SCIEX QTRAP® 4500 LC-MS/MS System

Compendium of food testing applications

SCIEX

The Power of Precision

SCIEX QTRAP® 4500 LC-MS/MS System

Compendium of food testing applications

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Challenges faced by analytical testing labs

Have you ever had second thoughts on whether the data you acquired is true?

Are you sure that your peak is truly your analyte of interest?

Do you ever wish you could confirm your result apart from MRM, retention times and ion ratios?

Are you experiencing matrix interferences that affect your ability to detect and quantify?

Addressing the challenges

The solution to these common challenges: SCIEX QTRAP® technology delivers the gold standard in quantification and offers confirmational experiments to enhance your workflows. Used in laboratories all over the world, the QTRAP system is the instrument of choice for many companies and institutions that rely on its power, accuracy, reproducibility and robustness to acquire the best possible data across key applications in pharmaceutical drug discovery and development, food testing, environmental monitoring, protein quantification and more.

In this compendium you will discover the functions of the QTRAP system supported by methods and application notes which will show why the QTRAP system is more than just a triple quad.

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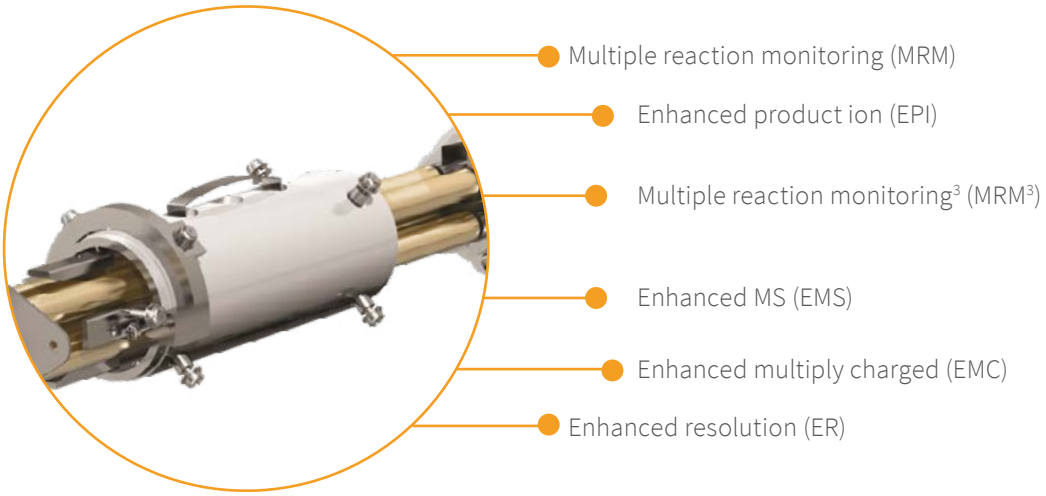
QTRAP® technology explained

What makes a QTRAP system so remarkable? While having the capability to function like a standard triple quad LC-MS/MS, it also doubles as a linear ion trap (LIT), proprietary technology that can perform a multitude of additional workflows beyond basic multiple reaction monitoring (MRM) for better specificity and quantitative performance.

QTRAP technology packs more than twice the functionality of a standard triple quad system.

Scan type	Triple quad	QTRAP
Precursor	•	•
MRM	•	•
Neutral loss	•	•
Product ion	•	•
Enhanced MS (EMS)		•
Enhanced multiply charged (EMC)		•
Enhanced resolution		•
Enhanced product ion		•
MS^3 (MS/MS/MS) and MRM^3		•



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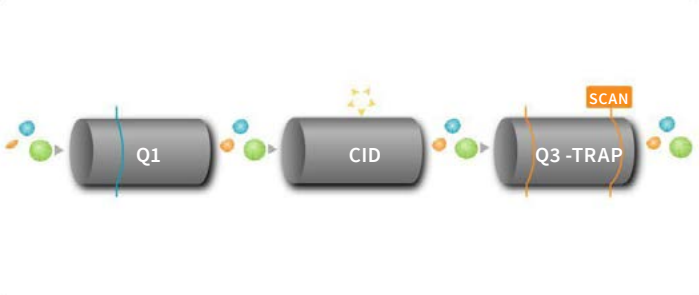
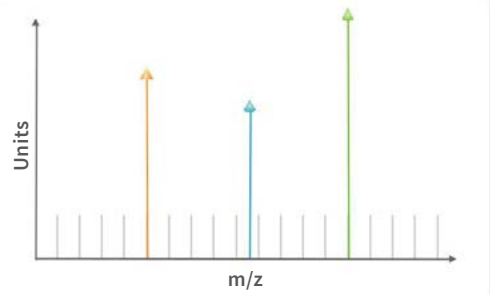
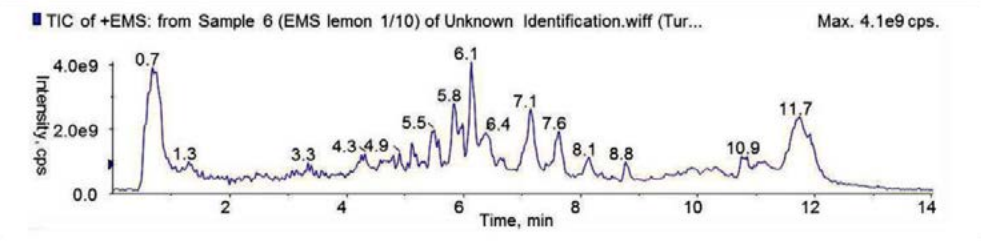
Unique scan functions to accelerate your lab's performance

Enhanced MS scan (EMS)

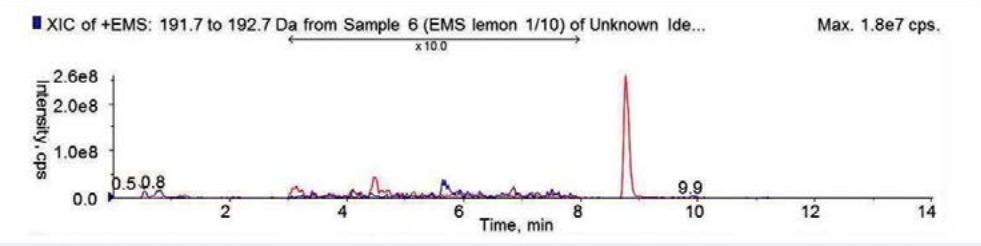
The enhanced MS scan is the standard QTRAP® system MS scan where ions are transmitted from the source (Turbo V® Ion Source or IoDrive® System Technology) through the RF mode quadrupoles into the ion trap. The ion trap is filled, and the ions are scanned out axially to the detector. The scan type delivers a highly sensitive full scan for the detection of unknown analytes when your conventional MS/MS system struggles to see a response.



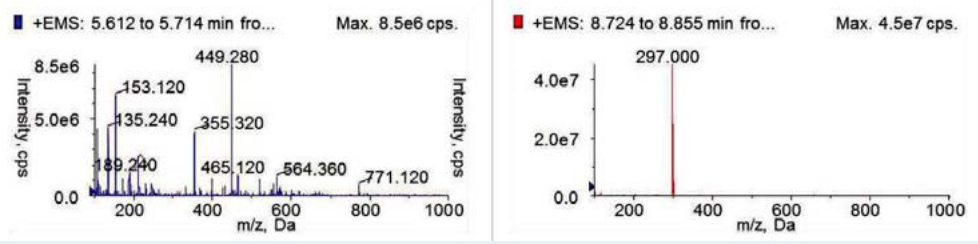




The total ion chromatogram (TIC) for the sample



The extracted ion chromatogram (XIC) from the EMS using a range of 191.7 to 192.7 Da



The spectra acquired from the EMS which can be used to understand the potential structure of the detected compound

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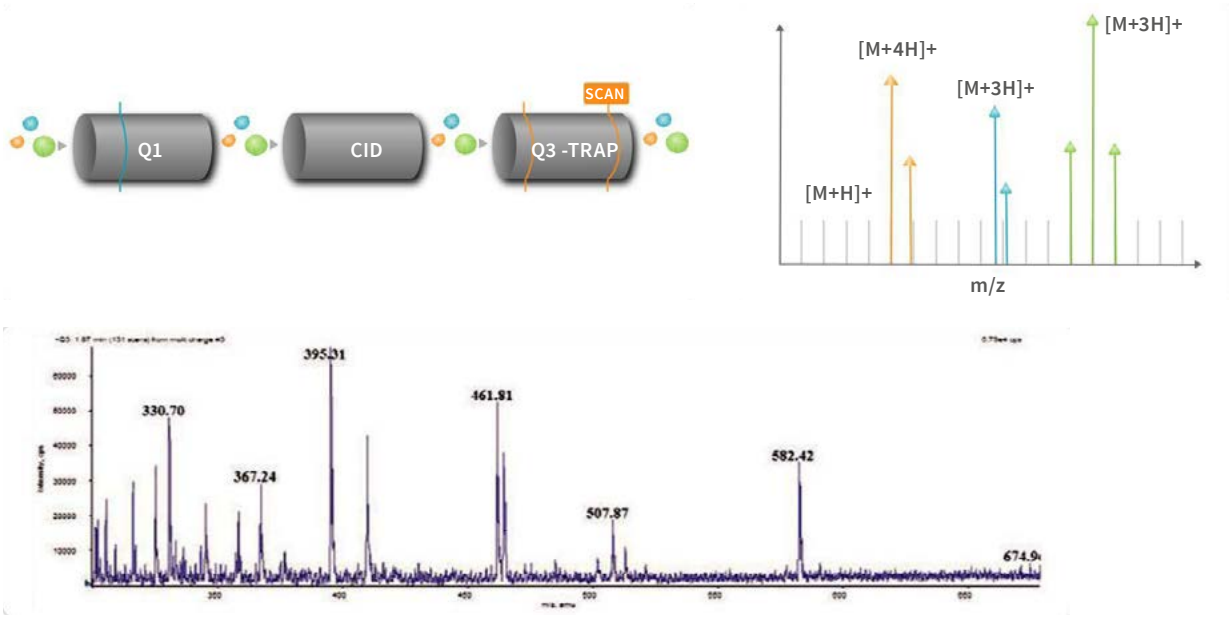
Enhanced multiply charged scan (EMC)

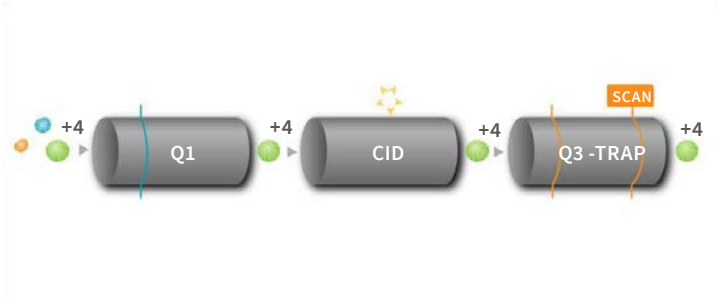
An EMC scan is a function of the QTRAP system that can be used to improve the signal/noise ratio on ions which are multiply charged. As in the EMS, the Ions are transmitted from the source to the LIT. Once the ion trap has been filled, the singly charged ions are removed, leaving the multiply charged ions behind. The ions are then scanned out axially to the detector.



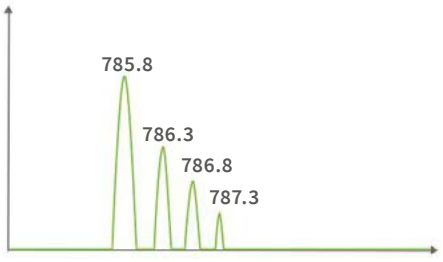
Only see what you need to see, the EMC scan filters out all of the single charged species so only the multiply charged ions remain.

Enhanced resolution scan (ER)

Using the enhanced resolution scan mode allows for high resolution MS to be obtained for an ion of interest. This scan is important in the acquisition of an accurate molecular weight and to obtain both the value of the charge state and the accurate mass to charge ratio (m/z). This scan type is most useful when trying to determine structural information, perform database searches, or perform peptide sequencing, among many other applications.



Schematic of the path of the ions through the QTRAP system



Example of how the QTRAP system will resolve the analyte

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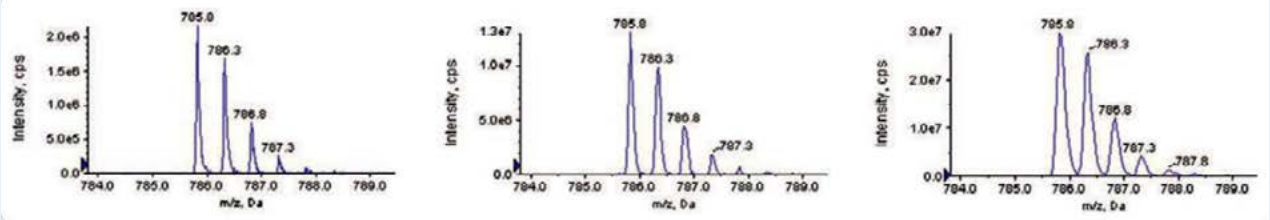
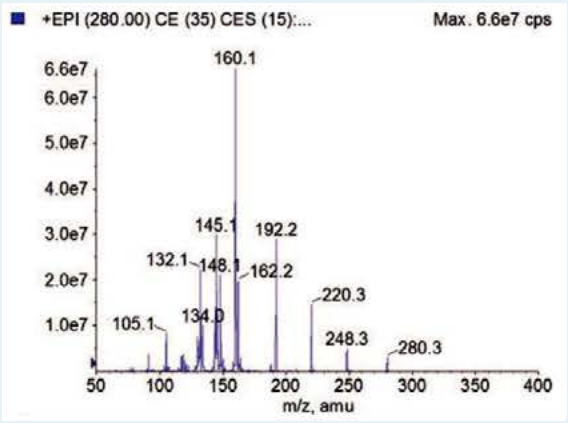


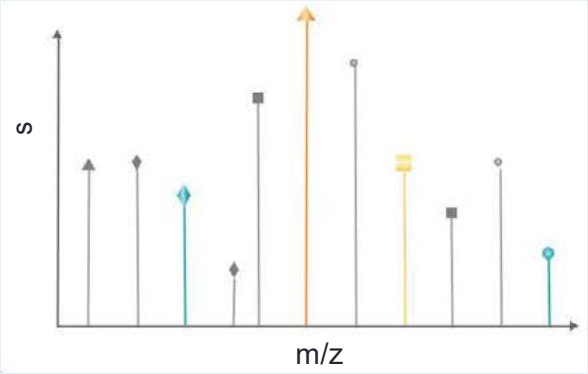
Acquired data using various parameters for an ER scan at 50, 250 and 1000 Da/sec

Enhanced product ion (EPI)

The enhanced product ion scan is a trap scan used to obtain high-quality MS/MS spectrum on a specific ion. The fragmentation is done in the collision cell and provides information-rich MS/MS spectral data. The fragmented ions generated are captured in the LIT and then scanned out. This scan type is key to identification experiments and perfectly complements a quantitative workflow. The EPI scan delivers high sensitivity, high mass accuracy and fast scanning when compared to a non-enhanced basic product ion scan.



Acquire high-quality MS/MS spectra so you have a unique thumbprint of your analyte and build a vast library of compound structural information.



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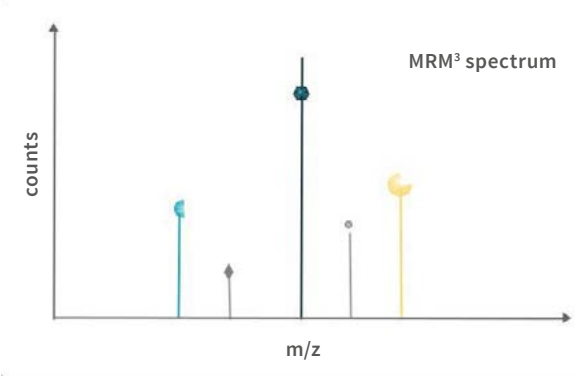
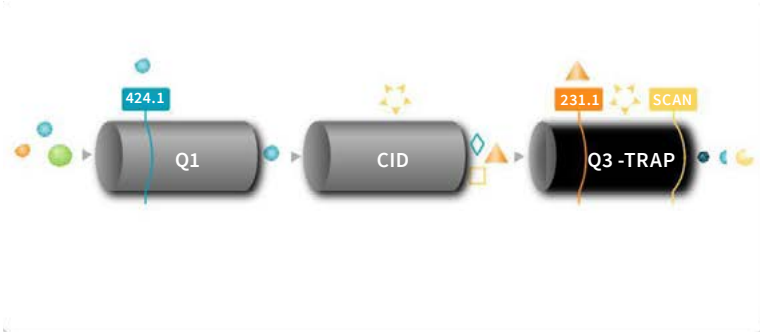
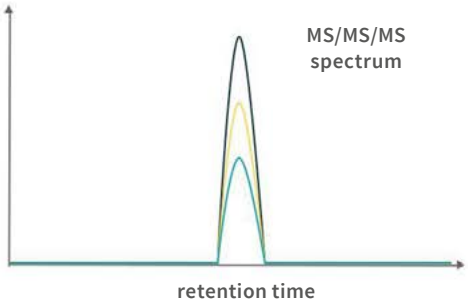
MS³ (MS/MS/MS) and MRM³

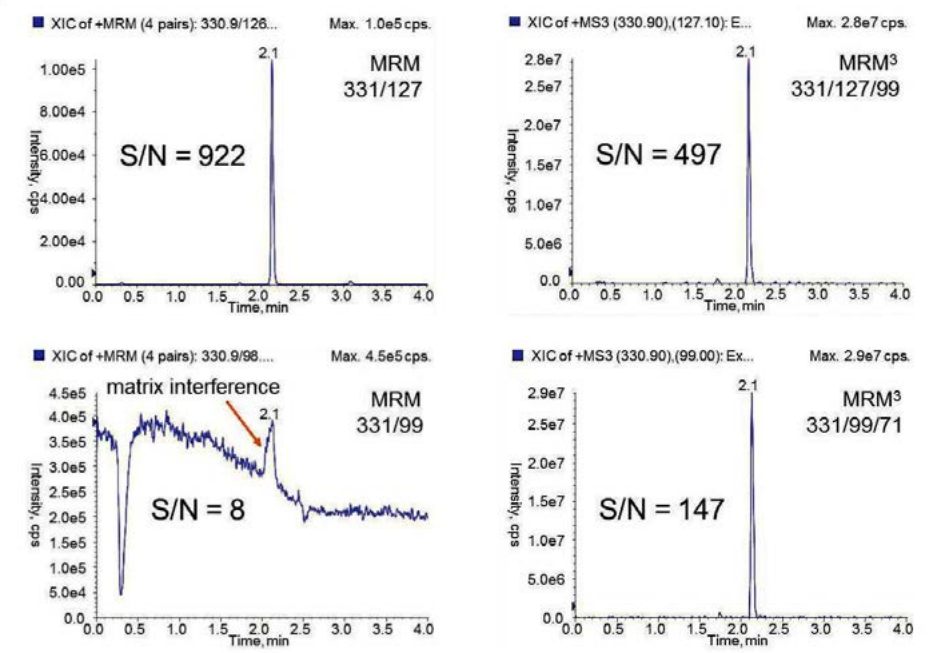
MRM³ is an effective scan solution for the quantification of analytes when high background and interferences make standard MRM quantification difficult. MRM³ can remove the interference and enable a much lower detection of the particular analyte.

This very powerful tool allows you to obtain additional structural information from your sample. In particular this scan mode is important in the characterization of modified peptides such as glycopeptides.









Left panel shows 2 MRM transitions for a compound, where the confirmation MRM transition (bottom panel) shows high background from matrix interferences. By monitoring the second generation MRM, or MRM³, the matrix interferences are virtually eliminated, and the analyte is detected with high S/N for accurate quantification.

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Extraordinary strength. Legendary performance.

Introducing the LC-MS/MS workhorse

Real-world labs need an industrious LC-MS/MS workhorse for trustworthy results hour after hour, day after day.

The QTRAP 4500 System delivers 100X more full-scan MS/MS sensitivity than standard triple quads for unmatched simultaneous quantification and library searching.

Whether your research is focused on food and environmental contaminant screening, clinical research, regulated bioanalysis or targeted quantitative proteomics, the 4500 series will deliver reliable, robust and definitive results with a new level of confidence. Combined with accelerated lab integration packages, which merge LC, application software and validation services into comprehensive workflows specific to your application, the SCIEX 4500 series more than carries its weight.

Explore the workhorse at sciex.com/qtrap-4500system

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LC-MS/MS Analysis of Emerging Food Contaminants

Detection of Peanut and Almond Allergens in Spices

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Introduction

Recent findings (in February 2015) of allergens in spices caused the recall of many food products in North America and Europe. The US Food and Drug Administration (FDA) advised people who are highly allergic to peanuts to consider avoiding products that contain ground cumin or cumin powder, because some shipments of these products have tested positive for undeclared peanut protein. The Food Standards Agency (FSA) has issued a further allergy alert following confirmation that a batch of paprika was the most likely source of undeclared almond protein in three food products which had been recalled. According to the European Rapid Alert System for Food and Feed (RASFF) portal, additional food products containing Cayenne pepper and Pilli-Pilli powder were found to contain undeclared traces of peanuts. Another recall of cumin containing product was triggered by the Canadian Food Inspection Agency (CFIA).[1-4]

This was the latest in a string of spices being recalled for possible nut protein findings. It remains unclear whether the contamination is accidental or deliberate.

It is important that consumers know food is safe and authentic. Potential weaknesses in the food supply chain need to be identified and counter measures need to be taken to strengthen consumer protection. Accurate and reliable analytical methods are needed to monitor the food supply chain and to allow correct labeling of food products.

Traditionally enzyme-linked immunosorbent assay (ELISA) based methods are used for food allergen testing. However, it is well known that ELISA can generate variable results, including false negative and false positive results that can occur due to the technique's limited sensitivity and selectivity. In addition, each allergen requires a separate test kit for the identification of an individual allergen. A multi-allergen screening method would be invaluable to increase the throughput and efficiency in allergen testing.

Here we present a method to detect the presence of peanut and almond in spices. Samples were extracted and then the allergenic proteins were reduced, alkylated and digested using trypsin. The extract containing peptides from the digested

proteins were filtered and analyzed by LC-MS/MS using a reverse phase chromatography and positive polarity electrospray ionization (ESI). The SCIEX QTRAP® 4500 system used for this study was operated in Multiple Reaction Monitoring (MRM) mode to achieve high selectivity of detection. In MRM mode characteristic transitions of peptides breaking into compound-specific fragment ions are monitored. At least 12 transitions (3 transitions for 4 peptides) were monitored per allergen to minimize potential false positive results caused by matrix interferences. The QTRAP® 4500 system also allows the acquisition of full scan MS/MS spectra which can be searched against mass spectral libraries to further increase the confidence in identification.

Experimental

Samples

Samples of cumin and paprika were obtained from local supermarkets. Store-bought roasted and raw peanuts and almonds were used for spiked experiments.

Sample preparation

The sample preparation method was based on previous work of Lock et al. The complete protocol is available in the iMethod™ Application for Allergens in Baked Goods (version 1.0).[5-6]

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The analytical workflow is shown in Figure 1.

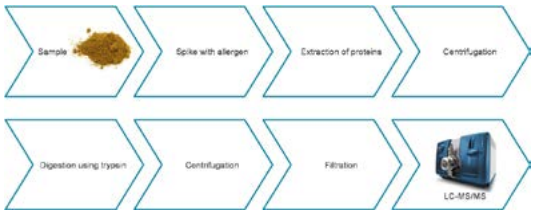

Figure 1. Sample preparation workflow

LC Separation

A Shimadzu UFLC$_{XR}$ system was used for analysis. Separation was achieved using a Phenomenex Kinetex 2.6u XB-C18 100A (30 x 1.0 mm) column with a mobile phase consisting of water and acetonitrile containing 0.1% formic acid and a 15 min gradient from 98/2 to 2/98 (A/B%). The LC column was held at 30°C. The flow rate was set to 300 µL/min and the injection volume to 30 µL.

MS/MS Detection

A SCIEX QTRAP® 4500 system with Turbo V™ source with ESI probe was used in positive polarity. The ion source temperature was set to 500°C.

MRM transitions were obtained from *in-silico* and protein ID experiments. Specificity and cross reactivity was evaluated by injecting extracts of roasted and raw almonds and peanuts as well as spiked extracts of spices. The final list of MRM transitions used in this study is shown in Table 1.

Table 1. Retention times (RT) and MRM transitions used for the detection of almond and peanut

Allergen (Peptide)	RT (min)	Q1	Q3
Almond (Peptide 1)	9.7	830.4	738.4
Almond (Peptide 1)	9.7	830.4	1035.5
Almond (Peptide 1)	9.7	830.4	922.5
Almond (Peptide 2)	8.3	571.8	369.2
Almond (Peptide 2)	8.3	571.8	858.5
Almond (Peptide 2)	8.3	571.8	743.4
Almond (Peptide 3)	7.7	698.3	732.4
Almond (Peptide 3)	7.7	698.3	879.5
Almond (Peptide 3)	7.7	698.3	936.5
Almond (Peptide 4)	10.1	780.8	1154.7
Almond (Peptide 4)	10.1	780.8	848.5
Almond (Peptide 4)	10.1	780.8	1186.7
Peanut (Peptide 1)	8.2	688.8	300.2
Peanut (Peptide 1)	8.2	688.8	930.6
Peanut (Peptide 1)	8.2	688.8	1077.5
Peanut (Peptide 1)	8.2	688.8	833.4
Peanut (Peptide 2)	8.4	564.4	686.6
Peanut (Peptide 2)	8.4	564.4	557.5
Peanut (Peptide 3)	8.5	793.9	827.5
Peanut (Peptide 3)	8.5	793.9	612.4
Peanut (Peptide 3)	8.5	793.9	726.4
Peanut (Peptide 4)	8.9	571.3	913.5
Peanut (Peptide 4)	8.9	571.3	669.3
Peanut (Peptide 4)	8.9	571.3	506.3

Results and Discussion

Qualitative Allergen Screening using MRM

Example chromatograms of spiked extracts are presented in Figure 2.

Figure 2a shows the results for 10 mg of roasted and raw almond spiked into 1 g of paprika, and Figure 2b shows the results for 10 mg of roasted and raw peanut spiked into 1 g of cumin.

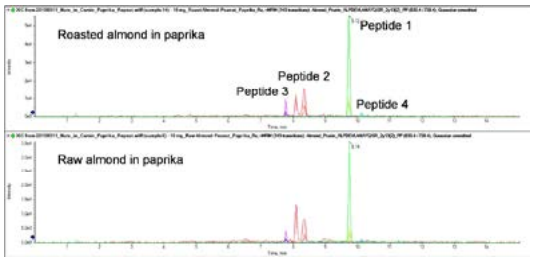

Figure 2a. Detection of almond in extracts of paprika (spiked at 10 mg/g)

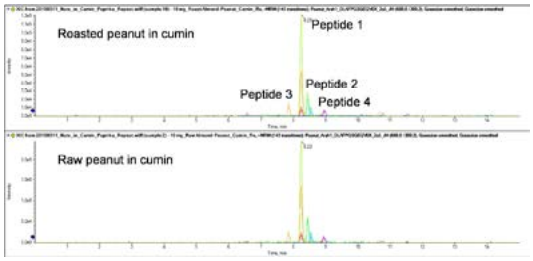

Figure 2b. Detection of peanut in extracts of cumin (spiked at 10 mg/g)

Identification of target compounds or peptides is typically based on MRM ratio calculation when utilizing LC-MS/MS.

There is the possibility of protein modification during food product, transportation, storage, and processing. The monitoring of 12 MRM transitions corresponding to 4 different peptide fragments per allergen provides high confidence in identification since different characteristic peptides of the allergen are monitored simultaneously. This procedure greatly reduces the possibility of false negative results.

MultiQuant™ software automatically calculates MRM ratios and MRM ratio tolerances. MRM transitions outside the tolerance will be flagged to identify outliers quickly. The MRM tolerances are also displayed in the Peak Review (see Figures 3a and 3b).

The MRM ratio measured from raw and roasted almonds and peanuts spiked into spices was typically well below 30%.

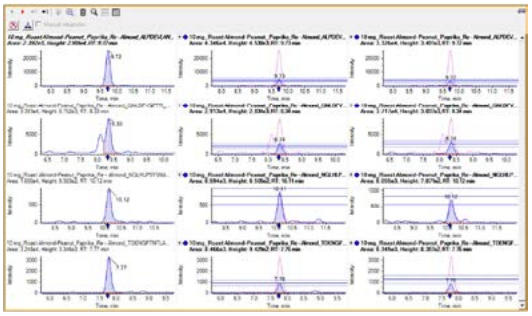

Figure 3a. Identification of almond in a paprika extract based on multiple MRM ratios

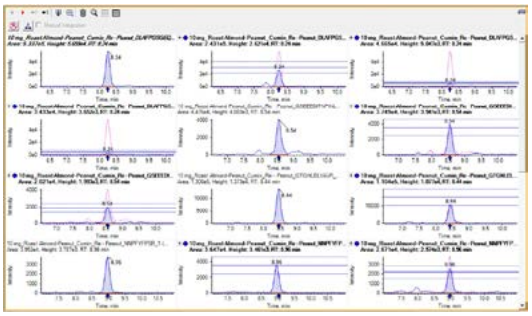

Figure 3b. Identification of peanut in a cumin extract based on multiple MRM ratios

Quantitation of Allergens in Spices

LC-MS/MS is a well know technique for the accurate and reproducible quantitation.

In this study initial quantitative results were obtained by spiking almond and peanut in spices (1, 10, and 100 mg/g) and analyzing samples following the complete sample preparation and LC-MS/MS workflow.

Example calibration lines are presented in Figure 4.

Figure 4a shows the results for roasted almond spiked into paprika and Figure 2b shows the results for roasted peanut spiked into cumin. Good accuracy and coefficients of correlation >0.999 were achieved for all transitions.

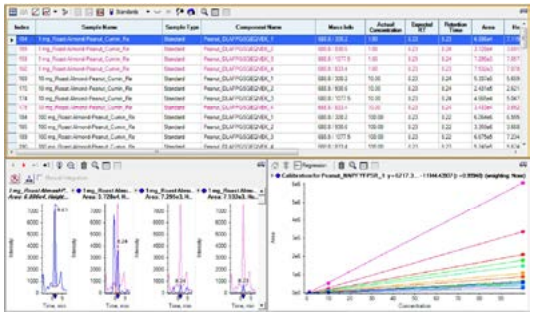

Figure 4a. Quantitative results of analyzing almond spiked into paprika powder

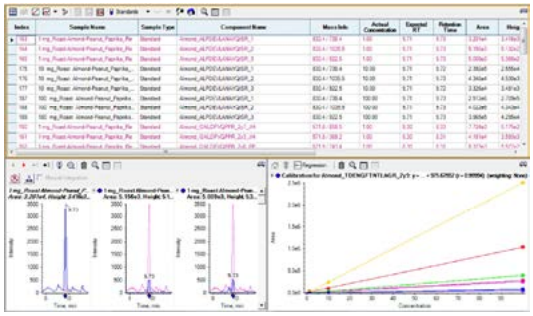

Figure 4b. Quantitative results of analyzing peanut spiked into cumin powder

Identification using MS/MS Scanning

The SCIEX QTRAP® 4500 system allows collecting MRM and MS/MS full scan data simultaneously using information dependent acquisition (IDA).

An example chromatogram with acquired MS/MS spectra for two peptides of peanut is presented in Figure 5. The spectra can be searched against mass spectral libraries which increases the confidence in identification when analyzing complex food samples.

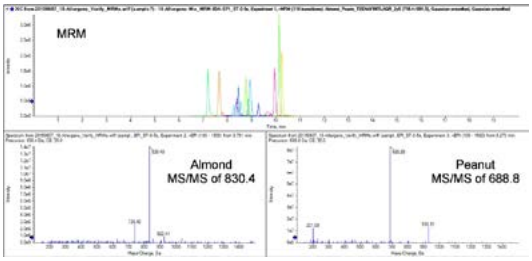

Figure 5. High confidence in identification using MS/MS full scan

Multiplexing of Allergens by LC-MS/MS

LC-MS/MS has the additional advantage of performing multi-allergen screening, unlike ELISA where different allergens are detected by separate kits.

In our laboratory LC-MS/MS was successfully applied to simultaneously screen for multiple food allergens, including egg, milk, gluten, peanut, tree nuts, soy, sesame, and mustard. An example of detecting a total of 18 allergens with a single analysis is presented in Figure 6.

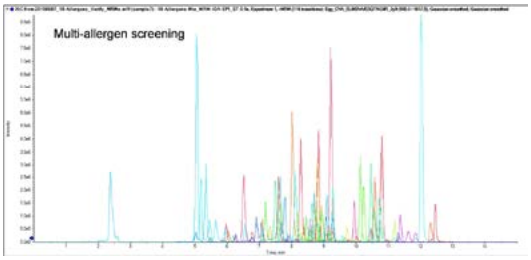

Figure 6. Multi-allergen screening by LC-MS/MS, detection of a total of 18 allergens in a single analysis

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Summary

An LC-MS/MS method for the detection of almond and peanut in spices was presented.

Samples were extracted and then the allergenic proteins were reduced, alkylated and digested using trypsin. The digested extract was filtered and analyzed by LC-MS/MS using a SCIEX QTRAP® 4500 system operated in MRM mode.

Good linearity for quantitation was achieved when analyzing almond and peanut spiked into paprika and cumin at different concentrations.

Allergen identification was achieved through the monitoring of 12 characteristic MRM transitions per allergen. MRM ratios were calculated automatically using MultiQuant™ software and MRM ratios were typically well below 30%. The QTRAP® 4500 system also allows the acquisition of full scan MS/MS spectra which further increase the confidence in identification.

LC-MS/MS has the additional advantage of performing multi-allergen screening, unlike ELISA where different allergens are detected by separate kits.

References

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2 http://www.food.gov.uk/news-updates/news/2015/13596/undeclared-almond-incident-update

3 https://webgate.ec.europa.eu/rasff-window/portal

4 http://www.inspection.gc.ca/about-the-cfia/newsroom/food-recall-warnings/eng/1299076382077/1299076493846

5. Stephen Lock et al.: 'The Detection of Allergens in Bread and Pasta by Liquid Chromatography Tandem Mass Spectrometry' Application Note AB SCIEX (2010) # 1830610-01

6 http://sciex.com/products/methods/imethod-application-for-allergens-in-baked-goods-version-1-for-cliquid-software

Publication number: 221115-01

LC-MS/MS Analysis of Emerging Food Contaminants

Quantitation and Identification 4-Methylimidazole (4-MEI) in Beverages

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SCIEX Concord, Ontario (Canada)

Introduction

4-Methylimidazole, also known as 4-MEI, is a by-product produced during the manufacturing of caramel coloring used to darken food products and can be found in carbonated beverages, such as cola, as well as in a variety of other products such as coffee, beer, soy sauce, and baked goods.

The International Agency for Research on Cancer (IARC) classified the 4-MEI as group 2B compound 'possibly carcinogenic to humans'.[1]

The use of caramel coloring in sodas, in particular, and the overall safety of 4-MEI has been the center of some recent controversy after the California Office of Environmental Health Hazard Assessment (OEHHA) included 4-MEI on its Proposition 65 list of potential human carcinogens on January 7, 2011 and proposed establishing a no significant risk level (NSRL) of 29 micrograms per day.[2-3] In response, large beverage producers have directed their caramel suppliers to modify their manufacturing processes to reduce the levels of 4-MEI.

The European Food Safety Authority (EFSA) considers 4-MEI as safe and established a maximum level for 4-MEI in caramel coloring (E 150c ammonia caramel and E 150d sulphite ammonia caramel) of 250 mg/kg.[4]

Different analytical techniques, including GC, GC-MS, and LC-UV are in use for the analysis of 4-MEI. The official method for caramel analysis written by the Joint FAO/WHO Expert Committee on Food Additives is based on a method of Wilks et al. using GC.[5] All of these method require long time for sample preparation and analysis. An online SPE-LC-UV method was developed by Moretton et al. which allows automation and accurate quantitation at low ppm levels.[6]

Here we present a new and improved method using LC-MS/MS to significantly simplify sample preparation, decrease chromatographic run time, and allow accurate and reproducible quantitation down to sub ng/mL (ppb) levels in beverages.

Experimental

Sample Preparation

The sensitivity and selectivity of the SCIEX QTRAP® 4500 System allows minimal sample preparation for this analysis. Beverage samples were centrifuged and diluted 10x with water before LC-MS/MS analysis.

LC

LC separation was achieved using the Shimadzu UFLC$_{XR}$ system with a Hypercarb 5 µm (100 x 2.1 mm) column with a gradient of water and methanol containing 0.1% formic acid at a flow rate of 0.5 mL/min. The injection volume was set to 20 µL.

MS/MS

The SCIEX QTRAP 4500 was operated in Multiple Reaction Monitoring (MRM) mode. The Turbo V™ source was used with an Electrospray Ionization (ESI) probe in positive polarity. Two selective MRM transitions were monitored for 4-MEI (83/56 and 83/42) using the ratio of quantifier and qualifier ion for compound identification (Table 1).

LC-MS/MS data was processed using the MultiQuant™ Software version 2.1.

Table 1. MS/MS Parameters for the detection of 4-MEI using the SCIEX QTRAP® 4500 system

MRM	Q1/Q3	DP (V)	CE (V)
4-MEI 1	83/56	26	25
4-MEI 2	83/42	26	35

Results and Discussion

First, limit of detection (LOD), limit of quantitation (LOQ), linearity, and reproducibility were evaluated using injections of 4-MEI ranging in concentration from 0.1 to 100 ng/mL.

Signal to noise (S/N) was calculated using the 3x standard deviation algorithm. By this approach, the LOD was determined to be 0.1 ng/mL (S/N > 3) and the LOQ, 0.5 ng/mL (S/N > 6). The MRM ratio was calculated using all standard injection resulting in an average of 0.193 (Figure 1).

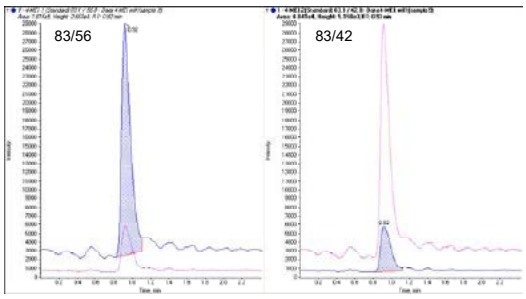

Figure 1. Injection of 1 ng/mL of 4-MEI, LOD was found at 0.1 ng/mL and LOQ at 0.5 ng/mL, the average ratio of quantifier and qualifier ion was 0.193

Linearity was observed from 0.1 to 100 ng/mL with accuracy values between 88.7 and 111.2% and a regression coefficient of 0.997 (Figure 2). Quality control samples at 1 ng/mL were injected 10 times resulting in a coefficient of variation (%CV) of 2.6%.

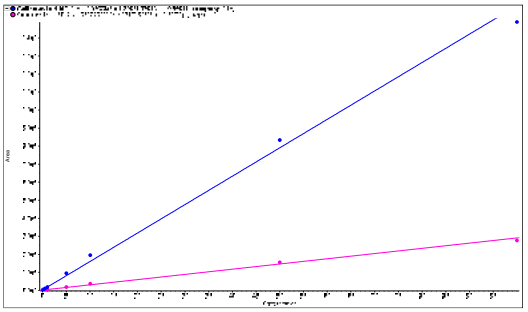

Figure 2. Linear range of the detection of 4-MEI from 0.1 to 100 ng/mL with an r of 0.997 for both MRM transitions

This level of sensitivity allows the direct injection of beverage samples without using time-consuming and extensive sample cleanup. Samples were injected directly after centrifugation and a simple 10x dilution to minimize possible matrix effects.

Several cola samples were purchased from a local store and analyzed by the method described. The MRM chromatograms of two samples are shown in Figure 3. When quantified and corrected for dilution, the samples were determined to contain 46.6 ng/mL and 78.2 ng/mL of 4-MEI, respectively.

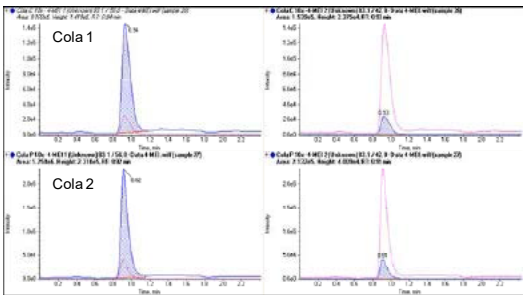

Figure 3. Quantitation and identification of 4-MEI in store bought cola

MRM ratios for compound identification were automatically calculated and compared in the MultiQuant™ Software. The MRM ratios of 0.169 and 0.166, respectively, were well in between the 20% tolerances of 0.154 and 0.232 clearly confirming the identity of 4-MEI.

Ideally, an isotopically labeled internal standard of 4-MEI should be used to eliminate matrix effects (ion suppression or ion enhancement) and to improve accuracy of detection in unknown samples, but such an internal standard was not available.

To evaluate matrix effects in this analysis, we performed standard additions to more accurately quantify 4-MEI in the matrix itself. For our standard addition analysis, we added defined concentrations of 4-MEI to aliquots of the unknown sample. These standards, along with an aliquot of the unknown sample which does not contain any added standard, were analyzed. The resulting calibration curve was extrapolated and the absolute value of the intercept with the concentration axis was used to determine the concentration of the target compound in the unknown sample.

Standard addition can be calculated automatically in the MultiQuant Software using the 'Standard Addition' query (Figure 4).

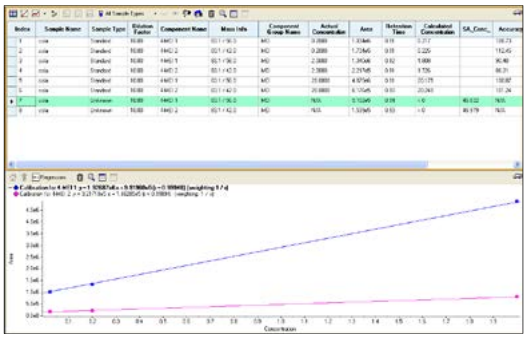

Figure 4. Automatic quantitation using the 'Standard Addition' query in the MultiQuant Software

Summary

The method and data presented here showcase the fast and accurate solution for the quantitation and identification of 4-MEI in beverage samples by LC-MS/MS. The SCIEX QTRAP® 4500 System provides excellent sensitivity and selectivity for this analysis, with minimal sample preparation allowing maximized throughput for the analysis of many samples in a short time period.

4-MEI was quantified in store bought cola samples. Automatic MRM ratio calculation in MultiQuant Software was used for compound identification.

Standard addition was also used to assess any possible quantitation errors caused by ion suppression or ion enhancement. Calculations were automatically performed using the 'Standard Addition' query.

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1 Agents Classified by the IARC Monographs, Volumes 1-104 (last update March 27, 2012) http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf

2 Proposition 65: 'Chemicals known to the state to cause cancer or reproductive toxicity' (last update March 16, 2012) http://oehha.ca.gov/prop65.html

3 Proposition 65: 'No significant risk level (NSRL) for the proposition 65 carcinogen 4-methylimidazole' (October 2011) http://oehha.ca.gov/prop65/law/pdf_zip/1007114MEI.pdf

4 EU Commission Directive 2008/128/EC 'Specific purity criteria concerning colours for use in foodstuffs' (December 22, 2008)

5 R. A. Wilks et al.: 'An Improved Method for the Determination of 4-Methylimidazole in Caramel Color' J. Agric. Food Chem. 25 (1977) 605-608

6 C. Moretton et al.: 'Quantification of 4-Methylimidazole in Class III and IV Caramel Colors: Validation of a New method Based on Heart-Cutting Two-Dimensional Liquid Chromatography (LC-LC)' J. Agric. Food Chem. 59 (2011) 3544-3550

Publication number: 5470212-02

Comprehensive Quantitation and Identification of Pesticides in Food Samples Using the SCIEX UltraLC 100 and the SCIEX QTRAP® 4500 System

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Overview

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) is a widely used analytical tool for the screening of food residues and contaminants. Here we present a new method using QuEChERS extraction, separation using the SCIEX UltraLC 100 system with a Phenomenex Synergi™ 2.5u Fusion-RP column, and the SCIEX QTRAP 4500 System. The mass spectrometer was operated in highly selective and sensitive Multiple Reaction Monitoring (MRM) mode. The *Scheduled* MRM™ algorithm was used to obtain the best data quality and combined with fast polarity switching to cover the broadest range of pesticides possible. In addition, MS/MS spectra were acquired to enable compound identification with highest confidence based on mass spectral library matching.

Introduction

LC-MS/MS is a powerful analytical tool capable of screening samples for numerous compounds. MRM is typically used because of its excellent sensitivity, selectivity, and speed. As LC-MS/MS technology continues to evolve, demands in the food testing industry to detect and quantify an increasing number of compounds in a single run are becoming more prevalent.

Generic extraction procedures, like QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and ultra high performance LC systems combined with polar embedded C18 phases with small particles, providing good resolution and excellent peak shape, made it possible to detect pesticides of a wide variety of compound classes and chemical properties in each sample.[1-3]

State-of-the-art LC-MS/MS systems make it possible to detect hundreds of pesticides and other food residues in a single run.

The SCIEX UltraLC 100 is a UHPLC system designed specifically for use with SCIEX mass spectrometers sustaining pressure of up to 18000 psi at any flow rate up to 5 mL/min. It contains a unique injector valve to maximize column life time, a side port injector needle for increased ruggedness, and a programmable needle wash to greatly reduce carry-over.

The SCIEX QTRAP 4500 system combines the legendary sensitivity, reproducibility, and accuracy of the 4000 series with the speed and trapping capabilities of the QTRAP 5500 system. The Turbo V™ source and Curtain Gas™ interface provide exceptional robustness and successfully reduce chemical noise. The advanced eQ™ electronics and Qurved LINAC® collision cell were designed for unparalleled speed of MRM detection and fast polarity switching for comprehensive multi-component analysis.

In addition, advanced software tools like the *Scheduled* MRM algorithm intelligently uses information of retention times to automatically optimize MRM dwell time of each transition and total cycle time of the experiment resulting in highest data quality.

To further increase confidence in analytical results QTRAP® technology is used to automatically acquire fast and sensitive MS/MS spectra in Enhanced Product Ion (EPI) mode and search them against mass spectral libraries for compound identification. The information of the complete molecular fingerprint saved into EPI spectra significantly reduces the risk of false positive results.

Here we present a new LC-MS/MS method utilizing the Ultra LC 100 and the QTRAP 4500 system using the *Scheduled* MRM algorithm in combination with fast polarity switching, and acquisition of MS/MS spectra for compound identification. The method was successfully applied to quantify

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and identify pesticides in a QuEChERS extracts of fruit and juice samples.

Method Details

- Different fruit samples were extracted using Restek QuEChERS kits (Q110, Q210 and Q213) and diluted 5 times with water to optimize chromatographic peak shape and minimize possible matrix effects and interferences. Juice samples were injected directly after centrifugation and 5x dilution. The injection volume was set to 10 µL.
- The SCIEX iD*Quant*™ Standards Kit for Pesticide Analysis was used for method setup and preparation of calibration standards. Additional pesticides were added to cover all compounds of interest.
- LC separation was achieved on the SCIEX UltraLC 100 with a Phenomenex Synergi-Fusion 2.5u 50x2 mm column and a fast gradient of water and methanol with 10 mM ammonium formate buffer at a flow rate of 0.5 mL/min.
- The new SCIEX QTRAP® 4500 System was operated with Turbo V™ source and Electrospray Ionization (ESI) probe.
- Approximately 500 MRM transitions were monitored in both positive and negative polarity. Optimized transitions for all compounds were obtained through the MRM catalogue of the iMethod™ Test for Pesticide Screening version 2.1.
- The *Scheduled* MRM™ algorithm was used in combination with fast polarity switching using Analyst® 1.6.1 Software.
- For increased confidence in compound identification, EPI spectra were acquired at a scan speed of 10000 Da/s using dynamic fill time for best spectral quality and Collision Energy Spread (CES) to ensure a characteristic MS/MS pattern independent of the compound's fragmentation efficiency. MS/MS spectra were search against the iMethod™ Pesticide Library version 2.1.
- MultiQuant™ 2.1 Software was used for quantitative data processing.

Results

Sensitivity, Reproducibility, Linearity and Accuracy

The *Scheduled* MRM algorithm uses knowledge of the retention of each analyte to monitor the MRM transition only in a short time window. Thus at any one point in time, the number of concurrent MRM transitions are significantly reduced resulting in much higher duty cycles for each analyte. The software computes maximum dwell times for the co-eluting compounds while still maintaining the desired cycle time for best data quality.[3] Combining *Scheduled* MRM™ with fast polarity switching further allows extending the target list of pesticides while maintaining throughput.

An example chromatogram of a solvent standard at 1 ng/mL is shown in Figure 1. Approximately 500 MRM transitions were monitored in both polarities throughout the entire chromatographic run. The total target cycle time of 0.7 sec ensures the collection of at least 12 data points across the LC peak resulting in excellent accuracy and reproducibility.

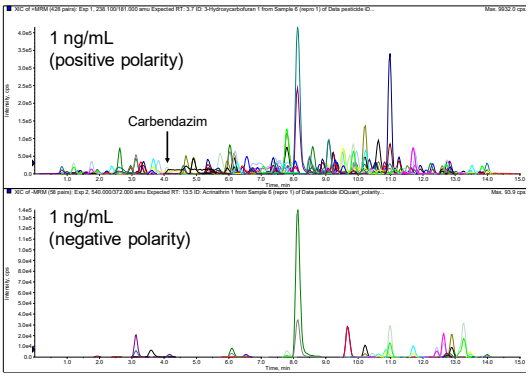

Figure 1. Comprehensive pesticide screening using the *Scheduled* MRM™ algorithm and fast polarity switching, ~500 MRM transition were detected with a total target cycle time of 0.7 sec

Figure 2 shows example chromatograms of 10 repeat injections at 1 ng/mL of early to late eluting pesticides in both polarities. The %CV values of 10% or less highlight the speed and effectiveness of *Scheduled* MRM combined with fast polarity switching. The developed method enables quantitation of all target pesticides with an LOD of at least 1 ng/mL and, thus, allowing sample extract dilution to minimize possible matrix effects.

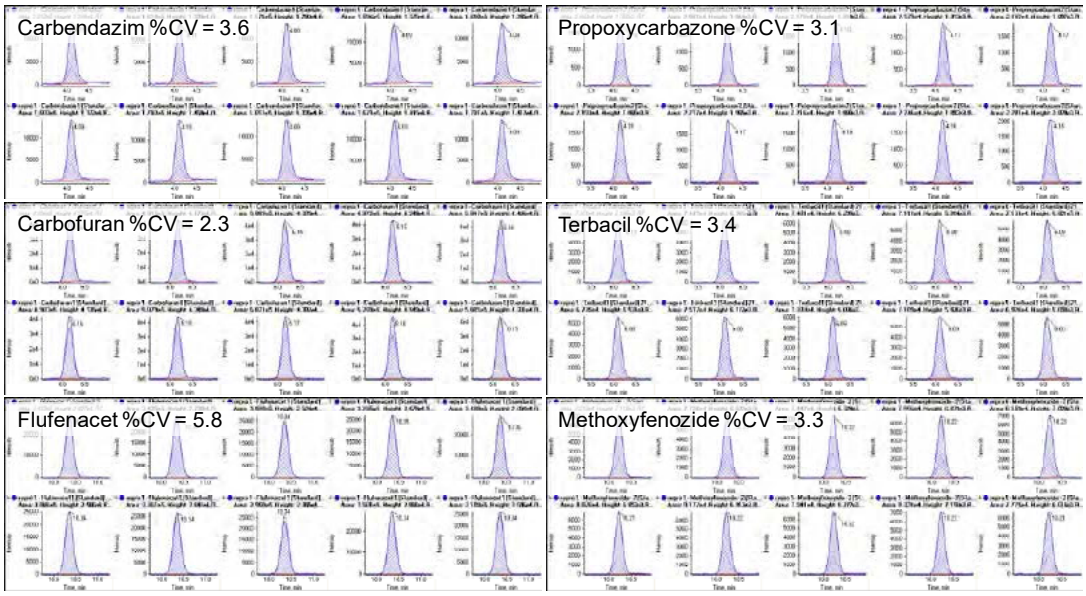

Figure 2. Repeat injections of pesticides at a concentration of 1 ng/mL detected in positive (left) and negative (right) polarity in a single run using *Scheduled* MRM™ and fast polarity switching (Carbendazim and Propoxycarbazone at 4.1 min, Carbofuran and Terbacil at 6.1 min, and Flufenacet and Methoxyfenozide at 10.3 min)

Linearity was obtained for most pesticides over 4 orders of magnitude (0.1-100 ng/mL). An example calibration line of Carbendazim is shown in Figure 3. Both MRM transitions have a regression coefficient of > 0.999 with accuracies between 97 and 109%.

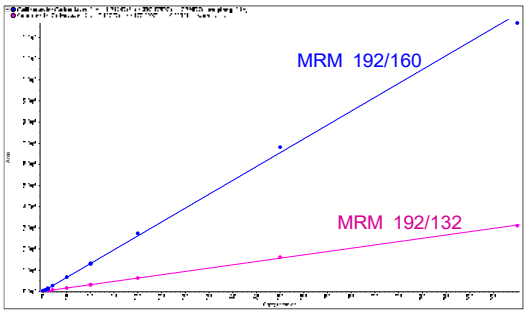

Figure 3. Calibration lines of both MRM transitions of Carbendazim

Accuracy between 80 and 120% were achieved for all targeted pesticides over the entire calibration range. Data points of the lowest or highest standards were excluded for a few pesticides with weak or strong ionization, respectively.

Findings in Fruit and Vegetable Samples

The developed method was applied to the quantitation and identification of pesticides in real food extracts. QuEChERS extracts of fruits and vegetables were diluted 5x prior LC-MS/MS analysis. Juice samples were injected directly after centrifugation and 5x dilution.

Sample data was processed using MultiQuant™ Software version 2.1 with the 'Multicomponent' query. Query files are customizable commands to perform custom querying of the result table. The 'Multicomponent' query automatically calculates and compares MRM ratios for compound identification and highlights concentrations above a user specified maximum residue level. An example of the results and peak review after running the query file is shown in Figure 4.

Contents

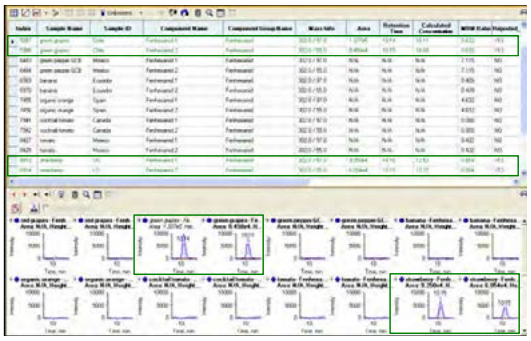

Figure 4. Automatic reporting of pesticides using the 'Multicomponent' query in MultiQuant™ software: Fenhexamid was positively identified using MRM ratio calculation in two samples and quantified in green grapes at 18.1 µg/kg and in strawberry at 12.5 µg/kg, respectively.

Example chromatograms of analyzed samples are shown in Figures 5a-e. The findings are also summarized in Table 1.

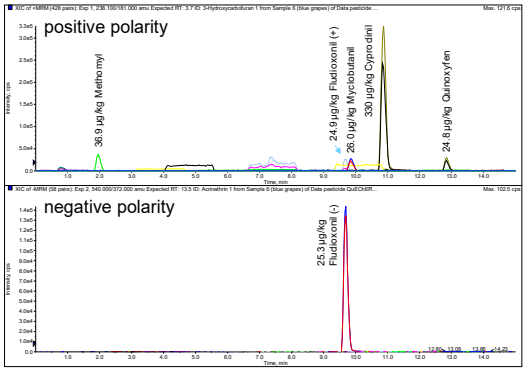

Figure 5a. Pesticides identified and quantified in a red grape sample

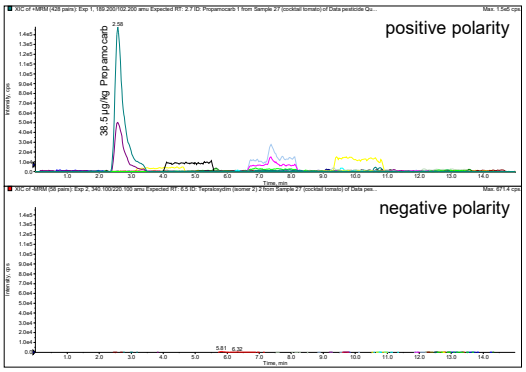

Figure 5b. Pesticides identified and quantified in a cocktail tomato sample

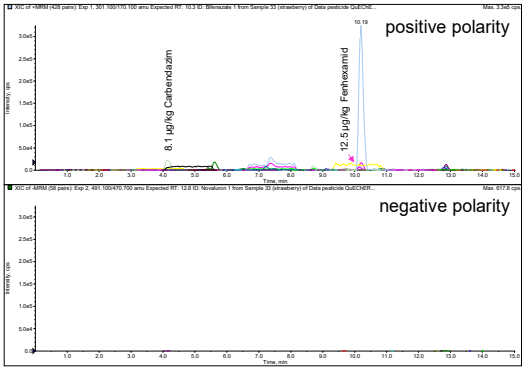

Figure 5c. Pesticides identified and quantified in a strawberry sample

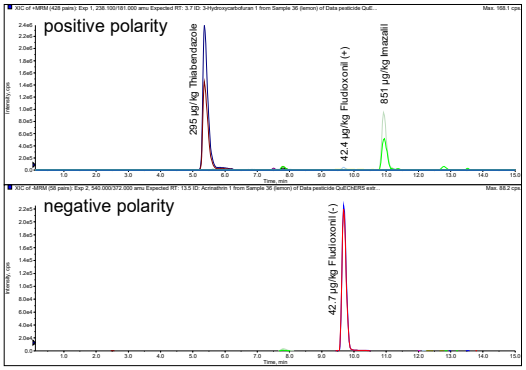

Figure 5d. Pesticides identified and quantified in a lemon sample

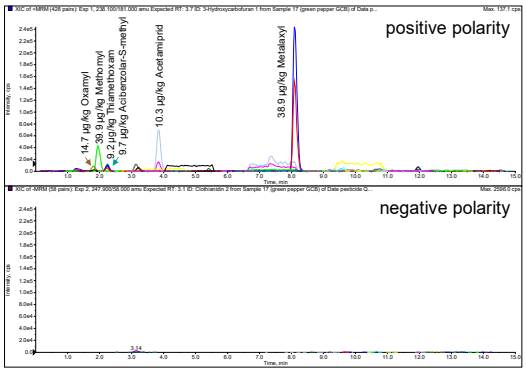

Figure 5e. Pesticides identified and quantified in green pepper sample

Table 1. Summary of pesticide findings in store bought food and orange juice samples above a concentration of 5 µg/kg

Sample	Pesticide	Concentration (µg/kg)
Red grapes	Cyprodinil	330
	Fludioxonil	24.9
	Methomyl	36.9
	Myclobutanil	26.0
	Quinoxyfen	24.8
Cocktail tomato	Propamocarb	38.5
Strawberry	Carbendazim	8.1
	Fenhexamid	12.5
Lemon	Fludioxonil	42.4
	Imazalil	851
	Thiabendazole	295
Green pepper	Acetamiprid	10.3
	Acibenzolar-S-methyl	9.7
	Metalaxyl	38.9
	Methomyl	39.9
	Oxamyl	14.7
	Thiamethoxam	9.2
Banana	Imazalil	40.7
	Thiabendazole	18.5
Clementine	Imazalil	1250
Green grapes	Boscalid	10.8
	Fenhexamid	18.1
	Imidacloprid	32.0
	Myclobutanil	7.2
	Quinoxyfen	12.5
Organic orange	no pesticides detected above 5 µg/kg	
Raspberry	Azoxystrobin	35.5
	Cyprodinil	71.0
	Fludioxonil	7.2
	Pyrimethanil	22.7
Red pepper	Flutriafol	44.0
Tomato	Difenoconazole	61.0
	Buprofezin	97.8
Orange juice 1	Carbendazim	13.0 ng/mL
Orange juice 2	Carbendazim	67.0 ng/mL

Compound Identification using MS/MS Library Searching

Despite the high selectivity of MRM detection, there is always a risk of false positive findings due to interfering matrix signals. Typically, a second MRM is monitored per analyte and the ratio of quantifier to qualifier transition is calculated for each unknown sample and compared to the MRM ratio of standards for identification. However, it has been reported that relying only on MRM ratios for identification can result in a significant number of false positive results for compound identification.

To increase confidence in identification full scan MS/MS experiments can be performed, and unknown spectra can be searched against mass spectral libraries. Here MS/MS spectra acquired in the EPI mode of the QTRAP® 4500 system were searched against the iMethod™ pesticide library (version 1.1). Example spectra and library search FIT values to identify Carbendazim in orange juice samples and Cyprodinil and Fludioxonil in a raspberry sample are shown in Figures 6 and 7. These examples highlight that MS/MS library searching increases confidence in identification, especially if the targeted analytes have low fragmentation efficiency (many low intensity product ions).

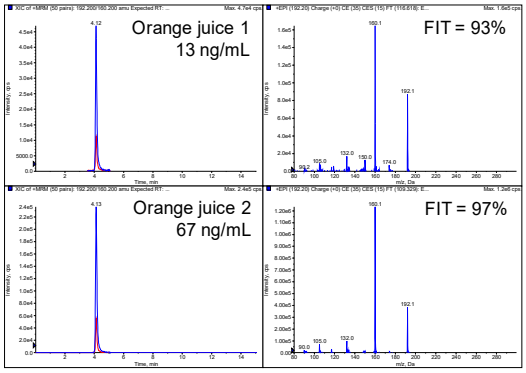

Figure 6. Identification of Carbendazim in two orange juice samples using MS/MS library searching: The samples were injected directly after 5x dilution, and FIT values above 90% clearly confirm the identity of Carbendazim.

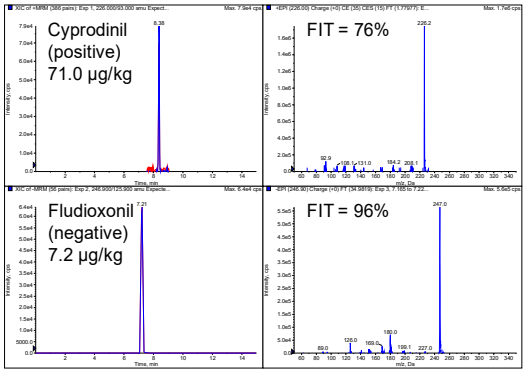

Figure 7. Identification of Cyprodinil and Fludioxonil in a raspberry sample using MS/MS library searching: The samples were injected after QuEChERS extraction and 5x dilution, and MS/MS spectra were acquired in positive polarity and negative polarity.

Summary

This new and unique LC-MS/MS method using the SCIEX UltraLC 100 and QTRAP® 4500 system utilizing the *Scheduled* MRM™ algorithm in combination with fast polarity switching and acquisition of MS/MS spectra for compound identification has significant advantages. The method was successfully used to quantify and identify pesticides covering a broad range of chemical properties, including the acquisition of positive and negative polarity spectra.

The automatic method setup based on the *Scheduled* MRM algorithm resulted in excellent quantitative data. LOQ were measured for all pesticides at 0.1 ng/mL or below. This allows the dilution of sample extracts to significantly reduce possible matrix effects and interferences. Accuracies were typically found between 80 and 120% with %CV of less than 10%.

Different food and juice samples were analyzed after QuEChERS extraction and dilution to minimize possible matrix effects.

Results were processed using MultiQuant™ Software with the 'Multicomponent' query. This query automatically highlights findings above a user specified threshold and when identification based on MRM ratio comparison was positive.

In addition full scan MS/MS spectra were acquired using the QTRAP 4500 system. MS/MS spectra contain the complete molecular fingerprint of each analyte, and when searched against a spectral library, reduce the possibility of false positive and negative results.

References

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[2] J. Wong et al.: J. Agric. Food Chem. 58 (2010) 5897-5903 [3] A. Schreiber et al.: Application Note SCIEX (2010) 1282310-01

Publication number: RUO-MKT-03-5620-A / 5090212-02

Qualitative LC-MS/MS Analysis of 13 Food Allergens in a Single Injection on the QTRAP® 4500 System

Detection of Multiple Signature Peptides of Food Allergens in Bakery-products and Raw Food Matrices

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Introduction

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

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

Herein, we present an LC-MS/MS method using the QTRAP® 4500 LC-MS/MS system that detects and screens 13 separate allergenic proteins simultaneously in a single injection. The allergens detected in this method were selected from the guidelines presented in the Codex Alimentarius, a resource developed by the United Nations' Food and Agriculture Organization (FAO) and the World Health Organization (WHO) to harmonize international food standards.[3]

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

To evaluate a range of food products (both raw and bakery goods) for their allergenic content, several unique signature peptides specific to each allergen were identified from tryptic digests of food homogenate extracts. A mixture of 13 allergens was added to bakery product food matrices (either bread or cookie) over a range of known concentrations, and several MRM transitions corresponding to allergenic signature peptides were evaluated simultaneously using the *Scheduled* MRM™ algorithm. Presently, this method can detect allergenic peptides from five of the major classes of allergenic foods at a detection limit of 10 ppm (except for those from macadamia nuts) in a variety of food matrices.

Contents

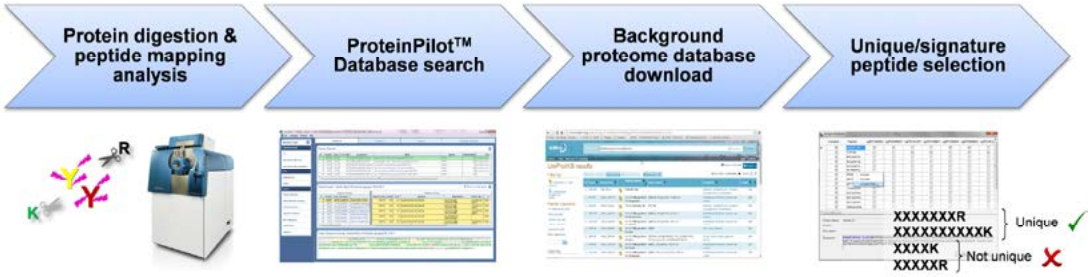

Figure 1. Signature peptide selection workflow using the SCIEX TripleTOF® 6600 system and ProteinPilot™ software

Experimental

Sample Preparation

To prepare bread and cookie homogenates, unbaked gluten-free bread or cookie mixes (100 g) were supplemented with 10 to 500 ppm (by weight) of each of the following 13 allergenic foods: eggs, milk, peanuts, soy, almonds, Brazil nuts, cashew nuts, hazelnuts, macadamia nuts, pecans, pine nuts, pistachios, and walnuts. The fortified foods were then cooked according to manufacturer's specifications. The food samples (raw nuts, baked goods) were finely homogenized using a coffee grinder. Each homogenate (1 g) was defatted by extracting twice with hexane and dried by evaporation in the fume hood. Extraction buffer (4 mL) was added to the defatted homogenates, which were then centrifuged prior to the removal of supernatants (500 µL). Reductant (50 µL) was added to supernatants at 60°C for 1 hr. After cooling (20°C), samples were alkylated using a cysteine blocking reagent (25 µL). Trypsin (20 µg) was added to modified proteins (3 to 12 hr) in calcium chloride/ammonium bicarbonate buffer to obtain tryptic peptides for signature peptide analysis prior to neutralization with formic acid (30 µL). Digested samples (500 µL) were vacuum filtered using a 10 kDa MWCO filter prior to LC-MS/MS analysis.

LC Separation

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

MS/MS Detection

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

To screen foods for allergens, a SCIEX QTRAP® 4500 system with Turbo V™ source in positive ESI mode was employed using an ion source temperature of 500°C. The *Scheduled* MRM™ algorithm was used to analyze food samples for 13 allergens in a single injection by multiplexing the detection of multiple MRM transitions for allergenic signature peptides.

Results and Discussion

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

For each allergen, two unique proteins, three unique peptides per protein, and three MRM transitions per peptide were chosen to ensure confidence in the identification of an allergen. To monitor many MRM transitions during a single injection, the *Scheduled* MRM™ Algorithm was employed, where individual MRM transitions were monitored for a short period during their expected retention time, decreasing the total number of concurrent MRM experiments during a cycle and allowing

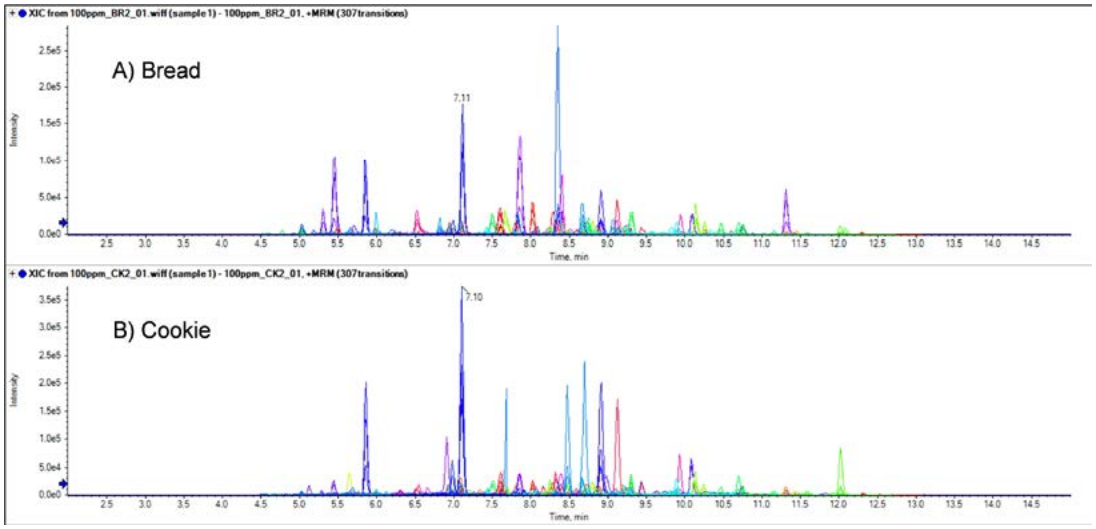

Figure 2. Extracted ion chromatograms (XIC) from LC-MS/MS analysis of bread (top) and cookie (bottom) homogenates fortified with egg, milk, peanut, soy, and nut proteins at100 ppm. Multiple peaks corresponding to allergenic tryptic peptides are displayed.

cycle time and dwell time to be maintained. This approach maximized the S/N for signature peptide detection and allows the method to be expanded as new allergenic markers are identified.

To identify multiple allergens in the same food sample, a total of 234 MRM transitions corresponding to 75 allergenic peptides, from eggs, milk, peanuts, soy beans, and tree nuts, were characterized (Figure 2). Of these 75 peptides, 68 transitions corresponded to peptides with unique sequences not shared by background proteins. The LC-MS/MS-based screening method deployed here simultaneously detected 13 allergenic proteins from 5 major classes of food allergens (egg, milk, peanut, soy and tree nuts) that had been fortified into bakery products at varying concentrations.

To show that signature peptide signals were linear in response to increasing allergen levels, calibration curves for each peptide and its three transitions were generated over a wide dynamic range (0 to 500 ppm) with good reproducibility in matrix (Figures 3a and 3b). MRM transitions were linear over a broad dynamic range and resulted in regression values over 0.95 for all allergens.

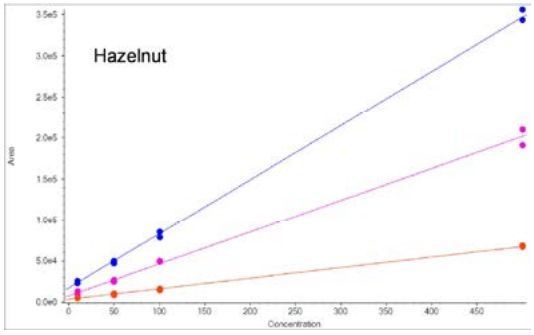

Figure 3a. Calibration lines of a hazelnut peptide form 0 to 500 ppm. Three MRM transitions were monitored: fragment 1 (blue), fragment 2 (pink), fragment 3 (orange).

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

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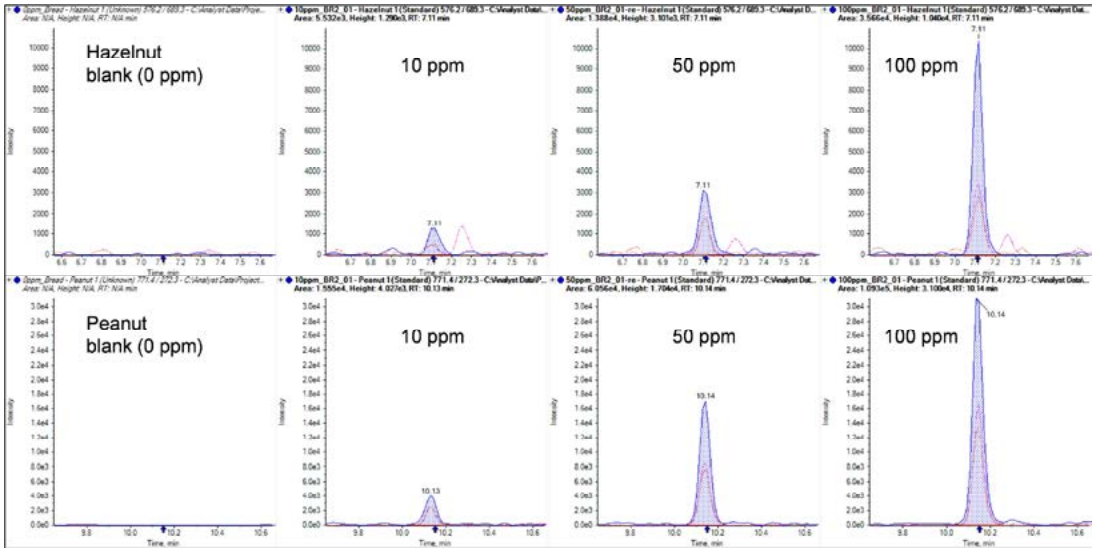

Figure 4. Extracted ion chromatograms for the signature peptide, protein 1 peptide 1, from hazelnut (top) and peanut (bottom). Varying concentrations of allergen (0, 10, 50 and 100 ppm) were added to bread samples. Three different MRM transitions for protein 1, peptide 1 are shown (blue, orange, and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

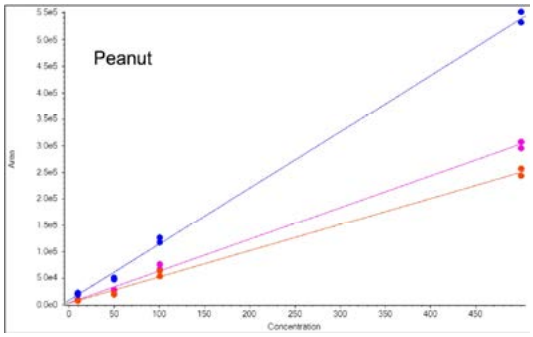

Figure 3b. Calibration lines of a peanut peptide form 0 to 500 ppm. Three MRM transitions were monitored: fragment 1 (blue), fragment 2 (pink), fragment 3 (orange).

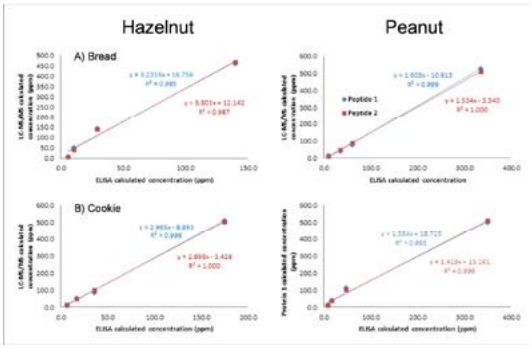

Figure 5. Comparison of allergen concentrations detected using ELISA vs. LC-MS/MS methods for two peptides (blue and orange) and two matrices, bread (top) and cookie (bottom)

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

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ELISA-based tests underestimated the concentrations of hazelnut and peanut supplements in bread and cookie matrices, especially at higher concentrations.

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

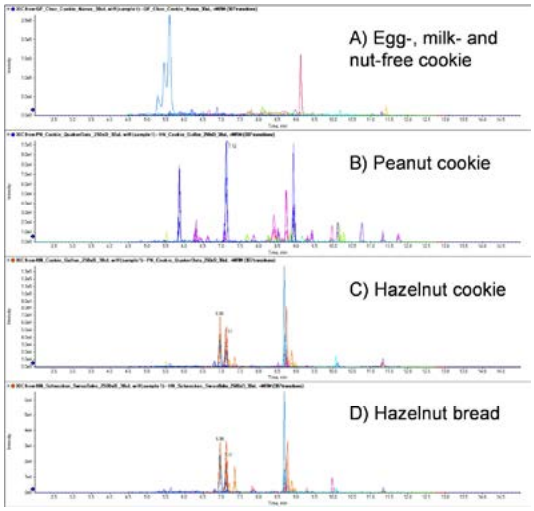

Figure 6. Extracted ion chromatograms of (A) Egg-, milk-, and nut-free cookie, (B) peanut cookie, (C) hazelnut cookie, and (D) hazelnut bread.

Summary

We have developed a multi-allergen screening tool using an LC-MS/MS method that can detect 13 food allergens in commercial products by identifying several MRM transitions corresponding to unique signature peptides for each allergen and multiplexing their detection into a single injection. In total, there are 234 MRM transitions representing peptides from the egg, milk, peanut, soy, and tree nut allergen groups. Unlike ELISA methods, this LC-MS/MS analysis detects multiple peptides from each allergic protein, thus improving method specificity and minimizing the potential for false positive and false negative results. Using only a single sample preparation method and a multiplexed data acquisition, more allergens than previously reported[4] were screened and differentiated from other food ingredients contained in baked food matrix.

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Publication number: RUO-MKT-02-2846-B

Automated Derivatization, SPE Cleanup and LC-MS/MS Determination of Glyphosate and Other Polar Pesticides

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Overview

Glyphosate and glufosinate are widely used herbicides and, thus, there is an interest in the reliable and sensitive determination of glyphosate in water and food. These pesticides are difficult to extract and analyze because of their high polarity. Here we describe an automated workflow for the FMOC-derivatization, sample cleanup, and LC-MS/MS detection using a GERSTEL Multi Purpose Sampler (MPS) 2XL configured with an online solid phase extraction (SPE^{XOS}) module coupled to an SCIEX QTRAP® 4500 system for the identification and quantitation of glyphosate, its major metabolite AMPA, and glufosinate in water and food samples.

Introduction

Glyphosate (N-phosphonomethyl glycine) and glufosinate [ammonium (S) -2-amino-4-[hydroxyl (methyl) phosphinoyl] butyrate] are non-selective post emergence herbicides used for the control of a broad spectrum of grasses and broad-leaf weed species in agricultural and industrial fields. Aminomethyl-phosphonic acid (AMPA) is the major metabolite of glyphosate and also included into the pesticide residue definition.[1, 2]

There is interest in the reliable and sensitive determination of residues of these pesticides in water and food. Due to their high polarity it is difficult to extract these pesticides from samples and to retain them on LC phases. Derivatization with fluorenylmethyloxycarbonyl chloride (FMOC-Cl) is a common procedure to improve extraction and separation for the analysis of glyphosate and related compounds. Previously reported methods using derivatization with FMOC-Cl have inherent limitations, such as long derivatization times, long LC run times, and often suffer from lack of repeatability and reproducibility.

Here we present an automated workflow to derivatize and analyze water and food samples for glyphosate, glufosinate and AMPA by LC-MS/MS using a GERSTEL Multi Purpose Sampler (MPS) 2XL with SPE^{XOS} coupled to a SCIEX QTRAP® 4500 system (Figure 2).

Water samples were injected directly into the LC-MS/MS system providing sufficient sensitivity to identify and quantify targets at sub 100 µg/L concentrations. Food samples can be injected directly after automatic derivatization followed by extensive dilution or can be cleaned up using online SPE prior LC-MS/MS analysis. Target compounds can be easily identified and quantified at 10 µg/kg levels with excellent repeatability.

Experimental

Derivatization and Sample Preparation

Water samples were analyzed directly and food samples were extracted using the QuPPe (Quick Polar Pesticides) method developed by the EU Reference Laboratories for Residues of Pesticides.[3] QuPPe results were compared to results obtained when using an extraction method reported by Miles et al.[4]

Derivatization and cleanup was performed using the GERSTEL MPS 2XL with SPE^{XOS} system configured for automatic sample handling, derivatization, and online SPE. The Gerstel system is fully controlled using the GERSTEL MAESTRO version 1.4 coupled to Analyst® software version 1.6.

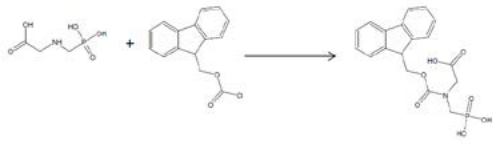

Figure 1. Derivatization of glyphosate using FMOC-Cl

Figure 2. GERSTEL MPS 2XL with SPE^{XOS} coupled to an QTRAP® 4500 system

Automated Derivatization Procedure (Figure 1)

1. Add 100 µL of borate buffer (pH=9) to 1 mL of sample.
2. Add 200 µL of 10mM FMOC-Cl solution.
3. Agitate for 20 min at 50°C.
4. Cool to bring to ambient temperature.
5. Add 130 µL 2% H_3PO_4.

Water samples were derivatized and injected directly (10 µL) into LC-MS/MS.

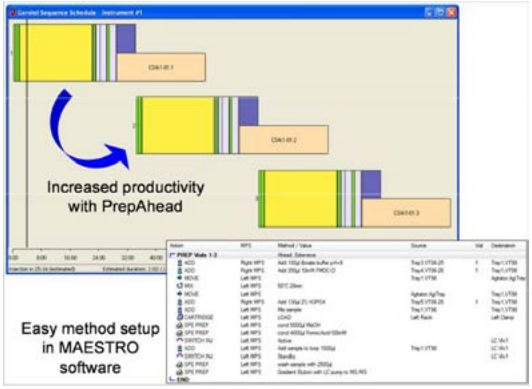

Figure 3a. Sequence of scheduled events in the Maestro software for online SPE: green - adding buffer and FMOC-Cl, yellow - derivatization, light blue - online SPE, orange LC-MS/MS analysis, dark blue washing of the autosampler, the PrepAhead function increases productivity by simultaneously preparing the following sample while perming LC-MS/MS analysis of the previous sample

Automated Online-SPE Cleanup Procedure

1. Condition GERSTEL SPE^{XOS} C8EC-SE (18.5 mg) cartridge with methanol and water + 100 mM formic acid.
2. Load 1 mL of derivatized sample onto SPE.
3. Wash with water + 100 mM formic acid.
4. Elute with LC pump gradient

Food sample extracts were diluted extensively to minimize possible matrix effects and automatically cleaned up using SPE^{XOS} system. Here we injected 1 mL of the diluted sample extract onto the SPE cartridge. (Figures 3a and 3b)

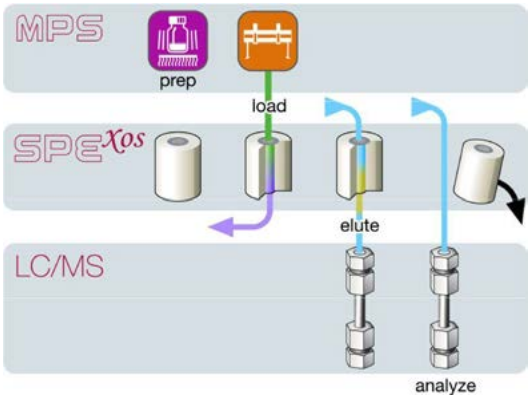

Figure 3b. Sequence of scheduled events when using the automated workflow of FMOC-derivatization, SPE cleanup, and LC-MS/MS detection

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LC Separation

The analyses were performed using a Phenomenex Gemini 3µ C18 (150 x 2 mm) column with a gradient of (A) 50 mM ammonium acetate adjusted to pH= 9 and (B) Acetonitrile. The gradient conditions are listed in Table 1.

Table 1. LC gradient used for separation

Time (min)	Flow (mL/min)	A (%)	B (%)
0	0.25	80	20
10	0.25	5	95
15	0.25	5	95
15.1	0.25	80	20
25	0.25	80	20

MS/MS Detection

The analyses were performed on a SCIEX QTRAP® 4500 LC/MS/MS system using the Turbo V™ source operated in electrospray ionization and negative polarity with an IS voltage of -4200 V.

The Curtain Gas™ interface (CUR) was set to 30 psi, nebulizer gas (Gas 1) set to 50 psi, drying gas (Gas 2) set to 70 psi, and the source temperature set to 400ºC.

The MRM transitions used for the detection of pesticides are shown in the table below. Each MRM was monitored with a dwell time of 100 ms.

Table 2. MRM transitions used for detection

Compound	Q1	Q3	CE (V)
Glyphosate	390	168, 150	-18, -34
Glufosinate	402	180, 206	-16, -20
AMPA	322	110, 136	-12, -22

Analyst® version 1.6.1 was used for data acquisition and MultiQuant™ version 3.0 software was used for qualitative and quantitative processing.

Results and Discussion

A standard chromatogram after automatic derivatization is shown in Figure 4.

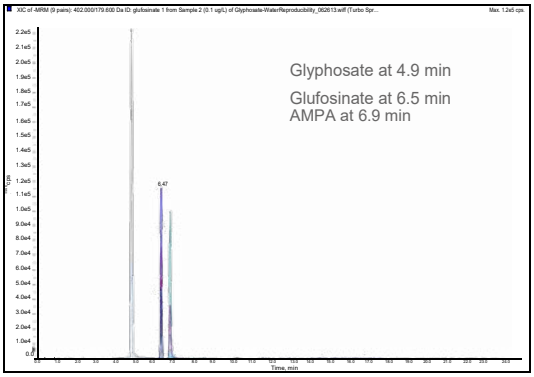

Figure 4. Standard chromatogram at a concentration of 10 ng/mL

A drinking water sample was spiked at 0.1 and 10 µg/L, automatically derivatized, and analyzed in triplicates. The method allowed accurate quantitation of all target compounds well below 0.1 µg/L with excellent repeatability (Figure 5 and Table 3).

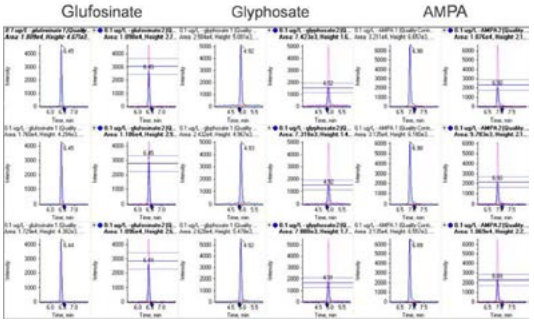

Figure 5. Triplicate analysis of polar pesticides in a spiked water sample at 0.1 µg/L (injection volume of 10 µL), ion ratios for compound identification were calculated automatically in MultiQuant™ software version 3.0

Table 3. Triplicate analysis of polar pesticides in a spiked water sample at 0.1 µg/L (injection volume of 10 µL)

Compound	Concentration (µg/L)	%CV of MRM 1	%CV of MRM 2
Glyphosate	0.1	4.0	3.9
	10	7.7	8.9
Glufosinate	0.1	2.3	4.5
	10	4.6	5.4
AMPA	0.1	1.4	5.3
	10	5.1	5.4

Different food matrices (corn and soy bean) where spiked with glyphosate, glufosinate, AMPA at 10 and 100 µg/kg and extracted using the QuPPe (Quick Polar Pesticides) method:

1. Add 10 mL water to 5 g of homogenized sample, shake and soak for 10 min.
2. Add 10 mL of acidified methanol (1% formic acid).
3. Shake vigorously for 1 min and centrifuge (at 3000 rpm) for 10 min.
4. Load 1 mL onto the Gerstel MPS 2XL system for automated dilution, derivatization, and SPE cleanup followed by LC-MS/MS analysis.

Corn and soy samples were spiked at 10 and 100 µg/kg and analyzed in triplicates using the automated derivatization and cleanup procedure. The method allowed accurate quantitation of all target compounds well below the target concentration of 100 µg/kg with excellent repeatability (Table 4, Figures 6 and 7).

Table 4. Triplicate analysis of polar pesticides spiked into corn and soy samples 100 µg/kg

Compound	Concentration (µg/kg)	%CV of MRM 1	%CV of MRM 2	Ion ratio (%RSD)
Glyphosate	100 (in corn)	3.6	6.0	0.36 (1.9%)
	100 (in soy)	5.1	5.9	0.31 (1.9%)
Glufosinate	100 (in corn)	1.6	12.5	0.71 (8.9%)
	100 (in soy)	5.2	7.7	0.67 (3.9)%
AMPA	100 (in corn)	5.7	4.8	0.43 (0.9%)
	100 (in soy)	5.3	6.2	0.38 (2.2%)

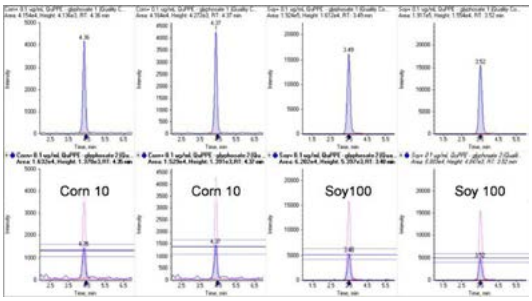

Figure 6. 10 and 100 µg/kg of glyphosate spiked into corn and soy and analyzed using automatic derivatization, dilution, and cleanup followed by LC-MS/MS, ion ratios for compound identification were calculated automatically in MultiQuant™ software version 3.0

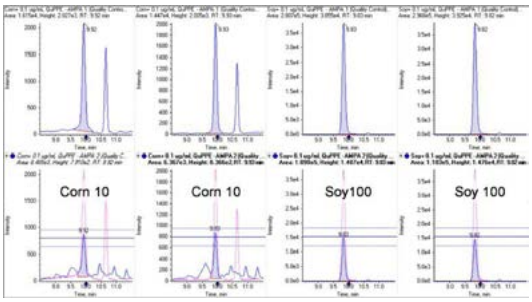

Figure 7. 10 and 100 µg/kg of AMPA spiked into corn and soy and analyzed using automatic derivatization, dilution, and cleanup followed by LC-MS/MS, ion ratios for compound identification were calculated automatically in MultiQuant™ software version 3.0

Ion ratios for compound identification where automatically calculated in the result table in MultiQuant™ software version 3.0. The quantifier and qualified ratio was found to be a valuable tool to identify all target pesticides in matrix samples with excellent reproducibility and values well in between ± 20% (Table 4).

The slightly higher %RSD of the ion ratio of glufosinate in corn can be explained by interfering matrix signals (Figure 8). Stable LC separation was essential for confident identification and accurate quantitation of glufosinate.

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The results using the QuPPe extraction where compared to results obtained when using the none QuPPe procedure based on extraction with 0.1 M HCl.[8] In general, recoveries were between 70-120% for both matrices when using the QuPPe protocol with slightly better recoveries in corn due to the lower protein content.

Recoveries using the none QuPPe extraction where found to be lower in all cases. However, in the case of corn this extraction resulted in cleaner MRM chromatograms for glufosinate (Figure 8).

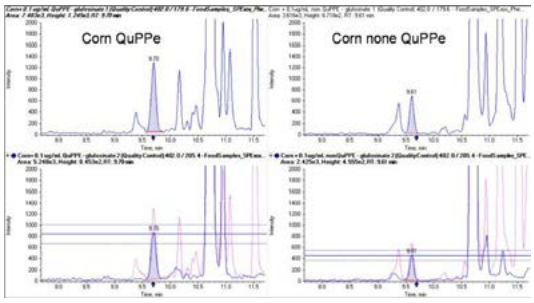

Figure 8. Corn analyzed for glufosinate using the QuPPe and a none QuPPe extraction procedure with higher recoveries but more matrix interferences when using the QuPPe protocol

The total cycle time per sample for the automated sample derivatization and online SPE was approximately 25 minutes, enabling "just in time" sample preparation using the GERSTEL MAESTRO software PrepAhead function. Using this automated procedure for derivatization, extraction and analysis over 55 samples can be processed per day.

Future studies will include the use of isotopically labeled standards to compensate for possible matrix effects. Also retention time shifts were observed when analyzing glyphosate in matrix samples with high protein content. The use of an internal standard will increase confidence in identification using relative retention times.

Summary

As a result of this study, we were able to show:

- Glyphosate, glufosinate, and AMPA can be detected after automatic derivatization using FMOC-Cl at relevant concentration in drinking water and food samples[5-7]
- The described workflow using the GERSTEL MPS 2XL with SPE^{XOS} coupled to a SCIEX QTRAP® 4500 system enabled automated derivatization, dilution, and SPE cleanup and analysis of water and QuPPe extracts of food for LC-MS/MS of polar pesticides.
- The method is highly repeatable with %CV well below 10% due to the automation of sample handling and derivatization.
- Sensitivity was sufficient to inject water samples directly and detect all target compounds below 0.1 µg/L. Food samples can be diluted prior SPE cleanup using the online SPE to monitor at 10 µg/kg.

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6 Commission Regulation (EU) 'regards maximum residue levels' No 441/2012

7 Council Directive 'concerning the quality of water intended for human consumption' 98/83/EC

8 O. Cabrices et al.: 'Automated Derivatization, SPE Cleanup and LC-MS/MS Determination of Glyphosate and Other Polar Pesticides' Application Note Gerstel (2013) #AN9/2013

Publication number: 8013813-01

An Automated Sample Preparation and Analysis Workflow for Mycotoxin Contamination in Different Food Matrices

André Schreiber[1] and Oscar G. Cabrices[2]
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Overview

In this publication, we describe a completely automated sample preparation workflow for the extraction and screening of multi-mycotoxin contamination in different food matrices (corn, wheat) by LC-MS/MS. The extraction methodology was performed using a GERSTEL Multi Purpose Sampler (MPS) 2XL followed by analysis with a SCIEX QTRAP® 4500 system. The automated sample preparation workflow involved centrifugation, dispersive solid phase extraction (dSPE) and evaporative concentration, providing extraction efficiencies greater than 70% with relative standard deviations (RSD) less than 15% for most analytes.

The LC-MS/MS method was developed for screening a panel of 14 mycotoxins (aflatoxins, trichotecenes and fuminosins) using the *Scheduled* MRM™ algorithm in combination with fast polarity switching, achieving excellent linearity (r^2 values of 0.98 or greater), average accuracies greater than 88% and limits of quantitation lower than the action levels established by the EU and US FDA.

Introduction

One of the major challenges in food safety is the abundance of natural toxic contaminants known as mycotoxins. Mycotoxins are secondary metabolites (by-products) that are produced by different types of filamentous fungi genus such as Aspergillus (Aflatoxins), Penicillum (Ochratoxin A) and Fusarium (trichothecenes, fumonisins, deoxynivalenol and zearalenone).[1] The presence of these compounds in agriculturally grown products is an important concern due to the health risks they pose to humans and livestock.[2] For this reason, it is crucial to have monitoring and surveillance methods that screen for mycotoxin presence in a variety of food and feeds.

These analytical methods typically require many manual steps making it a quite labor-intensive and time consuming process. In this publication, we describe a completely automated sample preparation workflow using a GERSTEL MPS 2XL configured for the extraction of multi-mycotoxin residues in different food products for analysis by LC-MS/MS. This workflow features centrifugation, spiking and calibration line generation, a dSPE technique referred to as disposable pipette extraction (DPX), and evaporative concentration for subsequent LC-MS/MS analysis.

DPX is based on sorbent loosely contained inside pipette tips, which is used to remove matrix interferences and provide a cleaned sample. We have evaluated two different automated sample cleanup strategies using DPX in order to efficiently extract a panel of 14 different mycotoxins for analysis with a SCIEX QTRAP® 4500 system. The LC-MS/MS method utilized the *Scheduled* MRM™ algorithm in combination with fast polarity switching. The method was successfully applied to quantify and identify multi-mycotoxin residues in a number of DPX extracts of agricultural commodities (corn, wheat). In addition full scan MS/MS spectra were acquired to allow library searching to increase confidence in identification.

Experimental

Materials

1 mL ampoules of the following mycotoxins were obtained from Romer Labs for this study: Mix 1 Aflatoxins B1, B2, G1 and G2 (AFB1, AFB2, AFG1, AFG2), Fumonisin B1 (FB1), Ochratoxin A (OTA), and Zearalenone (ZON). A trichothecenes A&B dry standard containing 0.2 mg of the following mycotoxins was obtained from Trilogy Analytical Laboratory: Fusarenon-X (FUS-X), Deoxynivalenol (DON), 3-Acetyldeoxynivalenol (3-AcDON), HT-2 Toxin (HT-2), T-2 Toxin (T-2), Diacetoxyscirpenol (DAS) and Neosolaniol (NEO).

MycoSpin 400 multitoxin columns were purchased from Romer Labs. 100 mg of the cleanup sorbent contained in the MycoSpin columns were packed in empty 1mL DPX tips, and are referred as DPX-MYCO tips. DPX-WAX-1mL (30 mg, 10-20 µ) tips were obtained from DPX Labs. Mycotoxin free and Quality Control (QC) corn and wheat midds samples with known concentrations

Contents

Figure 1. The GERSTEL MPS 2XL equipped with a CF-100 dual position centrifuge, mVAP multi-evaporation station, mVORX vortex and DPX option and the SCIEX QTRAP® 4500 system

of mycotoxins were donated by the Maryland Department of Agriculture. All solvents used were LC grade.

Automated Sample Preparation

All automated mycotoxin sample preparation PrepSequences were performed using a GERSTEL MPS 2XL equipped with a CF-100 dual position centrifuge, mVAP multi-evaporation station, mVORX vortex and DPX option as shown in Figure 1.

The automated sample preparation workflow performed by the MPS 2XL is described below:

1. Weigh 1 g of homogenized sample into a sealed 10 mL vial and place on MPS.
2. MPS adds 4 mL of acetonitrile/water (84/16) to vial and moves it to agitator for mixing and extraction (1 h at 55°C and 500 rpm).
3. MPS moves sample to centrifuge for centrifugation (10 min at 1900 rpm).
4. MPS transfers 500 µL of supernatant into test tube for DPX cleanup.
5. MPS transfers 200 µL of cleaned extract to a sealed 2 mL vial for evaporation (5 min at 55°C) and reconstitution in 500 µL of mobile phase A.
6. Cleaned Sample is injected for LC-MS/MS analysis.

Using the GERSTEL MAESTRO software, it is possible to enhance the productivity of the proposed sample preparation workflow by using the PrepAhead feature, which allows staggering different stages of the sample preparation workflow (Figure 2).

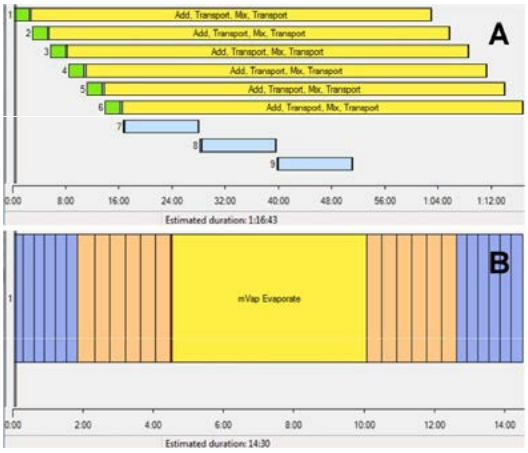

Figure 2. Graphical representations of sample preparation using PrepAhead with the MAESTRO software

A: Mixing, extraction, and centrifugation of samples, centrifugation of first sample batch occurs while the following batch is being mixed and extracted

B: Batch evaporation and reconstitution of 6 extracts in less than 15 min

LC Separation

The LC-MS/MS analyses were performed using an Agilent 1200 Series LC pump configured with a Phenomenex Gemini 5 µm (110 Å, 150 x 4.6 mm) column. Mobile phase A consisted of water/methanol/acetic acid (89/10/1) + 5mM ammonium acetate and mobile phase B of methanol/water/acetic acid (97/2/1) + 5mM ammonium acetate. Gradient conditions are listed in Table 1.

Contents

Table 1. LC gradient used for separation

Time (min)	Flow (µL/min)	A (%)	B (%)
0.00	700	90	10
2.00	700	90	10
12.00	700	5	95
16.00	700	5	95
16.01	700	90	10
20.00	700	90	10

The column oven was set to 40°C.

The GERSTEL MPS 3C autosampler configured with a Modular Active Washstation and Cheminert C2V injection valve was used to inject 50 µL of samples.

MS/MS Detection

The SCIEX QTRAP® 4500 system was operated with Turbo V™ source and Electrospray Ionization (ESI) probe. Approximately 29 MRM transitions were monitored in both positive and negative polarity. The *Scheduled* MRM™ algorithm was used in combination with fast polarity switching using Analyst® 1.6.1 software.

For increased confidence in compound identification QTRAP® MS/MS spectra at a scan speed of 10000 Da/s were acquired using dynamic fill time for optimal MS/MS quality. MS/MS spectra were generated using standardized Collision Energy (CE) of ±35 V with Collision Energy Spread (CES) of 15 V to ensure a characteristic MS/MS pattern independent of the compound's fragmentation efficiency. MS/MS spectra were searched against the iMethod™ Mycotoxin Library version 1.0.

MultiQuant™ software version 3.0 was used for quantitative data processing. LibraryView™ software version 1.0 was used for MS/MS library searching.

Results and Discussion

Automated DPX Strategies

DPX differs from other SPE approaches in that sample solutions are dynamically mixed with the sorbent within the pipette tip. The extraction efficiency is dependent on the equilibration time between solutions and sorbent, rather than flow rates through a packed bed.

Two strategies were evaluated to ensure maximum recovery of all mycotoxins described in Table 3.

Table 2. MRM transitions and retention times (RT) used for detection using the *Scheduled* MRM™ algorithm

Compound	RT (min)	Polarity	Q1	Q3
3-AcDON	10.3	negative	397	59 / 337
AFB1	11.8	positive	313	285 / 241
AFB2	11.4	positive	315	287 / 259
AFG1	11.0	positive	329	243 / 200
AFG2	10.6	positive	331	245 / 313
DAS	11.9	positive	384	307 / 105
DON	7.2	negative	355	59 / 295
FB1	12.5	positive	722	704 / 334 / 352
FUS-X	8.7	negative	413	59 / 353
OTA	14.1	positive	404	239 / 102
HT-2	12.8	positive	447	345 / 285
NEO	8.8	positive	400	215 / 185
T-2	13.6	positive	484	215 / 185
ZON	14.4	negative	317	131 / 175

Table 3. DPX strategies evaluated in this study

(1) DPX using 100 mg DPX-MYCO	(2) DPX using 30 mg DPX-WAX and 100 mg DPX-MYCO
500 µL of sample is aspirated and trapped with sorbent inside tip.	500 µL of sample is aspirated with DPX-WAX and dispensed back to test tube.
	~500 µL of sample is then aspirated with DPX-MYCO and trapped inside tip.
MPS moves tip to mVORX for cleanup (30 s, 2500 rpm).	MPS moves tip to mVORX for cleanup (30 s, 2500 rpm).
Sample is eluted back to test tube.	Sample is eluted back to test tube.
	MPS adds 250 µL of 4% acetonitrile to top of DPX-WAX and elutes acidic mycotoxins to the same test tube combining both eluents.
MPS transfers 200 µL to sealed 2 mL vial for evaporation and reconstitution.	MPS transfers 200 µL to sealed 2 mL vial for evaporation and reconstitution.

Relative recoveries were determined by comparing results from spiked corn samples (5 replicates) with matrix-matched samples prepared by adding the neat mixture of mycotoxin standards directly to the eluent of the extracted blank matrix. The total DPX extraction time ranged between 5-9 minutes per sample allowing high throughput sample preparation and analysis.

Strategy (1) averaged analyte recoveries between 15%-110% with relative standard deviations (RSD) of 4%-15%, whereas Strategy (2) averaged slightly higher recoveries ranging between 45%-120% with RSD of 2%-15% (Figure 3).

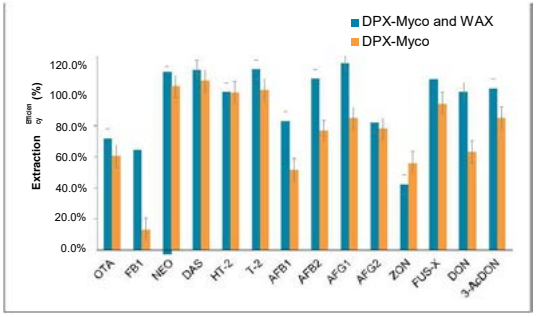

Figure 3. Recoveries and relative standard deviation for all mycotoxins after automated DPX extraction

It was observed that using strategy (1), the FB1 irreversibly bound to the resin in the DPX-MYCO, however, by initially using DPX-WAX in strategy (2) the FB1 selectively binds to the resin and can be eluted off improving its recovery, suggesting the possibility of including the rest of the fumonisin mycotoxin family. This WAX sorbent also has reversed phase characteristics, which can selectively extract some the mycotoxins of interest. It should be noted that using an internal standard would significantly improve the reproducibility. In this preliminary study no internal standards were used.

Validation of the Automated Workflow

Figure 4 shows MRM chromatograms from a mycotoxin-fortified corn sample extract at 10 µg/kg using fast polarity switching. All 14 mycotoxins were successfully monitored from this sample matrix at low concentrations using the automated DPX-LC-MS/MS sample preparation and analysis workflow.

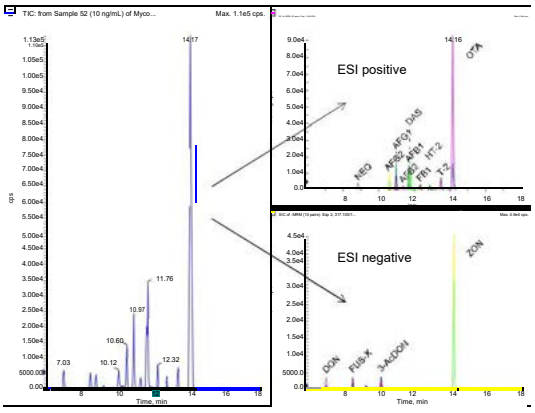

Figure 4. Representative MRM chromatograms from a matrix matched corn sample extract at 10 µg/kg

Figure 5 shows representative calibration lines for DON, AFB1, and OTA obtained after analysis of solvent standards.

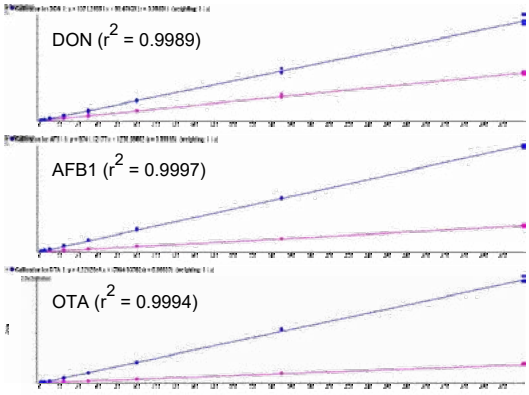

Figure 5. Calibration lines of the quantifier and qualifier MRM for DON (top), AFB1 (middle), and OTA (bottom)

The resulting calibration lines were shown to be linear from at least 2 to 500 ng/mL for the mycotoxins monitored, using a linear fit with 1/x weighting. Limits of quantitation (LOQ) lower than the action levels established by the FDA and EC.[3,4]

Accuracies were well between 80 and 120% with %CV of less than 15% (n=3).

LC-MS/MS Analysis of QC and Incurred Samples

Figures 5a and 5b show MRM chromatograms for from QC samples (wheat middlings and corn) containing 250 µg/kg DON and 20 µg/kg OTA, respectively.

Sample data were processed using MultiQuant™ software version 3.0. Built-in queries of MultiQuant™ software can be used to calculate ion ratios and flag outliers. Ion ratio tolerances for each analyte are defined in the quantitation method editor. The ion ratio is also visualized using tolerance bars in the Peak Review pane (Figure 5).

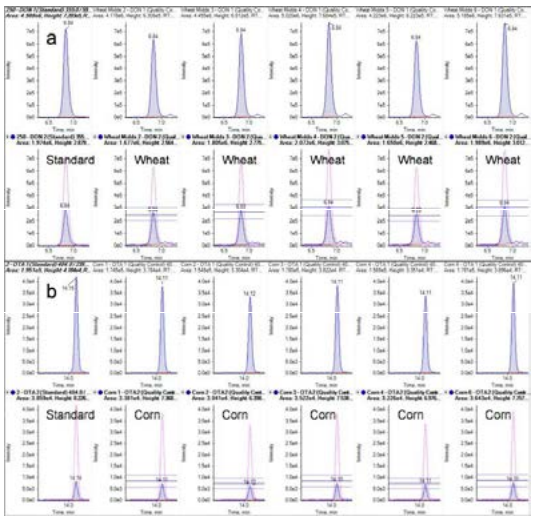

Figure 5. Peak review of wheat middlings containing 250 µg/kg DON **(a)** and corn containing 20 µg/kg OTA **(b)**, MRM ratio are automatically calculate and displayed with tolerance levels

DON was quantified in wheat middlings with an accuracy of 99.2% with %CV of 9.9 (n=5). The average ratio of quantified and qualifier MRM was 0.399 with %CV of 2.6 (expected ratio 0.398) was well inside the 25% tolerance of the guideline SANCO/12495/2011.[LIT]

OTA was quantified in corn with an accuracy of 104.6% with %CV of 4.5 (n=5). The average ratio of quantified and qualifier MRM was 0.200 with %CV of 2.3 (expected ratio 0.221) was well inside the 25% tolerance.

For improved confidence in compound identification full scan MS/MS spectra were automatically acquired using information dependent acquisition with the *Scheduled* MRM™ experiment as survey scan. The rapidly collected high quality MS/MS data were used for mass spectral library searching, using LibraryView™ software version 1.0. An example of identification of FB1 with a Purity of 96.4% is shown in Figure 6.

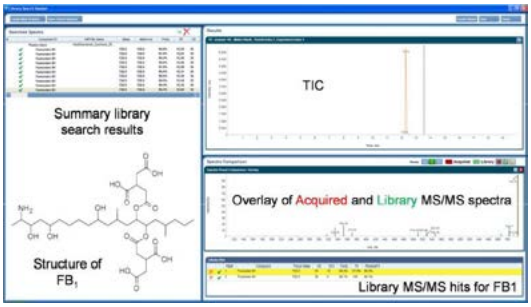

Figure 6. Automated identification of FB1 in a contaminated corn sample using MS/MS library searching

Summary

As a result of this work, we were able to demonstrate:

- A completely automated sample preparation workflow for the efficient extraction and analysis of multi-mycotoxin residues in different food matrices using the GERSTEL MPS 2XL and the SCIEX QTRAP® 4500 system.
- Automated dispersive SPE using DPX requires only small volumes of sample (~500 µL), which allowed for fast sample preparation (5-9 min/sample) with average extraction efficiencies greater than 70% and good reproducibility %CV less than 15%) using 2 different cleanup strategies for all mycotoxins analyzed.
- The method for the quantitation of 14 mycotoxins was successfully developed using the *Scheduled* MRM™ algorithm in combination with fast polarity switching, achieving excellent linearity, accuracies, and reproducibility, and limits of quantitation lower than the action levels established by the EU and US FDA.
- QTRAP® *Scheduled* MRM™ and MS/MS scanning allowed high confidence compound identification based on MRM ratios but also using mass spectral library searching.

References

1 M. Sulyok et al.: 'Development and Validation of a Liquid Chromatography/Tandem Mass Spectrometric Method for the Determination of 39 Mycotoxins in Wheat and Maize' Rapid Commun. Mass Spectrom. 20 (2006) 2649-59

2. K. Zhang et al.: 'Determination of Mycotoxins in Milk-Based Products and Infant Formula Using Stable Isotope Dilution Assay and Liquid Chromatography Tandem Mass Spectrometry' J. Agric. Food Chem. 61 (2013), 6265-6273

3 U.S. Food and Drug Administration: In Compliance Program Guidance Manual, Program 7307.001, Chap 7. (2004)

4 Commission Regulation (EC) No. 1881/2006 of December 2006. Off. J. Eur. Communities

5 Commission Regulation (EC) No 1881/2006 'maximum levels for certain contaminants in foodstuffs'

6 Document N° SANCO/12495/2011 'Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed'

8 O. Cabrices et al.: 'Development of an Automated Sample Preparation and Analysis Workflow for Mycotoxin Residues in Different Food Matrices' Application Note Gerstel (2013) #AN/2013/10

Automated Sample Preparation and Analysis Workflows for Pesticide Residue Screening in Food Samples using DPX-QuEChERS with LC-MS/MS

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Overview

QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation methods have been developed to help monitor pesticides in a range of food samples. These methods require many manual steps, such as shaking, centrifugation, and dispersive SPE cleanup, making it a quite labor-intensive process. There is a need for automating parts of QuEChERS extraction in order to improve laboratory productivity for monitoring pesticide residue in foods.

In this publication, we describe an automated sample preparation and analysis workflow for the screening of over 200 pesticides in different food matrices by LC-MS/MS. The automated cleanup of the QuEChERS extracts was performed using Disposable Pipette Extraction (DPX) with a GERSTEL MultiPurpose Sampler (MPS) 2XL interfaced to an SCIEX QTRAP® 4500 LC/MS/MS system. The sensitivity and selectivity of the LC-MS/MS system enabled us to identify and quantify with limits of detection which meet acceptance criteria for reporting Maximum Residue Levels (MRL) as established by regulatory agencies. The ability to automate the dispersive SPE cleanup of QuEChERS extracts followed by direct LC-MS/ MS analysis resulted in improved laboratory productivity by streamlining the complete analytical process.

Introduction

QuEChERS protocols are widely used to prepare samples for the monitoring of pesticide residues in food. These methods require many manual steps, such as shaking, centrifugation, and dispersive SPE cleanup, making it a quite labor-intensive process. A laboratory's productivity and efficiency can be greatly improved by automating parts of the QuEChERS procedure, the dispersive SPE cleanup step and subsequent dilution prior LC-MS/MS analysis.

A simpler and more practical way to perform the dispersive SPE cleanup method is to use DPX tips. These tips have a screen that retains loose sorbent material inside the pipette tip. The DPX tips used for this project contain anhydrous magnesium sulfate ($MgSO_4$) and primary and secondary amine (PSA) as cleanup sorbents and are denominated "QuEChERS Tips".

Here we present a new automated sample preparation and analysis workflow for pesticide residue screening of food samples using DPX-QuEChERS with LC-MS/MS. The use of QuEChERS tips has been reported previously[1-3] and has been found to provide comparable results to those obtained using manual methods based on dispersive SPE. A GERSTEL MPS 2XL equipped with DPX option coupled to an SCIEX QTRAP® 4500 system was used for the automated cleanup of QuEChERS extracts and extract dilution. The LC-MS/MS method utilized the *Scheduled* MRM™ algorithm to obtain the best data quality in combination with fast polarity switching to cover the broadest range of pesticides possible. In addition, QTRAP® full scan MS/MS spectra were acquired to allow library searching in order to increase confidence in identification.

The method was successfully applied to identify and quantify over 200 pesticides in QuEChERS extracts of fruit, vegetable, herb and spice samples.

Contents

Figure 1. GERSTEL MPS 2XL with DPX option coupled to an QTRAP® 4500 system

Experimental

Materials

- Fruit, vegetable, herb, and spice samples, including organic produce, from a local supermarket
- SCIEX iD*Quant*™ standards kit for pesticide analysis plus additional pesticides of interest (Table 1)
- Serial dilutions to prepare calibration standards with concentration of 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000 ng/mL
- Acetonitrile extracts of blank matrix samples, incurred samples and fortified samples using commercial QuEChERS kits following the AOAC method 2007.1
- DPX QuEChERS tips provided by DPX labs containing PSA (75 mg), $MgSO_4$ (25 mg) and GCB (12.5 mg) for dispersive SPE cleanup

QuEChERS Pretreatment

1. Pipette 1 mL of the acetonitrile extract obtained following the 1st centrifugation step of the QuEChERS sample preparation method, into an autosampler vial.
2. Place the sample onto a tray on the dual head GERSTEL MPS XL configured for automated DPX-QuEChERS LC-MS/MS analysis.

Automated QuEChERS Sample Preparation Sequence[5]

1. MPS transfers 500 µL of QuEChERS extract to an open test tube.
2. DPX-QuEChERS tip is picked up and transported to the test tube for sample cleanup.
3. Sample is aspirated into the tip, mixed for 30 sec and discharged to test tube. Repeat 3 times.
4. MPS transfers 50 µL of cleaned extract to a sealed vial, where it is diluted with 450 µL of mobile phase A.
5. The diluted cleaned extract is injected to the LC-MS/MS for analysis.

A schematic of the automated DPX-QuEChERS procedure is shown in Figure 2.

Table 1. Pesticides monitored using the automated DPX-QuEChERS-LC-MS/MS method

3-Hydroxycarbofuran	Acephate	Acetamiprid	Acibenzolar-S-methyl	Alanycarb	Aldicarb
Aldicarb sulfone	Aldicarb sulfoxide	Aspon	Avermectin B1a	Avermectin B1b	Azadirachtin
Azoxystrobin	Benalaxyl	Bendiocarb	Benfuracarb	Benoxacor	Benthiavalicarb
Benzoximate	Bifenazate	Bifenthrin	Bitertanol	Boscalid	Bromuconazole
Bupirimate	Buprofezin	Butafenacil	Butocarboxim	Butoxycarboxim	Cadusafos
Carbaryl	Carbendazim	Carbetamide	Carbofuran	Carboxin	Carfentrazone-ethyl
Chlordimeform	Chlorfenvinphos	Chlorfluazuron	Chlortoluron	Chloroxuron	Clethodim
Clofentezine	Clothianidin	Coumaphos	Cumyluron	Cyanazine	Cyanophos
Cyazofamid	Cycluron	Cymoxanil	Cyproconazole	Cyprodinil	Cyromazine
D_{10}-Diazinon	D_6-Dichlorvos	D_6-Dimethoate	D_6-Diuron	D_6-Linuron	D_6-Malathion
Daimuron	Dazomet	Deltamethrin	Diazinon	Dichlorvos	Dicrotophos
Diethofencarb	Difenoconazole	Diflubenzuron	Dimethenamid	Dimethoate	Dimethomorph
Dimoxystrobin	Diniconazole	Dinotefuran	Dioxacarb	Disulfoton	Dithiopyr
Diuron	Dodemorph	Fenpyroximate	Emamectin B1a	Emamectin B1b	Epoxiconazole
Eprinomectin B1a	EPTC	Esprocarb	Ethidimuron	Ethiofencarb	Ethion
Ethiprole	Ethirimol	Ethofumesate	Ethoprophos	Etobenzanid	Etofenprox
Etoxazole	Famoxadone	Fenamidone	Fenarimol	Fenazaquin	Fenbuconazole
Fenhexamid	Fenoxanil	Fenoxycarb	Fenpropathrin	Fenpropimorph	Fenuron
Flonicamid	Flucarbazone	Fludioxonil	Flufenacet	Flufenoxuron	Flumetsulam
Flumioxazin	Fluometuron	Fluquinconazole	Flusilazole	Fluthiacet-methyl	Flutolanil
Flutriafol	Forchlorfenuron	Formetanate	Fuberidazole	Furalaxyl	Furathiocarb
Heptenophos	Hexaconazole	Hexaflumuron	Hexythiazox	Hydramethylnon	Imazalil
Imazapyr	Imibenconazole	Imidacloprid	Indanofan	Indoxacarb	Ipconazole
Iprovalicarb	Isocarbamid	Isofenphos	Isopropalin	Isoproturon	Isoxaben
Isoxaflutole	Kresoxim-methyl	Lactofen	Leptophos	Linuron	Lufenuron
Mandipropamid	Mefenacet	Mepanipyrim	Mepronil	Metalaxyl	Metconazole
Methabenzthiazuron	Methamidophos	Methiocarb	Methomyl	Methoprotryne	Methoxyfenozide
Metobromuron	Metribuzin	Mevinphos	Mexacarbate	Molinate	Monocrotophos
Monolinuron	Moxidectin	Myclobutanil	Neburon	Nitenpyram	Norflurazon
Novaluron	Nuarimol	Omethoate	Oxadixyl	Oxamyl	Paclobutrazol
Penconazole	Pencycuron	Phenmedipham	Picoxystrobin	Piperonyl butoxide	Pirimicarb
Pirimicarb-desmethyl	Pirimicarb-desmethyl-formamide	Prochloraz	Promecarb	Prometon	Prometryn
Propachlor	Propamocarb	Propargite	Propazine	Propham	Propiconazole
Propoxur	Pymetrozine	Pyracarbolid	Pyraclostrobin	Pyridaben	Pyrimethanil
Pyriproxyfen	Quinoxyfen	Rotenone	Sebuthylazine	Secbumeton	Siduron
Simazine	Simetryn	Spinosyn A	Spinosyn D	Spirodiclofen	Spiromesifen
Spiroxamine	Sulfentrazone	Tebuconazole	Tebufenozide	Tebufenpyrad	Tebuthiuron

Contents

Table 1. (cont.)

Teflubenzuron	Temephos	Terbumeton	Terbutryn	Terbuthylazine	Tetraconazole
Tetramethrin	Thiabendazole	Thiacloprid	Thiamethoxam	Thiazopyr	Thidiazuron
Thiobencarb	Thiofanox	Thiophanate-methyl	Triadimefon	Triadimenol	Trichlamide
Trichlorfon	Tricyclazole	Trifloxystrobin	Triflumizole	Triflumuron	Triticonazole
Uniconazole	Vamidothion	Zoxamide			

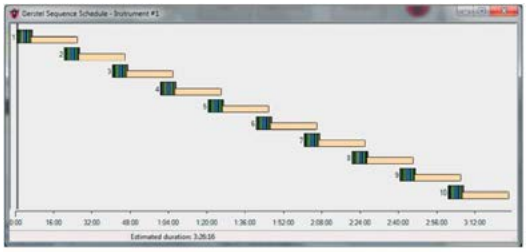

Figure 2. Example sample preparation sequence for automated DPX-QuEChERS LC-MS/MS analysis

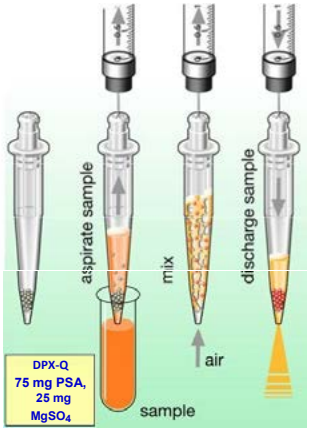

Figure 3. Example sample preparation sequence for automated DPX-QuEChERS LC-MS/MS analysis

Figures 3 and 4 show the automated sample preparation sequence used to perform DPX-QuEChERS.

Preparation of Solvent Standards and Matrix Matched Standards

1. Transfer 100 µL of previously extracted matrix blank or 100% acetonitrile into an empty autosampler vial.
2. Transfer 250 µL of mobile phase A into the vial.
3. Transfer 150 µL of the respective standard stock solution into the vial and mix.

LC-MS/MS Analysis

All analyses were performed using an Agilent 1200 Series LC system and a GERSTEL MPS MPS 2XL equipped with DPX option and a 10 µL stainless steel loop with active wash station.

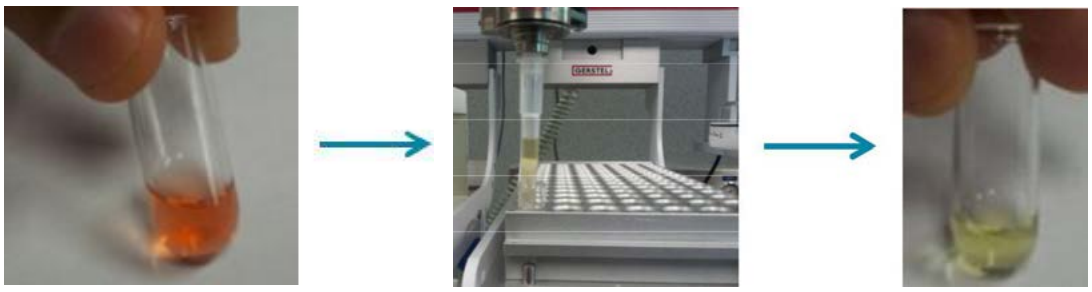

Figure 4. Schematic of the automated DPX-QuEChERS procedure, 500 µL of apple extract (left) and after DPX-QuEChERS cleanup (right)

A Phenomenex Synergi-Fusion 2.5u (50x2 mm) column was used with a gradient of water / methanol + 5 mM ammonium formate at a flow rate of 0.4 mL/min was used with a total run time of 20 min.

The SCIEX QTRAP® 4500 system was used with Turbo V™ source and Electrospray Ionization (ESI) probe operated in both positive and negative polarity. The *Scheduled* MRM™ algorithm was used for enhanced Signal-to-Noise (S/N), accuracy and reproducibility.[6]

Optimized MRM transitions for all pesticides were obtained through the MRM catalogue of the iMethod™ application for pesticide screening version 2.1. Two MRM transitions were monitored for each target pesticide to allow quantitation and identification using the MRM ratio. In addition, QTRAP® full scan MS/MS spectra were acquired to allow library searching in order to increase confidence in identification.

MultiQuant™ and LibraryView™ software was used for qualitative and quantitative data processing.

Results and Discussion

The automated DPX-QuEChERS cleanup method was performed to automatically remove matrix components from sample extracts prior to LC-MS/MS analysis. The removal of water ($MgSO_4$) and fatty acids (PSA) is necessary to ensure reproducible peak intensities for quantitative analysis. GCB is used to remove pigments, particularly chlorophyll and carotenoids.

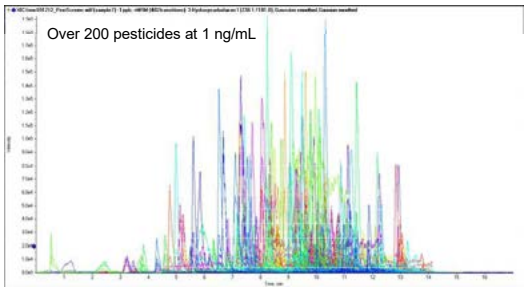

Figure 5. Detection of over 200 pesticides in a fortified cucumber sample at 1 ng/mL

Figure 5 shows a representative MRM chromatogram from a pesticide-fortified cucumber sample QuEChERS extract at 1 ng/mL. Over 200 pesticides were successfully detected in this sample matrix using the automated DPX-QuEChERS LC-MS/MS method.

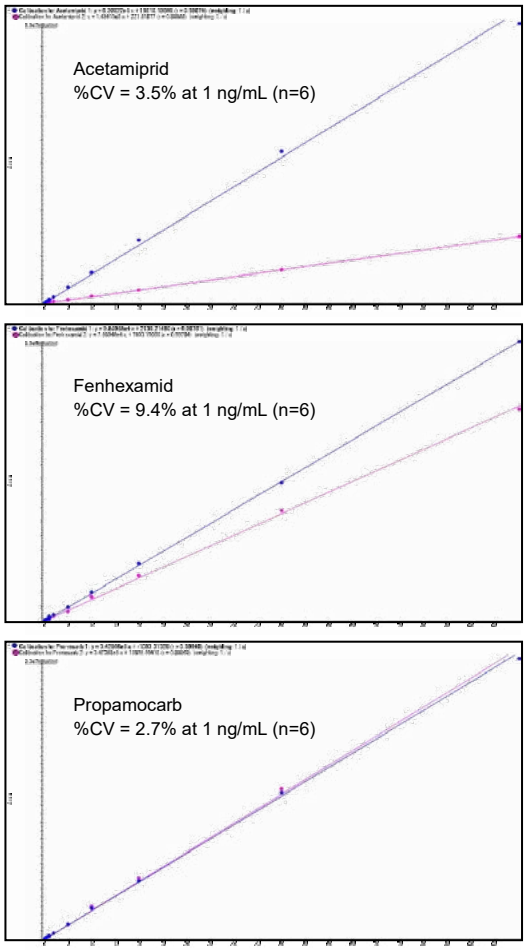

Figure 6. Representative calibration lines for Carbendazim, Propiconazole, and Thiacloprid from 0.1 to 100 ng/mL with an regression coefficient r^2 > 0.997 with excellent repeatability of %CV < 10%

Figure 6 shows calibration curves obtained using automated solvent standards. The resulting calibration curves were shown to be linear from at least 0.1 to 100 ng/mL with excellent repeatability for the pesticides monitored.

Contents

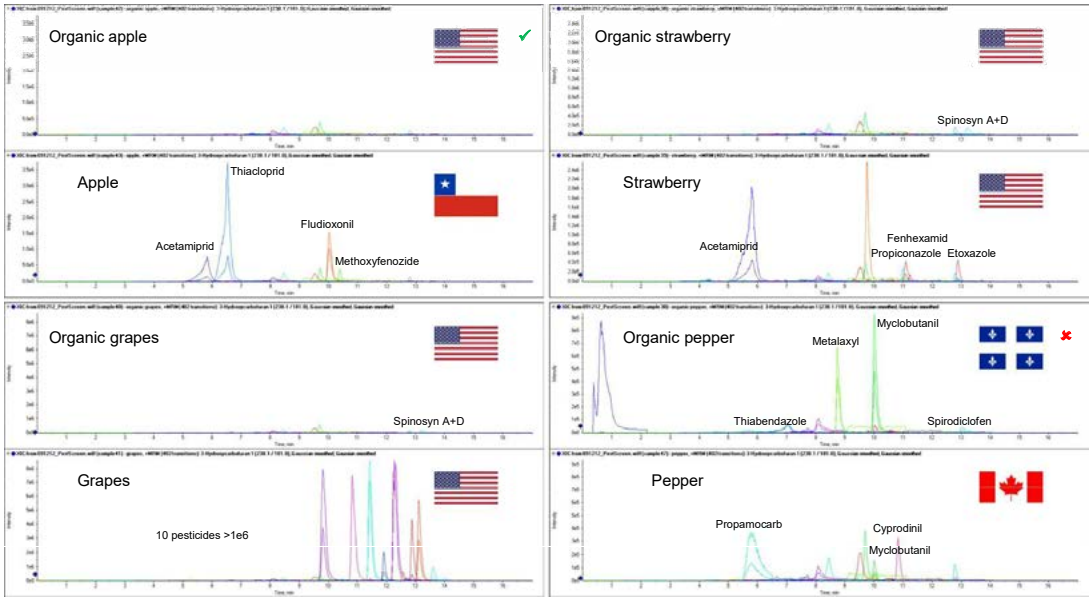

Figure 7. MRM chromatograms of pesticides identified in different food samples, including organic produce, from a local super market

The developed method was applied to the detection of pesticides in extracts of real food samples obtained from a local supermarket. QuEChERS extracts were cleaned using the DPX-QuEChERS method and diluted 10x for LC-MS/MS analysis (Figure 7).

Sample data was processed using MultiQuant™ software with the 'Multicomponent' query. Query files are customizable commands to perform custom querying of the result table. Figures 8a and b show examples of using the 'Multicomponent' query to flag pesticides present in sample extracts above a user

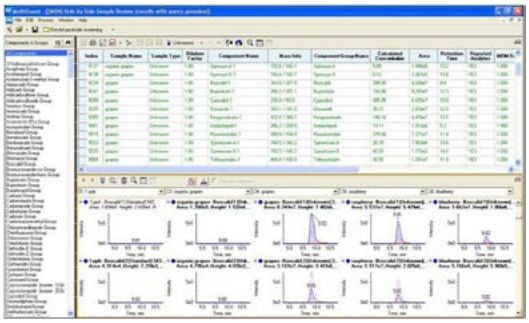

Figure 8a. Results review in MultiQuant™ software after using the 'Multicomponent' query with peak review for the pesticide Boscalid (bottom): 1 ng/mL standard, organic grapes, grapes, raspberry, and blueberry

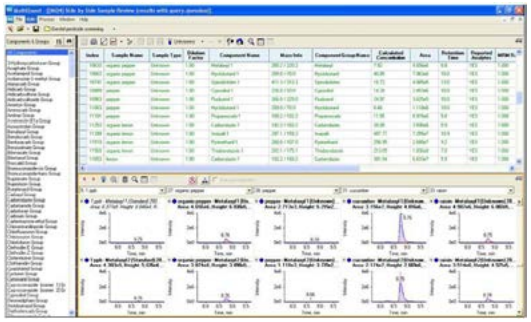

Figure 8b. Results review in MultiQuant™ software after using the 'Multicomponent' query with peak review for the pesticide Metalaxyl (bottom): 1 ng/mL standard, organic pepper, pepper, cucumber, and raisin

specified concentration level and with positive identification using the MRM ratio.

To increase confidence in identification additional full scan MS/MS experiments were performed and spectra were searched against the iMethod™ pesticide library. Extracted spectra and library search Purity score values using the LibraryView™ software are shown in Figures 9a and b for an paprika and tarragon with low analyte concentrations.

Figure 9a. Automated library identification for Pyrimethanil butoxide detected in a paprika extract after DPX-QuEChERS LC-MS/MS

Figure 9b. Automated library identification for Desmethyl-pirimicarb detected in a paprika extract after DPX-QuEChERS LC-MS/MS

Summary

As a result of this study, we were able to show:

- The described DPX-QuEChERS LC-MS/MS workflow using the GERSTEL MPS 2XL equipped with DPX option coupled to an SCIEX QTRAP® 4500 system enabled automated cleanup and analysis of QuEChERS extracts for screening and confirmation of over 200 pesticides in a single LC-MS/MS run.
- Quantitative analysis was performed in the same run allowing for both quantitation and qualitative data to be collected simultaneously. Linear calibration curves resulting in r^2 values of 0.99 or greater were achieved for the samples analyzed.
- With this configuration a 15 min/sample cycle time is achieved, including "just-in-time" PrepAhead sample preparation, for LC-MS/MS analysis of QuEChERS extracts.

References

1 U. Koesukwiwat, S. Lehotay, S. Miao, N. Leepipatpiboon: 'High throughput analysis of 150 pesticides in fruits and vegetables using QuEChERS and low-pressure gas chromatography time-of-flight mass spectrometry' J. Chromatogr. A 1217 (2010) 6692

2 L. Podhorniak: 'Tubes or Tips?' Poster presentation at Florida Pesticide Residue Workshop (2010) St. Pete Beach, FL (USA)

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4 AOAC Official Method 2007.01: 'Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate' AOAC Int. (2007)

5 O. Cabrices et al.: 'Automated Sample Preparation and Analysis Workflows for Pesticide Residue Screenings in Food Samples using DPX-QuEChERS with LC/MS/MS' Application Note Gerstel (2013) #AN/2013/08-9

6 A. Schreiber et al.: 'Comprehensive Quantitation and Identification of Pesticides in Food Samples using LC-MS/MS with *Scheduled* MRM™, Fast Polarity Switching, and MS/MS Library Searching' Application Note SCIEX (2011) 3370411-01

Publication number: 8013613-01

The Use of Micro Flow LC Coupled to MS/MS in Veterinary Drug Residue Analysis

Stephen Lock
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Overview

A rapid, robust, sensitive and specific LC-MS/MS method has been developed for the simultaneous detection of veterinary drug residues in milk and meat. The method uses an Eksigent ekspert™ microLC 200 and the SCIEX QTRAP® 4500 system utilizing the *Scheduled* MRM™ algorithm with a simplified sample preparation to detect veterinary residues below EU screening requirements.

Introduction

Traditionally, in veterinary drug residue screening of food samples, samples are extracted and analyzed by LC-MS/MS usually at LC flow rates in excess of 500 µL/min and in combination with smaller particle size LC columns result in high UHPLC pressure separations. These conditions result in short chromatographic run times with excellent efficiency and peak shape, but have a drawback in that they require higher volumes of mobile phase. The consumption of organic LC solvents, such as acetonitrile and methanol, is a growing cost of analysts and its disposal has an environmental impact. Therefore, ways to reduce solvent consumption in food residue testing will be beneficial to the environment and reduce running costs of a testing laboratory.

Here we present new data using micro flow LC, running below 40 µL/min, in combination with a LC-MS/MS method developed on the SCIEX QTRAP® 4500 system which utilizes the *Scheduled* MRM™ Pro algorithm. Initially this approach has been applied to a screen of veterinary residues including sulfonamides and beta-lactam antibiotics to show its applicability in food analysis. Data presented shows a comparison of micro flow LC-MS/MS with traditional high flow LC-MS/MS and show that low limits of detection (LOD) below legislated levels[1] are easily possible by this approach.

Experimental

Standards and Samples

For this work the target compounds were commercially available and purchased from Sigma Aldrich. Milk and meat samples for spiking experiments were obtained from a local supermarket.

Sample Preparation

The milk samples (2 mL) was simply mixed with acetonitrile (8 mL) and roller mixed for 20 minutes. After mixing the sample extracts was centrifuged for 5 minutes at 2500 rpm. The supernatant (4 mL) was evaporated to dryness (Eppendorf vacuum concentrator at 60°C) and then reconstituted into 0.1% formic acid in water(2 mL). The reconstituted sample was centrifuged for 1 min at 13,000 and the top layer was decanted into plastic HPLC vials ready for LC-MS/MS analysis.

Contents

For meat samples the extraction protocol was exactly the same except the initial extraction solvent was acetonitrile/water (87.5/12.5).

LC

All microLC method development and analysis was done using an Eksigent ekspert™ microLC 200 UHPLC system. Final extracted samples (5 µL) were separated over a 3.5 minute gradient (shown in Table 1 where A = water and B = acetonitrile both containing 0.1 % formic acid) on a reversed-phase Triart C18 2.7 µm (50 x 0.5 mm) column (YMC) at 30 µL/min and at a temperature of 60ºC.

For the high flow LC comparison a Shimadzu UFLCXR system was used at a flow rate of 600 µL/min using a Kinetex 2.6 µm XDB-C18 (50 x 2.1 mm) column (Phenomenex). The gradient conditions are shown in Table 2.

Table 1. Gradient conditions used for micro flow LC separation at a flow rate of 30 µL/min

Step	Time	A (%)	B (%)
0	0	98	2
1	0.5	98	2
2	1.7	35	65
3	1.8	0	100
4	2.3	0	100
5	2.4	98	2
6	3.5	98	2

Table 2. Gradient conditions used for traditional high flow LC separation at a flow rate of 600 µL/min

Step	Time	A (%)	B (%)
0	0	98	2
1	2	98	2
2	7	40	60
3	7.2	5	95
4	8	5	95
5	8.1	98	2
6	10	98	2

MS/MS

All analyses were performed on a SCIEX 4500 QTRAP® system using the Turbo V™ source in electrospray ionization (ESI) mode. For micro flow LC analysis the electrode was changed to a microLC hybrid electrode (50 µm ID) designed for micro flow rates.[2] In the final micro flow LC method the ion source conditions used were Gas 1, Gas 2 and the Curtain Gas™ interface was set to 30 psi, the temperature (TEM) was set at 350°C and the IS voltage was set to 5500 V.

The veterinary drugs were analyzed using Multiple Reaction Monitoring (MRM) using the *Scheduled* MRM™ algorithm to obtain high selectivity, sensitivity, accuracy and reproducibility. The *Scheduled* MRM™ Pro algorithm in Analyst® software version 1.6.2 allows setting the MRM detection window separately for each compound based on the LC peak width for more efficient scheduling of dwell time (Figure 1).

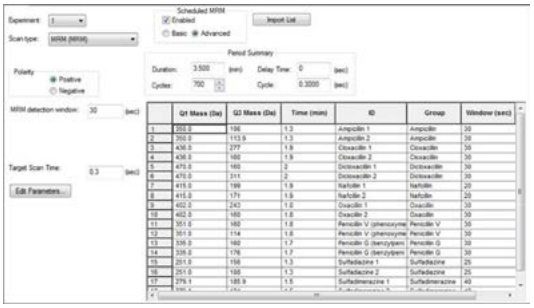


Figure 1. Method editor in Analyst® software version 1.6.2 used to setup the *Scheduled* MRM™ Pro experiment

A total of 32 MRM transitions (Table 3) were monitored to quantify and identify 15 veterinary drug residues and internal standards over a 3.5 minute run time. Only a small set of residues were tested in this project but there is scope to add more compounds to this method. In all the analyses Q1 and Q3 resolution were set to unit.

Table 3. MRM transitions and retention times (RT) of veterinary drug residues investigated in this

Compound	RT (min)	Q1 (amu)	Q3 (amu)	DP (V)	CE (V)
Ampicillin	1.3	350	106, 114	56	23, 41
Cloxacillin	1.9	436	277, 160	51	19, 17
Dicloxacillin	2	470	160, 311	66	19, 21
Nafcillin	1.9	415	199, 171	61	19, 47
Oxacillin	1.8	402	243, 160	46	19, 17
Penicillin V	1.8	351	160, 114	50	19, 45
Penicillin G	1.7	335	160, 176	50	15, 19
Sulfadiazine	1.3	251	156, 108	66	26, 30
Sulfadimerazine	1.5	279	186, 124	80	23, 31
Sulfadimethoxine	1.7	311	156, 92	71	29, 45
Sulfamerazine	1.4	265	108, 92	80	33, 35
Sulfamethaxazole	1.55	254	156, 92	120	21, 35
Sulfamethazine	1.5	279	186, 124	120	23, 31
Sulfaquinoxaline	1.9	301	156, 108	80	27, 37
Sulfathiazole	1.4	256	156, 92	80	19, 33

Results and Discussion

Before the micro LC was used for residue analysis the method was compared against a traditional high flow method that had previously been developed for residue detection in meat and milk. A 1 ng/mL standard of a mixture of different veterinary residues was prepared and analyzed (Figure 2).

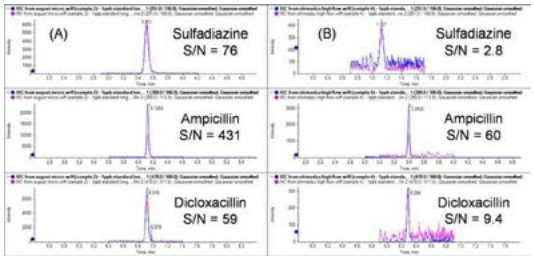


Figure 2. Comparison of microLC (A) with traditional high flow LC (B) using a 1 ng/mL standard.

For the high flow separation a Kinetex 2.6 µm XDB-C18 column at a flow rate of 600 µL/min was used and a Triart C18 column was used for microLC at 25 µL/min. The gradient conditions (Table 2) were kept the same as was the injection volume and column temperature. The results showed sensitivity increases of factors greater than 4 fold to over 10 fold for the veterinary drugs tested with none of the compounds showing a sensitivity loss.

The gradient on the microLC was then adjusted and the flow rate increased to 30 µL/min, to shorten the run time down to 3.5 minutes (Figure 3).

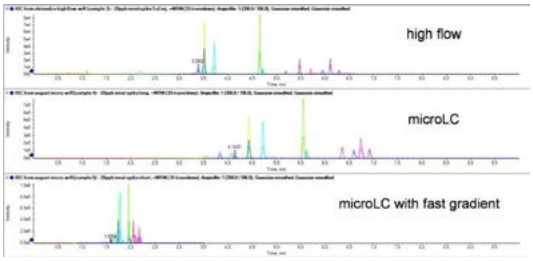


Figure 3. Comparison of meat sample spiked at 20 µg/kg and analyzed by traditional high flow LC and micro flow LC-MS/MS,. In this example analysis time was decreased from 10 min to 3.5 min using micro flow LC and by speeding up the gradient. In all methods peak widths at the base were 3 seconds or less.

Table 4. Results for the calibration lines for a selection of veterinary drug residues and the repeat analysis of spiked milk and meat samples. Displayed are the coefficient of regression (r), coefficient of variation (CV), and signal-to-noise (S/N) obtained. Linearity ranged from 0.1 to 100 ng/mL with linear fit and no weighting used except for sulfamerazine where linear fit and 1/x weighting was used.

Compound	r	CV (%) at 20 µg/kg spiked into milk (n=20)	S/N at 2 µg/kg spiked into milk	S/N at 20 µg/kg spiked into milk	CV (%) at 20 µg/kg spiked into meat (n=20)	S/N at 20 µg/kg spiked into meat
Ampicillin	0.999	5.8	67	712	3.6	285
Cloxacillin	0.999	4.7	94	934	9.1	591
Dicloxacillin	1.000	5.7	50	389	9.0	508
Nafcillin	0.999	2.7	39	379	10.2	800
Oxacillin	0.999	5.6	39	337	8.4	299
Penicillin V	0.999	4.3	101	1162	5.5	272
Penicillin G	0.991	5.8	19	150	14.0	175
Sulfadiazine	0.997	11.1	24	208	6.9	196
Sulfadimerazine	0.995	6.1	30	2131	8.3	1119
Sulfadimethoxine	0.999	4.2	152	1549	1.4	539
Sulfamerazine	0.996	3.5	44	366	3.0	333
Sulfamethaxazole	0.993	7.2	40	356	5.7	189
Sulfamethazine	0.997	10.4	55	662	2.8	357
Sulfaquinoxaline	0.998	4.8	25	275	3.7	705
Sulfathiazole	0.998	3.4	25	290	5.2	131

The results showed that for the late eluting compounds there was some sensitivity loss due to peak broadening but again sensitivity gains were also observed for early eluting compounds. Generally speaking increasing the speed of analysis three fold did not have a negative effect on the response observed for these veterinary residues.

Calibration standards were analyzed for all compounds using the shortened microLC method and three examples of calibration lines for different compounds are shown in Figures 4a to 4c. In each figure the calibration lines were linear and the residues could be detected at a level of 0.1 ng/mL or below (see peak review in each figure).

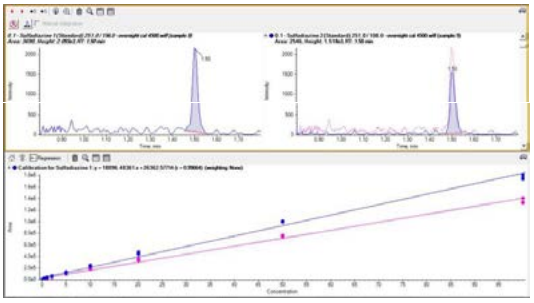


Figure 4a. Quantifier, qualifier MRM transition at 0.1 ng/mL (top), and calibration line of sulfadiazine from 0.1 to 100 ng/mL (bottom), the linearity is provided without the use of any internal standards

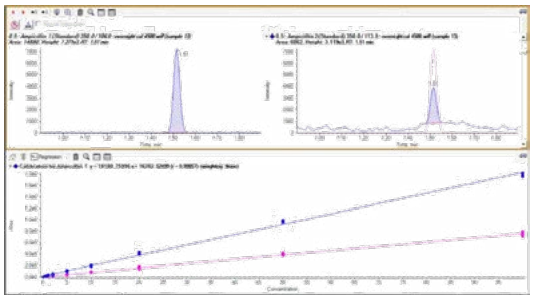


Figure 4b. Quantifier, qualifier MRM transition at 0.5 ng/mL (top), and calibration line of ampicillin from 0.1 to 100 ng/mL (bottom), the linearity is provided without the use of any internal standards

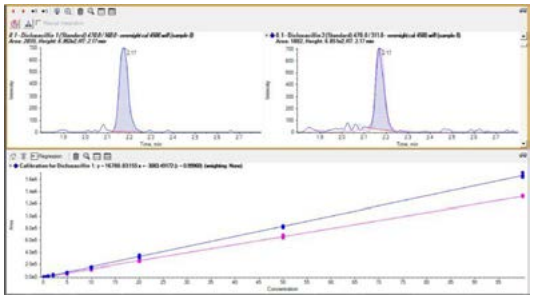


Figure 4c. Quantifier, qualifier MRM transition at 0.1 ng/mL (top), and calibration line of dicloxacillin from 0.1 to 100 ng/mL (bottom), the linearity is provided without the use of any internal standards

The calibration data for each compound is shown in Table 4. Following on from the assessment of linearity milk, meat samples were spiked and extracted and repeatedly analyzed to assess reproducibility with the results displayed in Table 4. For both the calibration lines and the spiking experiments no internal standards were used.

From the results displayed in Table 4 it can be seen that the method can easily provide detection limits which comply with current EU legislation. Linearity was excellent from 0.1 to 100 ng/mL with coefficients of regression greater than 0.99. The repeatability observed and signal-to-noise (S/N) measured varied with the matrix showing the need of internal standards to counter matrix effects from the simplified sample extraction protocol used. However, no coefficient of variation (CV) was over 15% which mirrored a previous study of pesticide residue analysis using microLC[3] with most generally below 10%. All S/N (calculated using 3x standard deviation algorithm in Analyst® software) were greater than 15/1 even in the 2 µg/kg spike into milk.

Summary

This study has clearly demonstrated that using microLC is a valid approach in veterinary residue analysis. The method developed using Eksigent ekspert™ microLC 200 and the SCIEX QTRAP® 4500 system was rapid, sensitive, reproducible, and easily reached the requirements of current EU legislation. Micro flow LC offers the opportunity to cut the analysis time by over half without a loss in performance and in the majority of cases a gain in signal by over a factor of 5 was observed.

Micro LC also provides huge cost saving to laboratories. With LC grade acetonitrile running at a cost of £100/L this 3 day study could have cost about £ 100 with conventional chromatography (0.6 mL/min running for 24hrs a day) and less than £10 with microLC. Over a year this amounts to savings of over £4000 (£90 x 50 weeks) in solvent consumption alone.

Although this method is still under development, with plans to expand the number of compounds in this screen, this work has shown the clear potential of Micro LC in this application area.

References

1. COMMISSION REGULATION (EU) No 37/2010 'on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin' December 2009
2. K. Mriziq et al.: 'Higher Sensitivity and Improved Resolution Microflow UHPLC with Small Diameter Turbo V™ Source Electrodes and Hardware for use with the ExpressHT™-Ultra System' Technical Note Eksigent (2011) # 4590211-01
3. Stephen Lock: 'The Use of Micro Flow UHPLC in Pesticide Screening of Food Samples by LC-MS/MS' Application Note SCIEX (2012) # 6330212-01

Publication number: 8880414-01

Using Your QTRAP® LC/MS/MS System at Full Potential

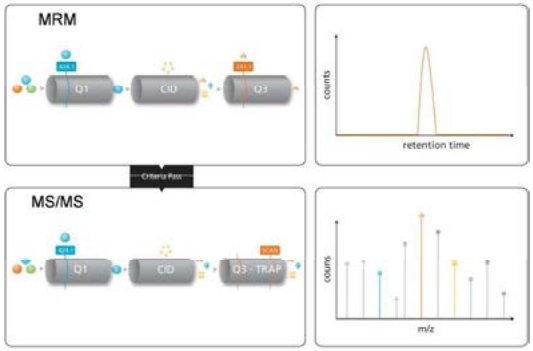
A Quick-Start Guide to Activate and Perform MS/MS Library Searching for Identification and Confirmation MasterView™ and MultiQuant™ Software

Overview

This document outlines the 6 easy steps you can follow to analyze full scan MS/MS spectra (MRM-triggered MS/MS scans collected on a QTRAP® LC/MS/MS system) and compare those results to MS/MS compound libraries to identify and confirm positive peaks in unknown samples.

The benefits of this workflow include:

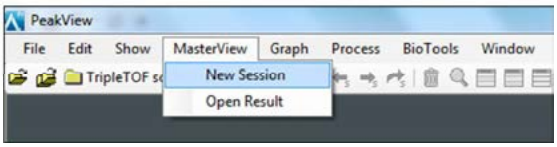
- **Improved selectivity** – multiple fragment ions are detected (beyond just 2 MRM transitions) meaning additional confidence in identification of positive findings
- **Improved sensitivity** – Enhanced MS/MS scans are called 'enhanced' because fragment ions are accumulated in Q3 of your QTRAP®, giving you better signal-to-noise for the detected MS/MS spectra
- **Improved data processing** – dual injection approach with automatic quantitation, identification and confirmation using MasterView™ software and MultiQuant™ software
- **Improved confidence** – ability to automatically calculate ion ratios and compare results to MS/MS mass spectral libraries



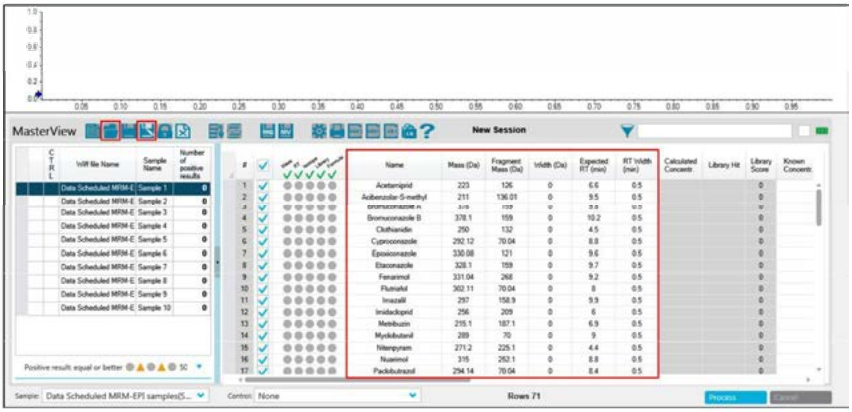
The QTRAP® Software Workflow in MasterView™ Software

Start a new session in MasterView™ and open your sample data file(s)

Select 'New Session' in the MasterView™ menu and select sample(s) to process in the browser window.

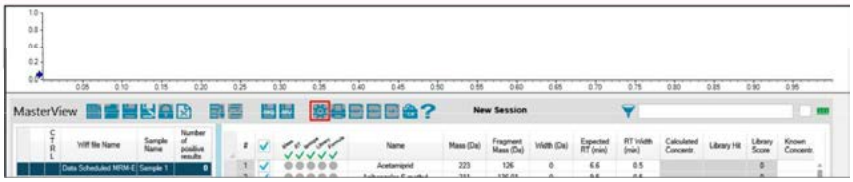


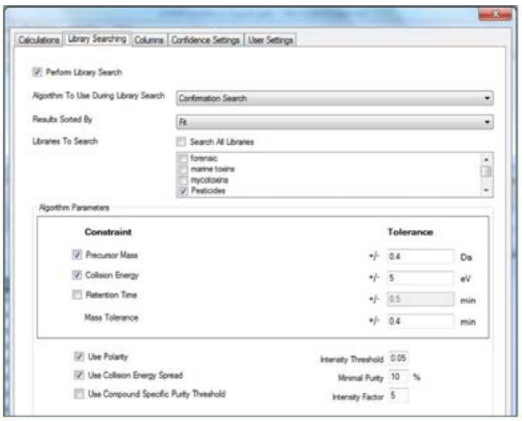
Create a new or open an existing XIC list



- Create a new XIC list by simply copying Name, Mass, Fragment Mass and Retention Time (RT) from your *Scheduled* MRM™ acquisition method into the MasterView™ software.
- The XIC list can be saved and opened for future processing.

Edit settings for data processing and library searching

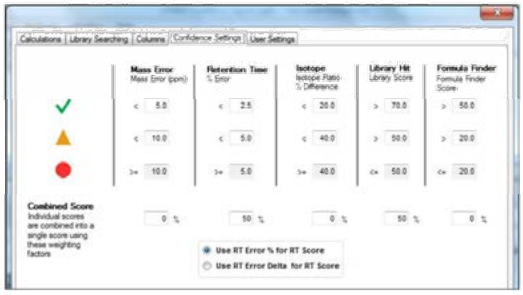




- Data processing settings can be edited by clicking the 'Settings…' button at the top of the XIC list
- Define thresholds for intensity and S/N in the calculations tab.
- In the 'Library Searching' tab select the libraries to search, the search algorithm, and specify other criteria such as mass tolerance, Collision Energy tolerance, and the use of polarity and Collision Energy Spread when searching

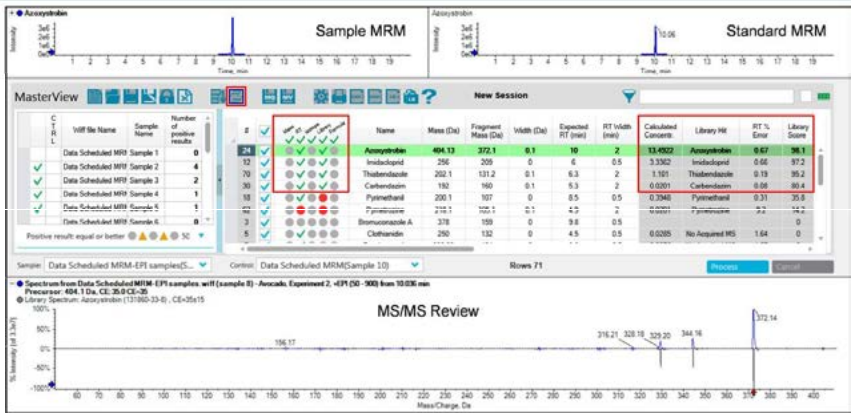
Contents

Define confidence settings for compound identification



- Define criteria for compound identification for RT and library searching in the 'Confidence Settings' tab.
- Note: All settings will be saved with the XIC list.

Review qualitative results



- Confidence in identification is visualized using the RT and Library 'traffic light'
- Numeric values can be found in the RT % Error and Library Score column. Any of these columns can be used to sort results.
- The MS/MS spectrum can be visually compared to the library spectrum by clicking the 'Show MS/MS' button.

Data example: Identification of Azoxystrobin, Carbendazim, Imidacloprid, and Thiabendazole in an avocado sample (QuEChERS extract 10x diluted)

Food and Environmental

Review quantitative results

- Findings in unknown samples are automatically compared against a standard injection of known concentration.
- The MRM transitions can be normalized for easy comparison of peak intensity.
- Results with a concentration higher than the standard are highlighted in green. Results can be filtered using the 'Display highlighted XIC only'.
- Results can be reported using customizable report templates or can be exported in MultiQuant™ for further processing including the automatic calculation of ion ratios.

Data example: Identification of Carbendazim, Cyprodinil, Fenhexamid, Pyrimethanil, Quinoxyfen, and Trifloxystrobin in a grapes sample (QuEChERS extract 10x diluted), however, only Fenhexamid and Pyrimethanil were present at a concentration higher than 10 µg/kg)

Confirmation of Fenhexamid using a second analysis and automatic ion ratio calculation in MultiQuant™ software

Additional library searching and reporting functionality is available in the LibraryView™ and Cliquid® software.

For additional support on implementing this workflow in your own lab, or for support on other SCIEX products, visit our website or email us at support@sciex.com.

Publication number: 10160414-01

Contents

MRM^3 Quantitation for Highest Selectivity in Complex Matrices

Innovations on the QTRAP® 4500, 5500, 6500 and 6500+ LC-MS/MS Systems

Christie Hunter
SCIEX, USA

Mass spectrometry has transformed quantitative analysis to become the method of choice for many assays. More recently, LC-MS/MS has revolutionized quantitative bioanalysis. While single MS filtering offers advantages over non-mass selective techniques, the use of tandem mass spectrometry (MS/MS, or MS2) eliminates interferences and results in a dramatic increase in selectivity which yields a very low baseline, excellent limits of quantification, and very good linearity. As a result, the Multiple Reaction Monitoring (MRM) experiment performed on triple quadrupole mass spectrometers has become the technique of choice for highly sensitive and selective quantification in biological matrices.

In some cases, interferences cannot be eliminated using MRM. More elaborate sample cleanup and chromatography is required to eliminate these interferences. If a high baseline or matrix interference cannot be eliminated, the result is a compromised Lower Limit of Quantification (LOQ) as the detection of compounds in complex matrices is limited by signal-to-noise rather than by raw instrument response. In such cases, the addition of a third MS stage has been shown to greatly increase selectivity and eliminate the high baseline or chromatographic interference. The result is a lower LOQ and better chromatographic peak shape.

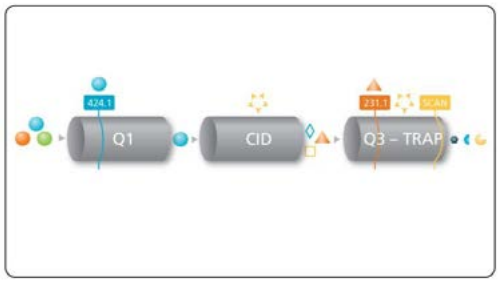


Figure 1. MRM^3 for Quantitative Analysis by LC-MS. Analyte ion is first selected in the Q1 quadrupole, then fragmented in Q2 collision cell. Fragment ions are trapped then isolated in the linear ion trap, followed by excitation to perform the second fragmentation step. Second generation product ions are scanned out to the detector.

Key Features of the QTRAP® 4500, 5500, 6500 and 6500+ Systems for MRM^3 Quantitation

- MRM^3 quantification - An MS^3 scan is performed with a fast cycle time and using a narrow scan range centered at the second generation product ion m/z which is being used for quantification. This type of scan is referred to as MRM^3 (Figure 1).
- Faster linear ion trap scan speeds – Scan speeds up to 20,000 Da/sec enable MS^3 scans with an HPLC compatible cycle time such that extracted ion chromatograms (XICs) of second generation product ions can be extracted and integrated with a sufficient number of data points across the chromatographic peak.
- Better in-trap fragmentation – The new Linear Accelerator™ Trap with pulsed gas valve implemented in the QTRAP® systems provides faster, more efficient in-trap fragmentation (Figure 2)
- High ion trap sensitivity – The QTRAP systems feature high sensitivity when operating in linear ion trap mode
- High selectivity – Unit isolation of precursor ion in Q1 followed by excitation and fragmentation at unit resolution in the ion trap provides the very high selectivity in MRM^3 analysis (Figure 3).

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Speed

The speed and efficiency of ion-trap fragmentation has also been greatly enhanced on the QTRAP® 4500, 5500, 6500 and 6500+ systems. Collision gas is now introduced through a high speed pulsed gas valve that enables a rapid increase in pressure in the LIT (Figure 2). Together with an increase in the RF drive frequency, this pulsed gas results in increased fragmentation efficiency and reduced excitation time of 25 ms or less.

In addition, the scan speed of the linear ion trap has been increased to a maximum of 20,000 through the use of the faster eQ™ electronics. This enabling fast MRM^3 scan cycles at very high sensitivity.

Selectivity

Because of the configuration of the QTRAP® systems, the MRM^3 quantification workflow is highly flexible and leverages the sensitivity and selectivity of the system. For the MRM^3 workflow, the analyte ion of interest is first isolated in the Q1 quadrupole with a user–selected resolution, usually unit resolution (0.7 Th FWHH). It is then fragmented in the Q2 collision cell, providing a broad range of product ions to be selected in the ion trap.

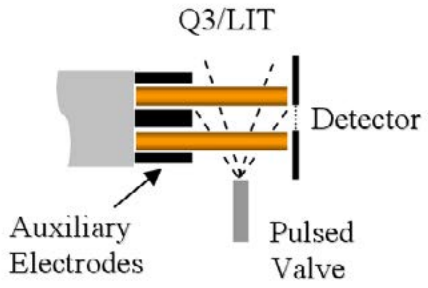

Figure 2. Pulsed Gas Valve. Gas is introduced into the linear ion trap using a high speed pulsed gas valve which rapidly increases LIT pressure and reduces required excitation time for ion trap fragmentation.

The in-trap fragmentation is achieved through the application of a single wavelength / narrow band excitation. As shown in Figure 3, this allows very selective fragmentation. The C12 isotope of a product ion can be specifically excited and fragmented to completion with minimal fragmentation of the C13 isotope. This provides further selectivity advantages in the removal of interfering background.

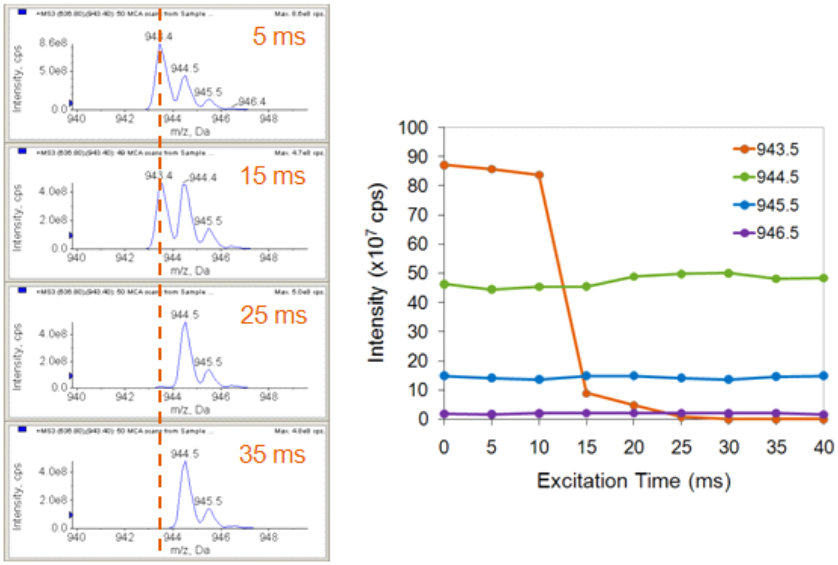

Figure 3. Single Frequency Excitation for High Selectivity. Narrow band excitation is used to specifically excite and fragment just the C12 isotope of the ion isolated in the LIT (left). This isotope can be fragmented to completion with no impact on the nearby C13 isotopes (right).

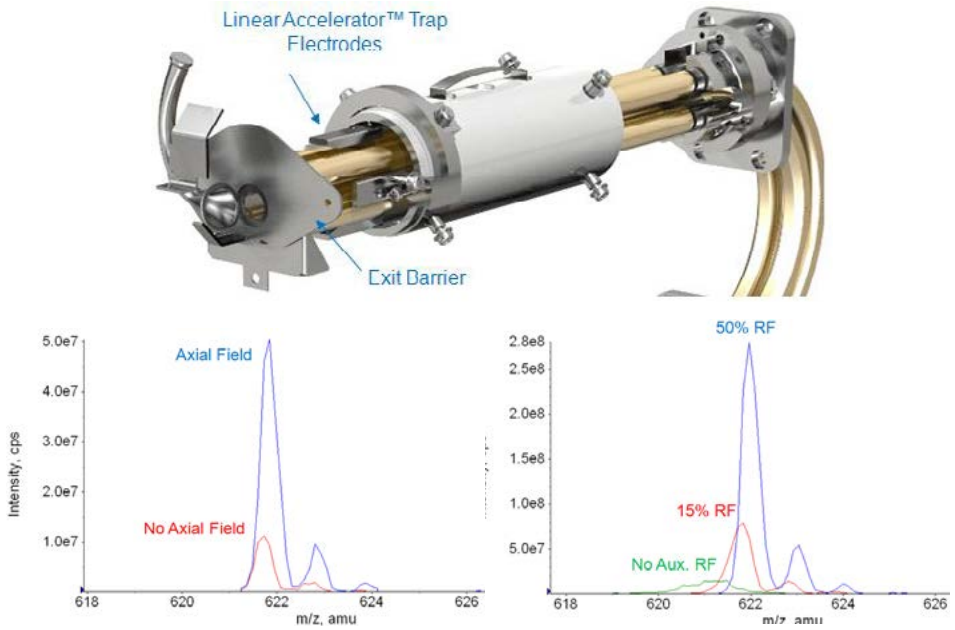

Figure 4. Linear Accelerator™ Trap Innovations for Sensitivity. Addition of electrodes in this new trap design (top) significantly improves the sensitivity of trap scanning by moving the trapped ions into the extraction region before axial ejection from the trap (bottom left). Further sensitivity gains are achieved by the addition of RF to the exit barrier of the trap (bottom right).

Sensitivity

Linear Accelerator™ Trap technology has resulted in ground breaking improvement in the handling of ions inside the linear ion trap of the QTRAP® 4500, 5500, 6500 and 6500+ systems, resulting in up to 100x more sensitivity. Trapped ions are manipulated within the linear ion trap through the use of auxiliary DC fields provided by the addition of small electrodes (Figure 4, top). Ions are gently moved toward the extraction region of the linear ion trap during the cooling period by a voltage applied to the trap collar. A potential barrier is created by increasing the potential on the auxiliary electrodes just before the mass scan to complete the ion concentration process. The application of this axial field has a significant effect on sensitivity (Figure 4, bottom left).

In addition, a radio frequency is applied to the exit lens of the Linear Accelerator Trap resulting in further sensitivity gains (Figure 4, bottom right). These two innovations enable better than unit resolution to be obtained in the trap scan modes at these very high scan speeds.

Removal of Tough Interferences

Innovations in scanning speed, selectivity and sensitivity on the QTRAP systems enable successful implementation of the MRM^3 workflow for a wide range of analytes [3,4]. Sometimes, background noise or interferences can limit the detection of an analyte. Shown in Figure 5 is an example of an interference that has the same MRM transition as Clenbuterol and elutes at the same retention time. Use of MRM^3 can completely remove this interference and enable a much lower detection of this analyte.

Contents

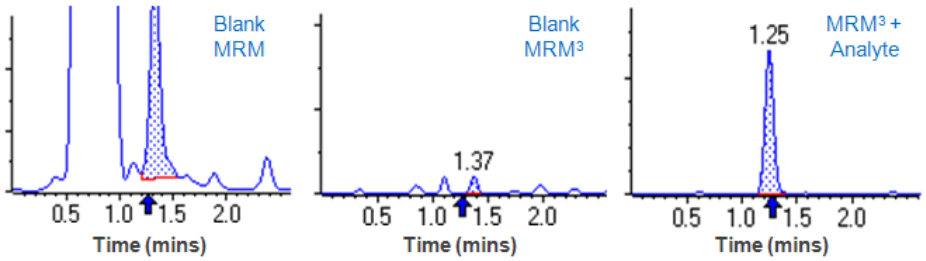

Figure 5. Analysis of Clenbuterol in Urine. Analysis of Clenbuterol in urine by MRM is plagued by the presence of a large co-eluting interference. Left – MRM for Clenbuterol used to analyze the urine blank. Middle – MRM^3 analysis of the urine blank shows the interference is completely gone. Right – MRM^3 analysis of 0.5 ng/mL clenbuterol spiked into urine. 10x better LOQ obtained with MRM^3 than MRM due to substantial reduction in interference (data not shown).

Conclusions

- MRM^3 is an effective strategy for quantitation of analytes when high background or interferences make standard MRM quantitation difficult (Figure 5).
- MRM^3 can be used to achieve similar LOQ's with less sample preparation and simplified or faster chromatography.
- MRM^3 has been successfully applied to the detection and quantitation of small molecules, peptides, and protein biomarkers.
- MRM^3 is significantly improved on the QTRAP® systems, making them useful tools for quantitation in tough matrices.
- The QTRAP® 4500, 5500, 6500 and 6500+ LC-MS/MS systems are high performance triple quadrupole and linear ion trap systems providing users with many powerful quantitative and qualitative tools.

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Document number: RUO-MKT-02-2739-A

Moving Your Old GC Methods to LC-MS/MS Technology to Enhance and Accelerate Environmental and Food Testing

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Overview

This paper highlights benefits of advanced LC-MS/MS technology over GC for the analysis of environmental and food contaminants, which include:

- Less time-consuming and labor-intensive sample preparation
- Direct injection of water samples
- Typically no need for derivatization
- Short chromatographic run times
- Quantitation of trace levels (parts per trillion) due to superior selectivity and sensitivity of multiple reaction monitoring (MRM)
- Screening for a wider range of compounds in a single analysis
- Compound identification using MRM ratio calculation and library searching of enhanced product ion spectra

Introduction

Gas chromatography (GC) and liquid chromatography (LC) are essential analytical tools in modern food and environmental laboratories. A gas chromatograph equipped with element-selective detectors or mass spectrometry (MS) has traditionally been the method of choice for residue and contaminant analysis, with GC-MS being the preferred technique for the analysis of less polar and more volatile compounds, such as organochlorine pesticides, dioxins, and polychlorinated biphenyls (Figure 1). But time-consuming and labor-intensive sample preparation and derivatization steps and long chromatographic run times have led to the increased adoption of LC with tandem mass spectrometry (LC-MS/MS).

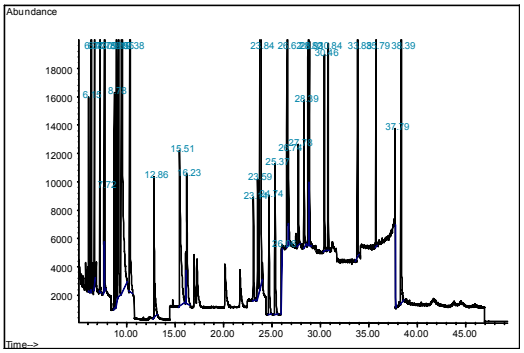

Figure 1. GC-MS chromatogram of 65 targeted organochlorine pesticides and polychlorinated biphenyls

Higher Selectivity and Sensitivity

Initially, LC-MS/MS was mostly used as a complementary tool for the analysis of compounds that were difficult or impossible to analyze by GC. But LC-MS/MS has gained popularity for quantitation due to its high selectivity and sensitivity in multiple reaction monitoring (MRM) mode. MRM works like a double mass filter; only molecules with the correct molecular ion filtered in Q1 and the correct product ion filtered in Q3 can reach the detector, and thus very little noise and matrix interferences are detected. This high selectivity typically results in improved limits of detection and quantitation (Figure 2) and also simplifies sample preparation.

Analysis Across Different Compound Classes

LC-MS/MS using electrospray ionization (ESI) has quickly become the technique of choice for the analysis of polar, semi-volatile, and thermally labile compounds, such as pesticides, mycotoxins, pharmaceuticals, personal care products, perfluorinated compounds (Figure 3), drugs of abuse, etc. In addition, alternative ion sources, like atmospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI), allow the analysis of compounds of lower

polarity, such as steroids, brominated flame retardants (Figure 4), and even polycyclic aromatic hydrocarbons.

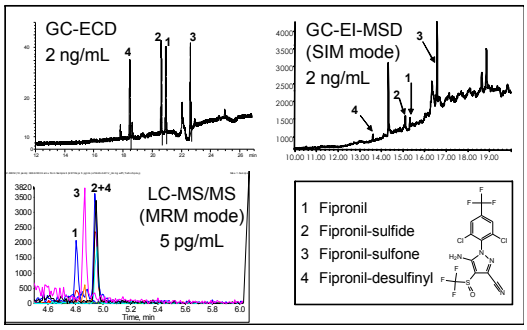

Figure 2. Increased selectivity and sensitivity of LC-MS/MS operating in multiple reaction monitoring (MRM) mode, in comparison to GC for the detection of Fipronil and its metabolites using a 4000 QTRAP® LC-MS/MS System

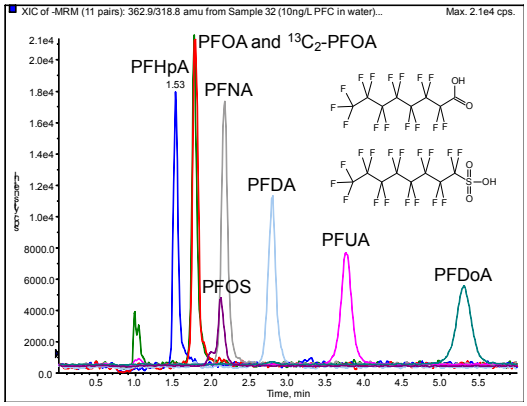

Figure 3. Detection of perfluorinated compounds (C_7–C_{12} perfluorocarboxylic acids and perfluorooctanesulfonic acid) at a concentration of 10 ng/L by directly injecting water into an API 4000™ LC-MS/MS System

Multi-Compound Screening

More recently, LC-MS/MS replaces traditional GC methods for multi-compound screening because of its ability to analyze a wider range of food and environmental contaminants in a single analysis.

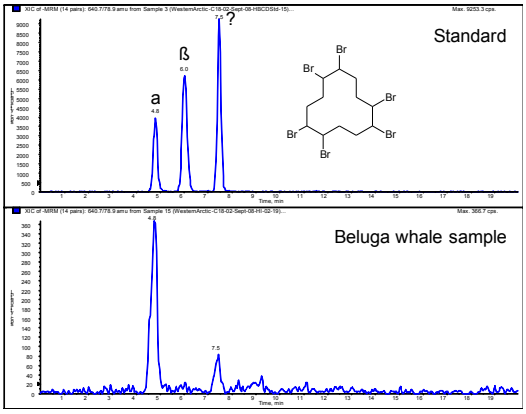

Figure 4. Detection of isomers of hexabromocyclododecane (HBCD) in a beluga whale from the Canadian Arctic using an API 4000™ LC-MS/MS System

Alder et al. compared the use of GC-MS and LC-MS/MS for multi-residue pesticide analysis and concluded, "…the benefits of LC-MS/MS in terms of wider scope, increased sensitivity, and better selectivity are obvious".[1-2] Figure 5 shows the number of pesticides, out of 500 investigated pesticides, that could be detected with a signal-to-noise ratio (S/N) of at least 10. Many pesticides were reliably quantified by LC-MS/MS at a concentration of 0.1 or 1 ng/mL. In comparison, the median limit of quantitation using GC-MS is distinctly higher, at approximately 100 ng/ mL. Only most organochlorine pesticides and a few organophosphorus and pyrethroid pesticides showed higher sensitivity using GC-MS.

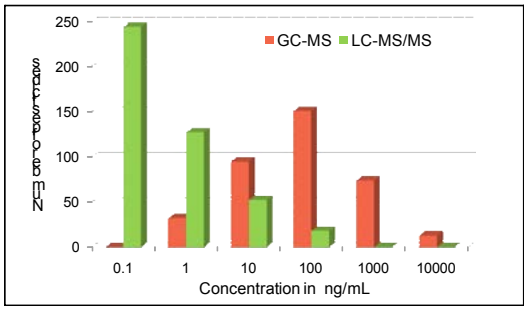

Figure 5. Distribution of limits of quantitation (S/N ≥ 10) of 500 pesticides detected with GC-MS and LC-MS/MS using an API 4000™ LC-MS/MS System

The demand to extend the scope of methods to screen for hundreds of targeted analytes requires using very generic extraction procedures. For instance, QuEChERS (quick, easy, cheap, effective, rugged, and safe) has become a very popular method to extract matrices with low fat content, such as fruits and vegetables. QuEChERS is based on acetonitrile extraction with partitioning using $MgSO_4$ followed by a dispersive SPE cleanup. This procedure has successfully been applied to extract pesticides, antibiotics, mycotoxins, and other compounds effectively and reproducibly from a variety of food commodities.[3-5] The high selectivity and sensitivity of LC-MS/MS detection in MRM mode allows the injection of crude extracts. In many applications, sample extracts can be even further diluted to minimize possible matrix effects during ionization.

The analysis of drinking water and surface water also benefits from the power of modern LC-MS/MS. Such samples can be injected directly into the LC-MS/MS system, and concentrations as low as nanograms per liter (parts per trillion) are detectable (Figure 6). Replacing sample preparation with a direct injection approach helps to save time and money and also minimizes the risk of concentrating humic acids during the extraction, which can cause ion suppression and, thus, incorrect analytical results.

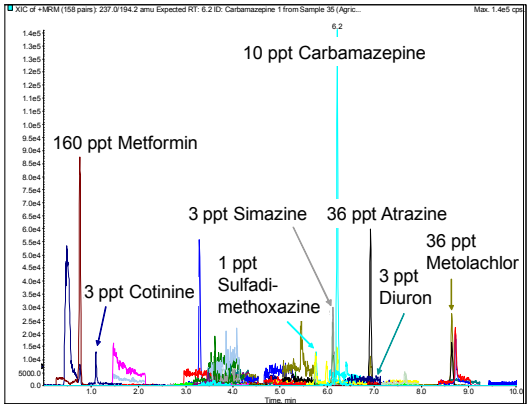

Figure 6. Direct injection of 100 µL of an urban water sample to detect trace levels of pesticides, pharmaceuticals, and personal care products using a QTRAP® 5500 LC-MS/MS System

Compound Identification

Despite the high selectivity of multiple reaction monitoring, there is always a risk of false positive or false negative findings due to interfering matrix signals. Accordingly, additional qualitative information is needed for compound identification. Typically a second MRM is monitored per analyte, with the ratio of quantifier to qualifier transition calculated for each unknown sample and compared to the MRM ratio of standards. As an alternative, identification can be performed using full-scan MS/MS experiments and library searching to compare the unknown with a standard spectrum. QTRAP® LC-MS/MS systems are hybrid mass spectrometers combining the benefits of triple quadrupole MS/MS, such as MRM, with full-scan capabilities of linear ion traps. A QTRAP® system operated in enhanced product ion (EPI) scan mode provides higher sensitivity and faster scanning than triple quadrupole instruments and more characteristic spectra and shorter cycle times than traditional ion traps.[6]

Figure 7 illustrates the benefit of using an EPI spectrum and library searching versus an MRM ratio for identification. The MRM ratio suggested the presence of Metalaxyl in strawberry extract, but the corresponding MS/MS spectrum and the library search result clearly identified a false positive finding—Metalaxyl was not present in this strawberry sample. This example shows that the molecular fingerprint of the MS/MS contains much more compound information than a single MRM ratio and thus is a useful tool to minimize false positive and false negative findings.

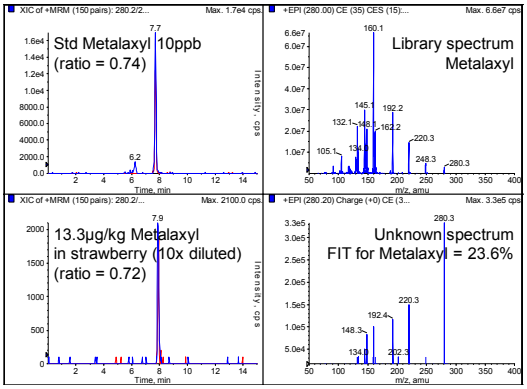

Figure 7. Quantitation of 13.3 µg/kg Metalaxyl in strawberry extract using a 3200 QTRAP® LC-MS/MS System. This MRM ratio of 0.72 was within tolerance levels of 0.59–0.89, but the full-scan MS/MS spectrum and the library search clearly indicated a false positive finding.

Summary

GC-MS and LC-MS/MS are powerful analytical tools for the quantitation and identification of food and environmental contaminants, such as pesticides, mycotoxins, pharmaceuticals, personal care products, and drugs of abuse.

LC-MS/MS with electrospray ionization operating in the multiple reaction monitoring mode is a standard technique for targeted quantitation because of its well-known selectivity and sensitivity. Furthermore, its popularity has increased due to the ability to screen for, quantify, and identify large panels of analytes across different compound classes in a single analysis.

Simple extraction procedures, such as QuEChERS, allow the efficient and reproducible extraction of hundreds of compounds from simple to complex matrices. The dilution of extracts helps to minimize possible matrix effects. In addition, the direct injection of water samples into LC-MS/MS has gained popularity to avoid time-consuming and labor-intensive sample preparation. High-sensitivity LC-MS/MS instrumentation is needed to allow extract dilution and direct injection while still matching the required and challenging limits of detection.

The capability to perform MS/MS fragmentation is a great tool to positively identify detected compounds. Typically, the ratio of two MRM transitions is used for identification, but the acquisition of enhanced product ion spectra and library searching provide an added degree of confidence and reduce the risk of false positive and false negative results.

Finally, advances in electronics have resulted in economical and robust benchtop LC-MS/MS instrumentation, and recent software developments make LC-MS/MS as easy to use as standard GC instrumentation.

Acknowledgment

We wish to thank Gordia Macinnis and Chris Marvin (Environment Canada, Burlington, ON, Canada), Paul Yang (Ministry of Environment, Toronto, ON, Canada), and Thomas Anspach (Eurofins, Hamburg, Germany) for providing GC example chromatograms and samples for LC-MS/MS analysis.

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Document number: RUO-MKT-02-4119-A 1281310-02

Confirmation of Pesticides in Jalapeno Peppers Using QTRAP® MS/MS Data and Library Matching in SCIEX OS-Q

Combining Enhanced Product Ion Scan with the Latest Software for High Confidence Confirmation

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SCIEX, Redwood City, CA, USA

What: Food testing labs are frequently confronted with the need for more confirmatory information when it comes to analyte identification in real- world samples. In complex matrices, interferences may complicate interpretation of data resulting in false positives or reported residue levels to come into question. Combining QTRAP® system with the latest software tools of SCIEX OS-Q allows for the collection of full-scan MS/MS spectra for targeted pesticides in complex food samples. Those MS/MS data can be subsequently searched against SCIEX verified libraries for unambiguous qualitative confirmation of pesticide detection and ID.

How: The MS method was set up with a targeted MRM list of 200+ pesticides. IDA (Information Dependent Acquisition) criteria were defined such that Enhanced Product Ion (EPI) acquisition would be triggered when an MRM signal exceeded a threshold of 2000 cps. This type of data acquisition results in the collection of MS/MS spectral information in addition to the MRM signal (Fig. 1). Samples of dried, canned, and fresh jalapeno peppers were extracted and analyzed for the pesticide panel. Data were processed in SCIEX OS-Q for quantitation by MRM and spectral identification by library matching. Figure 2 shows collected MS/MS spectra being used to confirm the identity of the MRM peak in the pepper extract. This additional stringency in identity confirmation could help protect against reporting false positive detections for residues in complex samples.

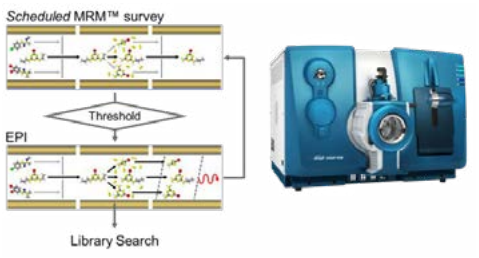

Figure 1. The QTRAP® 4500 System with EPI Scan Type Triggered from MRM Signal from Target Pesticides. The resulting MS/MS spectra were matched to library spectra for confirmation.

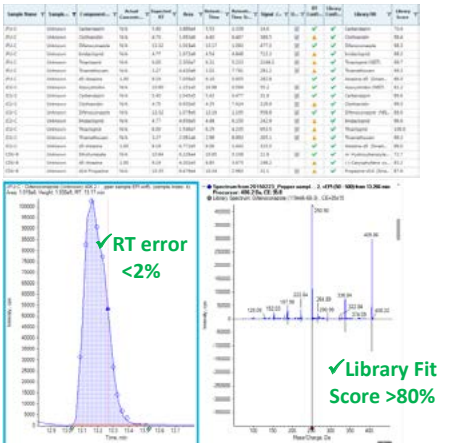

Figure 2. High Confidence in Positive Detection Possible with QTRAP System and SCIEX OS-Q. ID and quantitation of pesticides can be achieved with high confidence using QTRAP system to collect MS/MS spectra in addition to MRM peaks. Retention time and MS/MS pattern are compelling evidence that the identified peak is Difenoconozole. Circles on the chromatographic peak represent where EPI scan has been triggered; the software automatically chooses which spectrum is the highest quality and displays it on the right. The colored circle denotes which scan is being displayed. SCIEX OS-Q results table quickly shows with green check marks in the Confidence columns where peaks have been confirmed with RT error <2% and library fit score of >80%. Rapid visual assessment of large sample sets is possible, ultimately resulting in greater efficiency and increased qualitative confidence in reported detections during data processing for residues in food samples.

Document number: RUO-MKT-02-8199-A

Contents

A Highly Selective and Sensitive LC-MS/MS Method for the Quantification of Gluten Proteins

Measuring Multiple, Unique Signature Peptides to Determine Gluten Levels in Diverse Food Matrices

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[1]SCIEX Gurgaon, Haryana (India); [2]SCIEX Singapore; [3]SCIEX Concord, Ontario (Canada); [4]SCIEX Darmstadt (Germany); [5]SCIEX Redwood City, California (USA)

Introduction

Gluten is a multi-protein complex located in the endosperm portion of wheat, rye, and barley grains that are commonly found in Western diets and are steadily becoming more prevalent in Eastern diets. Gluten ingestion has been linked to a number of gastrointestinal disorders, including celiac disease, wheat allergies, and non-celiac gluten sensitivity, with the epidemiologically relevant prevalence of these disorders estimated to be around 5% of the global poplulation.[1]

Gluten-related disorders result from diverse mechanisms: celiac's disease is caused by an autoimmune reaction to gliadin, a glycoprotein that, along with glutenin, helps to form the gluten complex; wheat allergies are mediated by IgE antibodies to allergenic gluten proteins; while the cause of non-celiac gluten sensitivity is not yet well understood. However, for all these gluten-related disorders, consuming gluten can have serious health consequences, making the detection and quantitation of dietary gluten in pre-packaged foods extremely important.

In 2013, the FDA established, among other criteria, a gluten limit of less than 20 parts-per-million (ppm) for foods that carry a gluten-free label (e.g., gluten-free, no gluten, free of gluten, without gluten). Before issuing this regulation, there were no U.S. standards or definitions for the food industry to reference when labeling a food's gluten levels, leaving many consumers unsure of the gluten content. Typically, gluten content has been determined using an ELISA assay, but these tests have a limited linear response and often generate false negative or false positive results.

Herein, we have developed and verified a selective and sensitive LC-MS/MS-based method for detecting and quantifying gluten signature peptides in a variety of food matrices. This method relies on the use of three MRM transitions for each unique gluten signature peptide released from the glutenin subunit (DY10). To increase assay precision, a stable isotope-labeled gluten standard was added to food homogenates with unknown gluten levels prior to enzymatic protein digestion. Assay performance was evaluated using raw cereal grains, as well as baked, dehydrated, and fermented products.

A major advantage of LC-MS/MS analysis over the ELISA assay is that multiple, unique peptide markers can be monitored simultaneously in a single injection, providing information on gluten content, as well as the identity of other grains (rye, barley, oats). Using this LC-MS/MS method, an accurate gluten concentration as low as 5 ppm can be obtained, with excellent repeatability (%CV) of less than 20%, along with information on a food product's grain composition.

Contents

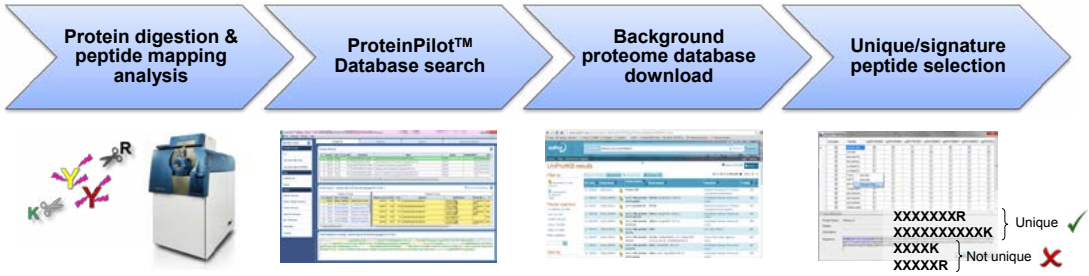

Figure 1. Signature peptide selection workflow using the SCIEX TripleTOF® 6600 system and ProteinPilot™ software

Experimental

Sample Preparation and Digestion of Bakery Products and Baby Formula

Baked goods (1 g) were homogenized by grinding and then defatted with hexane (5 mL). Excess hexane was removed by oven-drying samples at 60ºC. Eight-point calibration lines were constructed over a broad range of concentrations (5 to 1000 ppm gliadin in 1 g gluten- free homogenate). Food samples were extracted (3 mL of gluten extraction solvent) and centrifuged. Supernatants (1 mL) were then collected and dried under vacuum to 0.25 mL.

After adding diluent (100 µL), samples were denatured and reduced with denaturant (5 µL) and reductant (5 µL) at 60ºC for 1 hr. After cooling to room temperature, samples were alkylated with a cysteine blocking reagent (15 µL) at 20ºC for 1 hr. Samples were incubated with a reductant (2.5 µL) at 20 ºC for 1 hr to stop alkylation,. The reduced and alkylated protein samples were digested with trypsin (50 µg) for 3 hr at 37ºC to release signature peptides.

Sample Preparation and Digestion of Fermented Beverages

An eight-point calibration line was constructed over a broad range (5 ppm to 1000 ppm of gliadin in 1 mL gluten- free beverage). Fermented beverage samples were dried to 0.25 mL, prior to dilution, denaturation and reduction of proteins as described for bakery products. Beverage samples were digested following the above protocol for bakery products.

Sample Cleanup

A stable isotope-labeled internal standard (IS) was added to the sample after digestion, followed by solid phase extraction to remove excess polar and non-polar contaminants. Strong cation-exchange cartridges (Phenomenex) were used for sample cleanup following the manufacturer's protocol.

LC Separation

Peptides were chromatographically separated using a C18 column (2.6 µm, 100 x 2.1 mm) and a 9-min gradient at a flow rate of 300 µL/min. The mobile phases were 0.1% formic acid in water and 0.1% formic acid in acetonitrile. The injection volume was set to 5 µL.

MS/MS Detection

A SCIEX TripleTOF® 6600 LC-MS/MS system was used for identifying allergen proteins and selecting signature peptides. The peptide mapping experiments were performed by analyzing protein digests of rye, barley, oats and wheat. The detailed selection strategy is discussed in the results section.

For quantitative analyses of signature peptides of glutenin, food samples were evaluated using a SCIEX QTRAP® 4500 LC-MS/MS system with Turbo V™ source in positive electrospray ionization mode.

Results and Discussion

During method development, signature peptides were chosen based on: 1) the uniqueness of their sequence; and 2) their sensitivity of detection. Information on peptide sequences, relative abundance, and post-translational modifications was generated using a ProteinPilot™ software database search and LC-MS/MS analysis of peptides using a TripleTOF® 6600 System (Figure 1). Results were compared to information on the tryptic peptides generated from the food homogenate's background proteins.

Contents

After peptide selection, the effects of acute conditions during baking (such as high acidity and heat) on the stability of signature peptides were evaluated using the QTRAP® 4500 System. Similarly, the efficiency of the protein extraction protocol was optimized by testing various solvents and enhancing SPE procedures and monitoring peptide levels.

To gauge the linearity of the response, signals generated from the MRM transitions of signature peptides from various grains (wheat, rye, barley, and oats) were compared to peak areas generated from known concentrations of gluten standards added to food homogenates. MRM transitions that produced linear signals over the broadest dynamic range were selected for the final method. Three signature peptides and three MRM transitions for each peptide were selected for the final quantitation and screening of proteins from various food homogenates.

To quantify gluten peptides, calibration curves were constructed over a broad range of gluten concentrations (5 to 1000 ppm). The signature peptide MRM transitions were tested for selectivity and linearity in three different food matrices (Figures 2a-c). The most selective transition with the best regression value was employed for the remaining quantitation experiments. The other two MRM transitions were used in qualitative screens for gluten.

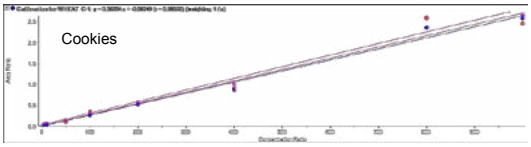

Figure 2a. Calibration lines for gluten quantification. A gluten signature peptide, selected for its stability under acute conditions, was detected over a broad range of concentrations (5 to 1000 ppm) in cookies. Three different MRM transitions were evaluated for linearity for each food product: Blue) wheat peptide C-1, Pink) wheat peptide C-2, and Orange) wheat peptide C-3.

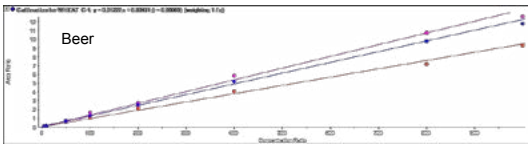

Figure 2b. Calibration lines for gluten quantification. A gluten signature peptide, selected for its stability under acute conditions, was detected over a broad range of concentrations (5 to 1000 ppm) in beer. Three different MRM transitions were evaluated for linearity for each food product: Blue) wheat peptide C-1, Pink) wheat peptide C-2, and Orange) wheat peptide C-3.

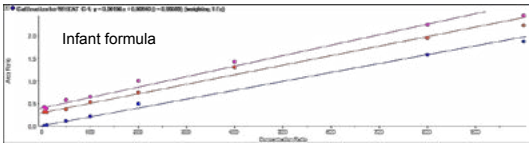

Figure 2c. Calibration lines for gluten quantification. A gluten signature peptide, selected for its stability under acute conditions, was detected over a broad range of concentrations (5 to 1000 ppm) in infant formula. Three different MRM transitions were evaluated for linearity for each food product: Blue) wheat peptide C-1, Pink) wheat peptide C-2, and Orange) wheat peptide C-3.

Quality control samples were tested at three different levels, LQC (10 ppm), MQC (400 ppm), and HQC (800 ppm), for each food homogenate (bakery products, fermented beverages, baby formula). Table 1 shows that intra-batch precision and accuracy results were within the specified limits for each matrix type and QC sample, with %CVs < 20% for all gluten levels tested. To ensure stability, solutions of gluten standards and IS were also tested over time and found to be stable for seven days at 4 ºC.

To identify other major grains (aside from wheat) in food homogenates, signature peptides for rye, barley and oats were identified. Three MRM transitions were chosen for each unique peptide from each grain type, and the highest performing transition for the most stable peptide was used to quantity levels of each grain type, while the remaining two MRM transitions were used for screening samples qualitatively.

The most selective gluten MRM transition was used to evaluate the effects of various food matrices (cookies, beer, and baby formula) on gluten detection. Increasing amounts of gluten standard (0, 5, and 1000 ppm) were added to food homogenates, which were then analyzed by LC-MS/MS for the gluten signature peptide content. Representative chromatograms for the selected gluten MRM transition showed a corresponding increase in instrument response when gluten levels were increased with no interference from the matrix regardless of food homogenate type (Figure 3).

Contents

Table 1. Within-batch precision and accuracy for the repeat measurement of gluten signature peptide levels in various food matrices (n=6)

Food Matrix	QC Sample	Concentration (ppm)	Mean (ppm)	Accuracy (%)	%CV
Cookies	LQC	10.0	11.2	111.7	8.1
	MQC	400	391.4	97.8	4.0
	HQC	800	735.9	92.0	3.2
Beer	LQC	10.0	9.4	93.7	17.4
	MQC	400	355.6	88.9	2.6
	HQC	800	924.0	115.5	2.5
Baby formula	LQC	10.0	9.9	98.8	18.8
	MQC	400	447.3	111.8	7.2
	HQC	800	767.4	95.9	3.5

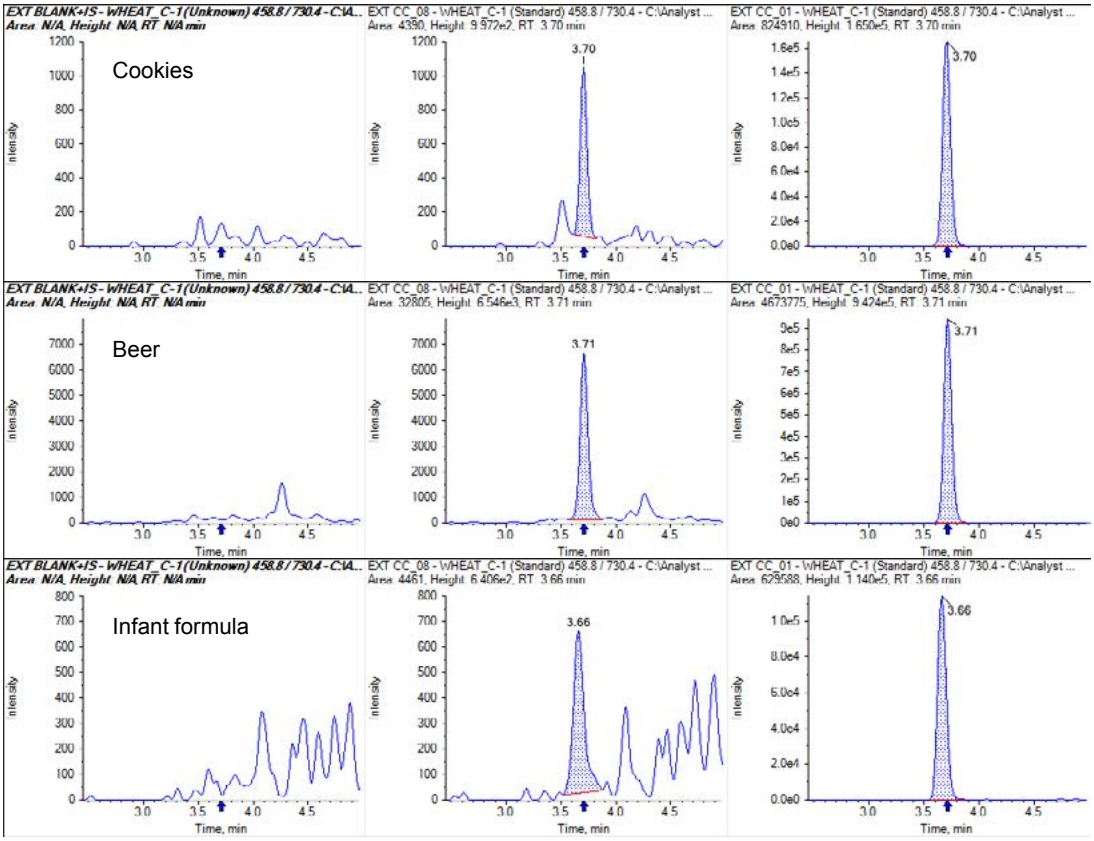

Figure 3. Representative chromatograms from MRM detection of gluten in A) cookies, B) beer, and C) infant formula. To detect a range of gluten levels, increasing amounts of gluten standard were fortified to the food homogenate: 0 ppm (left), 5 ppm (middle), and 1000 ppm (right).

Contents

Table 2. Gluten concentration in different food samples

	Labeled as Gluten-free		Gluten containing	
Food Matrix	**Sample ID**	**Gluten (ppm)**	**Sample ID**	**Gluten (ppm)**
Cookies	Choco Chip	1.3	Biscuit 1	7990
	Lemon	1.1	Biscuit 2	557
	Nutty Fibre Choco Chip	1.9	Biscuit 3	7970
Baby formula	Baby rice	n.d.	Infant Cereal multi-grain	493
	Baby rice	n.d.	Infant Cereal wheat apple	833
			Infant Cereal wheat honey	538
			Infant Cereal wheat mix fruit	504

The protein extraction efficiency for each matrix was calculated by comparing the gluten peak areas for food homogenate samples prior to the addition of gluten standards with gluten peak areas obtained post-extraction. The peak areas of six samples were averaged, and extraction efficiency was found to be around 50%, 80%, and 90% for bakery products, baby formula, and beer, respectively.

The cleanup efficiency of the SPE procedure was calculated by comparing peak areas from extracted samples to peak areas obtained from food homogenates prior to extraction. The SPE cleanup efficiency (from an average of six samples) was approximately 90%, 85% and 75 % for bakery products, fermented beverages, and baby formula, respectively.

A diverse range of food products, including gluten-free and gluten-containing foods with unconfirmed gluten levels, were analyzed for each food matrix. To simulate blinding, samples with unknown gluten levels were interspersed amongst QC samples for each batch of replicates.

Table 3. Gluten concentration in different beer samples

Food Matrix	Sample ID	Gluten (ppm)
Beer	Brand 1	2.4
	Brand 2	5.7
	Brand 3	n.d.
	Brand 4	2.3
	Brand 5	4.9
	Brand 6	1.4

Table 2 shows that food products that were advertised as gluten-free met those standards, and consistently revealed a gluten concentration under 20 ppm, while gluten-containing foods displayed much higher levels of gluten (86- to 1400-fold greater than the highest gluten level found in the food labeled gluten-free).

Table 3 shows the gluten concentration measured in different beer samples. The gluten concentration was calculated based on the signature peptides of glutenin was below 20 ppm, however, much higher concentrations of hordein were detected.

Figures 4 shows the peak area of a signature peptide of hordein for different beers.

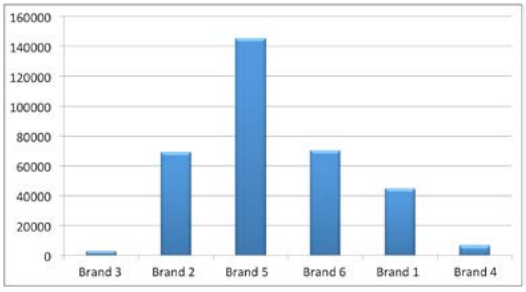

Figure 4. Peak area for a signature peptide of hordein in different beers

Summary

A highly selective and sensitive LC-MS/MS method was developed to quantitate and screen for wheat gluten proteins in various food matrices, including bakery products, fermented beverages, and baby formula. This method measures unique, stable signature peptides using pre-determined MRM transitions for accurate quantitation and detection. The method was able to detect accurately gluten protein concentration as low as 5 ppm with a CV less than 20%.

Signature peptides for rye, barley, and oats were also established so that other major grains could be identified in various food matrices. LC-MS/MS methods permit the analysis of several MRM transitions simultaneously, offering a significant advantage over ELISA-based assays by supporting the detection of multiple grain species from the same food homogenate in a single injection.

References

1 L. Elli, et al.: 'Diagnosis of gluten related disorders: Celiac disease, wheat allergy and non-celiac gluten sensitivity' World J Gastroenterol. 6 (2015) 7110-7119

2 S. Lock: 'Gluten Detection and Speciation by Liquid Chromatography Mass Spectrometry (LC-MS/MS)' Foods 3 (2014) 13-29

Document number: RUO-MKT-02-2847-C

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

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

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[1]SCIEX Redwood City, California (USA); [2]SCIEX Singapore; [3]SCIEX Beijing, China

Introduction

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

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

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

Experimental

Sample Preparation

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

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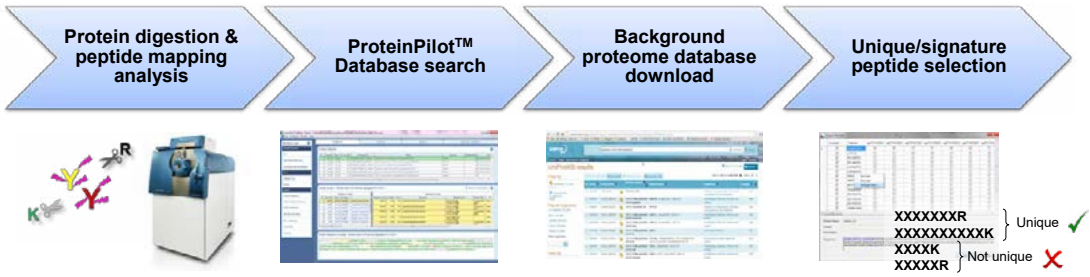

Figure 1. Signature peptide selection workflow using the SCIEX TripleTOF® 6600 system and ProteinPilot™ software

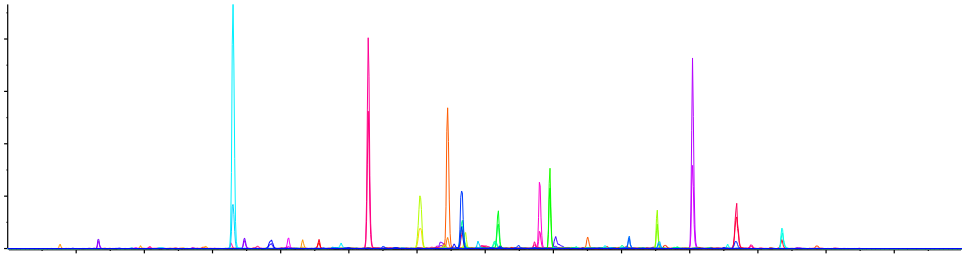

Figure 2. Extracted ion chromatograms from the LC-MS/MS analysis of raw meat mixture containing pork, beef, lamb, chicken, duck and horse (10, 20, 20, 20, 20 and 10% w/w, respectively). Multiple peaks corresponding to tryptic marker peptides are displayed.

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

LC Separation

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

MS/MS Detection

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

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

Results and Discussion

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

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

Contents

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

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

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

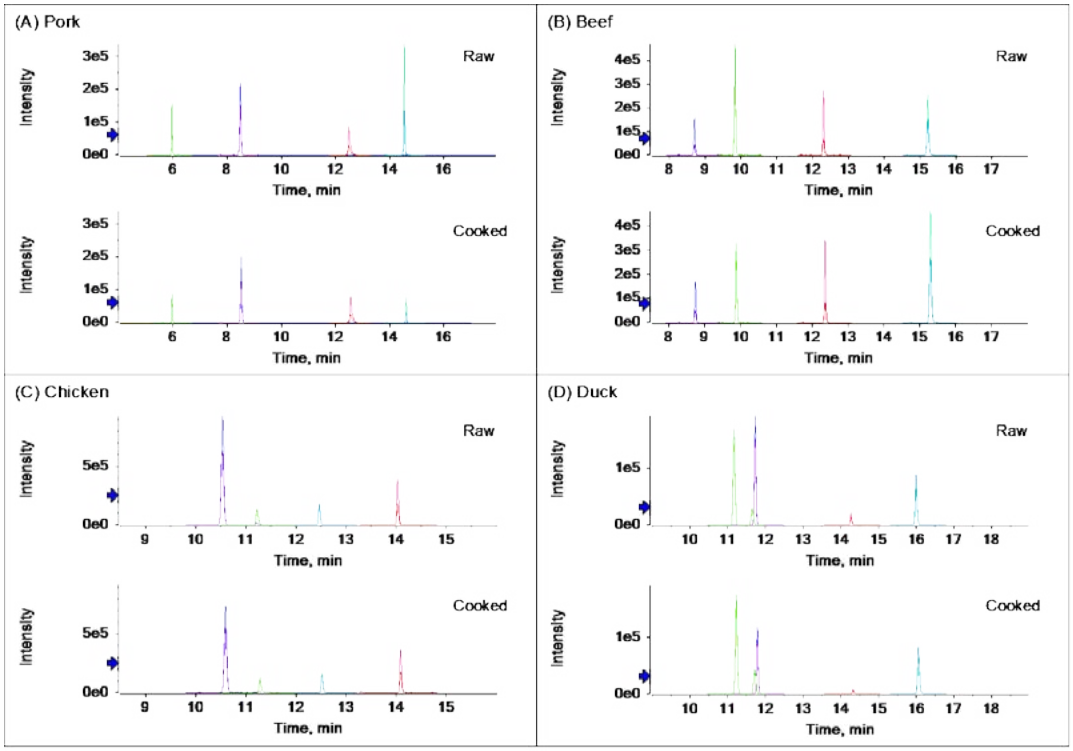

Figure 3. Extracted ion chromatograms (XIC) from the LC-MS/MS analysis of raw (top) and cooked (bottom) meat mixture containing pork, beef, chicken, duck and lamb (data not shown).

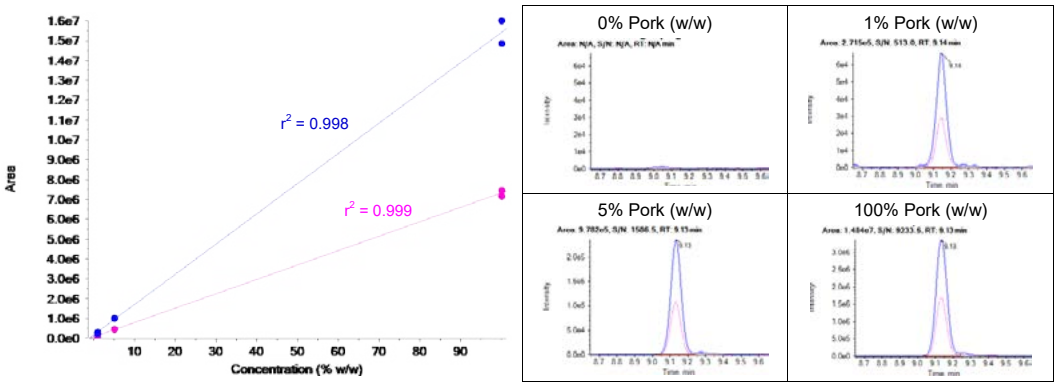

Figure 4. Calibration curves and XICs of Protein_1.Peptide_A from 0 to 100% raw pork (w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored.

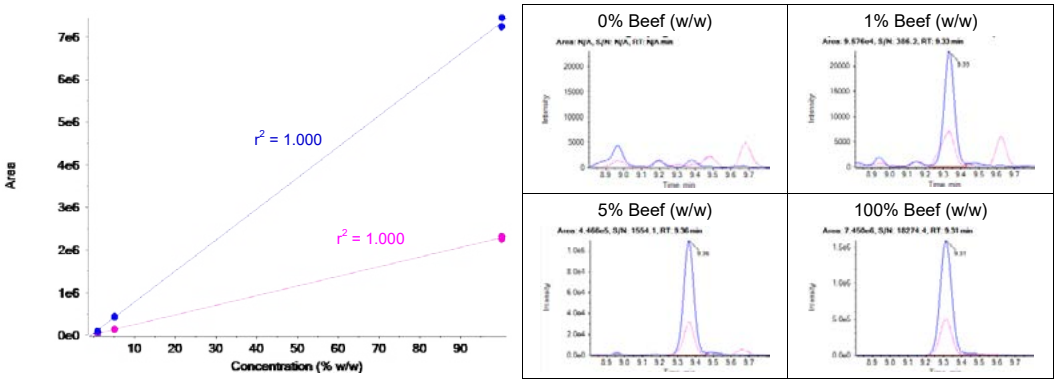

Figure 5. Calibration curves and XICs of Protein_1.Peptide_A from 0 to 100% raw beef (w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored.

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

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

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

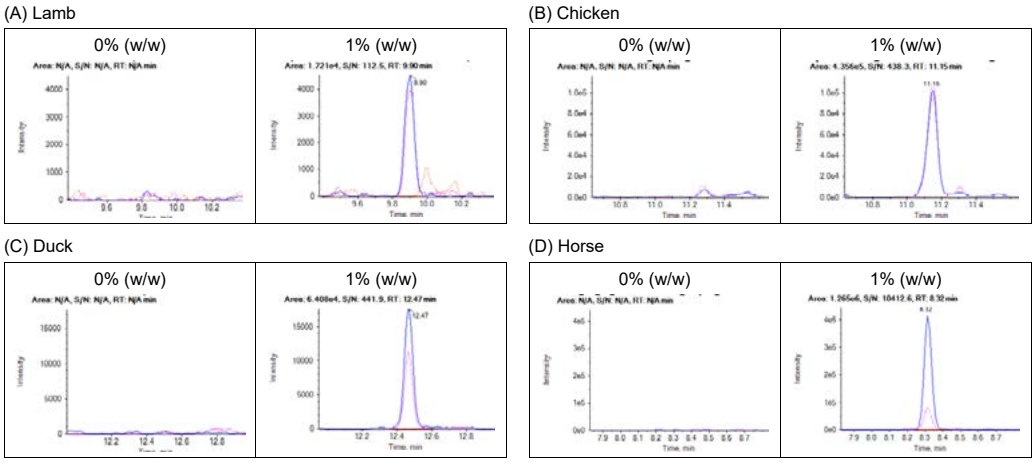

Figure 6. XICs of Protein_1.Peptide_A for 0 and 1% (w/w) of lamb, chicken, duck and horse in combined meat matrix (refer to Figure 5 for detection of pork and beef at 0 and 1% w/w). Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored for each marker peptide.

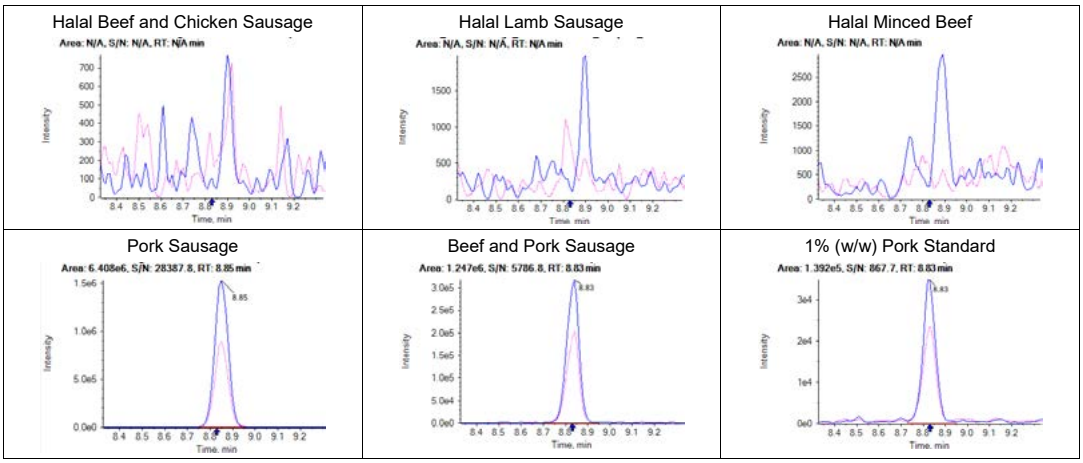

Figure 7. XIC of Pork.Protein_1.Peptide_A in commercial sausage products. Two MRM transitions, fragment 1 (blue) and fragment 2 (pink), were monitored for the marker peptide.

Summary

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

References

1. Department for Environment Food & Rural Affairs (2013). Processed beef products and horse meat. Retrieved from https://www.gov.uk/government/news/processed-beef-products-and-horse-meat

2. S. Wearne. Setting a threshold for contamination of processed meat products with undeclared meat species (2014). Retrieved from https://www.food.gov.uk/sites/default/files/multimedia/pdfs/board/fsa-140104v1.pdf

Document number:RUO-MKT-02-5606-A

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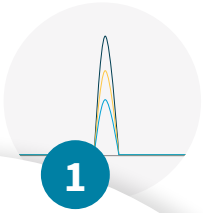
QTRAP Top 5

QTRAP technology delivers equivalent or better data, and more of it, than you can capture on an ordinary triple quadrupole system. Overcome these 5 common challenges by taking the leap to the QTRAP system.



1

Quantification challenges due to complex matrices?

QTRAP technology can decrease the worry for accurate quantification, as it is free from matrix interferences.

Increase data selectivity beyond triple quadrupole technology. The integrated linear ion trap’s (LIT) MRM^3 scan functionality enables quantification from second generation fragment ions, decreasing matrix interferences without added sample prep.

2

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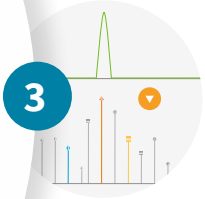
QTRAP technology can reduce the doubt for definitive compound identification.

Increase specificity beyond triple quadrupole technology. QTRAP technology’s enhanced product ion (EPI) functionality gives you complete MS/MS spectra to cross reference with an integrated library for ultimate confirmation when reporting your results.

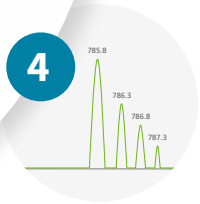


3

Looking to increase throughput by decreasing sample rechecks?

QTRAP technology produces more high-quality results in every run.

Increase throughput beyond triple quadrupole technology. By capturing MRM and enhanced product ion (EPI) MS/MS confirmation scans in one injection, you get high-quality MRM quantification with MS/MS confirmation at once for ultimate throughput and fewer rechecks.



4

Needing to find unknowns in your samples?

QTRAP technology has the tools for your search.

Screen for unknowns better than you can with triple quadrupole technology. With the enhanced mass spec (EMS), enhanced resolution (ER) and enhanced product ion (EPI) scanning capabilities, the QTRAP system can help you find and characterize components in your sample.

5

Being asked to do more than just quantification?

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Better selectivity. Complete confidence. Unrivalled efficiency. Ultimate performance.

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QTRAP Top 5

Residue analysis

The biggest benefits:

The ability to capture full MS/MS spectra for library search comparisons when an MRM peak is detected means you not only get more confidence in your compound identification to complement your MRM ion ratios, but you reduce the potential for sample rechecks to help increase your lab's throughput.

Mycotoxin analysis

The biggest benefits:

Experience the power of polarity switching and scheduled MRM™ algorithm to screen and identify multi-toxins even at trace level, within a single analysis.

Quantification in complex matrices

The biggest benefits:

Sometimes MRM does not offer enough selectivity to eliminate background interferences from complex matrices, making quantification impossible. MRM³ offers the added selectivity needed to remove those interferences for clear peaks that allow reliable quantification in complex matrices.

General chemical screening and confirmation E.g. naturally occurring toxic substances

The biggest benefits:

QTRAP technology delivers true combined qualitative and quantitative (qual/quant) analysis: Qualitative screening is powered by the enhanced product ion MS/MS scans with library searching, with quantification using the MRM peak—both from the same sample injection.

The enhanced mass spec scanning function is also powerful for the sensitive qualitative screening for unknowns across these application areas.

Veterinary drugs and antibiotic analysis

The biggest benefit:

The QTRAP 4500 system provides optimum results and accurately screens, identifies and quantifies residues at trace levels, enabling the detection of both regulated and banned substances.

The QTRAP 4500 System easily meets regulatory requirements for identification and quantification of dozens of residues in a single run.

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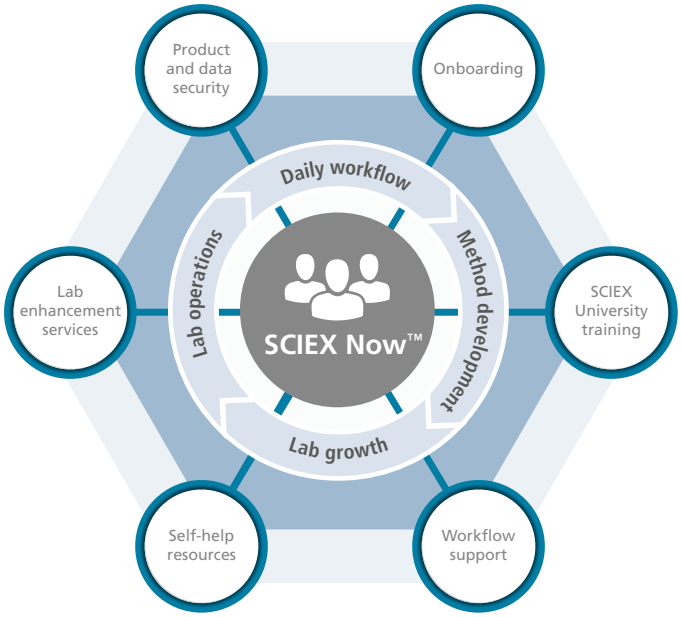

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Compendium of food testing applications

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