Affordable
Mass Spectrometry

Make the leap to LC-MS/MS with this compendium of food testing applications

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Knowledge for Life.™

Contents

Affordable Speed, Accuracy and Sensitivity

Mass spectrometry is more affordable than you might think.

Historically, mass spectrometry was beyond the technology budget of many labs. It's also perceived to be too complicated and constantly needs intricate maintenance to maintain the uptime you demand for your high throughput of samples.

Common misconceptions of mass spectrometry

Too expensive

You can do more testing with mass spec technology and improve efficiencies even more by condensing multiple tests into one. Now is the time to expand your lab's capabilities as you investigate a huge range of food analytical methods.

Too complicated

Apprehension can be high when your staff has never used mass spec instruments. However, training and implementation can happen with little disruption to your lab's daily workflow.

Too long to result

Assays require separate kits for multiple allergens and separate workflows for pesticides depending upon the functional group. Using mass spec you can simply combine your pesticides methods into one, even testing a large mycotoxin suite. One injection does it all.

HPLC is good enough

Perhaps you already use mass spectrometry, but hesitant to make the leap from HPLC to LC-MS/MS. Although effective, HPLC involves complicated sample prep techniques and is labor intensive. With food testing, traditional assays are known for producing false negatives and positives due to limited sensitivity and selectivity – forcing re-tests. The need for more chemicals and human interaction also drive up your costs and limit testing to finite amounts of compounds. Using LC-MS/MS opens your lab up to more extensive molecule tests, so you can enhance and accelerate your environmental and food testing.
Both HPLC and LC-MS/MS are powerful analytical tools. However, LC-MS/MS lends greater capabilities for classifying analytes in different compound classes in a single run.

With LC-MS/MS you will achieve

- Reduced sample preparation and handling
- Improved detection limits
- Faster analytical run times
- Increased confidence

Analysis of Pesticides in Food Samples Using the SCIEX Triple Quad™ 3500 System

André Schreiber
SCIEX Concord, Ontario (Canada)

Overview

Pesticides are widely used in agriculture to protect crops and to improve efficiency of production. Pesticide residues may pose a potential threat to human health. Modern analytical techniques, such as LC-MS/MS allow the screening for hundreds pesticide residues in food samples quickly, efficiently, and with excellent sensitivity and selectivity to meet global food trade guidelines and regulations.[1-3]

Mass spectrometers are typically considered to be expensive and complex instruments. However, the SCIEX Triple Quad™ 3500 System, combined with an extensive compound MRM catalog, provides labs with robust and reliable mass spec technology and method starting points, at an affordable price.

Here we present a method using QuEChERS extraction with Phenomenex roQ kits, filtration with Thomson filter vials, separation using a Kinetex Biphenyl 2.6u (50 x 2.1mm) column, and the SCIEX Triple Quad™ 3500 System. The mass spectrometer was operated in highly selective and sensitive Multiple Reaction Monitoring (MRM) mode. The *Scheduled* MRM™ Pro algorithm was used to obtain the best data quality. Compound identification and quantitation was achieved by monitoring two MRM transitions for each pesticide. The MRM ratio was automatically evaluated in MultiQuant™ software.

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.

Generic extraction procedures, like QuEChERS, ultra high performance LC systems combined with core-shell particle columns, 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. 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 Triple Quad™ 3500 System takes the best features of the API 3200™ system and enhances them with

modern engineering and electronics. The proven design of Turbo V™ source and Curtain Gas™ interface provide exceptional robustness and ruggedness. The advanced eQ™ electronics and the curved LINAC® collision cell were designed for ultra-fast speed of MRM detection and fast polarity switching for comprehensive multi-component analysis.

Advanced software tools like the *Scheduled* MRM™ Pro 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 best data quality. Two MRM transitions were monitored for each pesticide to use the ratio of quantifier and qualifier ion for compound identification.

Experimental

- The SCIEX iD*Quant*™ standards kit for pesticide analysis was used for method setup and preparation of calibration standards.[4]
- Store-bought fruit and vegetable samples were extracted using Phenomenex roQ QuEChERS kit buffer-salt mix and dSPE kits following the European standard method 15662.[5]
- Extracts were diluted 5 times with water in Thomson filter vials, filtered using the 0.45 µm PVDF membrane and directly

placed into the autosampler for LC-MS/MS analysis. The injection volume was set to 2 µL.

- LC separation was achieved using a Phenomenex Kinetex Biphenyl 2.6u (50 x 2.1mm) column and a fast gradient of water and methanol with 5 mM ammonium formate buffer at a flow rate of 0.5 mL/min (see Table 1 for the gradient profile).

Table 1. Gradient conditions used for the separation of pesticides

Step	Time (min)	A (%)	B (%)
0	0.0	90	10
1	0.5	90	10
2	2.0	70	30
3	9.0	40	60
4	11.0	20	80
5	12.0	5	95
6	15.0	5	95
7	16.0	90	10
8	20.0	90	10

- The SCIEX Triple Quad™ 3500 System was operated with Turbo V™ source and Electrospray Ionization (ESI) probe set to 400°C.
- Approximately 400 MRM transitions were monitored in positive polarity. Optimized transitions for all compounds were obtained through the MRM catalogue of the iMethod™ application for Pesticide Screening version 2.1.
- The *Scheduled* MRM™ Pro algorithm was used with a target cycle time of 0.5 sec and compound dependent detection windows and thresholds (Figure 1).

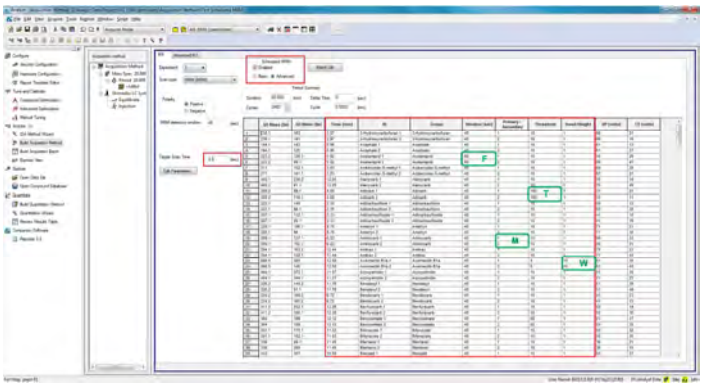

Figure 1. *Scheduled* MRM™ Pro algorithm allowing: Flexible Window Width (F), Dynamic Window Extension (T), MRM-triggered MRM (M, T), Dwell Time Weighting (W)

- MultiQuant™ software version 3.0 was used for quantitative and qualitative data processing.

Results and Discussion

Sensitivity, Reproducibility, Linearity and Accuracy

Chromatograms of a solvent standard at 10 ng/mL analyzed using the API 3200™ and Triple Quad™ 3500 are shown in Figure 2. An average gain in sensitivity of 3x was observed.

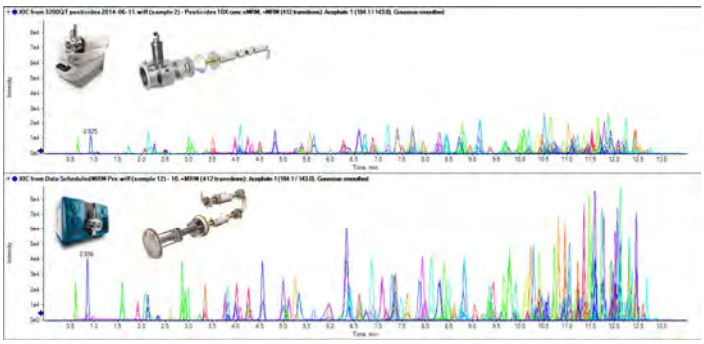

Figure 2. Sensitivity comparison of a 10 ng/mL standard analyzed using the API 3200™ system (top) and SCIEX Triple Quad™ 3500 System (bottom) with an average sensitivity gain of 3x

Most pesticides were detectable at a concentration below 1ng/mL and all pesticides had a limit of detection (LOD) of 2 ng/mL or lower. Example chromatograms at a concentration of 5 ng/mL are shown in Figure 3. The achieved sensitivity allows sample extract dilution by 5x to minimize possible matrix effects.

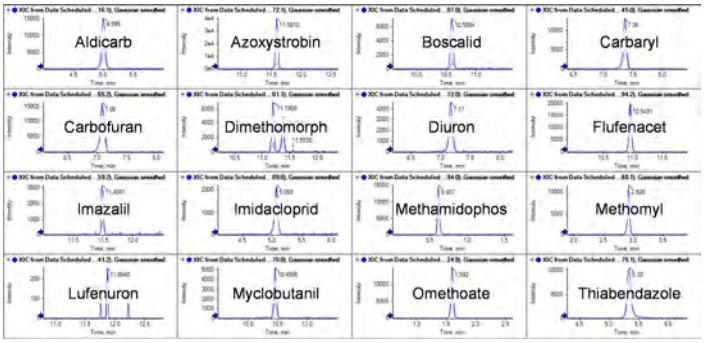

Figure 3. Sensitivity of selected pesticides detected at a concentration of 5 ng/mL using the Triple Quad™ 3500 system

Linearity was obtained over 3 to 4 orders of magnitude for most pesticides with accuracies between 80 and 120%. Data points of

the lowest or highest standards were excluded for a few pesticides with weak or strong ionization, respectively. Repeatability was studied at 1 and 10 ng/mL (n=5). The coefficient of variation (%CV) was typically below 10%.

An example calibration line of Acephate is shown in Figure 4. Both MRM transitions had a regression coefficient of > 0.998 and excellent repeatability of 2.9 and 3.2% at 1 and 10 ng/mL respectively (n=5).

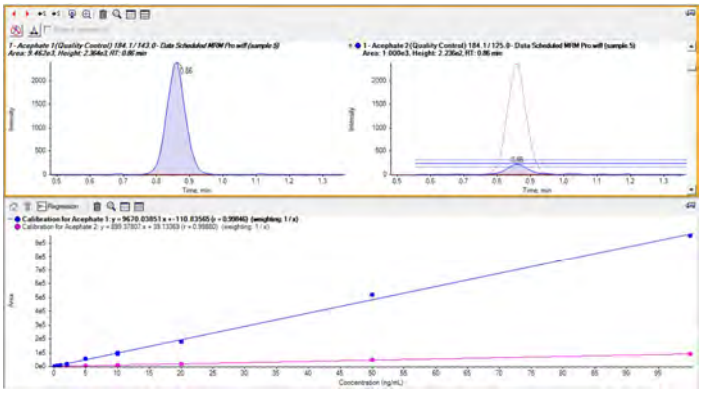

Figure 4. Peak review quantifier-qualifier ratio of Acephate at 1 ng/mL and calibration line from 0.1 to 100 ng/mL with %CV of 2.9% and 3.2% at 1 and 10 ng/mL, respectively, and.

Findings in Fruit and Vegetable Samples

The developed method was applied to the quantitation and identification of pesticides in real food extracts. Different dispersive SPE kits of Phenomenex (roQ KS0-8913, 8914, 8915, 8916) were used for sample cleanup depending on the type of matrix following the European standard method 15662. Extracts were diluted 5 times with water to minimize possible matrix effects. The diluted extracts were filtered using the Thompson 0.45 µm PVDF membrane and directly placed into the autosampler for LC-MS/MS analysis.

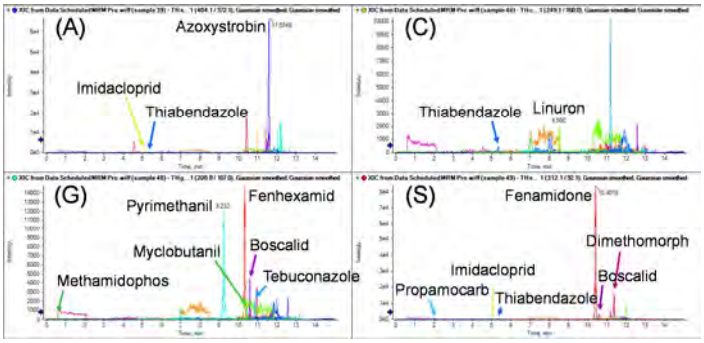

Figure 5. Detection of pesticides in filtered QuEChERS extracts of avocado (A), carrot (C), grapes (G), and spinach (S)

Example chromatograms of different type of food samples with detected compounds are presented in Figure 5. Qualitative and quantitative results are summarized in Table 2. Compound identification was based on the criteria of SANCO/12571/2013 (retention time tolerance of ± 0.02 min and maximum tolerances for ion ratios ± 30%). All quantitative and qualitative results were automatically calculated in MultiQuant™ software (Figure 6).[6]

Figure 6. Quantitation and identification based on MRM ratios in MultiQuant™ software, the example shows the side-by-side peak review for Boscalid with positive findings in grapes and spinach samples

Table 2. Summary of pesticide findings in store bought food above a concentration of 1 µg/kg

Sample	Pesticide	Concentration (µg/kg)	RT Error (min)	MRM Ratio (Expected)
Avocado	Azoxystrobin	55.0	0.01	0.146 (0.126)
	Imidacloprid	6.2	0.03	0.823 (0.818)
	Thiabendazole	2.9	0.06	1.035 (0.820)
Carrot	Linuron	14.3	0.00	0.613 (0.742)
	Thiabendazole	5.3	0.04	0.995 (0.820)
Grapes	Boscalid	17.3	0.00	0.240 (0.242)
	Fenhexamid	363	0.04	0.973 (1.053)
	Methamidophos	1.2	0.01	0.873 (0.698)
	Myclobutanil	14.2	0.02	0.811 (0.830)
	Pyrimethanil	687	0.05	0.482 (0.435)
	Tebuconazole	7.1	0.03	0.030 (0.261)
Grapefruit	Imazalil	899	0.07	0.410 (0.348)
	Imidacloprid	1.3	0.03	1.052 (0.993)
	Thiabendazole	7.6	0.03	0.812 (0.820)
Lemon	Imazalil	981	0.06	0.266 (0.348)
	Thiabendazole	7.6	0.04	0.782 (0.820)
Orange	Imazalil	1830	0.06	0.282 (0.348)
	Thiabendazole	>3000	0.04	0.812 (0.820)
Spinach	Boscalid	12.3	0.00	0.264 (0.242)
	Dimethomorph	53.7	0.08	0.537 (0.541)
	Fenamidone	755	0.01	0.749 (0.672)
	Imidacloprid	217	0.03	0.907 (0.993)
	Propamocarb	3.1	0.06	0.260 (0.336)
	Thiabendazole	3.6	0.05	0.917 (0.820)

Improving data acquisition quality with Scheduled MRM Pro algorithm

Figures 7 and 8 show results of pesticides detected in food samples to explain different features of *Scheduled* MRM™ Pro algorithm.

The detection window can be set differently for each compound depending on LC peak width and potential retention time shifts. This allows a more effective scheduling of MRM transitions resulting in better data quality. The example in Figure 7 shows Boscalid detected with a window of 45 sec, while the window of Dimethomorph was set to 120 sec to detect both isomers together.

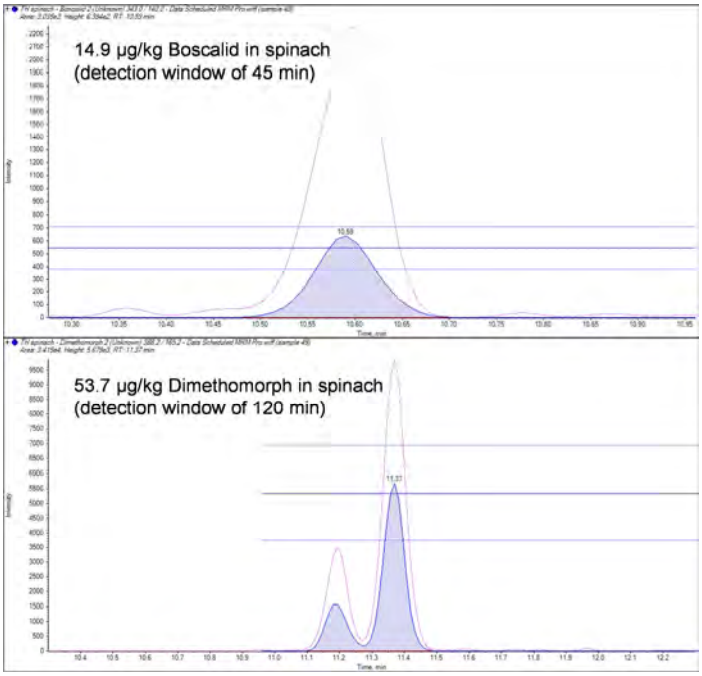

Figure 7. Examples of using the Flexible Window Width in a *Scheduled* MRM™ Pro method: the window for Boscalid was set to 45 sec and Dimethomorph was detected using a wider window to detect both isomers together

The *Scheduled* MRM™ Pro algorithm also allows automatic triggering of qualifier MRM transitions when a quantifier transitions is present (Figure 8). This feature further optimizes the MRM scheduling. The threshold is also used to automatically extend the detection window if an MRM signal is still present at the end of the default detection window.

Figure 8 shows an example of dynamic window extension for the detection of Thiabendazole in an orange sample. The sample contained Thiabendazole at more than 3000 µg/kg resulting in peak tailing. The automatic extension of the detection window enabled to capture the complete peak area for accurate quantitation and identification based on the MRM ratio.

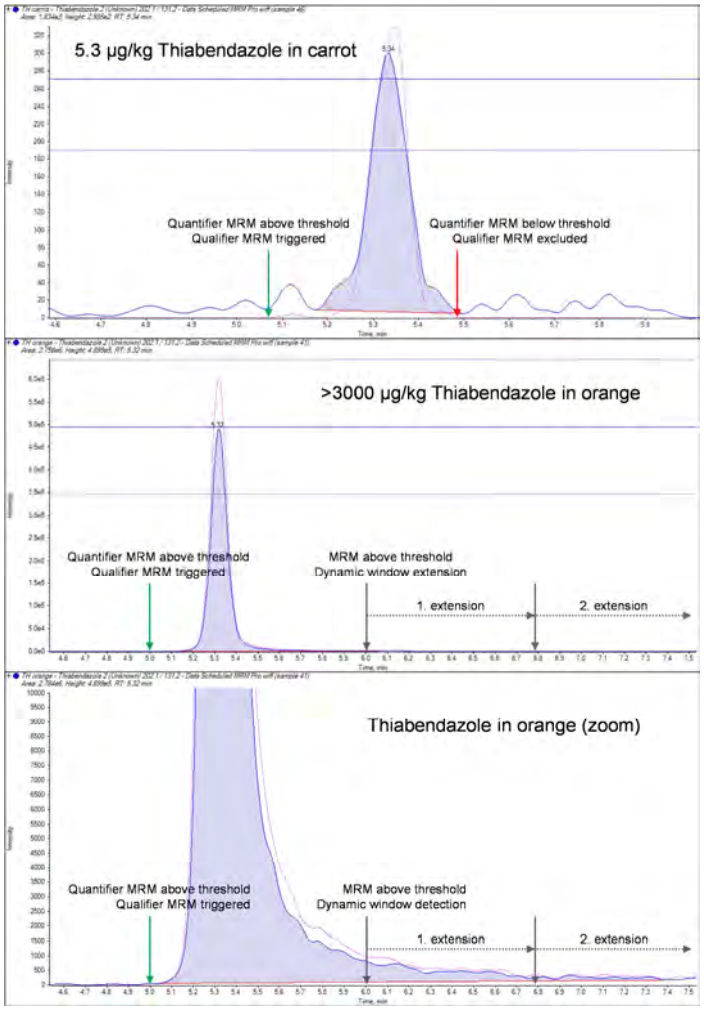

Figure 8. Examples of MRM-triggered MRM and Dynamic Window Extension: the qualifier MRM transition is automatically triggered when the quantifier MRM transitions exceeds the threshold set in the *Scheduled* MRM™ Pro method, the detection window is automatically extended if the MRM signal is above the threshold at the end of the detection window

Summary

A new LC-MS/MS method for the identification and quantitation of pesticides was developed and successfully applied to fruit and vegetable samples.

Samples were extracted using a QuEChERS protocol following the European standard method 15662 with Phenomenex roQ kits. Sample extracts were diluted 5x to minimize potential matrix effects and filtered using Thomson filter vials. The SCIEX Triple Quad™ 3500 System operated in MRM mode and utilizing the *Scheduled* MRM™ Pro algorithm was used for detection. Two MRM transitions were monitored for each analyte and the ratio of quantifier and qualifier transition was used for identification.

Qualitative and quantitative data processing was performed in MultiQuant™ software. Criteria of SANCO/12571/2013 were used for identification. All pesticides had an LOD of 2 ng/mL or lower and good linearity of 3-4 orders of magnitude with repeatability well below 10%.

References

1 M. Anastassiades et al.: 'Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce' J. AOAC Int. 86 (2003) 412-431

2 St. Lehotay: 'Determination of Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate: Collaborative Study' J. AOAC Int. 90 (2007) 485-520

3 J. Wong et al.: 'Development and Interlaboratory Validation of a QuEChERS-Based Liquid Chromatography−Tandem Mass Spectrometry Method for Multiresidue Pesticide Analysis' J. Agric. Food Chem. 58 (2010) 5897-5903

4. A. Schreiber et al.: 'Using the iD*Quant*™ Standards Kit for Pesticide Analysis to Analyze Residues in Fruits and Vegetable Samples' Application Note SCIEX (2011) #3370211-0

5 CSN EN 15662: 'Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method' (2008)

6 SANCO/12571/2013: 'Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed.'

Simultaneous Analysis of Chloramphenicol and Tetracycline Antibiotics in Food Samples Using the SCIEX Triple Quad™ 3500 System

André Schreiber
SCIEX Concord, Ontario (Canada)

Overview

Utilizing liquid chromatography with tandem mass spectrometry (LC-MS/MS) to analyze for antibiotic residues in a food samples offers many benefits to routine food testing labs, including the ability to screen for many compounds at once, the selectivity to meet regulatory guidelines, and the sensitivity to reduce sample preparation time to get to results faster. The SCIEX Triple Quad™ 3500 System enables labs performing antibiotic testing in foods to upgrade to LC-MS/MS and capitalize on its many benefits, at an affordable price.

Here we present a method using QuEChERS extraction (for the analysis of milk, meat and shrimp samples) with Phenomenex roQ kits and dilute-and-shoot (for honey samples), separation using a Kinetex Biphenyl 2.6u (50 x 2.1mm) column, and the SCIEX Triple Quad™ 3500 System for the detection of Chloramphenicol and Tetracyclines. The mass spectrometer was operated in highly selective and sensitive Multiple Reaction Monitoring (MRM) mode. Limits of detection (LOD) met regulatory limits. Compound identification and quantitation was achieved by monitoring two or three MRM transitions for each analyte. The MRM ratio was automatically evaluated in the MultiQuant™ software.

Introduction

Antibiotics are widely used as growth promoting agents and therapeutics against microbial infections. The presence of antibiotics in food of animal origin is of concern due to the potential of increasing bacterial resistance and to hypersensitivity for some individuals. Tolerance limits and maximum residue limits (MRL) have been established around the world and agencies monitor the food supply to ensure that antibiotic residue concentrations do not exceed these levels.

LC-MS/MS based methods for single-residue and single-class residues are used to monitor veterinary drugs in food. Recently multi-class multi-residue methods have been introduced to further increase monitoring efficiency.[1-3]

Generic extraction procedures[4-5], ultra high performance LC systems combined with core-shell particles columns, providing good resolution and excellent peak shape, made it possible to detect a variety of antibiotics in a single method. The LC-MS/MS system is typically used in MRM mode because of its excellent sensitivity, selectivity, and speed.

The SCIEX Triple Quad™ 3500 System takes the best features of the API 3200™ system and enhances them with modern engineering and electronics. The proven design of Turbo V™ source and Curtain Gas™ interface provide exceptional robustness and ruggedness. The advanced eQ™ electronics and the curved LINAC® collision cell were designed for ultra-fast speed of MRM detection and fast polarity switching for comprehensive multi-component analysis.

A triple quadrupole based method for the quantitation of Chloramphenicol and three selected tetracyclines was developed using selective Multiple Reaction Monitoring (MRM) with the *Scheduled* MRM™ algorithm activated. The ratio of quantifier and qualifier transition was used for compound identification. Sensitivity of detection met existing regulatory requirements, such as Codex Alimentarius' Maximum Residue Limits (MRL) of 200 µg/kg (tissue) and 100 µg/L (milk) for tetracyclines, the MRL

of 50 µg/kg set by Chinese government, and the Minimum Required Performance Limit (MRPL) for Chloramphenicol set by the European Union of 0.3 µg/kg.[6-8]

The method was successfully applied to the analysis of store-bought milk, meat, shrimp, and honey samples.

Experimental

- Store-bought food samples (milk, meat, shrimp) were extracted following the protocol of the European standard method 15662[5] using the Phenomenex roQ QuEChERS kit buffer-salt mix and the dSPE kit (#KS0-8913) containing 150 mg $MgSO_4$, 25 mg PSA, and 25 mg C18.
- QuEChERS extracts were diluted 10 times with water to minimize possible matrix effects.
- Honey samples were diluted with 5 times water and injected directly.
- The injection volume was set to either 10 or 50 µL, depending on targeted LOQ.
- LC separation was achieved using a Phenomenex Kinetex Biphenyl 2.6u (50 x 2.1mm) column and a fast gradient of water and acetonitrile with 0.1% formic acid at a flow rate of 0.5 mL/min (see Table 1 for the gradient profile).
- The SCIEX Triple Quad™ 3500 System was operated with Turbo V™ source and Electrospray Ionization (ESI) probe set to 500°C.
- Two MRM transitions were monitored for Chloramphenicol and three transitions were monitored for each tetracycline (Table 2).
- The *Scheduled* MRM™ algorithm was activated to achieve best data quality.
- Fast polarity switching of 50 msec was used. The IS voltage was to -4000 V and +5000 V, respectively.
- MultiQuant™ software version 3.0 was used for quantitative and qualitative data processing.

Table 1. Gradient conditions used for the separation

Step	Time (min)	A (%)	B (%)
0	0.0	80	20
2	4.0	5	95
3	7.0	5	95
4	7.1	80	20
5	10.0	80	20

Table 2. MRM transitions and retention times (RT) used for the detection of Chloramphenicol and tetracyclines

Compound	Polarity	RT (min)	Q1 (amu)	Q3 (amu)
Chloramphenicol 1	negative	1.32	321	152
Chloramphenicol 2	negative	1.32	321	257
Chlortetracycline 1	positive	1.30	479	444
Chlortetracycline 2	positive	1.30	479	462
Chlortetracycline 3	positive	1.30	479	154
Oxytetracycline 1	positive	0.57	461	426
Oxytetracycline 2	positive	0.57	461	444
Oxytetracycline 3	positive	0.57	461	201
Tetracycline 1	positive	0.76	445	410
Tetracycline 2	positive	0.76	445	427
Tetracycline 3	positive	0.76	445	154

Results and Discussion

Sensitivity, Reproducibility, Linearity and Accuracy

The LC-MS/MS chromatogram of a 10 ng/mL solvent standard is shown in Figure 1 highlighting the excellent separation and peak shape achieved using the Phenomenex Kinetex Biphenyl with a fast gradient of water and acetonitrile containing 0.1% formic acid. Fast polarity switching was required to detect all compounds in a single method since Chloramphenicol (negative polarity) and Chlortetracycline (positive polarity) are not chromatographically separated by this method.

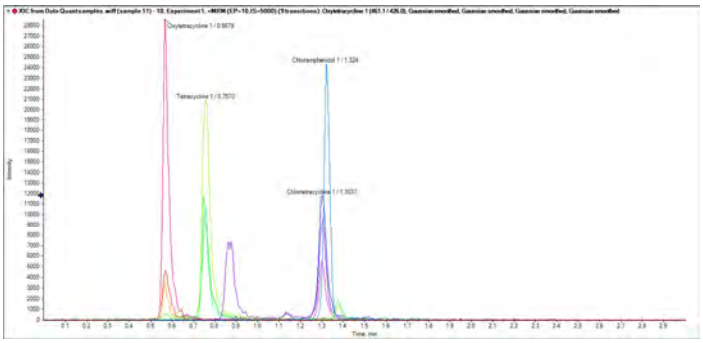

Figure 1. LC separation and detection in MRM mode of three tetracyclines and Chloramphenicol at 10 ng/mL

Figures 2 and 3 show the achieved sensitivity for all targeted antibiotics. Tetracyclines can be easily quantified at the target MRL using a small injection volume of 10 µL reducing the matrix

load for the mass spectrometer to increase robustness and to reduce potential ion suppression.

However, Chloramphenicol sometimes requires a larger injection volume to match the target MRPL while still allowing sufficient dilution to minimize potential matrix effects. In these cases, 50 µL injection volumes were utilized.

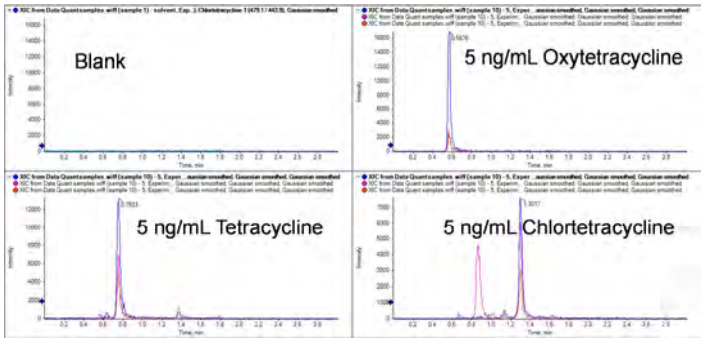

Figure 2. Sensitivity of a 5 ng/mL standard of tetracyclines (injection volume of 10 µL)

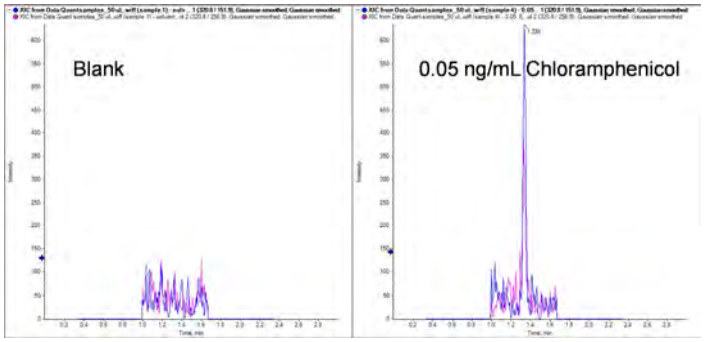

Figure 3. LOQ for Chloramphenicol of less than 0.05 ng/mL with an injection volume of 50 µL, allowing 10x dilution of matrix extracts

Calibration lines are shown in Figure 4, over the range of 0.05 to 100 ng/mL for Chloramphenicol and 0.1 to 100 ng/mL for tetracyclines, respectively, with a coefficient of regression > 0.997.

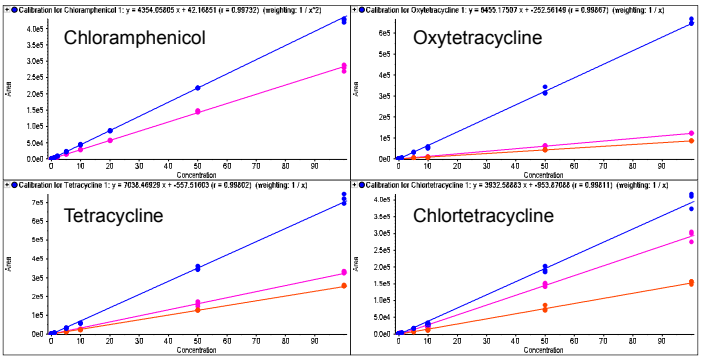

Figure 4. Calibration lines for all 4 compounds analyzed in this study

Accuracies for all calibration standards were between 80 and 120%, and repeatability was found to be better than 5% CV and 10% at the LOQ (n=3).

The achieved method performance allowed diluting sample extracts by a factor of 10 to reduce possible matrix effects. The additional use of isotope labeled internal standards is recommended to compensate matrix effects.

Findings in Food Samples

Figures 5 and 6 show matrix samples tested negative for Chloramphenicol and tetracyclines. The honey sample had a trace contamination with Chloramphenicol below the LOQ of 0.05 ng/mL (0.25 µg/kg in matrix after accounting for the 5x dilution during sample preparation).

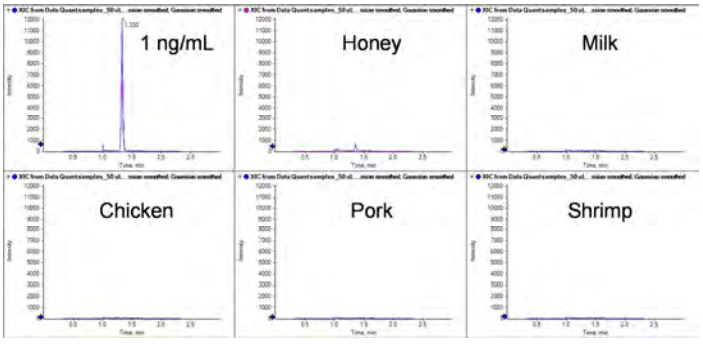

Figure 5. Blank matrices tested for Chloramphenicol (50 µL injection), the honey sample had a trace contamination with Chloramphenicol below the LOQ of 0.05 ng/mL (0.25 µg/kg in matrix after 5x dilution)

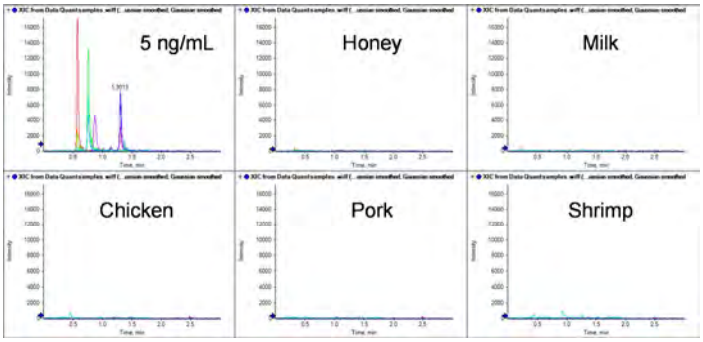

Figure 6. Blank matrices tested for tetracyclines (10 µL injection)

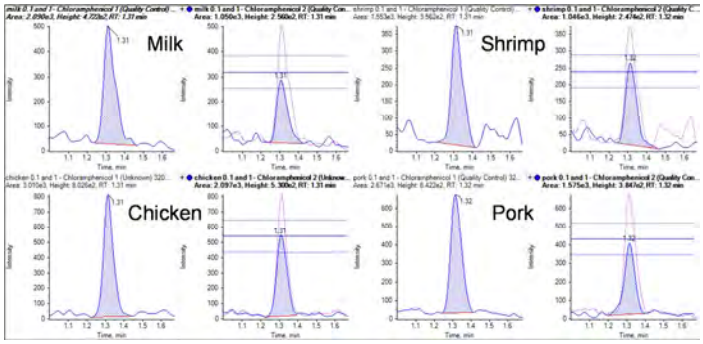

Figure 7. Different food extracts spiked with Chloramphenicol at 0.1 µg/kg (50 µL injection), the MRM ratio tolerances are displayed in the peak review window

Example chromatograms of different food samples spiked with antibiotics are presented in Figures 7 and 8. Compound identification was based on the criteria of directive 2002/657/EC[9] (retention time tolerance of ± 2.5% and maximum tolerances for ion ratios of ± 20 to 50% depending on the ratio). All quantitative and qualitative results were automatically calculated in MultiQuant™ software (Figure 6).[10]

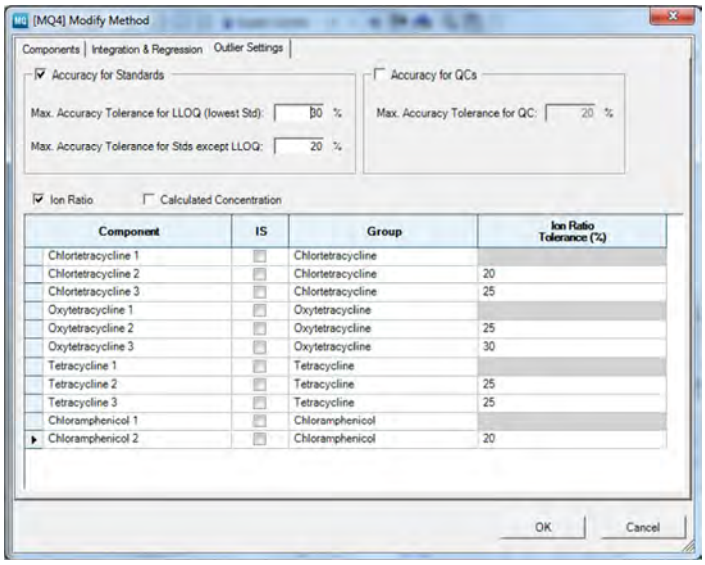

Figure 6. MRM ratio tolerances setup in the method editor of MultiQuant™ software

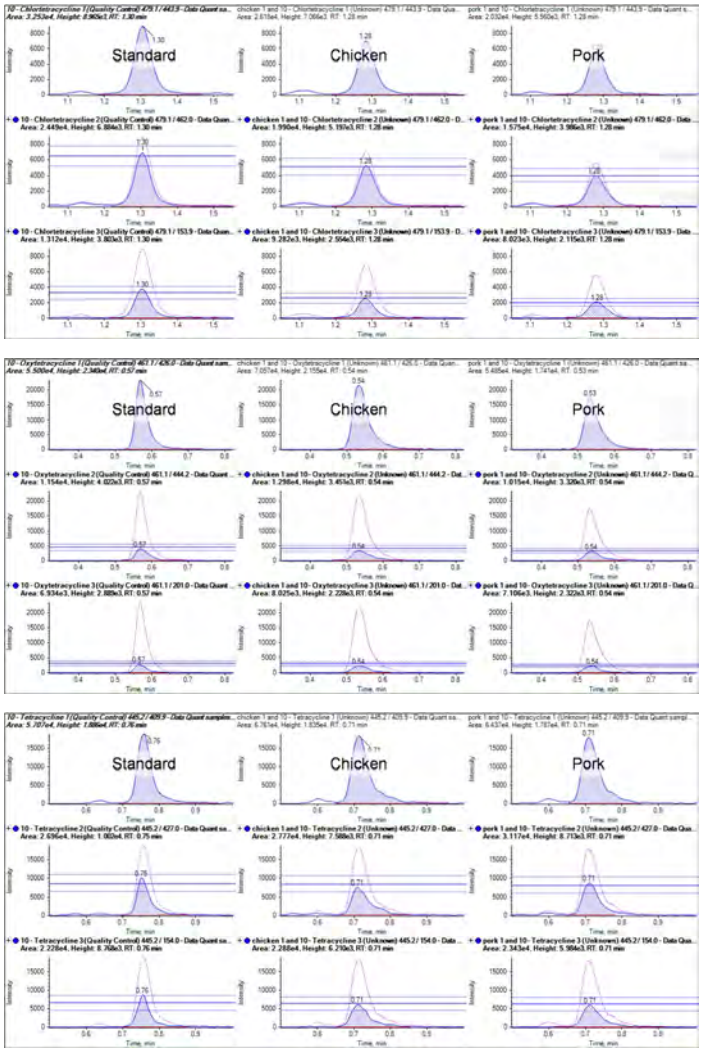

Figure 8. Side-by-side peak review of a standard injection (left) and spiked meat extracts (middle and right) with automatic calculation of MRM ratios, the MRM ratio tolerances are displayed in the peak review window

Summary

A new LC-MS/MS method for the identification and quantitation of antibiotics was developed and successfully applied to different food samples, including honey, milk, shrimp and meat.

The method consists of QuEChERS extraction followed by dilution to minimize possible ion suppression and a dilute and shoot approach for honey. The SCIEX Triple Quad™ 3500 System operated in MRM mode and utilizing the *Scheduled* MRM™ algorithm was used for detection. Limits of detection (LOD) met regulatory requirements. Two to three MRM transitions were monitored for each analyte and the ratio of quantifier and qualifier transition was used for identification. Data processing was performed in MultiQuant™ software. Identification criteria of directive 2002/657/EC were used for identification.

Acknowledgement

The author thanks Cheryl Stephenson (Eurofins Central Analytical Laboratories US) New Orleans, LA for providing standards.

References

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3 Marilyn Schneider et al.: 'Evaluation of a multi-class, multi-residue liquid chromatography – tandem mass spectrometry method for the analysis of 120 veterinary drugs in bovine kidney' Drug test. Analysis 4 (2012) 91-102

4. B. Kinsella et al.: 'New method for the analysis of flukicide and other anthelminthic residues in bovine milk and liver using liquid chromatography-tandem mass spectrometry' Analytica Chimica Acta 637 (2009) 196-207

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6 Codex Alimentarius Commission CAC/MRL 2-2012: 'Maximum Residue Limits for Veterinary Drugs in Foods' 2012

7 GB/T 21317-2007: 'Determination of tetracyclines residues in food of animal origin. LC-MS/MS method and HPLC method' (2007)

8 2003/181/EC: 'setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin' (2003)

9 2002/657/EC: 'concerning the performance of analytical methods and the interpretation of results' (2002)

10 A. Schreiber: 'MultiQuant™ Software Version 3.0 - Improving Data Quality and Processing Throughput with Better Peak Integration, Quantitative and Qualitative Compound Review for the Analysis of Food, Drinking Water, and Environmental Samples' Application Note SCIEX (2013) #8160213-01

Using the MRM Catalogue of Cliquid® Software to Quickly Build LC-MS/MS Methods for Pesticide Analysis Matching the Japanese Positive List

André Schreiber[1], Yuriko Ozeki[2], Dorothée Elbert[3], Birgit Schlutt[3], Daniel Leigh[4], Nadine Harding[4], and Byron Kieser[1]

[1]SCIEX, Concord, ON, Canada; [2]SCIEX, Tokyo, Japan; [3]SCIEX, Darmstadt, Germany; [4]SCIEX, Warrington, Cheshire, UK

Overview

This application note gives an example of the quick and easy method development of three LC-MS/MS pesticide screening methods containing a total number of 159 pesticides matching new Japanese food testing regulations.[1] All methods were generated using the MRM catalogue of pesticides in the Cliquid® Software. The developed LC-MS/MS methods were successfully used to analyze pesticides in a variety of food products.

Introduction

Recent regulations on food and environmental analysis especially in Europe and Asia require the screening for pesticides using confirmatory techniques, such as GC-MS and LC-MS/MS. With more than 1000 pesticides and their metabolites and degradation products of more than 100 compound classes in use or present in the environment there is a demand for powerful and rapid analytical methods, which can detect very low concentrations of pesticides.

Alder et al. compared the use of GC-MS and LC-MS/MS for multi residue pesticide analysis and concluded with "…the benefits of LC-MS/MS in terms of wider scope, increased sensitivity, and better selectivity are obvious."[2]

But presently, no analytical technique is able to detect all pesticides in a single method. Here the Cliquid® Software with preconfigured iMethod™ Tests provides an easy way of customizing such screening methods for a multitude of potential residues or pollutants. Built into the software is an MRM catalogue containing more than 500 compounds which can be used to quickly build LC-MS/MS methods. Compound names and information, optimized MRM transitions, and compound dependent parameters together with retention times are saved into this catalogue. The MRM catalogue can be adjusted and extended easily with new compounds and more parameters.

Experimental

Chemicals and Samples

Pesticide standards used to build the MRM catalogue were obtained at highest available purity from Sigma Aldrich (PESTANAL, analytical standard) and Wako Pure Chemical Industries (for Pesticide Residue Analysis). Solvents and buffers were obtained from Sigma Aldrich (LC-MS grade).

Fruit and vegetable samples (apple, apricot, banana, cucumber, grape, grapefruit, kiwi, lemon, orange, pear, pepper, raisin, strawberry, tea, and tomato) were obtained from a supermarket.

Sample Preparation

A modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure was used to extract fruits and vegetables.[3-5]

HPLC Separation

A Shimadzu Prominence LC system consisting of system controller (CBM-20A), 2 pumps (LC-20AD) with semimicro gradient mixer, degasser (DGU-20A3), autosampler (SIL-AC), and column oven (CTO-20AC) were used Separation was performed on a Phenomenex Synergi 4u Fusion-RP 80A 50x2 mm column with an eluent of H_2O + 5 mM ammonium formate (A) and CH_3OH + 5 mM ammonium formate (B). The gradient conditions are shown in Table 1. A flow rate of 250 µL/min was used. The column oven temperature was set to 25°C. An injection volume of 20 µL was used.

Table 1. LC gradient using water + 5mM ammonium formate (A) and methanol + 5mM ammonium formate (B)

Step	Time (µL/min)	A (%)	B (%)
1	5	80	20
2	8	10	90
3	14	10	90
4	15	80	20

MS/MS Detection

A hybrid triple quadrupole linear ion trap mass spectrometer 3200 QTRAP® LC/MS/MS system with Turbo V™ source and Electrospray Ionization (ESI) probe was used. The mass spectrometer was operated in Multiple Reaction Monitoring (MRM) mode. MRM mode allowed quantifying targeted compounds with highest selectivity and sensitivity by monitoring the transition from the precursor ion (filtered in Q1) to a product ion (generated in a collision cell Q2 and filtered in Q3). Compound identification was performed based on the ratio of two MRM transitions detected for each analyte.

Cliquid® Software

Cliquid® Software was used to build LC-MS/MS methods, to analyze samples, and to automatically generate reports. Cliquid® Software was specifically designed for ease-of-use of LC-MS/MS technology in routine testing laboratories. It simplifies the operation of an LC-MS/MS system using preconfigured iMethod™ Tests, a simple four step wizard to setup the analysis and automatic reporting. In addition an MRM catalogue containing more than 500 pesticides is available to quickly build methods.[6]

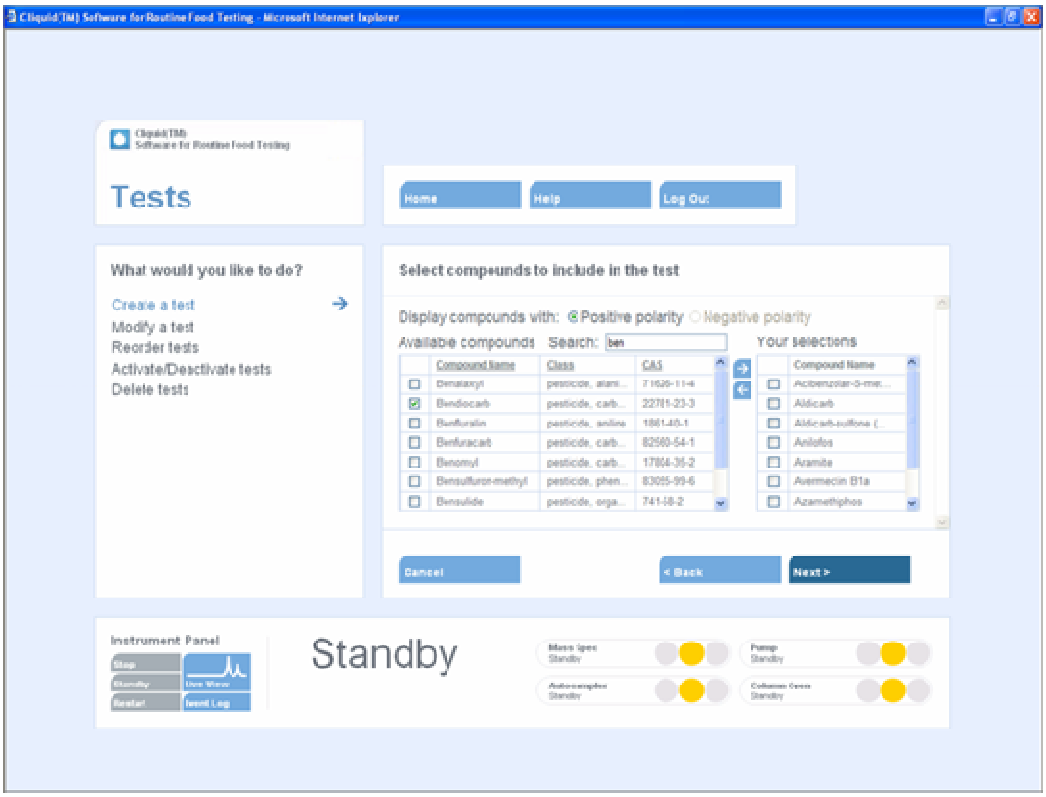


Figure 1. Screenshot of Cliquid® Software showing the MRM catalogue test wizard

The MRM catalogue of Cliquid® Software was used to build the following multi-residue methods matching the Japanese Positive List.1 Two MRM transitions were detected for all pesticides. Dwell times were adjusted automatically depending on the number of monitored compounds allow collecting enough data points across the LC peaks. Complete LC-MS/MS parameters are available in the method documentation imbedded into the Cliquid® Software.

Japanese Positive List I (202 MRM transitions with 5 ms dwell time in positive polarity): Acibenzolar-S-methyl, Aldicarb, Aldicarb-sulfone, Anilofos, Aramite, Avermectin B1a, Azamethiphos, Azinphos-methyl, Azoxystrobin, Bendiocarb, Benzofenap, Boscalid, Butafenacil, Carbaryl, Carbofuran, Carpropamid, Chloridazon, Chloroxuron, Chromafenozide, Clofentezine, Clomeprop, Cloquintocet-mexyl, Clothianidin, Cumyluron, Cyazofamid, Cycloate, Cycloprothrin, Cyflufenamid, Cyprodinil, Daimuron, Diallate, Diflubenzuron, Dimethirimol, Dimethomorph, Diuron, Epoxiconazole, Fenamidone, Fenobucarb, Fenoxaprop-ethyl, Fenoxycarb, Fenpyroximate, Ferimzone, Flufenacet, Flufenoxuron, Fluridone, Furametpyr, Furathiocarb, Hexaflumuron, Hexythiazox, Imazalil, Imidacloprid, Indanofan, Indoxacarb, Iprodione, Iprovalicarb, Isoxaflutole, Lactofen, Linuron, Lufenuron, Mepanipyrim, Methabenzthiazuron, Methiocarb, Methomyl, Methoxyfenozide, Milbemectin A3, Milbemectin A4, Monolinuron, Naproanilide, Novaluron, Oryzalin, Oxamyl, Oxaziclomefone, Oxycarboxin, Pencycuron, Pentoxazone, Phenmedipham, Pirimicarb, Propaquizafop, Pyraclostrobin, Pyrazolynate, Pyriftalid, Quizalofop-ethyl, Quizalofop-P-tefuryl, Silafluofen, Simeconazole, Spinosyn A, Spinosyn D, Tebufenozide, Tebuthiuron, Teflubenzuron, Tetrachlorvinphos, Thiabendazole, Thiacloprid, Thiamethoxam, Thiodicarb, Tralkoxydim (2 isomers), Tridemorph (2 isomers), Triflumuron, Triticonazole

Japanese Positive List II (36 MRM transitions with 50 ms dwell time in negative polarity): 1-Napthaleneacetic acid, 2,4-D, 2,4-DP (Dichlorprop), 4-Chlorophenoxyacetic acid, Acifluorfen, Bromoxynil, Cloprop, Cyclanilide, Fluazifop, Fluroxypyr, Fomesafen, Gibberellin, Ioxynil, MCPA, MCPB, MCPP (Mecoprop), Thidiazuron, Triclopyr

Japanese Positive List II (86 MRM transitions with 15 ms dwell time in positive polarity): Azimsulfuron, Bensulfuron-methyl, Chlorimuronethyl, Chlorsulfuron, Cinosulfuron, Clodinafop acid, Clofencet, Cloransulam-methyl, Cyclosulfamuron, Diclomezine, Diclosulam, Ethametsulfuronmethyl, Ethoxysulfuron, Fenhexamid, Flazasulfuron, Florasulam, Fluazifop, Flumetsulam, Fluroxypyr, Foramsulfuron, Forchlorfenuron, Halosulfuron-methyl, Haloxyfop, Imazaquin, Imazosulfuron, Iodosulfuron-methyl, Mesosulfuron-methyl, Metosulam, Metsulfuron-methyl, Naptalam, Penoxsulam, Primisulfuron-methyl, Propoxycarbazone, Prosulfuron, Pyrazosulfuron-ethyl, Sulfentrazone, Sulfosulfuron, Thidiazuron, Thifensulfuron-methyl, Triasulfuron, Tribenuron-methyl, Trifloxysulfuron, Triflusulfuron-methyl

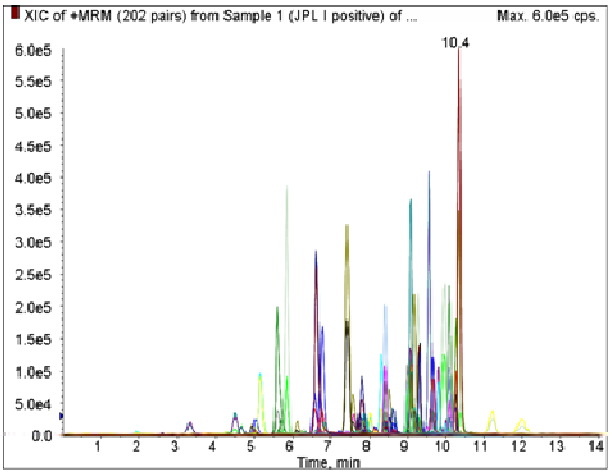


Figure 2. LC-MS/MS chromatogram of 101 pesticides of Japanese Positive List I method in positive polarity

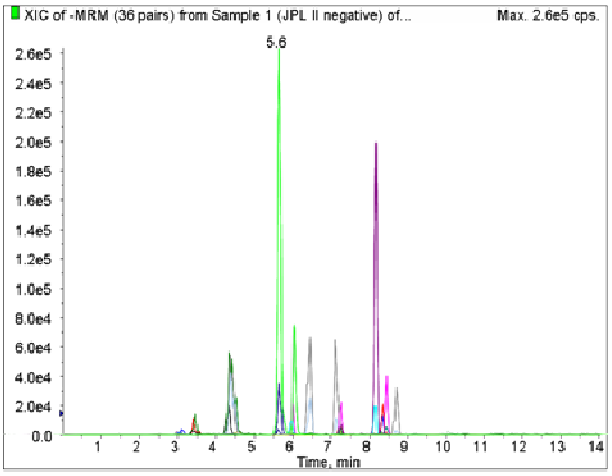


Figure 3. LC-MS/MS chromatogram of 18 pesticides of Japanese Positive List II method in negative polarity

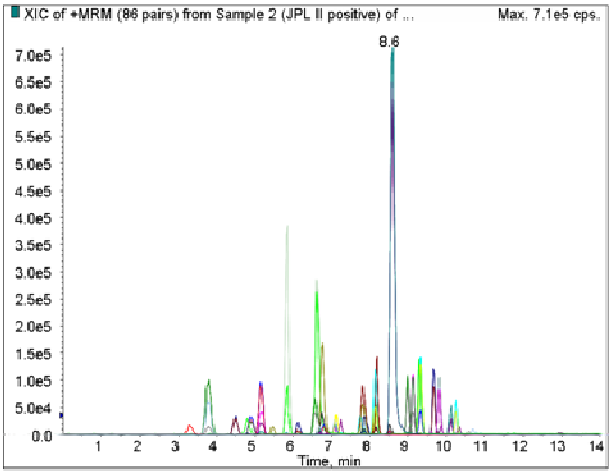


Figure 4. LC-MS/MS chromatogram of 43 pesticides of Japanese Positive List II method in negative polarity

Results and Discussion

Standard chromatograms of all developed methods are given in Figure 2-4 to illustrate chromatographic separation and mass spectrometric detection using Electrospray Ionization in positive and negative polarity, respectively. Two MRM transitions were monitored to allow quantitation and identification. The Linear Accelerator (LINAC®) collision cell of the 3200 QTRAP® system enables the detection of all MRM transitions in a single detection window using short dwell times (5 ms to 50 ms) without loss in sensitivity. Most studied pesticides were detectable at a concentration below 1ng/mL and all pesticides were detectable at 5 ng/mL using the LC-MS/MS methods built with the MRM catalogue of Cliquid® Software. Example chromatograms highlighting the superior sensitivity of the 3200 QTRAP® system are given in Figure 5.

The LOD values in Table 2 demonstrate that the developed LC-MS/MS methods provide enough sensitivity to test for pesticides at the required 10 µg/kg level in food samples. The linear range, determined based on accuracy between 85 and 115% with linear regression and 1/x weighting, was 2.5 to 3.5 orders of magnitude starting from the LOD for each analyte.

The standard deviation of the ratio of quantifier and qualifier MRM transition was typically in between ±15%.

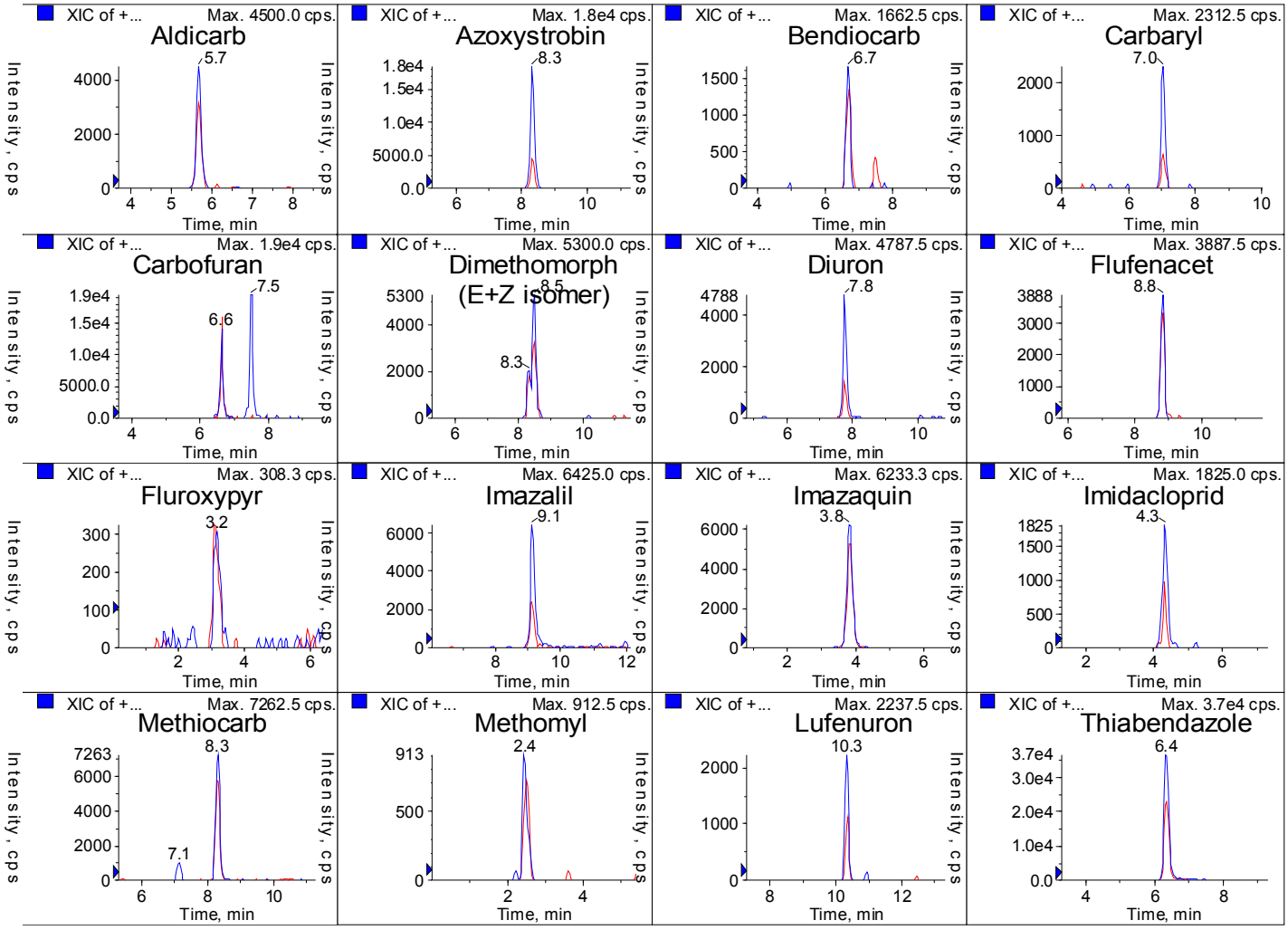


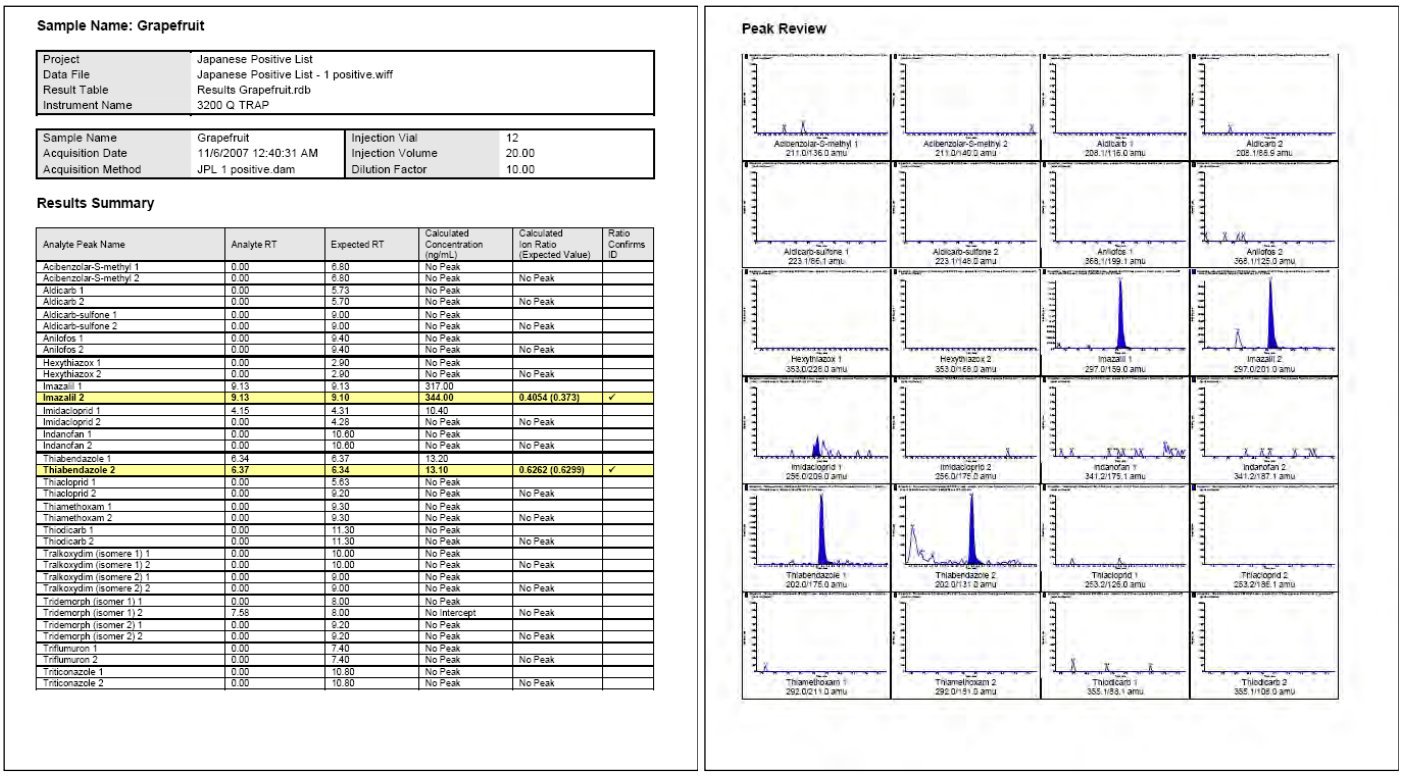
Figure 5. Sensitivity of selected pesticides detected by LC-MS/MS at a concentration of 10ng/mL

Table 2. Limits of Detection (LOD) with Signal-to-Noise = 3

Pesticide	LOD (ng/mL)	Pesticide	LOD (ng/mL)
Aldicarb	0.05	*Fluroxypyr*	1.76
Azoxystrobin	0.04	*Imazalil*	0.16
Bendiocarb	0.33	*Imazaquin*	0.13
Carbaryl	0.23	*Imidacloprid*	0.18
Carbofuran	0.04	*Lufenuron*	0.09
Dimethomorph	0.12	*Methiocarb*	0.08
Diuron	0.19	*Methomyl*	0.19
Flufenacet	0.10	*Thiabendazole*	0.11

The developed LC-MS/MS methods were used to monitor pesticides in various fruit and vegetable samples after simple QuEChERS extraction. Figure 6-8 show example reports of the analysis of selected samples.

These reports were generated automatically after data acquisition by Cliquid® Software. The software provides a large variety of preconfigured report styles to report, for instance, calibration lines, statistical data, concentrations of analyzed residues in unknown samples including MRM ratio calculation and chromatograms.



Sample Name: Grapefruit

Project	Japanese Positive List
Data File	Japanese Positive List - 1 positive.wiff
Result Table	Results Grapefruit.rdb
Instrument Name	3200 Q TRAP

Sample Name	Grapefruit	Injection Vial	12
Acquisition Date	11/6/2007 12:40:31 AM	Injection Volume	20.00
Acquisition Method	JPL 1 positive.dam	Dilution Factor	10.00

Results Summary

Analyte Peak Name	Analyte RT	Expected RT	Calculated Concentration (ng/mL)	Calculated Ion Ratio (Expected Value)	Ratio Confirms ID
Acibenzolar-S-methyl 1	0.00	6.80	No Peak		
Acibenzolar-S-methyl 2	0.00	6.80	No Peak	No Peak	
Aldicarb 1	0.00	5.73	No Peak		
Aldicarb 2	0.00	5.70	No Peak	No Peak	
Aldicarb-sulfone 1	0.00	9.00	No Peak		
Aldicarb-sulfone 2	0.00	9.00	No Peak	No Peak	
Anilofos 1	0.00	9.40	No Peak		
Anilofos 2	0.00	9.40	No Peak	No Peak	
Hexythiazox 1	0.00	2.90	No Peak		
Hexythiazox 2	0.00	2.90	No Peak	No Peak	
Imazalil 1	9.13	9.13	317.00		
Imazalil 2	**9.13**	**9.10**	**344.00**	**0.4054 (0.373)**	✓
Imidacloprid 1	4.15	4.31	10.40		
Imidacloprid 2	0.00	4.28	No Peak	No Peak	
Indanofan 1	0.00	10.60	No Peak		
Indanofan 2	0.00	10.60	No Peak	No Peak	
Thiabendazole 1	6.34	6.37	13.20		
Thiabendazole 2	**6.37**	**6.34**	**13.10**	**0.6262 (0.6299)**	✓
Thiacloprid 1	0.00	5.63	No Peak		
Thiacloprid 2	0.00	9.20	No Peak	No Peak	
Thiamethoxam 1	0.00	9.30	No Peak		
Thiamethoxam 2	0.00	9.30	No Peak	No Peak	
Thiodicarb 1	0.00	11.30	No Peak		
Thiodicarb 2	0.00	11.30	No Peak	No Peak	
Tralkoxydim (isomere 1) 1	0.00	10.00	No Peak		
Tralkoxydim (isomere 1) 2	0.00	10.00	No Peak	No Peak	
Tralkoxydim (isomere 2) 1	0.00	9.00	No Peak		
Tralkoxydim (isomere 2) 2	0.00	9.00	No Peak	No Peak	
Tridemorph (isomer 1) 1	0.00	8.00	No Peak		
Tridemorph (isomer 1) 2	7.58	8.00	No Intercept	No Peak	
Tridemorph (isomer 2) 1	0.00	9.20	No Peak		
Tridemorph (isomer 2) 2	0.00	9.20	No Peak	No Peak	
Triflumuron 1	0.00	7.40	No Peak		
Triflumuron 2	0.00	7.40	No Peak	No Peak	
Triticonazole 1	0.00	10.80	No Peak		
Triticonazole 2	0.00	10.80	No Peak	No Peak	

Figure 6. Report (partial) of positive finding of Imazalil (317 µg/kg) and Thiabendazole (13.2 µg/kg) in a grapefruit sample including a result table with highlighted positive identification based on MRM ratio calculation (left) and chromatograms (right)

Sample Name: Grape

Project	Japanese Positive List
Data File	Japanese Positive List - 1 positive.wiff
Result Table	Results Grape.rdb
Instrument Name	3200 Q TRAP

Sample Name	Grape	Injection Vial	11
Acquisition Date	11/6/2007 12:19:31 AM	Injection Volume	20.00
Acquisition Method	JPL 1 positive.dam	Dilution Factor	10.00

Results Summary

Analyte Peak Name	Analyte RT	Expected RT	Calculated Concentration (ng/mL)	Calculated Ion Ratio (Expected Value)	Ratio Confirms ID
Mepanipyrim 1	0.00	7.20	No Peak		
Mepanipyrim 2	0.00	7.20	No Peak	No Peak	
Methabenzthiazuron 1	0.00	7.48	No Peak		
Methabenzthiazuron 2	0.00	7.48	No Peak	No Peak	
Methiocarb 1	0.00	8.33	No Peak		
Methiocarb 2	0.00	8.29	No Peak	No Peak	
Methomyl 1	2.61	2.43	1.910		
Methomyl 2	2.60	2.49	4.950	2.2112 (0.8519)	
Methoxyfenozide 1	0.00	2.90	No Peak		
Methoxyfenozide 2	0.00	2.90	No Peak	No Peak	
Milbemectin A3 1	0.00	9.70	No Peak		
Milbemectin A3 2	0.00	9.70	No Peak	No Peak	
Milbemectin A4 1	0.00	11.30	No Peak		
Milbemectin A4 2	0.00	11.30	No Peak	No Peak	
Monolinuron 1	0.00	7.11	No Peak		
Monolinuron 2	0.00	7.11	No Peak	No Peak	
Naproanilide 1	0.00	9.40	No Peak		
Naproanilide 2	0.00	9.40	No Peak	No Peak	
Novaluron 1	0.00	5.90	No Peak		
Novaluron 2	0.00	5.90	No Peak	No Peak	
Oryzalin 1	0.00	7.60	No Peak		
Oryzalin 2	0.00	7.60	No Peak	No Peak	
Oxamyl 1	0.00	9.70	No Peak		
Oxamyl 2	0.00	9.70	No Peak	No Peak	
Oxaziclomefone 1	0.00	9.80	No Peak		
Oxaziclomefone 2	0.00	9.80	No Peak	No Peak	
Oxycarboxin 1	10.20	10.30	No Intercept		
Oxycarboxin 2	0.00	10.30	No Peak	No Peak	
Pencycuron 1	0.00	4.70	No Peak		
Pencycuron 2	0.00	4.70	No Peak	No Peak	
Pentoxazone 1	0.00	11.00	No Peak		
Pentoxazone 2	0.00	11.00	No Peak	No Peak	
Phenmedipham 1	0.00	8.80	No Peak		
Phenmedipham 2	0.00	8.80	No Peak	No Peak	
Pirimicarb 1	0.00	10.10	No Peak		
Pirimicarb 2	0.00	10.10	No Peak	No Peak	
Propaquizafop 1	0.00	15.40	No Peak		
Propaquizafop 2	0.00	15.40	No Peak	No Peak	
Pyraclostrobin 1	0.00	9.70	No Peak		
Pyraclostrobin 2	0.00	9.70	No Peak	No Peak	

Peak Review

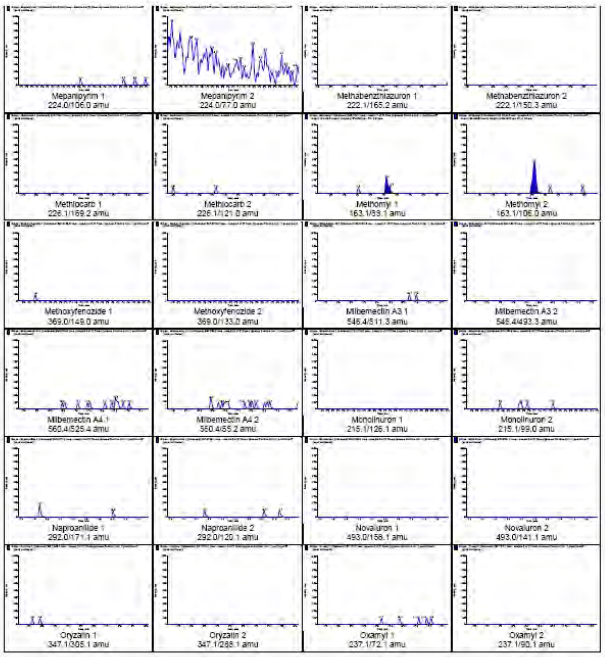


Figure 7. Report (partial) of positive finding of Methomyl (1.9 µg/kg) in a grape sample including a result table (left) and chromatograms (right) — this finding was discounted based on an incorrect MRM ratio

Sample Name: Orange

Project	Japanese Positive List
Data File	Japanese Positive List - 1 positive.wiff
Result Table	Results Orange.rdb
Instrument Name	3200 Q TRAP

Sample Name	Orange	Injection Vial	15
Acquisition Date	11/6/2007 1:43:27 AM	Injection Volume	20.00
Acquisition Method	JPL 1 positive.dam	Dilution Factor	10.00

Results Summary

Analyte Peak Name	Analyte RT	Expected RT	Calculated Concentration (ng/mL)	Calculated Ion Ratio (Expected Value)	Ratio Confirms ID
Daimuron 1	0.00	9.70	No Peak		
Daimuron 2	9.47	9.70	141.00	No Peak ( )	
Diallate 1	0.00	9.50	No Peak		
Diallate 2	0.00	9.50	No Peak	No Peak ( )	
Diflubenzuron 1	0.00	9.03	No Peak		
Diflubenzuron 2	0.00	10.80	No Peak	No Peak ( )	
Dimethirimol 1	0.00	11.20	No Peak		
Dimethirimol 2	0.00	11.20	No Peak	No Peak ( )	
Dimethomorph 1	8.83	8.46	23.30		
Dimethomorph 2	**8.83**	**8.46**	**21.15**	**1.4419 (1.5568)**	✓
Diuron 1	0.00	7.75	No Peak		
Diuron 2	0.00	7.75	No Peak	No Peak ( )	
Epoxiconazole 1	0.00	8.70	No Peak		
Epoxiconazole 2	0.00	8.70	No Peak	No Peak ( )	
Fenamidone 1	0.00	8.50	No Peak		
Fenamidone 2	0.00	8.50	No Peak	No Peak ( )	
Imazalil 1	9.13	9.13	274.00		
Imazalil 2	**9.13**	**9.10**	**283.00**	**0.3857 (0.373)**	✓
Imidacloprid 1	0.00	4.31	No Peak		
Imidacloprid 2	0.00	4.28	No Peak	No Peak ( )	
Indanofan 1	0.00	10.60	No Peak		
Indanofan 2	0.00	10.60	No Peak	No Peak ( )	
Indoxacarb 1	0.00	8.20	No Peak		
Indoxacarb 2	0.00	8.20	No Peak	No Peak ( )	
Iprodione 1	0.00	7.40	No Peak		
Iprodione 2	0.00	7.40	No Peak	No Peak ( )	
Iprovalicarb 1	0.00	10.10	No Peak		
Iprovalicarb 2	0.00	10.10	No Peak	No Peak ( )	
Isoxaflutole 1	0.00	9.20	No Peak		
Isoxaflutole 2	0.00	9.20	No Peak	No Peak ( )	
Lactofen 1	0.00	9.90	No Peak		
Lactofen 2	0.00	9.90	No Peak	No Peak ( )	
Linuron 1	0.00	8.22	No Peak		
Linuron 2	0.00	8.26	No Peak	No Peak ( )	
Lufenuron 1	0.00	10.30	No Peak		
Lufenuron 2	0.00	10.30	No Peak	No Peak ( )	
Mepanipyrim 1	0.00	7.20	No Peak		
Mepanipyrim 2	0.00	7.20	No Peak	No Peak ( )	
Methabenzthiazuron 1	0.00	7.48	No Peak		
Methabenzthiazuron 2	0.00	7.48	No Peak	No Peak ( )	
Methiocarb 1	0.00	8.33	No Peak		
Methiocarb 2	0.00	8.29	No Peak	No Peak ( )	

Peak Review

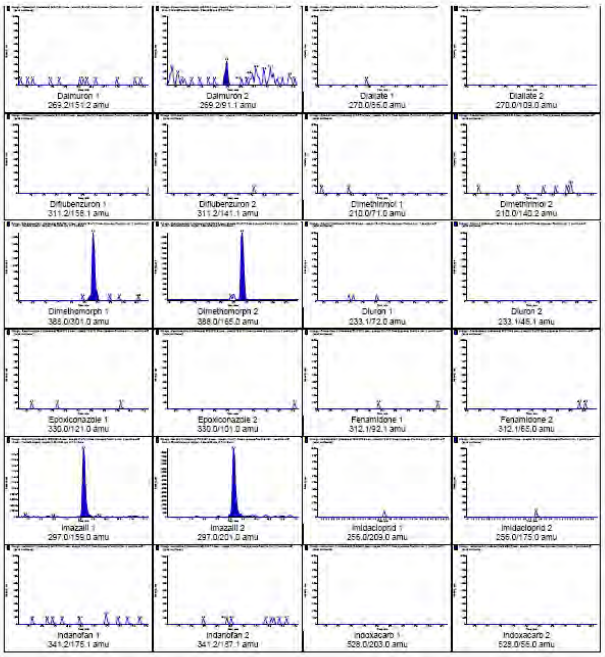


Figure 8. Report of positive finding of Dimethomorph (23.3 µg/kg) and Imazalil (274 µg/kg) in an orange sample including a result table with highlighted positive identification based on MRM ratio calculation (left) and chromatograms (right)

Summary

Three LC-MS/MS methods for the analysis of pesticides matching new Japanese Positive List were developed and successfully applied to the analysis of fruits, vegetables. The MRM catalogue of Cliquid® Software was utilized to build these MRM methods. The catalogue enables quick method customization depending on the analytical problem without time consuming method optimization. The presented method are available as iMethod™ Tests to download into Cliquid® Software. Visit www.absciex.com/iMethods

Future studies will include a complete validation of different sample preparation methods to minimize matrix effects. In addition the use of internal standards in such multi targeted methods will be investigated.

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The Quantitation and Identification of Artificial Sweeteners in Food and Drink by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

Stephen Lock

[1]*SCIEX, Warrington, Cheshire (U.K.)*

Overview

Artificial sweeteners are food additives whose use has been controlled by European Parliament guidelines. The method described in this application note, shows how LC-MS/MS can be used to simultaneously detect and confirm the presence of several artificial sweeteners. The method is both quicker than conventional non LC-MS/MS methods and more sensitive. This has meant that these compounds can be detected in samples below ingredient levels using a simple dilute and shoot approach.

Introduction

As we aim to eat less sugar, many of us are turning more and more to alternative sweeteners. Intense sweeteners such as Acesulfame (E950), Aspartame (E951), Cyclamate (E952), Saccharin (E954), and Sucralose (E955) are very low in calories and are safer for teeth (Figure 1). As with all additives, sweeteners are thoroughly assessed for safety before they are permitted, and are only then permitted in a limited range of products. The European Parliament has set out guidelines for the labeling of food containing artificial sweeteners (Directive 94/35/EC 'on sweeteners for use in foodstuff' with several amendments 96/83/EC, 2003/115/EC, and 2006/52/EC) and it has deemed that the presence of Aspartame and Aspartame-Acesulfame salt should state that the food 'contains a source of phenylalanine'. In addition some sweeteners cannot be used in foods for infants and young children, mentioned in Directive 89/398/EC.

At present standard methods, for the detection of sweeteners in food, use LC with evaporating light scattering detection.[1] This work shows where LC-MS/MS can be used to detect seven commercially available artificial sweeteners in diet drinks and baby food which were obtained from local supermarkets. The method has several advantages over the existing methodology in that it is five times faster as well as more than 100 to 1000 fold more sensitive. In all cases, due to the sensitivity of the technique and the level of artificial sweeteners, the samples had to be diluted at least 100 fold before analysis thus reducing the

effects of matrix on the analysis and simplifying sample preparation.

Experimental

Sample Preparation

Samples of soft drinks such as cola, orange flavored fizzy drink and lemonade were diluted 100 or 1000 fold in water. To test the method on baby food an 'off the shelf' sample of fruit was spiked with artificial sweeteners at 10 parts per million (ppm) and mixed with distilled water in a ratio of 1 part baby food to 9 parts water and shaken for one minute. The extract was centrifuged and then diluted 1 in 10 with water before LC-MS/MS analysis.

LC

Samples were separated by reversed-phase LC on a polar end capped column (4 µm, 150 x 2.1 mm), at 800 µL/min using a Shimadzu UFLC system. The gradient was over 6 minutes from 5% to 100% methanol in water. Both the water and methanol mobile phases had been modified by the addition of triethylamine and formic acid.

MS/MS

Analysis was performed using an SCIEX 3200 QTRAP® LC-MS/MS System fitted with a Turbo V™ source in Electrospray Ionization (ESI) mode and run in negative polarity. The detected Multiple Reaction Monitoring (MRM) transitions are listed in Table 1.

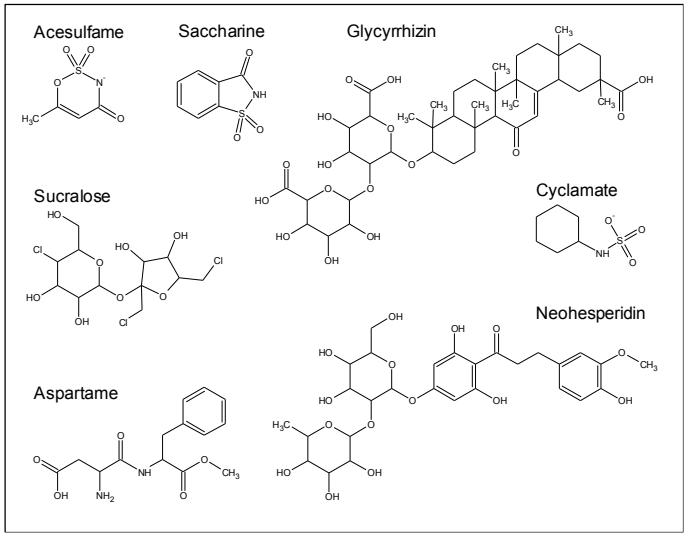

Figure 1. Structures for seven commercially available artificial sweeteners in the present method

Table 1. MRM transitions used in the method

Compound	Q1 (amu)	Q1 (amu)
Acesulfame	162	82
	162	78
Aspartame	293	200
	293	261
Cyclamate	178	80
	178	79
Glycyrrhizin	821	351
	821	113
Neohesperidin	611	303
	611	166
Saccharin	182	42
	182	106
Sucralose	395	359
	397	361

Confirmation of the identity of the compound has been further enhanced by the automatic generation of an Enhanced Product Ion (EPI) scan triggered by the MRM transition of a sweetener.

Results and Discussion

It can be seen that all the artificial sweeteners can be detected at concentrations of low parts per billion (ppb), Figure 2, with no carry over observed.

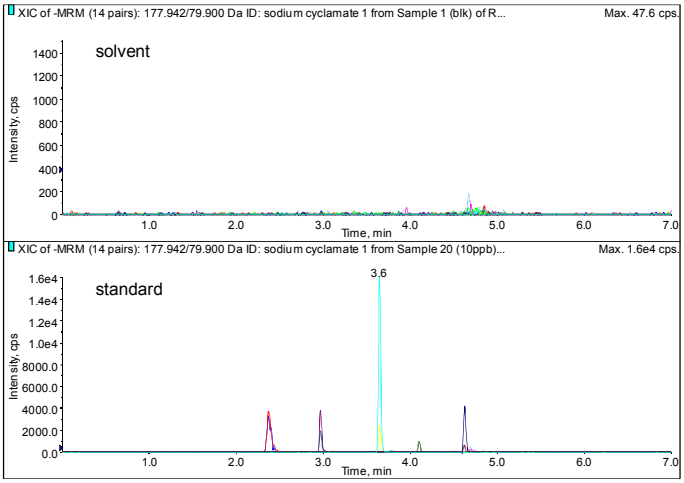

Figure 2. An example of the chromatogram obtained from a water blank (top) and a 10 ppb standard of artificial sweeteners in water (bottom)

When this method was applied to real samples it was found that drinks taken off supermarket shelves had to be diluted 100 or even a 1000 times to be within the range of the calibration standards (Figure 3). All the artificial sweeteners found in the samples corresponding to those which were listed on the ingredient labels. When this method was applied to a spiked baby food sample again all the sweeteners were observed at the spike level which was similar to the level used in drink manufacture.

From the peak heights shown in Figure 2 it can be seen that the sensitivity for the artificial sweeteners vary by over 2 orders of magnitude, with the acidic Cyclamate the most sensitive and Sucralose the least. This wide ranging sensitivity is down to the structural differences between these compounds which not only produces a wide range of different molecular weights but also a wide range pKa.

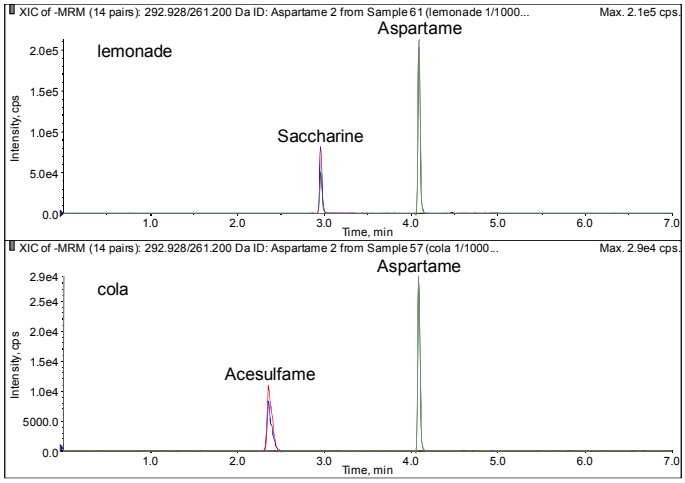

Figure 3. Chromatograms obtained from a 1000 dilution of a lemonade sample (top) and of cola sample (bottom). The two sweeteners detected corresponded to those listed on the drink's label.

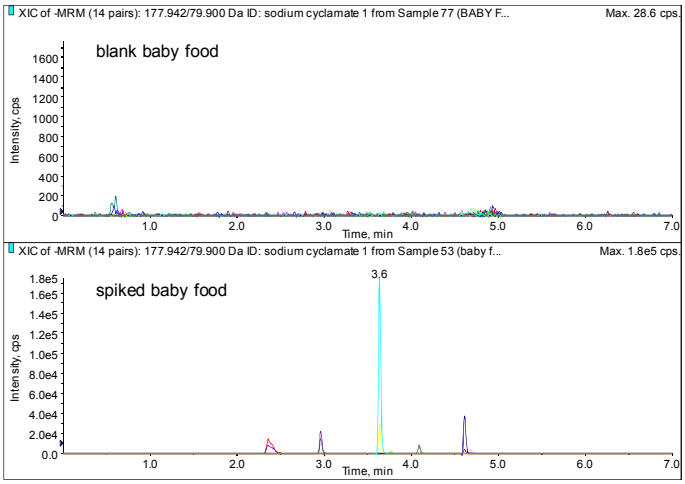

Figure 4. An example of the chromatogram obtained from a baby food sample (top) and 10ppm spike of sweeteners into baby food (bottom)

Little or no retention was found with standard reversed phase columns (C8 and C18) or a polar end-capped columns using a standard ammonium acetate buffered gradient making the use of an ion pairing reagent necessary.

The early elution and complex nature of some sweeteners also leads to some quadratic calibration curves (Figures 5). The non linearity has also been observed by other groups using ammonium acetate buffered LC conditions[2] and was improved in this work by the addition of triethylamine into the mobile phase. The non linearity starts below the point of normal detector saturation and seems to be a result of ionization efficiency and possibly the pH of sample and could probably be corrected further by the use of deuterated internal standards.

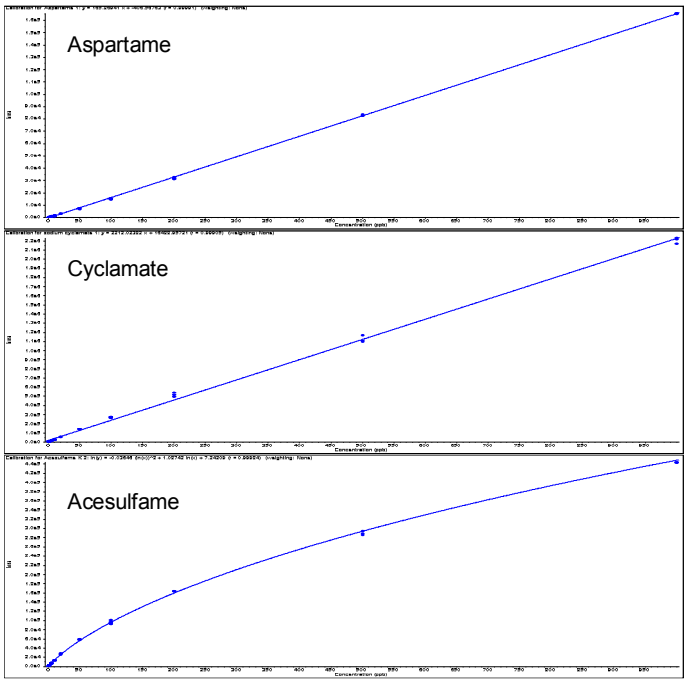

Figure 5. Examples of calibration curves for three commonly detected artificial sweeteners [Aspartame (top), Cyclamate (middle) and Acesulfame (bottom)], as it can be seen some compounds produce a non linear response over the range from 1 to 1000 ppb,

Even with the varying intensities and the complex nature of these compounds good robustness and reproducibility was observed. The coefficients of variation (%CV) observed from the repeat analysis of solvent standards are all less than 15% (except for Sucralose which was 15.2%) at 10 ppb and less than 10% at 100 ppb even with no internal standard present for any of the compounds (Table 2).

An additional advantage of using the SCIEX 3200 QTRAP® System is the possibility to confirm the identity of compounds based on automatically acquired EPI spectra. EPI spectra contain a complete molecular fingerprint of the detected analyte resulting in increased confidence of identification. An example of this is shown in Figure 6 where Acesulfame and Aspartame where identified using EPI spectra which were identical to those generated from standards.

Table 2. Reproducibility from the repeat injections (n=6) at 10 ppb and 100 ppb

Compound (# of MRM transition)	MRM Transition	Concentration (ppb)	%CV
Acesulfame	1	10	8.0
	2	100	4.1
	1	10	3.9
	2	100	1.9
Aspartame	1	10	6.0
	2	100	5.4
	1	10	11.2
	2	100	4.0
Cyclamate	1	10	2.9
	2	100	3.2
	1	10	9.7
	2	100	3.9
Glycyrrhizin	1	10	6.7
	2	100	2.1
	1	10	9.4
	2	100	1.5
Neohesperidin	1	10	4.0
	2	100	4.7
	1	10	11.9
	2	100	8.0
Saccharin	1	10	5.6
	2	100	4.6
	1	10	5.7
	2	100	3.4
Sucralose	1	10	11.1
	2	100	2.9
	1	10	15.2
	2	100	4.6

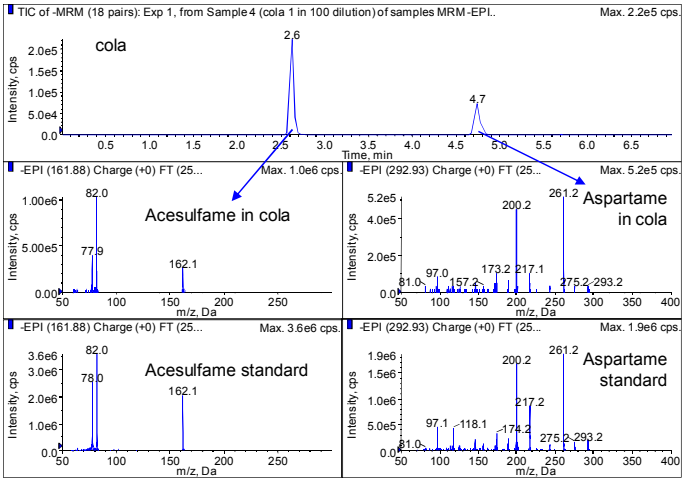

Figure 6. Examples of identification of sweeteners in a cola flavored drink by the automatic generation of EPI spectra

Summary

The work to date shows that artificial sweeteners can be easily detected in negative polarity LC-MS/MS using Electrospray Ionization and well below current levels used in the drink industry. The method is more than five times faster than non LC-MS/MS methods currently available and due to the high sensitivity a much reduced sample pre-treatment is possible.

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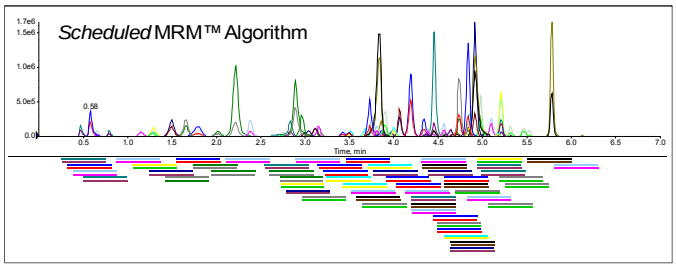
Intelligent Use of Retention Time during Multiple Reaction Monitoring for Faster and Extended Compound Screening with Higher Sensitivity and Better Reproducibility

André Schreiber and Nadia Pace
SCIEX Concord, Ontario (Canada)

Key Features of *Scheduled* MRM™ Algorithm

- Intelligent use of retention times to maximize dwell times and optimize cycle time of MRM methods
- Increased number of monitored MRM transitions to screen and quantify more analytes per analysis
- Better Signal-to-Noise due to higher dwell times
- Greatly improved reproducibility and accuracy by detecting more data points across chromatographic peaks
- Faster sample analysis by applying UHPLC without compromising data quality



Introduction

LC-MS/MS instruments operating in Multiple Reaction Monitoring (MRM) are widely used for targeted quantitation and screening on triple quadrupole and hybrid triple quadrupole linear ion trap (QTRAP®) systems because of their well known selectivity and sensitivity. Extensive panels with a few hundred MRM transitions are used routinely in many laboratories, for example to screen for food contaminants and environmental pollutants or to identify drugs in intoxication cases in forensic laboratories.

However, the current limit of a few hundred transitions per chromatographic run limits the number of analytes that can be monitored per injection. This is further complicated by the demand for faster analysis through Ultra High Pressure Liquid Chromatography (UHPLC) without reducing the number of monitored analytes and without compromising reproducibility and accuracy.

With the new *Scheduled* MRM™ Algorithm offered in the Analyst® software version 1.5, MRM transitions of the targeted analytes are monitored only around the expected retention time. Thus, automated MRM scheduling decreases the number of concurrent MRM transitions, allowing both the cycle time and the dwell time to be optimized for highest sensitivity, accuracy, and reproducibility. In addition *Scheduled* MRM™ allows the monitoring of many more MRM transitions in a single acquisition or to speed up the analysis by the use of UHPLC or to combine both concepts without compromising data quality.

Key Principles of MRM and *Scheduled* MRM™ Algorithm

Dwell time is the time spent acquiring the targeted MRM transition during each cycle. While very short dwell times can be used (5-10 ms) for extended compound screening, higher dwell times are desirable for better Signal-to-Noise (S/N).

Duty cycle is effectively the amount of time spent monitoring an analyte, therefore the higher the duty cycle the better the data quality. Duty cycle is inversely proportional to the number of, concurrent MRM transitions monitored.

Therefore, an increase in multiplexing resulting in more concurrent MRM transitions can decrease the analytical reproducibility.

The ideal cycle time for an MRM method is a chromatographic consideration. A cycle time which provides 10-15 data points across the LC peak is optimal for accurate quantitation and reproducibility, especially for low abundant analytes. The relationship between number of MRM transitions, dwell time, duty cycle, and cycle time is illustrated in Figure 1.

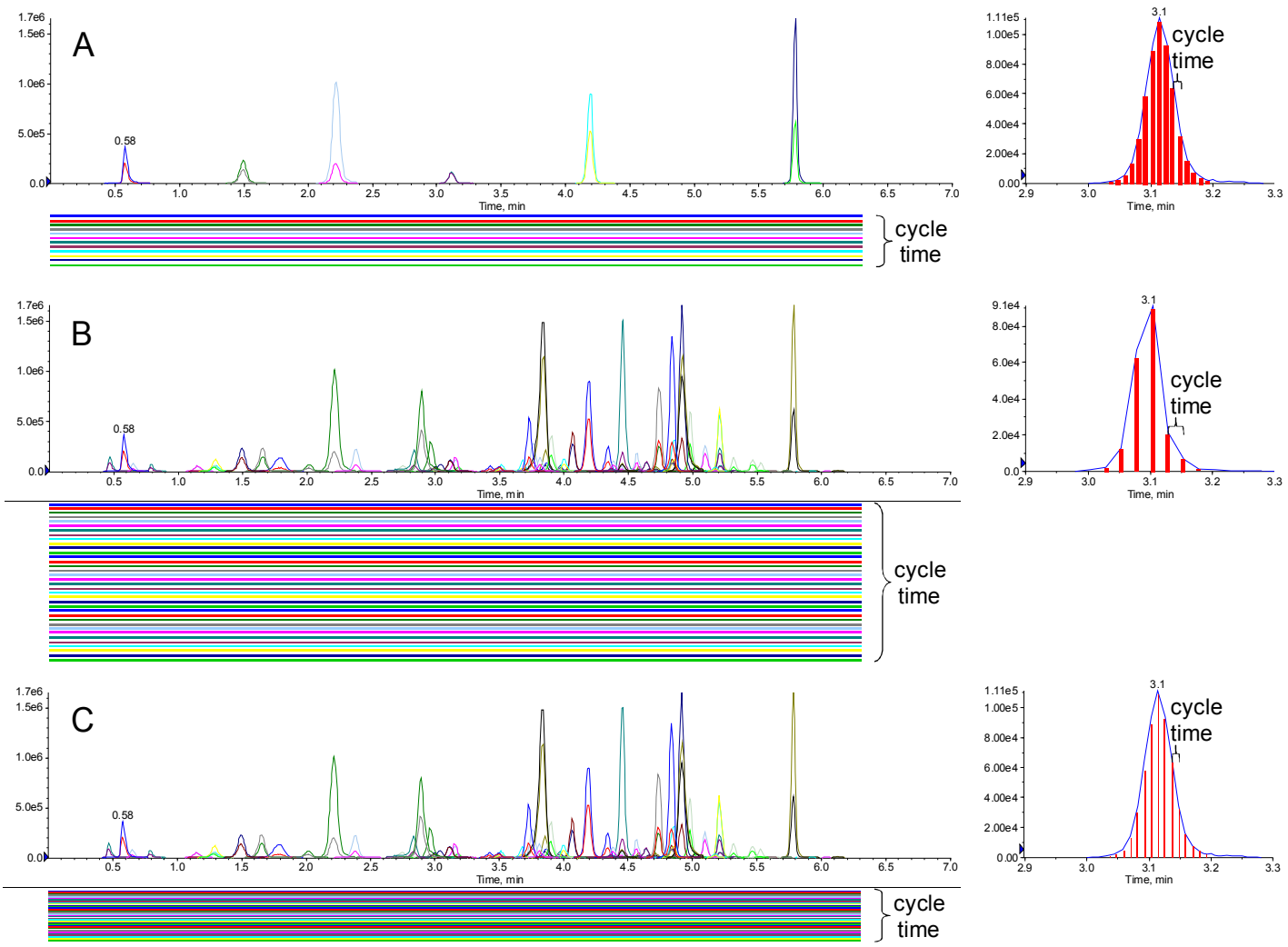


Figure 1. Considerations for Multiple Reaction Monitoring

(A) Traditionally, few MRM transitions are detected to quantify targeted analytes with high dwell times for best S/N and cycle times to collect enough data points across the LC peak for accurate and reproducible data (the width of the bars indicate the dwell time and the space between bars indicate the cycle time).

(B) Increasing the number of MRM transitions by maintaining the dwell time extends the cycle time resulting in very poor quantitative results because of an insufficient number of data points across the LC peak.

(C) Increasing the number of MRM transitions by decreasing the dwell time results in lower duty cycle and, thus, in lower S/N and higher limits of detection.

The *Scheduled* MRM™ Algorithm is illustrated in Figure 2. Prior knowledge of the retention of each analyte allows the MRM transition to be monitored only in a short time window. 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.

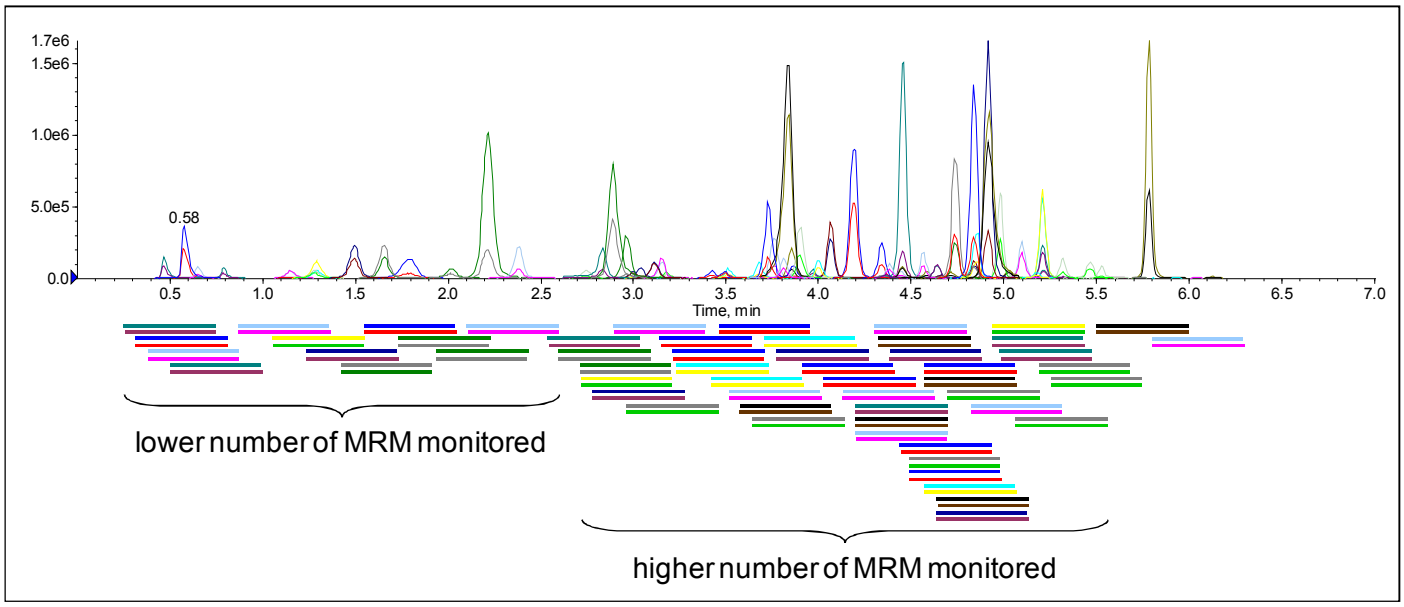


Figure 2. The *Scheduled* MRM™ Algorithm uses the knowledge of the elution of each analyte to monitor MRM transitions only during a short retention time window. This allows many more MRM transitions to be monitored in a single LC run, while maintaining maximized dwell times and optimized cycle time.

Good Chromatography is the Key for the Best LC-MS/MS Data

The key to the highest order multiplexing and optimal MS/MS performance is high quality and highly reproducible LC separation.

One of the user inputs to the software to automatically create the *Scheduled* MRM™ methods is the MRM Detection Window. This is an estimate of the LC peak width and chromatographic reproducibility expected, and should therefore reflect the time window around the supplied retention time which will contain the entire LC peak plus any shifts in chromatography. The narrower the peak widths and the more reproducible the elution, the tighter this MRM detection window can be and, thus, less concurrent MRM transitions are monitored. Reduced concurrency also means that higher dwell times will be used for each MRM, improving the data quality.

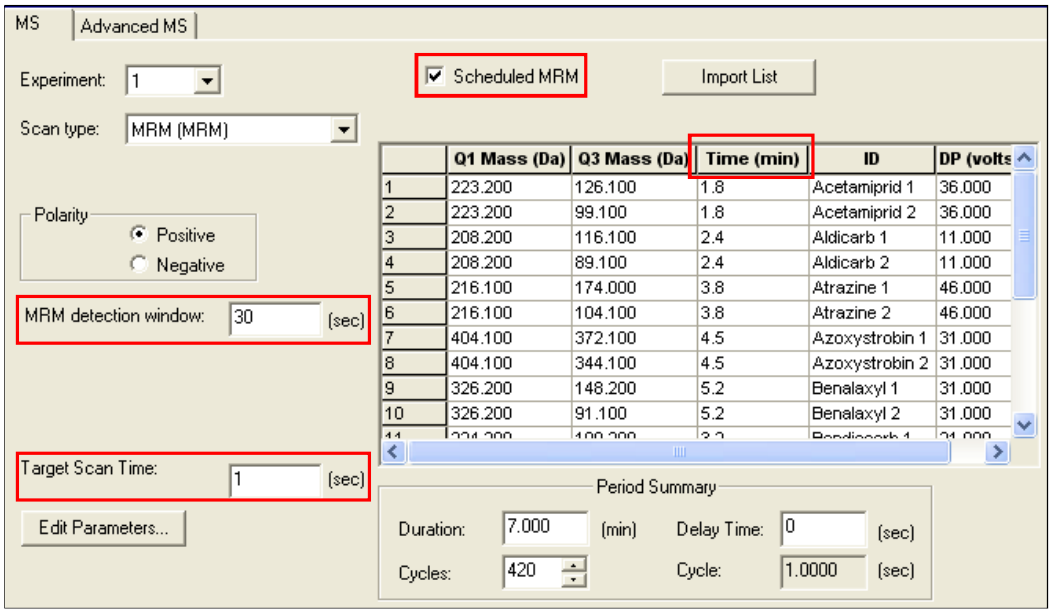


Figure 3. Acquisition method interface for *Scheduled* MRM™, in addition to traditional MRM parameters, the user provides retention times of all analytes, an MRM detection window, and a Target scan time. The software then automatically designs and optimizes the Scheduled MRM™ acquisition method.

Easy Method Creation

Another key advantage in *Scheduled* MRM™ is the ease at which powerful quantitative MRM acquisition methods can be created. The user is required to specify a few key parameters (Figure 3):[1]

- MRM transition: (Q1, Q3) and any compound dependent parameters
- Expected retention time for each MRM transition
- MRM detection window must be wide enough to allow the MRM peak to stay entirely within the window across all injections – consider the width of the LC peak at the base and the retention time stability
- Target scan time is effectively the cycle time – how often the chromatographic peak should be sampled. This is determined from the peak width at the base. The best accuracy and reproducibility is between 10-15 points across the peak
- Additionally, MRM ID, like compound name, for easier data processing and reporting

The software algorithm then automatically builds an acquisition method that schedules the appropriate MRM transitions to be screened over the chromatographic analysis at the appropriate times. Instead of monitoring all transitions all of the time, it will only look for those transitions within the targeted time window.

Results of Using the *Scheduled* MRM™ Algorithm

Increased Number of MRM Transitions

The number of MRM transitions which can be monitored in a single analysis depends on chromatographic peak width and required S/N (dwell time). Several publications show that SCIEX systems equipped with Linear Accelerator® collision cell can be used to detect several hundred transitions using traditional LC configurations.[2-4]
The automated MRM scheduling decreases the number of concurrent MRM transitions. Thus *Scheduled* MRM™ allows the monitoring of many more MRM transitions per cycle without the need to sacrifice data quality.

The example in Figure 4 shows an injection of more than 750 compounds typically analyzed in forensic laboratories to screen for toxic substances, such as drugs of abuse, pharmaceuticals and their metabolites.

Such screening methods are used frequently to screen for a large number of targeted compounds. The Scheduled MRM™ survey was used to automatically acquire Enhanced Product Ion (EPI) spectra on a 3200 QTRAP® LC-MS/MS system. The characteristic and high sensitivity spectra can be searched against a mass spectral library for compound identification.

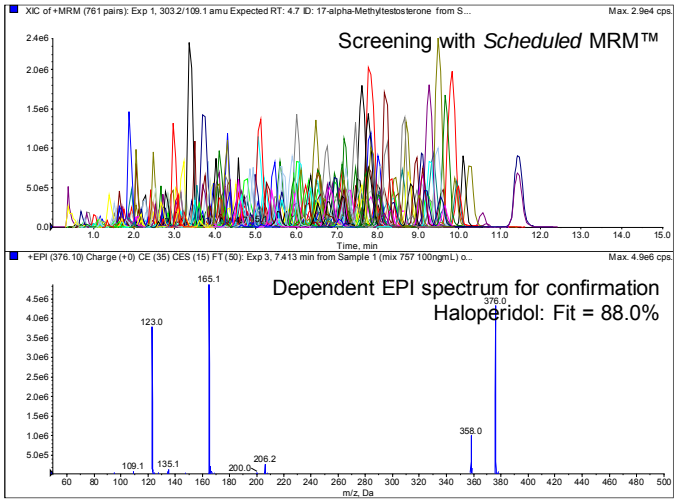


Figure 4. Using *Scheduled* MRM™ to increase the number of monitored MRM transitions for screening applications. The example shows an injection of more than 750 compounds relevant in forensic toxicology. The *Scheduled* MRM™ survey was used to automatically acquire EPI spectra for identification by library searching.

Better Sensitivity and Reproducibility

Figure 5 shows a comparison of using traditional MRM and *Scheduled* MRM™ detection for the screening of pesticides in fruit and vegetable samples. A 4000 QTRAP® LC-MS/MS system was used to detect 150 MRM transitions.

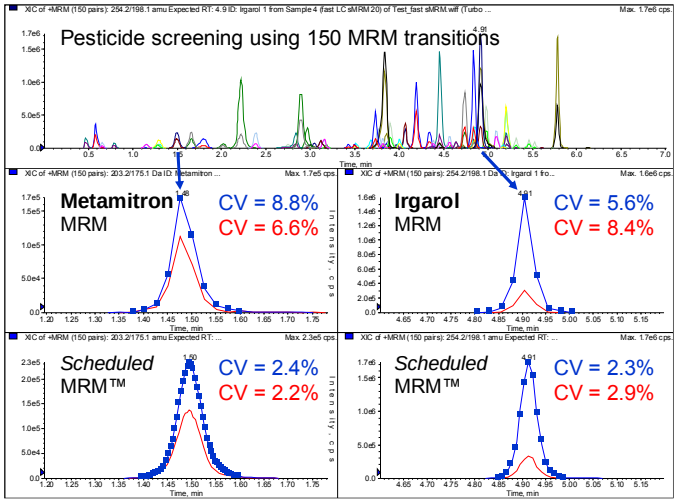


Figure 5. Using *Scheduled* MRM™ to optimize dwell times and number of data points across the LC peak in a pesticide screening method with 150 MRM transitions. The *Scheduled* MRM™ method shows significantly better sensitivity and reproducibility.

The *Scheduled* MRM™ Algorithm automatically optimizes dwell times enabling detection with higher sensitivity and better

reproducibility by collecting more data points across the LC peak. The improvement in sensitivity and reproducibility depends on the number of concurrent MRM transitions. Narrow LC peaks and highly stable retention times allow setting a smaller MRM detection window for best *Scheduled* MRM™ performance.

Faster analysis using UHPLC without compromising data quality

The use of small particle size columns and faster gradients results in narrower LC peaks. Traditional MRM would require decreasing the number of transitions or compromising quality to maintain the number of transitions.

The chromatograms in Figure 6 show examples of traditional, fast and ultra fast LC to monitor 150 MRM transitions. *Scheduled* MRM™ allows accelerated analysis without the need to compromise the number of monitored compounds and/or data quality. The data were acquired using a 4000 QTRAP® LC-MS/MS system. A Phenomenex Synergi 2.5u Fusion-RP 50x2 mm column with different gradients of water/methanol and 5 mM ammonium formate was used. The gradient conditions are shown in Table 1.

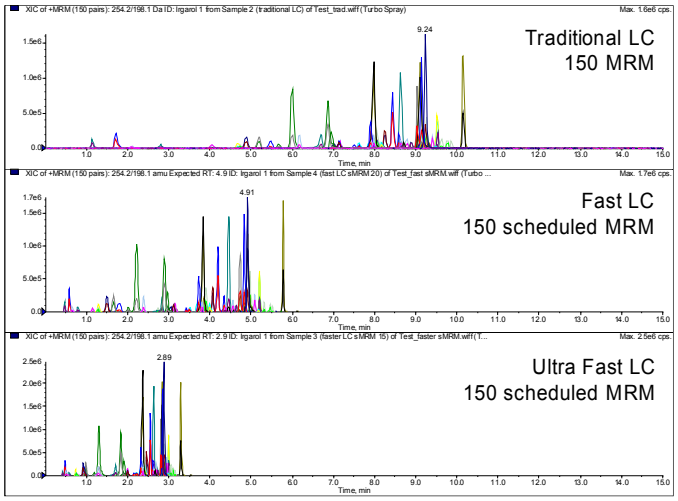


Figure 6. *Scheduled* MRM™ allows fast and ultra fast LC separation using small particle column while maintaining the number of monitored MRM transitions without compromising data quality.

Table 1. Traditional, fast and ultra fast LC gradients to detect 150 MRM transitions of pesticides on a 4000 QTRAP® LC-MS/MS system

	Traditional LC (2150 psi)			Fast LC (4330 psi)			Ultra Fast LC (4570 psi)		
Step	**Time (min)**	**Flow (µL/min)**	**A%/B%**	**Time (min)**	**Flow (µL/min)**	**A%/B%**	**Time (min)**	**Flow (µL/min)**	**A%/B%**
0	0	250	80/20	0	500	70/30	0	500	60/40
1	8	250	10/90	5	500	10/90	2	500	10/90
2	14	250	10/90	6	500	10/90	4	500	10/90
3	15	250	80/20	7	500	70/30	5	500	60/40
4	20	250	80/20	10	500	70/30	8	500	60/40

Figure 7 shows results of the analysis of fruit extracts analyzed with a traditional LC and MRM method in comparison to a fast LC and *Scheduled* MRM™ method. The samples were extracted using a QuEChERS procedure before analysis.

Several pesticides were detected, quantified and identified using MRM ratio calculation, including Imazalil at 42 µg/kg and Thiabendazole at 3.4 µg/kg in grapefruit, Metazachlor at 8.9 µg/kg in apricot, and Methomyl at 4.7 µg/kg in grapes. The use of *Scheduled* MRM™ for this analysis allowed faster sample analysis with better sensitivity and reproducibility. In addition, data exploration was easier because of a more selective acquisition.

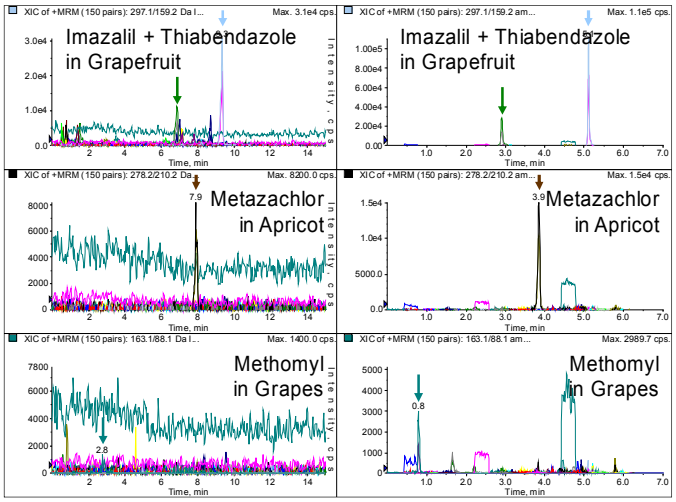


Figure 7. Comparison of traditional LC and MRM with fast LC and *Scheduled* MRM™ for the analysis of pesticides in fruit extracts, the new method allowed faster analysis with better sensitivity and reproducibility. Also cleaner data display made data exploration easier.

Summary

The new *Scheduled* MRM™ Algorithm offered in Analyst® software version 1.5 automatically monitors MRM transitions of the targeted analytes only around the expected retention time. The scheduling decreases the number of concurrent MRM transitions, allowing both the cycle time and the dwell time to be optimized for highest sensitivity, accuracy, and reproducibility. In addition, *Scheduled* MRM™ allows the monitoring of many more MRM transitions in a single acquisition and/or accelerating the analysis by the use of UHPLC maintaining highest data quality.

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