Food Compendium

Volume 3

SCIEX

The Power of Precision

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SCIEX Food Compendium Volume 3

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Introduction

SCIEX Food Compendium Volume 3

Food is an essential component of life, and its analysis relies on two processes: quantification and identification of analytes. These analytes can be nutrients, vital components to health and well-being, with quantification potentially identifying where supplementation might be necessary.

But analysis is also important in maintaining consumer confidence. Produce available from suppliers must be safe for consumption, free from pesticides, mycotoxins and other potential contaminants.

Mass spec is now established as the go-to characterization method that can detect and quantify both nutrients and contaminants. From vitamin quantitation in infant milk formula or vitamin supplementation medicines, to the detection of ultra-low contaminant concentrations to ensure food meets the strict regulatory body requirements, liquid chromatography-tandem mass spectrometry is capable of delivering reliable qualitative and quantitative results.

SCIEX offers a comprehensive portfolio of instruments and software that simplifies analytical workflows by making data easy to interpret and analyses easy to perform. Across the globe, the requirements of food detection vary depending on the common contaminants and cultural food trends. This Food Compendium demonstrates the utility of SCIEX instruments in analyzing the nutritional levels of food, as well as analyzing contaminants such as herbicides and pesticides, highlighting specific applications utilizing mass spec in the food industry.

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Alex Liu

Market Manager, Food and Environmental Testing

The food industry is a complex market that faces a myriad of challenges in its goal to deliver safe food. Any produce available to the public must be both nutritious and safe for consumption.

This has bred an industry with a strong desire to identify and quantify everything in a sample of food. Throughout the journey from field to fork, there are various opportunities for contaminants such as pesticides or herbicides to be introduced into the supply chain. Therefore, it is essential for food scientists to have robust tools for reliable detection and accurate quantitative analysis of contaminants or nutrients in food samples.

Food contains a diverse mix of molecules, such as sugars, oils and fats. Within this mixture of chemical compounds are those molecules whose identification and quantitation are vital. These could be nutrients or vitamins – essential to provide consumers with accurate nutritional information – or trace pesticide or herbicide contaminants, which must be identified before their contamination results in an unexpected recall. Therefore, any analytical instrument needs to assist the food scientist by providing information to help answer the question: Is this food fit for public consumption?

Contaminants in particular represent a challenge for food scientists. These compounds are varied in structure and typically present at trace concentrations. Thus, instruments capable of ultra-sensitive detection are needed that can screen samples against a library of potential contaminants within a single sample. SCIEX instruments are capable of delivering these requirements. For instance, the X500R QTOF system provides revolutionary detection capabilities for samples with unknown molecules, making it a future-proof solution that addresses the needs of a food detection lab.

"Throughout the journey from field to fork, there are various opportunities for contaminants such as pesticides or herbicides to be introduced into the supply chain"

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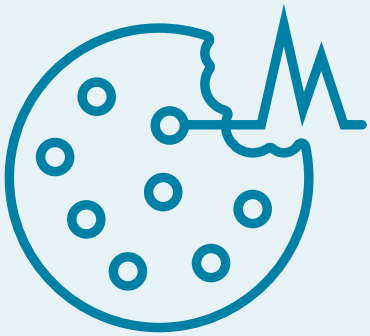

Over the years, many of the barriers that prevented or hindered mass spec adoption in the food industry have been overcome. Originally, mass spec was accompanied by the common misconception that the technology was unintuitive or difficult to use. Now it is widely considered to be the best characterization method for food analyses, with SCIEX software and hardware earning its reputation as simple to learn and use. Processes are continually being developed to ease the user experience, including automated data analysis or interpretation to deliver a simple report directly to the user that portrays exactly what compounds are present in the food sample.

Food analysis is a challenging process. New pesticides are being used to treat produce across the globe. These must be identified to prevent penetration into the food supply chain and avoid contamination scares, such as in the example of 2008's melamine and 2017's fipronil scandals. In addition, changes in regulation and legislation in both developed and developing nations mean that detection methods must continue to evolve.

Within the next few years, we expect the demand for more innovative technology to increase. With the introduction of new pesticides comes the need for novel detection methods and procedures. In addition, there is the industrial demand for more field deployable instruments that can analyze samples as soon as they are obtained, or can be integrated directly into the supply chain. However, current portable instruments must sacrifice accuracy and reliability, compromising the detection capabilities in an industry where such mistakes must be avoided at all costs. But, portable equipment will facilitate a change in how and where food is analyzed. Analysis is expected to move from the lab directly to the field or the supply chain to directly monitor the levels of contaminants in food closer to its source. The changing demands of the food industry suppliers, consumers and regulators will result in changes to how mass spec is used and SCIEX will be there to provide the instruments needed to facilitate these changes.

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A Global Perspective

Food testing requirements vary from country to country, one region may be seeing a surge in analysis in emerging testing areas such as cannabis in North America or the analysis of food product for allergens in Japan. Here we present a global perspective looking at regional food testing trends from our experts.

Ashley Sage
Senior Market Development Manager, EMEAI

Oscar G. Cabrices
Market Development Manager, Americas

Paul Winkler
Market Development Manager, North America

Yuka Ikoma
Market Development Manager, Japan

Kerong Zhang
Senior Market Development Manager, China

Jason Neo
Director, Marketing & Field Applications Support, ROA

Chris Hodgkins
Market Development Manager & Sales, Australia

Global Trends in Food Analysis

Oscar Cabrices, Marketing Development Manager, Food, Beverage and Forensic Testing, Americas at SCIEX

Food safety in the Americas relies on comprehensively identifying the most common contaminants: pesticides, veterinary drugs, mycotoxins and other types of residues. But as the maximum permissible levels of residual contaminants continues to evolve with new FDA regulations, it is important that laboratories are confident that their instruments are capable of detecting every analyte, even those present at ultra-low concentrations. Therefore, scientists are interested in exploring the workflows and methods that provide the ability to analyze multiple analytes in a single streamlined workflow.

In terms of specific equipment, the food testing community is continuing to embrace the shift towards liquid chromatography mass spectrometry technologies. In particular, the most widely adopted mass spectrometers in food testing laboratories are the Triple Quadrupole (QQQ) detectors, which offer a targeted and selective approach to contaminant detection. As consumer trends change, there is growing desire in the food testing industry to obtain a comprehensive picture of the commodities society consumes on a daily basis, which is leading scientists in this space to look towards next generation of technology, which is High-resolution mass spectrometry. Here at SCIEX, one of our teams' most important responsibilities is to listen to our customers, thanks to their feedback we have engineered the revolutionary X500R Quadrupole Time of Flight (QTOF) mass spectrometer, offering scientists a benchtop sized solution that provides that extra mile of specificity needed to capture every relevant piece of information from a food sample, such as: contaminants, residues, allergens, nutrients and more.

Paul Winkler, Marketing Development Manager for Environment, Industry and Cannabis

Cannabis containing food commodities have risen in popularity across America and other countries across the globe. This increase is a direct result of the steady legalization of medicinal and recreational cannabis. In response to these changes, many foodstuffs, such as chocolates, cookies and beverages are being infused with cannabis extracts. There are two major analyses that food scientists must perform when analyzing cannabis-containing products. The first is the need to determine the cannabidiol (CBD) and tetrahydrocannabinol (THC) amounts in the products for labelling requirements and to inform customers about the products they consume. Secondly is the need to detect residual pesticide contamination. LC-MS/MS techniques are now commonly being employed to address both of these challenges. One difficulty is that government directed legislation varies according to the different states and nations, which complicates detection and necessitates the use of sensitive, adaptable and robust methods.

To address the analytical needs of this market, SCIEX has developed methods that are capable of meeting the reporting requirements of all compounds usingonly LC-MS/MS. The market for cannabis testing is becoming increasingly focused on contaminant testing and LC-MS/MS is the most appropriate technique for this analyte class. It has the capacity to simplify existing laboratory procedures and, with the use of novel SCIEX instrumentation and methodology, users are equipped with the best tools to tackle future challenges.

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Ashley Sage, Senior Manager, Applied Markets Development for the EMEAI region

The principle aim of routine food safety testing is consumer protection. In recent years, several high-profile public food scandals have resulted in more stringent legislation that dictates maximum residue limits of pesticides, mycotoxins and veterinary drugs in food. In 2017, it was uncovered that a common pesticide, fipronil, was present at above safe limits in eggs and chickens across Europe, resulting in the destruction of millions of eggs. Another area of concern for the food industry is food adulteration, where foodstuffs or commodities are intentionally changed to a lower value product before being sold off. One high-profile case from the UK in 2013 was the 'horse meat scandal', where horse meat was found to be present in many meat products advertised as containing higher value beef.

To combat such cases of food contamination, food fraud and adulteration is detection via mass spectrometry, which has revolutionized the food analysis industry. Today, mass spec is able to screen over 700 pesticides from a single sample analytical run, with high-end instruments capable of detecting analytes to the concentration of parts per trillion. Such analyses are essential in an industry where future-proof methodology and instrumentation helps to minimize the potential occurrence of future food scandals.

Kerong Zhang, Senior Manager for Food, Environment and Forensic Market Development in China

Globalization has resulted in a more international food trade, but one in which detecting pesticides and veterinary drugs using multi-residue panel analyses are vitally important. The major food concerns in China relate to residual pesticides in grains and oils, but recently the presence of mycotoxins has garnered attention owing to the threat their presence poses to humans and animals. In response to these challenges, in 2017 the Chinese government made several important arrangements about grain and food safety, aimed at improving the supervision level of grain quality and safety by constructing a dedicated inspection and monitoring team.

As a result of legislative changes and the establishment of new regulatory bodies, SCIEX provides instrumentation and methodology specifically developed to meet the requirements of food scientists. Of particular note are the various vMethods™ designed for the detection of mycotoxins, pesticides, veterinary drugs and other residual contaminants that have been easily implemented and used within food laboratories.

Jason Neo, Director, Marketing and Applications, in the Asia Pacific region

In response to many emerging contaminants in foodstuffs and commodities, food scientists in the Asia Pacific region are beginning to recognize that mass spectrometry is the best characterization method available to analyze food. In this region, most food and beverage screening is performed in order to detect residual contaminants such as pesticides and veterinary drugs. In particular, there is increased demand to detect emerging contaminants such as PFAS (per- and poly-fluoroalkyl substances) or glyphosates. Furthermore, analytical trends are influenced by events across the world. For example, the 2017 fipronil scandal in Europe led to a similar demand in Korea for methodology that is capable of detecting potential fipronil contamination.

As researchers in developing nations shift from traditional HPLC methods to mass spectrometry methodology, SCIEX will continue to align its instrumentation and methodology to meet the needs of customers and users. For instance, the X500R instrument is the ideal companion to screen against potentially unknown contaminants. Mass spec is, and will continue to be, a key technology that will help scientists combat future food fraud.

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Yuka Ikoma, Marketing Development Manager for Food, Environment and Forensics for SCIEX Japan

Mass spec usage in Japan for the food industry is mainly for pesticide and veterinary drug detection. However, instrument use varies between the different disciplines of food analysis, and in recent years, there are clear trends in the desired detection methods. There is increased demand for more sensitive detection of allergens, which has led to a rise in popularity of the high-powered, sensitive SCIEX instruments and methodology that is capable of detecting these analytes. Furthermore, Japan's aging society has led to rise in interest in extending life expectancy solutions. This means food research areas such as FOSHU (food for specified health uses) are becoming more popular, and demand in this sector is only expected to increase in the coming years.

Generally, the needs for food scientists in Japan mirror those across the globe. There is strong desire for non-targeted analysis of known/unknown contaminants, which makes instruments such as the X500R and data acquisition capabilities such as SWATH® Acquisition essential for any analytical laboratory.

Chris Hodgkins, Marketing Development Manager for SCIEX Australia and New Zealand

Across both Australia and New Zealand, and the world in general, there is increased awareness and a stronger desire for high-quality foods and foodstuffs catering to specific diets. The market for gluten-free products continues to grow, along with food contaminants and other allergens, and given the risks involved for consumers, accurate quantitation of these analytes is key. Australia and New Zealand have long been known for export of high-quality food products, but this is increasingly translating into a local desire for the same quality products. As a result, there is now increased demand for understanding potential contact contamination from food packaging, so we are seeing an increase in enquiries related to testing of packages, in addition to the food products themselves.

To answer the needs of consumers and scientists in this region, SCIEX is helping to produce methodology that can address the needs of the industry, along with high-performance, robust instrumentation our customers have come to expect. The QTRAP® and Triple Quad™ systems, particularly the 6500+ models, are popular with food testing scientists. There is also increased demand for the X500R system, which brings non-targeted analysis to any routine food testing laboratory.

Technical

Overview

Food scientists around the globe need to be confident in their ability to detect everything in their food sample, meaning they need equipment that is fit for the challenge.

A typical working day can entail using a powerful analytical instrument to monitor samples of food for the smallest traces of deadly contaminants, or identifying and quantifying the nutrients in food in order to provide accurate information to consumers.

Therefore, efficient, accurate and reliable instruments have become vital components to the food supply chain, ensuring the food made available to the public is safe and nutritious.

To ensure the continued quality of food that fulfils these essential criteria, we at SCIEX have designed and developed some of the foremost advanced mass spectrometers in the industry that deliver reliable and accurate results. Through our own research, these tools have been tested in a variety of contexts across the entire food supply chain, from difficult polar pesticide detection to the quantitation of cannabinoids in cannabis-containing products.

Here, we offer an overview of some of our latest technologies used in the rapidly expanding field of food science and the exciting results they can produce:

The X500R QTOF Series

Food scientists require techniques that can offer high-throughput and sensitive detection capabilities in order to quickly validate food samples. To meet these needs, SCIEX developed the revolutionary X500R Series of QTOF mass spectrometers. Its key features include enhanced mass accuracy to deliver improved selectivity for routine mass spectrometry (MS) quantitation of targeted compounds, combined with sensitive and high speed MS/MS data acquisition to enhance quality of data and ensure comprehensive detection of unknowns. This accuracy is due to the X500R series' Turbo V™ ion source, which has a renowned ionization performance among industry professionals.

In addition, the SCIEX X500R Series has an independent calibrant delivery path, which maintains highly reliable mass calibration through long runs. Together, these features provide the platform with the speed and mass accuracy required for the high throughput identification and quantitation of food analytes. These advantages make the X500R system well-suited for screening investigations where samples contain unknown analytes. For detail on accomplishments using the X500R Series, please see the relevant Technology Notes in this compendium.

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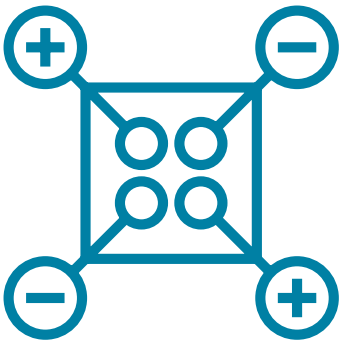


The SCIEX Triple Quad™ 3500 System

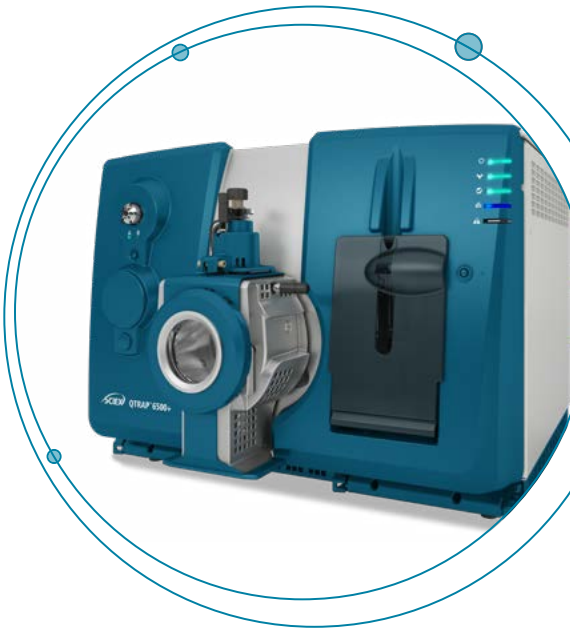
Specifically designed for the food and environmental testing laboratories, the Triple Quad 3500 System delivers consistent results across multiple samples. Powered by the legendary Turbo V ion source, samples are efficiently ionized to deliver reliability to sample analysis. Even dirty samples can be used in this machine, with the Curtain Gas™ interface protecting from contamination and reducing the need for routine maintenance. Using a single injection, hundreds of analytes can be analysed, making it the ideal companion for any high-throughput testing laboratory.

The QTRAP® 4500 Series

Intelligently re-engineered from the 4000 QTRAP platform, the SCIEX QTRAP 4500 LC-MS/MS system offers food scientists vigorous and reliable high-throughput screening for a wide range of analytes. Designed for robustness, rapidity, and the quantitative sensitivity, the QTRAP 4500 is well-suited for situations where trace analyte detection is needed, a common need for pesticide or contaminant detection. Like the X500R Series, it possesses a Turbo V ionization source - the gold standard for LC-MS/MS ionization. Plus, with the 4500, investigators can maximize the capacity of their laboratories and deliver quality quantitation every time. This is due to its powerful Scheduled MRM™ algorithm and Curved LINAC® collision cell design, which, together, improve the quality of data to ensure fewer peaks are missed and optimal sensitivity is achieved.

The QTRAP 6500+ System

For the most difficult, challenging food applications involving complex food groups, you need the most sensitive instrument available. The QTRAP 6500+ System is the fastest and most sensitive QTRAP system available, delivering enhanced selectivity and improved levels of quantitation. Featuring the ability to eliminate background interferences to make quantitative analyses easier, as well as IonDrive™ technology to boost performance in those situations where exceptional selectivity is a necessity. This single instrument pushes the boundaries of LC-MS/MS farther than ever before and provides a simple route to achieving comprehensive food residue monitoring.



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The CESI 8000

Despite its origins dating back to the 1990s, capillary electrophoresis (CE) has proved to be a vital instrument in the analysis of pesticides in food. The SCIEX CESI 8000, in particular, has been used as a platform for transformative chiral separation techniques. The CESI 8000 provides a unique multi-segment injection system, which offers a ten-fold increase in the throughput of an analysis.

CE is well suited to the separation of polar samples and Capillary Electrophoresis Electrospray Interface for Mass Spectrometry (CESI-MS) offers a methodology that can improve retention times of samples for better detection. As will be detailed later in this compendium, the technique has been used to develop quantitation and identification methodology for polar pesticides, commonly considered to be amongst the most difficult to quantitate.

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Food Testing

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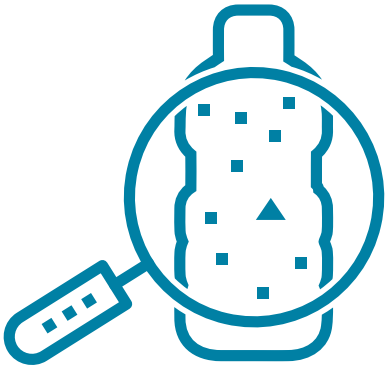

KC Hyland

Staff Scientist, Food and Environmental Testing

KC Hyland is the Global Staff Scientist for SCIEX Food and Environment, placing her at the forefront of innovation at SCIEX. Here, she addresses how mass spec detection has changed over the years.

Consumer safety is the driving-force behind food analysis, with mass spec being a common characterization tool used to detect many different classes of residual contaminants in food. One such class is mycotoxins, which are toxins produced by fungi and molds in foods. Mycotoxin analysis also helps illustrate how mass spec technology has, and will continue to, evolve with advances and innovation in instrumentation and methodology.

Originally, mycotoxin detection relied on a targeted analysis, with mass spec only analyzing a panel of characterized mycotoxins which may be present in the sample. This legacy approach of detection and quantitation of a target suite was both time-consuming in method development, and limited in the scope of what answers could be reported. While this is still a dominant approach for mycotoxins and other residues analysis, advancements in technology continue to expand the capabilities of these analytical methods.

Nowadays, innovation has advanced mass spec technology from targeted analysis to a non-targeted or screening analysis. This change has begun through two simultaneous changes in technology. First, instrumentation has become more sensitive, meaning lower-abundance mycotoxins can be identified and the library of known toxins expanded. Secondly, a non-targeted analysis overcomes the need to analyze each toxin individually. Scientists can instead use instrumentation specifically designed to screen samples and identify unknown analytes. For instance, the SCIEX X500R QTOF system has been successfully applied to multiple analyte screening methods detailed in this compendium.

"Consumer safety is the driving-force behind food analysis, with mass spec being a common characterization tool used to detect many different classes of residual contaminants in food."

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However, this decades-long journey from targeted analysis, to screening analyses and onto metabolite detection is not limited to the food industry nor mycotoxin detection. In fact, there are a multitude of other instances including veterinary drug detection, pesticide identification and environmental analysis that can benefit from non-targeted analysis. It is becoming increasingly apparent that mass spec has a fundamental role in any future food analysis.

But, innovation is not limited to the present day. Metabolomics of food is an area gaining popularity amongst food researchers. Once these toxins have entered foodstuffs and have metabolized, it is possible for scientists to identify and quantify these analytes, which are often present at ultra-low concentrations. The connection between metabolomics research and food analysis is novel to this community. Although full integration of these two disciplines will take time, food omics highlights an area of potential collaboration and will be an area of impending food research and analysis over the coming years.

Training and services for SCIEX

Realize your lab's potential

It can be difficult to manage the everyday challenges of an analytical lab, contending with an increasing volume of food samples for analysis while ensuring any potential contaminant is detected. SCIEX is here to help. Our Lab Optimization Services can help you increase your throughput, reduce your costs and improve quality by identifying and removing unproductive activity.

Building on over 15 years of experience working in food testing labs, we can help identify areas to eliminate unproductivity across various workings including method development and refinement, routine troubleshooting, data or sample processing, and other areas. We have a proven track record, with our clients reporting productivity increases as high as 50% and a corresponding saving of capital.

Discover more > sciex.com/professional-laboratory-services/laboratory-optimization-services

Sharpen your skills

In addition to maintaining laboratory equipment, it's vital to maintain your employees' knowledge. In a testing environment where every analyte must be identified and quantified using a variety of different mass spectrometers, your technicians must have the knowhow to accurately assess results and deliver concise summaries.

SCIEXUniversity Success Programs offer a series of comprehensive training courses that are designed specifically to maximize learning retention. The programs allow you to choose a personalized learning plan tailored to your instrument and employee experience. Our courses can even help to teach you how to maintain and troubleshoot your system, so you can further reduce unscheduled downtime.

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Anytime solutions to any analytical problem

If you run into issues, you want to know that the solution is only a short, simple step away. At SCIEX we have worked to minimize the time needed to provide you with the solutions to your complex problems. That's why we have a team of experts on hand to help with your technical or application-related challenges. Our online support hub, SCIEXNow™ Online, is available 24/7 offering service and support cases, individual training courses and a vast array of pre-solved user questions in the Knowledge Base. If your query hasn't been solved, you can create a new support case, or contact SCIEX experts directly using the Technical Live Chat to connect you with the experts that can help solve your challenges as quickly as possible.

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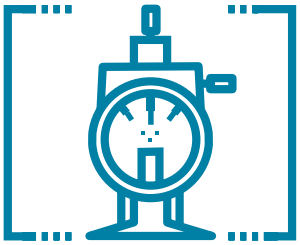

An Overview of SCIEX Technology Notes

Simultaneous Analysis of 25 Mycotoxins in Grain by LC-MS/MS

Produced by fungi, mycotoxins are capable of causing health issues and death if consumed through contaminated food and agricultural commodities. This risk has led to many countries implementing strict regulations controlling mycotoxin concentrations.

To ensure the highest accuracy and reliability, LC-MS/MS is rapidly becoming the method of choice for such analyses. However, the many different classes of mycotoxins necessitate standardizing sample preparation techniques, which can be time consuming.

To address this issue, SCIEX researchers have developed a new, fast purification method, which allows up to 25 mycotoxins in the same sample to be detected using the SCIEX QTRAP® 4500 LC-MS/MS system.

Simultaneous analysis of 12 food allergens in baked and raw food products using the LC-MS/MS QTRAP® 4500 system

Food allergies are the leading cause of anaphylaxis, a severe and potentially deadly allergic reaction. As there is currently no cure for allergies, those who suffer from them must rely on the accurate testing and labelling of food products to avoid health issues.

Although they are the most commonly used tests for screening allergens, enzyme-linked immunosorbent assays (ELISAs) have limited selectivity and can produce false positive or false negative results.

To combat the potential risk associated with mislabeled allergens, SCIEX researchers developed a method using the QTRAP® 4500 LC-MS/MS system that detects and screens 12 separate allergenic proteins simultaneously in a single injection.

Improving Identification and Quantification of Polar Herbicides by CESI-MS

Glyphosate is a common herbicide that has been associated with various health risks. As a result, more stringent regulation has been introduced to restrict its presence in the food chain to safe-for-consumption levels, most recently by the European Union.

However, current LC-MS methods of analysis can have difficulty distinguishing between different degradation products of these herbicides. Thus, SCIEX researchers endeavored to develop a new, more effective CESI-MS method for this separation and identification.

The new CESI-MS method not only demonstrates an excellent ability to distinguish between glyphosate and its degradants, but also between similar degradation products of another widely-used herbicide, fosetyl aluminum.

Combining Non-Targeted SWATH® MS/MSALL Acquisition with Highly Selective MRMHR for the Analysis of Veterinary Drugs in Tissue Using the SCIEX X500R QTOF System

Due to the associated risks of antimicrobial resistance and possible allergenic reactions, the European Union has strict guidelines concerning veterinary drugs in animal products.

The SCIEX X500R QTOF system is a powerful instrument capable of performing the sensitive analysis of veterinary drugs in complex matrices. When analyzing veterinary drugs in a liver extract, the SCIEX system displays the mass errors of the precursor and fragment ions and the ion ratio as a traffic light system. This allows users to review of large volumes of data simply and be confident in the identification of a detected signal, which meets the European Union's criteria of identification points.

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Pesticide Residues in Produce Analyzed by Targeted MRMHR "Full-Scan" Acquisition and Processing

Food and environmental sample analysis is a field of great importance to both local and worldwide economies. Positive hits or results above tolerance limits can lead to the delay or destruction of products, with massive impacts on the import, export, sale or distribution of goods, and millions of dollars at stake.

For such tests on pesticides the standard method for many organizations has been LC-MS/MS coupled with Multiple Reaction Monitoring (MRM). While this practice has a high degree of sensitivity, SCIEX researchers explored potential improvements by incorporating High Resolution Accurate Mass (HRAM) mass spectrometry technology.

The research effort concluded that MRMHR provides high resolution monitoring of known ion transitions as well as full scan product ion spectrum collection.

Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS

The increasing legal use of cannabis in the USA now requires a more robust and reproducible analytical method, quantifying both residual pesticides as well as psychotropic cannabinoid content.

In an effort to contribute to the creation of such a method, SCIEX researchers analyzed cannabis samples with two different SCIEX Triple Quad™ platforms to detect all the pesticides compromising the Oregon Pesticide List (the most comprehensive list of pesticides allowed in plant products in the USA).

This method is superior to previous practices in multiple ways. For one, the SCIEX vMethod™ can analyze ten cannabinoids within the same sample. When verified, the method was shown to offer a simpler form of sample preparation and optimized LC-MS conditions.

In addition, the final version of the vMethod is accompanied by a disc that contains a comprehensive a quantitation methods and reporting template that may be directly loaded on to the instrument. This convenient feature can allow laboratories to become fully operational for pesticide and cannabinoid analysis in a matter of days.

X500R QTOF System with SWATH® Acquisition for Pesticide Residue Screening in Fruits and Vegetables

Chinese agriculture is often subjected to excessive and potentially toxic levels of chemical fertilizers, pesticides, and herbicides. The most commonly used method for detecting these compounds is based on the QTRAP® system and high resolution TOF-IDA-MS/MS technology.

In an attempt to provide an even better service, SCIEX researchers used the SCIEX X500R QTOF System with SWATH® Acquisition to screen for pesticide residues in six varieties of Chinese vegetables and fruits. By the end of the analysis it was found that although pesticide residues were extremely high, they could be easily washed off with detergent.

Ultimately, the experiment's goal was achieved and SWATH screening was established as a highly accurate method for scanning the residues of the 190 most commonly used types of pesticides according to the Ministry of Agriculture.

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A Selective and Robust LC-MS/MS Method for Multiple Meat Speciation and Authentication on the QTRAP® 4500 System

Following the identification of horsemeat in certain beef products in early 2013 and its subsequent publicity, the Food Safety Authority (FSA) and Department for Environment Food & Rural Affairs (DEFRA) set the threshold for undeclared meat species in meat products to 1%.

Thus, it is imperative that the previously adequate analytical methods, such as polymerase chain reactions (PCR) and enzyme-linked immunosorbent assays (ELISA), are superseded by methods that offer greater sensitivity and accuracy.

One such method, as presented by SCIEX researchers, is an LC-MS/MS method, using the QTRAP® 4500 LC-MS/MS system, that can detect and screen pork, beef, lamb, chicken, duck and horse to a threshold limit of 1% simultaneously, in a single injection.

A Robust and Sensitive Method for the Direct Analysis of Polar Pesticides in Food and Environmental Samples Without Derivatization

In recent years, multi-residue LC-MS/MS analyses have become the minimum requirement for the quantification of pesticides in food and environmental samples. However, some highly polar compounds can only be analyzed using single-residue methods, which often involve derivatization – a time consuming technique used to transform a chemical compound into a product – to improve detection.

However, NofaLab, an independent Dutch sampling laboratory, has developed a method that can analyze many of these highly polar pesticides in a single analysis without derivatization. Along with a technique that utilized the SCIEX 6500+ QTRAP® mass spectrometer, the methods were found to be considerably more robust and sensitive than contemporary approaches and have achieved the target limits of detection required to meet existing and proposed regulations.

Use of X500R QTOF for Monitoring Unexpected Additives in Nutritional Supplements

Nutritional supplement manufacturers often claim that their products can support an individual's recovery from illness. But in order to maximize these functions, the companies may add related drugs to the supplement to increase its efficacy without including them as a listed ingredient. This uncertainty creates a potential risk to consumer's health.

The SCIEX X500R QTOF high-resolution mass spectrometry system can qualitatively confirm the presence of over 50 additives, and provides an efficient means for rapid, high-throughput monitoring of nutritional supplements for additives.

To prove this efficacy, this SCIEX study randomly selected 19 nutritional supplements commonly found on the market. Screening results showed that blood pressure-lowering and glucose-lowering products commonly contained additives, especially those products advertised to use Chinese medicine extracts to lower blood sugar. Many of the additives detected were present in amounts several times greater than therapeutic doses. Thus, they could be quite hazardous to consumers' health.

Analyzing Different Compositions of Polygala from Different Regions using the X500R QTOF System

Authentic herbs from regions across China have commonly been used as herbal medicines. There is now interest in studying these herbal medicines, including Polygala, to identify and analyze compounds, and further understand their pharmacodynamic efficacy.

However, this is currently difficult to achieve owing to the need for easy identification of active ingredients and differentiate authentic medicines from fraudulent herbs. To make identification more efficient, this study analyzed 24 different Polygala samples from 4 regions across China. Using the X500R QTOF mass spectrometer and SCIEX OS and MarkerView™ software, the different compounds were easily identified, obtaining high resolution spectrometric data that support identification of Chinese medicinal components.

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SCIEX OS SOFTWARE

Delivering clear, accurate and concise results for Food testing laboratories around the world.

Whether your laboratory is doing high throughput regulatory analysis of pesticides, mycotoxins and veterinary drugs or you are leading the way in foodomics research, SCIEX OS is the software platform for you. SCIEX OS enables you to process your acquired sample data in a quickly and efficiently without the need for time consuming manual integrations and spectral interpretation.

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Tech Notes

Contents

Simultaneous Analysis of 25 Mycotoxins in Grain by LC-MS/MS

Xiaoyuan Shi[1]; Wan Wang[2]; Jeremy Dietrich Netto[3]
[1]SCIEX, China; [2]Bonna Agela, China; [3]SCIEX, Singapore

Mycotoxins are secondary metabolites produced by a wide range of fungi known to contaminate a variety of food and agricultural commodities worldwide and has been recognized as a potential health threat to humans and animals. Many countries have regulations in place for mycotoxin detection and identification and their permissible limits. In China, the limits of mycotoxins in certain products are regulated by GB 2761 and in EU, mycotoxin limits are harmonized in the regulation for contaminants in foodstuffs EC 1881/2006 and the amended regulation EC 1126/2007. Regulations on food and environmental analysis require the analysis of contaminants using confirmatory techniques. Thus, there is a demand for powerful and rapid analytical methods that can detect very low concentrations of mycotoxins in a variety of sample matrices. In recent years, LC-MS/MS has gained popularity of becoming the method of choice, leveraging its ability to analyze a wider range of compounds in a single analysis coupled together with the high selectivity and sensitivity of Multiple Reaction Monitoring (MRM).

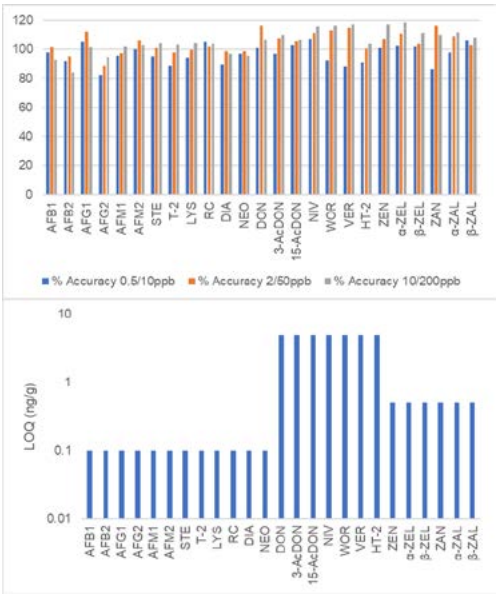

Figure 1. Accuracy and LOQ values shown for the panel of mycotoxins. Limits of Quantitation (LOQ) of all mycotoxins were found between 0.1 ng/g and 5 ng/g. Accuracy assessed over three concentrations ranged from 82% to 118%. These measurements of performance demonstrate excellent sensitivity and accuracy for this assay.

Traditionally, different classes of mycotoxins required different sample preparation techniques, making the process laborious and time consuming. Presented is an integrated workflow to analyze 25 compounds simultaneously in one sample. This includes a simplified extraction procedure that does away with additional clean-up steps by immunoaffinity columns and couples it to high resolution LC separation and high sensitivity MS detection.

Key Assay Attributes

- A fully integrated LC-MS/MS solution is presented to analyze 25 common mycotoxin residues simultaneously in relevant grain samples. Polarity switching ensures best coverage of relevant analytes.
- Simplified extraction procedure is described which does away with additional clean-up steps, saving time and labor at the front end of analysis.
- The method was validated for performance including sensitivity and robustness in different grain matrices.
- Limits of Quantitation (LOQ) of all mycotoxins were found between 0.1µg/kg and 5µg/kg. All LOQ meet the requirements of the GB methods.

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Experimental

Sample Preparation

Sample preparation was carried out in accordance to the vMethod SOP (P/N 5060674). Grain samples (corn, rice, wheat etc.) were first homogenized and 2.5g of sample was extracted using a mixture of acetonitrile and water. Once sonicated and centrifuged, the supernatant was passed through a Cleanert® MC SPE Cartridge (Agela Technologies, P/N ZS-MYT10-B) which contains a sorbent chemistry specially optimized for mycotoxins. The filtrate was then dried down and reconstituted for LC-MS analysis.

LC Conditions

Liquid chromatography analysis was performed using a SCIEX ExionLC™ AD UHPLC system. 20µL was injected onto a Phenomenex Kinetex C_{18} column (100mm X 2.1 mm, 1.7µm, P/N 00D-4475-AN). Mobile phase A contained water with 0.1% formic acid and mobile phase B contained methanol with 0.1%.

Table 1. LC Gradient time program. Flow rate at all steps was 0.3 mL/min, and the total run time was 13 minutes including re-equilibration.

Time (min)	%B
1.0	*3*
2.0	*10*
4.0	*50*
9.0	*80*
9.1	*99*
11.0	*99*
11.1	*3*
13.0	*3*

MS/MS Conditions

Electrospray ionization was carried out on a SCIEX QTRAP® 4500 system with fast polarity switching. The Turbo V™ source was kept at a temperature of 500°C and the Scheduled MRM™ algorithm was used to analyze grain samples for 25 mycotoxins in a single injection by multiplexing the detection of multiple MRM transitions for signature fragments.

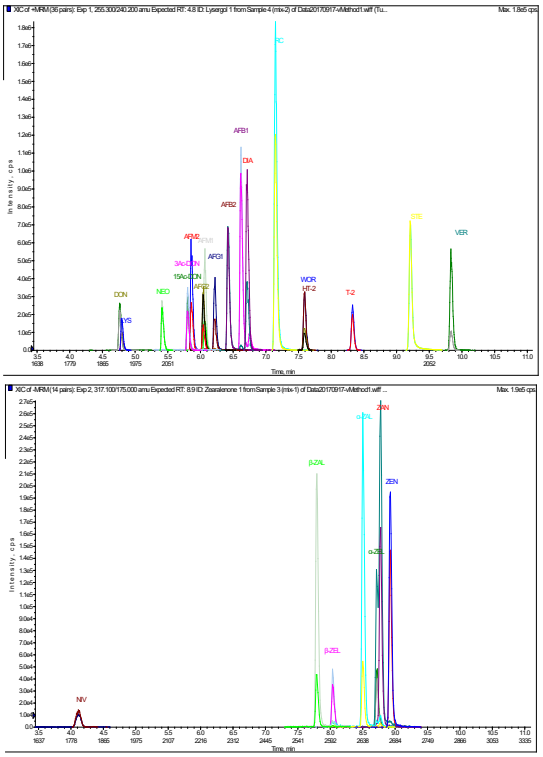

Figure 2. Chromatographic profile is shown for those 18 mycotoxins collected in ESI positive mode (top) and those 7 mycotoxins collected in ESI negative mode (bottom). Both positive and negative modes were analyzed simultaneously during a single sample injection, allowing all 25 mycotoxins to be analyzed in one data acquisition method.

Results and Discussions

For each analyte, two signature MRM transitions were chosen to ensure confidence in the identification of each mycotoxin (Table 2). To monitor many MRM transitions during a single injection, the Scheduled MRM algorithm was employed, where individual MRM transitions were monitored for a short time window during their expected retention time. Thus, at any one point in time, the number of concurrent MRM transitions were significantly reduced resulting in much higher duty cycles for each analyte. Combining Scheduled MRM with fast polarity switching further allowed extending the target list of mycotoxins while maintaining sample throughput by eliminating need for multiple injections. Typical chromatograms of solvent standard were shown in Figure 2. The total target cycle time of 0.6 sec ensured the collection of at least 12 data points across the LC peak resulting in excellent accuracy

and reproducibility. The system suitability was tested with the concentration of 5 or 50ng/mL standards (some compounds spiked at 5 and some at 50 depending on relative sensitivity) and the standard solution was injected three times. The %CV of each analyte peak was calculated to less than 15%.

For sample preparation, a simplified sample clean-up method was developed. Instead of immunoaffinity columns, one mycotoxin specialized solid phase extraction (SPE) column (Cleanert® MC, Agela) was used. This column proved advantageous in that it doesn't need to be activated, washed, and eluted. It not only shortened the sample preparation time, but also saved cost. Figure 3 shows the comparison of the sample clean-up step before and after. Combing with LC-MS/MS analysis it could be quantified more accurately.

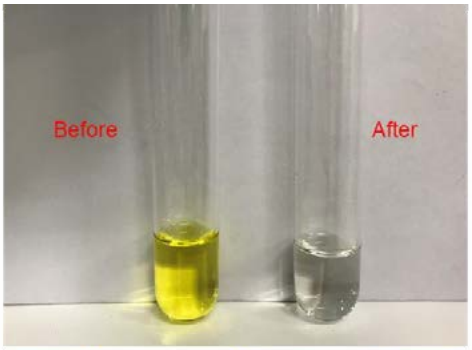

Figure 3. Sample preparation and clean up. Visual comparison of a grain sample before and after the Cleanert® SPE column clean-up step. Cleaning up the sample can provide reduction of matrix interferences as well as help in maintaining instrument performance.

The limit of quantitation and matrix matched linearity were evaluated. Because of the matrix inhibitory effects, the matrix matched curves were used to quantify the unknown samples. For AFB1 and DON as example, the method was found to be good reproducibility, linear regression coefficient was found to be greater than 0.99 (Figure 4). According to the different sensitivity of each compound on the instrument, the LOQ of all target mycotoxins were from 0.1ng/g to 5ng/g. The accuracy of low, medium and high concentration spiked sample was between 80% and 120% (Figure 1).

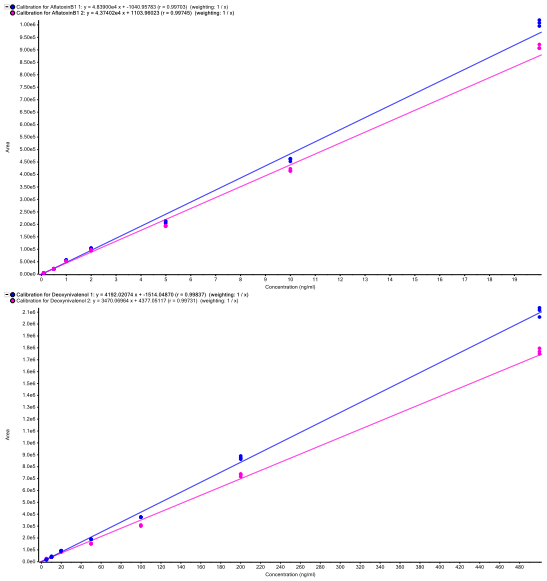

Figure 4. Calibration lines of AFB1 (top) and DON (bottom) from 5 to 500 ng/mL. Two MRM transitions were monitored: fragment 1 (blue) and fragment 2 (pink). R-values shown for both transitions for both representative analytes are >0.99, demonstrating excellent linear range and response for the assay.

CONCLUSIONS

A fast, robust, and reliable method, for the detection 25 mycotoxins in the matrix grain was developed and validated. A fast purification method was used to cover the 25 kinds of mycotoxins. High resolution LC using a small particle size column was combined with high sensitivity detection using a SCIEX QTRAP® 4500 LC-MS/MS system. Multiple Reaction Monitoring (MRM) was used because of its high selectivity and sensitivity. The Scheduled MRM™ algorithm used automatically optimized dwell times and cycle times for best sensitivity and reproducibility. The method was validated in different grain matrices. Limits of Quantitation (LOQ) of all mycotoxins were found between 0.1µg/kg and 5µg/kg. All LOQ meet the requirements of the GB methods.

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Table 2. MRM transitions and retention times are provided for two transitions for each mycotoxin in the 25-analyte panel. Shown are the 18 compounds analyzed in positive ion mode.

Compounds name	RT(min)	MRM (primary, quantifier)	MRM (secondary, qualifier)
AflatoxinB1(AFB1)	6.62	313.1>285.1	313.1>241.1
AflatoxinB2(AFB2)	6.43	315.1>287.1	315.1>259.1
AflatoxinG1(AFG1)	6.22	329.1>243.2	329.1>214.9
AflatoxinG2(AFG2)	6.05	331.1>245.1	331.1>189.1
AflatoxinM1(AFM1)	6.07	329.0>273.1	329.0>268.9
AflatoxinM2(AFM2)	5.86	331.1>273.1	331.1>285.1
T-2 toxin(T-2)	8.32	484.2>305.3	484.2>185.1
Verruculogen(VER)	9.84	534.3>392.3	534.3>191.1
Neosolaniol(NEO)	5.41	400.2>185.1	400.2>305.2
Wortmannin(WOR)	7.59	447.2>345.2	447.2>285.2
Roquefortine C(RC)	7.13	390.3>193.1	390.3>322.2
Sterigmatocysin(STE)	9.19	325.1>310.1	325.1>281.0
Lysergol(LYS)	4.8	255.3>240.2	255.3>197.2
Diacetoxyscirpenol (DIA)	6.7	384.2>307.2	384.2>105.1
HT-2 Toxin(HT-2)	7.59	442.1>263.1	442.1>215.0
Deoxynivalenol(DON)	4.76	296.9>249.1	296.9>231.1
3-Acetyl Deoxynivalenol (3-AcDON)	5.8	339.0>231.0	339.0>203.0
15-Acetyl Deoxynivalenol (15-AcDON)	5.8	339.1>321.3	339.1>137.2

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Pesticide Residues in Produce Analyzed by Targeted MRM^{HR} "Full- Scan" Acquisition and Processing

MRM^{HR} for Concurrent Quantitation, Library Searching, and High-Confidence ID Confirmation

KC Hyland
SCIEX, Redwood City, CA

Food and environmental sample analysis represents an impossibly large universe of potential matrices and hundreds of potential contaminant residues, including chemically alike (even isomeric) species, as well as those which may be widely chemically diverse. In addition to robust routine quantitation, testing laboratories are increasingly tasked with confirmation of positive detections. In addition to the paramount importance of protecting consumers and the environment, positive hits or above-tolerance limit results can also lead to the delay or destruction of products, with massive impacts to import, export, sale or distribution, and millions of dollars, at stake.

Application of LC-MS/MS with multiple reaction monitoring (MRM) has represented the principal workflow for pesticide residues analyses due to the high degree of sensitivity and selectivity imparted by the monitoring of unique MRM transitions. The work presented explores the additional advantages gained when leveraging High Resolution Accurate Mass (HRAM) mass spectrometric technology.

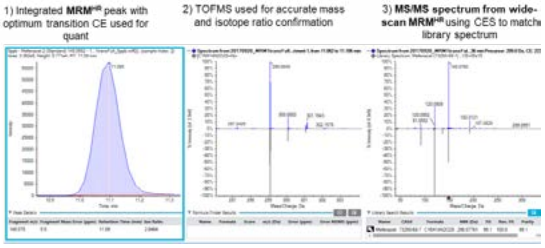

Figure 1. Simplified Quantitation and Confirmation Combined. An MRM^{HR} experiment type was employed to collect pesticide data in food matrices. MRM^{HR} acquisition allows monitoring of both optimized transitions as well as full-scan product ion spectrum collection. This approach provides the capability for concurrent quantitation (using a highly specific MRM transition optimized for maximum sensitivity) and identity confirmation by MSMS spectral matching, with a single acquisition method and a single processing step. In this example, an MRM^{HR} peak for Mefenecet is shown, including its retention time error and fragment mass error, alongside confirmatory TOFMS spectrum and MSMS library matched spectrum with a Purity score of 99.

The SCIEX X500R QTOF system and SCIEX OS software combined provide the ability to perform both routine targeted quantitation as well as screening. The key advantages of this HRAM approach are realized in the streamlined MRM^{HR} workflow which achieves sensitive and selective quantitative MRM data collection and processing with practical, concurrent collection and searching of MSMS data.

Key Advantages of MRM^{HR} Analysis

- Data acquisition with MRM^{HR} in conjunction with the simultaneous collection of TOF MS data provides access to **multiple approaches** for achieving accurate and sensitive quantitative analyses.
- MRM^{HR} takes advantage of monitoring a transition for specificity. Defining optimized voltages for each transition maximizes sensitivity. MRM^{HR} specificity leads to **reduced background and increased signal to noise ratios**. Retention time scheduling allows data collection only during known elution windows for best peak quality.
- **Full scan MSMS can be collected in MRM^{HR} mode**, and the resulting spectra can be searched against a compound library for qualitative ID.
- High confidence in compound identification is achieved through **multiple points of matching** including accurate precursor ion mass, isotope pattern matching, accurate fragment mass, ion ratio, chromatographic retention time, and library matching.

Contents

Experimental

Sample Preparation:

The iDQuant™ Standards Kit for Pesticide Analysis includes 209 well characterized pesticides. Here we present example data where we used the iDQuant™ Kit to screen for, quantify, and identify pesticides in extracts of fruits and vegetables using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) with an AB SCIEX QTRAP® 5500 system. Organic produce samples were extracted using QuEChERS. The iD Quant Kit Pesticides mixture, containing 209 characterized pesticides, was used as a spiking solution in some samples and to build standard calibrators for external quantitation.

HPLC Conditions:

Analytical liquid chromatography (LC) separation was achieved using a SCIEX ExionLC™ AD system and a Phenomenex Kinetex XB-C18 LC Column (100 x 3 mm) with mobile phases consisting of A) Water + 5 mM ammonium formate + 0.1% formic acid and B) Methanol + 5 mM ammonium formate. Column oven temperature was 50°C and a 20 µL injection was used. Gradient conditions were used with a run time of 21 minutes for the full gradient with a flow rate of 0.4 mL/min. An example elution profile of the MRM transitions is shown in Figure 2.

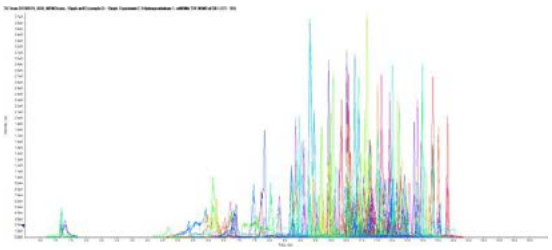

Figure 2. Chromatographic profile of MRM^{HR} transitions in a standard solution of the 209-pesticide mixture in the iD quant kit. Separation was achieved using Phenomenex Kinetex XB-C18 column and a 21-minute LC gradient.

MS Conditions:

The SCIEX X500R QTOF system with the Turbo V™ source was operated in positive mode electrospray ionization (ESI). Source parameters are listed in Table 1. The TOF MS scan was conducted over a range of 50 to 1000 *m/z*. Two different MS acquisition methods are demonstrated. Targeted analysis of the pesticide panel was conducted using an MRM^{HR} experiment including two transitions monitored for each analyte. Additionally, retention time (RT) values were specified for each MRM^{HR} transition, with RT tolerance values of 15 s for each, and the Extended Linear Dynamic Range feature was turned on (Figure 3).

Table 1. Ion Source Parameters. Electrospray Ionization (ESI) conducted in positive ion mode.

Parameter	Setting
Curtain Gas (CUR)	*30*
Collision Gas	*10*
Ion Spray voltage (IS)	*5500*
Temperature (TEM)	*650*
Nebulizer Gas (GS1)	*50*
Heater Gas (GS2)	*50*

The second acquisition method demonstrated was the Data Independent Acquisition known as SWATH® Acquisition. TOF MS scan parameters were identical to the MRM^{HR} method. Variable window SWATH® acquisition was employed to cover the precursor mass ranges from 50 to 800 *m/z*. A total of 20 nominal mass SWATH® windows were defined, and total scan time for this acquisition method was approximately 1.7 seconds.

MRM^{HR} Data Acquisition and Full-scan MSMS Collection

For each target transition in the acquisition method, the nominal mass precursor ion was defined for the target analyte, and a mass range was defined which would encompass the expected fragment ion. Optimized declustering potential (DP) and collision energy (CE) voltages were designated for the primary transition, around which a narrow (20 Da) TOF mass range was defined. A second MRM^{HR} transition was also defined for each target, with the same nominal mass precursor ion, but which collects a "full scan" range of product ion masses from 40 to 1000 *m/z*. A generic CE (35 V) with Collision Energy Spread (CES) of 15 V was defined to achieve a more robust MSMS spectrum for searching against database spectra. Additionally, scan scheduling was applied to all transitions by assigning the known retention time to each; in this mode of operation, data for each transition will only be acquired within the defined chromatographic time window, this preserving total instrument cycle time to maintain peak quality, sensitivity, and ability to potentially add large numbers of additional transitions. Figure 3 shows a portion of the MS acquisition method in the SCIEX OS software, highlighting the differences between the two defined transitions for each compound.

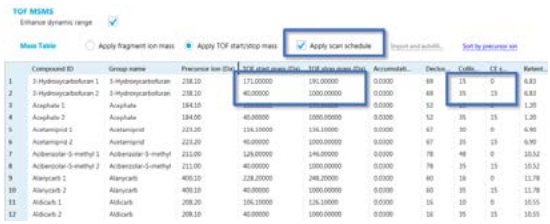

Figure 3. MRM^{HR} Data Acquisition for Combined Quantitation and Library Matching. This MS method setup in SCIEX OS software includes each pesticide compound with two MRM^{HR} transitions with different acquisition parameters. Example components for this type of workflow are shown, and the columns utilized to set up the method. From left to right, these are: Compound ID, Group Name, Precursor Ion, TOF Start Mass, TOF Stop Mass, Accumulation Time, Declustering Potential, Collision Energy, Collision Energy Spread, and Retention Time. "Apply Scan Schedule" is checked, so that data acquisition of each compound occurs only around its known RT. The first MRM^{HR} transition of each compound includes a narrow TOF range for product ion collection, an optimized CE, and CES of 0. The second MRM^{HR} transition includes a generic CE of 35 and a CES of 15 to generate a robust MSMS spectrum.

Quantitation with TOFMS and MRM^{HR}

Matrix interferences are an obstacle and confidence in identification of residues is paramount. The increased specificity of monitoring an MRM^{HR} transition is one approach which can be utilized to reduce matrix background, baseline, or interferences which may be observed in the TOFMS data trace. However, the signal intensity and peak quality of the transition relies on the efficient formation of the monitored fragment ion. Reduction in signal during precursor transmission and fragmentation results in a lower absolute intensity observed when monitoring an MRM^{HR} transition versus extracted TOFMS ions. Despite this, reduced baseline can still provide greater perceived sensitivity due to drastically reduced baseline and subsequently increased signal to noise ratio. In the presented MRM^{HR} acquisition method, both scans happen simultaneously in a single injection, and processing can utilize either or both, thus reducing or eliminating the need for multiple confirmatory injections or re-injections.

Method Performance:

Table 2 shows some example method performance data for a subset of pesticides, comparing quantitation achieved using extracted TOFMS data and MRM^{HR} transitions. In general, the sensitivity achieved for most pesticides in the iD Quant Kit mixture was <0.1 ng/mL in neat solvent and most analytes also exhibited >3.5 orders of linear dynamic range.

Table 2. Method performance measurements for a small set of analytes using TOF MS data for quantitation. XIC width around theoretical mass of 0.02 Da was used.

Analyte	~LLOQ, ng/mL (S/N > 10)	CV % LLOQ	CV % 10x LOQ	Cal Range (ng/mL)	Dynamic Range (log[ULOQ/LLOQ])
Quinoxyfen	*0.05*	*6%*	*5%*	*0.05 – 500*	*4*
Carboxim	*0.01*	*22%*	*11%*	*0.01 – 100*	*4*
Isoproturon	*0.05*	*2%*	*4%*	*0.05 – 500*	*4*
Tebuconazole	*0.1*	*13%*	*1%*	*0.1 - 500*	*3.7*

Method performance measurements for a same subset of analytes, shown for quantitation using MRM^{HR} data.

Analyte	~LLOQ, ng/mL (S/N > 10)	CV % LLOQ	CV % 10x LOQ	Cal Range (ng/mL)	Dynamic Range (log[ULOQ/LLOQ])
Quinoxyfen	*0.05*	*9%*	*3%*	*0.05 – 500*	*4*
Carboxim	*0.01*	*22%*	*1%*	*0.01 – 100*	*4*
Isoproturon	*0.05*	*6%*	*9%*	*0.05 – 500*	*4*
Tebuconazole	*0.1*	*7%*	*12%*	*0.1 - 500*	*3.7*

When comparing the method performance of extracted TOFMS ions to MRM^{HR} transitions in a complex matrix such as a plant extract, three scenarios represent the most commonly observed behavior. Identifying which compounds in a panel exhibit which of these three behaviors can help in assessing which type of scan is best used for optimal quantitation method performance. The three potential observed behaviors are:

1. Despite a higher absolute signal in TOFMS data, the MRM^{HR} data provides a reduced baseline, increased signal to noise, and results in greater observed sensitivity in matrix.
2. TOFMS data is drastically more sensitive than MRM^{HR} data. Poor fragmentation is a potential reason for this, and the result is that the greater signal for TOFMS peak provides improved sensitivity and method performance over the MRM^{HR} peak.
3. Isobaric matrix peaks which elute close to or overlapping with the target analyte make peak integration in the TOFMS trace challenging and impact the accuracy and reproducibility of the quantitation; the MRM^{HR} trace, however, does not show the interferences and therefore has improved sensitivity and quantitative method performance.

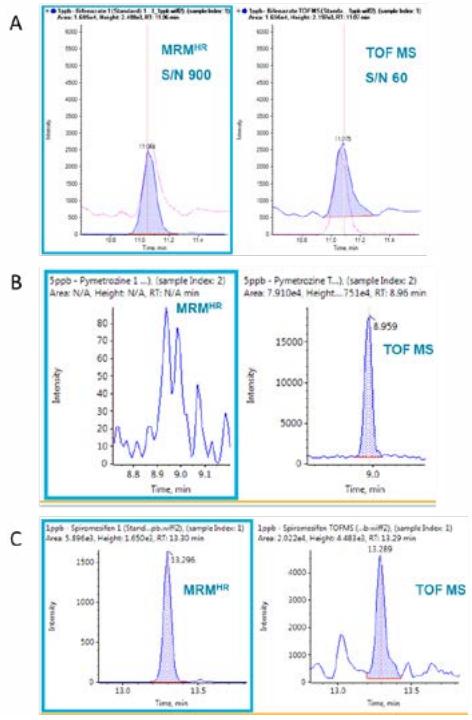

Figure 4. Example behavior of different analytes comparing MRM^{HR} data and TOFMS data. A.) Bifenezate example. The baseline is greatly reduced in the MRM^{HR} data compared to TOFMS, leading to a higher signal to noise ratio observed for the MRM^{HR} peak. B.) Pymetrozine example. No signal is observed in the MRM^{HR} trace at all at the 5ppb concentration level, however, for this same concentration a distinct TOFMS peak can be observed. For this analyte, TOFMS signal is vastly improved over MRM^{HR}. C.) Spiromesifen example. Interfering peaks and background in the TOFMS data make integration challenging; these a greatly reduced and both baseline and integration improvement can be seen in the MRM^{HR} data for this analyte in this matrix.

In an analyte panel which can be very diverse (such as a pesticide suite) and a matrix or matrices which can be very complex and have high concentrations of endogenous background species, there is potential for these differing behaviors to be observed not only between analytes (for example, some analytes do not provide sensitive fragments) but also between different types of matrix (i.e., not all matrices will produce the same interfering peaks at the same masses). It may be important, then, to consider assessing quantitative method performance of both TOFMS data and MRMHR data until a better understanding of the behaviors in the desired panels/matrices is attained. Table 3 breaks down some of the pesticides in the iD quant kit mixture by which of these behaviors each of them demonstrates in the QuEChERS arugula extract. A subset of these examples can also be seen in Figure 4.

Ion Ratios:

Many triple quadrupole- based MRM quantitative workflows include the reporting of signal ratios between multiple MRM transitions. To do so, however, requires the collection of a secondary MRM transition during data acquisition, adds to the number of transitions in the method and which, without stringent method optimization, can impact method parameters such as cycle time, data points collected across a peak, and ultimately sensitivity and reproducibility. Utilizing the described data acquisition approach of monitoring two MRM^{HR} channels per compound, there are multiple ways in which ion ratios can be derived and reported to gain further confirmation in analyte detection and identification. Multiple MRM^{HR} traces can be generated without having multiple specific transitions defined during acquisition, because the full- scan product ion range in the second monitored MRM^{HR} channel allows for extraction of any fragment or fragment within that range. Additionally, the extracted TOFMS peak, when grouped together with an MRM^{HR} transition, can also produce ion ratio values which can be reported (Figure 5).

Table 3. Comparing MRM^{HR} data to extracted accurate mass from the TOFMS data reveals differences in the optimum type of monitoring for each analyte. For some compounds, MRM^{HR} is an improvement over TOFMS due to reduction of interferences or lowered baseline. These behaviors might also be expected to differ when observed in a variety of matrices.

MRM^{HR} Baseline reduction from TOFMS	Much greater sensitivity for TOFMS	Shows interferences in TOFMS but not in MRM^{HR}
Bifenezate	*Acibenzolar-S-methyl*	*Bupirimate*
Amitraz	*Alanycarb*	*Diclobutrazol*
Benfuracarb	*Ametryn*	*Dimoxystrobin*
Bitertanol	*Dioxacarb*	*Fenbuconazole*
Carbetamide	*Ethiofencarb*	*Flusilazole*
Cycluron	*Fenoxycarb*	*Ipconazole*
Fenarimol	*Fenpropimorph*	*Prometryn*
Fenuron	*Hydramethylnon*	*Spiromesifen*
Fluometuron	*Imazalil*	*Terbutryn*
Iprovalicarb	*Indoxacarb*	
Isoprocarb	*Mandipropamid*	
Metalaxyl	*Omethoate*	
Methamidophos	*Oxadixyl*	
Methiocarb	*Phenmedipham*	
Methoxyfenozide	*Prometon*	
Metribuzin	*Propham*	
Nitenpyram	*Pymetrozine*	
Propamocarb	*Pyrimethanil*	
Propargite	*Spiridiclofen*	
Pyracarbolid	*Sulfentrazone*	
Tebufenozide	*Tebufenpyrad*	
	Terbumeton	
	Thiofanox	
	Triadimefon	

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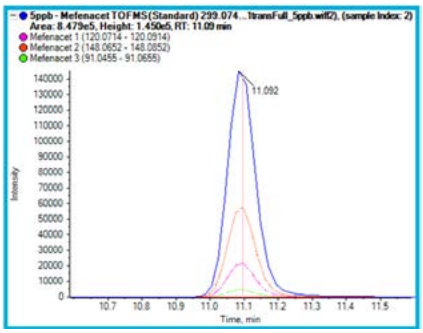

Figure 5. Ion ratios. Ion ratios for compound identity confirmation can be generated for multiple MRM^{HR} transitions. In this example for Mefenacet, the TOFMS peak is overlaid with the MRM peaks extracted for three transitions, the first of which comes from the optimized acquisition channel and the last two extracted from the full- scan acquisition channel.

Library Searching and Confirmation of Compound ID from MRM^{HR} Acquisition

Collection of full MSMS spectrum allows for spectral library searching and matching, without performing a separate sample acquisition. Use of the Collision Energy Spread (CES) ensures that the collected MSMS spectrum includes an enriched range of fragment masses collected over multiple collision energy values, which can be searched against a compound library or database for more dependable spectral matching. Data processing methods were built in the SCIEX OS software which incorporated both the integration and quantitation parameters for the primary MRMHR transitions, but also dictated that MSMS library searching be performed on the processed data. The results table displays, for review, the chromatographic peak for quantitation; the TOF MS mass spectrum and isotopic distribution; and the MSMS product ion spectrum mirrored with the matching database spectrum for confirmation (Figure 1).

Identification of these pesticides in unknown samples were achieved with high confidence by leveraging HRAM analysis to provide multiple points of matching using accurate mass of the precursor ion, MRM^{HR} transition monitoring (including accurate mass of the fragment ion), isotope pattern matching, ion ratio, and chromatographic retention time (Figure 7). This extremely high degree of confidence in analyte identification provides failsafe against reporting false positive hits, by ensuring that multiple points of independent confirmation are satisfied.

Summary

For quantifying a targeted analyte suite or confirming suspect target detections or identities, MRM^{HR} provides high resolution monitoring of known ion transitions as well as full scan product ion spectrum collection. MRM^{HR} can deliver lower baseline and increased specificity for some target compounds, resulting in better signal- to- noise ratios and improved sensitivity in complex

Sample Name	Component Name	Component Group Na...	Used	Calculated Concentration	Precursor Mass	Fragment Mass	Ion Ratio...	Mass Error...	Frag... Mass...	RT Confi...	Isotope Confi...	Library Confi...	Found At Mass	Mass Error (pp...	Fragment Mass Err...	Library Hit	Library Score
Arugula_S_10	Metalaxyl 1	Metalaxyl	☑	14.260	280.154	160.112	✓	✓	✓	✓	✓	✓	280.1541	-0.7	3.6	Metalaxyl	84.6
Arugula_S_10	Metalaxyl TOFMS	Metalaxyl	☑	9.463	280.154	N/A	✓	✓	■	✓	✓	✓	280.1541	-0.7	N/A	Metalaxyl	85.6
Arugula_S_10	Metalaxyl 1	Metalaxyl	☑	16.522	280.154	160.112	✓	✓	✓	✓	✓	✓	280.1543	-0.2	-1.8	Metalaxyl	87.7
Arugula_S_10	Metalaxyl TOFMS	Metalaxyl	☑	10.326	280.154	N/A	✓	✓	■	✓	✓	✓	280.1543	-0.2	N/A	Metalaxyl	87.7

The TOF MS and MRM^{HR} data are shown together in the table

The expected precursor mass and fragment masses have been defined for this targeted analyte

Green check marks are shown for all the positive points of confirmation; each point "passes" within the user-defined tolerance limits to produce a confident confirmation of this analyte ID in an unknown arugula sample.
1.) Ion ratio within 20% of standard.
2.) Precursor mass error with 2ppm of expected.
3.) Fragment mass error (for MRM^{HR}) within 5ppm of expected.
4.) RT within 0.2 minutes of expected.
5.) Isotope distribution matches expected with 70% or better
6.) MSMS spectrum matches library spectrum with 70% or better purity score

The accurate masses found for precursor and fragment are within 1ppm and 4ppm mass error, respectively.

Library search returns confirmation of compound ID with a corresponding score.

Figure 7. Target Identification Points of Confirmation: Some example rows from SCIEX OS results table are shown. Identification and quantitation of pesticides in unknown samples can be achieved with high confidence by utilizing the breadth of information available for processing from MRM^{HR} full scan acquisition.

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matrices. TOF MS quantitation can also be used when greater signal is needed and there is no isobaric interference from the matrix. This combination of scans in a single acquisition allows for selection of the most advantageous quantitation options for analyte and matrix combinations. Full scan MSMS can be collected in MRM^{HR} mode and searched against a compound library for compound identification or confirmation. Multiple ion ratios for compound confirmation can be generated and can also include TOF MS data in their calculation.

References

1. Schreiber, *et al*. "Using the iDQuant™ Standards Kit for Pesticide Analysis to Analyze Residues in Fruits and Vegetable Samples." SCIEX, 2011.
2. Anastassiades, *et al*. "Fast and easy multiresidue method employing acetonitrile extraction/partitioning and 'dispersive solid-phase extraction' for the determination of pesticide residues in produce." J. AOAC Int., 2003.

X500R QTOF System with SWATH® Acquisition for Pesticide Residue Screening in Fruits and Vegetables

Xiaojing Sun, Haiyan Cheng , Lijun Li , Wenhai Jin
SCIEX, Asia Pacific Application Support Center (Shanghai), China

Introduction

It is widely accepted that modern Chinese agriculture has a long history of excessive chemical fertilizer, pesticide, and herbicide use, and this has not only resulted in reduced nutrient content in food, but also in a variety of chemical residues that have harmed human health. It has been confirmed that pesticide residues may interfere with the body's endocrine effects on the immune system and hematopoietic system, and can even cause in-vivo fetal visceral hypoplasia or deformity in pregnant women. Weighed against the benefits of consuming more fruits and vegetables, people have recently become more heavily concerned about the widespread existence of pesticide residues and the excessive damage they can cause to the human body.

With the continued development and popularization of liquid chromatography / mass spectrometry, more and more pesticide residue detection technologies are being developed based on the LC-MS/MS system. The most commonly used pesticide residue screening method includes the MRM-IDA-EPI system, which is based on QTRAP® system and high resolution TOF-IDA-MSMS technology. While the SWATH technology is based on high-resolution systems, it also combines the advantages of IDA and MRM by dividing the mass range of the parent ion into multiple mass windows and allowing all ions in each window to collide with each other and fragment, resulting in fragmentation information for all ions in the entire mass range. SWATH® technology's measurement of second-order fragmentation differs from the IDA, in which only the selected ions are triggered, ensuring the continuity of all ion debris and achieving SWATH's second degree of quantification. By customizing the unique variable window settings, the size of the mass window is automatically adjusted according to the quantity of ions, ensuring the collection of high-quality data.

The SCIEX High Resolution Mass Spectrometry X500R QTOF system provides high resolution, high accuracy, high sensitivity and high linearity range scan speeds, making SCIEX SWATH technology not only popular for protein macromolecules but also for small molecule pesticide residue screening. The X500R QTOF system uses newly designed SCIEX OS software to achieve an all-in-one whole process analysis with instrument control, data acquisition and data processing. The software has the built-in SWATH® method of setup and powerful automatic deconvolution capabilities. This simple and convenient design meets food safety field use requirements.

Experimental considerations:

1. Collect and process samples of fruits and vegetables, and measure the actual SWATH data
2. Prepare Standard Curve, Test 190 Pesticide Standard SWATH data
3. Screening of Pesticide Residues in Vegetables and Fruits
4. The pesticide residue was quantified at two levels

Sample treatment:

1. Weigh10g of mashed sample into a clean tube
2. Add 10mL of Acetonitrile with 1% acetic acid, votex for 1min
3. Add 1.5g of NaAC, 6g of MgSO4, votex, then Centrifuge for 5 mins
4. Precipitate 8mL of supernatant with Agela clean package
5. Centrifuge for 5 mins, transfer supernatant for analysis

The QuEChERS method was used to pretreat received samples: 1 leek, 2 cauliflower, 3 bean, 4 jujube (after washing), 5 jujube (not cleaned), 6 pear.

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Chromatographic Methods

Chromatography column: Phenomenex Kinetex C18, 100*2.1 mm, 2.6μm

Mobile phase: A: Contains 5mM ammonium acetate in water; B: Contains 5mM ammonium acetate in methanol gradient elution

Flow rate: 0.4mL/min

Column temperature: 40℃

Input volume: 10μL

Time (min)	B%
0	*3*
1	*3*
2	*45*
19	*95*
22	*95*
22.1	*5*
25	*5*

Mass Spectrometry Method

Scanning method: SWATH® Acquisition methods

Ion source: ESI+source CDS automatic calibration

Table 1: Ion source parameters

IS Voltage: 5500V	*Air curtain gas CUR: 35psi*
Atomizing gas GS1: 55 psi	*Auxiliary gas GS2: 55 psi*
Source Temperature TEM: 550°C	*Collision Gas CAD: 7*
Collision energy CE ± CES: 35 ±15V	*Air curtain gas CUR: 35psi*

Data acquisition and SWATH setup process

IDA (Information Dependent Acquisition) uses TOF/MS Survey Scan to pre-scan. When a peak ion is successfully triggered and detected, the scan mode is switched to Q1 and the parent ion is selected to acquire a high sensitivity MS/MS secondary spectrum of the target ion. SWATH distributes all the ions into successive windows, and all the ions in each window are transferred to the collision chamber and broken into second-order MS/MS debris and then traced back to the parent ion through the software's powerful de-convolution function. Thus all of the second-order fragments of all abundant ions can be obtained through this technique, which ensures that the secondary information of the low-content target is included, allowing the trace residue screening to become more complete and accurate.

Unique intelligent variable windows, according to the distribution of ions in the sample, set narrow windows in the high density distribution areas and set up wider windows in the regions with fewer ions to ensure high-quality secondary mass spectra are collected for all ions.

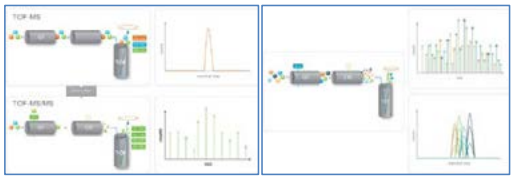


Figure 1 Left IDA schema; Right SWATH schema

Figure 2 SWATH method settings

In the SCIEX OS Software, choose "Experiment" and then pick the SWATH Acquisition mode. The software then automatically lists the required parameters for the SWATH mode. Mass Table is for the Q1window.

The Variable Window Calculator can be based on TOF/MS's parent ion to automatically calculate the SWATH smart variable window. The mode can be established by copying and pasting to the Mass Table, which is a method that is simple, rapid, and easy to use.

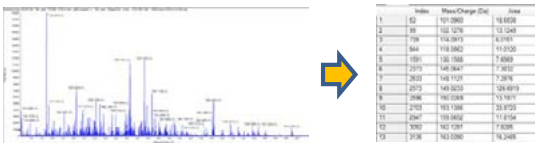


Figure 3 TOF/MS's parent ion

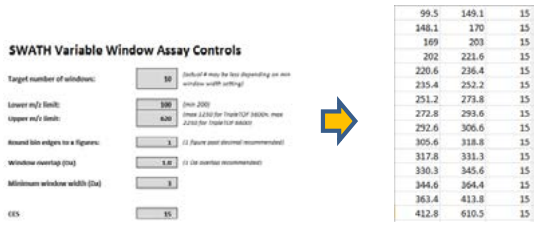
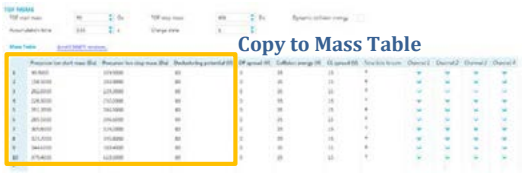


Figure 4 Variable Window Calculator



Establish SWATH® Acquisition method and initial test of 190 varieties of standard pesticide products.

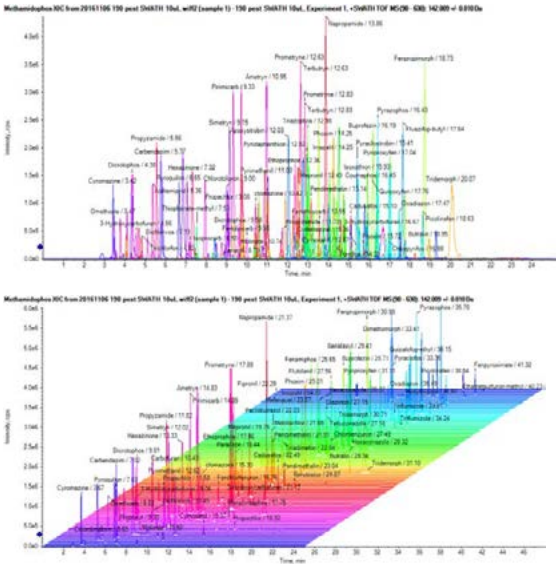


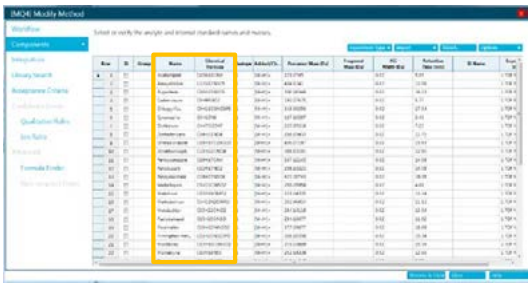
Figure 5 Chromatogram of 190 varieties of pesticide residue standard products collected by SWATH.

Data Analysis

1. Qualitative screening

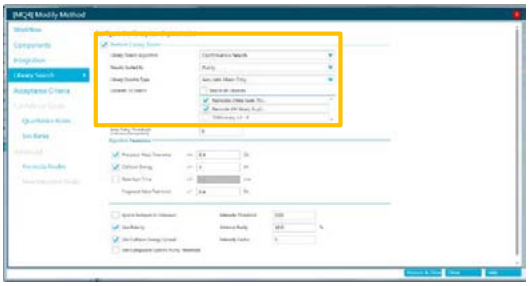
Test SWATH data of 1 (leek), 2(cauliflower), 3 (kidney beans), 4 (jujube, washed), 5 (winter jujube, unwashed), and 6 (pear). Use X500R SCIEX OS Software to perform data analysis by passing four confidence conditions: mass accuracy, retention time, isotope distribution and secondary library matching to screen pesticide residues in the 6 samples.

1. Select the standard product data to establish screening methods; import the screening list



2. Set the quantitative integration parameters

3. Set the library search criteria



4. Set the screening confidence conditions

5. One time import of all standard product and samples' SWATH® data to perform screening

6. Filter results through the Mass error, RT, Isotope, Library

7. Obtain he results of screening for each sample

Chromatogram isotope pattern MS/MS FIT

2. SWATH second degree quantification

Using TOF/MS's first degree quantitative data in complex matrix samples has disadvantages such as high baseline noise and a narrow linear range, etc. The SCIEX OS Software in the X500R QTOF system can be used to directly copy and paste the ion pairs of compounds when a quantitative method is established, obtaining the results of the second quantification by using the MRM^{HR} method to process SWATH data.

Preparation of 190 kinds of pesticide on the standard curve1ng/mL~100ng/mL established the second degree quantitative SWATH® method to obtain second degree quantitative linear relationships, see Fig. 6.

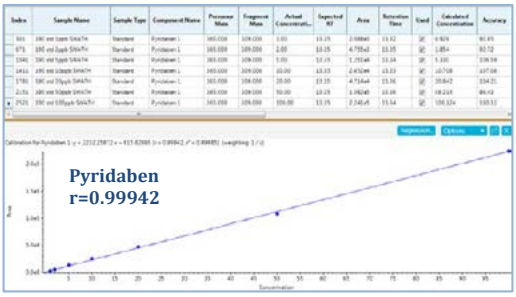


Figure 6 Quantitative linearity using pyridaben as an example

For use of the high sensitivity and high selectivity SWATH second degree quantitative method to quantify the pesticide residues contained in leek, cauliflower, kidney bean, winter jujube (washed), winter jujube (unwashed), and pear, please see the table below for the pesticide residues contained in the above samples.

Detected pesticides (unit: ng / mL)

	Leek	Cauliflower	Kidney Bean	Winter Jujube (washed)	Winter Jujube (unwashed)	Pear
Carbendazim	*8.7*		*1*		*1.4*	
Insecticide	*4.5*					
Methylpyrimidine	*3.5*					
Prometryn	*33*					
Pyrimethanil	*23*		*270*			
Thiophanate-methyl	*2.1*					
Imidacloprid		*1.2*			*580*	*3.4*
Propoxur		*50*	*13.7*			
Tebuconazole		*1.1*	*4.5*			*3.4*
Acetamiprid			*3.7*		*5.1*	*86*
Kresoxim			*8.2*	*15*	*22*	
Streptozotocin			*35*	*18*	*74*	
Buprofezin					*160*	
Fenpyroximate					*61*	
Paclobutrazol					*140*	
Triadimefon					*23*	

Summary

The experiment used the SCIEX X500R QTOF system's SWATH technology to screen pesticide residues in six varieties of vegetables and fruits, among which leeks, kidney beans and

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jujube contained 6 or more types of pesticide residue. In particular, in the jujube, the imidacloprid content reached 580 ppb, far exceeding the limits of pesticide residue standards; buprofezin and paclobutrazol content also exceeded100ppb. Through the analysis of the washed jujube samples we found that although the pesticide residue is extremely high, it is fortunately possible to be washed off with detergent. Even so, the washed jujube still contains more than 10ppb of kresoxim-methyl and pyraclostrobin.

This experiment established the SWATH® screening and quantitative methods for residues of the190 most commonly used types of pesticides for the Ministry of Agriculture risk assessment. SWATH® technology obtained the primary and secondary data of all pesticide residues by entering the samples only a single time. The highly sensitive secondary spectra were still able to identify each compound in the spectral library and obtained the secondary spectra even when the pesticide content in the sample was very low, using four confidence conditions: mass accuracy, isotope distribution, retention time, and secondary library matching to corroborate.

Any ion's continuous chromatographic peak data in the SWATH® secondary spectrum can be used as the basis for quantitative data analysis. When the sample in the first class chromatographic peak has matrix interference, SWATH® second degree quantification can effectively reduce the background noise and eliminate interfering ions so that the quantitative results are more accurate and reliable. 190 kinds of standard curve pesticide residues were profiled, and qualitative and quantitative analysis of the 6 samples was performed.

Setting up the SWATH® method is very easy. One can directly establish methods by going to SCIEX OS software's built-in SWATH® options, and through the Variable Windows, the settings allow the user to intelligently partition the ions' Q1 mass window, to ensure that each ion can receive high quality SWATH® data, in order to meet the qualitative and quantitative needs.

References

1. *André Schreiber, SCIEX, Concord, Ontario (Canada),* X500R and SWATH for pesticide screening RUO-MKT-11-4711-A
2. *SCIEX*, European Union Reference Laboratory (EURL-FV) Almeria, Spain and the EMEA team, Analysis of Regulated Pesticides in Baby Food Using SCIEX X500R QTOF

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A Robust and Sensitive Method for the Direct Analysis of Polar Pesticides in Food and Environmental Samples Without Derivatization

Wim Broer[1], Ugo Chiuminato[2], Jianru Stahl-Zeng[2], Daniel McMillan[3] and Phil Taylor[3]
[1] Nofalab Laboratories, Schiedam, NL; [2] SCIEX, Darmstadt, DE; [3] SCIEX, Warrington, UK

The Challenge of Polar Pesticides

The prevalence of multi-residue LC-MS/MS analyses for the quantification of pesticides in food and environmental samples has been steadily increasing for many years, and they are now considered to be a minimum requirement of most laboratories working in these fields. Modern tandem quadrupoles are capable of detecting such regulated compounds at very low levels with minimal sample preparation, such as QuEChERS, thereby enabling labs to process large numbers of samples for many analytes with a fast turnaround. However, some very polar compounds which are not amenable to the extraction procedure, chromatographic method or are poor ionizers require additional single-residue methods which involve time-consuming preparation and separation and often involve derivatization to improve detection.

Key Advantages Presented

- All analytes were well retained, allowing detection of the majority of background components which could otherwise interfere. Separation between the analytes was also sufficient to allow unambiguous identification, and retention times were reproducible. Sensitivity in spiked environmental waters was found to be similar to that in standards, and the target limit of detection of 20 ng/L was easily achieved with real drinking water samples.
- Matrix effects were largely eliminated in both the NofaLab method for food sample extracts and the modified method for direct injection of water samples. Use of QTRAP® is expected to confirm positive results by their full-scan MS/MS spectra, but future work will investigate different or additional clean-up.

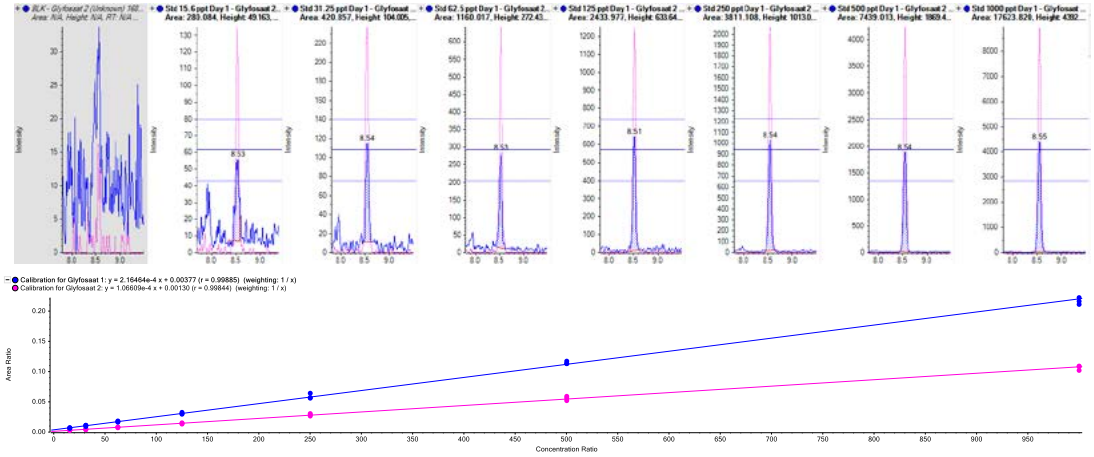

Figure 1. **Method sensitivity and linearity of glyphosate.** Calibration standards in concentrations from 15.6 to 1000 ng/L of glyphosate achieved using the modified method for water samples. Ion ratios were all well within the specified ±20% tolerance.

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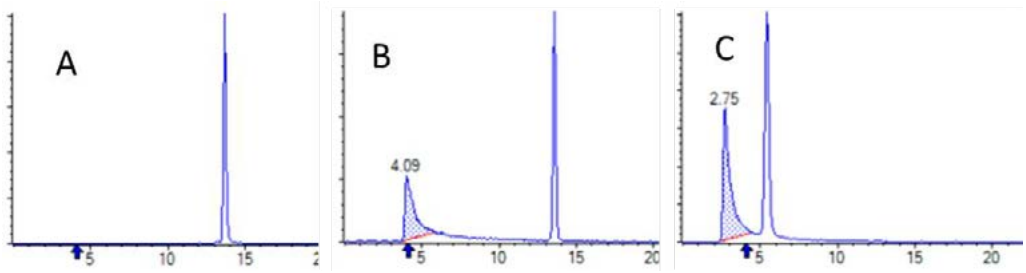

Figure 2. Use of a preferred column means: Install, Prime, Repeat, and finally Replace. Image A shows the performance of the preferred column after installation, no glyphosate peak is present. Image B shows the same column after it has been conditioned with 30 spinach extracts, a glyphosate peak can be detected at 4.09 mins. Image C shows retention time (RT) drift of the glyphosate peak on the same column after 100 injections.

Growing Concerns

Recent increase in public concern regarding the presence of glyphosate has significantly increased the requirement to analyse it and its metabolites in food, feed and the environment, so has accelerated the need for a more efficient and robust analytical method. The extraction and chromatography of these compounds is well described in the EURL-QUPPE method, but the separation is not robust in practice, so system and method maintenance are intensive. Several different HPLC or HILIC based methods have failed to address the issues of reproducibility and sensitivity, so FMOC derivatization prior to analysis is often still employed for glyphosate, AMPA and glufosinate. Although possible to automate, this procedure is still time consuming or expensive, and is not applicable to the other polar pesticides of interest.

Creating a High Throughput Method

NofaLab is an independent sampling and testing laboratory based near Rotterdam, Netherlands, specializing in the fields of food, feed and environmental safety. The increasing pressure to provide fast, quantitative analysis has driven NofaLab to add to their portfolio of LC-MS/MS instrumentation and develop a new method which covers as many of these polar pesticides in a single analysis as possible. Ion chromatography has been shown to be beneficial for separation, but the need for a suppressor is detrimental to MS analysis and the inefficiencies of changing inlet systems on a heavily used mass spectrometer makes it impractical in a busy lab performing primarily reverse-phase LC.

So, the final method, presented here, makes use of an LC column in a method-switching reverse phase (RP) system with MS amenable mobile phases at around pH 9. Such conditions configure glyphosate ideally for MS detection with good retention and separation of the other analytes and matrix interferences. The method meets the DG-SANTE[1] requirements of reproducibility (<20%) and recovery (80-110%), and the LOD of the method is below 0.01 mg/kg. Excellent long-term stability and robustness were achieved throughout the validation of this method for food samples extracted by the QUPPE procedure.

Where environmental samples require testing, the regulatory limits are much lower5 and interference from matrix more problematic in traditional analyses with a short retention time, so derivatization is often the only option. However, since glyphosate is well retained in this new method, the potential to further develop it for direct large-volume injection was investigated in collaboration with SCIEX. By modifying the gradient conditions and optimizing the injection parameters, a second method specific to environmental water samples has been developed. Although the large volume injection (LVI) is more susceptible to changes in pH (for example, due to evaporation of mobile phase) robustness has been shown to be similarly good, and allows detection of the same suite of analytes with a LOD of <0.02 ng/l.

Experimental Considerations

Food samples

The QuPPe method for extraction of polar pesticides from samples of plant and animal origin developed by Anastassiades et al. at CVUA Stuttgart[2] are well described and have undergone several revisions. Since the analytes are water soluble, it is based on aqueous extraction with addition of methanol and formic acid to improve efficiency.

The addition of internal standards is essential to compensate for the shifting retention times in most chromatographic method and helps to counter matrix effects where present. This was particularly important for grain and seed samples, where

chromatographic performance deteriorates, and the MS source becomes dirty, losing sensitivity quickly, so dispersive C18 cleanup as described in the QuPPe-AO3 method was attempted before finalizing on a push-through method with two sorbents using SPE filters.

Various chromatographic methods have been investigated and found to have several limitations. Figure 2 illustrates the common practice of extensive conditioning prior to analysis, which after relatively few (typically 30-50) sample injections in order to maintain peak shape and retention time Ion chromatographic methods showed most promise, but the eluents' incompatibility with electrospray ionization sources requires the use of a suppressor, which is detrimental to peak width. However, by employing a polyvinyl alcohol based column with quaternary ammonium groups and using an ammonium bicarbonate buffer prior to detection by a very sensitive quadrupole mass spectrometer, the need for a suppressor is removed.

Table 1. List of food matrices used for method verification.

Lists of Validated Commodities	
A	*Fruit and Vegetables*
B	*Seeds*
C	*Vegetable oil, Fat and Fatty Acids*
D	*Grain*
E	*Herbs and spices*
F	*Meat and Seafood*
G	*Animal Oil, Fat and Fatty Acids*
H	*Eggs and Eggs products*
I	*Milk and Milk products*
V	*Fatty acids*

Method verification was performed on a variety of food matrices (Table 1), all subject to clean-up as described above. Performance was robust and reproducible with 10µl injections, but peak shape started to deteriorate after around 200 samples, with significant distortion appearing by the 350th injection due to the limited capacity of the 2mm i.d. column. The final chromatographic method uses a 150 x 4mm column and employs a guard column of the same material and a 0.5µm filter, both of which are replaced every 250 samples to maintain performance and to keep the MS source clean.

Water samples

Environmental and drinking water samples varied widely in the degree of comprised particulate matter, which causes difficulties for LC injection and is detrimental to reproducibility. However, minimal sample preparation is desirable in a high throughput laboratory situation and SPE type clean-up would add significant time and financial cost. In order to overcome these challenges, a simple filtration step using Chromacol 17-SF-02 (RC) from 17 mm syringe filters was performed when transferring samples to the LC vials. Internal standards to a final concentration of 1ppb were added to samples and standards, and QC samples in tap water were prepared in a similar fashion. Experiments were also performed using standard addition to the samples to investigate any potential matrix effects.

Separation was achieved using a Shimadzu Nexera UHPLC system comprising LC-30AD pumps, a SIL-30AC autosampler fitted with a 500µL loop and a CTO-20A column oven. An injection volume of 500µL was employed in a chromatographic method similar to that used for the food samples. During verification of the method, the primary focus was on achieving stable peak shapes and retention times for all analytes. Loop size (irrespective of injection volume), initial conditions, gradient and pH of the mobile phase had very significant effects, so the final optimized method should be fixed, and fresh mobile phases prepared regularly.

Method verification was performed with real drinking water samples, testing for both AMPA and Glyphosate, a LOQ of 20ng/L could be reached.

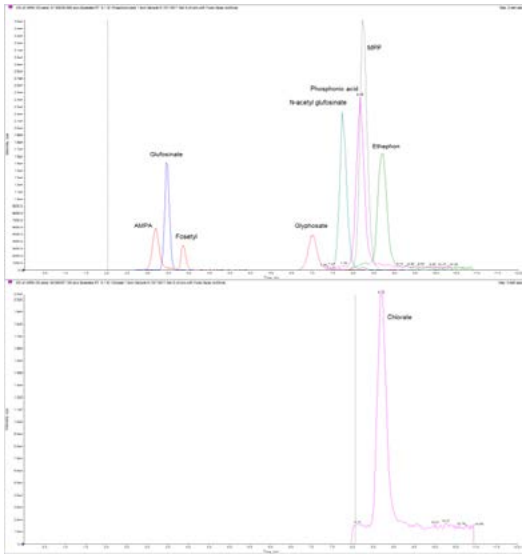

Figure 3. Example chromatograms shown for polar pesticides suite. Chromatographic separation using the hypercarb column was an integral component of the described method.

Table 2. Source parameters for the SCIEX QTRAP®® 6500+ System.

Source Parameters	
Curtain Gas (CUR)	*30 psi*
Collison Gas (CAD	*9 psi*
IonSpray Voltage (IS)	*-3000v*
Temperature (TEM)	*500°C*
Ion Source Gas (GS1)	*55 psi*
Ion Source Gas (GS2)	*65 psi*

MS-MS Analysis

Analyses were performed using a SCIEX QTRAP®® 6500+ mass spectrometer in negative electrospray ionization mode. At least two MRM transitions were optimized for each analyte as outlined in Table 3 in order to quantify and confirm their concentration in all samples. Data was acquired using Analyst® 1.6.3 .and processed for quantitation and confirmation with reference to internal standards using MultiQuant™ 3.0.2 software.

Table 3. List of analytes with MRM transitions employed. Internal standards are crucial to this method and must be used.

Analyte	Q1 m/z	Q3 m/z
Glyphosate 1	167.9	150.0
Glyphosate 2	167.9	78.8
Glyphosate 3	167.9	62.8
Ethephon 1	142.9	106.8
Ethephon 2	142.9	79.0
N-ac Glufosinate 1	222.0	136.0
N-ac Glufosinate 2	222.0	62.8
N-ac Glufosinate 3	222.0	59.1
AMPA 1	110.0	81.2
AMPA 2	110.0	79.1
AMPA 3	110.0	62.9
Glufosinate 1	180.0	136.0
Glufosinate 2	180.0	95.0
Glufosinate 3	180.0	85.0
Glufosinate 4	180.0	63.1
3-MPPA 1	151.0	132.9
3-MPPA 2	151.0	107.0
3-MPPA 3	151.0	63.1
Phosphonic Acid 1	81.0	62.9
Phosphonic Acid 2	81.0	79.0

Contents

Results and Discussion

Food samples

Chromatographic performance using both the NofaLab method for QuPPe extracts of food samples and the modified method for water samples achieved good separation between the analytes and from matrix interferences, and excellent repeatability in terms of peak profile and retention time. The EU maximum residue limits for these compounds in food samples range from 10 to 2000 µg/kg, depending on the commodity and compound[4], so the target for each is variable. Although water regulations are under discussion, a detection limit of 20 ng/L for environmental samples is desirable in anticipation of future regulation. Some analyte/matrix combinations proved to be particularly difficult, but these target concentrations were easily achieved for all samples in the verification of the methods. Over 1000 food samples from a variety of commodities were analyzed at NofaLab without maintenance of the system, and the stability in terms of retention time, peak width, peak area and tailing factor was found to be excellent. Figure 1 shows several measures of reproducibility based on the glyphosate internal standard.

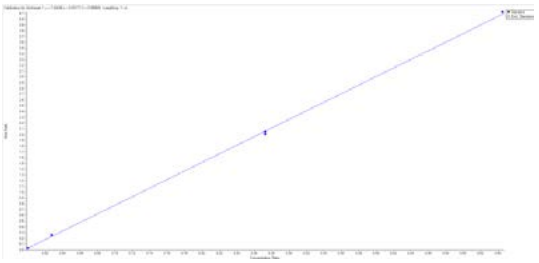

Figure 4. Glyphosate calibration standards. Linear calibration regression for glyphosate with 1/x weighting, showing r-value of 0.9997 and excellent precision for duplicate calibrators.

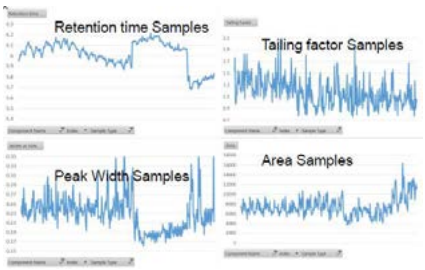

Figure 5. Reproducibility data for glyphosate IS. NofaLab method for food samples, tested over 1000 injections of extracts from fruit/veg, seeds, veg oil/fat, grains, herbs/spices, meat/fish, animal oil/fat, eggs/egg products, milk/milk products and other fatty acids.

Table 4. Summary of Limits of Detection achieved in various food matrices using the NofaLab method. Shown along with their EU Maximum Residue Limits[1].

Product	Glufosinate sum			Fosetyl sum			Glyphosate			Chlorate			Ethephon		
	LOD	MRL	%RSD at MRL	LOD	MRL	%RSD at MRL	LOD	MRL	%RSD at MRL	LOD	MRL	%RSD at MRL	LOD	MRL	%RSD at MRL
Fruit and Vegetables	16	30	11%	25	2000	13%	5	100	15%	8	10	15%	18	50	11%
Seeds	12	30	12%	90	2000	15%	8	100	15%	3	10	10%	6	50	14%
Vegetable oil, Fat and Fatty Acids	15	30	19%	40	2000	12%	7	100	22%	2	10	6%	3	50	7%
Grain	18	30	12%	71	2000	14%	8	100	7%	7	10	14%	9	50	6%
Herbs and spices	25	100	8%	87	2000	13%	23	100	6%	8	10	15%	8	100	16%
Meat and Seafood	19	30	15%	23	100	12%	9	50	23%	4	10	8%	4	50	10%
Animal Oil, Fat and Fatty Acids	14	30	20%	51	100	11%	9	50	25%	10	10	16%	7	50	12%
Eggs and Eggs products	18	30	12%	33	100	11%	4	50	13%	12	10	9%	6	50	17%
Milk and Milk products	17	30	9%	20	100	6%	8	50	22%	5	10	12%	5	50	13%
Fatty acids	21	100	14%	70	1000	14%	3	100	18%	4	10	9%	3	100	10%

Water samples

To achieve the target sensitivity for environmental water samples, it was necessary to inject increase the amount of sample, so trials with increasing injection volume and different loop sizes were carried out. With each incremental change, the composition of eluent in the loop was altered, thereby changing initial conditions of the analysis and the retention times and peak shapes of the analytes. To compensate, modification of the stating composition of the mobile phase was required, but when final parameters had been fully developed, the method was found to be as stable and robust as the NofaLab method for food samples. All analytes were well retained, allowing detection after the majority of background components which could otherwise interfere had eluted. Separation between the analytes was also sufficient to allow unambiguous identification, and retention times were reproducible. Sensitivity in spiked environmental waters was found to be similar to that in standards, and the target limit of detection of 20 ng/L was easily achieved with real drinking water samples. In order to verify the results, analyses with standard addition of the target compounds were also performed.

Matrix effects were largely eliminated in both the NofaLab method for food sample extracts and the modified method for direct injection of water samples. However, MRM ion ratios were found to be outside of the normal ±20% tolerance in some very complex sample matrices. Use of the QTRAP® will be advantageous to confirm positive results by their full-scan MS/MS spectra, but future work will investigate different or additional clean-up of samples in order to remove background interferences.

Conclusions

This ion chromatographic approach to the analysis of polar pesticides offers the ability to include multiple analytes in a single injection without derivatization. Deviating from traditional LC buffers has enabled detection by MS/MS and the sensitivity of the SCIEX 6500+ QTRAP® mass spectrometer allowed the analysis to be performed without the need for an ion suppressor using a standard reverse-phase LC based system. Therefore, the need to change inlets between typical pesticide analyses is eliminated, allowing high-throughput laboratories to manage samples efficiently and minimize running costs. System maintenance was found to be within expectations, with a change of guard column only required after approximately 250 sample injections.

The methods were found to be considerably more robust and sensitive than other approaches described in various publications and have achieved the target limits of detection required to meet existing and proposed future regulations. The separation has been found to minimize matrix interferences in most samples, but further work will investigate possible improvements to clean-up in order to achieve confirmatory results in even very complex matrices.

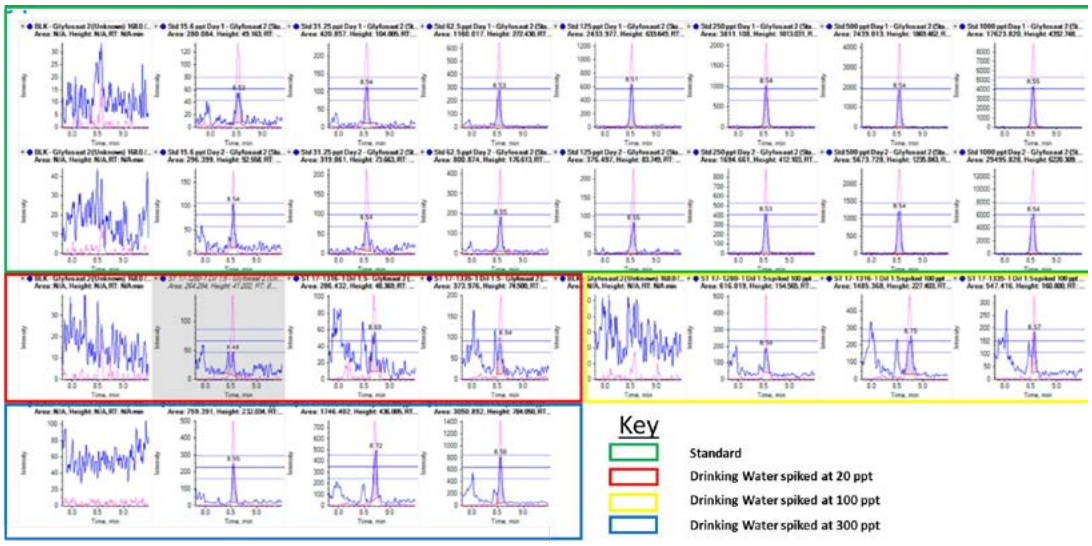

Figure 6. Example chromatography from drinking water samples using the modified water method.

References

1. https://ec.europa.eu/food/plant/pesticides/glyphosate_en
2. http://www.crl-pesticides.eu/library/docs/srm/meth_QuPPe.pdf
3. http://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN
4. http://ec.europa.eu/environment/archives/ppps/pdf/ma_reding_annex4.pdf
5. Glyphosate and AMPA in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality

Contents

Quantitation of Oregon List of Pesticides and Cannabinoids in Cannabis Matrices by LC-MS/MS

Diana Tran[1], KC Hyland[1], Simon Roberts[1], Scott Krepich[3], Paul Winkler[1], Craig Butt[2], and Christopher Borton[1]
[1] SCIEX (Redwood City, CA); [2] SCIEX (Framingham, MA); [3] Phenomenex (Torrance, CA)

Overview

Increased legalization of cannabis for medical and recreational use substantiates the need for a standardized robust and reproducible method for quantitation of pesticide residues and relevant psychotropic cannabinoids in cannabis products. A fully verified method is presented using two different SCIEX triple quadrupole platforms for the analysis of those pesticides comprising the Oregon Pesticide List. The QET 4500 presents a cost-effective platform for achieving the majority of the Oregon List Maximum Residual Limits (MRL) in cannabis flower matrix. The highly sensitive Triple Quad/QTRAP 6500+ is capable of meeting the MRLs for the full list in cannabis flower matrix. Cannabis flower demonstrated the most severe matrix- induced ion suppression on our target analytes and, therefore, the performance of this method in flower represents performance in the most difficult matrix. The SCIEX vMethod utilizes dilution with six pesticide deuterated internal standards and two cannabinoid internal standards in its sample preparation method to maximize recoveries for the most analytes as well as to correct for analyte recovery efficiency. A 16 minute gradient maximizes separation of endogenous isobaric interferences for pesticide analysis. A five-minute gradient separates all isobaric cannabinoids from each other and ensures precision of quantitative analysis.

Introduction

Pesticide application in agricultural industries is intended to protect crop yield from pests or pathogens. Insecticides, acaricides, fungicides or other protective chemical reagents on crops pose potential health risks both to field employees via exposure as well as consumers through consumption. Pesticides and pesticide action levels may be regulated differently by state. Currently, the most comprehensive list of pesticides and their respective MRLs allowed in plant products is known as the Oregon List of Pesticides.

Several pesticides on the Oregon List have been historically monitored by GC-MS including complicated sample preparation with derivatization and relatively long sample run times. The SCIEX vMethod Application for Quantitation of Pesticide Residues in Cannabis Matrices 1.0 presents a simplified sample preparation protocol complete with analysis of all 59 compounds using electrospray ionization (ESI) and LC-MS/MS. Additionally, the method can be used to analyze ten cannabinoids with the same sample extract using a 5 minute acquisition method that utilizes our triple quadrupole's fast polarity switching to monitor targets in both negative and positive polarities.

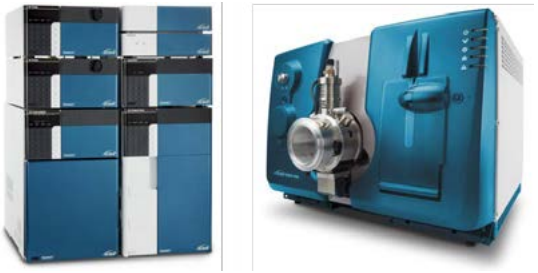

Figure 1: The SCIEX ExionLC™ AC HPLC system (left) and the SCIEX QET 4500 LC-MS/MS System (right).

The complete vMethod provides a step by step SOP that is suitable for use for ISO 17025 compliance, acquisition methods with optimized source and analyte parameters as well as a quantitation method using MultiQuant software. This vMethod is designed to have a new user running samples in days.

Experimental

Standards and Internal Standards (IS)

Pesticide standards were purchased from RESTEK (Bellefonte, PA). The complete list of pesticides monitored can be found in the SCIEX vMethod Application for Quantitation of Pesticide

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Residues in Cannabis Matrices 1.0. Deuterated internal standards were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada).

Cannabinoid standards and deuterated internal standard were purchases from Cerilliant (Round Rock, TX). The complete list of cannabinoids monitored can be found in SCIEX vMethod Application for Quantitation of Pesticide Residues in Cannabis Matrices 1.

Acetonitirile, methanol, water, formic acid, acetone and ammonium formate were purchased from Sigma-Aldrich (St.Louis, MO).

Sample preparation

Calibrators and quality controls were made in acetonitrile and then diluted with 75:25 (v/v) methanol:water.

Unknown cannabis matrices were analyzed using 0.2 gram of cannabis flower or 0.02 gram of cannabis concentrates diluted in 5mL of acetonitrile which was sonicated, vortexed and centrifuged.

The extract was then diluted in 1:6 (v/v) using 75:25 (v/v) methanol and water.

LC-MS/MS injection volumes are 20µL for a 4500 system and 25µL for a 6500+ system. The maximum injection volume for this method is 25 µL in order to maintain symmetrical peak profiles of early eluting Daminozide and Acephate.

The sample extract was also used for cannabinoid potency analysis by further diluting 1:2000 (v/v) serially. The suggested LC-MS/MS injection volumes are 5 µL for a 4500 system and 1 µL for a 6500+ system

An outline of the sample preparation procedure is shown in Figure 2A for pesticides and 2B for cannabinoid analysis.

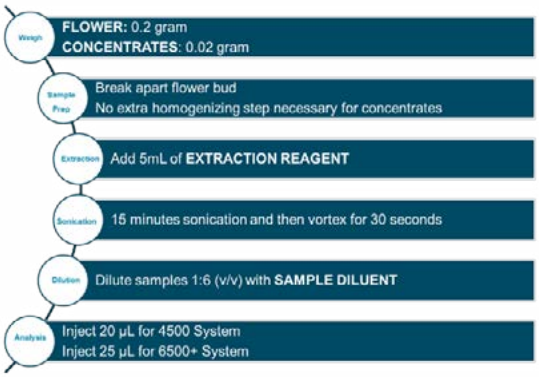

Figure 2A An overview of sample preparation for cannabis flower and concentrates for pesticide residue analysis

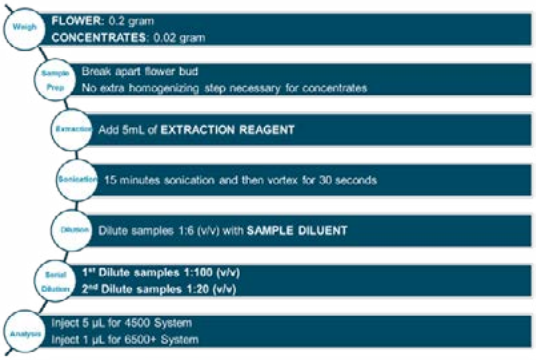

Figure 2B An overview of sample preparation for cannabis flower and concentrates for cannabinoid analysis.

HPLC System

Chromatographic separation was achieved using Shimadzu LC-20AD binary pumps or with a SCIEX ExionLC™ AC LC system and a Phenomenex Kinetex Biphenyl Column (2.6µm, 4.6 x150mm) at flow rate of 1 mL/min.

The analytical column is heated to 30°C for analysis using the CTO-20AC integrated column oven for pesticide analysis and 35°C for cannabinoid testing respectively. The eluents used for the separation are shown in Table 1 and the gradient profile is

Contents

shown in Figure 3A for pesticide residue testing and Figure 3B for cannabinoid testing.

LC Reagent	Composition
Mobile Phase A	5mM Ammonium Formate (100:0.1, Water: Formic Acid)
Mobile Phase B	5mM Ammonium Formate (98:2, Methanol: Water)
Autosampler Wash	(70:20:10) (Acetonitirile: Isopropanol: Acetone)

Table 1: LC reagents for LC-MS/MS analysis

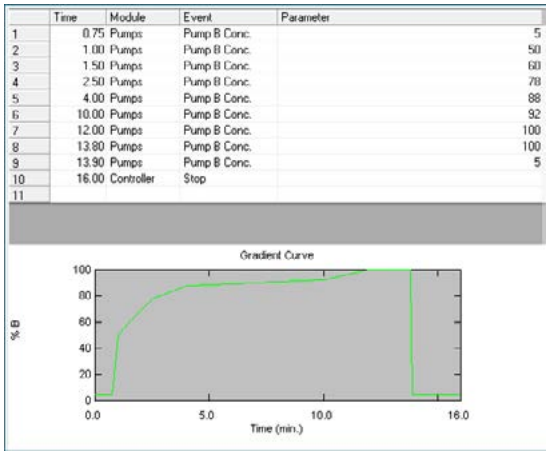

Figure 3A: LC Gradient is detailed using % Mobile Phase B as the parameter

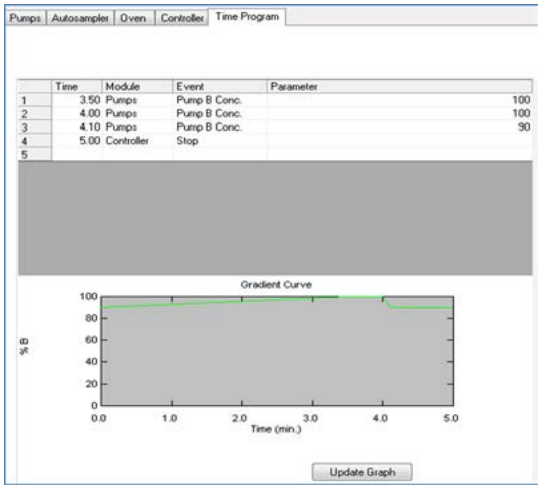

Figure 3B: LC Gradient is detailed using % Mobile Phase B as the parameter

MS/MS Detection

Optimized source parameters for a SCIEX QET 4500(**Tables 2A and Table 2B**) and a QTRAP 6500+(**Tables 3A and 3B**) coupled to a TurboV and IonDrive source respectively are detailed below.

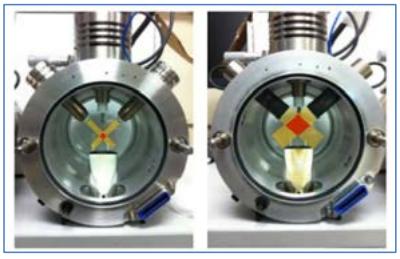

Figure 4: SCIEX TurboV source(**left**) and SCIEX IonDrive source(**right**) have different ion source temperatures that are optimal for each model.

Table 2A. Ion source parameters for QET 4500 - pesticide analysis

Parameter	Value
Curtain Gas (CUR)	35 psi
IonSpray voltage (IS)	3500 V
Temperature (TEM)	500°C
Nebulizer Gas (GS1)	60 psi
Heater Gas (GS2)	60 psi

Table 2B. Ion source parameters for QET 4500 - cannabinoid analysis

Parameter	Value
Curtain Gas (CUR)	35 psi
IonSpray voltage (IS)	4500 V/-4500
Temperature (TEM)	600°C
Nebulizer Gas (GS1)	60 psi
Heater Gas (GS2)	60 psi

Table 3A. Ion source parameters for Triple Quad/QTRAP 6500+ - pesticide analysis

Parameter	Value
Curtain Gas (CUR)	35 psi
IonSpray voltage (IS)	3500 V
Temperature (TEM)	400°C
Nebulizer Gas (GS1)	60 psi
Heater Gas (GS2)	60 psi

Table 3B. Ion source parameters for Triple Quad/QTRAP 6500+ - cannabinoid analysis

Parameter	Value
Curtain Gas (CUR)	35 psi
IonSpray voltage (IS)	4500 V/-4500
Temperature (TEM)	500°C
Nebulizer Gas (GS1)	60 psi
Heater Gas (GS2)	60 psi

Two MRM transitions were monitored for each analyte while one transition was monitored for each of the internal standards. In the pesticide panel, the *Scheduled* MRM™ algorithm was activated to monitor compounds during a 60 second expected retention time window to maximize dwell times and optimize the cycle time such that all analytes have at least 12 scans across the baseline of the peak. For a complete list of all target analytes monitored, refer to SCIEX vMethod Application for Quantitation of Pesticide Residues in Cannabis Matrices 1.0. Due to the variable ionization efficiencies of the different pesticide groups and the commercial standards being at the same concentration, a 9-point calibration curve is coupled with 2 quality controls to ensure accuracy for quantitation analysis. (**Table 4A**).

Cannabinoid results are reported as % by weight and the calibration level for each standard as well as quality control in solvent are listed in **Table 4B** below.

STANDARD	Concentration (ppb or ng/mL)
STD 1	0.075
STD 2	0.25
STD 3	1
STD 4	2
STD 5	3
STD 6	5
STD 7	9
STD 8	12.5
STD 9	15
QC 1	0.125
QC 2	7.5

Table 4A. **Calibration and quality control scheme for pesticide residue analysis**

STANDARD	% by weight
STD 1	0.3
STD 2	1.5
STD 3	6
STD 4	15
STD 5	24
STD 6	30
QC 1	0.75
QC 2	22.5

Table4B. Calibration and quality control scheme for cannabinoid analysis

Quantitation was performed using MultiQuant™ 3.0.2 using 1.5 Gaussian smoothing and 1/x weighted variable quadratic or linear regression for the QET 4500. The detector on the QTRAP 6500+ allows for a greater dynamic range compared to the QET 4500, therefore all calibration curves are analyzed with 1/x weighted linear regression.

Several pesticides containing different isomers were integrated with a peak split factor of 10 and a noise percentage level of 50% in MultiQuant™ 3.0.2. Examples of this integration are found in **Figures 5-7** for Propiconazole, Cyfluthrin, and Cypermethrin.

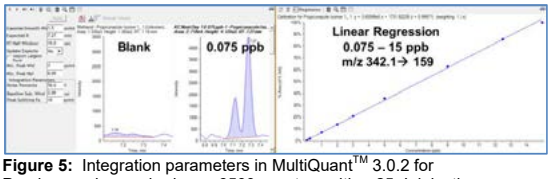

Figure 5: Integration parameters in MultiQuant™ 3.0.2 for Propiconazole acquired on a 6500+ system with a 25µL injection showing multiple isomers.

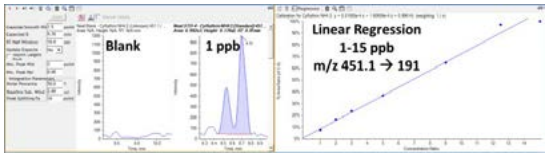

Figure 6: Integration parameters in MultiQuant™ 3.0.2 for Cyfluthrin acquired on a 6500+ system with a 25µL injection showing multiple isomers.

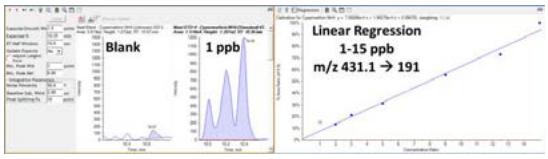

Figure 7: Integration parameters in MultiQuant™ 3.0.2 for Cypermethrin acquired on a 6500+ system with a 25µL injection showing multiple isomers.

Results and Discussion

Chromatography

The biphenyl column chemistry provides retention of early eluting pesticides as well as chromatographic separation of endogenous pyrethrin-like compounds found in cannabis flower. A representative elution profile of target analytes in solvent can be found in **Figure 8A for pesticides and Figure 8B for cannabinoids.** An example of the isobaric interferences surrounding Pyrethrin, Pyrethrin I and II are detailed in **Figure 9** when comparing a solvent standard to standards spiked into flower extract. The ability to chromatographically separate isobaric interferences allows for both easier visual and quantitative analysis of the pyrethrins in an unknown sample.

Carryover analysis was done by analyzing the highest calibrator standard, followed a solvent blank. The absence of any analyte peaks ≥20% of the low calibrator areas demonstrated that the method is free from carryover.

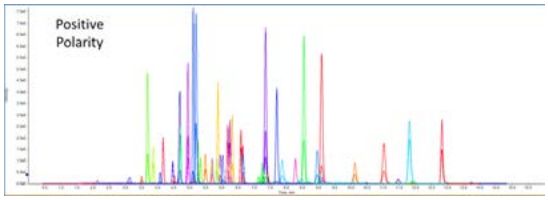

Figure 8A: Elution profile of target analytes in solvent

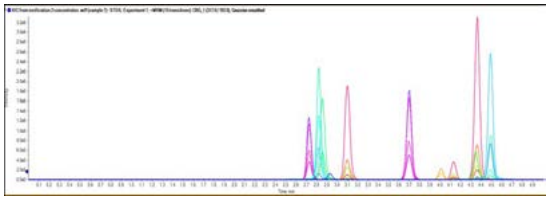

Figure 8B: Elution profile of target cannabinoid analytes in solvent using the same mobile phases and analytical column as the pesticide panel

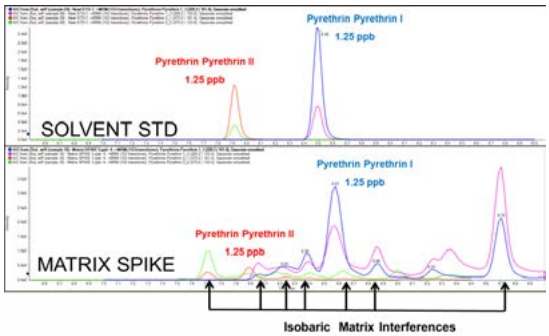

Figure 9: Comparison of standards in solvent compared to Pyrethrin Pyrethrin I and II spiked into cannabis flower extract showing interferences with similar ion ratios of both quantifier and qualifier MRM transitions.

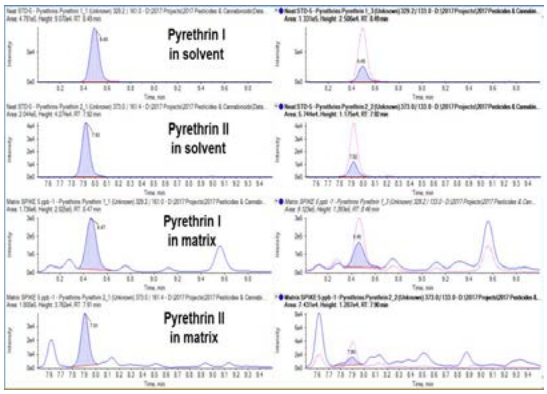

Figure 10: Example chromatograms in MultiQuant™ 3.0.2 showing chromatographic separation of Pyrethrin Pyrethrin I and II in cannabis flower extract. The chromatograms on the left are the quantifier ions while the chromatograms on the right are the qualifier ions. The qualifier ions also show overlaid quantifier ions for ion ratio analysis (pink trace).

Matrix Recovery

Matrix induced ion suppression was observed in cannabis flower more so than the three concentrates tested (shatter, kief/pollen and hash). To correct for ion suppression, deuterated internal standards were assigned to each pesticide based on a combination of retention time, chemical structure and back-calculated concentrations from solvent calibration curves. A table outlining the recoveries from solvent standards can be found in **Table 1 in the Appendix** for targeted pesticides. Several pesticides showed recoveries greater than ±20% deviation from the target concentration, potentially because the compound did not have its own deuterated internal standard to correct for suppression or ion enhancement.

Limit of Quantitation Analysis

Solvent LOQs were determined by analyzing 5 solvent spiked replicates over the course of two days. The parameters for determining LOQ was %CV of ≤ 20% and a %Recovery of 80 to 120% of the target spike concentration.

The SCIEX vMethod for pesticide analysis outlines the concentrations of calibration standards to be used, with the lowest of these at a concentration of 0.075 ppb. The instrument LOQ for the majority of pesticides is lower than this concentration, both in solvent as well as spiked into cannabis flower matrix. A complete table of the LOQ analysis for solvent using the QTRAP6500+ can be found in **Table 1 in the Appendix**. The LOQ tables for pesticides in cannabis flower matrix acquired on the QTRAP 6500+ are found in **Table 2 in the Appendix.**

The cannabinoid analysis in the SCIEX vMethod has six calibration standards that range from 0.3-30% by plant weight. The %CV of the ten cannabinoids ranged from 6.24-19.09% at the first calibration level (0.3% by weight). The %Recovery of the LOQ standard range from 82-116%.

Linear Dynamic Range

The dynamic range was established across five calibration curves acquired through method verification. All curve fittings used a linear regression with 1/x weighting. Calibration points below the LOQ of the method were excluded. Figures 11-15 show examples of dynamic range for some representative

Contents

pesticide analytes Refer to Figures 16-17 for representative calibration curves of cannabinoid analytes.

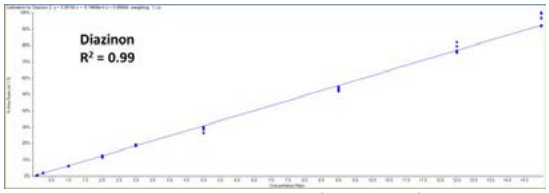

Figure 11: 5 calibration curve replicates for Diazinon from 0.75-15 ppb on a QTRAP 6500+ system.

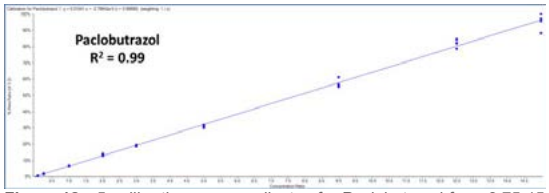

Figure 12: 5 calibration curve replicates for Paclobutrazol from 0.75-15 ppb on a QTRAP 6500+ system.

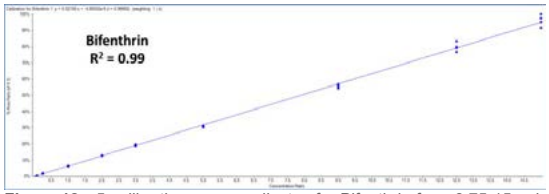

Figure 13: 5 calibration curve replicates for Bifenthrin from 0.75-15 ppb on a QTRAP 6500+ system.

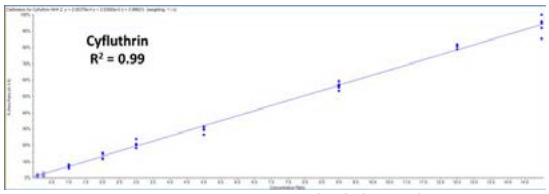

Figure 14: 5 calibration curve replicates for Cyfluthrin from 1-15 ppb on a QTRAP 6500+ system.

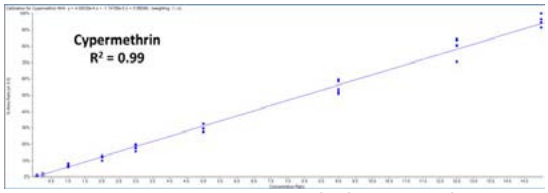

Figure 15: 5 calibration curve replicates for Cypermethrin from 0.75-15 on a QTRAP 6500+ system.

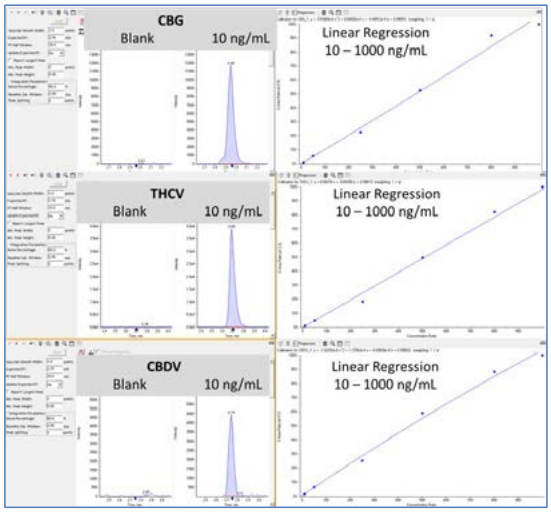

Figure 16: Representative solvent blanks, first LOQ standard and calibration linearity of CBG, THCV and CBDV.

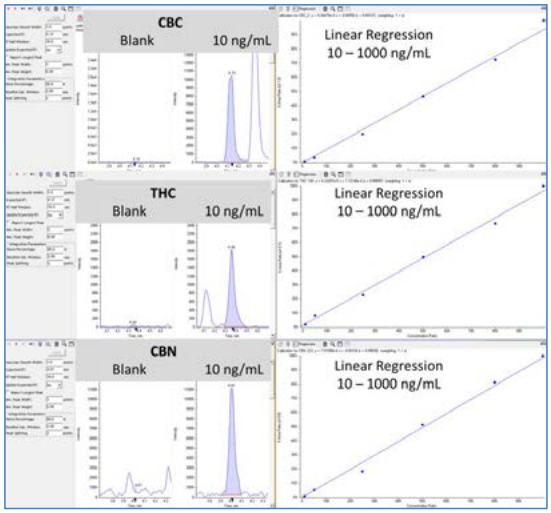

Figure 17: Representative solvent blanks, first LOQ standard and calibration linearity of CBC, THC and CBN. The first calibrator also shows separation of isobaric CBC and THC.

Summary

The SCIEX vMethod was optimized and verified on two different triple quadrupole models. The LOQ's were established in both solvent as well as extracted cannabis flower which proved to give the most matrix effects when compared to the three concentrates tested for this method.

It was observed that there were many differences between cannabis flower strains that could potentially alter the ion ratios due to ion enhancement or ion suppression. However, during development, ten different matrix strains were analyzed and the quantifier ion was found to be chromatographically separated from endogenous interferences in 9 of the strains.

LOQ's in cannabis flower were achieved with ±20 %CV for all pesticides on the Oregon list. The vMethod offers a simple sample preparation, optimized LC-MS conditions as well as verified linearity, precision, LOQ and matrix spike recoveries for pesticides and cannabinoid analysis. This is accompanied by a disc that contains a comprehensive SOP, a method that may be directly loaded on to the instrument, quantitation methods and reporting template for true plug and play operation for the purpose of getting a laboratory fully operational for pesticide and cannabinoid analysis in days.

Acknowledgements

SCIEX acknowledges Phenomenex (Torrance, CA) for providing HPLC columns and expertise for this application note.

APPENDIX

Appendix Table 1: Solvent LOQ analysis on a Triple Quad/QTRAP 6500+ System. Pesticides annotated with * is based on the most abundant isomer.

Compound	LOQ (ppb)	%CV	%Recovery
Abamectin*	0.25	6.84%	107.33%
Acephate	0.075	6.63%	98.89%
Acequinocyl	0.25	17.54%	104.00%
Acetamiprid	0.075	2.88%	94.44%

Contents

Aldicarb	0.075	6.48%	101.11%
Azoxystrobin	0.075	7.11%	92.22%
Bifenazate	0.075	6.63%	98.89%
Bifenthrin	0.075	2.88%	94.44%
Boscalid	0.075	9.07%	90.00%
Carbaryl	0.075	6.63%	98.89%
Carbofuran	0.075	9.78%	103.33%
Chlorantraniliprole	0.25	7.96%	107.33%
Chlofenapyr	2	16.00%	96.58%
Chlorpyrifos	0.075	8.39%	105.56%
Clofentezine	0.075	8.39%	105.56%
Cyfluthrin	1	13.96%	103.83%
Cypermethrin	1	13.54%	104.83%
Daminozide	3	9.12%	103.73%
Diazinon	0.25	13.54%	120.00%
Dichlorvos	0.075	7.40%	98.67%
Dimethoate	0.075	7.40%	98.67%
Ethoprophos	0.075	7.40%	98.67%
Etofenoprox	0.075	6.21%	96.00%
Etoxazole	0.075	2.83%	97.87%
Fenoxycarb	0.075	1.99%	94.67%
Fenpyroximate	0.075	5.73%	104.00%
Fipronil	0.25	15.96%	109.28%
Flonicamid	0.075	6.43%	97.87%
Fludioxinil	0.25	13.39%	103.36%
Hexythiazox	0.075	3.20%	94.93%
Imazalil	0.075	10.58%	102.93%
Imidacloprid	0.075	6.96%	108.40%
Kresoxim-methyl	0.125	7.25%	106.56%
Malathion	0.075	2.12%	95.47%
Metalaxyl	0.075	2.35%	86.13%
Methiocarb	0.075	6.90%	91.20%
Methomyl	0.075	2.40%	104.00%
MGK 264*	0.075	6.07%	101.07%
Myclobutanil	0.075	18.22%	88.53%
Naled	0.075	10.82%	96.80%
Oxamyl	0.075	2.32%	94.40%
Parathion Methyl	0.075	13.76%	92.27%
Permethrins*	2#	8.47%	83.80%
Phosmet	0.075	8.87%	92.00%
Piperonyl Butoxide	0.075	11.32%	84.27%
Prallethrin	0.075	3.07%	90.13%

Propiconazole	0.075	5.02%	87.20%
Paclobutrazol	0.075	4.99%	90.67%
Propoxure	0.075	4.15%	105.07%
Pyrethrins*	0.075	8.30%	88.00%
Pyridaben	0.075	3.11%	88.80%
Spinosad*	0.075	5.32%	92.00%
Spiromesifen	0.075	9.26%	102.13%
Spirotetramat	0.075	5.31%	96.27%
Spiroxamine	0.075	2.18%	92.80%
Tebuconazole	0.075	5.08%	94.67%
Thiaclomprid	0.25	3.91%	107.52%
Thiamethoxam	0.075	3.64%	93.33%
Trifloxstrobin	0.075	5.99%	89.60%

Appendix Table 2:. LOQ analysis spiked into cannabis flower extracts and analyzed against a solvent calibration curve on a QTRAP 6500+.

*Analytes is based on the most abundant isomer.

≠Analytes have %recoveries that can be improved using their deuterated internal standards.

Compound	LOQ (ppb)	%CV	%Recovery
Abamectin*≠	0.25	13.00%	135.00%
Acephate	0.25	4.00%	119.00%
Acequinocyl	1	7.00%	88.00%
Acetamiprid	0.25	4.00%	96.00%
Aldicarb	0.25	9.00%	105.00%
Azoxystrobin	0.25	5.00%	109.00%
Bifenazate	0.25	5.00%	112.00%
Bifenthrin	0.25	13.00%	116.00%
Boscalid	1	3.00%	110.00%
Carbaryl	1	10.00%	94.00%
Carbofuran	0.25	19.00%	97.00%
Chlorantraniliprole	0.25	13.00%	98.00%
Chlofenapyr	5	22.00%	104.00%
Chlorpyrifos	1	2.00%	108.00%
Clofentezine ≠	0.25	12.00%	75.00%
Cyfluthrin	5	16.51%	100.32%
Cypermethrin	2	10.32%	113.24%
Daminozide ≠	5	4.19%	70.58%
Dichlorvos	0.25	9.00%	86.00%
Diazinon	0.25	11.00%	113.00%
Dimethoate	0.25	3.00%	91.00%
Ethoprophos ≠	1	6.00%	60.00%
Etofenoprox	0.25	2.00%	94.00%
Etoxazole	0.25	1.00%	90.00%
Fenoxycarb ≠	0.25	6.42%	129.12%
Fenpyroximate	1	3.00%	91.00%
Fipronil	1	14.00%	90.00%
Flonicamid	0.25	2.00%	90.00%
Fludioxonil	0.25	2.00%	105.20%
Hexythiazox ≠	0.25	4.76%	75.36%
Imazalil	0.25	3.00%	94.00%
Imidacloprid ≠	0.25	6.16%	124.56%
Kreosim-methyl	0.25	18.00%	113.00%
Malathion ≠	0.25	6.66%	73.92%
Metalaxyl	0.25	2.29%	104.88%
Methiocarb	0.25	3.28%	127.52%

Methomyl	0.25	4.46%	116.72%
MGK 264* #	0.25	10.10%	53.68%
Myclobutanil	0.25	18.00%	112.00%
Naled	0.25	17.89%	111.68%
Oxamyl	0.25	5.67%	88.32%
Paclobutrazol	0.25	5.42%	91.28%
Parathion Methyl #	1	19.87%	72.36%
Permethrins*	0.25	5.62%	108.96%
Phosmet	0.25	6.43%	86.12%
Piperonyl Butoxide	0.25	2.00%	120.00%
Prallethrin #	0.25	6.09%	124.28%
Propiconazole	1	8.10%	94.98%
Propxure	0.25	3.00%	106.00%
Pyrethrins*	0.25	18.41%	116.96%
Pyridaben	1	2.00%	90.00%
Spinosad*	0.25	3.00%	104.00%
Spiromesifen	1	11.00%	116.00%
Spirotetramat	0.25	7.55%	97.36%
Spiroxamine	0.25	6.00%	100.00%
Tebuconazole	1	14.00%	99.00%
Thiacloprid	0.25	2.00%	93.00%
Thiamethoxam	0.25	4.00%	106.00%
Trifloxystrobin	0.25	4.00%	107.00%

Contents

Improving Identification and Quantification of Polar Herbicides by CESI-MS

Achieving better differentiation of glyphosate, fosetyl aluminum and their degradation products

Bryan Fonslow,[1] Stephen Lock,[2] Wiley Hall,[3] and Spencer Walse[4]
SCIEX Separations, [1]Brea, CA and [2]Warrington, UK; [3]Safe Food Alliance, Fresno, CA; [4]USDA-ARS, Parlier, CA

Overview

Who Should Read This: Senior Scientists, Lab Directors

Focus: Advantages of CESI-MS for separating, identifying and quantifying the polar herbicides glyphosate and fosetyl aluminum, and their degradation products.

Goals: Develop an effective CESI-MS method for separating, identifying and quantifying polar herbicides and compare the selectivity, accuracy and reproducibility of that method to those of an approved, currently-used LC-MS method.

Problem: Concerns about the safety of glyphosate-based herbicides (GBHs) have made it essential to be able to detect glyphosate in foods (especially fruits and nuts) and distinguish it from other alternative herbicides such as fosetyl aluminum. Current LC-MS methods have significant limitations, including ion suppression, retention time instability and problems in distinguishing between degradation products of these herbicides (Figure 1). Both glyphosate and fosetyl aluminum are regulated but false positive identification (ID) and inaccurate quantitation of their degradation products, phosphate and phosphonate, is possible using current LC-MS methods. Therefore, a method is needed that provides accurate ID and quantitation of these degradation products.

Results: The developed CESI-MS method demonstrated an excellent ability to distinguish between glyphosate and its degradants, and between similar degradation products of another widely-used herbicide, fosetyl aluminum. It also demonstrated better migration/retention time stability and quantitative linearity than the LC-MS method.

Key Challenges:

- Separation of highly polar molecules by LC requires either time-consuming analyte derivatization prior to reverse-phase LC, or reliance on less reliable LC techniques, e.g. HILIC or anion exchange chromatography
- LC-MS methods suffer from a variety of issues, including: derivatization selectivity, ion suppression due to matrix effects, and retention time instability

- LC-MS methods frequently have difficulty resolving phosphate and phosphonate, the final degradation products of glyphosate and fosetyl aluminum, respectively

Key Features:

- Capillary electrophoresis is well suited to the separation of polar herbicides
- The CESI-MS method provided excellent specificity, easily resolving and identifying glyphosate, fosetyl aluminum, and many of their degradation products
- The CESI-MS method demonstrated very good migration time stability over more than 160 runs
- The CESI-MS exhibited excellent quantitative linearity when analyzing phosphonate, the degradation product of fosetyl aluminum, in matrices

Differentiating Glyphosate, Other Herbicides and Their Degradation Products

Glyphosate is a common, broad-spectrum, systemic herbicide widely used to kill weeds that compete with crops. Concerns about the safety of glyphosate[1,2] have led to increasing restrictions on glyphosate-based herbicides (GBHs), most recently in the European Union.[3] As such, it is increasingly important to develop robust analytical methods with the sensitivity and selectivity to identify and quantify glyphosate and its degradation products in foods and differentiate them from other herbicides such as fosetyl aluminum.

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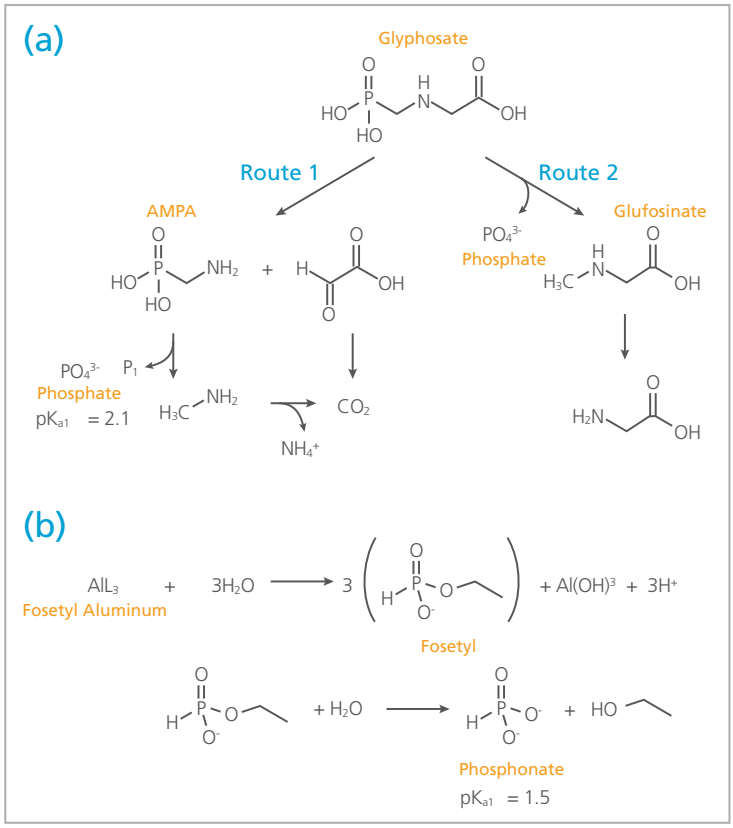

Figure 1: Degradation pathways for (a) glyphosate and (b) fosetyl aluminum.

Limitations of LC-MS Methods

Several LC-MS -based methods are currently used to analyze glyphosate and its degradation products, with many listed in the Quick Polar Pesticides (QuPPe) Method document created by the EU Reference Laboratories for Residues of Pesticides.[4] The methods use anion-exchange, porous-graphitized carbon, or HILIC liquid chromatography coupled with mass spectrometry. Reverse-phase LC methods have also been used but require analyte derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) before sample analysis.[5] While LC-MS methods generally can differentiate glyphosate and fosetyl aluminum (Figure 1), these methods can suffer from derivatization selectivity, matrix effects, ion suppression, and poor retention time reproducibility. Additionally, LC-MS methods are generally not suitable for resolving phosphate and phosphonate, the final degradation products of glyphosate and fosetyl aluminum, especially in real-world matrices.[4]

Advantages of CESI-MS

Capillary electrophoresis (CE) is well-suited to the analysis of polar ions and has already proven useful in the analysis of pesticides.[6] The mechanism of separation is by differences in pKa and hydrodynamic radii. In the case of phosphate and phosphonate, they differ in pKa by 0.6 units (Figure 1). Integration of capillary electrophoresis and electrospray ionization (CESI) into a single dynamic process facilitates the mass spectrometric use of CE detection and analysis. The developed CESI-MS method readily differentiated glyphosate, fosetyl aluminum and their degradation products (Figure 2). It demonstrated far better migration/retention time stability than a corresponding LC-MS method (Figure 3). Finally, quantitative CESI-MS/MS analysis of phosphonate in real-world nut extracts showed exceptional linearity while the corresponding anion-exchange LC-MS/MS method exhibited significant ion suppression due to matrix effects (Figure 4).

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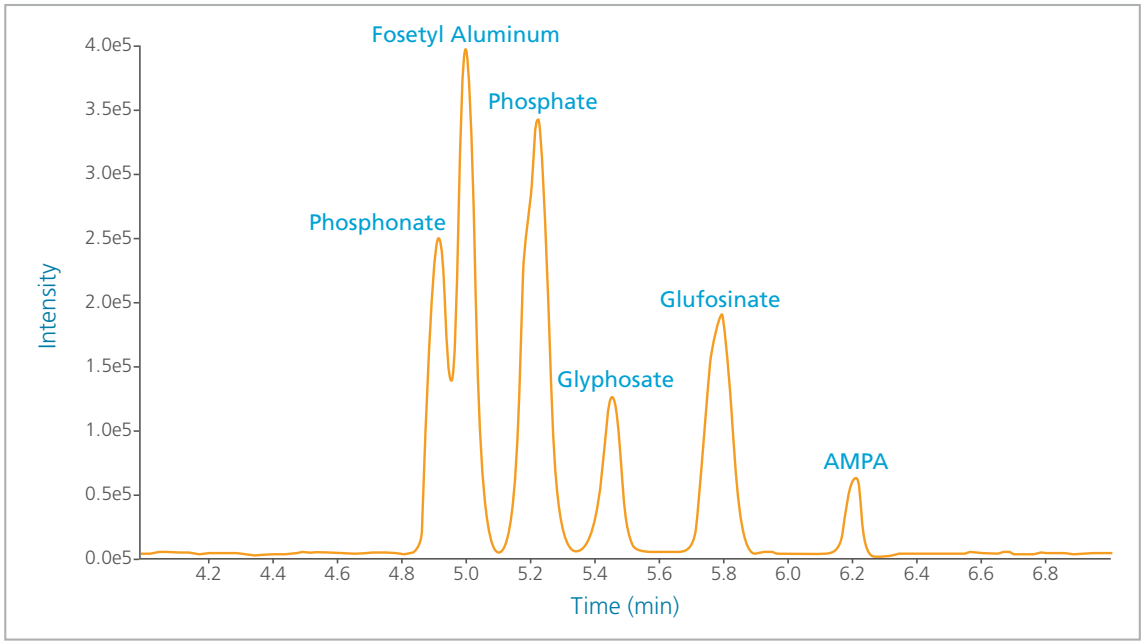

Figure 2: CESI-MS provides clear separation and detection of glyphosate and three of its degradation products: glufosinate, AMPA and phosphate, along with fosetyl aluminum and one of its degradation products, phosphonate.

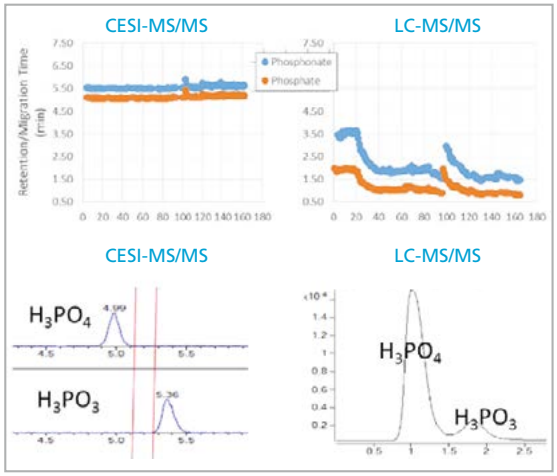

Figure 3: Migration/retention times for phosphonate and phosphate across more than 160 analyses, with sample electropherograms (CESI-MS/MS) and chromatograms (LC-MS/MS). CESI-MS proved far more stable over time and baseline separation was achieved only in the CESI-MS analysis.

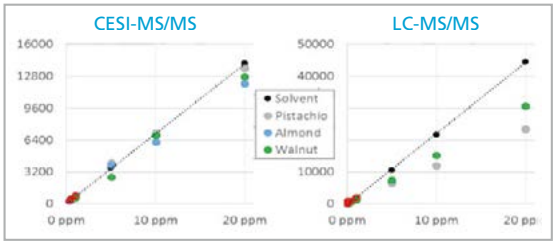

Figure 4: CESI-MS/MS and anion-exchange LC-MS/MS quantification of phosphonate spiked into 0.1% formic acid (solvent) and nut extracts. Significant ion suppression due to matrix effects is clearly visible in the LC-MS/MS results, but not in the CESI-MS/MS results. Fit lines are for the solvent curves only.

Contents

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Combining Non-Targeted SWATH® MS/MSALL Acquisition with Highly Selective MRM^{HR} for the Analysis of Veterinary Drugs in Tissue Using the SCIEX X500R QTOF System

Jasmin Meltretter
SCIEX, Landwehrstraße 54, Darmstadt, Germany

Overview

A highly flexible, selective and sensitive LC-MS/MS method for the analysis of veterinary drugs in liver extract is presented, using the SCIEX X500R QTOF high resolution mass spectrometer together with the SCIEX OS software for a combined non-targeted and targeted screening workflow.

Introduction

Veterinary drugs are commonly used in livestock breeding to prevent or treat infections of the animals and to ensure their optimal growth. Legal regulations define waiting periods between the application of active pharmaceutical ingredients and the release of the animals for food manufacturing. Veterinary drugs which still find their way into human nutrition represent a potential risk to human health, e.g. in terms of possible allergenic reactions or reproductive dysfunctions. Furthermore, abuse of antibiotics in animals may also contribute to the development of antimicrobial resistance.

Therefore, European guidelines require to carefully and sensitively control residues of veterinary drugs in animal products [1]. Here we present a versatile and sensitive workflow on the SCIEX X500R QTOF system which combines a non-targeted screening workflow using SWATH® data acquisition looped with highly selective MRM^{HR} acquisition. Confident identification of veterinary drug residues according legal requirements [2] is achieved by accurate precursor and fragment mass measurement and their compound specific ion ratios, as reported in the SCIEX OS software.

Materials and Methods

Sample Preparation

Liver tissue was mixed with extraction solution (acetonitrile, water, formic acid) and homogenized. Following centrifugation for 5 minutes, a 5 mL aliquot from the supernatant was concentrated under nitrogen flow. After addition of 2.5 mL of solvent A, the extract was vortexed, centrifuged and filtered prior to injection. Aliquots of the extracts were spiked with a standard solution yielding final concentrations of 0.2, 1, 5, 10, and 50 ng/mL (corresponding to 0.08, 0.4, 2, 4, and 20 µg/kg liver).

Figure 1: SCIEX X500R QTOF system

LC Method

Veterinary drugs were chromatographically separated on a SCIEX ExionLC™ AD UHPLC system, using a Phenomenex Kinetex C18 column (150 x 2.1 mm, 2.6 µm). Mobile phase A was water with 5% acetonitrile and 0.3% formic acid. Mobile phase B was acetonitrile with 5% water and 0.3% formic acid. Chromatographic separation was achieved using the gradient below. Oven temperature was set to 30 °C. Injection volume was 5 µL.

	A [%]	B [%]	Flow [mL/min]
0.0 min	100	0	0.4
2.0 min	100	0	0.4
7.0 min	70	30	0.4
11.0 min	0	100	0.4
11.1 min	0	100	0.8
12.5 min	0	100	0.8
12.6 min	100	0	0.4
14.0 min	100	0	0.4

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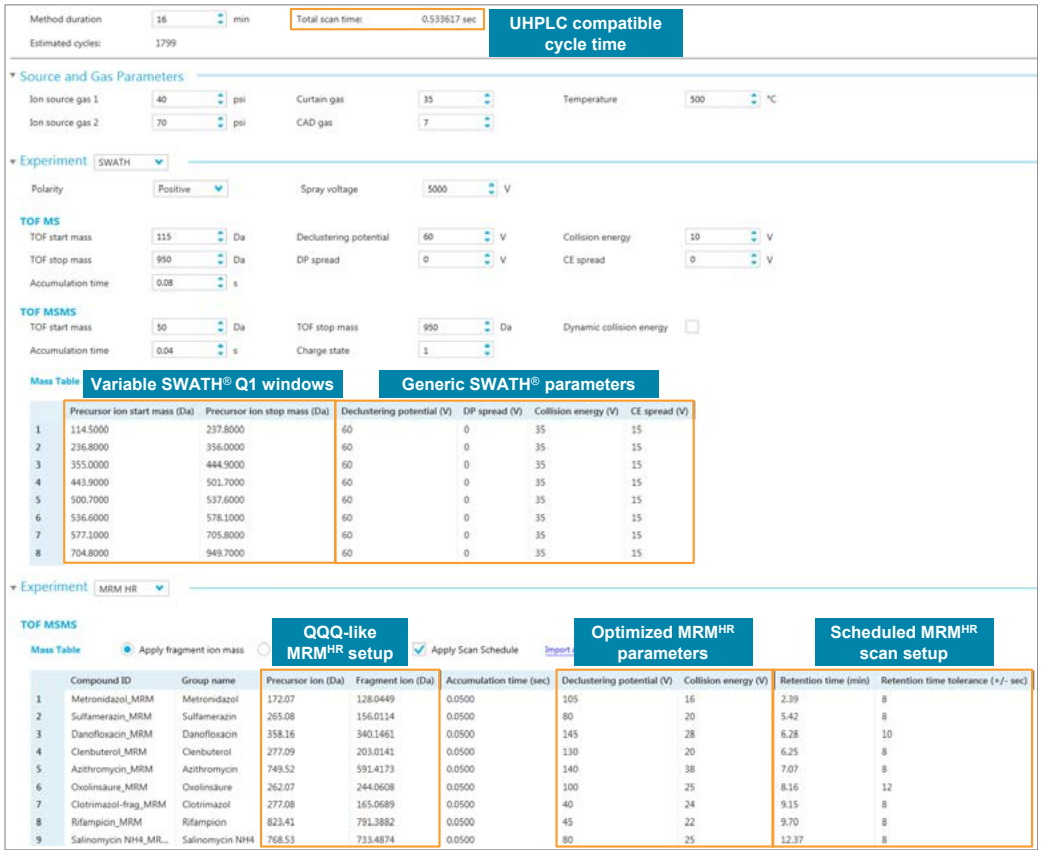

Figure 2: MS Method in SCIEX OS.

MS Method

The SCIEX X500R QTOF system was operated in positive mode with electrospray ionization. Data acquisition was performed using TOF-MS mode looped with eight SWATH® MS/MS experiments and scheduled MRM^{HR} acquisition. Variable SWATH® Q1 windows were used, calculated with the SCIEX SWATH® Variable Window Calculator. MRMHR experiments were acquired in fragment mode with a TOF scan window of 20 Da. Figure 2 shows the MS method as displayed in SCIEX OS. Data processing was done in SCIEX OS version 1.3.

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Results & Discussion

Quantitative Results

On the SCIEX X500R QTOF system, TOF-MS mode is the standard acquisition mode for quantitation, providing non-targeted data collection which can be subsequently processed in SCIEX OS using a list of targeted compounds. For the 27 analytes of interest, TOF-MS mode provides excellent sensitivity in the standard solution at 1 ng/mL, as shown in figure 3.

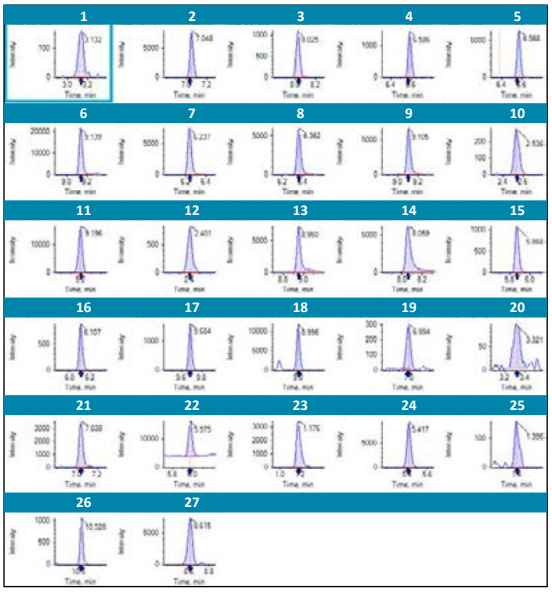

Figure 3: Extracted ion chromatograms of a standard solution of veterinary drugs at 1 ng/mL. 1 Amoxicillin. 2 Azithromycin. 3 Ceftiofur. 4 Chlortetracycline. 5 Clenbuterol. 6 Clotrimazole. 7 Danofloxacin. 8 Enrofloxacin. 9 Flumequine. 10 HMMNI. 11 Josamycin. 12 Metronidazole. 13 Nalidixic acid. 14 Oxolinic acid. 15 Oxytetracycline. 16 Penicillin G. 17 Rifampicin. 18 Roxythromycin. 19 Spiramycin. 20 Sulfacetamide. 21 Sulfachlorpyridazine. 22 Sulfadimidine. 23 Sulfagunidine. 24 Sulfamerazine. 25 Sulfanilamide. 26 Triclabendazole-sulfone. 27 Tylosin A.

However, in very complex matrices such as liver extracts, interferences may hamper the sensitive detection of certain analytes. For example, the signal for azithromycin in matrix spiked at 0.2 ng/mL shows a shoulder from a matrix interference which is not chromatographically resolved, and which makes an accurate integration and thus quantitation difficult (left panel in figure 4). In such a case, quantitation can be alternatively performed using the comprehensive MS/MS traces from SWATH® acquisition, a unique – as low matrix interfered – MS/MSALL technology. Using the MRM-like higher selectivity of SWATH® fragments, the interference observed in the TOF-MS trace can be removed (middle panel in figure 4). If even higher selectivity and sensitivity is needed, true MRM^{HR} provides even better signal-to-noise ratios (right panel in figure 4). The increase of signal-to-noise performance is due to the fact that MRM^{HR} uses compound specific collision energy, CE, and declustering potential, DP, voltages, while SWATH® is a generic method. Furthermore, transmission of the precursor ion as well as the fragment ion on their way through the mass spectrometer is optimized. Finally, the high selectivity in MRM^{HR} decreases the noise in the chromatogram to its minimum.

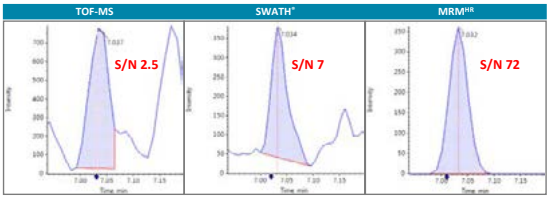

Figure 4: Extracted ion chromatograms of azithromycin spiked at 0.2 ng/mL in liver extract from different acquisition experiments. Left panel: TOF-MS (m/z 749.5158). Middle panel: SWATH®-MS/MS (749.52 > 591.4215). Right panel: MRMHR (749.52>591.4215).

Qualitative Results

SCIEX OS displays several parameters allowing the confident identification of a detected signal, meeting the European Union criteria of identification points [2]. First, it calculates mass errors of the precursor ion as well as of the fragment ions. Second, the ion ratio measured in unknown samples is compared to the one calculated from standards. Both the mass error and the ion ratio confidences are clearly displayed with a traffic light system, using a green checkmark for signals which meet the identification criteria. This allows the user to easily review large data sets and filter for positively detected compounds (figure 5).

Typically, the ion ratio can be calculated from the area of the precursor ion and the area of one fragment. Alternatively, if the TOF-MS trace is disturbed by interferences, two MS/MS fragments can be used. MS/MS fragments can be taken either from the SWATH® experiment or, if higher selectivity is needed, from a looped MRM^{HR} experiment.

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Figure 5: Quantitative and qualitative results for Danofloxacin as shown in SCIEX OS. Upper left panel: Results table with confidence display for ion ratio and mass errors of precursor and fragment. Upper right panel: Calibration curve. Lower Panel: Extracted ion chromatograms of standard solutions and matrix samples. Quantifier (TOF-MS) is displayed in pink. Qualifier (MS/MS fragment from SWATH®) is displayed in blue. Expected ion ratio is shown as blue solid line, tolerances (±30%) as dotted line.

Conclusion

The SCIEX X500R QTOF system is a powerful instrument for the sensitive analysis of veterinary drugs in complex matrices, with a unique combination of versatile acquisition modes for different requirements:

1) TOF-MS data as standard trace used for quantitation.

2) Concurrent acquisition of untargeted SWATH® MS/MS data, used for identification with the help of accurate fragment masses and compound specific ion ratios as required by official guidelines. Furthermore, SWATH® MS/MS fragment can be used for quantitation, if the TOF-MS trace shows interferences.

3) Concurrent acquisition of targeted MRM^{HR} data increased selectivity for analytes which show interferences both in TOF-MS and SWATH® MS/MS mode.

References

1) European Commission, Commission Decision 37/2010/EU of 22 December 2002. ABl. :L15/1-72 (2002)

2) European Commission, Commission Decision 2002/657/EC of 12 August 2002. ABl. L221, 8-36 (2002)

Acknowledgements

We would like to thank Anton Kaufmann from the Cantonal Laboratory of Zurich, Switzerland, who provided the liver extracts and the chromatographic method.

Simultaneous analysis of 12 food allergens in baked and raw food products using the LC-MS/MS QTRAP® 4500 system

Detection of multiple signature peptides of 12 priority food allergens

Lee Sun New[1], Rahul Baghla[2], André Schreiber[3], Jianru Stahl-Zeng[4], and Hua-Fen Liu[5]
[1]SCIEX Singapore; [2]SCIEX Gurgaon, Haryana (India); [3]SCIEX Concord, Ontario (Canada); [4]SCIEX Darmstadt (Germany); [5]SCIEX Redwood City, California (USA)

Introduction

A food allergy is an immune-mediated, adverse reaction to an antigenic protein. Even limited exposure to an antigen can provoke a significant reaction in sensitive individuals, causing rashes, itching and swelling in the mouth, nausea, vomiting, and asthma. Additionally, food allergies are the leading cause of anaphylaxis, an acute, potentially deadly allergic reaction. The prevalence and severity of food allergies are rising, with approximately 150 million people suffering from food allergies worldwide.[1, 2] Presently, there is no cure for food allergies, and sufferers must rely on the correct labeling of foods to avoid consuming allergens. Hence, the development of sensitive and accurate analytical methods to screen for the presence of allergens in food products is necessary for the prevention of potentially life-threatening health problems for allergy sufferers.

Enzyme-linked immunosorbent assays (ELISA) are the most commonly used tests for screening allergens. Although relatively quick and simple to perform, ELISA tests are limited in selectivity and susceptible to cross-reactivity, which can lead to false positive or false negative results. Additionally, most ELISA tests are capable of detecting only one allergen at a time, requiring multiple tests to screen for more than one allergen in a food sample. Therefore, a method that can unambiguously confirm and identify multiple allergens would be invaluable for food screening.

Herein, we present an LC-MS/MS method using the QTRAP® 4500 LC-MS/MS system that detects and screens 12 separate allergenic proteins simultaneously in a single injection. The allergens detected in this method were selected from the guidelines presented in the Codex Alimentarius, a resource developed by the United Nations' Food and Agriculture Organization (FAO) and the World Health Organization (WHO) to harmonize international food standards.[3]

The Codex recommends eight allergenic food groups be declared on the labels of pre-packaged foods: grains, shellfish, eggs, fish, legumes, milk, sulfite, tree nuts.[3] Five of these allergens are detected with this method including eggs, milk, peanuts, soy beans, and tree nuts (almonds, Brazil nuts, cashew nuts, hazelnuts, pecans, pine nuts, pistachios, and walnuts).

To evaluate a range of food products (both raw and bakery goods) for their allergenic content, several unique signature peptides specific to each allergen were identified from tryptic digests of food homogenate extracts. A mixture of 12 allergens was added to bakery product food matrices (either bread or cookie) over a range of known concentrations, and several MRM transitions corresponding to allergenic signature peptides were evaluated simultaneously using the *Scheduled* MRM™ algorithm. Presently, this method can detect allergenic peptides from five of the major classes of allergenic foods at a detection limit of 10 ppm in a variety of food matrices.

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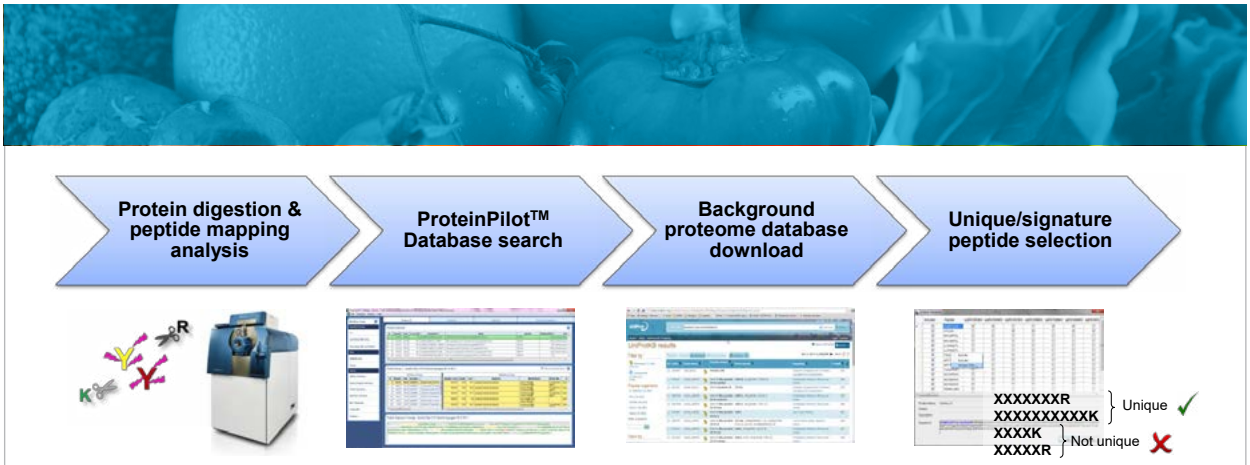

Figure 1. Signature peptide selection workflow using the SCIEX TripleTOF® 6600 system and ProteinPilot™ software

Experimental

Sample Preparation

To prepare bread and cookie homogenates, unbaked gluten-free bread or cookie mixes (100 g) were supplemented with 10 to 500 ppm (by weight) of each of the following 12 allergenic foods: eggs, milk, peanuts, soy, almonds, Brazil nuts, cashew nuts, hazelnuts, pecans, pine nuts, pistachios, and walnuts. The fortified foods were then cooked according to manufacturer's specifications. The food samples (raw nuts, baked goods) were finely homogenized using a coffee grinder. Each homogenate (1 g) was defatted by extracting twice with hexane and dried by evaporation in the fume hood. Extraction buffer (4 mL) was added to the defatted homogenates, which were then centrifuged prior to the removal of supernatants (500 µL). Reducing reagent (50 µL) was added to supernatants at 60°C for 1 hr. After cooling (25°C), samples were alkylated using a cysteine blocking reagent (25 µL). Trypsin (20 µg) was added to modified proteins (3 to 12 h) in calcium chloride/ammonium bicarbonate buffer to obtain tryptic peptides for signature peptide analysis prior to neutralization with formic acid (30 µL). Digested samples

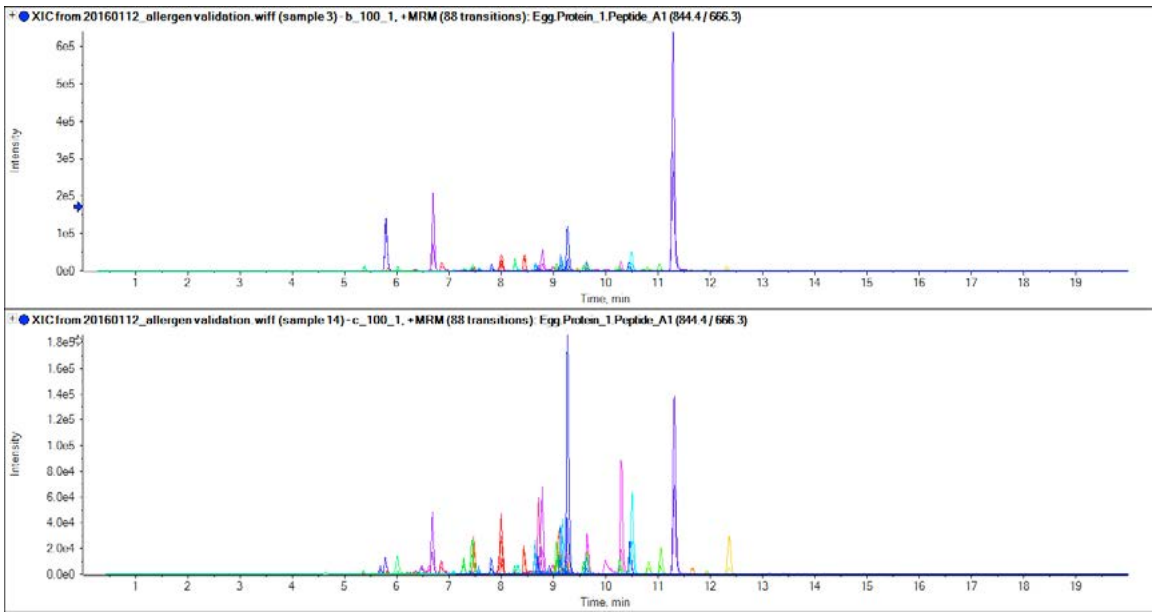

Figure 2. Extracted ion chromatograms (XIC) from LC-MS/MS analysis of bread (top) and cookie (bottom) homogenates fortified with egg, milk, peanut, soy, and nut proteins at100 ppm. Multiple peaks corresponding to allergenic tryptic peptides are displayed.

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(500 µL) were centrifuge-filtered using a 10 kDa MWCO filter prior to LC-MS/MS analysis.

LC Separation

Tryptic peptides (30 µL injection volume) were chromatographically separated using a Shimadzu Prominence UFLC$_{XR}$ system equipped with a Phenomenex Kinetex C18 column (2.6 µm, 100 x 3 mm). A linear gradient was employed over 12 min at a flow rate of 300 µL/min using 0.1% formic acid in water and 0.1% formic acid in acetonitrile.

MS/MS Detection

To identify signature peptides for allergen screening, peptide maps of various allergenic foods (eggs, milk, peanuts, soy beans, and tree nuts) were acquired using a TripleTOF® 6600 LC-MS/MS System (Figure 1). The strategy for the selection of signature peptides can be found in more detail in the Results and Discussion.

To screen foods for allergens, a SCIEX QTRAP® 4500 system with Turbo V™ source in positive ESI mode was employed using an ion source temperature of 500°C. The *Scheduled* MRM™ algorithm was used to analyze food samples for 12 allergens in a single injection by multiplexing the detection of multiple MRM transitions for allergenic signature peptides.

Results and Discussion

Signature peptides were chosen for each allergen based on: 1) their uniqueness compared to background proteins; and 2) their sensitivity of detection. Further details on peptide sequences, their relative abundance, and possible post-translational modifications were generated using the ProteinPilot™ software's protein database search features after LC-MS/MS analysis of peptides on a TripleTOF® 6600 System (Figure 1). The list of selected peptides was refined by removing peptide sequences susceptible to further reaction (e.g., post translational modification, Maillard reaction) during food processing or baking.

For each allergen, two unique proteins, two unique peptides per protein, and two MRM transitions per peptide were chosen to ensure confidence in the identification of an allergen. To monitor many MRM transitions during a single injection, the *Scheduled* MRM™ Algorithm was employed, where individual MRM transitions were monitored for a short period during their expected retention time, decreasing the total number of concurrent MRM experiments during a cycle and allowing cycle time and dwell time to be maintained. This approach maximized the S/N for signature peptide detection and allows the method to be expanded as new allergenic markers are identified.

To identify multiple allergens in the same food sample, a total of 88 MRM transitions corresponding to 44 allergenic peptides, from eggs, milk, peanuts, soy beans, and tree nuts, were characterized (Figure 2). Of these 44 peptides, 40 transitions corresponded to peptides with unique sequences not shared by background proteins. The LC-MS/MS-based screening method deployed here simultaneously detected 12 allergenic proteins from 5 major classes of food allergens (egg, milk, peanut, soy and tree nuts) that had been fortified into bakery products at varying concentrations.

To show that signature peptide signals were linear in response to increasing allergen levels, calibration curves for each peptide and its three transitions were generated over a wide dynamic range

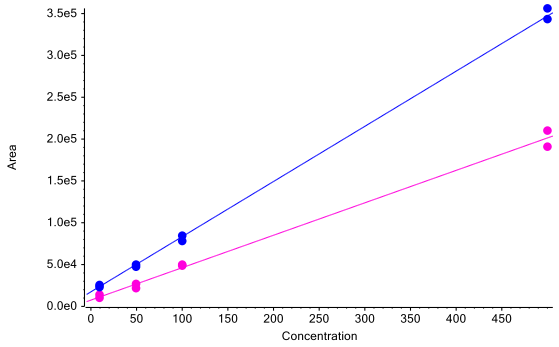

Figure 3a. Calibration lines of a hazelnut peptide from 0 to 500 ppm. Two MRM transitions were monitored: fragment 1 (blue) and fragment 2 (pink)

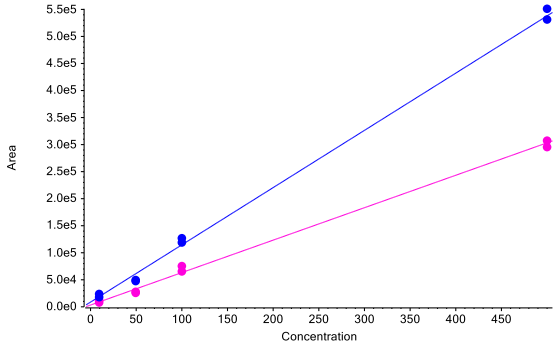

Figure 3b. Calibration lines of a peanut peptide form 0 to 500 ppm. Two MRM transitions were monitored: fragment 1 (blue) and fragment 2 (pink)

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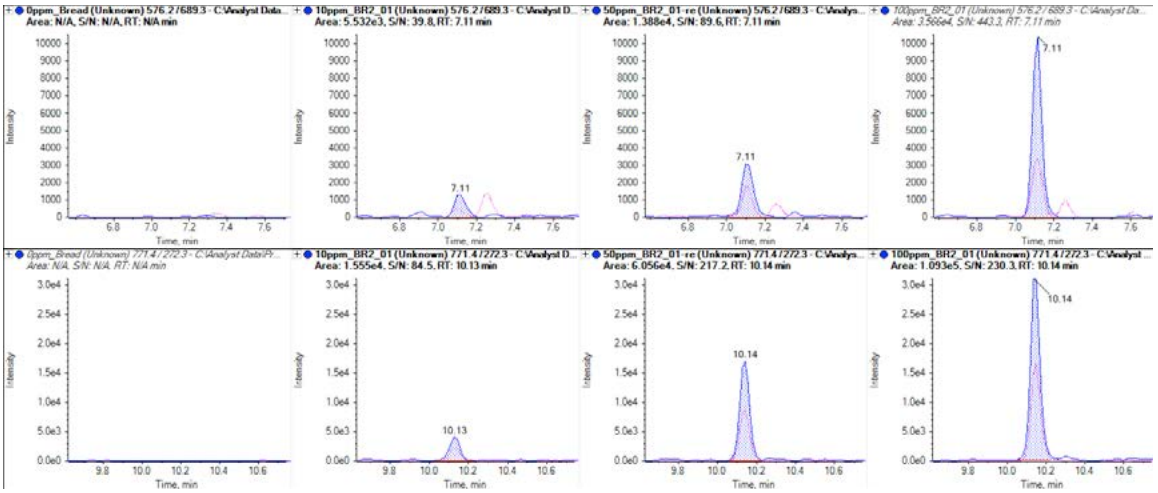

Figure 4. Extracted ion chromatograms for the signature peptide, protein 1 peptide 1, from hazelnut (top) and peanut (bottom). Varying concentrations of allergen (0, 10, 50 and 100 ppm) were added to bread samples. Two different MRM transitions for protein 1, peptide 1 are shown (blue, and pink traces).

(0 to 500 ppm) with good reproducibility in matrix (Figures 3a and 3b). MRM transitions were linear over a broad dynamic range and resulted in regression values over 0.95 for all allergens.

All allergenic peptides were detected at concentrations as low as 10 ppm (Figure 4) and generated signals proportional to the quantity of supplemented allergen.

One advantage of the LC-MS/MS method over ELISA-based detection methods is that multiple allergens can be detected in the same sample with one injection. To ensure that a high standard of performance was maintained as throughput increased with the multiplexed LC-MS/MS method, two separate allergen detection methods were directly compared. Signature peptides for select allergens (hazelnut and peanut) were analyzed using two separate ELISA kits and with the LC-MS/MS based method. In general, there was good correlation between the calculated concentrations obtained from ELISA and LC-MS/MS with $r^2 \geq 0.99$ (Figure 5). However, results from the ELISA-based tests underestimated the concentrations of hazelnut and peanut supplements in bread and cookie matrices, especially at higher concentrations.

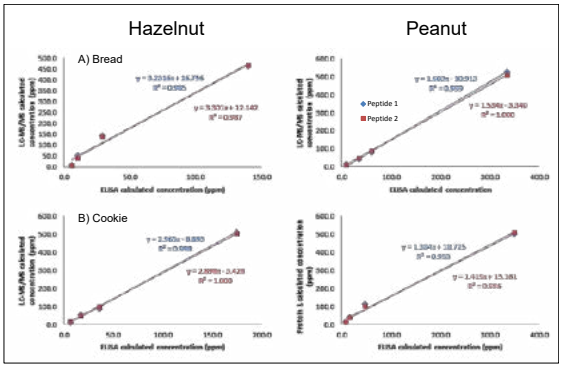

Figure 5. Comparison of allergen concentrations detected using ELISA vs. LC-MS/MS methods for two peptides (blue and orange) and two matrices, bread (top) and cookie (bottom)

To verify the effectiveness of the LC-MS/MS method for detecting allergens in commercial food samples, bakery products (cookies) containing a variety of allergens were screened using the signature peptide method (Figure 6). Allergen-related signals were not detected in cookie samples that were egg-, milk- and nut-free. However, cookies and bread products that listed hazelnuts and peanuts as ingredients tested positive using the LC-MS/MS method. Other allergens were identified, including egg and milk.

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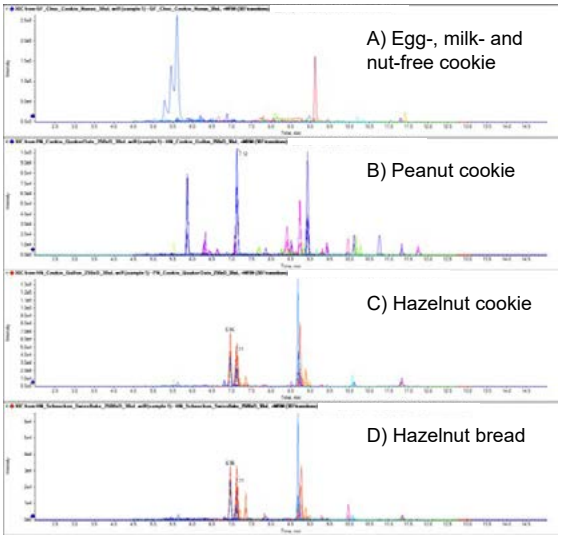

Figure 6. Extracted ion chromatograms of (A) Egg-, milk-, and nut-free cookie, (B) peanut cookie, (C) hazelnut cookie, and (D) hazelnut bread.

Summary

We have developed a multi-allergen screening tool using an LC-MS/MS method that can detect 12 food allergens in commercial products by identifying several MRM transitions corresponding to unique signature peptides for each allergen and multiplexing their detection into a single injection. In total, there are 88 MRM transitions representing peptides from the egg, milk, peanut, soy, and tree nut allergen groups. Unlike ELISA methods, this LC-MS/MS analysis detects multiple peptides from each allergic protein, thus improving method specificity and minimizing the potential for false positive and false negative results. Using only a single sample preparation method and a multiplexed data acquisition, more allergens than previously reported[4] were screened and differentiated from other food ingredients contained in baked food matrix.

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2 A. Sage: 'Food Allergen Analysis Using LC-MS-MS' LCGC The Column 10 (2015) 13-29

3 Codex Alimentarius Commission: 'Report of the Twenty-Second Session of the Codex Committee on Food Labelling' (1993) downloaded at http://www.codexalimentarius.org/

4 S. Lock et al.: 'The Detection of Allergens in Bread and Pasta by Liquid Chromatography Tandem Mass Spectrometry' Application Note SCIEX (2010) #1830610-0

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A Selective and Robust LC-MS/MS Method for Multiple Meat Speciation and Authentication on the QTRAP® 4500 System

Rapid and Reliable Detection of Multiple Meat Species in Food Products in a Single Injection

Si Mou[1], Lei Xiong[1], Lee Sun New[2], Peibin Qin[3], Guo Lihai[3], and Hua-Fen Liu[1]
[1]SCIEX Redwood City, California (USA); [2]SCIEX Singapore; [3]SCIEX Beijing, China

Introduction

In early 2013, horse and pig DNA were identified in beef products sold in several supermarket chains. Further testing across Europe and beyond had revealed widespread incidences of such contamination.[1] This type of contamination not only misleads the consumers, but also has health, religious, and ethical implications. In response to this, the Food Safety Authority (FSA) and Department for Environment Food & Rural Affairs (Defra) have set the threshold for undeclared meat species in meat products to 1% (w/w).[2] Therefore, it is imperative that analytical methods are sensitive and accurate enough to screen for the presence of meat adulteration in food products.

Traditionally, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA) are used for meat speciation. PCR amplifies fragments of DNA extracted from food samples and demonstrates good sensitivity in unprocessed products. However, DNA can be easily disrupted or removed during food processing and manufacturing, thus limiting the use of PCR for processed or cooked meat products. ELISA is relatively quick and simple to perform, but has poor selectivity and is susceptible to cross-species reactivity which can lead to false positive or false negative results. Moreover, most ELISA tests lack multiplexing capabilities. Hence, LC-MS/MS provides an excellent alternative to these methodologies to identify and confirm different meat species with more accuracy and reliability.

Herein, we present a robust and sensitive LC-MS/MS method using the QTRAP® 4500 LC-MS/MS system that detects and screens pork, beef, lamb, chicken, duck and horse simultaneously in a single injection. The optimized sample preparation procedure is easy to follow and can be used for analyzing raw, cooked and processed meat products. Signature marker peptides unique to each species were identified and verified to ensure that they do not present any cross-species reactivity. Presently, this method can detect peptides from each meat species at a threshold detection limit of 1% w/w (10 mg/g) in a variety of food products.

Experimental

Sample Preparation

Meats or meat products (10 g) were frozen for 1 hour and grounded using a food processor or a coffee grinder. As an optional step, each grounded meat (1 g) was defatted with hexane and dried under a gentle flow of nitrogen. Extraction buffer was added to each defatted meat sample and the mixture was homogenized at high speed using a probe homogenizer to extract the proteins. Standard samples were prepared by combining different amounts of pork, beef, lamb, chicken, duck and horse homogenates to final concentrations of 0% and 1% (w/w) for each meat species (single-point calibration). The mixed meat homogenates (2 mL) were centrifuged and 0.4 mL of supernatant was diluted with ammonium bicarbonate buffer. Reducing reagent was added and the samples were incubated at 60°C for 1 hour. After cooling to room temperature, samples were alkylated using a cysteine blocking reagent. The modified proteins were digested with trypsin (4 to 12 hours). After which, the enzymatic activity was quenched with formic acid. Digested samples were desalted and concentrated using Agela

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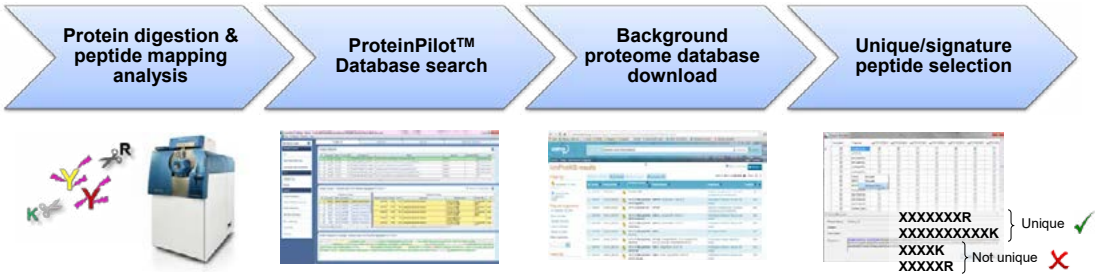

Figure 1. Signature peptide selection workflow using the SCIEX TripleTOF® 6600 system and ProteinPilot™ software

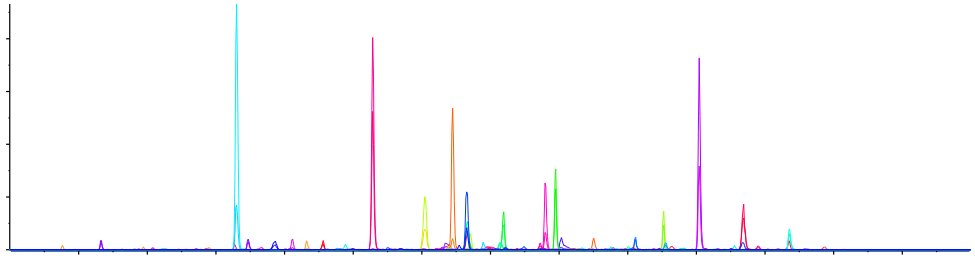

Figure 2. Extracted ion chromatograms from the LC-MS/MS analysis of raw meat mixture containing pork, beef, lamb, chicken, duck and horse (10, 20, 20, 20, 20 and 10% w/w, respectively). Multiple peaks corresponding to tryptic marker peptides are displayed.

Technologies Cleanert PEP SPE cartridges (60 mg/3 mL). The SPE eluents containing the peptides were dried and reconstituted for LC-MS/MS analysis.

LC Separation

Analytes (10 µL injection volume) were chromatographically separated using a ExionLC™ AC system equipped with a Phenomenex Kinetex C18 column (2.6 µm, 100 x 4.6 mm i.d.). A linear gradient was employed over 15 min at a flow rate of 500 µL/min using 0.1% formic acid in water and 0.1% formic acid in acetonitrile.

MS/MS Detection

Ion-dependent acquisitions (IDA) on a TripleTOF® 6600 LC-MS/MS System were performed to identify the proteins and peptides representative of pork, beef, lamb, chicken, duck and horse meats (Figure 1). The strategy for the selection of signature peptides can be found in more detail in the Results and Discussion.

Meat speciation and screening analysis was performed on a SCIEX QTRAP® 4500 system with Turbo V™ source in positive ESI mode using an ion source temperature of 650 °C. The Scheduled MRM™ algorithm was used to analyze food samples for 6 meats in a single injection by multiplexing the detection of multiple MRM transitions for unique signature peptides.

Results and Discussion

Comprehensive information of protein/peptide IDs was generated using the ProteinPilot™ software's protein database search features after LC-MS/MS analysis of digested meat samples on a TripleTOF® 6600 System (Figure 1). Selections of signature peptides for each meat species were performed using the Skyline software and NCBI Protein BLAST to ensure that the shortlisted peptides were unique and not found in other common livestock.

Signature peptides were finalized for each meat based on their: 1) specificity for each meat species; 2) uniqueness compared to the cross-species background; 3) sensitivity of detection; and 4) ability to be detected in both raw and cooked or processed meat samples.

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For each meat species, two unique proteins, two unique peptides per protein, and two unique MRM transitions per peptide were chosen to ensure confidence in positive identification (Table 1). This corresponds to 24 marker peptides or a total of 48 MRM transitions representing pork, beef, lamb, chicken, duck and horse, for the simultaneous identification of multiple meat species in the same food sample (Figure 2). To monitor many MRM transitions during a single injection, the Scheduled MRM™ algorithm was employed, where each MRM transition was monitored for a short period during its expected retention time, decreasing the total number of concurrent MRM experiments during a cycle and allowing cycle time and dwell time to be maintained. This approach maximized the sensitivity for signature peptide detection and allows the method to be expanded as markers from other meats are identified.

LC-MS/MS analyses of raw and cooked (pan-fried) meat mixtures were performed to evaluate the thermal stability of the marker peptides. As shown in Figure 3, each meat marker peptide was detected without significant changes in sensitivity before (raw) and after cooking.

To demonstrate that signature peptide signals were linear in response to increasing meat concentrations, calibration curves for each peptide were generated over a wide dynamic range (0 to 100% w/w) with good reproducibility in combined meat matrix. For all meat species tested (pork, beef, lamb, chicken, duck and horse), MRM transitions were linear over a broad dynamic range with correlation coefficient values of over 0.99 for both MRM transitions. Figures 4 and 5 show examples of pork and beef with good linear response in meat matrix.

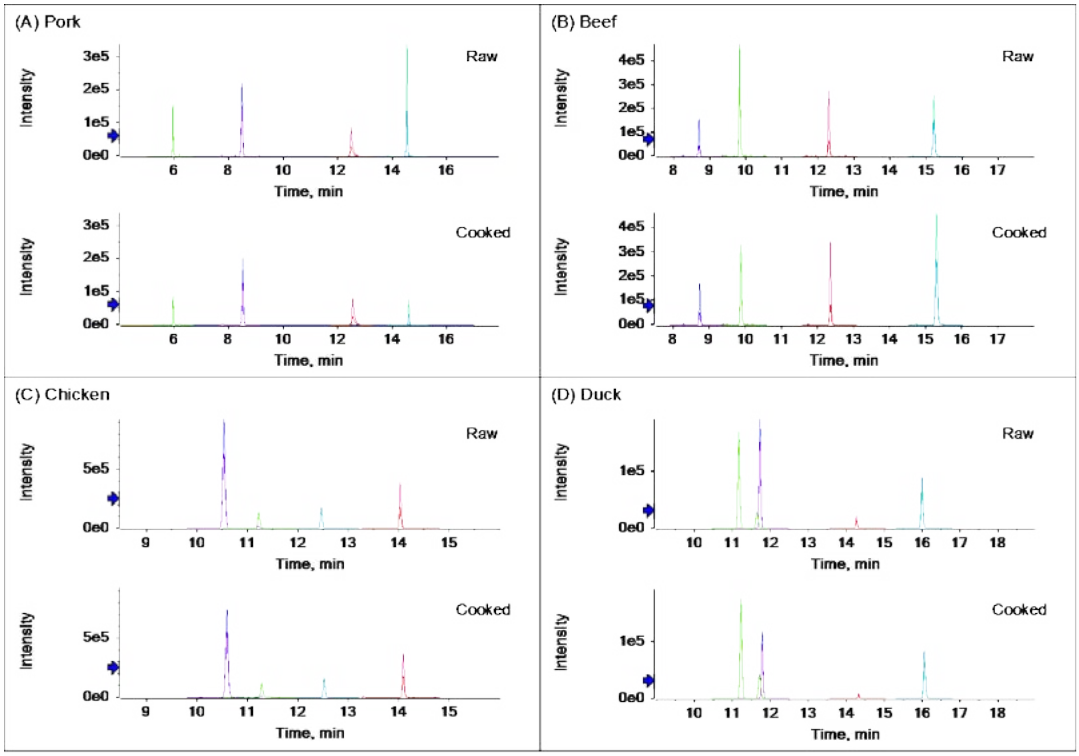

Figure 3. Extracted ion chromatograms (XIC) from the LC-MS/MS analysis of raw (top) and cooked (bottom) meat mixture containing pork, beef, chicken, duck and lamb (data not shown).

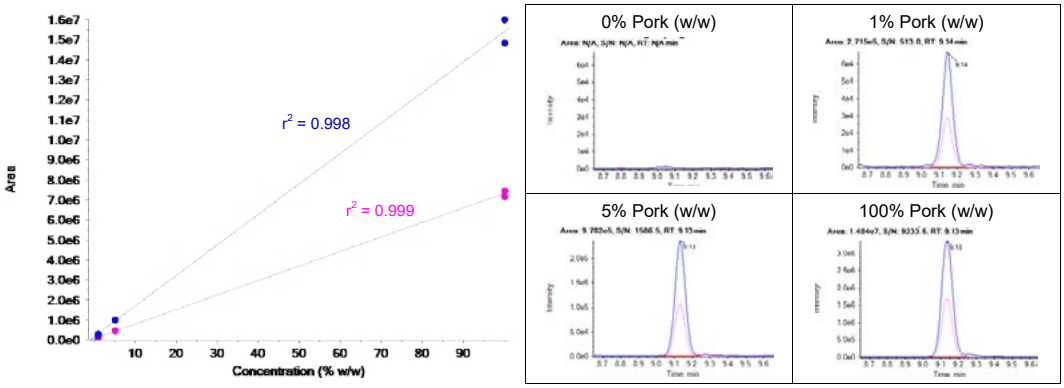

Figure 4. Calibration curves and XICs of Protein_1.Peptide_A from 0 to 100% raw pork (w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored.

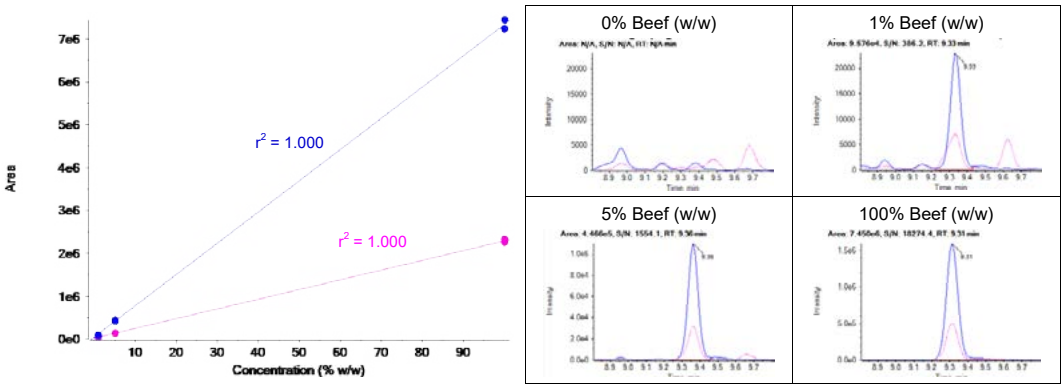

Figure 5. Calibration curves and XICs of Protein_1.Peptide_A from 0 to 100% raw beef (w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored.

The 1% (w/w) detection threshold limit of meat species in the combined meat matrix was verified on a SCIEX QTRAP® 4500 system by analyzing the 0% and spiked 1% (w/w) meat species in meat matrix. All marker peptides for each meat species were reliably detected at 1% spiked and no interference signals were observed in the background matrix (0%). Figures 5 and 6 show example XICs of quantifier ion (Protein_1.Peptide_A1) for each meat in 0% and 1% (w/w) samples, demonstrating high sensitivity and reliability of detection. It's worth noting that 0.1% (w/w) detection threshold limit of meat can also be achieved with a SCIEX QTRAP® 6500+ system (data not shown).

To verify the effectiveness of the method for detecting meat contamination or adulteration, various raw and processed food products purchased from supermarkets were screened. As an

example in Figure 7, no significant pork marker peptides were detected in the halal certified products. Pork was tested positive only in products that had this meat labeled as one of the ingredients.

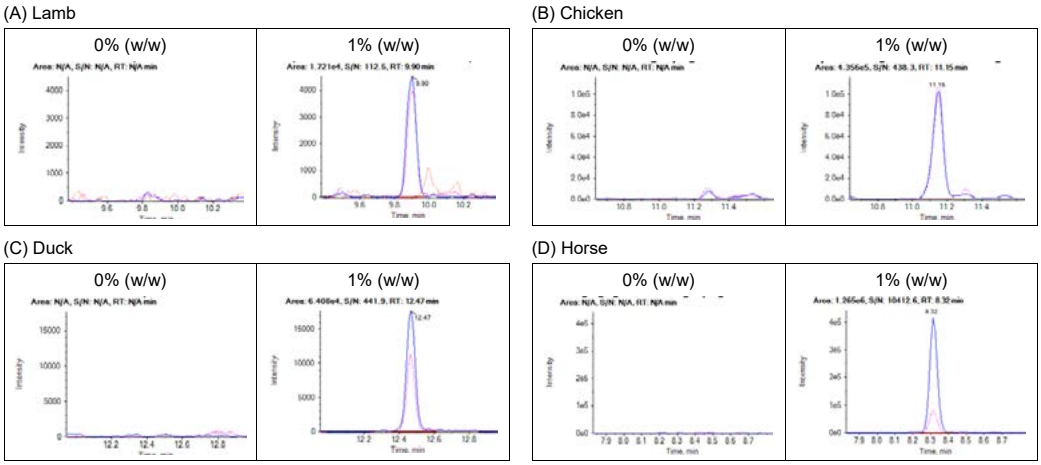

Figure 6. XICs of Protein_1.Peptide_A for 0 and 1% (w/w) of lamb, chicken, duck and horse in combined meat matrix (refer to Figure 5 for detection of pork and beef at 0 and 1% w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored for each marker peptide.

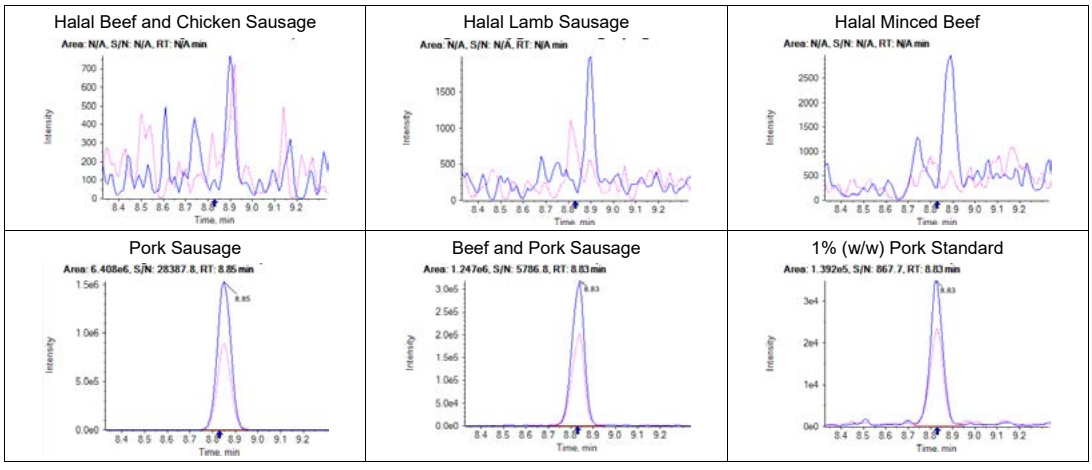

Figure 7. XIC of Pork.Protein_1.Peptide_A in commercial sausage products. Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored for the marker peptide.

Summary

We have developed an LC-MS/MS-based meat speciation method for screening meat adulteration at 1% (w/w) for pork, beef, lamb, chicken, duck and horse. This method identifies MRM transitions corresponding to unique peptides for each meat species, and multiplexes their detection into a single injection. Unlike PCR and ELISA, the method is applicable to both unprocessed and processed meat matrices, providing high specificity and sensitivity in a single analysis. In addition to 1% meat adulteration screening on a SCIEX QTRAP® 4500 system, the method also demonstrates good linear responses at different meat concentrations in meat matrix, indicating its potential capability for relative quantitation. The vMethod package includes an easy-to-follow and robust sample preparation procedure, an optimized LC-MS/MS acquisition method, established templates for data processing and reporting to facilitate the rapid detection and identification of meat adulteration or contamination in food products.

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Use of X500R QTOF for Monitoring Unexpected Additives in Nutritional Supplements

Zhao Xianglong, Cheng Haiyan, Li Lijun, Jin Wenhai
SCIEX, Pacific Applications Support Center (Guangzhou), China

Introduction

Nutritional supplements can supplement necessary nutrients and are believed to support recovery from illness. Generally, these products promote a particular effect or claimed function[1]; thus, in typical use, people often incorrectly believe they have a definite treatment efficacy. They are often linked to the alleviation of certain illnesses. In order to maximize these functions, manufacturers may add related drugs in order to increase their efficacy without including them as a listed ingredient. According to reports and discoveries from actual monitoring cases, unexpected additives to nutritional supplements are generally selected because they relate to the health product effects or address the additive side effects or functions; the additive usually takes the form of one or more drug additives, drug derivatives, etc.[5] Because these additives are generally high-dose, drug interactions can be unclear. Thus, a great potential hazard exists for human health [2-4]; the China Food and Drug Administration (CFDA) “Health product potential illegal additives list” clearly stipulates monitoring processes for additives in 6 different types of nutritional supplements: those with weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening functions. The purpose is to protect consumers’ health.

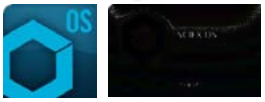
SCIEX’s X500R QTOF high resolution mass spectrometry system can be used for rapid monitoring of additives in nutritional supplements; after sample injection, a first order mass accuracy number and second order fragmentation spectrum are simultaneously obtained. Currently, over 50 additives can quickly be qualitatively confirmed in this way. Matrix interference in complex matrices can be overcome for specific screening of additives; preprocessing is even simpler and more convenient. The new SCIEX OS software fully integrates instrument control, data collection, data handling, and other processes. The workflow is more intuitive and smarter; this method provides an efficient means for rapid, high-throughput monitoring of nutritional supplements for additives.

Experimental Process

1. Collect samples of 6 types of nutritional supplements currently on the market - those with weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening functions. Perform simple preprocessing.
2. Use TOF MS-IDA MS/MS mode for data collection; after sample injection, obtain first order ion and second order ion fragmentation spectrograms.
3. The mass accuracy number, isotope distribution, retention time, and standard library alignment are used for positive verification of samples and checking the accuracy of sample monitoring results.
4. Monitoring reports systematically summarize sample screening results; the report content can be tailored to specific requirements.

X500R high-resolution mass spectrometry screening workflow:

1. Both TOF-MS-IDA-MS/MS And TOF-MS/MS data gathered in the same injection



2. SCIEX OS is the integral software used to perform this analysis

3. Screening results and report generation

Contents

Preprocessing Method

1. Use tablets ground into a powder, granules from inside capsules, or liquid samples; weigh accurately a 1.0gsample, and place in a 10mLcentrifuge tube;
2. Add 5mL acetonitrile and agitate 2 min;
3. Vortex 2 min;
4. Centrifuge at 4℃ at 10000 Rpm for 15min;
5. Dissolve the supernatant 1-fold;
6. Pass through a 0.22μm filter and directly inject sample;

Liquid Phase Conditions

Chromatographic Column: Phenomenex Kinetex C18, 2.1*100mm, 2.6μm;

Elution gradient

Time (min)	A%	B%
0	*95*	*5*
5.0	*55*	*45*
15.0	*20*	*80*
20.0	*5*	*95*
25.0	*5*	*95*
25.1	*95*	*5*
30	*95*	*5*

Positive ion mode: A: 0.1% Formic acid Water; B: 0.1% Formic acid Acetonitrile;

Negative ion mode: A: Water; B: Acetonitrile;

Flow rate: 0.3mL/min;

Column temperature: 40℃;

Amount inserted: 10 μL;

Mass Spectrometry Method

Scanning method: TOF MS-IDA MS/MS

Ion source: ESI source

Scanning range: m/z 50-2000

CUR gas: 30 PSI

Collision gas CAD: 7

IS voltage: 5500V/-4500V

Source temperature: 600℃

Atomizing gas GAS1: 55 PSI

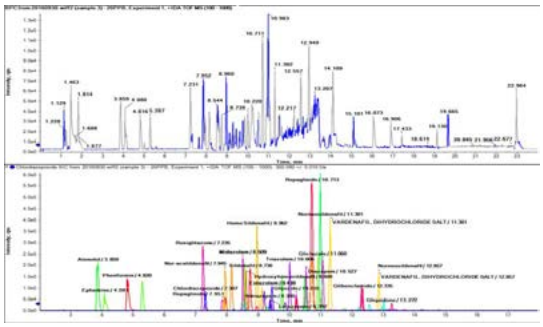
Auxiliary gas GAS2: 70 PSI

DP voltage: ± 60V

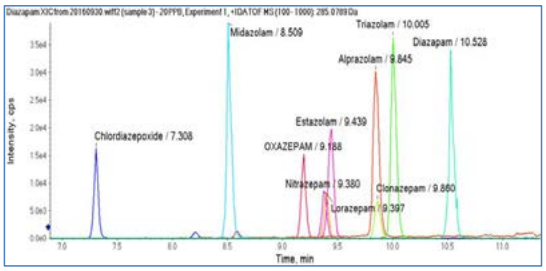
Collision energy: 35 ± 15V

Unexpected Additive Screening Method

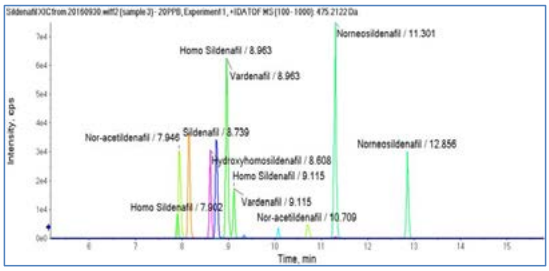
Injection of a single sample simultaneously monitors for over 50 unexpected additives:



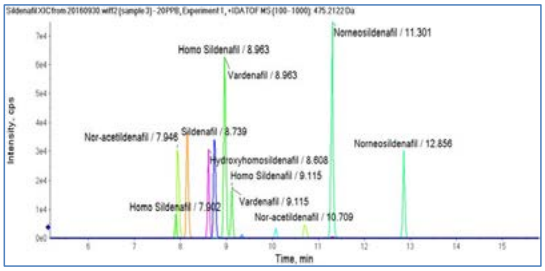
1. 10 sedative-hypnotic mixtures (20ppb), ion extraction flow diagram (XIC) appears below:



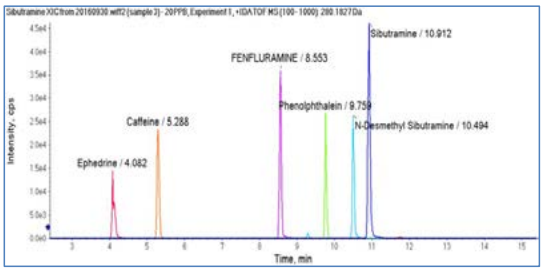
2. 7 blood glucose-lowering drugs (concentration 20ppb); ion extraction flow diagram (XIC) appears below:



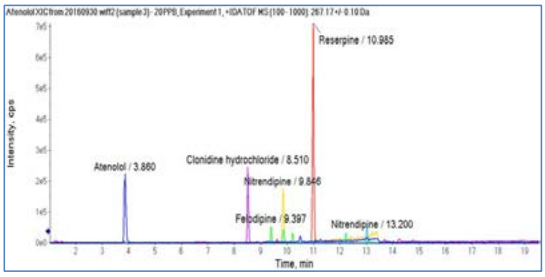
3. 8 impotence drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



4. 6 weight loss drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



5. 5 blood pressure-lowering drug mixtures (20ppb), ion extraction flow diagram (XIC) appears below;



Sample Information

Following the CFDA's "Health product potential illegal additives list" 6 different nutritional supplements were randomly selected, including those for weight loss, blood sugar reduction, blood pressure reduction, anti-fatigue, sleep improvement, and immune strengthening. Samples came from 19 different brands;

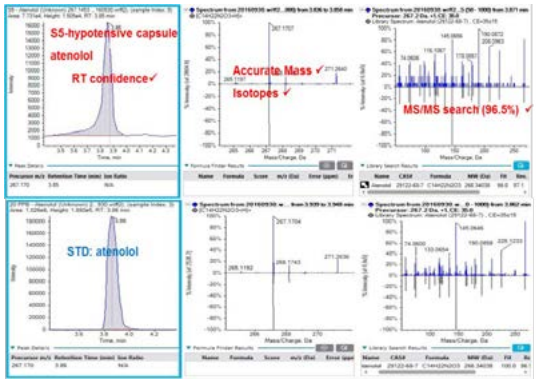
Sample No	sample type	name
Sample 1	sleeping	epiphysis pacify
Sample 2	hypoglycemic action	glycolipids safe
Sample 3	hypoglycemic action	hypoglycemic extract
Sample 4	anti-hangover	prime power
Sample 5	hypotensive	hypotensive capsule
Sample 6	sleeping	pacify syrup
Sample 7	hypoglycemic action	hypoglycemic TCM
Sample 8	slimming	slimming capsule
Sample 9	hypotensive	Hypotensive pill
Sample 19	.....	.....

Experimental Results

Blood Pressure-Lowering Drugs

1. Sample no. 5 - atenolol positive

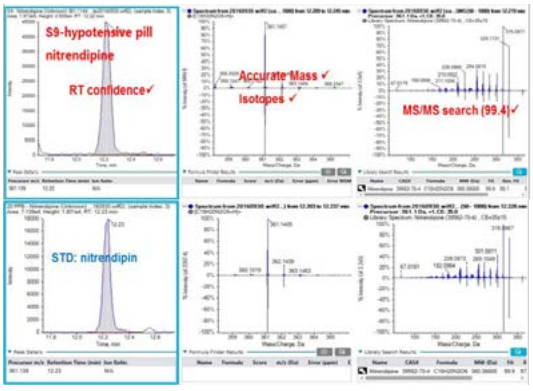
Sample no. 5 is a blood pressure-lowering capsule; it claims to have a rapid effect and prolonged use can control blood pressure.



Screening with the X500R QTOF system showed Sample no. 5 contains large amounts of the additive atenolol. Prolonged use of high-dose atenolol can lead to serious side effects including decreased vision, breathing difficulties, weakness, depression, unexplained rash and ankle swelling and other symptoms.

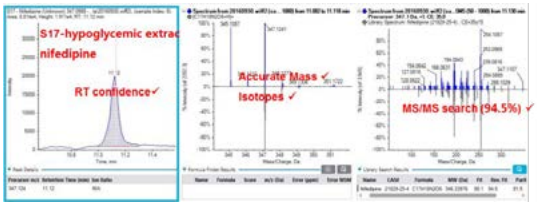
2. Sample no. 9 - nitrendipine positive

Sample no. 9 is from a brand of blood pressure-lowering tablet; screening shows a definite quantity of nitrendipine. The product claims to contain pure and natural extracts with no side effects, but prolonged oral nitrendipine can cause diseases like allergic hepatitis, rash, and even exfoliative dermatitis.



3. Sample no. 17 - nifedipine positive

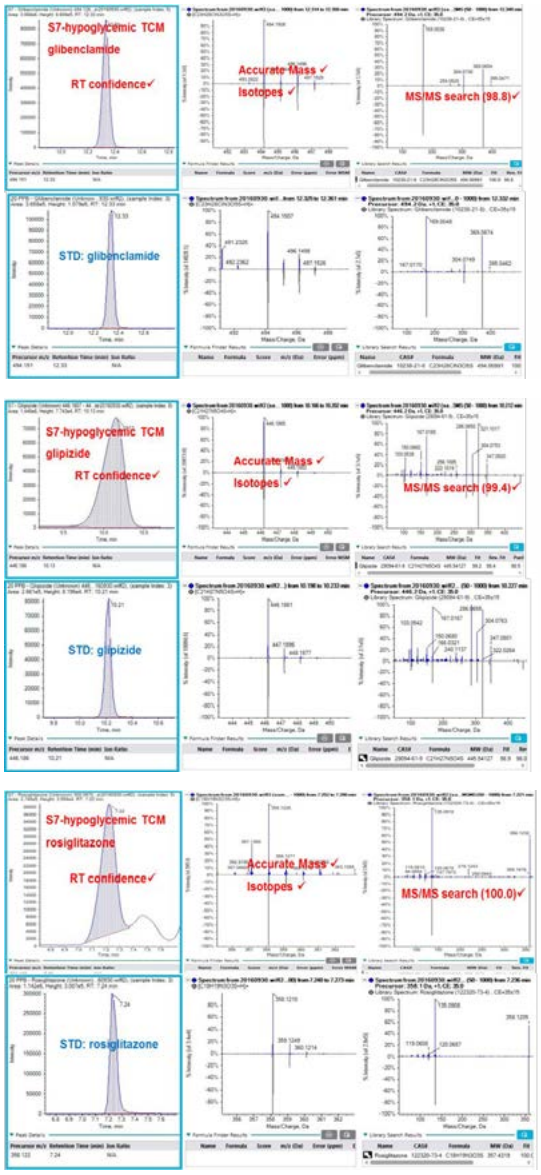
Sample no. 17 is from a brand of blood pressure-lowering Chinese medicine; screening shows a nifedipine additive. It claims to lower blood pressure with Chinese medicine, falsely advertising an anti-hypertensive effect.



Glucose-Lowering Drugs

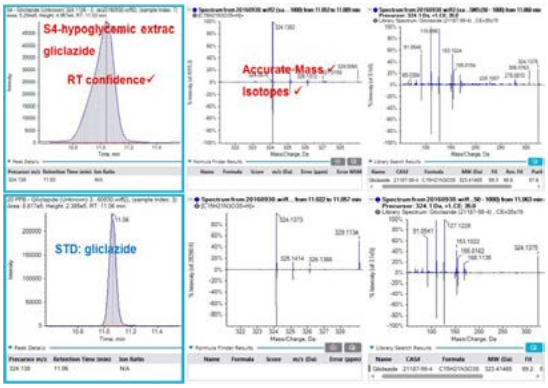
1. Sample no. 7 - glibenclamide, glipizide, rosiglitazone positive

Sample no. 7 is a brand of glucose- and lipid-lowering capsule; test results show sample no. 7 contains the 3 glucose-lowering drugs glibenclamide, glipizide, and rosiglitazone as additives. Improper use of sulfonylureas such as glibenclamide and glipizide can cause hypoglycemia; patients can rarely develop rash, erythema multiforme, edema, and liver and kidney damage. Thiazolidinediones like rosiglitazone can cause slight hypersensitivity and mild headache when used incorrectly or at improper doses.



2. Sample no. 4 - Gliclazide positive

Sample no. 4 is a brand of plant extract; it is mainly used to stabilize blood sugar. Screening results show an addition of glicazide, which produces a definitive glucose-lowering effect.

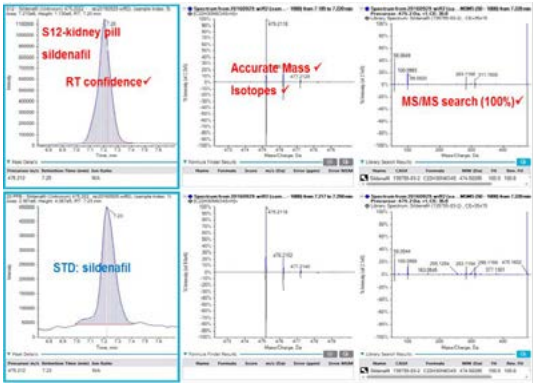


Glucose-lowering drugs are low-cost; they are common "functional components" added to nutritional supplements. These chemical drugs are often used to treat diabetes, as they have a clear hypoglycemic effect. However, their side effects are also quite evident; prolonged use can lead to hypoglycemia and kidney damage, even leading to death.

Anti-Fatigue/Impotence

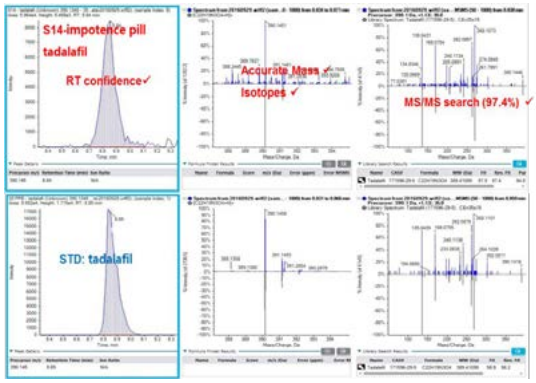
1. Sample no. 12 - sildenafil positive

Sample no. 12 is a kidney health product for the elderly; its description states it is pure Chinese medicine and contains several flavors of medicine. Screening shows an addition of large quantities of sildenafil in order to achieve its claimed kidney effects.



2. Sample no. 14 - tadalafil positive

Sample no. 14 is a brand of impotence health product. Impotence products are the most frequently found to contain additives. In order to accelerate the speed of onset, additives are generally used in large quantities; screening results showed sample no. 14 contained tadalafil.



When not used under the guidance of a specialized physician, prolonged use of nutritional supplements containing "impotence" additives can severely harm the body. Side effects can include dizziness, fainting, and even hearing loss.

Screening results appear in the table:

1. The problem of additives in nutritional supplements is widespread; additives appear in many samples;

2. Blood sugar- and pressure-reducing products contain many different additives; they generally take the form of multiple drugs, and use of Chinese medicine is especially serious.

3. Anti-fatigue and impotence health care products generally contain large amounts of additives;

Sample No	sample name	positvie results
Sample 1	epiphysis pacify	--------
Sample 2	glycolipids safe	--------
Sample 3	hypoglycemic action	--------
Sample 4	hypoglycemic extrac	gliclazide
Sample 5	hypotensive capsule	atenolol
Sample 6	pacify syrup	--------
Sample 7	hypoglycemic TCM	glipizide、rosiglitazone、glibenclamide
Sample 8	slimming capsule	--------
Sample 9	hypotensive pill	nitrendipine
Sample 12	kidney pill	sildenafil
Sample 14	impotence pill	tadalafil
Sample 17	hypoglycemic extrac	nifedipine

Contents

Summary

This study randomly selected 19 nutritional supplements commonly found on the market; these covered 7 glucose- and blood pressure-lowering products, 5 anti-fatigue, anti-impotence products, 4 sleep aids, and 3 weight loss products. Screening results showed that blood pressure-lowering and glucose-lowering products most commonly contained additives, especially those products advertised to use Chinese medicine extracts to lower blood sugar. Representative samples of blood pressure-lowering capsules showed a high rate of positive results. The main additives were atenolol, nitrendipine, nifedipine, glibenclamide, glipizide, rosiglitazone, gliclazide and other inexpensive and readily available glucose- and blood pressure-lowering drugs, impotence, anti-fatigue/immune system-enhancing additives were generally sildenafil or tadalafil. Additives take the form of one or many drugs; some additives are present in amounts several times therapeutic doses. Thus, they can be quite hazardous to consumer health.

The SCIEX X500R QTOF high resolution mass spectrometry system was used for rapid monitoring of 50 different additives in 6 types of nutritional supplements. Its high sensitivity detected small concentrations of additives, its rapid scanning and effective overcoming of complex matrix interference ensure that after sample injection, a first order mass accuracy number (TOF-MS) and second order fragmentation spectrum (TOF-MS/MS) are simultaneously obtained. Combined with the high-quality additive library, accurate qualitative screening for additives in complex matrices can be performed.

Health product additive screening methods using the X500R QTOF system are reliable, simple, and rapid. The system provides an efficient approach to additive screening of nutritional supplements, and it ensures health and safety product quality; it is critical in the fight against the use of potentially harmful additives.

References

[1] State Bureau of Technical Supervision. GB 16740-1997 General Standards for Health Food (Functions) [S]. Beijing: China Standards Press, 1997.

[2] Yin Shi'an, Wang Yin. Discussion of Health Food Safety [J]. Chinese Journal of Preventive Medicine, 2006, 40(2): 141-143.

[3] Wang Lanming. Analysis and Thoughts on China's Food Safety Management Work [J]. Food Science, 2004, 25(7): 187-192.

[4] Huang Xianglu, Wang Jingwen, Cao Jin. Monitoring and analysis of illegal additives in blood sugar-lowering health foods [J]. Food Science, 2014, 35(4): 149-151.

[5] Virginia M. Wheatley, John Spink. Defining the Public Health Threat of Dietary Supplement Fraud, Comprehensive Reviews in Food Science and Food Safety, 2013, 10.1111/1541-4337.12033.

Analyzing Different Compositions of Polygala from Different Regions Using the X500R QTOF System

Li Zhiyuan, Cheng Haiyan, Liu Ting, Li Lijun, Jin Wenhai
SCIEX, Pacific Applications Support Center (Beijing), China

Introduction

Authentic herbs come from specific locations that are traditionally known for these high-quality products. Authentic herbs have become synonymous with traditional Chinese medicine and form a comprehensive material standard for evaluating the quality of Chinese herbal medicines. Authentic herbs thus play a unique and important role in authentication and quality control of Chinese herbal preparations. Authenticity of Chinese medicine has become an important guarantee of high herbal quality.

Polygala is one of the main Chinese herbal medicines, one of 85 traditional Chinese herbal medicine exports, and one of 42 species of level 3 protected wild products in China[1]. The 2010 "Chinese Pharmacopoeia" divides Polygala herbs into those derived from the plant leaves of Polygalaceae and those made from dried Polygala leaves and roots. They have the properties of sedation, promoting heart and kidney circulation, acting as an expectorant, and decreasing swelling. They are used to treat insomnia, excessive dreaming, forgetfulness, and fear caused by poor heart and kidney circulation[2]. The commercial Polygala industry depends on the Polygala supply, which is found in an area bounded by the desert to the south and the Yangtze River to the north. It is grown mainly in Shanxi, Shaanxi, Henan, and Hebei, under the traditional notion of "Shanxi - large quantity, Shaanxi - high quality"[3].

Currently, the identification and analysis of Chinese herbal medicine components is quite challenging. These components underlie the pharmacodynamic efficacy of Chinese medicinal products. Herein lies the key to modernizing Chinese medicine. How to quickly identify the active ingredient and its structure, as well as how to identify the differences between the active ingredients of authentic and inauthentic herbs, are urgent problems that must be solved.

This study used the SCIEX high resolution X500R QTOF mass spectrometer for data acquisition and used the accompanying MarkerView™ analytic software to statistically analyze differences between components. This study involved Chinese medicine (e.g., Polygala) that includes components from different regions. This method makes component identification more effective, faster and a better reflection of the integrity and unique nature of the sample tested. In turn, it highlights the differences between Polygala components from different sources and provides a new framework for quality evaluation of Chinese herbal medicines.

The high resolution X500R's hardware design, including N-type ion path technology, time of flight tube design, and a stable and durable Turbo V™ ion source, ensures that under routine testing conditions, sample identification is more stable, higher quality, and more reliable for the long term. The X500R's high-sensitivity, high-resolution analysis and accurate mass-to-charge ratio analysis, combined with the intelligent TOF-MS-IDA-MS/MS acquisition mode, truly achieve the goal of collection of high-quality, accurate primary and secondary mass spectrometry data by single injection, and quickly provide the most accurate qualitative screening results.

SCIEX X500R QTOF mass spectrometry system with ExionLC™ liquid chromatography system and SCIEX OS workstation

Study Design

1. Samples of Polygala herbs from different sources were obtained and assigned to groups, each containing 6 samples.
2. TOF-IDA-MS/MS mode was used for data acquisition; one injection allowed simultaneous collection of various components' primary ions and secondary daughter ions.
3. The MarkerView™ Software was used to analyze differences in components and identify statistically significant differences between groups for use as markers.
4. After entering mass spectrometry data on primary ions and secondary daughter ions into SCIEX OS Software, the components were matched with the SCIEX high resolution MS/MS Chinese medicine database or the ChemSpider online database; differences in components were identified.

Contents

Study Design Workflow

Materials and Methods

This study collected Polygala herbs from 4 regions: Chengcheng, Shaanxi; Shangluo, Shaanxi; Shanxi; and Hebei. After the samples were dried, they were cut into small pieces and dried in the oven at 40 degrees C for 18 h. After removal, they were crushed and filtered through a 20 mesh sieve, placed in the dryer, and then used.

Sample Preparation

Carefully weigh out about 1.0 g of Polygala powder of consistent weight, add 50 mL of 70% methanol aqueous solution, ultrasonicate 30 min., centrifuge for 10 min at 13000 rpm, and take the supernatant for injection.

Chromatographic Conditions

Chromatographic Column: Phenomenex Kinetex F5, 100*3.0 Mobile phase: A is ultrapure water/B is acetonitrile;

Gradient elution was performed as shown below:

Time (min)	A%	B%
0.0	95	5
5.0	90	10
15.0	85	15
20.0	80	20
25.0	75	25
30.0	70	30
35.0	65	35
40.0	10	90
45.0	10	90
45.1	90	5
50.0	90	5

Flow rate: 0.4 mL/min ;

Column temperature: 40℃ ;

Amount inserted: 5 µL

Mass Spectrometry Method

Scanning method: TOF-IDA MS/MS qualitative screening;
Ion source: ESI source
Mass spectrum parameters are established in 4 steps:

Chromatogram

Typical ion base peak chromatograms (BPC) for four groups of polygala samples from different sources; see fig. 1 below:

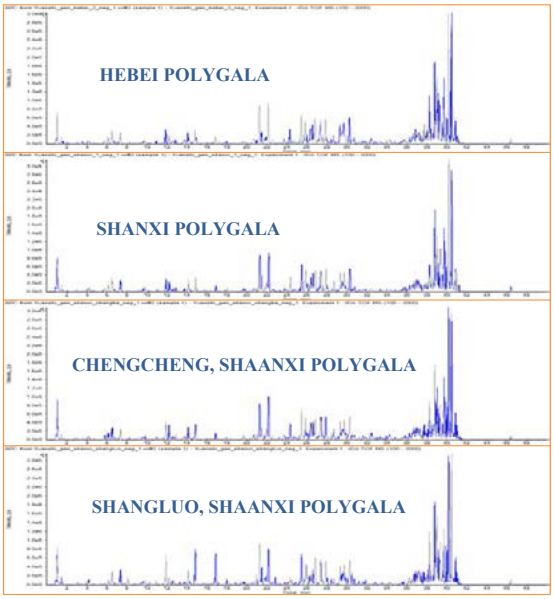


Figure 1. Typical BPC for four polygala samples from different sources

Chromatographic peak retention reproducibility was very good among the four Polygala samples from different sources. Many baseline analysis separation peaks were obtained on the BPC, showing good chromatographic separation.

Contents

MarkerView™ Data Processing

The MarkerView™ Software was used for preliminary data extraction of chromatographic peaks. Identification and integration were performed on chromatographic peaks with a retention time of 0 - 50 min; the three-dimensional data was transformed into a two-dimensional data matrix, including variables (*m/z*_RT), number observed (24 samples), and the integral area. This study found 994 variables (*m/z*_RT).

PCA-DA processing and Library Database Search

All samples underwent supervised PCA analysis, and their Score and Loading chart is as shown in Fig.2:

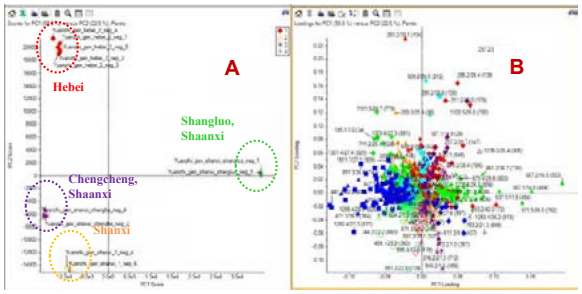


Figure 2 A) Polygala samples from different sources, PCA Score Plot; B) Polygala samples from different sources, PCA Loading Plot;

Fig. 2 Score Plot shows Polygala samples from the 4 different areas are well separated, meaning that there are large differences between groups.

Using Polygala products sourced from different areas, take m/z 667.2 (RT=16.9 min) as an example. For *m/z* 667.2 in the figure below, showing content differences in samples from different areas, the line plot shows that Chengluo, Shaanxi Polygala has a Tenuifoliside B2 content that is approximately 5 times that of the 3 other areas, as in Fig. 3:

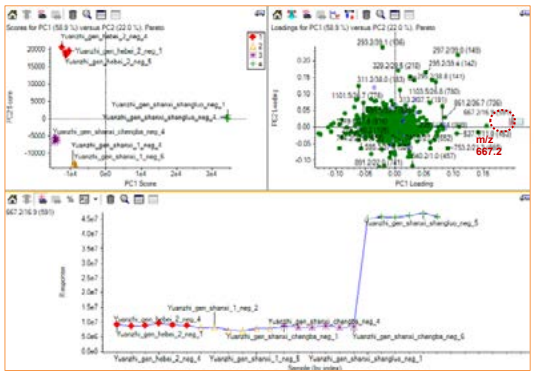


Figure 3 Polygala Marker: *m/z* (667.2), (RT=16.9 min)

Polygala characteristic marker *m/z* 667.2, retention time 16.9 min, SCIEX OS identification of the marker is: Tenuifoliside B2, $C_{30}H_{36}O_{17}$, *m/z* (MS)= 667.1875, *m/z* (MS/MS) = 461.1288, 367.1035, 239.0557, 205.0498, 190.0265. Using Library search, identification results in
Fig. 4-1 shows that secondary fragment matching is good, with the main fragment structural analysis shown in Fig. 4-2:

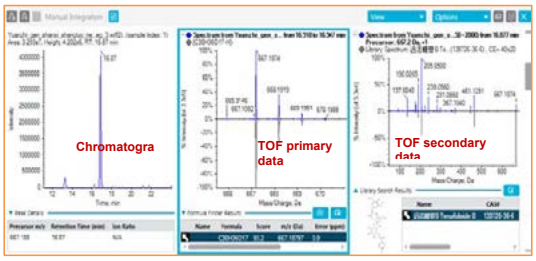


Figure 4-1. Polygala Marker m/z 667.2 via SCIEX OS structural attribution results

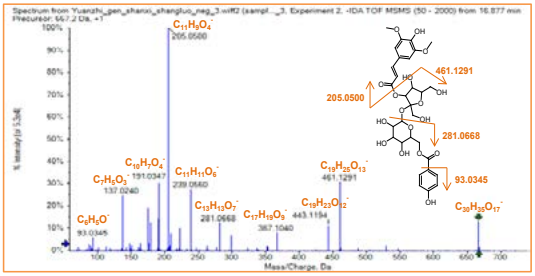


Figure 4-2. Polygala Marker m/z 667.2 secondary fragment attribution and main fragment structural analysis

T-test data processing

All samples underwent T-test data processing; results are in Fig. 5. Fig. A is the volcano plot, expressed as log fold change vs. p-value; as the X axis is approached, more ions are located at both ends of the X axis, indicating a greater difference between them. Fig. B is a line plot, and Fig. C is a box plot, showing the content relationships between the samples.

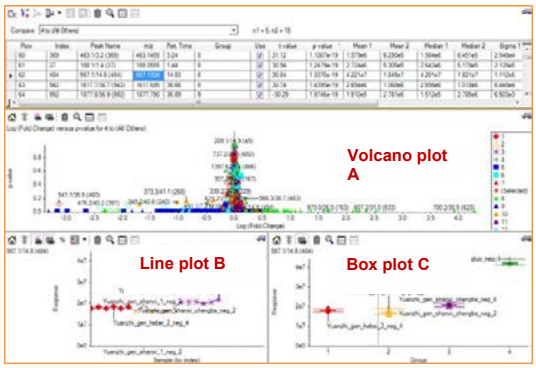


Figure 5 Log (Fold Change) versus p-values data processing

T-experimentally ($p < 0.005$) differentiated ion scans appear in line plot B and box plot C. Compound *m/z* 567.1 (RT 14.8 min) is significantly different in the Shangluo, Shaanxi Polygala, so it is used as a marker. Its structure is identified with SCIEX OS software's ChemSpider online structural identification for markers. Results are in Fig. 6:

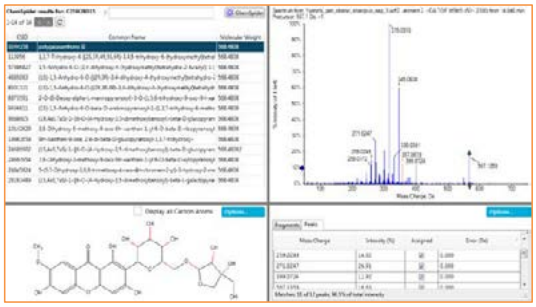


Figure 6-1. Polygala Marker: m/z (567.1), (RT 14.8 min)

Marker identified as: Polygalaxanthone III, $C_{25}H_{28}O_{15}$, *m/z* (MS)= 567.1359, *m/z* (MS/MS) = 345.0608, 315.0510, 399.0724, 271.0247; its online secondary fragment matching is good.

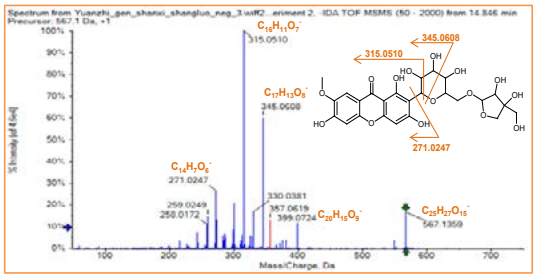


Figure 6-2. Polygala Marker m/z 567.1 secondary fragment attribution and main fragment structure analysis

SCIEX OS compound structural identification process

Using the SCIEX OS Formula Finder function, based on this ion's primary mass spectrum exact mass number and isotope ratio, the likely molecular formula was identified. At the same time, mass spectrometry fragmentation patterns and the ion's secondary mass spectrum mass number verified the molecular formula.

Using Polygala products sourced from different areas, take m/z 1379.4083 (RT 30.22 min) as an example. With the Formula Finder function, based on an exact mass number and isotope distribution, the molecular formula was determined to be $C_{62}H_{76}O_{35}$. Its TOF MS mass deviation was -0.8 ppm, and 17 TOF MS/MS fragments' mean mass deviation was 0.9 ppm. Results are shown in Fig. 7:

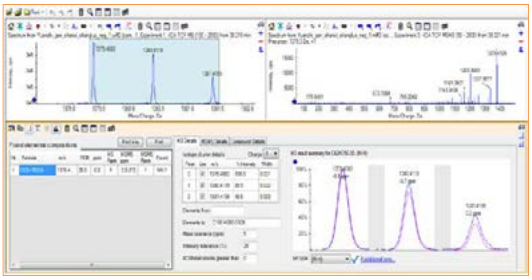


Figure 7. Polygala Marker m/z (1379.4083) molecular formula calculated with Formula Finder

The MS/MS fragmentation molecular formula is shown with green dots; see Fig. 8 for the fragmentation molecular formula, which is consistent with the mass spectrum fragmentation pattern.

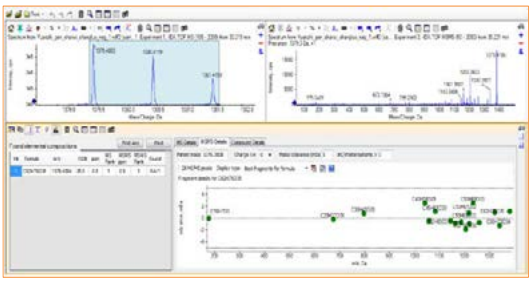


Figure 8. Polygala Marker m/z (1379.4083) secondary mass spectrum element component fitting

Contents

ChemSpider online structural identification, an additional verification of the structure, identified this marker as: Tenuifoliose A2, $C_{62}H_{76}O_{35}$, *m/z* (MS)= 1379.4083, *m/z* (MS/MS) = 1203.3603,1337.3977, 1161.3507, 1143.3406, 795.2362, 175.0401. Results are shown in Fig. 9.

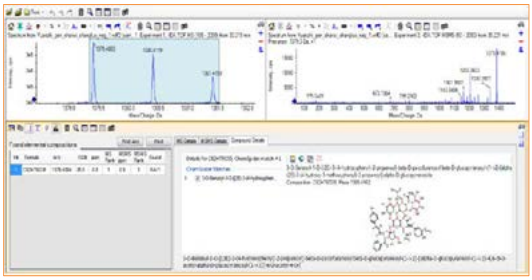


Figure 9-1. Polygala Marker m/z (1379.4083)

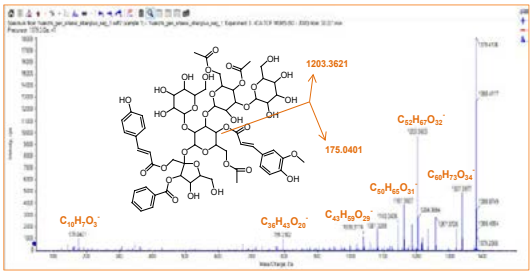


Figure 9-2. Polygala Marker m/z (1379.4083) secondary fragment attribution and main fragment structure analysis

Data Processing Results

Loading results and T-experimental results are combined to find the differentiated ions and the exact mass number and retention time of the potential biomarker are shown in Table 1.

This study identified 11 signature markers in Polyagala from different areas; of these, No. 1-2 are sucrose esters, No. 3–8 are oligosaccharides, No. 9-10 are triterpenoid saponins, No. 11 is xanthone. A summary is shown in Table 1-1. Table 1-2 shows the content differences in the 11 signature markers.

Table 1-1: Summary of signature ions of 4 Polygala samples from different sources

No.	m/z (MS)	RT (min)	Ion	Formula	Mass error (ppm)	Tentative identification	Major fragment ion (MS/MS)
1	667.1874	16.9	[M-H]⁻	C30H36O17	-0.8	Tenuifoliside B2	461.1291, 367.1040, 239.0560, 205.0500, 190.0265, 137.0240
2	753.2243	17.24	[M-H]⁻	C34H42O19	-0.7	3, 6'-Disinapoyl sucros	547.1647, 529.1542, 367.1024, 223.0602, 205.0507, 190.0263
3	1223.3681	24.7	[M-H]⁻	C55H68O31	0.8	Tenuifoliose S	1077.3292, 1059.3173, 955.2944, 931.2927, 753.2247
4	1233.3725	34.8	[M-H]⁻	C53H70O33	-0.1	Tenuifoliose V	1057.3263, 1015.3101, 839.2826, 465.2248
5	1265.3776	27.3	[M-H]⁻	C57H70O32	-0.1	Tenuifoliose K	1119.3386, 1101.3335, 1077.3305, 997.3054, 145.0299
6	1379.4083	30.22	[M-H]⁻	C62H76O35	-0.8	Tenuifoliose A2	1203.3603,1337.3977, 1161.3507, 1143.3406, 795.2362, 175.0401
7	1495.4586	25.79	[M-H]⁻	C67H84O38	0.6	Tenuifoliose L	1349.3947, 1307.3890, 1203.3589, 1161.3475, 1143.3385, 795.2381
8	1525.4692	25.84	[M-H]⁻	C68H86O39	0.5	Tenuifoliose F	1379.4050, 1337.4001, 1203.3599, 1185.3515, 1161.3516, 795.2363
9	1571.6874	36.87	[M-H]⁻	C75H112O35	-0.5	Onjisaponin B	1566.3539, 1542.6812, 1347.6365, 567.1939, 425.3044
10	1469.6571	37.06	[M-H]⁻	C71H106O32	-0.8	Onjisaponin Z	1439.6490, 1245.6042, 455.3163, 405.1402, 237.0766
11	567.1359	14.85	[M-H]⁻	C25H28O15	-0.7	Polygalaxanthone III	399.0724, 345.0608, 315.0510, 271.0247

No.	Compound	Response			
		Chengcheng of Shaanxi	Shangluo of Shaanxi	Shanxi	Hebei
1	Tenuifoliside B2	1	5	1	1
2	3, 6'-Disinapoyl sucrose	1	5	1	1
3	Tenuifoliose S	4	1	4	4
4	Tenuifoliose V	4	1	4	4
5	Tenuifoliose K	10	1	10	10
6	Tenuifoliose A2	1	6	1	1
7	Tenuifoliose L	4	1	4	4
8	Tenuifoliose F	8	1	8	8
9	Onjisaponin B	1	4	1	1
10	Onjisaponin Z	10	1	10	10
11	Polygalaxanthone III	1	3	1	1

Note: Numbers within the table show only the fold-relationship of the same compound (e.g., Shangluo, Shaanxi Tenuifoliside B2 content is 5 times that of Chengcheng, Shaanxi; Shanxi; and Hebei).

Experimental Conclusion

TheX500R high-resolution LC system was used to analyze 24 Polygala samples, and grouped analysis found 11 signature markers responsible for sample variation. These included 2 sucrose esters, 6 oligosaccharides, 2 triterpenoid saponins, and 1 xanthone.

Analysis of the differences among the 11 markers revealed that the content of Shangluo, Shaanxi Polygala is clearly different from that of other locations. Main component analysis software MarkerView™ helped to differentiate between Polygala samples, for example using the 11 signatures that differentiate Shangluo, Shaanxi Polygala from that of the 4 other locations.

Contents

Summary

This study showed the value of applying the X500R high resolution LC system to Chinese medicine component analysis. It obtained high resolution spectrometric data (TOF-MS and TOF-MS/MS) and delivered high efficiency, rapid, integrated solutions, giving users stronger data to support their Chinese medicinal component identification. The main technical features of this method are as follows:

5. SCIEX OS is a comprehensive software package for data acquisition and analysis. Its simple data acquisition and processing features avoid the need for tedious switching between multiple software packages.
6. SCIEX OS software brings together simpler analytic methods; following automated calculation of a molecular formula, it can be matched to items in the SCIEX high resolution Chinese medicine MS/MS database, or the structure may be deduced using ChemSpider. Secondary fragment analysis can be performed to dramatically reduce time and effort laboratory personnel must invest to identify compounds.
7. Data entry and PCA analysis in MarkerView™ software is rapid. T-experimental statistical analysis enables users to quickly identify differentiated ions among groups and alleviates the difficulty of analyzing massive data sets. The X500R QTOF is an important tool for origin analysis and group analysis of Chinese medicines.

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2. Chinese pharmacopeia [S]. Part 1. 2010.
3. Hu Shilin. Authentic Chinese Herbs [M]. Haerbin: Heilongjiang Science and Technology Publishing. 1989

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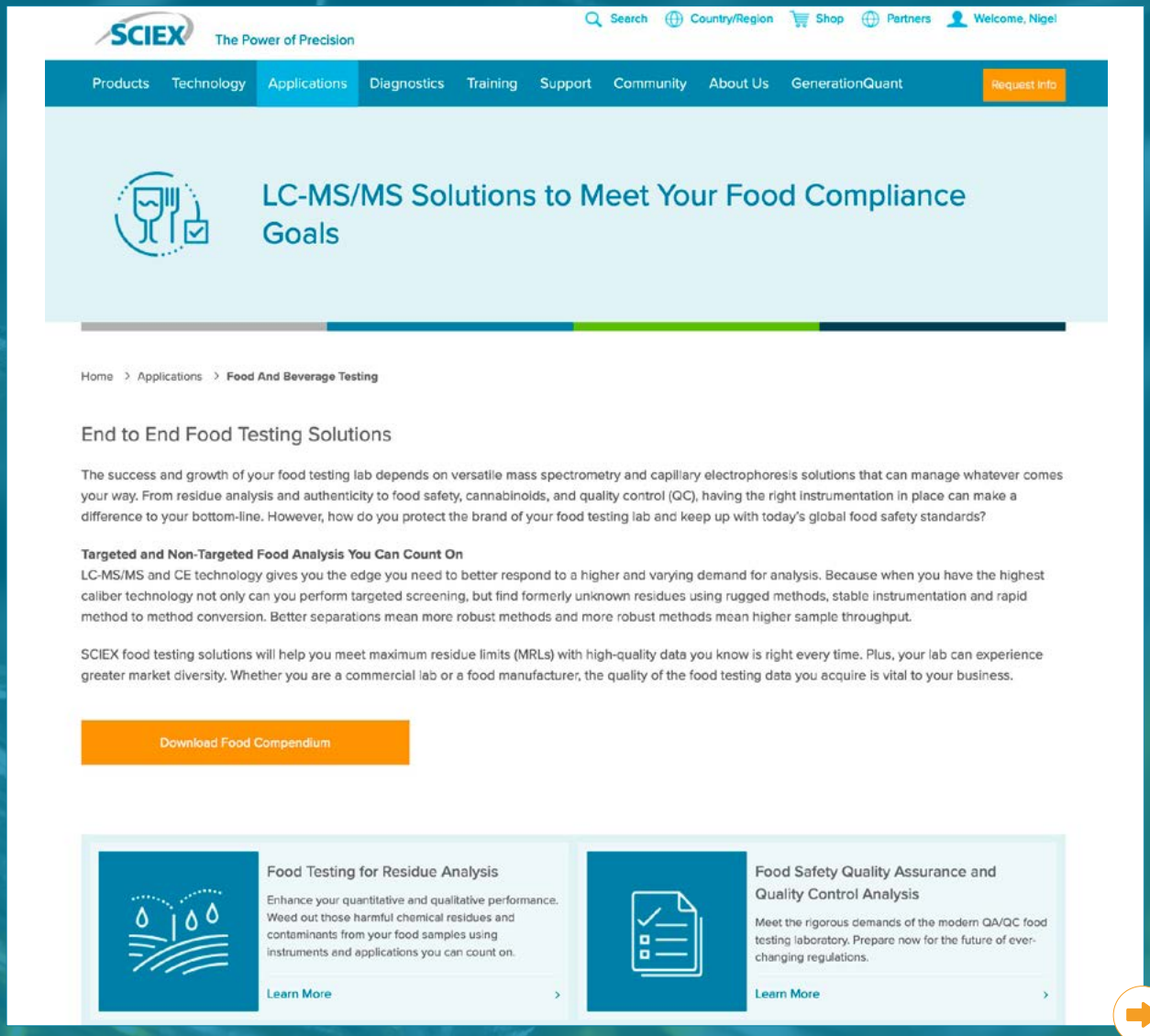

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Headquarters
500 Old Connecticut Path
Framingham, MA 01701 USA
Phone 508-383-7700
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