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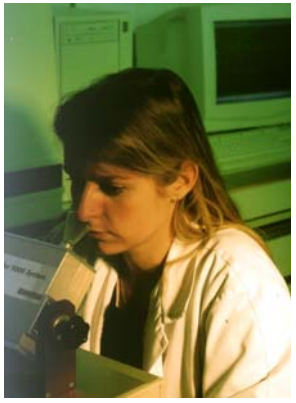
Switzerland

Gas Chromatography-Olfactometry GC-O

The state of the Art



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GC-O Preface

- Smell figures prominently in our enjoyment of life.
- Flavors and aromas contribute much to the appeal of food, whereas perfumes or fragrances can evoke powerful emotions and memories.
- Unquestionably then, flavors and fragrances are important to consumers.
- As a consequence, they are also important to researchers, developers and manufacturers of foods, perfumes as well as household and beauty products.

GC-O Preface

- The discovery of volatile compounds really matter to our overall perception of the complex mixtures that typify natural flavors and fragrances.

- Identification and quantification of such aroma or odor-active compounds has many important motivations, including the following……

GC-O: Important motivations

- Correlating sensory responses with volatile chemicals
- Selecting compounds useful for monitoring flavor changes during shelf life, processing and so on
- Resolving off-flavor problems
- Understanding flavor release during eating
- Augmenting creative flavor compounding
- Targeting flavor compounds for thermal or biological generation
- Assessing olfactory acuity of individuals
- Elucidating the mechanism of odor receptors

GC-O: GC-O in brief

- Gas Chromatography-Olfactometry or GC-O refers to the sniffing of GC effluent to determine which components posses odor.
- Many of the peaks detected by the GC do not actually contribute to our perception of flavors or fragrances, because they are present below our thresholds for detecting them.
- The emergence of GC-O in the 1960s and 1970s was a landmark development in flavor, aroma and fragrance research
- It provides valuable information about the areas of gas chromatogram on which to focus attention and resources.

GC-O: GC-O technical history I

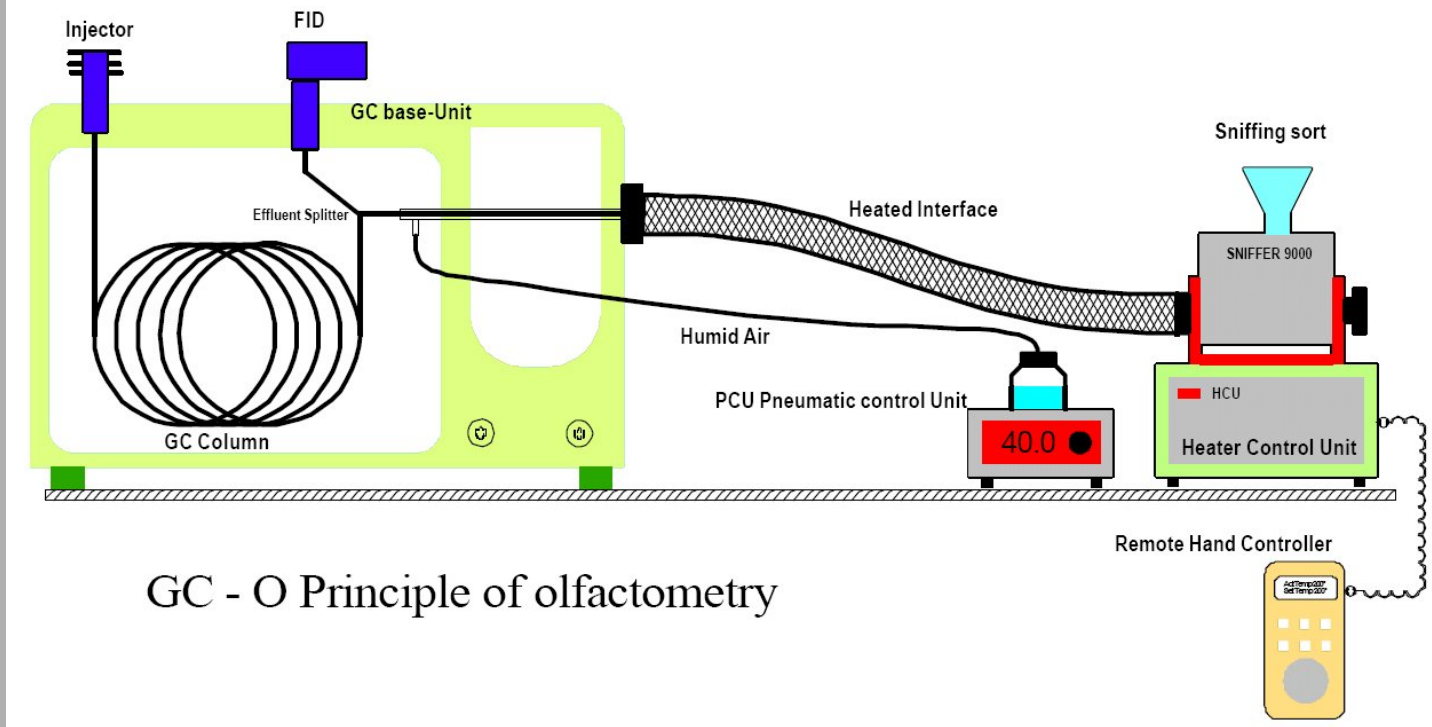
- In the early 1970s the most of the GC Olfactive ports have been mounted directly on the top or on the side of a GC.
- The idea was to have a short way from the Splitt in the oven to the Olfactive port.
- The inconvenience of this mounting technique was the fact that the uncomfortable position of the panelist did not allow to concentrate on the smelling and in most cases the temperature of the detector outlets as well as the oven was to high

GC-O: GC-O technical history II

- Between 1970 and 1995 the most of the olfactive systems has been installed in the way mentioned above and nobody did really developing a real comfortable system to help the flavor chemists to make their life much more easier.

- Brechbühler AG well known in the GC and GC/MS market started in 1996 to redesign their complete niche product range for the GC market and one of these products is the SNIFFER 9000.

GC-O: SNIFFER 9000
The principle



GC - O Principle of olfactometry

GC-O: SNIFFER 9000
the Instrument

- The Sniffer 9000 has been designed to be installed on any GC available on the market.
- Focusing on the aspect that panelists have to be comfortable during their work.
- And the fact that sometimes the need of more then one panelists one one GC run is required.
- Brechbühler AG did develop a unique system dedicated to the real needs of flavor, fragrances and aroma chemistry

GC-O: SNIFFER 9000
3 port system

- The Sniffer 9000 can be installed up to three systems to one GC.
- In some cases there is the need to have more then one panelist's to smell on one analysis.
- Therefore Brechbühler AG did develop a special installation kit to mount up to three SNIFFER 9000 systems to one GC
- This helps to increase efficiency in developing new products.

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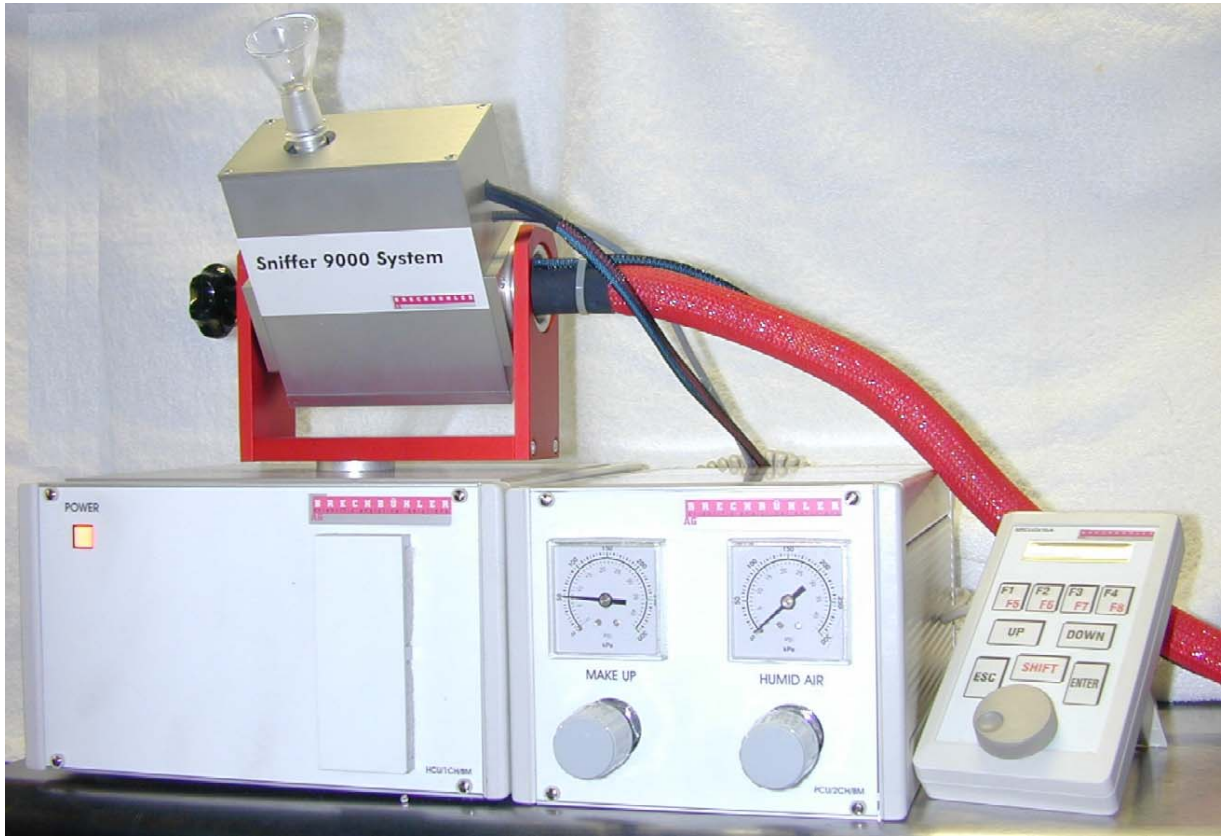
GC-O Challenges

- Challenging technique
  - *Combine scientific response and human perception…*
  - *Human factor is very important*
  - *Panelist has to stay focus*

The Sniffer 9000: GC-O in mind

The Sniffer 9000



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Sniffer 9000: Complete GC-O

The Trace GC and Sniffer 9000

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Sniffer 9000: Multi port

The Sniffer 9000: Multi port system

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The Sniffer 9000: Options

Fingerspan

Software

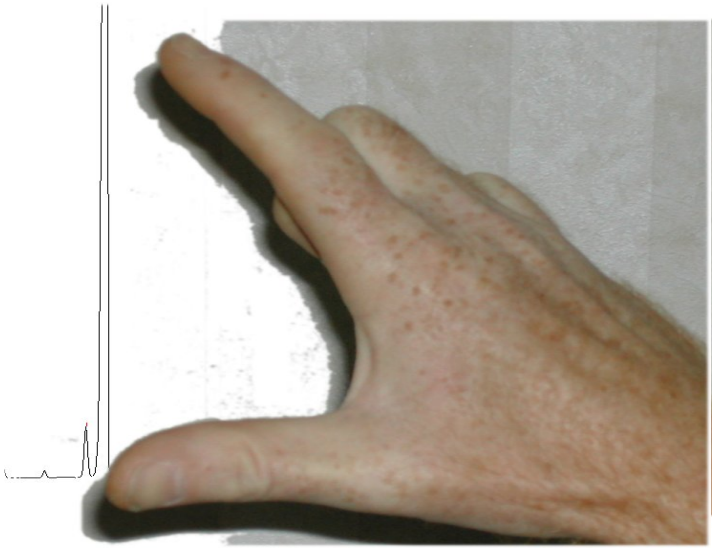
Training kit

Nose to Text

Sniffer 9000: Fingerspan option

Accurate and intuitive measurement of odor intensity

The intensity is proportional to the spread of the fingers



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Sniffer 9000: Software option

Software

Programming of the interface temperature

Full support of the Fingerspan with dynamic scale adjustment

Additional output range

One key intensity recording (on or off)

Sniffer 9000: Training/Test Kit

Training kit

Ideal for novice to GC-O

Includes:

column and fittings

Compound mixture

Documentation including method and compound description

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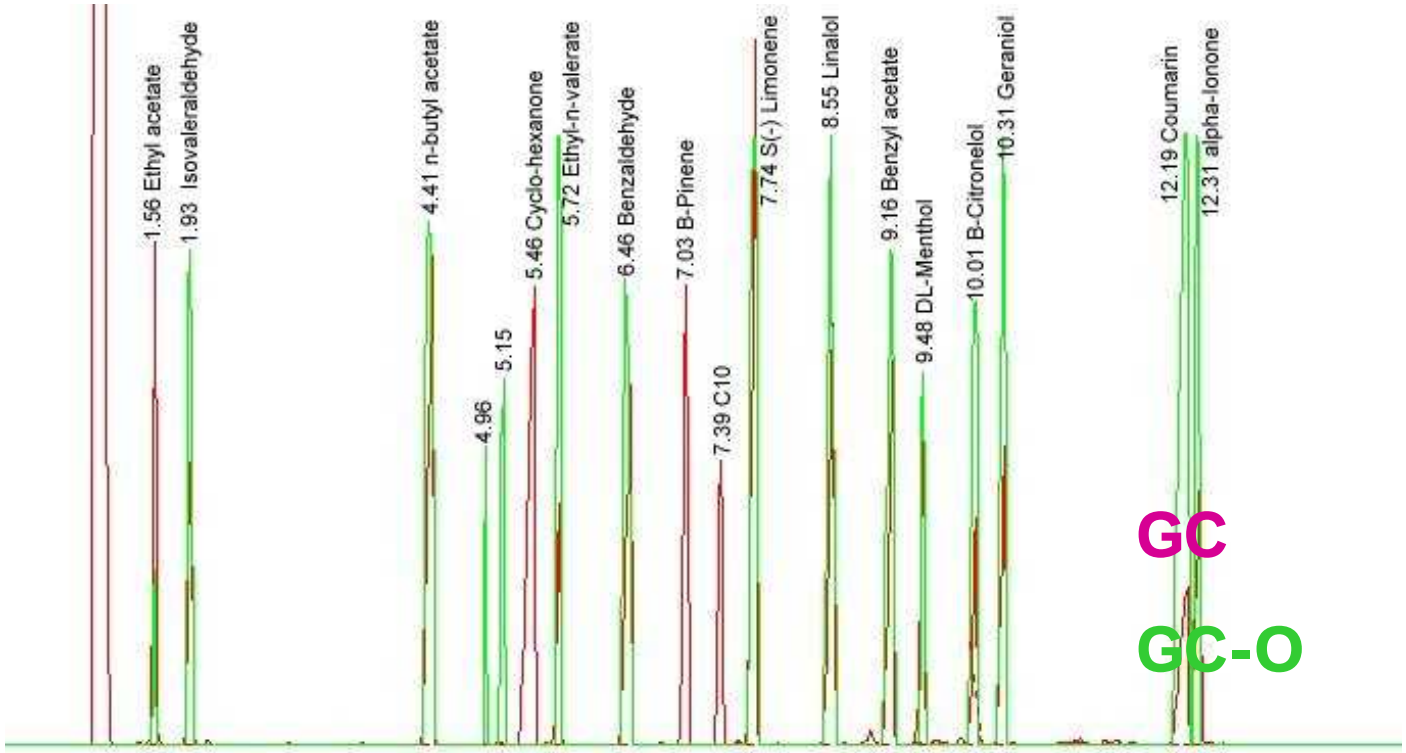
Sniffer 9000: Training/Test kit

Results from Test and training kit



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Sniffer 9000: Training/Test kit

Results from Test and training kit

N°	Name	Formula	Other name	Concentration	Odor
1	Ethyl Acetate	$C_4H_8O_2$	Acetic acid ethyl ester	6,1 µg/µl	Fruity Faint
2	Isovaleraldehyde	$C_5H_{10}O$	3-methylbutylraldehyde	4,9 µg/µl	Acid, Rancid
3	n-Butyl Acetate	$C_6H_{12}O_2$	Acetic acid butyl ester	6,1 µg/µl	Candy, pinapple Faint
?	Valeric Acid?		Decomposition? Very small peak		Acid, Sweat
4	Cyclo-Hexanone	$C_6H_{10}O$	$C_6H_{10}O$	6,1 µg/µl	Peppermint, acetone ND
5	Ethyl n-Valerate	$C_7H_{14}O_2$	Pentanoic acid ethyl ester	3,7 µg/µl	Apple Strong
6	Benzaldehyde	C_7H_6O	Aldéhyde Benzoïque	6,1 µg/µl	Almond, Sweet
7	β-Pinene	$C_{10}H_{16}$	6,6-dimethyl-2-methylenbicyclo[3.1.1] Heptane ou 2,6,6-Trimethylbicyclo[3.1.1]hept-2ene;2-pinene;	7,3 µg/µl	Wood, pine ND
8	C_{10}		Alcane	2,6 µg/µl	No odor
9	S(-) Limonene	$C_{10}H_{16}$	(S)-4-isopropenyl-1-methyl cyclohexene; (-)-p-Mentha-1,8-diene ou 1-Methyl-4-(1-methylthenyl)cyclohexene; p-mentha-1,8-diene;	4,9 µg/µl	Lemon like. Mild
10	Linalool	$C_{10}H_{18}O$	3,7dimethyl-1,6-octadien-3-ol	6,1 µg/µl	Citrus, orange Mild
11	Benzyl acetate	$C_9H_{10}O2$	Acetic acid benzyl ester	6,1 µg/µl	Pear like
12	DL-Menthol	$C_{10}H_{20}O$	Hexahydrothymol;2-isopropyl-5-methylcyclohexanol ou 5-Methyl-2-(1-methylethyl)cyclohexanol;3-p-menthanol	3,7 µg/µl	Mint persistent
13	β-Citronellol	$C_{10}H_{20}O$	3,7-Diméthyl-6-octen-1-ol	2,6 µg/µl	Lemon Strong
14	Geraniol	$C_{10}H_{18}O$	Trans-3,7-dimethyl-2,6-octadien-1-ol	3,7 µg/µl	Rose Strong
15	Coumarin	$C_9H_6O_2$	1-Benzopyran-2-one	3,7 µg/µl	Vanilla Persistent
16	α-ionone	$C_{13}H_{20}O$	4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	3,7 µg/µl	Cedar, forest Sweet

Sniffer 9000: Nose to text

Voice recognition software

for GC-O

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Nose to Text

Personal assistant for GC-O

- Voice recognition software based on the best recognition software available
- Intuitive, easy to use interface
- Dedicated to GC-O
- Reports data in multiple types
- Library search capabilities

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Nose to Text: Interface

Nose to Text

File Edit View Vocabulary Send... Help

Philippe

RT (min)	Data
1.20	orange
1.50	almond
1.88	fruity
2.07	musky
2.51	coumarin
2.92	dill
3.57	Strawberry
3.70	pineapple
3.83	blackcurrent
3.98	forest
4.38	nice smell
4.55	woody
4.92	coffee
5.12	cigarette
5.39	tobacco
5.86	fresh
5.95	Acquisition completed

Ready

Inject

Stop

GC-O Comments

Done 8/8/01 5:16 I

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Nose to Text: Reports

Reporting: Straight GC-O data reporting

datafile.txt - Notepad

File Edit Format Help

```
Report generated by Nose to text
Speaker:        Philippe
Report created on       8/24/01 at 1:06:02 PM

Comments        My comments.

RT      Data

0.04    banana
0.17    faint
0.24    nice
0.63    Apple
0.71    strong
0.74    Fruity
2.62    strawberry
2.66    strong
2.71    Fruity
3.31    forest
3.46    mild
3.51    earthy
4.00    Acquisition completed
```

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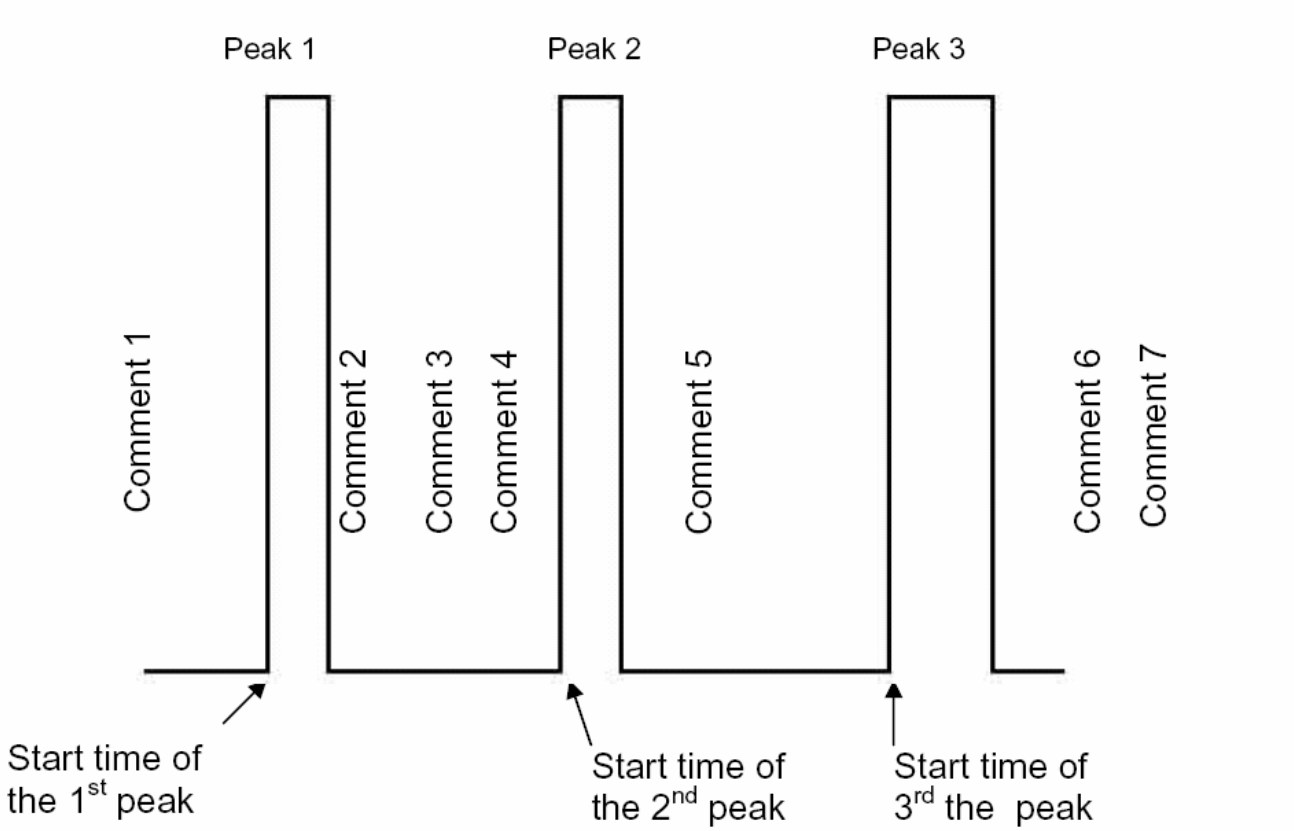
Nose to Text: Odorogram

Example applying NTT results to the Odorogram



Nose to Text: GC Reports

Merging of GC-O data and GC report

Chrom-Card Report - Modified by Nose to Text

```
Method Name    :
Method File    : C:\...\Thermo Finnigan\Chrom-Card 32 bit for TRACE\data\
Chromatogram   :
GC Method      :
Operator ID    :                          Company  Name : C.E. Instruments
Analysed       : 25.06.1991  10:22        Printed       : 26.07.2001  18:19
Sample ID      :             (# 1)        Channel       : Channel A
Analysis Type  : Calibration (Area)       Calc.  Method : External STD

Calib. method : using Response Factors
```

Component Name	Ret.Time	Area	Resp. Fact.	Rel.Ret.Time	GC-O Results
1	0.29	341716	0.0000	0.5062	herbaceous
2	0.80	68727	0.0000	0.5551	citrus
3	1.05	80736	0.0000	0.5795	orange almond
Peak aaa	1.75	901622	901622.0000	0.7419	fruity musky
Peak bbb	2.44	1856328	185633E+07	1.0000	coumarin dill
Peak ccc	3.16	2914266	.291427E+07	0.7198	
7	3.29	54130	0.0000	0.8364	Strawberry pineapple
Peak ddd	3.78	3575910	.357591E+07	0.8632	blackcurrent forest
Peak eee	4.28	3654779	.365478E+07	1.0000	
10	4.36	80449	0.0000	1.0591	nice smell woody coffee
11	4.93	189235	0.0000	1.0903	cigarette tobacco fresh
Totals		28402010			

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Nose to Text reporting

Display of recognized data on the GC trace

Custom macro available to display data on the Chromatogram.

Available for Agilent's chemstation

Reads the data from Nose to Text report and display the data recognized on the chromatogram displayed.

Under development for Thermoquest's Chromcard

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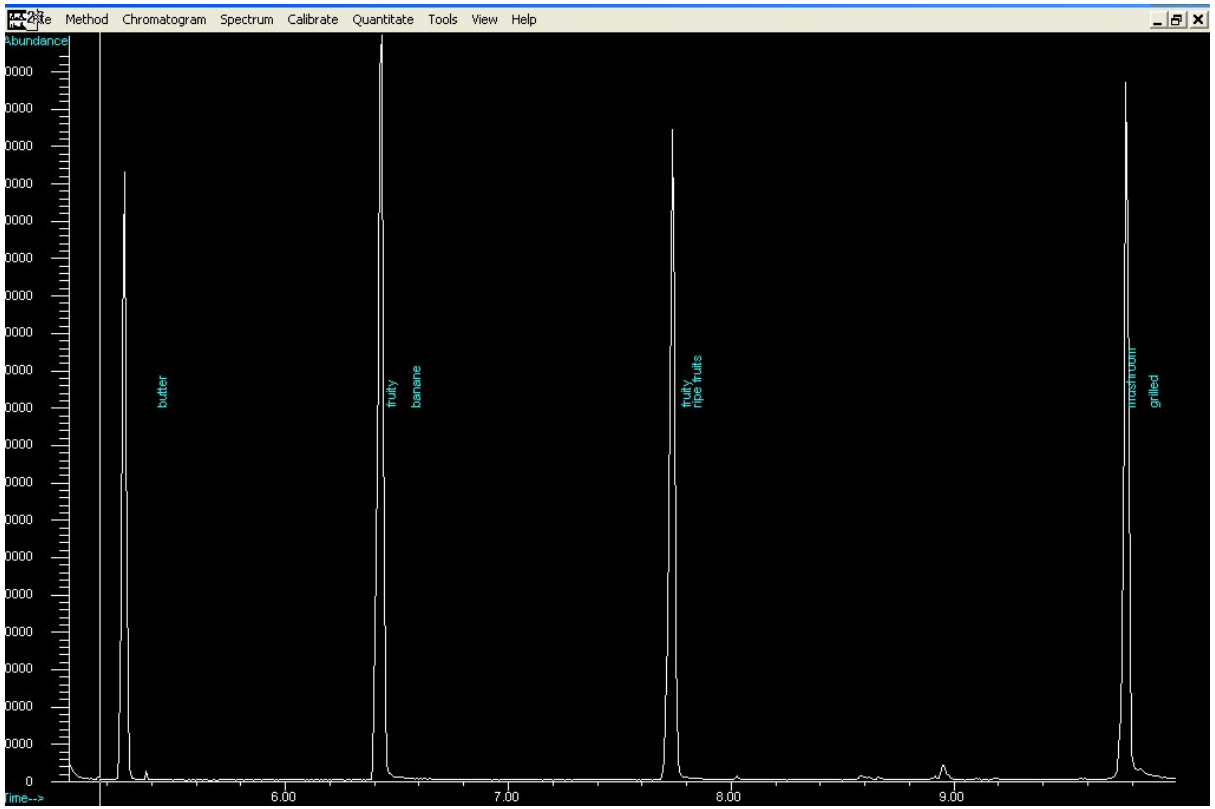
Nose to Text reporting

Display of recognized data on the GC trace



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Nose to Text

Library search capability

Custom odor library created from text file or other libraries:

Available parameters

- Compound name
- Odor description
- 6 custom fields for synonyms, MW, formula, CAS, etc…
- Retention indices for up to 10 columns

Nose to Text Library

Search within search

Narrow the search by using retention indices

GC-O Search - C:\Program Files\DevStudio\VB\Project\Nose to text\Ntt1.gco

Search by: Odor

keyword: Banana

search within results

use index from: 700 to: 1300

Column selected: DB5

Search

Print

Save as...

Calculate index

68	banana	3-methylbutyl acetate	876
116	green banana	(Z)-3-hexenylacetate	1009
162	banana	pentyl butanoate	1094

Search results

3 matches found

Library: Ntt1.gco - 356 entries
Kovats retention index

Load library

Edit Library

Exit

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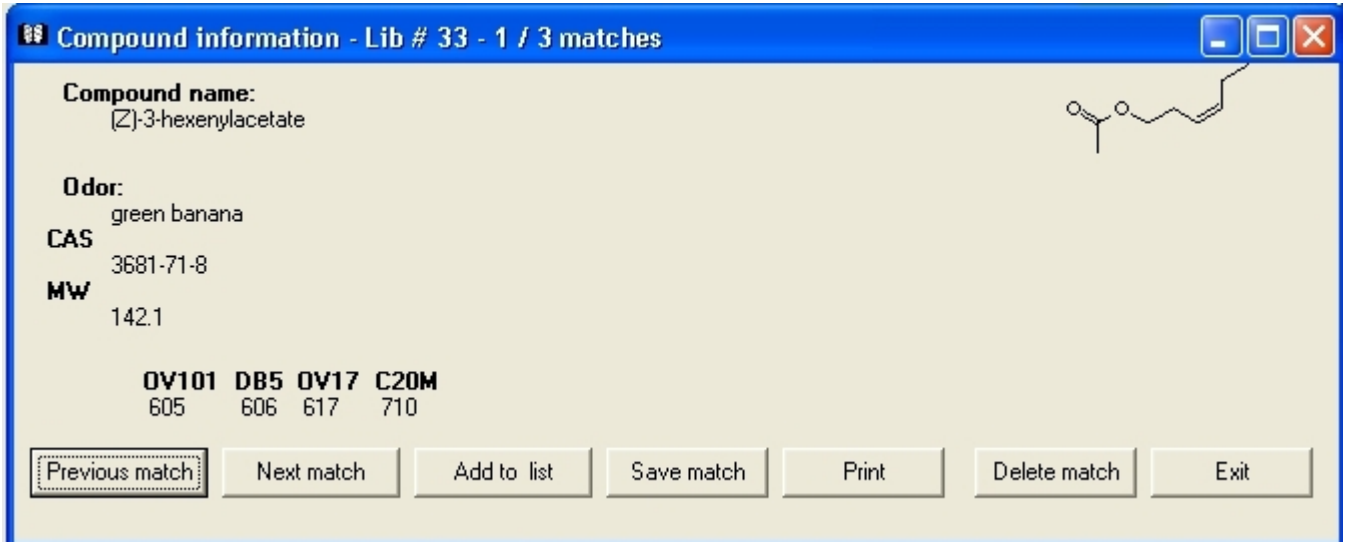
Nose to Text Library



Compound information

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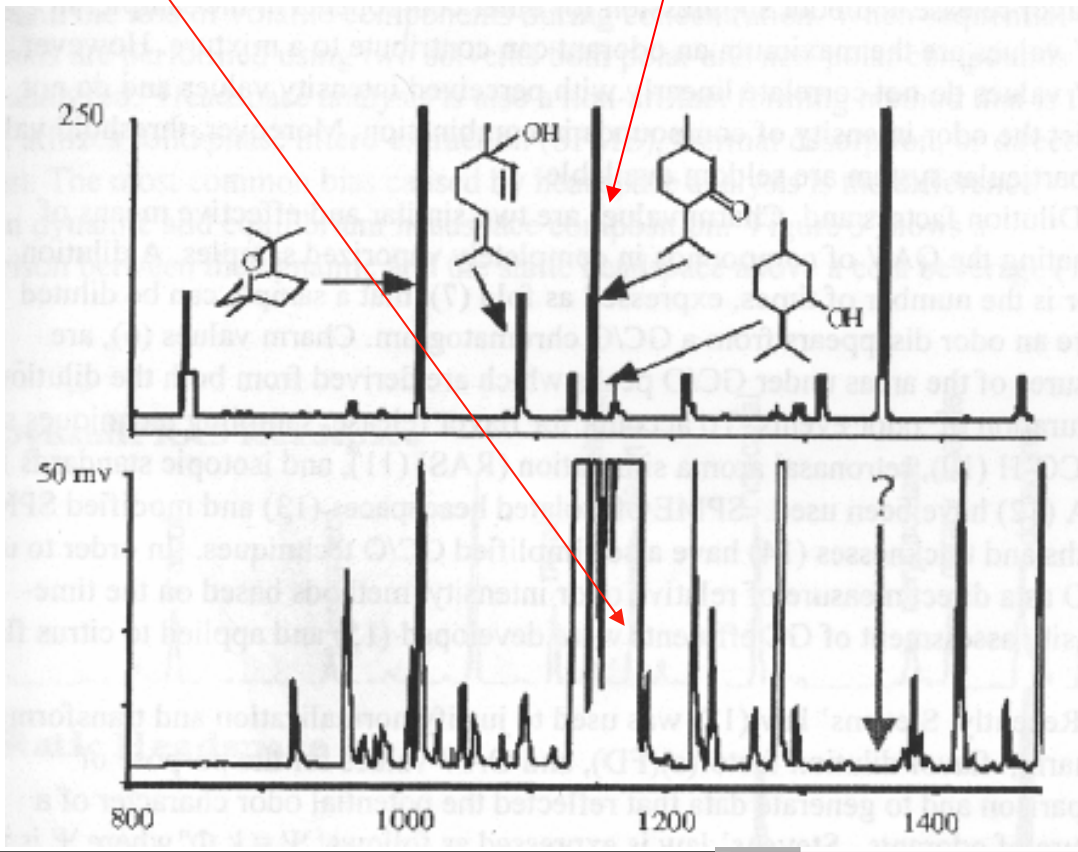
Applications

Applications can be applied to:

- Flavor and Fragrances industries
- Food and Aroma analysis industries
- Water industries
- Polymer industries
- Packaging industries
- And many others……

Applications
Example Peppermint oil

- The most abundant Peak in the FID Trace, Menthol, Shows very little odor potency in the GC-O Odorogramm

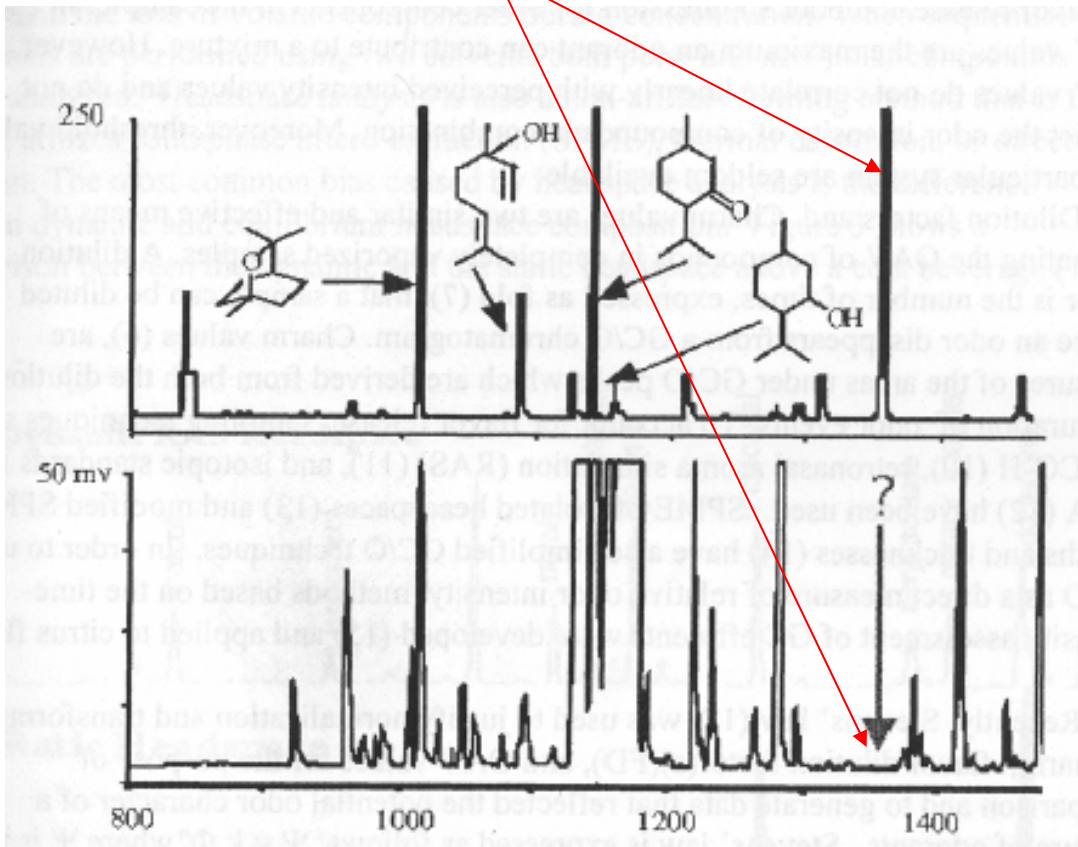


GC-O

FID

Applications
Example Peppermint oil

- In contrast the most odour potent compound in the GC-O Odorogramm shows no or just little response in the FID



GC-O

FID

Applications
Example Water

- DESIGN AND APPLICATION OF A GC-SNIFF/MS SYSTEM FOR SOLVING TASTE AND ODOR EPISODES IN DRINKING WATER

- C. Hochereau and A. Bruchet
- CIRSEE - Ondeo Services, 38 rue du Président WILSON - 78230 Le Pecq

Applications
Example Water

ONDEO Services is the new name of
Lyonnaise des Eaux worldwide activities

Local companies within ONDEO Services,
will not change their name,
but will **endorse** the new brand to reinforce their
belonging to one same group:

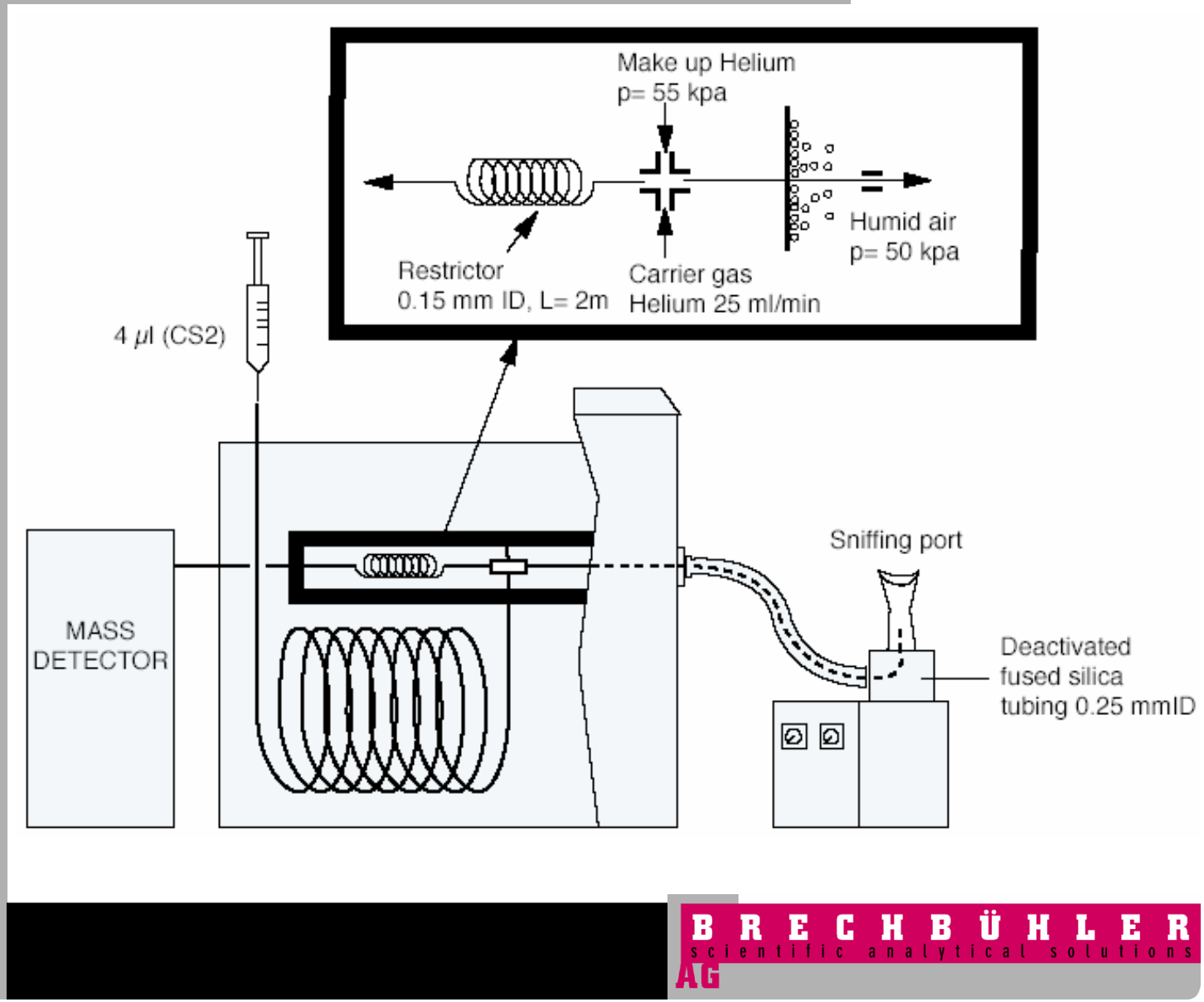
- Lyonnaise des Eaux France
- Northumbrian Water -- UK
- United Water – USA
- Palyja (Indonesia), Aguas do Amazonas (Brazil),
- Antsu (Turkey), AWS (Australia), LEMA, LEKA,

etc.

Applications
Example Water

- Chromatographic sniffing is usually carried out in two separate steps:
  - *A first Injection on GC-Olfactive (FID) Instruments to detect odorous peaks*
  - *A second injection on a GC-MS Instrument. Attempts to identify the odorous peaks are based on the similary of retentiontimes or retention indices*

Applications
Example Water



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Applications, Example Water Validation with known standards

N°	Standard GC/(SNIFF)/MS	Detection limits by GC/(SNIFF)/MS (full scan)	Detection limits by GC/SNIFF/(MS)
1	Menthone*	100 ng/L	ND
2	Methylisoborneol	2 ng/L	0.5 ng/L
3	Iodoform	> 0.5 µg/L	< 0.1 µg/L
4	Geosmin	2 ng/L	0.5 ng/L
5	2,6-Dibromophenol	10 ng/L	< 0.1 ng/L

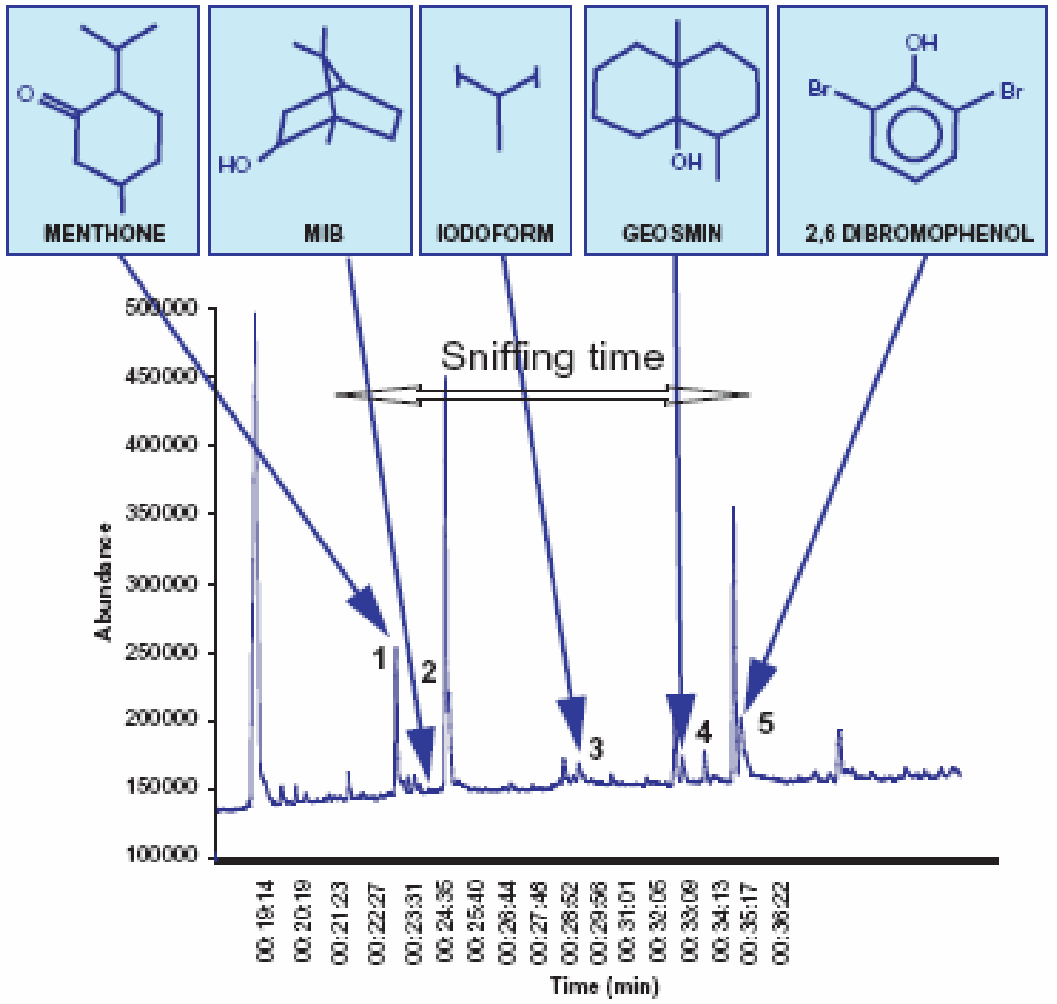
** internal standard*

Fig 3. Compared detection limits by GC-SNIFF and by GC-MS

- The performance of the GC-Sniff/MS coupling was initially tested with a mixture of menthone (used as an internal standard with sample extracts), MIB, geosmin and iodoform.

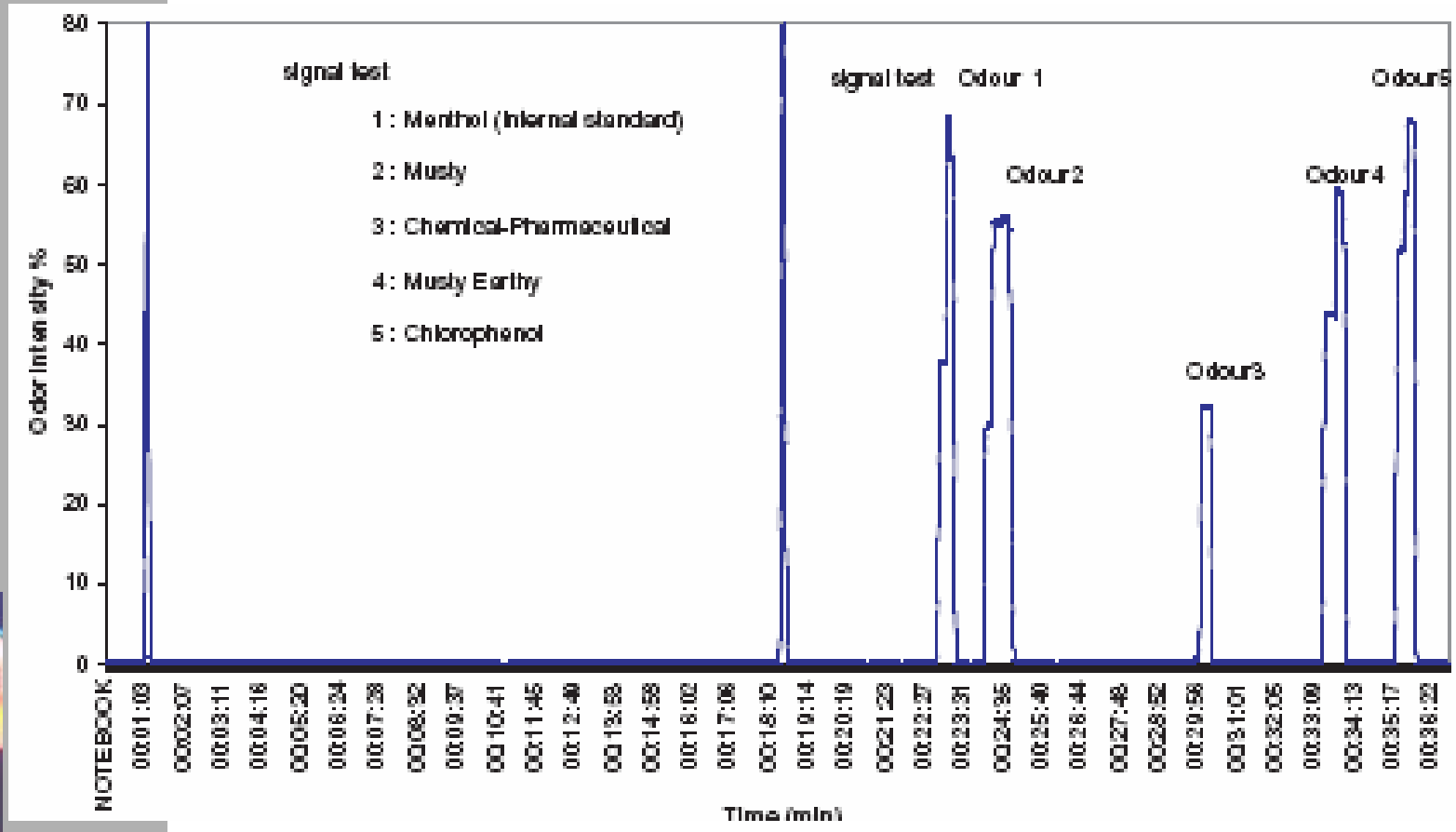
Applications, Example Water Validation with known Standards

- **GC-sniff/MS chromatogram (standard solution)**



Applications, Example Water Validation with known Standards

- **GC-sniff/MS odorogram (standard solution)**



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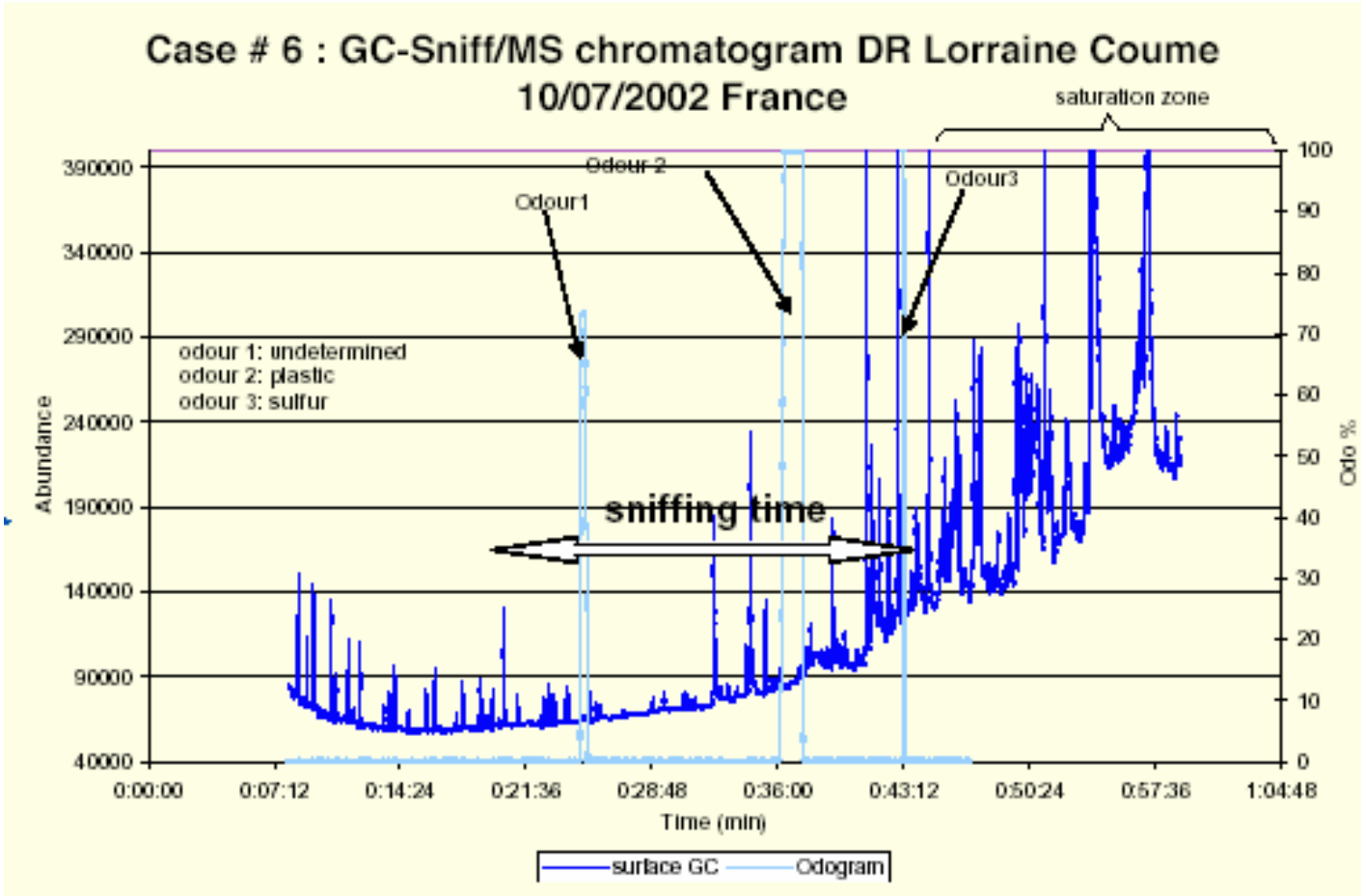
Applications, Example Water
Comparison DC/MS – GC-O



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Applications, Example Water Conclusion

- GC-SNIFF/MS provides simultaneous structural and organoleptic information. Olfactometric detection remains 10 times as sensitive as full scan MS detection
- The technique described allowed us to identify unexpected odorous causative

Applications Instrumentation

– *GC/SNIFF/MS system*

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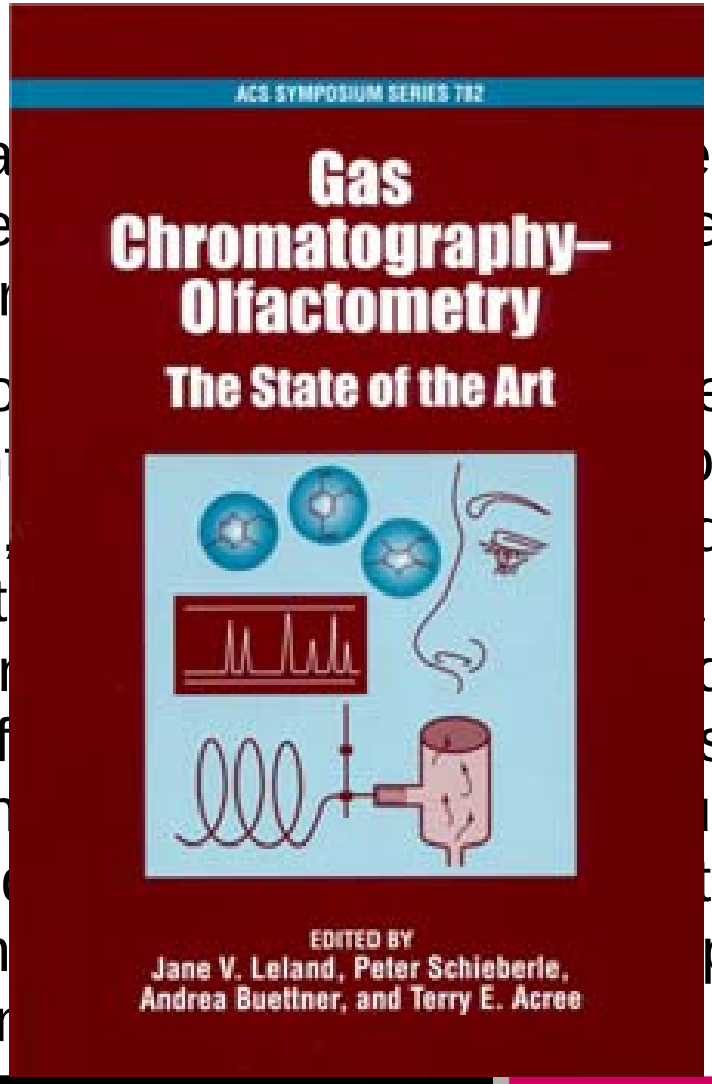
Applications, GC-O Analysis of Blanco,Reposado and Anejo Tequilas

- Tequila
  verage
  obtaine
  es from 8 to
  12 year
- Traditio
  e cooked,
  ferment
  produce
  Blanco,
  quilas.
  Recent
  flavor
  compor
  d. The main
  aims of
  sh the
  differen
  ila types
  and to
  odorants
  in each
  phy-
  olfactor



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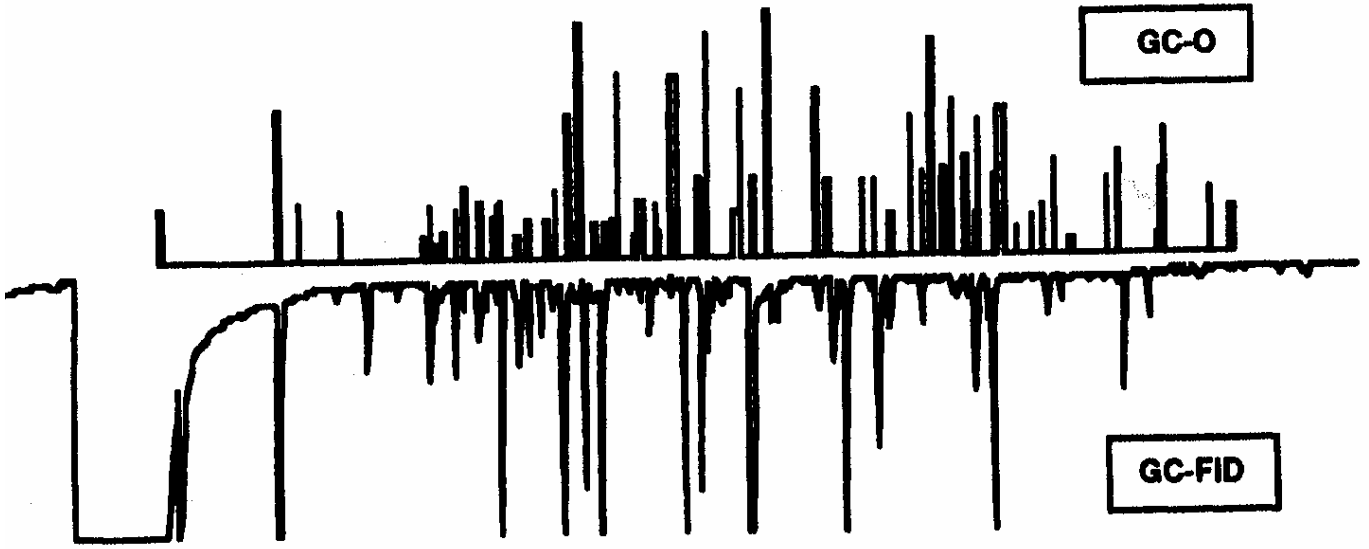
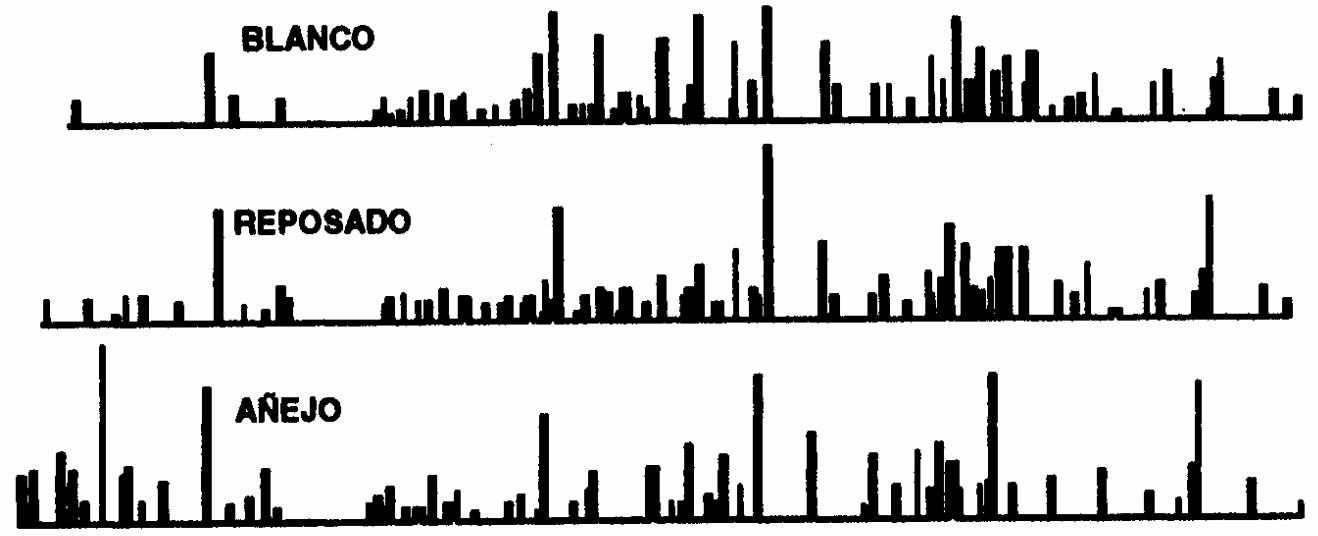
Applications, GC-O Analysis of Blanco, Reposado and Anejo Tequilas

- The main differences among the an
  tequilas were essentially quantitativ
  than qualitative. Figure 3 shows a typical
  aromagram/chromatogram profiles of a
  Blanco tequila extract.



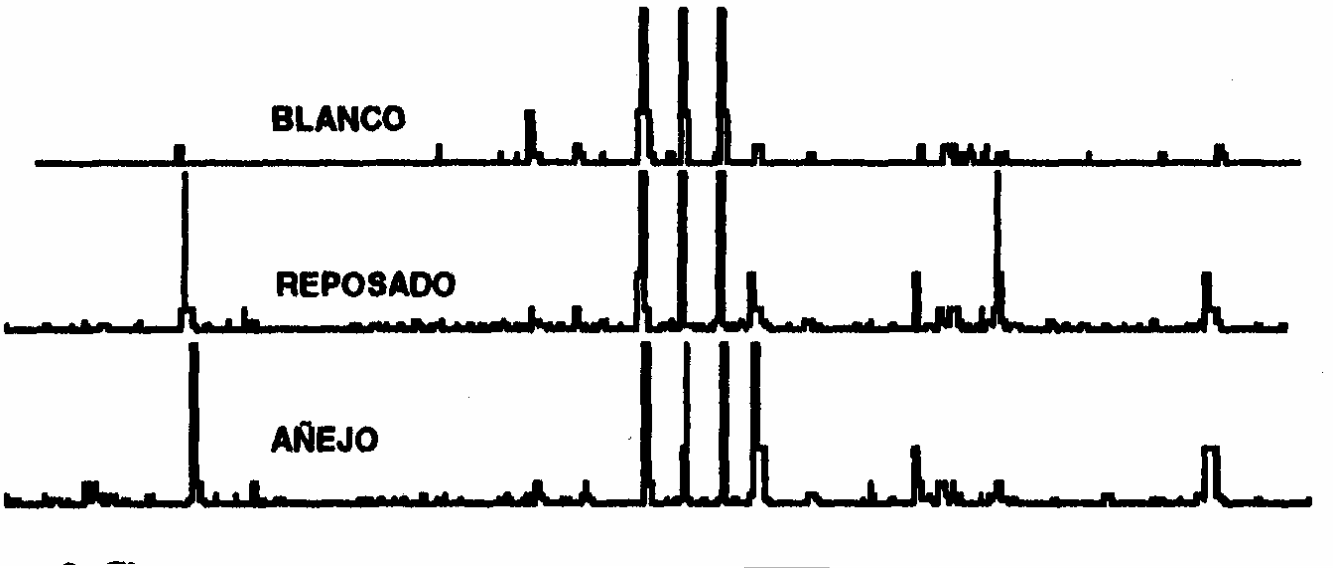
Applications, GC-O Analysis of Blanco, Reposado and Anejo Tequilas

- The figure below shows the original aromagrams of all tequilas. It is important to mention than the main differences are observed at the beginning of the aromagrams, therefore, more highly volatiles compounds are present in Reposado and Anejo tequila classes that in the non aged tequila. However, many odorants are common to all samples. Table I lists most of the volatiles that were completely characterized in all the tequila extracts.



Applications, GC-O Analysis of Blanco, Reposado and Anejo Tequilas

- The figure below presents the Odorograms of the three Tequilas a a series of dilution steps.
- It is obvious that Reposado and Anéjo have more complex aroma profile than Blanco Tequila



Applications, GC-O Analysis of Blanco, Reposado and Anejo Tequilas

- This table lists the most potent odorants in all tequilas.

KI	Compounds	Descriptor	Charm values		
			Blanco	Reposado	Añejo
1030	Unknown	Solvent	748	845	2842
1200	Butanol, 3-methyl	Alcohol, vinous	2407	2065	6515
1659	Decanoic acid ethyl ester	Fatty	267	400	357
1809	Phenylethyl acetate	Tepache, floral	1564	2415	3035
1862	Unknown	Medicinal	880	1501	2221
1906	Phenlylethyl alcohol	Sweet, floral	6083	4560	7771
1953	Unknown	Plastic		1644	16956
2166	Eugenol	Medicinal, sweet	941	1498	2403
2201	Terpenoid	Chicken	1259	2241	4733
2266	Decanoic acid	Fatty		411	2102
2555	Vainillin	Vainilla, sweet	1959	3641	5510

Applications, GC-O Analysis of Blanco, Reposado and Anejo Tequilas

- Conclusion
- It can be concluded that the Añejo class has more complex overall aroma. Most of these odorants must be generated during the aging process in white oak barrels.
- However, it is also important to mention an this point, that three of these compounds are still unknown, therefore, it is crucial to carry out more experiments using larger tequila volumes to be able to characterize these compounds by GC-MS

Sniffer 9000 Customer references

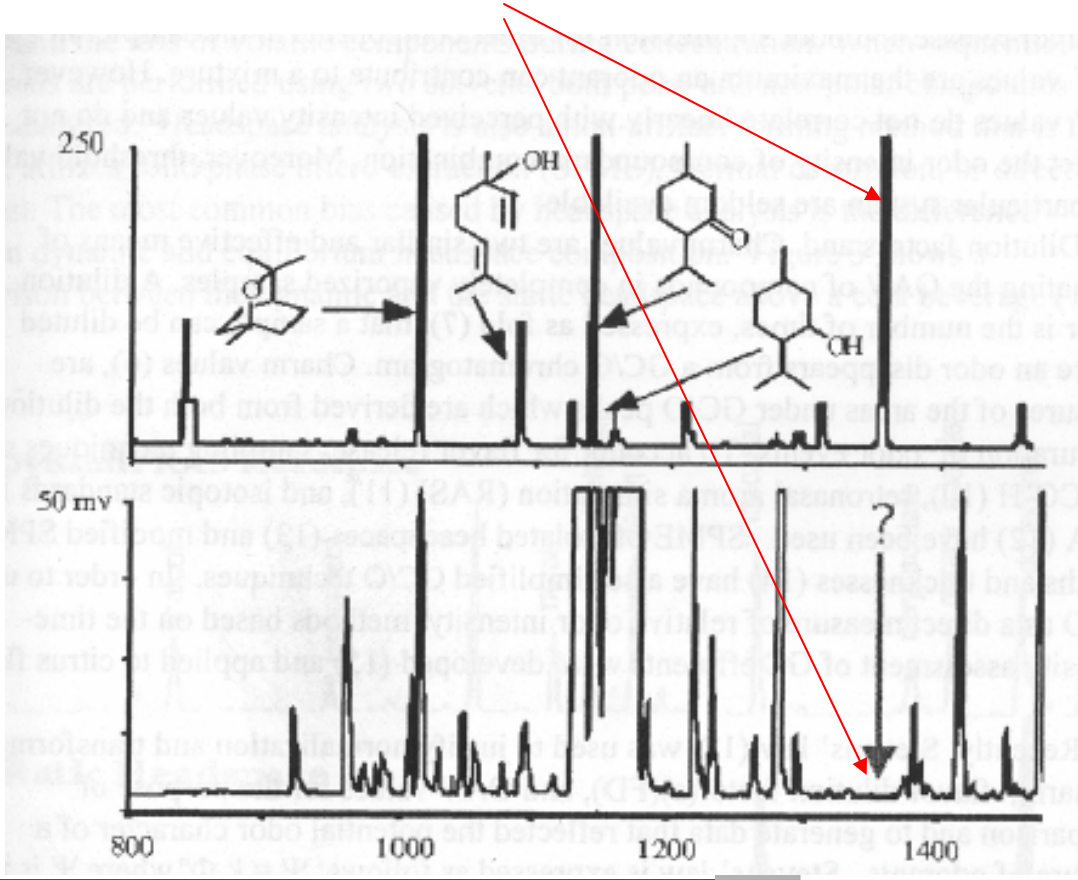
- P + G, USA
- Firmenich, Switzerland, France, USA
- Givaudan, Switzerland, France, USA
- Takasago, USA, Japan
- International Paper, USA
- Philippe Morris, USA
- Loreal, France
- Ciba Specialty Chemicals, Switzerland

Sniffer 9000 Customer references

- Kirin Beer, Japan
- Dainippon Ink and Chemicals, Japan
- Aohata Inc., Japan
- CACS, Japan

What do we do if this case happens?

- The Panellist smells a odour but the FID or the MS can’t detect anything



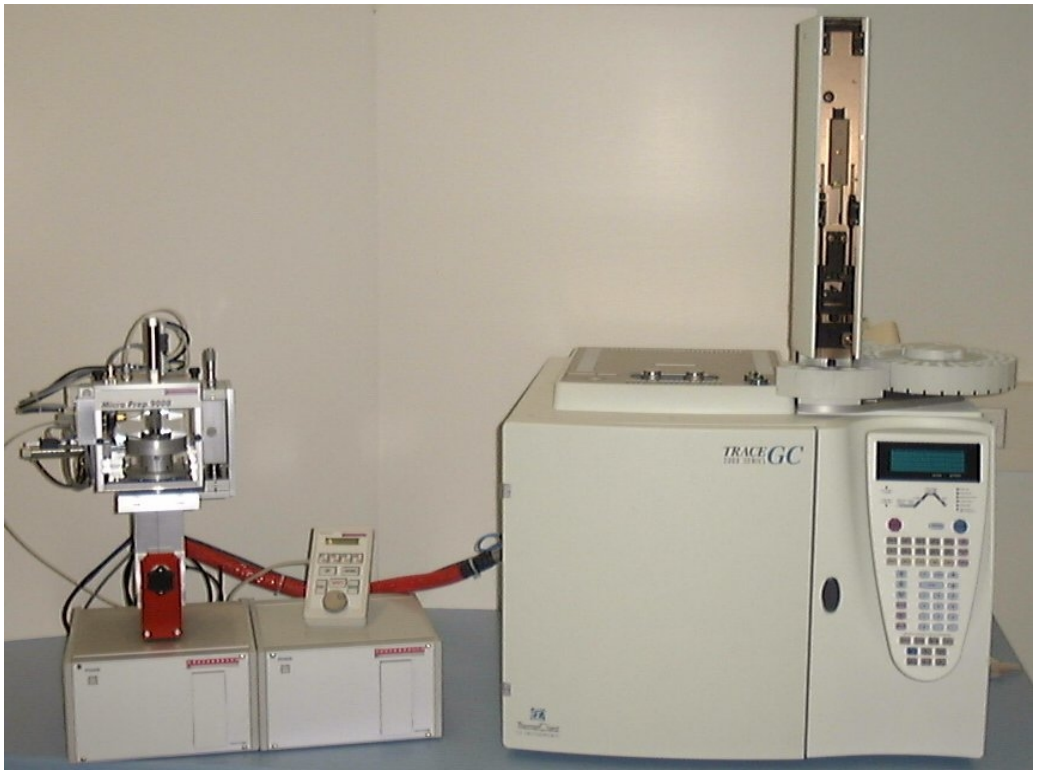
GC-O

FID

Prep9000: Fraction collection

GC Fraction collector

Prep 9000



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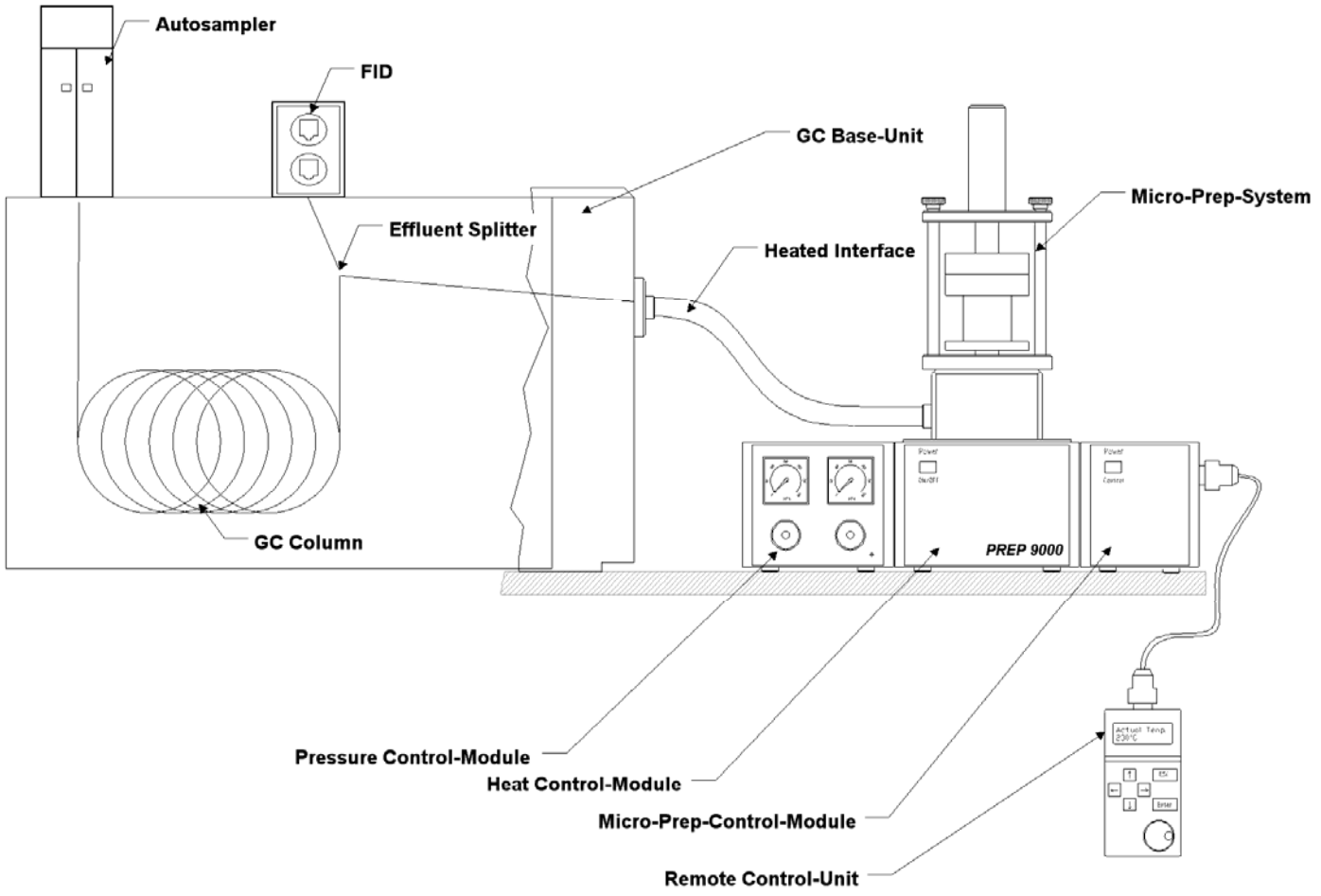
Prep9000: Application

- Enrichment of impurity
- Enrichment of low concentration compounds
- Isolation of compounds for standard
- Isolation of compounds for study with other technique, i.e. confirmation of results

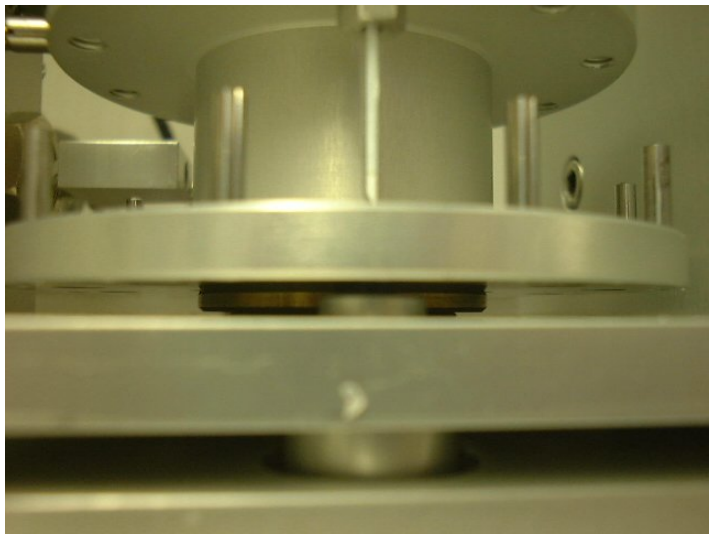
Prep9000: Description

- Split at the end of the column
- Heated transfer line
- Easy upgrade to the fraction collector
- Fast conversion from Sniffer to Prep



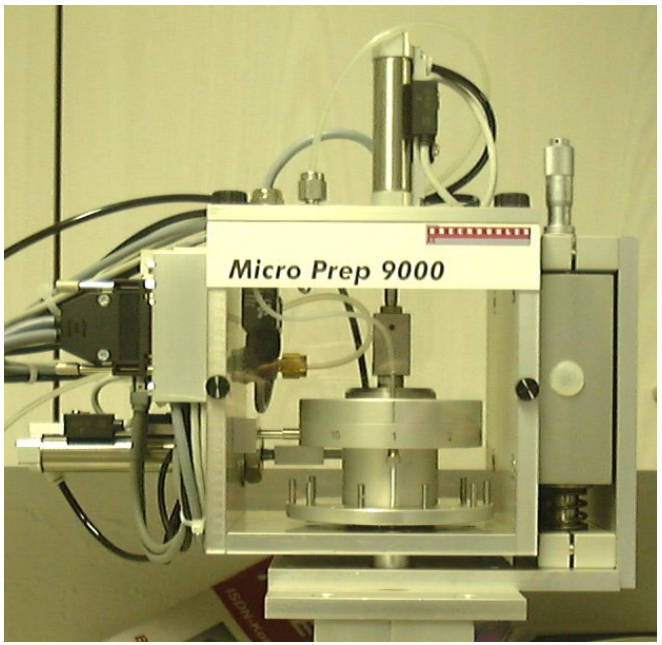
Prep 9000: Description

- Collection on adsorbtion tubes
- Up to 10 fractions per run
- Fully automated



Prep9000 Description

- No effect when not collecting
- Vacuum to draw sample
- Pneumatics to change collection tube



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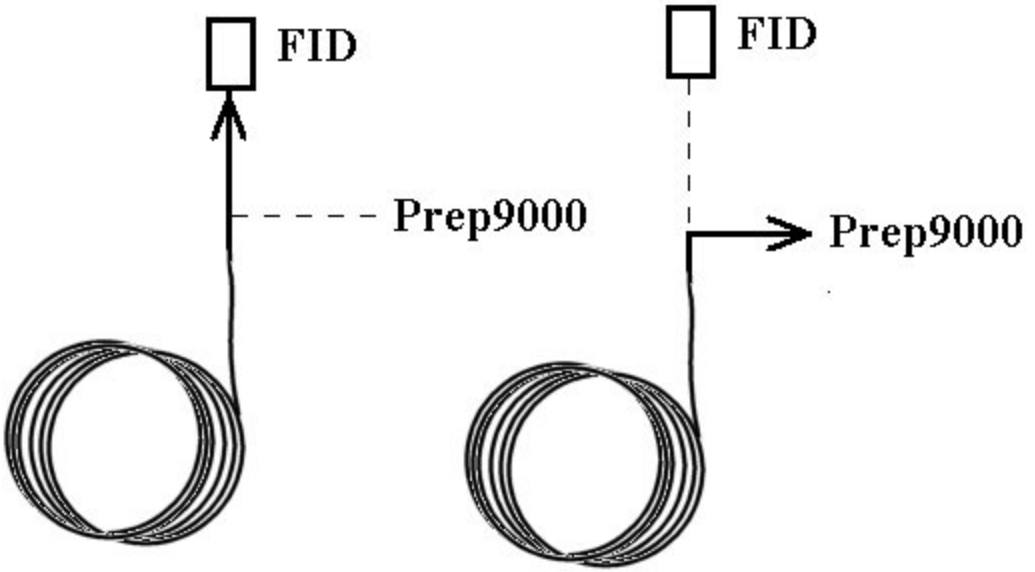
Prep 9000: Description

- Tubes filled with adsorbent and placed on a carrousel
- Choice of adsorbent
- Switching time less than 0.1 minute
- Follow the FID trace
- Easily programmed
  - *GC data system*
  - *Internally*

Prep 9000…Description



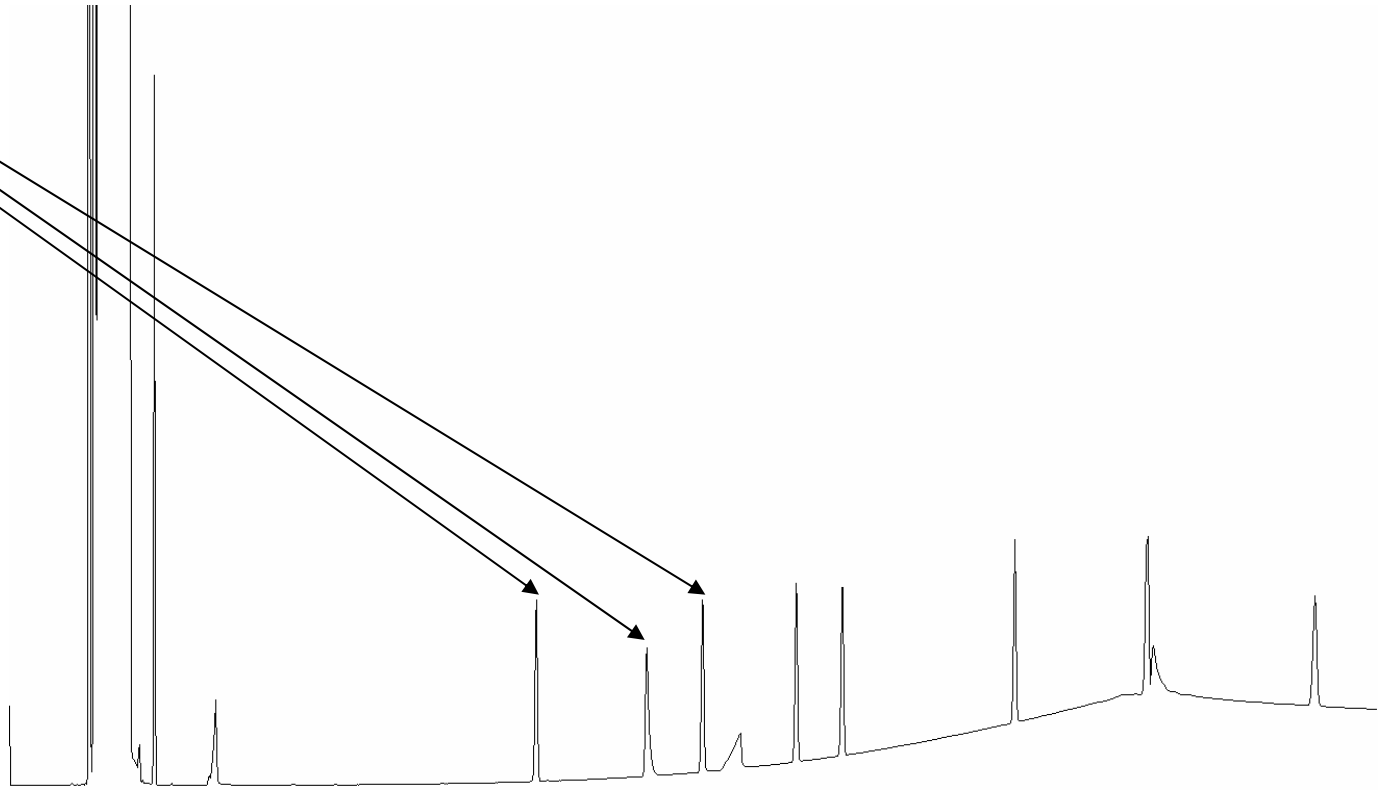
Normal

Collection

Prep 9000
Reference Chromatogram

- 1. Step establishing a reference Chromatogram

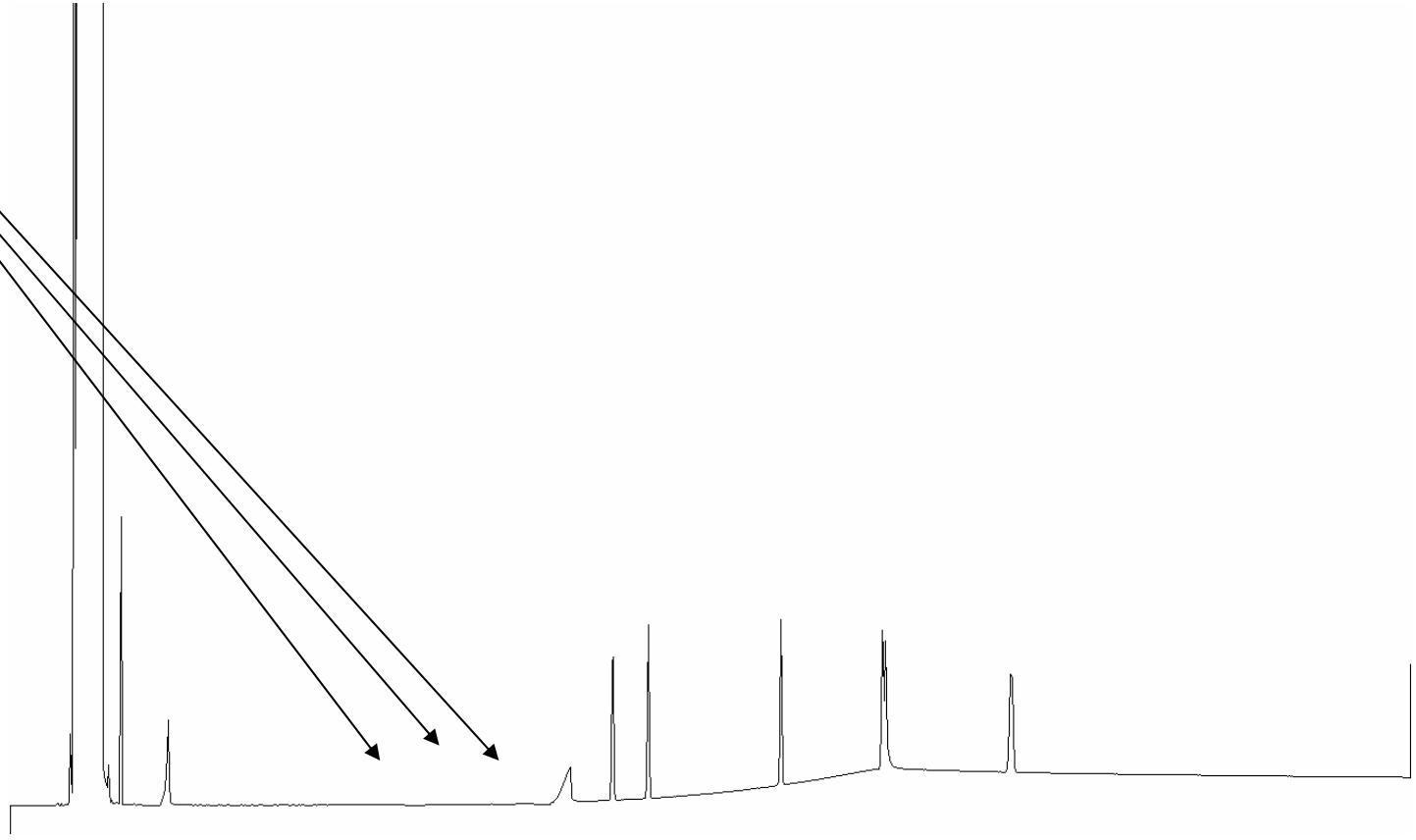
Peaks of interest
To be collected



Prep 9000
Collecting Chromatogram

- 2. Collecting Peaks, the 3 peaks don't appear anymore in the Chromatogram

Peaks of interest
To be collected



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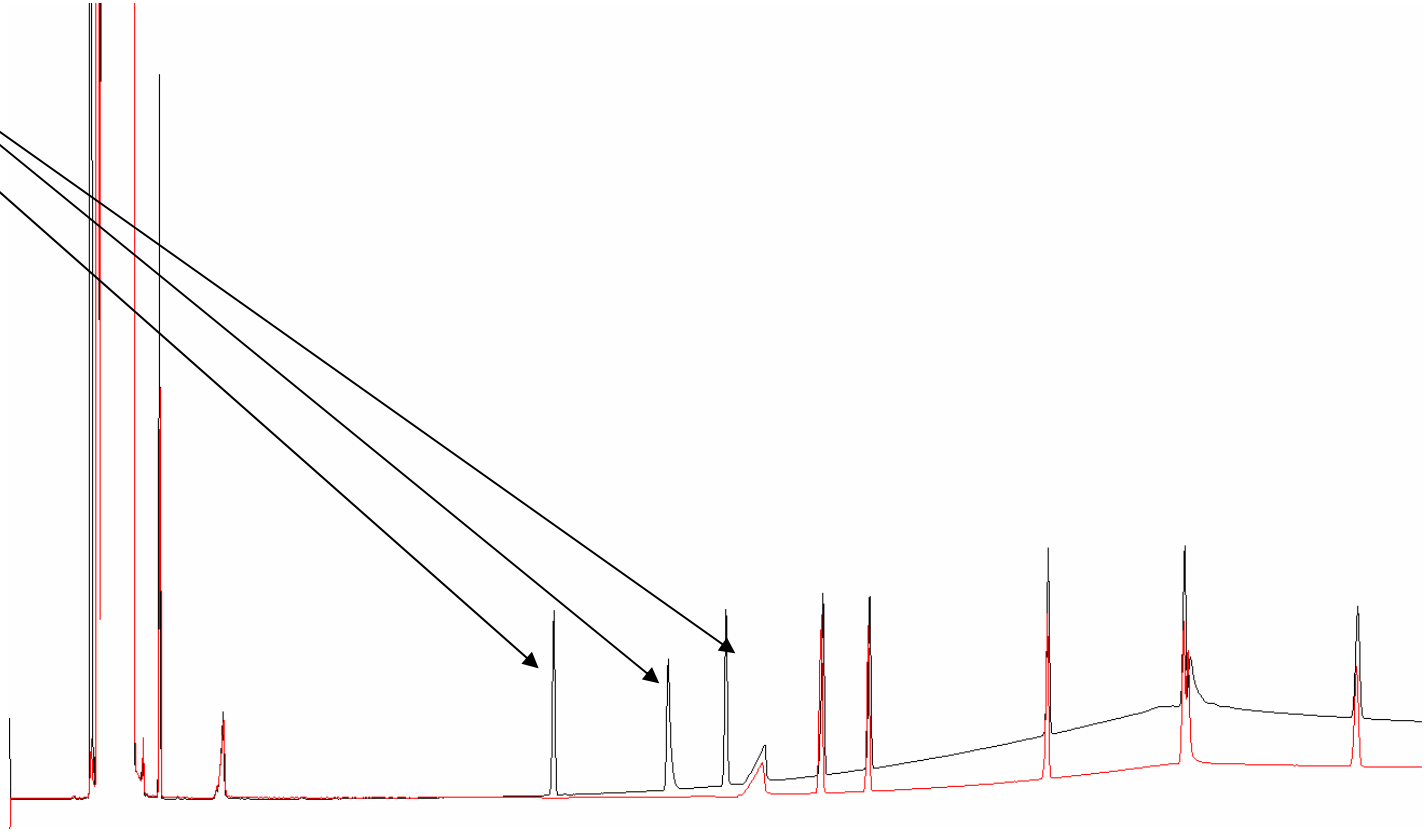
Prep 9000
Overlay Reference with Collecting

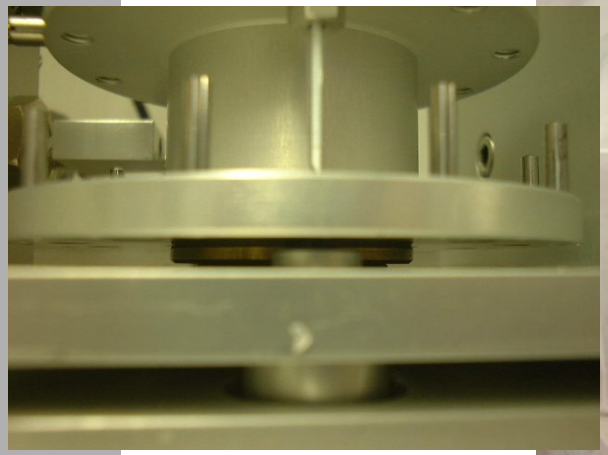
- 3. Comparison of reference Chromatogram with collected Chromatogram

Peaks of interest
To be collected



Prep 9000…Features

- Collection on adsorbtion tubes
- Up to 10 fractions per run
- Fully automated



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Prep 9000…Features

- Easy to setup
- Sample easily transposed to other instruments
- very short switching time
- Easy to program
- Very efficient

PREP 9000 Customer references

- P + G, USA
- Firmenich, Switzerland, France, USA
- Givaudan, Switzerland, France, USA
- Takasago, USA, Japan
- International Paper, USA
- Philippe Morris, USA
- Loreal, France
- Ciba Specialty Chemicals, Switzerland

PREP 9000 Customer references

- Kirin Beer, Japan
- Dainippon Ink and Chemicals, Japan
- Aohata Inc., Japan
- CACS, Japan

Thank You for your audience

- Any Questions?

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