SCIEX
The Power of Precision

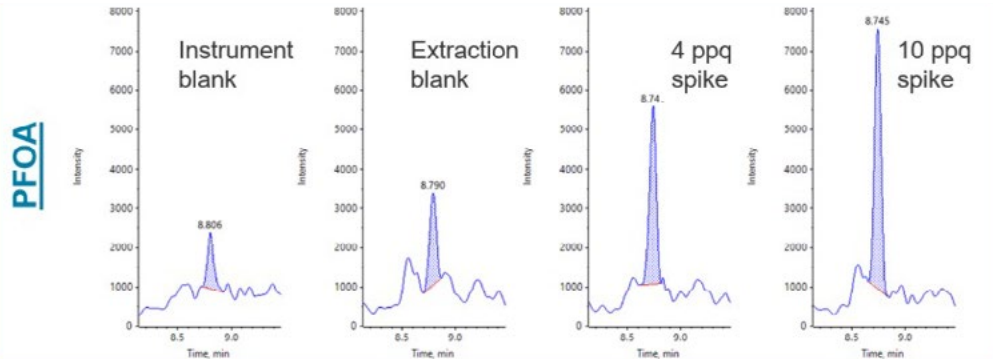
Food testing:
the LC-MS/MS collection

Contents

Introduction

By Gitte Barknowitz,
Global Market Development Manager,
Food and Environmental Testing

Food has a profound effect on our health, development, and overall wellbeing as well as on that of generations to come. A realistic healthy diet nowadays is nutritious and low in potentially harmful chemicals that can enter the food chain in various stages of production like farming, preserving, and packaging. With a growing global population, we face pressure on food supply chains and the need for automation and processing in food production. Food producers make use of potentially harmful crop-, and livestock-protection chemicals to enhance efficiency. Preservation with food additives and packaging can add further harmful substances to our diet. Laboratory food testing ensures our diets meet the standards governments have set. Food testing laboratories rely on high performance technology to meet requirements for low level detection of residues like pesticides, natural toxins, or veterinary drugs to keep food safe. Scientists conducting these analyses need robust technology, but they also need software that can adhere to the growing challenges of a modern laboratory as well as best-in-class service and application support to keep their laboratory running efficiently...



Eurofins collaborated with SCIEX to deliver 4ppq detection levels of PFOA on the 7500 system

Go to Eurofins webpage →

Go to technical note →

In recent years we have seen a shift in conventional food production and growing interest in producing food in more sustainable ways. Vertical farming and alternative protein production are focus areas to reduce water, land and chemical use and improve animal welfare and ecological footprint. The production of animal protein like meat, seafood, or dairy from cells rather than from animals poses an array of research challenges to the manufacturers from who benefit from the use of mass spectrometry to characterize new products. As government legislations for these novel foods are coming into place, food produced with innovative technologies will have to meet safety criteria just as conventional food does.

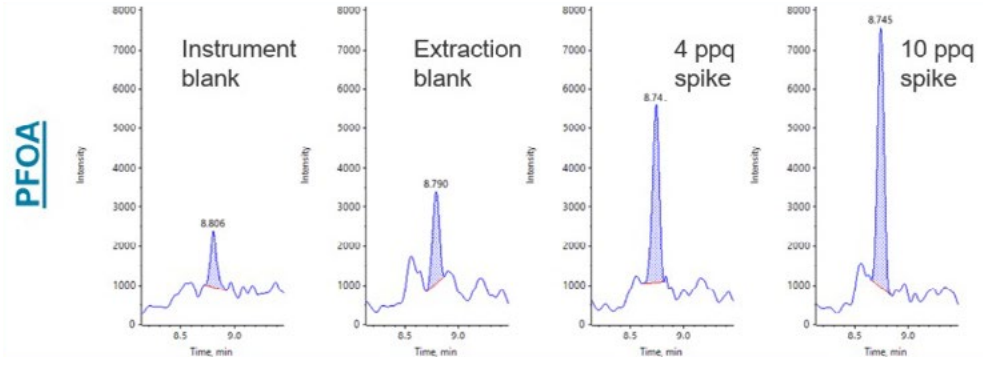
Evolving food production and testing pose region-specific challenges that will need to be overcome together to make our food safer and 'greener'. Whatever your main food-related application area might be: SCIEX' experts around the globe have developed a deep understanding of the food landscape through customer collaboration and can offer comprehensive support in market trend discussions and applications to future-proof your laboratory.

German translation on next page >

Einführung

By Gitte Barknowitz,
Global Market Development Manager,
Food and Environmental Testing

Lebensmittel haben umfassenden Einfluss auf unsere Gesundheit, Entwicklung, und die von zukünftigen Generationen. Eine realistische, gesunde Ernährung ist reich an wichtigen Nährstoffen und hat niedrige Gehalte schädlicher Stoffe, die in verschiedenen Stadien in die Nahrungskette eingebracht werden können, wie beim Anbau, der Prozessiereung oder Verpackung. Mit einer wachsenden Weltbevölkerung sehen wir uns mit zunehmendem Druck auf die Nahrungsmittelversorgung konfrontiert. Das erfordert Automatisierungund industrielle Verarbeitung und Haltbarmachung um der Nachfrage gerecht zu werden. Produzenten von Lebensmitteln nutzen oft Pestizide oder veterinärmedizinische Produkte um Effektivitätssteigerungen zu erzielen.Laboranalysen stellen sicher, dass unsere Nahrung den jeweiligen gesetzlichen Richtlinien der Länder entspricht und Konzentrationen von Chemikalien sehr niedrig gehalten werden. Lebensmittelanalyselabore sind auf hochsensitive Geräte angewiesen um geringe Spuren von Pestiziden, natürlichen Toxinen oder veterinärmedizinischen Arzneistoffen nachweisen zu können.



Eurofins collaborated with SCIEX to deliver 4ppq detection levels of PFOA on the 7500 system

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Wissenschaftler, die diese Analysen durchführen, benötigen neben verlässlichen Technologien aber auch Software, exzellente Wartung und Applikationsunterstützung. In den letzten Jahren haben wir Trends in der Nahrungsmittelproduktion hin zu nachhaltigeren Lösungen beobachten können. "Vertical Farming" und alternative Proteinproduktion wie "Cellular Agriculture" reduzieren die Nutzung von Wasser, Land und Chemikalien und verbessern damit sowohl das Tierwohl als auch den ökologischen Fußabdruck. Die Produktion von tierischem Protein wie Fleisch, Fisch und Meeresfrüchten oder Milchprodukten durch Zellen anstelle von lebenden Tieren stellt jedoch neue Herausforderungen an Produzenten. Massenspektrometrie bietet gute Möglichkeiten diese neuen Produkte zu charakterisieren. Lebensmittelrechtliche Anforderungen sind im Moment noch in der Entwicklung, aber es ist absehbar, dass neuartige

Lebenmittel den gleichen Sicherheitsanforderungen wie konventionelle Lebensmittel unterliegen werden. Veränderungen in der Nahrungsmittelproduktion und der –testung stellen regionalspezifische Anforderungen dar, die für Wissenschaftler in Zusammenarbeit überkommen werden müssen um unsere Nahrung sicherer und nachhaltiger zu machen. Was auch immer Ihr Applikationsgebiet ist: Die Experten von SCIEX haben global durch Zusammenarbeit mit Kunden ein tiefes Verständnis für die Branche entwickelt und teilen dieses gerne um Marktentwicklungen zu diskutieren und Ihr Labor für die Zukunft auszurichten.

SCIEX map

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.

Matt Noestheden
Market Development Manager, Americas

Daniel McMillian
Market Development Manager, EMEAI

Kai Uchiumi
Market Development Manager, Japan

Haiyan Cheng
Market Development Manager, China

See-Chung Yip
Market Development Manager, Southeast Asia

Charlie Liu
Applications Specialist and Market Development, Australia and New Zealand

Never miss a thing

Global trends in food analysis

Global trends in food analysis

Australia and New Zealand

By Charlie Liu,
Applications Specialist and Market Development,Australia and New Zealand

Across both Australia and New Zealand, there is expanding awareness of and desire for high-quality foods and foodstuffs that cater to specific diets, such as gluten-free products. Given the risks involved for consumers with dietary needs, accurate quantitation of potential food contaminants and other allergens is critical. Local demand for the high-quality products that Australia and New Zealand are known for exporting is also on the rise. This has led to increased interest in understanding the potential for contact contamination from food packaging and an uptick in inquiries about testing packaging in addition to food products.

SCIEX is helping to deliver methodologies that meet the food-safety needs of consumers and scientists in this region while providing the high-performance, robust instrumentation our customers have come to expect. The QTRAP and SCIEX Triple Quad systems, and the SCIEX Triple Quad 6500+ system in particular, are popular with food-testing scientists, and the ZenoTOF 7600 system brings non-targeted analysis and industry-leading sensitivity in accurate mass instrumentation that can

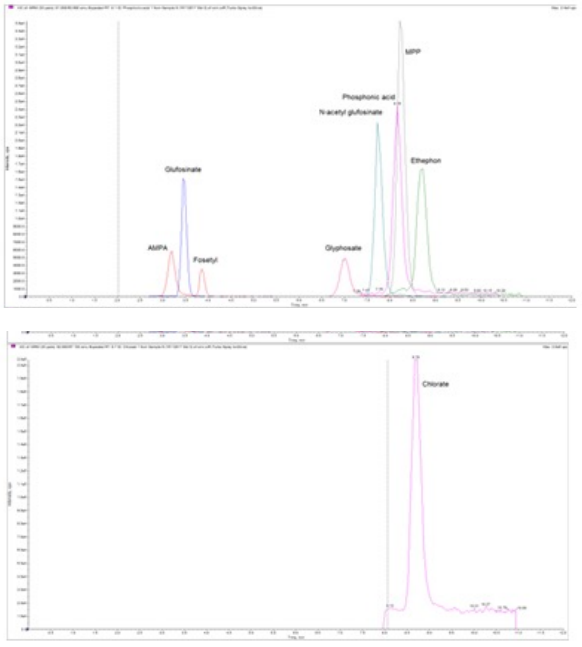
help any food-testing laboratory. The ZenoTOF 7600 system can also be useful in the global effort to meet net-zero commitments to cut greenhouse gas emissions. Many novel food development companies have made reducing the impact of animal agriculture on the planet, without compromising on nutrition and flavor, a central focus. The ZenoTOF 7600 system has demonstrated robust proteomic analysis and post-translational modification (PTM) characterization capabilities that can help with product development in support of achieving this goal.

Global trends in food analysis

EMEAI

By Dan McMillan,
Market Development Manager, EMEAI

Throughout the EMEAI region, food-safety testing is driven by regulations. While these regulations are often those defined by the European Commission, many countries outside of the EU set their own or follow the rules of other regulatory bodies, especially if they are exporting produce to the countries in which those regulations apply. Food-safety legislation is under constant review, and although maximum residue limits (MRLs) for contaminants are not regularly changed, the scope of products and analytes to which they apply is often extended. Currently, pesticide regulations specific to baby foods are undergoing significant modifications, from applying to a small subset of pesticides to encompassing all regulated compounds at low concentrations, which can pose challenges for testing laboratories. For example, polar pesticides are a major area of concern but are difficult to quantify due to their properties and related matrix effects. To detect these pesticides at the required low concentrations, laboratories need a way to perform robust, high-throughput analysis of complex matrices with minimal sample preparation.



Direct analysis of polar pesticides in food and environmental samples

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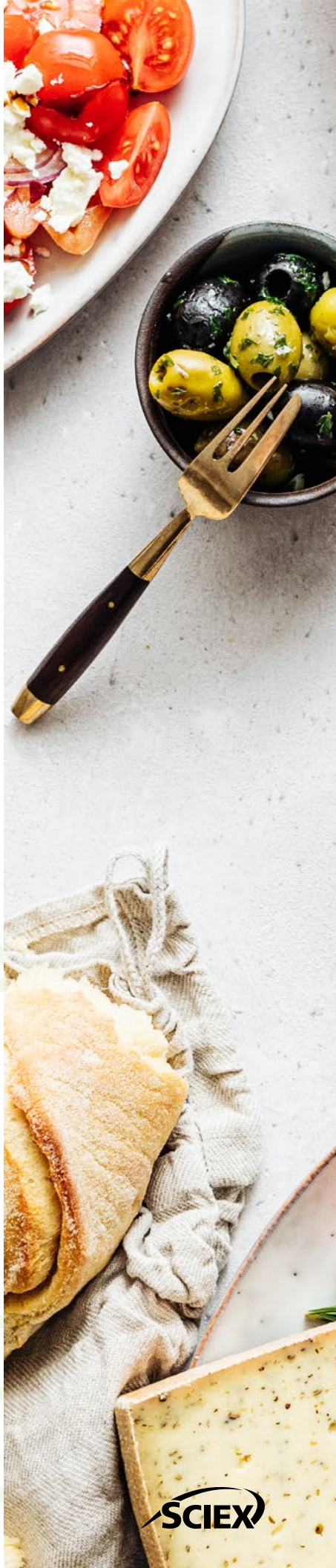
Global trends in food analysis

EMEAI, continued...

Another prominent area of concern is per- and polyfluoroalkyl substances (PFAS). While water and environmental researchers in Europe have monitored and analyzed PFAS for many years, the recent high level of worldwide focus on these chemicals and the growing understanding of their potential harmful effects have driven serious reconsideration of how they are regulated in food and consumer products. Several countries have proposed a total ban on PFAS, and many laboratories are looking to start testing for PFAS in food or expand their testing coverage in anticipation of strict regulations in the future. Again, challenging matrices combined with low required limits of quantitation (LOQs) are driving the need for ever higher-performing analytical instrumentation and methods.

Food legislation is also becoming a high priority for governments throughout the Middle East and Africa that are eager to ensure the health and safety of their citizens, leading to a rise in the need to detect traditional pesticides, mycotoxins and veterinary drug residues, along with newer compounds of concern such as alkaloids and allergens. Where high sample volumes are not required, sample preparation can be more efficient in achieving results using less advanced, routine instruments. However, to ensure laboratories in these regions can quickly and confidently meet demand, robust LC-MS/MS technologies and associated support from SCIEX, including verified methods with predefined SOPs, are essential.

India is another region where food legislation is important. While we see the same demands for food-safety testing in India that we see in the rest of the region, food authenticity has become an additional focus here. The enhanced capabilities of accurate mass and QTRAP systems can play a critical role in meeting various testing needs—such as ensuring high-value commodities like basmati rice are authentic and that honey is not adulterated with corn syrup—and making these analyses routine.



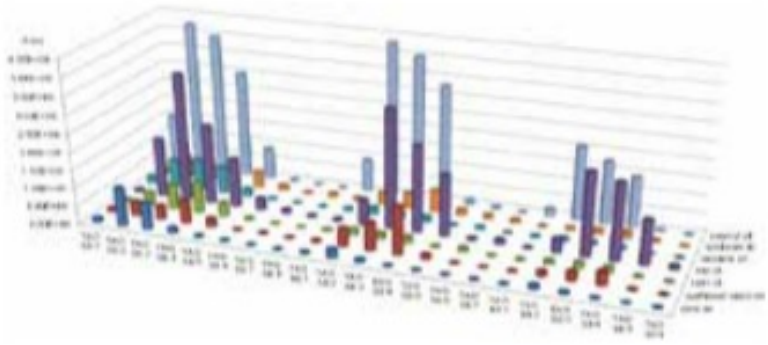
Global trends in food analysis

China

Haiyan Cheng,
Market Development Manager, China

Food testing and food research are the two major applications in the field of food safety in China, and mass spectrometry has a significant role to play in these areas—from safety to health, from farm to fork, from testing to research and from standards to solutions

In the field of food testing, the market in China is segmented according to national or industry standards. Monitoring and risk assessment focus on pesticide residues, veterinary drugs, food packaging, toxins and other contaminants. To help with these tasks, SCIEX collaborates with key thought leaders to develop standard methods for the simultaneous detection of multiple residues and challenging compounds. In addition, SCIEX provides overall solutions for meeting relevant national food standards and an industry application series to streamline residue testing, reduce costs, increase productivity and elevate food testing in China.



Discover how China is employing a holistic approach, 'Foodomics', for their food analysis

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The field of food research in China is focused on food nutrition and food metabolomics. Customers are paying increasing attention to traceability, fraud, identification of nutritional components and building nutritional databases. SCIEX is meeting these customer needs with established food metabolomics workflows, targeted nutritional databases and efficient data-processing methods in multiple metabolomics fields, such as lipidomics and proteomics. SCIEX also contributes to the field of food research by collaborating with customers to publish high-impact articles on topics such as specialty foods, 360-degree lipid analysis and targeted identification of comprehensive nutritional compounds. **Translation on next page 14 >**

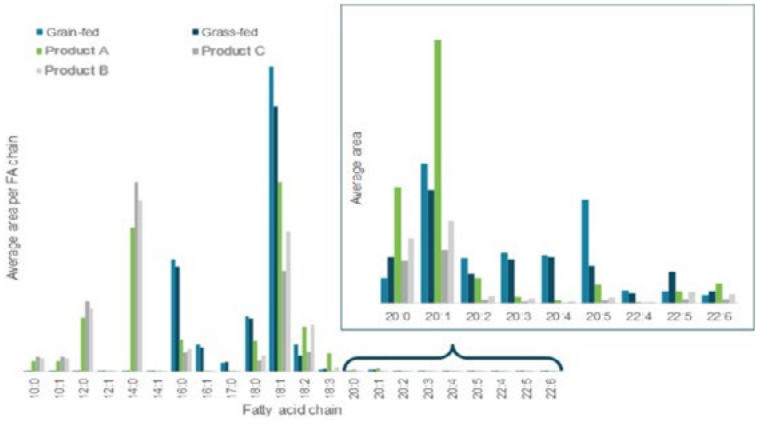
Global trends in food analysis

Japan

Kai Uchiumi,
Market Development Manager, Japan

Globalization has had a significant impact on food safety. International cooperation is essential to the import and export of food, and equally important is understanding and adhering to food safety regulations and recommendations that can vary across the globe.

One example of how regulatory approaches can differ is Japan. While the Japanese market is not large, it is unique. For instance, the QuEChERS method that is widely used around the world is not a national regulatory method in Japan—instead, this method is used in self-inspections. In addition, an accreditation system for testing laboratories already existed in Japan before the establishment of ISO/IEC 17025, so Japan has both the global laboratory accreditation and a national accreditation system in place. Private companies are also making efforts to comply with international regulations for compounds that have no domestic regulations. In recent years, the safety of novel foods—such as cultured meat, alternative proteins and insect-based food—and the detection of functional food ingredients have become hot topics, and Japan might take a unique approach to regulations in these areas in the future as well.



Japan is a leader in novel food and alternative protein. Discover how lipids can help in analysis of alternatively sourced meat

Go to technical note →

SCIEX offers support in all these areas. The robust triple quadrupole and QTRAP systems from SCIEX are useful for meeting the diverse demands of food safety, QTOF systems from SCIEX can help discover and identify functional ingredients and SCIEX OS software helps simplify data analysis and regulatory compliance.

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Global trends in food analysis

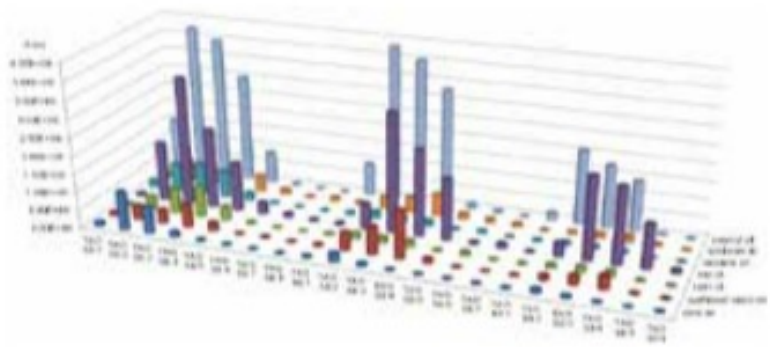
China

Haiyan Cheng,
Market Development Manager, China

食品检测和食品科研是中国食品领域的两大应用方向，聚焦检测，聚力科研，是中国本土市场的市场策略，从安全到健康，从农田到餐桌，从检测到科研，从标准到方案，让质谱改变每个人的生活。

在食品检测领域，细分检测市场，遵循细分行业的国标或者行业标准，进行农残、兽药、食品包材、毒素以及其它污染物的监测和风险评估。SCIEX致力于与重点客户合作开发标准方法，重点在多种物质同时检测以及疑难化合物的标准建立。除此之外，开发相关食品标准的整体解决方案，并形成行业应用文集，助力客户解决疑难物质检测，降本增效。

食品营养和食品组学是食品科研领域的研究重点，从安全到健康，客户越来越关注于食品溯源、食品打假、食品营养成分的鉴定、营养数据库的构建等，SCIEX在代谢组学、脂质组学、蛋白组学等多组学领域建立食品组学工作流程，靶向数据库的构建，以及高效数据处理方法。已与客户合作发表多篇高影响因子的文章，在特殊食品、360度脂类分析、拟靶向全方位营养物质鉴定等前沿领域，助力食品科研。在中国，为中国，服务本土客户，创升中国。



Discover how China is employing a holistic approach, 'Foodomics', for their food analysis

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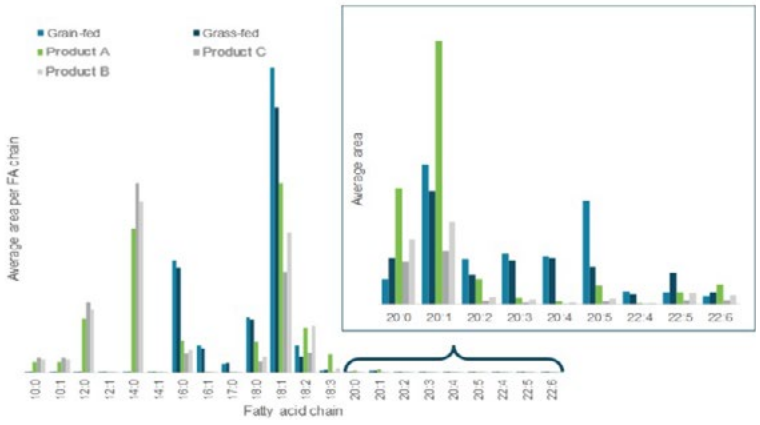
Global trends in food analysis

China & Japan

Kai Uchiumi,
Market Development Manager, Japan

グローバリゼーションは食の安全にも影響をもたらしています。食品の輸出入を考えた場合、国際社会との協調は不可欠であり、当然日本の規制にもその影響はあります。しかし、日本の市場は比較的小さい割にはユニークです。例えば、現在世界中で広く採用されているQuEChERS法は、日本国内の規制法では採用されていませんが、自主検査では使用されています。ISO/IEC 17025よりも以前から、独自の分析機関の認定制度が存在することから、この試験所認定と国内の認定制度の両方が存在する形になっています。また、国内に基準規制がない化合物では、国際的な規制に対応すべく企業努力がおこなわれているケースもあります。さらに、近年、培養肉、代替タンパク質、昆虫食などの新規食品が話題となっていますが、ここでも今後日本はユニークな方向へ進むかもしれません。また、食の安全だけではなく、食品の機能性成分も日本では関心を集めていいます。

食の安全についてはその多様な要求にSCIEXの頑健で高感度なTriple Quadあるいは QTRAP®が、機能性成分などの側面ではQTOFがその発見や同定に役立つでしょう。そしてSCIEX OSはデータ解析の簡略化や法規制の遵守に役立つでしょう。



Japan is a leader in novel food and alternative protein. Discover how lipids can help in analysis of alternatively sourced meat

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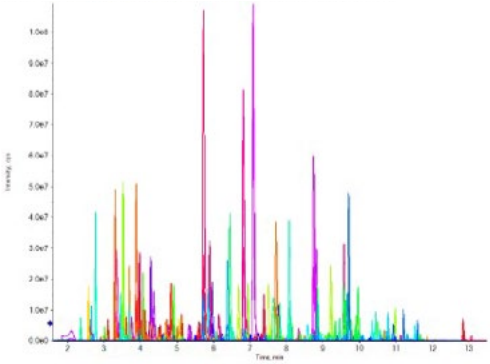
Global trends in food analysis

Americas

Matt Noestheden,
Market Development Manager, AMER

The infrastructure for food-safety testing in the Americas is built on the quantitation of a variety of chemical classes, with pesticides, veterinary drugs and mycotoxins as the major analytes of interest. In recent years, the scope of testing has expanded to include per- and polyfluoroalkyl substances (PFAS), food authenticity, allergens and process impurities such as acrylamide. Ingredient analysis is also a focus, as manufacturers of food products and dietary supplements, for example, need to verify the concentrations of key nutritional components and active ingredients in addition to meeting regulatory requirements for chemical contaminants.

As the maximum permissible concentrations of residual contaminants (typically in the low parts-per-billion range, but can reach the parts-per-trillion level) and the quantitation requirements for food ingredients continue to evolve, laboratories must be able to reliably quantify these disparate analyte classes—day-to-day, month-to-month and year-to-year. In addition, continued pressure for analytical efficiency has scientists seeking complete workflow solutions that can minimize per-sample turnaround time and cost, often by expanding the panel of analytes they can cover in a single analysis. To meet these demands, many laboratories have turned to liquid chromatography coupled with mass spectrometry, which offers the sensitivity, selectivity, throughput and robustness they need to be successful.



Everything from mycotoxins, pesticides, to veterinary drugs can be detected and analysed in a single run

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At SCIEX, we are committed to working with the food-testing community to support its critical role in consumer safety. We realize this commitment through customer consultations that inform how we bring our next-generation food workflows to market. The results of this commitment can be seen in the throughput and uptime made possible by the sensitivity of the SCIEX 7500 system, the confidence in unknown identification enabled by using the ZenoTOF 7600 system and the processing efficiencies that come from utilizing SCIEX OS software.

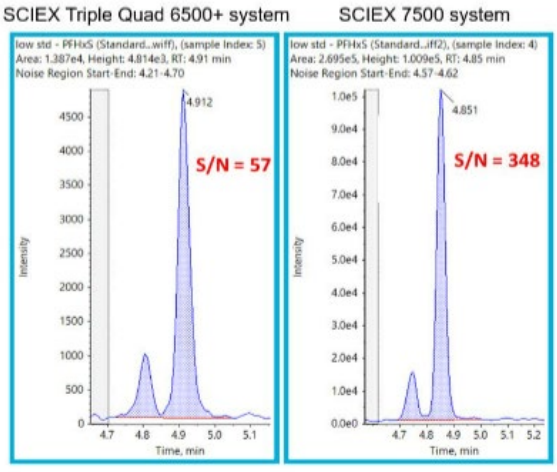
Global trends in food analysis

Southeast Asia

By See-Chung Yip,
Market Development Manager, Southeast Asia

As of June 2022, Singapore is the first and only country in the world to have approved cultivated meat for sale. This approval is based on guidance published (initially in 2019) by the Singapore Food Agency (SFA) that details the requirements for the safety assessment of novel foods, including specific requirements for the information that must be submitted for approval of cultivated meat and fermentation-enabled products.

In recent years, many new novel food manufacturers have emerged in the Southeast Asia market. Leading mass spectrometry solutions from SCIEX can help regulatory labs, research labs and novel food manufacturers adhere to regulations related to novel foods, such as the SFA guidance, by enabling testing for allergens, cell culture media and nutrition.



The SCIEX 7500 system data (right) shows a 7.1x increase in S/N compared with the SCIEX Triple Quad 6500+ system data (left), for perfluorohexanesulfonic acid (PFHxS)

Go to technical note →

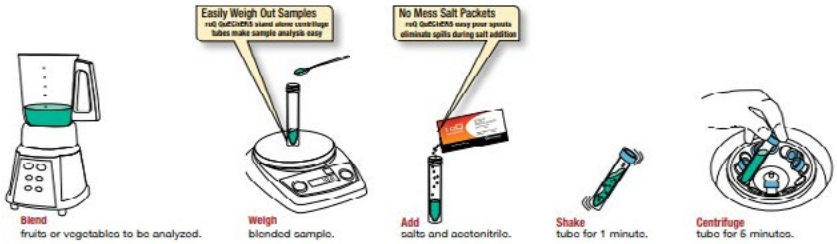
While per- and polyfluoroalkyl substances (PFAS) have been manufactured for over 80 years, their effects on human and environmental health were poorly understood for a long time. In September 2020, the European Food Safety Authority (EFSA) published a new health risk assessment related to PFAS in food, and in October 2022, the first annual AOAC Southeast Asia Section Meeting took place with a notable emphasis on testing for PFAS in food. Since then, regulatory laboratories across the Southeast Asia region have taken a keen interest in PFAS testing, and they are turning to SCIEX for its expertise in this area.

Consumables for food testing

Richard Jack,
Market Development Manager, Phenomenex

Food is a lifeline of our society. At Phenomenex, a primary focus is supporting our local and global food safety and quality organizations with tools and resources to help ensure that food is safe and food labels are accurate. Phenomenex is committed to supporting industry needs through a comprehensive product portfolio of sample preparation products, HPLC/UHPLC columns, LC-MS columns and GC-MS columns and accessories, along with application and method development services for meeting food industry guidelines.

The full portfolio of sample preparation products and filters provided by Phenomenex—including roQ QuEChERS, Strata SPE and Phenex and Claricep filters for extraction and removal of particulates to sub-micron levels—play a critical role in preventing the injection of any drug, or dirty sample into an HPLC, GC or mass spectrometer. This helps ensure low-level detection and keeps columns and systems up and running to maintain robust analysis capabilities.



Discover the various sample preparation options from Phenomenex

Our wide variety of column technologies provide coverage for a full range of particle sizes—from core shell to microporous particles—for complementary and orthogonal selectivity. For example, with our Kinetex EVO C18 columns, you can achieve lower back pressure and similar or better performance with three options: keep the lower pressure for less system strain, increase the flow for higher productivity or utilize a longer column length to increase potential resolving power. Our Luna column is a leading reversed-phase column because it provides measurable improvement over many HPLC columns for two important chromatographic properties: resolution and peak shape. The high efficiencies and bonded-phase surface coverage provide for sharp peaks.

With over 30 years of experience with developing products in collaboration with food manufacturers and global regulatory bodies and tailoring solutions to emerging MS technologies, from MS/MS to HRMS, at Phenomenex we know that success in food safety relies on working together as partners.

Our internal experts

Craig Butt,
Applied Markets Manager,
Global Technical Marketing

The Future of Food Analysis. Expanding analyte list demands.

In the next several years, analyte panels for food testing will continue growing, putting pressure on both the instrumentation as well as analytical chemists to adapt. For example, the evolution of the pesticide industry may introduce new compound classes or chemistries not previously seen. Alternatively, chemicals that were previously thought of being "safe" may come under new regulatory spotlight. Efficiency and productivity targets will drive the desire to analyze these new chemicals within existing methods, ultimately building towards so-called "mega" methods. Sample preparation methods must be universal to ensure good analyte coverage while minimizing matrix effects and maintaining data quality. Further, LC and mass spectrometry methods will need to adapt to the increased analyte demands.

How can mass spectrometry help? Improved instrument sensitivity inherently allows for lower detection limits. In addition, greater instrument sensitivity allows for more innovative solutions to our customer's problems. For example, the ability to dilute out matrix interferences to improve data quality or to simplify sample preparation methods for greater analyte coverage. Non-target analysis expands analyte coverage, improves confidence.

The upcoming years will also witness the continued adoption of accurate mass spectrometry workflows for routine food analysis. Non-target acquisition workflows provide additional lines of evidence – and therefore greater confidence – for the detection and identification of food contaminants. These include precursor and fragment mass accuracy, as well as MS/MS library matching. High-end QTOF instruments are sensitive enough to allow for routine food testing. In addition, the greater specificity helps to resolve complex matrix interferences, leading to greater data quality. Further, accurate mass workflows can be used to evaluate food authenticity such as honey and spices.

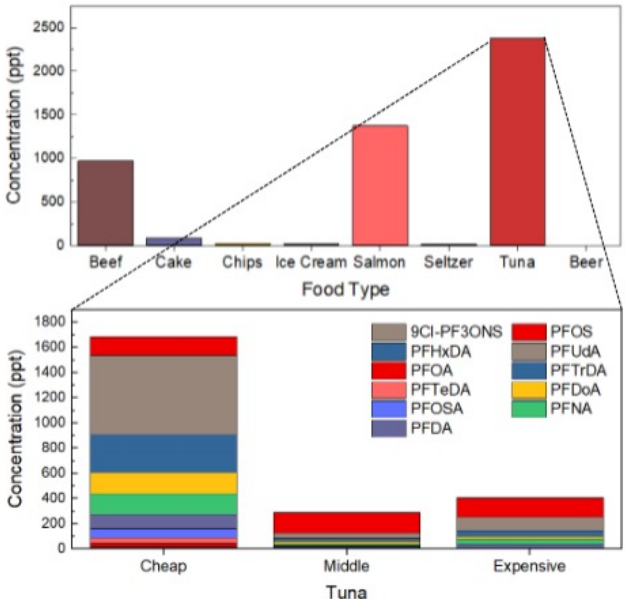
Watch fireside chat videos →

Our internal experts

Holly Lee,
Food LC-MS Scientist, Global Technical Marketing

"Food is essential to life, so make it good."

As a food LC-MS scientist, my definition of "good" is layered, and it extends beyond typical sensory characteristics—such as appearance, aroma, taste and texture—to less perceivable traits, such as nutrition, safety and authenticity. The importance of a sustainable and healthy diet cannot be overstated. We face malnutrition issues and the burden of feeding a global population of 8 billion people and growing amidst declining natural and environmental resources. New and alternative methods are being actively researched in the agri-food sector to increase the productivity of urban food production and develop novel foods from alternative protein sources.



Holly shows us how PFAS detection in food matrices is performed

Cellular agriculture, precision fermentation and plant molecular farming all rely on animal-free sources to produce recombinant proteins, which in turn are either used as ingredients themselves or ultimately transformed into meat, seafood or dairy products. Given that the application of these technologies in food production is still largely in the research and development phase, questions concerning the safety and hazard profiles of these novel foods and their production processes must be addressed by regulatory agencies, food safety testing labs and food companies, many of which have turned to mass spectrometry (MS) as a solution.

Our internal experts

Holly Lee continued...

MS is widely regarded as the gold standard for food safety testing to ensure that chemicals such as pesticides, veterinary drugs, natural toxins and additives and preservatives in food and food packaging are compliant with regulatory limits. In addition to testing for these known hazards, the use of MS in foodomics applications is gaining ground. This is especially true for detecting unexpected and potentially deleterious chemicals from adulteration and transformation products derived from the production, storage and processing conditions for both conventional and novel foods. MS-based metabolomics, lipidomics and proteomics will be critical for profiling the differences in nutritional components, additives, contaminants and allergens between conventional and novel foods.

The identification of novel biomarkers to detect new allergens coming from alternative protein sources and to trace the origin of protein sources will help regulators and food producers enhance consumer confidence in the safety and authenticity of novel food products. We must also widen our lens to look beyond North America and Europe as the center of gravity in future food production and look toward the Asia-Pacific region, where Singapore has become the first country to legally approve lab-cultivated chicken meat for consumption.

Detecting food adulterants in China

Jiukai Zhang, Ph.D
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At CAIQ, we focus on food authentication studies, such as species identification, origin traceability, and quality identification, that are based on mass spectrometry. In China, the most common adulteration practice is species substitution, especially in high value-added food and supplements. For example, edible bird's nest - an expensive, traditional Chinese ingredient harvested from swiftlet nests - is commonly substituted with cheaper materials such as egg white. Mixing berry fruit juice with inexpensive substitutes is also a common practice.

"The major challenge in our work is that you never know what will be added next, and targeted analysis of just a few constituents or even a single ingredient is very insufficient for food authenticity."

We have found that authentication technologies are often playing catch-up to adulteration techniques. The major challenge in our work is that you never know what will be added next, and targeted analysis of just a few constituents, or even a single ingredient, is very insufficient for food authenticity. The SCIEX TripleTOF 6600 allows us to carry out high-throughput metabolomics and proteomics studies for food authentication. In addition, the omics analysis software, such as MarkerView software, ProteinPilot software, and UpidView software are used for database searching and data processing. The global food trading market makes the food chain longer than ever before, increasing novel ways of adulteration. With this high-resolution mass spectrometry (HRMS) set-up, we can achieve large-scale analysis of numerous targeted or non-targeted characteristic markers in food samples.

Products

SCIEX OS software

By April Quinn-Paquet,
Global Product Marketing Manager,
SCIEX OS software

Mass spectrometry has become the gold standard solution for quantitative analytical assays in many applications, from food testing to pharmaceutical drug research and development. Software plays a key role in all these applications, because without it, we can't drive the instrument or process the data it generates.

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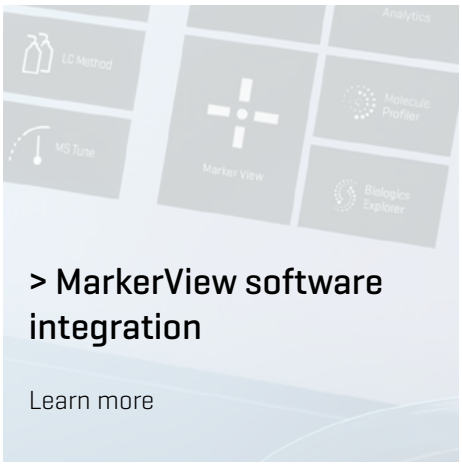

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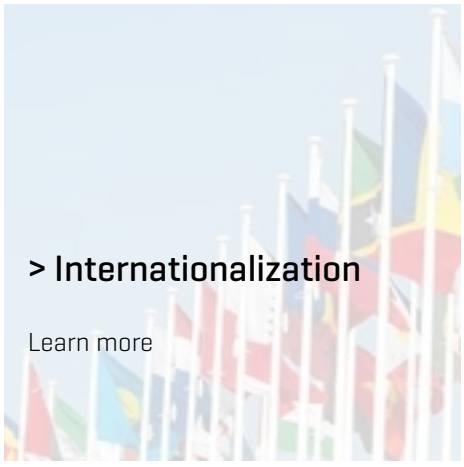

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Precise testing of pesticides in food using the SCIEX Triple Quad™ 7500 LC-MS/MS System – QTRAP® Ready

Highly sensitive analysis of multi-compound panels in various matrices for food regulations

Jianru Stahl-Zeng1, Ian Moore2, Thomas Biesenthal2, Jack Steed3, Wim Broer4 1SCIEX, Germany; 2SCIEX, Canada;
3 SCIEX, UK, 4Nofagroup, The Netherlands

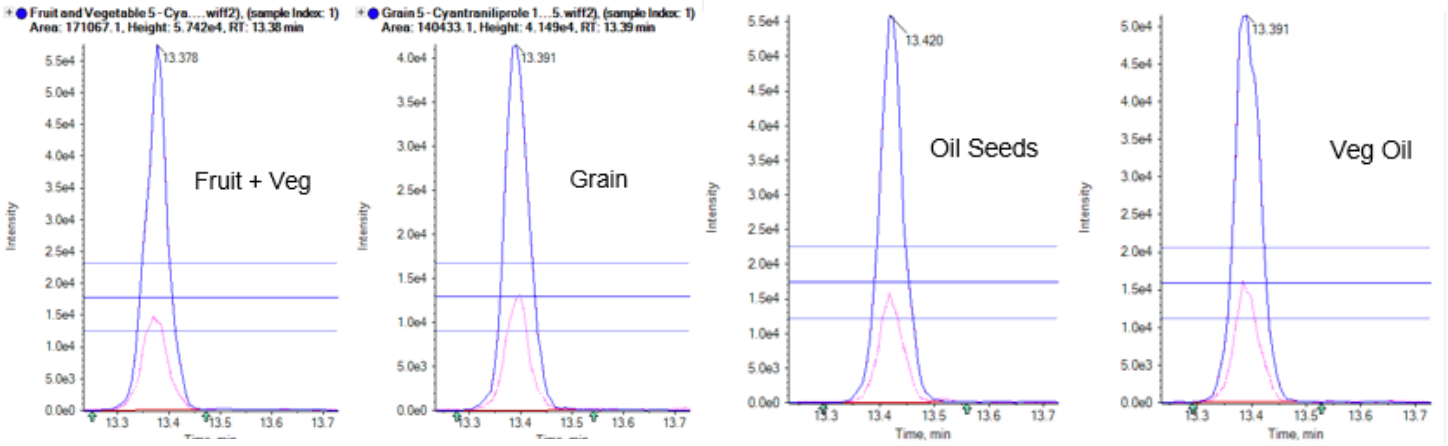
Abstract

The SCIEX Triple Quad 7500 LC-MS/MS System - QTRAP Ready provides impressive levels of sensitivity, robustness and accuracy for trace level analysis of pesticide residues in food matrices. Here, over 1400 MRM transitions for 700 compounds were analyzed in a single analysis achieving quantification limits of 0.2 ng/mL for the majority of the pesticides tested.

Introduction

The intensive use of pesticides in agriculture has led to the need for rigorous and extensive use of analytical technologies to ensure that there is no impact on human populations. Depending on the class of compounds, this has been primarily achieved through LC-MS/MS. Maximum residue limits (MRL) are set for regulated residues that define the highest level of a pesticide residue that is legally tolerated in food such that it is safe for consumers. Often these MRLs are set very low for some pesticides, to ensure highest safety, requiring very sensitive instrumentation to accurately quantify these compounds down to their MRL. Therefore LC-MS/MS solutions must be robust and sensitive to meet the needs of food testing labs.

In this method, over 700 compounds have been analyzed, covering required testing for numerous regions including Europe, North America and various areas within Asia. This breadth of coverage of pesticides has been achieved in part due to the increased sensitivity observed when utilizing the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready relative to previous generation instruments.[1] Improvements in both the generation of ions and the sampling of ions has resulted in significant sensitivity gains.[2] For residue testing in food testing, this means that more analytes can be combined into a single analysis, complex matrices can be further diluted to reduce matrix effects, while still being able to achieve or exceed the necessary levels of sensitivity.



Key results for large panel pesticide testing:

> Quantification of more than 700 pesticide compounds within 10 different food matrices

> Simple sample preparation was used, food matrices were diluted and analyzed without further SPE purification

> Improved sensitivity over previous assays with an LLOQ of 0.2 ng/mL in solvent and 5 µg/kg in matrices possible for the majority of compounds analyzed.

> Ion ratio values can confirm identity and improve the specificity of the analysis within all 10 food matrices at the LLOQ of 5 µg/kg.

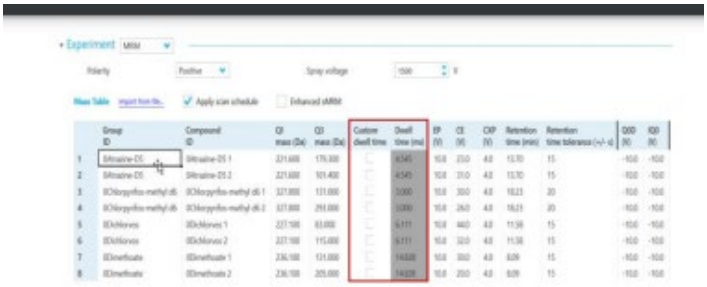
> Positive and negative switching was performed to improve the throughput of the analysis without any compromise on data quality.

> The ability to analyze such a broad panel of residues in a single injection will save significant time and resources in testing labs over the lifetime of the instrument

Methods:

Sample preparation: Ten representative commodities were chosen according to SANTE/12682/2019—fruit and vegetables, grain, oil containing seeds, vegetable oil, spices, milk, eggs, meat, fish oil and fatty acids. Stock solutions were made that contained over 700 different pesticides. Samples were then prepared using the simple protocol described in Supplementary information.[3] In brief, 1 gram of food matrix was mixed well with 10 mL of water, then 10 mL of acetonitrile. QuEChERS salt was added and sample was vortexed for 10 mins. Sample was centrifuged for 10 mins, and then frozen. When ready to analyze, samples were thawed, centrifuged for 1 min and the supernatantwas ready to inject. Calibration curves were constructed invarious prepared food matrices by spiking in pesticides atconcentrations of 0.2 to 20 ng/mL.

Chromatography: Chromatographic separation was performed using the ExionLC™ AD System which provides very low carryover and full UHPLC capabilities. The column used was a Phenomenex Luna Omega C18 (1.6µm, 100 x 2.1mm). A 1 µL sample injected for each HPLC run. Details of chromatography used are outlined in the Supplementary information.[3] Using a 30min run time, good separation was obtained across a very broadrange of analytes (Figure 3).



Mass spectrometry: These experiments were performed using the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready. The system was operated in electrospray ionization (ESI) mode using OptiFlow® Pro Ion Source. To cover the full range of pesticides, both positive and negative ionization mode were used, with rapid polarity switching. Data was acquired using SCIEX OS Software and the Scheduled MRM™ Algorithm to analyze over 1400 MRM transitions in a single injection (Figure 2). Details for MS conditions are outlined in Supplementary information.[3]

Data processing: Data was processed using SCIEX OS Software.

Method development and optimization

When performing a method with such a vast number of compounds it is important to ensure that the quality of the data is not compromised and that every compound can be effectively quantified. In this assay with over 1400 MRM transitions to analyze, time scheduling of MRMs was key to develop a method that was fast, comprehensive and maintained high quantitative quality. First retention times for each compound were determined, then a final optimized method was built using the Scheduled MRM Algorithm (Figure 2 and 3).

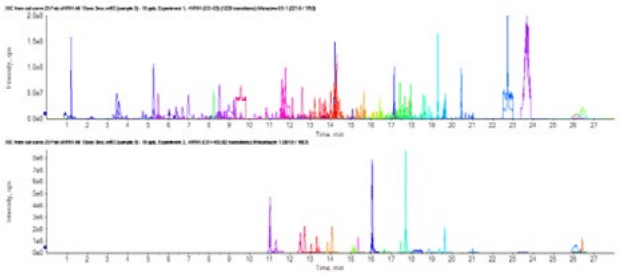


Figure 3. Good separation of large panel of pesticides. Overlays of all MRM transitions from single injection, including both positive mode data (top) and negative mode data (bottom). A total of 1400 transitions were monitored in a single injection.

Fast polarity switching

In addition to time scheduling of data acquisition, the ability to rapidly switch between positive and negative ionization modes is key for broad compound coverage in a single method. Fast polarity switching time is critical to ensure good data sampling rates are obtained across the LC peaks for accurate quantification of analyte signals. Data sampling is shown for 6 compounds to highlight data quality (Figure 4).

This allows for a broad range of compounds to be analyzed in a single method, providing both efficiency in data acquisition as well as data processing and reporting. Quantifier and qualifier transitions are also shown, again highlighting data quality (Figure 5).

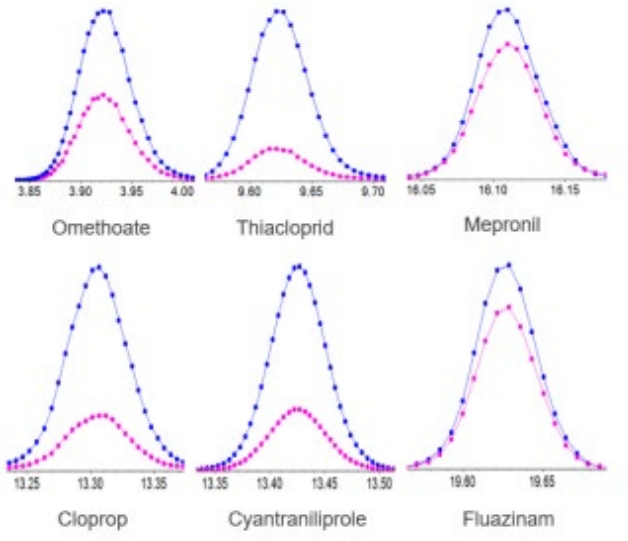


Figure 4. Importance of fast data sampling. Speed of analysis is illustrated by showing the number of data points achieved across the LC peak for three compounds analyzed in positive ionization mode (top) and negative ionization mode (bottom). This is made possible by both the Scheduled MRM Algorithm and the fast polarity switching of the SCIEX 7500 System.

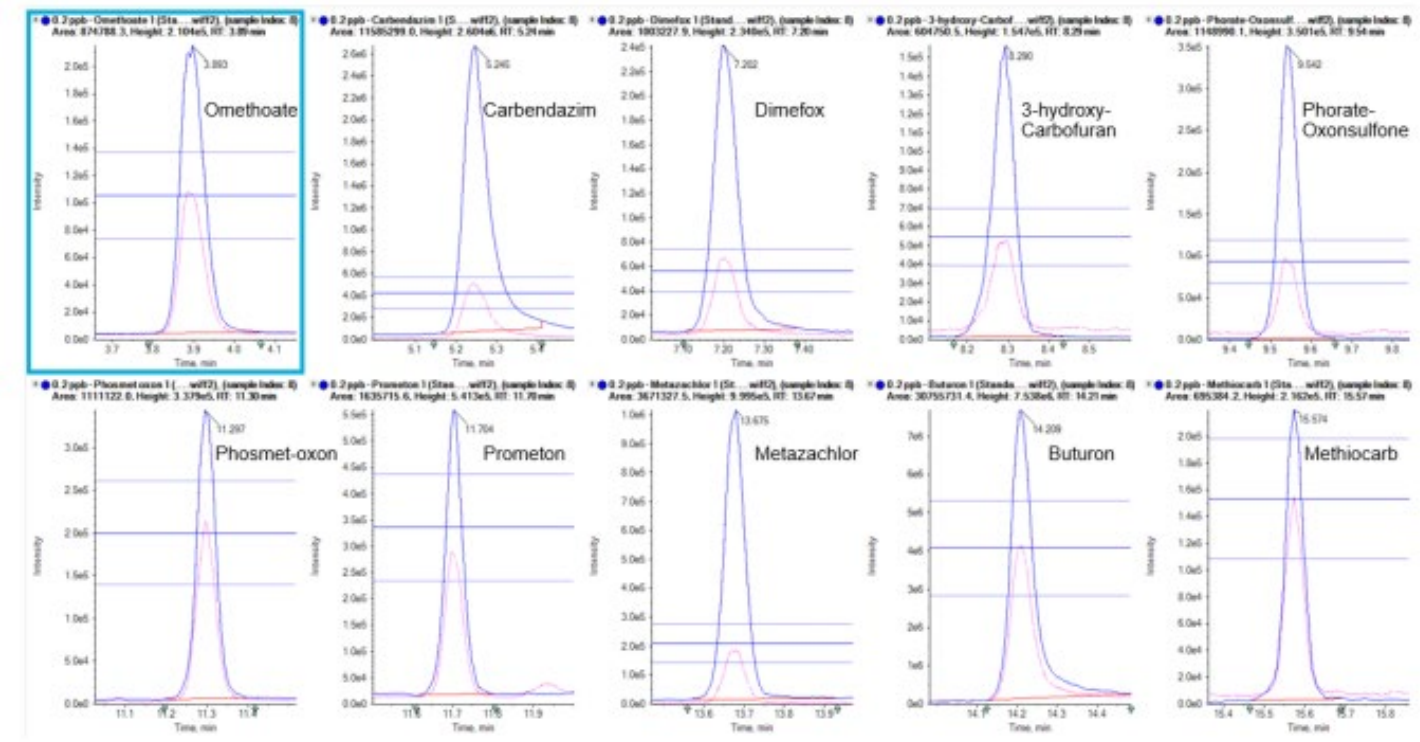


Figure 5. Chromatography of 10 selected compounds. Here both quantifier and qualifier MRM transitions for 10 compounds in solvent are overlaid for data obtained at the LLOQ of 0.2 ng/mL. Ion ratios are displayed showing the tolerance limits. Data for omethoate, carbendazim, dimefox, 3-hydroxycarbofuran and phorate-oxonsulfone is shown (top row). Data for phosmet oxon, prometon, metazachlor, buturon, and methiocarb is shown (bottom row).

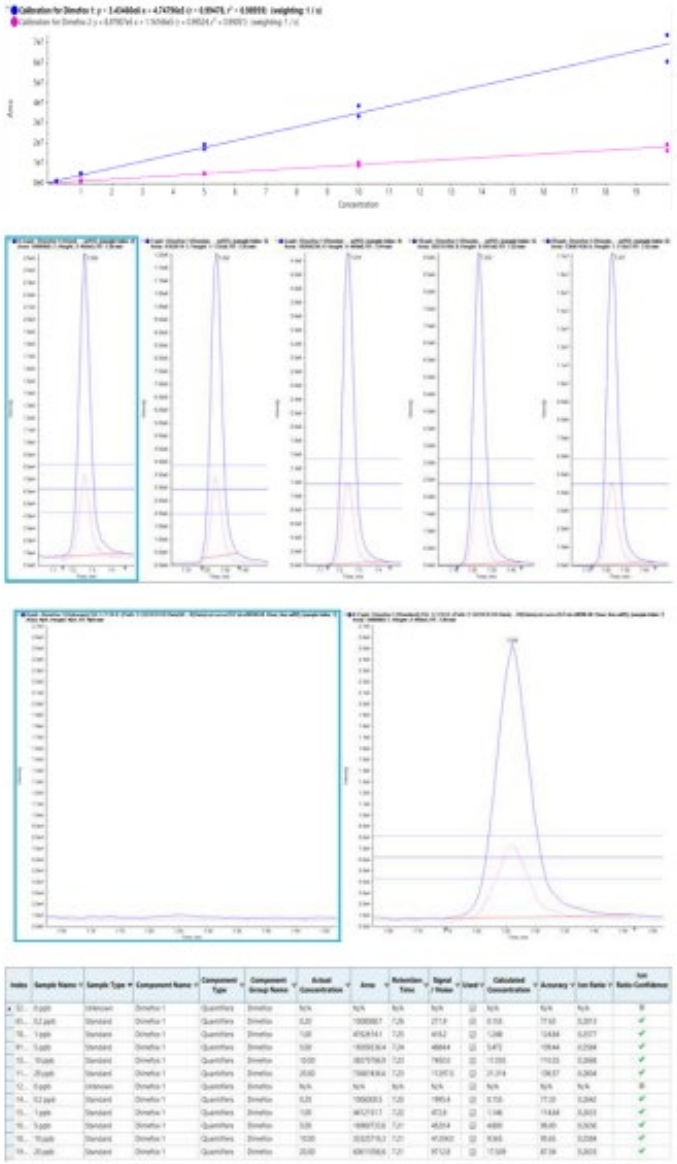


Figure 6. Calibration curve results for dimefox. (Top) Calibration curve in solvent across full concentration range (0.2 – 20 ng/mL). (Middle panes) XICs for concentrations of 0.2, 1, 5,10, 20 ng/mL, as well as a comparison of the data at the LLOQ (0.2 ng/mL) and blank. (Bottom) Results table for dimefox. The data was acquired in positive ionization mode, with ion ratios and confidence values shown for the two MRM transitions analyzed.

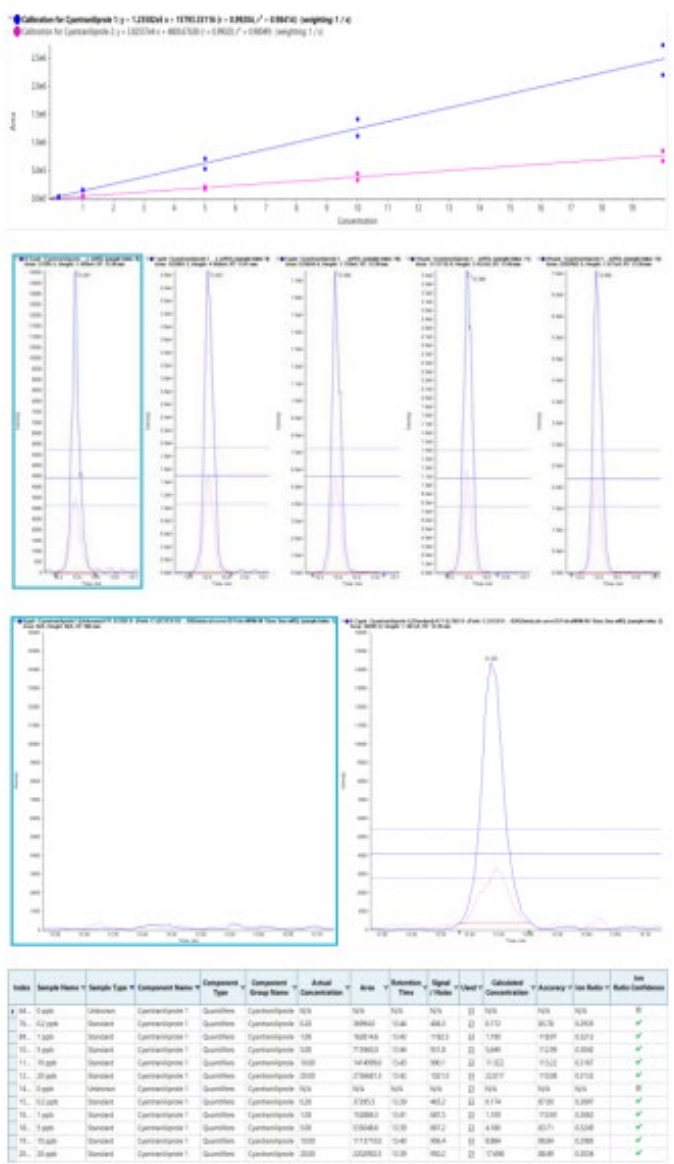


Figure 7. Calibration curve results for cyantraniliprole. (Top) Calibration curve in solvent across full concentration range (0.2 – 20 ng/mL). (Middle panes) XICs for concentrations of 0.2, 1, 5,10, 20 ng/mL, as well as a comparison of the data at the LLOQ (0.2 ng/mL) and blank. (Bottom) Results table for cyantraniliprole. The data was acquired in negative ionization mode, with ion ratios and confidence values shown for the two MRM transitions analyzed.

Sensitivity and accuracy

Calibration curves for this broad panel of pesticides were generated in solvent to determine the sensitivity achievable on the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready. The calibration curves showed high data quality across the concentration range of 0.2 to 20 ng/mL, with an r value above 0.99 and accuracy values at each level being well within acceptable tolerances when concerning trace analysis, 80 to 120% for high concentration and 70 to 130% for low concentration. Good linearity was observed for both transitions across the concentration curves (Figure 6 and 7). Data for selected pesticides is shown in Figure 6 and 7.

Carryover and interference are common problems observed in many analytical methods and should be characterized to ensure assay fidelity. Examples of clean blanks observed at the LLOQ (0.2 ng/mL) are shown in Figures 6 and 7.

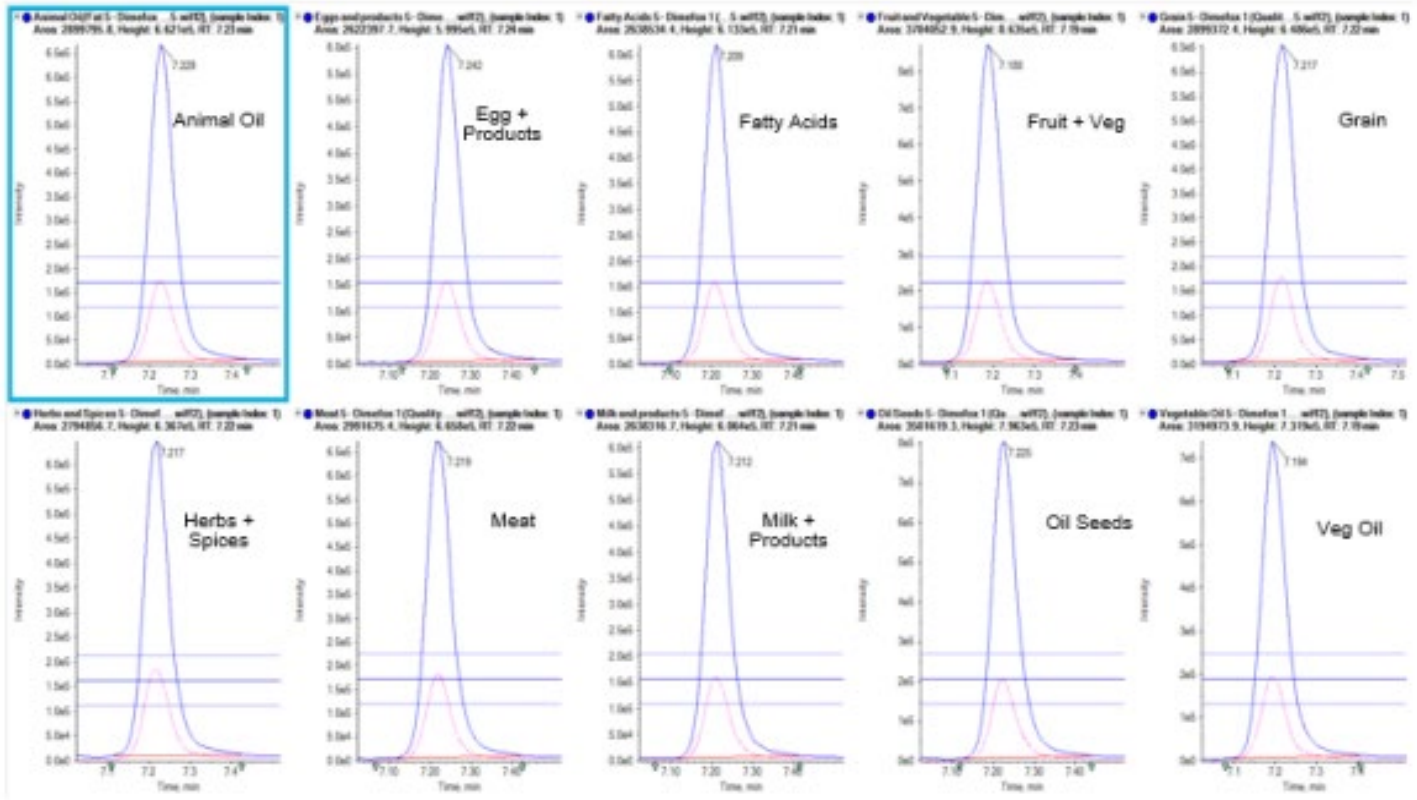
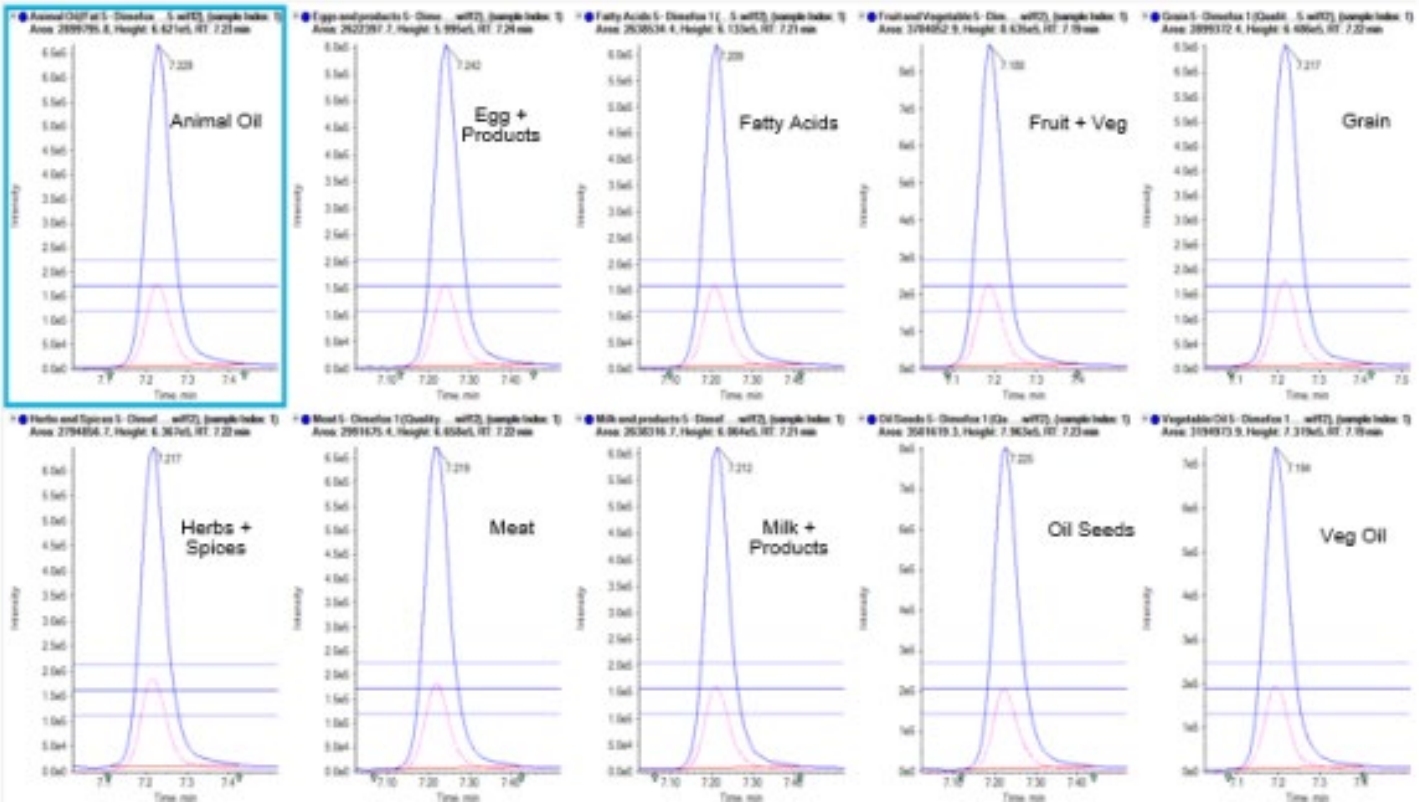


Figure 8. Quantification of dimefox in different food matrices. Representative spectra for dimefox (positive ionization) within each tested matrix, at the LLOQ of 5 µg/kg.

Next, the pesticide mixture was spiked into the prepared food matrices to evaluate sensitivity in real matrices. Monitoring two MRM transitions per analyte, both the quantifier and qualifier ions, provides added confidence in the detection of specific analytes in these complex matrices. Ion ratios were also computed and easily tracked throughout the study using the results table. Examples of two pesticides (dimefox and cyantraniliprole) are shown across the 10 matrices to illustrate the data quality. All ion ratios were found to stay within the specified tolerances within each of the 10 matrices analyzed (Figure 8 and 9) highlighting the power of the SCIEX 7500 System for high sensitivity residue testing in food matrices.



^ Figure 9. Quantification of cyantraniliprole in different food matrices. Representative spectra for cyantraniliprole (negative ionization) within each tested matrix, at the LLOQ of 5 µg/kg.

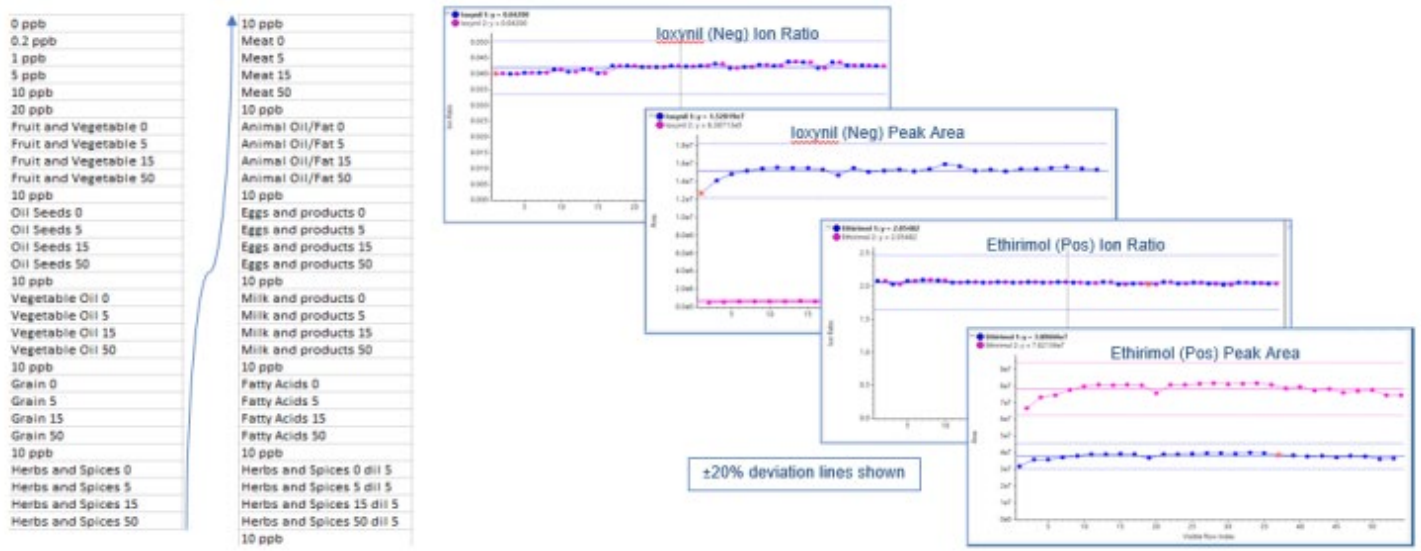


Figure 10. Batch reproducibility. A long batch was established to run the different food matrices and repeated 3 consecutive times (3.75 days, 90 hours of acquisition). The peak areas and ion ratios were tracked across the batch and show good reproducibility across the batch, highlighting the

Robustness

To determine the method robustness, a long batch with the representative matrices was run. The peak areas and ion ratios were tracked for the QC samples (Figure 10) and showed excellent reproducibility.

Conclusions

To summarize, the SCIEX Triple Quad 7500 LC-MS/MS System – QTRAP Ready provides impressive levels of sensitivity, robustness and accuracy for trace level analysis of pesticide residues in food matrices. In this study, over 1400 MRM transitions for 700 compounds were analyzed in a single analysis, utilizing the fast polarity switching functionality and the powerful Scheduled MRM Algorithm. Multiple MRMs per analyte also enabled ion ratio monitoring to ensure confident detection. Quantification limits of 0.2 ng/mL were observed for the majority of the pesticides tested. Extended batch analysis demonstrated the robustness of the method for the analysis of pesticides in complex food matrices.

References

Enabling new levels of quantification. SCIEX technical note RUO-MKT-02-11886-A.

2. Sensitivity gains for the evolution of routine bioanalysis. SCIEX technical note RUO-MKT-02-11885-A.

3. Download supplementary information.

4. C. Tomlin, The Pesticide Manual – A World Compendium, 13th ed., [British Crop Protection Council [BCPC], Alton, Hampshire, UK, 2003].

5. European Commission EU Pesticides Database, https://ec.europa.eu/food/plant/pesticides_en

6. European Commission Maximum Residue Levels, https://ec.europa.eu/food/plant/pesticides/max_residue_levels_en

Determination of pyrethroids and macrocyclic lactone insecticides in spices and tea

Using the SCIEX 7500 System

Cathy Lane,1 Sara Cheikh Ibrahim,2 Tino Schroeder,2 Susanne Hergett,2 Jack Steed,1 Jianru Stahl-Zeng,3 Roy Sperling2
1SCIEX, UK; 2Bilacon, Germany; 3SCIEX, Germany

Abstract
Pyrethroids and macrocyclic lactones are insecticides widely used in the agriculture/horticulture industries and have been designed based on the naturally occurring family of pyrethrins. To monitor the levels of these insecticides in final food products, an robust and sensitive method is required. Here an assay has been optimized for the SCIEX Triple Quad 7500 system using a total run time of 17 mins, monitoring eleven different compounds. Excellent sensitivity in solvent was been demonstrated with LLOQ values down to 0.02 ng/mL. Detection levels of 5-10 ppb for most insecticides were also demonstrated in tea and spice.

Introduction
Pyrethroids and macrocyclic lactones are groups of commonly used insecticides in the agriculture and horticulture industries. Macrocyclic lactones are naturally occurring, or semisynthetic, compounds produced as fermentation products in soil-dwelling Streptomyces avermitilis.[1] Pyrethroids, on the other hand, are synthetic, and were designed based on the naturally occurring family of pyrethrins, which were originally derived from chrysanthemum flowers.[2]

Due to the widespread use of these compounds in the environment, a comprehensive quantitative method is necessary to monitor and control their concentration in final food products destined for human consumption.

Here, a method has been developed using the SCIEX 7500 system for the simultaneous identification and quantification of pyrethroid and macrocyclic lactone insecticides at detection levels below the maximum residue level defined by the European Commission under regulation 2018/1514.3

Key features of the SCIEX 7500 system for the quantification of pesticides in spices and green tea:
> Highly sensitive detection and quantification of avermectin (containing 96% avermectin B1a and 4% avermectin B1b), bifenthrin, cyfluthrin, cypermethrin, deltamethrin, fenvalerat, λ -cyhalothrin, milbemectin A3 and A4 and permethrin from QuEChERS extracts of spices and green tea
> Improved sensitivity over previous assays with lower limits of quantification (LLOQs) down to 0.02 ng/mL in solvent (Figure 1)
> Optimization of new parameter QOD for milbemectin A3 in spices resulted in greatly reduced background and increased signal to noise
> Increased sensitivity allows for the use of lower sample injection volumes, increasing assay robustness and significantly reducing ion suppression in matrix

Go to full technical note →

Measuring PFAS in a variety of food samples at different price points

Karl Oetjen, Simon Roberts, Igor Zakharevich

SCIEX, USA

Abstract

Recent studies have shown that per- and polyfluoroalkyl substances (PFAS) accumulate in the food chain and are found in a wide variety of food products, including fish, meat, dairy and vegetables.[1] As a result, there is a growing concern about the potential health risks associated with consuming PFAS-contaminated foods. As the public becomes more aware of these compounds, they might make purchases that they believe would decrease the amount of potential exposure to PFAS. Many people may assume that more expensive alternatives might represent a healthier or less PFAS-contaminated option.

Introduction

This study explored the differences in PFAS concentrations in several food types at different price points. Eight different food products were selected to represent a wide range of food types, including ground beef, cake, salmon, tuna, potato chips, seltzer, beer and ice cream. For each type of food, low-, mid- and high-priced options were purchased for analysis. The samples were analyzed using the SCIEX 7500 system using a large volume injection, PFAS compounds were detected in food in the low parts per trillion (ppt) or picogram per gram (pg/g) depending on the analyte.

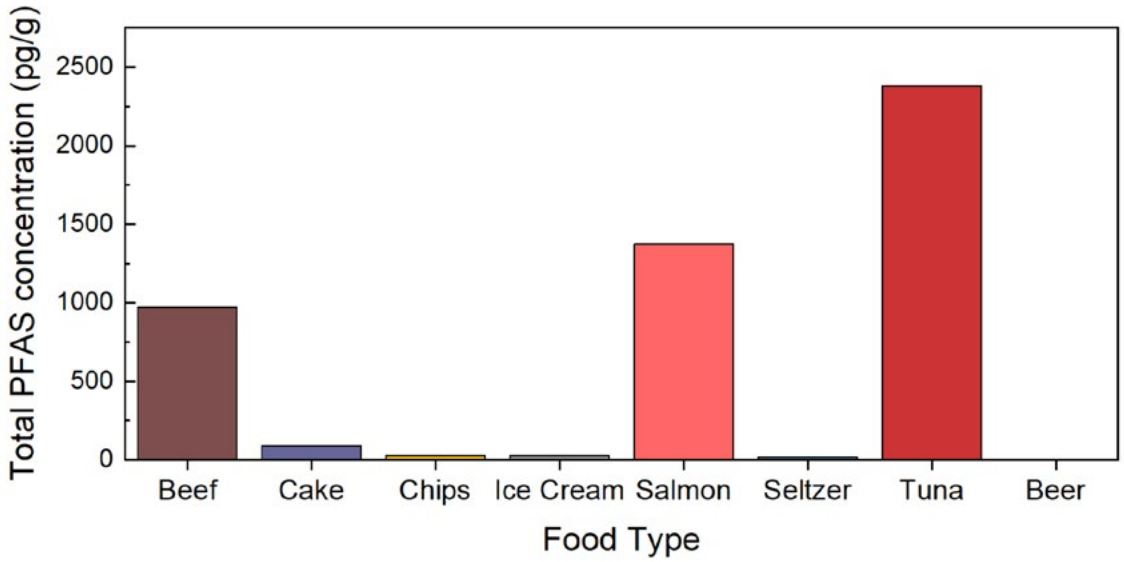


Figure 1. The foods sampled for this study ranged from solid, protein-rich foods, like fish and ground beef to liquid beverages like beer and seltzer and highly processed foods like chips and cake. The study sought to determine PFAS levels across very different matrices and whether price plays a factor in the distribution.

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The production of more ions with the IonDrive Turbo V source is just one of the enhancements you will see with the Triple Quad 6500+ system. Also added is the ability to capture and transmit more ions with the unique IonDrive QJet guide and greater detection with the new IonDrive High Energy Detector+.

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The legendary Turbo V source increases ion production by using enhanced gas flow dynamics and optimized heater configuration to improve reliability, reproducibility, and robustness.

> IonDrive QJet ion guide

With its dual-stage design, the IonDrive QJet ion guide improves sensitivity and ion capture rate while focusing ions more efficiently than funnel approaches.

> IonDrive High Energy Detector+

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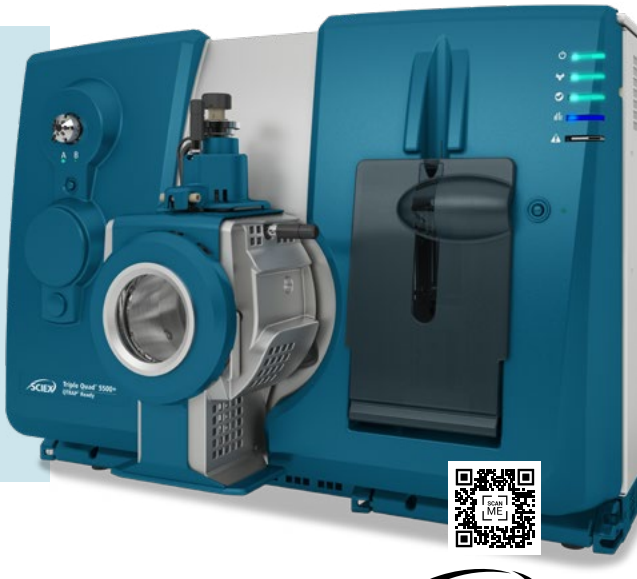
Regulations determine the allowed levels of veterinary drugs in food products. These low-level residues are detected easily with MRM workflows. Activate QTRAP functionality to have even more confidence in your results.

> Toxins in food

This high-throughput workflow for mycotoxin testing is critical to achieving regulatory compliance. Use a combined MRM and QTRAP library workflow to achieve a new level of confidence in your data.

> Pesticides

A variety of complex matrices are involved when testing for pesticides in fruit, vegetables, water, soil and other samples from emerging markets like cannabis. Quantify and confirm large suites of pesticides using these robust workflows.



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> Low EAD
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Protein based therapeutics come with a rich assortment of varying structures. Those modifications exist in complex heterogeneous mixtures.

> Mid EAD
post-translational modifications

The EAD cell brings fresh insight and completes the picture for biomolecule characterization. EAD, fragmentation of large multiply-charged produces those differential C & Z ions to garner those critical insights.

> High EAD
small molecules

CID fragmentation of small molecules can produce limited and/ or non-specific MS/MS information, giving rise to challenges in identification or lack of specificity in the development of quantitative assays.

Highly sensitive quantification and selective identification of pesticides in food with Zeno MRM^{HR}

Using the SCIEX ZenoTOF 7600 system, powered by SCIEX OS software

Robert A. Di Lorenzo1, Lukasz Rajski2, Jianru Stahl-Zeng3, Jason Causon1
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Abstract

Using the sensitivity enhancements of the Zeno trap, Zeno MRMHR is used to quantify low levels of pesticides in oilve oil and various fruits and vegetables to meet global regulations for pesticide residues. Over the mass range analyzed, the Zeno trap allowed for signal gains of 5-15x, with negligible increase in noise due to the selectivity of MRMHR analysis, hence gains were directly related to improved LLOQs. The selectivity and specificity afforeded by the accurate mass MS/MS quantitation on the ZenoTOF 7600 system allowed for highly confident identifications of pesiticdes in real food samples (MS and MS/MS mass accuracy, library matching, isotope ratio matching), while still maintaining triple quad-like ability to perfom ion ratios for confirmations.

Introduction

In order to ensure safety in the global food supply, testing for adherence to federal and international requirements is necessary. The tests monitor for chemical residues, including pesticides, microbial and fungal toxins, and microbiological hazards. Considering the ever-growing demand on the food supply, farmers and producers are tasked with optimizing yields, which means employing the use of pesticides to deter pests from harming their crops. For this reason, it is important to be aware of adherence to pesticide maximum residue limits set by the appropriate governing bodies.

Traditionally, pesticide residue analysis has been performed by triple quadrupole mass spectrometers, due to their sensitivity and quantitative power. Accurate mass instruments can afford additional levels of confirmation, however they have traditionally suffered from a lack of sensitivity and precision, especially when performing MS/MS experiments to meet the testing requirements for the regulatory guidelines. The technological enhancements afforded by the ZenoTOF 7600 system, however, bring QTOF systems into a new era of sensitivity and precision.

Key feature of Zeno MRMHR for pesticide quantification:

> Highly sensitive pesticide detection with a high degree of selectivity and identification confidence using Zeno MRMHR

> In-matrix LLOQs below 0.010 mg/kg to meet global pesticide regulations

> Quantification using accurate mass fragment extracted ion chromatograms cuts through noise and interferences, simplifying peak detection and integration to eliminate manual peak review

> Identification confidence using accurate mass precursor and fragments (< 5 ppm mass error or < 1mDa below 200 Da), ion ratios (± 30%) and MS/MS spectral library matching

> High speed analysis ensuring ample points across each chromatographic peak, even with highly-multiplexed analyses

>Easy data filtering and data review with SCIEX OS software.

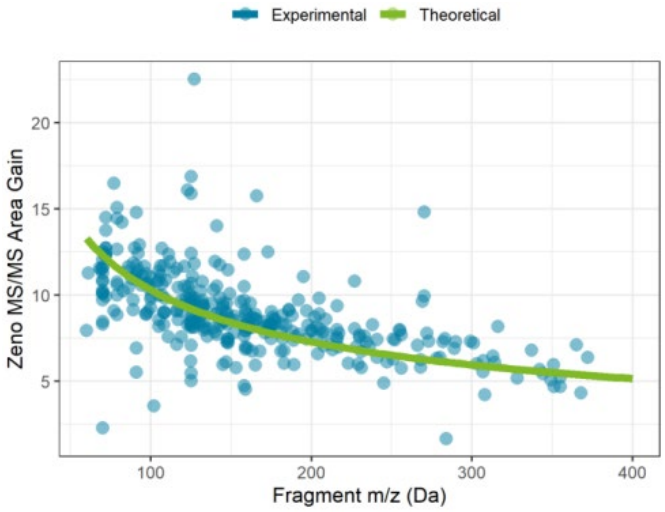

Figure 1. MS/MS peak area gains as a result of enabling the Zeno trap on the ZenoTOF 7600 system. Sensitivity improvements during MS/MS experiments using the Zeno trap are a result of regaining >90% of duty cycle related losses. For this reason, the improvements are dependent on the upper limit of scan range and the measured mass of the MS/MS fragment. The theoretical gain curve is constructed based the scan parameters acquired in this experiment.

Methods

Sample preparation: All samples were extracted according to the standard QuEChERS citrate protocol using a Phenomenex roQ QuEChERS EN Method kit. Briefly, 10 g of homogenized food product and 10 mL of LC-MS grade acetonitrile were combined in a 50 mL centrifuge tube and shaken for one minute. To separate aqueous and acetonitrile layers, 4 g MgSO4, 1 g NaCl, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate was added. The sample was shaken for an additional 1 minute and centrifuged for 5 min at 4000 rpm. A 5 mL aliquot of the acetonitrile layer was combined with 750 mg MgSO4 and 125 mg PSA in a 15 mL centrifuge tube for clean-up. The extract was shaken for 30 seconds and centrifuged for 5 min at 4000 rpm. A 200 µL aliquot of cleaned-up extract was added to an LC vial diluted with 800 µL of mobile phase A for sample analysis for an overall 5x dilution factor.

Chromatography: Analytes were separated on a polarembedded reverse phase C8 column (2.1 x 150 mm, 1.8 µm) using a SCIEX ExionLC AD system. Separation was performed at a flow rate of 300 µL/min with a column temperature of 35°C and an autosampler sample storage temperature of 5°C. Mobile phase solvents were (A) 98:2 water/methanol and (B) 2:98 water/methanol, both with 0.1% formic acid and 5 mM ammonium formate. The gradient program can be seen in Table 1. Total runtime was 17 minutes

Mass spectrometry: All compounds were analyzed using a SCIEX ZenoTOF 7600 system with scheduled Zeno MRMHR for optimal sensitivity. Source conditions can be observed in Table 2. Accurate mass precursor ions were scanned from 100-950 Da with an accumulation time of 100 ms, a declustering potential (DP) of 80 V and a collision energy (CE) of 10 V. Zeno MRMHR acquisition was retention time scheduled with a tolerance of ± 20 seconds. MS/MS data were acquired with DP = 80 V and compound optimized CE. Accumulation times for each compound were varied (minimum 10 ms) to maintain a total cycle time of 600 ms across the acquisition. Each MS/MS was acquired down to 50 Da to ensure all fragments were captured for identification.

Table 1. LC gradient conditions.

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
0.00	0.300	100	0	0
1.00	0.300	100	0	0
2.00	0.300	70	30	0
3.00	0.300	50	50	0
11.00	0.300	0	100	0
14.00	0.300	0	100	0
14.01	0.300	100	0	0
17.00	0.300	100	0	0

Data processing: All data were processed with SCIEX OS software 2.0.1 using the Analytics module and MQ4 integration algorithm. The monoisotopic mass and two accurate mass fragments were extracted for each analyte with a 20 mDa window, with 1-point gaussian smoothing applied. Retention time thresholds for peak identification were set to ± 15 s. Quantitative and qualitative thresholds were set according to SANTE/12682/2019. Briefly, positive identifications were determined such that two fully overlapping extracted ion chromatograms had mass error < 5 ppm (< 1 mDa for masses below 200 Da) and S/N > 3 (or 5 points of consecutive signal in the absence of noise). Additionally, ion ratio thresholds were set according to the nominal mass requirement of ± 30% relative deviation from standards. Quantitatively, LOQs were set at the lowest standard giving average accuracy and precision within ± 20% across the n=6 replicates. Along with the SANTE guidelines, flagging rules were set for library match and theoretical isotope ratio match for easy filtering in SCIEX OS software using the conditions in Figure 2.

Figure 2. Settings for applied Qualitative Rules. (Top) Built-in flagging rules for mass error, retention time, isotope ratio and library score applied for easy filtering during data analysis. (Bottom) Ion ratio threshold settings. Expected ion ratios are automatically calculated from standards in data set.

Sensitivity enhancement with Zeno MS/MS

The use of the Zeno trap on the ZenoTOF 7600 system allows for >90% of duty cycle related losses to be recovered during MS/MS experiments across the entire mass range. With traditional orthogonal injection QTOF mass spectrometers, ion losses occur as a result of mating a continuous ion beam coming from the quadrupole ion guides with the pulsed nature of the TOF accelerator. To overcome this on the ZenoTOF 7600 system, a Zeno trap was added at the end of the Q2 collision cell assembly, where an ordered release of ions based on their potential energy allows for all ions to arrive at the TOF accelerator at the same time and location. For this reason, the sensitivity enhancements are both dependent on the upper limit of the MS/MS scan range and the mass of the fragment ion. Figure 1 shows the experimental gains experienced during this analysis overlaid with the theoretical gains based on the fragment masses and scan parameters used in this experiment. The Zeno trap yields a minimum of 5x intensity gain, with gains up to 13x as fragment masses decrease.

With Zeno MS/MS, it is frequently the case that extracted ion chromatograms (XICs) show greater intensity compared to XICs from the precursor ion traces, as observed in Figure 3.

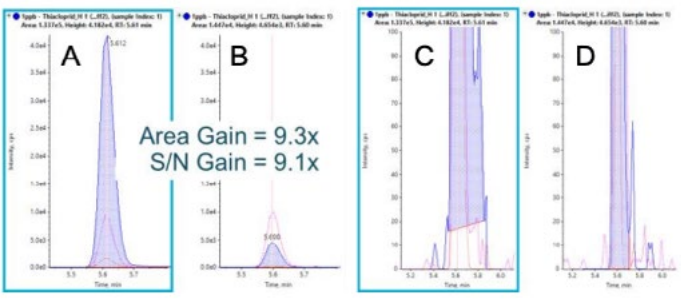

Figure 4. Example MS/MS sensitivity gains with Zeno MS/MS. A 9.3x area gain can be observed for thiacloprid between MS/MS acquired with (A) and without (B) the Zeno trap enabled, with negligible change to the noise regions (C, D, respectively). This results in a 9.1x gain in S/N.

This is a result of comparing an MS/MS spectrum with >90% duty cycle to an MS spectrum with a typical duty cycle of 5-25%. Improvements can also be observed in MS/MS spectral quality for greater confidence during library confirmation. Since duty cycle improvements are only a function of scan range and fragment mass, these gains are observed across all molecule types, independent of chemistry and ionization efficiency. Further, since the selectivity afforded by accurate mass MS/MS analysis often results in little to no chemical noise, the gains in signal-to-noise approach the gains observed in raw signal (Figure 4).

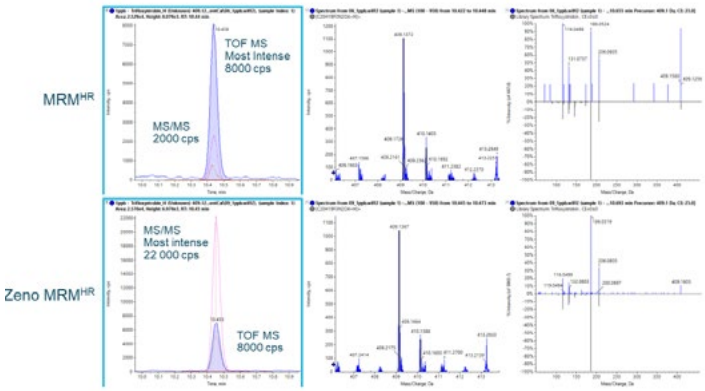

Figure 3. Example comparison between (top) normal MRMHR acquisition and (bottom) Zeno MRMHR acquisition for trifloxystrobin at 1 ng/mL. Between the two acquisitions, the TOF MS information remains constant: the TOF MS XICs (left, filled blue traces) have the same intensity, and the MS spectrum (middle) remains the same. However, the MS/MS XIC (left, pink trace) gains approximately 11x intensity and the MS/MS spectral quality (right) improves significantly.

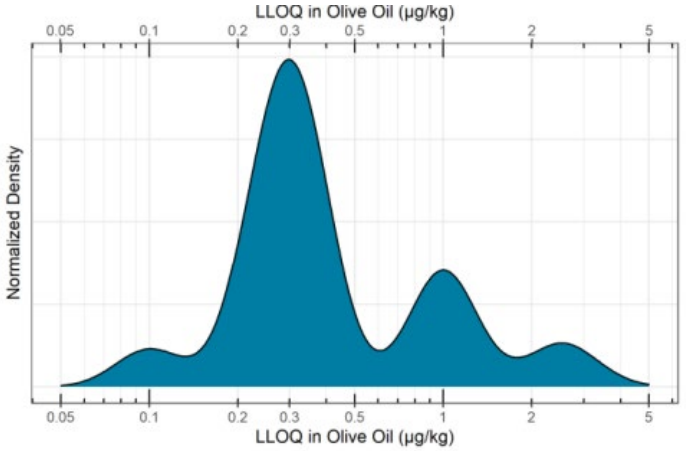

Figure 5. In-matrix LLOQ distribution for the ZenoTOF 7600 system. Calculated from n=6 replicate injections of the olive oil matrixmatched curve using quantifier ion and processed according to SANTE/12862/219 guidelines. >90% of analytes meet two ion criteria below 10 ppb.

LLOQ assessment in olive oil

To assess the in-matrix quantitative performance of the ZenoTOF 7600 system, n=6 replicates of a matrix matched curve was constructed in QuEChERS extracted olive oil ranging from 0.10 µg/kg to 0.10 mg/kg. Incorporating the 5x dilution during sample preparation, in-vial concentrations ranged from 0.02 to 20 ng/mL. The curve was assessed for linearity, accuracy and precision, mass accuracy and ion ratios according to SANTE/12862/2019 guidelines. Figure 5 shows the distribution of in-matrix LLOQs determined for the quantified pesticides. It can be observed that nearly all quantified pesticides have an LLOQ below 0.001 mg/kg, with the majority with LLOQs falling between 0.0002 and 0.0005 mg/kg. Also, all quantified pesticides showed LLOQs at or below 0.005 mg/kg, meaning that the ZenoTOF 7600 system has enough sensitivity to meet nearly any global pesticide residue regulation, as most maximumresidue limits are set as low as 0.010 mg/kg. Additionally, theuse of accurate mass fragment ions for quantification cutsthrough nearly all noise and interferences, simplifying peak detection and integration to eliminate the need for manual peak review.

At the calculated LLOQs, the quality of qualitative information acquired remains consistent. Figure 6 highlights three example pesticides at their respective LLOQs. In each case, the fragment XICs (left, filled blue traces) show ample S/N for detection and improved S/N over the precursor XICs (pink traces). The ion ratio thresholds, adopted from the SANTE nominal mass requirement of ± 30%, are maintained at these low concentrations. With the additional sensitivity, mass error on fragments can also be maintained due to the improved ion statistics during detection, as well as the generation of clear and unambiguous MS/MS spectra for library confirmations, even in dirty matrices.

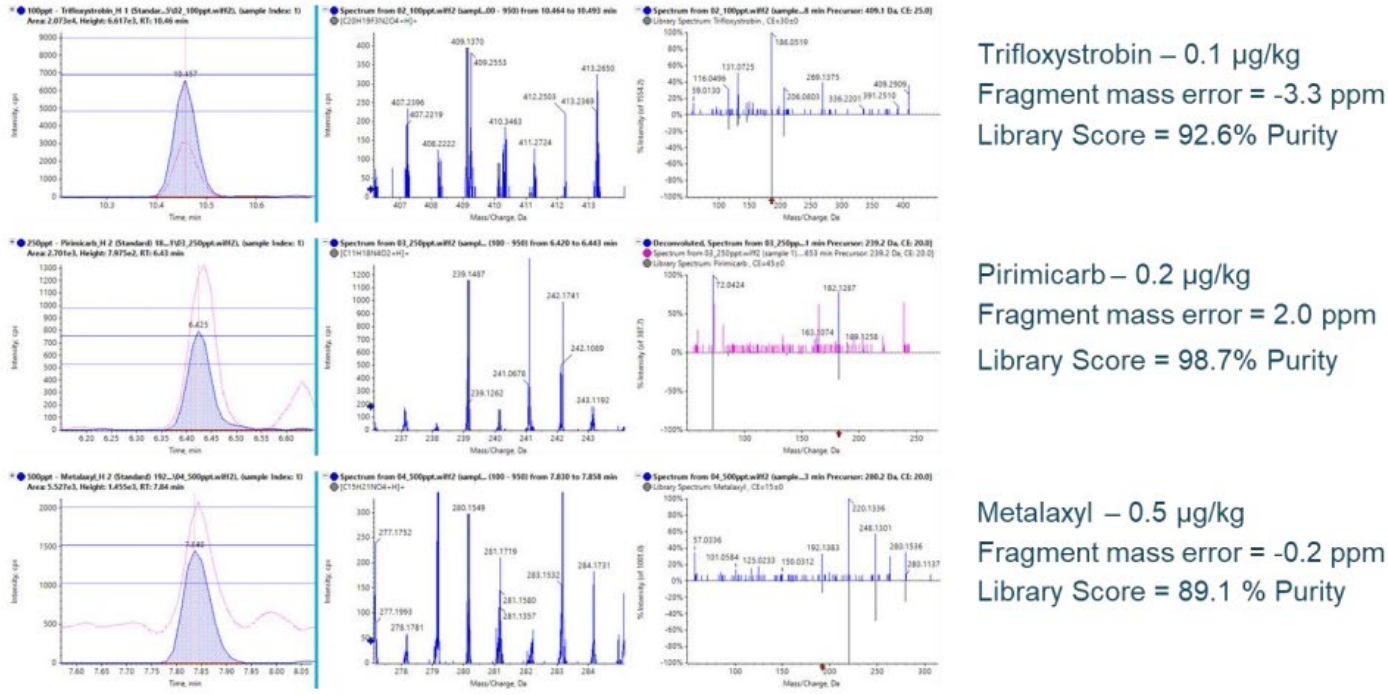

Figure 6: Zeno MRMHR acquisition for three pesticides. The TOF MS data XICs (left, pink trace), the MRMHR XIC (left, blue filled trace), the TOF MS spectrum (middle) and the MS/MS spectrum (right) is shown for three of the detected pesticides. Library matches are also shown for trifloxystrobin (top), Pirimicarb (middle) and Metalaxyl (bottom).

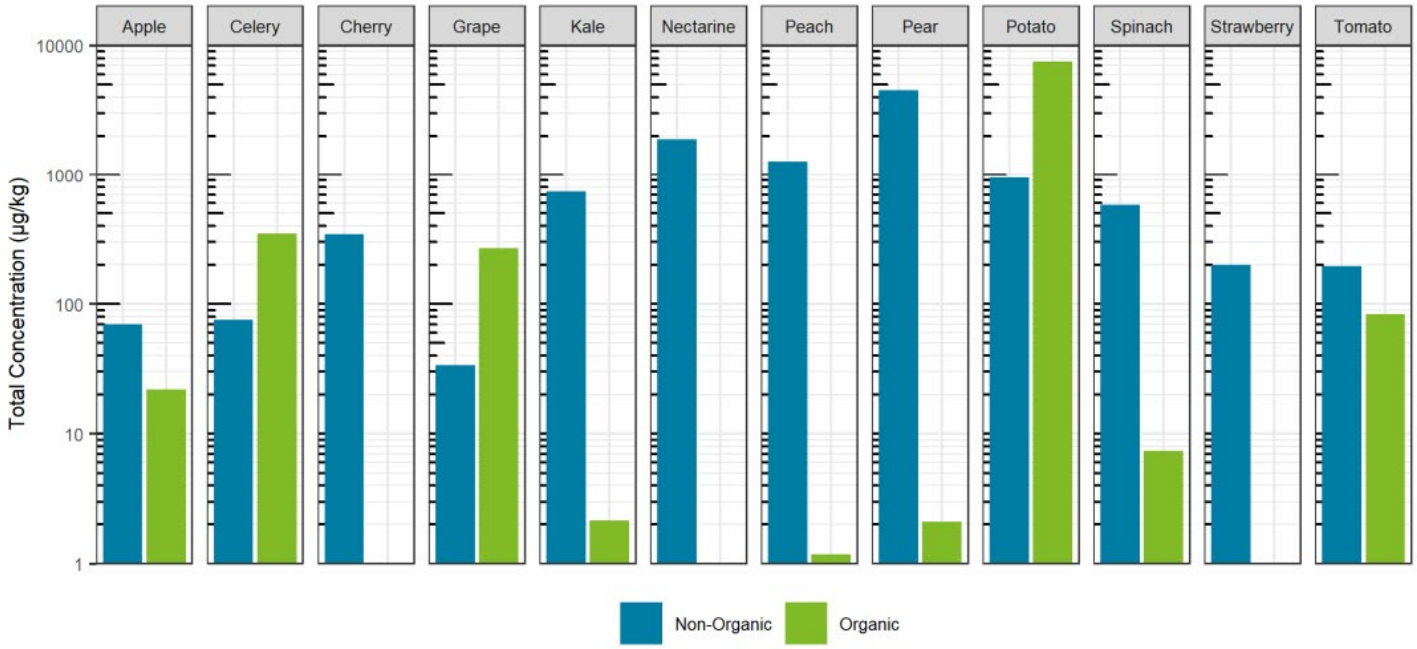

Figure 7. Comparison of sum total concentration of pesticides detected in non-organic and organic produce

Comparison of organic and non-organic produce using Zeno MRMHR

The developed quantitative method was also applied to a variety of fruits and vegetables in order to compare the relative pesticide burden between produce that was traditionally farmed and produce purchased from an organic grocer. The twelve fruits and vegetables chosen were from the Environmental Working Group's "Dirty Dozen" list, an annual list of produce on which pesticides are most frequently detected.

Comparing the sum total of pesticides detected for each specific food, traditionally farmed produce showed a larger total pesticide burden, with the exception of celery, grapes and potatoes. For these exceptions, the organic produce showed a larger total pesticide concentration (Figure 7). For the non-organic produce, pears, peaches and nectarines showed the largest total concentration, whereas potatoes showed the largest sum concentration for the organic produce and for produce overall. Sum total concentrations ranged from less than 0.001 mg/kg to greater than 7 mg/kg. Comparing the aggregate concentration distribution for each pesticide hit between all organic and nonorganic produce, the organic produce showed a slightly lower average concentration, as seen in Figure 8.

Looking at the individual pesticide hits in Figure 9, a few key observations can be made. First, the most contaminated food, organic potatoes, was a result of a single hit: chlorpropham, a germination inhibitor commonly applied to potato crops. Second, when the same pesticide was detected in both organic and nonorganic produce, the concentration was higher in the organic produce, with the exception of pyrimethanil in pears. Third, although concentrations were detected as high as mg/kg levels, each hit was below the maximum residue levels set by Health Canada, as all produce was purchased in Canada. Overall, the differences between the organic and non-organic produce were not as delineated as most advertisements and product packaging for organic products claim. The levels detected are far below any concentration that may trigger acute or chronic health effects.

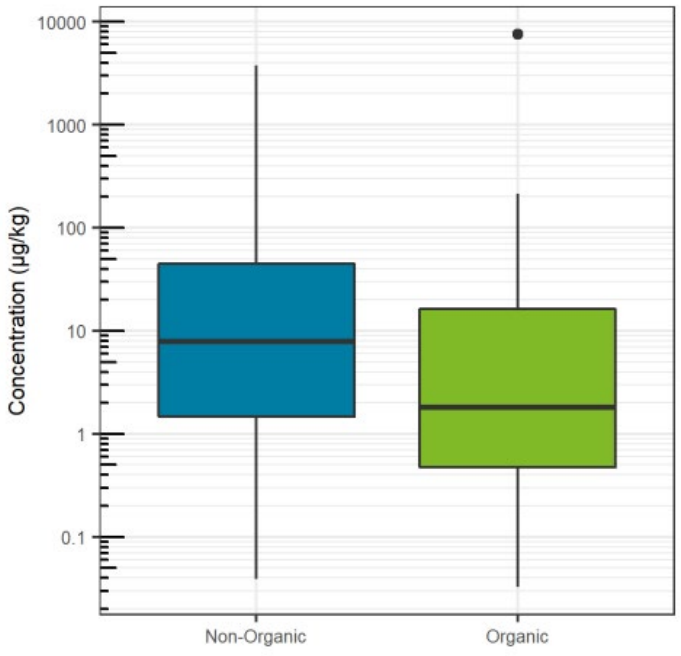

Figure 8. Aggregate concentration distribution of pesticides quantified in (blue) non-organic and (green) organic produce.

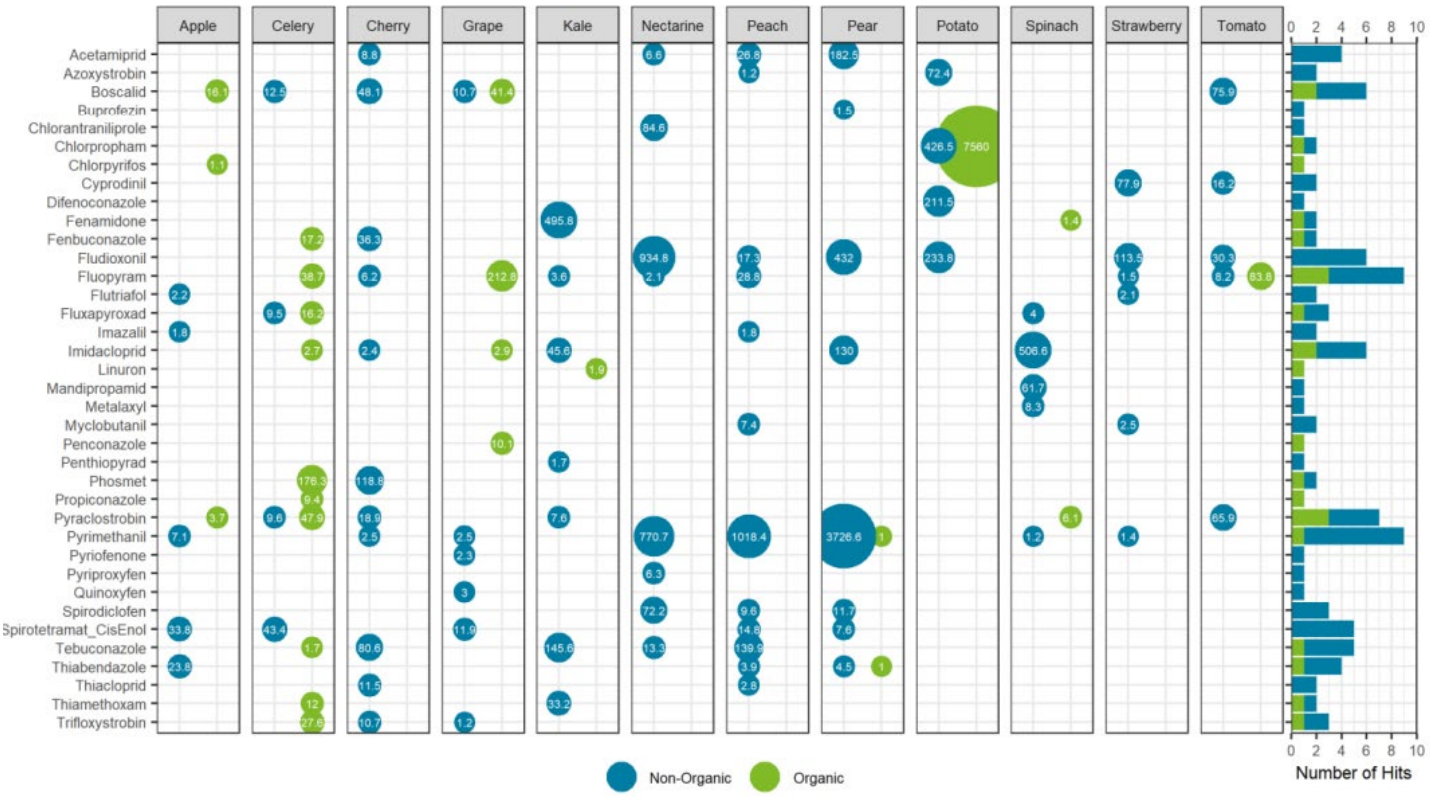

Figure 9. Individual pesticide hits in each food commodity. Inset numbers represent individual pesticide concentrations in µg/kg. Generally, nonorganic produce contained a greater number of pesticide hits than organic produce, but organic produce still contained pesticide residues. All pesticide hits were quantified below Health Canada maximum residue limits for their respective commodity. The right panel shows the number of times each pesticide was detected, where pyrimethanil, fluopyram, pyraclostrobin and fludioxonil were detected most 9.

Conclusions

> Highly sensitive, quantitative analysis using the ZenoTOF 7600 system, with ample sensitivity to meet global pesticide regulations

>Mass accuracy, library matching and ion ratios used for confirmation

>Quantification using accurate mass fragment ions reduces chemical noise and chromatographic interferences, ensuring accurate and precise peak integration, and minimizing manual peak review

References

1. Qualitative flexibility combined with quantitative power - Using the SCIEX ZenoTOF 7600 LC-MS/MS system, powered by SCIEX OS software. SCIEX technical note RUO-MKT-02-13053-A.

X500R QTOF system

Go to webpage →

Be ready for anything with quantitative and qualitative capabilities on the same instrument
For the modern researcher who needs flexible and scalable workflows that deliver reliable, high-quality, accurate mass results with confidence, the X500R will quickly become your workhorse. Built specifically to balance robust performance with triple-quad-like quantitation, you can perform everything from untargeted analysis to broad screens, comprehensive profiling, and identification of unknowns, all on the same instrument. With simple workflows, robust hardware, sensitivity, and precision, the X500R QTOF is the first high-resolution LC-MS/MS system designed for both characterization and quantitation.

Key features of the X500R system:

> Mass range TOF

Up to 40 kDa. Precursor ion selection: 5-2250 m/z.

> Mass accuracy over time, external

Less than 2 ppm RMS over 12 hours of LC-MS

> Ionization sources

Turbo V ion source with twin sprayer ESI probe and twin sprayer APCI probe

> TOF-MS resolution and speed

≥ 42,000 (FWHM) measured on the (M+6H)6+ charge isotope cluster for bovine insulin at m/z 956.

Technical content summaries

Technical content summaries
Contents

Robust, high-throughput, fast polarity switching quantitation of 530 mycotoxins, masked mycotoxins and other metabolites

Using the SCIEX Triple Quad™ 5500+ LC-MS/MS System – QTRAP® Ready

1Jianru Stahl-Zeng, 2Yoann Fillâtre, 3Daniel McMillan, 3Philip Taylor, 4Ian Moore 1Darmstadt, Germany.
2Paris, France. 3Warrington, U.K, 4Concord, Canada

Mycotoxins are toxic fungal metabolites, which are derived from certain molds and fungi. The growth of mold can occur before crops are harvested or under inappropriate storage conditions such as warm and humid conditions. Consumption of food products containing mycotoxins can have serious health implications. According to the World Health Organization (WHO), the effects of some foodborne mycotoxins are acute, with symptoms of severe illness appearing quickly after consumption.[1] Others have been linked to long term human health effects, such as cancers or immune deficiency. The most important classes of mycotoxins including the highly carcinogenic Aflatoxins (e.g. AFB1), trichothecenes (e.g. DON), Fumonisins (e.g.FB1), Ochratoxins (OTA) and Zearalenone (ZEN) and several others are regulated in many countries. In China, GB 2761 regulates mycotoxin limits in certain products; in the EU, mycotoxins in foodstuffs are regulated by the EC1881/2006.2,3,4 A living plant can change the chemical structure of toxins and produce so-called "masked mycotoxins". The plant might modify the chemical structure of the toxin with a glucose or sulfate moeity, which reduces its toxicity to the plant. The plant itself may now contain only the conjugated form of the toxin, but the original mycotoxin may emerge during human or animal digestion if the conjugate functional groups are cleaved, thus exposing the consumer to the dangers of the toxin.[5] The term 'masked mycotoxins' was coined to refer to this group of conjugated or otherwise transformed mycotoxins which become undetectable by targeted methods for the original compounds. Current knowledge of these "emerging mycotoxins" (e.g: NX-Toxins), as well as masked or other modified forms of mycotoxins, is limited but the number of compounds that need to be analyzed is increasing rapidly, requiring more comprehensive analytical LC-MS methods.[6] Mycotoxin analysis needs to be comprehensive and able to deliver accurate and consistent results across a wide range of matrices. This application note introduces an improved approach to testing Mycotoxins, their metabolites and emerging masked mycotoxin compounds using LC-MS/MS with fast polarity switching.

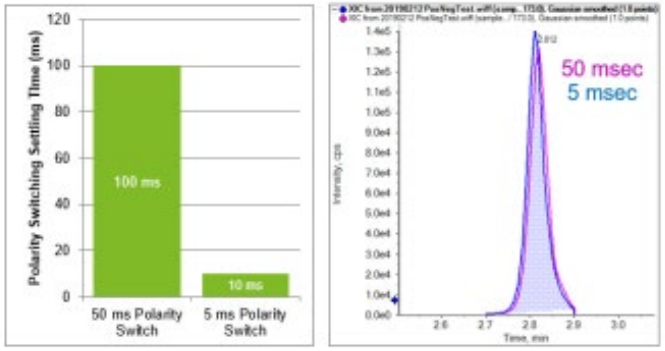

Figure 1. Advantages of fast polarity switching. Reducing the polarity switching time from 50 to 5 msec means a gain of 90 msec per cycle (Left). This time can be used to add more compounds to each scan, or dwell longer on existing MRMs to improve ion statistics. Similar peak areas are observed in the data collected for these mycotoxins between the 50 and 5 msec tests.

Key features of high-throughput mycotoxin analysis:

· Workflow which incorporates 530 mycotoxins, their metabolites and emerging masked mycotoxins in one comprehensive method using Scheduled MRM™ Algorithm.

· Demonstrated barley and corn extracts

· Utilizes the rapid 5 msec positive and negative polarity switching of the SCIEX Triple Quad 5500+ System to condense the standard two injection method into a single 22 min analysis.

· Reduced analysis time means results can be delivered quicker, and a cost reduction can be realized by the minimizing of consumables, such as mobile phase solvents, used in the analysis.

Methods

Sample preparation: Barley and corn samples were extracted with 4mL of Acetonitrile/Water/Acetic Acid at a ratio of 79/20/1 per gram of sample. Following the extraction, the samples are diluted to a 1:1 with Acetonitrile/Water/Acetic Acid at a ratio of 20/79/1.

Chromatography: Chromatographic separation was performed using the ExionLC™ AD System which was selected as it combines near zero carryover and full UHPLC capabilities to provide world-class performance under the most demanding analytical conditions. The column used was a Phenomenex Kinetex Polar C18 2.6µm, 100 x 2.1mm. Details are outlined in Table 1.

Table 1. Gradient profile and mobile phase composition.

Total Time (min)	Flow Rate (µL/min)	A%	B%
0.0	400	98	2
0.5	400	98	2
0.6	400	80	20
1.5	400	80	20
14	400	20	80
14.1	400	2	98
18	400	2	98
18.1	400	98	2
22	400	98	2

Mobile Phase A: H_2O +0.1% Acetic Acid + 5mM Ammonium Formate
Mobile Phase B: Acetonitrile +0.1% Acetic Acid + 5mM Ammonium Formate

Mass spectrometry: These experiments were performed using the SCIEX Triple Quad 5500+ LC-MS/MS System λ QTRAP Ready. This highly sensitive instrument incorporates the Turbo V™ Ionization Source which has the power and efficiency to easily handle the dirty matrices associated with mycotoxin analysis. Data was acquired using Analyst® Software 1.7.1. Table 2 outlines the MS conditions used.

Data processing: Data was processed using SCIEX OS-Q Software.

Table 2. Source and key MS parameters.

Parameter	Positive mode	Negative mode
CAD	8	8
CUR	25 psi	25 psi
GS1	60 psi	60 psi
GS2	70 psi	70 psi
IS	5500 V	-4500 V
TEM	400°C	400°C
Pause Time	3 msec	
Target Scan Time	0.5 sec	
MRM detection window	12 sec	
Number of MRMs	582	458
Switching Time	Tested 5, 10, 15, 50 msec (5msec used in final method)	

Analytical targets

This study analyzed a large suite of mycotoxins, masked mycotoxins and their metabolites in corn and barley extracts. The test was to observe trace detection and quantitation and to evaluate how the SCIEX Triple Quad 5500+ System handles a sample batch representative of the high throughput demands of the food testing industry.

The crucial elements of high-throughput mycotoxin analysis to beassessed were (in no particular order) linearity, sensitivity, data points, reproducibility, carryover, and robustness.
An acquisition method of 1040 MRM transitions was created using the Scheduled MRM™ Algorithm in Analyst® Software 1.7 to ensure the best quality data was acquired. This acquisition method combined both positive and negative polarity experiments. Approximately 40% of the target analytes require negative ionization.

Transforming samples into results

To process such a large amount of data, SCIEX OS-Q Software was used. SCIEX OS-Q Software is an integrated platform which can process high-throughput screening of time scheduled quantitative MRM data. The software can be customized to relay the processed data to the operator in a format which is familiar and appropriate to all analytical requirements.

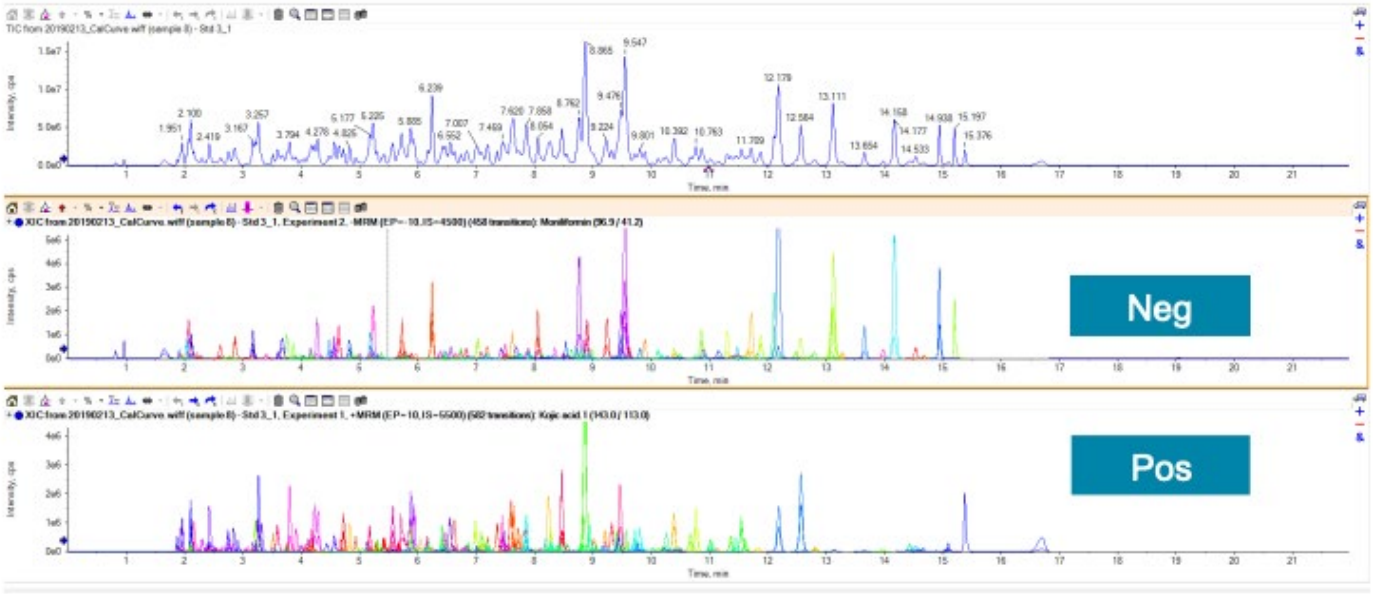

Figure 2. Chromatography for analyzing 530 mycotoxins. 1040 transitions for 530 mycotoxings were monitored using the Scheduled MRM Algorithm in Analyst Software 1.7. All analytes within the method are detected within a 16-minute window. The remaining time on the run is used to wash and equilibrate the system for the next injection.

Evaluation of switching time

Very good separation was achieved for the mycotoxins using the Phenomenex Kinetex Polar C18 2.6µm, 100 x 2.1mm (Figure 2). All analytes eluted within a 16 min window and the analytes were well spread out across the time window. There were 458 analytes run in negative ionization mode and 582 analytes requiring positive ionization mode.

In Figure 3, an example of different switching times for some Toxins on the SCIEX Triple Quad 5500+ System is shown. This extracted ion chromatogram (XIC) illustrates the compounds which use 50, 15, 10 and 5 msec polarity switching times. Here, extracted responses for Aflatoxin M1, Aflatoxin G1, Ochratoxin B, Aflatoxin B1, Ochratoxin A, and T-2 Toxin are shown. No significant differences either on signal intensity or signal/noise are observed. So, the method is usin g 5 msec switching time was selected for the final method.

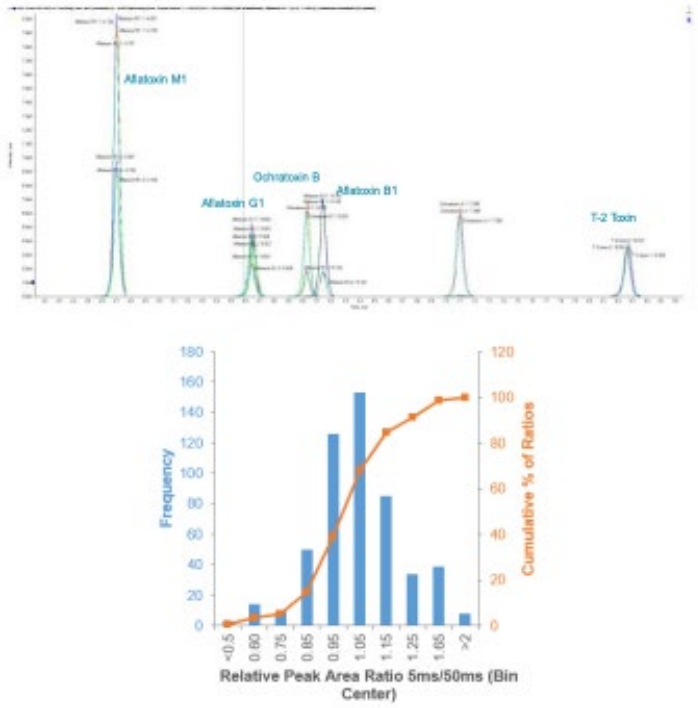

Figure 3. Minimal impact on peak area with fast polarity switching. (Top) XICs of select mycotoxins showing the peak areas at the various polarity switching times. (Bottom) Majority of peak area ratios between 5 and 50 msec showed equivalent or better data with 5 msec switching, with 85% of the analytes having 90% or better peak areas of that observed for 50 msec data.

Quantitative accuracy

Acceptance criteria will stipulate the minimum number of data points required for acquired results to be confirmed. Figure 4 illustrates the number of data points and Retention Time (RT) acquired across key positive compounds.

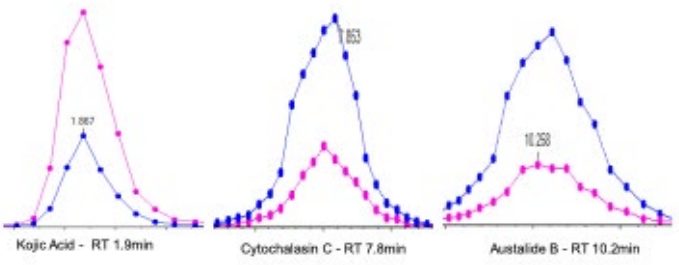

Figure 4. Good peak sampling for positive mode compounds. Kojic Acid, Cytochalasin C and Austalide B show sufficient data point coverage for confirmation reporting of results.

As detailed earlier in this application note, approximately 40% of the compounds in this acquisition list are ionized in negative mode. This method has 1040 sMRM transitions, so the 5 msec polarity switching time is critical to attaining comprehensive coverage and data quality in both positive and negative modes. The figure below (Figure 5) details the number of data points, and RT acquired for a selection of negative mode amenable mycotoxins.

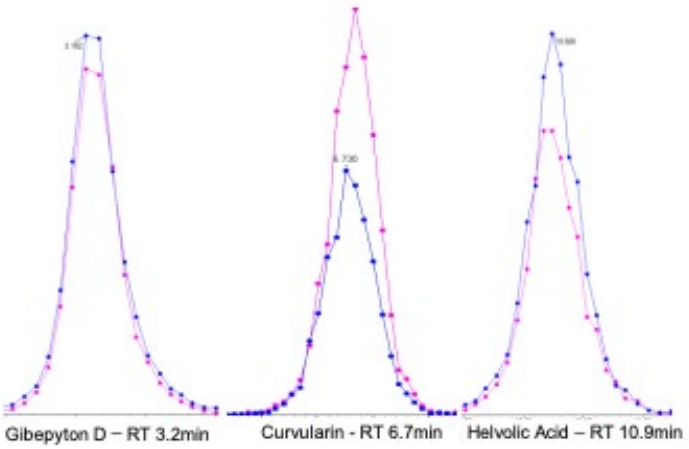

Figure 5. Good peak sampling for negative mode compounds. Gibepyton, Curvularin, and Helvolic Acid show sufficient data point coverage for confident result submission.

The SCIEX Triple Quad 5500+ λ QTRAP Ready LC-MS/MS System has demonstrated that even with a very large number of target compounds, the accuracy and number of data points acquired is sufficient to enable confident and accurate quantitation of mycotoxins, masked mycotoxins, and their metabolites.

All other compounds across the batch also showed sufficient data points to ensure accurate quantitation of mycotoxins in complex matrices such as the corn and barley that have been tested in this study.

Sensitivity, linearity and ion ratios

The sensitivity and linearity of calibration standards is essential to any analysis. Using the AutoPeak algorithm delivers consistent integrations that reduce the need for manual intervention. The Automatic Outlier Removal Algorithm calculates linear regression and highlights any point that fails to meet user-specified rules. This reduces the need for hands-on analysis and minimizes the time taken to create calibration curves. These features are unique to the SCIEX OS Software platform. The following are examples of calibration curves (Figure 6 and 7) which detail the range and various coefficients. Also in the report are the ion ratio qualification guides which are essential to delivering confirmed and approved positive results.

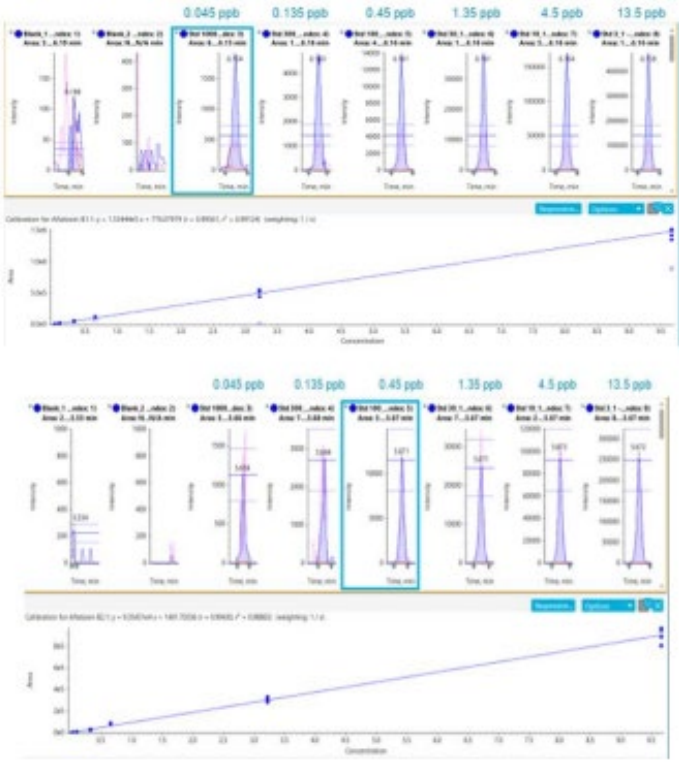

Figure 6. Standard concentration curves. The sensitivity and linearity achieved for Aflatoxin B1 (top) and Aflatoxin B2 (bottom) Aflatoxin B2 is shown.

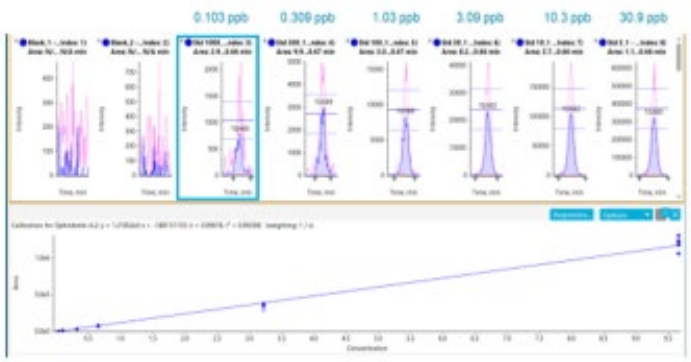

Figure 7. Standard concentration curve. The sensitivity and linearity achieved for Ophiobolin A is shown,

Repeatability

Having a method which consistently delivers the same quality and accuracy for every injection of every batch is imperative to delivering reliable analytical results. During this experiment, a series of replicate injections were run in order to evaluate the reproducibility of the method and SCIEX Triple Quad 5500+ System.

5 injections were made per standard and the Percent Coefficient of Variance (%CV) and the Accuracy for each concentration were calculated. Figure 8 highlights the results for two representative analytes.

		Mean	Standard Deviation	Percent CV	Accuracy
Aflatoxin B1.1	Std 1000_1	3.077e-2	3.281e-3	10.66	102.58
Aflatoxin B1.1	Std 300_1	1.058e-1	9.051e-3	8.55	105.80
Aflatoxin B1.1	Std 100_1	3.254e-1	2.886e-2	8.87	101.68
Aflatoxin B1.1	Std 30_1	7.472e-1	4.892e-2	6.55	114.95
Aflatoxin B1.1	Std 10_1	3.341e0	3.159e-1	9.46	103.74
Aflatoxin B1.1	Std 3_1	9.436e0	4.881e-1	5.17	97.68

		Mean	Standard Deviation	Percent CV	Accuracy
Pentoxyfylline.1	Std 1000_4	2.957e-2	2.531e-3	8.56	98.56
Pentoxyfylline.1	Std 300_4	9.488e-2	5.866e-3	6.18	94.88
Pentoxyfylline.1	Std 100_4	3.027e-1	3.005e-2	9.93	94.60
Pentoxyfylline.1	Std 30_4	7.341e-1	3.877e-2	5.28	112.94
Pentoxyfylline.1	Std 10_4	3.345e0	1.152e-1	3.44	103.88
Pentoxyfylline.1	Std 3_4	9.479e0	2.717e-1	2.87	98.13

Figure 8. Mycotoxin repeatability. Using SCIEX OS-Q Software, all necessary calculations are performed automatically. Here the %CV and accuracy across 5 replicates are computed for the standards 3x to 1000x. (Top) For Aflatoxin B1, all dilutions had %CV and accuracy well within the required limits. (Bottom) Pentoxifylline, a compound used in treating mycotoxin poisoning shows excellent %CV in this large multi- analyte method.

Sample Name	Component Group Na...	Accur...	Ion R...	Form...	Precu... Mass	Ion Ratio Confide...
Blank_3	Penicillic acid	N/A	N/A		171.200	
Matrix Barley 1:10 300	Penicillic acid	N/A	0.3152		171.200	✓
Matrix Corn 1:10 300	Penicillic acid	N/A	0.3241		171.200	✓
Blank_3	Penicillic acid	N/A	N/A		171.200	
Matrix Barley 1:10 300	Penicillic acid	N/A	0.3551		171.200	✓
Matrix Corn 1:10 300	Penicillic acid	N/A	0.3048		171.200	✓
Blank_3	Penicillic acid	N/A	N/A		171.200	
Matrix Barley 1:10 300	Penicillic acid	N/A	0.3069		171.200	✓
Matrix Corn 1:10 300	Penicillic acid	N/A	0.3202		171.200	✓
Blank_3	Penicillic acid	N/A	N/A		171.200	
Matrix Barley 1:10 300	Penicillic acid	N/A	0.2820		171.200	✓
Matrix Corn 1:10 300	Penicillic acid	N/A	0.3494		171.200	✓

Sample Name	Component Group Na...	Accur...	Ion R...	Form...	Precu... Mass	Ion Ratio Confide...
Blank_3	Xanthotoxin	N/A	N/A		217.00	
Matrix Barley 1:10 300	Xanthotoxin	N/A	1.0361		217.00	✓
Matrix Corn 1:10 300	Xanthotoxin	N/A	0.9838		217.00	✓
Blank_3	Xanthotoxin	N/A	N/A		217.00	
Matrix Barley 1:10 300	Xanthotoxin	N/A	0.7877		217.00	✓
Matrix Corn 1:10 300	Xanthotoxin	N/A	0.8568		217.00	✓
Blank_3	Xanthotoxin	N/A	N/A		217.00	
Matrix Barley 1:10 300	Xanthotoxin	N/A	1.0623		217.00	✓
Matrix Corn 1:10 300	Xanthotoxin	N/A	0.9519		217.00	✓
Blank_3	Xanthotoxin	N/A	N/A		217.00	
Matrix Barley 1:10 300	Xanthotoxin	N/A	1.2499		217.00	✓
Matrix Corn 1:10 300	Xanthotoxin	N/A	1.0897		217.00	✓

Figure 9. Ion ratios for confirmation of detection. (Top) Penicillic acid was positively confirmed using ion ratios at all of the concentrations in matrix tested. (Bottom) Similarly, the ion ratio confirms Xanthotoxin detection in the matrix.

Testing the unknown

As part of this experiment, a series of extracts were performed on regular corn and barley extracts, with no known mycotoxins present in the samples. The same extraction process detailed earlier in this application note was used, and standards were then spiked into the matrix extracts at a low concentration around the regulatory limits. The extracts were run in this batch and processed in SCIEX OS-Q Software against the standard calibration curves. Shown in Figure 9, as examples, are the results for penecillic acid and xanthotoxin, detected at the regulated concentration level in both the corn and barley matrices. All positive results were confirmed in SCIEX OS-Q Software with the ion ratio along with ion ratio confidence tool which allows a rapid review of results to confirm positive identifications.

The MRM chromatograms for aspinonene clearly showing both the quantitative and confirmatory transitions, and the blank response (Figure 10). This increases user confidence in results by providing a fast, visual cross check of both positive and negative results as well as a good indication of analytical performance since there is no observed carry-over.

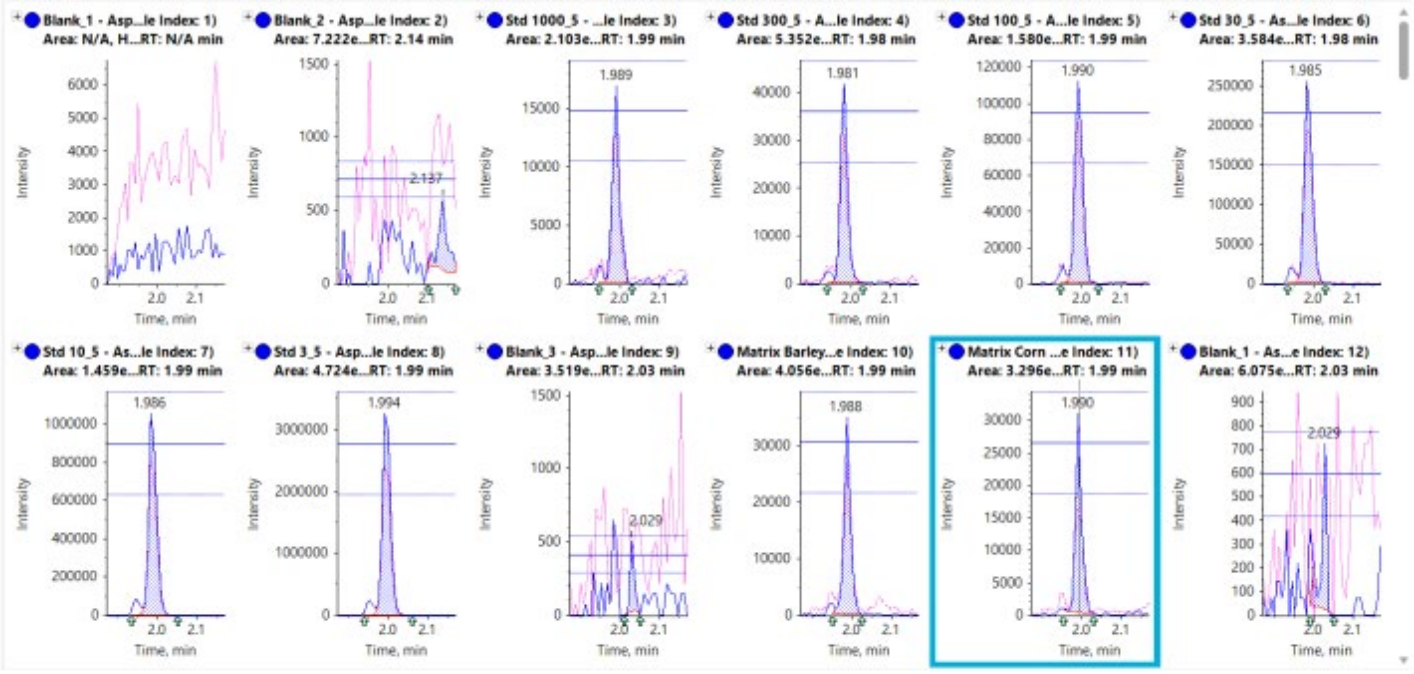

Figure 10. Visualization of results in SCIEX OS-Q Software. The AutoPeak algorithm has integrated the positively confirmed peaks for Aspinonene. Within SCIEX OS-Q Software, both transitions are overlaid along with the confirmatory ion ratio lines for quick review of data.

Injection order was: blank, blank, standards with 6 different concentrations, blank, barley as a matrix, corn as a matrix, blank.

This workflow demonstrates the key robustness and detection levels required for the screening of corn and barley for mycotoxins, masked mycotoxins, and their metabolites. With very little adjustment, this method can be adapted to the analysis of other matrices of interest such as milk, other grains, and ingredients.

Conclusions

This study has shown that the SCIEX Triple Quad 5500+ LCMS/MS System λ QTRAP Ready delivers high-quality quantitation of mycotoxins, masked mycotoxins and their metabolites. The method combines both positive and negative polarity electrospray ionization modes into one comprehensive quantitation/screening method which covers an unmatched range of analytes without any sacrifice or compromise to data quality and integrity.

Data quality is paramount to delivering reliable results. Assurances of data quality are found in the confirmatory ion ratio scoring and confidence reports. These reduce the risk of reporting false positive results which could have impact outside of the laboratory.

The scope for further enhancing data quality for this and many other methods on this instrument can be achieved with a simple license activation of the QTRAP System technology. By unlocking the power of the QTRAP System functionality, laboratories can incorporate additional scans such Enhanced Product Ion for spectral confirmation and MRM3 which enables MS/MS/MS analysis to effectively negate the problems often associated with matrix suppression.

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Mycotoxins

Technical content summaries

Quantification of Aflatoxin M1 in Milk Using the SCIEX QTRAP 4500 System

India, Germany

Aflatoxin M1 is a toxin that can be found in milk and milk products due to contaminated feed. This toxin can cause serious health effects in children, resulting in stringent monitoring requirements for aflatoxin M1, but determination is difficult due to the complexity of the sample matrix. This technical note describes a robust method developed using the QTRAP 4500 system for the quantitation of aflatoxin M1 in milk. The method was validated according to the European Commission Decision 2002/657/EC and meets local India guidelines for maximum residue levels of this toxin—0.5 ppb (500 pg/mL)—in milk.

Simultaneous analysis of 26 mycotoxins in grain on a SCIEX Triple Quad 3500 system

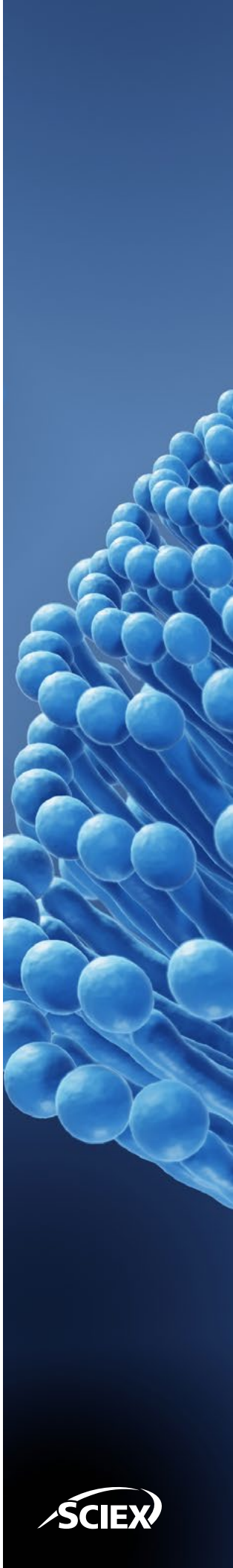
China, Singapore

Fungi are known to produce secondary metabolites, such as mycotoxins, that contaminate agricultural commodities and pose a threat to human health and animal safety. For this reason, many countries have regulations in place for mycotoxin detection and their permissible limits. This technical note—which focuses on mycotoxin limits in China and testing regulations that require using confirmatory techniques—describes a single workflow to analyze 26 compounds simultaneously that was validated in different grain matrices.

Analysis and Quantification of Mycotoxins in Cereals Using a Time-Scheduled MRM^{HR} workflow

China, England

Abnormal climate conditions and insect damage have caused extensive mycotoxin contamination of grain in recent years, leading to severe damage, huge losses and threats to human and animal health. This technical note describes the detection of common mycotoxins in grain using a targeted high-resolution MRM approach for quantitative product ion detection, qualitative product ion detection and qualitative confirmation at high scanning speeds. Using wheat and corn as testing matrices, this work demonstrated mycotoxin prevalence and its contamination of grain samples, highlighting the need for greater monitoring and oversight.



A robust and sensitive method for the direct analysis of polar pesticides in food and environmental samples without derivatization

Wim Broer1 , Ugo Chiuminato2 , Jianru Stahl-Zeng2, Daniel McMillan3 and Phil Taylor3
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.

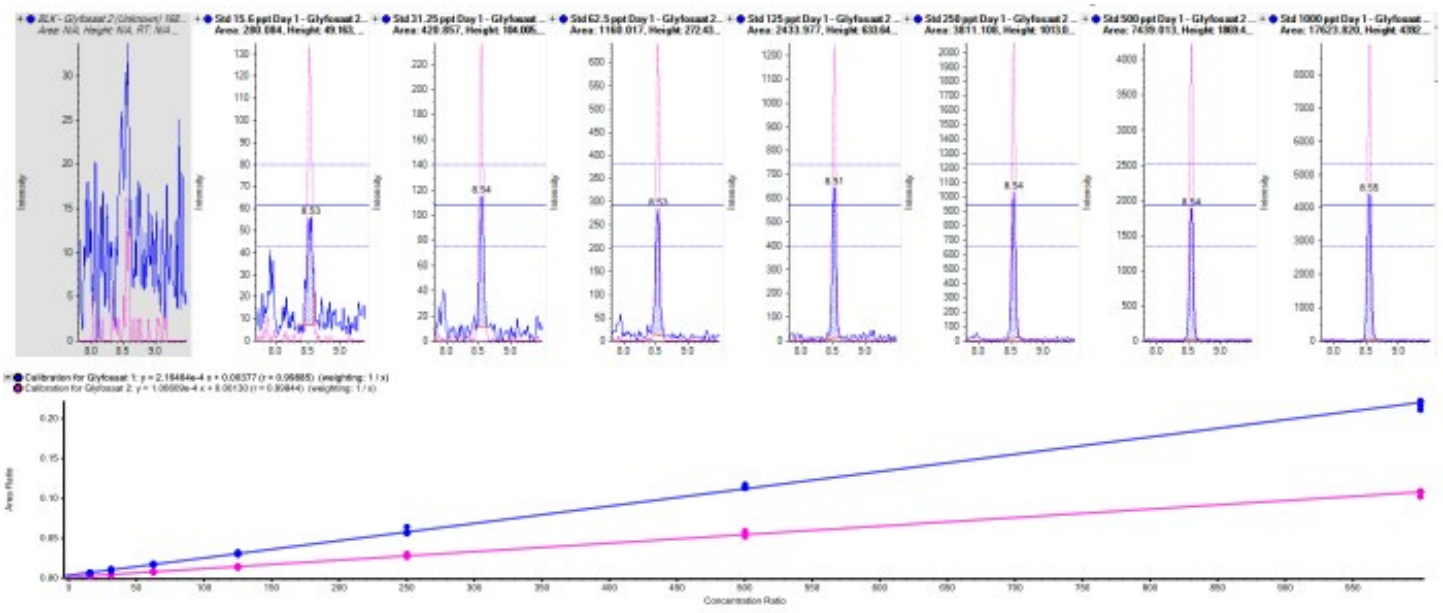


Figure 1. Method sensitivity and linearity of glyphosate. Calibration standards in concentrations from 15.6 to 1000 ng/L of glyphosate achieved using the modified method for water samples. Ion ratios were all well within the specified ±20% tolerance.

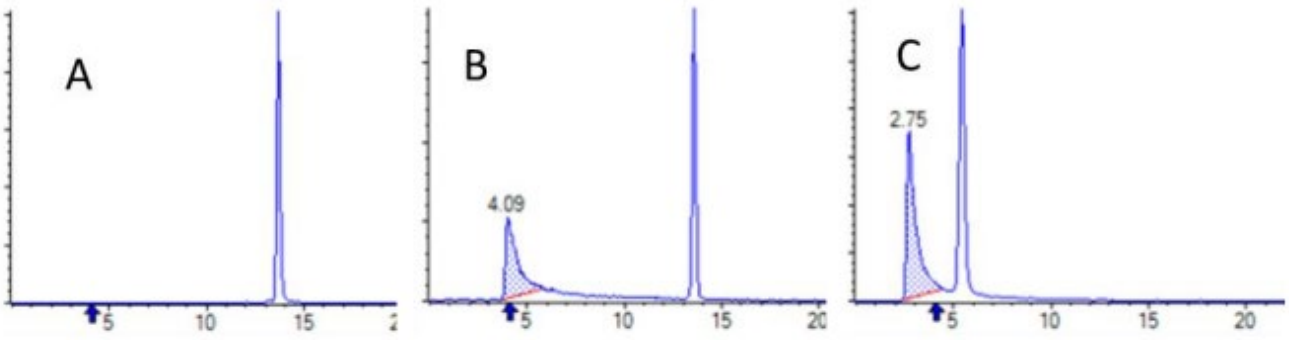


Figure 2. Use of Hypercarb Column means: Install, Prime, Repeat, and finally Replace. Image A shows the performance of the hypercarb column after installation, no glyphosate peak is present. Image B shows the same column after it has been conditioned with 30 spinach extracts, a glyphosate peak can be 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 itimpractical in a busy lab performing primarily reverse-phase LC.

So, the final method, presented here, makes use of an IC 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-SANTE1 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 Stuttgart2 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-A03 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.

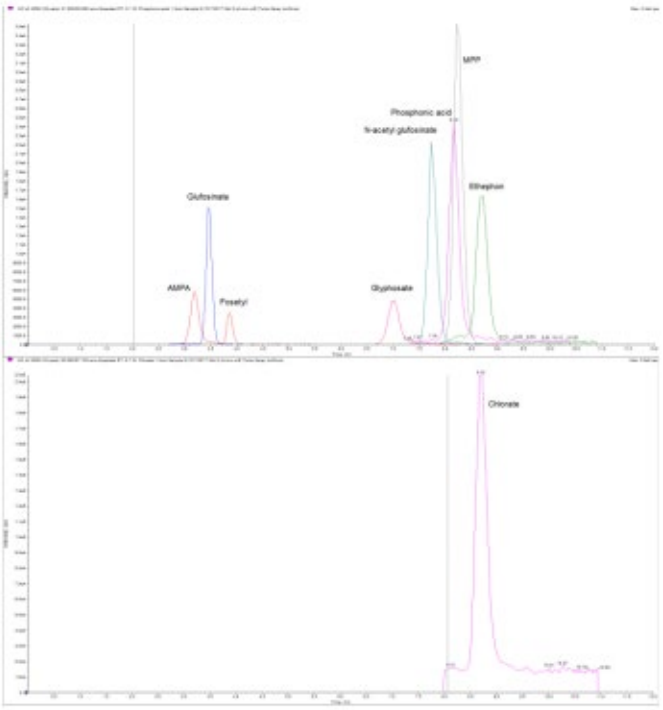


Figure 3. Example chromatograms shown for polar pesticides suite. Chromatographic separation using the hypercarb column was an integral component of the described method.

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

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 compound4 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.

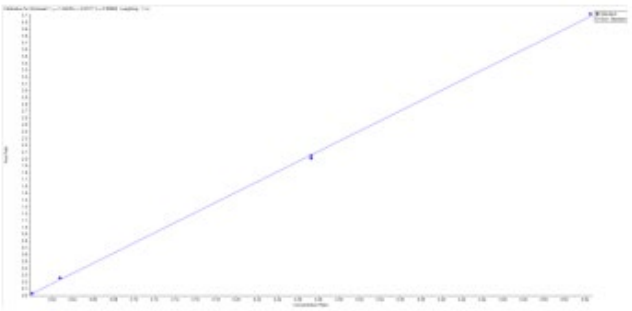


Figure 4. Glyphosate calibration standards. Linear calibration regression for glyphosate with 1/x weighting, showing r-value of 0.9997 and excellent precision for duplicate calibrators.

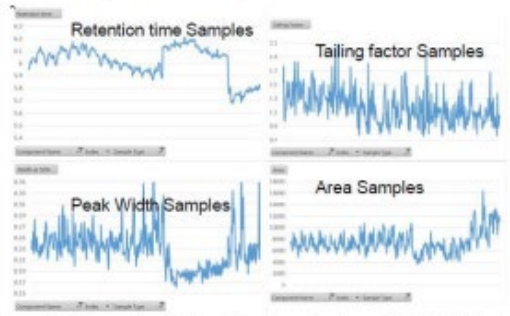


Figure 5. Reproducibility data for glyphosate IS. NofaLab method for food samples, tested over 1000 injections of extracts from fruit/veg, seeds, veg oil/fat, grains, herbs/spices, meat/fish, animal oil/fat, eggs/egg products, milk/milk products and other fatty acids.

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.

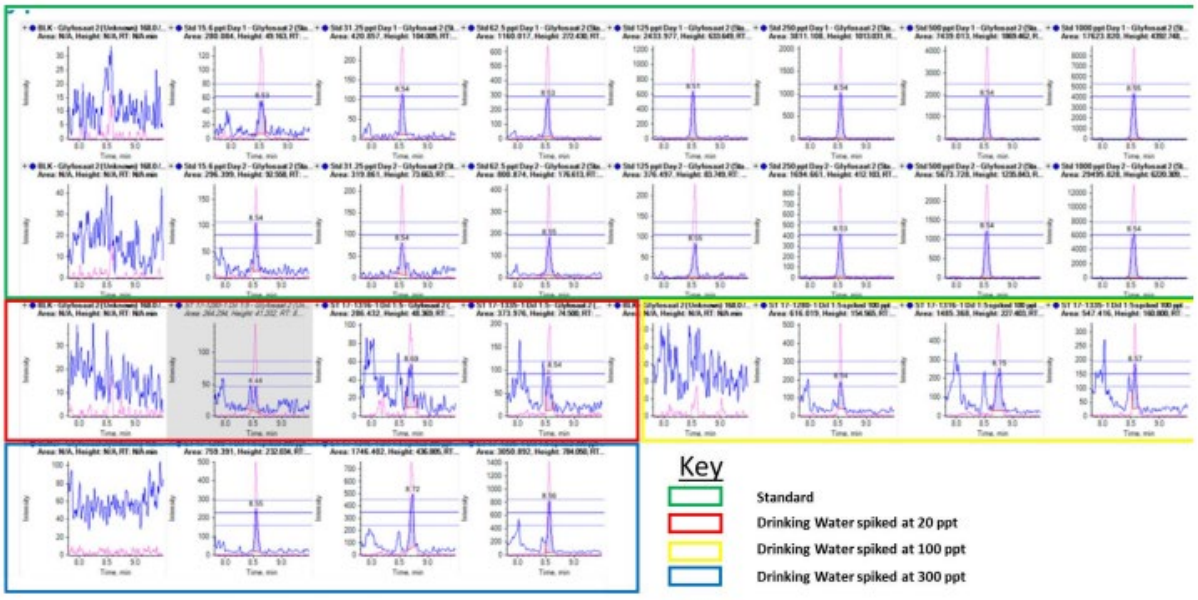


Figure 6. Example chromatography from drinking water samples using the modified water method.

Pesticides

Technical content summaries

Highly sensitive quantification and selective identification of pesticides in food with Zeno MRM^{HR}

Canada, Spain, Germany

The safety of the global food supply requires adherence to pesticide maximum residue limits set by governing bodies around the world. In the workflow described here, Zeno MRMHR is used to quantify low levels of pesticides in olive oil and various fruits and vegetables to meet global regulations for pesticide residues. The selectivity and specificity afforded by the accurate mass MS/MS quantitation on the ZenoTOF 7600 system allowed for highly confident identification of pesticides in real food samples (through MS and MS/MS mass accuracy, library matching and isotope ratio matching) while maintaining triple quadrupole-like ability to perform ion ratios for confirmations.

Quantification of Acetamiprid and Prochloraz in Black Pepper with the 3500 System

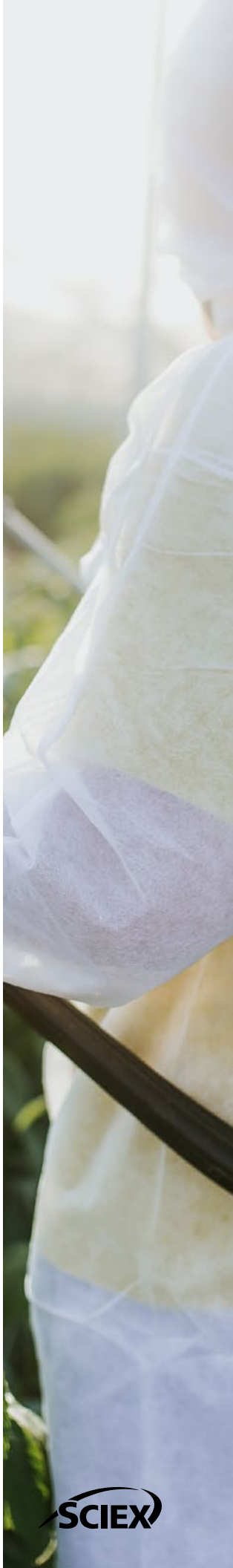
India, Germany

Single digit ppb levels were achieved on the 3500 System, below the Maximum Residual Limit (MRL) outlined by FSSAI (10ppb) and the European Union (100ppb). This method performed as per commission decision SANTE/11813/2017 directive recommendations and fulfils regulatory requirements fort sensitivity, prevision, and accuracy. Monitoring pesticide residues in pepper samples have never been easier.

Precise testing of pesticides in food using the SCIEX Triple Quad™ 7500

Canada, Germany, England, Holland

The SCIEX Triple Quad 7500 LC-MS/MS System - QTRAP Ready provides impressive levels of sensitivity, robustness and accuracy for trace level analysis of pesticide residues in food matrices. Here, over 1400 MRM transitions for 700 compounds were analyzed in a single analysis achieving quantification limits of 0.2 ng/mL for the majority of the pesticides tested.



Ultra-high sensitivity quantification of veterinary drug residues in products of animal origin

Using the SCIEX 7500 system

Jack Steed1, Yoann Fillatre2, Michael Scherer3, Aline Staub Spörri4 , Grégoire Bonvin4 and Jianru Stahl-Zeng5 SCIEX, UK; 2SCIEX, France; 3SCIEX, Switzerland; 4Official Food Control Authority and Veterinary Affairs of Geneva; 5SCIEX, Germany

The use of pharmacologically active substances in veterinary settings has been scrutinized for several years due to their sometimes inappropriate or excessive use. This is particularly worrying to authorities due to possible allergic reactions and increasing antibiotic resistance in both livestock and human populations.[1] Therefore, the use of these substances must be controlled and limited to mitigate these issues. One way this control is implemented is through testing products of animal origin. In these analyses, compounds of interest have a set maximum residue limit (MRL), intended to reduce the levels of these compounds. Some compounds have been prohibited altogether, due to their inherent toxicity. While performing this analytical testing, it is therefore crucial to achieve very low LLOQ values to limit the misuse of compounds in the food industry.[2] Outside of this, minimum method performance requirement (MMPR) values have also been set for certain compounds to ensure than an acceptable level of sensitivity is reached.[3]

Here, a method that reached LLOQ values as low as 0.005 ng/mL was developed and implemented to analyze more than 180 relevant compounds used in the veterinary industry

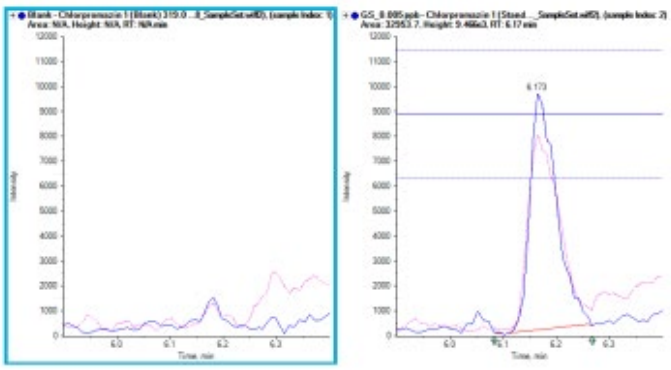


Figure 1. Overlaid XICs for chlorpromazine in solvent. Both the quantifier (blue) and qualifier (pink) MRM transitions for chlorpromazine are shown in the solvent blank (left) and in solvent at the LOQ of 0.005 ng/mL (right). The LLOQ image shows 2 MRM transitions for chlorpromazine overlaid with ion ratio lines that outline the acceptable height (±30%) of the qualifier peak.

Figure 1 highlights the sensitivity of the SCIEX 7500 system for the analysis of chlorpromazine, which is prohibited by the European Union[4] Tables 1 and 2, included at the end of this technical note, provide an overview of the LLOQs, precision data and linear ranges achieved for each compound analyzed.

Key features the SCIEX 7500 system for the analysis of veterinary drug residues in products of animal origin:

> The sensitivity of the SCIEX 7500 system allows for LLOQ values as low as 0.005 ng/mL

> Demonstrated ability to analyze more than 180 relevant compounds in pork, chicken and milk food matrices, at concentrations as low as 0.01 µg/kg in pork and chicken and 0.005 µg/kg in milk

> The use of quantifier and qualifier transitions allows for the use of ion ratios to increase specificity of the analysis

> Linear dynamic ranges span up to 4 orders of magnitude

> Scheduled MRM algorithm ensures accurate quantification with enough data points for each compound analyzed.

Methods

Standard preparation: A mixed standard solution was provided by the official food control authority and veterinary affairs of Geneva at a concentration of 1 µg/mL. A series of dilutions was performed to cover a range of concentrations from 0.005 to 100 ng/mL.

Sample preparation: Each sample was homogenized prior to weighing. A 5 g sample of pork, milk or chicken was combined with 0.1M EDTA-McIlvaine buffer (4 mL for pork and chicken, 3 mL for milk). Pork and chicken samples were then homogenized for 75 seconds using a FASTH21, whereas milk samples were homogenized for 10 minutes using a mechanical shaker. Pork and chicken solutions were then combined with 16 mL acetonitrile and 5 g ammonium sulfate. Milk solutions were combined with 8 mL acetonitrile and 5 g ammonium sulfate. All samples were then further homogenized and centrifuged for 5 minutes at 4,700 rpm at 4°C. A 4 mL sample of the upper layer was removed and transferred into an evaporative vial with 400 µL of DMSO. Nitrogen was used to evaporate the sample at 40°C until a final weight of approximately 0.5 g was achieved. Then, 1 g of H2O was added to each solution before vortexing for approximately 15 seconds, centrifuging for 5 minutes at 13,000rpm and filtering through a 0.45 µm nylon filter prior to analysis.

Spiked sample preparation: 90 µL of prepared pork, chicken or milk matrix sample was added to 10 µL of a relevant standard solution. Three spike concentrations were prepared at 0.01, 0.1 and 1 ng/mL with standard solutions at 0.1, 1 and 10 ng/mL being used as spiking solutions.

Chromatography: An ExionLC AD system was used to perform the chromatographic separation with a Phenomenex Kinetex Polar C18 (2.6 µm, 100 Å, 100 x 2.1 mm) column.

Mass spectrometry: The analysis was performed using the SCIEX 7500 system. The system was operated using the timescheduled multiple reaction monitoring (sMRM) mode (see Figure 2) using positive and negative electrospray ionization (ESI) switching. QOD optimization was performed to enhance the signal of some compounds. [5] Data were acquired using SCIEX OS software.

Data processing: Data were processed using SCIEX OS software with the AutoPeak algorithm for peak integration.[6]

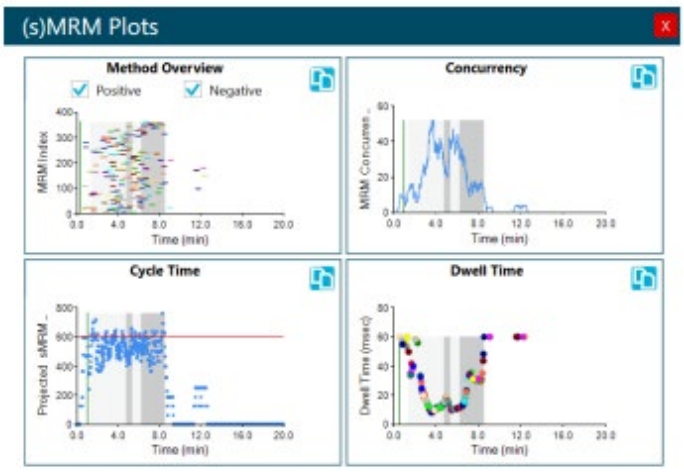


Figure 2. Scheduled MRM algorithm plots in SCIEX OS software. The scheduled MRM algorithm enables the analysis of hundreds of compounds in a single analysis with high quantitative accuracy. Each MRM transition is acquired during a short retention time window around its known elution time. This allows more MRM transitions to be monitored in a single LC run, while still maintaining maximized dwell times and optimized cycle times.[6] In SCIEX OS software, new visualization plots are available to help with method development and improve overall data quality.

Chromatographic separation

When analyzing many compounds in a single analytical method, chromatographic separation is an important factor to ensure that the number of compounds analyzed at a particular time is minimized. This allows the balance between the cycle time of the mass spectrometer and the dwell time for each analyte to be optimal. Figure 3 shows the chromatographic separation achieved in both positive and negative ion acquisition modes. It is important to note that baseline separation of compounds is not necessary in most cases, due to the specificity of a triple quadrupole mass spectrometer operated in MRM acquisition mode. Chromatography helps to minimize analyte concurrency and to reduce interferences that may occur from the sample matrix.

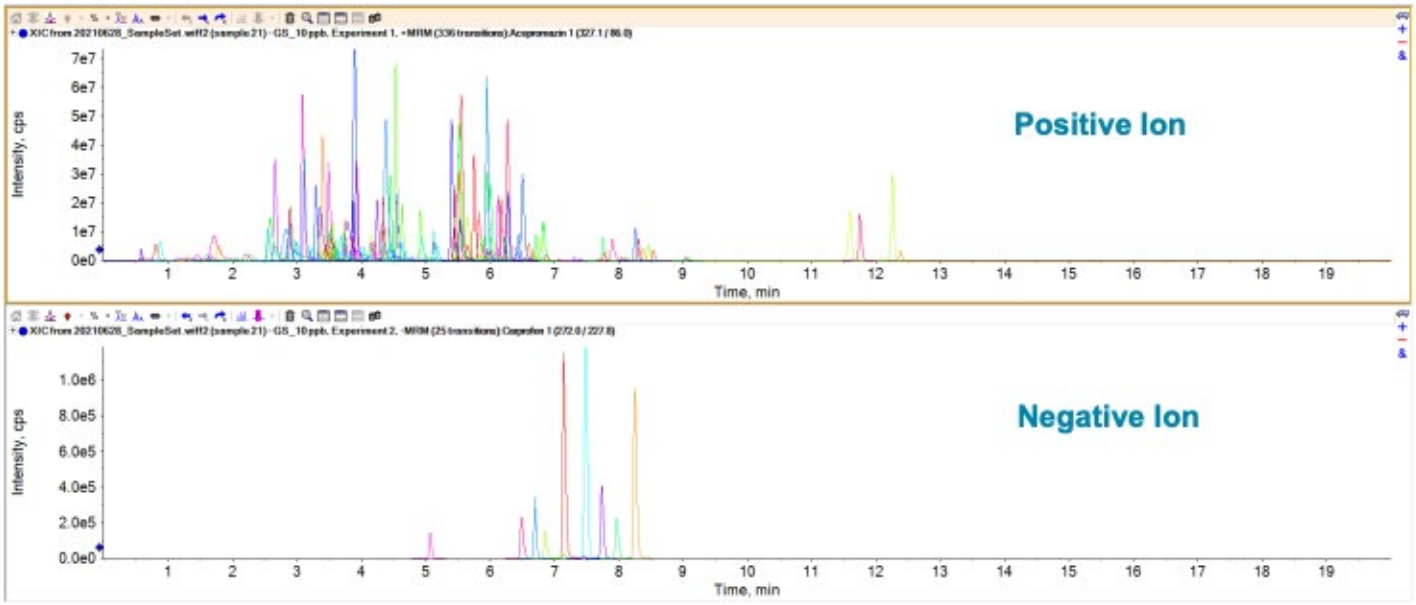


Figure 3. Positive and negative XIC overlays of all compounds analyzed. The above image illustrates the chromatographic separation achieved using the method, allowing for accurate quantification of each compound by achieving enough data points across each peak. Figure 6 highlights the amount of data points achieved for a subset of the compounds analyzed.

Sensitivity and specificity

Sensitivity and specificity are crucial for a successful analysis and triple quadrupole mass spectrometers are typically used to achieve high levels of these metrics. Figure 4 highlights the levels of sensitivity that can be achieved using the SCIEX 7500 system for 3 representative analytes at their respective LLOQs of 0.005 ng/mL in solution. The blank injection is free of interferences, therefore indicating that the peak observed at the LLOQ is genuine. The use of quantifier and qualifier MRM transitions enhances the specificity of the analysis in matrix by using ion ratios to better confirm the identity of the peak, relative to a single MRM transition. The ion ratio lines highlight the acceptable range within ±30% of the expected value for the qualifier peak.

Although sensitivity in solvent is an important factor for analysis, it is crucial to maintain the quality of analyses in complex matrices. The same 3 compounds analyzed in solvent in Figure 4 were spiked into the pork, milk and chicken matrices. The results of these analyses are shown in Figure 5. The XICs shown are for the lowest spike level analyzed (0.01 ng/mL), which equated to 0.01 µg/kg of compound in the pork or chicken samples and 0.005 µg/kg of compound in milk. These results demonstrate the ultra-high level of sensitivity that can be achieved in this assay.

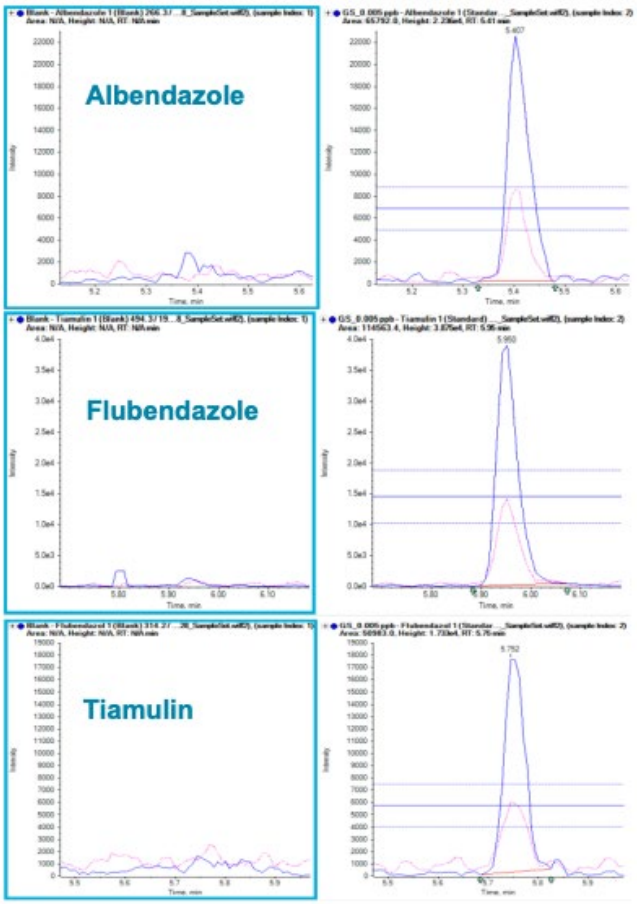


Figure 4. Overlaid XICs of the quantifier (blue) and qualifier (pink) MRM transitions of albendazole (top), flubendazole (middle) and tiamulin (bottom). The above images show the respective solvent blank (left) for each compound alongside the MRM transitions obtained at the LLOQ (right). Each compound achieved an LLOQ of 0.005 ng/mL.

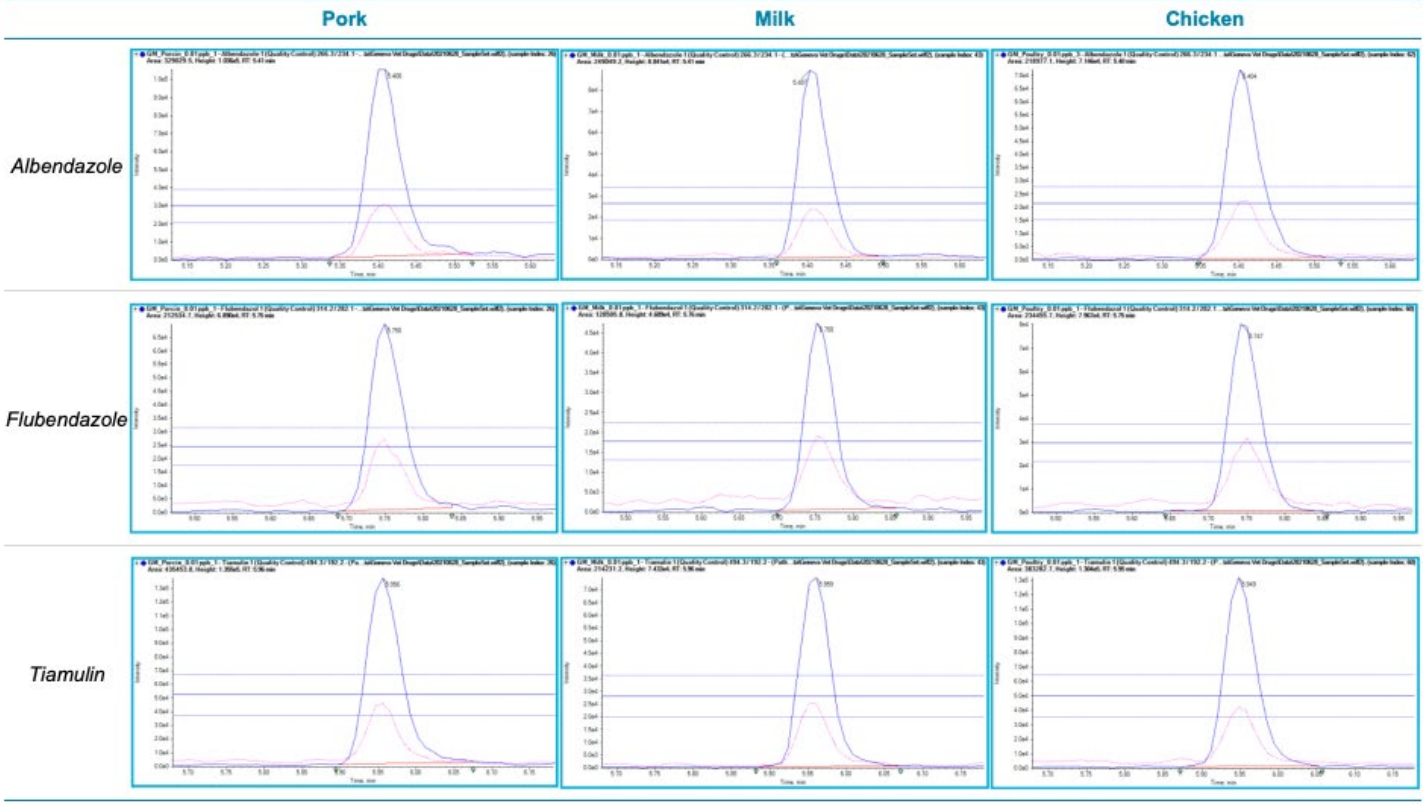


Figure 5. XIC overlays of 3 analytes spiked into 3 different food matrices at the lowest spike concentration analyzed (0.01 ng/mL). Quantifier and qualifier MRM transitions are shown in blue and pink, respectively, with ion ratio lines. The concentration analyzed equates to 0.01 µg/kg in sample for pork and chicken and 0.005 µg/kg for milk.

Linear dynamic range

The linear range of an analyte is the span at which quantification is possible while achieving acceptable levels of precision and accuracy. Each calibration curve analyzed achieved an r value >0.99 with accuracy between ±30% of the expected value. The linear dynamic ranges are indicated in Tables 1 and 2, included at the end of this technical note, for compounds analyzed in positive and negative ion modes, respectively. Representative examples of 3 compounds with linear dynamic ranges spanning 4 orders of magnitude are shown in Figure 6.

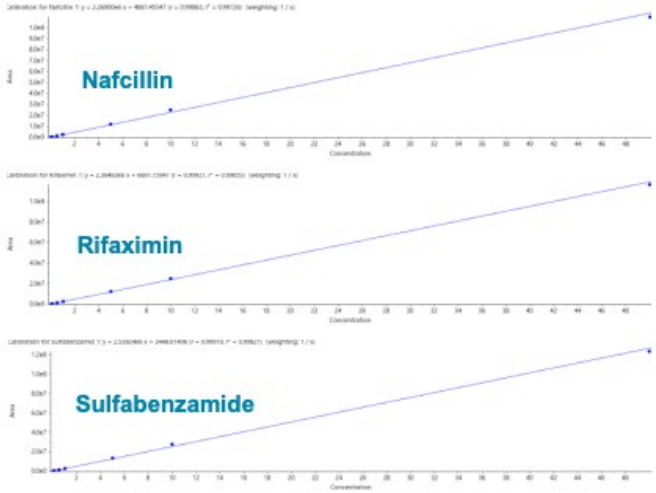


Figure 6. The linear ranges for 3 of the compounds analyzed. Each compound had a linear dynamic range that spanned 4 orders of magnitude, between 0.005 and 50 ng/mL. The r value for each compound was >0.99.

Accurate and precise quantification

To ensure accurate and precise quantification, individual peaks must have enough data points. For the compounds analyzed, each peak consisted of more than 10 data points. Representative quantifier and qualifier ion MRM transitions are shown in Figure 7 for 3 analytes at a concentration of 10 ng/mL in solution.

Accuracy was assessed at each point of the calibration curves. Accuracy values for all analytes were between ±30% of the expected value at each concentration.

Precision is paramount to show the consistency of the instrument for analyses in solution and in sample. Table 3 outlines the %CV values achieved for the 3 compounds highlighted in Figure 7.. Precision was assessed for compounds in solvent and spiked in matrix. Precision was calculated for the lowest concentrations tested: 0.01 ng/mL in solvent, 0.01 µg/kg in pork and chicken and 0.005 µg/kg in milk.

Table 3. The calculated concentration %CV values for chlorpromazine, metronidazole and triclabendazole sulfoxide.

Compound name	Solvent	Pork	Milk	Chicken
Chlorpromazine	*4.00*	*4.15*	*5.67*	*6.60*
Metronidazole	*5.33*	*5.24*	*5.84*	*6.99*
Triclabendazole sulfoxide	*12.11*	*9.89*	*11.36*	*5.99*

Precision was assessed for each compound at 0.01 ng/mL in solvent, 0.01 µg/kg in pork or chicken and 0.005 µg/kg in milk. (N=5).

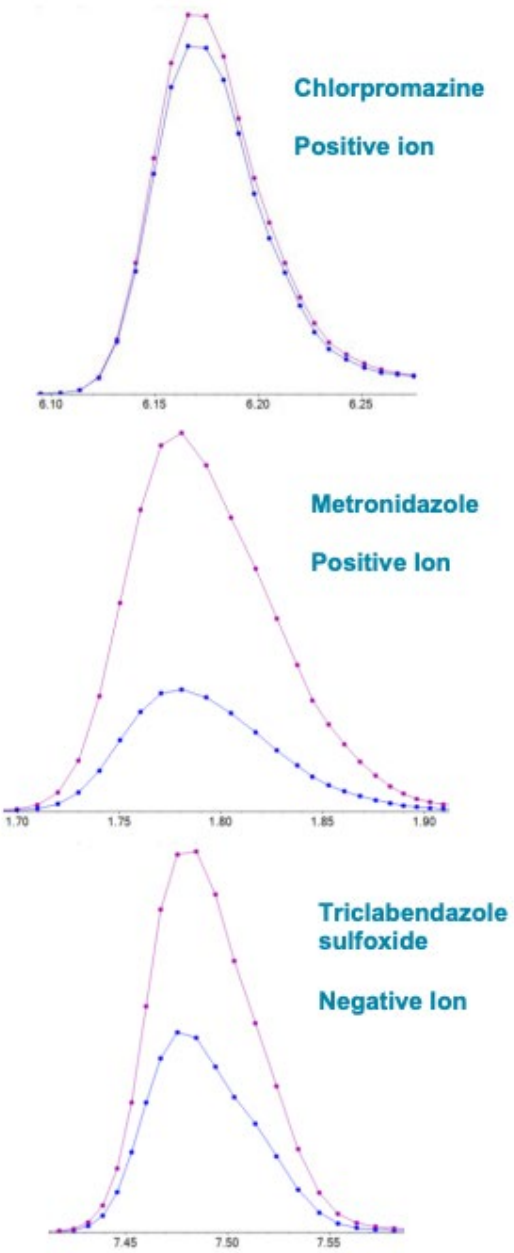


Figure 7. Overlaid XICs of quantifier (purple) and qualifier (blue) MRM transitions, highlighting the number of data points acquired for each of the 3 compounds shown. Over 10 data points were collected for both the quantifier and qualifier transitions across the peak, indicating that accurate and reproducible quantification can be performed.

Minimum method performance requirements (MMPRs)

Many of the compounds analyzed in this study are prohibited for use in veterinary settings. For these compounds, it is paramount to achieve high levels of sensitivity to ensure that they are not used illegally. Prohibited compounds do not have MRL values, as their presence at any level is not permitted. Some of these prohibited compounds have been detailed in a specific EURL guidance that defines MMPR values, which correspond to the minimum compound concentrations that should be detected by official laboratories. 3 Table 4 presents a compiled list of the prohibited compounds analyzed in this study that have MMPR values. The method developed was capable of detecting each of the prohibited compounds analyzed at concentrations lower or equal to the MMPR value.

Conclusions

An ultra-sensitive and fast method for the quantification of more than 180 pharmacologically active compounds was developed

· Analysis was performed in 3 relevant food matrices, at concentrations as low as 0.01 µg/kg for pork and chicken and 0.005 µg/kg for milk

· Linear dynamic ranges spanned up to 4 orders of magnitude without the use of internal standard

· Accurate and precise quantification were achieved, and ion ratios were used to increase the specificity of the analysis

· The assigned MRL or MMPR values were achieved for all compounds analyzed.

References

Estelle Dubreil et al. (2017) Validation approach for a fast and simple targeted screening method for 75 antibiotics in meat and aquaculture products using LC-MS/MS, Food Additives & Contaminants: Part A, 34:4, 453-468.\
2. Commission regulation (EU) No 37/2010 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, 22nd December 2009.
3. EURL guidance on minimum method performance requirements (MMPRs) for specific pharmacologically active substances in specific animal matrices – September 2020.
4. EFSA - Scientific opinion on chloramphenicol in food and feed, 26th November 2014.
5. Enabling new levels of quantification. SCIEX technical note, RUO-MKT-02-11886-A.
6. The Scheduled MRM algorithm Pro. SCIEX technical note, RUO-MKT-02-8539-A.
7. SCIEX OS software – Transforming your samples into meaningful analytical answers.
8. Commission regulation (EU) No 2019/1871, 7 th November 2019.
9. Commission implementing regulation (EU) No 2021/808, 22nd March 2021.

Table 4. A comparison of the MMPR value and the lowest quantifiable concentration in a chicken matrix. The chicken matrix was spiked at 0.01, 0.1 and 1 µg/kg.

Compound Name	Lowest quantifiable concentration (µg/kg)	MMPR value (µg/kg)
Brombuterol	*0.1*	*0.1*
Carbadox	*0.1*	*5*
Cimbuterol	*0.01*	*0.1*
Clenbuterol	*0.1**	*0.1*
Clenproperol	*0.1**	*0.1*
Dapsone	*0.1*	*5*
Dimetridazol-hydroxy	*0.1*	*1*
Flufenamic acid	*0.1*	*10*
Metronidazole-hydroxy	*0.1*	*1*
Mefenamic acid	*0.1*	*10*
Ronidazole	*0.1*	*1*
Tulobuterol	*0.1**	*0.1*
Zeranol	*1*	*1*
Phenylbutazone	*0.1*	*5*
Salbutamol	*0.1*	*0.5*

**= Peak observed in the blank matrix injection prevented assessment at lower concentrations.*

Veterinary drugs

Technical content summaries

High sensitivity and dynamic range for veterinary drugs analysis with the SCIEX 5500+ system

France, Germany, Switzerland, USA

Veterinary drugs are commonly used in livestock breeding to prevent or treat infections and to ensure optimal growth. For safety and regulatory reasons, the ability to quantify any residues of these drugs in products of animal origin is critical. Here, a highly selective and sensitive LC-MS/MS method for the analysis of veterinary drugs is presented that uses the SCIEX 5500+ system together with SCIEX OS software for a targeted quantitation/screening workflow. A mixture of 218 veterinary drugs with different compound classes—such as corticoids, quinolones, sulfamides, macrolides, tetracyclines and many others—are included in this study.

Combining SWATH® Acquisition with MRM^{HR} for the Analysis of Veterinary Drugs in Tissue

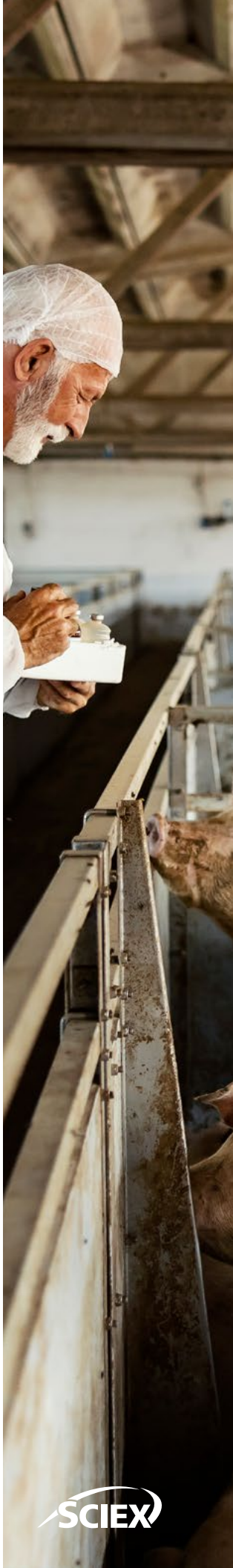
Germany

Veterinary drugs that find their way into human nutrition represent a potential risk to human health, and abuse of antibiotics in animals may also contribute to the development of antimicrobial resistance. For these reasons, regulatory bodies require careful control of the presence of veterinary drug residues in animal products. This technical note presents a highly flexible, selective and sensitive LC-MS/MS method for the analysis of veterinary drugs in liver extract using the X500R QTOF system and SCIEX OS software for a combined non-targeted and targeted screening workflow.

Analysis of Metronidazole and Ronidazole in Milk with the 3500 System

India/Germany

The use of antibiotics in cattle ranching, poultry and other farming is a major concern due to the potential impact of these substances on human health. This technical note describes the successful analysis of both metronidazole and ronidazole in a milk test matrix using the SCIEX Triple Quad 3500 system in combination with a simplified sample preparation protocol. A simple extraction procedure was used that detected the presence of these antibiotics down to sub-single-digit ppb levels to help ensure that recommended safety levels are met for local regulations.



Simultneous quantification of aminoglycoside antibiotics in milk with the SCIEX Triple Quad 3500 system

India/Germany

Aminoglycosides (AGs) are broad-spectrum antibiotics that have been extensively used in both human and veterinary medicine. However, AGs can be a risk to consumer health if they are present at levels that are too high. Here, to measure AG residues in milk, a fast and robust sample preparation procedure was developed based on buffer extraction and without the need for a cleanup step. Various analyses of milk samples confirmed the ability to detect trace-level concentrations of AG antibiotic residues.

Simultaneous determination of 88 veterinary drug residues in pork liver using LC-MS/MS

China

In recent years, the use of illicit drugs in animal breeding and husbandry has become increasingly widespread, and the presence of residues of these drugs in animal-derived foods is a growing safety concern. In this technical note, a quantitative method is described for detecting 88 kinds of prohibited veterinary drugs on the QTRAP 4500 system using pig liver as a test matrix. This method provides a simple and efficient solution for the detection of veterinary drugs and illegal added drugs in food of animal origin.

PFAS lifecycle

Where food plays a part

While exposure to per- and polyfluoroalkyl substances (PFAS) has been a dominating focus in health-related news cycles, awareness of the food component of the PFAS exposure journey has been growing—especially since food-related exposure extends beyond food consumption to include food contact materials (FCMs) and the potential long-term effects for humans.

The above infographic maps out the entire PFAS life cycle, where food testing and safety play both large and small roles. The next section evaluates various types of applications in food testing that can uncover how food and PFAS are linked in more ways than one.

RUO-MKT-18-15229-A

PFAS in me: which ones and how much?

Karl A. Oetjen [1], Andrew Patterson [2], Thep Phomsopha [2] [1] SCIEX, USA [2] Eurofins, Sacramento, CA

Introduction

Bioaccumulation of PFAS in the human body resulting from environmental exposure is a growing public health concern. Recent studies have linked PFAS exposure to adverse health outcomes, including childhood health complications, reduction in kidney functions, thyroid disease, hormone suppression, decreased fertility, increased cholesterol levels, and diabetes, among others.

In this study, we combined low volume blood sampling with the SCIEX QTRAP 7500 system for the analysis of trace level of PFAS. We present here a quantitative workflow capable of accurately quantifying sub-ng/mL levels of 42 PFAS compounds. The analysis was performed on the author, and the results of the analysis are shared to demonstrate what PFAS exposure looks like in a typical American.

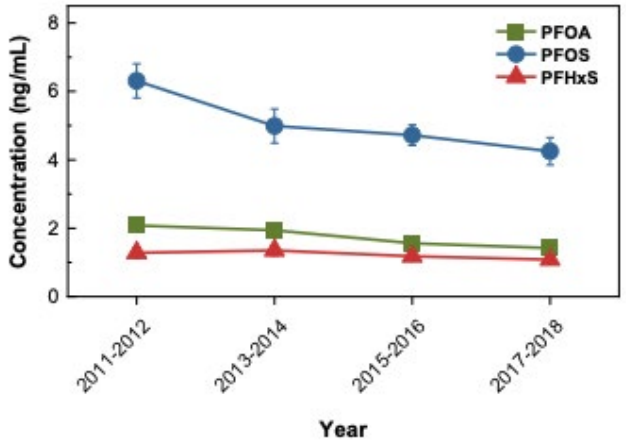


Figure 1. Mean concentration of PFAS in the US population (CDC, NHANES 1999-2018).

What are the current recommendations from the National Academy of Sciences?

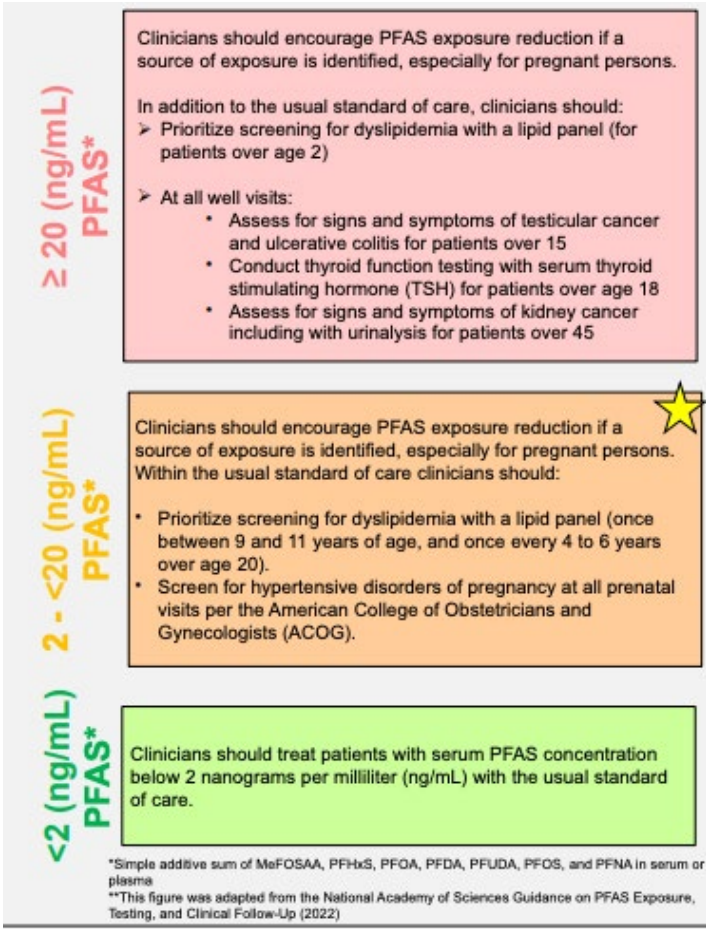


Figure 2. Guidance for clinicians on exposure determination, PFAS testing and clinical follow-Up (National Academy of Sciences, 2022)

A total of 42 PFAS compounds were screened for and quantified in serum at sub-ng/mL

Key takeaways:

· PFHxS in my sample was 1.5x higher than the average population and may be related to working with aqueous filmforming foams (AFFF) that contained high levels of this compound

· Limited exposure to PFOA and PFOS from drinking water facilities may have contributed to the lower-than-average levels of PFOA and PFOS

· The distribution of linear and branched PFOS was within the expected ratio (Schulz et al., 2020)

· PFOA in human serum is on average 2.9% branched (Schulz et al., 2020). Thus, the amount present in my sample was likely present but below the detection limit

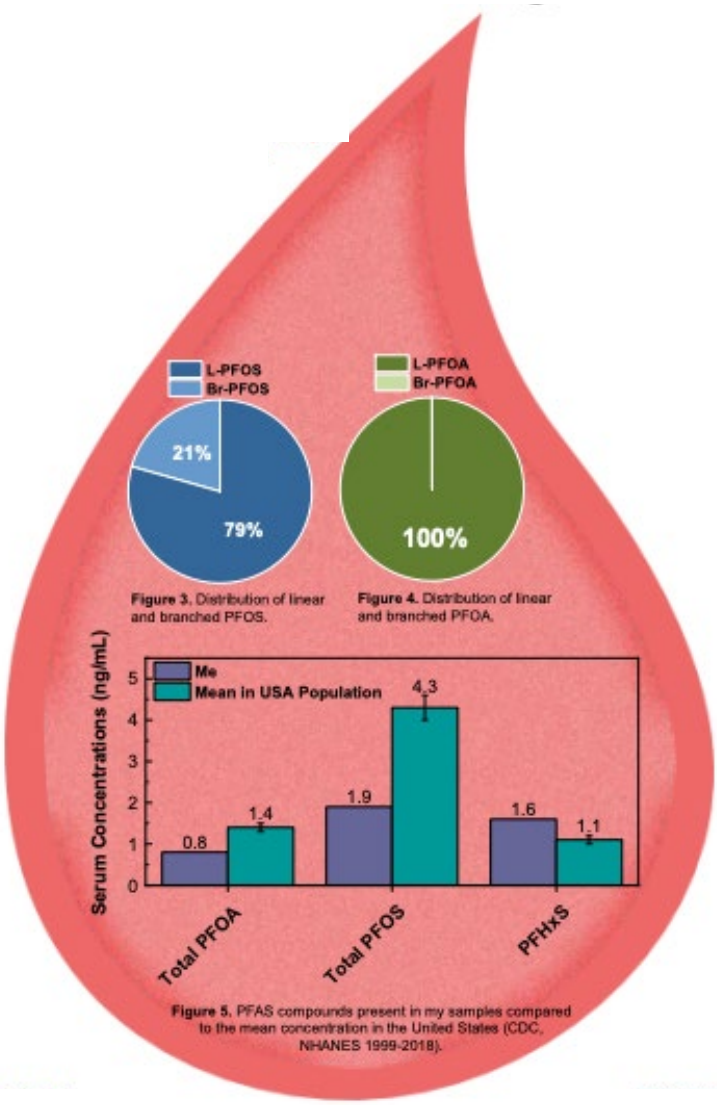


Figure 3. Distribution of linear and branched PFOS.

Figure 4. Distribution of linear and branched PFOA.

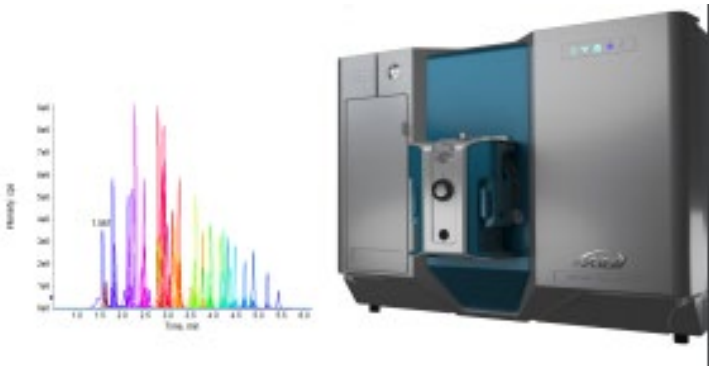
Figure 5. PFAS compounds present in my samples compared to the mean concentration in the United States (CDC, NHANES 1999-2018).

Methods

1 - A finger prick was used to drawcapillary blood. The first drop of blood was wiped away with a PFASfree gauze, and the Mitra device was applied to the subsequent drops of blood.

2 - The Mitra tips contained approximately 30 µL of blood and were placed in polypropylene vials with isotopically labelled internal standards or IDAs (Isotope Dilution Analytes) and acetonitrile to aid with protein removal.

3 - The extracts were combined, and solid phase extraction (SPE) was performed. Injection internal standards (ISs, or recovery standards) were added to the SPE extract immediately prior to placing it in a new polypropylene vial for analysis.



These extracts were injected onto a Luna Omega PS column at 30°C. Data were collected using a SCIEX 7500 system using electrospray ionization (ESI) in negative mode.

PFAS in food

Technical content

PFAS summit: a scientific series

Go to webpage →

Listen to SCIEX customers and industry experts explore how forever chemicals have shaped our environmental research. Get insight into topics ranging from the history of per-and polyfluoroalkyl substances (PFAS) to analytical testing methods.

Watch the presentations to:

> Learn about the history of PFAS and the need to increase testing for these "forever chemicals"

> Understand how PFAS regulations differ across the globe

> See what the future could look like in the PFAS testing world

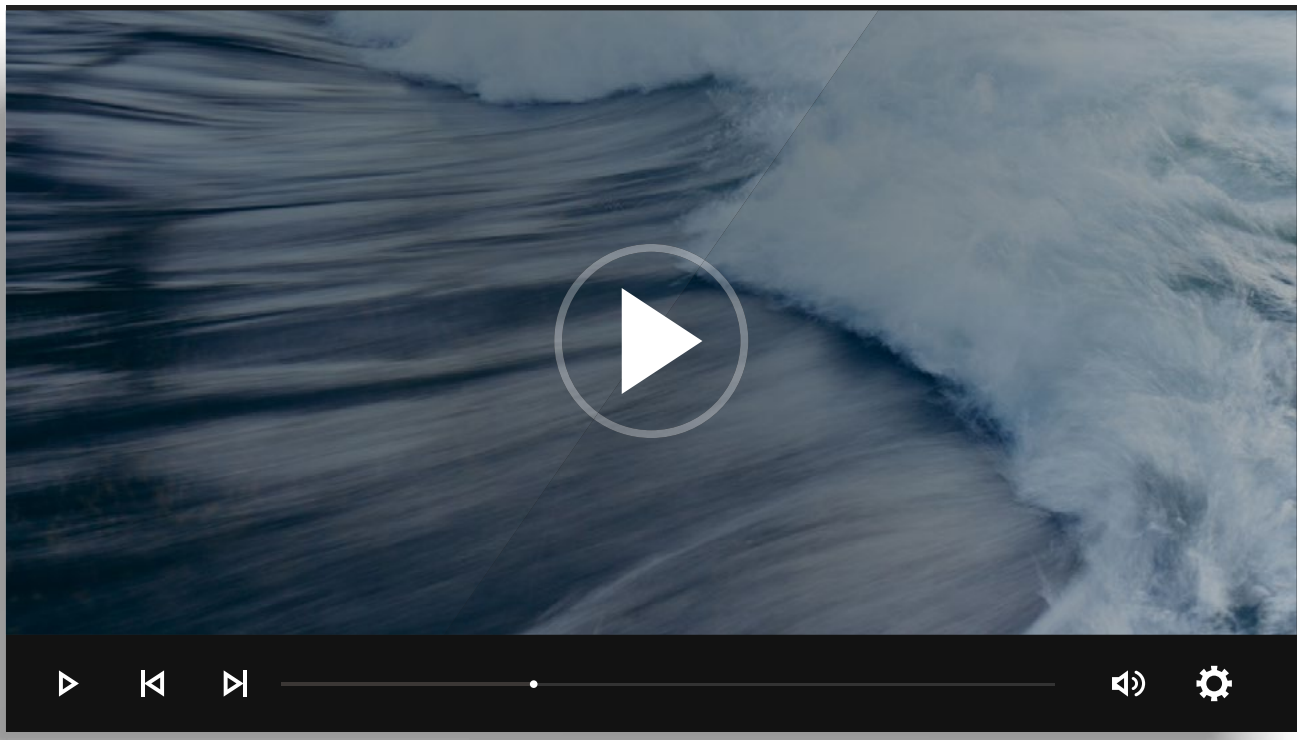
> Explore different matrices for PFAS testing

> Discover how LC-MS can help solve challenges related to testing for PFAS chemicals

> Get tips and tricks from seasoned PFAS testing experts

Finding PFAS in people

Watch the video →



Rapid identification and quantification of 27 primary aromatic amines in kitchen utensils

Parts-per-trillion (ppt) sensitivity using the SCIEX 7500 system

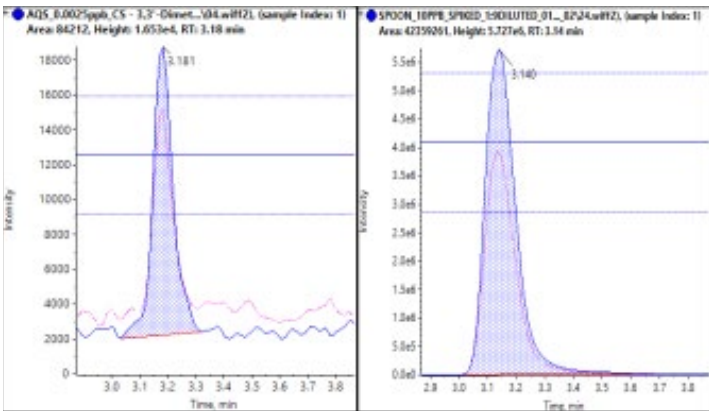
Sabarinathan1vSashank PillaivJessica Smith2vJack Steed2 and Jianru Stahl-Zheng3vSCIEX, India; 2SCIEX, UKv3SCIEX, Germany

Introduction

In this technical note, a method was developed to analyze 27 primary aromatic amines (PAAs) in food contact materials (FCMs). The method achieved LOQs of 0.001-0.50 ng/mL, which correspond to detection limits several orders of magnitude lower than EU regulation mandate. In addition, the high sensitivity of the SCIEX 7500 system permitted dilution of the sample extracts to reduce matrix effects and improve data quality, as shown by the high accuracy and precision (%CV <15%) of spiked matrix samples (Figure 1).

PAAs are widely used in the production of certain colorants and azo pigments used in a wide range of products such as kitchenware, paper napkins, and printed packaging. PAAs have raised concern because they can migrate FCMs into food 1 and be ingested. Most PAAs are considered safe and might not impact human health but some PAAs have been identified as possible carcinogens.

Due to rapidly changing global regulations, the detection and quantification of PAAs in FCMs is critical to consumer safety. Specific migration limits have been established by EU regulations for PAAs in FCMs. Therefore, a detectable limit of 10 ng/mL (EU 2020/1245 amending and correcting regulation EU No 10/2011) in food or a food simulant is applied to the sum of PAAs released.2



(bottom right) Figure 1. Representative extracted ion chromatogram (XIC) of 3,3'- dimethylbenzidine with ion ratio tolerance lines overlaid for the quantifier and qualifier ions. Compound identification was based on ion ratio calculation. The tolerance levels were set to ±30% for the quantifier and qualifier ions. This calculation was performed at the LOQ of 0.0025 ng/mL in solvent (left) and at 1 ng/mL in spiked spoon sample extract (10 ng/mL in-sample, 10x diluted) (right).

Key features of the SCIEX 7500 system for the analysis of PAAs

> Simple, robust, reproducible and rapid sample preparation

> Good chromatographic separation with a fast run time of 12 minutes

> Due to the excellent sensitivity of the SCIEX 7500 system, LOQs achieved in diluent were at sub-ng/mL levels

> A single standard calibration curve in diluent was used for 2 different FCM matrices. The majority of the compounds analyzed were within acceptable limits for accuracy (70-130%) and precision (%CV <15%)

>All quantification results used 2 transitions and were confirmed using both quantifier and qualifier ions PAAs released.2

Methods

Standard preparation: Individual standard stock solutions were prepared by dissolving 1 mg of neat standard into 1 mL of the appropriate solvent before vortexing for 1 min (Table 4). The individual stock solutions were used to prepare a mixed solution of 27 PAAs at 200 ng/mL in methanol, which was diluted with 70:30 water/methanol (diluent, Table 1) to cover a linear range of 0.001-10 ng/mL.

Sample preparation: A 6 g sample of spoon or cake mold was weighed into a 50 mL glass centrifuge tube and 12 mL of 3% acetic acid in water (simulant solution, Table 1) was added. The solution was vortexed for 2 minutes and then incubated for 2 hours at 80°C. After incubation, the samples were filtered using a 0.22 µm PTFE syringe filter (Phenomenex part #: AF8-6710-12) prior to analysis.

Post-spiked sample preparation: Samples were extracted following the sample preparation protocol described above. After filtration, 0.950 mL of the filtered sample and 0.050 mL of the 200 ng/mL stock solution were used to prepare a 10 ng/mL solution. The samples were vortexed and transferred into autosampler vials for the analysis. To evaluate the effect of sample dilution on recovery and accuracy, the 10 ng/mL postspiked sample was diluted 1:9 ratio with methanol to obtain a 1.0 ng/mL solution (i.e., 10x dilution)

Chromatography: An ExionLC AD system was used with a Phenomenex Kinetex F5 analytical column (2.6 µm, 100 x 3.0 mm). Table 2 outlines the gradient conditions that were used with a flow rate of 0.300 mL/min. A 5 µL injection volume was used and the column oven temperature was set to 40°C.

Mass spectrometry: The SCIEX 7500 system was used with electrospray ionization operating in positive mode. Data were acquired using scheduled MRM mode (sMRM) to optimize dwell time and the number of data points collected across the chromatographic peak. The source and compound-specific parameters used are presented in Tables 3 and 4, respectively. Individual QOD values were individually optimized for all 27 analytes (Table 4) to reduce the background noise and to improve sensitivity and selectivity.

Data processing: All data were processed using SCIEX OS software, version 2.1.6.

Table 1. Simulant, dilution solvent, and diluent for PAA

Simulant	Dilution solvent	Diluent
3% Acetic Acid (w/v)	*100 % Methanol*	*Water: Methanol (70:30 v/v)*

Table 2. Gradient program for the analysis of 27 PAA compounds

Time (min)	% A	% B
0.00	*70*	*30*
7.00	*10*	*90*
9.00	*5*	*95*
9.10	*70*	*30*
12.00	*70*	*30*

Mobile phase A: 0.05% formic acid in water
Mobile phase B: methanol

Table 3. Optimized source parameters for the analysis of 27 PAA compounds.

Source parameters	
Curtain gas	*45 psi*
CAD gas	*8 psi*
Ion spray voltage	*1500 V*
Temperature	*350°C*
Ion source Gas 1 and Gas 2	*60 and 70*

Table 4. Stock solution diluent, MRM conditions and compound-dependent parameters for 27 PAAs tested. Due to the variability of compound solubility, the 1 mg/mL stock was prepared with individual solvents.

Compound	Stock (1 mg/mL) solution diluent	Q1 (m/z)	Q3 (m/z)	EP	CE	CXP	Q0D
Aniline	*Methanol*	*94.0*	*77.0*	*6*	*25*	*10*	*55*
2-Anisidine	*Ethanol*	*124.1*	*109.0*	*10*	*25*	*14*	*10*
o-Toludine	*Methanol*	*108.0*	*91.0*	*10*	*25*	*12*	*50*
2,4-Dimethylaniline	*Methanol*	*122.1*	*105.0*	*8*	*25*	*14*	*15*
2,6-Dimethylaniline	*Ethanol*	*122.1*	*105.1*	*8*	*25*	*12*	*15*
1,3-Phenylenediamine	*Methanol*	*109.0*	*92.0*	*10*	*20*	*8*	*70*
1,4-Phenylenediamine	*Methanol*	*109.1*	*92.0*	*8*	*20*	*10*	*70*
2,4-Diaminotoluene	*Methanol*	*123.1*	*106.0*	*10*	*20*	*6*	*65*
2,6-Diaminotoluene	*Ethanol*	*123.1*	*106.0*	*10*	*20*	*6*	*65*
2,4,5-Trimethylaniline	*Methanol*	*136.1*	*121.1*	*4*	*25*	*6*	*20*
4-Chloro-2-methylaniline	*Ethanol*	*142.0*	*107.1*	*4*	*25*	*10*	*35*
4-Aminophenylthioether	*Methanol*	*217.1*	*124.0*	*10*	*30*	*5*	*25*
4,4'-Benzidine	*Methanol*	*185.1*	*168.1*	*6*	*25*	*20*	*35*
2-Aminonaphthalene	*Ethanol*	*144.1*	*127.1*	*6*	*30*	*16*	*30*
4-Chloroaniline	*Methanol*	*128.0*	*93.0*	*6*	*25*	*12*	*35*
4-Aminobiphenyl	*Ethanol*	*170.1*	*152.1*	*12*	*40*	*14*	*40*
4-Aminophenylether	*Acetone*	*201.1*	*108.0*	*8*	*30*	*6*	*15*
3,3'-Dimethylbenzidine	*Methanol*	*213.1*	*180.1*	*6*	*45*	*12*	*40*
3,3'-Dichlorobenzidine	*Methanol*	*253.0*	*217.0*	*8*	*30*	*12*	*45*
3,3'-Dimethoxybenzidine	*Methanol*	*245.1*	*230.1*	*10*	*25*	*16*	*25*
2-Methoxy-5-methylaniline	*Ethanol*	*138.1*	*123.1*	*10*	*25*	*16*	*10*
2-Amino-4-nitrotoluene	*Ethanol*	*153.1*	*89.0*	*10*	*50*	*10*	*5*
Bis-(4-aminophenyl) methane	*Methanol*	*199.1*	*106.1*	*6*	*30*	*12*	*25*
4,4'-Methylene-bis(2-chloroaniline)	*Methanol*	*267.1*	*231.1*	*10*	*30*	*12*	*65*
4-Amino-2',3-dimethylazobenzene	*Acetone*	*226.1*	*91.0*	*10*	*30*	*10*	*15*
4,4'-Diamino-3,3'-dimethyldiphenylmethane	*Toluene/methanol (1:9)*	*227.2*	*120.1*	*4*	*35*	*6*	*60*
4-Methoxy-1,3-phenylenediamine	*Methanol*	*139.1*	*124.0*	*6*	*20*	*10*	*20*

Table 5. Calibration curve correlation coefficient (r^2) and accuracy range across the calibration curve for quantifier ion.

Compound	Linear range (ng/mL)	LOQ	Correlation coefficient (r^2)	Accuracy (%) range of calibration standards
Aniline	*0.1000 - 10.00*	*0.1000*	*0.998*	*93.3 – 105*
2-Anisidine	*0.0050 - 5.00*	*0.0050*	*0.998*	*90.5 – 105*
o-Toludine	*0.2500 - 10.00*	*0.2500*	*0.999*	*95.5 – 103*
2,4-Dimethylaniline	*0.0250 - 10.00*	*0.0250*	*0.998*	*92.1 – 104*
2,6-Dimethylaniline	*0.0250 - 10.00*	*0.0250*	*0.998*	*91.8 – 104*
1,3-Phenylenediamine	*0.5000 - 10.00*	*0.5000*	*0.997*	*93.3 – 104*
1,4-Phenylenediamine	*0.2500 - 10.00*	*0.2500*	*0.999*	*95.9 – 104*
2,4-Diaminotoluene	*0.5000 - 10.00*	*0.5000*	*1.000*	*99.2 – 102*
2,6-Diaminotoluene	*0.5000 - 10.00*	*0.5000*	*1.000*	*98.2 – 103*
2,4,5-Trimethylaniline	*0.0250 - 10.00*	*0.0250*	*0.998*	*91.8 – 104*
4-Chloro-2-methylaniline	*0.0010 - 10.00*	*0.0010*	*0.996*	*82.9 – 105*
4-Aminophenylthioether	*0.0025 - 10.00*	*0.0025*	*0.997*	*86.6 – 105*
4,4'-Benzidine	*0.0100 - 10.00*	*0.0100*	*0.997*	*89.2 – 108*
2-Aminonaphthalene	*0.0100 - 5.00*	*0.0100*	*0.998*	*90.2 – 106*
4-Chloroaniline	*0.0100 - 10.00*	*0.0100*	*0.999*	*95.2 – 103*
4-Aminobiphenyl	*0.0050 - 5.00*	*0.0050*	*0.995*	*84.0 – 105*
4-Aminophenylether	*0.0050 - 10.00*	*0.0050*	*0.999*	*97.1 – 105*
3,3'-Dimethylbenzidine	*0.0025 - 10.00*	*0.0025*	*0.995*	*86.1 – 106*
3,3'-Dichlorobenzidine	*0.0050 - 10.00*	*0.0050*	*0.998*	*91.6 – 104*
3,3'-Dimethoxybenzidine	*0.0050 - 10.00*	*0.0050*	*0.991*	*84.0 – 107*
2-Methoxy-5-methylaniline	*0.0100 - 5.00*	*0.0100*	*0.996*	*85.9 – 105*
2-Amino-4-nitrotoluene	*0.2500 - 10.00*	*0.2500*	*0.996*	*93.4 – 108*
Bis-(4-aminophenyl) methane	*0.0250 - 10.00*	*0.0250*	*0.992*	*81.9 – 108*
4,4'-Methylene-bis(2-chloroaniline)	*0.0025 - 10.00*	*0.0025*	*0.999*	*93.9 – 103*
4-Amino-2',3-dimethylazobenzene	*0.0100 - 5.00*	*0.0100*	*0.998*	*90.0 – 104*
4,4'-Diamino-3,3'-dimethyldiphenylmethane	*0.0100 - 10.00*	*0.0100*	*0.999*	*91.7 – 103*
4-Methoxy-1,3-phenylenediamine	*0.1000 - 10.00*	*0.1000*	*1.000*	*98.2 – 103*

Note 1: The calibration curve was prepared using 70:30 water/methanol diluent

Note 2: The LOQ value was selected based on 2 selective MRM transitions, S/N ratio >10 for quantifier and qualifier of calibration standard, accuracy ±30%, %CV <15% and ion ratio tolerance ± 30%

Chromatographic separation of 27 PAAs using a 12 min LC gradient

The chromatographic gradient used resulted in good separation of the 27 PAAs in 12 min. The suite of PAAs covered a moderate range of compound polarity, requiring careful LC gradient development. For example, the method achieved the baseline separation of a pair of PAA isomers, 2,4- and 2,6-dimethylaniline (Figure 2). Although not all isomer groups were separated, this is acceptable, according to EU regulations. Further, the separation of the 27 PAA compounds was obtained while running at HPLC pressures due to the 2.6 µm particle size and relatively low flow rate

Linear dynamic range and sensitivity

Calibration curves for all 27 PAA compounds showed r2 values >0.99 with accuracies ranging between 80% and 110% (Table 5). The calibration curves generated for 4-aminophenylthioether and 3,3'-dimethylbenzidine are shown in Figure 3 and display a linear range of 0.0025 to 10.00 ng/mL.

The SCIEX 7500 system showed excellent sensitivity for the entire suite of 27 PAA compounds with LOQs ranging between 0.001 and 0.5 ng/mL for standards prepared in the 70:30 water/methanol diluent. The LOQ value was selected based on 2 selective MRM transitions, S/N ratio >10 for quantifier and qualifier of calibration standard, accuracy ±30%, %CV <15% and ion ratio tolerance ±30%. Overall, the observed sensitivity was several orders of magnitude greater than 10 ng/mL, as currently required by the EU regulation. Figure 4 shows the quantifier transition XIC for 4-aminophenylthioether at the LOQ concentration (0.0025 ng/mL). For comparison, the overlaid quantifier and qualifier ion transitions at 1 ng/mL in spoon extract matrix (post-spiked at 10 ng/mL and diluted 10x) are shown in the right panel. The ion ratio tolerance was within ±30%, which met the acceptance criteria.

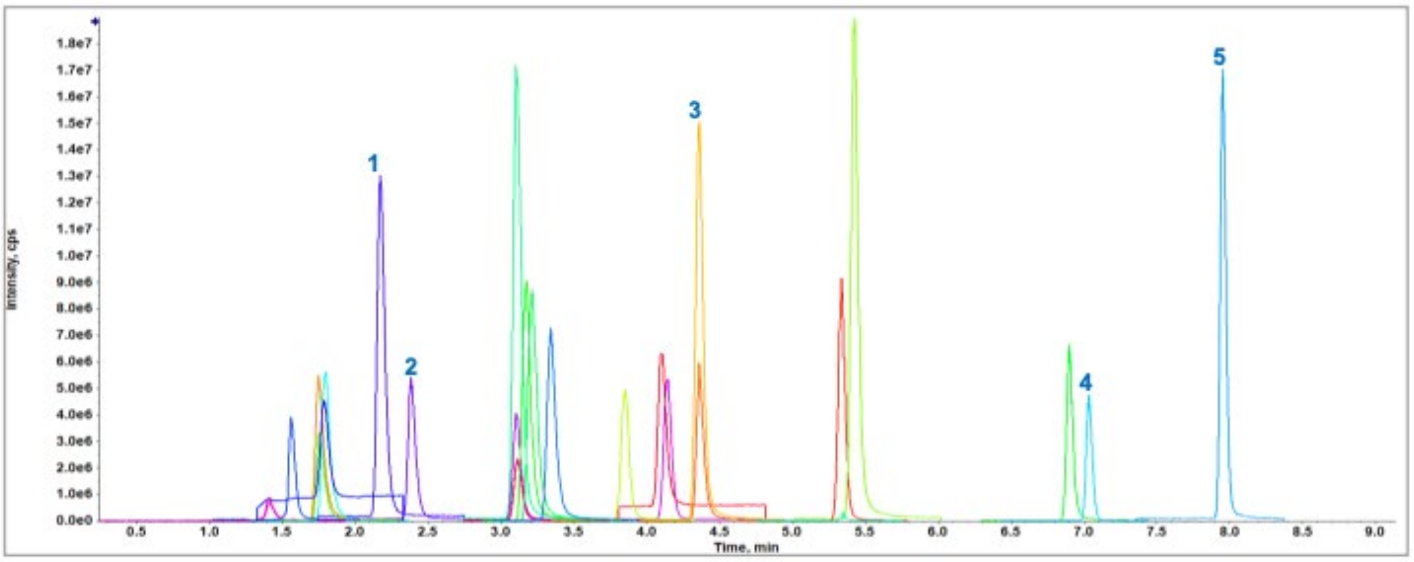


Figure 2. XIC of 27 PAAs at 1 ng/mL from the quantifier transitions. Good chromatographic peak-to-peak separation was achieved using the Phenomenex Kinetex F5 column. Highlighted traces indicate 1) Anisidine, 2) o-Toluidine, 3) 2-Aminonapthalene, 4) 4,4'-Methylene-bis(2-chloroaniline) and 5.) **4-Amino-2',3-dimethylazobenzene.**

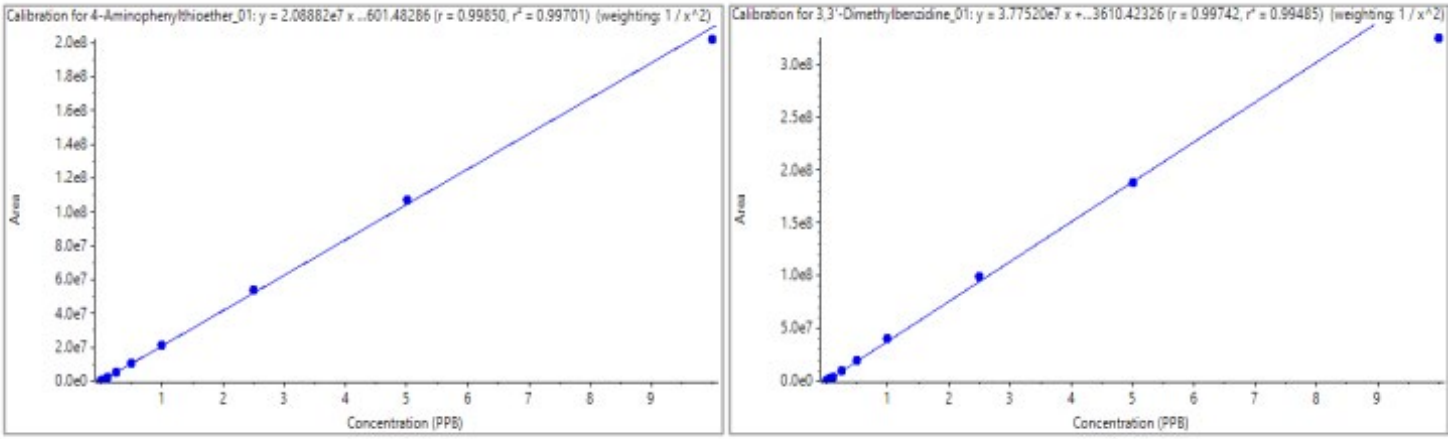


Figure 3. Representative calibration curve for 4-aminophenylthioether (left) and 3,3'-dimethylbenzidine (right). The $1/x^2$ weighting factor was used. A linear range of 0.0025-10 ng/mL with an r^2 value of >0.99 was achieved.

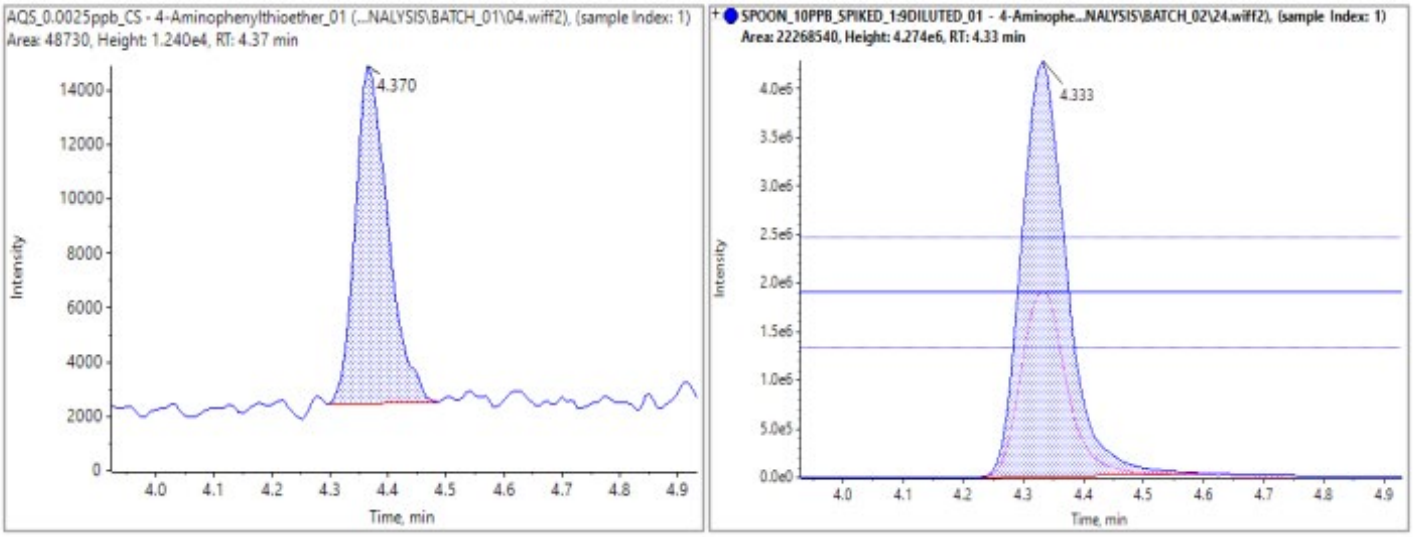


Figure 4. Chromatogram of 4-aminophenylthioether. Left) Representative XIC of 4-aminophenylthioether at the LOQ level of 0.0025 ng/mL from the quantifier transition (m/z: 217.1/ 124.0). Excellent peak shape and sensitivity were achieved. Right) Representative XIC for 4-aminophenylthioether with the ion ratio overlaid for the quantifier and qualifier ions at 1 ng/mL (post-spiked 10ng/mL solution diluted with methanol) in spoon sample.

Post-extraction spiked samples

A 10 ng/mL mixed standard solution was spiked into the processed spoon and cake mold samples and diluted 10x with methanol (1 ng/mL final concentration) to evaluate the analyte recovery in spoon and cake mold samples (n=2 per matrix). The accuracy data were calculated from 6 injections of quality control samples (1 ng/mL) and the calibration curves generated for all PAAs diluated in 70:30 water/methanol. The accuracy range achieved between 70-130% for most of the analytes and %CV <15% for all compounds analyzed (Table 6). In addition, the 10x diluted samples showed improved accuracy, compared to the undiluted samples, presumably due to reduced matrix effects.The enhanced sensitivity of the SCIEX 7500 system permitted sample dilution while maintaining low detection limits. These results highlight an additional benefit of the SCIEX 7500 system sensitivity, improved data quality from reducing potential matrix effects through sample dilution.

Table 6. Average accuracy and %CV (n=6) for all 27 PAAs in undiluted and diluted spoon and cake mold samples. All recovery measurements (n=6) were performed at 10 ng/mL (post-spiked) and 1 ng/mL (post-spiked 10 ng/mL, diluted with methanol in 1:9 ratio) against the single external calibration curve. The average accuracy and %CV were calculated from the quantifier ion.

Compound	Spoon				Cake mold			
	10x Diluted (n=6)		Undiluted (n=6)		10x Diluted (n=6)		Undiluted (n=6)	
	Average accuracy	%CV	Average accuracy	%CV	Average accuracy	%CV	Average accuracy	%CV
Aniline	89.1	5.2	63.0	1.48	87.5	2.8	51.4	0.6
2-Anisidine	95.3	1.3	N/A**	N/A**	86.2	1.8	N/A**	N/A**
o-Toludine	97.2	1.8	89.0	0.7	88.9	2.2	79.1	0.9
2,4-Dimethylaniline	97.3	2.1	82.6	1.2	87.8	2.3	74.3	1.1
2,6-Dimethylaniline	96.6	1.2	81.8	0.8	83.7	1.1	71.9	0.9
1,3-Phenylenediamine*	70.5	4.6	45.1	5.5	58.9	2.4	34.1	2.6
1,4-Phenylenediamine*	65.0	6.0	47.6	5.5	52.0	3.6	37.3	2.3
2,4-Diaminotoluene	96.8	1.9	90.2	0.7	93.1	1.2	81.7	1.3
2,6-Diaminotoluene	96.6	1.3	90.1	1.2	91.3	1.4	81.5	0.7
2,4,5-Trimethylaniline	97.8	1.4	87.6	0.4	90.3	1.2	81.5	0.9
4-Chloro-2-methylaniline	96.2	2.7	82.9	0.5	88.2	1.5	77.3	0.6
4-Aminophenylthioether	97.6	3.6	85.0	2.1	87.0	2.4	77.6	2.0
4,4'-Benzidine	88.6	2.1	79.8	2.3	76.8	2.6	58.2	2.7
2-Aminonaphthalene	97.2	1.7	N/A**	N/A**	87.9	1.7	N/A**	N/A**
4-Chloroaniline	94.2	3.9	92.8	2.6	88.6	1.8	82.6	2.1
4-Aminobiphenyl	97.9	2.0	N/A**	N/A**	86.9	1.2	N/A **	N/A**
4-Aminophenylether	90.0	4.2	94.7	1.7	80.6	2.3	72.9	1.8
3,3'-Dimethylbenzidine	98.6	2.7	71.7	1.9	84.4	1.4	55.6	1.4
3,3'-Dichlorobenzidine	97.7	2.9	78.9	1.3	82.1	1.4	71.1	1.0
3,3'-Dimethoxybenzidine	97.4	3.4	52.5	7.1	76.2	2.4	10.1	16.1
2-Methoxy-5-methylaniline	97.9	1.4	N/A**	N/A**	88.0	1.3	N/A**	N/A**
2-Amino-4-nitrotoluene	107.4	5.8	99.2	7.5	95.6	6.8	89.9	5.6
Bis-(4-aminophenyl) methane	88.5	3.6	68.7	1.5	81.1	1.2	56.6	1.8
4,4'-Methylene-bis(2-chloroaniline)	95.8	2.9	86.4	1.4	85.7	2.5	79.8	0.8
4-Amino-2',3-dimethylazobenzene	96.1	2.0	72.2	1.4	88.1	1.7	68.9	1.0
4,4'-Diamino-3,3'-dimethyldiphenylmethane	94.2	2.5	82.8	1.1	86.2	2.2	78.4	0.9
4-Methoxy-1,3-phenylenediamine*	60.6	4.1	14.5	13.0	40.2	3.8	0.5	14.1

*In acetic acid simulant, the analyte response was unstable, a lower recovery value was obtained and more dilution was required to reduce the acid level
**Calculated concentration was above the calibration curve
***Blank corrections were applied for the analytes in spiked samples that obtained >20% of blank (unspiked sample) area from the LOQ area count

Unspiked spoon and cake mold samples

Spoon and cake mold samples were processed using the sample preparation procedure described and diluted 10x with methanol before analysis to reduce potential matrix effects. The unspiked samples were analyzed against a single external calibration curve in solvent for the presence of the 27 PAA compounds. The values obtained in the unspiked samples were below the LOQ level for the 2 analytes detected (Table 7).

Table 7. Analysis of unspiked spoon and cake mold samples. Unspiked samples showed detection of 2 PAAs.

Compound	Standard data	Unspiked sample conc. (ng/mL)	
	LOQ ng/mL	*Spoon*	*Cake mold*
2-Anisidine	*0.0050*	*<0**	*0.017**
4,4'-Methylene-bis(2-chloroaniline)	*0.0025*	*0.194**	*0.574**

**Concentrations were above the LOQ level but less than the method reporting level (MRL) specified in the EU regulation (10 ng/mL)*

Conclusions

> The SCIEX 7500 system showed excellent sensitivity, linearity and reproducibility for the quantification of PAAs in kitchen utensils

> Using a calibration curve prepared in 70:30, water/methanol diluent, excellent accuracy (80-110%) and precision (%CV <15%) was achieved for all PAAs analyzed. LOQs were excellent, ranging between 0.001 and 0.50 ng/mL

> When quantifying the spiked samples against an external standard calibration curve, the recovery was between 70% and 130%, which meets the acceptance criteria for most PAAs

> The linear range was assessed across a concentration range of 0.001-10.00 ng/mL with an r2 value > 0.99 achieved for all compounds

> Good chromatographic separation was achieved with a fast run time of 12 minutes and an isomer pair was separated

References

1 Rapid Determination of 41 Primary Aromatic Amines in Water. SCIEX technical note, RUO-MKT-02-10038-ZH-A.

2. Commission Regulation (EU) 2020/1245 of 2 September 2020 amending and correcting Regulation (EU) No 10/2011 on plastic materials and articles intended to come into contact with food

3. Rapid Determination of 33 Primary Aromatic Amines in Kitchen Utensils, Textiles Dyes and Food Packaging Materials. SCIEX technical note, RUO-MKT-02-7950-B.

4. Patrick Kämpfer, Stéphanie Crettaz, Susanne Nussbaumer, Michael Scherer, Scott Krepich, Otmar Deflorin. Quantitative determination of 58 aromatic amines and positional isomers in textiles by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. J Chromatogr A. 2019 May 10;1592:71-81

Food contact materials

Technical content

Identification and quantification of PFAS in food contact materials using MRMHR workflow on X500R QTOF system

China

PFAS are widely used in plastic packaging materials for food and as coating in nonstick pans. Given the tremendous persistence of PFAS in the environment and their adverse effects on human health, monitoring of PFAS residue has gained traction in China and elsewhere. This technical note describes the use of the X500R QTOF system with the MRMHR acquisition mode to enable detection and quantitation of PFAS and to meet EU regulations and national standards in China.

Determination of Irganox compounds extracted from food packaging using 4 food simulants

India, England, Germany

Human health can be impacted by chemicals that migrate into foods and beverages from food contact materials (FCMs), such as wrappers and containers. Understanding the risk of FCM chemicals requires a sensitive and accurate analytical method. The method presented in this technical note accurately identified and quantified 16 Irganox compounds that were spiked into normal food matrices in extracts of plastic food packaging materials. This technique eliminated the need for matrix-matched standards while achieving accuracy and precision criteria, allowing for a simpler sample preparation procedure.

Detection of sesame peptides and 12 other food allergens using the SCIEX vMethod for food allergen testing

Sesame allergen screening using the SCIEX 7500 system

Simon Roberts1, Karl Oetjen1 and Jianru Stahl-Zeng2 1SCIEX, USA, 2SCIEX, Germany

In 2021, the Food Allergy Safety, Treatment, Education, and Research (FASTER) Act was passed in the United States. The FASTER Act requires all foods sold in the United States that contain sesame to declare it as an ingredient or state "Contains: Sesame" immediately after the ingredient list.[1]

The SCIEX vMethod application for food allergen testing previously provided a workflow for sample preparation and LCMS/MS detection of 12 distinct allergens, including egg, milk, almond, Brazil nut, cashew, hazelnut, pine nut, pistachio, pecan, walnut, peanut and soy[2,3]. After evaluating 24 different sesame peptides[4, 2] of the most sensitive peptides were selected (Figure 1) and added to the SCIEX vMethod (Table 1).

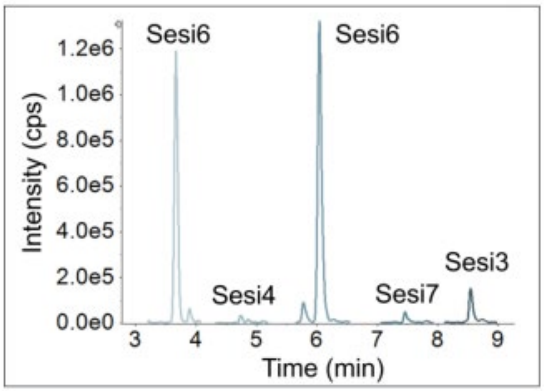


Figure 1. LC-MS/MS chromatograms for Sesi3, Sesi4, Sesi6 and Sesi7 peptides of sesame proteins.

Ten replicate injections of cookie dough spiked with 5 ppm sesame flour (Figure 2) showed good repeatability with a CV of 3.7%. Good linearity of r2 = 0.998 was observed in a curve prepared with 5, 25, 100 and 250 ppm sesame flour. By including the MRM parameters for sesame peptides in the SCIEX vMethod for food allergen testing, the SCIEX 7500 system was able to reach a detection limit of 5 ppm for both sesame peptides tested.

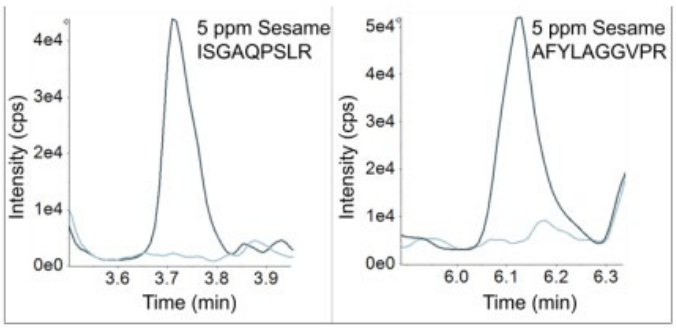


Figure 2. Chromatograms for 2 peptides in sesame-free cookie dough (light) and cookie dough spiked with 5 ppm sesame flour (dark).

Table 1. MRM parameters for 2 sesame peptides.

Peptide	RT (min)	Q1	Q3	CE
ISGAQPSLR_1	3.7	464.8	815.4	22
ISGAQPSLR_2	3.7	464.8	472.3	22
AFYLAGGVPR_1	6.1	525.8	556.3	23
AFYLAGGVPR_2	6.1	525.8	669.4	23

References

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2. SCIEX vMethod Application for Food Allergen Testing.

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Allergens

Technical content summaries

vMethod Application: Simultaneous Analysis of 12 Food Allergens in Baked and Raw Food Products

Singapore, India, Canada, Germany, USA

The ability to accurately and effectively screen for allergens in food products is critical for the prevention of potentially life-threatening allergic reactions. Here, an LC-MS/MS method is presented that uses the QTRAP 4500 system to detect and screen 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 and the World Health Organization to harmonize international food standards.

vMethod Application: A Highly Selective and Sensitive LC-MS/MS method for the Quantification of Gluten Proteins

Singapore, India, Canada, Germany, USA

For those with gluten-related disorders, consuming gluten can have serious health consequences, making the detection and quantitation of dietary gluten in prepackaged foods extremely important. Here, a highly selective and sensitive LC-MS/MS method is described that screens and quantifies wheat gluten proteins in various food matrices, including bakery products, fermented beverages and baby formula. This method measures unique, stable signature peptides using predetermined multiple reaction monitoring (MRM) transitions for identification and quantitation, and it accurately detects gluten protein concentrations as low as 5 ppm.

Cutting into tallow: A lipidomic exploration of animal-based and alternatively sourced meat

Using the SCIEX 7500 system

Robert D. Lorenzo1, Megan Danielewicz2 and Mackenzie Pearson2 1SCIEX, Canada; 2SCIEX, USA

Alternatively sourced meat has been available at the grocery store in many forms for several years. Black bean "burger" patties or tofu "turkey" have been choices of protein sources for consumers, but with the rising concern about global climate change, the call for more sustainable sources of protein is increasing. Since 2019, the retail sales of plant-based meat alternatives have grown 27%1, as these alternatives to meat are readily available at popular fast food chains, restaurants and local grocery stores.

In addition to their protein source, many factors define these alternatives to meat products besides just another source of protein. Synthetic biology companies investigate the smell, mouthfeel and visual qualities of their plant-based products. The taste and texture provide the consumer with an overall experience that is similar to the experience of eating meat. Fats, from either a single vegetable oil or from several sources, are often added to develop the flavor profile of the meat alternative. Depending on the source, however, the added fats may have beneficial or harmful effects on consumers' health.[2]

Figure 1. Supervised PCA analysis of various meat and alternative meat products. PC1 vs. PC2 loading plot shows clear differentiation between meat and alternative meat products. Biological replicates cluster tightly, highlighting the reproducibility of the method. Differentiation can also be seen between the various alternative meat products.

Here, the lipid profiles of 5 different types of meat and meat alternatives were analyzed for their lipid profiles. Using the global lipidomic profiling method3 , over 2000 lipids were screened and then narrowed down to quantify over 1000 lipid species in a single injection. A total of 21 lipid classes were monitored for this study, with the changes in carbon length composition, fatty acid composition and degree of desaturation are reported for select classes. The final lipid panel enabled clear differentiationbetween animal-based meat and meat alternatives (Figure 1).

Key features of lipidomic profiling in meat and alternatively sourced meat

· High-throughput lipidomic profiling method that provides a customizable MRM list that can be adapted based on matrix of interest or lipid class, such as oxidized lipids

· High sensitivity detection using the SCIEX 7500 system
· Enables user to profile and quantify changes in lipid profiles before and after cooking (Figure 1)

· Method can be utilized to report changes in chain length composition, fatty acid profiles and degree of desaturation by lipid class

· Enables users to identify potential markers for animal diet type in beef samples or added oils in meat alternative samples

Methods

Sample preparation: Raw 85% lean, 15% fat ground beef and grass-fed beef were tested alongside 3 commercially available products that are described as Products A, B and C. A Bligh-Dyer extraction was performed on 3 replicates of each sample type. To control for extraction efficiencies, 10 µL each of Lipidyzer and Avanti SPLASH Lipidomix standards were spiked in. Each replicate was normalized by tissue weight.

Chromatography: Separations were performed on an ExionLC AC system using a Phenomenex Luna 3.0 µm NH2 column (2 x 100 mm). PN: 00D-4377-B0

Mass spectrometry: A SCIEX 7500 system with an OptiFlow Pro ion source (electrospray ionization probe) was used for data acquisition in positive and negative mode. A targeted assay was built using the Scheduled MRM algorithm (sMRM) for quantification. For details on the LC-MS/MS methods, see references.[4]

Data processing: All data was processed using SCIEX OS software using the MQ4 algorithm. Automated computation of the time scheduled MRM methods was performed using the sMRM Pro Builder v1.4.5

Global lipidomic profiling

There are various LC-MS/MS methodologies in which to probe the lipidome, however many can be time consuming from method setup to data analysis. Here, a high-throughput, targeted lipid screening protocol was utilized to broadly and rapidly profile 21 lipid classes and screen more than 2200 lipid molecules. Afterusing the sMRM Pro Builder template to focus the analyses,[5] the final sMRM method screened more than 1100 lipids in a 17-minute chromatographic run time in a single injection. Althoughseveral lipid classes were included in the final analyses, this study focuses specifically on triacylglycerols (TAGs). The clustering observed in Figure 1 can be attributed to the changes in this lipid class. A total of 498 TAG species were monitored in the final sMRM method out of the total 520 transitions included in the panel. The final lipid count by class can be seen in Figure 2.

Triacylglycerol and fatty acid analysis

When analyzing lipid profiles in food samples the total carbon length of triacylglycerols (sum of all 3 fatty acid chain lengths on the glycerol backbone) can be indicative of the origin of the fat. Figure 3 shows the TAG profiles for each sample type, organized by carbon chain length. The meat alternatives show more diversity in their total carbon length profiles, spanning from C30 to C58, with largest summed average peak areas in carbon lengths C30 to C44. This difference in distribution can be attributed to the oils (often added for taste and texture) in the alternative meats being sourced from plants. Coconut oils are rich in short chain TAGs, whereas soybean, canola and corn oils have longer chain length lipids. 6 Soybean, canola and corn oils are rich in linoleic acid and can be observed in Figure 3 at sum composition C54 or in Figure 4 at fatty acid C18:1.

Total carbon length does not always indicate how healthy a fat source can be. The fatty acids monitored from all TAG molecules were summed based on average peak area for the replicates in Figure 4. While the meat alternatives had a higher proportion of saturated fats compared to the ground beef, the saturated fats were short-chain fatty acids, which have been shown to be beneficial. [7] The ground beefs profiles are mostly comprised of C16 and C18 fatty acids with an emphasis in saturated C16 and monounsaturated C18. The C20 and C22 series were predominantly present in the ground beef samples, especially as the number of double bonds increases. This is one health benefit of the ground beef samples, as foods with polyunsaturated fats tend to have positive health benefits.[8]

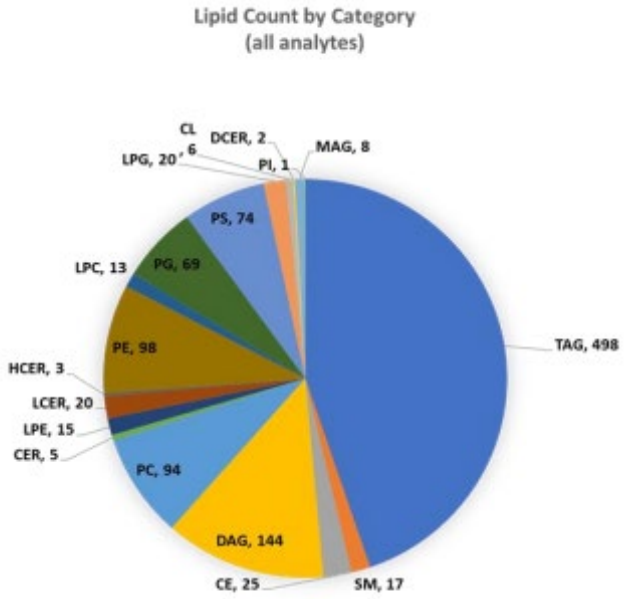

Figure 2. Distribution of lipids in final sMRM assay. Using the sMRM Pro Builder template, lipid species were filtered and the final sMRM list was curated and assayed. The final lipid count, by class, is shown.

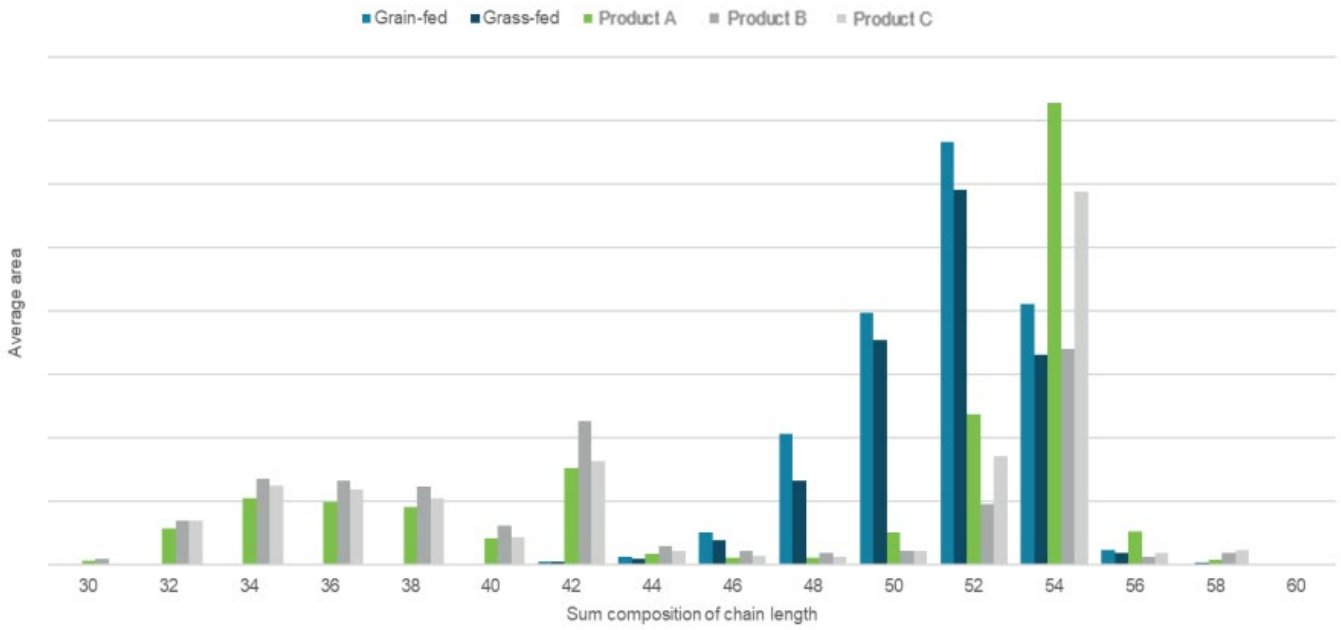

Figure 3. Sum composition of carbon length of triacylglycerols (TAG). The MRM peak areas of all TAGs within each chain length were summed for each sample then averaged within each group analyzed. The short chain lengths C30-44 are more abundant in the meat alternatives than in the ground beef, which is most abundant in chain lengths C46-52. All samples contain C54, with Product B containing the most.

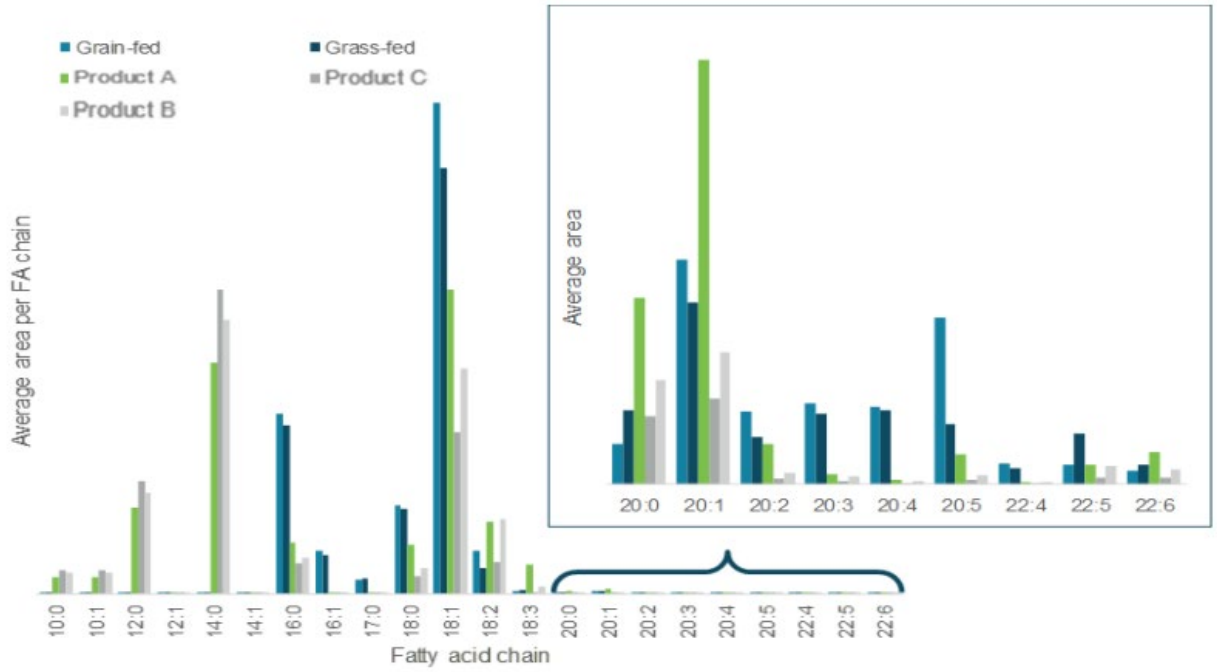

Figure 4. Fatty acid profiles of TAG species monitored. Each acyl chain length that was monitored in the TAG species was summed for the replicates and then averaged for each group and reported by that fatty acid chain. The grain-fed and grass-fed beef are shown in the light and dark blue bars and show more medium-chain saturated and monounsaturated and long-chain polyunsaturated fats. The meat alternatives have lipid profiles with more short-chain and long-chain saturated fats.

Unique markers for differentiation of lipid source

While looking at the overall composition of the lipidome profile can be a good indicator of the origin of the fat, a unique marker to identify the exact source can be utilized in targeted methods for origin identification or origin authentication. Figure 5 shows a unique marker for Product A meat alternative and Figure 6 shows two unique markers for grass-fed beef. Markers in the alternative meats could also be used to ensure the quality of the fats being added, as well as being able to monitor any potential changes in the lipid profiles from handling or treatment of the food that could affect the taste or texture of the product.
The markers for grass-fed beef could potentially be used to authenticate the diet the animal has been fed. Mammals do not produce the enzymes that synthesize fatty acid 18:3, which is present in the green leafy tissues of plants. Therefore, this fatty acid is unique to animals on a grass-fed diet. This fatty acid could likewise be a unique marker for oils or fats added to meat alternatives.

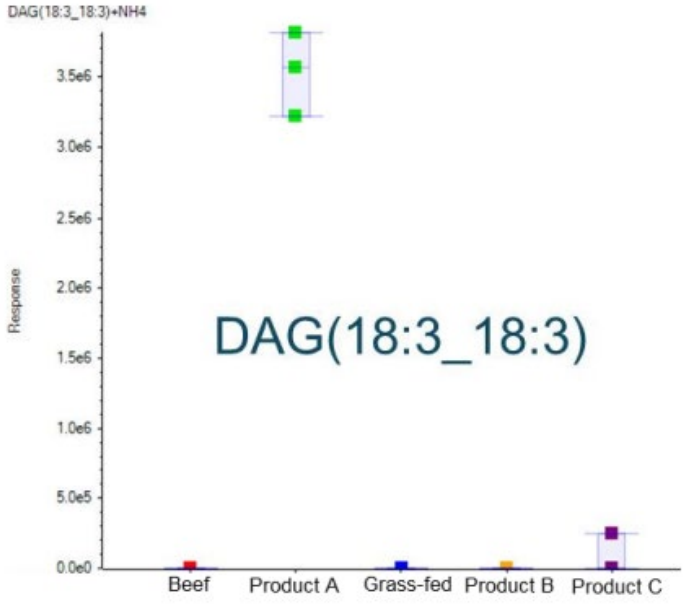

Figure 5. Unique marker in Product A. Using MarkerView software, a diacylglycerol (DAG) species, DAG(18:3_18:3) was found to be significantly increased in the Product A samples compared to the other samples.

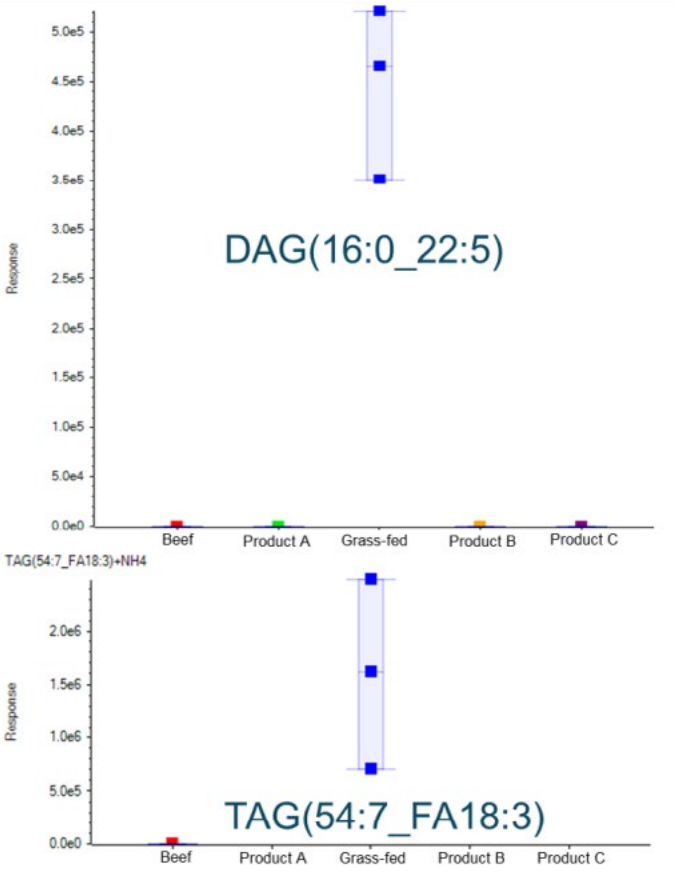

Figure 6. Increased neutral lipids in grass-fed beef. Two markers, DAG(16:0_22:5) and TAG(54:7_18:3), were identified only in grass-fed beef samples.

Conclusions

· The SCIEX 7500 system enables users to profile and identify the lipid composition of meat and meat alternative samples

· Utilizing the global lipidomic profiling method, users are able to report total carbon length profile changes, fatty acid profile changes, and lipid class changes in diverse set of fat sources

· MarkerView software provides useful visuals to determine lipid classes of interest for further study

· Fat sources from meat alternatives can be profiled to establish dietary benefits and to monitor for changes that could affect taste and texture

· The global profiling method can be used to authenticate the animal diet by targeting markers specific for grass-fed or grainfed meat

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2. DiNicolantonio JJ, O'Keefe JH. Good Fats versus Bad Fats: A Comparison of Fatty Acids in the Promotion of Insulin Resistance, Inflammation, and Obesity. Mo Med. 2017;114(4):303-307.

3. Achieve broad lipid quantitation using a high-throughput targeted lipidomics method. SCIEX technical note, RUOMKT-02-8482-B

4. Contact Mackenzie Pearson (Mackenzie.Pearson@sciex.com) for Standard Operating Protocol and LC-MS/MS details

5. Download the sMRM Pro Builder Template.

6. Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. Int J Mol Sci. 2015;16(6):12871-12890.

7. Tan J, McKenzie C, et al. The role of short-chain fatty acids in health and disease. Adv Immunol. 2014;121:91-119.

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Alternative proteins

Technical content summaries

High-throughput milk A2 -Casein Analysis by Capillary Zone Electrophoresis (CZE)

USA

Cow milk contains 13 types of λ -casein with A2 λ -casein found to cause the least symptoms of post-dairy digestive discomfort. This has led to increased demand for cow milk with high A2 λ -casein content and the need for authentication of high A2 λ -casein content in milk. This technical note describes the use of a CZE-UV application workflow on the BioPhase 8800 system to generate a fast and high-throughput application for quantifying A2 λ -casein in milk. The BioPhase 8800 system handled samples stored at different conditions with ease, as challenging collection methods can compromise testing and analysis.

Development of a targeted LC-MS/MS method for the detection of microbial transglutaminase from Streptomyces mobaraensis

UK, Germany

Transglutaminase can be used to cross-link smaller pieces of any type of meat, fish, or meat product to produce large, virtually intact sections. This work describes a targeted peptide methodology that was developed to ensure consumers can be confident in the origins of their food. It delivers reproducible quantitation of peptide targets found in microbial transglutaminase from Streptomyces mobaraensis in normal matrix. DiscoveryQuant software was used to optimize peptide standards coupled with a quick LC run time of 15 minutes to produce a high-throughput analytical method. .

A comprehensive method for in-depth profiling of secreted metabolites in cell culture media from human adipose stem cells

Italy, USA

Analyzing cell culture metabolites secreted from human adipose stem cells has become a key step in the development of therapeutics for regenerative medicine. A sensitive profiling and quantitation method for secreted metabolites analysis in cell culture media is presented here. Within 20 minutes, over 110 cell culture metabolites were monitored with a single method featuring fast polarity switching on the SCIEX 7500 system. Statistically significant quantitative trends demonstrated which components were upregulated or downregulated over the cell growth stages.

Honey authenticity analysis: a proposed workflow using the SCIEX X500R QTOF System

KC Hyland, Diana Tran, SCIEX, USA

Our global food supply chain is far reaching, complex, and profitdriven. A wide range of methods have been employed to screen food products and commodities to confirm authenticity and detect adulteration. Honey is one of the most commonly adulterated food commodities globally. One economically motivated adulteration practice which has been observed in the global sales and trades of honey commodities is the dilution of the honey products with a cheaper sugar syrup, such as corn syrup. Dilution or fraudulent labelling can also occur in instances where honey products have disparate levels of value due to rarity or other unique properties. Techniques to screen for fraudulent or diluted products include various analytical techniques such as physical, chemical, or morphological assessments; for example, morphological pollen analysis for botanical origin of honey, or the C4 isotope test for the presence of corn-derived sugars. Mass spectrometry for testing food authenticity can be utilized for targeted, and/or nontargeted analytical methods. Targeted methods by nature rely on there being a known adulterant or residue for which products must be screened; examples include illegal dyes, or melamine, and are typically analyzed using multiple reaction monitoring (MRM) transitions on a triple quadrupole mass spectrometer.

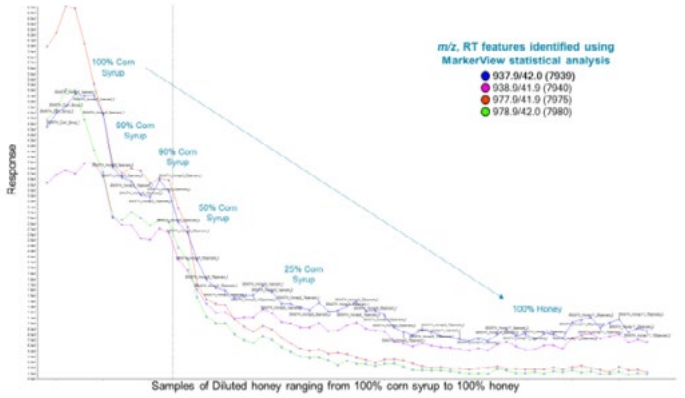


Figure 1: Marker compounds for identifying and quantifying corn syrup adulteration in honey. A series of dilutions of a honey sample with corn syrup is used to demonstrate the ability to plot the response of these m/z features and thus illustrate a quantitative capacity to measure honey dilution with corn syrup. The MarkerView Software was used to pick out four mass/retention time features unique to the corn syrup relative to the honey samples. While the exact structure identification of these four compound features is as yet unknown, the workflow proposed in this study was employed to achieve this information and be able to show this potential for developing methods for accurate honey screening.

Nontargeted mass spectrometry-based methods, common in the "Omics" disciplines (proteomics, metabolomics, lipidomics), can be employed in the identification and characterization of reliable marker compound(s) which would then be transferred to a targeted method for routine monitoring.1

This study shows the potential for the X500R QTOF System, SCIEX OS Software, MarkerView™ Software, and MS/MS libraries to be leveraged to develop and employ a nontargeted method for investigating honey chemical profiles and potential for adulterant screening.

Advantages of the X500R QTOF System for honey authenticity testing

· SWATH® Acquisition allows for the collection of spectral data for all ionizable, detectable constituents in the honey sample. These data are then available for characterization and profiling of honey commodities and identifying unique chemical markers.

· SCIEX OS Software and SCIEX validated MS/MS library allow for the tentative identification of naturally occurring constituents in honey

· MarkerView Software allows for the rapid visualization of PCA and t-test statistical analyses in order to discern the most unique, and therefore most valuable, chemical features in the complex honey and corn syrup matrices

· The proposed workflow was employed to show that markers unique to corn syrup can be identified and used to screen and quantify dilution of honey products with corn syrup

Methods

Sample preparation: Honey and corn syrup samples were purchased from local producers in order to best ensure that the samples were authentic in nature and also to capture different floral types of honey products. Eleven different honey samples were tested, and triplicate analyses were done for each. Honey was weighed out 1 gram and 10 milliliters of 50% methanol for LC-MS/MS analysis.

To mimic fraudulent honey products and assess the ability of the method to detect corn syrup adulateration, an experiment was performed in which honey samples were diluted with a series of increasing corn syrup concentrations, up to and including 100% corn syrup by mass, prior to sample extraction.

LC separation: A 10 µL volume of sample was injected using an ExionLC™ AD System coupled to the SCIEX X500R QTOF System. Separation was performed using a Phenomenex Luna Omega Polar C18 (150λ 4.6 mm, 3 um) analytical column. The LC mobile phases consisted of 0.1% formic acid in water (A) and 0.1% formic acid in methanol (B) at a flow-rate of 1 mL/min and column temperature of 25°C.

Mass spectrometry: Analysis was performed using the SCIEX X500R QTOF System, operated in both positive and negative polarity modes. The data independent acquisition strategy (SWATH Acquisition) was employed in order to collect high resolution precursor and product spectral information for all detectable constituents in the samples. The following MS source conditions were used: CUR=40 psi, CAD=11, IS =5500/-4500 V, TEM=500°C, GS1= 60 psi and GS2= 60 psi.

Variable window SWATH Acquisition was utilized to obtain high quality MS/MS spectra with 38 different precursor mass windows, the accumulation for the TOF MS is 0.1 sec and the accumuliation time for the TOF MS/MS is 0.025 sec.

Data processing: Data were processed using SCIEX OS-MQ Software 1.5 as well as MarkerView Software for statistical analyses. The SCIEX Natural Products 2.0 Library was used for searching database compound spectra for matches to experimentally derived spectra.

Table 1: Gradient conditions used for the LC separation. Flow rate of 0.3 mL/min was used.

Time (min)	B (%)
0	5
38	100
41	100
41.1	5
45	End

Results

The SCIEX X500R QTOF System was used with SWATH Acquisition to collect high resolution mass spectral data on constituents present in the honey samples of different floral origins and honey diluted with corn syrup. The ability to collect high resolution MS1 data allows statiscal analyses to be applied to the different chemical profiles of the different sample sets. The additional collection of the comprehensive MS/MS spectral information afforded by the SWATH Acquisition allows for further exploration of those chemical profiles - including identifying candidate structural matches through use of spectral libraries.

Differentiating between honey variants with PCA

Acquired data were imported and processed with MarkerView Software. In this workflow, the software will first pick all the features present in the TOF MS data, each feature identified as an m/z and retention time pair. The feature profiles across the different samples can then be statistically compared to find differences between sample sets, and identify features which are uniquely present (up-regulated) or uniquely absent (downregulated) in a particular sample. The Principal Component Analysis (PCA) shown in Figure 2 demonstrates the ability to use the SWATH Acquisition data acquired to statistically distinguish multiple honeys derived from three different floral origins.

Additionally, presenting the corn syrup data in the same PCA plot allows for a positive control; it is clearly observed that the corn syrup feature profile is highly differentiated from the honey samples in that it clusters far from the authentic honeys in the PCA plot. In practical applications, this type of analysis may serve two foreseeable purposes: to compare an unknown honey sample against a model built from data acquired for a large population of known authentic samples; or to profile honeys in order to investigate unique markers which may be present in different products of varying origins or processes.

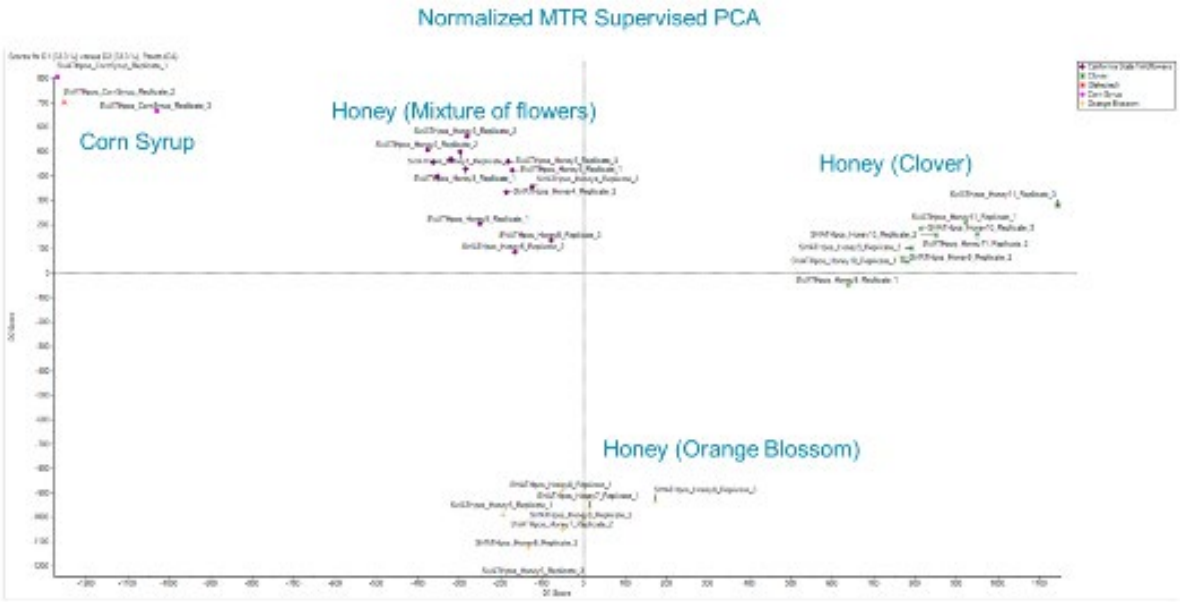


Figure 2. Principal Component Analysis of honey variants. PCA output from MarkerView Software of honey variants shows visually that the chemical profiles between honey samples of different floral origins are distinct. Three different honeys of orange blossom origin, three different honeys of clover origin, and four different honeys of wildflower/mixed flower origin were used show this distinction. Additionally, corn syrup is observed to have unique characteristics that place it on the plot distinct from the honeys.

Investigating unique marker features using T-tests

The next step in the workflow is to identify specifically which m/z features represent unique markers for a particular sample type. This might be accomplished in one of several ways, but Figure 3 shows a direct comparison between two groups using a t-test, plotting a volcano plot of the m/z features in order to quickly and visually find those with the greater difference in signal (log fold change between the two compared sets) and greatest statistical significance (lowest p-value).

Library searching for compound candidate ID

A primary advantage of acquiring data using SWATH Acquisition is the collection of MS/MS spectral information for every detectable precursor in the defined mass range. This allows product ion spectral information to be searched in a database for potential compound identification. For this study, the SCIEX Natural Products Library 2.0 was leveraged in order to attempt toidentify some of the characteristic components of the different honey samples and see how these components' occurrence varied between honey origins.

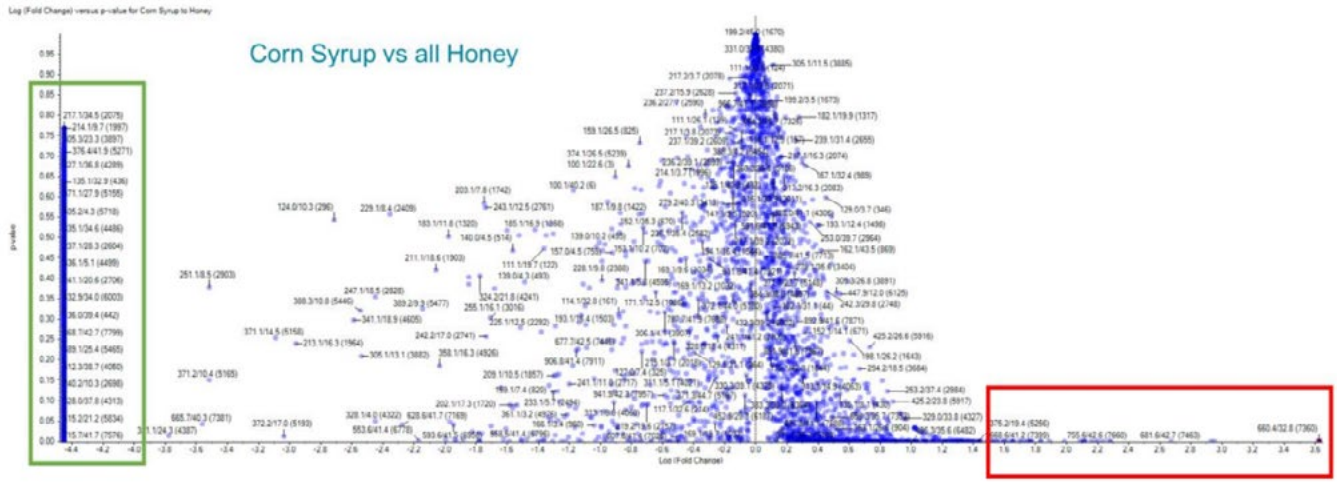
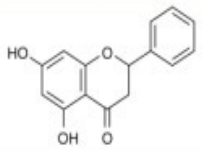
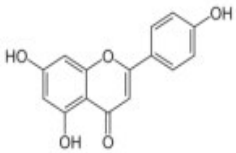
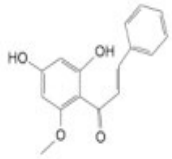
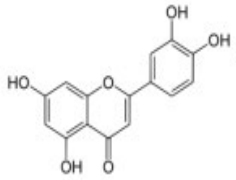
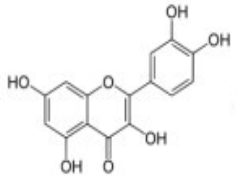
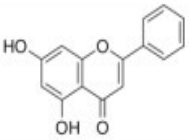
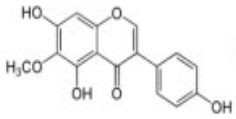


Figure 3. Volcano plot (p-value vs log fold change) constructed from T-test comparison of corn syrup sample set to combined set of all honeys. Green box shows features with the greatest upregulation in the honeys versus the corn syrup. Red box shows features with the greatest upregulation in corn syrup vs all honey.

Table 2. Natural products identified in honeys using MS/MS.

Compound Name	Structure	Description	Ref.
Pinocembrin		*Flavanone, antioxidant*	*2*
Apigenin		*Flavone, aglycone of natural glycosides*	*3*
Cardamomin		*Chalconoid*	*5*
Luteolin		*Flavonoid common to fruits, vegeatbles, and herbs used in traditional medicine*	*3*
Quercetin		*Plant flavonoid used as medicine*	*3*
Chrysin		*Flavone found in honey and flowers*	*3*
Tectorigenin		*Isoflavone found in some lilies*	*4*

There were seven natural products which were all detected in at least one honey sample with a library match score of at least 75 and a corresponding mass error within 5ppm (most within 1ppm except when the level was very close to noise). These were Pinocembrin, Apigenin, Cardamomin, Luteolin, Quercetin, Chrysin, and Tectorigenin (Table 2). Five of these were identified by Cianciosi et al. as being among the most common polyphenols detected in honey.[3] Figure 4 shows some example outputs from the SCIEX OS Software Analytics results table, in which can be seen the chromatographic peak, the TOF MS precursor data with empirical formula identification with FormulaFinder, and the MS/MS spectrum matched to a database entry and shown with a fit-based score representing how close the match is.

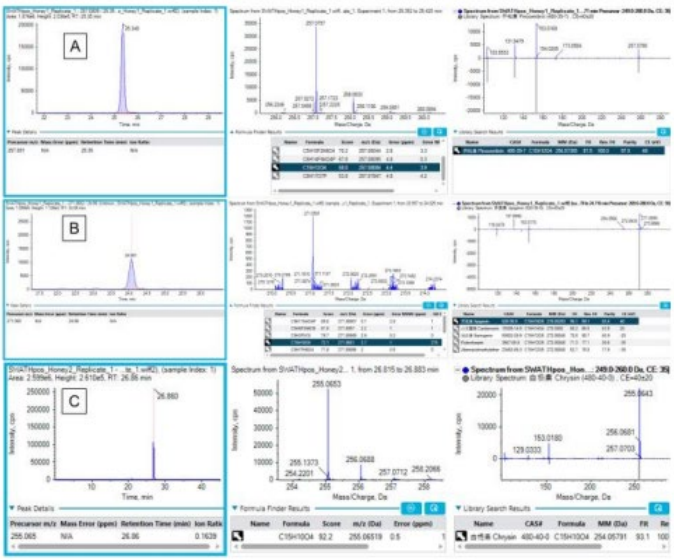


Figure 4. Screening the SWATH Acquisition data for the honey samples against the SCIEX Natural Products Library. Three identified natural products are shown as examples. From left to right is shown the chromatographic peak detected, the TOF MS spectrum and the corresponding empirical formula determination, and the acquired MS/MS spectrum mirrored with the matched MS/MS spectrum from the database with the compound name and fit score listed. A) Peak at 257.081 Da and 25.3 min. Formula match to $C_{15}H_{12}O_4$ within 4ppm mass error. MS/MS match to Pinocembrin, a known metabolite in honey, with a 97.5 Purity score. B) Peak at 271.060 Da and 24.0 min. Formula match to $C_{15}H_{10}O_5$ within 1ppm mass error. MS/MS match to Apigenin, a known metabolite in honey, with a 93.4 Purity score. C) Peak at 255.065 Da and 26.9 min. Formula match to $C_{15}H_{10}O_4$ within 1ppm mass error. MS/MS match to Chrysin, a known metabolite in honey, with a 93.1 Fit score.

There were seven natural products which were all detected in at least one honey sample with a library match score of at least 75 and a corresponding mass error within 5ppm (most within 1ppm except when the level was very close to noise). These were Pinocembrin, Apigenin, Cardamomin, Luteolin, Quercetin, Chrysin, and Tectorigenin (Table 2). Five of these were identified by Cianciosi et al. as being among the most common polyphenols detected in honey.[3]

Relative quantitation of identified natural products

At this point in the study, the seven natural products identified in the honey samples could then be imported into SCIEX OS Software as a targeted components list. This targeted components list could then be applied to honey samples to achieve relative quantitation of these species in the different honey varietals. Figure 5 shows the amount of each of these as represented by the chromatographic peak area in the different honeys as well as in the corn syrup and extraction blank. It can be seen that while the profile of these flavonoids and other compounds varies between honey types, they do not appear to be present in either corn syrup or extraction blank.

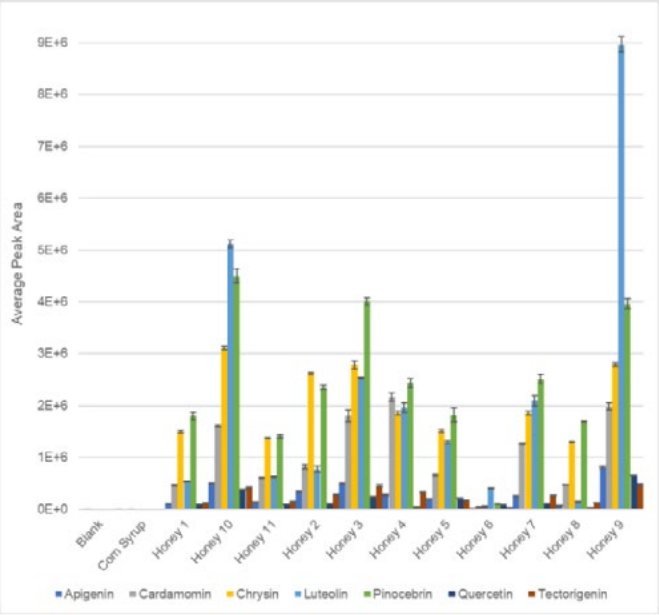


Figure 5. Relative amounts of polyphenols detected in 11 different honey samples. Both the corn syrup and the extraction blank showed an absence of the natural products associated with the honeys. Different honey samples appear to have different polyphenol profiles. Error bars represent a standard deviation about the mean of triplicate analyses.

Dilutions with corn syrup

This experiment focused on one of the primary questions in testing honey for authenticity: whether or not the presence of a corn syrup diluent can be discerned. One representative honey product was selected, and made into dilutions with corn syrup at a range of concentrations from 0% corn syrup (pure honey), 25%, 50%, 90% and 100% corn syrup. The previous MarkerView Software statistical comparison showing the corn syrup t-test versus all combined honeys was used to pick out mass/retention time features unique to the corn syrup and not present in any honeys. Plotting the response of these features across the series of diluted honey samples thus illustrates capacity to measure honey dilution with corn syrup (Figure 1). While the exact structure identification of these compound features was not confirmed with structural elucidation or matching with an analytical stardard, the workflow proposed in this study was able to achieve this information and show potential for developing methods for accurate honey screening for the known adulterant corn syrup.

Conclusions

This study shows the potential for the X500R QTOF System, SCIEX OS Software, MarkerView Software, and MS/MS libraries to be leveraged to develop and employ a nontargeted method for investigating honey chemical profiles and for adulterant screening.

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Authenticity testing & food fraud

Technical content summaries

Analysis of chloramphenicol in honey

Chloramphenicol is an antibiotic used to treat infections caused by bacteria. It is widely used as a human antibiotic and veterinary drug, and it is sometimes applied to beehives to reduce incidences of disease. This technical note describes a method for analyzing honey for the presence of chloramphenicol, which can have adverse effects on human health. Using the SCIEX Triple Quad 3500 system, multiple reaction monitoring (MRM) was used to quantify chloramphenicol in store-bought honey and the ion ratio was used to confirm its identification.

Authentication and geographical origin analysis of plant-derived edible oil using the X500R QTOF system

China

Plant-derived cooking oil is widely used and an important source of nutrition. The authenticity and traceability of these edible oils is critical for consumer safety. This technical note describes the use of LC-MS on an X500R QTOF system to establish a global profiling workflow for determining the authenticity and geographical origin of plants that are important to edible oil production. This type of vegetable oil quality analysis could be an important tool for assessing fraudulent or adulterated products in the global food supply.

Analysis of Nitrofuran Metabolites in Honey Using the SCIEX Triple Quad™ 3500 System

Nitrofurans are broad-spectrum antibacterial agents that were often used to treat bacterial infections in bee colonies until their use in food-producing animals was prohibited in the EU and other countries due to public health and safety concerns. While nitrofurans are unstable and easily metabolized within a few hours, nitrofuran metabolites are highly stable in nature. This technical note describes the quantitation of nitrofuran metabolites in honey using an LC-MS/MS method developed on the SCIEX Triple Quad 3500 system that meets the regulatory requirement of 1 µg/kg.

Precise Quantification of Melamine in Milk

India, Germany

Melamine is a compound that is sometimes used to artificially inflate the protein content of milk. The ability to quantify melamine in milk is critical to consumer safety, as it can cause tissue injury at high concentrations. This technical note describes a fast and simple method for melamine analysis in milk using the SCIEX Triple Quad 3500 system. The limit of quantitation (LOQ) achieved was 2.5 ng/mL, which was significantly less than the maximum residue limit (MRL) of 150 ng/mL set by the Food Safety and Standards Authority of India. Quality control samples (n=6) showed an accuracy range of 88–98% and precision (%CV) of <6% across the spiking range of 2.5–225 ng/mL.

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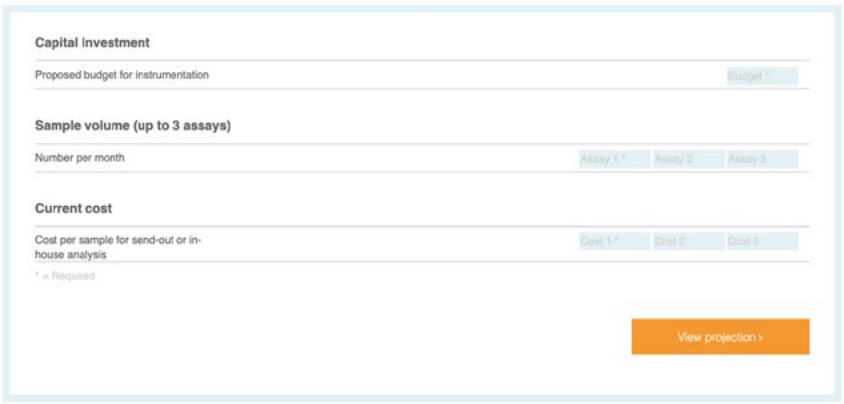

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Do you know what's really in your food?

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Nearly 4 billion tons of food are produced each year to feed our global population of nearly 7 billion people. The safety and quality of the global food supply is fundamental to human health and prosperity, and contaminated food has been linked to acute and long-term (and sometimes even life-threatening) health risks.

Food contamination affects everyone. Food producers are stressed to ensure the production of sufficient quantities of food to meet global demand. Chemical residues, such as pesticides or antibiotic drugs, are sometimes used to increase farming productivity, and those residues have the potential to accumulate in the resulting food products. Manufacturing plants are often required to process a variety of different products in a single facility, resulting in cross contamination risks (a potential cause of unclaimed allergens). Food suppliers, farmers, manufacturers, and regulatory agencies remain on a mission to ensure global food safety, and they want consumers to feel good about what they are eating.

Just what are food safety scientists testing for? Your food can contain contaminations of all kinds, including heavy metals, microbial contaminants, allergens, or chemical residues such as pesticides or adulterants. It is the job of the food industry to assess the food supply on a daily basis to ensure that our food is free of hazardous contaminants and is good enough to eat.

The trials of a food tester

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It is a great feeling to know that you are buying food that is safe to eat when you go to the grocery store. We have the food safety testing industry to thank for that. Food scientists work day-in and day-out testing food for contaminants and/or nutritional value, developing new methods to better test food for those assorted compounds, and surveying food samples for potential unknown residues, adulterants, or other components.

Food scientists have a number of things to consider when approaching a food testing workflow. The most critical is finding a contamination – an error could have devastating consequences, including consumer health impacts and product recalls.

Top considerations in food testing

Food samples are innately complex, making food analysis a challenge. Food products contain an assortment of chemical compounds, from proteins, carbohydrates, fats, and vitamins (healthy compounds) to residues and contaminants (unhealthy compounds). It is the job of the food tester to find anything unwanted, or potentially hazardous, among all the good.

Food samples are also perishable, so testwing must be done in an efficient and timely manner. Food testing labs are often faced with stringent sample turnaround deadlines – they are not only pressured to produce accurate results, but must do so under significant time constraints.

Technical steps in food testing

The food testing workflow contains many steps – first, residues of interest must be extracted from the food sample. Then, any interfering or unwanted matrix components (such as the proteins, sugars, or other endogenous compounds) must be removed to reduce their impact on the measurements. Next, the samples are analyzed using the appropriate technology. The data is processed, and any findings reported. Each of these steps must be strategically considered and optimized to ensure the best results for the analysis.

The Power of Precision