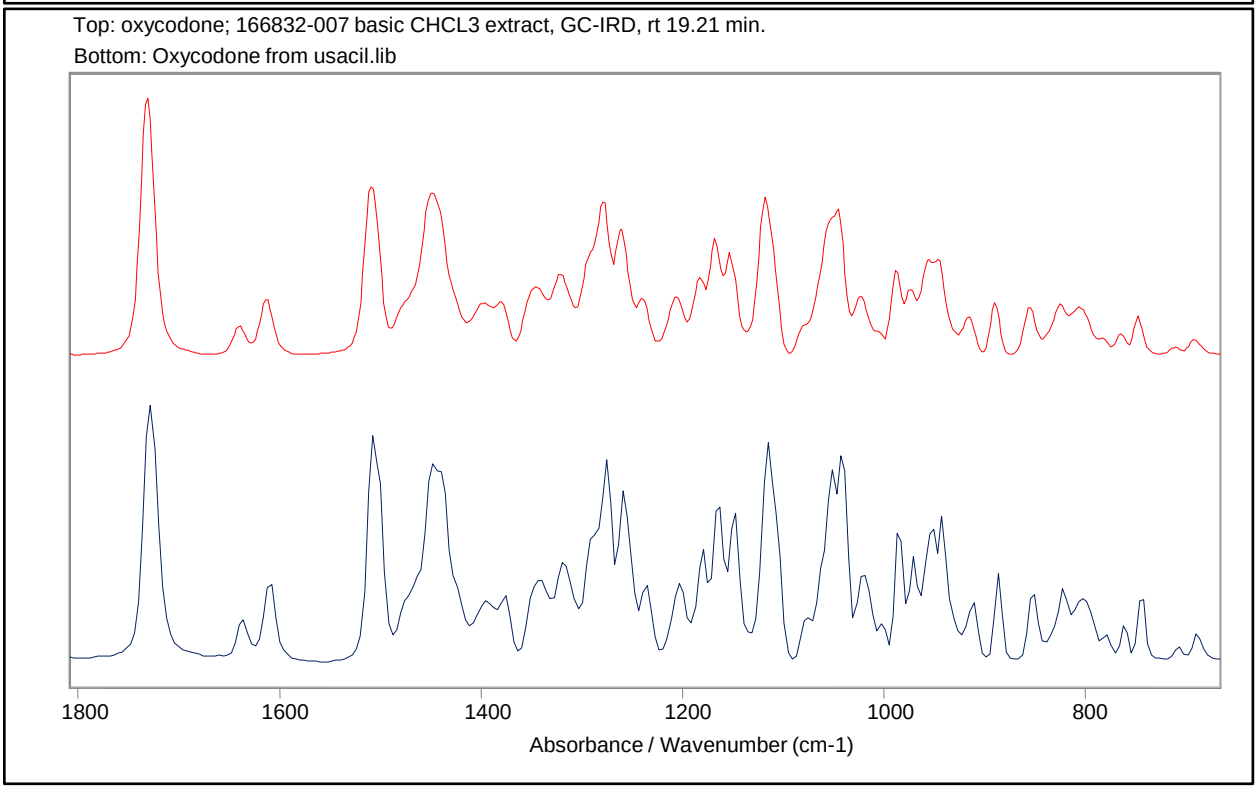


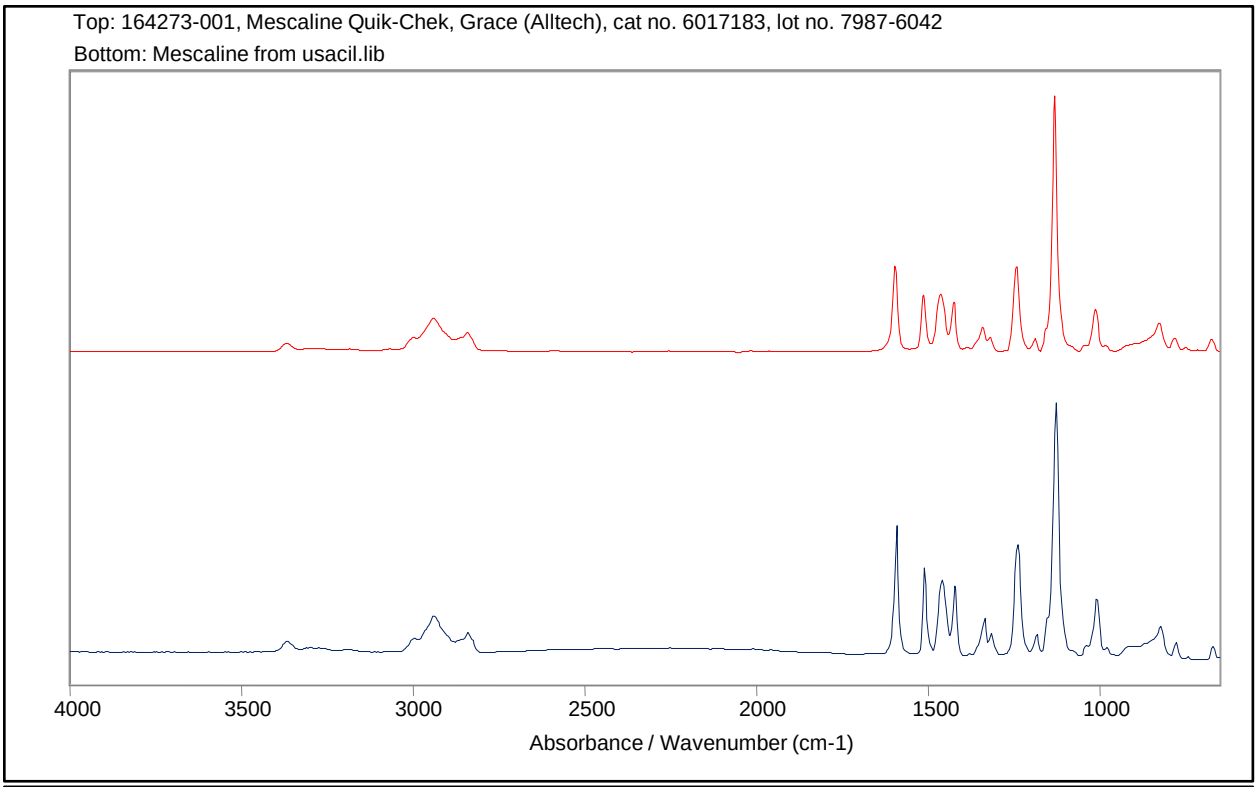


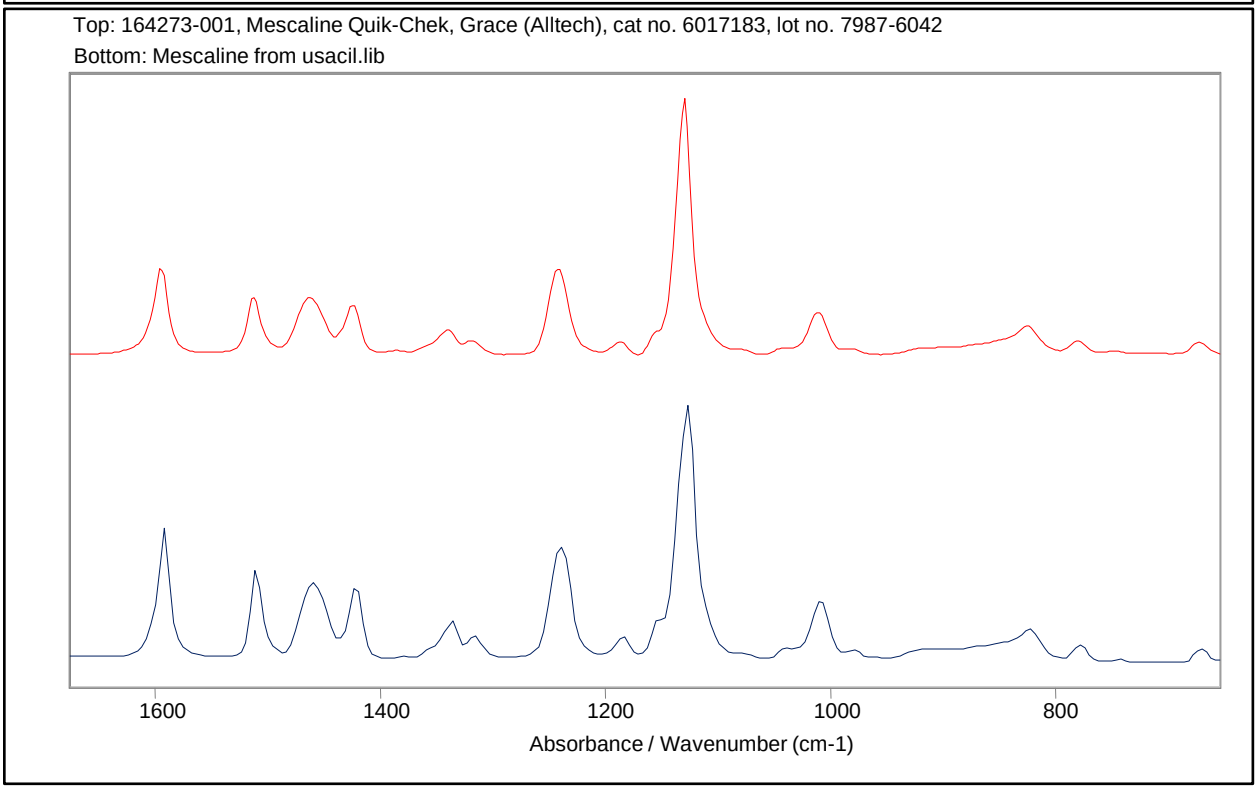


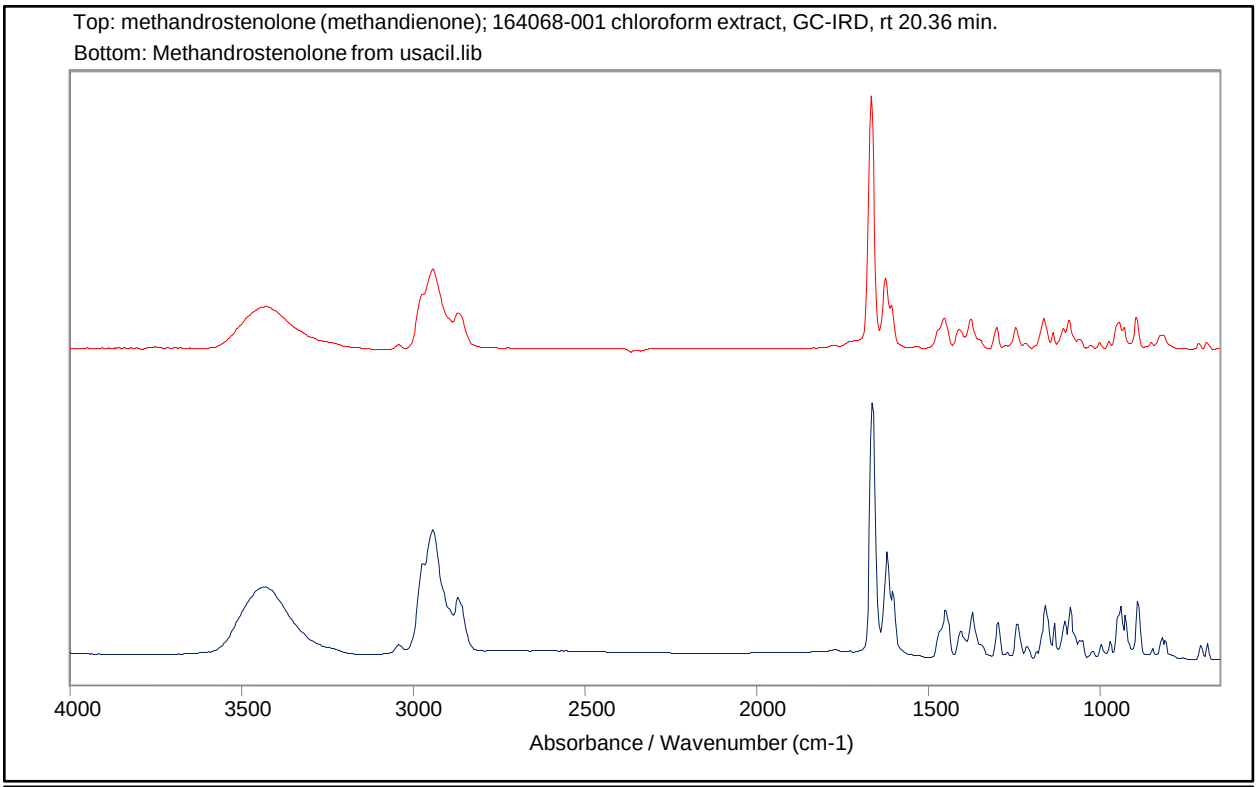


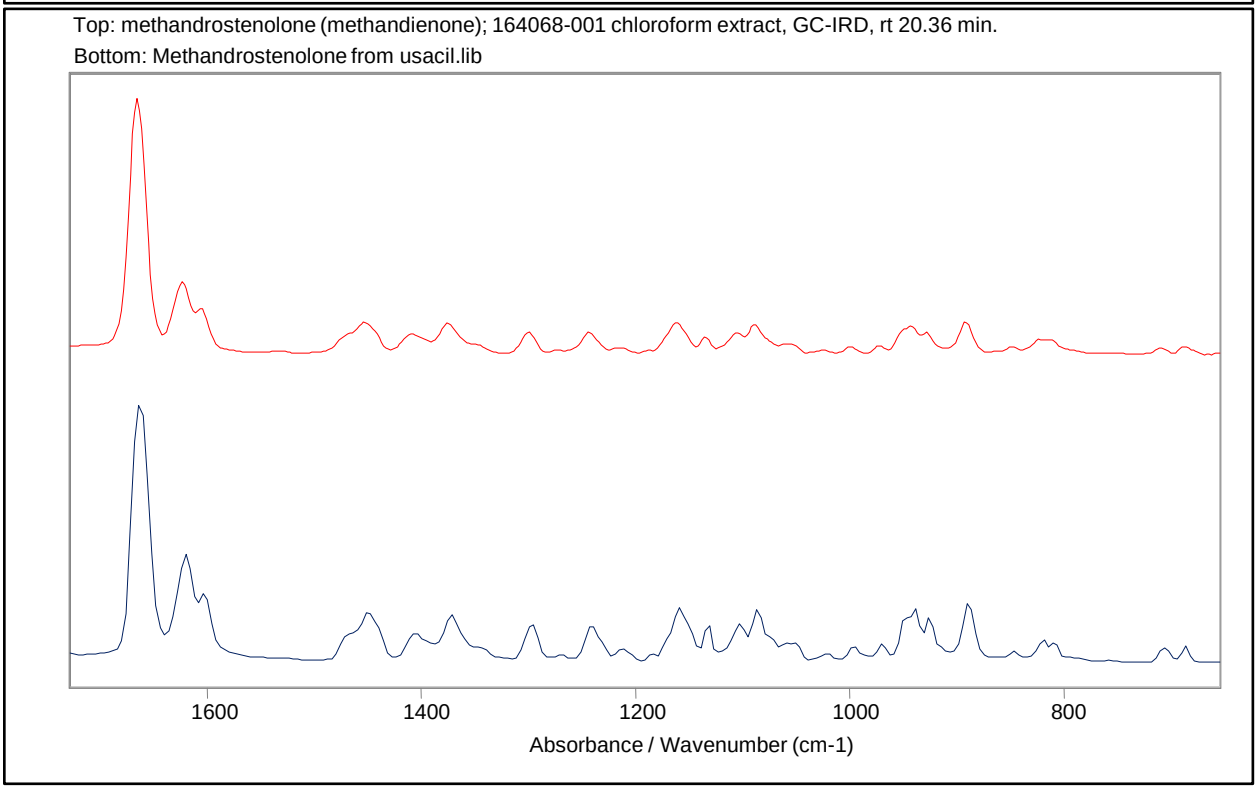


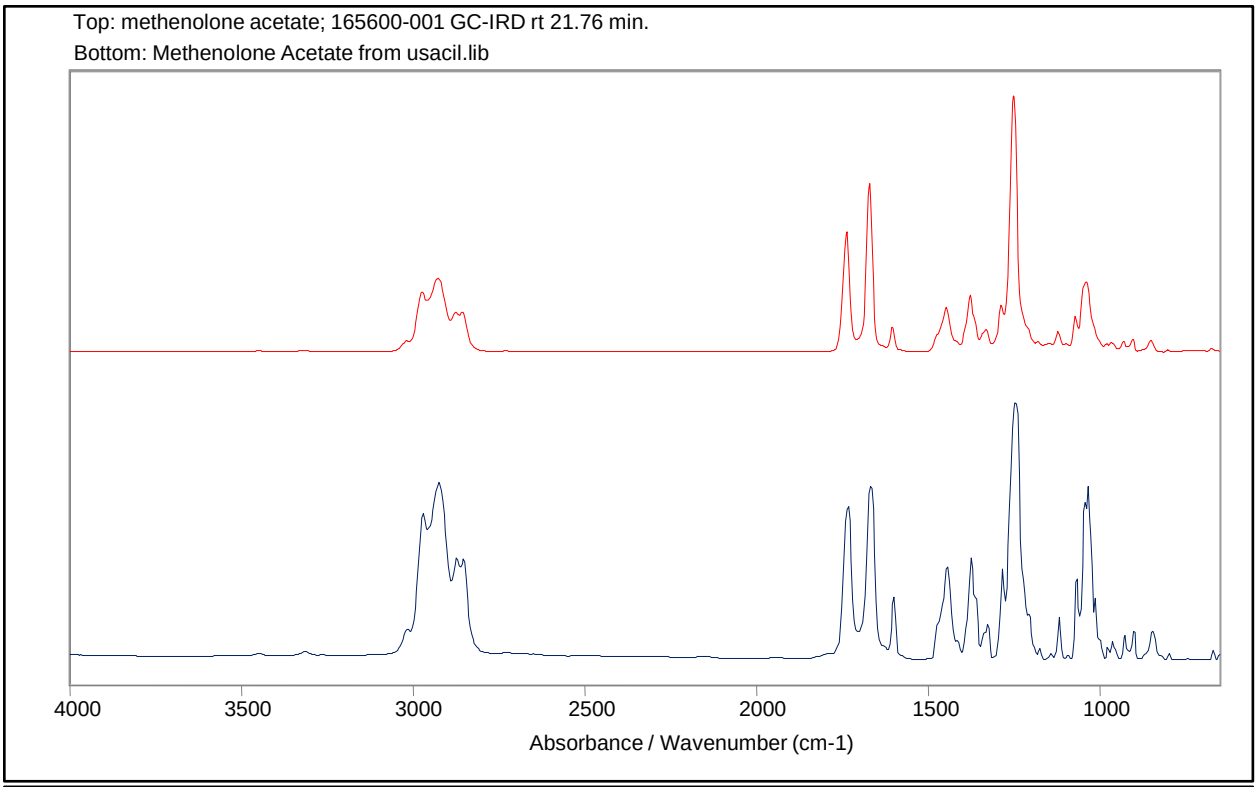


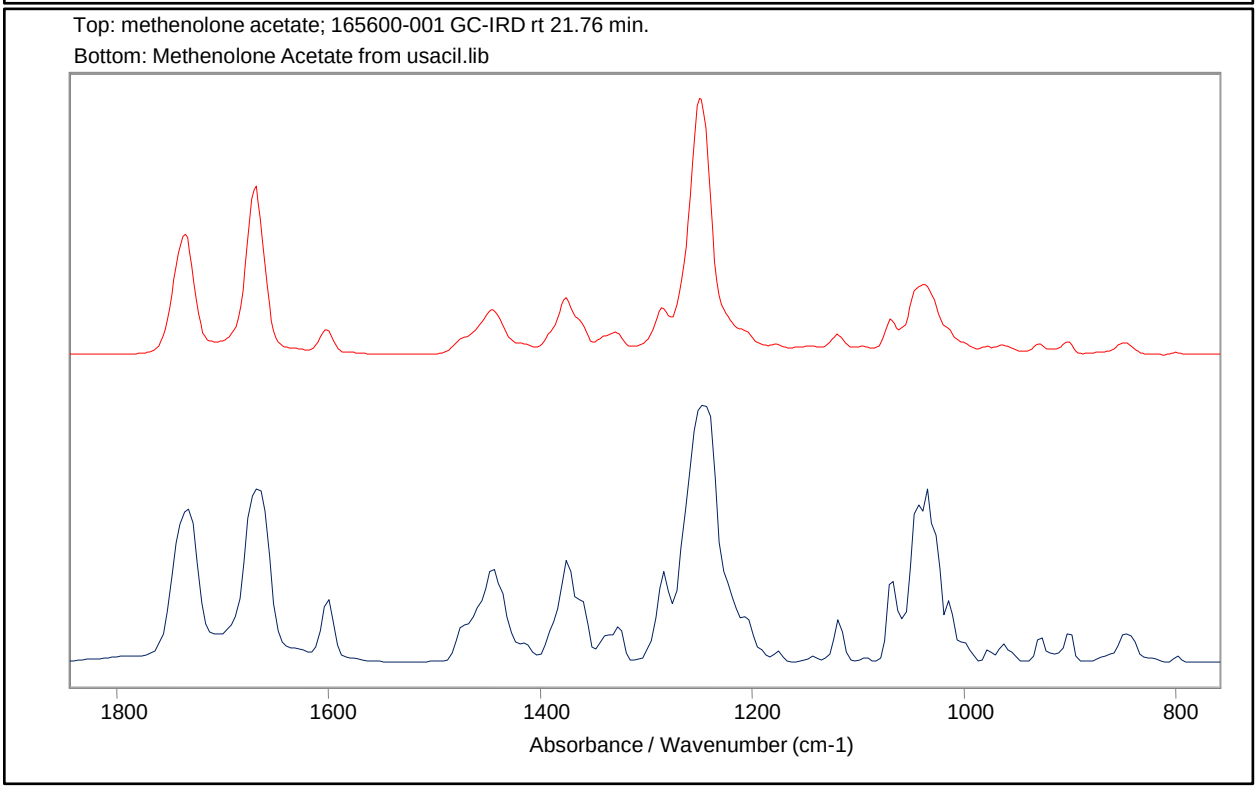


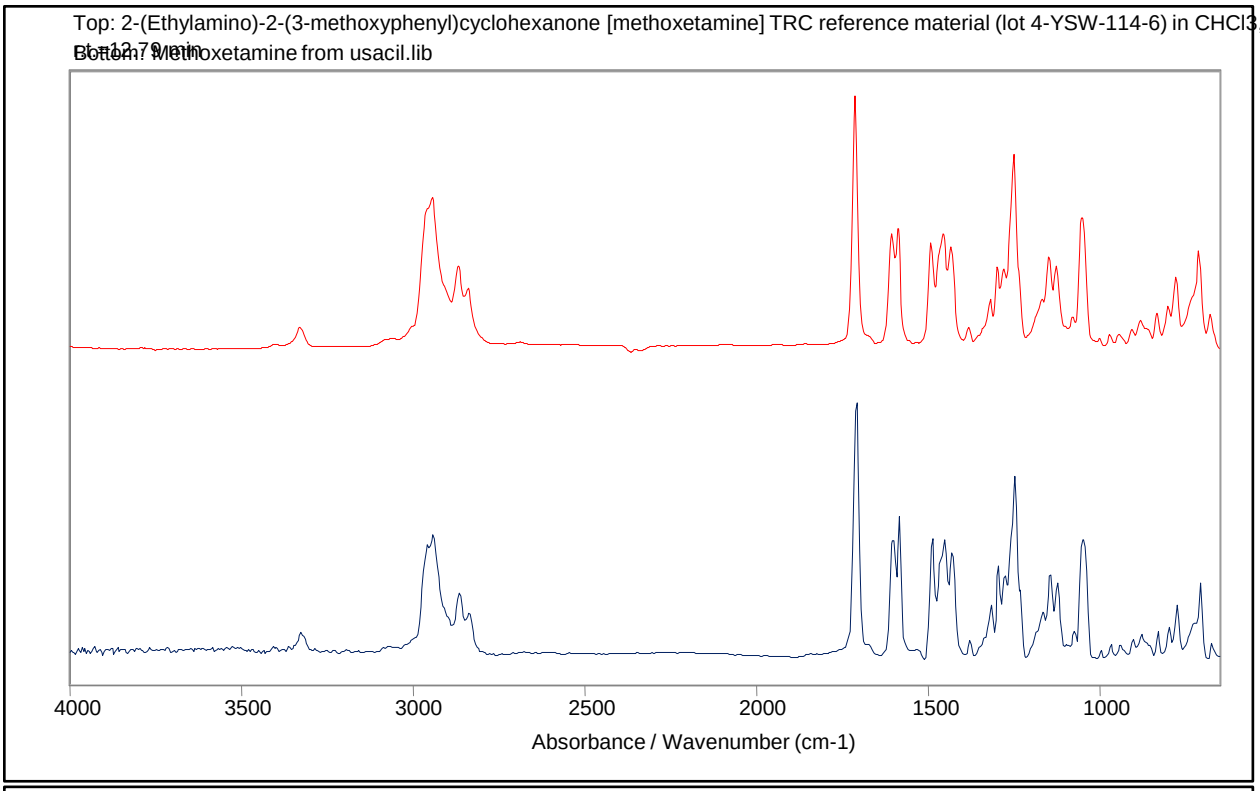


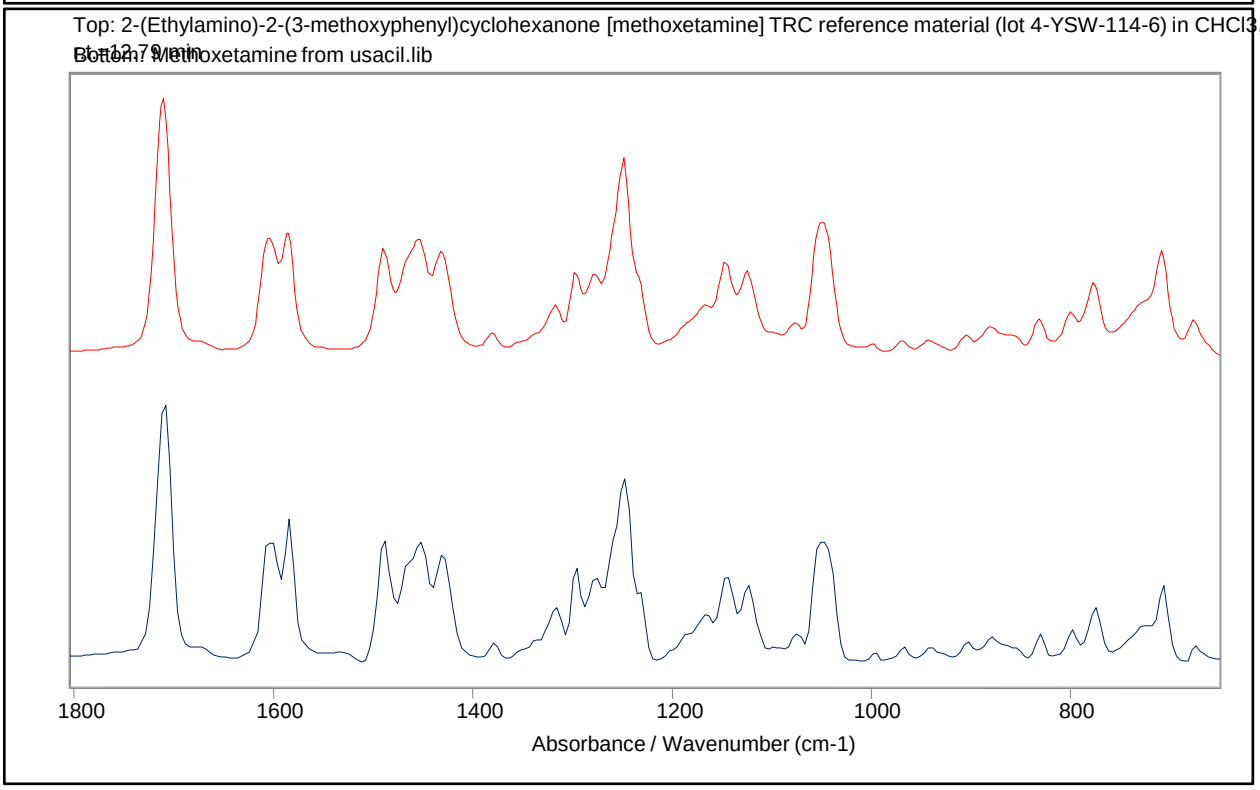


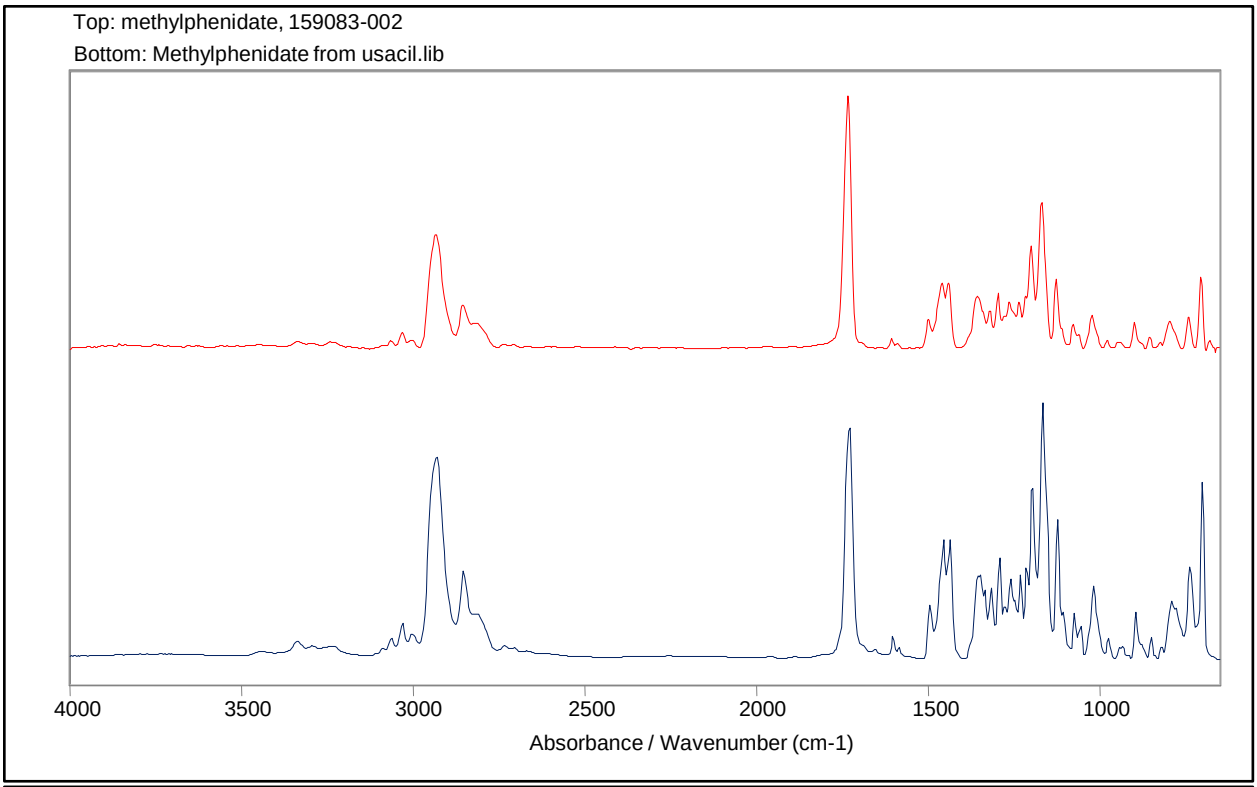


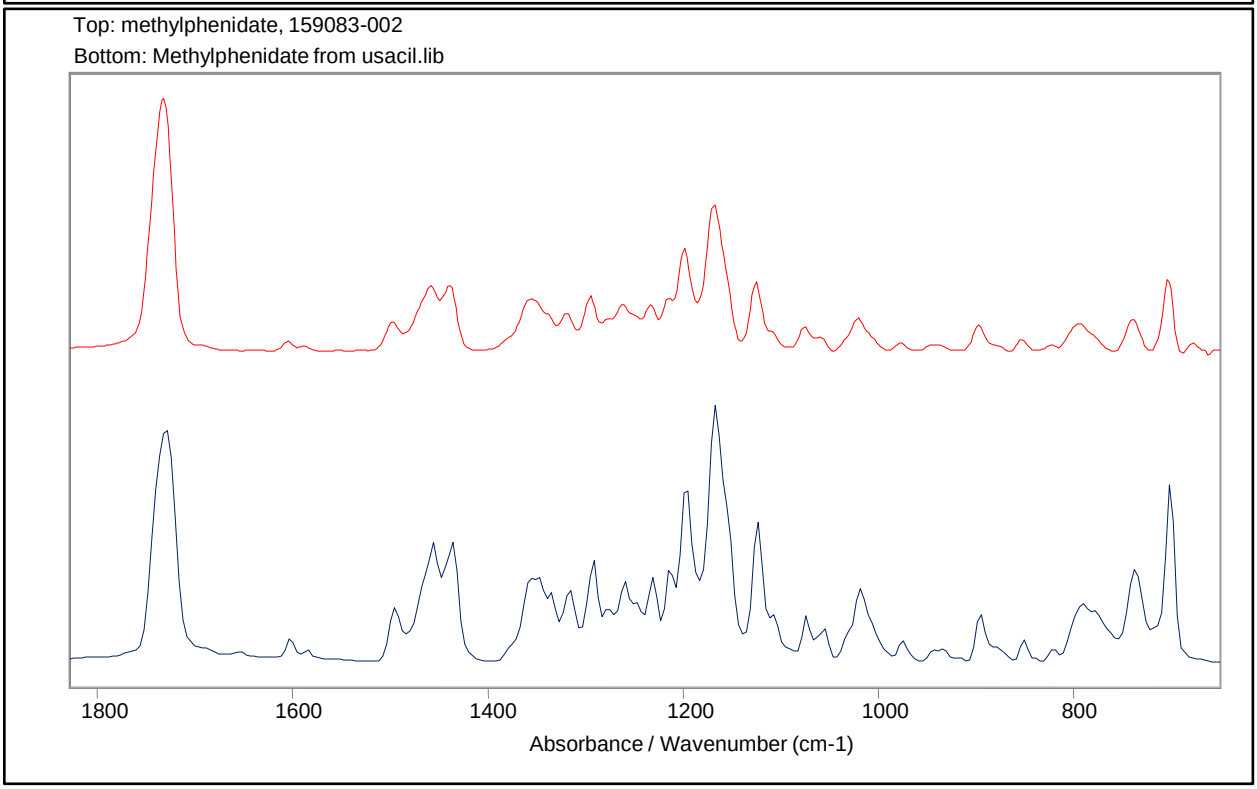


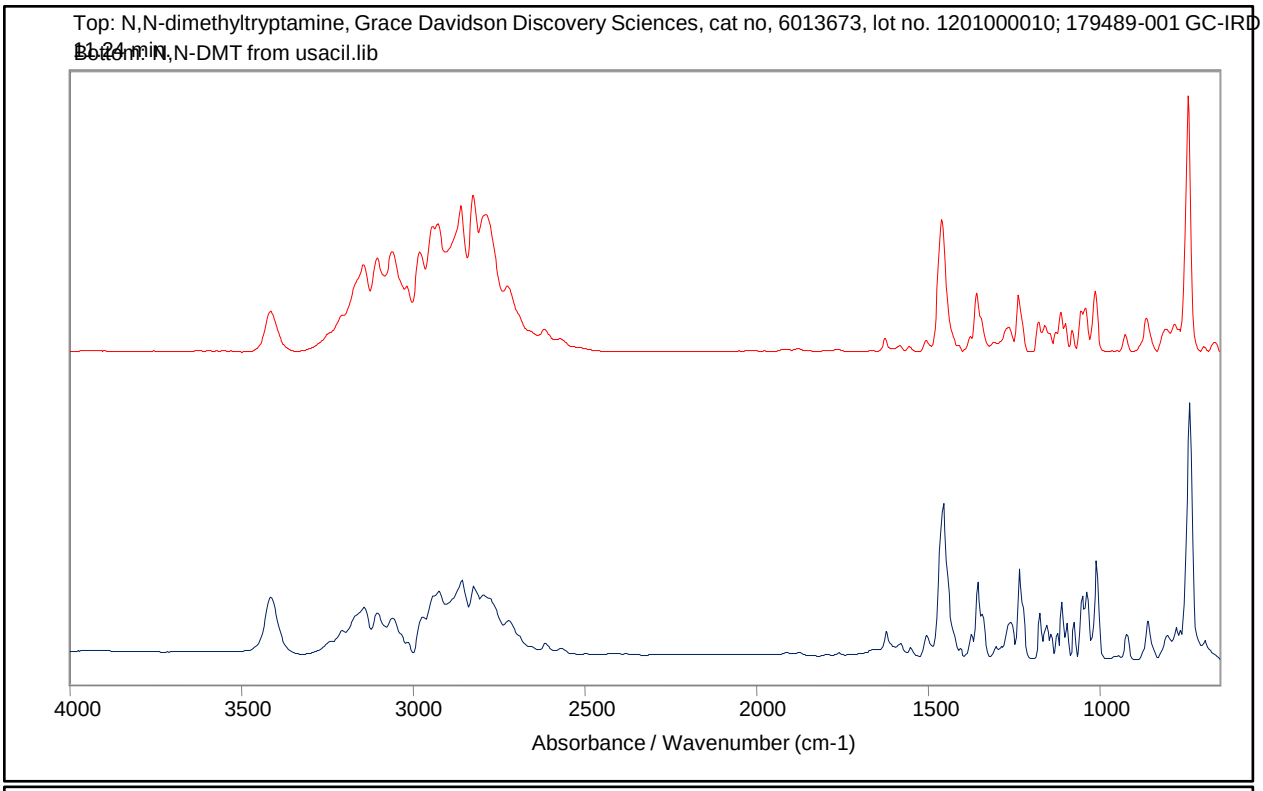


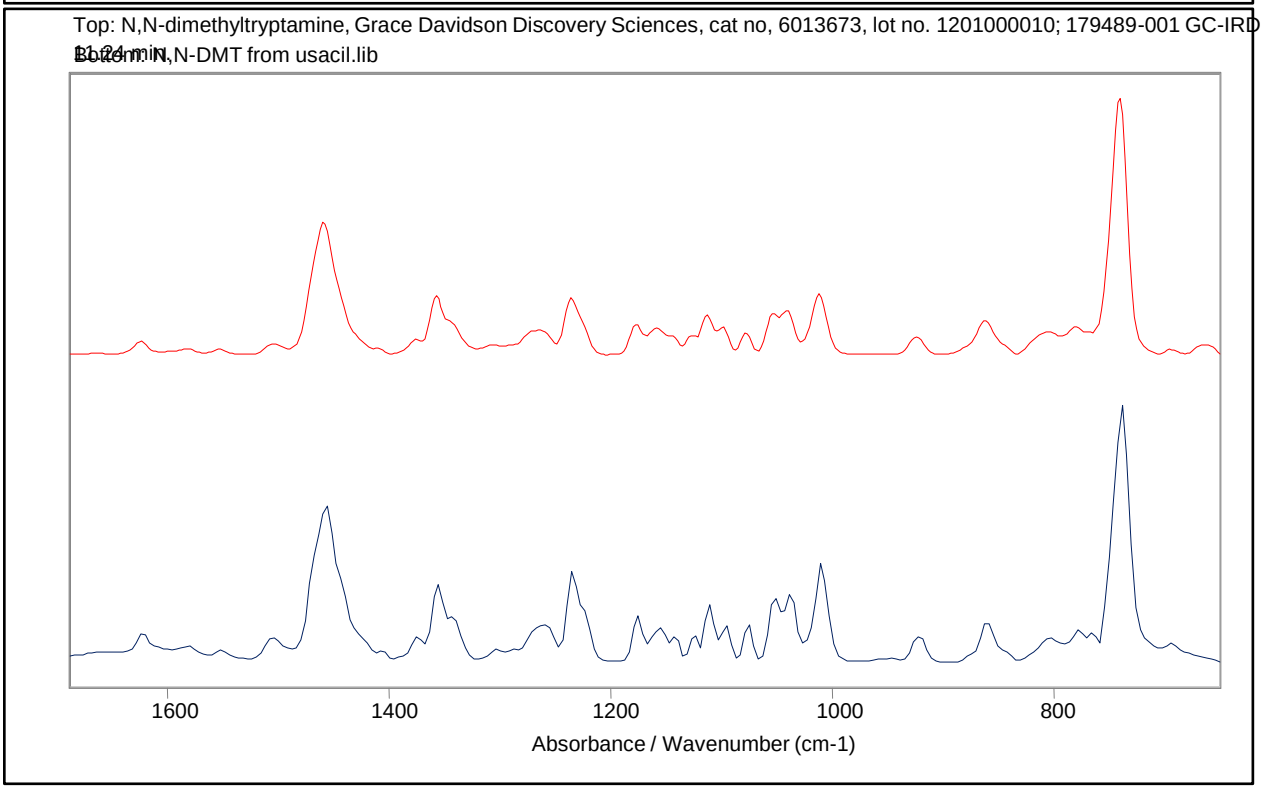


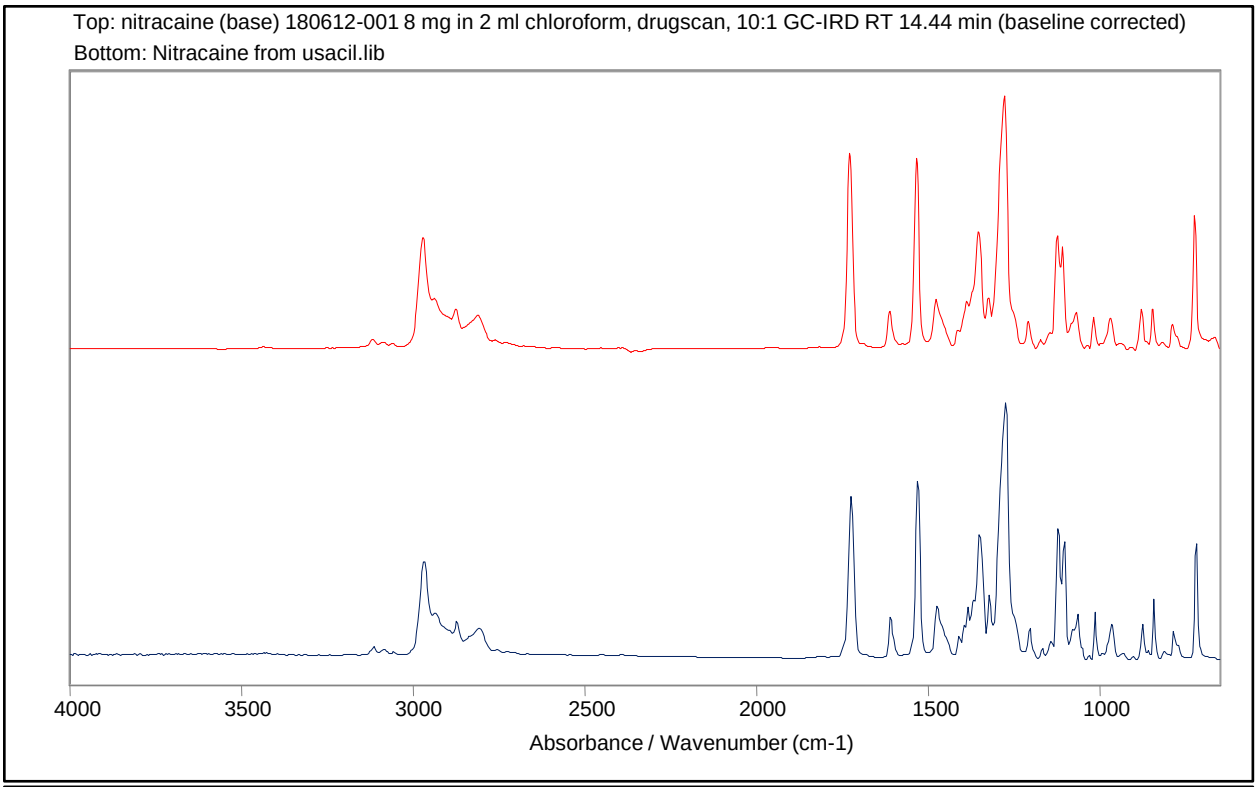


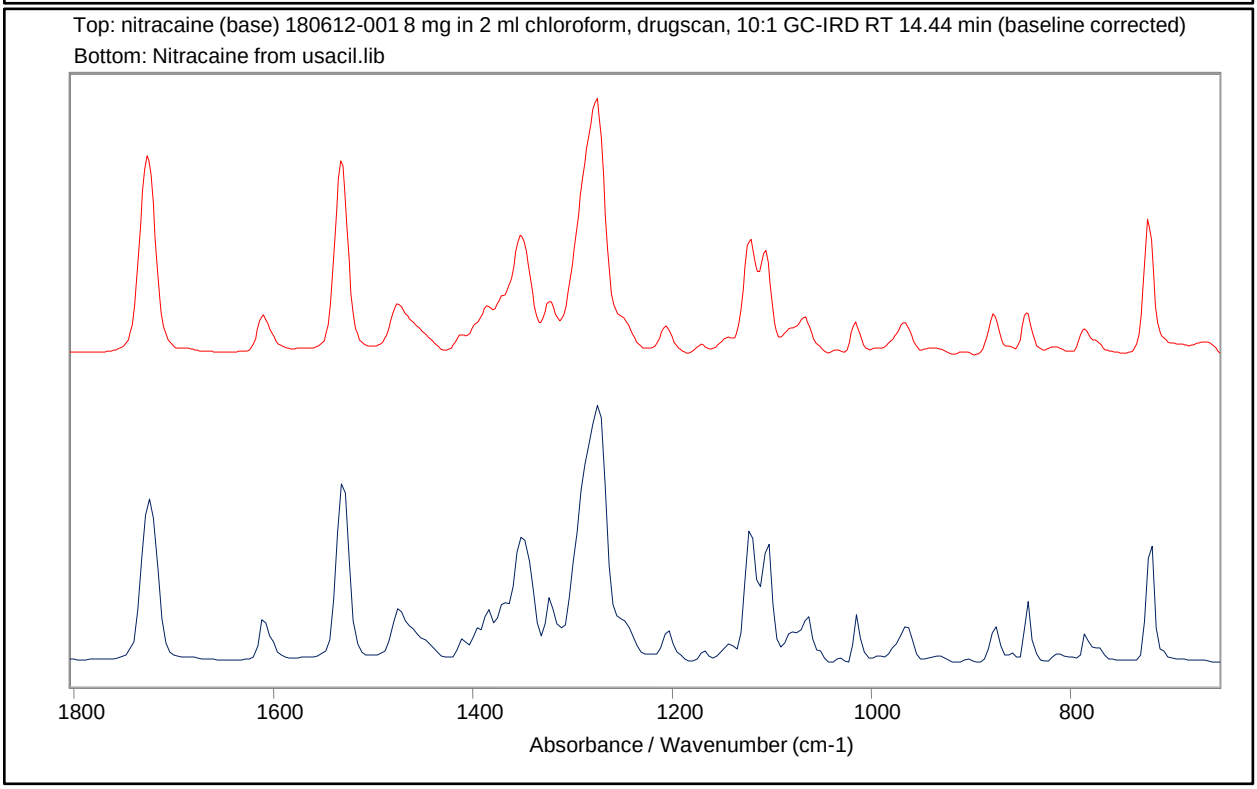


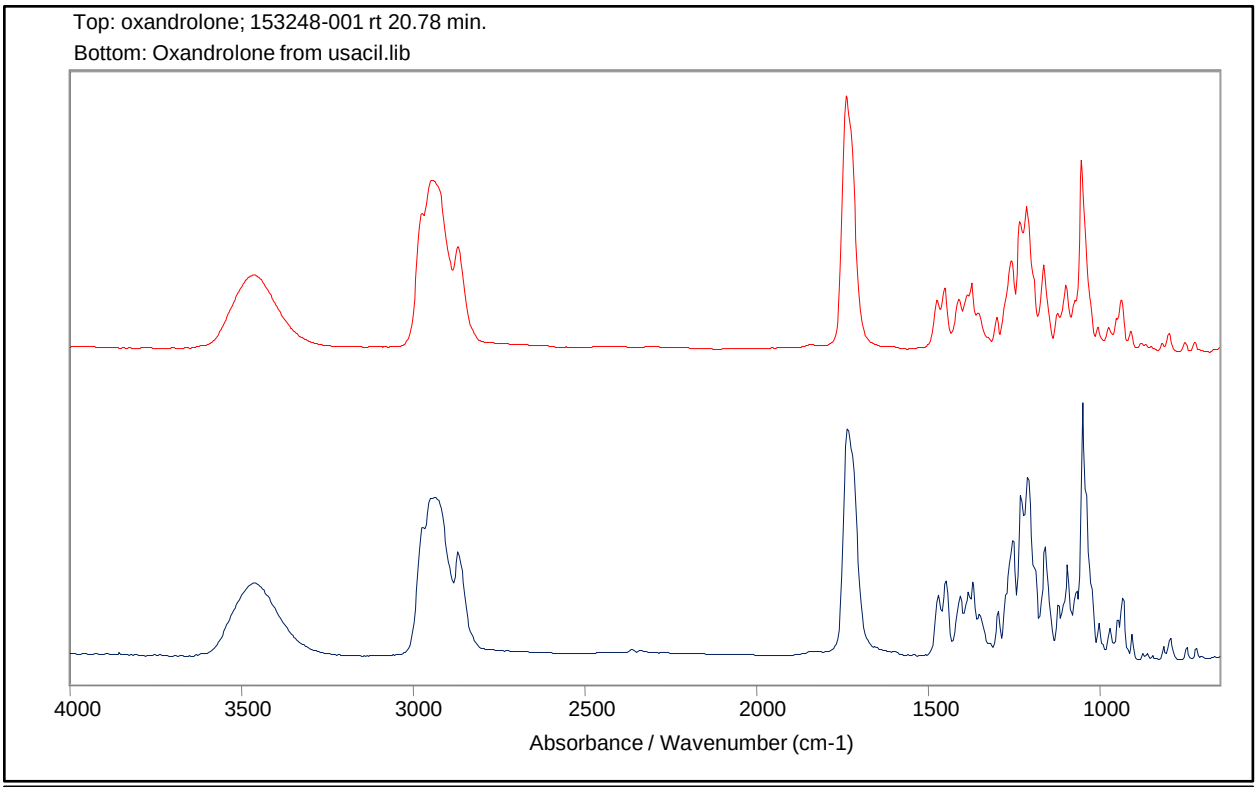


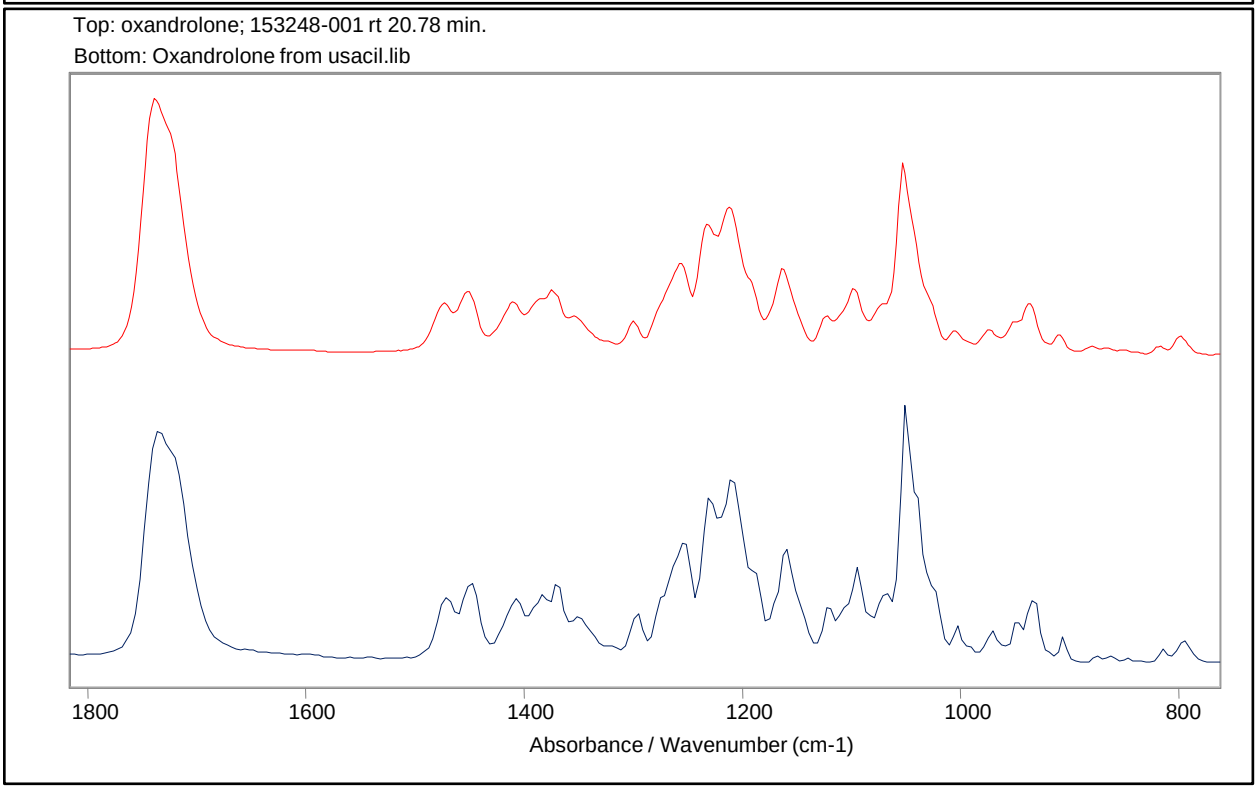


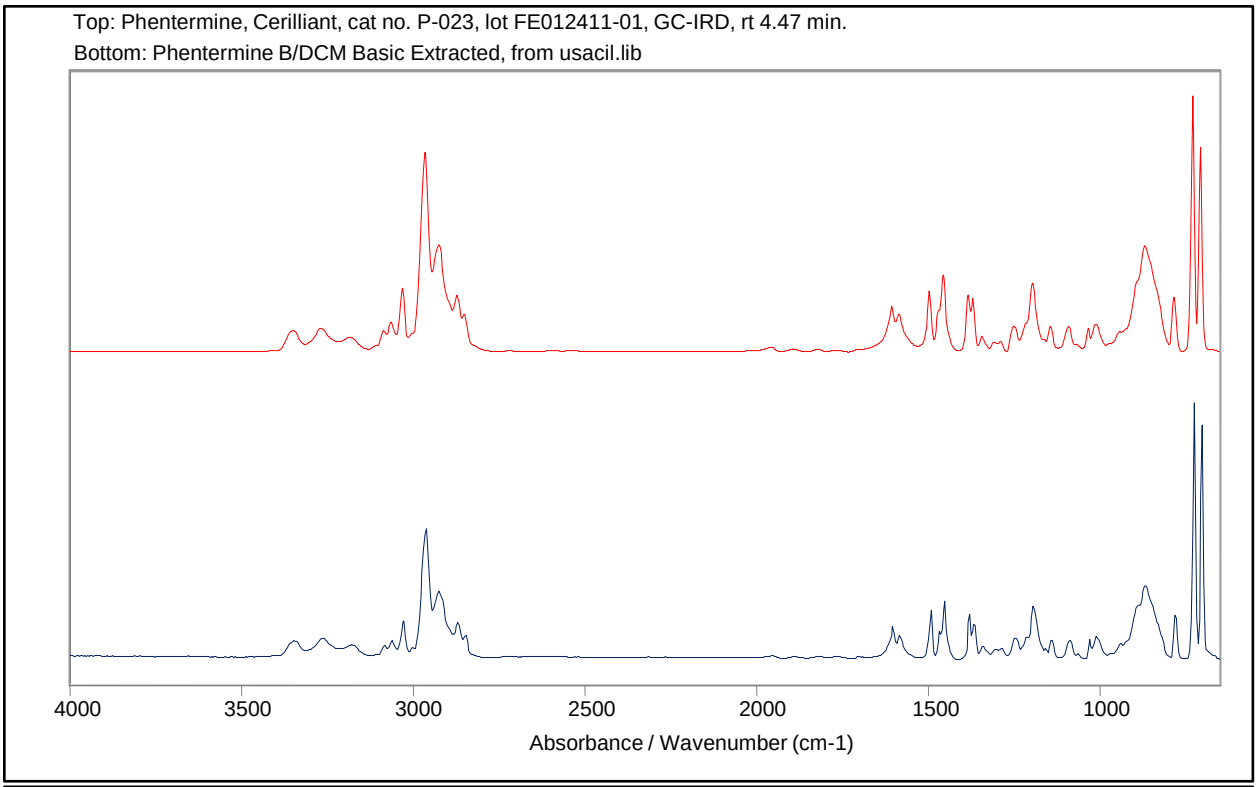


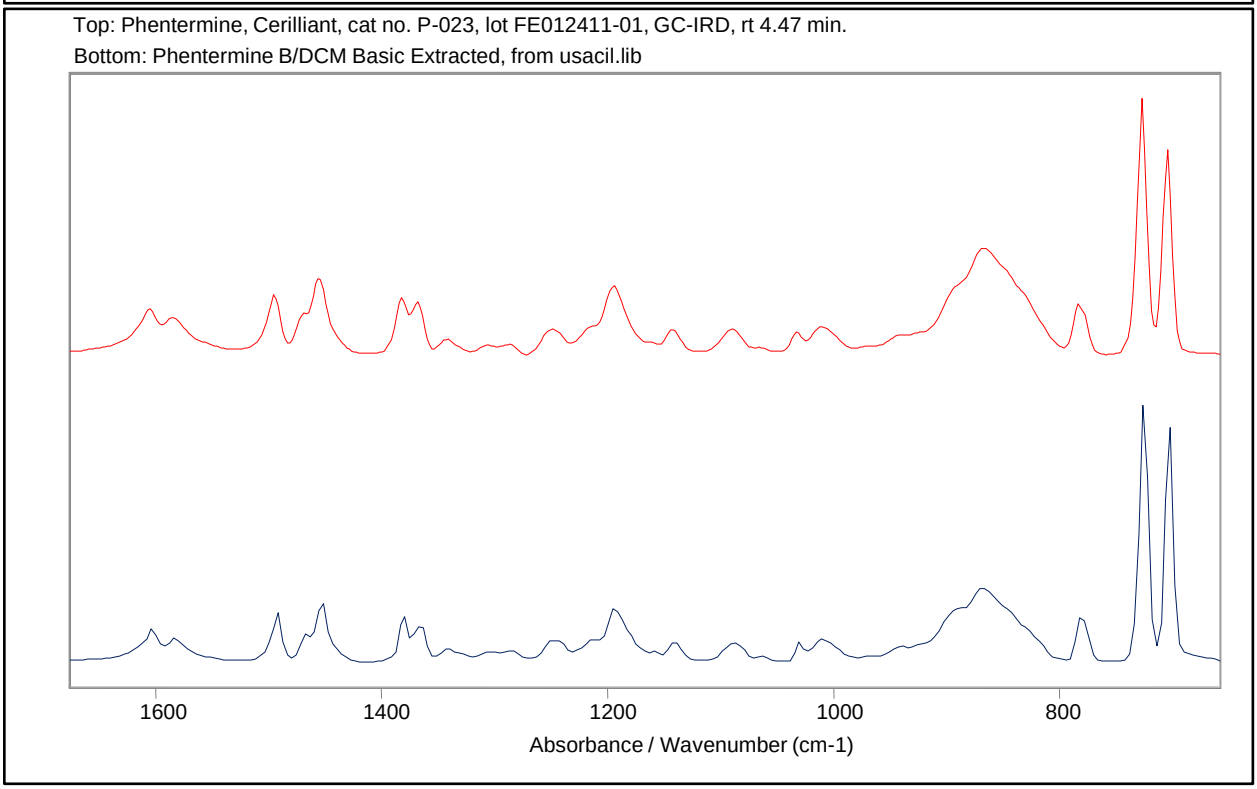


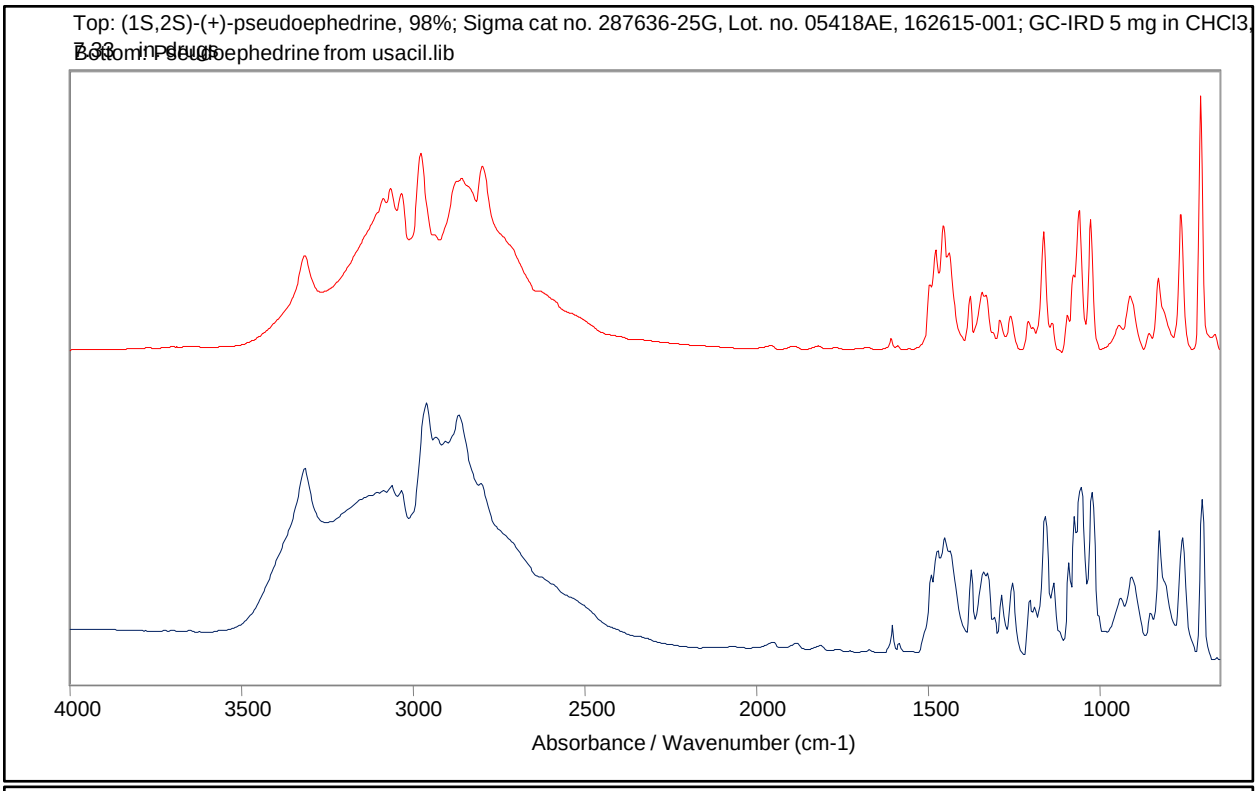


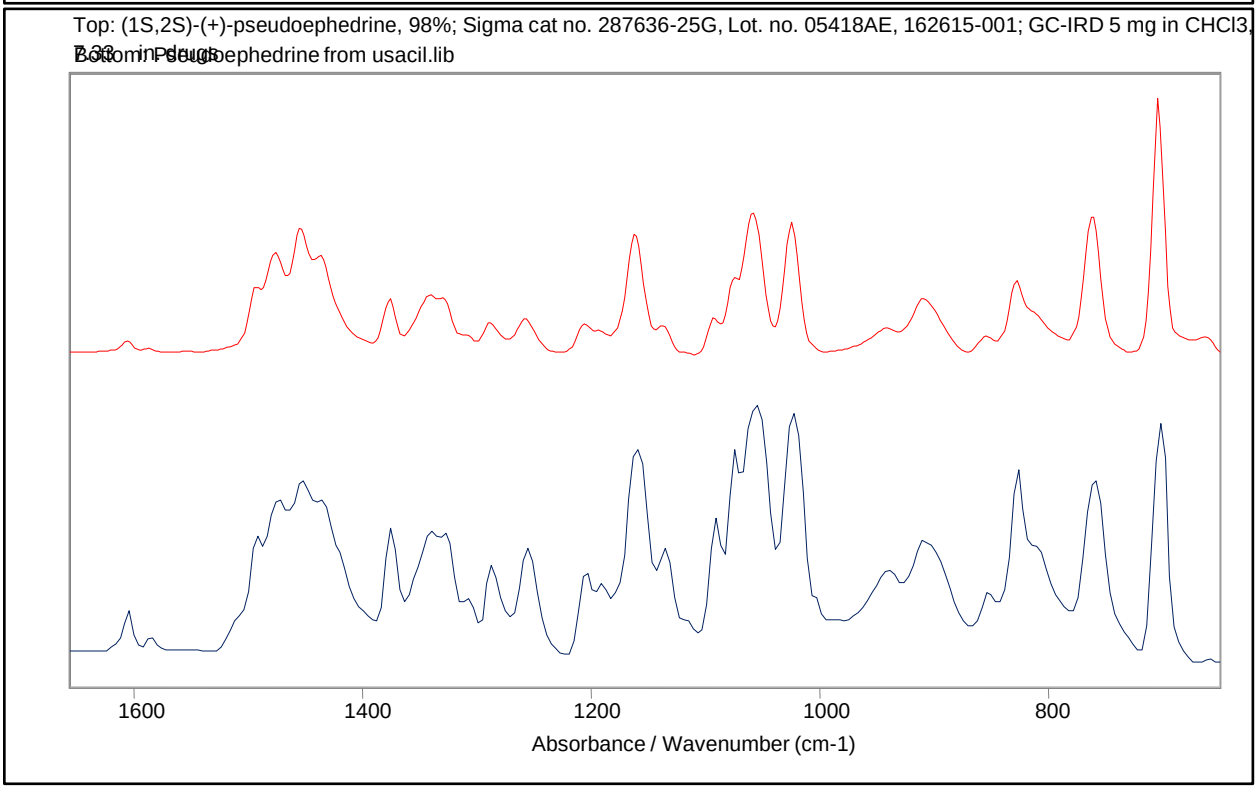


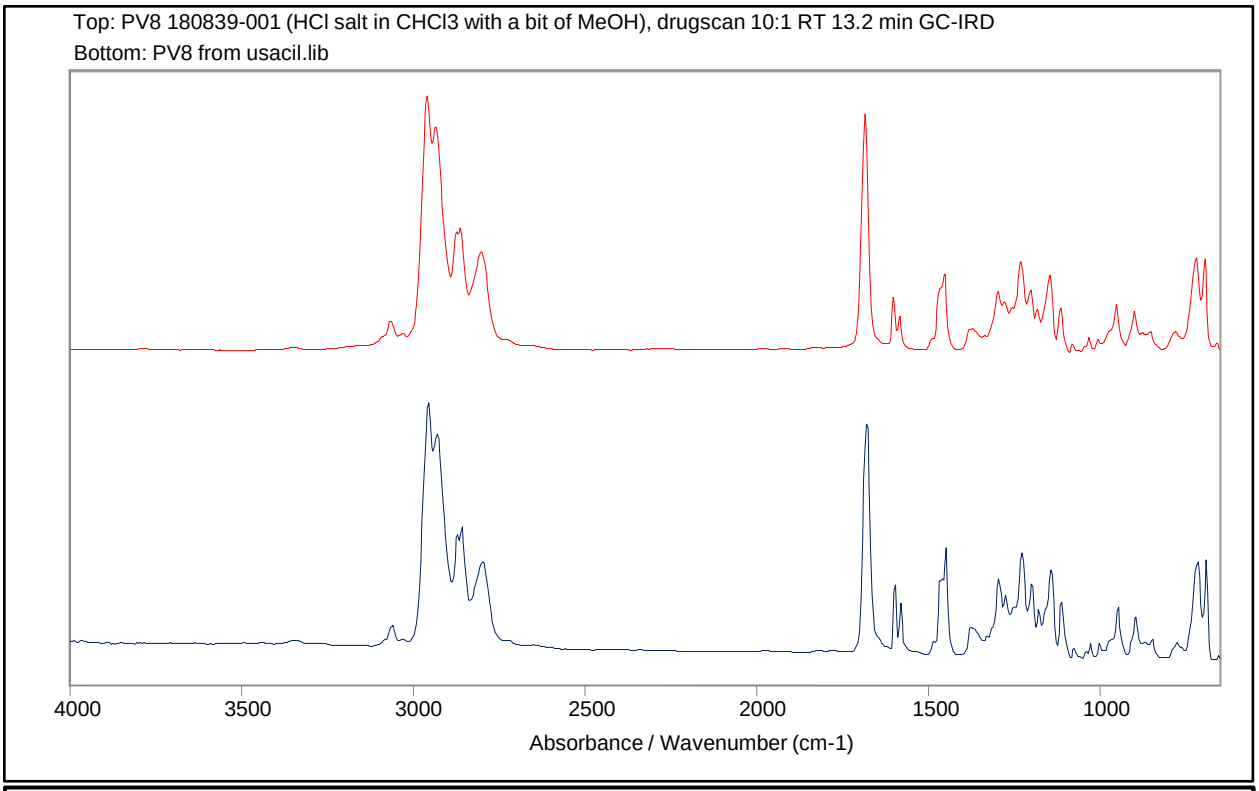


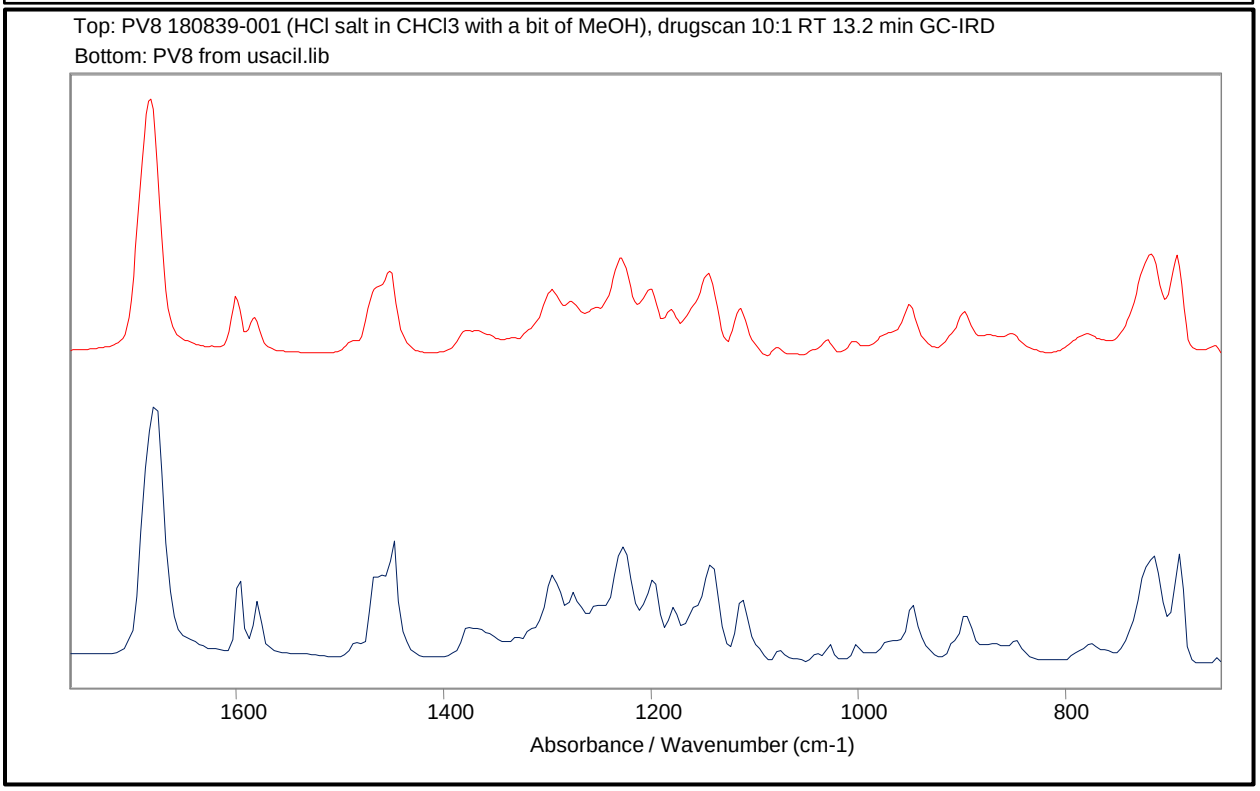


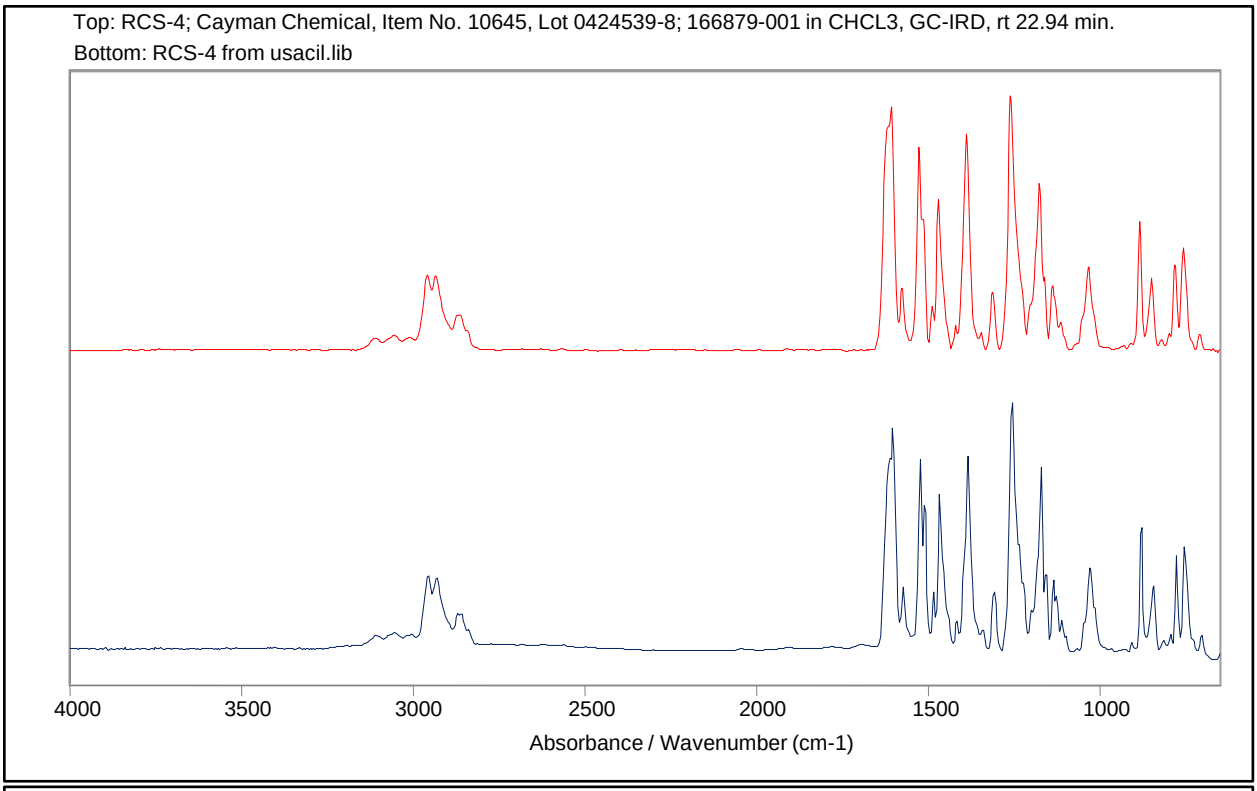


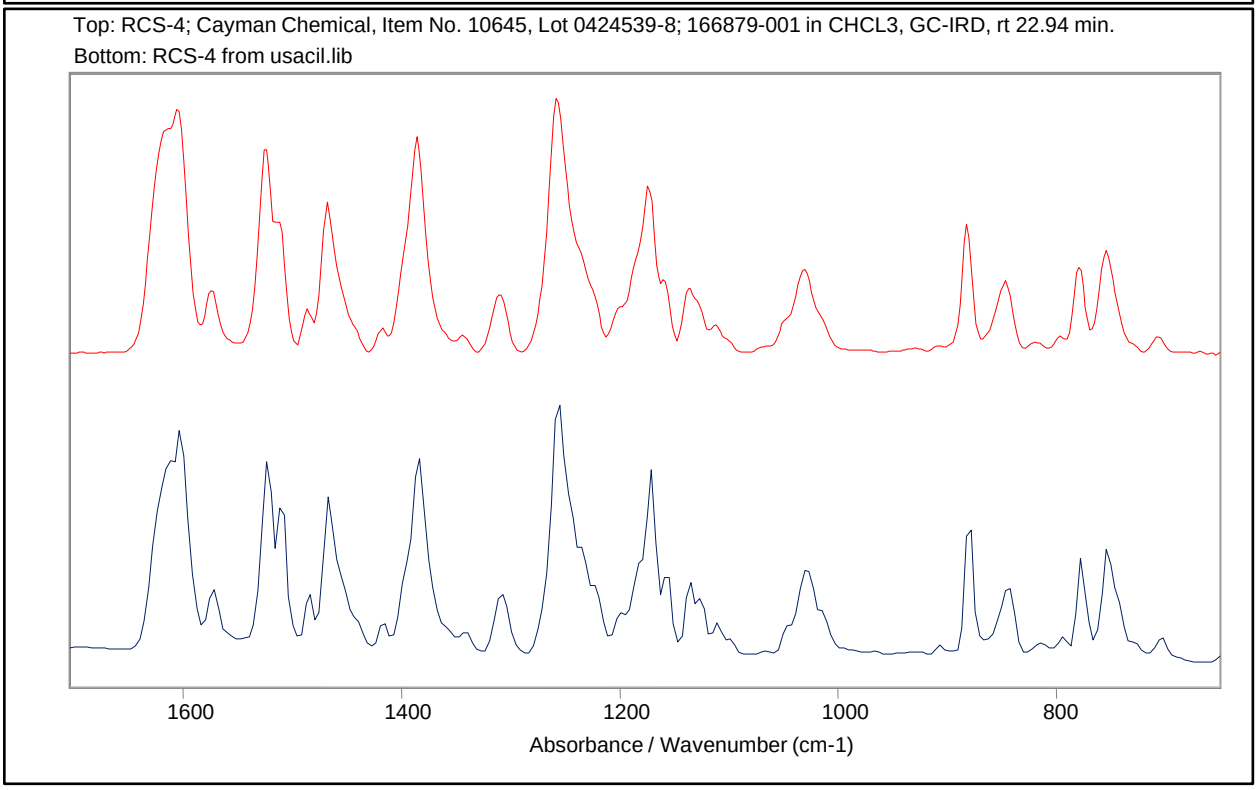


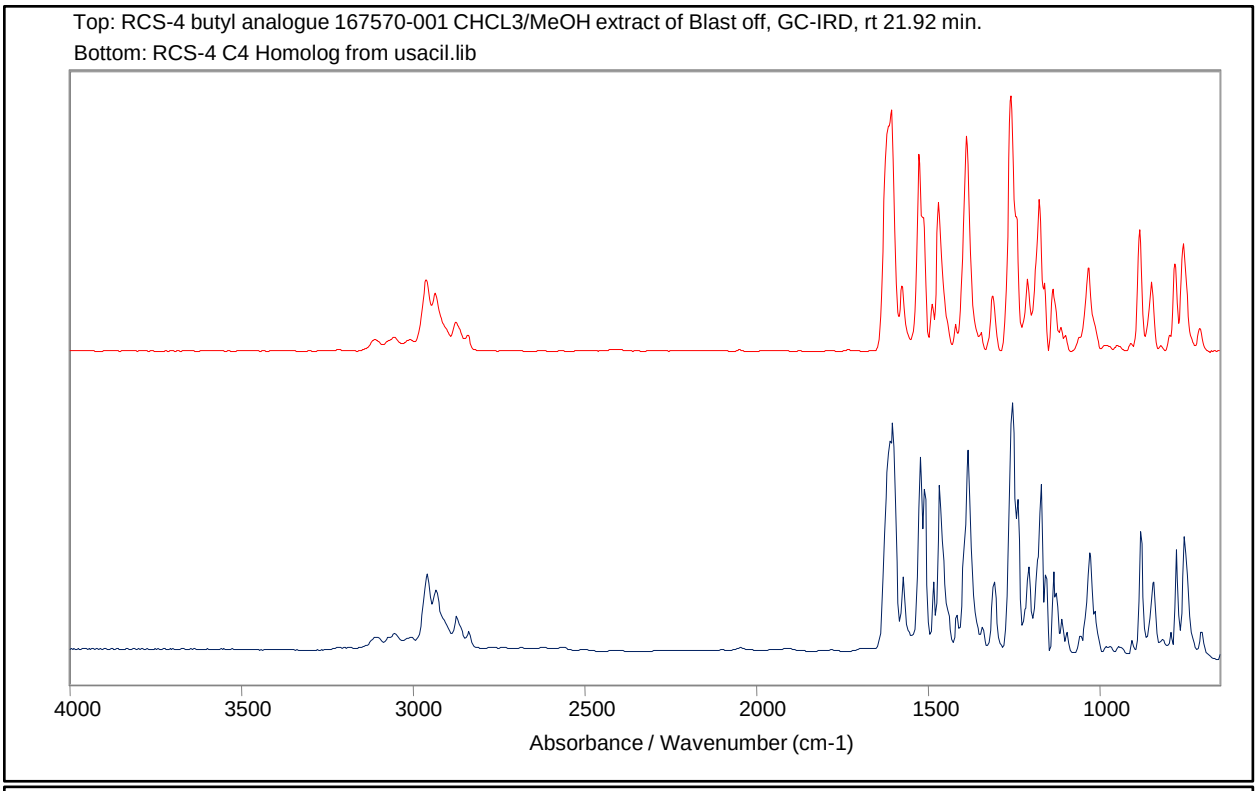


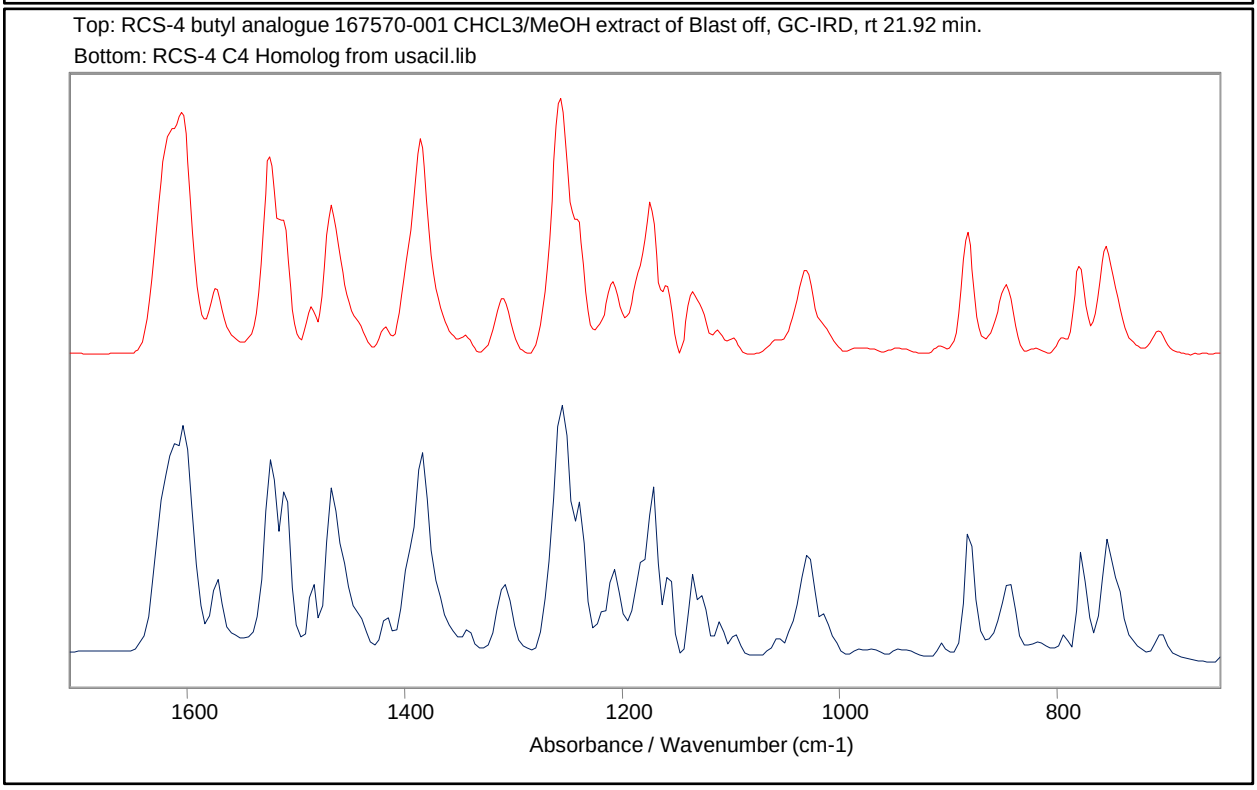


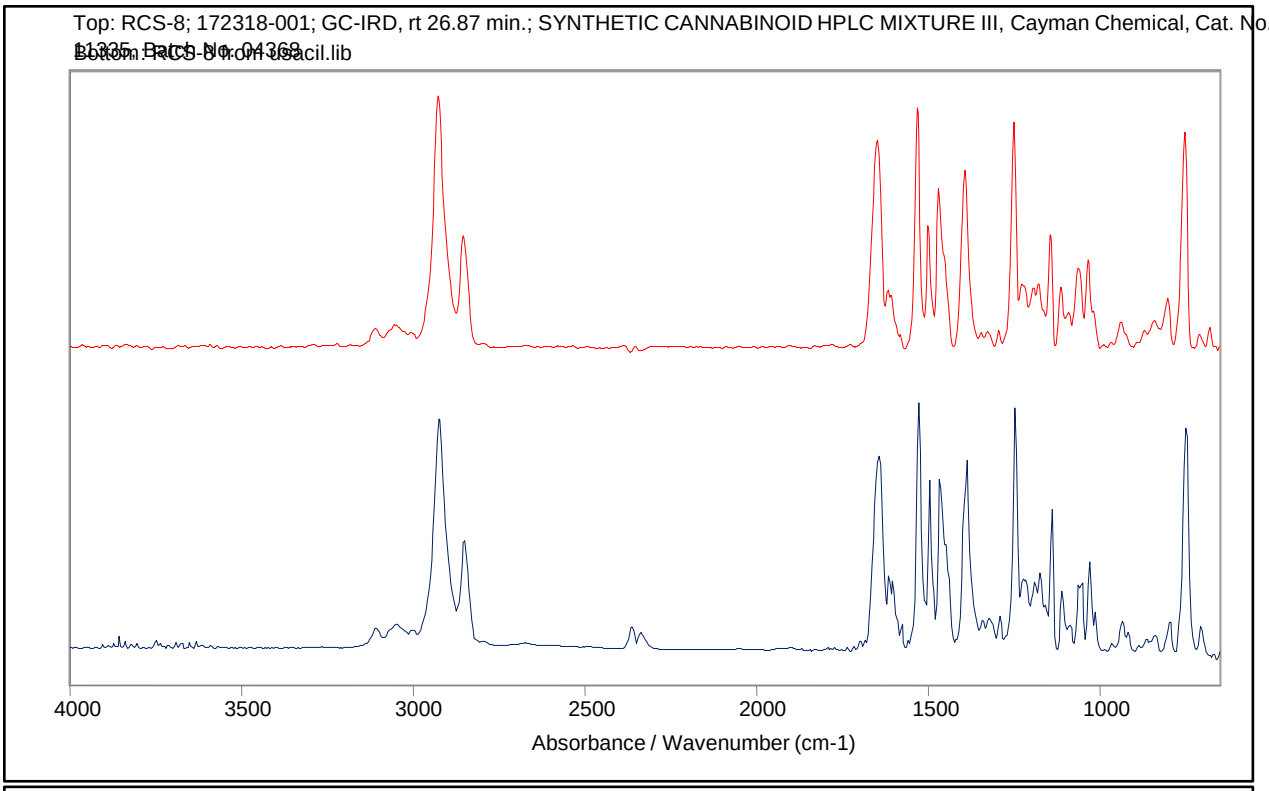


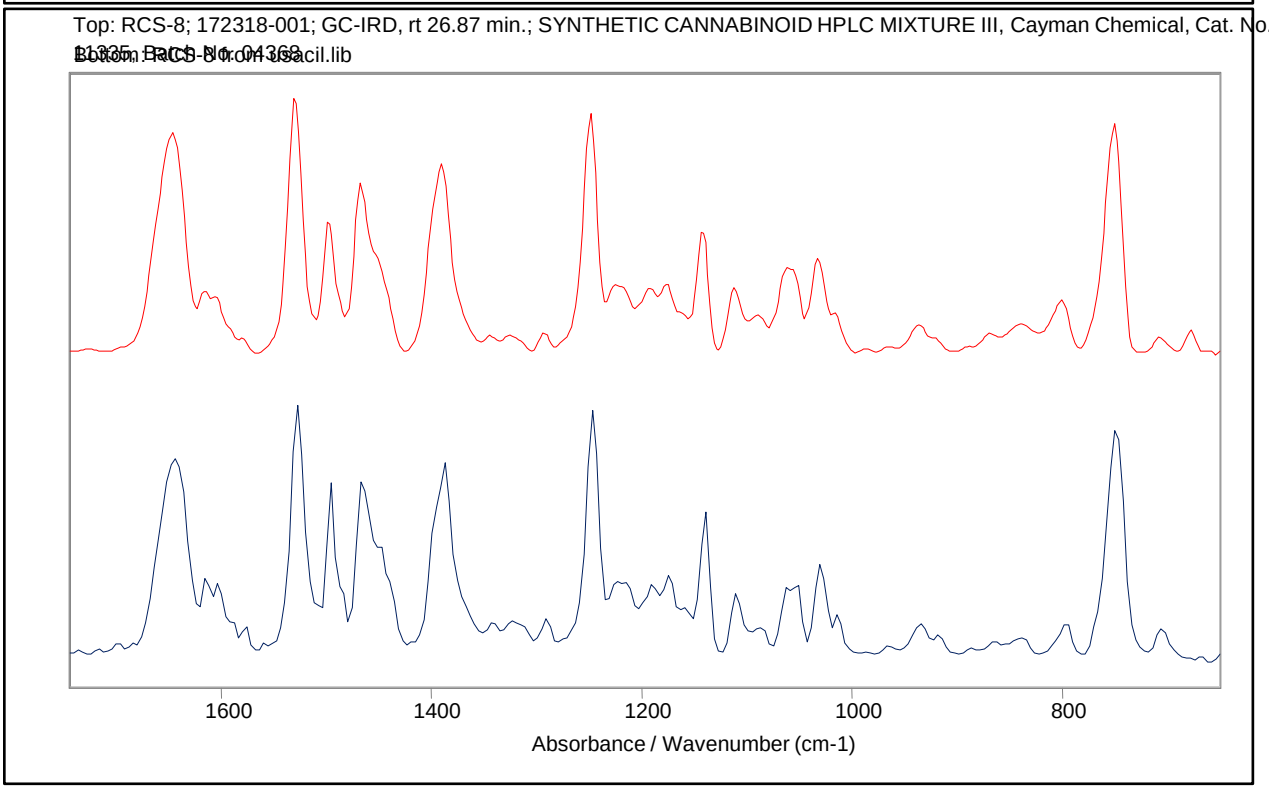


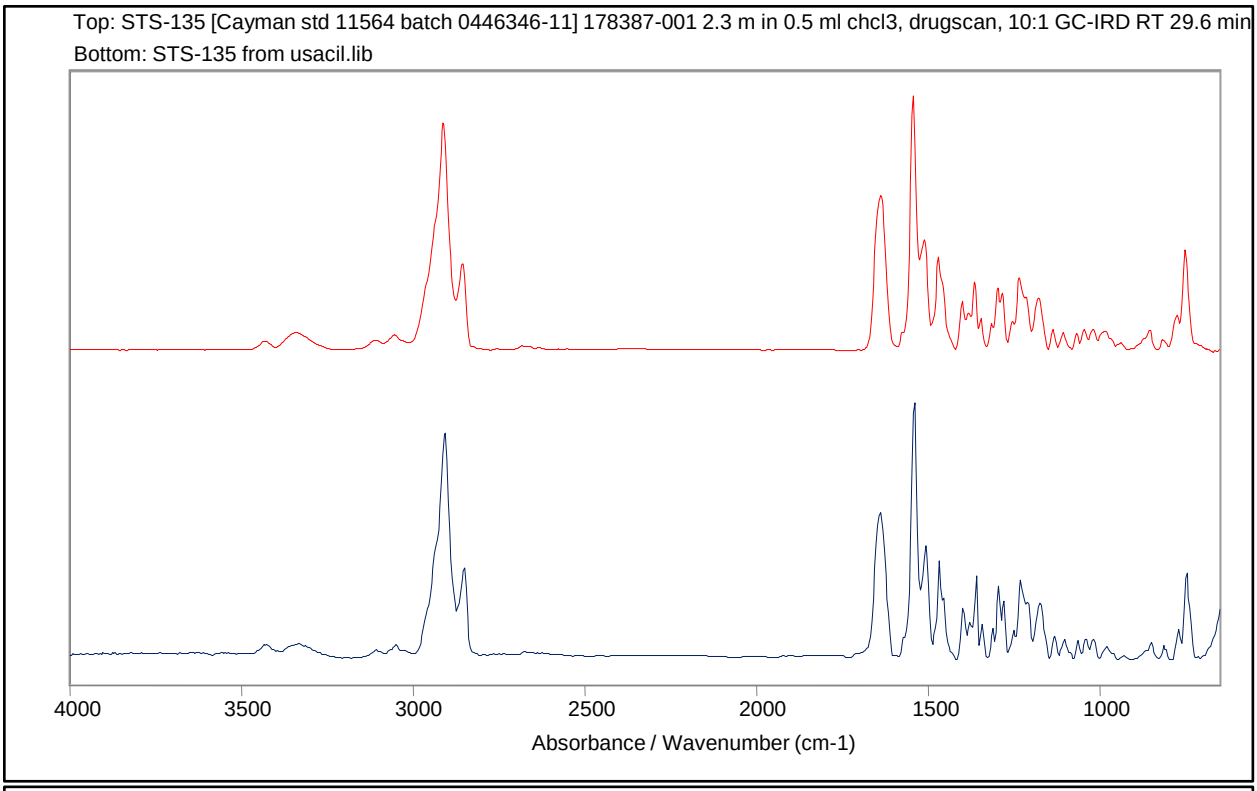


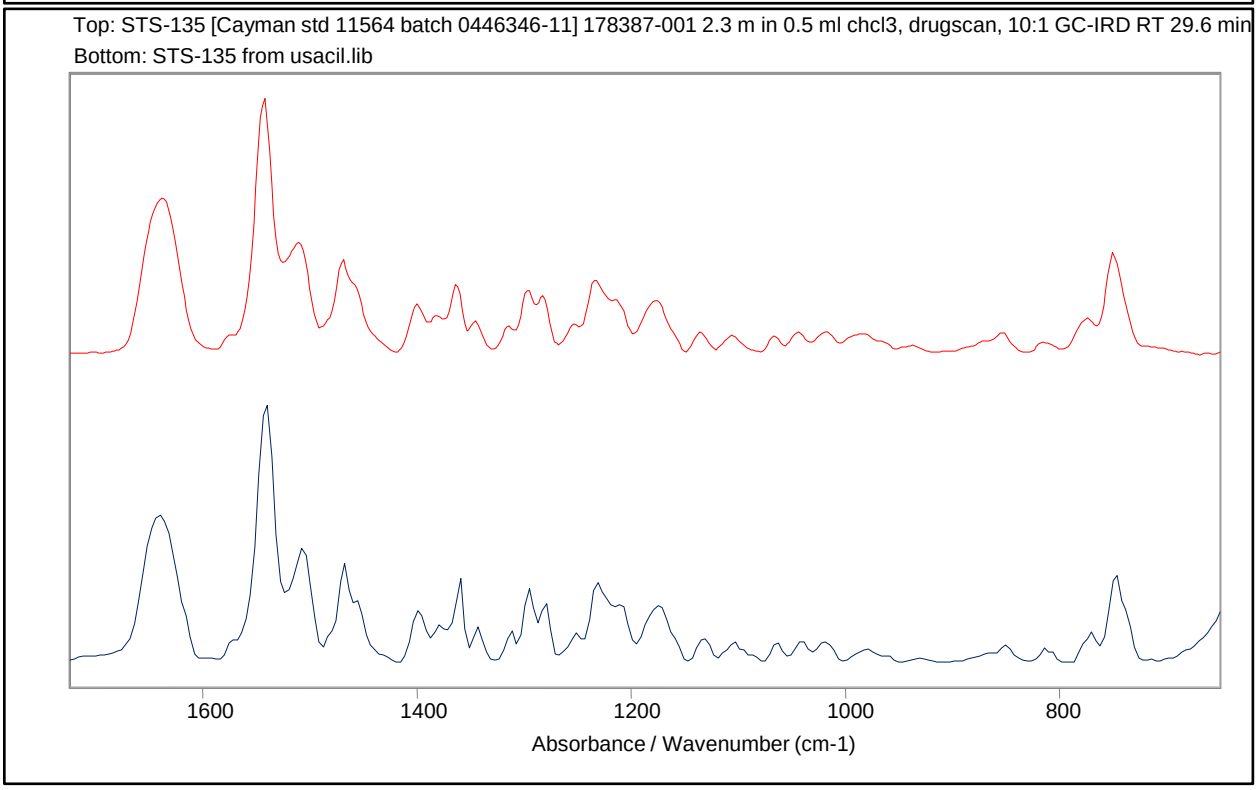


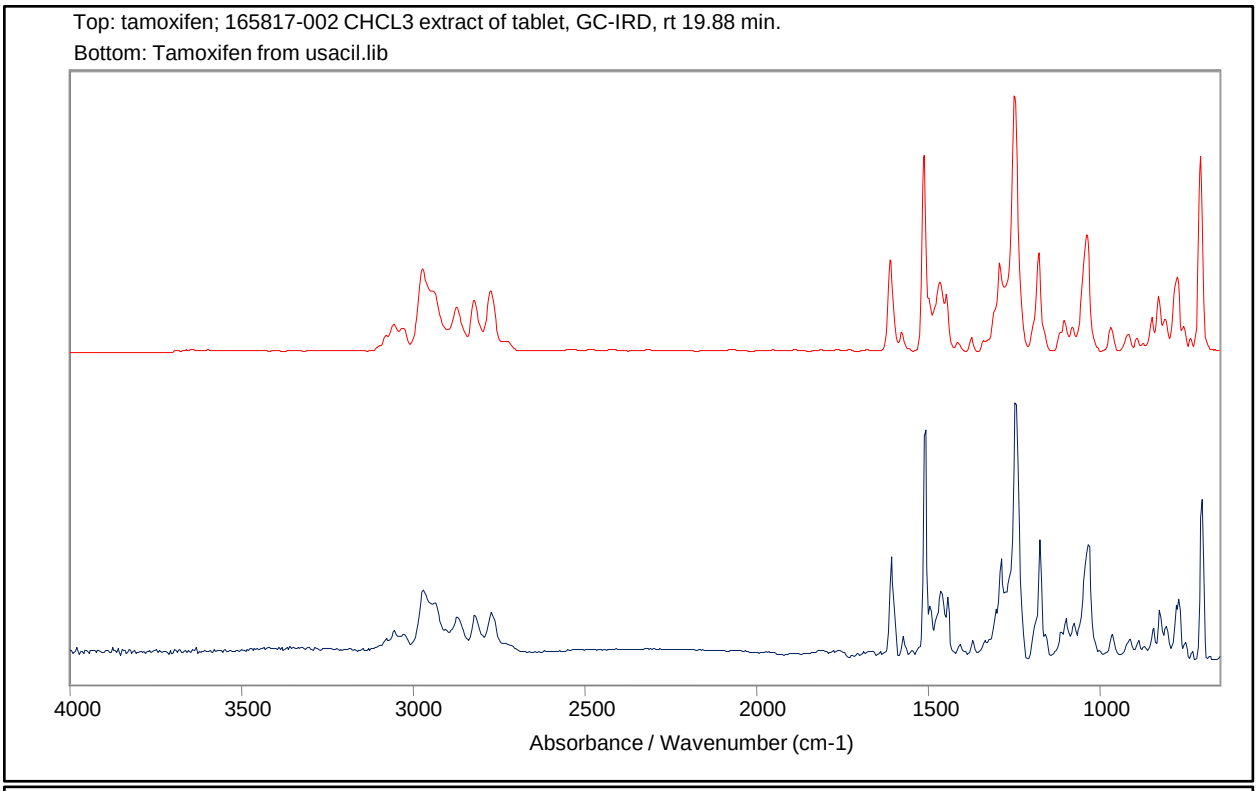


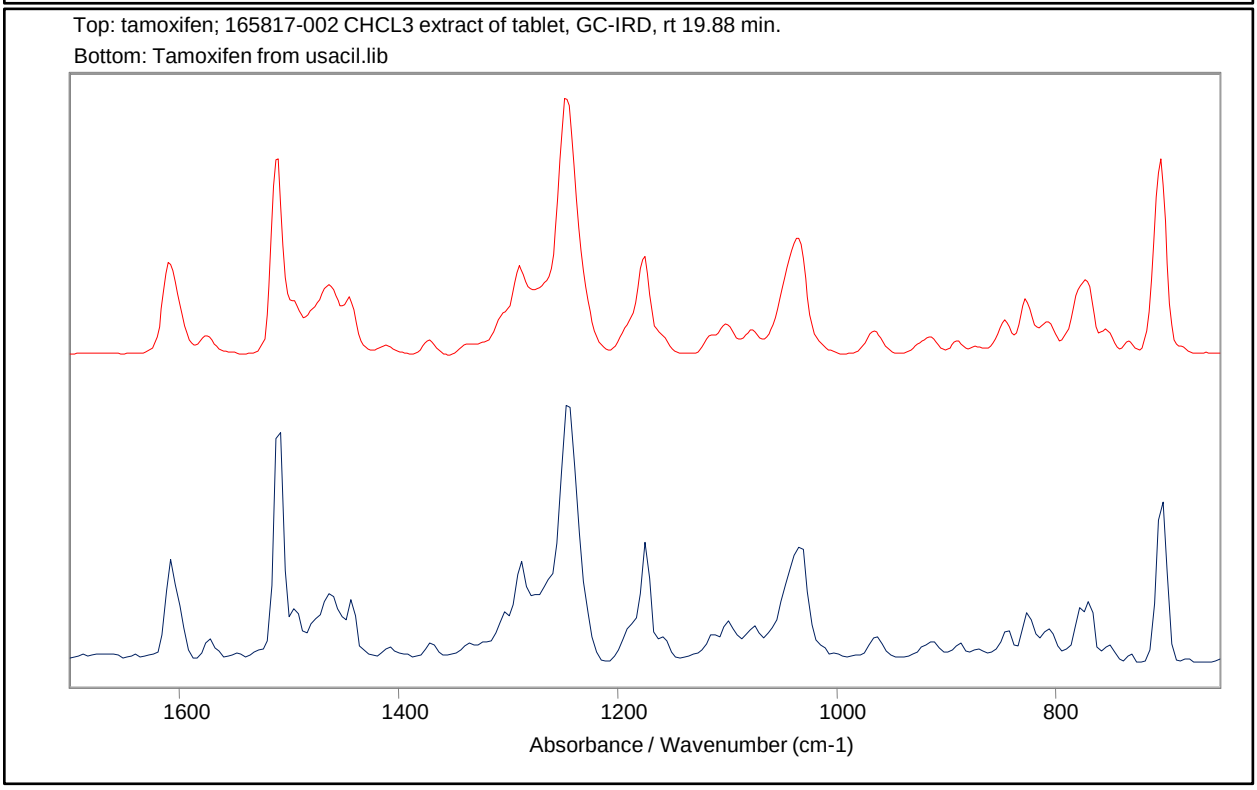




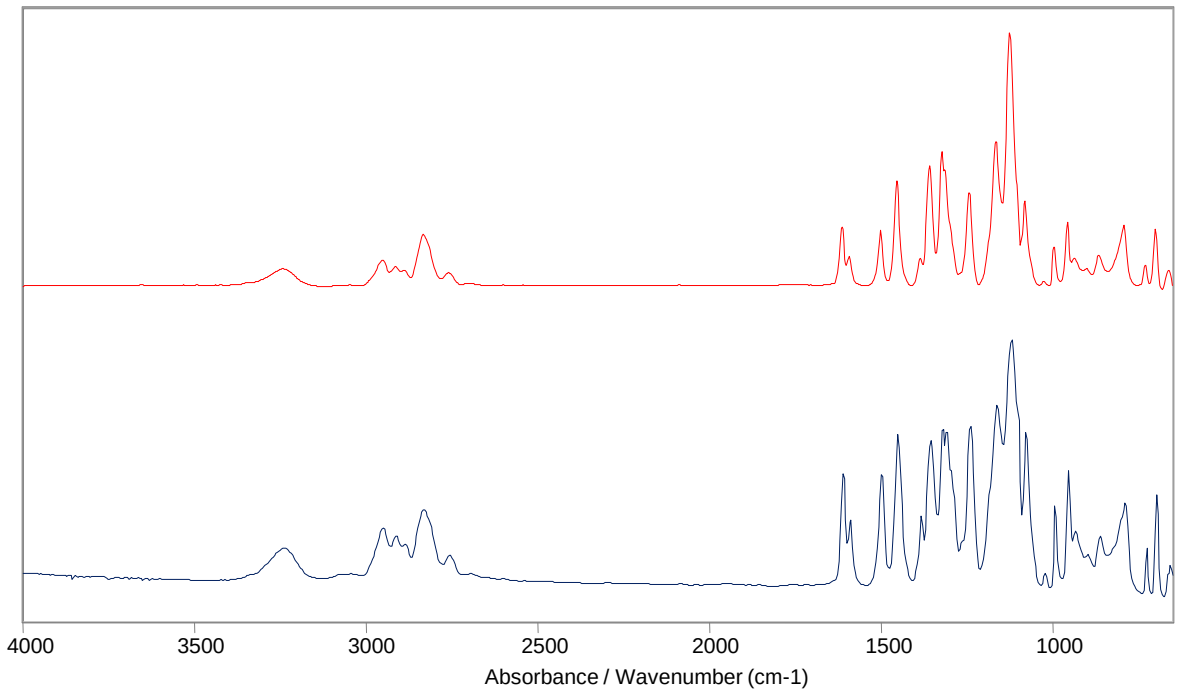




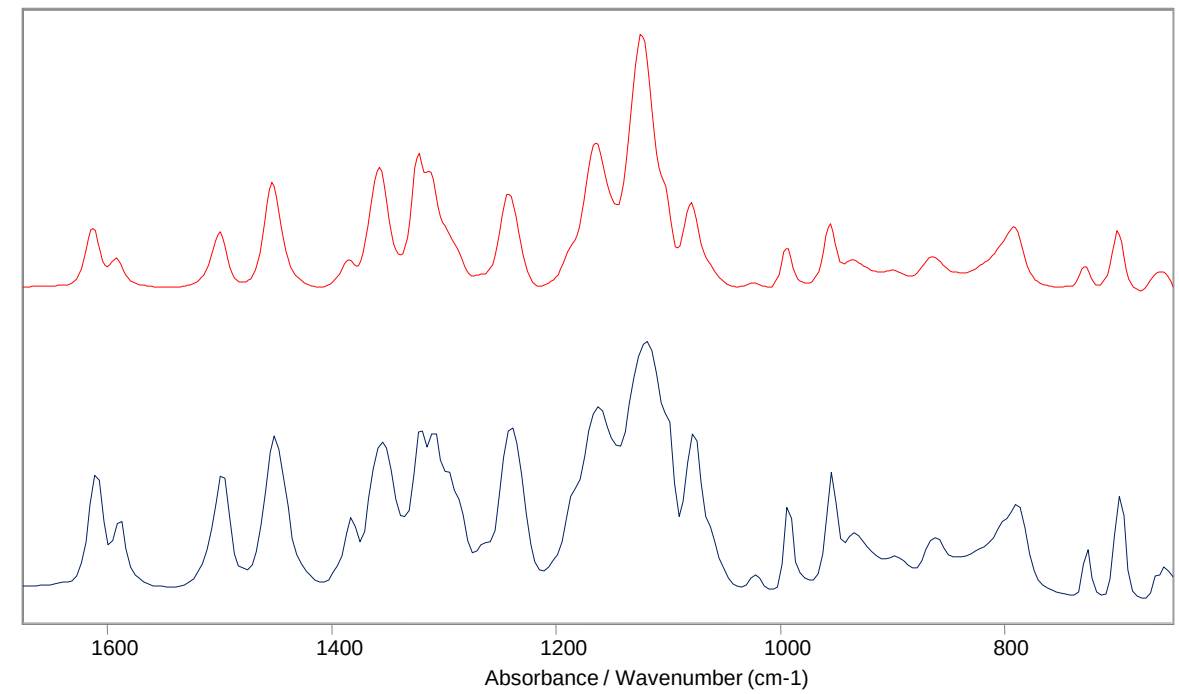


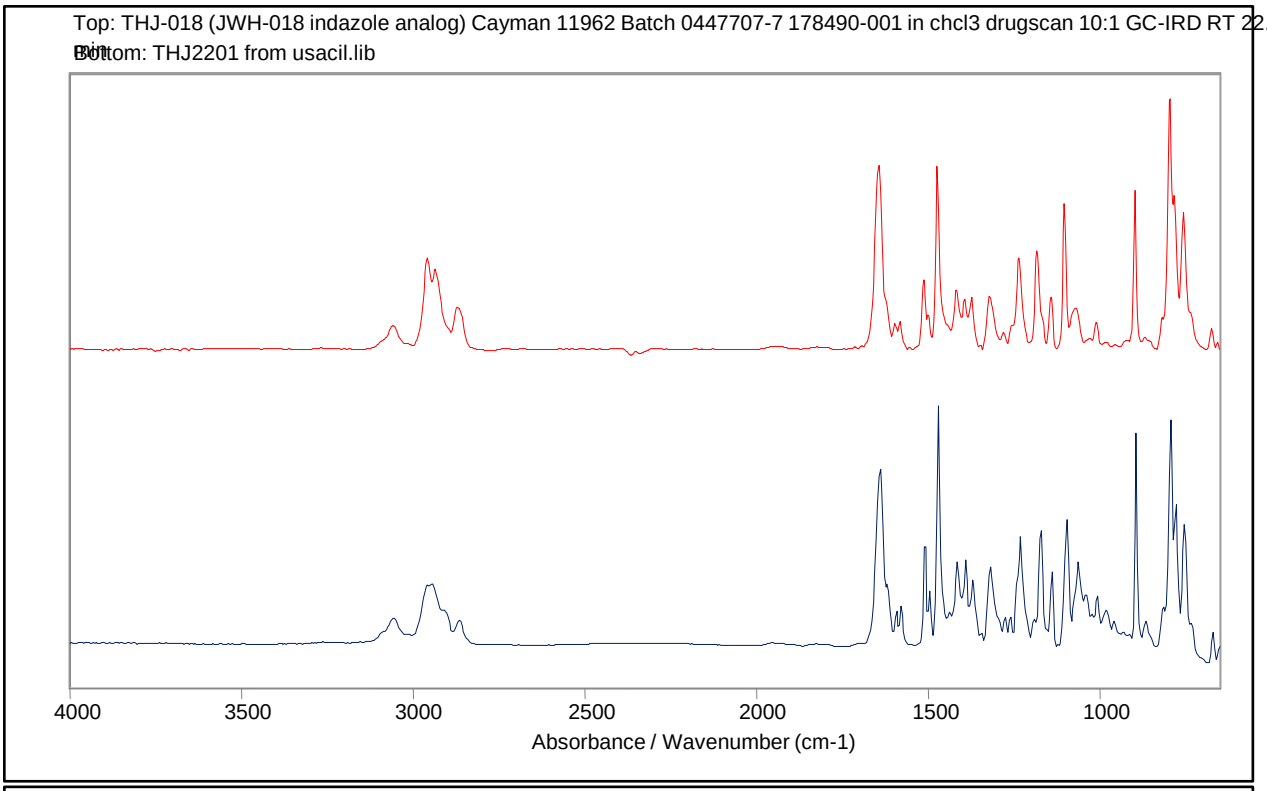


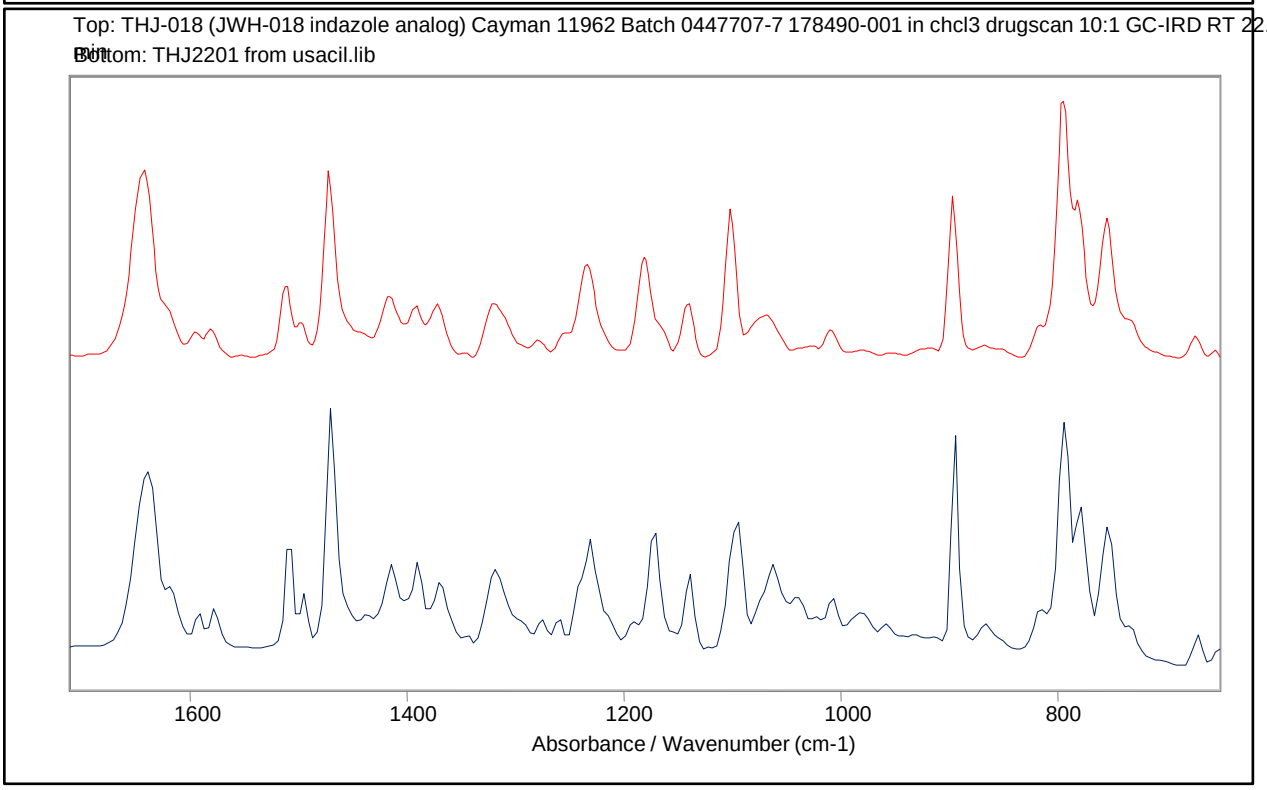
Top: 1-(3-trifluoromethylphenyl)piperazine HCl; National Measurement Institute, D906, 06-D-03; 162623-001 GC-IRD rt 9.18 min.
Bottom: TFMPP from usacil.lib

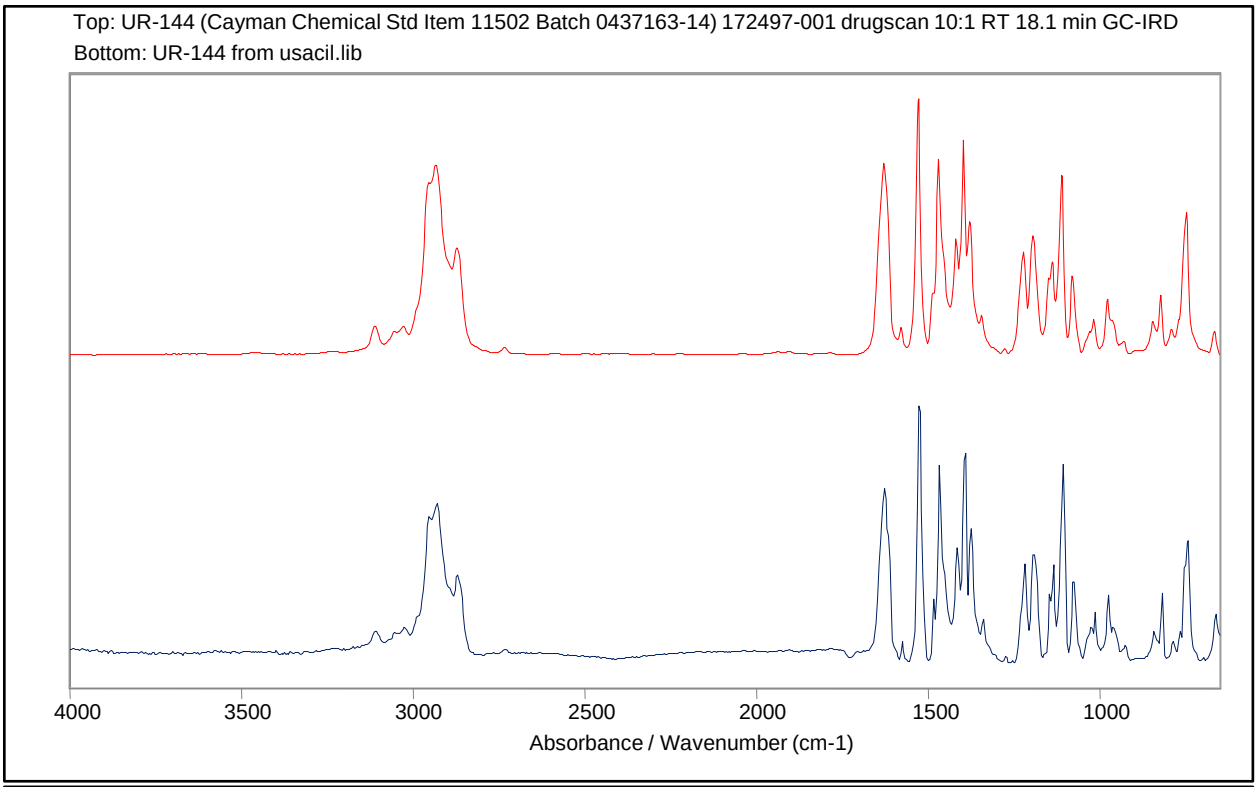


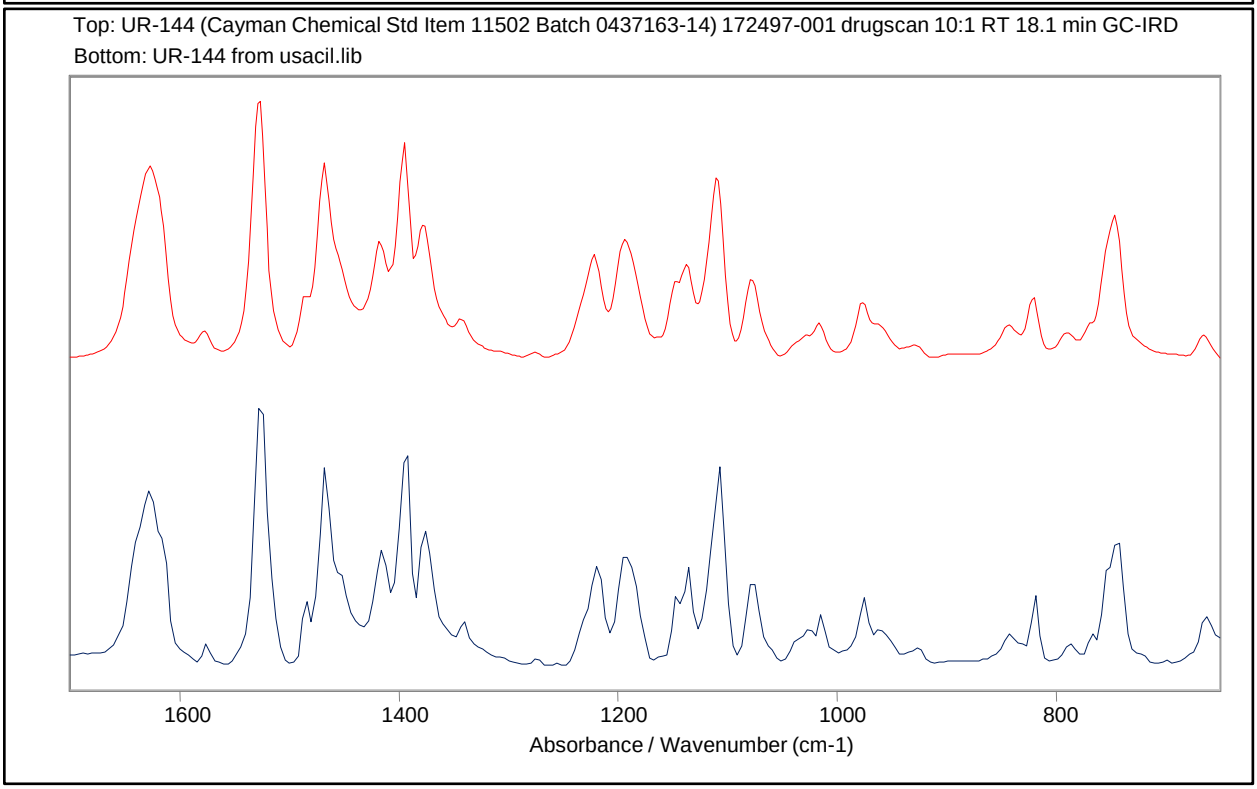
Top: 1-(3-trifluoromethylphenyl)piperazine HCl; National Measurement Institute, D906, 06-D-03; 162623-001 GC-IRD rt 9.18 min.
Bottom: TFMPP from usacil.lib



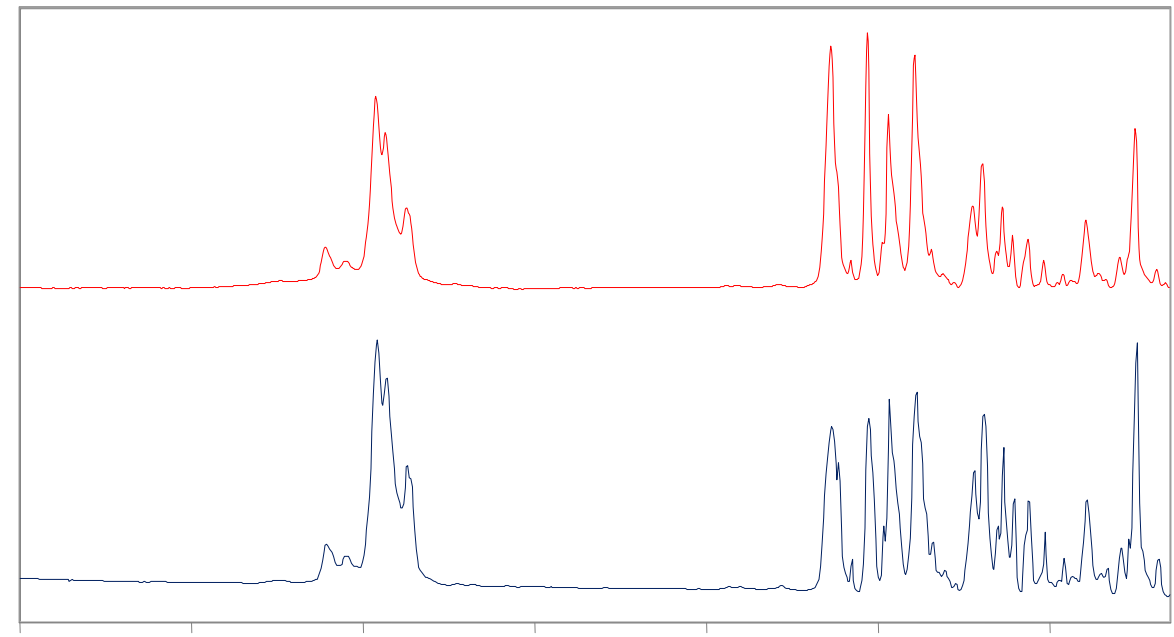




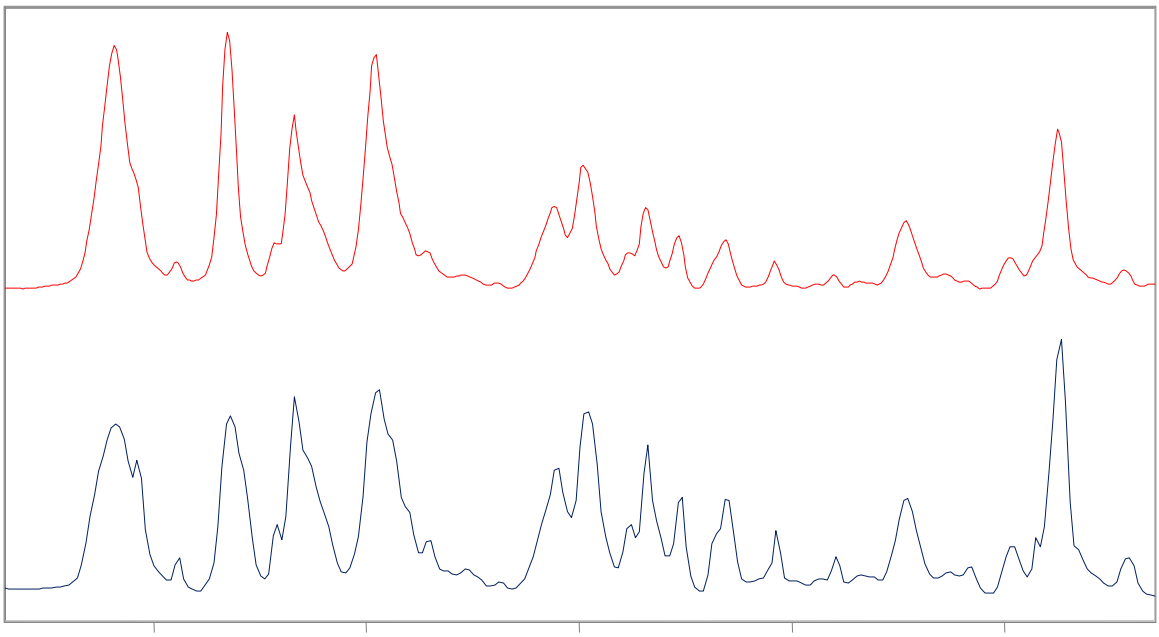


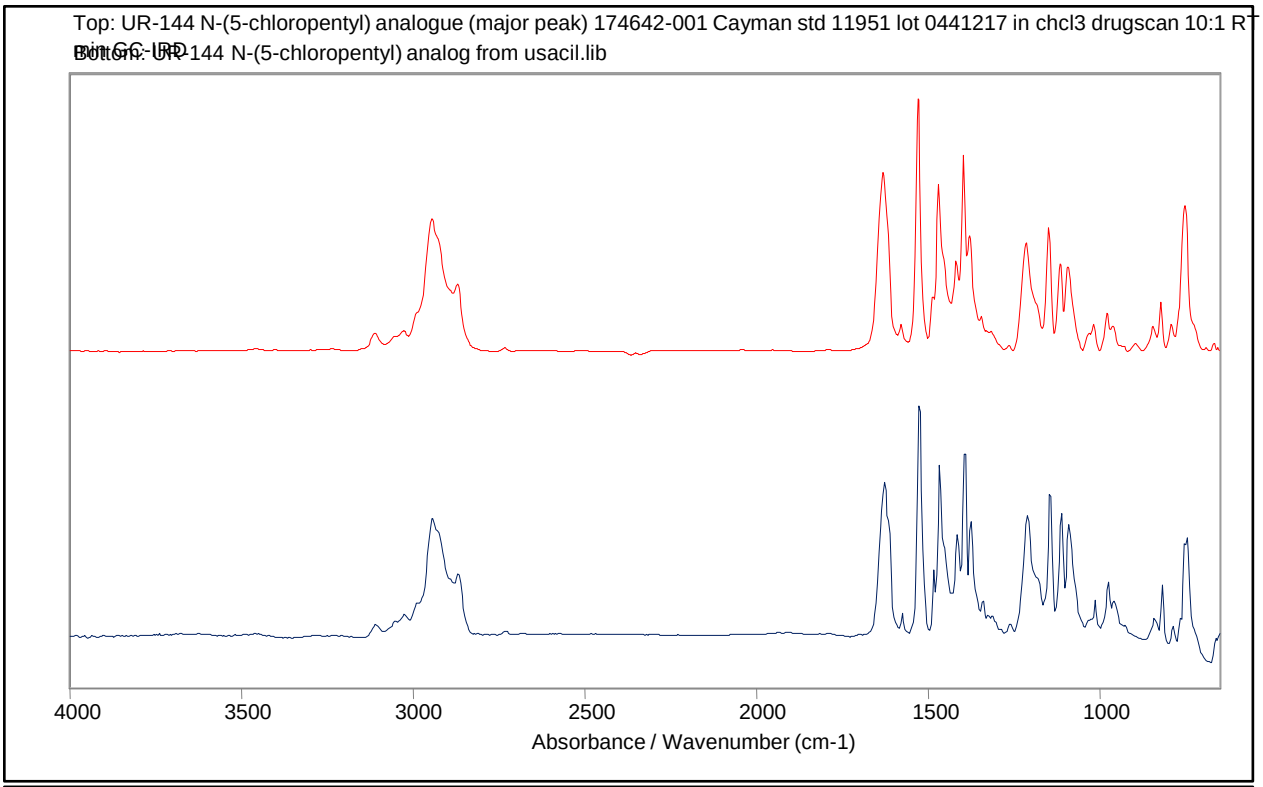


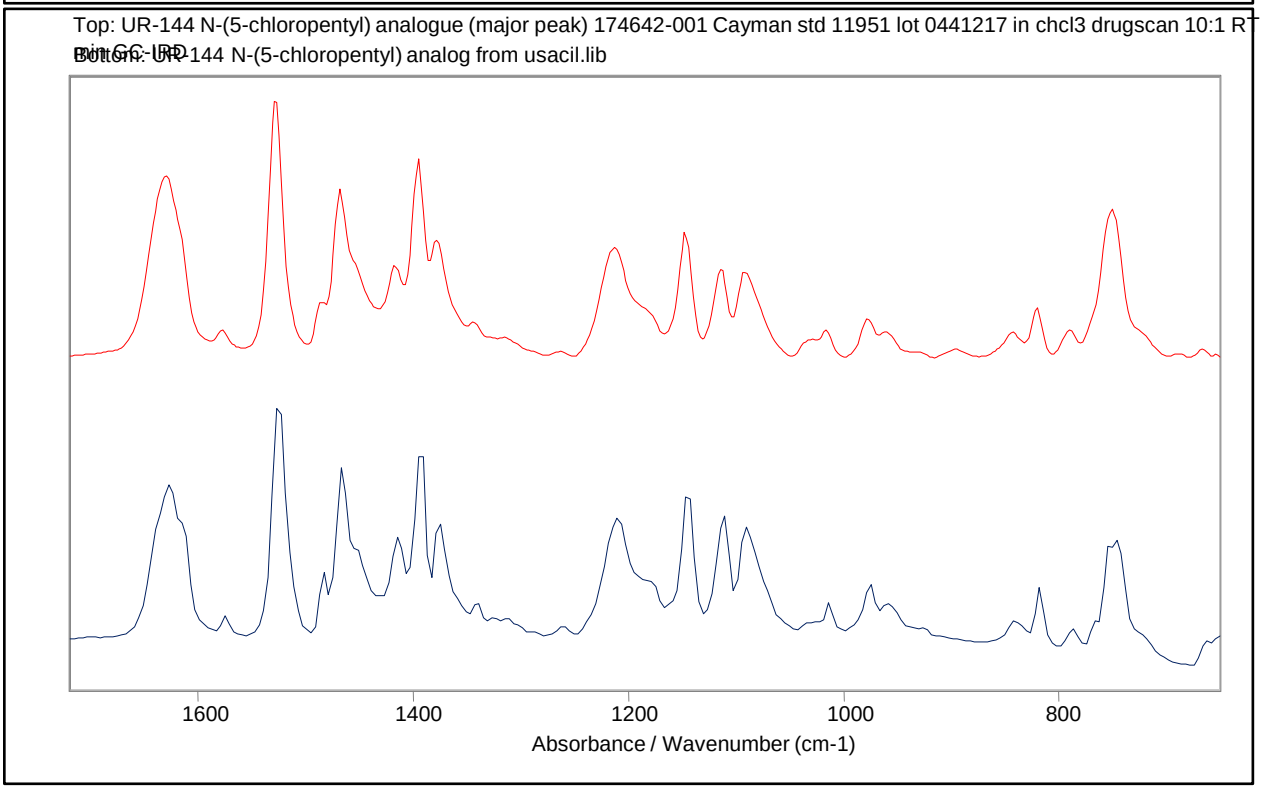
Top: UR-144 artifact (from Cayman Chemicals std Item 11502, Batch 0437163-14) 172497-001 in chcl3, drugscan 10:1 RT 18.3 min
Bottom: UR-144 Degradant from usacil.lib

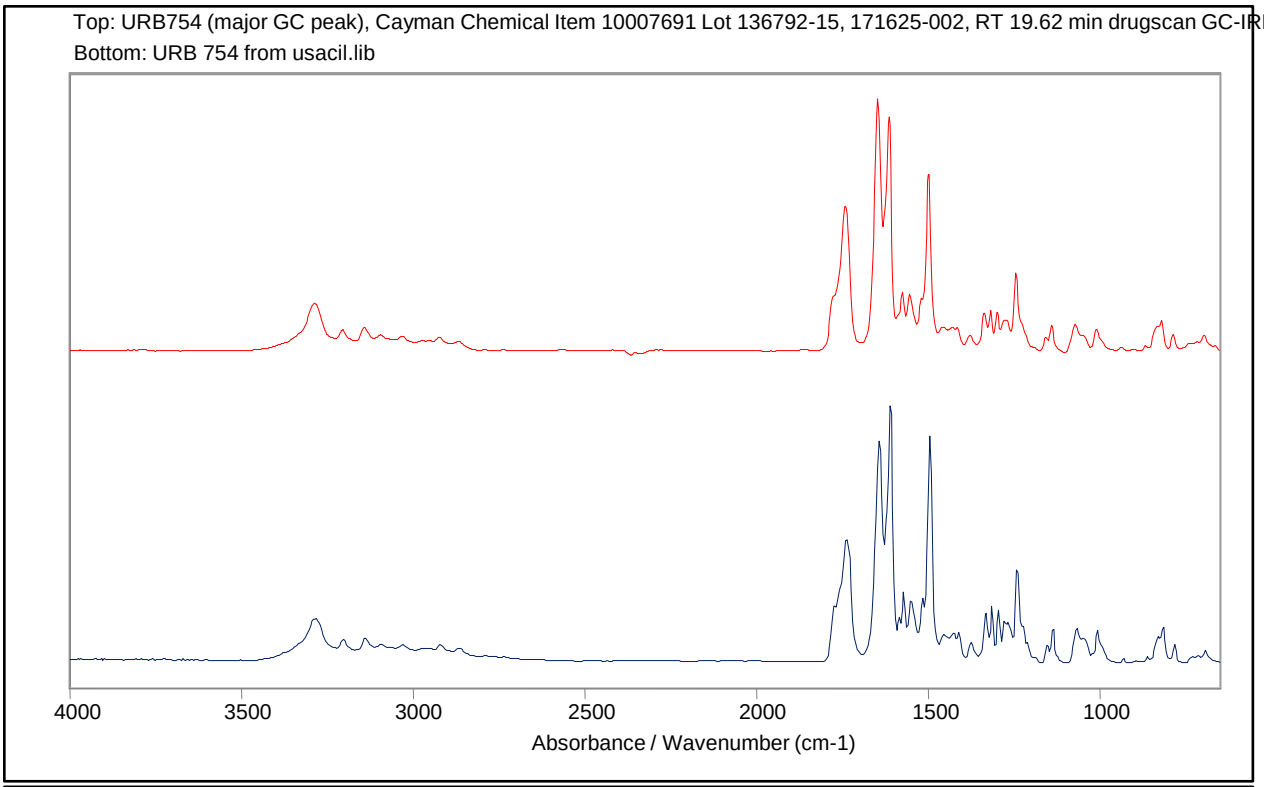


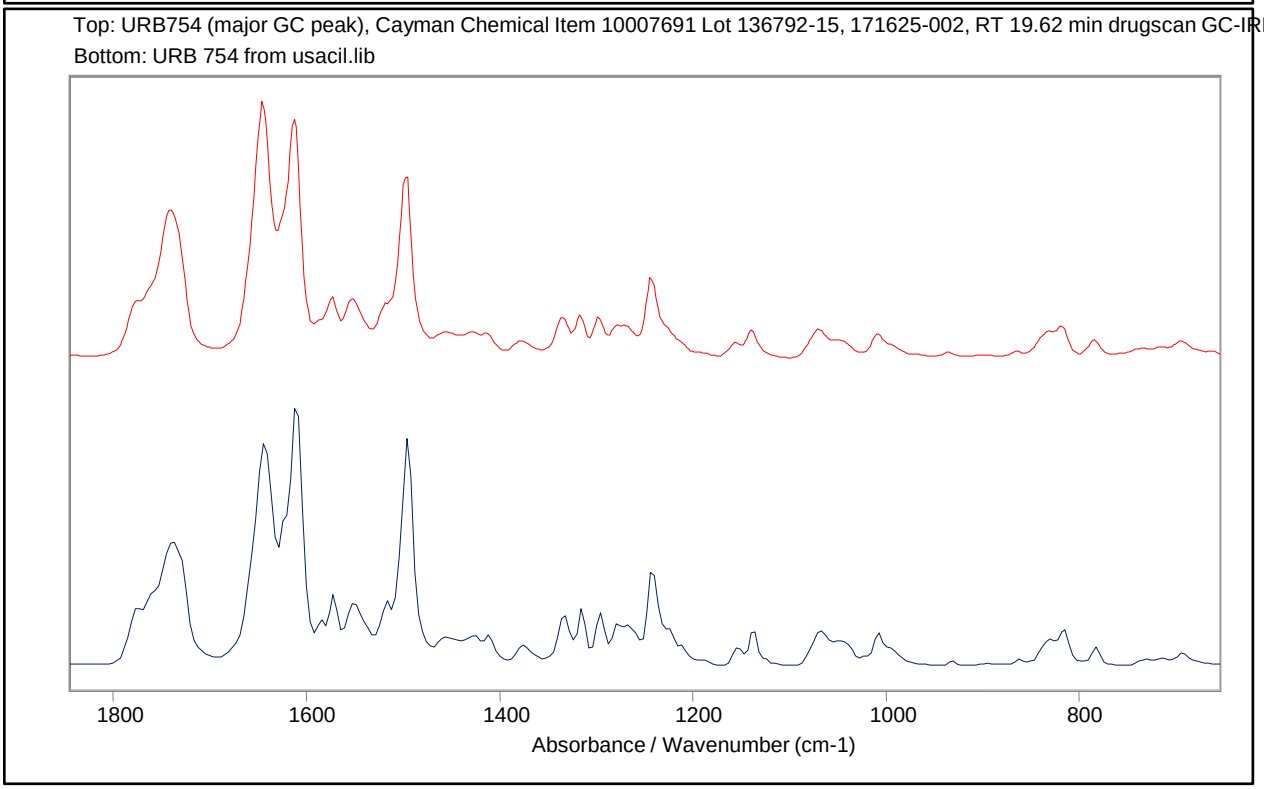
Top: UR-144 artifact (from Cayman Chemicals std Item 11502, Batch 0437163-14) 172497-001 in chcl3, drugscan 10:1 RT 18.3 min
Bottom: UR-144 Degradant from usacil.lib

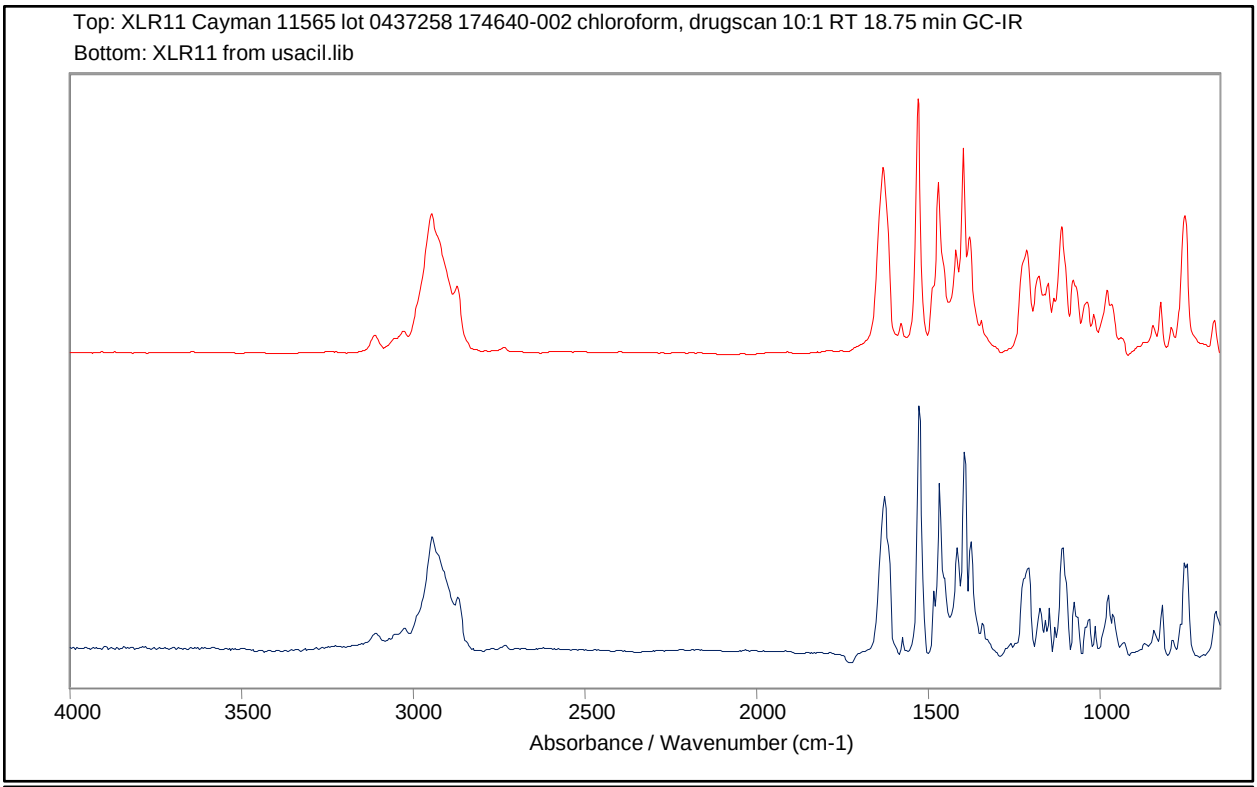


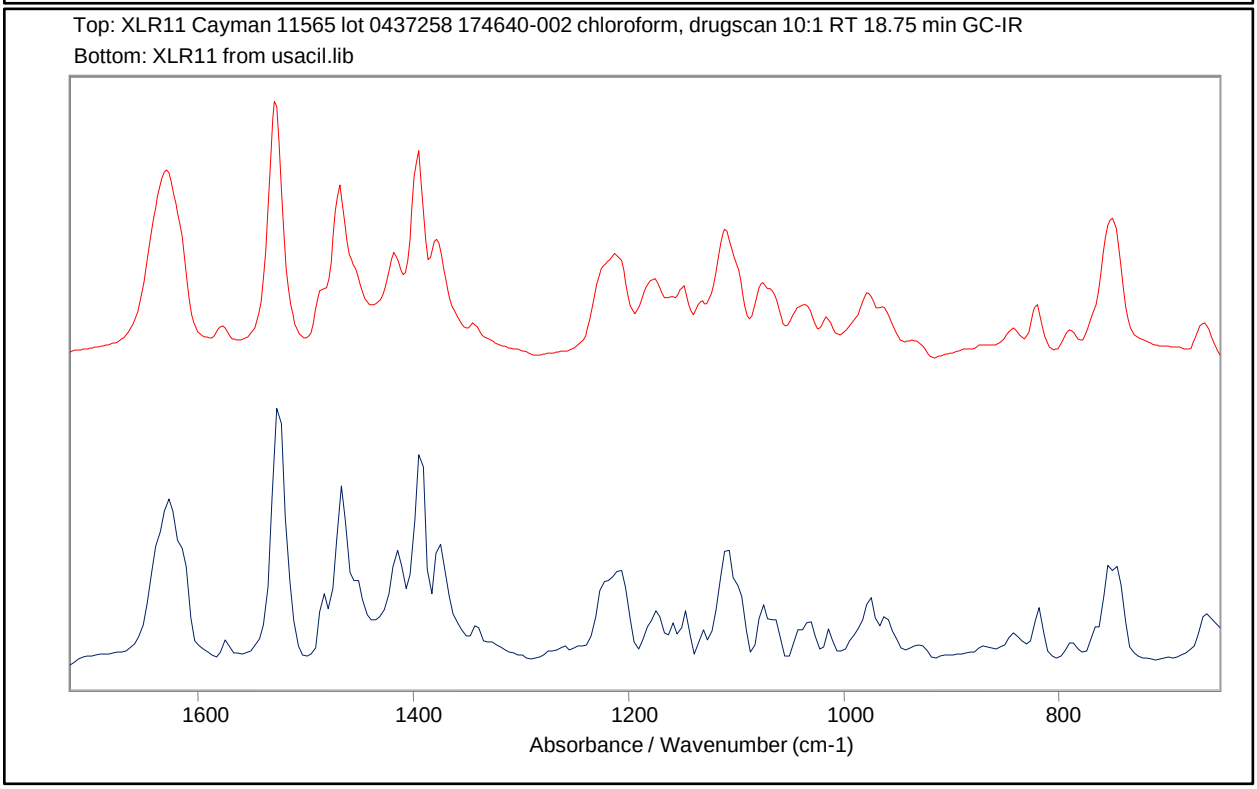


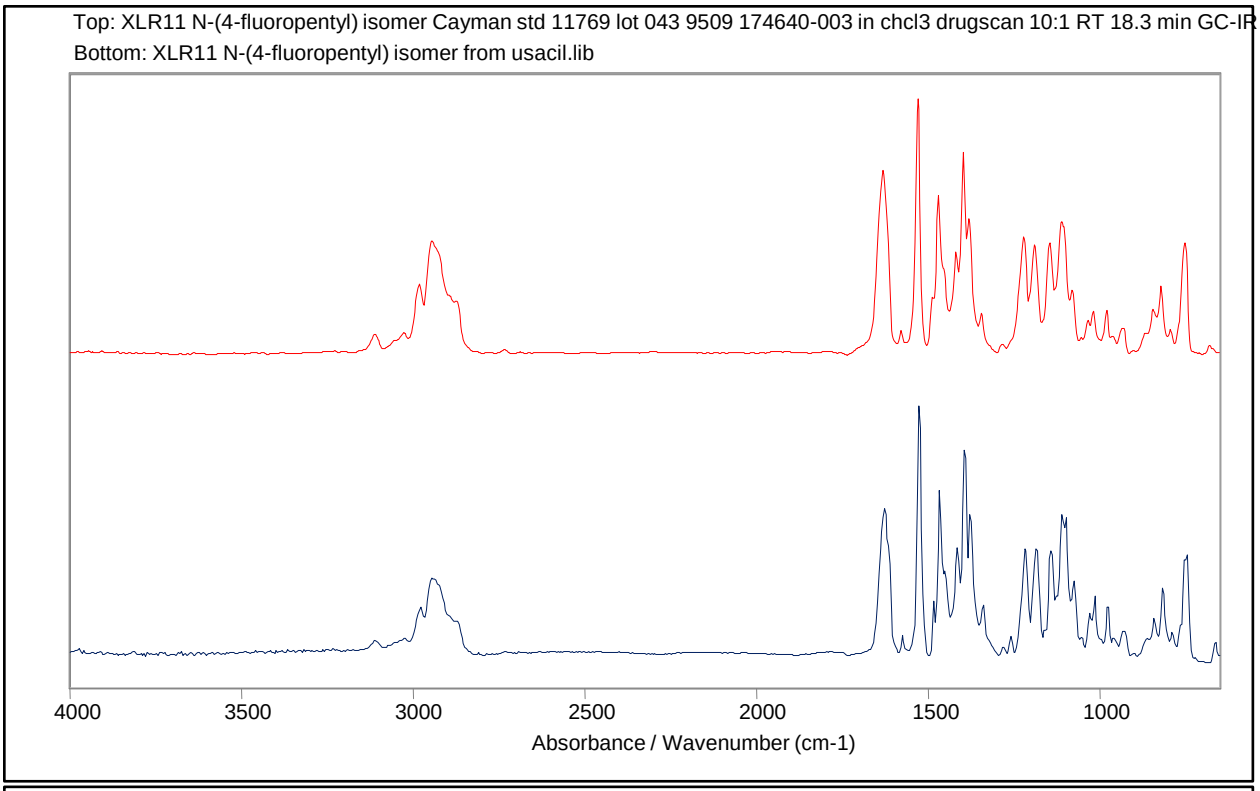


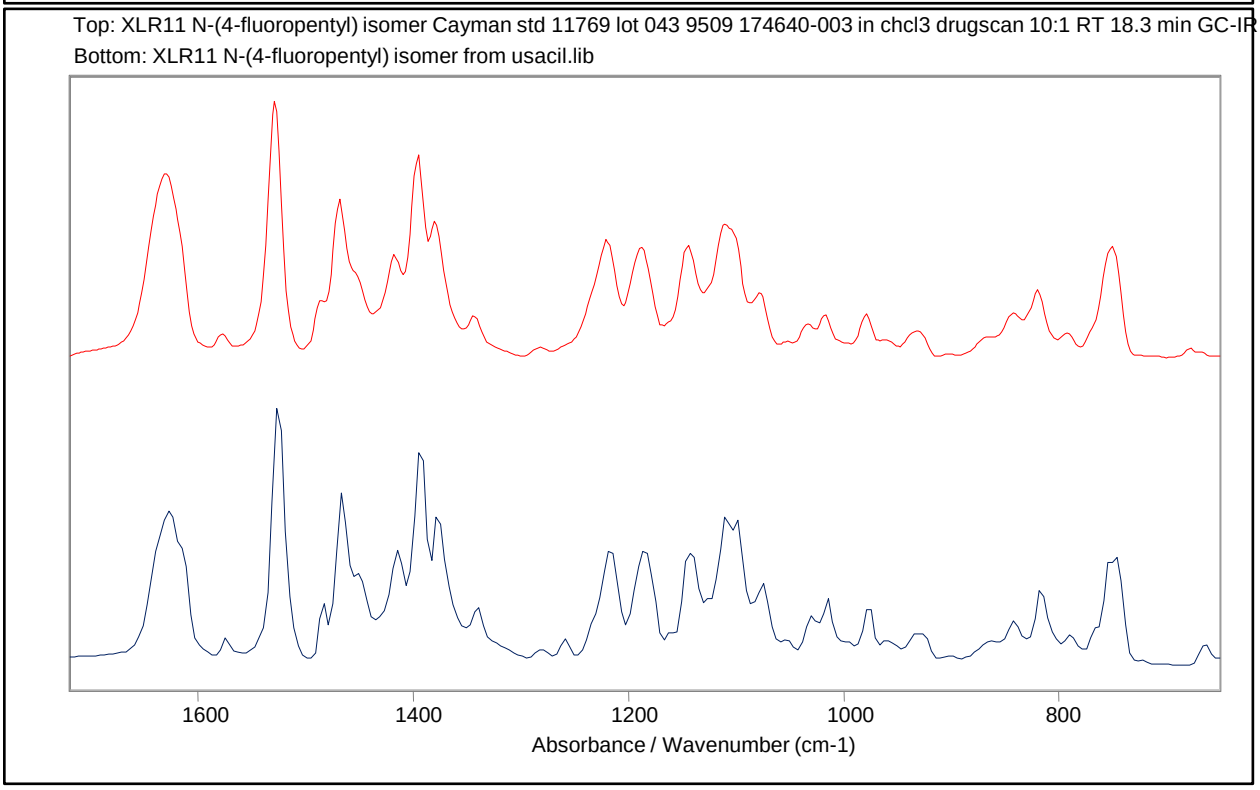


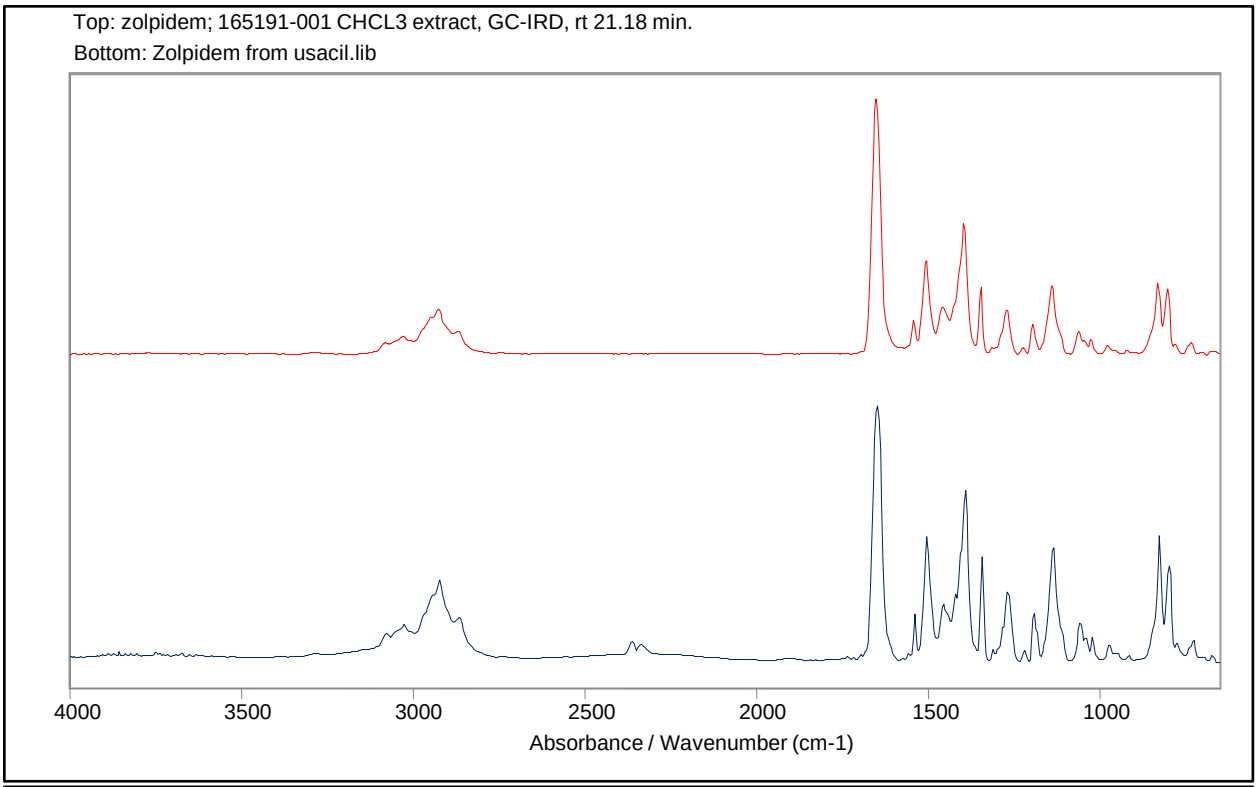


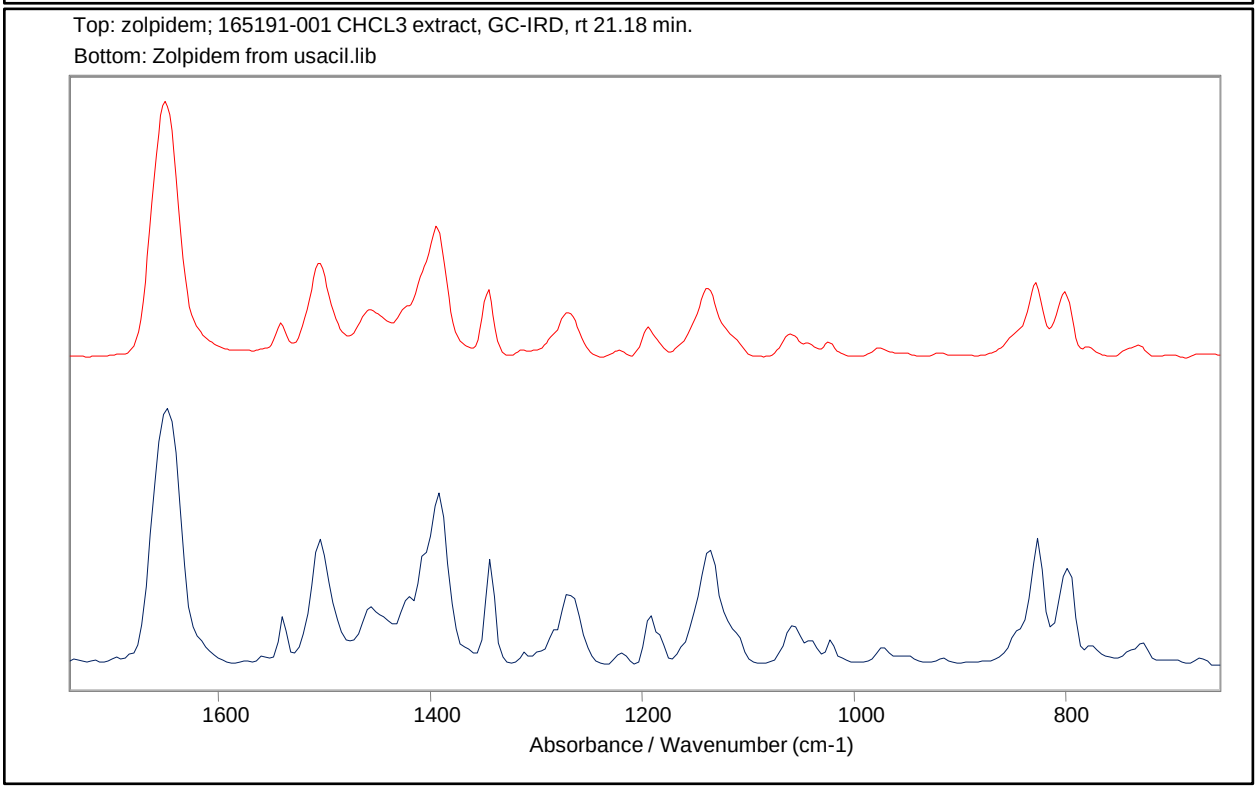










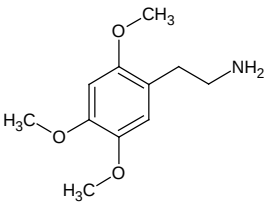


Appendix E: Drugs of Abuse Isomer Differentiation Bibliography

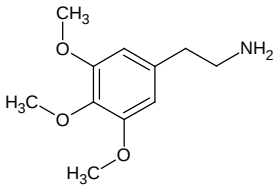
This bibliography was compiled in 2015 in order to act as a resource for the identification of drug of abuse isomers by forensic chemists.

1. Jansen, M. P. J. M. β-2,4,5-trimethoxyphenethylamine, an isomer of mescaline. Recueil des Travaux Chimiques des Pays-Bas. 50 (1931) 291-312. DOI: 10.1002/recl.19310500403.

Compounds



β-2,4,5-trimethoxyphenethylamine

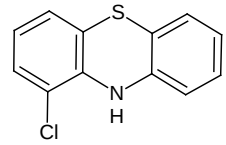


Mescaline

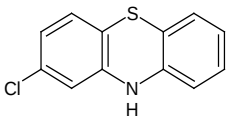
2. Eisciorfer, I.B., Warren, R.J., Thompson, W.E., Zarembo, J.E. Identification of 1-, 2-, 3-, and 4-chlorophenothiazine isomers. Journal of Pharmaceutical Science. 7 (1966) 734-735. DOI: 10.1002/jps.2600550715

The infrared and ultraviolet spectral data for four monochloroisomers of phenothiazine are presented and discussed. From these data it is possible to make a positive and rapid identification of any of the isomers with a minimum amount of sample. The method can be used to identify the isomers alone or in combination.

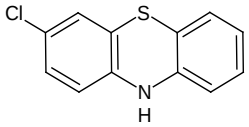
Compounds



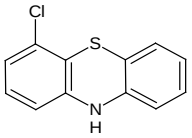
1-chlorophenothiazine



2-chlorophenothiazine



3-chlorophenothiazine



4-chlorophenothiazine

Instruments

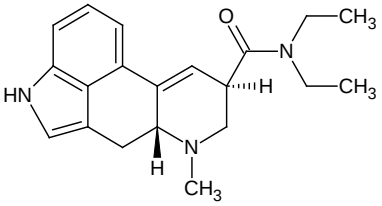
Perkin-Elmer model 21 double beam spectrometry and Cary model 14 recording spectrometry

3. Look, J. Determination of LSD and of ISO-LSD by Paper Chromatography. Microgram. Volume 1 No. 4. January 1968. Pages 18-19.

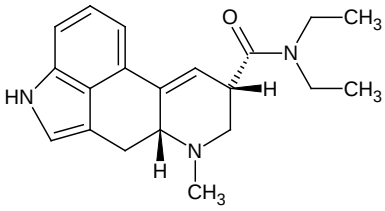
Paper chromatography can be used to identify and quantitatively measure LSD and ISO-LSD. Two qualitative tests and one quantitative method are given below.

Compounds of Interest:

LSD (Lysergic Acid Diethylamide)



ISO-LSD



Technique Used:

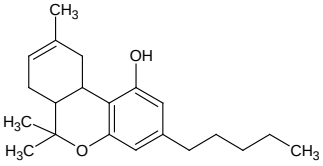
Paper Chromatography

4. Vree, T.B., Breimer, D.D., van Ginneken, C.A.M., van Rossum, J.M., and Nibbering, N.M.M. Gas chromatography of cannabinoids : Gas chromatographic behaviour of cis- and trans-tetrahydrocannabinol and isotethrahydrocannabinol. Journal of Chromatography. 79 (1973) 81-90. DOI: 10.1016/S0021-9673(01)85276-6.

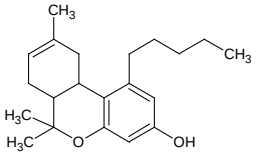
Synthetic side-products of tetrahydrocannabinols (1,6-, 1,2- and iso-THC) were submitted to gas chromatographic—mass spectrometric analysis and the compounds were identified by mass spectrometry. The gas chromatographic behaviour of the THC isomers is very characteristic and there are fixed ratios of the retention times for the *cis-trans*-isomers (1.85), *ortho/para*-isomers (1.20) and iso-THC/1,2-THC (2.67 and 4.80).

Compounds

cis, trans-(3,4)-para-1,6-THC



cis, trans-(3,4)-ortho-1,6-THC



Instruments

Gas Chromatography

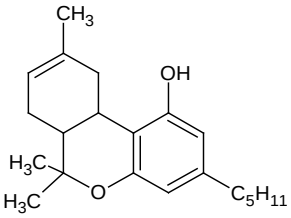
Mass Spectroscopy

5. Terlouw, J.K. et al. The use of metastable ion characteristics for the determination of ion structures of some isomeric cannabinoids. J.K. Terlouw et al. Tetrahedron. Volume 30, Issues 23–24, 1974, Pages 4243–4248. doi:10.1016/S0040-4020(01)97415-0

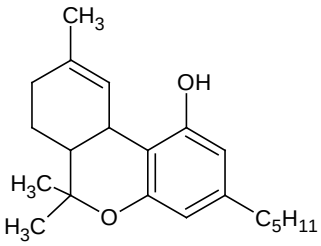
The mechanism of formation of the prominent $C_{15}H_{19}O_2$ ion at *m/e* 231 in the mass spectra of $\Delta^{1(6)}$-tetrahydrocannabinol and five isomeric cannabinoids has been investigated. Except via a

well-documented two step process, involving an RDA mechanism, a sizeable percentage of these ions is formed by a novel one-step route from the molecular ion. This was deduced from the spectra of deuterium-labelled compounds and measurements of the kinetic energy release of metastable ions. The latter value for the one step process varies from 25–44 meV for the six compounds investigated, attributed to two interdependent effects, different transition state geometries and common transition states differing in the time elapsing before their formation.

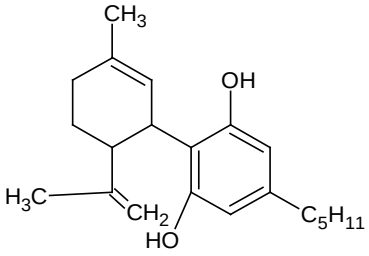
Compounds



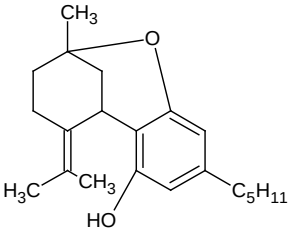
(1,6-THC)



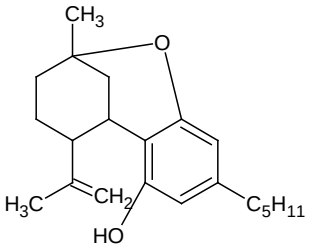
(1,2-THC)



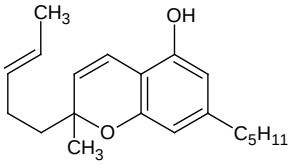
CBD



(4,8-iso-THC)



(8,9-iso-THC)



CBC

Instruments:

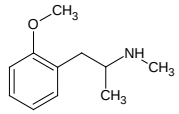
Mass Spectrometry

6. Bailey, K., By, A. W., Legault, D., and Verner, D. Identification of the N-methylated analogs of the hallucinogenic amphetamines and some isomers. J. Assoc. Off. Anal. Chem. 58 (1975) 62–69.

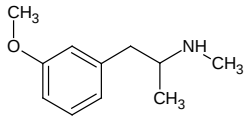
The drugs 2-, 3-, and 4-methoxy-N-methylamphetamine, 3-methoxy-4,5-methylenedioxy-N-methylamphetamine, and 3,4-methylenedioxy-N-methylamphetamine are identified by spectroscopic techniques. The ultraviolet and mass spectra of isomers are similar, but proton magnetic resonance and infrared spectra are distinctly different, and reference spectra and data are provided. Gas-liquid and thin layer chromatographic systems for the analysis are discussed

Compounds

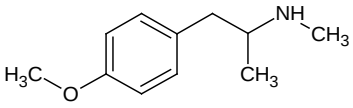
2- methoxy-N-methylamphetamine



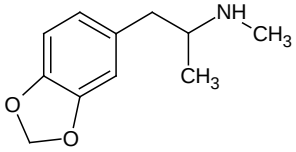
3- methoxy-N-methylamphetamine



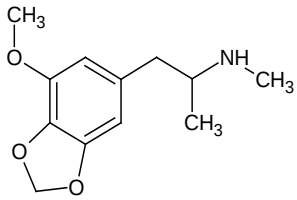
4-methoxy-N-methylamphetamine



3,4-methylenedioxy-N-methylamphetamine



3-methoxy-4,5-methylenedioxy-N-methylamphetamine



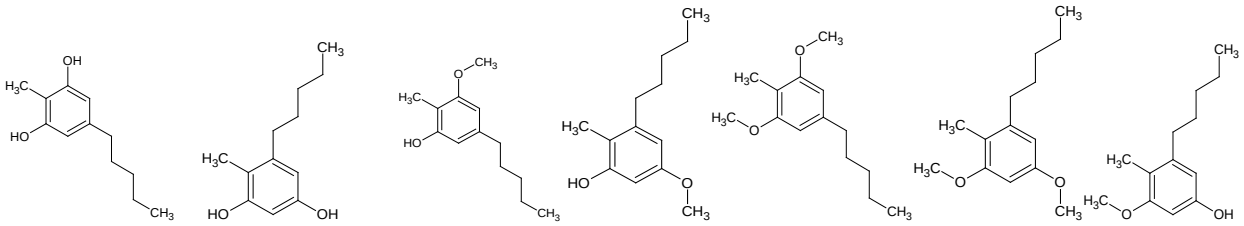
Instruments

UV Spectrometry
Mass Spectrometry
Proton Magnetic Resonance
Infrared Spectroscopy

7. Vree, T.B. Mass spectrometry of cannabinoids. Journal of Pharmaceutical Sciences. 66 (1977) 1444-1450. DOI: 10.1002/jps.2600661025.

The mechanism of fragmentation of cannabinoids to fragments m/e 314, 299, 271, 258, 246, 243, and 231 is given. Cannabidiol, cannabinodiol, cannabinol, Δ^6- and Δ^1-tetrahydrocannabinol, cannabichromene, cannabicyclol, derivatives with pentyl, propyl, and methyl side chains, their methyl ethers, and cis-trans and ortho-para isomers were analyzed by GLC-mass spectrometry using different energies for fragmentation during GLC elution. The following mechanism was distinguished: loss of a methyl radical, ring closure and rotation, McLafferty rearrangement, retro Diels-Alder, internal protonation, isomerization and internal bond formation, and one-step fragmentation to m/e 231

Compounds



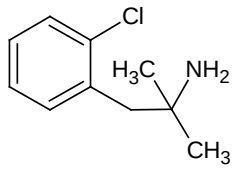
Instrument

Gas-Liquid Chromatography
Mass spectrometry

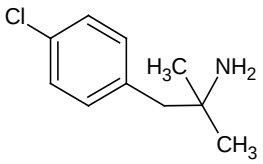
8. Hufsey, J. Special Characteristics of Chlorphentermine Isomers. Microgram. Volume 11 No. 9. September 1978. Pages 173-180.

Chlorphentermine represents an example in which forensic chemists are asked to distinguish between closely related structural isomers. This article presents spectral data of the three isomers, ortho-, meta-, and para- Chlorphentermine, and takes a close look at classical spectral analysis of di-substituted aromatic compounds using the above substances as examples.

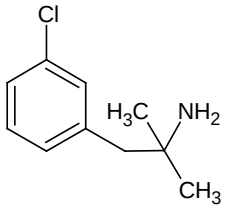
Ortho



Para



Meta

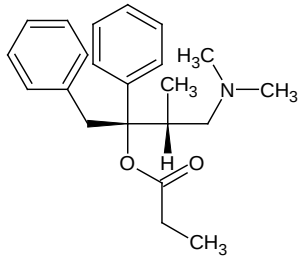


Instruments Used:
IR, NMR, GC/MS

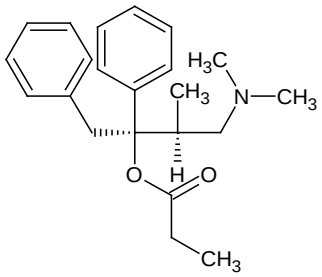
9. Kebabjian, D.E. Differentiation of the dextro and levo isomers of propoxyphene by mixed crystal test. Microgram. Volume 12 No 11. November 1979. Pages 199-201.

Objective: To provide relatively simple, quick and inexpensive method for the differentiation of dextropropoxyphene and levopropoxyphene.

Dextropropoxyphene



levopropoxyphene



Instruments/ Technique Used:
Crystal Test, GC/MS, IR

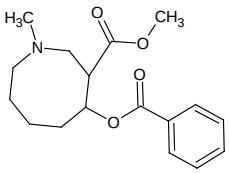
This resource was prepared by the author(s) using Federal funds provided by the U.S. Department of Justice. Opinions or points of view expressed are those of the author(s) and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

10. A.H. Lewin, S.R. Parker, and F.I. Carroll. Positive identification and quantitation of isomeric cocaines by high-performance liquid chromatography. Journal of Chromatography A. Volume 193, Issue 3, 30 May 1980, Pages 371–380. doi:10.1016/S0021-9673(00)87737-7

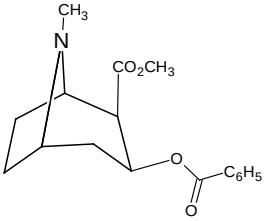
A high-performance liquid chromatographic procedure for separating and identifying the four isomeric cocaines has been developed. Use of this procedure with an internal standard allows for the determination of the quantity of any isomeric cocaine in an unknown sample. The pitfalls and problems encountered in the use of gas chromatography and mass spectrometry in the analysis of cocaines and ecgonine methyl esters are discussed.

Compounds

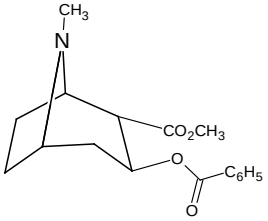
3-benzoyloxy-8-methyl-8-azabicyclo-octane-2-carboxylic acid methyl ester



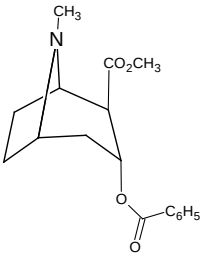
Cocaine



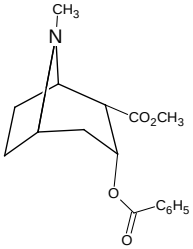
Pseudococaine



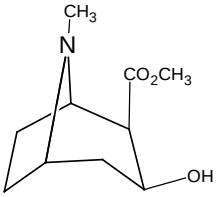
Allococaine



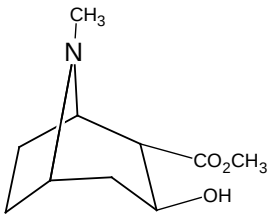
Allopseudococaine



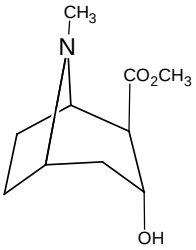
Ecgonine Methyl Ester (EMe)



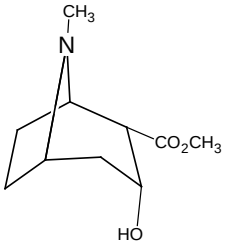
ϒ EMe



AEMe



AϒEMe



Instruments

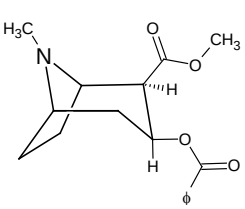
Gas Chromatography

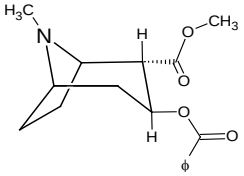
Mass Spectroscopy

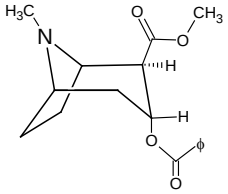
11. Allen, A. C., Cooper, D. A., Kiser, W. O., and Cottrell, R. C. The cocaine diastereomers. Journal of Forensic Sciences. 26 (1981) 12-26. DOI: 10.1520/JFS11325J.

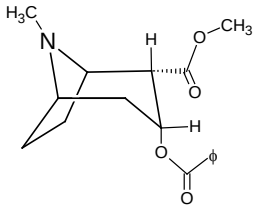
In the past, it has been argued in court, from a theoretical basis, that the techniques available to the forensic chemist would differentiate the "cocaines." This work has moved that argument from the realm of the theoretical into that of experimental fact. The techniques of infrared spectroscopy (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS) will unequivocally identify the racemic cocaine diastereoisomer. In addition, this work shows that the enantiomeric form of cocaine can be assigned by crystal tests, IR, and melting point techniques. The pure enantiomers of allococaine and pseudoallococaine were not isolated. This does not create a problem because the techniques of NMR and MS, as performed in this study, will not differentiate enantiomers. Therefore, the logical sequence of first identifying the diastereoisomer (via IR, MNR, or MS) and then determining the chirality by crystal tests, IR, melting points, or optical rotation measurements is valid

Compounds


Cocaine


Pseudococaine


Allococaine


Pseudoallococaine

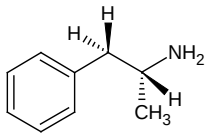
Instruments
NMR
Mass Spectrometry
Infrared Spectrometry

12. Wood. L.E., Daniels, R., Bauer, L. Gearien. J.E. Syntheses and biological evaluation of 2- and 9-aminobenzonorbornenes as conformationally rigid analogs of amphetamines. Journal of Pharmaceutical Science. 2 (1981) 199-204. DOI: 10.1002/jps.2600700222

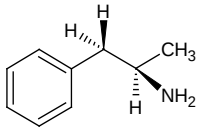
Isomers of the 2- and 9-aminobenzonorbornenes were prepared as rigid analogs of amphetamine and were employed to study the conformational requirements of indirectly acting sympathomimetic agents. Of this series of isomeric amines, the *exo*-2 and *anti*-9 isomers closely resemble the fully extended conformation of amphetamine. The other two amines, the *endo*-2 and *syn*-9 isomers, conformationally resemble the folded conformation of amphetamine. The isomers that resemble the extended conformation of amphetamine increased the spontaneous motor activity in mice while the isomers resembling the folded form either decreased or had no effect on motor activity. These compounds also were studied for their ability to accelerate the efflux of tritiated norepinephrine from vesicular and

nonvesicular storage sites of isolated perfused rabbit atria; either α-methyl-*p*-tyrosine- or reserpine-pretreated rabbits were used. Amphetamine and the *exo*-2 and *anti*-9 isomers of aminobenzonorbornene could accelerate norepinephrine efflux from either compartment while the *endo*-2 and *syn*-9 isomers could accelerate the efflux from only the nonvesicular compartment at the concentrations studied. Fenfluramine and methylphenidate also were studied for their ability to accelerate efflux. Fenfluramine and methylphenidate resembled the aminobenzonorbornenes that correspond to the folded conformation of amphetamine in their ability to accelerate the efflux from nonvesicular storage. However, fenfluramine also resembled amphetamine and the aminobenzonorbornenes corresponding to the extended conformation of amphetamine in its ability to accelerate efflux from vesicular storage sites. The response to methylphenidate was similar to that of the aminobenzonorbornenes resembling the folded conformation of amphetamine.

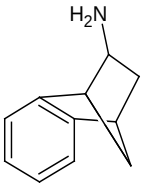
Compounds



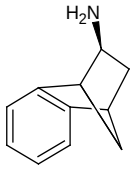
Amphetamine, extended



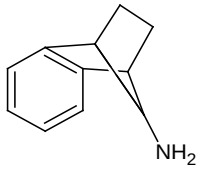
Amphetamine, folded



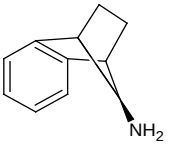
Exo-2-Aminobenzonorborenes



Endo-2-Aminobenzonorborene



Anti-9-Aminobenzonorborene

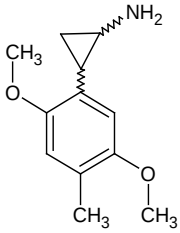


Syn-9-Aminobenzonorborene

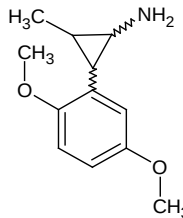
13. Jacob, J. N. and Nichols, D. E. Isomeric cyclopropyl ring-methylated homologues of trans-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine, an hallucinogen analogue. J. Med. Chem. 25 (1982) 526–530. DOI: 10.1021/jm00347a009

The hallucinogenic analogue trans-2-(2,5-dimethoxy-4-methylphenyl)cyclopropylamine was modified by adding a 3-methyl group, either cis- or trans- with respect to the amino group. These two isomeric cyclopropyl ring-methylated compounds were then tested for activity in the mouse ear-scratch assay and for a contractile effect in the rat fundus preparation. Neither compound was found to possess appreciable activity when compared to the nonmethylated parent, in either assay.

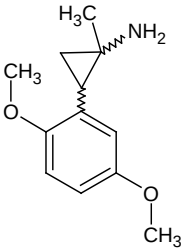
Compounds



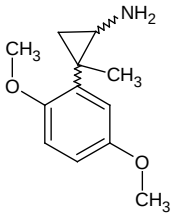
2-(2,5-dimethoxy-4-methylphenyl) Cyclopropylamine



2-(2, 5-dimethoxyphenyl)-3-methylcyclopropylamine



2-(2, 5-dimethoxyphenyl)-1-methylcyclopropylamine

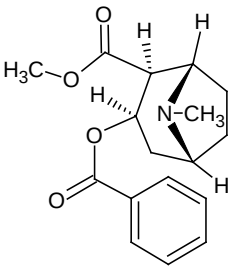


2-(2, 5-dimethoxyphenyl)-2-methylcyclopropylamine

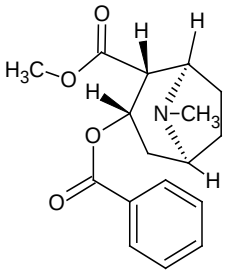
14. Differentiation of d and l- cocaine by microcrystalline test. Richard Ruybal. Microgram. Volume 15 No 9. September 1982. Pages 160-163.

It has become encumbent upon the forensic chemist to testify in court that the instrumental techniques performed in fact prove which enantiomeric form of cocaine is present. The techniques available rely primarily on the polarimeter which can sometimes be difficult on very small samples. Many laboratories do not possess polarimeter which compounds the problem even more. With this method the enantiometric forms of cocaine can be differentiated by microcrystalline test utilizing TDTA and TLTA.

D-Cocaine



L-Cocaine



Technique Used:
Microcrystalline Test

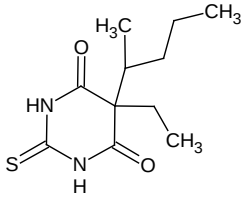
15. Stanski, D.R., et al. Pharmacokinetics and anesthetic potency of a thiopental isomer. Journal of Pharmaceutical Science. 8(1983)937-940. DOI: 10.1002/jps.2600720824

In developing a high-performance liquid chromatographic assay for thiopental [5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid], a thiopental isomer [5-ethyl-5-(1-ethylpropyl)-2-thiobarbituric acid] was found. This isomer occurs (6–7%) in supposedly pure thiopental and in the commercially available thiopental sodium administered to patients for induction of anesthesia. A similar type of isomer also

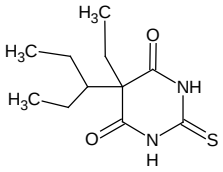
occurs in pentobarbital, the oxybarbiturate analogue of thiopental. Because the disposition and anesthetic potency of the isomer is unknown, its pharmacokinetic properties were determined in humans and its anesthetic potency in mice. In five surgical patients, the terminal elimination half-life, clearance, and volume of distribution at steady state of the isomer were not statistically different from those of thiopental. In mice, the isomer proved to be as effective as thiopental for induction of anesthesia. The LD_{50} and sleep time at one-half the LD_{50} did not statistically differ between the two compounds in mice. The close structural similarity of thiopental and the isomer results in similar pharmacokinetic and anesthetic properties. It does not appear critical that the isomer be separated from thiopental in subsequent pharmacological research.

Compounds

Thiopental



Thiopental isomer



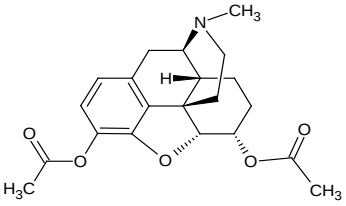
Instruments

Liquid Chromatography
UV spectrometer

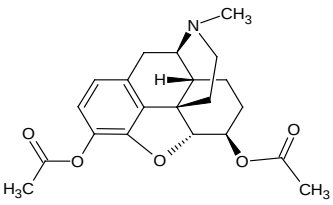
16. Beazley, W.D. Analytical characterization of isoheroin. Journal of Forensic Sciences 30 (1985) 915-921.

The synthesis of isoheroin is presented with the analytical data (mass spectroscopy [MS], nuclear magnetic resonance [NMR], infrared spectroscopy [IR], and gas liquid chromatography [GLC]) for this compound. Comparison between analytical results for heroin and isoheroin shows differentiation is possible.

Compounds



Heroin



Isoheroin

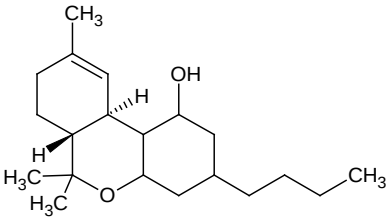
Instruments

NMR
IR Spectroscopy
Gas liquid Chromatography

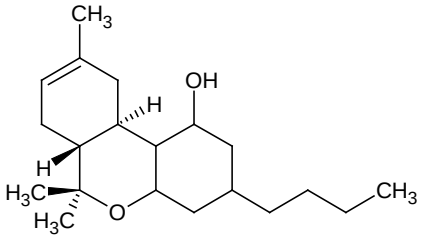
17. Banijamali, A.R. and Makriyannis, A. Separation of Tetrahydrocannabinol Isomers by Reverse-Phase High Pressure Liquid Chromatography. Journal of Liquid Chromatography. Volume 10, Issue 13, 1987. DOI: 10.1080/01483918708066836

The separation of three closely related tetrahydrocannabinol isomers differing only in the position of the double bond in ring C was achieved by HPLC using a µBondapak C18 column and a ternary mobile phase of acetonitrile/tetrahydrofuran/water. Near base line resolution was obtained on the first pass through the column and complete resolution was accomplished after one recycle.

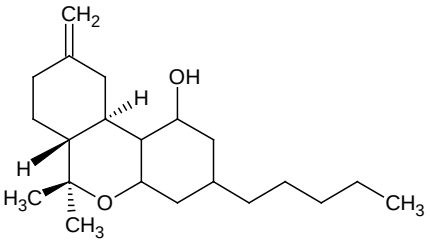
Compounds



Δ^9-THC



Δ^8-THC



$\Delta^{9,11}$-THC

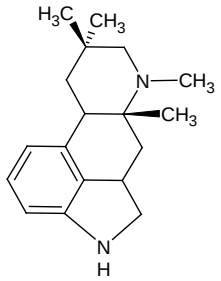
Instrument Used:

Reverse-Phase High Pressure Liquid Chromatography

18. Clark,C.C., "The Differentiation of Lysergic Acid Diethylamide (LSD) from N-Methyl-N-Propyl and N-Butyl Amides of Lysergic Acid." Journal of Forensic Science. JFSCA, Vol. 34, No. 3, May 1989 pp. 532-546.

The *N*-methyl-*N*-propyl, *N*-methyl-*N*-isopropyl, *N*-butyl, *N*-isobutyl, *N*-*sec*-butyl and *N*-*tert*-butyl amides of lysergic acid were synthesized to determine the specificity of electron impact mass spectroscopy (EI/MS), when combined with other analytical techniques, for the identification of lysergic acid diethylamide (LSD). After separation of the C_8 axial and equatorial isomers by preparative thin-layer chromatography, the amides were subjected to gas-liquid chromatography (GLC), thin-layer chromatography (TLC), high pressure liquid chromatography (HPLC), and EI/MS. EI/MS, when combined with other analytical techniques, is shown to be capable of differentiating LSD from any of the other compounds included in this study

Compounds



Lysergic Acid Diethylamide (LSD)

Instruments

Gas-Liquid Chromatography

Thin-Layer Chromatography

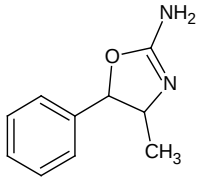
High Pressure Liquid Chromatography

Mass Spectroscopy

19. Klein, R. F. X., Sperling, A. R., Cooper, D. A., and Kram, T. C. The Stereoisomers of 4-Methylaminorex. Journal of Forensic Sciences. 34 (1989) 962-979.

Physical constants and instrumental data (melting point [mp], thin-layer chromatography [TLC] [R_f], gas chromatography [GC] [R_t], $[\alpha]_D^{25}$, ^{1}H- and ^{13}C-NMR, infrared (IR), 70-eV, electron impact - mass spectroscopy [EI-MS], color, and microcrystalline tests) are reported for the individual stereoisomers, racemates, and corresponding hydrochloride salts of 4-methylaminorex (2-amino-4-methyl-5- phenyl-Δ^2-oxazoline, 4,5-dihydro-4-methyl-5-phenyl- 2-oxazolamine, McN-822, "U4Euh", "ICE"). The data allow identification and differentiation of illicit samples of 4-methylaminorex

Compounds



4-methylaminorex

Instruments

Thin-layer chromatography

Gas chromatography

^{1}H- and ^{13}C-NMR

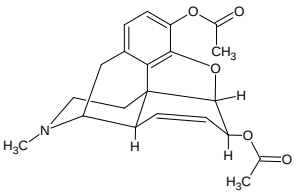
Infrared (IR) Spectrometry

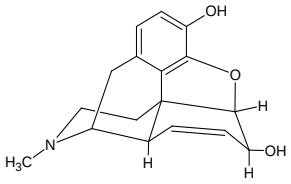
Electron impact-Mass Spectrometry

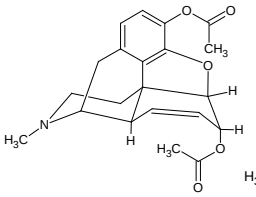
20. Medina,F., III. The Identification of Heroin and Three Structurally Related Isoheroins. Journal of Forensic Sciences. 34 (1989) 565-578.

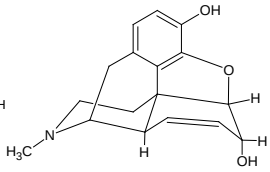
Heroin and three structurally related isomers were studied. The configurational assignments of the structural isomers supported from nuclear magnetic resonance and mass spectrometry data are discussed. Infrared spectroscopy spectra are also presented. Gas chromatography procedures using packed and capillary systems demonstrated how the isomers could be best fully resolved.

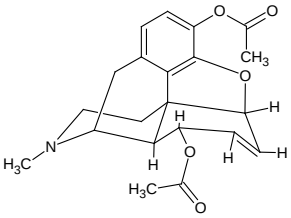
Compounds

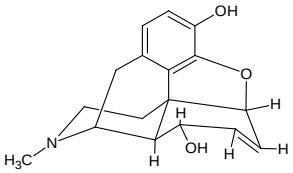

Heroin

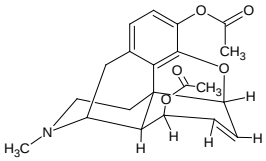

Morphine

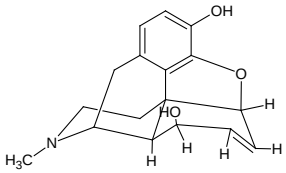

Alpha-Isoheroin


Alpha-Isomorphine


Gama-isoheroin


Gama-isomorphine


Beta-isoheroin


Beta-isomorphine

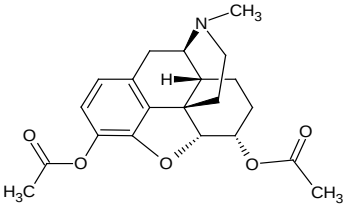
Instruments

Gas-Liquid Chromatography
Mass Spectrometry
NMR
Infrared Spectroscopy

21. Medina,F., III. The Identification of Heroin and Three Structurally Related Isoheroins. Journal of Forensic Sciences. 34 (1989) 565-578.

Heroin and three structurally related isomers were studied. The configurational assignments of the structural isomers supported from nuclear magnetic resonance and mass spectrometry data are discussed. Infrared spectroscopy spectra are also presented. Gas chromatography procedures using packed and capillary systems demonstrated how the isomers could be best fully resolved.

Compounds

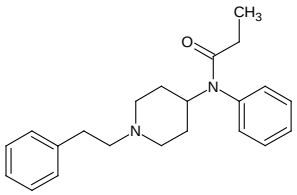

Heroin

Instruments
Gas Chromatography
Mass Spectrometry
NMR

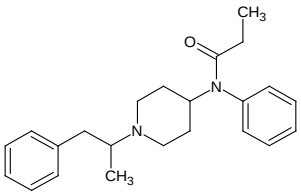
22. Suzuki, S. Studies on fentanyl and related compounds: II. Spectrometric discrimination of five monomethylated fentanyl isomers by gas chromatography/Fourier transform-infrared spectrometry. For. Sci. Int. 43 (1989) 15-19. DOI: 10.1016/0379-0738(89)90117-5.

A gas chromatograph/Fourier transform-infrared (GC/FTIR) spectrometric analysis of five monomethylated fentanyl related compounds, widely abused designer drugs, was evaluated, and spectra obtained were compared with those measured in condensed phase. Infrared spectra obtained in vapor phase showed much difference compared to those of condensed phase, especially in the fingerprint region. However, the discrimination of these five isomers by infrared spectra alone in vapor phase or condensed phase was considered to be difficult. In order to perform the accurate discrimination of these kinds of drugs, which possess high boiling points and very closely related structures, the combination of different analytical techniques must be used such as FTIF, GC/FTIR, and retention times.

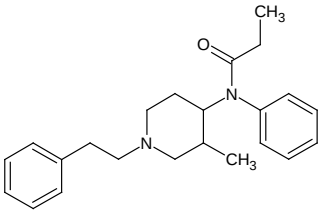
Compounds



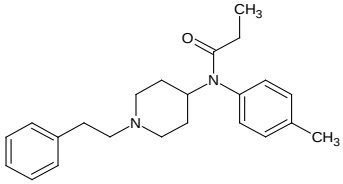
Fentanyl



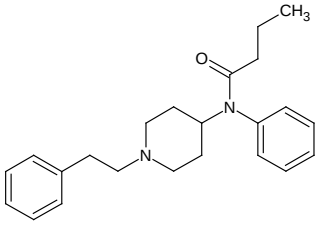
□-methylfentanyl



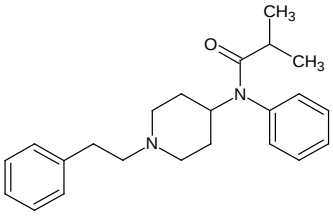
3-Methylfentanyl



p-Tolylfentanyl



n-Propylfentanyl



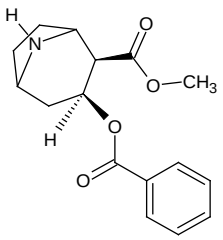
i-Propylfentanyl

Instruments
Gas chromatograph
Fourier transform-infrared (GC/FTIR)

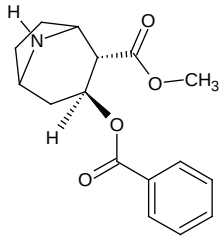
23. Chiarotti, M., Fucci, N. HPLC analysis of cocaine diastereoisomers by chiral stationary phase. For. Sci. Int. 44 (1990) 37-41. DOI: 10.1016/0379-0738(90)90164-T

A complete chromatographic resolution of cocaine and pseudococaine by liquid chromatography is described. The chromatographic analysis was carried out using a chiral stationary phase (Supelcosil LC-urea) and acetonitrile as mobile phase. The proposed method can be employed in forensic toxicology to investigate whether or not illicit cocaine samples are from a synthetic source.

Compounds



cocaine

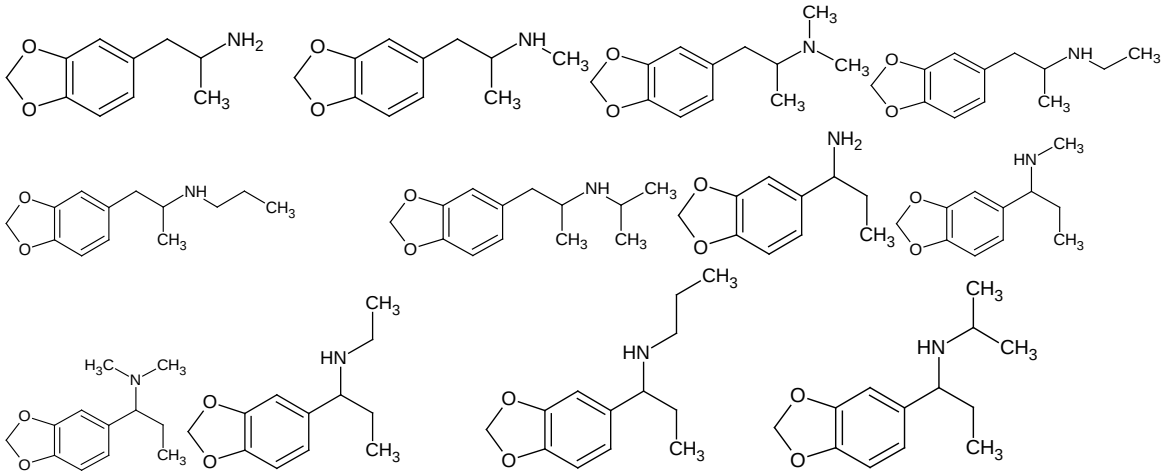


pseudococaine

Instruments
Gas Chromatography
Liquid Chromatography
Mass Spectroscopy

24. DeRuiter, J., Clark, C. R., and Noggle, Jr. F. T. Liquid Chromatographic and Mass Spectral Analysis of 1-(3,4-methylenedioxyphenyl)-1-propanamines: Regioisomers of the 3,4-Methylenedioxyamphetamines. Journal of Chromatographic Science. 28 (1990) 129-132.

Compounds

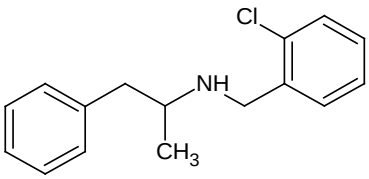


Instruments
Gas Chromatography
Liquid Chromatography
Mass Spectroscopy
Infrared Spectroscopy
UV Spectroscopy
Nuclear Magnetic Resonance

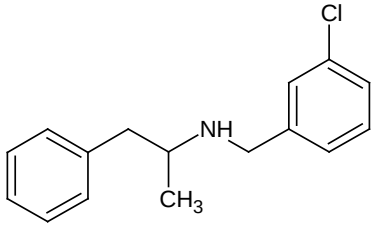
25. Noggle, Jr. F. T., Clark, C. R., Andurkar, S. V. and DeRuiter, J. Liquid Chromatographic Analysis of Regioisomers and Enantiomers of N-(Chlorobenzyl)-α-Methylphenethylamines: Analogues of Clobenzorex. Journal of Liquid Chromatography. 13 (1990) 763-777. DOI: 10.1080/01483919008051819.

The regioisomeric 2-, 3- and 4-chlorobenzylamphetamines were synthesized from racemic and (+)-amphetamine by reductive alkylation. The 2-, 3- and 4-chloro regioisomers were separated by reversed-phase liquid chromatography following phenylisothiocyanate derivatization. The indiviual enantiomers of each regio-isomer were identified by HPLC following derivatization with GITC. Normal phase liquid chromatographic analysis of the diastereomeric GITC derivatives produced α-values of approximately 1.0 for each racemic pair of regioisomers. These methods were developed in order to specifically identify the drug clobenzorex, d-N-(2-chlorobenzyl)-α-methylphenethylamine and distinguish it from its optical and regioisomers

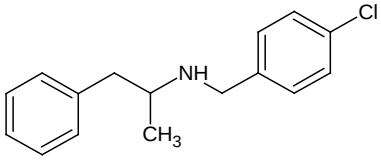
Compounds



2- chlorobenzylamphetamines



3-chlorobenzylamphetamines



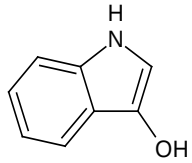
4-chlorobenzylamphetamines

Instruments
High-performance Liquid Chromatography (HPLC)

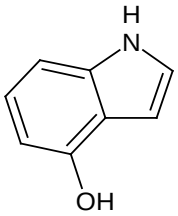
26. Evans, C., et al. Ion-trap mass spectrometry in ion structure studies. 1. Characterization of isomeric hydroxyindoles by electron ionization and energy-resolved collision-activated mass spectrometry. Rapid Communications in Mass Spectrometry. 9 (1990) 335-340. DOI: 10.1002/rcm.1290040908

Three hydroxyindole isomers were investigated by conventional electron ionization (EI) mass spectrometry and by collision-activation (CA) experiments. Although the EI mass spectra of the three isomers are virtually superimposable, 3-hydroxyindole can easily be differentiated from the others by single-energy collision spectroscopy. Only by energy-resolved mass spectrometry (ERMS) could the characterization of the 4- and 5- hydroxyindole isomers be achieved. ERMS, which in the ion-trap mass spectrometer can be performed by different methods, was achieved by changing both the AC supplementary voltage and the β_z value at which the collision experiments were performed. The first method proved to be the most effective in the present case. Further collision experiments, carried out at a range of AC voltage durations did not yield energy-resolved data

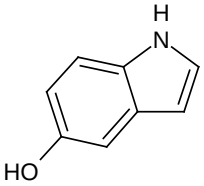
Compounds



3-hydroxyindole



4-hydroxyindole



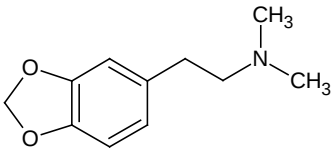
5-hydroxyindole

Instruments
Electron ionization Mass Spectroscopy
Ion-Trap Mass Spectroscopy

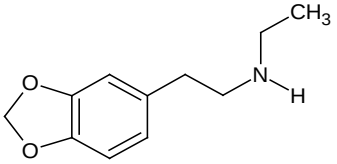
27. Noggle, T. et al. The differentiation of 3, 4-methylenedioxymethamphetamine from some regioisomers. Microgram. Volume 25 No 5. May 1991. Pages 114-131.

The analytical profiles are described for six amines, MDMA and five isomeric amines of MW=193.

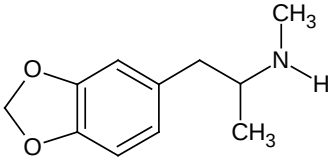
N,N-Dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine



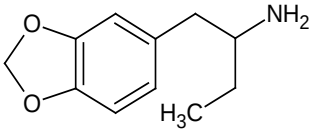
N-Ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine



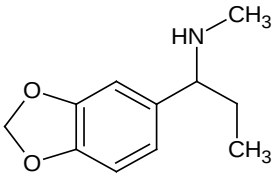
N-Methyl-1-(3,4-methylenedioxyphenyl)-2-propanamine (MDMA)



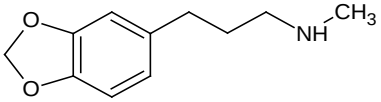
1-(3,4-methylenedioxyphenyl)-2-butanamine



N-Methyl-1-(3,4-methylenedioxyphenyl)-1-propanamine



N-Methyl-1-(3,4-methylenedioxyphenyl)-3-propanamine



Instruments Used:

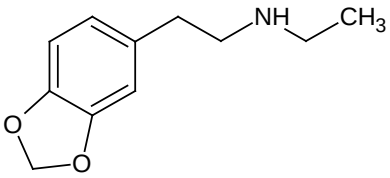
EI MS

FTIR

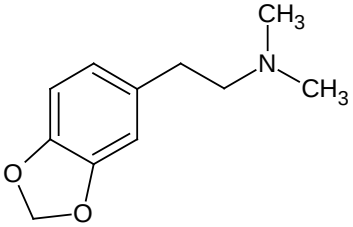
Reversed-Phase liquid chromatography

28. Noggle, F. T., DeRuiter, J., and Clark, C. R. Liquid Chromatographic and Spectral Methods for the Differentiation of 3,4-Methylenedioxymethamphetamine (MDMA) from Regioisomeric Phenethylamines. Journal of Liquid Chromatography. 14 (1991) 1913-1928. DOI: 10.1080/01483919108049662.

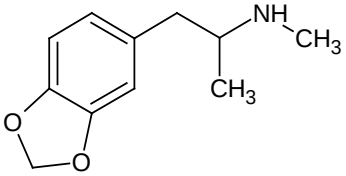
Compounds



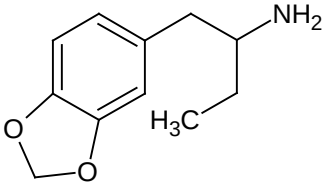
N-ethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine



N,N-dimethyl-1-(3,4-methylenedioxyphenyl)-2-ethanamine



MDMA



1-(3,4-methylenedioxyphenyl)-2-butanamine

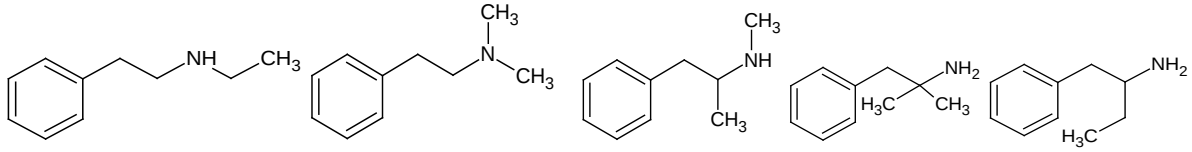
Instruments

Liquid Chromatography
Infrared Spectroscopy
Ultraviolet Spectroscopy
Nuclear Magnetic Resonance
Mass Spectrometry

29. Noggle, F. T., Clark, C. R., Bouhadir, K. H., and DeRuiter, J. Methods for the Differentiation of Methamphetamine from Regioisomeric Phenethylamines. Journal of Chromatographic Science. 29 (1991) 31-36. DOI: 10.1093/chromsci/29.1.31.

The analytical profiles are described for five amines, methamphetamine, and four isomeric phenethylamines of MW = 149. These five amines all contain an unsubstituted benzyl moiety, thus the regioisomerism is within the carbon-carbon bond located α- to the amine moiety. Therefore these phenethylamines are regioisomeric within the imine fragment (m/z = 58), which is the base peak in the electron impact (EI) mass spectrum of methamphetamine. The ultraviolet absorption spectra for these compounds show the characteristic phenethylamine absorption bands in the (250 – 260 nm) range. These amines are best differentiated by chromatographic separation and are well resolved by liquid chromatographic techniques. The five regioisomeric amines are separated using an isocratic reversed-phase system consisting of a C_{18} stationary phase and a mobile phase of pH 3 phosphate buffer and methanol. The elution order under these conditions appears to parallel the length of the carbon chain attached to the aromatic ring

Compounds



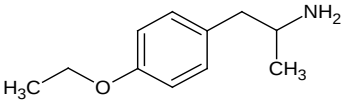
Instruments

Liquid Chromatography
UV Spectroscopy
Infrared Spectroscopy
Nuclear Magnetic Resonance

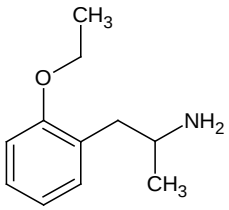
30. By, A. W., Duhaime, R., and Lodge, B. A. The synthesis and spectra of 4-ethoxyamphetamine and its isomers. Forensic Sci. Int. 49 (1991) 159–170. DOI: 10.1016/0379-0738(91)90075-T.

The appearance on the street of 4-ethoxyamphetamine (4-EA) led to the need for a reference standard to be synthesized. To satisfy concerns that this compound could be distinguished from the isomeric 2- and 3-ethoxyamphetamines (2-EA and 3-EA, respectively), standards for each of those substances were also synthesized, in each case as the hydrochloride salt. The UV, IR, ^{1}H-NMR, ^{13}C-NMR and GC/mass spectra are reported here for all three amphetamines

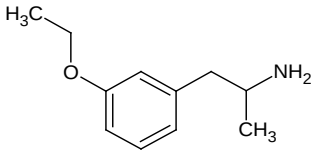
Compounds



4-ethoxyamphetamine



2- ethoxyamphetamine



3- ethoxyamphetamine

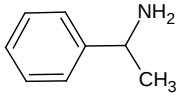
Instruments
UV Spectrometry
IR Spectrometry
^{1}H-NMR
^{13}C-NMR
Gas Chromatography
Mass Spectrometry

31. Xianwen Lou et al. Enantiomeric separation of α-phenylethylamine and its substituted isomers by gas chromatography. Journal of Chromatography A. Volume 586, Issue 1, 8 November 1991, Pages 139–144. doi:10.1016/0021-9673(91)80031-B

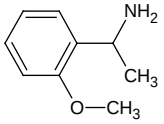
α-Phenylethylamine, o, m, p-methoxy-α-phenylethylamines and o, m, p-methyl-α-phenylethylamines were enantiomerically separated with four different diamide chiral stationary phases (CSPs) [monobenzyldi succinate-l-Val-tert.- butylamide (CSP-1), undecenoyl-l-Val-S-α-phenylethylamide (CSP-2), undecenoyl-l-Val-R-α-phenylethylamide (CSP-3) and cross-linked polycyanoethyl vinyl siloxane-l-Val-tert.-butylamide (CSP-4)] using capillary gas chromatography. The ortho-effect of the methoxy group on the enantiomeric separation was investigated. The elution order of the enantiomers on CSP-3 is reversed with respect to that on the other CSPs studied. The enantiomeric separation of α-phenylethylamine and its methoxy- and methyl-substituted isomers is illustrated.

Compounds

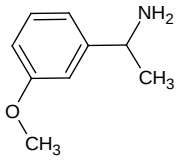
α-Phenylethylamine



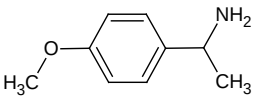
o-methoxy-α-phenylethylamine



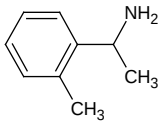
m-methoxy-α-phenylethylamine



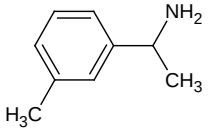
p-methoxy-α-phenylethylamine



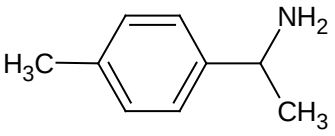
o-methyl-α-phenylethylamine



m-methyl-α-phenylethylamine



p-methyl-α-phenylethylamine



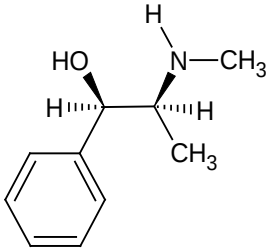
Instrument Used:

Capillary gas chromatography

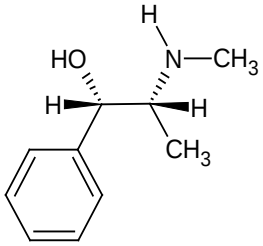
32. McKibben, T. Separation and identification of drug enantiomers via N-TFA-(S)-prolyl chloride derivitization. Journal of CLIC. 2 (1992) 13-20.

The ability to separate and identify drug enantiomers using gas chromatography/mass spectrometry (GC/MS) techniques can provide valuable intelligence information to the forensic chemist. This paper describes the separation and identification of twelve pairs of amphetamine-type enantiomers using the chiral acid chloride derivatizing agent, N-trifluoroacetyl-(S)-prolyl chloride.

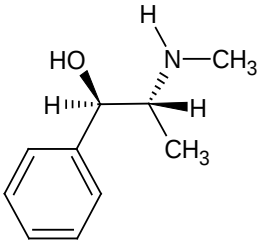
Compounds



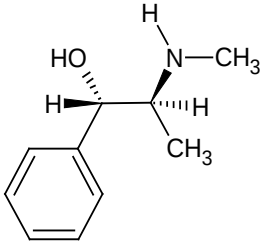
l-ephedrine



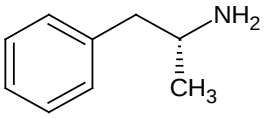
d-ephedrine



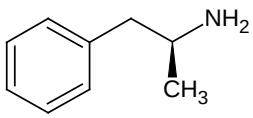
l-psuedoephedrine



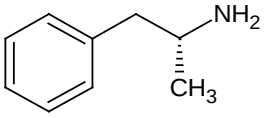
d-psuedoephedrine

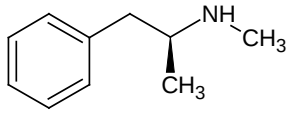


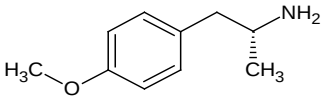
l-amphetamine

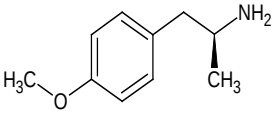


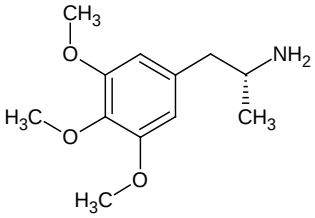
d-amphetamine

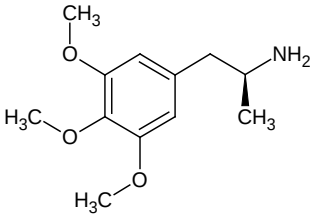

l-methamphetamine

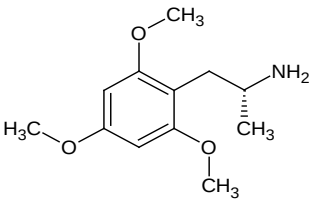

d-methamphetamine

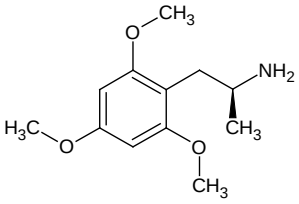

1-PMA

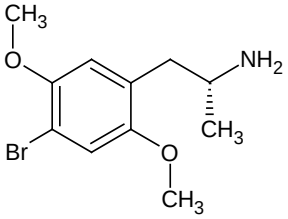

2-PMA

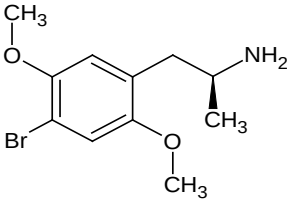

1-(3,4,5-TMA)

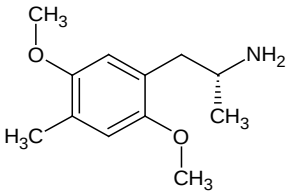

2-(3,4,5-TMA)

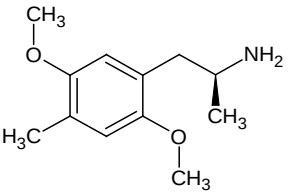

1-(2,4,6-TMA)

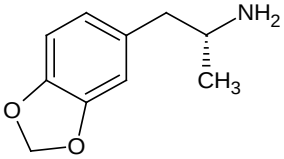

2-(2,4,6-TMA)

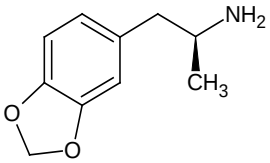

1-DOB


2-DOB


1-DOM


2-DOM


1-MDA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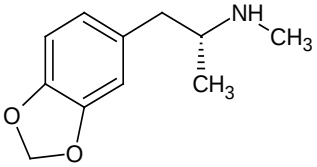
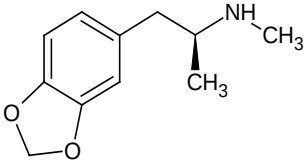
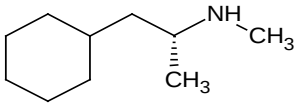
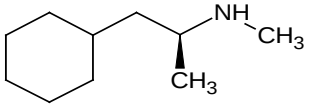

1-MDMA


2-MDMA


1-Propylhexedrine


2-Propylhexedrine

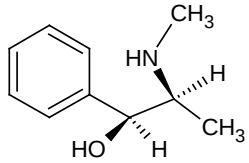
<u>Instruments</u>
Gas Chromatography
Mass Spectrometry

33. McKibben, T. SEPARATION AND IDENTIFICATION OF DRUG ENANTIOMERS VIA N-TFA-(S)-PROLYL CHLORIDE DERIVATIZATION. Journal of the Clandestine Laboratory Investigating Chemists Association. Volume 2, Issue 1, January 1992, Pages 13-20.

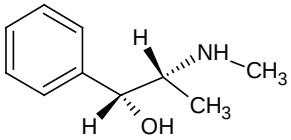
The ability to separate and identify drug enantiomers using gas chromatography/mass spectrometry (GC/MS) techniques can provide valuable intelligence information to the forensic chemist. This paper describes the separation and identification of twelve pairs of amphetamine-type enantiomers using the chiral acid chloride derivatizing agent, N-trifluoroacetyl-(S)-prolyl chloride.

Compounds of Interest:

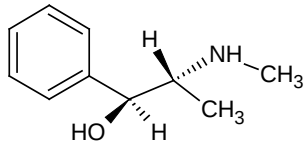
l -ephedrine



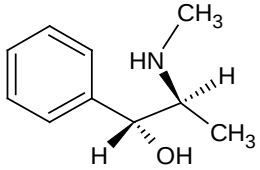
d -ephedrine



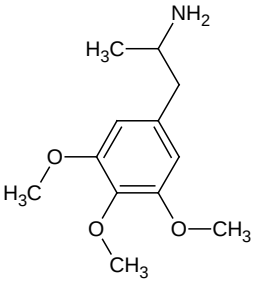
l -pseudoephedrine



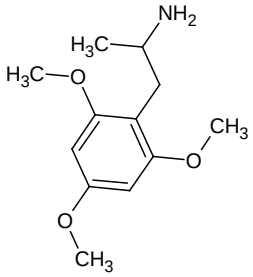
d –pseudoephedrine



3,4,5 trimethoxyamphetamine



2,4,6 trimethoxyamphetamine



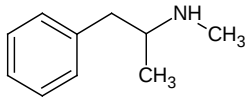
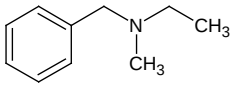
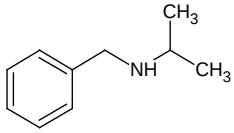
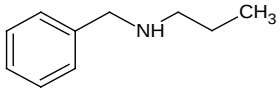
Instrument Used:

Gas chromatography/mass spectrometry

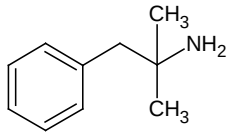
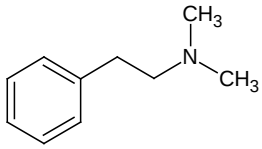
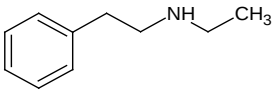
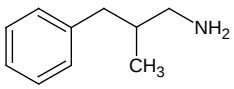
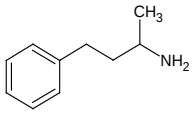
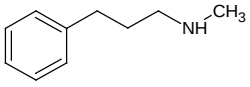
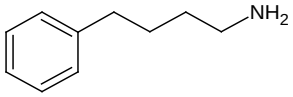
34. Madden, J. E., Pearson, J. R., and Rowe, J. E. Differentiation of side chain isomers of methamphetamine using gas chromatography, high performance liquid chromatography and mass spectrometry. For. Sci. Int. 61 (1993) 169-174. DOI: 10.1016/0379-0738(93)90223-W.

Eleven side chain positional isomers of methamphetamine can be distinguished from methamphetamine using a combination of the Marquis colour test, gas chromatography (GC), high performance liquid chromatography (HPLC) and mass spectrometry (MS). Many of the compounds gave identical colour tests, and several had similar mass spectral fragmentation patterns, but the combined technique of GC/MS unequivocally differentiates all the isomers.

Compounds

Methamphetamine

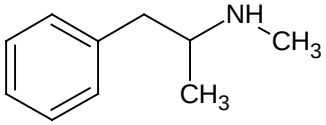
Instruments

Gas Chromatography
High Performance Liquid Chromatography
Mass Spectroscopy

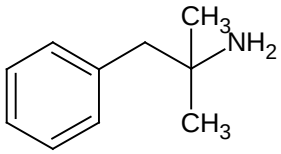
35. Clark, C. R. et al. GC-MS differentiation of acylated derivatives of methamphetamine and regioisomeric phenethylamines (Law Enforcement Restricted Publication). Microgram Bulletin. (1995).

The analytical profiles are described for five amines, methamphetamine and four isomeric phenethylamines of MW- 149. The pentafluoroppropionyl amide derivatives provided adequate GC resolution and mass spectra which can be used to differentiate among these regioisomeric amines.

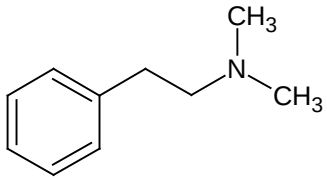
Compounds



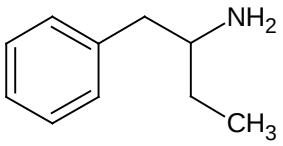
Methamphetamine



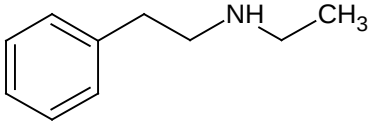
Phentermine



N,N-Dimethylphenethylamine



1-Phenyl-2-butanamine



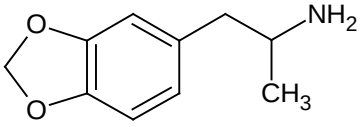
N-Ethylphenethylamine

Instrument
GC-MS

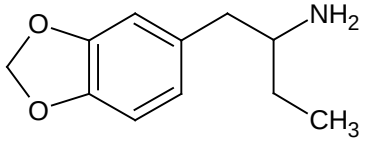
36. Clark, C. R. et al. Identification of 1-(3,4-methylenedioxyphenyl)-2-butanamines related to MDMA (Law Enforcement Restricted Publication). Microgram Bulletin. (1995).

A series of N-substituted 3,4-methylenedioxyphenyl-2-butanamines (MDP-2-Bs) were synthesized and their analytical properties compared to their structurally 3,4-methylenedioxyamphetamine (MDA) drugs of abuse as well as the corresponding 3,4-methylenedioxy-phenyl-3-butanamines ("homoMDAs", MDP-3-Bs).

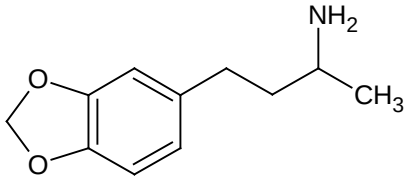
Compounds



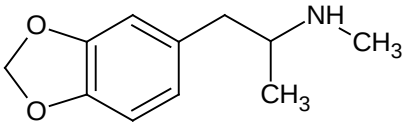
MDA



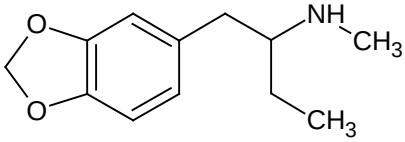
MDP-2-B



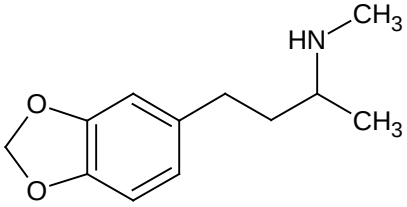
MDP-3-B



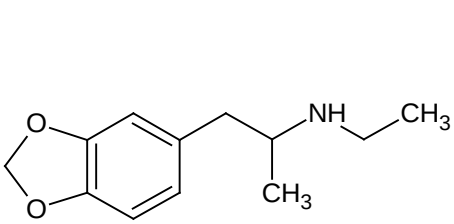
MDMA



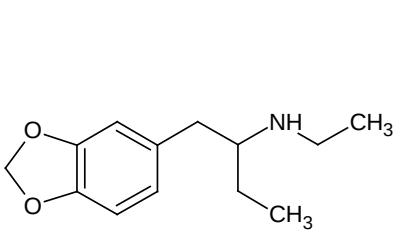
MDP-2-MB



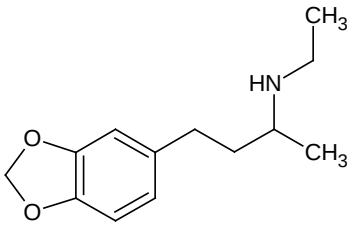
MDP-3-MB



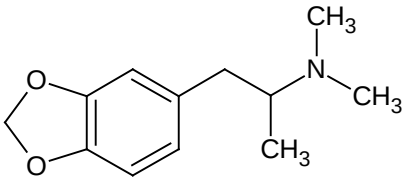
MDEA



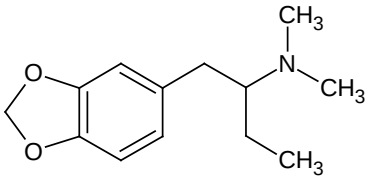
MDP-2-EB



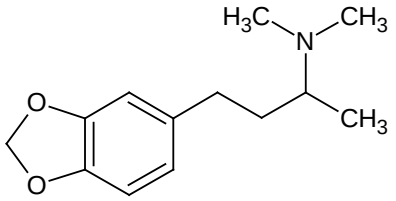
MDP-3-EB



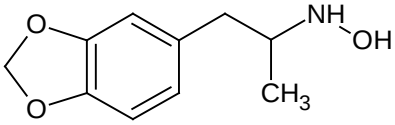
MDMMA



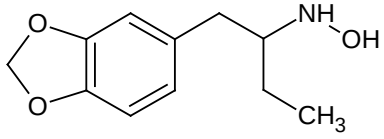
MDP-2-MMB



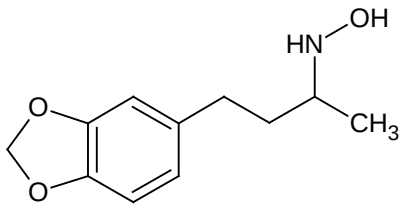
MDP-3-MMB



NOHMDA



MDP-2-OHB



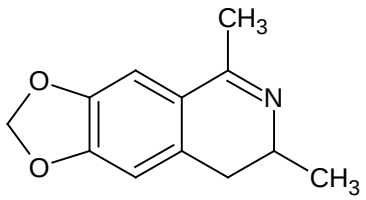
MDP-3-OHB

Instruments
EI-MS
LC-MS
FTIR

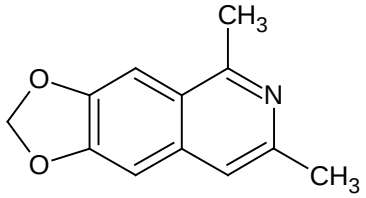
37. Robak, D. R. Identification of Clandestinely Produced Methylenedioxy-isoquinolines. Microgram Bulletin. (1995).

This laboratory recently encountered samples of clandestinely produced isoquinolines. The substances were identified as 1,3-dimethyl-3,4-dihydro-6,7-methylenedioxy-isoquinoline among with a small amount of 1,3-dimethyl-6,7-methylenedioxyisoquinoline. Isoquinolines have subsequently been encountered by at least one other DEA laboratory. This may indicate athe emergence of a "new"cladenstine process that could be encountered by cladenstine laboratory investigators.

Compounds



1,3-dimethyl-3,4-dihydro-6,7-methylenedioxyisoquinoline



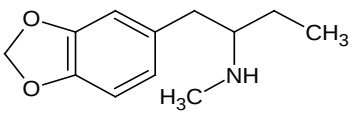
1,3-dimethyl-6,7-methylenedioxyisoquinoline

Instruments
GC-MS
FTIR
EI-MSD
NMR

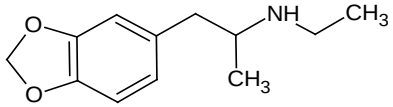
38. Clark, C.R., and DeRuiter, J., Chromatographic and Mass Spectrometry Methods for the Differentiation of N-Methyl-1-(3,4- methylenedioxyphenyl)-2-butanamine from Regioisomeric Derivatives. Journal of Chromatographic Science (1996) 34 (5): 230-237. doi: 10.1093/chromsci/34.5.230

Methods are described for the gas chromatographic—mass spectrometric identification of the street drug *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB or MDP-2-MB) and its differentiation from two uniquely isomeric drugs, *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA) and *N*,*N*-dimethyl-3,4-methylenedioxyamphetamine (MDMMA). These positional isomers have the same molecular weight (MW = 207) and fragment by a common mechanism under electron impact mass spectrometric conditions to yield a base peak of the same mass (*m/z* 72). Derivatization of the two secondary amines (MBDB and MDEA) with pentafluoropropionic anhydride (PFPA) yields amides with fragment ions which individualize their EI mass spectra. The PFPA derivative of MBDB yields diagnostic ions at *m/z* 160 and 176, whereas the PFPA derivative of MDEA produces ions at *m/z* 162 and 190. This EI spectra individualization for MBDB and MDEA is particularly significant since these two compounds have similar retention properties in the PFPA-derivatized and underivatized forms and since both are known street drugs.

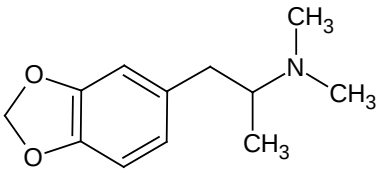
Compounds



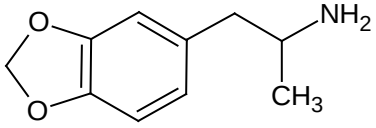
MBDB



MDEA



MDMMA



MDA

Instruments

Gas Chromatography

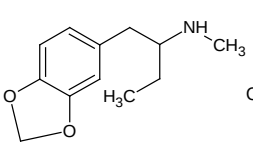
Liquid Chromatography

Mass Spectrometry

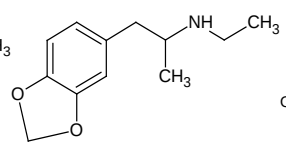
39. Noggle, F. T. et al. Chromatographic and mass spectrometric analysis of N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine and regioisomeric derivatives. Journal of Chromatographic Science. 34 (1996) 230-237. DOI: 10.1093/chromsci/34.5.230

Methods are described for the gas chromatographic—mass spectrometric identification of the street drug N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB or MDP-2-MB) and its differentiation from two uniquely isomeric drugs, N-ethyl-3,4-methylenedioxyamphetamine (MDEA) and N,N-dimethyl-3,4-methylenedioxyamphetamine (MDMMA). These positional isomers have the same molecular weight (MW = 207) and fragment by a common mechanism under electron impact mass spectrometric conditions to yield a base peak of the same mass (m/z 72). Derivatization of the two secondary amines (MBDB and MDEA) with pentafluoropropionic anhydride (PFPA) yields amides with fragment ions which individualize their EI mass spectra. The PFPA derivative of MBDB yields diagnostic ions at m/z 160 and 176, whereas the PFPA derivative of MDEA produces ions at m/z 162 and 190. This EI spectra individualization for MBDB and MDEA is particularly significant since these two compounds have similar retention properties in the PFPA-derivatized and underivatized forms and since both are known street drugs.

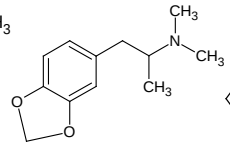
Compounds



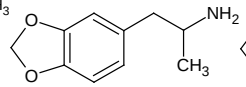
MBDB



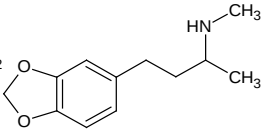
MDEA



MDMMA



MDA



HMDMA

Instruments

Gas Chromatography

Liquid Chromatography

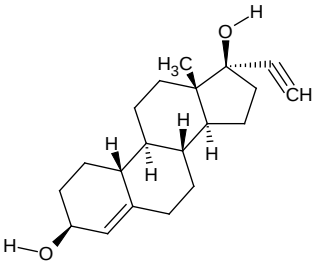
Mass Spectrometry

40. Kummer, M., Palm, H.J., and Werner, G. Resolution of enantiomeric steroids by high-performance liquid chromatography on chiral stationary phases. Journal of Chromatography. 749 (1996) 61- 68. DOI: 10.1016/0021-9673(96)00364-0.

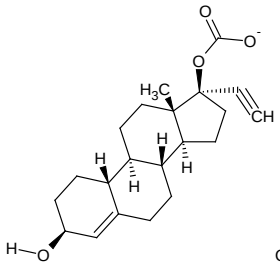
The chiral separation of 11 steroids was investigated on amyloae tris(3,5-dimethylphenyl carbamate). This chiral stationary phase was subjected to both normal-phase and reversed-phase conditions. Enantioselectivities were higher in the reversed-phase mode for the majority of steroids. The influence of structural modifications of the steroid molecule on the chiral separation was investigated. Acetylation of hydroxyl groups decreased enatioselectivity in the normal-phase mode. However, some acetate exhibited higher enantioselectivities in the reversed-phase confitions. The reversed-phase eluent were also chromatographed on permethylated β- and ϒ-cyclodextrin columns. Enantioselectivities were loqer compared to the amylase coulumn, with the β-cyclodextrin being superior to the ϒ-cyclodextrin.

Compounds

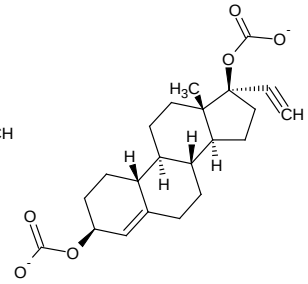
Ethynodiol



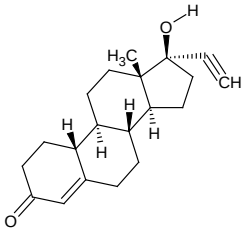
Ethynodiol-17-acetate



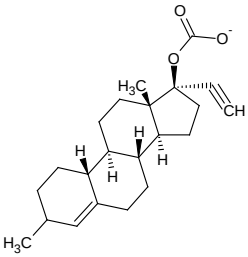
Ethynodiol diacetate



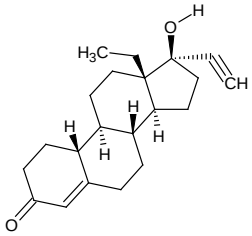
Norethisterone



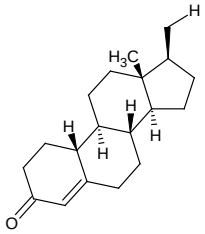
Norethisterone acetate



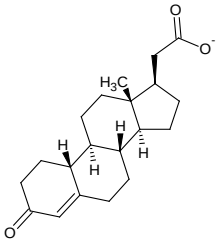
Norgestrel



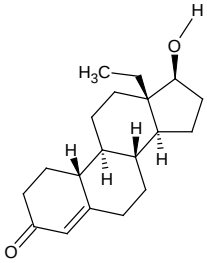
Nandrolone



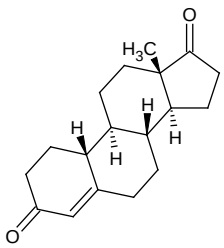
Nandrolone acetate



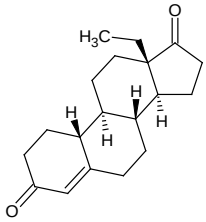
Ethylnandrolone



Norandrostenedione



Ethylgonenedione



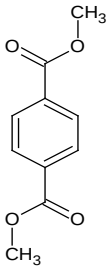
Instruments

High Performance Liquid Chromatography

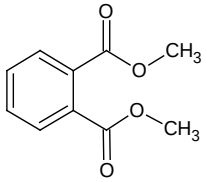
41. Cooper, S. Toskee, S. Identification and differentiation of dimethyl terephthalate and its geometrical isomer dimethyl phthalate. Microgram. Volume 29 No 12. December 1996. Pages 307-315.

One of the two geometrical isomers of dimethyl terephthalate is dimethyl phthalate, a liquid which is used as an ingredient in insect repellants. In this report, we provide analytical conditions and data using GC, GC/MS, IR and NMR that differentiates the two isomers.

dimethyl terephthalate



dimethyl phthalate



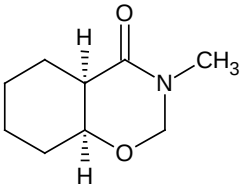
Instruments Used:

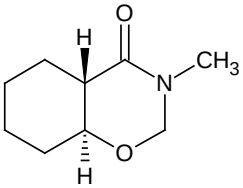
GC/MS, IR, NMR

42. Drahos, L., Vekey, K. Quantification of Isomeric Differences in Mass Spectra. Rapid Communications in Mass Spectrometry. 10 (1996) 1309-1315. DOI: 10.1002/(SICI)1097-0231(19960731)10:10<1309::AID-RCM624>3.0.CO;2-H.

Several indices have been developed to characterize isomeric differences, degree of isomerization and spectral similarity in mass spectra. An index (*S*), based on absolute value distance, is developed to describe the similarity of two spectra. Two indices (α and β) were developed to characterize the maximum degree of isomerization between two isomers under mass spectrometric conditions. Another index was derived from the former ones to characerize differences between spectra (*D*). The effect of random errors (i.e. reproducibility) on these indices was also checked. Comparison of spectra of various pairs of isomers indicated that the developed formulae are efficient for isomer characterization. The same indices can be used, not only for comparing isomers, but also for the quantitative comparison of complex mixtures (based either on mass spectra or on chromatograms).

Compounds



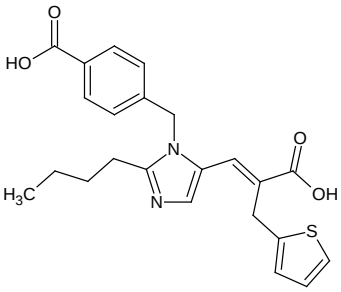


Instruments
Mass Spectroscopy

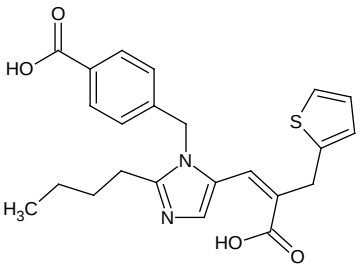
43. Brum, J., Hannah, R. Differentiation of Two Geometric Isomers of the Pharmaceutical Eprosartan Using Atmospheric Pressure Chemical Ionization. Rapid Communications in Mass Spectrometry. 13 (1997) 1430-1434. DOI: 10.1002/(SICI)1097-0231(19970830)11:13<1430::AID-RCM7>3.0.CO;2-5

The utility of an atmospheric pressure chemical ionization interface for distinguishing sterioisomers has been demonstrated. Two geometrical isomers of the pharmaceutical eprosartan ((*E,Z*)-3-[butyl-1-(4-carboxybenzyl)-1*H*-imidazole-5-yl]-2[2-thienyl)methyl]propenoic acid) were investigated in the positive- and negative-ion modes with in-source collision-induced dissociation (CID). In positive-ion mode, CID spectra display significant differences between the two isomers. Under identical collisional conditions several fragment ions present in the CID spectrum of the *E* isomer (SK&F 108566) are significantly suppressed in the spectrum of the *Z* isomer (SB 206328). Analysis of the fragmentation patterns of both isomers indicates that a pathway initiated by the loss of neutral thiophene from the *E* isomer is inhibited in the CID spectra of the *Z* isomer. In negative-ion mode, fragmentation and corresponding differences in spectra are not observed. Fragmentation is observed to result primarily from the ionization process

Compounds



Eprosartan E-isomer



Eprosartan Z-isomer

Instruments
Liquid Chromatography
Mass Spectroscopy

44. DeRuiter, J. et al. Analysis of the bromination products of the isomeric dimethoxyphenethylamines: differentiation of “nexus” from five positional isomers. Microgram. Volume 30 No 5. May 1997. Pages 96-111.

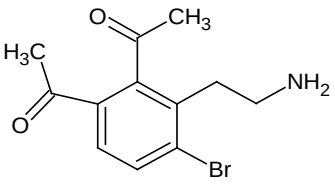
The brominated products from all six positional isomers of dimethoxyphenethylamines were prepared and their analytical properties evaluated. The mass spectra divide these compounds into two distinct groups: a group showing a strong m/z 180 ion via loss of bromine from the molecular ion (M-Br)+ and a second group showing no significant m/z 180 ion. The three compounds having no m/z 180 ion in their EI mass spectra are brominated 2,4-, 2,5- (Nexus) and 2,6 dimethoxyphenethylamine. These compounds are well resolved by reversed phase liquid chromatographic methods using a C_{18} stationary phase and a mobile phase of pH 3 phosphate buffer and methanol.

Compounds

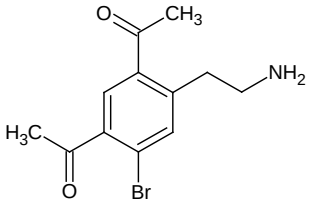
m/z 180 isomers

Non m/z 180 isomers

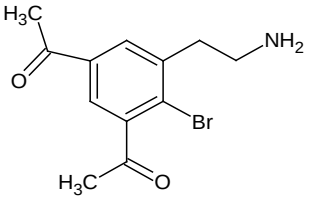
6-Bromo-2,3-dimethoxyphenethylamine



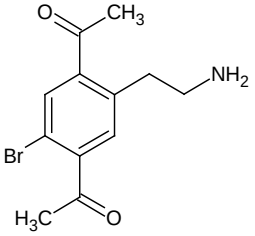
5-Bromo-2,4-dimethoxyphenethylamine



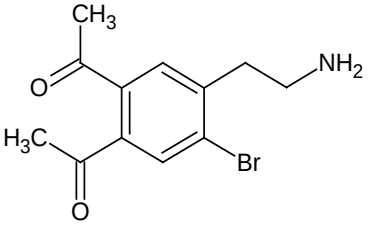
2-Bromo-3,5-dimethoxyphenethylamine



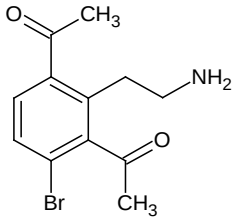
4-Bromo-2,5-dimethoxyphenethylamine



2-Bromo-4,5-dimethoxyphenethylamine



3-Bromo-2,6-dimethoxyphenethylamine

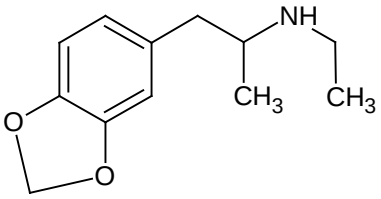


Instruments Used:

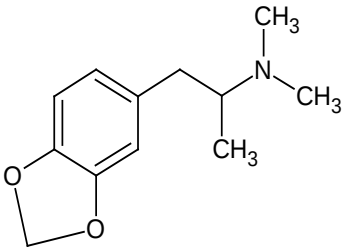
EI MS

45. Bovolento, A., and Morselli, O. Italian Clandestine Drug Market: MDEA, MDMMA, and MBDB in Street Tablets (Law enforcement restricted publication). Microgram Bulletin. (1997)

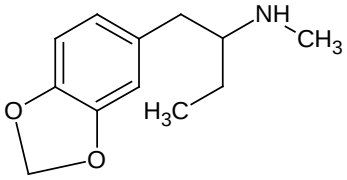
Compounds



MDEA



MDMMA



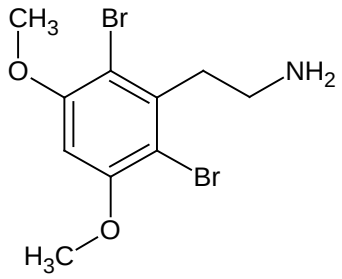
MBDB

Instruments:
GC-MS
IR
NMR

46. DeRuiter, J. et al. Analysis of the bromination products of the isomeric dimethoxyphenethylamines: differentiation of “Nexus” from five positional isomers. Microgram Bulletin. 30 (1997) 96-111.

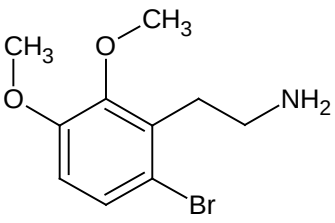
The brominated products from all six positional isomers of dimethoxyphenethylamine were prepared and theor analytical properties evaluated. The mass spectra divide these compounds into two distinct groups: a group showing a strong m/z 180 ion and a second groupd showing a no significant m/xz180 ion. The three compounds with no m/z 180 ion were resolved by reverse liquid chromatography.

Compounds

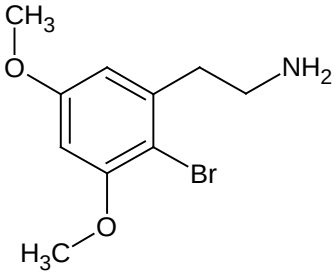


2,6-Dibromo-3, 5-dimethoxyphenethylamine

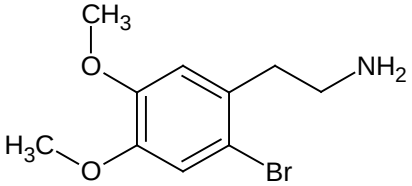
m/z 180 ion isomers



6-Bromo-2, 3-dimethoxyphenethylamine

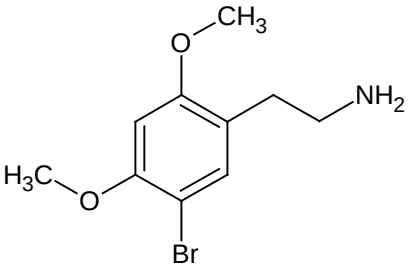


2-Bromo-3, 5-dimethoxyphenethylamine

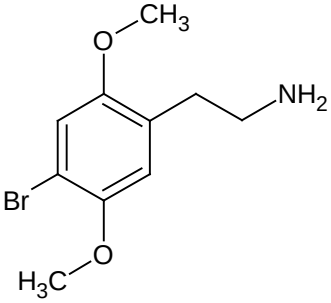


2-Bromo-4, 5-dimethoxyphenethylamine

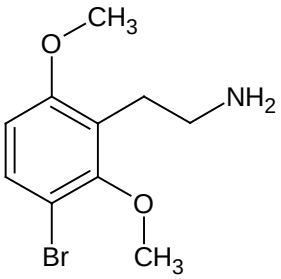
Non m/z 180 ion isomers



5-Bromo-2, 4-dimethoxyphenethylamine



4-Bromo-2, 5-dimethoxyphenethylamine



3-Bromo-2, 6-dimethoxyphenethylamine

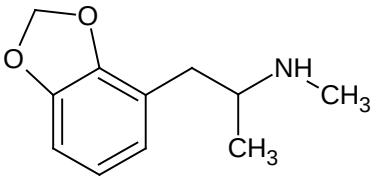
<u>Instruments</u>
GC-MS
LC-MS

47. Clark, C. R. et al. Methods of Differentiation for regioisomeric 2,3- and 3,4-methylenedioxyphenalkylamines by liquid chromatography and mass spectrometry (Law enforcement restricted publication). Microgram Bulletin. 36 (1998) 131-138. DOI: 10.1093/chromsci/36.3.131.

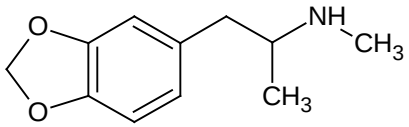
47. Clark, C. R. et al. Methods of Differentiation for regioisomeric 2,3- and 3,4-methylenedioxyphenalkylamines by liquid chromatography and mass spectrometry (Law enforcement restricted publication). Microgram Bulletin. 36 (1998) 131-138. DOI: 10.1093/chromsci/36.3.131.

A series of ring and side chain regioisomers of 3, 4-methylenedioxymethamphetamine are compared by chromatographic and spectroscopic methods.

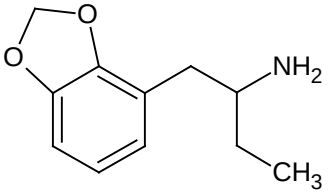
Compounds



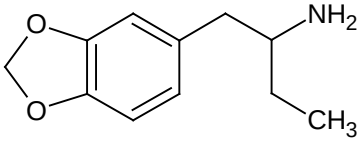
2,3-MDMA



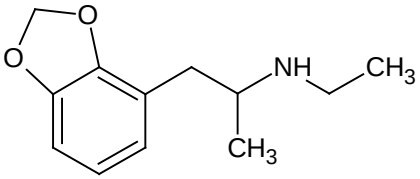
3,4-MDMA



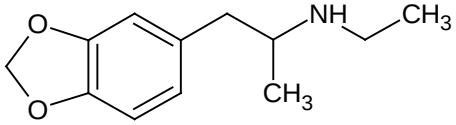
2,3-BDB



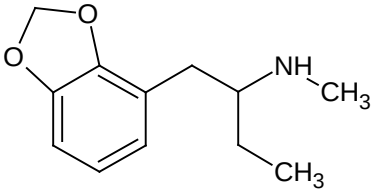
3,4-BDB



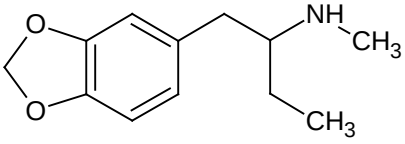
2,3-MDEA



3,4-MDEA



2,3-MBDB



3,4-MBDB

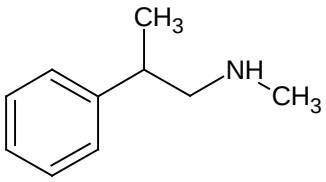
Instruments

GC-MS

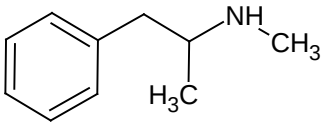
LC-MS

48. Hays, P. A. et al. Phenethylpropylamine [analytical data for positional isomer of methamphetamine] (Law enforcement restricted publication). Microgram Bulletin. (1998).

Compounds



Phenethylpropylamine



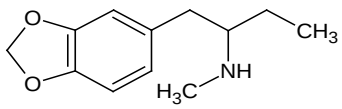
Methamphetaine

Instruments
GC/MS
FT-NMR
GC/IRD
FT-IR

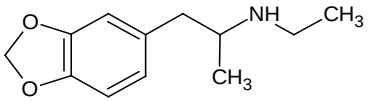
49. Garofano, L. et al. Ion trap mass spectrometry for the characterization of N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine and N-ethyl-3,4-methylenedioxyamphetamine, two widely distributed street drugs. Rapid Communications in Mass Spectrometry. 12(1998) 779-782. DOI: 10.1002/(SICI)1097-0231(19980630)12:12<779::AID-RCM233>3.0.CO;2-Q.

The potential of ion trap mass spectrometry has been evaluated for the characterization and distinction of two isomeric amphetamines drugs, namely N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine and N-ethyl-3,4-methylenedioxyamphetamine. Whereas the electron impact spectra of the two molecules lack specificity, collisional experiments on the ionic species at *m/z* 72 allows unequivocal distinction between the two isomers. Analogous results are achieved by positive ion chemical ionization and collisional experiments on the protonated molecules. All the different approaches have been successfully applied to the gas chromatography/mass spectrometry analysis of a tablet of illicit drug.

Compounds



MBDB



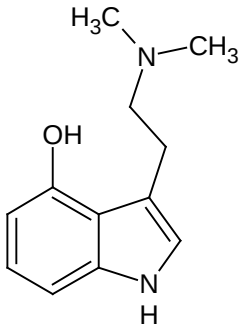
MDEA

Instruments
Gas Chromatography
Mass Spectroscopy

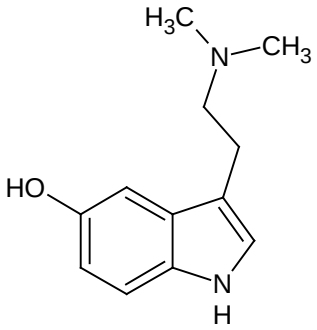
50. Phelan, C. P. Identification of psilocin and bufotenine via GC/IRD (Law enforcement restricted publication). Microgram Bulletin. (1999).

Psilocin and bufotenine are positional isomers of one another with very similar GC-MS spectra. An alternative method using GC-IRD was investigated.

Compounds



Psilocin



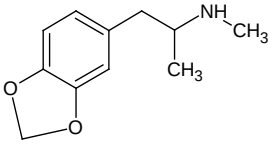
Bufotenine

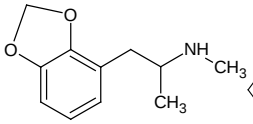
Instruments
GC-MS
GC-IRD

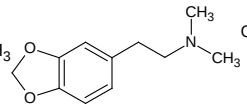
51. Aalberg, L., DeRuiter, J., Noggle, F. T., Sippola, E. and Clark, C. R. Chromatographic and Mass Spectral Methods of Identification for the Side-Chain and Ring Regioisomers of Methylenedioxymethamphetamine. Journal of Chromatographic Science. 38 (2000) 329-336. DOI: 10.1093/chromsci/38.8.329.

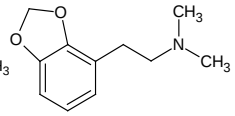
The popular drug of abuse 3,4-methylenedioxymethamphetamine (MDMA) is one of a total of 10 regioisomeric 2,3- and 3,4-methylenedioxyphenethylamines of MW 193 that yields regioisomeric fragment ions with equivalent mass (m/z 58 and 135/136) in the electron-impact (EI) mass spectrum. Thus, these 10 methylenedioxyphenethylamines are uniquely isomeric; they have the same molecular weight and equivalent major fragments in their mass spectra. The specific identification of one of these compounds (i.e., Ecstasy or 3,4-MDMA) in a forensic drug sample depends upon the analyst's ability to eliminate the other regioisomers as possible interfering or coeluting substances. This study reports the synthesis, chemical properties, spectral characterization, and chromatographic analysis of these 10 unique regioisomers. The ten 2,3- and 3,4-regioisomers of MDMA are synthesized from commercially available precursor chemicals. In the EI mass spectra, the side-chain regioisomers show some variation in the relative intensity of the major ions, with the exception of only one or two minor ions that might be considered side-chain specific fragments. The position of substitution for the methylenedioxy ring is not easily determined by mass spectral techniques, and the ultimate identification of any one of these amines with the elimination of the other nine must depend heavily upon chromatographic methods. The chromatographic separation of these 10 uniquely regioisomeric amines are studied using reversed-phase liquid chromatographic methods with gradient elution and gas chromatographic techniques with temperature program optimization

Compounds

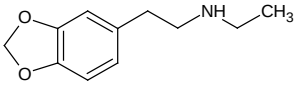


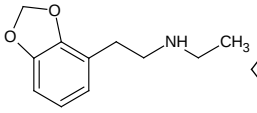


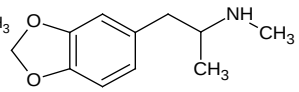


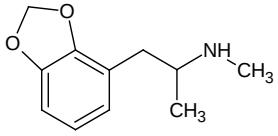


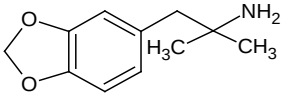
3,4-MDMA 2,3-MDMA

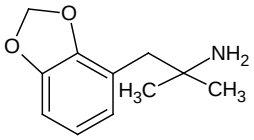


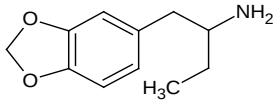


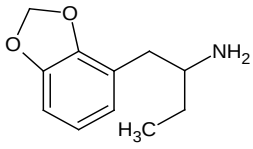












Instruments

Gas Chromatography

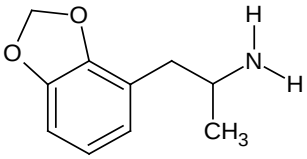
Reversed-phase liquid chromatography

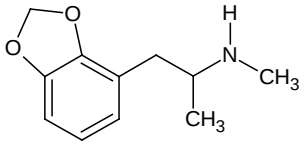
Mass Spectrometry

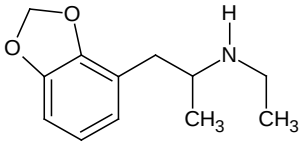
52. Borth, S., Hansel, W., Rosner, P., and Junge, Th. Regioisomeric differentiation of 2,3- and 3,4-methylenedioxy ring-substituted phenylalkylamines by gas chromatography/tandem mass spectrometry. Journal of Mass Spectrometry. 35 (2000) 705-710. DOI: 10.1002/1096-9888(200006).

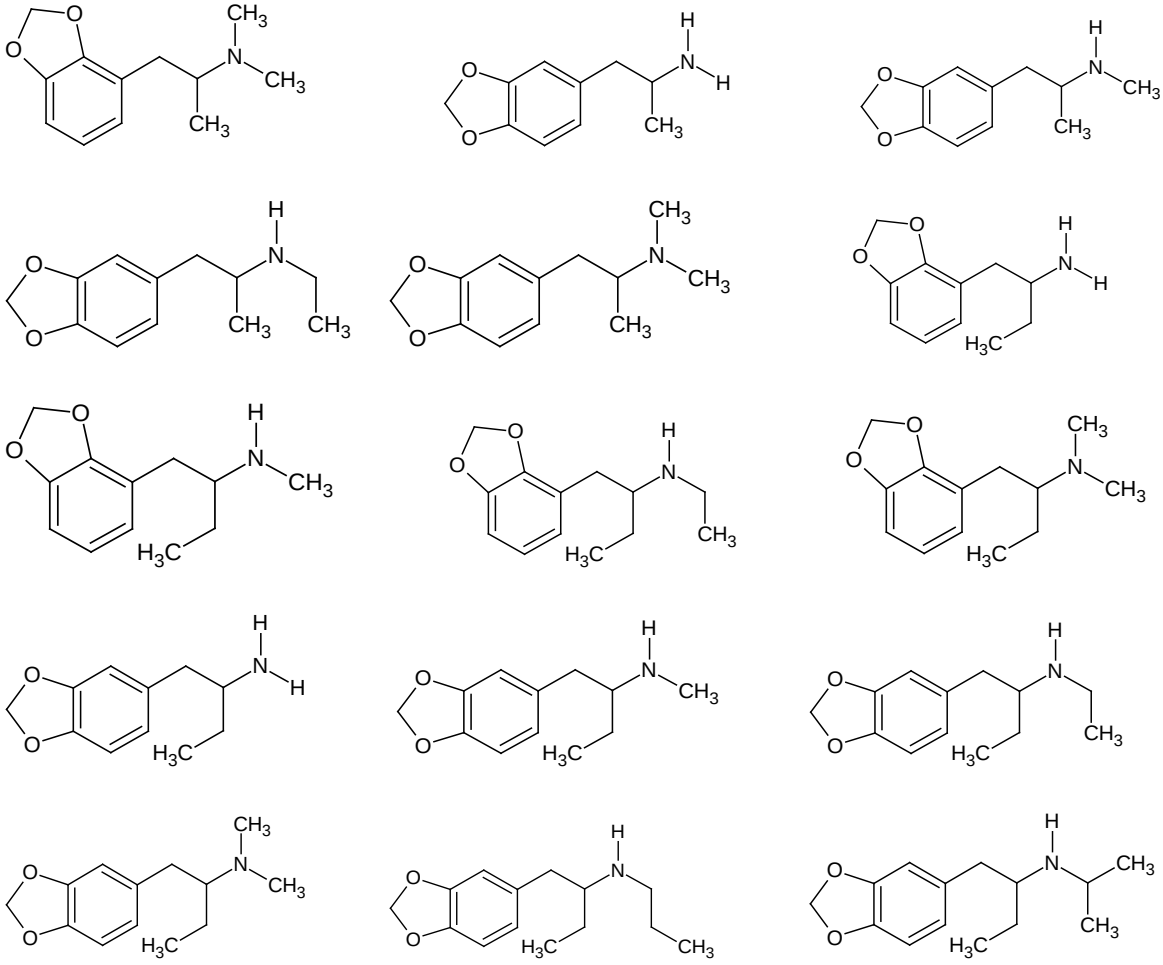
Numerous abused drugs of the 3,4-methylenedioxymetamphetamine (MDMA; Ecstasy; N-methyl-1-(3,4-methylenedioxyphenyl)-2-propaneamine) type and various alkyl chain- and aromatic ring-substituted isomers give very similar electron ionization (EI) mass spectra. This seriously affects the analysis of especially ring regioisomeric drug variants. Using collision-induced dissociation (CID) (argon) under EI and chemical ionization, the mass spectra of 18 2,3- and 3,4-methylenedioxy ring-substituted phenylethylamines were recorded. These techniques permitted an unequivocal differentiation of all studied ring regioisomeric methylenedioxyphenylethylamines. CID mass spectrometry therefore appear to be a reliable tool to establish the kind of ring substitution pattern in regioisomeric methylenedioxyphenalkylamines

Compounds







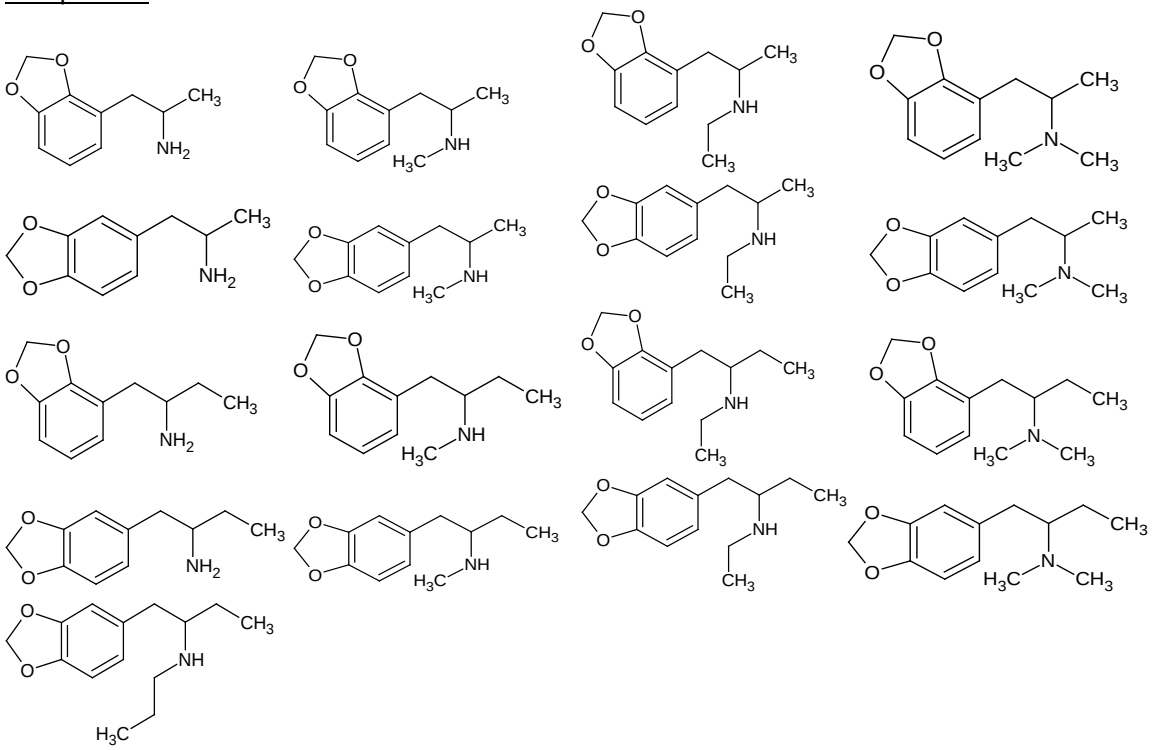


Instruments
Gas Chromatography
Mass Spectroscopy

53. Borth, S., Hansel, W., Rosner, P., and Junge, Th. Synthesis of 2,3- and 3,4-methylenedioxyphenylalkylamines and their regioisomeric differentiation by mass spectral analysis using GC-MS-MS. For. Sci. Int. 114 (2000) 139-153. DOI: 10.1016/S0379-0738(00)00296-6.

3,4-methylenedioxyamphetamine (MDA) derivatives are increasingly abused central nervous system stimulants with neurotoxic properties. In recent years a number of controlled substance analogs (designer drugs) with high structural variety reached the illegal market making their identification an arduous task. The underivatized compounds give very similar or even virtually identical electron impact mass spectra containing mainly intense $C_nH_{2n+2}N^+$ immonium ions. Using tandem mass spectrometry (MS-MS) the additional structural information contained in the collision induced dissoziation (CID) mass spectra of molecular ions using electron impact (EI) and especially chemical ionization (CI) allowed an unequivocal differentiation of 18 studied regioisomeric 1-(methylenedioxyphenyl)-2-propanamines and 1-(methylenedioxyphenyl)-2-butanamines

Compounds

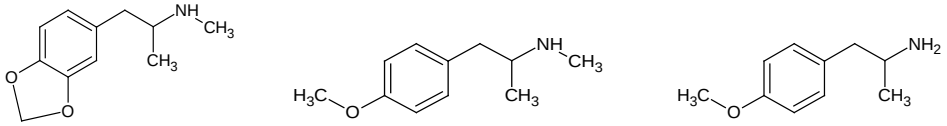


Instruments
Gas Chromatography
Colllision Induced Dissociation Mass Spectrometry
Electron Ionization Mass Spectrometry

54. Del Cason, T. A. A re-examination of the mono-methoxy positional ring isomers of amphetamine, methamphetamine and phenyl-2-propanone. For. Sci. Int. 119 (2001) 168-194. DOI: 10.1016/S0379-0738(00)00425-4.

Recently, tablets inscribed with the Mitsubishi 3-diamond logo, and sold as 3,4-methylenedioxymethamphetamine (MDMA), were found to contain p-methoxymethamphetamine (PMMA), a compound with MDMA-like effects. Shortly after this first submission, similarly inscribed tablets were encountered containing both PMMA and p-methoxyamphetamine (PMA). This second tablet composition has been implicated in several recent deaths in the US. Because two other positions are available for mono-methoxy substitution on the phenyl ring, it is essential that the correct identification be made for these compounds. Analytical data are supplied to enable differentiation of these ring isomers as well as the ketones that serve as their precursors

Compounds

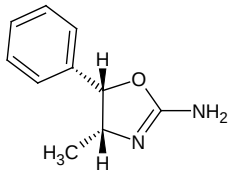


MDMA PMMA PMA

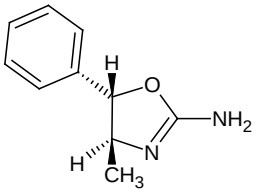
55. Kankaanpää, A. et al. Detection and assay of cis- and trans-isomers of 4-methylaminorex in urine, plasma and tissue samples. For. Sci. Int. 121 (2001) 57-64. DOI: 10.1016/S0379-0738(01)00453-4.

The 4-methylaminorex (4-MAX) is an amphetamine-related psychostimulant drug that has appeared on the clandestine market with a street name of "U4Euh". This compound exists as four stereoisomers, trans-4R,5R, trans-4S,5S, cis-4R,5S and cis-4S,5R, of which the cis forms have been classified as Schedule I substances in the US. The increasing variety of designer drugs has highlighted the importance of detection, identification, and quantitative measurement of these drugs, including 4-MAX, in biological samples. In the present study, the isomers of 4-MAX were detected in urine of rats treated with the drugs by some but not all of the on-site immunoassays tested, mainly as amphetamine or methamphetamine. To facilitate identification of 4-MAX by laboratories specialized in drug analysis, the electron-ionization mass spectrum and TLC data for underivatized 4-MAX using a routine laboratory drug-screening procedure is provided. In addition, a GC/MS method is described for the quantitative determination of cis- and trans-4-MAX as tert-butyldimethylsilyl-derivatives in plasma, urine and tissue

Compounds



Cis- 4-MAX



Trans- 4-MAX

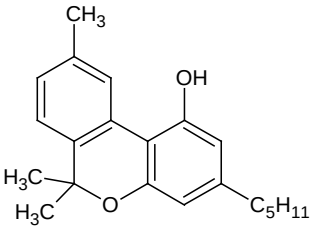
Instruments
Gas Chromotography
Mass Spectrometry

56. Wojtasik, E. Anyzewska, M. & Arent, I. THE OPTIMIZATION OF THE SEPARATION CONDITIONS FOR CANNABINOIDS FROM CANNABIS SATIVA L. VAR INDICA AND APPLICATION OF THE METHOD TO DETERMINE THE CONTENT OF Δ9-TETRAHYDROCANNABINOL IN PLANT MATERIAL. Journal of Liquid Chromatography & Related Technologies. Volume 25, Issue 6, 2002, pages 949-959. DOI: 10.1081/JLC-120003272

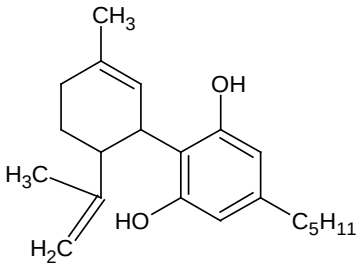
The method for determination of isomer Δ^9 of tetrahydrocannabinol in Cannabis sativa L. var indica has been developed. It is based on HPLC separation of isomers, Δ^9 and Δ^8, with resolution

factor Rs=1.00. The method validation involved specificity and precision. Based on analysis of the raw material – marihuana, the variation coefficient RSD was found to be 2.09%.

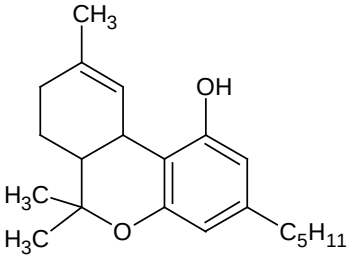
Compounds



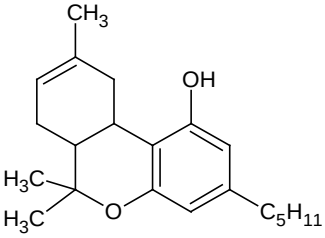
CBN



CBD



Δ^9-THC



Δ^8-THC

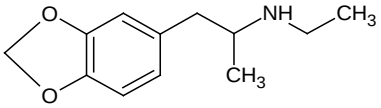
Instruments

High Performance Liquid Chromatography
Ultraviolet Spectroscopy

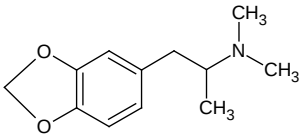
57. Aalberg, L. et al. Chromatographic and Spectroscopic Methods of Identification for the Side-Chain Regioisomers of 3,4-Methylenedioxyphenethylamines Related to MDEA, MDMMA, and MBDB. Journal of Chromatographic Science. 41 (2003) 227-233. DOI: 10.1093/chromsci/41.5.227.

Three regioisomeric 3,4-methylenedioxyphenethylamines having the same molecular weight and major mass spectral fragments of equivalent mass have been reported as components of clandestine drug samples in recent years. These drugs of abuse are 3,4- methylenedioxy-N-ethylamphetamine, 3,4-methylenedioxy-N, N-dimethylamphetamine, and N-methyl-1-(3,4- methylenedioxyphenyl)-2-butanamine. These three compounds are a subset of a total of ten regioisomeric 3,4-methylenedioxyphenethylamines of molecular weight 207, yielding regioisomeric fragment ions of equivalent mass (m/z 72 and 135/136) in the electron impact mass spectrum. The specific identification of one of these compounds in a forensic drug sample depends upon the analyst's ability to eliminate the other regioisomers as possible interfering or coeluting substances. This paper reports the synthesis, mass spectral characterization, and chromatographic analysis of these ten unique regioisomers. The ten regioisomeric methylenedioxyphenethylamines are synthesized from commercially available precursor chemicals. The electron impact mass spectra of these regioisomers show some variation in the relative intensity of the major ions with only one or two minor ions that might be considered side-chain specific

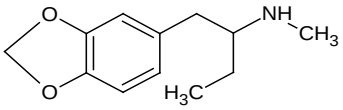
fragments. Thus, the ultimate identification of any one of these amines with the elimination of the other nine regioisomeric substances depends heavily upon chromatographic methods. Chromatographic separation of these ten uniquely regioisomeric amines is studied using gas chromatographic temperature program optimization.



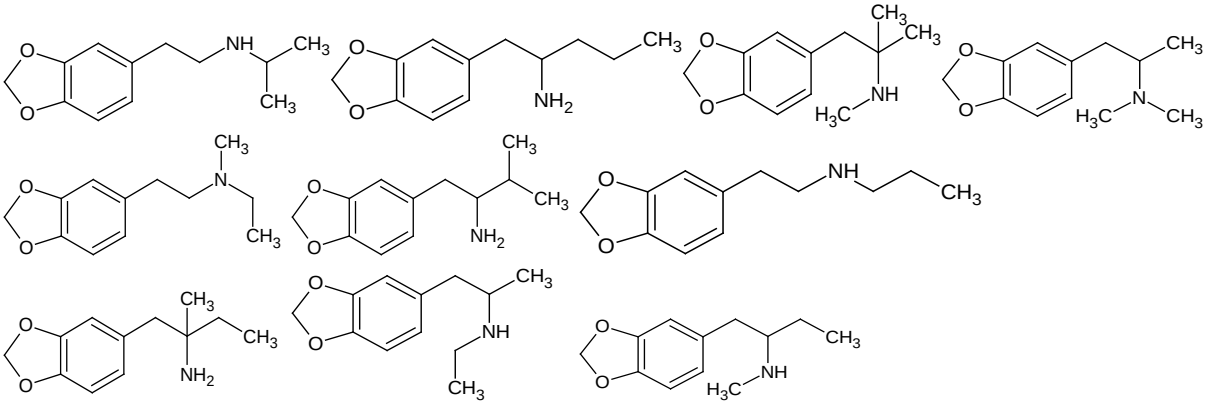
3,4- methylenedioxy-N-ethylamphetamine



3,4-methylenedioxy-N, N-dimethylamphetamine



N-methyl-1-(3,4- methylenedioxyphenyl)-2-butanamine



Instruments
Gas Chromatography
Mass Spectroscopy

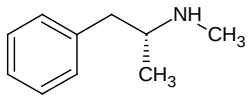
58. Ely, R.A. L–METHAMPHETAMINE AND NON–RACEMIC MIXTURES OF D– AND L–METHAMPHETAMINE IDENTIFIED IN 'ICE' METHAMPHETAMINE SAMPLES. Clandestine Laboratory Investigating Chemists Association. Volume 13, Issue 3, July 2003, Pages 6-7.

The DEA Western Laboratory (San Francisco, CA) recently received a 3.3 gram sample of clear crystalline material purchased in an undercover capacity as "ICE" methamphetamine. "ICE" methamphetamine has, in the past, been high-purity d-methamphetamine hydrochloride and is ingested by smoking. This particular sample, however, was identified as l-methamphetamine hydrochloride with a purity of 99% by weight. No synthetic route information was developed during the analyses [Figure 1]. The laboratory later received a 2670 gram submission obtained from the same defendant, packaged in six ziplock plastic bags that were further sealed in vacuum-heat sealed plastic bags. The crystalline material was clear, and the individual crystals

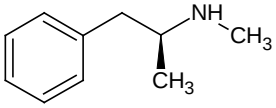
were large [Photo 1]. Each bag was screened using the Marquis color test and by gas chromatography with flame ionization detection (GC–FID) of the N–trifluoroacetyl–L–prolyl chloride (l–TPC) derivative to determine the isomeric form. Four of the bags were found to contain mixtures of d-methamphetamine hydrochloride and l–methamphetamine hydrochloride, with a higher concentration of the d–isomer [Figure 2]. The other two bags were found to have a similar mixture, except that the concentration of the l–isomer was greater [Figure 3]. The quantitative analysis of the composite sample from all 6 bags determined the purity to be 96% by weight.

Compounds of Interest:

L–methamphetamine



D–methamphetamine



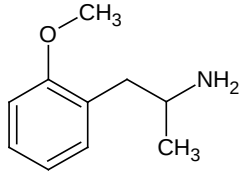
Instrument Used:

Gas chromatography with flame ionization detection (GC–FID)

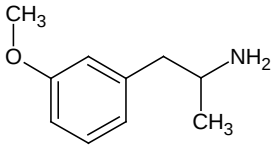
59. Liu, J., Sun, M., and Tsai, Y. Regioisomeric differentiation of mono-methoxy ring-substituted amphetamine and methamphetamine by GC-MS. Forensic Science Journal. 2 (2003) 59-68.

The 2-, 3-, 4-methoxyamphetamibe and methoxyamphetamines were prepared and analyzed by GC-MS. Regioisomerism at the aromatic ring in these compounds possess similar analytical properties, They show similar gas chromatographic retention properties on a column with phenylmethylsilicone (HP-5) stationary phase. The mass spectra for the underivatized amines are similar and fail to provide sufficient information to differentiate the ring regioisomers. Preparation of the pentafluorpropionylamide derivatives provides adequate GC resolution and distinct mass spectra that can be used to differentiate these regioisomeric amines.

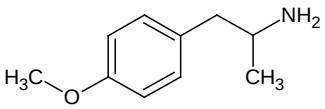
<u>Compounds</u>



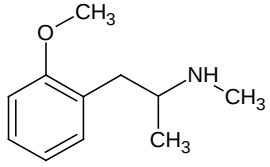
2-methoxyamphetamine

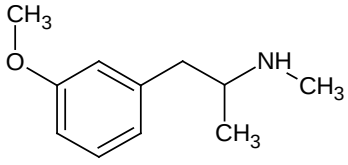


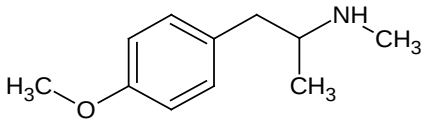
3-methoxyamphetamine



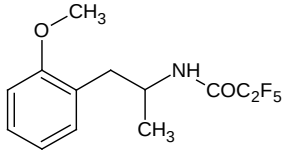
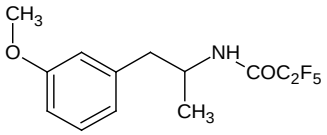
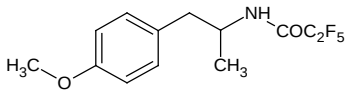
4-methoxyamphetamine



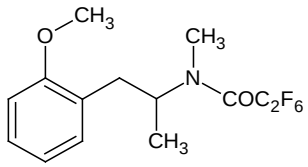
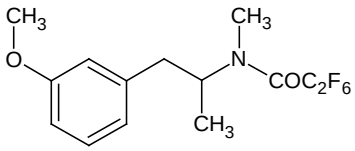




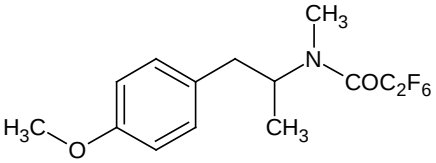
2-methoxymethamphetamine 3-methoxymethamphetamine 4-methoxymethamphetamine

2-methoxyamphetamine-PFPA 3-methoxyamphetamine-PFPA 4-methoxyamphetamine-PFPA

2-methoxymethamphetamine-PFPA 3-methoxymethamphetamine-PFPA



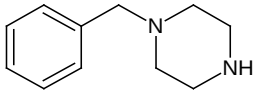
4-methoxymethamphetamine-PFPA

Instruments
Gas Chromatography
Mass Spectroscopy

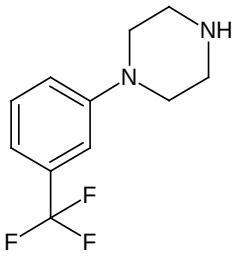
60. Inoue, H. et al. Analysis of Benzylpiperazine- like compounds. Japanese Journal of Science and Technology for Identification. 9 (2004) 165-184. DOI: 10.3408/jasti.9.165.

1-Benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine, newly controlled as narcotics in Japan on 2003, and their analogues were analyzed. The analytical data with color test, thin layer chromatography (TLC), infrared spectroscopy (IR), gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) are presented. The BZP-like compounds were less sensitive to Simon's reagent than amphetamine type stimulants on spot plates. Using on-site screening kit based on Simon's test (X-Checker®), BZP indicated almost the same result as methamphetamine. For TLC, the solvent system, methanol −25% aqueous ammonia (100 : 1.5), was the best among the systems examined. Iodoplatinate reagent was the most sensitive one to detect BZP. The IR spectra showed sufficient differences to make identification. Trimethylsilylation was the most appropriate choice for the GC/MS analysis of BZP-like compounds in terms of the peak shapes, separation and stability (using a J&W DB-5MS column). In LC/MS analysis, the gradient elution (10 mM formic acid and acetonitrile) using a Waters Symmetry Shield C18 column achieved discrimination of isomers except for 1-(2-fluorophenyl)piperazine and 1-(4-fluorophenyl)piperazine. The cone voltage of 30 V was recommended for the LC/MS screening. The information would be useful for identification of piperazines in confiscated powders, liquids or tablets.

Compounds



BZP



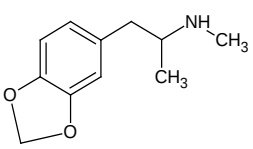
1-(3-trifluoromethylphenyl)piperazine

Instruments
Thin Layer Chromatography
Gas Chromatography
Infrared Spectroscopy
Mass Spectrometry

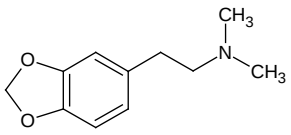
61. Aalberg, L., DeRuiter, J., and Clark, C. R. Chromatographic and Mass Spectral Studies on Isobaric and Isomeric Substances Related to 3,4-Methylenedioxymethamphetamine. Journal of Chromatographic Science. 42 (2004) 464-469. DOI: 10.1093/chromsci/42.9.464.

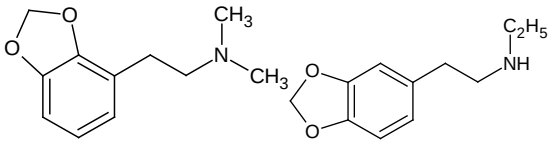
A series of isobaric and isomeric molecules related to 3,4-methylenedioxymethamphetamine (3,4-MDMA) are prepared and evaluated as potential mass spectral equivalents to this controlled substance. These compounds have the potential to produce a mass spectrum equivalent to 3,4-MDMA, thus making mass spectrometry a nonconclusive method for confirming the identity of any one of the substances. The various isomeric forms of the methoxymethylphenethylamines and the methoxymethcathinones have mass spectra essentially equivalent to 3,4-MDMA, but the ethoxy substituted phenethylamines show a unique fragment at m/z 107. Gas chromatographic separation on nonpolar stationary phases successfully resolved these compounds from 3,4-MDMA, however only a limited set of side chain regioisomers and ring substitution patterns are evaluated in this initial study

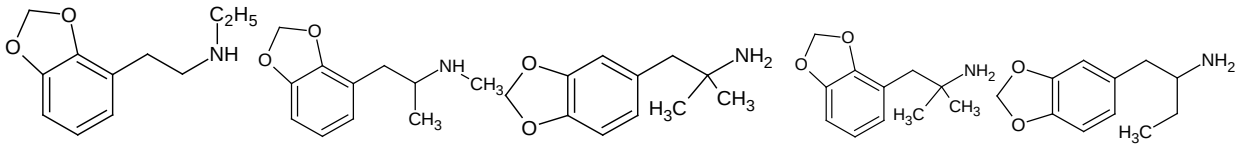
Compounds



3,4-MDMA





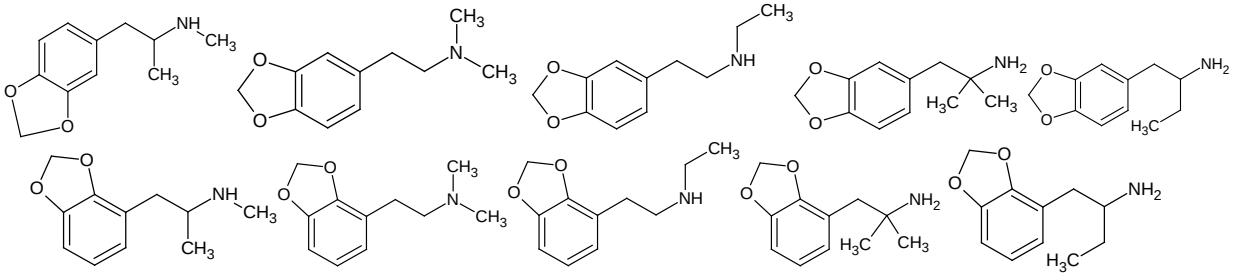


Instruments
Gas Chromtography
Mass Spectroscopy

62. Aalberg, L., DeRuiter, J., Sippola E., and Clark, C. R. Gas Chromatographic Optimization Studies on the Side Chain and Ring Regioisomers of Methylenedioxymethamphetamine. Journal of Chromatographic Science. 42 (2004) 293-298. DOI: 10.1093/chromsci/42.6.293.

Gas chromatographic (GC) optimization studies are conducted for the 10 methylenedioxyphenethylamine regioisomeric substances related to the drug of abuse 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy). These 10 compounds, having the same molecular weight and equivalent major mass spectral fragments, are not completely resolved using typical GC mass spectrometry screening methods for illicit drugs. MDMA coelutes with at least one nondrug regioisomer under standard drug screening conditions. Separation of the 10 regioisomers is studies using stationary phases of varying polarities. Resolution optimization shows that very slow program rates give the best separation for the nonpolar stationary phases, requiring analysis times of as much as 85 min. Narrow-bore columns containing the same nonpolar stationary phases improve the analysis time to approximately 29 min. The polar stationary phase DB-35MS allows high-temperature programming rates, yielding complete resolution of all 10 compounds in less than 7 min. Temperature program optimization studies on the DB-35MS phase allow the separation time to be reduced to approximately 4.5 min.

Compounds

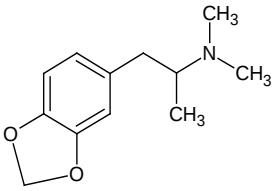


Instruments
Gas Chromatography
Mass Spectrometry

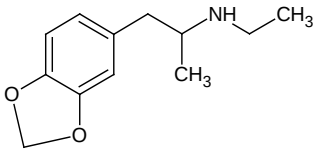
63. Casteele, S.R., Bouche, M.P., and Van Bocxlaer, J.F. LC-MS/MS in the elucidation of an isomer of the recreational drug methylenedioxy ethylamphetamine: methylenedioxy dimethylamphetamine. Journal of Separation Science. 28 (2005) 1729-1734. DOI: 10.1002/jssc.200500108.

This paper describes the surplus value of a quadrupole-orthogonal acceleration TOF mass spectrometer, coupled to a liquid chromatographic separation system, for the unequivocal identification and structural elucidation of an unknown compound in the field of designer drugs. In a patient sample set (blood, tissues, vitreous humor, etc.), analyzed with a dedicated liquid chromatographic–fluorescence detection method for the determination of methylenedioxy amphetamine, methylenedioxy methamphetamine, and methylenedioxy ethylamphetamine (MDEA), a “strange” inexplicable peak appeared at a retention time not corresponding to any of our reference materials. Based on the identical excitation and emission wavelengths in detection, and a retention behavior comparable to MDEA, it was assumed that this unknown compound was an isomer of the recreational drug MDEA. With a simple and straightforward methodological crossover between LC fluorescence detection and LC-MS/MS, additional information for structural elucidation was easily obtained. Chromatographic separation was achieved on a Hypersil BDS C18 column (fluorescence detection part) and on a Hypersil BDS phenyl column (mass spectrometric detection part). MS showed that the unknown compound's molecular mass was identical to that of MDEA, and, in addition, its fragmentation pattern too proved quite similar to that of MDEA. A thorough literature overview and study of the fragmentation pattern by means of the MS/MS spectrum led to an evidence-based hypothesis of 3,4-methylenedioxy N,N-dimethylamphetamine (MDDM) being the unknown compound. To confirm this hypothesis, MDDM was synthesized and its presence in our biological sample was finally demonstrated by co-injection with alternatively synthesized MDDM and MDEA. This application shows the synergism between LC and MS in the elucidation of unknown compounds, nevertheless emphasizing the essence of chromatographic separation when dealing with isomers

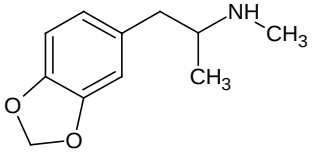
Compounds



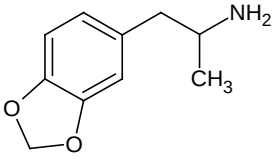
MDDM



MDEA



MDMA



MDA

Instruments

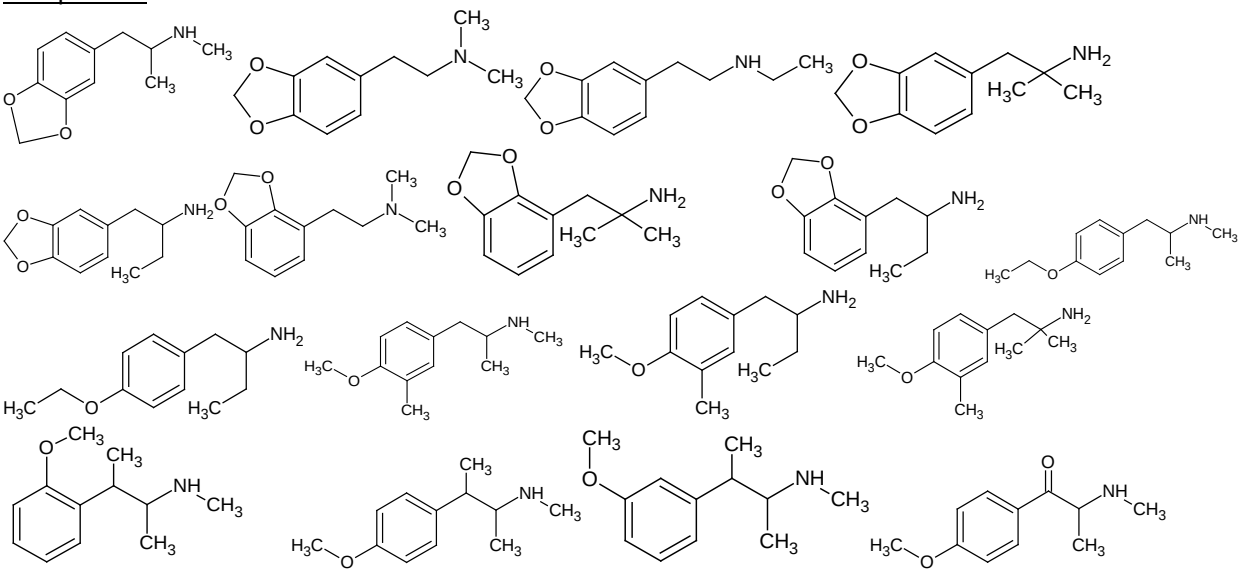
Liquid Chromatography

Mass Spectrometry

64. Pihlainen, K., Aalberg, L., Tepponen, M., Clark, C. R., and R. Kostiainen. The Identification of 3,4-MDMA from Its Mass Equivalent Isomers and Isobaric Substances Using Fast LC–ESI-MS–MS. Journal of Chromatographic Science. 43 (2005) 92-97. DOI: 10.1093/chromsci/43.2.92.

3,4-Methylenedioxymethamphetamine (3,4-MDMA, “Ecstacy”) and its 17 isomers and isobaric substances are studied using liquid chromatography (LC)-positive electrospray ionization-mass spectrometry (MS). 3,4-MDMA is a controlled substance, whereas in many countries the other studied isobaric compounds are not. A method for confirmation of the presence of 3,4-MDMA in drug seizures is developed and validated. Using single MS, the compounds produce an intense protonated molecule and some characteristic fragments; but tandem MS (MS-MS) is applied to enhance specificity. The MS-MS fragmentation is studied in order to distinguish 3,4-MDMA from the other 17 related compounds. However, the MS-MS spectra of 3,4-MDMA and six related compounds are very similar. Therefore, the LC-MS-MS method is developed for the unambiguous identification of 3,4-MDMA. The use of a monolithic column allows for 5-min gradient runs. This qualitative method is tested with 49 Ecstacy samples seized by the police. All results are congruent with the ones obtained with other methods.

Compounds



Instruments
Liquid Chromatography
Mass Spectrometry
MS-MS

65. Thevis, M., and Schänzer, W. Mass Spectrometric Analysis of Androstan-17β-ol-3-one and Androstadiene-17β-ol-3-one Isomers. Journal of the American Society for Mass Spectrometry. 16 (2005) 1660-1669. DOI: 10.1016/j.jasms.2005.06.007.

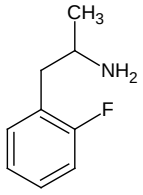
Mass spectrometric identification and characterization of steroids using electrospray ionization and tandem mass spectrometry has advantages in drug testing and doping control analysis attributable to limitations of gas chromatography followed by electron ionization mass spectrometry. Steroids with an

androstadiene-17β-ol-3-one nucleus and double bonds located either at C-1 and C-4, C-4 and C-9, or C-4 and C-6 were used to determine characteristic fragmentation pathways. Diagnostic dissociation routes are proposed using deuterium labeling, MS^3 experiments, and analyses of structurally closely related compounds. Steroids such as boldenone (androst-1,4-diene-17β-ol-3-one) produced characteristic product ions at m/z 121, 135, and 147. Compounds with double bonds at C-4 and C-9 generated abundant product ions at m/z 145 and 147. Conjugated double bonds at C-4 and C-6 gave rise to an intense and characteristic signal at m/z 133. Stereochemical differentiation between 5α- and 5β-isomers of androstan-17β-ol-3-ones was possible because of significant differences in relative abundance of product ions generated by elimination of acetone from α,β-saturated 3-keto steroids.

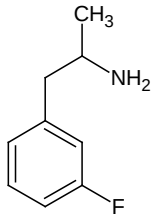
Instruments
Gas chromatography
Mass Spectrometry

66. Rösner, P., Quednow, B., Girreser, U., and Junge, Th. Isomeric Fluoro-methoxy-phenylalkylamines: a new series of controlled-substance analogues (designer drugs). For. Sci. Int. 148 (2005) 143-156. DOI: 10.1016/j.forsciint.2004.05.003.

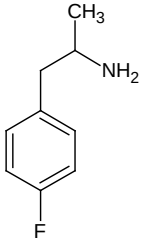
An impressively large number of clandestinely produced controlled-substance analogues (designer drugs) of amphetamine with high structural variety have been encountered in forensic samples in recent years. The continuous designer drug exploration and their widespread consumption results in an increasing number of reports regarding abuse and intoxication. This study presents the analytical properties of a series of new fluoro-methoxy-substituted controlled-substance analogues of amphetamine. Three ring positional isomeric fluoroamphetamines, two isomeric fluoromethoxyamphetmaines, two N-alkyl 4-fluoroamphetamines, and one 4-fluorophenylbutan-2-amine were identified and differentiated by gas-chromatography-mass spectrometry (GC-MS), ^1H- and ^{13}C-nuclear magnetic resonance (NMR), and gas chromatography-infrared spectroscopy (GC-IR).



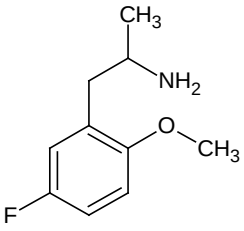
1-(2-fluorophenyl) propan-2-amine



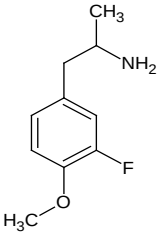
1-(3-fluorophenyl) propan-2-amine



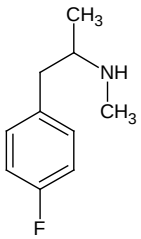
1-(4-fluorophenyl)propan-2-amine



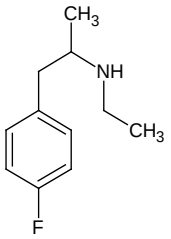
1-(5-fluoro-2-methoxyphenyl) propan-2-amine



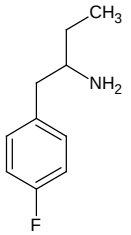
1-(3-fluoro-4-methoxyphenyl) propan-2-amine



1-(4-fluorophenyl)-*N*-methylpropan-2-amine



N-ethyl-1-(4-fluorophenyl)propan-2-amine



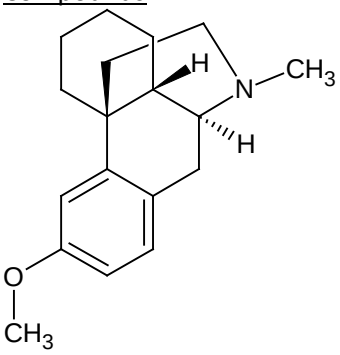
1-(4-fluorophenyl)butan-2-amine

Instruments
Gas Chromatography
Mass Spectroscopy
Nuclear Magnetic Resonance
Infrared Spectroscopy

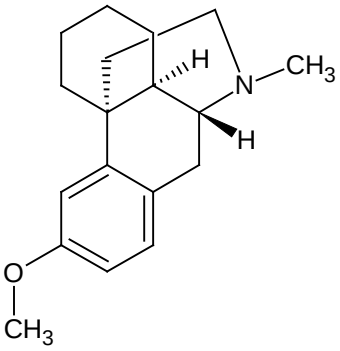
67. Lurie, I. S. and Cox, K. A. Rapid Chiral Separation of Dextro and Levo- Methorphan using Capillary Electrophoresis with Dynamically Coated Capillaries. Microgram Journal. 3 (2005) 138-141.

Dextromethorphan is an antitussive agent commonly found in Over-the-Counter (OTC) cough and cold pharmaceuticals (and more recently in Ecstasy (MDMA) mimic or combination tablets). Levomethorphan is a narcotic analgesic that is not commercially available, and therefore is not commonly submitted to forensic laboratories. Nonetheless, the differentiation and identification of these enantiomers is important in the United States, since Dextromethorphan is not controlled while Levomethorphan is a Schedule II controlled substance. However, the differentiation of Dextro- and Levo- Methorphan is challenging.

Compounds



Dextromethorphan

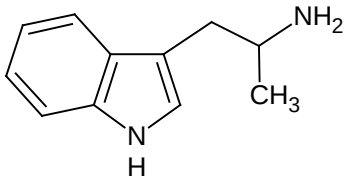


Levomethorphan

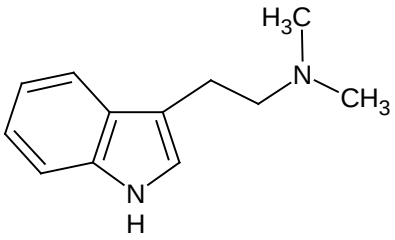
Instrument
Capillary Electrophoresis System

68. Rodriguez-Cruz, S. E. Analysis and Characterization of Designer Tryptamines using Electrospray Ionization Mass Spectrometry (ESI-MS). Microgram Journal. 3 (2005) 107-129.

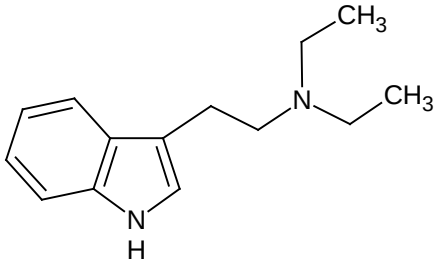
The analysis and characterization of 12 “designer” tryptamines by electrospray ionization mass spectrometry (ESI-MS) are presented. Molecular weights were confirmed based on the experimental observation of protonated and deprotonated pseudo-molecular ions in the positive and negative ion modes, respectively. Standard tandem mass spectrometry (MS^2) experiments were also performed, and the results provided for the characterization of various fragmentation signatures, useful for the future analysis of currently unknown, similar compounds. The fragmentation spectra obtained from collision-induced dissociation (CID) experiments (35 eV) were also compiled as part of an in-house mass spectral library. Results from selected MS^3 experiments are presented and their use in structural elucidation is discussed. For comparison, the gas chromatography/mass spectrometry (GC/MS) data for the tryptamines are also included and discussed.



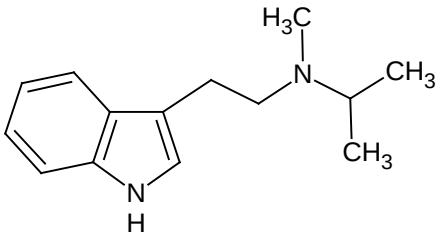
alpha-Methyltryptamine



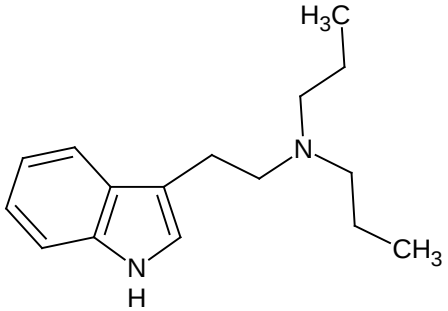
N,N-Dimethyltryptamine



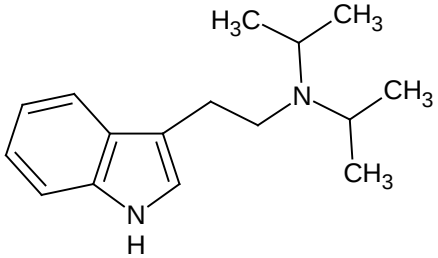
N,N-Diethyltryptamine



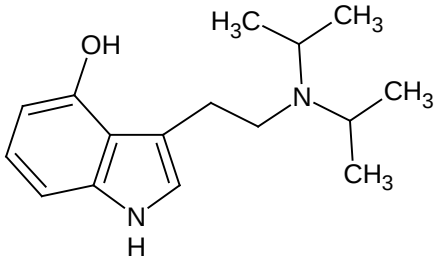
N,N-Methylisopropyltryptamine



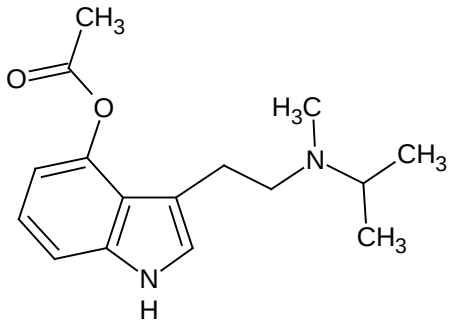
N,N-Dipropyltryptamine



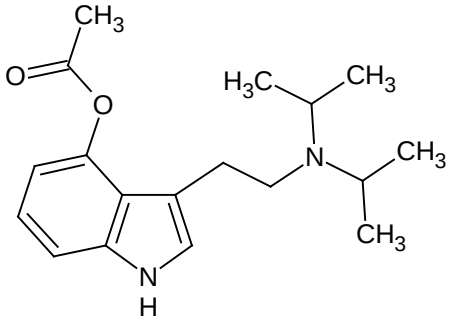
N,N-Diisopropyltryptamine



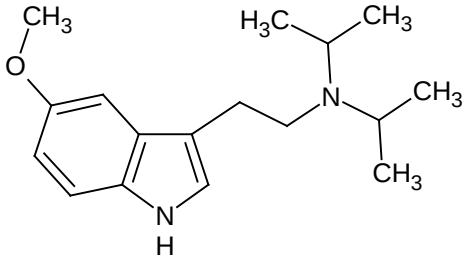
4-Hydroxy-*N,N*-diisopropyltryptamine



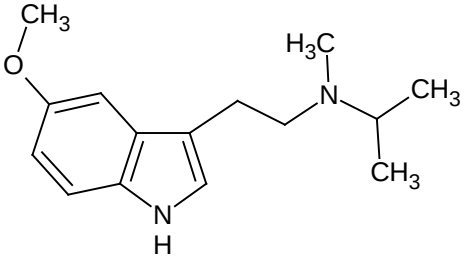
4-Acetoxy-*N,N*-methylisopropyltryptamine



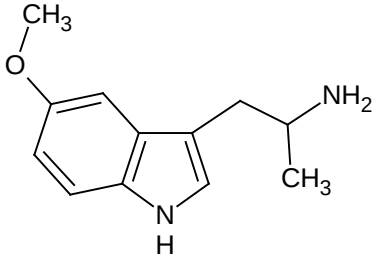
4-Acetoxy-*N,N*-diisopropyltryptamine



5-Methoxy-*N,N*-diisopropyltryptamine



5-Methoxy-*N,N*-methylisopropyltryptamine



5-Methoxy-*alpha*-methyltryptamine

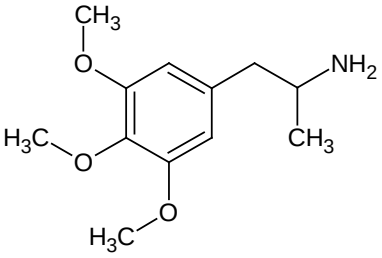
Instrument
EI-MS

69. Tsujikawa, K. et. al. Analytical Profiles for 3,4,5-, 2,4,5-, and 2,4,6-Trimethoxyamphetamine. Microgram Journal. 4 (2006) 12-23.

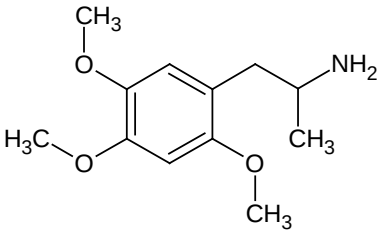
Analytical profiles (Marquis color testing, infrared spectroscopy, nuclear magnetic resonance, thin layer chromatography, high-performance liquid chromatography, and gas chromatography/mass spectrometry) are presented for 3,4,5-trimethoxyamphetamine, 2,4,5-trimethoxyamphetamine, and 2,4,6-trimethoxyamphetamine. The data allows identification and differentiation of these positional isomers.

Compounds

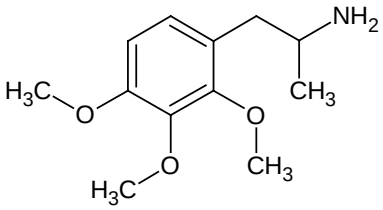
3,4,5-TMA



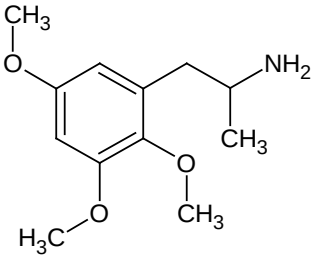
2,4,5-TMA



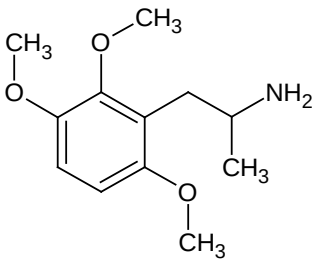
2,3,4-TMA



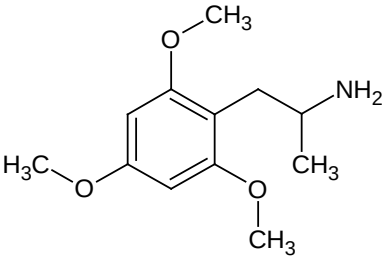
2,3,5-TMA



2,3,6-TMA



2,4,6-TMA

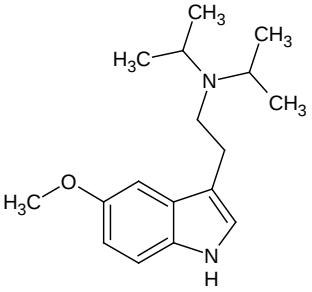


Instruments
IR
GC/MS
NMR
TLC

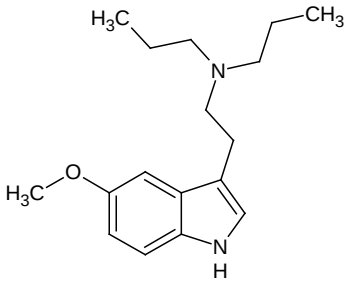
70. Doi, K., Miyazawa, M., Fuji H., Kojima, T. Analysis of the chemical drugs among structural isomer. Journal of the Pharmaceutical Society of Japan. 9 (2006) 815-823. DOI: 10.1248/yakushi.126.815.

Drugs that have a pharmacological effect similar to legal drugs such as narcotics and stimulants are available in the market and widely used. 5-methoxy-N,N-di-iso-propyl-tryptamine (5MeO-DIPT) and alpha-methyl-tryptamine (AMT) were categorized as narcotics and were specified as legal drugs in April 2005, and also 2,5-dimethoxy-4-n-propylthiophenethylamine (4C-T-7) and N-methyl-alpha-ethyl-3,4-methylenedioxy-phenethylamine (MBDB) were categorized as narcotics and were specified as legal drugs in April 2006, in Japan. We are analyzing these chemical drugs by investigating the market research. It is recognized that during the analysis of chemical drugs, drugs that resemble a structural isomer of a target substance, such as 5MeO-DIPT and 5-methoxy-N,N-di-n-propyl-tryptamine (5MeO-DPT) or 4C-T-7 and 2,5-dimethoxy-4-iso-propylthiophenethylamine (4C-T-4), should be distinguished. The results of TLC, IR, GC-MS and HPLC analyses were compared. 5MeO-DIPT and 5MeO-DPT could be distinguished by TLC and HPLC analyses, but not by IR and GC-MS analysis. The drugs 4-hydroxy-N-methyl-N-iso-propyl-tryptamine (4HO-MIPT) and 4-hydroxy-N,N-di-ethyl-tryptamine (4HO-DET) or could not be distinguished. Moreover, the isomers of 4-hydroxy-N-methyl-N-n-propyl-tryptamine (4HO-MPT) was not found to be present. Thus, we have demonstrated that the chemical drug could be distinguished from each other, and we have also shown that NMR data is essential for the analysis.

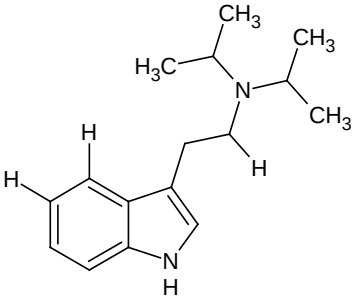
Compounds



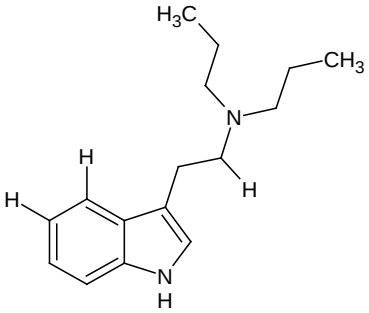
5MeO-DIPT



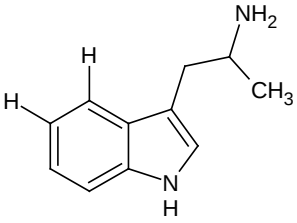
5MeO-DPT



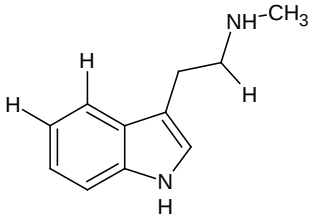
DIPT



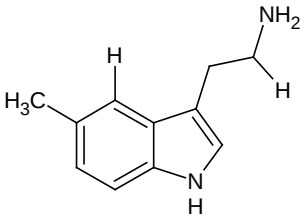
DPT



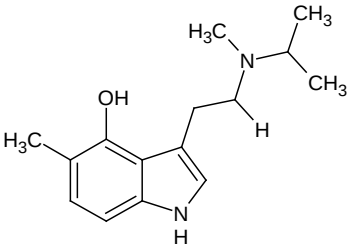
AMT



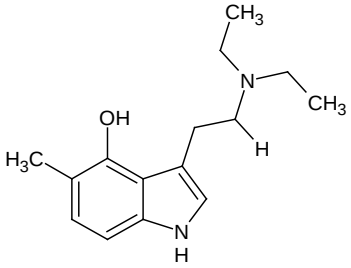
NMT



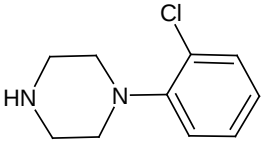
5MT



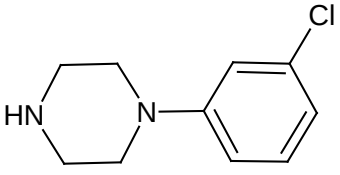
4HO-MIPT



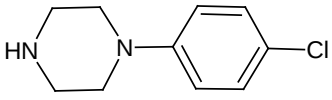
4HO-DET



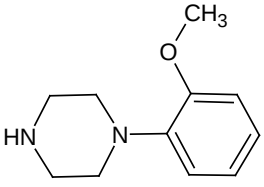
2CPP



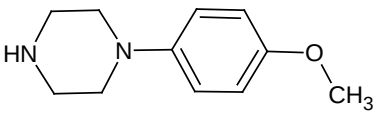
3CPP



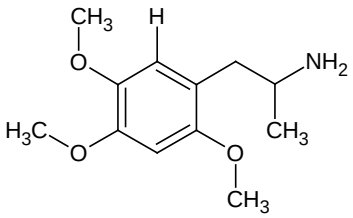
4CPP



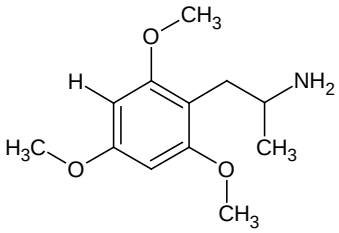
2MPP



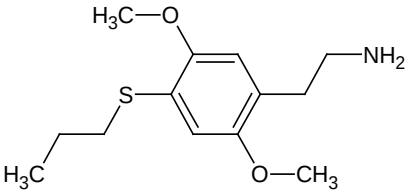
4MPP



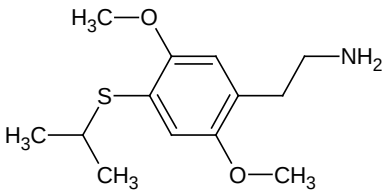
TMA-2



TMA-6



2C-T-7



2C-T-4

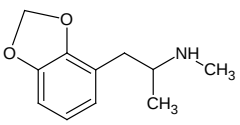
Instruments

Gas Chromatography
High-Performance Liquid Chromatography
Mass Spectroscopy
Infrared Spectroscopy
Nuclear Magnetic Resonance

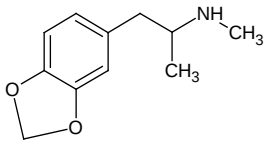
71. Awad, T., Clark, C. R., and DeRuiter, J. Chromatographic and Mass Spectral Studies on Methoxymethcathinones Related to 3,4-Methylenedioxymethamphetamine. Journal of Chromatographic Science. 44 (2006) 155-161. DOI: 10.1093/chromsci/44.3.155.

The methoxymethcathinones are uniquely regioisomeric with the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA) or Ecstacy. The various isomeric forms of the methoxymethcathinones have mass spectra essentially equivalent to 3,4-MDMA. They all have a molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136. Differentiation by mass spectrometry was only possible after formation of the perfluoroacyl derivatives, pentafluoropropionylamides (PFPA), and heptafluorobutrylamides (HFBA). Gas chromatographic separation on nonpolar stationary phases successfully resolved the three methcathinones from 2,3- and 3,4-MDMA as the PFPA and HFBA derivatives

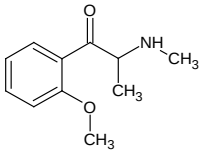
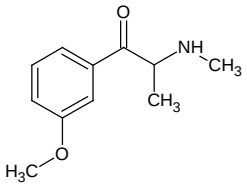
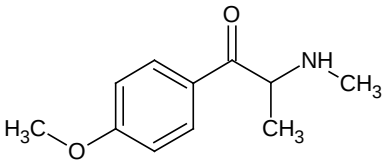
Compounds



2,3-MDMA



3,4-MDMA

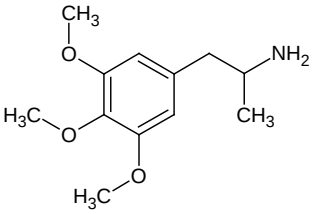




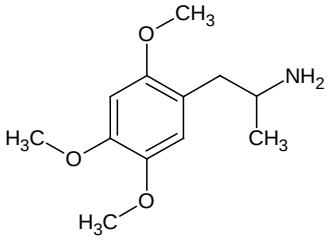
Instruments
Gas Chromatography
Mass Spectrometry

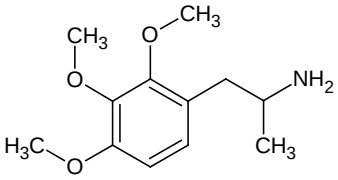
72. Zaitsu, K. et al. Discrimination and identification of the six aromatic positional isomers of trimethoxyamphetamine (TMA) by gas chromatography-mass spectrometry (GC-MS). Journal of Mass Spectrometry. 43 (2007) 528-534. DOI: 10.1002/jms.1347.

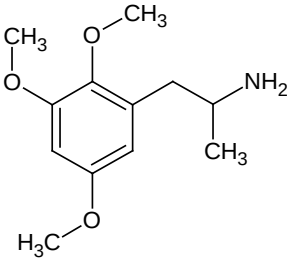
A reliable and accurate GC-MS method was developed that allows both mass spectrometric and chromatographic discrimination of the six aromatic positional isomers of trimethoxyamphetamine (TMA). Regardless of the trifluoroacetyl (TFA) derivatization, chromatographic separation of all the investigated isomers was achieved by using DB-5ms capillary columns (30 m × 0.32 mm i.d.), with run times less than 15 min. However, the mass spectra of the nonderivatized TMAs, except 2,4,6-trimethoxyamphetmine (TMA-6), showed insufficient difference for unambiguous discrimination. On the other hand, the mass spectra of the TFA derivatives of the six isomers exhibited fragments with significant intensity differences, which allowed the unequivocal identification of all the aromatic positional isomers investigated in the present study. This GC-MS technique in combination with TFA derivatization, therefore, is a powerful method to discriminate these isomers, especially useful to distinguish the currently controlled 3,4,5-trimethoxyamphetmine (TMA-1) and 2,4,5-trimethoxyamphetmine (TMA-2) from other uncontrolled TMAs

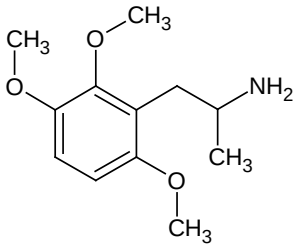
Compounds

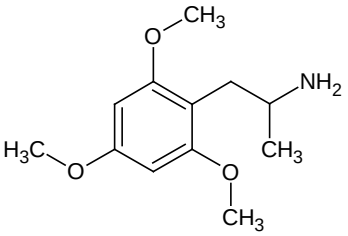

TMA-1


TMA-2


TMA-3


TMA-4


TMA-5

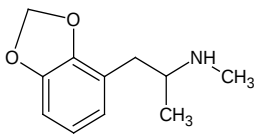

TMA-6

Instruments
Gas Chromatography
Mass Spectroscopy
Nuclear Magnetic Resonance

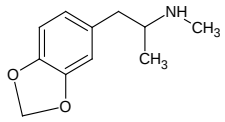
73. Awad, T. , Clark, C. R., and DeRuiter, J. GC-MS Analysis of Acylated Derivatives of the Side-Chain Regioisomers of 4-Methoxy-3-Methyl-Phenethylamines Related to Methylenedioxymethamphetamine. Journal of Chromatographic Science. 45 (2007) 477-485. DOI: 10.1093/chromsci/45.8.477.

The five side-chain regioisomers of 4-methoxy-3-methylphenethylamine constitute a unique set of compounds having an isobaric relationship with the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA or Ecstasy). These isomeric forms of the 4-methoxy-3-methylphenethylamines have mass spectra essentially equivalent to 3,4-MDMA, and all have a molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136. Mass spectral differentiation of 2,3- and 3,4-MDMA from primary and secondary amine regioisomeric side chains of 4-methoxy-3-methylphenethylamines was possible after formation of the perfluoroacyl derivatives, pentafluoropropionamides and heptafluorobutyrylamides. The mass spectra for these derivatives are significantly individualized, and the resulting unique fragment ions allow for specific side-chain identification. The individualization is the result of fragmentation of the alkyl carbon-nitrogen bond, which yielded unique hydrocarbon fragments. The heptafluorobutyrylamide derivatives offer more fragment ions for molecular individualization among these regioisomeric substances. Gas chromatographic separation on relatively non-polar stationary phases successfully resolves these derivatives

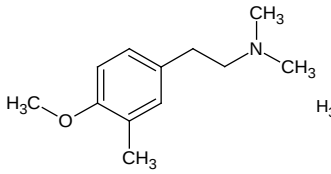
Compounds

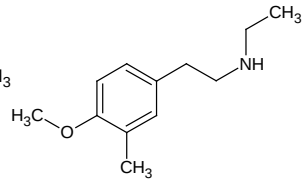


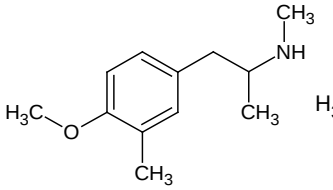
2,3-MDMA

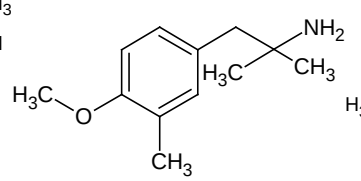


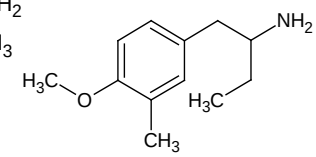
3,4-MDMA









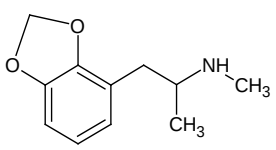


Instruments
Gas Chromatography
Mass Spectrometry

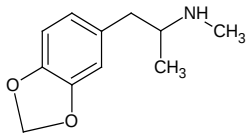
74. Awad, T., DeRuiter, J., and Clark, C. R. Chromatographic and Mass Spectral Studies on Methoxy Methyl Methamphetamines Related to 3,4-Methylenedioxymethamphetamine. Journal of Chromatographic Science. 45 (2007) 466-476. DOI: 10.1093/chromsci/45.8.466.

The methoxy methyl methamphetamines are a unique set of compounds having an isobaric relationship with the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA or Ecstasy). The various isomeric forms of the methoxy methyl methamphetamines have mass spectra essentially equivalent to 3,4-MDMA, all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136. Mass spectral differentiation of 3,4-MDMA from some of the methoxy methyl methamphetamines was possible after formation of the perfluoroacyl derivatives, pentafluoropropionamides (PFPA) and heptafluorobutyramides (HFBA). Perfluoroacyl derivatization provided unique and characteristic mass spectral fragment ions when the methoxy group is substituted at the 2- or 4-position of the aromatic ring relative to the alkylamine side chain group. Perfluoroacyl derivatization did not offer any characteristic ions for discrimination of 3,4-MDMA from the 3-methoxy ring substituted methyl methamphetamines. Gas chromatographic separation on non-polar stationary phases successfully resolved subsets of the methoxy methyl methamphetamines, based on ring position of the methoxy group, from 2,3- and 3,4-MDMA as the PFPA and HFBA derivatives

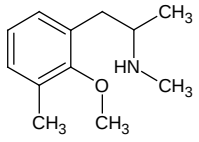
Compounds

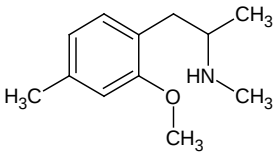


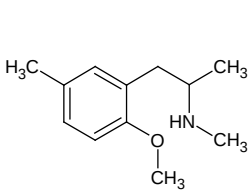
2,3-MDMA

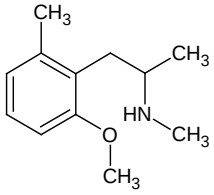


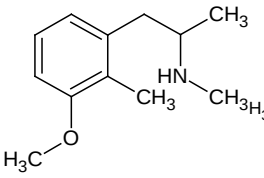
3,4-MDMA

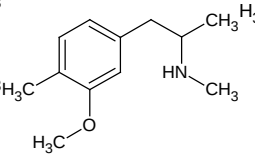


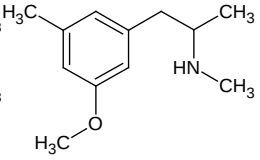


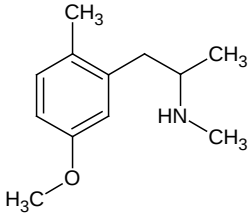


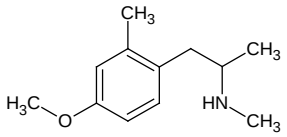


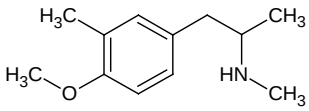










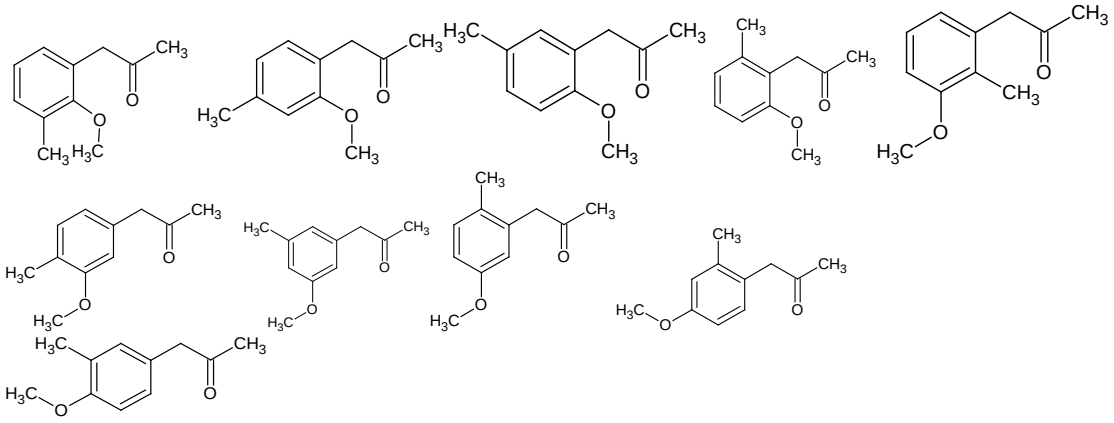


Instruments
Gas Chromatography
Mass Spectrometry

75. Awad, T., DeRuiter, J., and Clark, C. R. Gas Chromatography–Mass Spectrometry Analysis of Regioisomeric Ring Substituted Methoxy Methyl Phenylacetones. Journal of Chromatographic Science. 45 (2007) 458-465. DOI: 10.1093/chromsci/45.8.458

The methoxy methyl phenylacetones share an isobaric relationship (equivalent mass but different elemental composition) to the controlled precursor substance 3,4-methylenedioxyphenylacetone. The 10 methoxy methyl phenylacetones as well as the methylenedioxyphenylacetones show essentially equivalent mass spectra with major fragment ions at m/z 135 and 43. Those methoxy methyl phenylacetones with the methoxy group substituted ortho to the benzylic cation in the m/z 135 ion show a further fragmentation to lose formaldehyde (CH_2O) and yield a significant ion at m/z 105. The loss of formaldehyde from the ortho methoxy benzyl cation was confirmed using commercially available regioisomeric 2-, 3-, and 4-methoxyphenylacetones. The 10 regioisomeric methoxy methyl phenylacetones were prepared from the appropriately substituted benzaldehydes. Complete gas chromatographic resolution of all ten regioisomeric ketones was obtained on a stationary phase containing modified β-cyclodextrin. Using the cyclodextrin containing phase, the ortho methoxysubstituted ketones (K1-K4) eluted before the meta-methoxysubstituted ketones (K5-K8) and the para-methoxy-substituted ketones (K9-K10) showed the greatest affinity for the stationary liquid phase and eluted last. Complete separation of the 10 ketones was not obtained on Rtx-1 and Rtx-200 columns.

Compounds



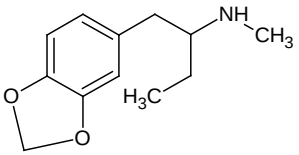
Instruments
Gas Chromatography
Mass Spectroscopy

76. Thigpen, A.L., DeRuiter, J., and Clark, C.R. GC–MS Studies on the Regioisomeric 2,3- and 3,4-Methylenedioxyphenethylamines Related to MDEA, MDMMA, and MBDB. Journal of Chromatographic Science. 45 (2007) 229-235. DOI: 10.1093/chromsci/45.5.229.

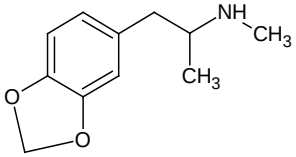
Three regioisomeric 3,4-methylenedioxyphenethylamines having the same molecular weight and major mass spectral fragments of equal mass have been reported as drugs of abuse in forensic studies in recent years. These compounds are 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxy-N-N-dimethylamphetamine (MDMMA), and N-ethyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB). The mass spectra of the regioisomers (2,3-methylenedioxyphenethylamines) are essentially equal to the three compounds reported as drugs of abuse. This paper reports the synthesis, mass spectral characterization, and chromatographic analysis of these six regioisomeric amines. The six regioisomeric methylenedioxyphenethylamines are synthesized from commercially available starting

materials. The electron impact mass spectra of these regioisomers show some variation in the relative intensity of the major ions with only a couple of minor ions that may indicate side chain specific fragments. Differentiation by mass spectrometry is only possible after the formation of the perfluoroacyl derivatives, pentafluoropropionylamides (PFPA) and heptafluorobutrylamides (HFBA). Gas chromatographic separation on non-polar stationary phases (Rtx-1 and Rtx-5) is not successful at resolving the three 3,4-methylenedioxyphenethylamines from the three 2,3-methylenedioxyphenethylamines as the underivatized amines. The six underivatized amines are resolved on the more polar trifluoropropylmethyl polysiloxane Rtx-200 stationary phase as well as a permethylated beta-cyclodextran Rtx-βDEX stationary phase. Gas chromatographic separation is successful at resolving the four PFPA and the four HFBA derivatives on the Rtx-200 stationary phase as well as the permethylated beta-cyclodextran stationary phase. The 2,3-methylenedioxyphenethylamine derivatives (compounds 4 and 6) eluted before the 3,4- methylenedioxyphenethylamine derivatives (compounds 1 and 3) as both the PFPA and HFBA derivatives

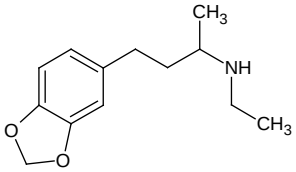
Compounds



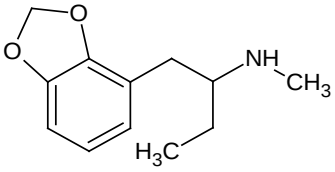
3,4-MBDB



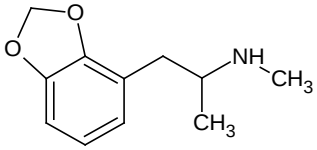
3,4-MDMA



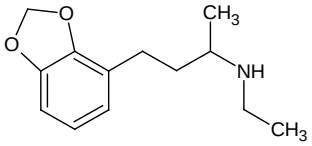
3,4-MDEA



2,3-MBDB



2,3-MDMA



2,3-MDEA

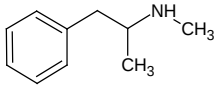
Instruments
Gas Chromatography
Mass Spectroscopy

77. Sachs, S. B. et al. A Detailed Mechanistic Fragmentation Analysis of Methamphetamine and Select Regioisomers by GC/MS. Journal of Forensic Sciences. 58 (2007) 308-319. DOI: 10.1111/j.1556-4029.2007.00401.x.

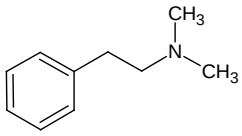
A novel ring-substituted methamphetamine regioisomer, N,α,4-trimethyl phenmethylamine, was synthesized in order to study the validity of proposed structures for various mass spectrometry (MS)-derived peaks in a methamphetamine fragmentation pattern. While other research efforts have studied aspects of methamphetamine in detail, a full fragmentation study has not been reported previously. In addition to showing molecular structures represented by fragment peaks, mechanisms for selected processes are detailed. An empirically derived procedure to easily determine by simple spectral peak pattern recognition the geometry of dimethyl- or ethyl-substituted immonium ions ($RRC=N^{+}RR$) where m/z=58 is outlined. These results are platform independent for electron ionization (EI) instruments, but have also proven to be helpful in explaining spectral peaks observed in spectra from ion trap systems. The spectrum for the synthesized methamphetamine regioisomer was accurately predicted using this methodology. While this approach is useful in some casework, the converse may be more useful: when

an unexpected or unusual peak pattern arises in a spectrum, being able to analyze it to determine the structure of the molecule. This paper gives an analyst the means to begin such retro-synthetic analyses

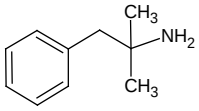
Compounds



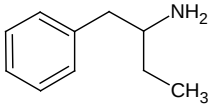
Methamphetamine



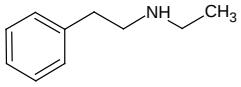
N, N-dimethyl phenethylamine



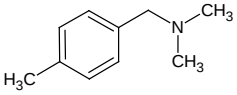
Phentermine



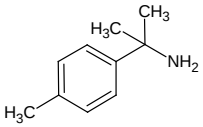
α-ethyl phenethylamine



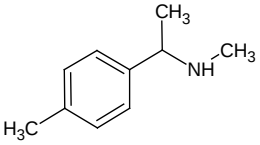
N-ethyl phenethylamine



N,N, 4-trimethyl phemethylamine



α,α, 4-trimethylphemethylamine



N,α,4- trimethyl phenmethyamine

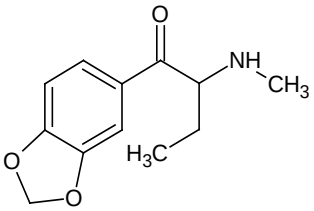
Instruments
Gas Chromatography
Mass Spectroscopy
Nuclear Magnetic Resonance

78. Zaitsu, K. et al. Discrimination and Identification of Regioisomeric B-Keto Analogues of 3,4-methylenedioxyamphetamines by Gas Chromatography-Mass Spectrometry. Forensic Toxicol. 26 (2008) 45-51. DOI: 10.1007/s11419-008-0050-1.

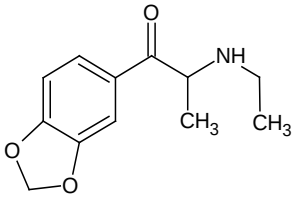
Very recently, β-keto derivatives of 3,4-methylenedioxyamphetamines (MDAs) have appeared on the illicit drug market. In the present study, we synthesized three isomers of β-keto derivatives of MDAs, 2-methylamino-1-(3,4-methylenedioxyphenyl)butan-1-one (bk-MBDB), 2-ethylamino-1-(3,4-methylenedioxyphenyl) propan-1-one (bk-MDEA), and 2-dimethylamino-1-(3,4-methylenedioxyphenyl)propan-1-one (bk-MDDMA), and measured their electron ionization mass spectra without and with trifluoroacetyl (TFA) derivatization using gas chromatography-mass spectrometry (GC-MS). Although the spectral profiles of the three isomers were very similar to each other in both the free and TFA-derivatized forms, there were characteristic peaks at m/z 44 and 140, for bk-MDEA without and with TFA derivatization, respectively; a peak at m/z 110 for bk-MBDB-TFA was also characteristic. These peaks are useful for discrimination of an isomer from others. All isomers could be well separated in both free and TFA-derivatized forms using a slightly polar fused-silica capillary GC column DB-5MS. The present data are likely to be very useful for actual identification and quantitation

of β-keto analogues of MDAs by GC-MS, because abuse of these materials is expected to spread worldwide in the near future

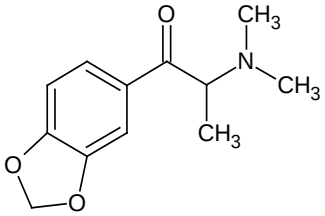
Compounds



bk-MDBD



bk-MDEA



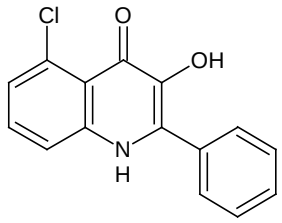
bk-MDDMA

Instruments
Gas Chromatography
Mass Spectroscopy

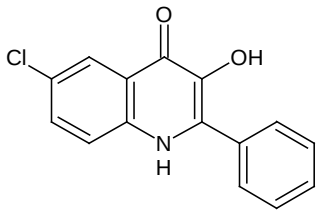
79. Grepi, M. Ionization and fragmentation of monochloro-isomers of 3-hydroxy-2-phenyl-4(1*H*)-quinolinone. Rapid Communication in Mass Spectrometry. 18(2008)2905-2914. DOI: 10.1002/rcm.3690.

Electron ionization (EI), methane chemical ionization (CI), and collision-induced dissociation (CID) mass spectra of complete series of positional monochloro-isomers of 3-hydroxy-2-phenyl-4(1*H*)-quinolinone are evaluated and discussed. EI, CI and CID spectra of the positional isomers show essentially the same fragmentation pathways but comparisons of the relative signal intensities of various product ions reveal some positional effects. Different isomers are also distinguished. The compounds can be divided into two groups using diagnostic ions (chloro substitution of the quinolinone moiety or the phenyl ring) or identified using a created spectral database. It was demonstrated that the reproducibility of the CID spectra is fully satisfactory for isomer identification, and that the created database can be applied for comparison of spectra measured over an extended time period (1 month) or spectra obtained during the direct analysis of a reaction mixture extract. Explanation of the fragmentation of the isomers is supported by exploratory density functional theory (DFT) calculations, e.g. rationalization of the relatively higher importance of the $M^{+\cdot}$-$H^{\cdot}$-$Cl^{\cdot}$-CO fragmentation pathway during EI than during CID, and vice versa for the pathway $M^{+\cdot}$-$Cl^{\cdot}$-CO.

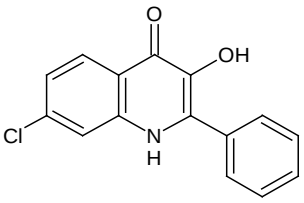
Compounds



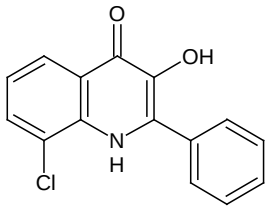
Chloro-3hydroxy-2-phenyl-4(1H)-quinoline



5-Chloro-3hydroxy-2-phenyl-4(1H)-quinoline



7-Chloro-3hydroxy-2-phenyl-4(1H)-quinoline



8-Chloro-3hydroxy-2-phenyl-4(1H)-quinoline

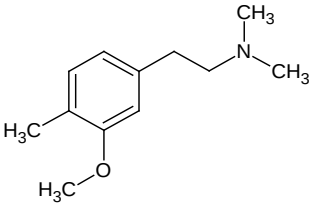
Instruments

Mass Spectroscopy

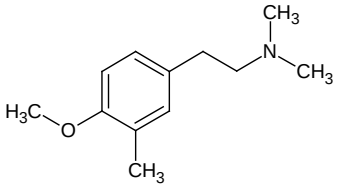
80. Awad, T., Belal, T., DeRuiter, J., and Clark, C. R. GC–MS studies on acylated derivatives of 3-methoxy-4-methyl- and 4-methoxy-3-methyl-phenethylamines: Regioisomers related to 3,4-MDMA. For. Sci. Int. 178 (2008) 61-82. DOI: 10.1016/j.forsciint.2008.02.002.

A series of side chain regioisomers of 3-methoxy-4-methyl- and 4-methoxy-4-methyl-phenethylamines have mass spectra essentially equivalent to the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA), all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136. The acetyl, propionyl and trifluoroacetyl derivatives of the primary and secondary regioisomeric amines were prepared and evaluated in GC-MS studies. The mass spectra for these derivatives were significantly individualized and the resulting unique fragment ions allowed for specific side chain identification. The trifluoroacetyl derivatives provided more fragment ions for molecular individualization among these regioisomeric substances. These trifluoroacetyl derivatives showed excellent resolution on a non-polar stationary phase such as Rtx-1

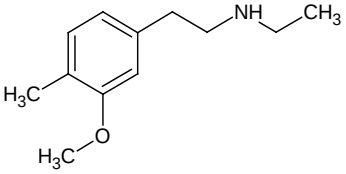
Compounds



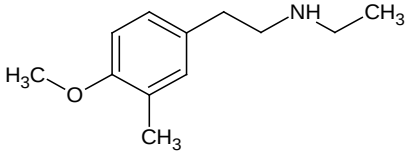
N,N-Dimethyl-3-methoxy-4-methyl-phenethylamine



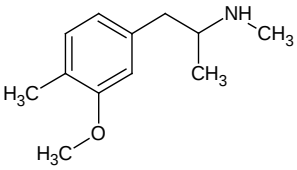
N,N-Dimethyl-4-methoxy-3-methyl-phenethylamine



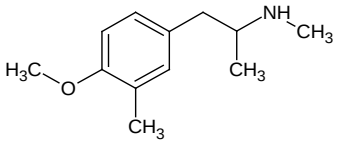
N-Ethyl-3-methoxy-4-methyl-Phenethylamine



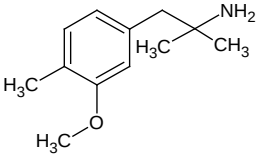
N-Ethyl-3-methoxy-4-methyl-phenthylamine



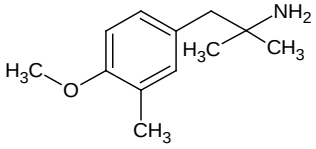
3-Methoxy-4-methylmethamphetamine



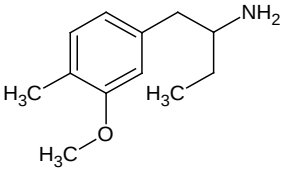
4-Methoxy-3-methylmethamphetamine



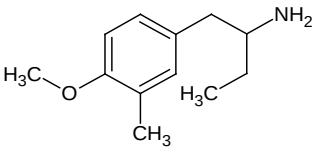
3-Methoxy-4-methylphentramine



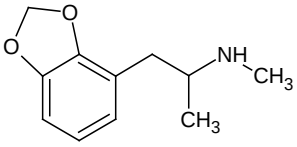
4-Methoxy-3-methylphentramine



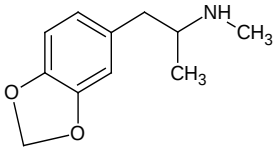
1-(3-Methoxy-4-methylphenyl)-2-Butanamine



1-(4-Methoxy-3-methylphenyl)-2-butanamine



2,3-MDMA



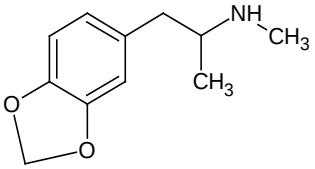
3,4-MDMA

<u>Instruments</u>
Gas Chromatography
Mass Spectroscopy

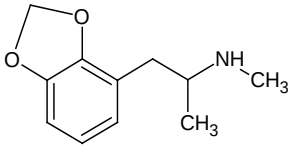
81. Awad, T., DeRuiter, J., and Clark, C. R. GC–MS Analysis of Acylated Derivatives of a Series of Side Chain Regioisomers of 2-Methoxy-4-Methyl-Phenethylamines. Journal of Chromatographic Science. 46 (2008) 375-380. DOI: 10.1093/chromsci/46.5.375.

Five side chain regioisomers of 2-methoxy-4-methylphenethylamine constitute a unique set of compounds having an isobaric relationship with the controlled drug substance 3,4-methylenedioxymethamphetamine (3,4-MDMA or Ecstasy). These isomeric forms of the 2-methoxy-4-methyl-phenethylamines have mass spectra essentially equivalent to 3,4-MDMA; all have molecular weight of 193 and major fragment ions in their electron ionization mass spectra at m/z 58 and 135/136. Mass spectral differentiation of 2,3 and 3,4-MDMA from primary and secondary amine regioisomeric side chains of 2-methoxy-4-methylphenethylamines was possible after formation of the perfluoroacyl derivatives, pentafluoropropionamides (PFPA) and heptafluorobutyrylamides (HFBA). The mass spectra for these derivatives are individualized and the resulting unique fragment ions allow for specific side-chain identification. The individualization is the result of fragmentation of the alkyl carbon-nitrogen bond yielding unique hydrocarbon fragments of varying mass. Gas chromatographic separation on relatively non-polar stationary phases gave essentially base line resolution for these compounds

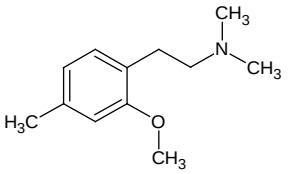
Compounds

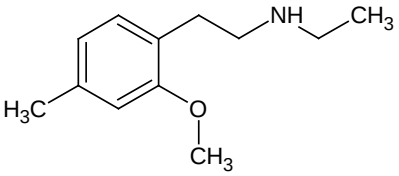


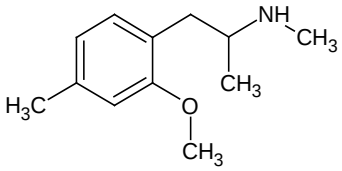
3,4-MDMA

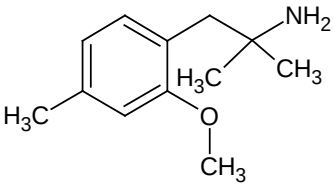


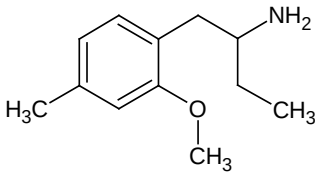
2,3-MDMA









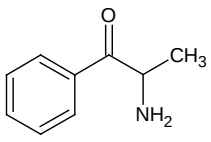


Instruments
Gas Chromatography
Mass Spectroscopy

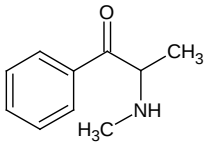
82. R.P. Archer. Fluoromethcathinone, a new substance of abuse. Forensic Science International. 185. 2009. Pages 10-20.

We have identified a new compound in capsules marketed as plant feeders available from internet suppliers. It is apparent from internet forums that these so-called plant feeders are being used as recreational drugs. The material is identified as being 30-fluoromethcathinone. The compound in the capsule was identified by GC–MS, 1H, 13C and 19F NMR as well as FTIR. Other materials identified in the tablet were caffeine and a methylamine salt. The exact position of the fluorine in the fluoromethcathinone was determined by comparison with materials synthesised in our laboratory. Internet-based companies are known to sell 40-fluoromethcathinone (flephedrone). We present GC–MS data for the three isomers of fluoromethcathinone and their N-acetyl derivatives and provide a rapid method for determining the positional isomers of fluoromethcathinone using FTIR or 19F NMR.

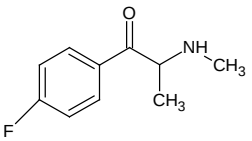
Compounds



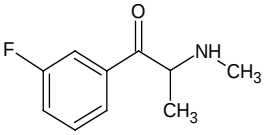
Cathinone



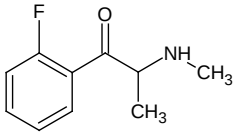
Methcathinone



4-Fluoromethcathinone



3-Fluoromethcathinone



2-Fluoromethcathinone

Instruments

Gas Chromatography

Mass Spectroscopy

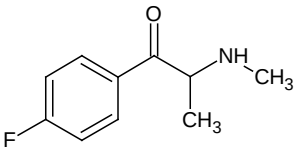
Fourier Transform Infrared Spectroscopy

Nuclear Magnetic Resonance

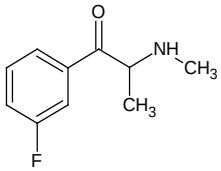
83. Archer, R. P. Fluoromethcathinone, a new substance of abuse. For. Sci. Int. 185 (2009) 10-20. DOI: 10.1016/j.forsciint.2008.11.013

We have identified a new compound in capsules marketed as plant feeders available from internet suppliers. It is apparent from internet forums that these so-called plant feeders are being used as recreational drugs. The material is identified as being 3'-fluoromethcathinone. The compound in the capsule was identified by GC-MS, 1H, (13)C and (19)F NMR as well as FTIR. Other materials identified in the tablet were caffeine and a methylamine salt. The exact position of the fluorine in the fluoromethcathinone was determined by comparison with materials synthesised in our laboratory. Internet-based companies are known to sell 4'-fluoromethcathinone (flephedrone). We present GC-MS data for the three isomers of fluoromethcathinone and their N-acetyl derivatives and provide a rapid method for determining the positional isomers of fluoromethcathinone using FTIR or (19)F NMR.

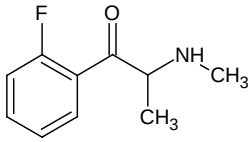
Compounds



Flephedrone



3-fluoromethcathinone



2-fluoromethcathinone

Instruments

Gas Chromatography

Mass Spectroscopy

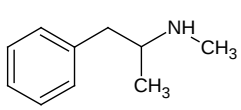
Infrared Spectroscopy

Nuclear Magnetic Resonance

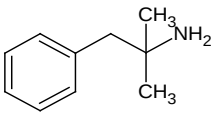
84. Awad, T., Belal, T., DeRuiter, J., Kramer, K., and Clark, C. R. Comparison of GC–MS and GC–IRD methods for the differentiation of methamphetamine and regioisomeric substances. For. Sci. Int. 185 (2009) 67-77. DOI: 10.1016/j.forsciint.2008.12.014.

Gas chromatography-mass spectrometry (GC-MS) and gas chromatography-infrared detection (GC-IRD) methods were developed and compared for the differentiation of regioisomeric phenethylamines related to methamphetamine. There are a total of five regioisomeric phenethylamines (methamphetamine and four regioisomers) that produce essentially equivalent mass spectra. This unique set of five phenethylamines having the same molecular weight and elemental composition yield major mass spectral fragments at equivalent mass. The trifluoroacetyl derivatives of the primary and secondary amines yield characteristic individual fragment ions allowing structural differentiation among these regioisomers. The vapor phase infrared spectra generated via capillary gas chromatography differentiated among these compounds without the need for derivatization. The regioisomeric phenethylamines are well resolved by GC with the elution order generally determined by the degree of molecular linearity

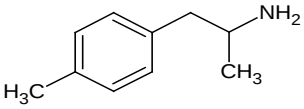
Compounds



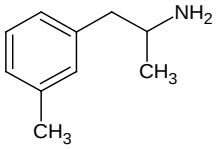
Methamphetamine



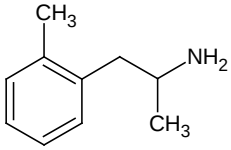
Phentermine



4-methylamphetamine



3-methylamphetamine



2-methylamphetamine

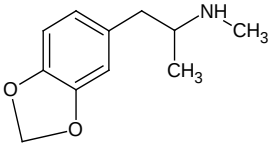
Instruments
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

85. Belal, T., Awad, T., DeRuiter, J., and Clark, C. R. GC–IRD methods for the identification of isomeric ethoxyphenethylamines and methoxymethcathinones. For. Sci. Int. 184 (2009) 54-63. DOI: 10.1016/j.forsciint.2008.12.003.

A series of 12 isomeric phenethylamines were evaluated by gas chromatography using vapor phase infrared spectrophotometric detection. The major mass spectral fragments for each of these unique isomers occur at equivalent mass and all have equal molecular weight. The infrared spectra for these compounds allow for identification of any one of these amines to the exclusion of all other isomers. This differentiation is accomplished without the need for chemical derivatization. The methoxymethcathinones show unique infrared absorption bands in the 1690-1700 cm(-1) range for the

carbonyl group and the ring substitution pattern in the ethoxymethamphetamines can be differentiated by several bands in the 700-1610 cm(-1) region. Side chain and degree of nitrogen substitution can be evaluated in the 2770-3000 cm(-1) region of the infrared range. All the studied regioisomers could be differentiated from 3,4-MDMA via their vapor phase IR spectra. Capillary gas chromatography on an Rxi-50 stationary phase successfully resolved the side chain regioisomers, the substituted methamphetamines and the methoxymethcathinones

Compounds



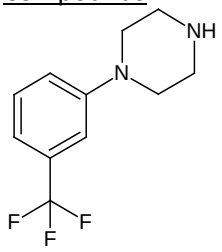
3,4-MDMA

Instruments
Gas Chromatography
Infrared Spectroscopy

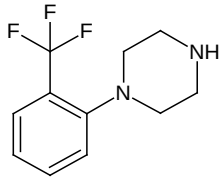
86. Maher, H. M., Awad, T., and Clark, C. R. Differentiation of the regioisomeric 2-, 3-, and 4-trifluoromethylphenylpiperazines (TFMPP) by GC–IRD and GC–MS. For. Sci. Int. 188 (2009) 31-39. DOI: 10.1016/j.forsciint.2009.03.009.

Gas chromatography with infrared detection (GC-IRD) provides direct confirmatory data for the identification of the psychoactive designer drug 3-trifluoromethylphenylpiperazine (3-TFMPP) from the regioisomeric 2- and 4-trifluoromethylphenylpiperazines. These three regioisomeric substances are well resolved by GC and the vapor phase infrared spectra clearly differentiate among the three trifluoromethylphenyl substitution patterns. However, the mass spectra for the three regioisomeric 2-, 3-, and 4-trifluoromethylphenylpiperazines are identical and do not provide structural confirmation for one of the three isomers to the exclusion of the other two compounds. Perfluoroacylation of the secondary amine nitrogen for each of the three regioisomers was conducted in an effort to individualize their mass spectra. The resulting derivatives were resolved by GC and their mass spectra showed some differences in relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation of structure

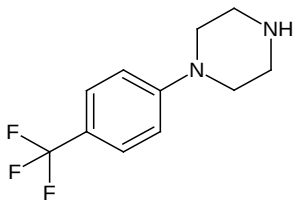
Compounds



3-TFMPP



2-TFMPP



4-TFMPP

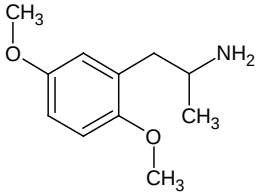
Instruments
Gas Chromatography

Mass Spectroscopy
Infrared Spectroscopy

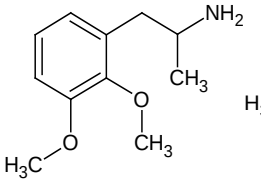
87. Maher, H. M. et al. GC–MS and GC–IRD studies on dimethoxyamphetamines (DMA): Regioisomers related to 2,5-DMA. Forensic Sci. Int. 192 (2009) 115-125. DOI: 10.1016/j.forsciint.2009.08.010.

The mass spectrum of the drug of abuse 2,5-dimethoxyamphetamine (2,5-DMA) is characterized by an imine fragment base peak at m/z 44 and additional fragments at m/z 151/152 for the dimethoxybenzyl cation and radical cation, respectively. Five positional ring isomers of dimethoxyamphetamines (DMA) have an isomeric relationship to 2,5-DMA. All six compounds have the same molecular weight and produce similar EI mass spectra. This lack of mass spectral specificity for the isomers in addition to the possibility of chromatographic coelution could result in misidentification. The lack of reference materials for the potential imposter molecules constitutes a significant analytical challenge. Perfluoroacylation of the amine group reduced the nitrogen basicity and provided individual fragmentation pathways for discrimination between these compounds based on some unique fragment ions and the relative abundance of common ions. GC–IRD studies provided additional structure–IR spectra relationships and yielded confirmation level identification for each of the six regioisomeric dimethoxyamphetamines. The amines and their perfluoroacylated derivatives were resolved by capillary gas chromatography and the amines showed excellent resolution on the more polar stationary phase, Rtx-200

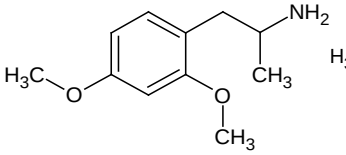
Compounds



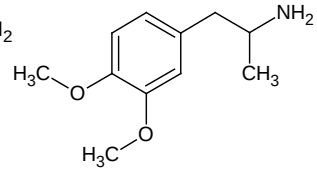
2,5-DMA



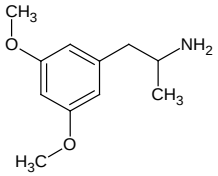
2,3-DMA



2,4-DMA



3,4-DMA

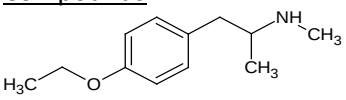


3,5-DMA

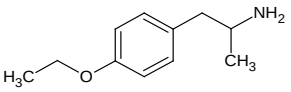
Instruments
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

88. Belal, T. et al. GC–IRD methods for the identification of isomeric ethoxyphenethylamines and methoxymethcathinones. Journal of Forensic Sciences. 184 (2009) 54-63. DOI: 10.1016/j.forsciint.2008.12.003

Compounds



Methoxymethcathinone



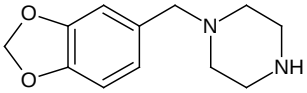
Ethoxyphenethylamine

Instruments
Gas Chromatography
Infrared Spectroscopy

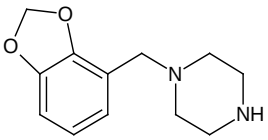
89. Abdel-Hay, K.M., Awad, T., DeRuiter, J., and Clark, C. R. Differentiation of methylenedioxybenzylpiperazines (MDBP) by GC–IRD and GC–MS. For. Sci. Int. 195 (2010) 78-85. DOI: 10.1016/j.forsciint.2009.11.016.

The substituted benzylpiperazine, 3,4-methylenedioxybenzylpiperazine (3,4-MDBP) and its regioisomer 2,3-methylenedioxybenzylpiperazine (2,3-MDBP) have almost identical mass spectra. Perfluoroacylation of the secondary amine nitrogen of these regioisomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions. However the spectra did not yield any unique fragments for specific identification of one regioisomer to the exclusion of the other compound. Gas chromatographic separation coupled with infrared detection (GC-IRD) provides direct confirmatory data for structural differentiation between the two regioisomers. The mass spectrum in combination with the vapor-phase infrared spectrum provides for specific confirmation of each of the regioisomeric piperazines. The underivatized and perfluoroacyl derivative forms of the ring substituted benzylpiperazines were resolved on a 30-m capillary column containing an Rxi-50 stationary phase

Compounds



3,4-MDBP



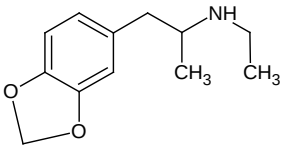
2,3-MDBP

Instruments
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

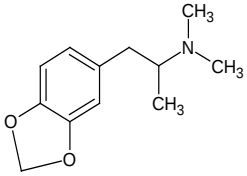
90. Al-Hossaini, A.M., Awad, T., DeRuiter, J., and Clark, C. R. GC–MS and GC–IRD analysis of ring and side chain regioisomers of ethoxyphenethylamines related to the controlled substances MDEA, MDMMA and MBDB. For. Sci. Int. 200 (2010) 73-86. DOI: 10.1016/j.forsciint.2010.03.033.

Three regioisomeric 3, 4-methylenedioxyphenethylamines having the same molecular weight and major mass spectral fragments of equal mass have been reported as drugs of abuse in recent years. These compounds are 3,4-methylenedioxy-N-ethylamphetamine (MDEA), 3,4-methylenedioxy-N,N-dimethylamphetamine (MDMMA), and N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB). Ring substituted ethoxy phenethylamines having the same side chain are compounds with an isobaric relationship to these controlled drug substances, all have molecular weight of 207 and major fragment ions in their electron ionization mass spectra at m/z 72 and 135/136. The three methylenedioxyphenethylamines were resolved from the ethoxyphenethylamines by capillary gas chromatography using an Rxi-50 stationary phase. The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of the secondary amines were evaluated in GC-MS studies. The mass spectra for these derivatives were significantly individualized and the resulting unique fragment ions allowed for specific side chain identification. The perfluoroacyl derivatives showed reasonable resolution on a non-polar stationary phase such as Rtx-1. GC-IRD studies provided structure-IR spectra relationships used for the discrimination of the three target drugs (MDEA, MDMMA and MBDB) from the other nine ring substituted ethoxyphenethylamine regioisomers

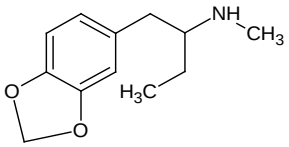
Compounds



MDEA



MDMMA



MBDB

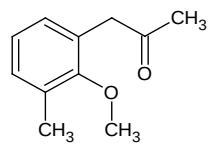
Instruments
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

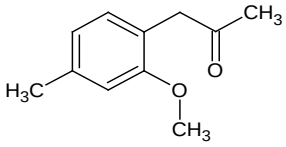
91. Awad, T., Belal, T., DeRuiter, J., and Clark, C. R. GC-IRD studies on regioisomeric ring substituted methoxy methyl phenylacetones related to 3,4-methylenedioxyphenylacetone. For. Sci. Int. 194 (2010) 39-48. DOI: 10.1016/j.forsciint.2009.10.005.

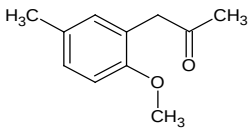
The methoxy methyl phenylacetones share an isobaric relationship (equivalent mass but different elemental composition) to the controlled precursor substance 3,4-methylenedioxyphenylacetone (3,4-methylenedioxyphenyl-2-propanone; 3,4-MDP-2-P). The ten ring substituted methoxy methyl phenylacetones are resolved by capillary gas chromatography on a modified cyclodextrin stationary phase. All ten regioisomeric ketones eluted before the controlled precursor substance 3,4-methylenedioxyphenylacetone. The vapor phase infrared spectra generated from the capillary column effluent clearly differentiated 3,4-MDP-2-P from the various methoxy methyl phenylacetones. Additionally the methoxy methyl phenylacetones provide unique individual infrared spectra. Infrared

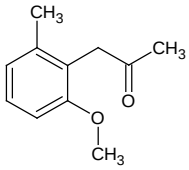
absorption frequencies and patterns confirmed the relative position of the methoxy-group and the acetone side-chain for the regioisomeric ketones

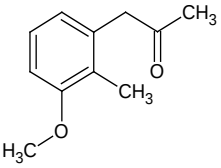
Compounds

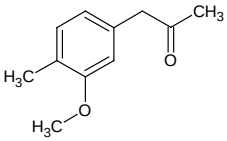

2-Methoxy-3-methyl Phenylacetone

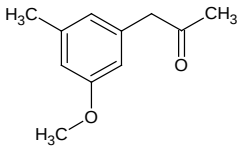

2-Methoxy-4-methyl phenylacetone

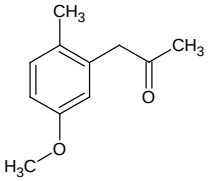

2-Methoxy-5-methyl phenylacetone

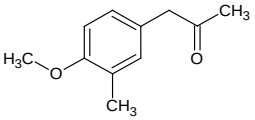

2-Methoxy-6-methyl phenylacetone

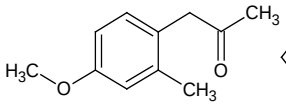

3-Methoxy-2-methyl Phenylacetone

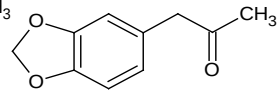

3-Methoxy-4-methyl phenylacetone


3-Methoxy-5-methyl phenylacetone


3-Methoxy-6-methyl phenylacetone


4-Methoxy-3-methyl Phenylacetone


4-Methoxy-2-methyl phenylacetone

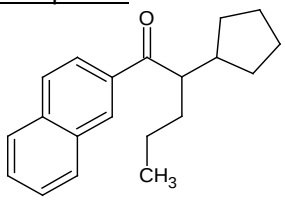

3,4-Methylenedioxyphenylacetone

Instruments
Gas Chromatography
Infrared Spectroscopy

92. Brandt, S. D., Wootton, R. C. R., De Paoli, G., and Freeman, S. The naphyrone story: The Alpha or Beta-naphthyl Isomer? Drug Testing and Analysis. 2 (2010) 496-502. DOI: 10.1002/dta.185.

Naphyrone (naphthylpyrovalerone, O-2482) has been recently advertised for purchase on a number of websites. This compound has been viewed as a so-called 'legal high' and was classified as a controlled drug under the UK Misuse of Drugs Act 1971 in mid-July 2010. So far, naphyrone is commonly equated with 1-naphthalen-2-yl-2-pyrrolidin-1-yl-pentan-1-one (β-naphyrone) but analytical characterization of two naphyrone samples revealed the existence of a novel isomer consistent with 1-naphthalen-1-yl-2-pyrrolidin-1-yl-pentan-1-one (α-naphyrone). Analyses of both α- and β-naphyrone were carried out using gas chromatography ion trap (EI/CI) mass spectrometry and 1D/2D nuclear magnetic resonance spectroscopy. This provides the first report of α-naphyrone in the scientific literature and the ability to differentiate it from the β-isomer should be of interest to forensic and clinical communities

Compound



naphyrone

93. Tyrkko, E., Pelander, A., Ojanpera, I. Differentiation of structural isomers in a target drug database by LC/Q-TOFMS using fragmentation prediction. Drug Testing and Analysis. 6 (2010) 259-270. DOI: 10.1002/dta.134.

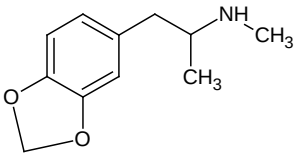
Isomers cannot be differentiated from each other solely based on accurate mass measurement of the compound. A liquid chromatography/quadrupole time-of-flight mass spectrometry (LC/Q-TOFMS) method was used to systematically fragment a large group of different isomers. Two software programs were used to characterize *in silico* mass fragmentation of compounds in order to identify characteristic fragments. The software programs employed were ACD/MS Fragmenter (ACD Labs Toronto, Canada), which uses general fragmentation rules to generate fragments based on the structure of a compound, and SmartFormula3D (Bruker Daltonics), which assigns fragments from a mass spectra and calculates the molecular formulae for the ions using accurate mass data. From an in-house toxicology database of 874 drug substances, 48 isomer groups comprising 111 compounds, for which a reference standard was available, were found. The product ion spectra were processed with the two software programs and 1–3 fragments were identified for each compound. In 82% of the cases, the fragment could be identified with both software programs. Only 10 isomer pairs could not be differentiated from each other based on their fragments. These compounds were either diastereomers or position isomers undergoing identical fragmentation. Accurate mass data could be utilized with both software programs for structural elucidation of the fragments. Mean mass accuracy and isotopic pattern match values (SigmaFit; Bruker Daltonics Bremen, Germany) were 0.9 mDa and 24.6 mSigma, respectively. The study introduces a practical approach for preliminary compound identification in a large target database by LC/Q-TOFMS without necessarily possessing reference standards.

94. Maher, H. M., Awad, T., DeRuiter, J., and Clark, C. R. GC–IRD methods for the identification of some tertiary amines related to MDMA. For. Sci. Int. 199 (2010) 18-28. DOI: 10.1016/j.forsciint.2010.02.022.

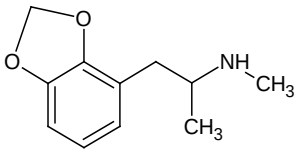
Gas chromatography with infrared detection (GC–IRD) provides direct confirmatory data for the identification of the drug of abuse; 3,4-MDMA and its regioisomer; 2,3-MDMA, from a set of seven tertiary amines which have an isobaric or regioisomeric relationship with the MDMAs. These compounds include three ring substituted regioisomers of 2-dimethylamino-1-(methoxyphenyl)ethanone, two ring regioisomers of N,N-dimethyl-2-(methoxymethylphenyl)ethanamine in addition to N,N-dimethyl-2-(2,3-

and 3,4-methylenedioxyphenyl)ethanamine. The major mass spectral fragments for each of these unique isomers occur at equivalent mass and all have equal molecular weight. Thus, gas chromatography with mass spectrometry detection (GC–MS) does not provide sufficient information for the confirmation of identity of any one of these isomers to the exclusion of the other compounds. The infrared spectra for these compounds allow for identification of any one of these amines. This differentiation is accomplished without the aid of chemical derivatization. The IR spectra served to divide the studied compounds into four groups depending on their absorption bands in the region 2700–3100 cm^{-1}. Moreover, compounds with different ring substitution pattern within each group can be differentiated by several bands in the 700–1700 cm^{-1} region. These regioisomeric substances are well resolved by GC on Rtx-1 stationary phase and the vapor-phase infrared spectra clearly differentiate among this set of compounds.

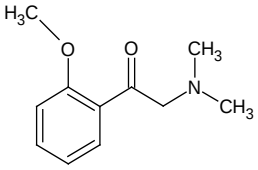
Compounds



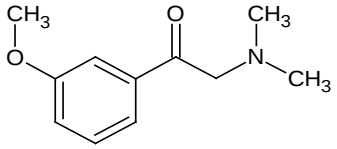
3,4-MDMA



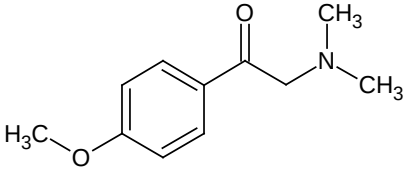
2,3-MDMA



2-dimethylamino-1-(methoxyphenyl)ethanone



2-dimethylamino-2-(methoxyphenyl)ethanone



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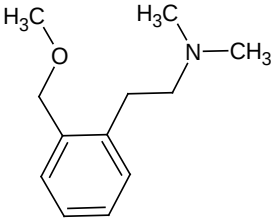
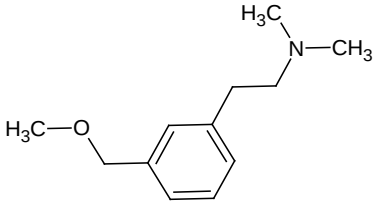
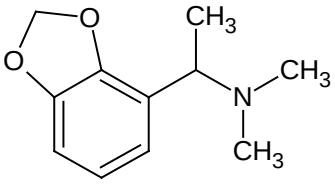


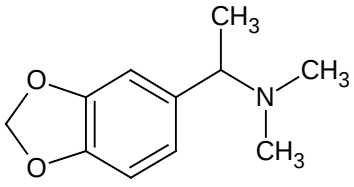
N,N-dimethyl-2-(methoxymethylphenyl) ethanamine



N,N-dimethyl-3-(methoxymethylphenyl)ethanamine



N,N-dimethyl-2- 2,3-methylenedioxyphenylethanamine



N,N-dimethyl-2- 3,4-methylenedioxyphenylethanamine

Instruments

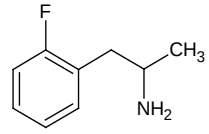
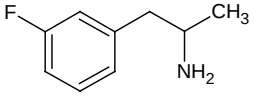
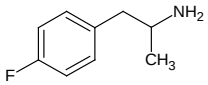
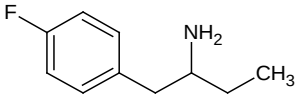
Gas Chromatography

Infrared Spectroscopy

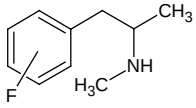
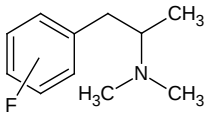
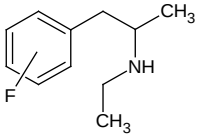
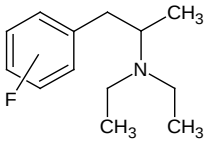
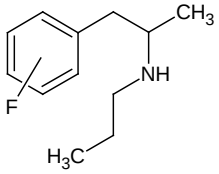
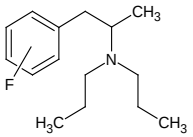
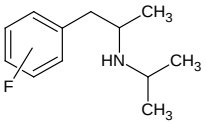
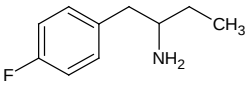
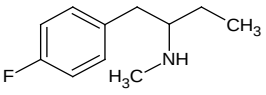
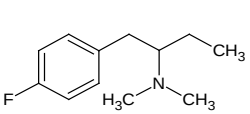
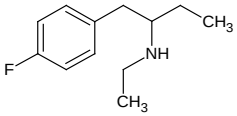
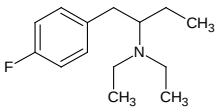
95. Westphal, F., Rosner, P., and Junge, Th. Differentiation of regioisomeric ring-substituted fluorophenethylamines with product ion spectrometry. For. Sci. Int. 194 (2010) 53-59. DOI: 10.1016/j.forsciint.2009.10.007.

The electron ionization (EI) of aromatic ring-substituted isomers gives virtual identical mass spectra which seriously affects their analysis. Especially regioisomeric meta- and para-ring-substituted compounds cannot show any ortho-effect reactions making their differentiation by mass spectrometry impossible. Furthermore o-, m- and p-substituted compounds can only be separated insufficiently by chromatography due to their very similar retention that do not allow univocal identification. Product ion mass spectrometry has proved to be a useful tool to differentiate structurally closely related fluorophenethylamines even in the case of the meta- and para-isomers. A series of N-alkylated o-, m- and p-fluoroamphetamines and 1-(4-fluorophenyl)butan-2-amines have been synthesized in microscale and studied by product ion spectrometry. The combination of chemical ionization (CI) and product ion spectrometry of hydrogen fluoride loss ions $[M+H-HF]^+$ allows a univocal differentiation of all studied fluoro-substituted phenethylamines without prior derivatization. This method with submicrogram detection limits provides great advantages for the differentiation between aromatic regioisomeric fluorophenethylamine designer drugs where other methods such as nuclear magnetic resonance (NMR) spectrometry lack sufficient sensitivity or might fail because complex mixtures have to be analyzed.

Compounds

2-fluoroamphetamine 3-fluoroamphetamine 4-fluoroamphetamine 1-(4-fluorophenyl)butan-2-amine

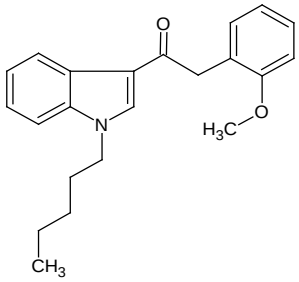
Instruments

Gas Chromatography

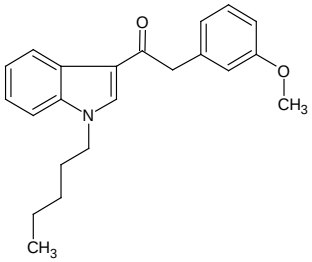
Mass Spectroscopy

96. Harris, D., Hokanson, S., and Miller, V. GC-MS Differentiation of Three Synthetic Cannabinoid Positional Isomers: JWH-250, JWH-302, and JWH-201. Journal of CLIC. 21 (2010) 23-32.

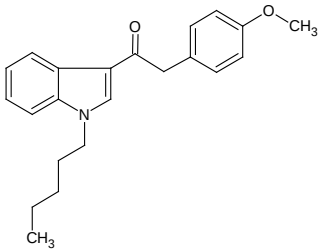
Compounds



JWH-250



JWH-302



JWH-201

Instruments

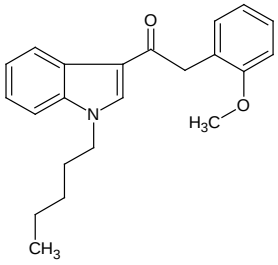
Gas Chromatography

Mass Spectroscopy

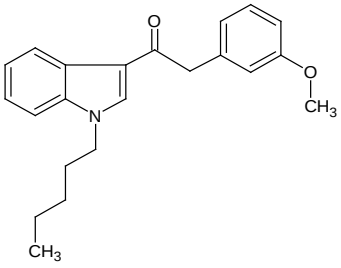
97. Spangler, M. R., and Benne, A. Synthetic Cannabinoid Isomer Differentiation. Journal of CLIC. 21 (2011) 17-22

The positional isomers JWH–250, JWH–302 and JWH–201 and the chain isomers JWH–018, JWH–018 N–(2–methylbutyl) isomer and JWH–018 N–(3–methylbutyl) isomer were analyzed by gas chromatography–mass spectrometry to determine if differentiation of the individual isomers was possible. It was determined that although JWH–250, JWH–302 and JWH–201 have nearly identical mass spectra, they all have different retention times and could therefore be distinguished from one another. It was also determined that although JWH–018 N–(2–methylbutyl) isomer and JWH–018 N–(3–methylbutyl) isomer have very similar retention times, they have different mass spectra and therefore can be distinguished from one another. In addition, the methyl butyl isomers can be distinguished from JWH–018 by both retention time and mass spectra.

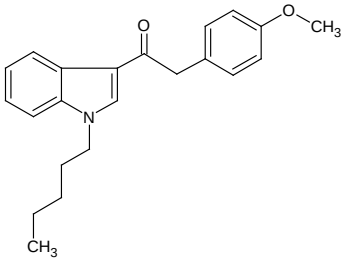
Compounds



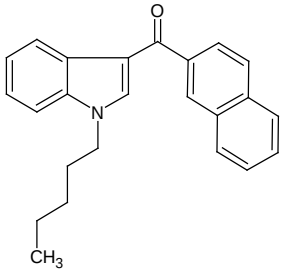
JWH-250



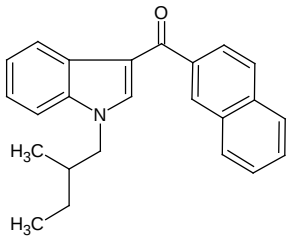
JWH–302



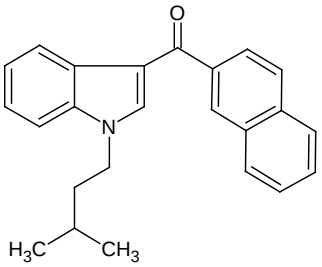
JWH–201



JWH–018



JWH–018 N–(2–methylbutyl)



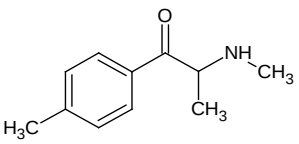
JWH–018 N–(3–methylbutyl)

Instruments
Gas Chromatography
Mass Spectroscopy

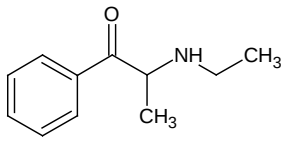
98. McDermott et al. The Analysis of Substituted Cathinones. Part 2: An Investigation into the Phenylacetone Based Isomers of 4-methylmethcathinone and N-Ethylcathinone. For. Sci. Int. 212 (2011) 13-21. DOI: 10.1016/j.forsciint.2011.06.030.

During the analysis of "seized samples", suspected of containing 4-methylmethcathinone (mephedrone) and N-ethylcathinone (ethcathinone) additional compounds were observed in the GCMS chromatogram. These compounds were suspected to be the corresponding phenylacetone isomers of mephedrone and ethcathinone respectively. These isomers are referred to as iso-mephedrone and iso-ethcathinone, respectively. The identity of these compounds was verified by synthesising the isomers from known starting materials and comparing them with the compounds found in the seized samples. Analytical data, GCMS, NMR and IR on these compounds are provided. Possible explanations for the presence of these compounds in the seized samples are explored. Contaminated starting material is one suggestion. Rearrangement of the propiophenone based product to the phenylacetone based product is also suggested. The reaction of the α-bromopropiophenone with a primary amine can also lead to the phenylacetone based product. The presence of these isomeric compounds in seized samples could be used to compare different samples and attempt to establish a common origin

Compounds



Mephedrone



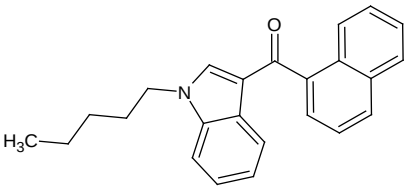
Ethcathinone

99. Spangler , M.R. Synthetic Cannabinoid Isomer Differentiation. Clandestine Laboratory Investigating Chemists Association. Volume 21, Issue 14, October 2011, Pages 17-22.

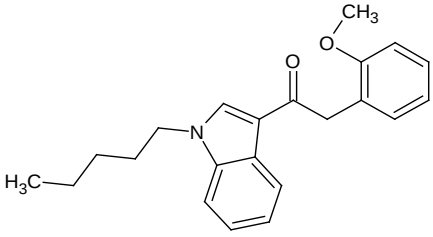
The positional isomers JWH–250, JWH–302 and JWH–201 and the chain isomers JWH–018, JWH–018 N–(2–methylbutyl) isomer and JWH–018 N–(3–methylbutyl) isomer were analyzed by gas chromatography–mass spectrometry to determine if differentiation of the individual isomers was possible. It was determined that although JWH–250, JWH–302 and JWH–201 have nearly identical mass spectra, they all have different retention times and could therefore be distinguished from one another. It was also determined that although JWH–018 N–(2–methylbutyl) isomer and JWH–018 N–(3–methylbutyl) isomer have very similar retention times, they have different mass spectra and therefore can be distinguished from one another. In addition, the methyl butyl isomers can be distinguished from JWH–018 by both retention time and mass spectra.

Compounds of Interest:

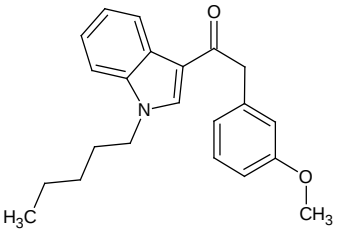
JWH–018



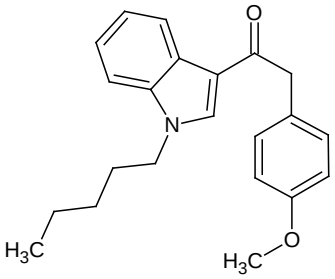
JWH–250



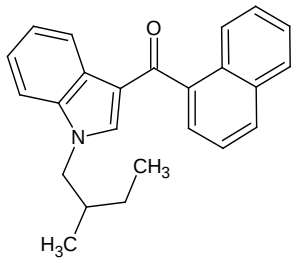
JWH–302



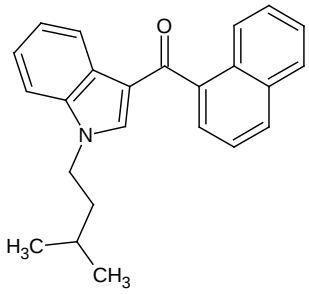
JWH–201



JWH–018 N–(2–methylbutyl) isomer



JWH–018 N–(3–methylbutyl)

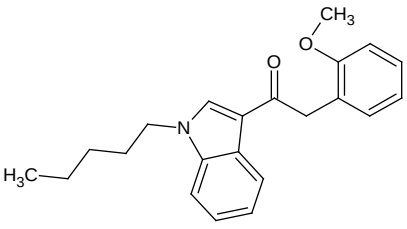


Instrument Used:
Gas chromatography–mass spectrometry (GC–MS)

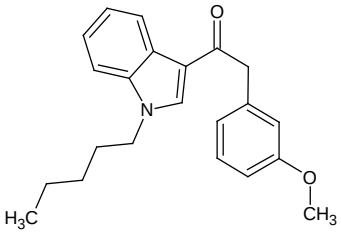
100. Harris, D. Hokanson, S. Miller, V. GC–MS Differentiation of Three Synthetic Cannabinoid Positional Isomers: JWH–250, JWH–302, and JWH–201. Clandestine Laboratory Investigating Chemists Association. Volume 21, Issue 14, October 2011, Pages 23-31.

The cannabinomimetic drug JWH–250 is found in herbal “spice” mixtures and is banned in many parts of the world. It is easy to misidentify JWH–250 with the positional isomers JWH–302 and JWH–201 using conventional gas chromatograph–mass spectrometry (GC–MS), as all three compounds have close GC retention times and nearly identical spectra. The isomers differ with ortho–, meta–, or para– position of a methoxy group. A method is presented to conclusively distinguish the three isomers by use of mass spectra fragmentation ratios using abundances of fragment m/z = 121 versus m/z = 91.

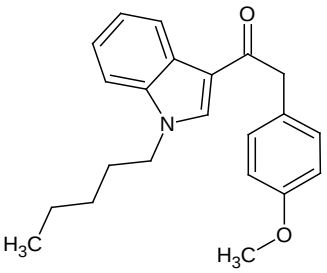
Compounds of Interest:
JWH–250



JWH–302



JWH–201



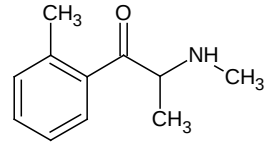
Instrument Used:

Gas chromatograph–mass spectrometry (GC–MS)

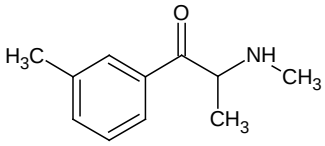
101. Power, J. D. et al. The Analysis of Substituted Cathinones. Part 1: Chemical Analysis of 2-, 3- and 4-methylmethcathinone. For. Sci. Int. 212 (2011) 6-12. DOI: 10.1016/j.forsciint.2011.04.020.

The ring substituted methyl isomers of methcathinone, 2-, 3- and 4-methylmethcathinone were analysed. The 2- and 3-isomers were synthesized. The 4-methylmethcathinone isomer is also known as mephedrone and has been widely studied. We present GCMS, NMR and IR data for the three isomers. We show that the three isomers can be separated by GCMS and that the IR spectra for the three compounds can be used to distinguish between them. A seized sample was analysed and it was found to contain 4-methylmethcathinone and benzocaine.

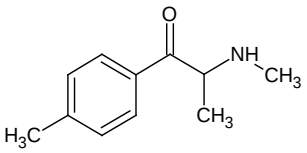
Compounds



2-methylmethcathinone



3-methylmethcathinone



4-methylmethcathinone

Instruments

Gas Chromatography

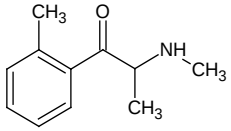
Mass Spectroscopy

Infrared Spectroscopy

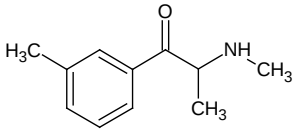
Nuclear Magnetic Resonance

102. Separation and identification of methyl-methcathinones. Provided by Kavanagh, P. (2011).

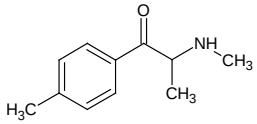
Compounds



2-methylmethcathinone



3-methylmethcathinone

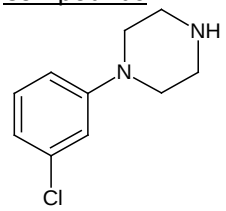


4-methylmethcathinone

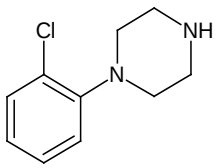
103. Romão, W. et al. Chemical profile of meta-chlorophenylpiperazine (m-CPP) in ecstasy tablets by easy ambient sonic-spray ionization, X-ray fluorescence, ion mobility mass spectrometry and NMR. Analytical and Bioanalytical Chemistry. 400 (2011) 3053-3064. DOI: 10.1007/s00216-011-4883-9.

Meta-chlorophenylpiperazine (m-CPP) is a new illicit drug that has been sold as ecstasy tablets. Easy ambient sonic-spray ionization mass spectrometry (EASI-MS) and X-ray fluorescence spectrometry (XRF) are shown to provide relatively simple and selective screening tools to distinguish m-CPP tablets from tablets containing amphetamines (mainly 3,4-methylenedioxymethamphetamine (MDMA)). EASI-MS detects the active ingredients in their protonated forms: [m-CPP + H](+) of m/z 197, [MDMA + H](+) of m/z 194, and [2MDMA + HCl + H](+) of m/z 423 and other ions from excipients directly on the tablet surface, providing distinct chemical fingerprints. XRF identifies Cl, K, Ca, Fe, and Cu as inorganic ingredients present in the m-CPP tablets. In contrast, higher Cl concentrations and a more diverse set of elements (P, Cl, Ca, Fe, Cu, Zn, Pt, V, Hf, Ti, Pt, and Zr) were found in MDMA tablets. Principal component analysis applied to XRF data arranged samples in three groups: m-CPP tablets (four samples), MDMA tablets (twenty three samples), and tablets with no active ingredients (three samples). The EASI-MS and XRF techniques were also evaluated to quantify m-CPP in ecstasy tablets, with concentrations ranging from 4 to 40 mg of m-CPP per tablets. The m-CPP could only be differentiated from its isomers (o-CPP and for the three isomers p-CPP) by traveling wave ion mobility mass spectrometry and NMR measurements.

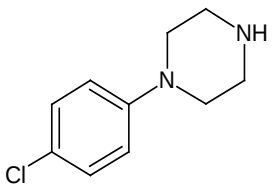
Compounds



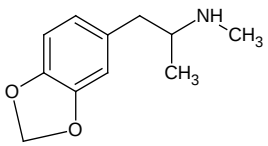
m-CPP



o-CPP



p-CPP



3,4-MDMA

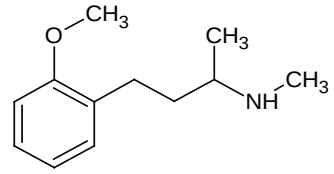
Instruments

Easy Ambient Sonic-ionization
Mass Spectroscopy

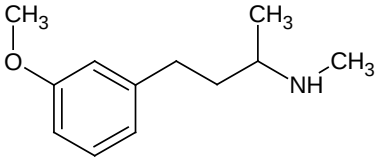
X-Ray fluorescence Spectroscopy
Nuclear Magnetic Resonance

104. Awad, T., Maher, H. M., DeRuiter, J., and Clark, C. R. GC–MS and GC–IRD Studies on the Ring Isomers of N-Methyl-2-Methoxyphenyl-3-Butanamines (MPBA) Related to 3,4-MDMA. Journal of Chromatographic Science. 49 (2011) 345-352. DOI: 10.1093/chromsci/49.5.345.

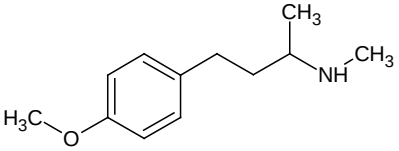
Compounds



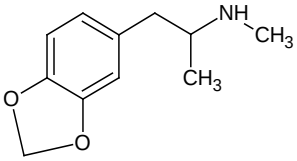
N-Methyl-2-Methoxyphenyl-3-Butanamine



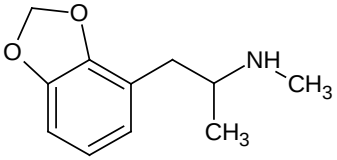
N-Methyl-3-Methoxyphenyl-3-Butanamine



N-Methyl-4-Methoxyphenyl-3-Butanamine



3,4-MDMA



2,3-MDMA

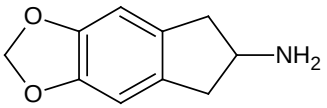
Instruments
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

105. Casale, J F., and Hays, P A. Characterization of the “Methylenedioxy-2-aminoindans”. Microgram Journal. 8 (2011) 43-52.

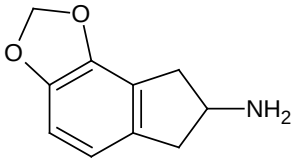
Spectroscopic and chromatographic data are provided for 5,6-(methylenedioxy)-2-aminoindan (commonly referred to as MDAI), 4,5-(methylenedioxy)-2-aminoindan (a positional isomer of MDAI), and their respective synthetic intermediates. Direct comparisons of the analytical data are made to assist forensic chemists in correctly differentiating between these isomers in illicit drug exhibits.

Compounds

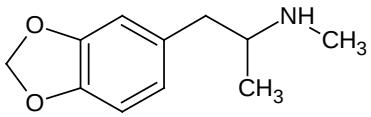
5,6-MDAI



4,5-MDAI



MDMA



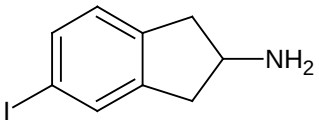
Instruments
GC/MS
FTIR
NMR

106. Casale, J F., and Hays, P A. The Characterization of 4- and 5-Iodo-2-aminoindan. Microgram Journal. 9 (2012) 18-26.

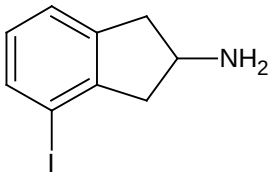
The synthesis, analysis, and characterization of 4- and 5-iodo-2-aminoindan (commonly referred to as “4-IAI” and “5-IAI”) are discussed. Analytical data (mass spectrometry, nuclear magnetic resonance spectroscopy, and infrared spectroscopy) are presented and compared.

Compounds

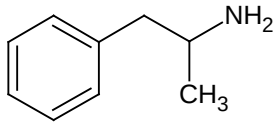
5-IAI



4-IAI



Amphetamine



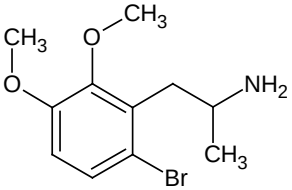
Instruments
GC/MS
NMR
FTIR

107. DeRuiter, J., Holston, P., and Clark, C. R. Liquid Chromatographic and Mass Spectral Methods of Identification for Regioisomeric Dimethoxyamphetamines and Brominated Dimethoxyamphetamines. Journal of Chromatographic Science. 4 (2012) 24-32.

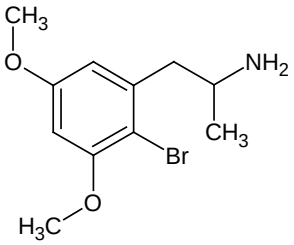
The six regioisomeric dimethoxyamphetamines are prepared from the commercially available dimethoxybenzaldehydes. The dimethoxyamphetamines show very similar mass spectra, and chromatographic methods must be used to differentiate the positional isomers. Bromination of the six isomeric dimethoxyamphetamines yields a monobromination product as the major component in all cases except for 3,5-dimethoxyamphetamine, which yields the 2,6-dibrominated species as the major product. Mass spectrometric analysis readily divides the regioisomeric bromodimethoxyamphetamines into two groups of three compounds each. Only those isomers having a bromine substituent "ortho-" to the alkylamine side-chain show a major fragment at m/z 194 from loss of bromine from the molecular

ion. The major drug of abuse 4-bromo-2,5-dimethoxyamphetamine (DOB) is one of three compounds that do not yield the m/z 194 ion. Though the mass spectra for the three "non-m/z 194" isomers show some subtle differences, these compounds are best differentiated by a reversed-phase liquid chromatographic system.

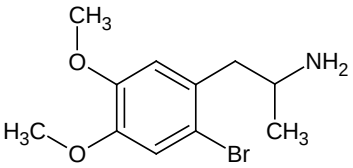
Compounds



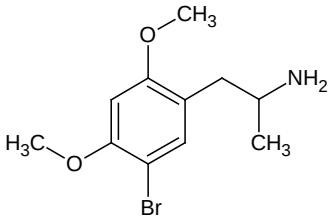
6-Bromo-2,3-dimethoxyamphetamine



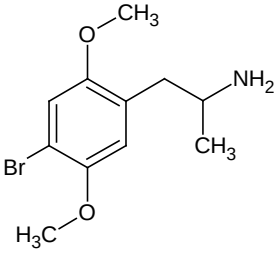
2-Bromo-3,5-dimethoxyamphetamine



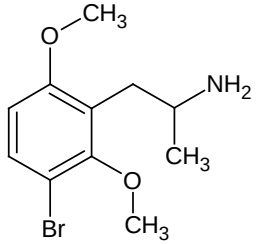
2-Bromo-4,5-dimethoxyamphetamine



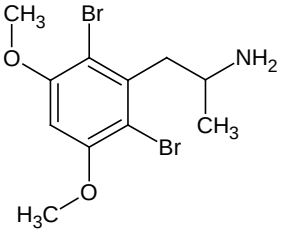
5-Bromo-2,4-dimethoxyamphetamine



4-Bromo-2,5-dimethoxyamphetamine



3-Bromo-2,6-dimethoxyamphetamine



2,6-Dibromo-3,5-dimethoxyamphetamine

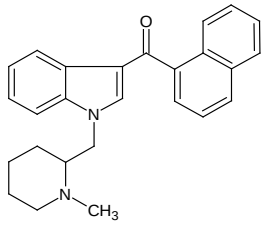
Instruments

Gas Chromatography
Liquid Chromatography
Mass Spectroscopy
Ultraviolet Spectroscopy

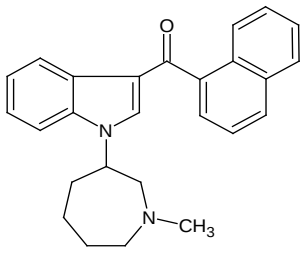
108. Kneisel, S. et al. Identification of the cannabimimetic AM-1220 and its azepane isomer (N-methylazepan-3-yl)-3-(1-naphthoyl) indole in a research chemical and several herbal mixtures. Forensic Toxicol. 30 (2012) 126-134. DOI: 10.1007/s11419-012-0137-6.

Recently, a large number of synthetic cannabinoids have been identified in herbal mixtures. Moreover, an even higher number of cannabimimetic compounds are currently distributed as research chemicals on a gram to kilogram scale via several online trading platforms. As this situation leads to a large number of new cannabimimetics and the occurrence of isobaric substances, the analysis of such compounds using mass spectroscopy (MS) involves the risk of incorrect assignments of mass spectra. In certain cases, this leads to considerable analytical challenges. In the majority of cases, these challenges can only be mastered by combining multiple analytical techniques. We purchased a so-called research chemical advertised as the cannabimimetic compound [(N-methylpiperidin-2-yl)methyl]-3-(1-naphthoyl)indole (AM-1220) via an Internet platform. Analysis of the microcrystalline substance using gas chromatography (GC)–MS indicated the presence of pure AM-1220. However, after further purity testing utilizing thin-layer chromatography we were surprised to see an additional spot indicating a mixture of two substances with highly similar physicochemical properties. After isolation, high-resolution mass spectroscopy (HR-MS) revealed an elemental composition of $C_{26}H_{26}N_2O$ for both substances, proving the presence of two isobaric substances. Moreover, GC–MS and LC-HR-MS/MS experiments indicated two naphthoylindoles featuring different heterocyclic substituents at the indole nitrogen. Nuclear magnetic resonance spectroscopy verified the presence of the highly potent cannabimimetic AM-1220 and its azepane isomer. Interestingly, only a few weeks after purchasing the powder we also detected both substances in a similar proportion in several herbal mixtures for the first time.

Compounds



AM-1220



AM-1220 Azepane isomer

Instruments

Gas Chromatography
Liquid Chromatography
Mass Spectroscopy
High Resolution Mass Spectroscopy
Nuclear Magnetic Resonance

109. Nakajima, J. et al. Identification and quantitation of two new naphthoylindole drugs-of-abuse, (1-(5-hydroxypentyl)-1H-indol-3-yl) (naphthalen-1-yl)methanone (AM-2202) and (1-(4-pentenyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone, with other synthetic cannabinoids in unregulated ''herbal'' products circulated in the Tokyo area. Forensic Toxicol. 30 (2012) 33-44. DOI: 10.1007/s11419-011-0130-5.

During our continual surveillance of unregulated drugs in May–June 2011, we found two new compounds as adulterants in herbal products obtained at shops in the Tokyo area. These compounds were identified by liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry, accurate mass spectrometry, and nuclear magnetic resonance spectroscopy. The first compound identified was a naphthoylindole (1-(5-hydroxypentyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone (AM-2202, 1), which is a side-chain hydroxyl analogue of JWH-018. The second compound was (1-(4-pentenyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone (2), which is side-chain double bond analogue of JWH-018. This is the first report to identify 1 and 2 in a commercial "herbal" product to our knowledge. For quantitation of the above compounds 1 and 2, and chemical analysis for previously reported compounds (AM-2201, 3; JWH-203, 4; JWH-019, 7; JWH-210, 8; mitragynine, 9), each product was extracted with methanol under ultrasonication to prepare solutions for analysis by liquid chromatography with ultraviolet detection. For the sake of identifying JWH-203 (4) and its positional isomers [JWH-203-3-chloroisomer (5) and 4-chloroisomer (6)] correctly, simultaneous liquid chromatography analysis on fluorocarbon-bonded silica gel column was performed. And a case report of commercially available products containing synthetic cannabinoids 7 and 8, and a natural occurring alkaloid 9, was also shown. Each of 6 commercially circulated products contained compounds 1–4 and 7–9; the amounts of the compounds ranged from 4.1 to 222 mg per pack

Compounds

Instruments
Liquid Chromatography
Gas Chromatography
Mass Spectroscopy
Accurate Mass Spectroscopy
Nuclear Magnetic Resonance

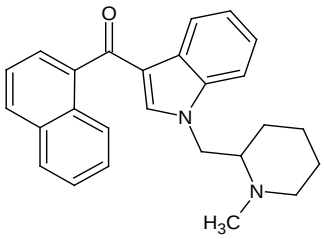
110. Kneisel, S. et al. Identification of the cannabimimetic AM-1220 and its azepane isomer (*N*-methylazepan-3-yl)-3-(1-naphthoyl)indole in a research chemical and several herbal mixtures. Forensic Toxicology. 2 (2012) 126-134.

Recently, a large number of synthetic cannabinoids have been identified in herbal mixtures. Moreover, an even higher number of cannabimimetic compounds are currently distributed as research chemicals on a gram to kilogram scale via several online trading platforms. As this situation leads to a large number of new cannabimimetics and the occurrence of isobaric substances, the analysis of such compounds using mass spectroscopy (MS) involves the risk of incorrect assignments of mass spectra. In certain cases, this leads to considerable analytical challenges. In the majority of cases, these challenges

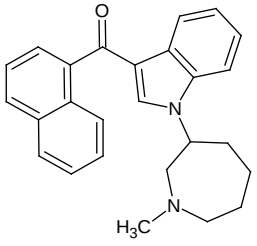
can only be mastered by combining multiple analytical techniques. We purchased a so-called research chemical advertised as the cannabimimetic compound [(*N*-methylpiperidin-2-yl)methyl]-3-(1-naphthoyl)indole (AM-1220) via an Internet platform. Analysis of the microcrystalline substance using gas chromatography (GC)–MS indicated the presence of pure AM-1220. However, after further purity testing utilizing thin-layer chromatography we were surprised to see an additional spot indicating a mixture of two substances with highly similar physicochemical properties. After isolation, high-resolution mass spectroscopy (HR-MS) revealed an elemental composition of C26H26N2O for both substances, proving the presence of two isobaric substances. Moreover, GC–MS and LC-HR-MS/MS experiments indicated two naphthoylindoles featuring different heterocyclic substituents at the indole nitrogen. Nuclear magnetic resonance spectroscopy verified the presence of the highly potent cannabimimetic AM-1220 and its azepane isomer. Interestingly, only a few weeks after purchasing the powder we also detected both substances in a similar proportion in several herbal mixtures for the first time.

Compounds

Am-1220



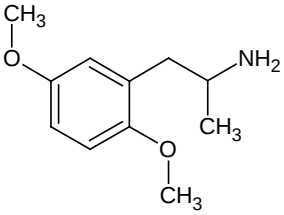
Am-1220 azepane isomer

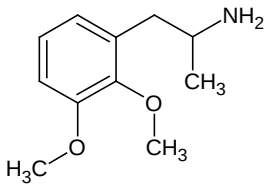


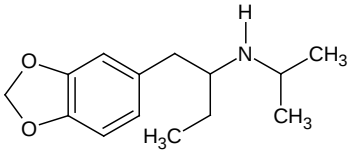
111. Maher, H. M., Awad, T., DeRuiter, J., and Clark, C. R. GC-MS and GC-IRD studies on dimethoxyphenethylamines (DMPEA): Regioisomers related to 2,5-DMPEA. Journal of Chromatographic Science. 50 (2012) 1-9. DOI: 10.1093/chromsci/bmr013.

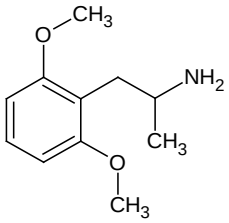
A series of regioisomeric dimethoxyphenethylamines have a mass spectra essentially equivalent to the drug substance 2,5-dimethoxyphenethylamine (2,5-DMPEA). These substances have a molecular weight of 181, and major fragment ions in their electron ionization mass spectra at m/z 151/152. The trifluoroacetyl, pentafluoropropionyl, and heptafluorobutryl derivatives of these primary amines were prepared and evaluated by gas chromatography with mass spectrometry detection (GC–MS). The mass spectra for these derivatives do not show unique fragment ions to allow the specific identification of a particular isomer. Thus, GC–MS does not provide for the confirmation of identity of any one of the six isomers to the exclusion of the other five compounds. However, GC–MS does divide the compounds into two groups depending on the mass of the base peak. GC with infrared detection provides direct confirmatory data for the identification of 2,5-DMPEA from the other regioisomers involved in the study. Perfluoroacylated derivatives of the six regioisomeric dimethoxyphenethylamines were successfully resolved via capillary GC on a non-polar stationary phase consisting of 50% phenyl and 50% methyl polysiloxane (Rxi-50).

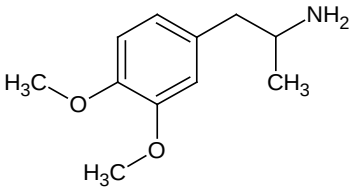
Compounds

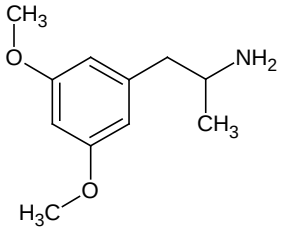

2,5-DMPEA


2,3-DMPEA


2,4-DMPEA


2,6-DMPEA


3,4-DMPEA


3,5-DMPEA

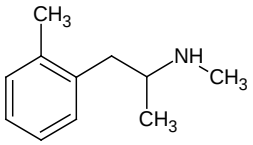
<u>Instruments</u>

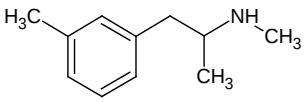
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

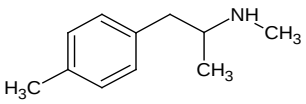
112. Davis, S., Blakey, K., and Rands- Trevor, K. GC–MS and GC–IRD analysis of 2-, 3- and 4-methylmethamphetamine and 2-, 3- and 4-methylamphetamine. For. Sci. Int. 220 (2012) 67-73. DOI: 10.1016/j.forsciint.2012.01.028.

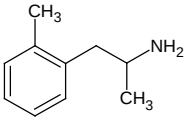
4-Methylmethamphetamine has been detected in samples submitted for analysis in several states throughout Australia. Six ring substituted methyl isomers of methamphetamine and amphetamine were synthesised and analysed. As the regioisomeric 2-, 3- and 4-methylmethamphetamine and 2-, 3- and 4-methylamphetamine have virtually identical mass spectra, the use of MS is an ineffective technique to discriminate between these closely related compounds. We set out to determine whether the regioisomers could be differentiated by a combination of GC-MS, acetyl derivatisation and GC-IRD. We demonstrate that the three isomers of methylmethamphetamine and methylamphetamine can be separated by GC, and a combination of acetyl derivatisation and vapour phase IR can identify the specific ring substituted compound

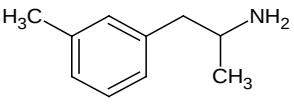
<u>Compounds</u>

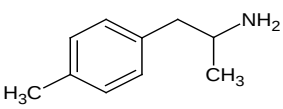

2-methylmethamphetamine


3-methylmethamphetamine


4-methylmethamphetamine


2-methylamphetamine


3-methylamphetamine

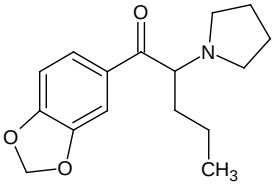

4-methylamphetamine

Instruments
Gas Chromatography
Mass Spectroscopy

113. Hamad Abeidalla, Y.F., Abdel-Hay, K. M., DeRuiter, J., and Clark, C. R. Synthesis and GC-MS analysis of a series of homologues and regioisomers of 3,4-methylenedioxypyrovalerone (MDPV). For. Sci. Int. 223 (2012) 189-197. DOI: 10.1016/j.forsciint.2012.08.040.

A series of ten homologous and regioisomeric aminoketones related to the designer synthetic cathinone derivative MDPV were evaluated in this study. These compounds were prepared from a common precursor chemical, piperonal (3,4-methylenedioxybenzaldehyde). These aminoketones show major peaks in their mass spectra corresponding to the regioisomeric and homologous immonium cation fragments from the loss of the methylenedioxybenzoyl radical species. All ten compounds in this study show equivalent EI MS fragments for the 3,4-methylenedioxybenzoyl fragments (m/z 149) and the methylenedioxybenzene fragment at m/z 121. The m/z 149 results from ionization of the carbonyl oxygen followed by an alpha-cleavage fragmentation. The loss of CO from this ion yields the m/z 121 fragments common to all spectra. The regioisomeric aminoketones yield equivalent mass spectra including mass equivalent regioisomeric immonium cation base peaks. A subset of these compounds has the same molecular weight and almost identical mass spectra to that of the designer drug MDPV. An evaluation of the effects of homologation on gas chromatographic retention showed that addition of a methylene (CH(2)) in the nitrogen-containing ring increases retention more than the equivalent group added to the alkyl side-chain

Compounds



3,4-methylenedioxypyrovalerone

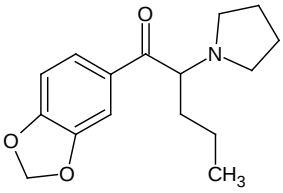
Instruments
Gas Chromatography
Mass Spectroscopy

114. Kavanagh, P. et al. The analysis of substituted cathinones. Part 3. Synthesis and characterisation of 2,3-methylenedioxy substituted cathinones. For. Sci. Int. 216 (2012) 19-28. DOI: 10.1016/j.forsciint.2011.08.011.

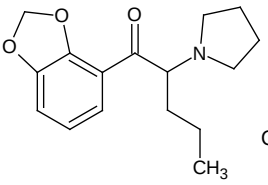
The first synthesis of the 2,3-isomers of MDPV, butylone and methylone is reported. The isomers were characterised by (1)H and (13)C NMR spectroscopy and compared to the corresponding 3,4-isomers. A

GC method is described which separates the 3,4- and the 2,3-isomers from each other. IR spectra of the 2,3-isomers are also compared with the corresponding 3,4-isomers. Two seized drug samples were analysed by GCMS and the samples were found to contain the 3,4-isomers.

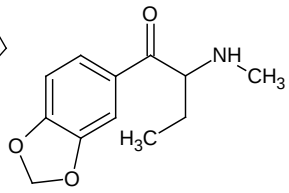
Compounds



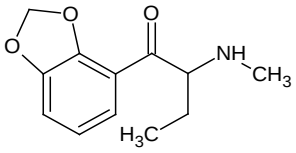
3,4-MDPV



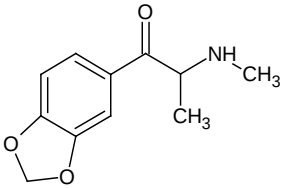
2,3-MDPV



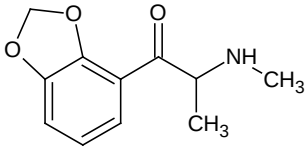
3,4-butylone



2,3-butylone



3,4-methylone



2,3-methylone

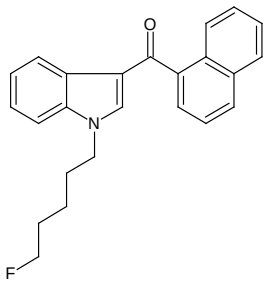
Instruments

Gas Chromatography
Mass Spectroscopy

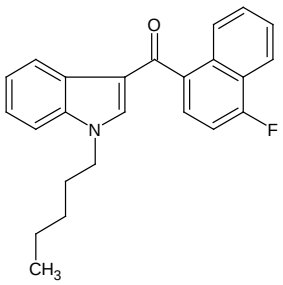
115. Moosmann, B. Separation and structural characterization of the synthetic cannabinoids JWH-412 and 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl)methanone using GC–MS, NMR analysis and a flash chromatography system. For. Sci. Int. 220 (2012) 17-22. DOI: 10.1016/j.forsciint.2011.12.010.

The 'herbal highs' market continues to boom. The added synthetic cannabinoids are often exchanged for another one with a high frequency to stay at least one step ahead of legal restrictions. While most of these substances were synthesized for pharmaceutical purposes and have been described in the scientific literature before, others originate from clandestine laboratories supplying this lucrative market. In this paper, the identification and structure elucidation of two synthetic cannabinoids is reported. The first compound, 1-[(5-fluoropentyl)-1H-indol-3yl]-(4-methylnaphthalen-1-yl)methanone, was found along with AM-2201 in a 'herbal mixture' obtained via the Internet. For isolation of the substance from the mixture, a newly developed flash chromatography method was used providing an inexpensive and fast way to gain pure reference substances from 'Spice' products for the timely development or enhancement of analytical methods in the forensic field. The second substance, 4-fluoronaphthalen-1-yl-(1-pentylindol-3-yl)methanone (JWH-412) was seized by German authorities as microcrystalline powder, making it very likely that it will be found in 'herbal mixtures' soon.

Compounds



AM-2201



JWH-412

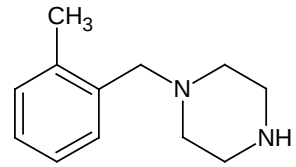
Instruments
Gas Chromatography
Flash Chromatography
Mass Spectroscopy
Nuclear Magnetic Resonance

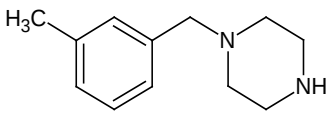
116. Abdel-Hay, K. M., DeRuiter, J., and Clark, C. R. Differentiation of methoxybenzoylpiperazines (OMeBzPs) and methylenedioxybenzylpiperazines (MDBPs) By GC-IRD and GC-MS. Drug Testing and Analysis. 4 (2012) 430-440.

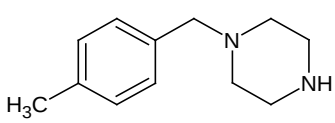
117. Abdel-Hay, K. M., DeRuiter, J., and Clark, C. R. Differentiation of methylbenzylpiperazines (MBPs) and benzoylpiperazine (BNZP) using GC-MS and GC-IRD. Drug Testing and Analysis. 4 (2012) 441-448. DOI: 10.1002/dta.383.

Three-ring substituted methylbenzylpiperazines (MBPs) and their isobaric benzoylpiperazine (BNZP) have equal mass and many common mass spectral fragment ions. The mass spectrum of BNZP yields a unique benzoyl-group containing fragment at m/z 122 and an additional major fragment at m/z 69 that allows its discrimination from the three MBP regioisomers. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in relative abundance of some fragment ions but acylation does not alter the fragmentation pathway and did not provide additional MS fragments of discrimination among these isomers.
Gas chromatography coupled with infrared detection (GC-IRD) provides direct confirmatory data for the structural differentiation between the four isomers. The mass spectra in combination with the vapour phase IR spectra provide for specific confirmation of each of the isomeric piperazines. The underivatized and perfluoroacyl derivatives of these four piperazines were resolved on a stationary phase of 100% trifluoropropyl methyl polysiloxane (Rtx-200). Gas chromatography coupled with time-of-flight mass spectrometry provides an additional means of differentiating between the isobaric MBP and BNZP which have equivalent nominal masses but are different in their elemental composition and exact masses

Compounds







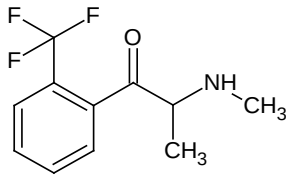
2-methylbenzylpiperazine 3-methylbenzylpiperazine 4-methylbenzylpiperazine

Instruments
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

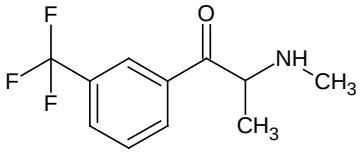
118. Brandt, S. D., Daley, P. F., and Cozzi, N. V. Analytical characterization of three trifluoromethyl-substituted methcathinone isomers. Drug Testing and Analysis. 4 (2012) 525-529. DOI: 10.1002/dta.382.

Cathinone derivatives display a wide range of pharmacological activities and uses; some of them are used as prescription medicines, while others are encountered within a recreational context and are available without a prescription over the Internet and in retail shops around the world. One of the difficulties involved in the unambiguous identification of these new psychoactive substances is the lack of suitable reference standards, particularly when dealing with unreported derivatives and positional isomers. In order to address this need, three trifluoromethyl analogues of the psychostimulant methcathinone, with a CF(3) substituent at the 2-, 3- and 4-position of the phenyl ring (2-TFMAP 1, 3-TFMAP 2 and 4-TFMAP 3), have been prepared for analytical characterization using ATR-FTIR, (1)H and (13) C NMR, and GC-(EI/CI)-ion trap-MS. Differentiation among isomers was feasible by IR, for example when assessing the carbonyl stretch at 1711 (1), 1693 (2) and 1688 (3) cm(-1) , respectively. In addition to the expected iminium base peak at m/z 58, EI-MS displayed key ions at m/z 173, 145, 125, 95, and 75. Separation of isomers was possible under GC conditions. A characteristic feature under CI conditions was the loss of water from the [M + H](+) yielding m/z 214 in addition to m/z 58. Studies currently underway show that the three CF(3) -methcathinone analogues have central nervous system effects and that the 4-CF(3) isomer 3 is more potent as a serotonin uptake inhibitor and releasing agent than the 3-CF(3) and 2-CF(3) counterparts.

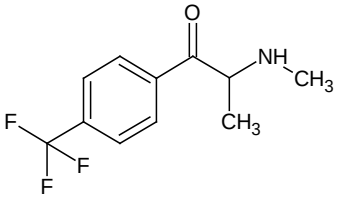
Compounds



2-TFMAP



3-TFMAP



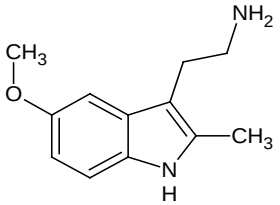
4-TFMAP

Instruments
Gas Chromatography
Mass Spectroscopy
Nuclear Magnetic Resonance
Infrared Spectroscopy
Atenuated Total Reflectance Spectroscopy

119. Brandt, S. D. et al. Synthesis and characterization of 5-methoxy-2-methyl-N,N-dialkylated tryptamines. Drug Testing and Analysis. 4 (2012) 24-32. DOI: 10.1002/dta.398.

The absence of reference material is a commonly experienced difficulty among medical and forensic professionals tasked with identifying new psychoactive substances that are encountered for the first time. The identification of newly emerging substances lies at the heart of forensic and clinical analysis, and a proactive public health policy calls for a thorough analysis of the properties of new psychoactive substances before they appear in the emergency clinic, where they may be noticed because of adverse reactions or toxicity. For example, a wide range of N,N-dialkyltryptamines show psychoactive properties in humans and these tryptamines are sometimes encountered as intoxicants. However, most of the existing reference data on new psychoactive tryptamines have been obtained retrospectively, after reports of acute toxicities. To address the need for reference standards for new tryptamines, thirteen 5-methoxy-2-methyl-N,N-dialkyltryptamines were prepared. Analytical characterization was based on ^{1}H and ^{13}C nuclear magnetic resonance (NMR), gas chromatography-electron ionization ion-trap mass spectrometry (GC-EI-IT-MS) and chemical ionization-ion-trap tandem mass spectrometry (CI-IT-MS/MS), respectively. Differentiation among isomers was feasible by NMR and MS. In addition to the expected iminium ion base peak, indole-related key ions were detected under EI-IT-MS conditions at m/z 174, 159, 131, 130, and 103. CI-IT-MS/MS analysis of the 5-methoxy-2-methyl derivatives revealed the presence of m/z 188 in addition to [M+H]+ and the iminium species. This study served as an extension from previous work on isomeric 5-ethoxylated counterparts and confirmed the ability to differentiate between the two groups. The data provided here add to the existing body of literature and aim to serve both forensic and clinical communities

Compounds



5-methoxy-2-methyltryptamine

Instruments

Gas Chromatography
Mass Spectroscopy
Nuclear Magnetic Resonance

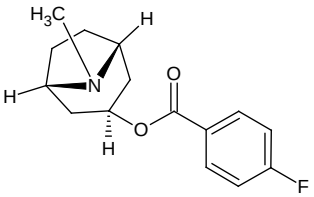
120. Kavanagh, P. et al. The syntheses and characterization 3b-(4-fluorobenzoyloxy) tropane (fluorotropacocaine) and its 3a isomer. Drug Testing and Analysis. 4 (2012) 33-38. DOI: 10.1002/dta.362.

3β-(4-Fluorobenzoyloxy)tropane (3β-FBT, fluorotropacocaine) was first reported by Finnish authorities to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) via the Early Warning System (EWS) in 2008 and our own laboratory tentatively identified it in 2010 in several products purchased from head shops. Very little is known about this cocaine-like drug and, as no reference standards were available, we have synthesized and characterized both 3β-FBT and its 3α isomer for use

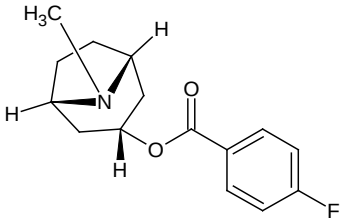
as reference standards. The two compounds are separable by gas chromatography (GC) but their electron-impact (EI) mass spectra were found to be almost identical. ^{19}F NMR spectroscopy was also found to be a useful technique for distinguishing the two isomers.

Compounds

3β-FBT



3α-FBT



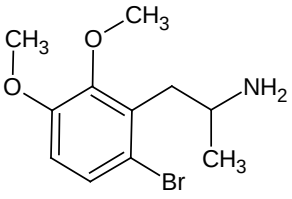
Instruments
Gas Chromatography
Mass Spectroscopy
High Performance Liquid Chromatography
Nuclear Magnetic Resonance

121. Maher, H. M., Awad, T., DeRuiter, J., and Clark, C. R. GC-MS and GC-IRD studies on brominated dimethoxyamphetamines: Regioisomers related to 4-Br-2,5-DMA (DOB). Drug Testing and Analysis. 4 (2012) 591-600. DOI: 10.1002/dta.409.

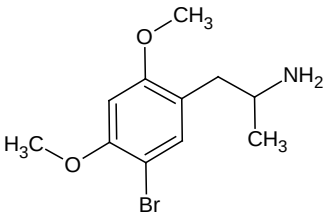
A series of regioisomeric bromodimethoxyamphetamines have mass spectra essentially equivalent to the controlled drug substance 4-Br-2,5-dimethoxyamphetamine (4-Br-2,5-DMA; DOB); all have molecular weight of 274 and major fragment ions in their electron ionization mass spectra at m/z 44 and m/z 230/232. The trifluoroacetyl, pentafluoropropionyl and heptafluorobutryl derivatives of the primary regioisomeric amines were prepared and evaluated in gas chromatography-mass spectrometry (GC-MS) studies. The mass spectra for these derivatives did not show unique fragment ions for specific identification of individual isomers. However, the mass spectra do serve to divide the compounds into three groups, depending on their base peak. Gas chromatography with infrared detection (GC-IRD) provides direct confirmatory data for the identification of the designer drug 4-bromo-2,5-dimethoxyamphetamine from the other regioisomers involved in the study. The perfluoroacylated derivatives of the six regioisomeric bromodimethoxyamphetamines were successfully resolved on non-polar stationary phases such as a 100% dimethylpolysiloxane stationary phase (Rtx-1) and 50% phenyl - 50% methyl polysiloxane (Rxi-50).

Compounds

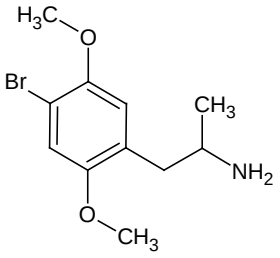
6-Br-2,3-DMA 5-Br-2,4-DMA 4-Br-2,5-DMA



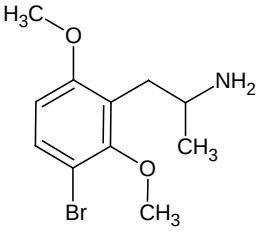
3-Br-2,6-DMA

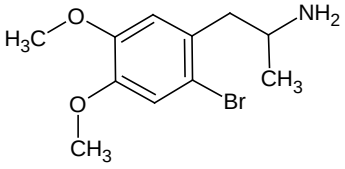


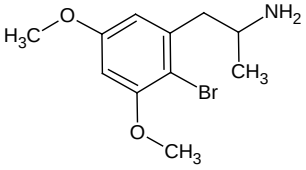
2-Br-4,5-DMA



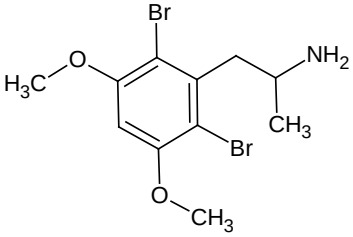
2-Br-3,5-DMA







2,6-DiBr-3,5-DMA



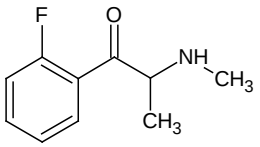
Instruments

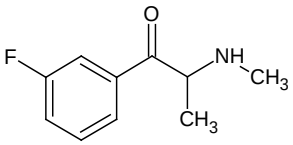
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

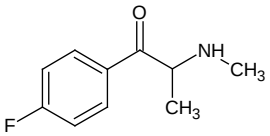
122. Westphal, F., and Junge, Th. Ring positional differentiation of isomeric N-alkylated fluorocathinones by gas chromatography/tandem mass spectrometry. For. Sci. Int. 223 (2012) 97-105. DOI: 10.1016/j.forsciint.2012.08.011.

In analogy to our previously published procedure for the differentiation of regioisomeric fluoroamphetamines a method was developed, to differentiate ring positional isomeric fluorocathinones by product ion spectrometry of ions generated by chemical ionization (CI) under GC-MS conditions using methane as reagent gas. N-alkylated ortho-, meta- and para-fluorocathinones could be unequivocally differentiated by product ion spectrometry of the hydrogen fluoride loss ions [M+H-HF](+) using a triple quadrupole mass spectrometer with argon as collision gas under normalized collision conditions. This method enables the differentiation of ring positional isomers of fluorocathinones even in complex

mixtures and low concentrations. The applicability of the method was shown by the analysis of synthesized N-alkylated ortho-, meta- and para-fluorocathinones and seized designer drug mixtures





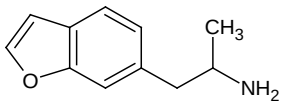


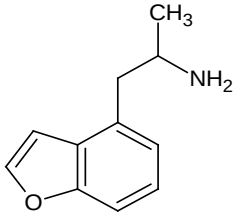
N-methyl-ortho-fluorocathinone N-methyl-meta-fluorocathinone N-methyl-para-fluorocathinones

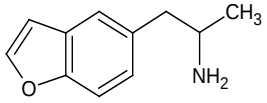
Instruments
Gas Chromatography
Mass Spectroscopy

123. Casale, J F., and Hays, P A. The Characterization of 6-(2-Aminopropyl)benzofuran and Differentiation from its 4-, 5-, and 7-Positional Analogues. Microgram Journal. 9 (2012) 61-74.

Compounds



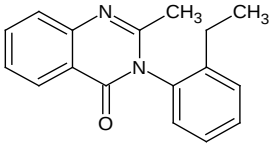


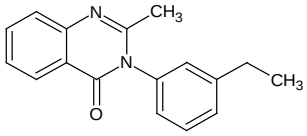


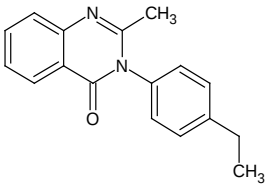
6-(2-Aminopropyl)benzofuran 4-(2-Aminopropyl)benzofuran 5-(2-Aminopropyl)benzofuran

124. Casale, J F., and Hays, P A. The Characterization of Etaqualone and Differentiation from its 3- and 4-Ethyl Analogues. Microgram Journal. 9 (2012) 47-51.

Compounds



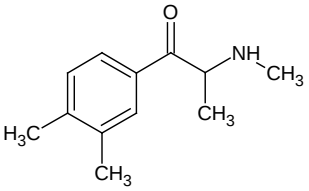




Etaqualone 3-ethyletaqualone 4-ethyletaqualone

125. Casale, J F., and Hays, P A. Differentiation of 3,4-Dimethylmethcathinone (3,4-DMMC) from its Dimethyl Aryl-Positional Isomers. Microgram Journal. 9 (2012) 75-83.

Compounds



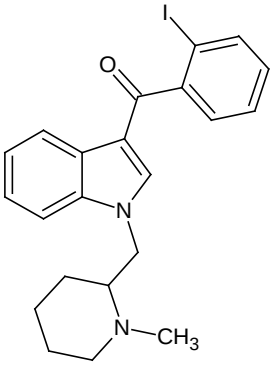
3,4-DMMC

126. Sekuta, K., Zuba, D., and Stanaszek, R. Identification of naphthoylindoles acting on cannabinoid receptors based on their fragmentation patterns under ESI-QTOFMS. Journal of Mass Spectrometry. 47 (2012) 632-643. DOI: 10.1002/jms.3004.

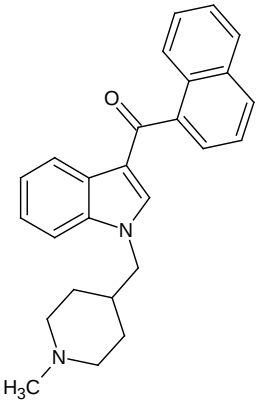
'Herbal highs' have been advertised as legal and natural substitutes to cannabis, but a detailed examination of these products has revealed that the herbal matrix is laced with synthetic substances that mimic the effects of marijuana. Producers select the ingredients based on the results of scientific studies on the affinities of different chemicals to cannabinoid receptors. Naphthoylindoles have turned out to be the most popular class of substances identified in the products. Legal actions taken in order to tackle the problem of uncontrolled access to one substance have usually resulted in the marketing of derivatives or analogues. In the study, the mass spectral behavior of twelve synthetic cannabinoids from the naphthoylindole family under electrospray ionization (ESI) was investigated. LC-QTOFMS experiments were performed in three modes (low fragmentor voltage, high fragmentor voltage with/without collision energy), and they enabled the identification of protonated molecules and main ions. A general fragmentation pattern under this ionization method was proposed, and mechanisms of ion formation were discussed. The developed procedure allowed the determination of substituent groups of the core naphthoylindole structure and distinction between positional isomers. The obtained results were used for the prediction of the ESI-MS spectra for many naphthoylindoles with a high affinity to cannabinoid receptors. Similarities and differences between ESI-MS and electron impact-MS spectra of naphthoylindoles were discussed. The developed identification process was presented on an example of an analysis of an unknown herbal material, in which JWH-007 was finally identified. Knowledge of the fragmentation mechanisms of naphthoylindoles could also be used by other researchers for identification of unknown substances in this chemical family

127. Nakajima, J. et al. Analysis of azepane isomers of AM-2233 and AM-1220, and detection of an inhibitor of fatty acid amide hydrolase [3′-(aminocarbonyl)(1,1′-biphenyl)-3-yl]-cyclohexylcarbamate (URB597) obtained as designer drugs in the Tokyo area. Forensic Toxicol. 31 (2013) 76-85. DOI: 10.1007/s11419-012-0169-y.

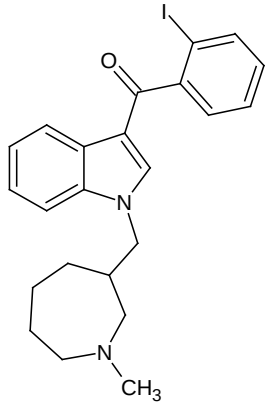
During our careful survey of unregulated drugs from November 2011 to January 2012 in the Tokyo area, we found two new compounds in commercial products. The first was identified as the benzoylindole (2-iodophenyl)[1-(1-methylazepan-3-yl)-1H-indol-3-yl]methanone (2), which is the azepane isomer of AM-2233 (1). Compound 2 was isolated by silica gel column chromatography, and was identified through a combination of liquid chromatography–mass spectrometry, gas chromatography–mass spectrometry, accurate mass spectrometry, and nuclear magnetic resonance spectroscopy. The second compound was identified as [3′-(aminocarbonyl)(1,1′-biphenyl)-3-yl]-cyclohexylcarbamate (URB597, 5) by comparing analytical data with that of the authentic compound. For quantitation of these three compounds, each commercial product was extracted with methanol under ultrasonication to prepare the solution for analysis by liquid chromatography with ultraviolet detection. The occurrence of compounds 1 and 2, and AM-1220 (3) and its azepane isomer (4) in 29 commercial products found in the Tokyo area are also shown in this report.



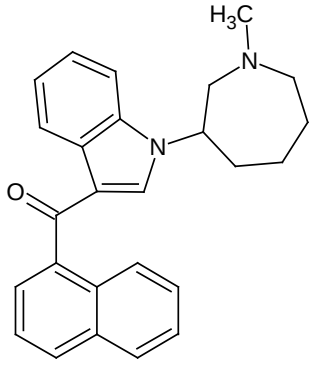
AM-2233



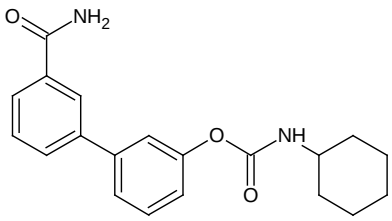
AM-1220



AM-2233 azepane isomer



AM-1220 azepane isomer



URB-597

Instruments

Liquid Chromatography

Mass Spectroscopy

Nuclear Magnetic Resonance

128. Nakazono, Y. et al. Differentiation of regioisomeric fluoroamphetamine analogs by gas chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry. Forensic Toxicol. 31 (2013) 241-250. DOI: 10.1007/s11419-013-0184-7.

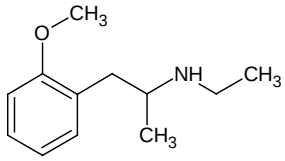
In recent years, a large number of clandestinely produced controlled-substance analogs (designer drugs) of amphetamine with high structural variety have been detected in forensic samples. Analytical differentiation of regioisomers is a significant issue in forensic drug analysis because, in most cases, legal controls are placed only on one or two of the three isomers. In this study, we used gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the differentiation of regioisomers of fluoroamphetamine analogs (fluoroamphetamines and fluoromethamphetamines), which were synthesized in our laboratories. Free bases and their acylated and silylated derivatives were subjected to GC–MS analysis using DB-1ms, DB-5ms, and DB-17ms capillary columns. The separation of free bases was incomplete on all columns. Trifluoroacetyl derivatives of 3- and 4-positional isomers showed slight separation on DB-1ms and DB-5ms. On the other hand, trimethylsilyl derivatization enabled baseline separation of six fluoroamphetamine analogs on DB-1ms and DB-5ms columns, which was sufficient for unequivocal identification. For LC–MS/MS, a pentafluorophenyl column was able to separate six regioisomeric fluoroamphetamine analogs but a conventional C18 column could not achieve separation between 3- and 4-positional isomers. These results show that a suitable choice of derivatization and analytical columns allows the differentiation of regioisomeric fluoroamphetamine analogs.

129. Zaitsu, K et al. Mass spectrometric differentiation of the isomers of mono-methoxyethylamphetamines and mono-methoxydimethylamphetamines by GC–EI–MS–MS. Forensic Toxicol. 31 (2013) 292-300. DOI: 10.1007/s11419-013-0193-6.

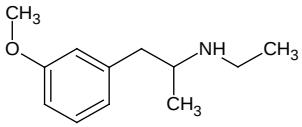
Mass spectrometric differentiation of the six isomers of mono-methoxyethylamphetamines (MeO-EAs) and mono-methoxydimethylamphetamines (MeO-DMAs) by gas chromatography–electron ionization–tandem mass spectrometry (GC–EI–MS–MS) was investigated. Based on their EI-mass spectra, the fragment ions at m/z 121 and 72 were selected as precursor ions for their regioisomeric and structurally isomeric differentiation, respectively. Collision-induced dissociation provides intensity differences in product ions among the isomers, enabling mass spectrometric differentiation of the isomers.

Furthermore, high reproducibility of the product ion spectra at the optimized collision energy was confirmed, demonstrating the reliability of the method. To our knowledge, this is the first report on mass spectrometric differentiation of the six isomers of MeO-EAs and MeO-DMAs by GC–EI–MS–MS. Isomeric differentiation by GC–EI–MS–MS has a high potential to discriminate isomers of newly encountered designer drugs, making GC–MS–MS a powerful tool in the forensic toxicology field

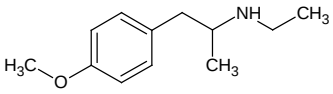
Compounds



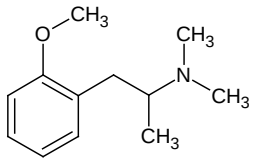
OMEA



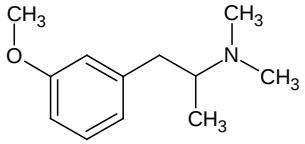
MMEA



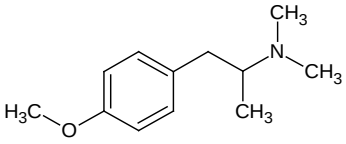
PMEA



OMDMA



MMDMA



PMDMA

Instruments

Gas Chromatography

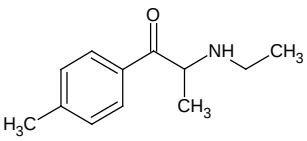
Mass Spectroscopy

GC-EI-MS-MS

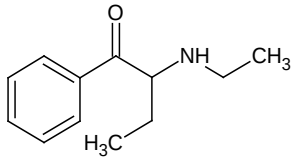
130. Lanza, M., et al. Distinguishing two isomeric mephedrone substitutes with selective reagent ionisation mass spectrometry (SRI-MS). Journal of Mass Spectrometry. 48 (2013) 1015-1018. DOI: 10.1002/jms.3253.

The isomers 4-methylethcathinone and N-ethylbuphedrone are substitutes for the recently banned drug mephedrone. We find that with conventional proton transfer reaction mass spectrometry (PTR-MS), it is not possible to distinguish between these two isomers, because essentially for both substances, only the protonated molecules are observed at a mass-to-charge ratio of 192 (C12 H18NO(+)). However, when utilising an advanced PTR-MS instrument that allows us to switch the reagent ions (selective reagent ionisation) from H3O(+) (which is commonly used in PTR-MS) to NO(+), O2(+) and Kr(+), characteristic product (fragment) ions are detected: C4H10N(+) (72 Da) for 4-methylethcathinone and C5 H12N(+) (86 Da) for N-ethylbuphedrone; thus, selective reagent ionisation MS proves to be a powerful tool for fast detection and identification of these compounds

4-methylethcathinone



N-ethylbuphedrone



Instruments
Gas Chromatography
Mass Spectroscopy

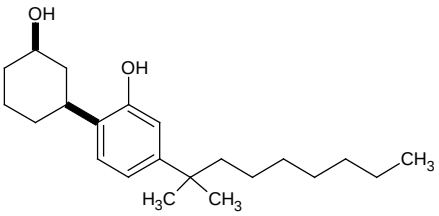
131. Abdel-Hay, K. M., Clark, C. R., and DeRuiter, J. Differentiation of trifluoromethylbenzylpiperazines (TFMBZPs) and trifluoromethylbenzoylpiperazines (TFMBOPs) by GC–MS. For. Sci. Int. 233 (2013) 113-120. DOI: 10.1016/j.forsciint.2013.09.002.

Two series of regioisomers - the trifluoromethylbenzylpiperazines (TFMBZPs) and the trifluoromethylbenzoylpiperazines (TMFBOPs) were synthesized and analyzed as potential "hybrid" derivatives of the benzylpiperazine (BZP) and 1-(3-trifluoromethylphenyl)piperazine (TMFPP) drugs of abuse. The TFMBZPs are readily differentiated from TMFBOPs by their mass spectra including differences in their mass, the base peaks in their mass spectra as well as several other unique fragment ions. However the mass spectra of each regioisomer in each of these two series have fragment ions of identical mass and thus cannot be differentiated by this analytical method alone. Furthermore, chemical derivatization by perfluoroacylation did not offer any additional unique marker fragment ions in the mass spectrum to allow identification of one regioisomer in a series to the exclusion of the other two regioisomers. The perfluoroacylamides of the regioisomers in the TFMBZP series and the regioisomers in the TMFBOP series were readily separated by GC on the stationary phase Rtx-200 and eluted in an order similar to other perfluoroacyl-derivatives of other benzyl- and benzoylpiperazine compounds reported earlier

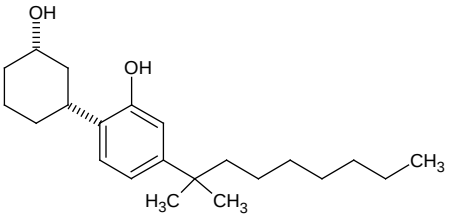
132. Uchiyama, N., et al, Isomeric analysis of synthetic cannabinoids detected as designer drugs. Journal of the Pharmaceutical Society of Japan. 7(2013) 1141-1147. DOI: 10.1248/yakushi.131.1141

Recently, many psychotropic herbal products, named such as "Spice", were distributed worldwide via the Internet. In our previous study, several synthetic cannabinoids were identified as adulterants in herbal products being available in Japan due to their expected narcotic effects. Among those, two derivatives of Δ(9)-tetrahydrocannabinol (Δ(9)-THC), which is major psychotropic cannabinoid of marijuana, cannabicyclohexanol (CCH, 3-[2-hydroxy-4-(2-methylnonan-2-yl)phenyl]cyclohexan-1-ol) and CP-47,497 (3-[2-hydroxy-4-(2-methyloctan-2-yl)phenyl]cyclohexan-1-ol), have been controlled as designated substances (Shitei-Yakubutsu) under the Pharmaceutical Affairs Law since November 2009. CCH was detected together with its trans-form (1-epimer) in many herbal products, and CCH and CP-47,497 have two chiral centers in the structures. However, the pharmaceutical activities of the isomers of CCH have not been reported. This study presents chiral separations of CCH, its trans-form and CP-47,497 in the products using LC-circular dichroism (CD) and LC-MS analyses. The enantiomeric pairs of CCH, its trans-form and CP-47,497 were separated, respectively. Subsequently, the analyses of the herbal products showed that CCH and its trans-form existed as mixtures of enantiomers and the relative ratios of CCH and the trans-form enantiomers ranged from 42/58% to 53/47% and from 33/67% to 52/48%, respectively.

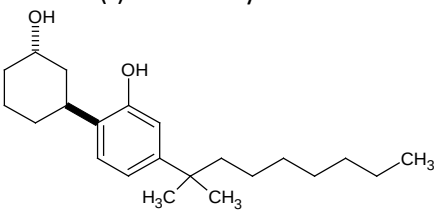
Compounds



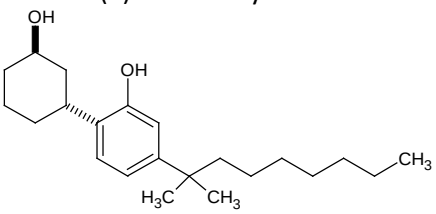
(-)-Cannabicyclohexanol



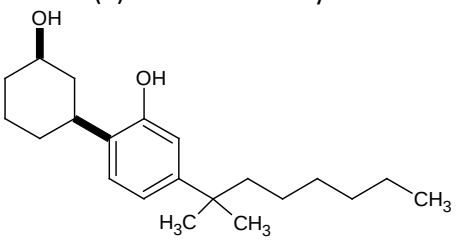
(+)-Cannabicyclohexanol



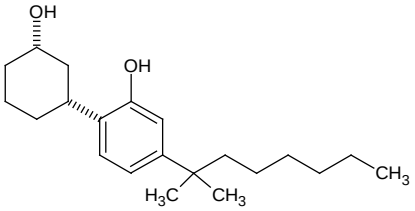
(+)-trans-Cannabicyclohexanol



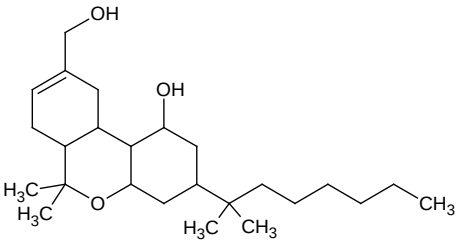
(+)-trans-Cannabicyclohexanol



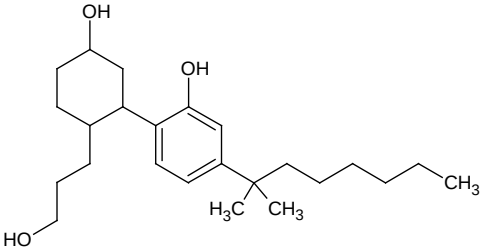
(-)-CP-47,497



(+)-CP-47,497



HU-210



(-)-CP-55,940

Instruments

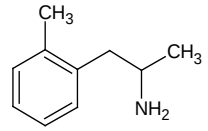
Gas Chromatography
High-Performance Liquid Chromatography
Mass Spectroscopy
Ultraviolet Spectroscopy

133. Power, J. D. et al. The identification of 4-methylamphetamine and its synthesis by-products in forensic samples. For. Sci. Int. 228 (2013) 115-131. DOI: 10.1016/j.forsciint.2013.02.039.

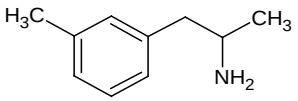
During the analysis of street samples for the suspected presence of controlled drugs, two samples were found to contain 4-methylamphetamine (4-MA). This is the first report of the drug in Ireland as

previously only N-methylamphetamine (N-MA) had been encountered. In Ireland, little attention had previously been paid to the possible presence of the isomeric forms of methylamphetamine in submitted samples. Two street level samples were analyzed and the presence of 4-MA was confirmed in both, the other major components of these samples were examined to establish the possible synthetic route employed. The identification of a single synthetic route which accounted for the major components found in the street samples has implications for previously thought route specific analogous compounds of 4-MA. The three ring substituted isomers of methylamphetamine, namely 2-, 3- and 4-MA, were synthesized and characterized by GCMS, HR-ESIMS, NMR and IR for use as reference standards.

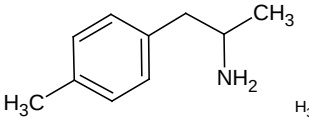
Compounds



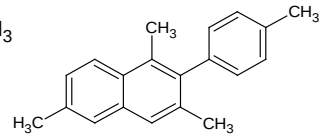
2-MA

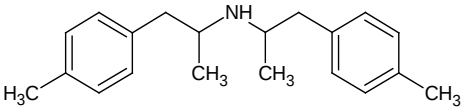


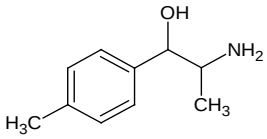
3-MA

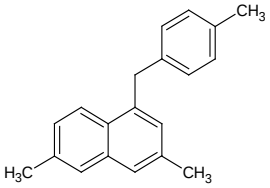


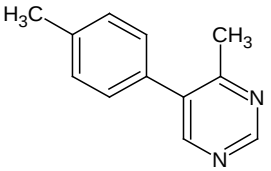
4-MA

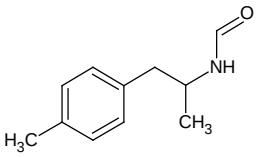


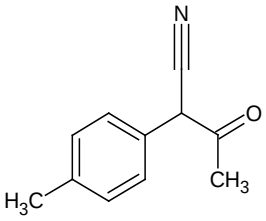












Instruments

G as Chromatography
Mass Spectrometry
Nuclear Magnetic Resonance
Infrared Spectrometry
HR-ESIMS

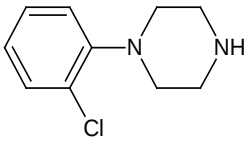
134. Siroka, J. et al. Separation and determination of chlorophenylpiperazine isomers in confiscated pills by capillary electrophoresis. Journal of Pharmaceutical and Biomedical Analysis. 84 (2013) 140-147. DOI: 10.1016/j.jpba.2013.05.042.

A simple capillary electrophoretic method with spectrophotometric UV detection at 236 nm has been developed for the selective separation and determination of 1-(2-chlorophenyl)piperazine (oCPP), 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-chlorophenyl)piperazine (pCPP) in confiscated pills. Several cyclodextrin derivatives were tested to compose the background electrolyte (BGE). The optimized BGE contained 20 mmol/L phosphoric acid adjusted to pH 2.5 with triethylamine and 10 mmol/L α-cyclodextrin, which provided acceptable resolution of analytes and candidate interferents in less than 15

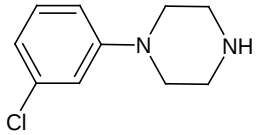
min. The analyses were performed at constant voltage of 25 kV in 60 cm (effective length 50 cm; 50 µm i.d.) uncoated fused-silica capillary maintained at 25°C with sample injection at 4,826 Pa for 8s. Procaine at a concentration of 0.1mg/mL was used as internal standard (IS). Possible interference from other drugs such as amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine, 3,4-methylenedioxymethamphetamine, 3,4-methylenedioxy-N-ethylamphetamine, 1-(3-trifluoromethylphenyl)piperazine and cocaine was also examined. The analytical curves were linear (R(2)=0.9994-0.9995) in the range of 10-200 µg/mL (for oCPP and mCPP) and 20-200 µg/mL for pCPP. Limits of detection (LODs) were 2.0 µg/mL (oCPP), 2.5µg/mL (mCPP) and 3.5 µg/mL (pCPP). Intraday precision at three concentration levels and six replicates of each level (10, 100, 200 µg/mL of each analyte; n=18) was evaluated for the corrected peak area ratio of analyte to IS and the migration times giving RSDs ≤ 4.9%. The accuracy was estimated for mCPP by a recovery test at the same three concentration levels and recoveries varied from 101.0 to 101.6%. The method has been successively applied to the analysis of 17 confiscated pills based mostly on mCPP.

Compounds

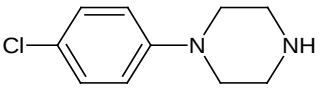
oCPP



mCPP



pCPP



Instruments

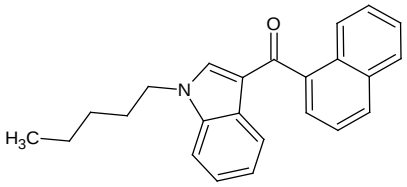
Capillary Electrophoresis

Diode Array Detector

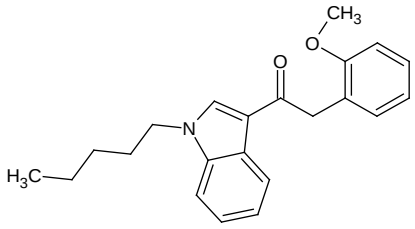
135. Thomas, B.F., Pollard, G.T., Grabenauer, M. Analytical surveillance of emerging drugs of abuse and drug formulations. Life Sciences. 8 (2013) 512-519. DOI: 10.1016/j.lfs.2012.10.031

Uncontrolled recreational drugs are proliferating in number and variety. Effects of long-term use are unknown, and regulation is problematic, as efforts to control one chemical often lead to several other structural analogs. Advanced analytical instrumentation and methods are continuing to be developed to identify drugs, chemical constituents of products, and drug substances and metabolites in biological fluids. Several mass spectrometry based approaches appear promising, particularly those that involve high resolution chromatographic and mass spectrometric methods that allow unbiased data acquisition and sophisticated data interrogation. Several of these techniques are shown to facilitate both targeted and broad spectrum analyses, the latter of which are often of particular benefit when dealing with misleadingly labeled products or assessing a biological matrix for illicit drugs and metabolites. The development and application of novel analytical approaches such as these will help to assess the nature and degree of exposure and risk and, where necessary, inform forensics and facilitate implementation of specific regulation and control measures

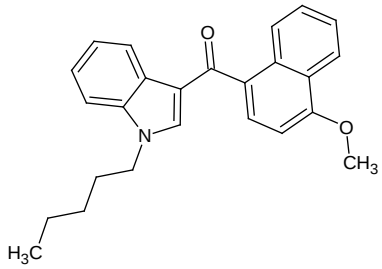
JWH–018



JWH–250



JWH-081



Instruments Used:
Direct ionization
Solid-phase micro extraction coupled to GC-MS
Ultra-high performance liquid chromatography and quadrupole time-of-flight

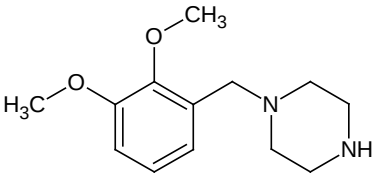
136. Abdel-Hay, K. M., DeRuiter, J, and Clark, C. R. GC-MS and GC-IRD studies on the six-ring regioisomeric dimethoxybenzylpiperazines (DMBPs). Drug Testing and Analysis. 5 (2013) 560-572. DOI: 10.1002/dta.1417.

Gas chromatography with infrared detection (GC-IRD) provides direct confirmatory data for the differentiation between the six regioisomeric aromatic ring substituted dimethoxybenzylpiperazines (DMBPs). These regioisomeric substances are resolved by GC and the vapour-phase infrared spectra clearly differentiate among the six dimethoxybenzyl substitution patterns. The mass spectra for these

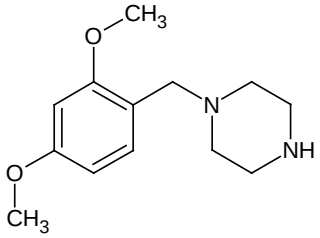
regioisomeric substances are almost identical. With only the 2,3-dimethoxy isomer showing one unique major fragment ion at m/z 136. Thus mass spectrometry does not provide for the confirmation of identity of any one of these compounds to the exclusion of the other isomers. Perfluoroacylation of the secondary amine nitrogen for each of the six regioisomers gave mass spectra showing some differences in the relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation of structure. Gas chromatography coupled with time-of-flight mass spectrometric detection (GC-TOF) provided an additional means of confirmation of the elemental composition of the major fragment ions in the mass spectra of these compounds.

Compounds

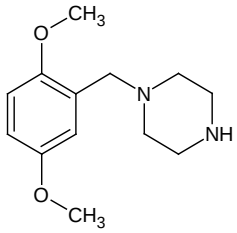
1-(2,3-dimethoxybenzyl)piperazine



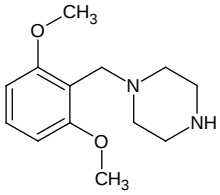
1-(2,4-dimethoxybenzyl)piperazine



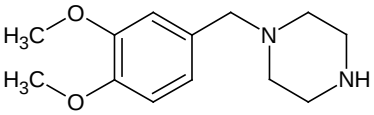
1-(2,5-dimethoxybenzyl)piperazine



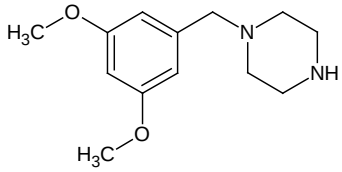
1-(2,6-dimethoxybenzyl)piperazine



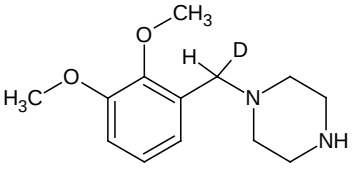
1-(3,4-dimethoxybenzyl)piperazine



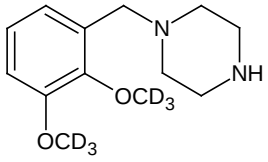
1-(3,5-dimethoxybenzyl)piperazine



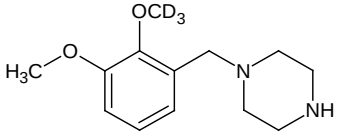
d1 -2,3-dimethoxybenzylpiperazine



d6 -2,3-dimethoxybenzylpiperazine



d3-2,3-dimethoxybenzylpiperazine



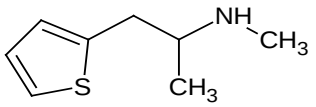
Instruments Used:
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

137. Angelov, D., O'Brien, J., and Kavanagh, P. The syntheses of 1-(2-thienyl)-2-(methylamino) propane (methiopropamine) and its 3-thienyl isomer for use as reference standards. Drug Testing and Analysis. 5 (2013) 145-149. DOI: 10.1002/dta.298.

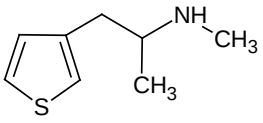
1-(2-Thienyl)-2-(methylamino)propane (methiopropamine, MPA), the thiophene analogue of methamphetamine, has recently appeared on a number of websites offering 'legal highs' for sale and has also been reported as a new psychoactive substance by the European Monitoring Centre for Drugs and Drugs Addiction (EMCDDA) Early Warning System. The drug is currently not controlled in the European Union (EU) but it would be expected that forensic laboratories will encounter it during routine analysis. As no reference standard was available, we have established a three-step protocol for its synthesis. We have also synthesized its 3-thienyl isomer and have established that this is separable from methiopropamine by gas chromatography using one of our routine protocols. The synthetic methodology presented here could be readily extended to the syntheses of analogous compounds.

Compounds

MPA



MPA 3-thienyl isomer



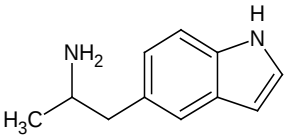
Instruments
Nuclear Magnetic Resonance
Gas Chromatography
Mass Spectrometry

138. Elliott, S. P., Brandt, S. D., Freeman, S., and Archer, R. P. AMT (3-(2-aminopropyl)indole) and 5-IT (5-(2-aminopropyl)indole): an analytical challenge and implications for forensic analysis. Drug Testing and Analysis. 5 (2013) 196-202. DOI: 10.1002/dta.1420

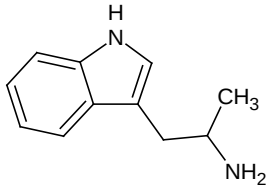
5-(2-Aminopropyl)indole (5-IT) and 3-(2-aminopropyl)indole (α-methyltryptamine, AMT) are isomeric substances and their differentiation can be a challenge under routine analytical conditions, especially when reference material is unavailable. 5-IT represents a very recent addition to the battery of new psychoactive substances that are commercially available from online retailers. This report illustrates how subtle differences observed under mass spectral and UV conditions can help to facilitate the differentiation between the two isomers. Analyses included ^{1}H and ^{13}C NMR, GC-EI/CI ion trap MS, applications of several U/HPLC-DAD and HPLC-MS methods. Investigations currently underway also

highlight the confirmation that AMT was detected in a number of fatal intoxications. These findings also demonstrate that there is a potential risk of misidentification when dealing with both substances

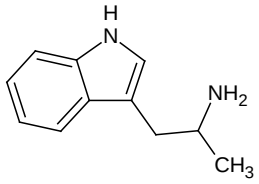
5-IT



α-methyltryptamine



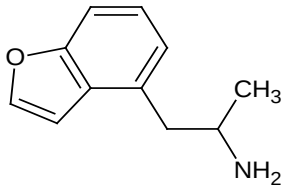
3-(2-aminopropyl)indole



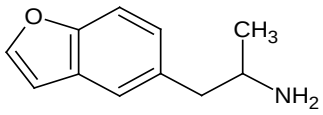
139. Stanczuk, A., Morris, N., Gardner, E. A., and Kavanagh, P. Identification of (2-aminopropyl)benzofuran (APB) phenyl ring positional isomers in Internet purchased products. Drug Testing and Analysis. 5 (2013) 270-276. DOI: 10.1002/dta.1451

5-(2-Aminopropyl)benzofuran (5-APB), a 'research chemical' that was first reported by UK authorities to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) in 2010, is anecdotally reported to produce a combination of stimulant and entactogenic effects. More recently, in 2011, 6-(2-aminopropyl)benzofuran (6-APB) was identified by Hungarian authorities. To confirm positional isomer identity in Internet purchased products, 4- 5- 6- and 7-APBs were synthesized and found to be separable by gas chromatography (as heptafluorobutyramide derivatives) and liquid chromatography. The analyses of products purchased from online vendors of 'research chemicals' identified the presence of 5- or 6-APBs. These findings were further confirmed by liquid chromatography-mass spectrometry and ^{1}H nuclear magnetic resonance spectroscopy. In products containing 6-APB, the 4- positional isomer was also identified and this may have arisen during the manufacturing process

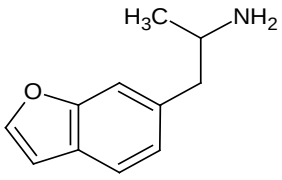
4-(2-Aminopropyl)benzofuran



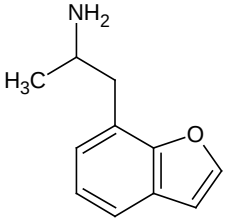
5-(2-Aminopropyl)benzofuran



6-(2-Aminopropyl)benzofuran



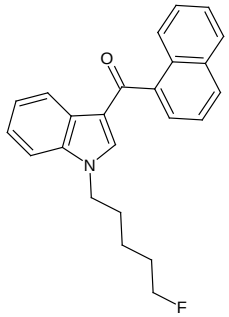
7-(2-Aminopropyl)benzofuran



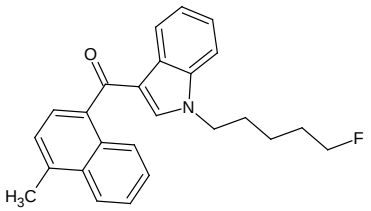
140. Langer, N., et al, Identification and quantification of synthetic cannabinoids in 'spice-like' herbal mixtures: A snapshot of the German situation in the autumn of 2012. Drug Testing and Analysis. 6 (2013) 59-71. DOI: 10.1002/dta.1499

Synthetic compounds mimicking cannabis-like effects are a recent trend. Currently, these so-called synthetic cannabinoids are the largest and fastest growing class of newly appearing designer drugs. Many national authorities are continuously adapting their regulations to keep pace with the permanently changing variety of compounds. We have analyzed eight herbal smoking blends containing synthetic cannabinoids. Altogether, nine compounds could be identified, namely AM-2201, AM-2201-pMe (MAM-2201), AM-1220, AM-1220-azepane, UR-144, XLR-11, JWH-122-pentenyl, AM-2232, and STS-135. Newly appearing compounds were isolated by column chromatography and their structures elucidated by 1D- and 2D-nuclear magnetic resonance (NMR) experiments. In addition, the compounds were investigated by electron ionization-mass spectrometry (EI-MS) and electrospray ionization-tandem mass spectrometry (ESI-MS/MS) to complete the physicochemical dataset. Based on the purified compounds a universal gas chromatography-mass spectrometry (GC-MS) method was developed for the identification and quantification of these compounds in commercial smoking blends. By applying this method, up to five different compounds could be found in such products showing total concentrations from 72 to 303 mg/g smoking blend while individual compounds ranged from 0.4 to 303 mg/g. ^{1}H NMR spectra of the chiral compounds AM-1220 and its azepane-isomer recorded in the presence of 1 equivalent of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA, Mosher's acid) showed them to be racemic mixtures

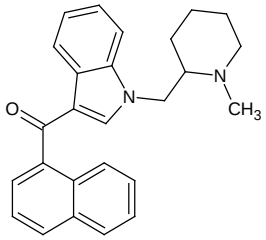
AM-2201



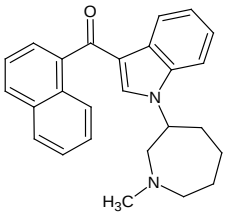
MAM-2201



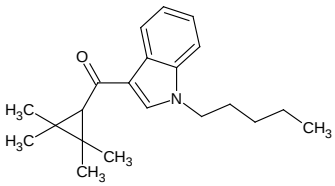
AM-1220



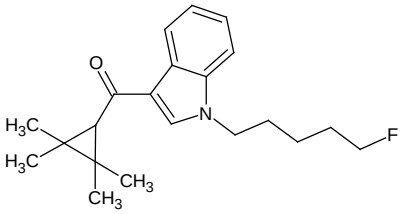
AM-1220-azepane



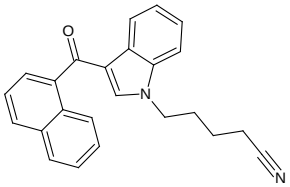
UR-144



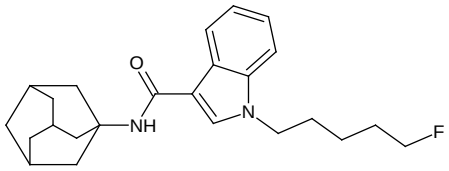
XLR-11



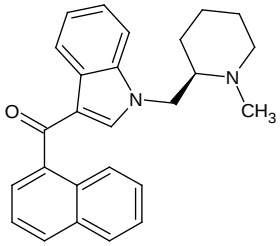
AM-2232



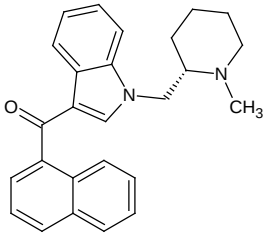
STS-135



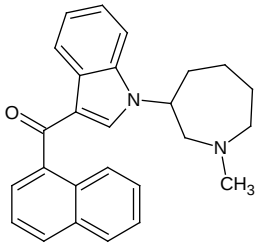
(R)-AM-1220



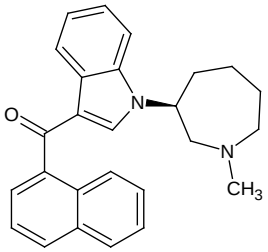
(S)-AM-1220



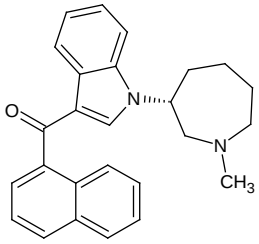
AM-1220-azepane



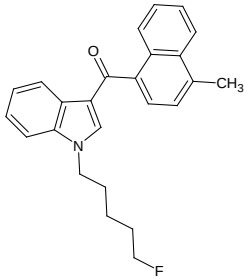
(S)-AM-1220-azepane



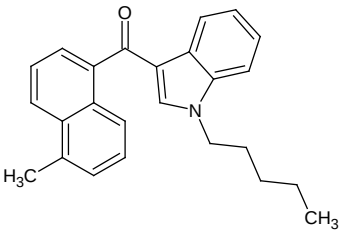
(R)-AM-1220-azepane



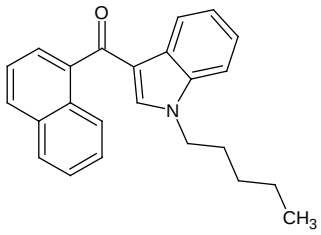
AM-2201-pMe



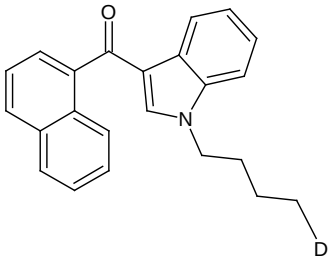
JWH-122-pentenyl



JWH-018



JWH-018-D_3



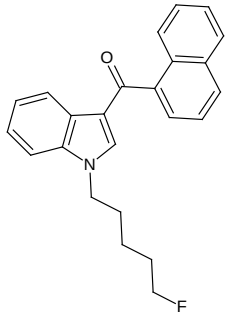
Instruments
Nuclear Magnetic Resonance
Gas Chromatography
Mass Spectroscopy

141. Langer, N., et al, Identification and quantification of synthetic cannabinoids in 'spice-like' herbal mixtures: A snapshot of the German situation in the autumn of 2012. Drug Testing and Analysis. 6 (2013) 59-71. DOI: 10.1002/dta.1499

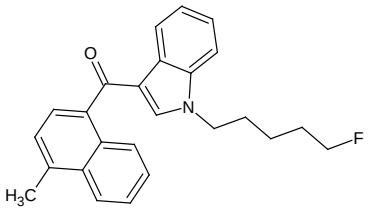
Synthetic compounds mimicking cannabis-like effects are a recent trend. Currently, these so-called synthetic cannabinoids are the largest and fastest growing class of newly appearing designer drugs. Many national authorities are continuously adapting their regulations to keep pace with the permanently changing variety of compounds. We have analyzed eight herbal smoking blends containing synthetic cannabinoids. Altogether, nine compounds could be identified, namely AM-2201, AM-2201-pMe (MAM-2201), AM-1220, AM-1220-azepane, UR-144, XLR-11, JWH-122-pentenyl, AM-2232, and STS-135. Newly appearing compounds were isolated by column chromatography and their structures elucidated by 1D- and 2D-nuclear magnetic resonance (NMR) experiments. In addition, the compounds were investigated by electron ionization-mass spectrometry (EI-MS) and electrospray ionization-tandem mass spectrometry (ESI-MS/MS) to complete the physicochemical dataset. Based on the purified compounds a universal gas chromatography-mass spectrometry (GC-MS) method was developed for the identification and quantification of these compounds in commercial smoking blends. By applying this method, up to five different compounds could be found in such products showing total concentrations from 72 to 303 mg/g smoking blend while individual compounds ranged from 0.4 to 303 mg/g. ^{1}H NMR spectra of the chiral compounds AM-1220 and its azepane-isomer recorded in the presence of 1

equivalent of (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA, Mosher's acid) showed them to be racemic mixtures

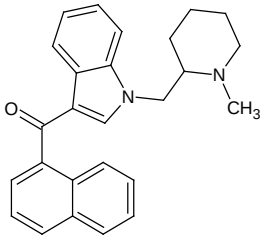
AM-2201



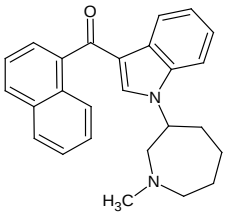
MAM-2201



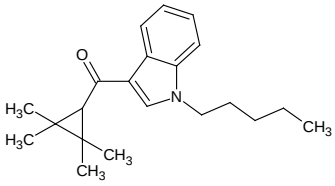
AM-1220



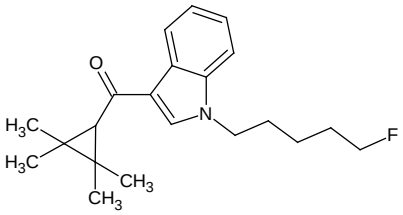
AM-1220-azepane



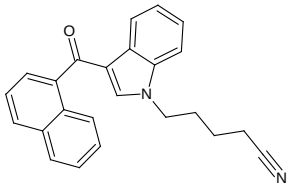
UR-144



XLR-11

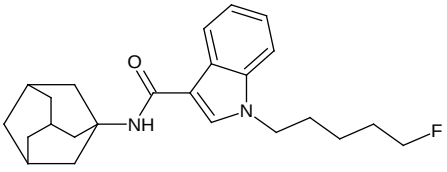


AM-2232

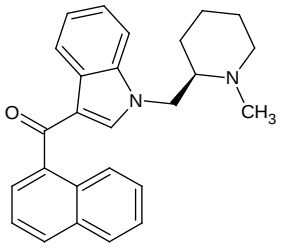


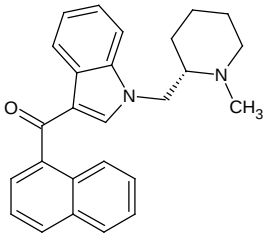
(R)-AM-1220

STS-135



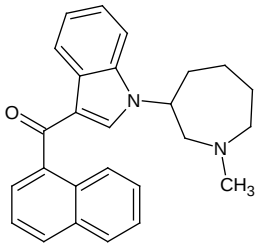
(S)-AM-1220

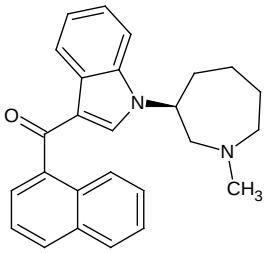




AM-1220-azepane

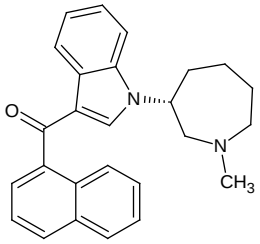
(S)-AM-1220-azepane

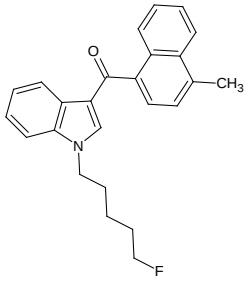




(R)-AM-1220-azepane

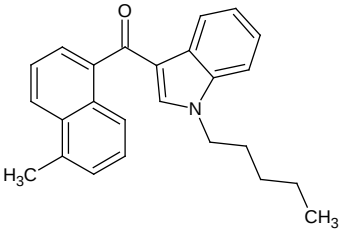
AM-2201-pMe

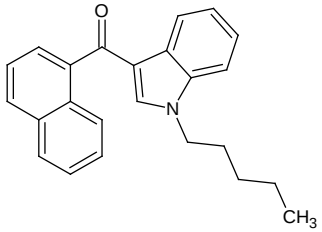




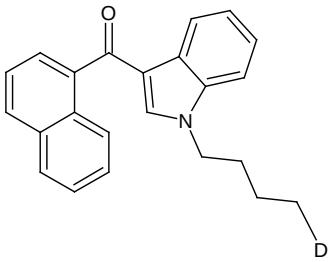
JWH-122-pentenyl

JWH-018





JWH-018-D_3

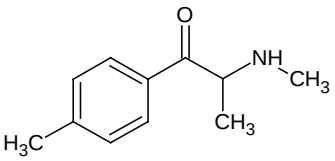


Instruments
Nuclear Magnetic Resonance
Gas Chromatography
Mass Spectroscopy

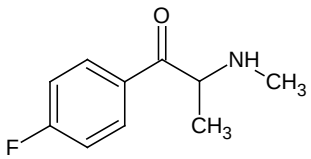
142. Tsujikawa, K., et al. Identification and differentiation of methcathinone analogs by gas chromatography-mass spectrometry. Drug Testing and Analysis. 8 (2013) 670-677. DOI: 10.1002/dta.1437

To overcome a number of challenges involved in analyzing methcathinone (MC) analogues, we performed gas chromatography-mass spectrometry (GC-MS) analysis, including sample preparation, of nine MC analogues – 4-methylmethcathinone, three positional isomers of fluoromethcathinones, 4-methoxymethcathinone, *N*-ethylcathinone, *N*,*N*-dimethylcathinone, buphedrone, and pentedrone. The MC analogues underwent dehydrogenation when the free bases were analyzed using splitless injection. Most of this thermal degradation was prevented using split injection. This indicated that a shorter residence time in the hot injector prevented decomposition. Uniquely, 2-fluoromethcathinone degraded to another product in a process that could not be prevented by the split injection. Replacing the liner with a new, clean one was also effective in preventing thermal degradation. Most of the analytes showed a substantial loss (>30%) when the free base solution in ethyl acetate was evaporated under a nitrogen stream. Adding a small amount of dimethylformamide as a solvent keeper had a noticeable effect, but it did not completely prevent the loss. Three positional isomers of fluoromethcathinones were separated with baseline resolution by heptafluorobutyrylation with a slow column heating rate (8 °C/min) using a non-polar DB-5 ms capillary column. These results will be useful for the forensic analysis of MC analogues in confiscated materials

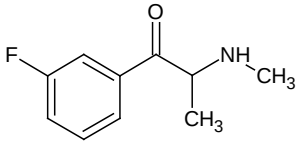
4-methylmethcathinone



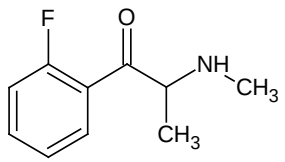
4-fluoromethcathinone



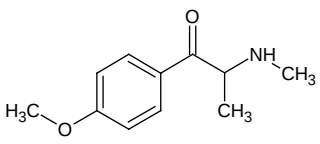
3-fluoromethcathinone



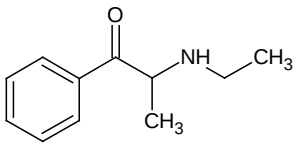
2-fluoromethcathinone



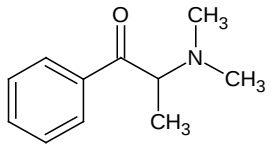
4-methoxymethcathinone



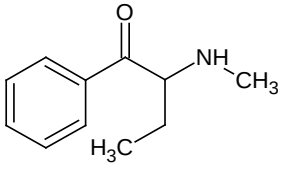
N-ethylcathinone



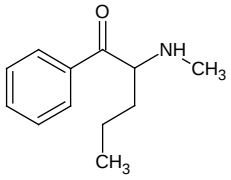
N,*N*-dimethylcathinone



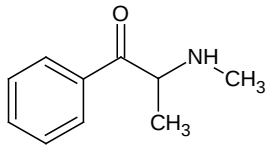
buphedrone



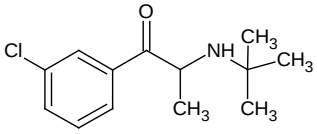
pentedrone



Methcathinone



Bupropion



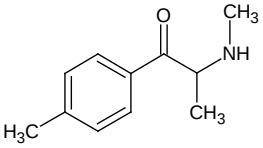
<u>Instruments</u>
Gas Chromatography
Mass Spectroscopy

143. Discrimination of cathinone regioisomers, sold as 'legal highs', by Raman spectroscopy. R. Christie et al. Drug Testing Analysis. 2013. DOI 10.1002/dta.1518

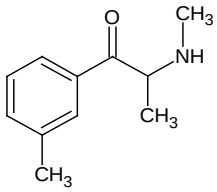
The discrimination of a cross section of cathinone regioisomers, sold as 'legal highs', using Raman spectroscopy, is reported here. Mephedrone and flephedrone were identified in 'legal high' products sold in Irish head shops, and their 2, 3 and 4-isomers were synthesized as reference standards. The 3,4-methylenedioxy substituted cathinones, methylone, butylone and methylenedioxypyrovalerone (MDPV), were also identified in 'legal highs' and their 2,3-isomers were synthesized for comparison. In addition, alpha- and beta-naphyrone were synthesized. Raman spectra of all the isomers were obtained using far-red excitation (785 nm) and it was found possible to discriminate the isomers of each substituted cathinone. In addition, Raman spectra were also recorded for a number of head shop products and, by comparison with the reference standards, correct isomer assignment for 4-mephedrone, 3-flephedrone, 3,4-methylone, 3,4-butylone, 3,4-MDPV, alpha-naphyrone and beta-naphyrone was achieved, thus providing a non-destructive, high-throughput and minimal sample preparation technique for the discrimination of such drug isomers.

Compounds of Interest:

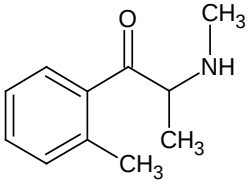
4-mephedrone



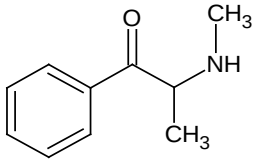
3-mephedrone



2-mephedrone



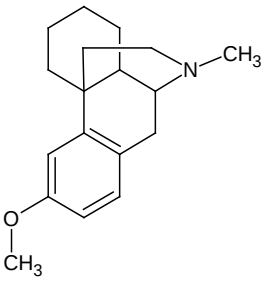
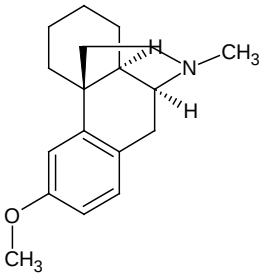
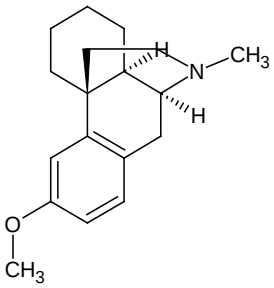
Mephedrone



144. Tedesco, D. et al. Determination of dextromethorphan and levomethorphan in seized heroin samples by enantioselective HPLC and electronic CD. Journal of Pharmaceutical and Biomedical Analysis. 81 (2013) 76-79. DOI: 10.1016/j.jpba.2013.03.024.

A new enantioselective HPLC method was developed for the resolution and determination of the enantiomers of methorphan, dextromethorphan (DXM) and levomethorphan (LVM), on a Chiralcel OJ column (250 mm × 4.6mm I.D.). The resolution of DXM and LVM was obtained using a mobile phase consisting of (n-hexane)-(2-propanol)-diethylamine (70:30:0.1, v/v/v) at a flow rate of 0.5 mL min(-1). The enantioselective method was found to be selective (α=1.92) and sensitive (LOD=2.8 μg mL(-1) for both DXM and LVM). The method was coupled with electronic circular dichroism (CD), allowing the determination of the elution order on the basis of the sign of CD signals of the single enantiomers at 285 nm (positive for DXM, negative for LVM). Under the optimized conditions, the validated method was applied to the identification and quantitation of the enantiomers of methorphan in samples of different sources of illicit drugs of abuse (heroin). DXM was found in eight seized samples of street heroin; two of these samples, for which the DXM content was found to be over 5% (w/w) and exceeding a 10% (w/w) ratio with respect to diacetylmorphine, were the cause of two deaths for overdose due to acute narcotism.

Methorphan dextromethorphan (DXM) levomethorphan (LVM)

Insturments

UV/VIS Spectroscopy

145. Lesiak, A. D and Shepard, J. R. E. Recent advances in forensic drug analysis by DART-MS. Bioanalysis. 6 (2014) 819-842. DOI: 10.4155/bio.14.31.

Mass spectrometry methods play a major role in many forensic applications. While gas chromatography–mass spectrometry methods are commonly used in crime laboratories and enforcement agencies, a variety of advanced techniques are now available that can improve upon standard methods and address emerging issues in forensic science. New mass spectrometry technologies include more versatile ionization sources, allowing the next generation of instrumentation to be more multipurpose and adaptable to the needs of the discipline. Direct analysis in real-time mass spectrometry is an ambient ionization method that allows direct testing of gas, liquid and solid samples without the need for any preparation or extraction, based on thermal desorption and ionization directly from the sample surface. This Review will provide an in-depth description of direct analysis in real-time time-of-flight mass spectrometry as applied to samples relevant to forensic science, with a focus on analysis and characterization related to forensic drug chemistry

Instrument

Gas Chromatography

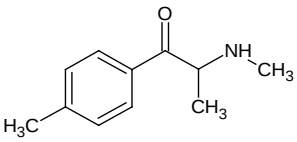
Mass Spectroscopy

146. Christie, R., Horan, E., Fox, J., O'Donnell, C., Byrne, H. J., McDermott, S., Power, J. and Kavanagh, P. Discrimination of cathinone regioisomers, sold as ‘legal highs’, by Raman spectroscopy. Drug Testing and Analysis. 6 (2014) 651-657. DOI: 10.1002/dta.1518.

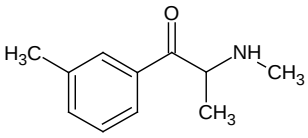
The discrimination of a cross section of cathinone regioisomers, sold as 'legal highs', using Raman spectroscopy, is reported here. Mephedrone and flephedrone were identified in 'legal high' products

sold in Irish head shops, and their 2, 3 and 4-isomers were synthesized as reference standards. The 3,4-methylenedioxy substituted cathinones, methylone, butylone and methylenedioxypyrovalerone (MDPV), were also identified in 'legal highs' and their 2,3-isomers were synthesized for comparison. In addition, alpha- and beta-naphyrone were synthesized. Raman spectra of all the isomers were obtained using far-red excitation (785 nm) and it was found possible to discriminate the isomers of each substituted cathinone. In addition, Raman spectra were also recorded for a number of head shop products and, by comparison with the reference standards, correct isomer assignment for 4-mephedrone, 3-flephedrone, 3,4-methylone, 3,4-butylone, 3,4-MDPV, alpha-naphyrone and beta-naphyrone was achieved, thus providing a non-destructive, high-throughput and minimal sample preparation technique for the discrimination of such drug isomers.

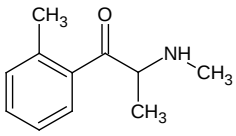
4-Mephedrone



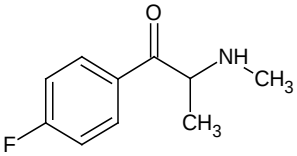
3-Mephedrone



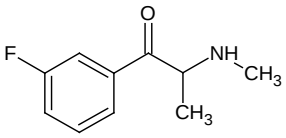
2-Mephedrone



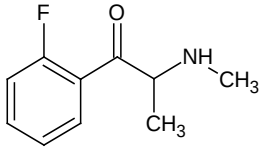
4-Flephedrone



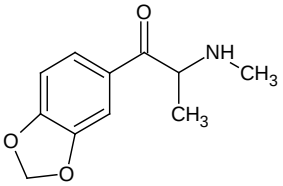
3-Flephedrone



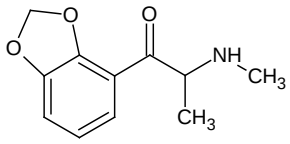
2-Flephedrone



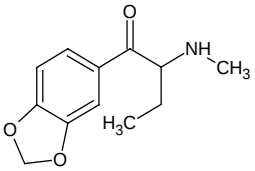
Methylone



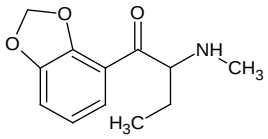
2,3-methylone



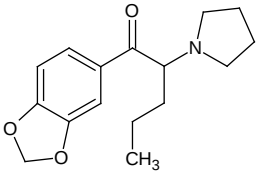
butylone



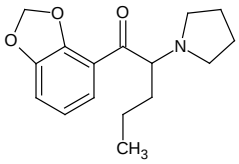
2,3-butylone



methylenedioxypyrovalerone (MDPV)



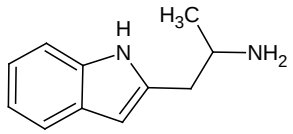
2,3-MDPV



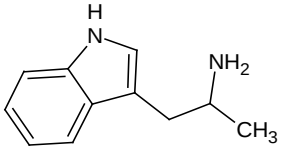
147. Scott, K. R. et al. Identification of (2-aminopropyl)indole positional isomers in forensic samples. Drug Testing and Analysis. 6 (2014) 598-606. DOI: 10.1002/dta.1508.

In 2012, 5-(2-aminopropyl)indole (5-API, 5-IT) was reported by Norwegian authorities to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) via the Early Warning System (EWS). The 3-isomer, 3-(2-aminopropyl)indole (3-API, AMT, alpha-methyltryptamine), has been available on the recreational drugs market for a somewhat longer time, having first been reported to the EMCDDA by Finnish authorities in 2001. Both isomers are available from online vendors of 'legal highs'. Recently, three forensic drug cases (two tablets and one powder) were presented for routine analysis and the active constituent was tentatively identified as an API isomer. The six positional isomers (2-, 3-, 4-, 5-, 6- and 7-(2-aminopropyl)indoles) were synthesized and analyses by a combination gas chromatography–mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS) showed that these could be readily discriminated thus facilitating the identification of 3-API in the tablets and 5-API in the powder. With exception of 5- and 6-APIs, which co-eluted, it was found possible to separate the isomers by GC without derivatization. LC separation also proved to be a feasible method for the discrimination of the isomers. Although the 2- and 7- isomers were not fully resolved by LC, it was found possible to distinguish them using their product ion spectra as the 2- isomer produced the m/z 132 fragment ion formed by loss of vinylamine, whereas the 7- isomer formed m/z 158 through loss of methylamine. In the synthesis 2-API, a novel tricyclic by-product was formed in an annulation reaction where the reaction solvent, tetrahydrofuran, was incorporated into the molecule

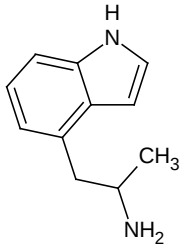
2-(2-aminopropyl)indole



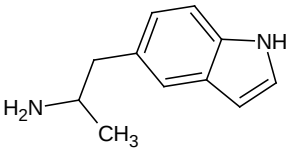
3- (2-aminopropyl)indole



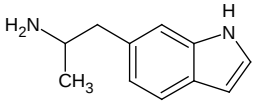
4- (2-aminopropyl)indole



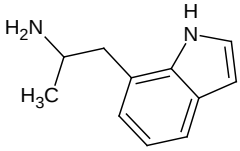
5- (2-aminopropyl)indole



6- (2-aminopropyl)indole



7-(2-aminopropyl)indole

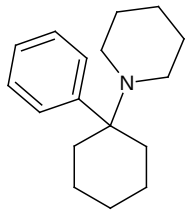


148. Wallach, J. et al. Preparation and characterization of the 'research chemical' diphenidine, its pyrrolidine analogue, and their 2,2-diphenylethyl isomers. Drug Testing and Analysis. (2014). DOI: 10.1002/dta.1689

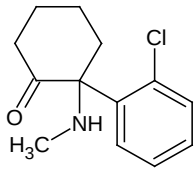
Substances with the diphenylethylamine nucleus represent a recent addition to the product catalog of dissociative agents sold as 'research chemicals' on the Internet. Diphenidine, i.e. 1-(1,2-diphenylethyl)piperidine (1,2-DEP), is such an example but detailed analytical data are less abundant. The present study describes the synthesis of diphenidine and its most obvious isomer, 1-(2,2-diphenylethyl)piperidine (2,2-DEP), in order to assess the ability to differentiate between them. Preparation and characterization were also extended to the two corresponding pyrrolidine analogues 1-(1,2-diphenylethyl)- and 1-(2,2-diphenylethyl)pyrrolidine, respectively. Analytical characterizations included high-resolution electrospray mass spectrometry (HR-ESI-MS), liquid chromatography ESI-MS/MS, gas chromatography ion trap electron and chemical ionization MS, nuclear magnetic resonance

spectroscopy (NMR) and infrared spectroscopy. Differentiation between the two isomeric pairs was possible under GC-(EI/CI)-MS conditions and included the formation of distinct iminium ions, such as m/z 174 for 1,2-DEP and m/z 98 for 2,2-DEP, respectively. The pyrrolidine counterparts demonstrated similar phenomena including the expected mass difference of 14 Da due to the lack of one methylene unit in the ring. Two samples obtained from an Internet vendor provided confirmation that diphenidine was present in both samples, concurring with the product label. Finally, it was confirmed that diphenidine (30 µM) reduced N-methyl-D-aspartate-mediated field excitatory postsynaptic potentials (NMDA-fEPSPs) to a similar extent to that of ketamine (30 µM) when using rat hippocampal slices. The appearance of 1,2- diphenylethylamines appears to reflect the exploration of alternatives to arylcyclohexylamine-type substances, such as methoxetamine, PCP and PCPy-based analogues that also show NMDA receptor activity as demonstrated here for diphenidine

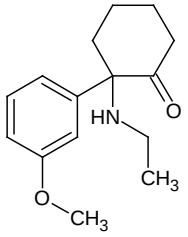
Phencyclidine



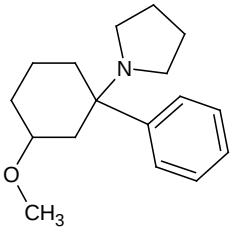
Ketamine



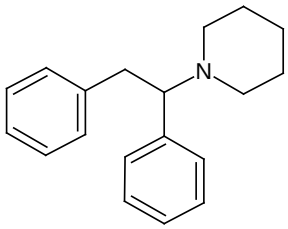
Methoxetamine



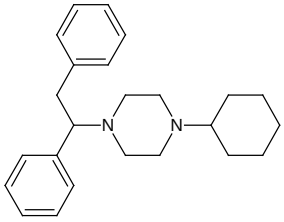
3-MeO-PCPy



1,2-DEP



MT-45



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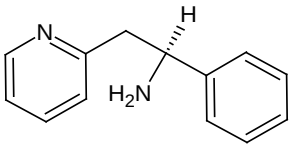
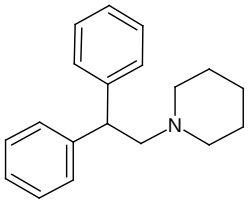
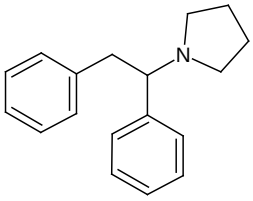


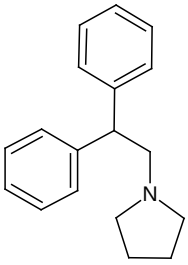
2,2-DEP



1,2DEPy



2,2-DEPy



Instruments

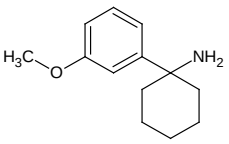
Gas Chromatography
Mass Spectrometer
High Performance Liquid Chromatography
Nuclear Magnetic Resonance

149. Wallach, J. et. al. Preparation and analytical characterization of 1-(1-phenylcyclohexyl)piperidine (PCP) and 1-(1-phenylcyclohexyl)pyrrolidine (PCPy) analogues. Drug Testing and Analysis. 7-8 (2014) 633-650. DOI:10.1002/dta.1468.

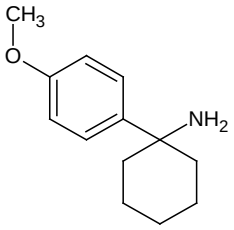
Classic examples of psychoactive arylcycloalkylamines include ketamine and 1-(1-phenylcyclohexyl)piperidine (PCP) and many others serve as important structural templates for neuropharmacological research. The recent emergence of PCP analogues that can be obtained from internet retailers requires the implementation of appropriate monitoring strategies for harm reduction purposes. Access to analytical data plays a key part when encountering these substances, especially if reference material is not available. The present study describes the synthesis of three substituted 1-(1-phenylcyclohexyl)piperidines, (3-MeO-, 4-MeO- and 3-Me-PCP) and three substituted 1-(1-phenylcyclohexyl)pyrrolidine analogues (3-MeO-, 4-MeO- and 3-Me-PCPy). Analytical characterizations of all six arylcyclohexylamines and their primary 1-phenylcyclohexanamine intermediates included gas chromatography ion trap electron- and chemical ionization and high resolution mass spectrometry, liquid chromatography electrospray hybrid triple-quadrupole linear ion trap tandem mass spectrometry, infrared, diode array detection and ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Solvent ($CDCl_3$ vs. d_6-DMSO) and protonation effects (free bases *vs* hydrochloride salts) were studied in order to investigate the impact on shifts and splitting patterns, for example, when attempting to assign separate axial and equatorial proton chemical shifts of NMR spectra. Differentiation between the isomeric 3-MeO-/4-MeO-PCP and PCPy analogues was feasible under mass spectral conditions. Gas chromatography analysis appeared to induce notable degradation of the 4-MeO-substituted analytes, especially when dealing with the HCl salts which led to the detection of the substituted 1-phenylcyclohex-1-ene nucleus. This phenomenon was observed to be less pronounced with the 3-MeO isomers, possibly due to the resonance properties of the *para*-methoxy group followed by more facile elimination of the amine.

Compounds

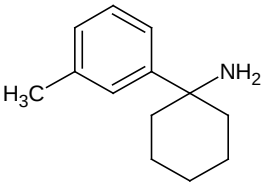
3-MeO-PCA 4-MeO-PCA 3-Me-PCA



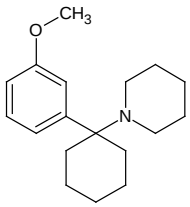
3-MeO-PCP

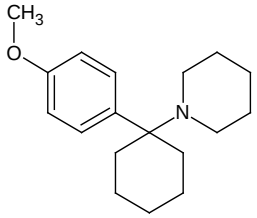


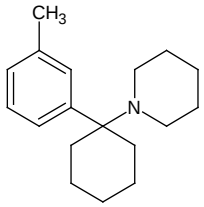
4-MeO-PCP



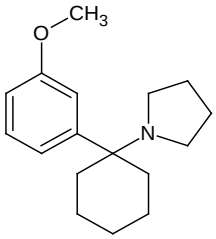
3-Me-PCP



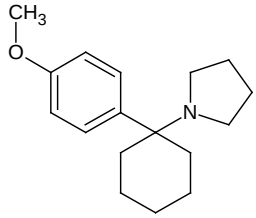




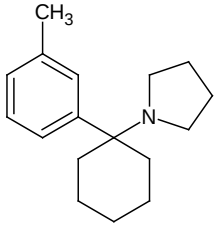
3-MeO-PCPy



4-MeO-PCPy



3-Me-PCPy



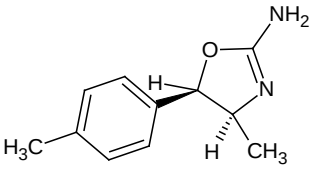
<u>Instruments</u>
Gas Chromatography
Mass Spectroscopy
High Performance Liquid Chromatography

150. Brandt, S.D., et al. Characterization of a novel and potentially lethal designer drug *(±)-cis-para*-methyl-4-methylaminorex (4,4'-DMAR, or 'Serotoni'). Drug Testing and Analysis. 7(2014) 684-695. DOI: 10.1002/dta.1668

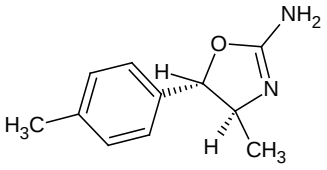
During the second half of 2013, a total of 26 deaths involving *para*-methyl-4-methylaminorex (4,4'-DMAR) were reported to the European Monitoring Centre for Drugs and Drug Addiction. While aminorex and 4-methylaminorex (4-MAR) are known psychostimulants, nothing is known about the comparatively new *para*-methyl analog. Analytical characterization of two independent samples obtained from online vendors confirmed the presence of the (±)-*cis* isomer that also appeared to be associated with at least 18 of the 26 deaths. Extensive characterizations included crystal structure

analysis, single, tandem, and high-resolution mass spectrometry, liquid and gas chromatography, and nuclear magnetic resonance spectroscopy. For the work described here, both the (±)-*cis* and (±)-*trans* racemates were also synthesized, confirming that the differentiation between these two forms was straight-forward. Monoamine transporter activity was studied using rat brain synaptosomes which included the comparison with *d*-amphetamine, aminorex and (±)-*cis*-4-MAR. (±)-*cis*-4,4'-DMAR was a potent, efficacious substrate-type releaser at transporters for dopamine, norepinephrine and serotonin with EC_{50} values of 8.6 ± 1.1 nM (DAT), 26.9 ± 5.9 nM (NET) and 18.5 ± 2.8 nM (SERT), respectively. The potency of (±)-*cis*-4,4'-DMAR at DAT and NET rivalled that of other psychomotor stimulant drugs like *d*-amphetamine and aminorex. However, (±)-*cis*-4,4'-DMAR had much more potent actions at SERT and activity at SERT varied more than 100-fold across the four drugs. The potent releasing activity of (±)-*cis*-4,4'-DMAR at all three monoamine transporters predicts a potential for serious side-effects such as psychotic symptoms, agitation, hyperthermia and cardiovascular stimulation, especially after high-dose exposure or following combination with other psychostimulants.

(±)-*cis*-4,4'-DMAR



(±)-*trans*-4,4'-DMAR

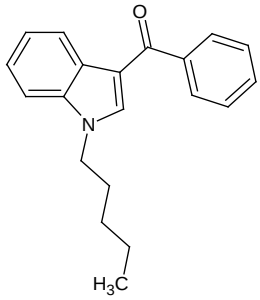


Instruments
Gas Chromatography
Mass Spectroscopy
Nuclear Magnetic Resonance
High Performance Liquid Chromatography

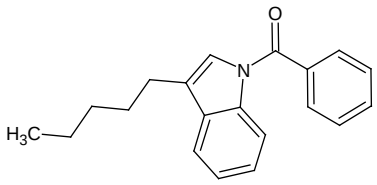
151. Smith, F. T., DeRuiter, J., Abdel-Hay, K., and Clark, C. R. Analytical Differentiation of 1-Alkyl-3-acylindoles and 1-Acyl-3-alkylindoles: Isomeric Synthetic Cannabinoids. Analytical Chemistry. 86 (2014) 3801-3808. DOI: 10.1021/ac500316x.

The 1-alkyl-3-acylindoles and the inverse regioisomeric 1-acyl-3-alkylindoles can be prepared directly from a common set of precursor materials and using similar synthetic strategies. The EI mass spectra for these isomers show a number of unique ions which allow for the differentiation of the 1-alkyl-3-acylindole compounds from the inverse regioisomeric 1-acyl-3-alkylindoles. The base peak at m/z 214 in the 1-n-pentyl-3-benzoylindole represents the M-77 cation fragment resulting from the loss of the phenyl group, and this ion is not observed in the inverse isomer. The 1-benzoyl-3-n-pentylindole inverse regioisomer shows a base peak at m/z 105 for the benzoyl cation. Thus, these two base peaks are the result of fragmentation initiated at the carbonyl-oxygen for both isomers. The 1-pentyl-3-benzoylindole is characterized by the strong intensity carbonyl band at 1703 cm^{-1}, while the amide carbonyl appears as a strong band of equal intensity at 1681 cm^{-1} in the 1-benzoyl-3-pentyl regioisomer

1-n-pentyl-3-benzoylindole



1-benzoyl-3-n-pentylindole



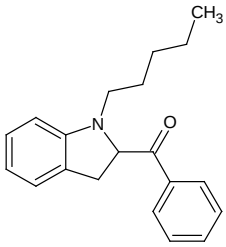
Instrument Used:

Electron Ionization Mass Spectrometry

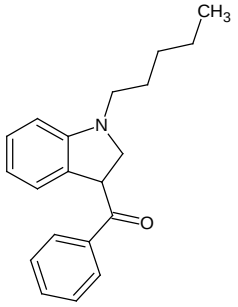
152. Smith, F. T., DeRuiter, J., Abdel-Hay, K., and Clark, C. R. GC–MS and FTIR evaluation of the six benzoyl-substituted-1-pentylindoles: Isomeric synthetic cannabinoids. Talanta. 129 (2014) 171-182. DOI: 10.1016/j.talanta.2014.05.023.

This report compares the GC-MS and FTIR properties of all 6 regioisomeric benzoyl substituted-1-n-pentylindoles. These compounds have the benzoyl-group attached at each of the possible ring substituent positions of the indole ring. The six compounds have the same elemental composition C20H21NO yielding identical nominal and exact masses. Additionally, the substituents attached to the indole ring, benzoyl- and 1-n-pentyl-groups, are identical for all six isomers. The electron ionization mass spectra show equivalent regioisomeric major fragments resulting from cleavage of the groups attached to the central indole nucleus. Fragment ions occur at m/z 77 and 105 for the phenyl and benzoyl cations common to all six regioisomeric substances. Fragmentation of the benzoyl and/or pentyl groups yields the cations at m/z 234, 220, 214, 186 and 144. While the relative abundance of the ions varies among the six regioisomeric substances the 1-n-pentyl-3-benzoylindole and 1-n-pentyl-5-benzoylindole share very similar relative abundances for the major fragment ions. Chromatographic separations on a capillary column containing a 0.5µm film of 100% trifluoropropyl methyl polysiloxane (Rtx-200) provided excellent resolution of these six compounds. The elution order appears related to the relative distance between the two indole substituted groups. The latest eluting compounds (highest retention time) have the two substituents on opposite sides of the indole nucleus. Infrared absorption spectral data show the carbonyl absorption band for each of the benzoylindoles and provide distinguishing and characteristic information to individualize each of the regioisomers in this set of compounds.

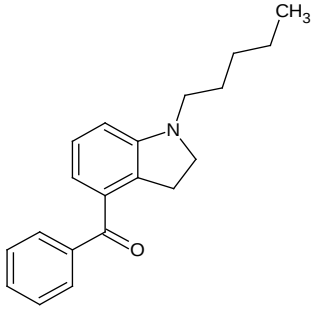
2-benzoyl-1-pentylindole



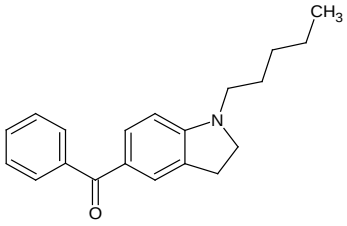
3-benzoyl-1-pentylindole



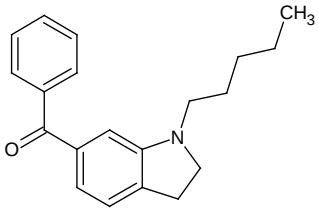
5-benzoyl-1-pentylindole



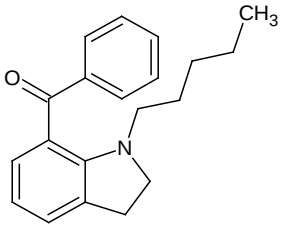
6-benzoyl-1-pentylindole



7-benzoyl-1-pentylindole



8-benzoyl-1-pentylindole

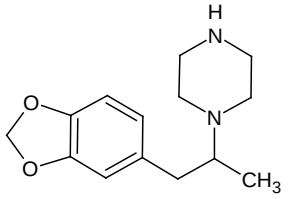


153. Abdel-Hay, K. M., DeRuiter, J., and Clark, C. R. Differentiation of the 1-(methylenedioxyphenyl)-2-piperazinopropanes and 1-(methoxyphenyl)-2-piperazinopropanones by GC-IRD and GC–MS. For. Sci. Int. 235 (2014) 40-51. DOI: 10.1016/j.forsciint.2013.11.015.

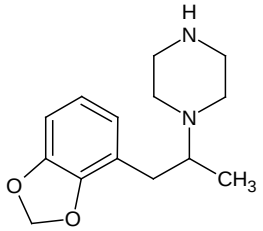
Two amphetamine-like piperazine-containing compounds, 1-(3,4-methylenedioxyphenyl)-2-piperazinopropane (3,4-MDPPP), its positional isomer 1-(2,3-methylenedioxyphenyl)-2-piperazinopropane (2,3-MDPPP) and three methcathinone-like piperazine-containing regioisomeric ring substituted 1-(methoxyphenyl)-2-piperazinopropanones (OMePPPOs) have identical elemental composition and no marked differences in their mass spectra. Perfluoroacylation of the secondary amine nitrogen of these isomeric piperazines gave mass spectra with differences in the relative abundance of some fragment ions but did not alter the fragmentation pathway to provide unique ions for discrimination among these isomers.

Gas chromatography coupled to infrared detection (GC-IRD) provides direct confirmatory data for the identification of the carbonyl containing compounds and the differentiation of the 3,4-MDPPP from its direct (2,3-MDPPP) and indirect (OMePPPOs) regioisomers. The vapor phase infrared spectra provide for specific confirmation of each of the isomeric piperazines. The perfluoroacyl derivative forms of the five piperazines involved in this study were resolved on two stationary phases, the first is composed of 100% dimethyl polysiloxane (Rtx-1) and the second of 5% diphenyl and 95% dimethyl polysiloxane (Rtx-5).

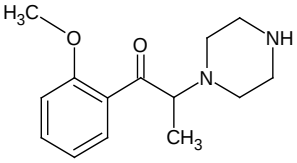
Compounds



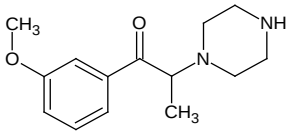
3,4-MDPPP



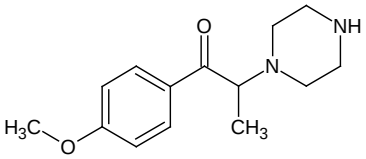
2,3-MDPPP



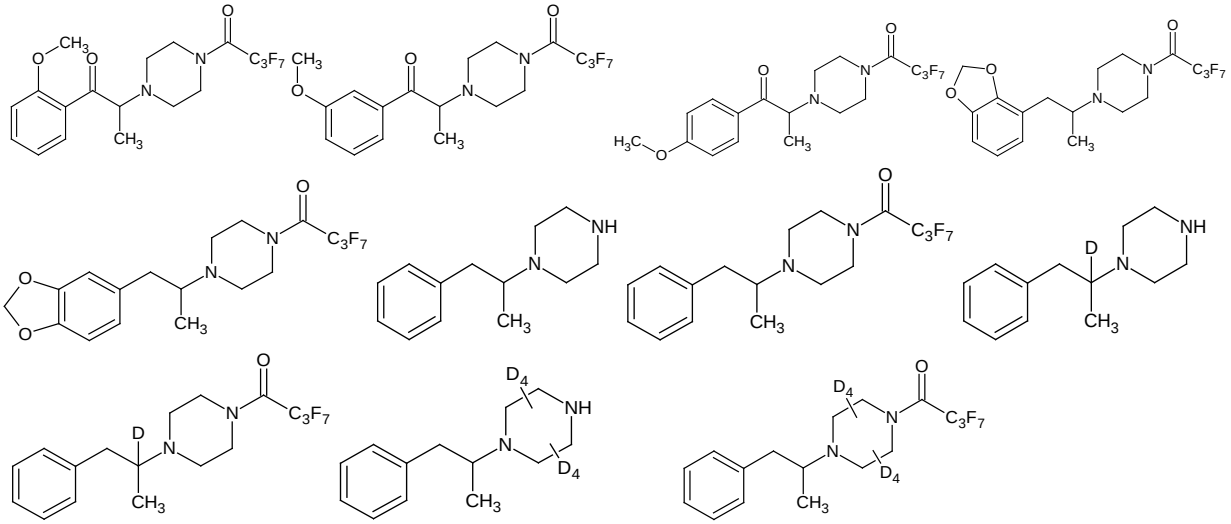
o-OMePPPO



m-OMePPPO



p-OMePPPO



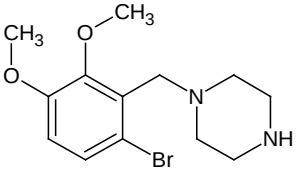
Instruments
Gas Chromatography
Mass Spectroscopy
Infrared Spectroscopy

154. Abdel-Hay, K. M., DeRuiter, J., and Clark, C. R. Regioisomeric bromodimethoxy benzyl piperazines related to the designer substance 4-bromo-2,5-dimethoxybenzylpiperazine: GC–MS and FTIR analysis. For. Sci. Int. 240 (2014) 126-136. DOI: 10.1016/j.forsciint.2014.04.019

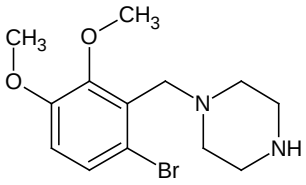
A series of seven regioisomeric bromodimethoxy benzyl piperazines including the designer benzylpiperazine (4-bromo-2,5-dimethoxybenzylpiperazine) were synthesized and their analytical profiles evaluated using GC-MS and FT-IR. The mass spectra for the seven regioisomeric

bromodimethoxy benzyl piperazines are almost identical with only the two 2,3-dimethoxy isomers showing one unique major fragment ion at m/z 214/216. Thus, mass spectrometry alone does not provide for the confirmation of identity of any one of the seven compounds to the exclusion of the other isomers. Perfluoroacylation of the secondary amine nitrogen for each of the seven regioisomers gave mass spectra showing some differences in the relative abundance of fragment ions without the appearance of any unique fragments for specific confirmation of structure. Attenuated total reflection infrared spectroscopy provides direct confirmatory data for differentiation between the seven regioisomeric aromatic ring substituted bromodimethoxy benzyl piperazines. Mixtures of the seven piperazine PFP derivatives were successfully resolved via capillary gas chromatography using a relatively polar stationary phase composed of 100% trifluoropropyl methyl polysiloxane

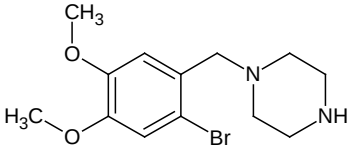
Compounds



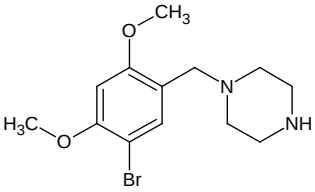
6-Br-2,3-DMBP



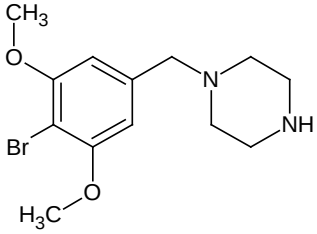
5-Br-2,3-DMBP



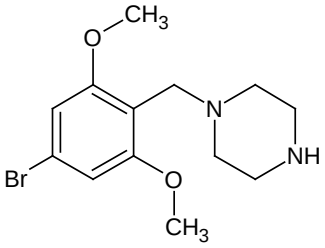
2-Br-4,5-DMBP



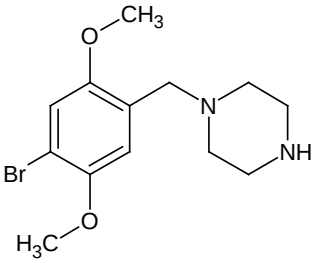
5-Br-2,4-DMBP



4-Br-3,5-DMBP



4-Br-2,6-DMBP



4-Br-2,5-DMBP

Instruments

Gas Chromatography
Mass Spectroscopy
Atenuated Total Reflectance
Fourier Transmission Infrared Spectroscopy

155. Abdel-Hay, K. M., Terrell, C. M., DeRuiter, J., and Clark, C. R. GC–MS and IR studies on the six ring regioisomeric dimethoxybenzoyl-N-methylpiperazines (DMBzMPs). For. Sci. Int. 237 (2014) 53-61. DOI: 10.1016/j.forsciint.2014.01.010.

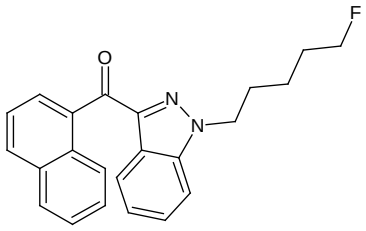
The complete series of regioisomeric dimethoxybenzoyl-N-methylpiperazines were synthesized and evaluated in GC-MS and FTIR studies. The EI mass spectra show fragment ions characteristic of both the dimethoxybenzoyl and the N-methylpiperazine portions of the molecules. These characteristic fragments include the dimethoxybenzoyl cation at m/z 165 as well as the m/z 99 N-methylpiperazine cation and the low mass cation species at m/z 56 (C3H6 N(+)) and the m/z 70 ion (C4H8N(+)). Unique radical cations characteristic for the benzoyl-N-methylpiperazines were observed at m/z 83 (C5H9 N(+)) and m/z 207 (C11H13NO3(+)) Deuterium labeling experiments were used to characterize the mechanism of formation of these fragment ions. Attenuated total reflection infrared spectroscopy provides direct confirmatory data for the differentiation between the six regioisomeric aromatic ring substituted dimethoxybenzoyl-N-methylpiperazines. Gas chromatographic separation of this series of compounds was accomplished on an Rtx-200 stationary phase and relative retention appears related to the degree of steric crowding of the aromatic ring substituents. The most crowded patterns of substitution elute first while the more symmetrical 1-, 3-, 5-substitution pattern has the highest retention time.

156. Li, L. and Lurie, I. S. Screening of seized emerging drugs by ultra-high performance liquid chromatography with photodiode array ultraviolet and mass spectrometric detection. For. Sci. Int. 237 (2014) 100-111. DOI: 10.1016/j.forsciint.2014.01.018.

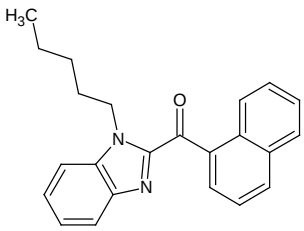
The use of psychoactive "designer drugs" has increased rapidly due to their varying and sometimes ambiguous legal status and their ready access via the Internet and at local "headshops." A quick screening method for samples containing these substances, using ultra-high performance liquid chromatography with photodiode array UV and mass spectrometric detection (UHPLC-PDA/UV-MS), is presented. The method enables the screening of a variety of samples containing emerging/reemerging drugs, including β-keto phenethylamines (cathinone derivatives), synthetic cannabinoids/cannabimimetics, and phenethylamine derivatives. The use of dual detectors not only provides molecular weight information but also differentiates the drugs by their categories and in some cases even their sub-categories. Moreover, ring positional isomers of cathinone and phenethylamine derivatives can be easily differentiated by their retention times and UV spectra

157. Shevyrin, V. et al. 3-Naphthoylindazoles and 2-naphthoylbenzoimidazoles as novel chemical groups of synthetic cannabinoids: Chemical structure elucidation, analytical characteristics and identification of the first representatives in smoke mixtures. For. Sci. Int. 242 (2014) 72-80. DOI: 10.1016/j.forsciint.2014.06.022.

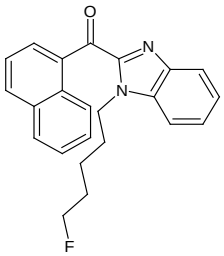
By means of gas chromatography with mass spectrometry detection (GC-MS), including high resolution mass spectrometry (GC-HRMS) together with ultra-high performance liquid chromatography in combination with high resolution tandem mass spectrometry (UHPLC-HRMS), nuclear magnetic resonance spectroscopy (NMR) and Fourier transform infrared spectroscopy (FT-IR), structure of novel synthetic cannabinoids, namely, 1-(5-fluoropentyl)-1H-indazol-3-yl(naphthalen-1-yl)methanone, naphthalen-1-yl(1-pentyl-1H-benzo[d]imidazol-2-yl)methanone and 1-(5-fluoropentyl)-1H-benzo[d]imidazol-2-yl(naphthalen-1-yl)methanone was established. Analytical data obtained in the paper enable reliable identification of these compounds during qualitative analysis of seizures, including smoke mixtures.



AM-2201



BIM-018

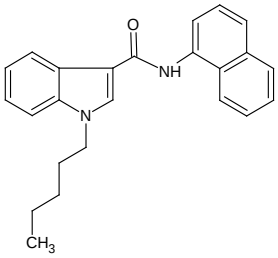


BIM-2201

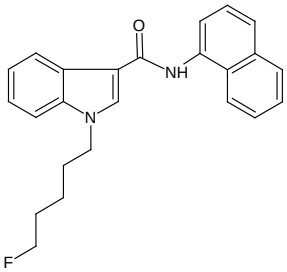
Compounds
Gas Chromatography
Mass Spectroscopy
Nuclear Magnetic Resonance
Infrared Spectroscopy

158. Uchiyama, N. et al. Characterization of four new designer drugs, 5-chloro-NNEI, NNEI indazole analog, α-PHPP and α-POP, with 11 newly distributed designer drugs in illegal products. For. Sci. Int. 243 (2014) 1-13. DOI: 10.1016/j.forsciint.2014.03.013.

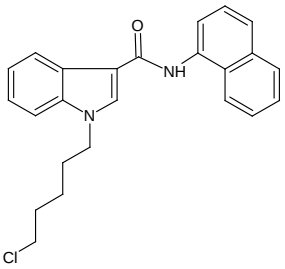
Our continuous survey of illegal products in Japan revealed the new distribution of 15 designer drugs. We identified four synthetic cannabinoids, i.e., NNEI (1), 5-fluoro-NNEI (2), 5-chloro-NNEI (3) and NNEI indazole analog (4), and seven cathinone derivatives, i.e., MPHP (5), α-PHPP (6), α-POP (7), 3,4-dimethoxy-α-PVP (8), 4-fluoro-α-PVP (9), α-ethylaminopentiophenone (10) and N-ethyl-4-methylpentedrone (11). We also determined LY-2183240 (12) and its 2'-isomer (13), which were reported to inhibit endocannabinoid uptake, a methylphenidate analog, 3,4-dichloromethylphenidate (14), and an MDA analog, 5-APDB (15). No chemical and pharmaceutical data for compounds 3, 4, 6 and 7 had been reported, making this the first report on these compounds.



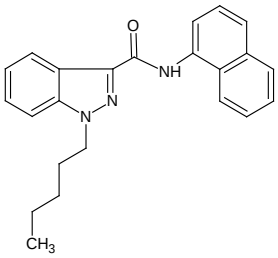
NNEI



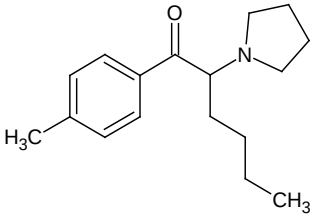
5-fluoro-NNEI



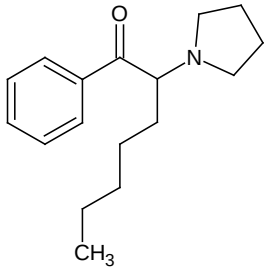
5-chloro-NNEI



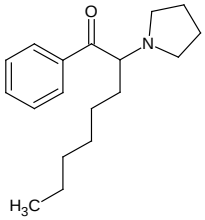
NNEI indazole analog



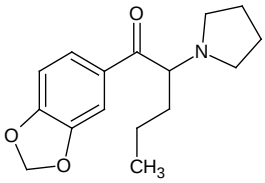
MPHP



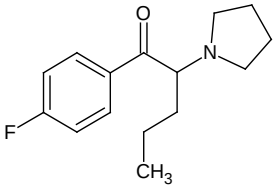
α-PHPP



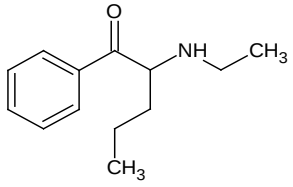
α-POP



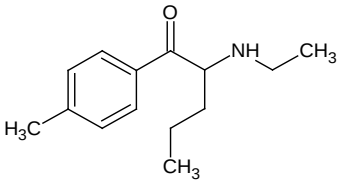
3,4-dimethoxy-α-PVP



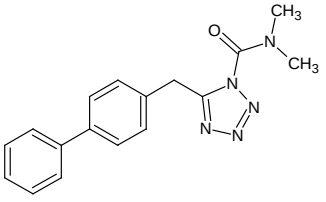
4-fluoro-α-PVP



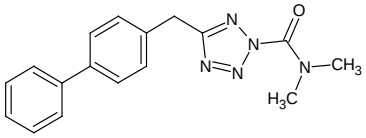
α-ethylaminopentiophenone



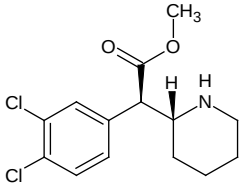
N-ethyl-4-methylpentedrone



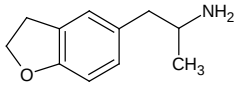
LY-2183240



LY-2183240 2'-isomer



3,4-dichloromethylphenidate



5-APDB

159. Chartrand, M., et al, Compound specific isotope analysis of hexachlorocyclohexane isomers: a method for source fingerprinting and field investigation of *in situ* biodegradation. Rapid Communication in Mass Spectrometry. 6 (2015) 505-514.

RATIONALE

The manufacturing and uses of hexachlorocyclohexane (HCH) have resulted in a serious environmental challenge and legacy. This study highlights the ability of compound specific isotope analysis (CSIA) to distinguish among various HCH sources and to support the evaluation of the potential for *in situ* biodegradation in contaminated groundwater.

METHODS

Tests were conducted to verify the absence of significant isotope fractionation during HCH sample pre-concentration including dichloromethane extraction, solvent exchange into iso-octane, and H_2SO_4 clean-up, and analysis by gas chromatography/combustion-isotope ratio mass spectrometry (GC/C-IRMS). The method was then applied to four Technical Grade (TG) HCH mixtures procured from different sources and to groundwater samples from a contaminated site.

RESULTS

The pre-concentration method enabled determination of carbon isotope ratios ($\delta^{13}C$ values) of HCH isomers with no significant isotopic fractionation. The TG-HCH mixtures had significantly different $\delta^{13}C$ values. Moreover, for any given TG-HCH, all isomers had $\delta^{13}C$ values within 1.1‰ of each other – a distinctly uniform fingerprint. At the HCH-contaminated field site, compared with source wells, downgradient wells showed significant (up to 5.1‰) enrichment in ^{13}C and the $\delta^{13}C$ values of the HCH isomers were significantly different from each other.

CONCLUSIONS

A method was successfully developed for the CSIA of HCH isomers that showed potential for HCH source differentiation and identification of HCH *in situ* biodegradation. At the HCH-contaminated site, the observed preferential isotopic enrichment of certain isomers relative to others for a given source allows differentiation between biodegraded and non-biodegraded HCH

<u>Instruments</u>

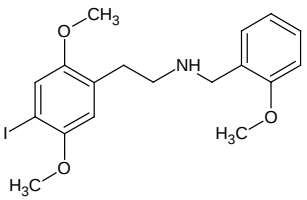
Gas chromatography

Combustion-isotope ratio mass spectrometry

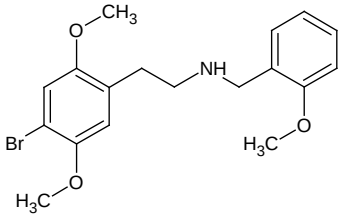
160. Brandt, S.D. Analytical characterization of bioactive *N*-benzyl-substituted phenethylamines and 5-methoxytryptamines. Rapid Communications in Mass Spectrometry. 7 (2015) 573-584. DOI: 10.1002/rcm.7134

The characterization of 18 'NBOMe' compounds provided a comprehensive collection of chromatographic and spectral data. Four groups of three positional isomers, i.e. 25I-NB2OMe, 25I-NB3OMe, 25I-NB4OMe, 25I-NB2B, 25I-NB3B, 25I-NB4B and their 5-methoxytryptamine counterparts, were included and assessed for ability to obtain differentiation. Six *meta*-substituted *N*-benzyl derivatives of 5-methoxytryptamine (CF_3, F, CH_3, Cl, I, SCH_3) were also studied.

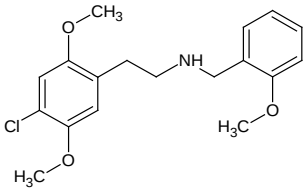
Compounds



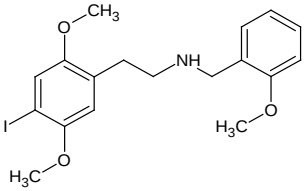
25I-NBOMe



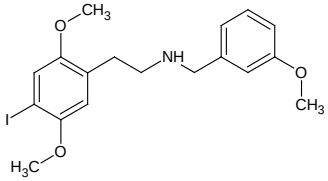
25B-NBOMe



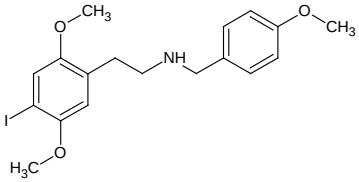
25C-NBOMe



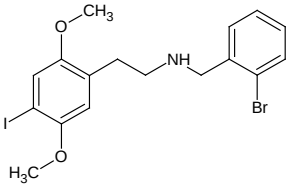
25I-NB2OMe



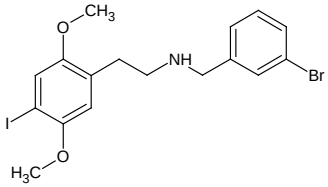
25I-NB3OMe



25I-NB4OMe



25I-NB2B



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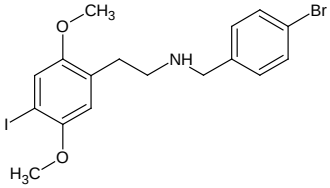

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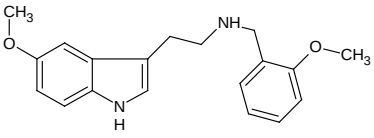
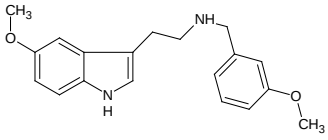
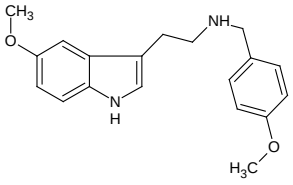


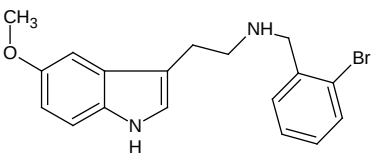
5MT-NB2OMe



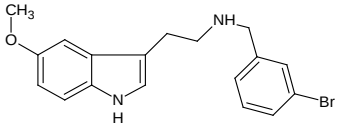
5MT-NB3OMe



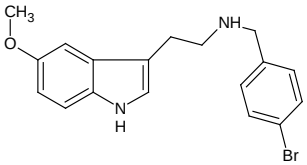
5MT-NB4OMe



5MT-NB2B



5MT-NB3B



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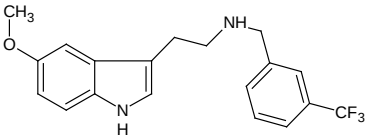
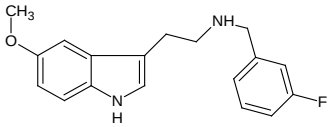
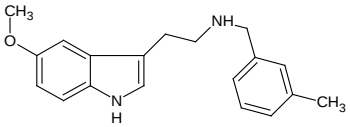


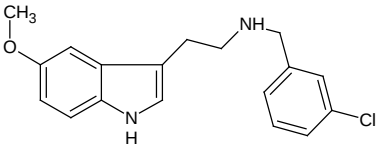
5MT-NB3TFM



5MT-NB3F



5MT-NB3Me



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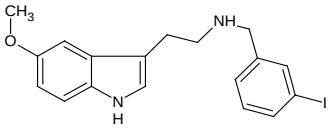

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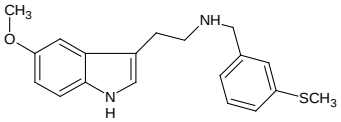


5MT-NB3SMe

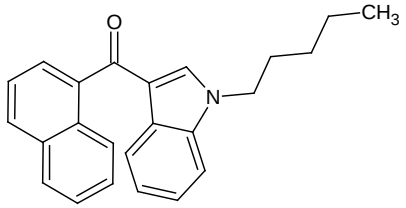
Instruments
High Performance Liquid Chromatography
Gas Chromatography
Mass Spectrometry

161. Amber Thaxton, Tarek S. Belal, Forrest Smith, Jack DeRuiter, Karim M. Abdel-Hay and C. Randall Clark. Mass spectral studies on 1-n-pentyl-3-(1-naphthoyl)indole (JWH-018), three deuterium-labeled analogues and the inverse isomer 1-naphthoyl-3-n-pentylindole. Rapid Communications in Mass Spectrometry. 2015, 29, 871–877. DOI: 10.1002/rcm.7171

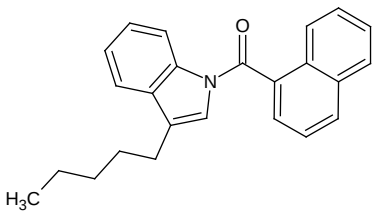
A number of synthetic cannabinoids such as the 1-alkyl-3-acylindoles are the target of significant designer drug activity. One of the first waves of these compounds identified in clandestine samples was 1-n-pentyl-3-(1-naphthoyl)indole, JWH-018. These totally synthetic molecules can be prepared in a number of regioisomeric forms.

Compounds

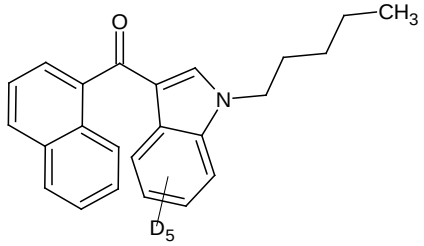
1-n-pentyl-3-(1-naphthoyl)indole (JWH-018)



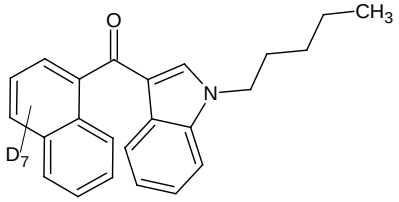
1-naphthoyl-3-n-pentylindole



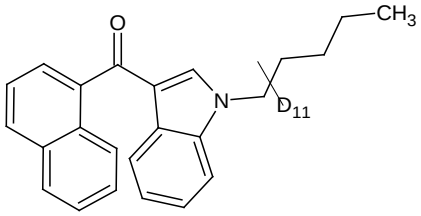
1-n-pentyl-3-(1-naphthoyl)-d5-indole



1-n-pentyl-3-(1-d7-naphthoyl)indole



1-d11-n-pentyl-3-(1-naphthoyl)indole



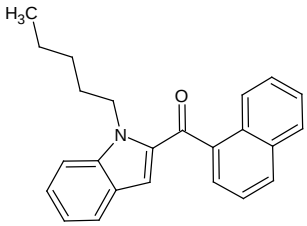
Instruments
Gas Chromatography
Mass Spectroscopy

162. Amber Thaxton, Tarek S. Belal, Forrest Smith, Jack DeRuiter, Karim M. Abdel-Hay , C. Randall Clark. GC–MS studies on the six naphthoyl-substituted 1-n-pentyl-indoles: JWH-018 and five regioisomeric equivalents. Forensic Science International 252 (2015) 107–113.

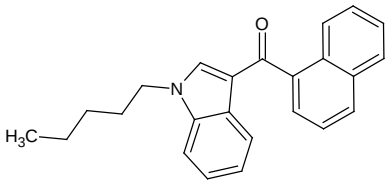
The GC–MS properties of the synthetic cannabinoid drug of abuse 3-(1-naphthoyl)-1-pentylindole (JWH-018) and all 5 of its' regioisomeric 1-naphthoyl substituted 1-n-pentylindoles are compared in this report. These compounds have the 1-naphthoyl-group attached at each of the possible substituent positions of the indole ring. The six compounds have the same elemental composition C24H23NO and the same substituents attached to the indole ring. The electron ionization mass spectra showed equivalent regioisomeric major fragment ions resulting from cleavage of the groups attached to the central indole nucleus. The characteristic (M_17)+ fragment ion at m/z 324 resulting from the loss of an OH group was significant in the EI-MS of 3-, 4-, 5- and 6-(1-naphthoyl)-1-pentylindole. Fragment ions occurred at m/z 127 and 155 for the naphthyl and naphthoyl cations common to all six regioisomeric substances. Indole containing fragments produced the cations at m/z 284, 270, 214 and 186. The unique fragment at m/z 141 observed in the 1,2- and 1,7-isomers resulted from a rearrangement involving the two indole substituents to yield the C10H7CH2+ cation. The major points of EI-MS differentiation of the synthetic cannabinoid JWH-018 from the other five isomers are the high

relative abundance of both the m/z 144 ion and the m/z 324 ion in the JWH-018 spectrum. GC separations on a capillary column containing a trifluoropropyl methyl polysiloxane (Rtx-200) stationary phase provided excellent resolution of these six compounds. The elution order appears related to the relative distance between the two indole substituents with the lowest retention associated with minimum distance between the groups attached to the indole nucleus.

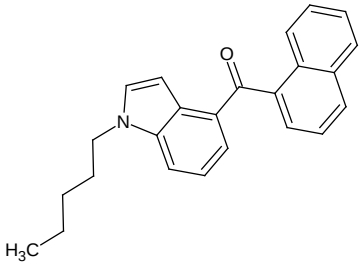
Compounds



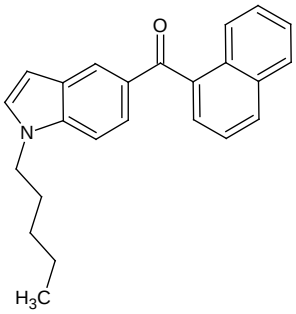
2-(1-naphthoyl)-1-pentylindole



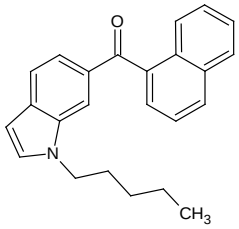
3-(1-naphthoyl)-1-pentylindole



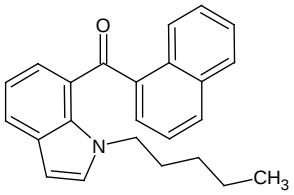
4-(1-naphthoyl)-1-pentylindole



5-(1-naphthoyl)-1-pentylindole



6-(1-naphthoyl)-1-pentylindole



7-(1-naphthoyl)-1-pentylindole

Instruments

Gas Chromatography

Mass Spectroscopy