SCIEX Triple Quad™ 3500 LC-MS/MS System

compendium, volume 2

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SCIEX The Power of Precision

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Introduction: making the right choice

You always start from the bottom when you climb a mountain. The message is simple: you need to master your basics before progressing to the next level. This applies to everything in life.

It's no different in the world of analytical science. Building a solid foundation is vital. Having a well-trained team is at the core of a laboratory's success, whether it's delivering accurate results, improving productivity or maintaining a safe environment.

Gaining competence and confidence is no easy task with complex interdisciplinary projects and an array of laboratory methods. It all begins with hands-on experience and on-site resources that can provide practical application and industry-related knowledge, and enrich and foster a better understanding of concepts that prepare you to thrive in modern laboratories.

Choosing the right route up the mountain

There is a wide range of analytical methods that qualitatively analyze the chemical makeup of samples as well as each component within a sample. The type of analytical instrumentation to invest in depends on the requirements for experiments planned for not only the near term, but also the long term.

The options are seemingly endless, from gas chromatography-mass spectrometry (GC-MS) to liquid chromatography-tandem mass spectrometry (LC-MS/MS). With so many choices, how do you decide on the best technique for you and your laboratory?

The following factors are key considerations as you evaluate your choices: multi-parameter testing capabilities, ease of use, robustness, maintenance requirements, expert support availability, cost and mass adoption ability.

While it's not a one-size-fits-all solution, it's important to note that these days, most laboratories seem to be dabbling in mass spectrometry. It is a workhorse in various fields, including food and beverage, environmental, forensic and pharmaceutical analysis. For this reason, students interested in a career in science must be acquainted with the fundamental concepts of mass spectrometry. It's also essential for both startup and academic laboratories to seek out robust analytical instrumentation to tackle challenging research.

There is no denying that a mass spectrometer is still a significant financial commitment, so we understand that research universities need to see value while assessing potential analytical techniques.

An affordable mass spec solution from SCIEX to expand your lab's potential with LC-MS/MS

It's time to get the best quality data you can from your samples and grow your lab's capabilities with an introductory SCIEX Triple Quad™ system.

1 Worthwhile investment

Increased ROI potential

LC-MS/MS expands your lab's portfolio of tests, and with its ease of use and dedicated support and training, you will be up and running and quickly delivering valuable analysis to new and existing customers.

2 See your analytes with confidence

Accuracy and speed combined

The unique scan functions of LC-MS/MS enable you to see the qualifying components of your compounds of interest, so you can quantify and rapidly report to on your regulatory compliance with more accuracy and confidence than you can achieve with HPLC.

3 More compounds, more samples and less time

Expand your lab's offerings

LC-MS/MS enables you to combine laborious workflows associated with HPLC and put them into 1 suite without compromising data quality or requiring expensive staffing. By employing LC-MS/MS, you can grow your lab, offer more services and stay ahead of your competition.

4 Get more data even in tough matrices

See what your HPLC assay can't

With LC-MS/MS, the matrix doesn't get in the way of the data. Our mass spectrometers are engineered to handle the most complex food matrices and provide you with highly accurate results, injection, after injection with maximum uptime. See more, test more and earn more.

SCIEX mass spectrometry:
Better accuracy. Superior confidence. Unrivalled speed.

Why LC-MS/MS?

Historically, LC-MS/MS has been used as a complementary tool for analyzing compounds that were difficult or impossible to analyze by GC.

Traditionally, GC-MS has been the preferred technique for analyzing less polar and more volatile compounds. While this technique can offer good separation, the high temperatures used for sample vaporization can alter many of the analytes in a sample.[1] This is the primary concern for life science researchers who deal with relatively labile biological molecules that break down easily at higher temperatures. GC-MS also becomes a challenge because it can involve labor-intensive sample preparation and long chromatographic run times.

These factors have led to the rapid adoption of LC-MS/MS in analytical and routine testing labs across various applications. LC-MS/MS offers specific advantages over GC-MS, such as the ability to identify and measure a broader range of compounds with minimal sample preparation. The technology is broadly compatible with both polar and nonpolar compounds. It offers exceptionally high selectivity and sensitivity, which typically results in radically improved limits of detection, quantification and accuracy over GC-MS. The transition from a niche tool for analytical chemists to standard equipment in life science labs has been further aided by greatly simplified sample preparation. Many analyses throughout the industry are now being transferred from GC-MS to LC-MS/MS, enabling labs to reduce costs and minimize the potential for errors or contamination.

Part of the enhanced sensitivity and selectivity of LC-MS/MS comes from the coupling with tandem MS, which allows experiments such as multiple reaction monitoring (MRM). LC-MS/MS with MRM can be utilized to analyze various drug classes in a single assay for quantification/confirmation and for comprehensive drug screening. These workflows also allow trace level quantification of analytes (at parts per trillion) in complex matrices such as food, tissues and soil.

The versatility delivers the simplified sample preparation and handling of LC-MS/MS instrumentation. Unlike GC-MS experiments that require organic or chlorinated injection solvent and the derivatization of samples for optimum peak shape, ionization and volatility, LC-MS/MS typically does not have these requirements.

This robust system can handle dirtier and more challenging samples and a wider variety of underivatized analytes. While LC-MS/MS sample preparation methods can include solid-phase extraction or liquid-liquid extraction, they are often as simple as direct injection, dilution or protein precipitation.

But is LC-MS/MS the right tool for your laboratory?

Mass spectrometers are frequently combined with separation techniques to form robust integrated LC-MS/MS systems. And here's where triple quadrupole systems offer good trace level quantification in complex, dirty samples. Whether it's analyzing water samples with low-level contamination, trace level pesticides in food or even key biomarkers, triple quads are ideal. Put simply, LC-MS/MS systems offer these primary benefits:

- Faster, simpler sample preparation
- Direct injection of aqueous samples
- Less need for derivatization
- Shorter chromatographic run times
- Increased selectivity, specificity and sensitivity with multiple reaction monitoring (MRM)
- Screening for a broader range of compounds in a single analysis

If your students work on papers and posters that get your institution noticed, or your students are looking to propel their careers in analytical chemistry, having hands-on mass spec experience with industry instruments is undoubtedly an asset.

And it's all possible with the SCIEX Triple Quad™ 3500 LC-MS/MS System.

The SCIEX Triple Quad™ 3500 LC-MS/MS System

The SCIEX Triple Quad 3500 System combines modern hardware, powerful software and robust engineering to enable lower detection and quantification levels than conventional GC and LC workflows. Across a broader range of analytes, it delivers consistent results across multiple samples.

The system incorporates the legendary Turbo V™ Ion Source, which ionizes samples efficiently, and the Curtain Gas™ Interface, which protects from contamination, to give labs consistent data quality over long runs with minimal downtime. Using a single injection, hundreds of analytes can be analyzed on the SCIEX Triple Quad 3500 System, making it the ideal companion for any high-throughput testing laboratory. With the SCIEX Triple Quad 3500 System, you will experience faster scan speeds, improved polarity switching, enhanced sensitivity and data acquisition algorithms to maximize throughput and help ensure that all targeted compounds are reliably detected in every injection.

Take 5 with Alex Liu

Alex Liu

SCIEX Senior Product Manager for Entry-Level Nominal Mass

Mass spectrometry has often been described as the smallest scale in the world. And it's not because of its actual physical size. It's because of the size of what it weighs: molecules. This analytical technique enables the detection of an ionized target molecule, the analyte and its mass measurement.

Over the past decade, we've witnessed tremendous technological advancements in mass spectrometry that have enabled its application to proteins, peptides, carbohydrates, drugs and many others.

To make your journey easier, we caught up with our in-house expert, Alex Liu, to gather his thoughts on mass spectrometry as a promising technique that is transforming analytical science.

What are the factors and application considerations involved in buying a new mass spectrometer?

Buying a new mass spectrometer is a significant investment, and when considering options, I recommend going with the 4 S's as your guide: sensitivity, specificity, selectivity and speed.

Sensitivity

These days, many analytes are measured at parts per billion and parts per trillion levels. This is especially the case in food testing, pharmaceuticals, forensics and even clinical labs. So, having the means to accurately detect analytes at these ultra-low concentrations is a crucial component.

Specificity

High specificity leads to better data, especially when dealing with complex matrices. You need to look for a system that can effectively discriminate isobaric species, and reduce false positives and negatives to deliver accurate, precise results.

Selectivity

Interferences are a big problem when you work with complex matrices. Having the means to offer good chromatographic separation is a valuable criterion. Ideal mass spectrometry gives you the ability to separate your analyte of interest from the matrix and detect and accurately quantify it.

Speed

With analytical methods, speed is often tied to an instrument's ease of use. Faster analysis, from faster scan speeds to faster results, means greater benefits. An instrument powered by intuitive software can bring the speed you need to get to the crux of your analytical problem.

And then there's the price factor. With limited resources, cost is always at the center of the conversation. Be sure to look at the value in terms of upfront cost vs. output. When you look at prices, weigh the system costs against the benefits of higher throughput, which can translate into lower analysis costs.

Why are people wary when considering mass spectrometry for their laboratory?

There are a few misconceptions that hold people back from even considering mass spectrometry as a solution for them.

Mass specs are too expensive and complex

This is a fair assumption. But putting things into context is key. There's no denying the upfront cost, but you can do more testing with mass spectrometry. By condensing multiple tests into a single test, your lab will improve its efficiency and turnaround time for delivering results. In a way, although the investment costs are substantially higher than most other techniques, subsequent costs for test development and operation are low. What's more, mass spectrometry allows your lab to respond to changing demands and offers the highest quality analysis level. In a way, it is a more sustainable investment.

Similarly, while there are some practical aspects of learning new technical skills, with the appropriate training, mass spec implementation can happen with little disruption to your lab's daily workflow. With expert support, especially in methodological development, your team's skills will eventually grow naturally and contribute to your lab's capital.

Time to results takes forever

To me, this assumption is a little misleading. This goes back to my previous point about speed. Since a mass spec distinguishes compounds based on mass, the chromatographic method does not have to separate every single component in the sample.

Assays require separate kits for multiple samples, and separate workflows for things like pesticides depend upon the functional group. Co-elution of non-isobaric analytes is possible. This allows fast LC analysis times and reduced sample preparation. You can combine your methods into a single injection, even when testing a large mycotoxin suite. These steps get you a faster turnaround time.

HPLC is good enough

Perhaps you already use mass spectrometry, but you are hesitant to make the leap from HPLC to LC-MS/MS. Although effective, HPLC involves complicated sample prep techniques and is labor-intensive. With something like food testing, traditional assays are known to produce false negatives and positives due to limited sensitivity and selectivity, which forces retests.

The need for more chemicals and human interaction also drives up your costs and limits testing to finite amounts of compounds. Using LC-MS/MS opens your lab up to more extensive molecule tests so that you can enhance and accelerate your environmental and food testing. Both HPLC and LC-MS/MS are powerful analytical tools. However, LC-MS/MS provides greater capabilities for classifying analytes in different compound classes in a single run.

What makes the SCIEX Triple Quad™ 3500 LC-MS/MS System a game-changer?

The 3 biggest reasons the SCIEX Triple Quad 3500 System is a game-changer are its economic price, rapid deployment ability and robustness.

The price is right

The SCIEX Triple Quad 3500 System is an entry-level triple quadrupole mass spectrometer, complete with a liquid chromatography system capable of handling a particular sample class. It's ideal, especially if you're on a tight budget.

Get your charge on at the get-go

The simplicity of the SCIEX OS-Q Software makes taking off much easier than before, and with SCIEX, you get a support structure that gets you up and running even faster. This includes training your people on the instrument and tackling ongoing challenges.

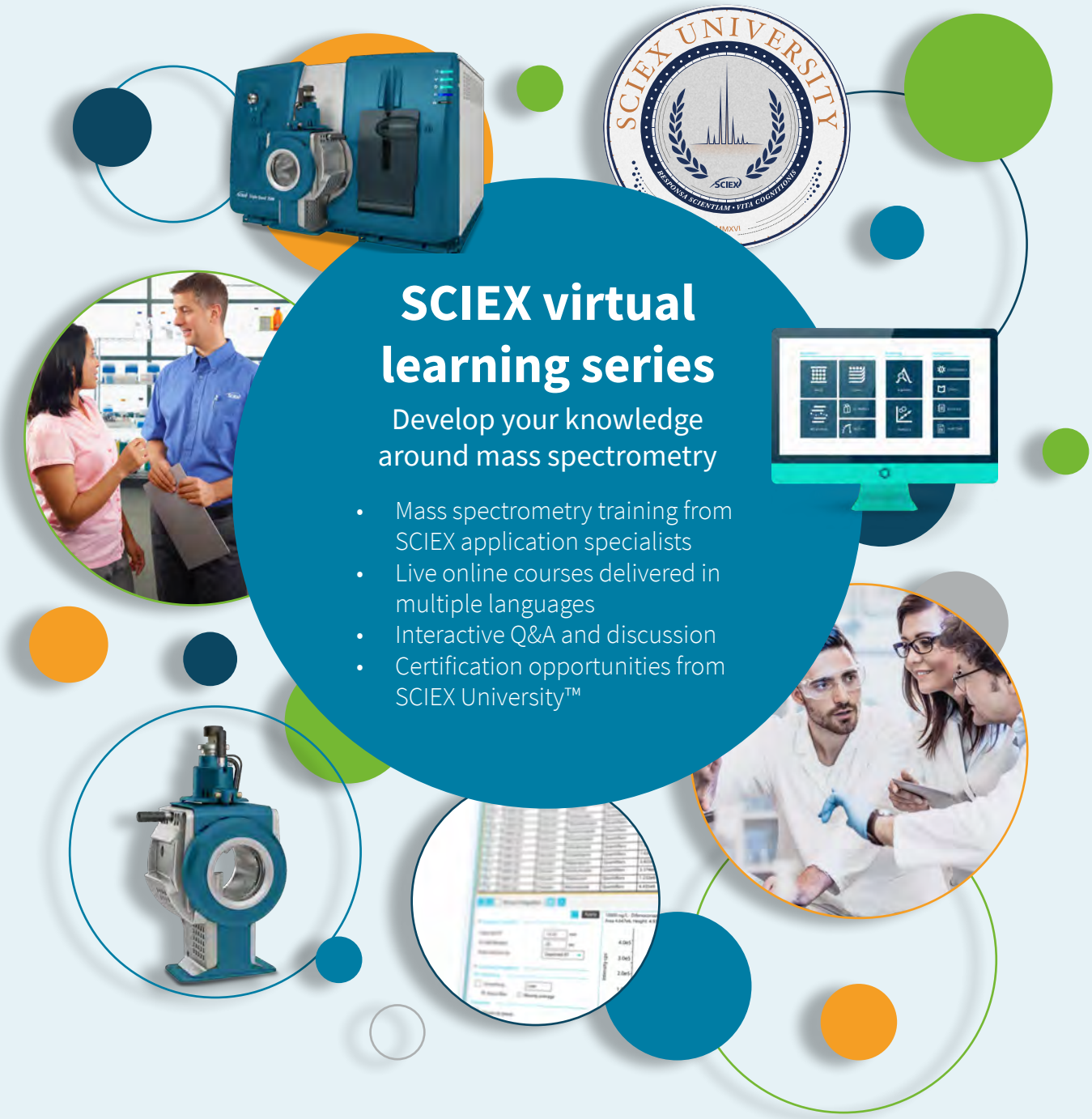
Pocket-sized power for exceptional robustness and ruggedness

Built with the legendary Turbo V™ Ion Source, samples are efficiently ionized to deliver sample analysis reliability. Even dirty samples can be used in this machine, with the Curtain Gas™ Interface protecting them from contamination and reducing the need for routine maintenance. With a single injection, hundreds of analytes can be analyzed.

Is MS software complex?

Software can certainly be daunting when you're talking about a new system. We often hear complaints about new systems being complex, tedious and challenging to navigate. Well, I'd like to introduce you to SCIEX OS-Q Software. Relying on customer and industry feedback, we developed intuitive software that powers the SCIEX Triple Quad 3500 System.

The tile layout enables you to easily navigate through steps you need to apply an existing method or a new method, with intelligent functions that rapidly get you to reliable results. From performing high-throughput screening MRM data processing to non-targeted investigations, all the tools you need are at your fingertips without sacrificing time, valuable samples or results.



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Application and technical summaries

Food testing

LC-MS/MS rapid quantification and screening method for 222 pesticide residues in tea

China is the world's home to tea and was the first country to discover, cultivate, make and drink tea. However, the use of pesticides on tea crops, driven by profit, is creating a crisis in China that is becoming increasingly alarming. This has mainly been caused by the "poisoning" of tea quality, and the dramatic drop in a large number of tea product prices due to repeated prohibition of "pesticide tea." This application note highlights a a method that can screen tea for 222 pesticides in 20 minutes using the SCIEX Triple Quad™ 3500 LC-MS/MS System.

Read technical note

LC-MS/MS rapid quantification and screening method for 30 mycotoxins in feed

Mycotoxins are a serious global problem that significantly impacts a variety of feedstuffs. From economic impacts because of rejection of commodities in the market to acute toxicity with lethal outcomes, such as mycotoxicosis when ingested by animals, mycotoxins are a major food safety hazard.

This application note describes a method that was developed in alignment with China's Hygienic Standard for Feeds (GB13078-2017) and takes a positive and negative simultaneous collection approach to detecting 30 types of mycotoxins within a single injection in 13 minutes.

Read technical note

Quantification of acetamiprid and prochloraz in black pepper using the SCIEX Triple Quad™ 3500 LC-MS/MS System

Pepper is a common food ingredient with some of the highest production and export values. Therefore, it's commonly laced with pesticides that are used to protect against various pests to keep them from damaging pepper crops. Because of the adverse effects of pesticide residues on human health and the environment, monitoring for food quality and safety requires suitable analytical methods for pesticides and herbicide residues.

As such, regulators such as the European Commission and the Food Safety and Standards Authority of India (FSSAI) have established maximum residue levels (MRLs) to ensure product quality and protect the public.

This application note outlines a robust, rapid, selective and sensitive LC-MS/MS method that quantifies acetamiprid and prochloraz residues in a complex pepper matrix using the SCIEX Triple Quad 3500 System. Due to the matrix's complexity, a modified QuEChERS method was used to reduce the matrix effect and improve the sensitivity. A detection limit of 0.05 ppb was achieved in the aqueous solution. A calibration range of 1.00–100 ppb was attained in the matrix. The method achieved 10 times below the MRL level specified by FSSAI.

Read technical note

A sensitive, robust method for quantification of sodium picosulfate in food

Stimulant laxatives such as sodium picosulfate, also known as 4,4'-(pyridin-2-ylmethylene) bisphenol-based bisulfate sodium salt, can offer some people relief. However, misuse in the market has led to stricter regulations on the sale of laxatives. China's "Food Supplementary Inspection Method Work Regulations" established an LC-MS/MS quantitative detection method for sodium picosulfate in foods (including jelly, preserves, candies, beverages, health foods, etc.).

This application note demonstrates a fast and sensitive MRM quantitative approach that uses the SCIEX Triple Quad 3500 System. The test substance has no obvious matrix effect in 5 different food matrices. The elution time of the method is only 4 minutes, which is very suitable for high-throughput analysis of food samples.

Read technical note

Quantification of multiple antibiotics in milk using the SCIEX Triple Quad™ 3500 LC-MS/MS System

The administration of antibiotics to livestock often helps to maintain their nutritional well-being. The use of these antibiotics is a significant threat to human health, however. Overuse creates the risk of developing resistant microorganisms. Individuals can be allergic to certain antibiotics. Additionally, the presence of these antibiotics in milk can affect the manufacturing of products such as cheese and yogurt by inhibiting the starter culture.

This has led to countries setting a permissible limit of antibiotics that can be present in their food products, typically in micrograms per kilogram. Given that these levels are challenging to quantify using traditional methods such as HPLC or microbial techniques, SCIEX developed a method in accordance with the recommendations of the EU's 2002/657/EC directive.

This application note explains how this method uses the SCIEX Triple Quad 3500 System to screen milk for residual levels of albendazole, fenbendazole and their metabolites (albendazole sulfone, albendazole sulfoxide, albendazole-2-amino sulfone, fenbendazole sulfone), along with tylosin and tilmicosin.

Read technical note

Ensuring quality and freshness in animal nutrition products

Biogenic amines (BAs) are found naturally in human foods, including meat, fish, cheese, wine, beer and fermented foods. These compounds can be formed in food during processing or storage when specific amino acids are released by the action of decarboxylases produced by microorganisms. Some of the most common BAs found in food are putrescine, cadaverine, histamine, 2-phenylethylamine, tyramine, spermidine, tryptamine and spermine. BAs are organic bases with aliphatic, aromatic and heterocyclic structures. While many amines can play important roles in human and animal physiological functions, a high accumulation of biogenic amines ingested from food can become a health hazard.

This is especially an issue with pet food. Pets on a fixed pet food diet that is high in BAs may experience harmful effects due to continuous exposure. Healthy adult cats and dogs may detoxify biogenic amines present in their diet, but kittens and puppies, reproducing females and ill animals could potentially be more prone to adverse effects.

This application note presents a sensitive, robust and fast LC-MS/MS method that was developed to quantify 8 biogenic amines, which allows for low-level environmentally relevant concentrations to be accurately quantified.

Read technical note

Cannabis testing

Quantification of psilocybin and psilocin in mushroom by LC-MS/MS

Historically, these mushrooms were used for spiritual and religious ceremonies in central and southern America. Still, recently there has been research related to using psilocybin and psilocin to treat addiction and depression. While mushrooms that produce the psychoactive compounds psilocybin and psilocin were historically used for spiritual and religious ceremonies in central and southern America, recent research has examined using them to treat addiction and depression. Currently, psilocybin and psilocin are defined as Schedule 1 illicit drugs under the Controlled Substances Act in the US, and the sale and purchase of these drugs remain illegal. However, many see a roadmap to the eventual legalization of these compounds, similar to cannabis, and in Denver, Colorado, for example, the possession of psychotropic mushrooms has been decriminalized.

Due to the increased interest in these compounds, robust and sensitive analytical methods are needed. This application note details a 5-minute reproducible quantitative LC-MS/MS method that was developed for psilocybin and psilocin in mushroom matrices. With a simple dilute and shoot sample preparation, this approach demonstrates limits of detection (LOD) below 1 ppb in the mushroom extract.

Read technical note

Analysis of vitamin E and vitamin E acetate in vape oils

A condition known as e-cigarette or vaping product use-associated lung injury (EVALI) has sickened thousands and killed many. These frightening cases of respiratory illness and death related to vaping have been linked to vitamin E and vitamin E acetate compounds in vape oils.

In collaboration with Cannalysis, the SCIEX team has put together this application note, which details an LC-MS/MS workflow for quantifying vitamin E and vitamin E acetate in vape oils using the SCIEX Triple Quad 3500 System. The method offers high specificity using 2 multiple reaction monitoring (MRM) transitions for each analyte, and an isotopically labeled internal standard reduces the effects of interferences.

This method is also highly sensitive in detecting vitamin E and vitamin E acetate in vaping oils at concentrations between 0.01% and 0.5%. A linear response for quantification is measured at 10 ppb to 500 ppb with precision (5% CV) for both analytes.

Read technical note

Quantification of terpenes in cannabis products using the SCIEX Triple Quad™ 3500 LC-MS/MS System

While terpenes have a well-defined role in the perceived aroma of specific cannabis strains, recent studies suggest that in addition to playing a sensory perception role, many cannabis terpenes have pharmacological properties of their own and may act synergistically with cannabinoids. Sensitive, selective, accurate and economical analytical methods are needed to assess these compounds to help manufacturers ensure lot-to-lot consistency between cannabis products.

Historically, terpenes have been analyzed by gas chromatography-mass spectrometry (GC-MS) due to their predominantly aliphatic composition. However, labs performing routine testing of cannabis will need to test the potency and, once regulations are established, test for pesticides and herbicides. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) represents an ideal analytical platform to address all of these testing needs. This technical note presents an LC-MS/MS method that uses atmospheric pressure chemical ionization (APCI) and the budget-friendly SCIEX Triple Quad 3500 System to analyze terpenes in cannabis products. The price-to-performance ratio of SCIEX Triple Quad 3500 System is ideally suited to the analysis of terpenes, cannabinoids and pesticides/herbicides in cannabis products. The method focuses on the 7 terpenes typically found in cannabis with examples of accurate quantification shown for a variety of cannabis products.

Read technical note

Environmental testing

Rapid and quantitative analysis of 14 sulfonylurea herbicides residues in water by direct injection

Sulfonylurea herbicides are broad-spectrum, high-efficiency, high-selectivity and low-toxicity herbicides, and they are currently among the most widely used classes of herbicides in the world. They are used to control rice, wheat, soybean, corn and rape field weeds as well as lawn and other non-cultivated weeds. This kind of herbicide is not easy to volatilize and does not photodegrade, so it remains in crops for a long time.

At present, the detection of sulfonylurea herbicides in soil, surface water and drinking water mostly requires sample concentration before analysis. The main methods of concentration are liquid-liquid extraction (LLE) and solid-phase extraction (SPE), but pre-treatment is time-consuming and uses a lot of organic solvents. These approaches also rely heavily on operators for sample handling, which can lead to precision and accuracy risks.

This application note details an experiment that uses the SCIEX Triple Quad 3500 System to screen and quantify 14 sulfonylurea herbicides in water. This method takes only 6 minutes for 1 injection, and the positive and negative ions are detected within the same run. Both qualitative and quantitative analysis of 14 sulfonylurea herbicides is quickly completed, and isomers are well separated. This approach demonstrates the excellent stability and sensitivity of the SCIEX Triple Quad 3500 System when switching between positive and negative modes to meet various regulations.

Read technical note

LC-MS/MS solution for national quality standards for drinking water, surface water and groundwater

The demand for water worldwide has increased substantially over the past decades, and protecting water sources

is critical to safeguarding drinking water. Measures to control water pollution are in full force in the US and Europe, and also in China, where economic expansion has driven a growing demand for water alongside a rise in threats to water quality. It's against this backdrop that the Chinese government has established various standards that stipulate the limit values of

several compounds, including standards that regulate surface water environmental quality (GB3838-2002), national standards for drinking water (GB5749-2006) and groundwater quality standards (GB14848-2017).

This application note explains how the SCIEX team used the SCIEX Triple Quad 3500 System to develop an LC-MS/MS method for quantifying 22 compounds in water (including 2 additional microcystin compounds), which provides a simple and rapid way to address these regulations in aqueous environments.

Read technical note

Forensic testing

Rapid and sensitive detection of 8 organic explosives in soil

The widespread use of explosive materials by the military, blasting, mining and other industries has fueled the continuous development of explosive production technology. The ability to quickly and easily manufacture explosive devices has also enabled terrorists to construct and use explosives to clandestinely pursue their criminal endeavors. As a result, the presence of explosives in geographical war zones or in crime scenes has important political and legal implications.

This technical note presents a comprehensive workflow that combines the SCIEX Triple Quad 3500 System with a fast and easy sample preparation procedure for the sensitive detection of 8 organic explosives is described. The method shows a simple solution for high-throughput detection of trace explosives in soil samples.

Read technical note

Rapid DUID screening of 17 drugs of abuse in plasma and saliva

The offense of driving under the influence of drugs (DUID) typically relates to driving while being intoxicated on mind-altering substances such as prescribed or illegal drugs. Law enforcement requires comprehensive drug screening approaches to support the prosecution of DUID cases by confirming the presence of these substances. Traditionally, DUID screens are performed by either immunoassay or GC-MS. However, immunoassays are known to suffer from cross-reactivity, have poor sensitivity and are prone to a high rate of false positives. On the other hand, GC-MS requires lengthy sample preparation and derivatization, which considerably slows the analytical process. As a result, there is a critical need to develop rapid, robust and comprehensive drug screening methods to identify and accurately quantify these substances from biological specimens.

This technical note presents a simple 8-step extraction procedure that uses protein precipitation to efficiently extract drugs from plasma and saliva samples and a Scheduled MRM™ Algorithm to achieve picogram to sub-nanogram per mL levels of 17 different drugs in a single 10-minute method.

Read technical note

Pharmaceutical testing

Targeted analysis of extractables/leachables in drug product, containers and closure systems

Due to the ways in which drug products are formulated and stored, they will come into contact with various containers and packaging systems that could result in contamination of the products. The US Food and Drug Administration's 21 CFR 211.94(a) regulation monitors levels of impurities caused by contact with containers, closure systems and packaging materials. These impurities are categorized as extractables and leachables. Extractables are compounds that can be extracted from the container or closure system when in the presence of a solvent or other harsh condition. Leachables are compounds that leach into the drug product formulation from the container or closure system due to direct contact with the formulation over time.

This application note presents a sensitive and selective method that uses the SCIEX Triple Quad 3500 System with an ExionLC™ AD System to simultaneously look at a set of 15 commonly monitored extractable and leachable compounds in drug product packaging, containers and closure systems.

Read technical note

LC-MS/MS rapid quantification and screening method for 222 pesticide residues in tea

SCIEX Triple Quad™ 3500 LC-MS/MS System

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SCIEX, China

China is the world's home of tea and is the first country in the world to discover, cultivate, make and drink tea.[1] Tea has become the "coffee" of the Chinese people. Drinking tea is not only a habit, but also reflects a culture. However, driven by profit, the tea crisis is becoming increasingly important. This is mainly highlighted by the "poisoning" of tea quality[2], and the repeated prohibition of "pesticide tea" has caused a large number of tea product prices to drop dramatically.

In order to ensure the quality of tea and avoid a greater crisis in the tea market, on 18 December, 2016, the Ministry of Agriculture of China and four other departments issued the "National Maximum Food Safety Pesticide Residue Limits" (GB2763-2016) (formally implemented on 18 June, 2017) which stipulated quantity limitations on 48 pesticides in tea[3].

This application note focuses on the problem of pesticide residues in tea. On the SCIEX Triple Quad 3500 System, a rapid screening method of 222 pesticides has been established to provide a simple and quick solution to the problem of pesticide residues in tea.

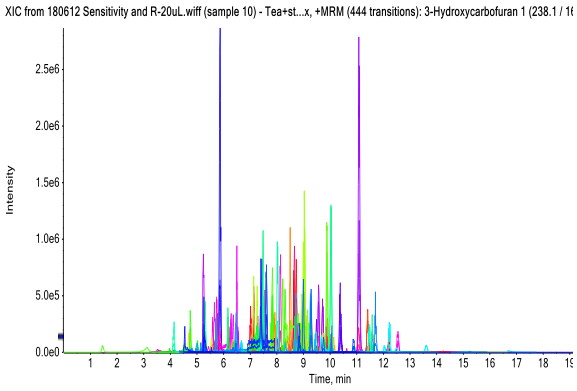

Figure 1. 222 pesticides in tea (10 µg/L).

Delivering accuracy and precision for pesticide residues in tea extracts

- This method analyses 222 pesticides simultaneously in a single injection
- Analysis time is only 20 minutes, which can provide high sample throughput
- This method provides mass spectrometric and chromatographic conditions for 222 pesticides, which saves method development time and improves work efficiency
- This method provides details for simple sample preparation for tea leaves. It has a high extraction recovery rate and is ready to use.

Methods

Sample preparation: Tea samples were extracted using the QuEChERS method, the process is detailed below:

1. Start with 1g tea sample after weighing and homogenization.
2. Add 5 mL water, vortex for 30 seconds, soak for 10 min.
3. Add 10 mL of acetonitrile/water/acetic acid = 70/29/1 (v/v/v) and vortex for 30 seconds.
4. Add QuEChERS extraction reagent kit and shake for 1 min.
5. Centrifuge at 6,000 rpm for 5 minutes.
6. Take 2 mL of supernatant, add QuEChERS purification kit and shake for 1 min.
7. Centrifuge at 6,000 rpm for 5 minutes.
8. Take 1 mL of supernatant and dry under nitrogen.
9. 200 µL acetonitrile/water = 10/90 (v/v) reconstituted residue.

Chromatography: The liquid chromatography for this workflow was performed using a SCIEX ExionLC™ System and a Kinetex Biphenyl column (100 x 3.0 mm, 2.6 µm, Phenomenex). The gradient details are outlined in Table 1. The column temperature was 40°C and the injection volume was 20 µL.

Mass spectrometry: Analysis was performed using the SCIEX Triple Quad 3500 System using the Turbo V™ Ion Source. Parameters are outlined in Table 2. MRM transitions for 222 compounds were optimized and analyzed.

Table 1: Details of the gradient elution.

Time (min)	A (%)	B (%)
0.00	*95*	*5*
0.50	*95*	*5*
3.00	*40*	*60*
7.00	*20*	*80*
15.00	*10*	*90*
16.00	*5*	*95*
16.50	*3*	*97*
17.0	*3*	*97*
17.10	*95*	*5*
20.00	*95*	*5*

Mobile phase A : water with 5mM ammonium formate)
Mobile phase B: acetonitrile with 5mM ammonium formate)

Table 2: Source and mass spectrometer parameters.

Parameter	Setting	Parameter	Setting
Acquisition mode	*MRM*	*GS1*	*50 psi*
Ionization mode	*ESI*	*GS2*	*60 psi*
Polarity	*Positive*	*CAD gas*	*Medium*
Ion Source Voltage	*5500v*	*Source Temperature*	*300°C*
CUR Gas	*30 psi*		

Results

This workflow demonstrates a single injection method for the detection and quantification of 222 compounds within 20 minute run time (Figure 1). The method shows consistency and robustness as well as sufficient sensitivity and precision to enable high throughput analysis of tea extracts.

The method has a high extraction recovery rate, the extraction recovery rate of more than 95% of the compounds is greater than 70%, the figure below (Figure 2) details the recoveries.

The method demonstrated good reproducibility for a series of replicate injections from the same sample. The statistical performance of the percent coefficient of variation (%CV) was calculated and shows acceptable reproducibility (Figure 3).

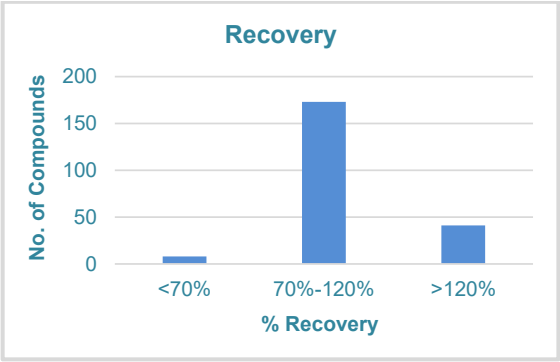

Figure 2: Statistics on the extraction and recovery rate from tea.

Row	Component Name	Sample Name	Num. Values	Mean	Standard Deviation	Percent CV
1	Acephate 1	10ppb	3 of 3	8.278e4	1.875e3	2.26
2	Acetamiprid 1	10ppb	3 of 3	9.411e6	1.302e5	1.38
3	Buprofezin 1	10ppb	3 of 3	1.047e7	3.817e5	3.65
4	Carbendazim 1	10ppb	3 of 3	2.880e6	9.352e4	3.25
5	Carbofuran 1	10ppb	3 of 3	5.168e5	2.237e4	4.33
6	Difenoconazole 1	10ppb	3 of 3	1.798e5	1.216e4	6.76
7	Imidacloprid 1	10ppb	3 of 3	3.113e5	8.899e3	2.86
8	Omethoate 1	10ppb	3 of 3	8.353e4	5.049e3	6.04
9	Imidaclothiz 1	10ppb	3 of 3	9.546e4	2.531e3	2.65
10	Cartap 1	10ppb	3 of 3	7.173e5	5.624e3	0.78

Figure 3. Reproducibility of typical compounds at a concentration of 10 µg/L.

Summary

Due to the very high consumption of tea in many cultures including china, the safety of tea is very important to human health. Here, a LC-MS/MS rapid screening and detection method was established for the determination of 222 pesticides in tea leaves using the SCIEX Triple Quad 3500 System. This method uses the QuEChERS method for sample pre-treatment. It was shown to have both a high extraction recovery rate and good reproducibility, and thus can be used for the determination of pesticide residues in actual tea samples. In addition, this method enables the detection of 222 pesticides in a single injection providing high efficiency, high throughput and broad screening for analytes of concern.

References

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LC-MS/MS rapid quantification and screening method for 30 mycotoxins in animal feed

Using the SCIEX Triple Quad™ 3500 LC-MS/MS System

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In recent years, animal mycotoxin poisoning incidents have occurred frequently in China and around the world[1], causing huge economic losses to the agriculture industry. Feed mycotoxin contamination is an ongoing global problem that seriously affects animal health and production and poses major food safety hazards. Controlling the mycotoxin contamination of animal feed is a common goal in the industry.

On October 14, 2017, China's General Administration of Quality Supervision, Inspection and Quarantine and the National Standardization Management Committee issued the "Feed Hygiene Standards"[2] (GB13078-2017), which stipulates seven mycotoxin limit requirements in animal feed. This added to the list of global regulations of mycotoxins in feed that already exists in the European Union and the United States. This is significant, as China's role in the global food supply is ever-growing. To not only meet regulatory guidelines, but to also maintain consumer

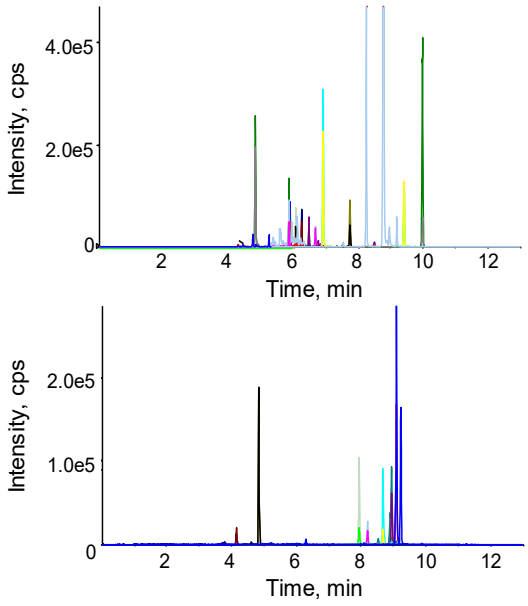

Figure 1. Typical chromatograms of 30 mycotoxins in pig feed. (Top) Extracted ion chromatograms (XICs) from the positive ionization mode. (Bottom) XICs from negative mode experiment.

protection and promote safe practices during the growth of agriculture and animal husbandry industries, it is important to have methods that monitor for known mycotoxins outside of the regulatory guidelines.

This work focuses on the detection of mycotoxins in animal feed. On the SCIEX Triple Quad 3500 System, a rapid determination method of 30 mycotoxins was established. This method provides a simple and quick solution for the detection of mycotoxins in the feed.

Key features of the mycotoxin workflow

- Efficient sample preparation for animal feed, providing high extraction recovery rates
- Easy to implement
- Fast polarity switching: the SCIEX Triple Quad 3500 System enables the use of a single injection workflow, with both positive and negative mode acquisition to cover all 30 analytes
- Fast run time of 13 minutes, providing high sample throughput
- Acquisition method details provided, including chromatographic and mass spectrometry conditions for 30 mycotoxins
- Method sensitivity that fully meets the requirements of global mycotoxin regulations for animal feed

Methods

Sample preparation: Samples were prepared using solid phase extraction (SPE) with a Cleanert MC (400 mg/2 mL) or a Cleanert SAX SPE Column (500 mg/6 mL) from Agela Technologies.

Sample extraction:

1. Weigh 2.5 g of homogenized sample into a 50 mL centrifuge tube.
2. Add 10 mL of acetonitrile/water/formic acid solution (85:15:0.1).
3. Extract by ultrasonication, then vortex to homogenize.
4. Centrifuge the extracted sample and remove the supernatant for further cleanup.

Sample cleanup:

Method 1 — for fumonisin B1, B2, B3 and ochratoxin:

1. To a 2 mL aliquot of the extracted supernatant solution, add ammonia to adjust to pH 6–8.
2. Activate the Cleanert SAX column with 5 mL methanol, and then equilibrate with 5 mL water.
3. Load the extracted supernatant and then rinse the column with 8 mL methanol/water (60:40), followed by 3 mL methanol, and then elute with methanol containing 1% acetic acid.
4. Blow down the sample to dryness with nitrogen and reconstitute with 125 µL acetonitrile/water = 5/95 (v/v).

Method 2 — for the other 26 toxins

1. Pass the remaining 1 mL aliquot of the original sample extract through a Cleanert MC column
2. Blow down the purified sample to dryness with nitrogen and reconstitute with 125 µL acetonitrile/water = 5/95 (v/v).
3. Combine the reconstituted sample with that from Method 1 for LC-MS/MS analysis

Chromatography: Sample separation was performed using an ExionLC™ System and a Phenomenex Kinetex C18 column (100 × 3.0 mm, 2.6 µm). The flow rate was 0.5 mL/min, the column temperature was held at 40 °C and the injection volume was 20 µL. The gradient elution program is listed in Table 1.

Mass spectrometry: MS analysis was performed on the SCIEX Triple Quad 3500 System equipped with the Turbo V™ Ion Source. Acquisition parameters are detailed in Table 2.

Table 1: LC gradient details.

Time (min)	A (%)	B (%)
0.0	97	3
1.0	97	3
2.0	90	10
4.0	50	50
9.0	20	80
9.1	0	100
11.0	0	100
11.1	97	3
13.0	97	3

Mobile phase A: water with 0.1% formic acid
Mobile phase B: methanol with 0.1% formic acid

Table 2. Mass spectrometer settings.

Parameter	Setting	Parameter	Setting
Acquisition mode	MRM	GS1	50 psi
Ionization mode	ESI	GS2	65 psi
Polarity	Positive and Negative	CAD gas	Medium
Ion Source Voltage	5500 V -4500 V	Source Temperature	550°C
CUR Gas	30 psi		

Results

Using standards, the MRM transitions and MS parameters for the 30 mycotoxins were optimized. Then the chromatographic conditions for the analysis were optimized to provide fast run times and good separation (Figure 1). Polarity switching was used to enable sensitive detection of all 30 compounds in a single injection, with a total run time of 13 minutes.

Two different sample preparation strategies were explored to ensure the highest efficiency and recovery for all 30 mycotoxins. Best results were obtained using a combination of two different SPE columns, as described in the methods section.

After optimization, recovery experiments were performed to characterize the final sample preparation strategy, where the extraction recovery rate of all compounds was found to be within 70–120% (Figure 2).

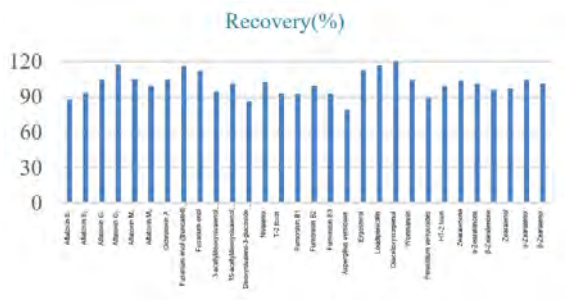

Figure 2: Extraction recovery for each of the mycotoxins analyzed. All calculated recoveries fell within 70–120% .

Calibration curves were generated to determine the lower limits of quantification (LLOQs) for each of the 30 mycotoxins. Very good linearity and reproducibility were obtained across the dataset. Example data for Fumonisin B1 is shown in Figure 3. Good linearity was observed across the interrogated concentration range of 1–40 µg/kg, with %CV across replicates <5%.

LLOQs for each of the compounds are outlined in Table 3, along with regulatory requirements from China, the EU and the US. The sensitivity of this complete method fully meets all outlined requirements for the detection sensitivity of mycotoxins in animal feed, and can monitor a suite of mycotoxins that are not yet regulated.

Table 3: List of method LLOQs and regulatory guidelines

Compound	Method LLOQ (µg/kg)	Regulation China[2] (µg/kg)	Regulation EU[4] (µg/kg)	Guidance USA[5-7] (µg/kg)
Aflatoxin B1	1	2	5	Total = 5
Aflatoxin B2	1			
Aflatoxin G1	0.5			
Aflatoxin G2	0.5			
Aflatoxin M1	0.5			
Aflatoxin M2	0.5			
Aspergillus versicolor	0.5			
T-2 toxin	0.1	2		
Ergosterol	1			
Loudi penicillin	0.1			
Diacetoxyscirpenol	10			
Fusarium enol	20			
Deoxynivalenol	20	100	900	1000
3-acetyldeoxynivalenol	10			
15-acetyldeoxynivalenol	10			
Deoxynivalenol-3-glucoside	20			
Zearalenone	5		100	NOG#
α-zearalenone	5			
β-zearalenone	2			
Zearalenol	2			
α-zearalenol	2			
β-zearalenol	2			
Ochratoxin A	1	5	50	NOG#
Fumonisin B1	1	B1+B2 = 50	B1+B2 = 5	B1+B2+B3 = 5000
Fumonisin B2	1			
Fumonisin B3	1			
Wortmannin	10			
HT-2 toxin	20			
Nivalenol	20			
Penicillium verrucosum	5			

*- Lowest MRL across all feed types, #-FDA has no official guidance (NOG). Any sample positives for these mycotoxins are referred to the Center for Veterinary Medicine to determine if regulatory action is required

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Fumonisin B1 1	1.0000	3 of 3	1.0142	0.0397	3.92	101.42
2	Fumonisin B1 1	2.0000	3 of 3	1.8915	0.0266	1.40	94.58
3	Fumonisin B1 1	5.0000	3 of 3	5.1004	0.2222	4.36	102.01
4	Fumonisin B1 1	10.0000	3 of 3	11.3009	0.4347	3.85	113.01
5	Fumonisin B1 1	20.0000	3 of 3	18.9296	0.3081	1.63	94.65
6	Fumonisin B1 1	40.0000	3 of 3	37.7365	0.8932	2.37	94.34

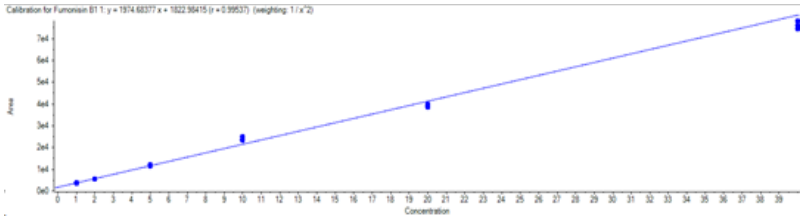

Figure 3: Reproducibility and linear results of Fumonisin B1. Calibration curves were generated for all mycotoxins across the concentration range of 1 – 40 µg/kg. Data shown above for Fumonisin B1. Statistics table for calibration curves showing high accuracy and %CV< 5%

Summary

A complete method was developed for the detection of mycotoxins in animal feed using the SCIEX Triple Quad 3500 System. Sample preparation was optimized for good recoveries of the 30 analytes from matrix, with 70–120% recovery. Single injection workflow with polarity switching enabled detection of all 30 mycotoxins within 13 minutes with high efficiency and high throughput. The method meets regulatory standards, while adding the ability to quantify mycotoxins that currently are not part of animal feed regulations for additional safety and security for consumers.

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Quantification of acetamiprid and prochloraz in black pepper using the SCIEX Triple Quad™ 3500 LC-MS/MS System

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Today, several pesticides are available in the market, which are used to protect against various pests that damage plant food products such as spices, cereals, pulses, fruits and vegetables, etc. Due to the adverse effects of pesticide residues on human health and to the environment, the use of pesticides must be controlled and monitored. Therefore, maximum residue levels (MRLs) for pepper have been fixed by regulations (European Commission, FSSAI and other regulatory bodies) to assess food safety.[1,2] Monitoring of food quality and safety requires suitable analytical methods for pesticides and herbicide residues.

Pepper is a common food ingredient with the highest production and export values. The presence of pesticide residues more than the MRL concentration in pepper samples need to be analyzed to ensure quality of the product and protect the public.[3,4]

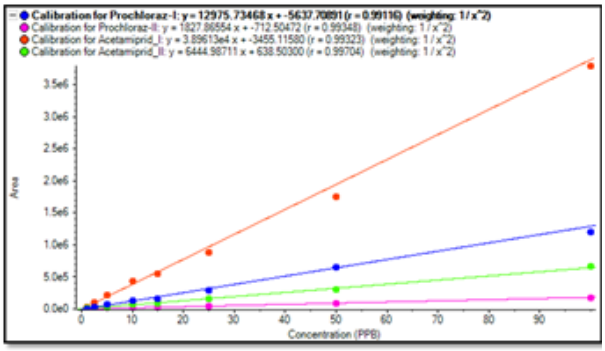


Figure 1: Representative calibration curves. Good linearity was observed for acetamiprid (red and green) and prochloraz (blue and pink) range for the concentration range of 1 to 100 ppb. Lower limits of quantification was 1 ppb for both pesticides in black pepper matrix.

Here, a sensitive, rugged and robust method was developed for the quantification of pesticide residues in pepper matrix using the SCIEX Triple Quad 3500 System.

Key features of targeted quantification method for pesticide residues in pepper

- A targeted quantitative method has been developed on the SCIEX Triple Quad 3500 System using two MRM transitions
- A modified QuEChERS method was developed for preparation of the black pepper samples
- The method achieved performance below the MRLs of acetamiprid and prochloraz residues in pepper as mentioned in the regulatory guidelines
- Experiments were performed with the five different concentration levels (1.0, 2.5, 5.0, 10.0 and 15.0 ppb) which meet the validation parameters as per the accuracy % requirements set by the global regulations (80%-120%).

Methods

Sample preparation: Pesticides standards were purchased from Sigma Aldrich and had ≥ 99% purity. All other chemicals used were of LC-MS grade, and were commercially available. Black pepper samples were procured from the local markets of Delhi and Gurgaon, India and were stored at room temperature until analysis.

1. Add 2 g of sample to a 50 mL Tarson tube and then add 0.5 g of disodium hydrogen citrate sesquihydrate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 2 g magnesium sulphate (anhydrous)
2. Add 10 mL of acetonitrile, and then vortex for 10 minutes. Centrifuge at 4500 rpm for 10 minutes
3. Collect the organic layer and evaporate with N_2 to dryness.
4. Reconstitute the samples with 1 mL of water/acetonitrile/formic acid (90:10:0.2% v/v/v) and vortex well, then transfer into 2 mL centrifuge tubes. Centrifuge at 12000 rpm for 10 minutes
5. After centrifugation, collect the upper layer and transfer into autosampler vial for LC-MS/MS analysis.

Chromatography: LC separation was performed with a Phenomenex Kinetex® C18 LC column (100 Å, 100×4.6 mm, 2.6 µm) using the gradient outlined in Table 1. A 20 µL injection volume was used and the column oven temperature was set to 40°C.

Table 1. Gradient profile and mobile phase composition.

Total Time (min)	Flow Rate (µL/min)	A%	B%
0.01	*800*	*90*	*10*
0.50	*800*	*90*	*10*
3.00	*800*	*40*	*60*
10.00	*800*	*10*	*90*
12.00	*800*	*10*	*90*
13.00	*800*	*90*	*10*
15.00	*800*	*Controller*	*Stop*

Mobile phase A: 5mM ammonium formate in water + 0.1% formic acid
Mobile phase B: 5mM ammonium formate in methanol + 0.1% formic acid

Mass spectrometry: The SCIEX Triple Quad 3500 System was operated in multiple reaction monitoring (MRM) mode. The Turbo V™ Ion Source was used with an electrospray ionization (ESI) probe in positive ion mode. Two selective MRM transitions were monitored (Table 2). Analyst® Software 1.7 was used for method development and data acquisition. Ionization voltage was 5500 V, source temperature was 550 ºC, GS1 was 55 and GS2 was 50.

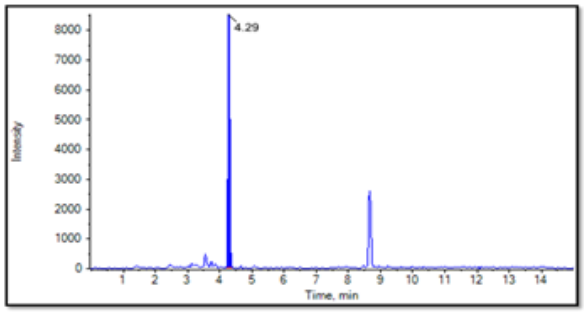
Table 2. MRM transitions of Acetamiprid and Prochloraz.

Compound	Precursor Ion	Product Ion (Quantifier)	Product Ion (Qualifier)
Acetamiprid	*223.0*	*126.0*	*56.0*
Prochloraz	*376.1*	*308.1*	*266.1*

Data processing: LC-MS/MS data was processed using the MultiQuant™ Software 3.0.2. Ion ratio is calculated automatically by the software for compound identification.

Results

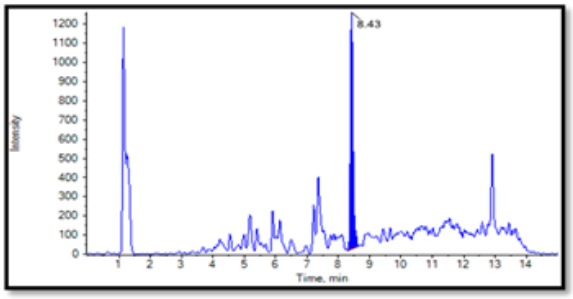
Due to the complexity of the pepper matrix, a modified QuEChERS method was used in this study to reduce the matrix effects and improve the assay sensitivity for the quantification of pesticide residues. The matrix matched calibration curve showed excellent linearity (1.0 to 100.0 ppb), with a correlation coefficient r≥0.99 for pesticide residues in pepper using linear regression and weighing factor $1/X^2$ (Figure 1). The lowest calibration point for both pesticide residues was 1 ppb in matrix (Figure 2 and 3).



Sample Name	Sample Type	Component Name	Mass Info	Actual Concentration	Area	Calculated Concentration	Accuracy
RCS	Blank	Acetamiprid-I	223.0 / 126.0	N/A	N/A	N/A	N/A
SAMPLE BLANK	Blank	Acetamiprid-I	223.0 / 126.0	N/A	2650	0.11	N/A
EXT_STD CC_01	Standard	Acetamiprid-I	223.0 / 126.0	1.00	34792	0.94	93.91
EXT_STD CC_02	Standard	Acetamiprid-I	223.0 / 126.0	2.50	103256	2.71	108.29
EXT_STD CC_03	Standard	Acetamiprid-I	223.0 / 126.0	5.00	215657	5.61	112.20
EXT_STD CC_04	Standard	Acetamiprid-I	223.0 / 126.0	10.00	438309	11.36	113.60
EXT_STD CC_05	Standard	Acetamiprid-I	223.0 / 126.0	15.00	538477	13.95	92.98
EXT_STD CC_06	Standard	Acetamiprid-I	223.0 / 126.0	25.00	888125	22.98	91.91
EXT_STD CC_07	Standard	Acetamiprid-I	223.0 / 126.0	50.00	1723967	44.56	89.12
EXT_STD CC_08	Standard	Acetamiprid-I	223.0 / 126.0	100.00	3792892	97.99	97.99

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
Acetamiprid-I	1.00	6 of 6	3.554e4	5.714e2	1.61

Figure 2. Acetamiprid results. (Top) Representative chromatogram at LLOQ level of 1 ppb acetamiprid in matrix. (Middle) Statistical data on calibration curve for acetamiprid from 1 to 100 ppb. The correlation coefficient r ≥ 0.99 for both the transitions (quantifier and qualifier Ions). (Bottom) High reproducibility was observed at the lowest concentration, 1.61%CV.



Sample Name	Sample Type	Component Name	Mass Info	Actual Concentration	Area	Calculated Concentration	Accuracy
RCS	Blank	Prochloraz-I	376.1 / 308.1	N/A	N/A	N/A	N/A
SAMPLE BLANK	Blank	Prochloraz-I	376.1 / 308.1	N/A	N/A	N/A	N/A
EXT_STD CC_01	Standard	Prochloraz-I	376.1 / 308.1	1.00	6463	0.94	94.10
EXT_STD CC_02	Standard	Prochloraz-I	376.1 / 308.1	2.50	28456	2.74	109.57
EXT_STD CC_03	Standard	Prochloraz-I	376.1 / 308.1	5.00	65651	5.56	111.23
EXT_STD CC_04	Standard	Prochloraz-I	376.1 / 308.1	10.00	136229	11.07	110.71
EXT_STD CC_05	Standard	Prochloraz-I	376.1 / 308.1	15.00	161273	13.03	86.84
EXT_STD CC_06	Standard	Prochloraz-I	376.1 / 308.1	25.00	285966	22.76	91.04
EXT_STD CC_07	Standard	Prochloraz-I	376.1 / 308.1	50.00	650147	51.19	102.38
EXT_STD CC_08	Standard	Prochloraz-I	376.1 / 308.1	100.00	1200160	94.13	94.13

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
Prochloraz-I	1.00	6 of 6	9.726e3	1.040e2	1.07

Figure 3. Prochloraz results. (Top) Representative chromatogram at LLOQ level of 1 ppb prochloraz in matrix. (Middle) Statistical data on calibration curve for prochloraz from 1 to 100 ppb. The correlation coefficient r ≥ 0.99 for both the transitions (quantifier and qualifier Ions). (Bottom) High reproducibility was observed at the lowest concentration, 1.07%CV.

Using the developed method, pepper samples from local markets were tested for the presence of these pesticide residues (Figure 4).

Conclusions

The method and data acquired demonstrate the assay provides sensitive and accurate results for the quantification and confirmation of pesticide residues in pepper samples using the SCIEX Triple Quad 3500 System. For both the analytes, a LLOQ of 1 ppb was achieved, which was easily below the MRL level as mentioned by FSSAI (MRL – 10 ppb) and European Union (MRL – 100 ppb) regulatory bodies. The developed method achieved acceptable accuracy (80-120%) for the calibration curves in matrix without any significant matrix interferences.

Sample Name	Sample Type	Component Name	Mass Info	Area	Calculated Concentration
Sample_01	Unknown	Acetamiprid-I	223.0 / 126.0	107438	2.82
Sample_02	Unknown	Acetamiprid-I	223.0 / 126.0	59777	1.58
Sample_03	Unknown	Acetamiprid-I	223.0 / 126.0	11250	0.33
Sample_04	Unknown	Acetamiprid-I	223.0 / 126.0	3452	0.13

Sample Name	Sample Type	Component Name	Mass Info	Area	Calculated Concentration
Sample_01	Unknown	Prochloraz-I	376.1 / 308.1	3521	0.71
Sample_02	Unknown	Prochloraz-I	376.1 / 308.1	3881	0.73
Sample_03	Unknown	Prochloraz-I	376.1 / 308.1	4200	0.76
Sample_04	Unknown	Prochloraz-I	376.1 / 308.1	3814	0.73

Figure 4. Analysis of unknown pepper samples from local markets. Results for acetamiprid and prochloraz from four unknown samples showed detection of some pesticide residue but all were below the MRL level of 10 ppb.

The method performed as per commission decision SANTE/11813/2017 directive recommendations and fulfilled regulatory requirements for of sensitivity, precision, and accuracy. Overall, the high-throughput, accurate, and sensitive method on the SCIEX Triple Quad 3500 System has demonstrated required ability to monitor pesticide residues in pepper samples.

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A sensitive, robust method for quantification of sodium picosulfate in food

Using the SCIEX Triple Quad™ 3500 LC-MS/MS System

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SCIEX Application Technology Center, China

Sodium picosulfate, also known as 4,4'-(pyridin-2-ylmethylene) bisphenol-based bissulfate sodium salt, is a special laxative, which has a mild laxative effect on the large intestine mucosa. It is sometimes used clinically for patients experiencing constipation following surgery and barium meal.

Recently, in accordance with the relevant requirements of the "Food Supplementary Inspection Method Work Regulations", the State Administration for Market Supervision has approved the release of the "Determination of Sodium Picosulfate in Food" food supplementary inspection method.[1] This standard mainly describes the LC-MS/MS quantitative detection method of sodium picosulfate in foods (including jelly, preserves, candies, beverages, health foods, etc.).

Here the above-mentioned measurement method was established using the SCIEX Triple Quad 3500 LC-MS/MS System to establish a fast and sensitive MRM quantitative method. The linearity, recovery and repeatability of the method was verified. This general method can be applied across different SCIEX Triple Quad systems.

Key advantages

- ***Fast and sensitive:*** A liquid-phase elution time only needs 4 minutes, and the quantification limit of sodium picosulfate is as low as 0.4 ng/mL (Figure 1), providing sensitivity which meets the standard requirement (5 ng/mL).[1]
- ***Wide linear range:*** Assay provided wide linear dynamic range for sodium picosulfate, across a concentration range of 0.4~1000 ng/mL (Figure 3).
- ***Wide application range:*** This method verifies the recovery rates of the five substrates mentioned in the standard (Table 2) with minimal interferences.

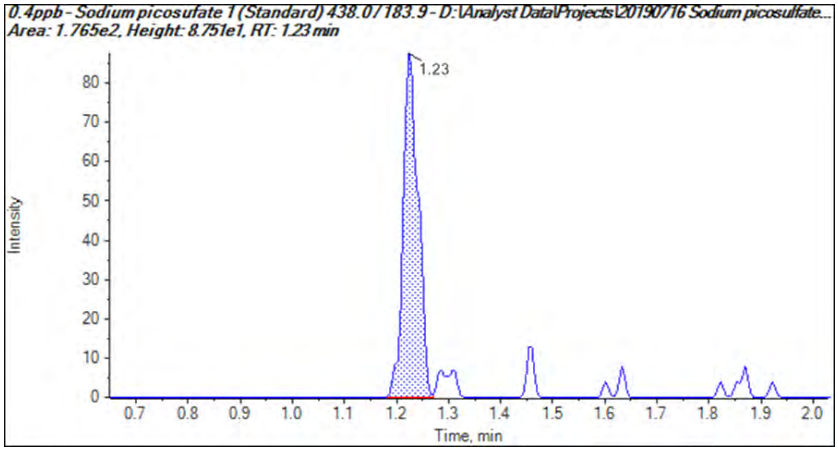

Figure 1. Chromatogram of the compound at the lower limit of quantification (0.4 ng/mL).

Methods

Sample and standard preparation: According to the sample preparation process in the food supplement inspection method of "Determination of sodium picosulfate in food", pre-treatment of a certain tablet health product, candy, solid beverage, plum and jelly was carried out.[1]

The standard curve samples were diluted with pure water and prepared into solutions with concentrations of 0.4, 1, 2, 6, 20, 60, 200, 600, and 1000 ng/mL, with a total of 9 concentration points.

Chromatography: SCIEX ExionLC™ AD System was used with Phenomenex Kinetex C18, 2.6 µm, 2.1 mm × 50 mm analytical column. A gradient elution was used with flow rate of 0.6 mL/min, injection volume of 5 µL, and column temperature of 40 ºC (Table 1).

Table 1. Chromatography.

Time (mins)	% A	% B
0	*95*	*5*
1.5	*50*	*50*
1.6	*10*	*90*
2.5	*10*	*90*
2.6	*95*	*5*

Mobile phase A: water (5 mM ammonium formate)
Mobile phase B: acetonitrile

Mass Spectrometry: SCIEX Triple Quad 3500 LC-MS/MS System was operated using the ESI source in positive ion mode.

Ion source parameters were:

- IS voltage: 5500V
- Curtain gas: 35psi
- Atomizing gas GS1: 50psi Auxiliary gas GS2: 55psi
- Source temperature (TEM): 500℃
- Collision gas CAD: 7

Two ion pairs were monitored for the sodium picosulfate; both using the precursor ion of 438 *m/z*; the quantifier product ion was 183.9 *m/z* and the qualifier product ion was 277.9 *m/z*.

Sensitivity and linear range

The detection concentration of this method is as low as 0.4 ng/mL (Figure 2), and it has a good linear dynamic range across a concentration range of 0.4~1000 ng/mL, with a correlation coefficient is r>0.999 (Figure 3). There is no residual signal found in a blank injection performed right after an injection of the 1000 ng/mL high concentration point.

Table 2. The recovery rate of sodium picosulfate in five different matrices.

Health Products	Recovery at 3 Concentrations (ng/mL)		
	6	60	600
Candy	*109.0*	*94.8*	*95.0*
Solid beverage	*101.0*	*96.7*	*96.1*
Huamei	*108.5*	*99.7*	*90.9*
Plum	*102.8*	*106.3*	*91.5*
Jelly	*105.7*	*97.2*	*93.2*

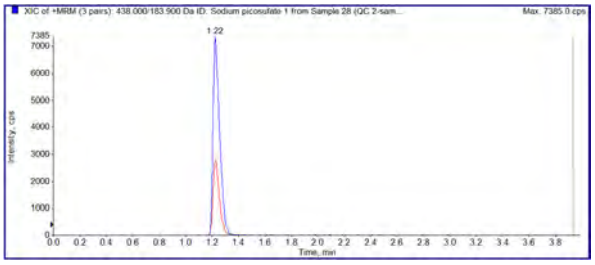

Figure 2. Typical chromatogram of sodium picosulfate. Signal obtained in sweet plum matrix for sodium picosulfate at 60 ng/mL concentration.

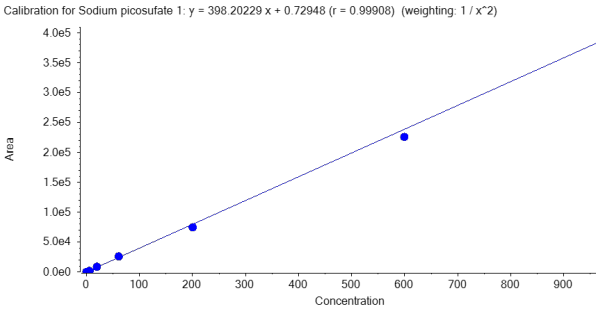

Figure 3. Standard curve of sodium picosulfate. Good linear dynamic range was obtained, from 0.4~1000 ng/mL with good correlation coefficient r>0.999.

Table 3. Repeatability of sodium picosulfate in plum matrix.

	Concentration (ng/mL)		
	6	60	600
1	*101.44*	*97.57*	*97.06*
2	*102.87*	*97.47*	*97.10*
3	*98.53*	*96.97*	*95.57*
4	*97.73*	*94.99*	*93.82*
5	*89.92*	*95.6*	*95.42*
6	*95.08*	*94.78*	*92.51*
RSD %	*4.78*	*1.31*	*1.90*

Recovery

Taking the plum matrix as an example, the repeatability of the compound at three different concentration points of 6, 60 and 600 ng/mL was investigated. The results are shown in Table 3. Three spiked samples with different concentrations were injected for 6 consecutive times. The peak area RSD was within 2% except for the low concentration point of 6 ng/mL, which was 4.78%.

Measurement in food samples

Sodium picosulfate was not detected in the five sample matrices, and the relevant chromatograms are shown in Figure 4.

Summary

Here, the SCIEX Triple Quad 3500 LC-MS/MS System was used to establish a quantitative method for sodium picosulfate in food. This method has high sensitivity, good repeatability, and a wide linear dynamic range of 0.4~1000 ng/mL. The test substance has no obvious matrix effect in 5 different food matrices. The elution time of the method is only 4 min, which is very suitable for high throughput analysis of food samples.

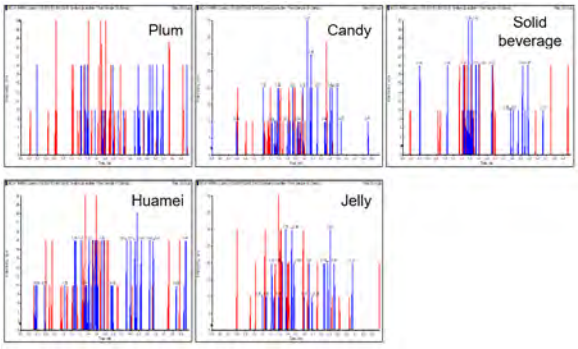

Figure 4. Chromatograms of five sample extracts. No peaks for sodium picosulfate were detected in the five food samples tested, plum, candy, solid beverage, Huamei and jelly.

References

1. Announcement of the General Administration of Market Supervision on Issuing the Supplementary Inspection Method of Food for the Determination of Sodium Picosulfate in Food. BJS 201911.

Quantification of multiple antibiotics in milk using the SCIEX Triple Quad™ 3500 LC-MS/MS System

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The nutritional well-being of the livestock is often maintained by the administration of antibiotics. However, the usage of these antibiotics is a major threat to human health. Over-usage creates the risk of creating resistant microorganisms. Individuals can be allergic to certain antibiotics. Additionally, the presence of these antibiotics in milk can affect the manufacturing of products like cheese, yogurt, etc. by inhibiting the starter culture.[1]

Each country had set a permissible limit of antibiotic that can be present in their food products based on human studies to determine tolerance limits. The permissible limit set for an antibiotic is typically in microgram per kg, which is difficult to quantify accurately using traditional methods like HPLC or microbial techniques. For the dairy industry, a simple assay that provides the accurate determination of various antibiotics at low levels in milk plays a vital role in helping them ensure the safety of their product and the health of their consumers.[2,3]

Here, a method was developed using SCIEX Triple Quad 3500 System, to determine residual antibiotics in milk. Local market milk samples were analyzed to check the presence of albendazole, fenbendazole, tylosin and tilmicosin and some metabolites. Sufficient sensitivity was provided with this assay to meet the MRL levels for these analytes and good assay performance was observed.

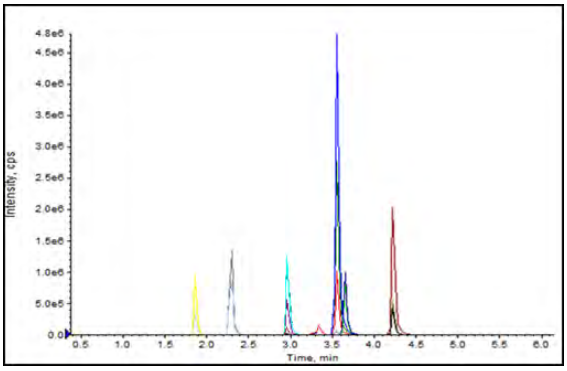


Figure 1: Chromatographic separation of antibiotics. LC separation is shown for albendazole, fenbendazole (and its metabolites), tilmicosin, tylosin at 100 ppb.

Key features for the multi-antibiotic assay in milk

- A quantitative method for the analysis of multiple veterinary drugs in milk was developed, namely albendazole, fenbendazole and its metabolites (albendazole sulfone, albendazole sulfoxide, albendazole-2-amino sulfone, fenbendazole sulfone) tylosin and tilmicosin
- Using the SCIEX Triple Quad 3500 System, sufficient sensitivity was provided to meet the regulatory requirements
- A linear dynamic range of 2.5-300 ppb was analyzed and excellent quantitative performance was achieved
- The robustness of the Turbo V™ Ion Source with the Curtain Gas™ Interface ensures high uptime and maximum productivity
- The method development was performed and partially validated per the regulatory guidelines described in 2002/657/EC directive recommendations

Methods

Chemicals: Standard albendazole, fenbendazole and their metabolites (albendazole sulfone, albendazole sulfoxide, albendazole-2-amino sulfone, and fenbendazole sulfone), tylosin and tilmicosin were obtained from collaborator. All other chemicals used were of LC-MS grade and commercially available. Milk samples were purchased from the local market of Delhi, and Gurgaon and stored in refrigerator at 2 to 8 ºC until the analysis was completed.

Sample preparation: A generalized extraction procedure was performed in which 1 mL of milk was mixed with 5 mL of water and vortexed. 10 mL of acetonitrile with 0.1% formic acid was added and vortexed for 10 min. Then, 2 g of NaCl was added to the solution, the solution was mixed well and centrifuged at 2598 g (4000 rpm) for 5 min. After centrifugation, the supernatant was collected and evaporated to dryness. The sample were reconstituted with 1 mL of acetonitrile/water (20:80, V/V) with 0.1% formic acid.

LC conditions: LC separation was performed on an ExionLC™ System using a Phenomenex Luna C18(2) (4.6×150 mm, 5.0 µm) column. The injection volume of the method was 10 µL. See Table 1 for gradient information.

Table 1. Gradient profile and mobile phase composition.

Total Time (min)	Flow Rate (µL/min)	A%	B%
0.0	*600*	*90*	*10*
7.0	*600*	*10*	*90*
8.5	*600*	*10*	*90*
8.6	*600*	*90*	*10*
10.0	*600*	*90*	*10*

Mobile phase A: water + 0.1% formic acid
Mobile phase B: acetonitrile + 0.1% formic acid

MS/MS conditions: The SCIEX Triple Quad 3500 LC-MS/MS System was operated in positive ion mode, using the Turbo V™ Ion Source with an electrospray ionization (ESI) probe. Data were collected using multiple reaction monitoring (MRM) with two transitions per analyte using Analyst® Software 1.6.

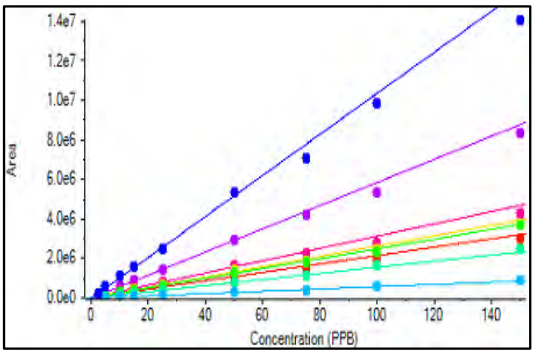
Data processing: LC-MS/MS data were processed using the Multiquant™ Software 3.0.2.

Results

The SCIEX Triple Quad 3500 System showed very good sensitivity for multi-residue antibiotic analysis in milk. The experimental data were acquired and partially validated in accordance with 2002/657/EC directive recommendations. The antibiotic mix was prepared and spiked at 5% in the milk matrix to determine the accuracy, precision, and reproducibility. The linearity in matrix was assessed from the range of 2.50 to 300 ppb for albendazole, fenbendazole and its metabolites (albendazole sulfone, albendazole sulfoxide, albendazole-2-amino sulfone, fenbendazole sulfone) tylosin, tilmicosin, trimethoprim, and tilmicosin (Figures 2-4). For all the analytes a regression coefficient was found to be above 0.99, where the weighing factor used was $1/x^2$.

Table 2. MRM transitions of antibiotics.

Compound	Precursor Ion	Product Ion (Quantifier)	Product Ion (Qualifier)
Albendazole	*266.0*	*234.3*	*191.1*
Albendazole Sulfone	*298.0*	*266.0*	*159.1*
Albendazole Sulfoxide	*282.0*	*240.0*	*208.1*
Albendazole-2-Amino Sulfone	*240.0*	*133.2*	*197.9*
Fenbendazole	*300.0*	*268.0*	*158.7*
Fenbendazole Sulfone	*332.0*	*159.0*	*300.1*
Tilmicosin	*869.8*	*174.2*	*696.7*
Tylosin	*916.7*	*174.1*	*772.4*



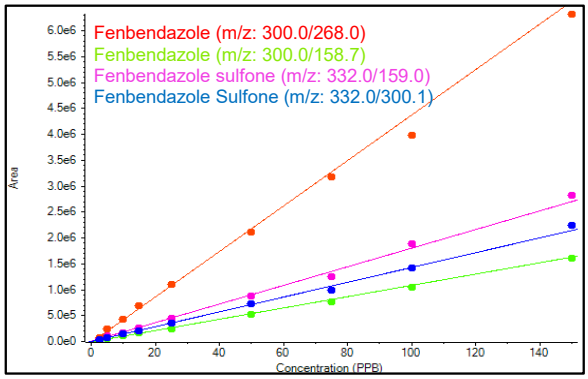
Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
ALBENDAZOLE_01	0.05	6 of 6	1.699e4	1.308e3	7.70

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
ALBENDAZOLE SULFONE_01	0.10	6 of 6	3.900e3	2.544e2	6.52

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
ALBENDAZOLE SULFOXIDE_01	0.30	6 of 6	7.652e3	6.072e2	7.93

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
ALBENDAZOLE 2 AMINO SULFONE_01	1.00	6 of 6	1.025e4	7.313e2	7.13

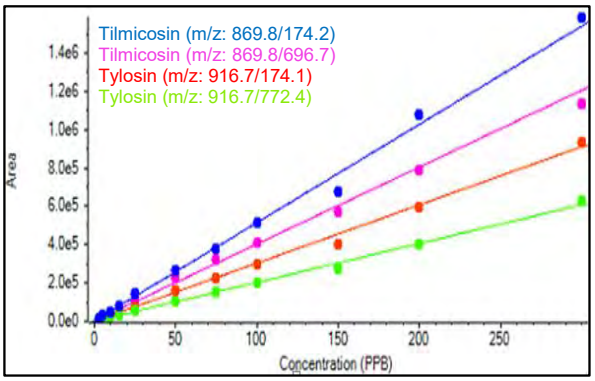
Figure 2: Calibration curve of albendazole and its metabolites. Good linearity was observed for these analytes from 2.5-150 ppb. Albendazole (m/z: 266.0/234.3), Albendazole (m/z: 266.0/191.1), Albendazole sulfone (m/z: 298.0/266.0), Albendazole sulfone (m/z: 298.0/159.1.0), Albendazole Sulfoxide (m/z 282.0/240.0), Albendazole Sulfoxide (m/z 282.0/208.1), Albendazole-2-amilno sulfone (m/z:240.0/133.2),Albendazole-2-amilno sulfone (m/z:240.0/197.9). Good reproducibility was observed at the lowest concentration of each for 6 replicate injections.



Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
FENBENDAZOLE_01	0.05	6 of 6	1.030e4	9.120e2	8.85

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
FENBENDAZOLE SULFONE_01	0.10	6 of 6	2.019e3	1.463e2	7.25

Figure 3: Calibration curve of fenbendazole and its metabolites. Good linearity was observed for these analytes from 2.5-300 ppb. Good reproducibility was observed at the lowest concentration of each for 6 replicate injections.



Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
TILMICOSIN_01	0.50	6 of 6	1.461e3	1.182e2	8.09

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
TYLOSIN_01	0.50	6 of 6	1.903e3	1.667e2	8.76

Figure 4: Calibration curve of tilmicosin and tylosin. Good linearity was observed for these analytes from 2.5-300 ppb. Good reproducibility was observed at the lowest concentration of each for 6 replicate injections.

The accuracy for each analyte across the concentration curve was 90-110% , which is in compliance with EU guidelines and the repeatability %CV were found to be less than 10% (n=6), at the respective MRL level of each analyte. Two MRM transitions were used for each analyte as quantifier and qualifier ions, to allow the determination of the ion ratio for analyte. MultiQuant Software was used for the data processing, for automatic calculation of statistics of the calibrations curves as well as the MRM ratios for the analytes.

Table 3. %CV at LOD level in aqueous standard (AQS) and MRL level in extracted samples.

Compound	LOD in AQS (ppb)	%CV at LOD (n=6)	MRL Value (ppb)	%CV at MRL (n=6)
Albendazole	*0.05*	*7.70*	*100*	*3.38*
Albendazole Sulfone	*0.10*	*6.52*	*100*	*5.37*
Albendazole Sulfoxide	*0.30*	*7.93*	*100*	*5.69*
Albendazole-2-Amino Sulfone	*1.00*	*7.13*	*100*	*5.94*
Fenbendazole	*0.05*	*8.85*	*10*	*4.94*
Fenbendazole Sulfone	*0.10*	*7.25*	*10*	*4.73*
Tilmicosin	*0.50*	*8.09*	*50*	*5.75*
Tylosin	*0.50*	*8.76*	*25*	*4.49*

Analysis of milk samples

Milk samples collected from local markets of Delhi and Gurgaon, India, were tested for the presence of multi-residue analyte. Albendazole, fenbendazole and its metabolites, tilmicosin and tylosin were absent in all the tested samples.

Conclusions

The method presented here on the SCIEX Triple Quad 3500 System shows a fast and accurate solution for the quantification of 8 antibiotics in milk samples using simple sample preparation combined with LC-MS/MS. The method developed as per 2002/657/EC directive recommendations showed acceptable accuracies (80%-120%) for analysis in matrix samples, linearity with r ≥ 0.99 for both the MRM transitions, and %CV for repeatability was ≤10% at the LLOD levels. No significant matrix interferences were observed. Automatic MRM ratio calculation provides quick confirmation of each analyte for increased assay confidence.

References

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3. Martins MT et al. (2016) Multiclass and multi-residue determination of antibiotics in bovine milk by liquid chromatography-tandem mass spectrometry: Combining efficiency of milk control and simplicity of routine analysis. *Int. Dairy J.* **59**, 44-51.

Ensuring quality and freshness in animal nutrition products

Streamlined quality assurance workflow to quantitate biogenic amines using LC-MS/MS Technology

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Biogenic amines (BAs) can exist in a variety of foods and food products, such as, meat, cheese, vegetables, and wine. BAs are organic bases with aliphatic, aromatic, and heterocyclic structures. These compounds can be formed in food during processing or storage when specific amino acids are released by the action of decarboxylases produced by microorganisms.[1,2] Some of the most common BAs found in food are putrescine, cadaverine, histamine, 2-phenylethylamine, tyramine, spermidine, tryptamine, and spermine.[1,3]

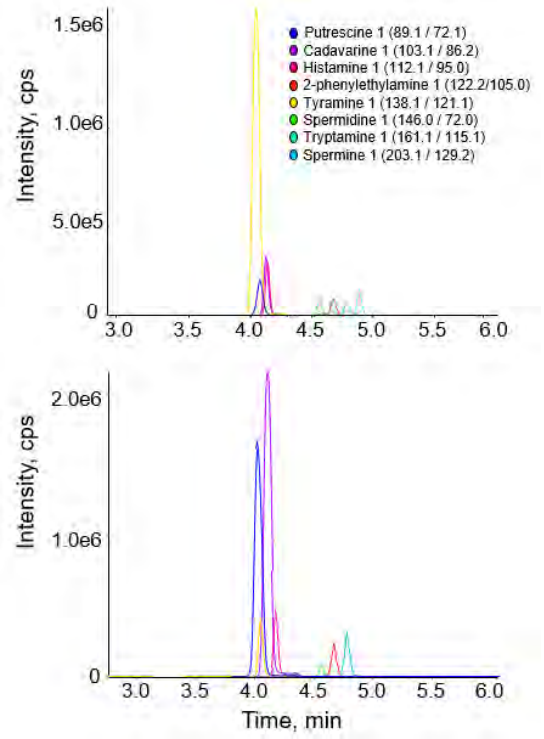

Figure 1. Rapidly detect biogenic amines from a single analysis. Extracted Ion Chromatograms showing the detection of biogenic amines contained in commercially available animal nutrition products. A rapid LC-MS/MS method was used to screen dog food (top) and blood meal (bottom). Compounds like Putrescine, 2-phenylethylamine and Cadavarine were easily detected at concentrations ranging from 1 ppm to 50.6 ppm.

Humans may consume an occasional meal where biogenic amines are elevated however pets on a fixed pet food diet that is high in biogenic amines may have ill effects due to steady exposure. Healthy adult cats and dogs may be able to detoxify biogenic amines present in the diet; however, kittens and puppies, reproducing females and ill animals could potentially be more prone to adverse effects.[1]

In this study, a sensitive, robust, and fast LC-MS/MS was developed to quantify eight biogenic amines which allows for low level environmentally relevant concentrations to be accurately quantified.

Features of the SCIEX Triple Quad™ 3500 LC-MS/MS System for quality assurance in animal nutrition

- The SCIEX Triple Quad 3500 LC-MS/MS System is designed for routine food testing assays, enabling the analysis of more compounds in a single injection, lower quantitation limits, and increased sample volume and throughput productivity over conventional HPLC or GC workflow
- Industry leading Turbo V™ source efficiently ionizes biogenic amines, delivering highly efficient desolvation for stable and sensitive performance while analyzing complex nutritional products (e.g., Blood Meal)
- Proprietary Curtain Gas™ interface reduces the need for routine maintenance and ensures maximum productivity and robustness

Figure 2. Overview of sample preparation.

Methods

Sample preparation: Approximately 0.5 g of sample was placed in a conical 15 mL tube with 10 mL of 5% trichloroacetic acid (TCA). Samples were then vortexed for 10 min and centrifuged for 10 min. The supernatant was saved, and the extraction repeated using the pellet. The supernatant was combined, and the pH was adjusted to 6 - 7.5 with NaOH. A 1 mL aliquot of methanol (MeOH) was added and the sample was filtered. The solid phase extraction (SPE) was performed using a Strata-XL-CW, 200 mg/6 mL cartridge with a 20 mL reservoir attachment and eluted in methanol and evaporated. The sample was then reconstituted with 0.5% formic acid in water and n-heptane-1-sulfonate.

Commercial pet food samples: Six corn flour samples were prepared with biogenic amines spiked at concentrations ranging from 0.5 to 100 ppm, these samples were analyzed to determine the limits of quantitation and linearity. Additionally, unknown samples of cat food, dog food, chicken meal, blood meal, and chicken-by-product were analyzed.

LC-MS/MS analysis: A 25 µL aliquot of the samples was injected onto an ExionLC™ AC System coupled to a SCIEX Triple Quad 3500 LC-MS/MS System. Chromatographic separation was performed using a Phenomenex Kinetex C18 (100×2.1 mm, 5 mm) analytical column. Mobile phases consisted of formic acid in water (A) and formic acid in methanol (B). Mass spectrometry analysis was performed using positive electrospray ionization.

Data processing using Standard Addition: Results were processed using the SCIEX OS Software. The embedded standard addition algorithm was used. The column “Standard Addition Calculated Concentration” was activated in the Results Table to show the calculated matrix spiked standards.[5]

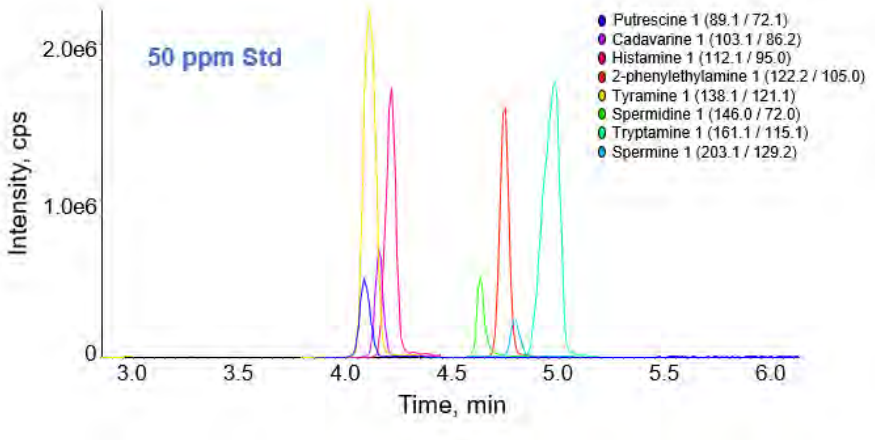

Figure 3. Obtain maximum ionization efficiency with LC-MS/MS to ensure best product quality possible. High pressure liquid chromatography (HPLC) coupled with an ultraviolet detector has been successfully employed but has a limited linear dynamic range and requires derivatization steps. In contrast, the approach applied in this study relies on tandem mass spectrometry (MS/MS), thereby added considerable sensitivity and provided structural information, which added certainty in identification. An acidic ion pairing agent, n-heptane-1-sulfonate, was also used, resulting in improved chromatographic separation as shown above.

Implementing a rapid biogenic amines analysis method for quality assurance

The use of the ion pairing agent on the samples was a key element to enable the selective chromatographic separation of all target BAs (Figure 3). Quantitative analysis for all the analytes tested showed good linearity covering 3 orders of magnitude in concentration. Although detection levels lower than the listed Limits of Quantitation were achieved (Table 1), priority was given to levels that represented relevant concentrations in food. Furthermore, the addition of an internal standard would likely widen the linear dynamic range for quantitation.

Table 1. Calibration range of biogenic amines analyzed.

Compound	Calibration range (ppm)	R^2
Putrescine	0.5 - 100	0.996
Cadavarine	1 -100	0.999
Histamine	1-100	0.999
2- phenylethylamine	0.5-100	0.992
Tyramine	5-100	0.997
Spermidine	5-100	1.000
Tryptamine	0.5-50	0.999
Spermine	10-100	0.998

Compound identification was achieved using the ratio of quantifier and qualifier MRM transition. SCIEX OS-MQ Software 1.4 automatically calculates ion ratios, displays tolerance levels, and flags outliers in the result table. An example for the 0.5 ppm standard of histamine is shown in Figure 4.

Proven robustness on toughest food matrices

The SCIEX Triple Quad 3500 System demonstrated excellent reproducibility in matrix. Triplicate injections were performed at 0.5, 1, 5, 10, 25. 50 and 100 mg/L. Relative Standard Deviation (%RSD) was typically below 10% for most compounds (Table 2).

In this study samples several matrices including cat food, dog food, chicken meal, blood meal, and chicken-by-product were extracted and analyzed for select BAs.

The analyzed dog food sample (Figure 1) had concentrations of putrescine, cadaverine, and tryptamine at 46.2 ppm, 50.6 ppm, and 1.7 ppm respectively. Additionally, the concentrations of histamine, tyramine, spermine were 8.0 ppm, 32.1 ppm, and 25.2 ppm. Spermidine and 2- phenylethylamine were below the Limits of Quantitation.

The blood meal samples had concentrations of putrescence, cadaverine, and spermine greater than 100 ppm. While the histamine, 2- phenylethylamine, and tyramine concentrations of 13.7 ppm, 3.4 ppm and 5.1 ppm respectively.

Conclusions

In this study, a robust LC-MS/MS method for the analysis of BAs in food and food products was described. Samples were extracted using straightforward SPE method utilizing an ion pairing agent to improve chromatographic separation. Samples were analysed using the SCIEX Triple Quad 3500 System. Overall, this method demonstrated good repeatability and linearity for quantitation (over 3 orders of magnitude).

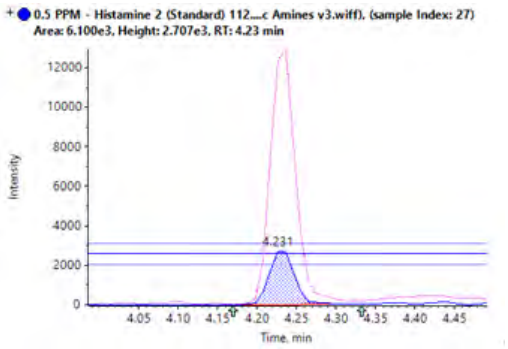

Figure 4. Quickly deliver analyte specific information present in an animal nutrition product. View of all compounds present in using SCIEX OS-MQ Software. Here an extracted ion chromatogram obtained for histamine showcasing both MRM transitions overlaying qualifier and quantifier ions with ion ratio lines, at a concentration of 0.5 ppm with maximum tolerances of 20%, which is the industry standard for analyte quantitation and identification.

Table 2. %CV for standard curve samples (n=3).

Conc. (ppm)	Putrescine	Cadavarine	Histamine	2-Phenylethylamine	Tyramine	Spermidine	Tryptamine	Spermine
0.5	13.1%	7.9%	4.2%	4.4%	4.7%	4.0%	8.7%	1.6%
1	3.7%	2.3%	5.4%	4.3%	2.9%	2.7%	7.8%	2.6%
5	7.4%	8.8%	7.7%	5.1%	5.7%	7.5%	5.8%	4.6%
10	9.8%	10.9%	7.8%	5.6%	7.3%	5.4%	8.9%	1.8%
25	10.6%	10.7%	8.3%	7.1%	6.4%	17.9%	6.2%	5.5%
50	8.7%	6.5%	6.6%	4.8%	4.4%	4.5%	6.7%	7.9%
100	9.9%	8.5%	3.8%	6.1%	4.9%	5.7%	5.7%	4.9%

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4. Download the details for the Bioamine quantitation method.
5. Standard addition processing simplified with SCIEX OS Software. SCIEX technical note RUO-MKT-07-7660-B.

Quantification of Psilocybin and Psilocin in Mushroom by LC-MS/MS

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Psilocybin and psilocin are psychoactive compounds produced by numerous mushroom species. Historically, these mushrooms were used for spiritual and religious ceremonies in central and southern America, but recently there has been research related to using psilocybin and psilocin to treat addiction and depression. Currently, psilocybin and psilocin are defined as Schedule 1 illicit drugs under the United States Controlled Substances Act.[1,2] However, in Denver, Colorado, the possession of psychotropic mushrooms has been decriminalized. While the selling and purchase of these drugs remain illegal,[3] many see this as a roadmap to eventual legalization, similar to cannabis. Due to the increased interest in these compounds, robust and sensitive analytical methods are needed.

In this study, a 5-minute LC-MS/MS method has been developed for the quantification of psilocybin and psilocin in mushroom matrices.

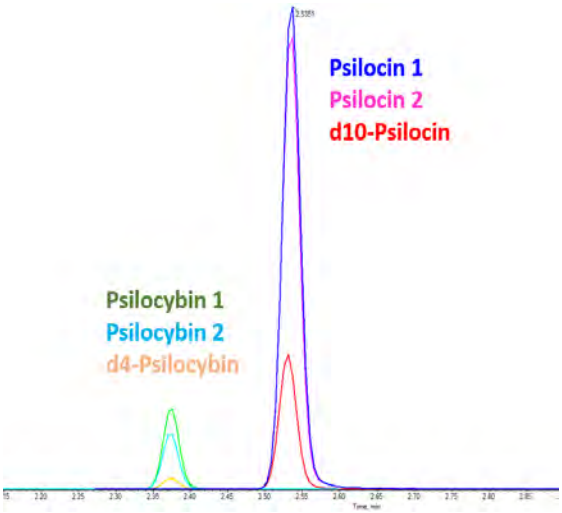

Figure 1. Chromatogram of Psilocybin and Psilocin in Mushroom Matrix (M).

Key Features of LC-MS/MS System for Psilocin and Psilocybin Analysis

- Limits of detection below 1 ppb in mushroom extract using the SCIEX Triple Quad™ 3500 LC-MS/MS system
- Simple dilute and shoot sample preparation
- Fast analysis time of 5 minutes
- Retention of polar psilocybin and psilocin
- Reproducible quantitative results

Experimental

Sample Preparation: Analytical standards for both native and deuterated psilocybin and psilocin were purchased from Cerilliant (TX, USA). Two different mushroom extractions are detailed: the first screens for illicit psilocybin and psilocin in unknown mushroom matrices and the second confirms and quantifies psilocybin and psilocin in actual hallucinogenic mushrooms.

1) Screening and Detection of Psilocybin and Psilocin in Unknown Mushroom Matrices (M): An initial sample weight of 100 mg fresh homogenized shiitake mushroom is extracted in 10 mL of methanol and vortexed. The mushroom matrix was left to further extract at 4ºC overnight. The extract was then spiked with psilocybin and psilocin, with the spiked amount represented as concentration in-vial. Deuterated internal standards were added to each vial.

2) Diluted Mushroom (DM) for Quantification and Confirmation: The diluted mushroom (DM) matrix was diluted to replicate the dilution factor needed to accurately quantify psilocybin and psilocin in real hallucinogenic mushrooms.[1] The 1:100 mushroom extract is further diluted another 1:400 for a total dilution factor of 1:40,000. This large dilution volume is necessary to linearly quantify 3% by sample weight psilocybin and psilocin in hallucinogenic mushrooms.

Chromatography: An injection volume of 2 µL was separated on a Phenomenex Luna Omega Polar C18 (4.6 µm x 150 mm) using mobile phases of formic acid, water, and acetonitrile at a flow rate of 1.2 mL/min.

Table 1. LC Gradient.

Time (min)	B (%)
0	*5*
3	*100*
4	*100*
4.1	*5*
5	*End*

Mobile Phase A - 0.1% formic acid in water
Mobile Phase B - 0.1% formic acid in acetonitrile

Mass Spectrometry: Both compounds were analyzed in positive polarity on a SCIEX Triple Quad 3500 System using MRM scan mode. Each analyte had a peak width of less than six seconds and at least ten scans for accurate quantification.

Table 2. Source Parameters Optimized for Analysis.

Source Parameter	Optimized Value
Curtain Gas	*40*
Ion Spray Voltage	*3500*
CAD Gas	*11*
Heater Temperature	*600*
Nebulizer Gas (GS1)	*50*

Discussion

The matrix matched calibration curve exhibited good accuracy within +/- 30% of the expected values for all points, accuracy within +/- 10% for the lowest calibrator, and R^2 coefficients of >0.990. The linear range for psilocybin was from 1 to 250 ppb and for psilocin was 0.1 to 250 ppb in vial (Figure 2). The calibration curve for method one corresponds to 100 ng/mL to 25,000 ng/mL of psilocybin and 10 ng/mL to 25,000 ng/mL of psilocin in unknown mushroom matrices. The calibration curve for method two corresponds to 0.04 to 40 mg/mL of psilocybin (Figure 3) and 0.004 to 10 mg/mL of psilocin (Figure 4) in psychedelic mushrooms. This is an ideal range, as Gambaro *et al.* (2015) showed psychedelic mushrooms have an average of 2.1 mg/mL of psilocin and 1.1 mg/mL of psilocybin.[1] Sensitivity of the SCIEX Triple Quad 3500 System and the robust 5 minute method make this the ideal instrument for this type of analysis.

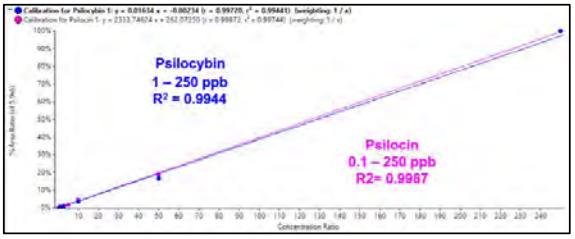

Figure 2. Representative Calibration Curves of Psilocybin and Psilocin.

References

1. Gambaro, Veniero *et al.* (2015) Identification of Hallucinogenic Mushrooms Seized on the Illegal Market Using a DNA-Based Approach and LC-MS/MS Determination of Psilocybin and Psilocin. *Journal of Analytical and BioAnalytical Techniques.* ***6*** **(6)**, 578–585.
2. Drug Enforcement Administration (DEA). The Controlled Substances Act. (n.d.). Retrieved from https://www.dea.gov/controlled-substances-act. Nov.2019.
3. Colorado Legal Defense Group. Retrieved from https://www.shouselaw.com/colorado/drugs/CO_psilocybin_mushrooms.html. Nov. 2019.

Psilocybin

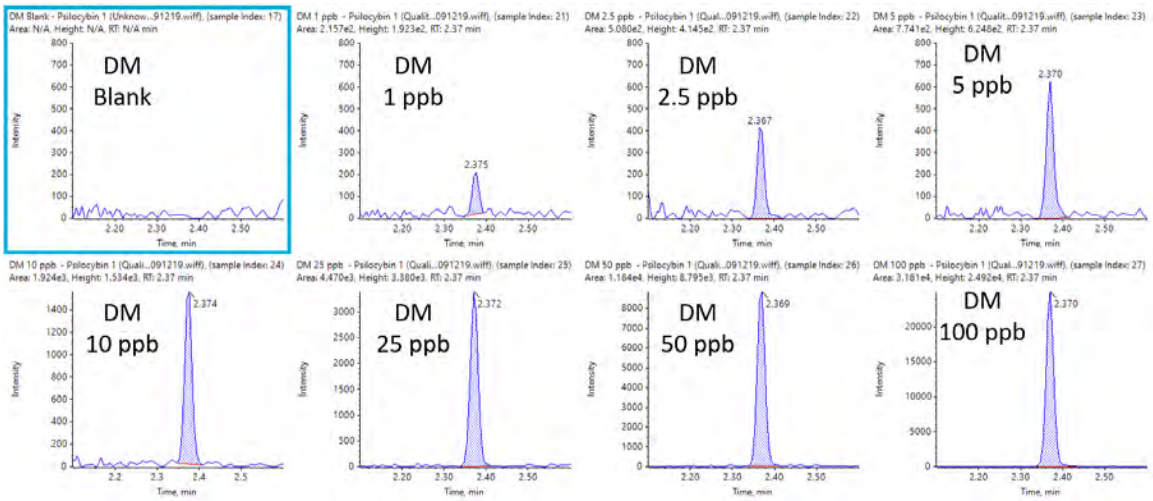

Figure 3. Psilocybin Spiked into Varying Concentrations in Diluted Mushroom Matrix. Limits of quantification were found to be 1 ppb in vial.

Psilocin

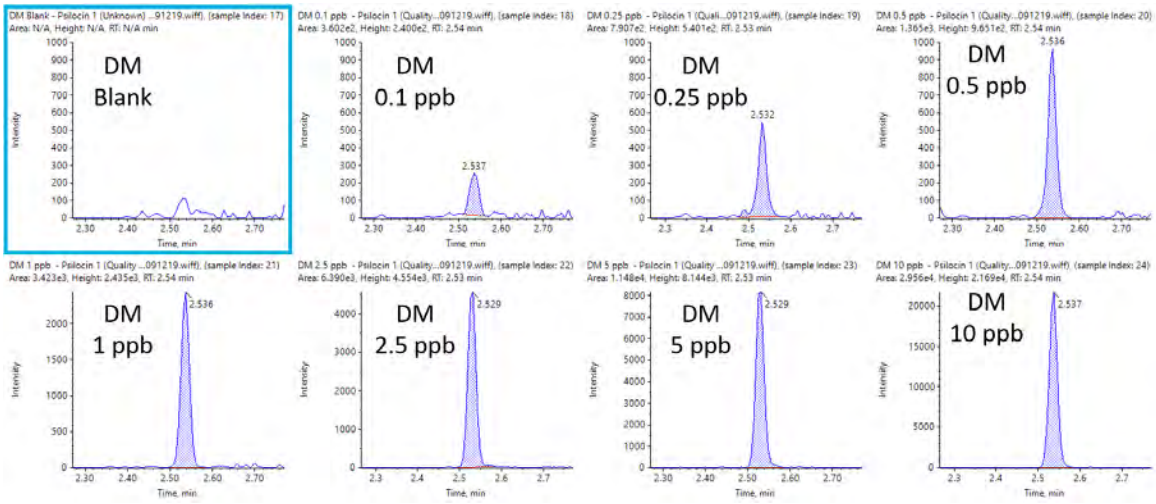

Figure 4. Psilocin Spiked into Varying Concentrations in Diluted Mushroom Matrix. Limits of quantification were found to be 0.1 ppb in vial.

Analysis of vitamin E and vitamin E acetate in vape oils

Triple quadrupole analysis of vape oils produces high quality quantitative results

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Recently, a drastic increase in vaping related lung illnesses has been observed.[1] The cause of this unprecedented number of people gravely affected from vaping is of urgent concern to the CDC and FDA.[2] Vitamin E acetate, a compound used to thicken vaping liquids, has been implicated in the rise in the rate of observed lung illnesses.[3] While the link between lung illness and vitamin E acetate is not certain[1], there has been an increase in requests to have products tested for the presence of vitamin E and vitamin E acetate.[4] LC-UV methods have historically been employed for these analytes, but the variability of the relevant matrices and the possibility of co-eluting interferences in a non-specific method demands that a more specific and reliable analytical approach be used to assure product safety. Increased specificity is a hallmark of Multiple Reaction Monitoring (MRM) analysis on a triple quadrupole mass spectrometer. The mass spectrometric approach using two MRM transitions for each analyte as well as an isotopically-labelled internal standard, ensures that the detected signal for the vitamin E and vitamin E acetate can indeed be attributed to the presence of these species and are not the artifact of complex matrix interferences.

This application note details a workflow for accurate and precise analysis of vitamin E and vitamin E acetate in vape oils. The SCIEX Triple Quad™ 3500 LC-MS/MS System was leveraged to produce quantitative results which are of high quality, robust, and time efficient.

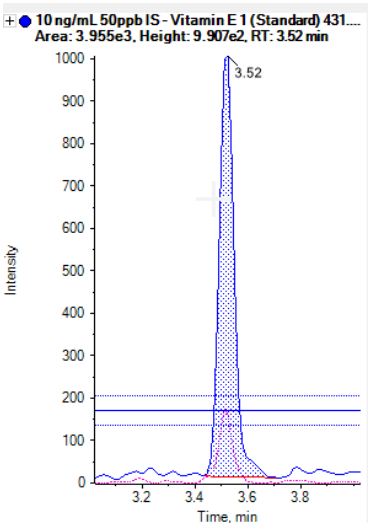

Figure 1. Vitamin E acetate detection. Vitamin E Acetate at 10ng/mL, showing acceptable ion ratio confirmation.

Key advantages of the vitamin E method

- Quantitative method combines analyses for vitamin E and vitamin E Acetate
- MRM analysis for high specificity of detection
- Highly simplified sample preparation: “dilute-and-shoot”
- Fast 7-minute analysis
- Linear response for quantitation from 10ppb up to 500ppb with excellent precision (5%CV) for both analytes

Experimental

Sample preparation: Samples were prepared for analysis by dissolving 500mg of sample in 40mL of methanol. The injection solvent contained vitamin E d6 at a concentration of 50ppb. This was used as the internal standard for quantitation. The diluted sample was analyzed without any further processing.

Chromatography: Chromatographic separation was achieved using an Agilent Poroshell 120 EC-C18, 2.7µm, 100 X 4.6mm column with a solvent flow rate of 1.2 mL/min. The column oven was set to 50°C. 5 µL injection volume was used. The chromatographic gradient and mobile phases are outlined in Table 1.

Table 1. Gradient for vitamin E separation.

Time (min)	Mobile phase B (%)
0.0	95.0
0.5	100.0
5.0	100.0
5.1	95.0
7.0	95.0

Mobile phase A: Water with with 5mM ammonium formate, 0.3% formic acid
Mobile phase B: Methanol with with 5mM ammonium formate, 0.3% formic acid

Mass spectrometry: Analysis was performed on the SCIEX Triple Quad 3500 System with a Turbo V™ Source using electrospray ionization (ESI) in the positive ion mode. Data were collected using the conditions shown in Table 2. Ion source and collision gas conditions were as follows: GS1 = 30, GS2 = 30, CUR = 35, CAD = 11, TEM = 300ºC.

Table 2. Compound-specific acquisition and data processing parameters.

	Precursor	Fragment	DP (V)	CE (V)	RT (min)
Vitamin E 1	431.1	165.1	121	37	3.53
Vitamin E 2	431.1	137.1	111	59	3.53
Vitamin E acetate 1	473.2	207.1	176	25	4.38
Vitamin E acetate 2	473.1	165.1	176	55	4.38
Vitamin Ed6	437.1	171.1	106	37	3.54

Linearity, precision and sensitivity

Calibration curves for Vitamin E and Vitamin E acetate were acquired from 10ppb to 500ppb. An example curve is shown in Figure 2. The top trace is the calibration curve for the primary MRM transition of vitamin E and the bottom calibration is the primary MRM transition for vitamin E acetate. Both compounds exhibit excellent linearity over this range (r-value >0.98).

The calibration was run 5 consecutive times to demonstrate the precision and stability of the method. Very good reproducibility was obtained and is shown in Table 3. The percent CV for the 5 injections was 6% except for vitamin E at 10ppb, which had a percent CV of 9%. The measured accuracy ranged from 83% to 118% and was generally within 10% of the expected value. These data demonstrate that highly reproducible analytical results are observed using this method. These values indicate that the accuracy expected to be obtained with this method will meet analytical requirements, based on the regulation in place for residues testing. Figure 3 shows the MRM group for vitamin E acetate for each of the five 10ppb injections. The ion ratios (ratio of primary MRM signal to secondary MRM signal) for each of the injections demonstrates that reliable ion ratios are consistently obtained even at low concentration.

Sample results

Thirty-three vape oils from a wide variety of sources were analyzed using the method. Typical results from a subset of these samples are shown in Table 4. These representative results show that vitamin E acetate is detectable in all the products, while vitamin E is not detected in any of the products. The data also demonstrate that there is no correlation between the concentration of vitamin E and vitamin E acetate.

Table 4: Typical values for vitamin E and vitamin E acetate from range of vape oils (subset of 33 samples analyzed).

Sample	Vitamin E (ppb)	Vitamin E acetate (ppb)
1	<0	0.415
2	74.1	0.461
3	76.4	0.383
4	<0	0.532
5	<0	2.32
6	<0	2.02
7	106.7	0.594
8	72.7	0.524

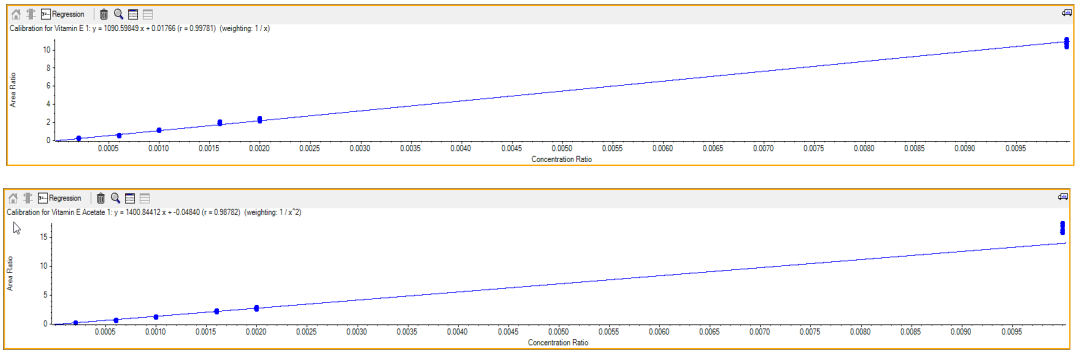

Figure 2: Example calibration curves. (Top) Calibration curve for vitamin E from 10 to 500ppb. (Bottom) Calibration curve for vitamin E acetate from 10 to 500ppb.

Table 3. Precision and accuracy for five consecutively analyzed calibration curves.

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Vitamin E 1	0.010	5 of 5	0.0	0.0	8.85	99.99
2	Vitamin E 1	0.030	5 of 5	0.0	0.0	5.22	85.57
3	Vitamin E 1	0.050	5 of 5	0.1	0.0	3.64	101.63
4	Vitamin E 1	0.080	5 of 5	0.1	0.0	4.15	109.59
5	Vitamin E 1	0.100	5 of 5	0.1	0.0	3.71	105.08
6	Vitamin E 1	0.500	5 of 5	0.5	0.0	2.67	98.15

Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy
1	Vitamin E Acetate 1	0.010	5 of 5	0.0	0.0	5.62	106.76
2	Vitamin E Acetate 1	0.030	5 of 5	0.0	0.0	5.54	83.03
3	Vitamin E Acetate 1	0.050	5 of 5	0.0	0.0	5.43	92.54
4	Vitamin E Acetate 1	0.080	5 of 5	0.1	0.0	2.90	101.75
5	Vitamin E Acetate 1	0.100	5 of 5	0.1	0.0	4.58	98.14
6	Vitamin E Acetate 1	0.500	5 of 5	0.6	0.0	4.40	117.78

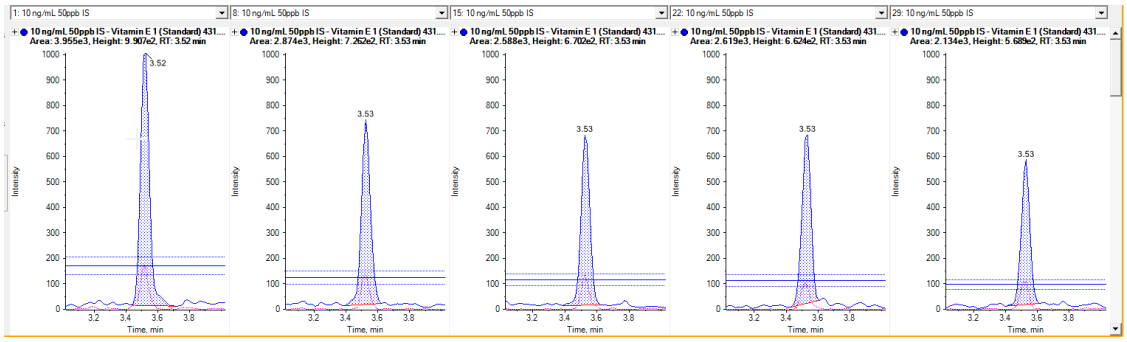

Figure 3: Example chromatography. Peaks for the primary and secondary ions overlaid at 10ppb vitamin E Acetate showing acceptable ion ratios for each injection.

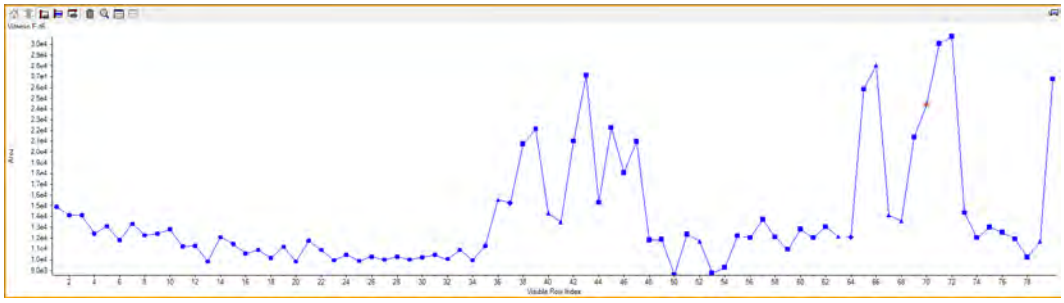

Figure 4: Peak areas for the internal standard, vitamin E d6. Circles represent calibration standards, Squares represent unknown samples, and triangles represent CCV standards.

The peak areas for the internal standard, vitamin E d6 are shown in Figure 4. The plot shows area values for the injected samples of a single batch. The standards (represented by closed circles) run at the start of the batch show a very stable response during the analysis of the calibration solutions with an RSD of 12%. The IS areas for the samples (represented as squares) show elevated areas for some of the standards resulting in an RSD for the sequence of 37%. Elevated areas for the internal standard were not observed for all samples and appear to be related to those samples that had high concentrations of vitamin E. The areas do, however, demonstrate the need for using an internal standard to achieve accurate quantitation.

The performance of the method was monitored during the run with the analysis of Continuing Calibration Verification standards (CCV). These samples were spiked with vitamin E acetate. The results for the method QC are shown in Table 5. The CCVs were stable during the sequence of injections with recoveries from 86 to 108%, which is within general acceptance criteria required for residue analysis methods. The CCVs were acquired with different concentrations throughout the sequence and further demonstrate that the method provides accurate quantitation across the calibration concentration range during sample analysis.

Conclusions

A method has been developed for the analysis of vitamin E and vitamin E Acetate that is suitable for the quantitative determination of these compounds in vaping oils from 0.01% to 0.5%. The method has been demonstrated to provide accurate and precise results during an extensive analysis of several actual samples of vaping oil.

Table 5: Continuing calibration verification results.

Sample type	Spike conc (ppm)	Calc conc (ppm)	Accuracy (%)
CCV 1	*0.050*	*0.054*	*108*
CCV 2	*0.100*	*0.101*	*101*
CCV 3	*0.050*	*0.043*	*87*
CCV 4	*0.100*	*0.101*	*101*

References

1. U.S. Food and Drug Administration. https://www.fda.gov/consumers/consumer-updates/vaping-illnesses-consumers-can-help-protect-themselves-avoiding-tetrahydrocannabinol-thc-containing (accessed September 29, 2019).
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3. David Downs. Amid Vape Pen Lung Disease Deaths: What Exactly Is Vitamin E Oil? https://www.leafly.com/news/health/vape-pen-lung-disease-vitamin-e-oil-explained (Accessed September 29, 2019).
4. Communication with Swetha Kaul, Cannalysis Laboratories, Santa Ana, CA (September 26, 2019).

Quantification of terpenes in cannabis products using the SCIEX Triple Quad™ 3500 LC-MS/MS System

KC Hyland,[1] Christopher Borton,[1] Paul Winkler,[1] Simon Roberts,[1] Matthew Noestheden[2]
[1]SCIEX, USA; [2]SCIEX, Canada

With the recent legalization of cannabis in several states, there is a growing need for robust, cost-effective analytical methods to facilitate routine testing. While testing of potency (and for pesticide and herbicide residues) is important, manufacturers of cannabis products also need fit-to-purpose analytical methods that provide information on the sensory profile of their products to ensure lot-to-lot consistency.

At least 200 terpenes have been identified in Cannabis sativa (cannabis), with unique strains presenting different terpene profiles.[1,2] The terpenes present have a well-defined role in the perceived aroma and user preference for specific cannabis strains. Moving beyond a role in sensory perception, recent studies suggest that many cannabis terpenes have pharmacological properties of their own and may also act synergistically with cannabinoids.[2] As such, sensitive, selective, accurate and economical analytical methods are needed to assess these key compounds.

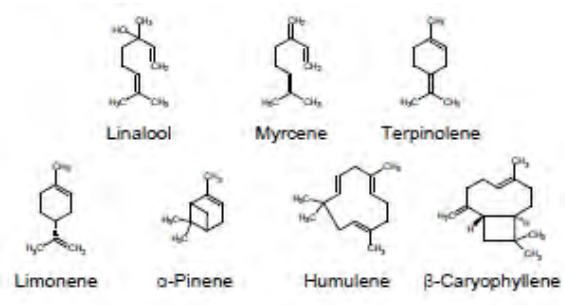

Figure 1. Chemical structures of terpenes evaluated in this study.

Historically, terpenes have been analyzed by gas chromatography-mass spectrometry (GC-MS) due to their predominantly aliphatic composition. However, labs performing routine testing of cannabis will need to test potency and, once regulations are established, test for pesticides and herbicides. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) represents an ideal analytical platform to address all of these testing needs.

This technical note presents an LC-MS/MS method that uses atmospheric pressure chemical ionization (APCI) and the budget-friendly SCIEX Triple Quad™ 3500 LC-MS/MS System for the analysis of terpenes in cannabis products. The price-to-performance ratio of the SCIEX Triple Quad 3500 System is ideally suited to the analysis of terpenes, cannabinoids and pesticides/herbicides in cannabis products. The method focuses on the 7 terpenes typically found in cannabis (see Figure 1), with examples of accurate quantification shown for a variety of cannabis products.

Key features of the SCIEX Triple Quad 3500 System for terpene analysis

- An affordable solution for quantification of terpenes in cannabis products
- Robust detection at 1 ppb levels in a variety of matrices
- Spike recoveries of 80% to 120% showed the quantitative accuracy of the method in a variety of cannabis matrices

Methods

Standards and internal standards (IS): Standards, internal standards (ISTDs), ACS grade ammonium acetate, formic acid and distilled-in-glass grade methanol were used as received. High purity water was produced by passing reverse osmosis water through a water purification system. All standard solutions and samples were stored at 5 ± 3°C and allowed to reach room temperature before analysis.

Sampling and sample preparation: A total of 10 cannabis samples were extracted by adding 0.1–2 g of cannabis product to 10 mL of methanol. Following mixing and filtration, samples were analyzed without further workup.

LC separation: Chromatography was performed on an Agilent 1290 Infinity LC system equipped with a binary pump, autosampler and thermostated column compartment. Chromatographic separation was achieved using a Kinetex Biphenyl column (100 x 4.6 mm, 2.6 µm) heated to 40°C. A 3 µL injection was performed. The gradient elution conditions are summarized in Table 1.

Table 1. LC gradient.

Time (mins)	Mobile phase A (%)	Mobile phase B (%)
0.0	*50*	*50*
3.0	*25*	*75*
4.0	*25*	*75*
5.0	*5*	*95*
6.0	*5*	*95*
7.0	*50*	*50*
12.0	*50*	*50*

Mobile phase A: 2 mM ammonium acetate + 0.1% formic acid
Mobile phase B: 2 mM ammonium acetate + 0.1% formic acid in methanol

MS/MS detection: Data were acquired in positive APCI mode using a SCIEX Triple Quad 3500 System and Analyst® Software 1.6.2 using the Scheduled MRM™ Algorithm. The MS and MRM parameters are provided in Tables 2 and 3. All data were smoothed prior to quantification using MultiQuant™ Software 3.0.2 and the MQ4 integration algorithm. Signal-to-noise (S/N) data were calculated using MultiQuant Software 3.0.2. All calibration curves were fit with linear regression and 1/x concentration weighting from 1 to 1,000 ppb.

Table 2. MS parameters.

Parameter	Value
Polarity and ionization mode	*Positive APCI*
Nebulizer current	*5*
Temperature (TEM)	*500 ºC*
Nebulizer gas (GS1)	*30 psi*
Heater gas (GS2)	*50 psi*
Collision gas (CAD)	*7*
Declustering potential (DP)	*70*
Entrance potential	*10*
Curtain gas (CUR)	*20 psi*

Results and discussion

Linearity and instrument sensitivity were evaluated for all compounds using external calibration from triplicate injections of solvent standards (1 to 1,000 ppb; Figure 2). The 1 ppb results demonstrate excellent precision, with all values < 10%. Linearity was demonstrated across the entire calibration range with all correlation coefficients > 0.995. The S/N data for the 1 ppb calibrators suggested that linalool (S/N 220) and humulene (S/N 70) can be analyzed with LOQs < 1 ppb (Table 4).

Table 3. MRM transitions.

Compound	RT (min)	Q1	Q3	CE	CXP
Limonene	*3.67*	*137.0*	*95.0*	*17*	*8*
		137.0	*81.0*	*16*	*10*
Terpinolene	*3.61*	*137.0*	*95.0*	*17*	*8*
		137.0	*81.0*	*16*	*10*
α-Pinene	*4.18*	*137.0*	*95.0*	*17*	*8*
		137.0	*81.0*	*16*	*10*
Myrcene	*3.45*	*137.0*	*95.0*	*17*	*8*
		137.0	*81.0*	*16*	*10*
Linalool	*1.21*	*137.0*	*95.0*	*17*	*8*
		137.0	*81.0*	*16*	*10*
Humulene	*5.69*	*205.0*	*149.0*	*17*	*8*
		205.0	*93.0*	*27*	*10*
β-Caryophylene	*5.91*	*205.0*	*149.0*	*17*	*8*
		205.0	*93.0*	*27*	*10*

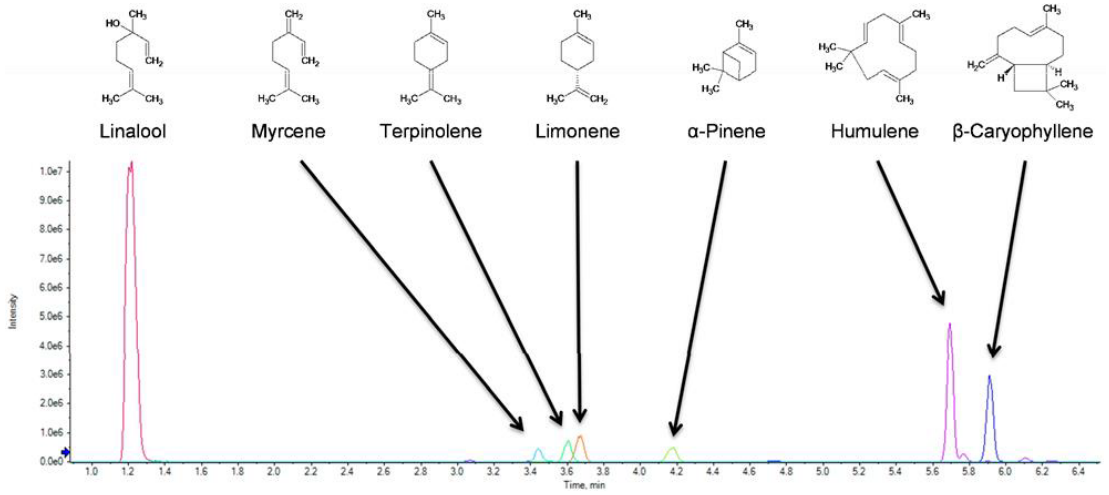

Figure 2. LC separation. Structures of some terpenes relevant to the production of cannabis products shown with a sample chromatogram from this method. Resolution of isobars was critical to the success of this application.

The chromatographic method was rapid and provided baseline resolution of all isobars except limonene and terpinolene (Figure 1). Despite this co-elution, acceptable method performance was observed for both compounds.

A series of 10 cannabis extracts were quantified using external calibration (Table 4). The extracts represented a cross-section of cannabis products, including flowers, edibles, extracts, a raffinate, a concentrate and a topical formulation. The relative levels of the terpenes detected were consistent with previous results obtained using GC with flame ionization detection (GC-FID) and gas chromatography-tandem mass spectrometry (GC-MS/MS). It is unclear why the edibles did not contain detectable terpenes, but it could be related to the extraction chemistry and/or the cooking process used to produce the edibles.

Chromatographically, the cannabis matrices presented isobaric interferences, but none that significantly affected accurate peak integration (Figure 3). To assess ion suppression, extracts were fortified to 100 µg/L (Table 5). Although several recoveries in sample 3 (extract) were > 120%, the majority of spike recoveries ranged from 80% to 120%. Given that internal standard correction was not used, these results demonstrate the quantitative potential of this method for the routine characterization of key aroma components in cannabis products.

Table 4. Sensitivity, repeatability and linearity. Sensitivity was computed with the S/N at 1 ppb (calculated using 3x standard deviation in PeakView® Software). Repeatability was also computed at 1 ppb. Linearity of the concentration curve from 1 to 1,000 ppb (linear fit with 1/x weighting) was also computed.

Compound	S/N at 1 ppb	%CV at 1 ppb	r
Limonene	*20*	*6.3*	*0.997*
Terpinolene	*34*	*2.9*	*0.996*
α-Pinene	*34*	*4.4*	*0.996*
Myrcene	*31*	*3.9*	*0.999*
Linalool	*220*	*4.2*	*0.999*
Humulene	*70*	*5.5*	*0.999*
β-Caryophylene	*37*	*8.2*	*0.999*

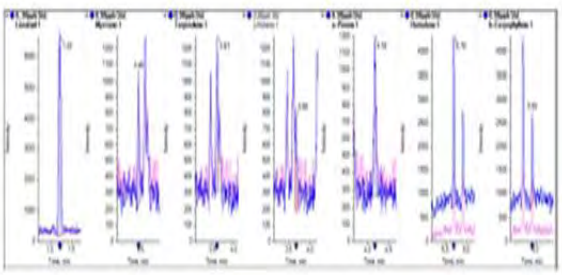

Figure 3. Instrument performance. Relative standard deviation (n = 3). All compounds were analysed from 1 to 1,000 ppb and were fit with linear regression and 1/x concentration weighting.

Table 5. Quantification in various cannabis products. Spike recoveries (with external calibration) are shown in parentheses. All concentrations reported as ppb. The sample matrix is shown below each sample number.

Compound	1 Flower	2 Extract	3 Extract	4 Flower	5 Edible	6 Raffinate	7 Topical	8 Edible	9 Concentrate	10 Flower
Limonene	13.1 (81%)	15.7 (91%)	14.2 (103%)	*16.1 (101%)*	- (96%)	*1.4 (100%)*	*3.5 (97%)*	- (94%)	*39.6 (87%)*	*15.2 (104%)*
Terpinolene	- (94%)	- (97%)	*6.3 (104%)*	- (90%)	- (94%)	- (96%)	- (96%)	- (83%)	- (84%)	- (90%)
α-Pinene	1.8 (89%)	2.1 (88%)	70.9 (137%)	*51.3 (87%)*	- (95%)	*2.5 (92%)*	- (100%)	- (87%)	*11.3 (97%)*	*21.1 (89%)*
Myrcene	2.7 (101%)	*90.2 (88%)*	*60.9 (140%)*	*2.4 (99%)*	- (93%)	- (97%)	- (98%)	- (90%)	*2.8 (104%)*	*117 (81%)*
Linalool	*6.5 (105%)*	*7.4 (110%)*	*9.8 (110%)*	*16.5 (101%)*	- (96%)	*1.4 (100%)*	*3.5 (97%)*	- (94%)	*39.6 (87%)*	*15.2 (104%)*
Humulene	*1.8 (98%)*	*3.5 (99%)*	*14 (114%)*	*2.7 (92%)*	- (99%)	- (94%)	- (97%)	- (95%)	*40.6 (93%)*	*3.9 (100%)*
β-Caryophylene	*12.7 (98%)*	*10.7 (102%)*	*21.8 (121%)*	*7.3 (97%)*	- (98%)	*3.2 (94%)*	*1.8 (98%)*	- (95%)	*84.3 (83%)*	*9.4 (91%)*

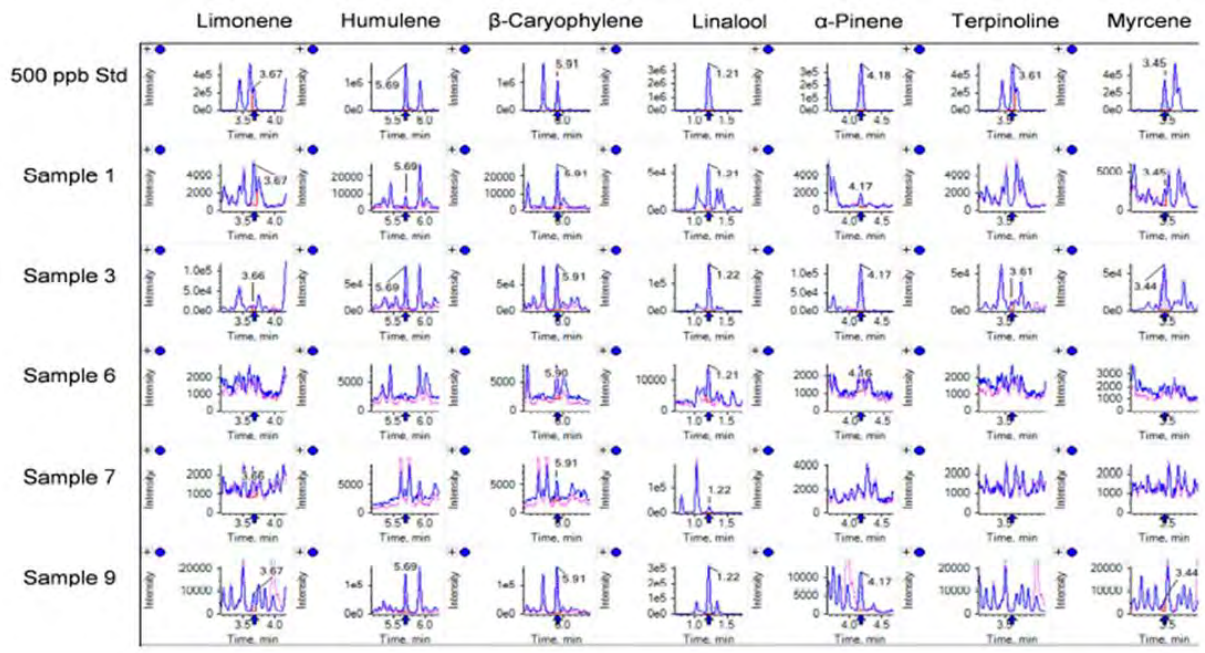

Figure 4. Sample chromatograms from a 500 ppb solvent standard and the unfortified cannabis matrices evaluated.

Summary

These results demonstrate the utility of the SCIEX Triple Quad 3500 System for the analysis of terpenes in cannabis products. Instrument performance was excellent, with precision within < ± 8% (n = 3) and S/N > 10 at 1 ppb for all target compounds. Spike recoveries of 80% to 120% showed the quantitative accuracy of the method in a variety of cannabis matrices.

Acknowledgments

The authors wish to thank Scott Churchill of MCR Labs in Framingham, MA, for providing the samples.

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Rapid and quantitative analysis of 14 sulfonylurea herbicides residues in water by direct injection

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Sulfonylurea herbicides are broad-spectrum, high-efficiency, high-selectivity, and low-toxicity herbicide developed in the late 20th century. They are currently one of the most widely used classes of herbicides in the world. They are widely used to control rice, wheat, soybean, corn, and rape field weeds as well as lawn and other non-cultivated weeds. This kind of herbicide is not easy to volatilize and does not photodegrade, so it remains in crops for a long time. With the washing of rain, there are also some residues in soil, sediment and water. At present, the detection of sulfonylurea herbicides in soil, surface water, and drinking water mostly requires sample concentration before analysis. The main methods of concentration are liquid-liquid extraction (LLE) and solid-phase extract (SPE). The pre-treatment is time-consuming and consumes a lot of organic solvent, in addition to relying on operator precision for sample handling, which can lead to unreliable precision and accuracy.

In this experiment, the SCIEX Triple Quad™ 3500 LC-MS/MS System was used to establish a rapid screening and quantitative method for 14 sulfonylurea herbicides. In comparison with the existing methods[1], this method covers all the commonly used domestic compounds and the direct injection approach has the advantages of simplicity, speed, high sensitivity, and improved accuracy and reproducibility. The detection of urea herbicides by LC-MS/MS provides a simple and fast solution.

Key advantages

- Fast and high throughput: This method only takes 6 minutes for one injection, and the positive and negative ions are detected in the same run. The qualitative and quantitative analysis of 14 sulfonylurea herbicides can be quickly completed, and isomers are separated well.
- No need for pre-treatment: Direct injection of water samples without complicated and tedious pre-treatments, such as solid phase extraction, drying and reconstitution, greatly reduces sample preparation time and is simple and efficient.
- High sensitivity: The quantitative limits in water fully meet various regulations[2,3] and reflect the excellent stability and sensitivity of the instrument when switching between positive and negative modes.

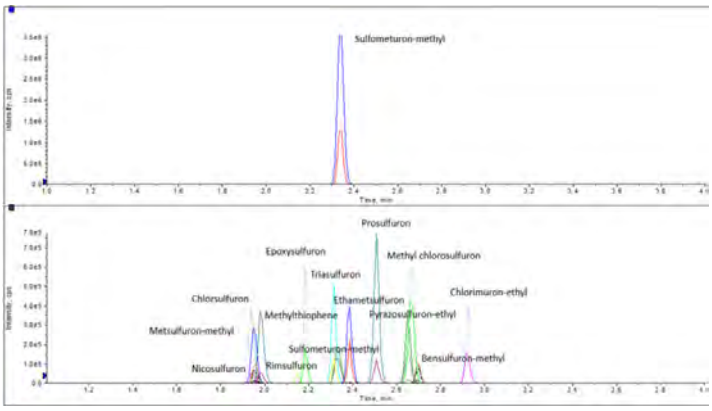

Figure 1. Typical chromatograms of 14 sulfonylurea compounds.

Methods

Sample preparation: The water sample is directly injected for analysis.

Chromatography: SCIEX Exion LC™ AD System was used with Phenomenex Kinetex C18 (2.1x100 mm, 1.7 µm) analytical column. A gradient elution with flow rate of 0.4 mL/min, injection volume of 50 µL, and a column temperature of 40℃ were used.

Mass spectrometry: SCIEX Triple Quad 3500 System was operated using the ESI source, and employing the positive/negative ion switching mode.

Ion source parameters:

IS voltage: -4500 V
Curtain gas: 30 psi
Atomizing gas GS1: 35 psi Auxiliary gas GS2: 40 psi
Source temperature (TEM): 400 ℃
Collision gas CAD: 7

Results

Linear range: The 14 kinds of sulfonylureas have good linearity in their respective linear ranges (r>0.995), and the linear range is wide, ensuring accurate quantification of samples at different concentration levels (Table 1).

Recovery: Both China and the European Union stipulate that the limits of sulfonylurea herbicide monomers in drinking water, surface water, and industrial wastewater are 0.1, 1, and 10 µg/L, respectively. Therefore, 0.1, 1, and 10 µg/L are added to the water samples. A mixture of standard sulfonylurea samples was directly injected and detected by LC-MS/MS. The recovery was 80.3 - 116.3.0% (Figure 2), the reproducibility (n=6), and the RSD was 1.34 - 2.94% (Figure 3).

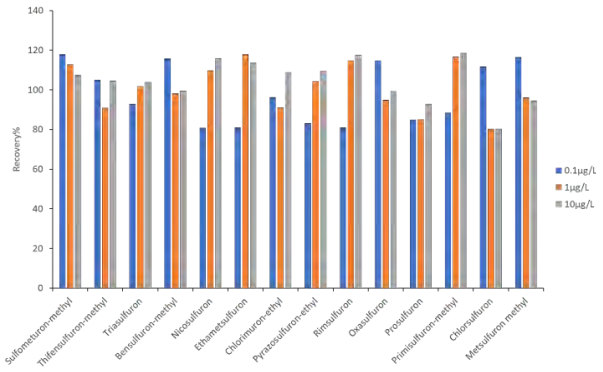

Figure 2. Standard addition recovery rates of 14 sulfonylureas in water samples.

Table 1. Linear range and linearity of response for 14 sulfonylurea herbicides in water samples.

	Linear concentration range	r^2
Thifensulfuron-Methyl	*0.05-200*	*0.9980*
Triasulfuron	*0.05-200*	*0.9963*
Bensulfuron-Methyl	*0.05-100*	*0.9973*
Nicosulfuron	*0.05-200*	*0.9953*
Ethametsulfuron-Methyl	*0.02-50*	*0.9993*
Chlorimuron-ethyl	*0.02-200*	*0.9962*
Pyrazosulfuron-ethyl	*0.02-100*	*0.9975*
Rimsulfuron	*0.02-200*	*0.9977*
Sulfometuron-Methyl	*0.02-10*	*0.9972*
Oxasulfuron	*0.02-200*	*0.9978*
Prosulfuron	*0.02-200*	*0.9979*
Primisulfuron-Methyl	*0.02-100*	*0.9991*
Chlorsulfuron	*0.02-200*	*0.9997*
Metsulfuron-Methyl	*0.02-50*	*0.9981*

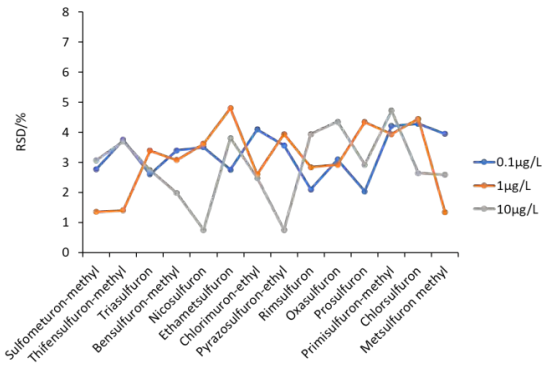

Figure 3. Reproducibility of spiked analysis of 14 sulfonylureas in water samples.

Conclusions

In this paper, a set of LC-MS/MS detection methods for the determination of 14 sulfonylurea herbicides in water samples was established on the SCIEX Triple Quad 3500 System.

The method removes the complicated pre-treatment process by directly injecting the sample, effectively reduces the loss of the target object, and greatly saves time and economic costs.

The analysis time of this method is only 6 minutes, and the detected compounds cover all the sulfonylurea herbicides commonly used in China. The method has high sensitivity, and the limit of quantification in water can reach ng/L level; it has good reproducibility and can meet the detection limit requirements of sulfonylurea herbicides in industrial wastewater, surface water and drinking water in China and the European Union.

References

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LC-MS/MS solution for national quality standards for drinking water, surface water and groundwater

Meets the national standard and limit requirements without the enrichment and with large volume injections

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The standards for surface water environmental quality (GB3838-2002), the national standards for drinking water (GB5749-2006) and groundwater quality standards (GB14848-2017) issued by China currently stipulate the limit values of several compounds determined by the LS-MS/MS method.

Here, the SCIEX Triple Quad™ 3500 LC-MS/MS System was used to develop a workflow for quantifying 22 compounds in water (including 2 additional microcystin compounds), which provides a simple and rapid solution to address these regulations in such aqueous environments.

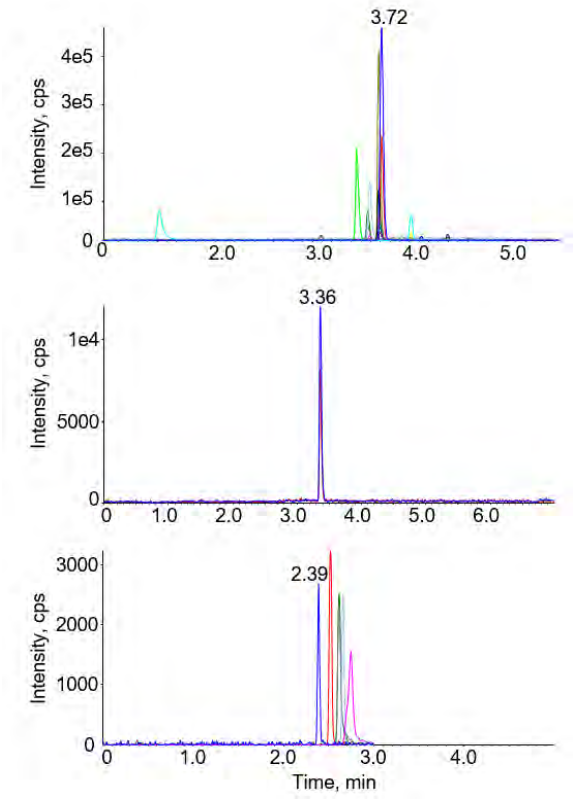

Figure 1. Chromatograms of 22 compounds in water. ESI + (upper) ESI - (middle) and APCI - (lower).

Key features of the LC-MS/MS method

- This method covers the 20 compounds in the Chinese national standards GB 3838-2002, GB 5749-2006, and GB 14848-2017 and two additional microcystin compounds of concern
- The determination limits of the 22 compounds fully meet the sensitivity requirements of the above standards
- Direct injection of water samples without enrichment or extraction
- A fast and simple method which improves laboratory efficiency

Methods

Sample preparation: The water sample was analyzed by direct injection, with no sample treatment.

Chromatography: Separation was achieved using the SCIEX ExionLC™ System with a Phenomenex Kinetex Biphenyl (50 × 3.0 mm, 2.6 µm). The flow rate was 0.4 mL/min with an injection volume of 100 µL. Gradient information is provided in Table 1.

Table 1. Chromatography.

Time (mins)	% A	% B
0	95	5
0.5	95	5
2.0	5	95
5.0	5	95
5.1	95	5
7.0	95	5

Mobile phase A: water (5 mM ammonium formate)
Mobile phase B: methanol

Mass spectrometry: Samples were analyzed using the SCIEX Triple Quad 3500 LC-MS/MS System. The method was divided into ESI positive, ESI negative and APCI negative ion modes of detection. Method parameters are described in Tables 2 - 4.

Data processing: The data was processed using SCIEX OS Software (version 1.4).

Table 2. MS parameters.

Parameter	ESI +	ESI -	APCI -
Collision gas CAD	Medium	Medium	Medium
Nebulizing gas GS1	60	60	60
Auxiliary gas GS2	70	70	- -
Corona needle current NC	- -	- -	3 µA
Air current gas CUR	25	25	25
Source temperature TEM (°C)	600	600	250
IS voltage	5500	-4500	- -

Table 3. MS parameters for 16 compounds in positive ion ESI.

Detection mode	Compound	Q1	Q3	DP	CE
ESI +	Acrylamide	72	55.1	30	15
		72	43.9	30	22
	Carbofuran	222.1	165	70	17
		222.1	123.1	70	29
	Aldicarb	116.1	89	47	10
		116.1	70	47	10
	Dichlorvos	221	109	70	23
		221	127	70	25
	Parathion-methyl	264	124.9	40	26
		264	231.9	40	24
	Malathion	331	127	64	17
		331	285	64	13
	Dimethoate	230	125	56	29
		230	199	56	13
	Chlorpyrifos	350	198	82	29
		350	97	82	49
	Atrazine	216.1	174	71	23
		216.1	104	71	39
	Parathion	292	236	80	20
		292	264	80	15
	Bentazone	241	199	20	15
		241	107	20	35
	Carbaryl	202.1	145	54	15
		202.1	127	54	40
	Deltamethrin	523.1	281	40	21
		523.1	506	40	13
	Microcystin-LR	498.4	135.1	40	16
		498.4	103.1	40	75
	Microcystin-RR	519.9	135.1	70	45
		519.9	103.1	70	90
	Microcystin-YR	523.4	135.1	65	20
		523.4	103.1	65	75

Table 4. MS parameters of 6 compounds in negative ion ESI and APCI.

Detection mode	Compound	Q1	Q3	DP	CE
ESI -	2,4-D	219	161	-40	-19
		221	163	-40	-19
APCI -	Chlorothalonil	244.8	181.8	-60	-39
		244.8	244.8	-60	-10
	2-Chlorophenol	126.9	35	-39	-40
	2,4-Dichlorophenol	160.9	35	-70	-40
	2,4,6-Trichlorophenol	194.9	35	-100	-50
		194.9	159	-100	-30
	Pentachlorophenol	264.7	35	-70	-61
		264.7	36.9	-70	-56

Results

The chromatographic separation achieved on the Kinetex Biphenyl is shown in Figure 1. Peaks are well defined and resolution is sufficient for accurate and reliable quantification.

Calibration curves were generated for all 22 compounds in the method. Results are summarized in Table 5 and compared to the requirements for the Chinese national standards. The lower limits of quantification achieved with this method meet or surpass the current requirements.

Very good linearity was achieved for each compound in water with r > 0.99, as shown in Figure 2. These calibration curves can be applied to the detection of analytes in actual samples.

Table 5. Lower limit of determination of this method and GB 3838-2002, GB 5749-2006, and GB 14848-2017.

Compound	GB3838-2002	GB5749-2006	GB14848-2017	This method
2,4-D		30	0.1	0.025
Carbofuran		7	0.05	0.005
Aldicarb			0.05	0.005
Dichlorvos	50	1	0.05	0.025
Parathion-methyl	2	20	0.05	0.05
Malathion	50	250	0.05	0.01
Dimethoate	80	80	0.05	0.005
Chlorpyrifos		30	0.05	0.01
Chlorothalonil	10	10	0.05	0.05
Atrazine	3	2	0.05	0.005
2-Chlorophenol				0.5
2,4-Dichlorophenol	93			0.5
2,4,6-Trichlorophenol	200	200		0.1
Pentachlorophenol	9	9		0.02
Parathion	3	3		0.1
Bentazone		300		0.025
Carbaryl	50			0.025
Acrylamide	0.5	0.5		0.025
Deltamethrin		20		1.0
Microcystin-LR	1	1		0.05
Microcystin-RR				0.05
Microcystin-YR				0.05

Conclusions

A workflow comprising LC-MS/MS methods on the SCIEX Triple Quad 3500 System was developed which can completely cover the current Chinese national standards for surface water environmental quality standards (GB3838-2002), national drinking water standards (GB5749-2006) and groundwater quality standards (GB14848-2017). The lower limit of the method was better than the current standard, providing an accurate quantitative LC-MS/MS detection method.

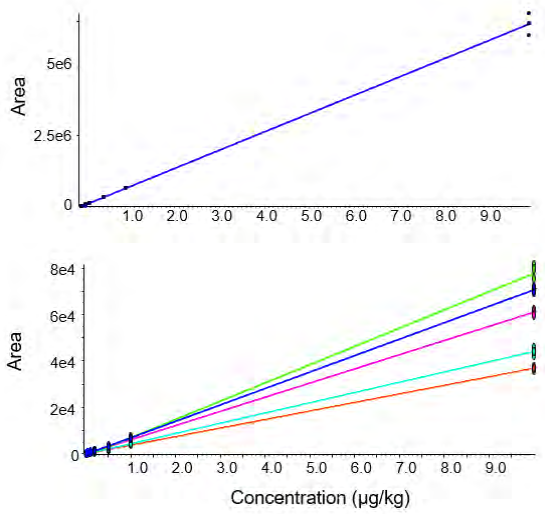

Figure 2. Typical curves of selected compounds in water. (Top) Calibration curve for compound analyzed in ESI – mode. (Bottom) Calibration curve for compound analyzed in APCI – mode.

Rapid and sensitive detection of 8 organic explosives in soil

Using the SCIEX Triple Quad™ 3500 LC-MS/MS System with an ExionLC™ AD System

Zhang Chong[1], Liu Bingjie[1], Li Lijun[1], Guo Lihai[1]
[1]*SCIEX, Shanghai*

The widespread use of explosive materials by the military, blasting, mining and other industries has fueled the continuous development of explosive production technology. The fast and simple process of manufacturing explosive devices has also enabled terrorists to clandestinally construct and use explosives to pursue their criminal endeavors. As a result, the presence of explosives in geographical warzones or in crime scenes has important political and legal implications.

Although their detection provides valuable legal evidence of their use as weapons, the daily occurrence of explosive materials is a rising concern for health and legal authorities alike. In addition to their destructive nature, the majority of organic explosive materials are carcinogenic to the human body. Inevitably, their production and use have serious impact on environmental pollution and contamination and can pose serious health issues to the population exposed to it. As a result, rapid and accurate screening techniques are critically needed to detect trace levels of explosives with a high level of sensitivity and selectivity.

Mass spectrometry is a sensitive analytical technique with the throughput and selectivity required for rapid and confident security screening. Because explosive compounds are intrinsically rich in electron-withdrawing nitrogen-containing groups and thermally instable in nature, they are suitable for analysis by LC-MS/MS technology.

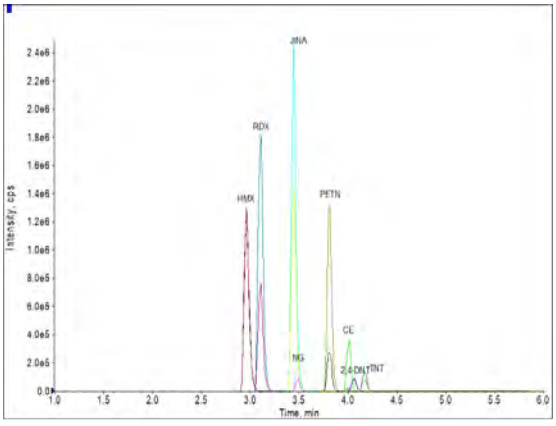

Figure 1: Chromatographic profile of the 8 organic explosives. Extracted Ion Chromatogram (XIC) shows rapid LC separation and identification of the 8 organic explosives used in this study.

In this technical note, a comprehensive workflow combining the use of the SCIEX Triple Quad 3500 System with a simple and fast sample preparation procedure for the sensitive detection of 8 organic explosives is described. The method is shown to provide a simple solution for high-throughput detection of trace explosives in soil samples.

Key features of the SCIEX Triple Quad 3500 LC-MS/MS System for high-throughput detection of organic explosives in soil

- Sample pre-treatment method is fast and simple, which greatly reduces sample preparation time while negating the need for time-consuming steps such as solid-phase extraction (SPE), evaporation and reconstitution
- Fast, 8 minute LC run provides high throughput and baseline separation of the 8 organic explosives used in this study
- MRM acquisition method on the SCIEX Triple Quad 3500 System provides accurate and sensitive quantitation of the organic explosives in soil samples
- Excellent specificity and sensitivity, with LODs in the pg/g levels for the organic explosives in soil samples
- Excellent linearity from 0.05 to 1000 ng/g is observed for all 8 organic explosives accurately identified in the soil samples
- Excellent negative ion mode sensitivity for explosive detection

Methods

Sample preparation: 10 g of surface soil samples were dissolved in 10 mL of methanol:acetonitrile (50:50, v/v), spiked at various concentration levels with a standard mixture containing the 8 organic explosives, vigorously vortexed for 30 seconds, sonicated for 5 minutes and centrifuged at 10,000 rpm for 10 minutes. 2 µL of the supernatant was injected for analysis. Table 1 lists the 8 organic explosives used in this study. The sample preparation procedure is summarized in Figure 2.

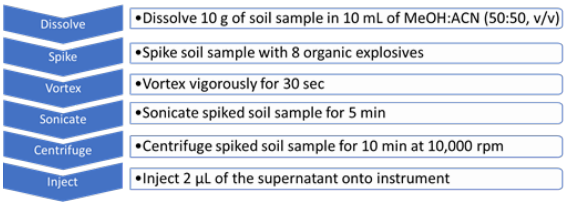

Figure 2. Sample preparation procedure for the 8 organic explosives in soil samples. A 6-step sample pre-treatment method was used for preparing the soil samples for analysis with the SCIEX Triple Quad 3500 System.

Liquid chromatography: LC separation was performed using a Phenomenex Kinetex biphenyl column (50 × 3 mm, 2.6µm, 00A-4723-AN) held at 40 °C on a SCIEX ExionLC™ AD System. Mobile phases used were ammonium formate, ammonium chloride, and methanol. The flow rate was 0.4 mL/min. The injection volume was 2 µL and the total LC runtime was 8 min.

Mass spectrometry: A SCIEX Triple Quad 3500 LC-MS/MS System with Turbo V™ Source and Electrospray Ionization (ESI) was used. The 8 organic explosives were detected in negative ion mode using two MRM tansitions per compound to allow quantification and confirmation of the explosive compounds.

Data analysis: Data was acquired in Analyst® Software 1.7 and processed in MultiQuant™ Software 3.0.3.

Table 1. List of the 8 explosive compounds analyzed in this study using the SCIEX Triple Quad 3500 System.

Compound	Abbreviation
2,4,6-trinitrobenzidine	*CE*
Octahydro-1,3,5,7-tetranitro-1,3,5,7-triazine	*HMX*
Pentaerythritol tetranitrate	*PETN*
Hexahydro-1,3,5-trinitro-1,3,5-triazine	*RDX*
2,4-Dinitrotoluene	*2,4-DNT*
Diethanolnitramine dinitrate	*DINA*
2,4,6-trinitrotoluene	*TNT*
Nitroglycerine	*NG*

Developing a robust workflow for the detection of 8 explosives in soil samples

10 g of surface soil samples were dissolved in 10 mL of methanol:acetonitrile (50:50, v/v) and spiked with the 8 organic explosives at various concentrations. The resulting samples were vortexed vigorously, sonicated for 5 minutes, centrifuged at 10,000 rpm for 10 minutes and 2 µL of the supernantant was injected to the instrument to build a data processing method.

Figure 1 shows the extracted ion chromatogram (XIC) for the 8 organic explosives. The combination of an appropriate mobile phase composition, column choice and optimized LC conditions resulted in baseline separation of all 8 organic explosives.

Accurate and sensitive quantification of organic explosives

The quantitative performance of the assay was investigated by injecting a series of soil samples spiked with the explosive compounds at various concentration levels ranging from 0.05 to 1000 ng/g. Calibration curves were generated to evaluate the sensitivity and linearity of the assay as well as the linear dynamic range of the instrument for each of the 8 explosive compounds.

Figure 3 shows the calibration curves for both the quantifier and qualifier ions of the 8 explosive compounds for concentrations ranging from 0.05 to 1000 ng/g. The calibrarion curves show excellent linearity, with R^2 values >0.99 for all the explosive compounds. Sensitivity down to sub ng/g was achieved and the linear dynamic range exceeded 4 orders of magnitude for the majority of the explosive compounds without internal standard correction. This demonstrates that the SCIEX Triple Quad 3500 System provides sensitive and accurate quantification of explosive compounds across a wide range of concentration levels. Table 2 summarizes the quantitative results for the assay

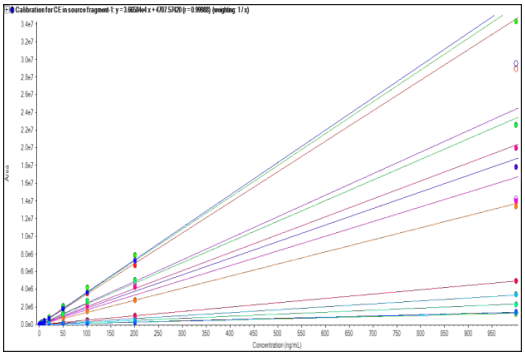

Figure 3. High sensitivity, linearity, and dynamic range for the 8 explosive compounds. Calibration curves resulting from the calibration series for both the quantifier and qualifier ions of the 8 explosive compounds from 0.05 to 1000 ng/g.

Table 2. Summary of the quantitative performance for the detection of the 8 organic explosive compounds analyzed in this study.

Compound name	Chemical formula	Precursor mass (Da)	Molecular species	Concentration range (ng/g)	R^2 Value
CE	$C_7H_5N_5O_8$	*288.0*	*In-source cleavage*	*0.05-200*	*0.9998*
HMX	$C_4H_8N_8O_8$	*331.0*	$[M+Cl]^{-1}$	*0.05-200*	*0.9987*
PETN	$C_5H_8N_4O_{12}$	*351.0*	$[M+Cl]^{-1}$	*0.5-1000*	*0.9997*
RDX	$C_3H_6N_6O_6$	*267.0*	$[M+FA]^{-1}$	*0.05-200*	*0.9995*
2,4-DNT	$C_7H_6N_2O_4$	*181.1*	$[M-H]^{-1}$	*1-1000*	*0.9999*
DINA	$C_4H_8N_4O_8$	*285.1*	$[M-H]^{-1}$	*0.2-1000*	*0.9993*
TNT	$C_7H_5N_3O_6$	*226.0*	$[M-H]^{-1}$	*1-1000*	*0.9997*
NG	$C_3H_5N_3O_9$	*262.1*	$[M-H]^{-1}$	*1-1000*	*0.9981*

and includes the concentration range and the R^2 values for the 8 explosive compounds analyzed in this study.

Fast and simple sample pre-treatment leads to high recovery of explosive compounds in soil samples

One of the challenges associated with the detection of analytes extracted from soil samples is the presence of many matrix components that can affect the detection performance of the assay. As a result, a reliable sample preparation procedure is critical to achieve the desired reproducibility and analytical performance of the assay.

To assess the efficiency of the sample preparation procedure used in this experiment, the recovery was calculated by spiking a soil sample with the standard mixture at two concentration levels (5 and 10 ng/mL) before and after the sample pre-treatment procedures. Three replicate injections were performed for each of the two concentrations. The recovery values (depicted as "RE") were calculated for each of the 8 organic explosive compounds at both concentration levels by dividing the average peak area of the pre-spiked sample (depicted as "A") by the average average peak area of the post-spiked sample (depicted as "B") as shown in the following equation:

$$RE\ (\%) = A/B \times 100 \qquad (1)$$

The sample preparation procedure used in this experiment demonstrated recovery values ranging between 51.7 and 106%, as shown in Figure 4. In addition, the assay showed excellent reproducibility across the three injections for the two concentration levels with RSD values between 1.31 and 4.59% (Figure 5) for all the explosive compounds used in this study. The result shown in Figure 4 and 5 illustrate the overall robustness and reproducibility of the assay.

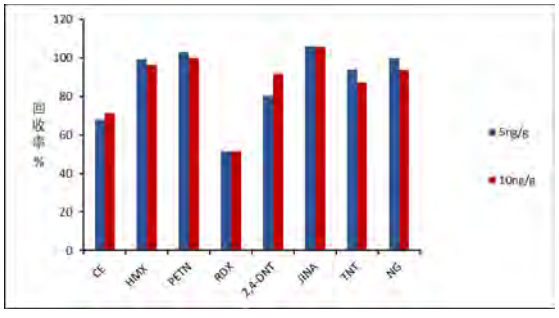

Figure 4. Reproducible and consistent recovery values observed for all 8 organic explosive compounds across the two concentration levels. Diagram showing the recovery (RE) values calculated for the 8 organic explosive compounds at 5 ng/g and 10 ng/g across three replicate injections.

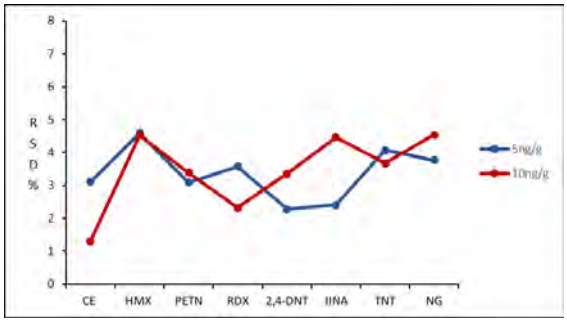

Figure 5. High reproducibility of injection for all 8 organic explosive compounds across the two concentration levels. Plot showing the RSD values values calculated for the 8 organic explosive compounds at 5 ng/g and 10 ng/g across three replicate injections.

Conclusions

A complete workflow for the detection of 8 organic explosives in soil samples was successfully developed using the SCIEX Triple Quad 3500 System. The combination of a simple sample preparation procedure and a robust LC-MS method enabled accurate detection of the organic explosives in soil samples.

- A simple sample preparation procedure enabled fast and reproducible extraction of 8 organic explosives from soil samples without the need for long and tedious sample extraction procedures commonly needed for this type of matrix
- Quantification of the 8 organic explosives in a single method
- Explosive compound extraction recovery values were found to be between 51.7 and 106%
- Reproducibility of injection (measured as RSD) across two contration levels (5 ng/g and 10 ng/g) was found to be between 1.31 and 4.59% for all the explosive compounds
- The optimized LC-MS/MS method on the SCIEX Triple Quad 3500 LC-MS/MS System enabled sensitive detection, with LODs in the pg/g levels for the organic explosives in soil samples
- Excellent accuracy and linearity for concentrations ranging between 0.05 and 1000 ng/g, with R^2 values above 0.99 for all 8 explosive compounds used in this study
- Overall, the developed workflow enabled reproducible, sensitive and reproducible detection of 8 organic explosives from soil samples

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Rapid DUID screening of 17 drugs of abuse in plasma and saliva

Using the SCIEX Triple Quad™ 3500 LC-MS/MS System with an ExionLC™ AD System

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The offense of driving under the influence of drugs, or DUID, typically relates to driving while being intoxicated on mind-aterning substances such as prescribed or illegal drugs. This serious crime can result in serious DUI charges and carries significant financial, legal, and social penalties. These substances are known to act on the central nervous system and impair the driver's alertness, concentration and judgment as well as their overall ability to operate a vehicle safely. Over the years, drug-impaired driving has become a prevalent cause of motor vehicle fatalities worldwide. As a result, detecting the presence of these drugs in the impaired driver's system is paramount to law enforcement. They require comprehensive drug screening approaches to confirm the presence of these substances and support evidence to prosecute the DUID charge.

Traditionally, DUID screens are either performed by immunoassay or GC-MS. However, immunoassays are known to suffer from cross-reactivity, have poor sensitivity and are prone to a high rate of false positives. On the other hand, GC-MS requires lengthy sample preparation and derivatization which considerably slows down the analytical process. As a result, there is a critical need to develop rapid, robust and comprehensive drug screening methods for positive identification and accurate quantification of these substances from biological specimens.

In this technical note, a simple and fast sample preparation procedure was used in combination with the SCIEX Triple Quad 3500 LC-MS/MS System for picogram to sub-nanogram per mL detection of 17 drugs in plasma and saliva samples. This targeted screening method is shown to provide a fast and sensitive quantitative solution for high-throughput detection of these substances typically screened in DUID cases.

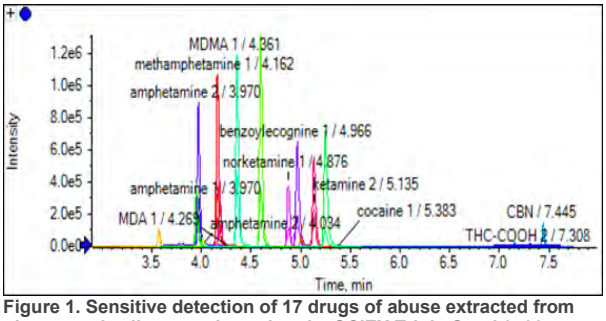

Figure 1. Sensitive detection of 17 drugs of abuse extracted from plasma and saliva samples using the SCIEX Triple Quad 3500 System. Extracted Ion Chromatogram (XIC) showing baseline separation of the 17 drugs of abuse screened in this DUID panel.

Key advantages for sensitive detection of 17 drugs of abuse in plasma and saliva samples

- Fast and simple sample preparation procedure enabled efficient extraction of drugs from plasma and saliva samples
- Rapid (10 minutes) and high-throughput MRM acquisition method provides accurate and sensitive quantitation of the 17 drugs in the panel, enabling pg to sub-ng/mL detection limits in plasma and saliva samples
- Fast polarity switching capabilities on the SCIEX Triple Quad 3500 System enabled detection of ions in positive and negative mode in a single run, negating the need for a two injection workflow traditionally performed
- Workflow demonstrated excellent linearity for concentrations ranging from 0.1 to 50 ng/mL with R values greater than 0.995
- Excellent precision and accuracy as demonstrated by RSD values less than 5% for multiple quality control samples acquired at three different concentration levels
- Analyte extraction recoveries were demonstrated to be between 70 and 120% for the majority of the drugs in the DUID panel
- Method enabled identification and quantification of drugs present in real plasma and saliva case samples, demonstrating the robustness of the developed workflow in complex biological matrices
- Method can be easily implemented by law enforcement authorities as a fast and robust screening method to test drivers suspected of DUID

Methods

Sample preparation: A total of 17 drugs commonly screened for in DUID cases were selected for this panel. The full list of the drugs included in this DUID panel is summarized in Table 1. Drugs were extracted from plasma and saliva samples by using a protein precipitation procedure. In short, 300 µL of plasma or saliva was added to a centrifuge tube to which 900 µL of acetonitrile was added and vigorously vortexed for 30 seconds. The samples were then centrifuged for 10 min at 12,000 rpm. 250 µL of the supernatant was transferred out to a glass tube and completely dried for 20 minutes at 60˚C under a gentle nitrogen flow. The residues were reconstituted with 100 µL of an acetonitrile/water (20:80, v/v) solution. The protein precipitation procedure is summarized in Figure 2.

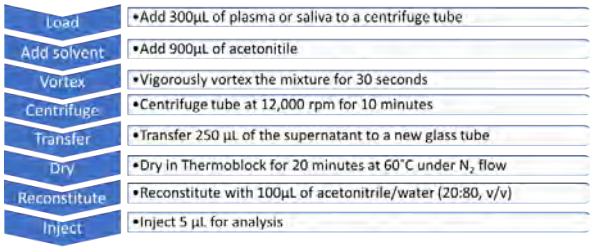

Figure 2. Protein precipitation procedure for plasma and saliva samples. An 8-step protein precipitation procedure was used for extracting the 17 drugs from plasma and saliva samples for MS analysis.

Table 1. List of the 17 drugs along with their molecular formula included in the DUID panel.

Compound Name	Molecular Formula
6-acetylmorphine	$C_{19}H_{21}NO_4$
Morphine	$C_{17}H_{19}NO_3$
Cocaine	$C_{17}H_{21}NO_4$
Benzoylecognine	$C_{17}H_{21}NO_4$
Cannabinol	$C_{21}H_{26}O_2$
11-nor-9-carboxy-THC	$C_{21}H_{28}O_4$
Methamphetamine	$C_{10}H_{15}N$
Amphetamine	$C_9H_{13}N$
MDMA	$C_{11}H_{15}NO_2$
MDA	$C_{10}H_{13}NO_2$
Ketamine	$C_{13}H_{16}ClNO$
Methcathinone	$C_{10}H_{13}NO$
Heroin	$C_{21}H_{23}NO_5$
3,4-Methylene dihydro-N-ethyl-amphetamine	$C_{12}H_{17}NO_2$
Norketamine	$C_{12}H_{14}ClNO$
Cannabidiol	$C_{21}H_{30}O_2$
Tetrahydrocannabinol	$C_{21}H_{30}O_2$

Liquid chromatography: UHPLC separation was performed on a Phenomenex Kinetex Biphenyl (100 × 3 mm, 2.6µm, 00D-4622-Y0) held at 40°C on a SCIEX ExionLC AC System. Mobile phases used consisted of ammonium formate, methanol, and appropriate additives. The flow rate was 0.4 mL/min. The injection volume was 5 µL and the total LC runtime was 10 minutes.

Mass spectrometry: MS and MS/MS data were collected using the Scheduled MRM™ Algorithm on the SCIEX Triple Quad 3500 LC-MS/MS System. The 17 drugs were detected in positive and negative electrospray ionization (ESI) modes using the polarity switching capabilities of the instrument. Two MRM transitions per compound were used to allow confirmation and quantification of the drugs. Samples were injected in triplicate.

Data analysis: Data was acquired in Analyst® Software 1.7 and processed in MultiQuant™ Software 3.0.3.

Scheduled MRM Algorithm ensures sensitive detection and accurate quantitation of drugs of abuse extracted from plasma and saliva

Control plasma and saliva samples spiked with the mixture of the 17 drugs of abuse were prepared at various concentrations ranging from 0.1 to 50 ng/mL. These standard mixtures were extracted using the aforementioned procedure and injected in triplicate to build a data processing method.

Figure 1 shows the chromatographic profile of the 17 drugs of abuse in the DUID panel in a control plasma sample at final drug concentration of 5 ng/mL. The 10-minute long gradient in combination with the column selection and the choice of mobile phase composition resulted in baseline separation of the 17 drugs, as seen in the extracted ion chromatogram (XIC) traces shown in Figure 1.

The linearity of the method was assessed for both the plasma-spiked and the saliva-spiked control samples. Calibration curves were generated for each of the drugs in the DUID panel and plotted across seven calibration levels ranging from 0.1 to 50 ng/mL. Figure 3 shows the resulting calibrations curves for the plasma-spiked (A) and the saliva-spiked (B) control samples. The regression curves showed excellent correlation with R values greater than 0.995 for all 17 drugs in the DUID panel, regardless of the matrix from which they were extracted. The results demonstrate the robustness of the extraction method across a wide variety of drug chemistries and the wide applicability of the protein precipitation procedure for both plasma and saliva samples.

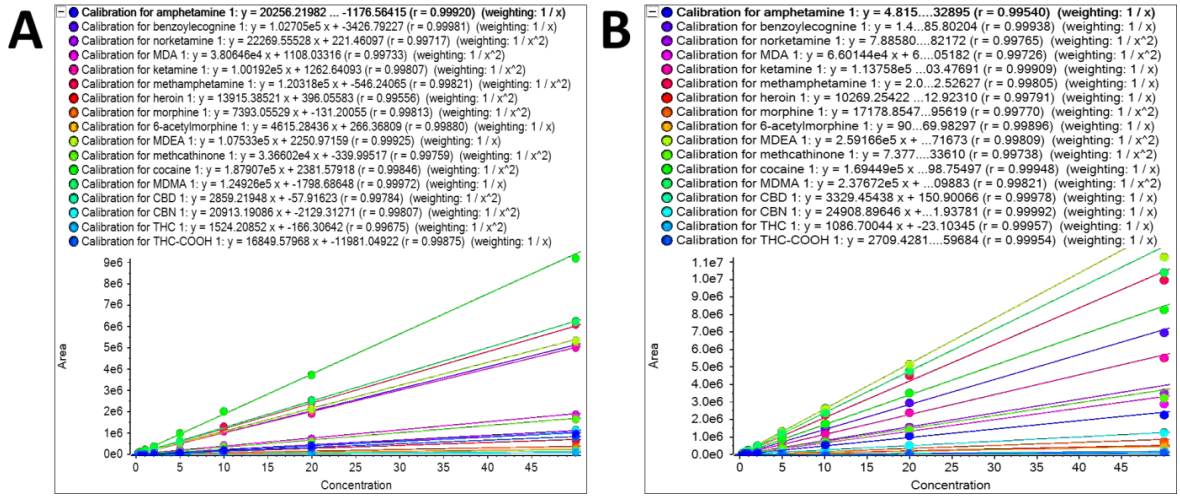

Figure 3: Excellent linearity for the 17 drugs in the DUID panel. Calibration curves resulting from the calibration series for the 17 drugs from 0.1 to 50 ng/mL extracted from (A) plasma and (B) saliva samples. R values greater than 0.995 were observed for all 17 drugs, regardless of the matrix from which they were extracted.

Achieving high sensitivity is essential to any toxicology workflow that requires accurate quantification of low levels of drugs in complex biological matrix. The sensitivity of the workflow was investigated by determining the lower limit of quantitation (LLOQ) for the 17 drugs of abuse in both plasma and saliva matrix. These values were determined based on the lowest concentration at which the integrated peak area of the analyte was quantifiable. Table 2 shows the LLOQ for each of the drugs extracted from plasma and saliva samples. The LLOQ values range from 0.0025 to 0.25 ng/mL for all the drugs in the DUID panel, demonstrating the sensitivity of the workflow on the SCIEX Triple Quad 3500 System.

Developing toxicology workflows that are both robust and reproducible is key to attaining reliable quantification of drugs in biological specimens. The reproducibility of the assay was assessed by spiking the control plasma and saliva samples at three concentration levels (1, 5 and 50 ng/mL). Three replicate injections were performed to determine the precision of measurement (expressed as percent variation coefficient, CV%). Table 3 shows the average reproducibility values at each of the three calibrator levels for each of the drugs of abuse in both plasma and saliva matrix. Overall, the assay showed great reproducibility at all three calibration levels with %CV values between 0.47 and 6.15% for the drugs in plasma and 0.6 and 5.74% for the drugs in saliva, respectively.

Table 2. LLOQ values for the 17 drugs of abuse included in the DUID panel in both plasma and saliva matrix.

Compound Name	LLOQ in plasma (ng/mL)	LLOQ in saliva (ng/mL)
6-acetylmorphine	*0.01*	*0.04*
Morphine	*0.01*	*0.05*
Cocaine	*0.0025*	*0.0025*
Benzoylecognine	*0.01*	*0.01*
Cannabinol	*0.25*	*0.25*
11-nor-9-carboxy-THC	*2*	*1*
Methamphetamine	*0.05*	*0.1*
Amphetamine	*0.1*	*0.25*
MDMA	*0.005*	*0.01*
MDA	*0.1*	*0.2*
Ketamine	*0.01*	*0.01*
Methcathinone	*0.1*	*0.2*
Heroin	*0.02*	*0.02*
3,4-Methylene dihydro-N-ethyl-amphetamine	*0.005*	*0.01*
Norketamine	*0.01*	*0.03*
Cannabidiol	*0.05*	*0.25*
Tetrahydrocannabinol	*0.025*	*0.05*

Table 3. Average results showing the precision of measurement (expressed as percent variation coefficient, % CV, n=3) for each of the 17 drugs of abuse at the three concentration levels (1, 5 and 50 ng/mL) extracted from plasma and saliva matrix.

Compound	% CV in Plasma Matrix			% CV in Saliva Matrix		
	1 ng/mL	5 ng/mL	50 ng/mL	1 ng/mL	5 ng/mL	50 ng/mL
6-acetylmorphine	5.02	2.9	0.48	1.05	2.16	0.64
Morphine	3.63	2.21	0.51	0.65	0.82	1.74
Cocaine	4.11	3	2.7	1.12	1.24	2.88
Benzoylecognine	3.17	1.57	1.4	1.87	2.98	2.26
Cannabinol	2.13	1.43	6.15	2.64	2.86	3.53
11-nor-9-carboxy-THC	3.11	1.37	1.38	1.17	0.6	2.96
Methamphetamine	1.01	2.22	1.42	3.91	3.18	1.53
Amphetamine	3.21	1.11	2.3	3.38	4.14	0.68
MDMA	3.67	1.35	1.64	2.34	1.26	0.75
MDA	1.24	1.96	1.35	1.73	5.74	2.13
Ketamine	2.89	2.77	1.42	2.65	3.16	1.92
Methcathinone	3.06	1.76	2.54	2.88	2.79	4.43
Heroin	1.45	2.42	1.71	1.84	1.96	1.74
3,4-Methylene dihydro-N-ethyl-amphetamine	4.5	0.47	1.02	1.5	2.21	1.87
Norketamine	1.86	1.55	0.87	2.79	1.55	3.12
Cannabidiol	3.12	3.23	1.66	4.35	3.41	2.38
Tetrahydrocannabinol	1.83	0.88	4.57	4.46	2.37	1.62

Fast and efficient sample preparation procedure leads to high recovery of drugs in plasma and saliva samples

Fast and reliable sample extraction procedures are critical to accurately measure the concentration of drugs from complex biological matrix. The recovery of the drugs included in this panel was calculated to assess the efficiency of the protein precipitation procedure for plasma and saliva samples. The recovery values were calculated for each of the three concentration levels (1, 10 and 50 ng/mL) by expressing ratio of the peak areas of each analyte spiked before and after the extraction procedure as a percentage. Table 4 lists the recoveries of each drug at the three concentration levels for plasma and saliva samples. The protein precipitation procedure used in this experiment demonstrated recoveries between 70.57 and 118.8% for the drugs in plasma and 55.54 and 114.97% for the drugs in saliva, respectively. The recovery values for all the drugs and drug metabolites in this panel enabled reliable and reproducible quantitation in both matrices.

Evaluating method in case samples

Developing a robust workflow is key to its full implementation to routine testing in the forensic laboratory. The robustness of the workflow was investigated by analyzing a plasma case sample from a driver suspected of operating a motor vehicle under the influence of drugs. The sample was subject to the protein precipitation procedure and screened using the developed method. Amphetamine and methamphetamine were detected in the case sample at concentrations of 21.42 and 155.73 ng/mL, respectively. Figure 4 shows the extracted ion chromatograms of each of the two drugs were detected in the driver's sample. The results from the analysis demonstrated the robustness of the DUID screening workflow and showed that the combined sample preparation procedure with Scheduled MRM Algorithm on the SCIEX Triple Quad 3500 System enabled accurate identification and sensitive detection of drugs in real case samples.

Table 4. Average (n=3) recovery values for each of the 17 drugs of abuse at the three concentration levels (1, 5 and 50 ng/mL) extracted from plasma and saliva matrix.

Compound	Recovery Values in Plasma Matrix			Recovery Values in Saliva Matrix		
	1 ng/mL	5 ng/mL	50 ng/mL	1 ng/mL	5 ng/mL	50 ng/mL
6-acetylmorphine	*91.63*	*111.30*	*114.97*	*86.94*	*99.23*	*82.31*
Morphine	*111.20*	*102.30*	*100.64*	*75.18*	*82.67*	*76.24*
Cocaine	*101.48*	*108.14*	*94.38*	*91.85*	*97.60*	*85.09*
Benzoylecognine	*100.34*	*93.70*	*102.83*	*92.65*	*97.03*	*86.97*
Cannabinol	*92.31*	*85.83*	*102.25*	*71.65*	*55.54*	*57.23*
11-nor-9-carboxy-THC	*101.62*	*89.04*	*76.65*	-	*73.70*	*85.60*
Methamphetamine	*80.90*	*70.88*	*81.26*	*93.39*	*97.67*	*85.32*
Amphetamine	*84.39*	*81.38*	*87.23*	*95.41*	*97.48*	*80.58*
MDMA	*99.02*	*87.38*	*96.05*	*98.15*	*101.27*	*82.84*
MDA	*106.69*	*84.77*	*92.78*	*101.04*	*100.69*	*83.91*
Ketamine	*98.41*	*88.26*	*95.31*	*91.60*	*93.89*	*85.78*
Methcathinone	*84.72*	*70.57*	*73.83*	*85.26*	*86.33*	*79.50*
Heroin	*107.58*	*85.58*	*93.54*	*93.32*	*94.37*	*88.46*
3,4-Methylene dihydro-N-ethyl-amphetamine	*95.99*	*91.26*	*92.93*	*107.48*	*107.76*	*87.98*
Norketamine	*94.54*	*90.54*	*95.50*	*108.60*	*99.62*	*87.41*
Cannabidiol	*98.13*	*85.54*	*97.30*	*76.80*	*71.10*	*65.07*
Tetrahydrocannabinol	*87.39*	*82.29*	*95.63*	*74.71*	*61.44*	*63.30*

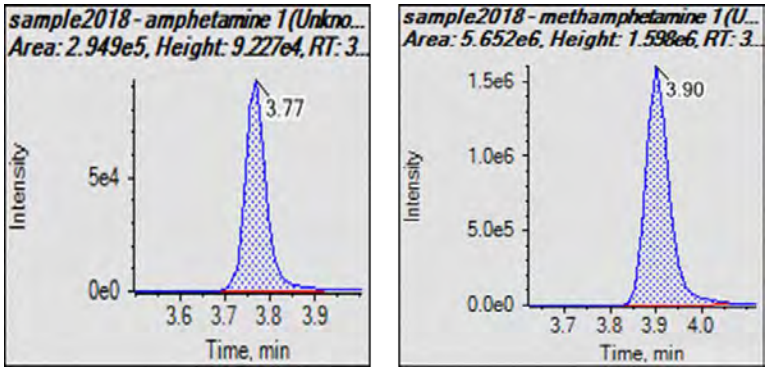

Figure 4. Positive identification of two drugs of abuse in a plasma case sample. Extracted Ion Chromatogram (XIC) traces showing positive identification of amphetamine and methamphetamine at concentrations of 21.42 and 155.73 ng/mL, respectively.

Conclusions

The combination of fast and robust protein precipitation procedure with optimized chromatography on the SCIEX Triple Quad 3500 System allowed efficient and sensitive detection of 17 drugs of abuse typically screened in DUID case. The broad applicability of the sample preparation procedure enabled extraction of these drugs from plasma and saliva matrix at pg to low ng/mL levels and with high recoveries.

- An 8-step extraction procedure using a protein precipitation efficiently extracted drugs from plasma and saliva samples
- Scheduled MRM Algorithm enabled analysis of 17 different drugs in a single 10 minute method
- Excellent linearity across seven calibration levels ranging from 0.1 to 50 ng/mL was achieved, with R values greater than 0.995 for all the drugs in the DUID panel
- Excellent reproducibility of measurement was achieved at all three calibration (1, 5 and 50 ng/mL) levels with %CV values between 0.47 and 6.15% for the drugs in plasma and 0.6 and 5.74% for the drugs in saliva, respectively
- Extraction method demonstrated recoveries between 70.57 and 118.8% for the drugs in plasma and 55.54 and 114.97% for the drugs in saliva, respectively
- Developed workflow enabled confident identification and accurate quantification of drugs in a real case plasma sample, demonstrating the applicability of the method for screening DUID sample
- This robust method provides the necessary evidence for toxicologists, law enforcement agencies and other traffic safety personel to successfully investigate and prosecute DUID cases
- Overall, the efficiency of the extraction method combined with the analytical performance of the SCIEX Triple Quad 3500 System is providing a robust method for screening DUID case samples, making it easily adaptable into a forensic toxicology laboratory

Targeted Analysis of Extractables / Leachables in Drug Product, Containers and Closure Systems

Using the SCIEX Triple Quad™ 3500 LC-MS/MS System

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The formulation and storage of drug products means that the product will come in contact with a variety of containers and packaging systems. Impurities from these packaging and transfer materials have the possibility of contaminating the drug product. Therefore it is very important to be able to measure whether any additional unwanted compounds have entered the drug product due to this contact. As per US FDA21CFR 211.94(a) "Drug product containers and closures shall not be reactive, additive, or absorptive so as to alter the safety, identity, strength, quality, or purity of the drug beyond the official or established requirements."

The US FDA monitors levels of impurities due to container, closures, and packaging material contact. These impurities are categorized as extractables and leachables. Extractables are compounds that can be extracted from the container closure system when in the presence of a solvent, or other harsh condition, while leachables are compounds that leach into the drug product formulation from the container and/or closure as a result of direct contact with the formulation over time. The objective of extractables analysis involves identification and quantitation of impurities from the container, closures and packaging material, while for leachables the drug product is analyzed for contaminants. A sensitive and selective method for determination of a set of commonly monitored extractable and leachable compounds was developed and is described here.

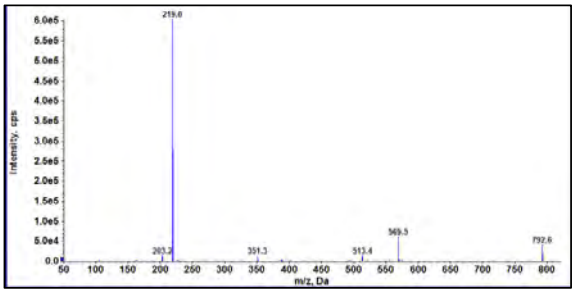

Figure 1. Product Ion Spectra of Irganox-330. Using the MS/MS spectra acquired on the 15 analyte standards, the MRM assay was developed including both quantifier and qualifier MRM transitions.

The goal was to development a sensitive analytical method using the SCIEX Triple Quad™ 3500 system with an ExionLC™ AD LC System for simultaneous detection of 15 leachable and extractables commonly found in drug product, packaging, containers and closure.

Key Feature of LC-MRM for Specific and Sensitivity Detection of E&L in Drug Product

- LC-MS/MS is an efficient platform for identification and quantitation of ppb levels of extractables and leachables
- The analytical method provides detection and quantification of the usual E&L found in the pharmaceutical containers and closures
- SCIEX TripleQuad 3500 System provides detection limits below regulatory requirements for these compounds.

Methods

Sample Preparation: The standards of Antioxidant 425, Bbot 150, Diethylphthalate, Diphenylphthalate, Irgafos 168, Irganox 1010, Irganox 330, Irganox 565, Methylparaben, Propylparaben, Tinuvin 120, Tinuvin 328, Tinuvin p, Uvinul 3030, Uvinul 408 were prepared as a cocktail in 100% acetonitrile in a concentration range of 50 – 400 PPB.

For extraction of extractables and leachable from containers, 1g of the sample (Nasal spray container, Ophthalmic solution container) was weighed. Added 10 ml of n-Hexane solution and vortex. The samples were sonicated for 3hrs. The supernatant was collected and evaporated to dryness under nitrogen stream. The dried samples were reconstituted with Diluent (100% ACN).

For extraction of extractables and leachable from drug product, 4ml of sample was taken. Added 4 ml of Dichloromethane and vortex. The supernatant was collected and evaporated to dryness under nitrogen stream. The samples were then reconstituted in diluent (100% ACN).

Chromatography: Liquid chromatography separation was performed on the SCIEX ExionLC™ AD HPLC system at 30°C. Separation was achieved using a Phenomenex Kinetex column, with mobile phase 2mM ammonium formate in water with 0.1% formic acid and acetonitrile. Column temperature was 30 ºC and the injection volume was 10 µL. More details on the chromatography can be found in the Supplementary Information.[4]

Mass Spectrometry: The SCIEX Triple Quad™ 3500 mass spectrometer was operated in positive electrospray ionization mode with unit resolution for both Q1 and Q3. The MS compound parameters (DP, EP, CE, CXP) were optimized individually for each analyte (Supplementary Information).[4]

Data Processing: The software program Analyst® Software 1.6.3 was used for instrument control, data acquisition and processing. A 1/x2 weighted linear regression was used to calculate the concentrations.

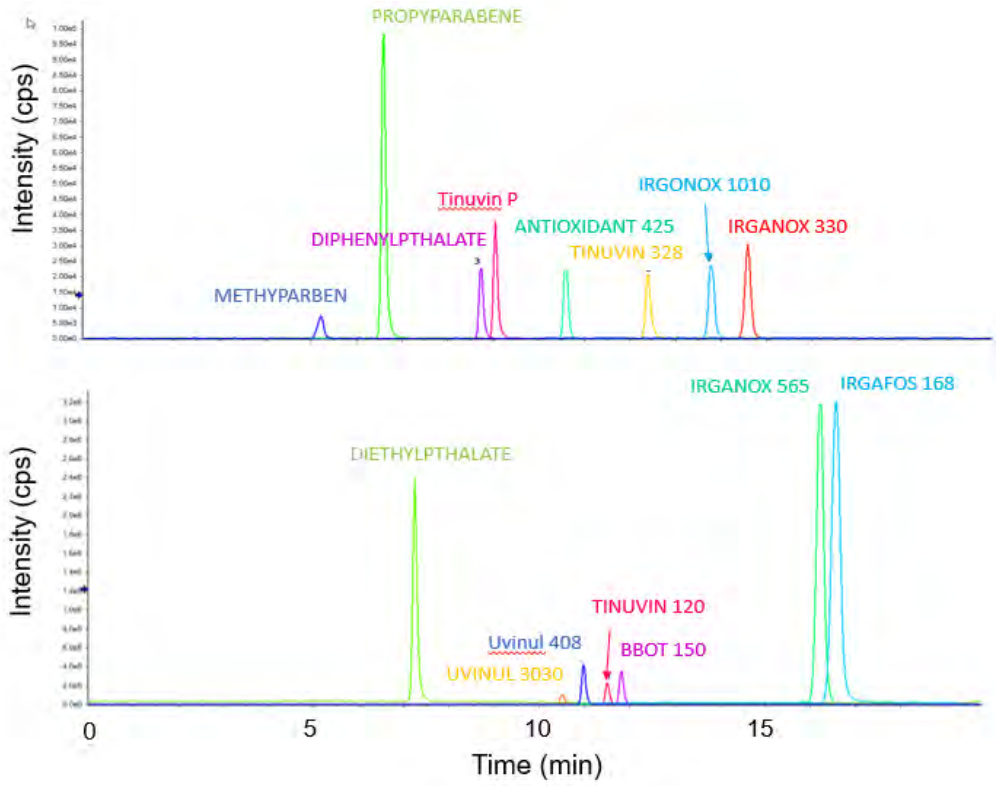

Figure 2. Chromatogram of Mixture of 15 Known Extractable and Leachable Compounds. Separation was achieved using the Kinetix column for these 15 analytes. Figure drawn in two parts to highlight the chromatographic peak shape and separation archived on all analytes.

Results

Good separation was achieved using the Kinetix column, the chromatogram (XIC) for all 15 analytes analyzed by using LC-MS/MS is shown in the Figure 2. The expected retention time when using this method is provided in the method information in the Supplementary information.[4]

The limit of quantification is different for each analyte as expected as the MS response varies per analyte. The calibration range used here was from 50 to 400 ppb due to usual reporting range for E&L, however for some analytes the lower level of quantitation (LLOQ) might be lower. Figure 3 shows the XIC for each analyte at a concentration of 50 ppb. Figure 4 shows the calibration curves of all the analytes. The method is highly selective and sensitive using analyte specific MRM transitions for detection in matrix.

Next, the method was tested using a variety of drug products, nasal spray, ophthalmic drug container and drug product of the liquid formulation of the ophthalmic drug. The results are summarized in Table 1. Most of the E&L analytes were not within the detection range of the developed method. Only six E&L compounds (Diethylpthalate, Tinuvin 120, BBOT 150, Tinuvin P, Irganox 1010, Irgafos 168) were found in detectable range in these drug products. However, the detected amount was much less than analytical evaluation threshold specific for E&L testing.

Conclusions

The developed LCMS method for analysis of 15 E&L was successfully applied for analysis in pharmaceutical drug product and containers and closures. Further developments may include addition of more analytes to the method of analysis to result into broader area of application, like food, water, and environmental monitoring.

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4. Detailed Method information – community link

Table 1. Results of Analytes in Detection Range from Different Pharmaceutical Products, Containers and Closures.

Sample	Diethylpthalate (ppb)	Tinuvin (ppb)	BBOT 150 (ppb)	Tinuvin P (ppb)	Irganox 1010 (ppb)	Irgafos 168 (ppb)
Nasal Spray Container	*62.94*	*14*	*13.23*	*15.7*	*151.44*	*958.74*
Ophthalmic drug container	*1277.09*	*10.84*	*13.55*	*30.5*	*N/A*	*248.16*
Ophthalmic drug	*N/A*	*N/A*	*5.82*	*N/A*	*N/A*	*12.25*

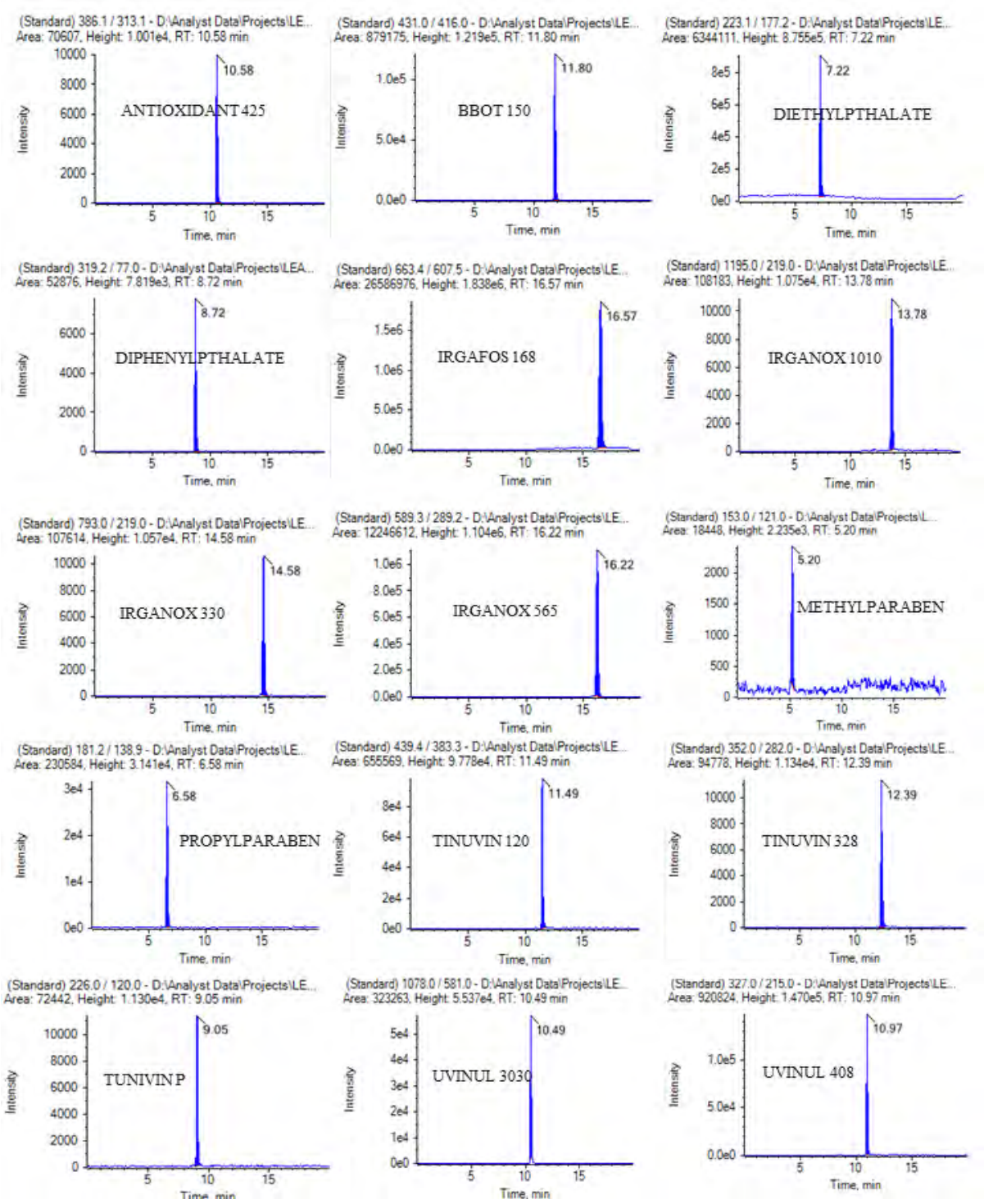

Figure 3. Extracted Ion Chromatogram of Each Analyte at 50 ppb Level.

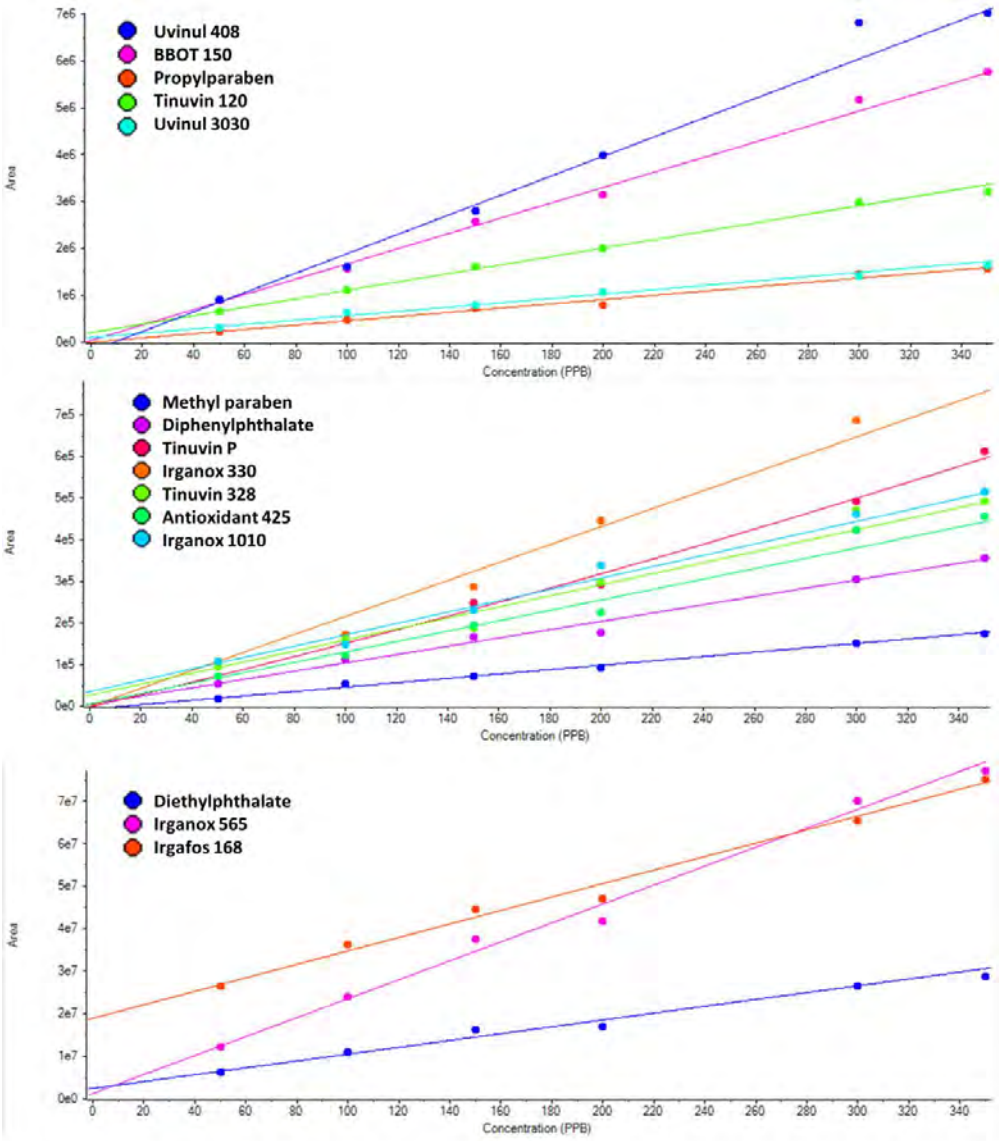

Figure 4. Calibration Curves for the 15 Different E&L Compounds. Calibration curves were generated from 50 to 400 ppb.

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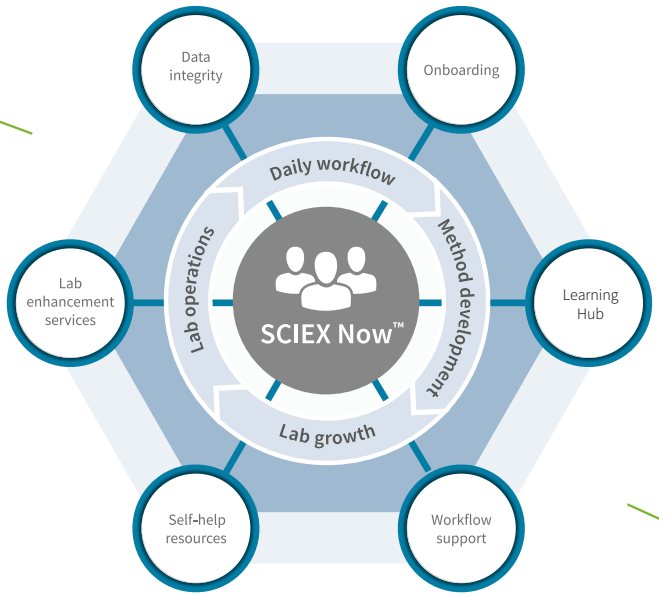

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