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Using the X500R QTOF System and SCIEX OS Software to Identify and Quantify Food Residues

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Overview

Here we present results using a new method to identify and quantify pesticide residues in food using the SCIEX X500R QTOF system. Samples were extracted using a QuEChERS method and analyzed by LC-HR-MS/MS. Limits of quantitation of 10 µg/kg were achieved for every compound after 10x dilution of the extract to minimize possible matrix effects.

Target compounds were automatically identified by matching retention time, accurate mass and isotope pattern of the molecular ion and MS/MS library searching using SCIEX OS software. In the same data processing step, compounds were quantified and unknown samples were flagged when a user-defined reporting level was exceeded.

Introduction

Recent advancements in LC-MS/MS technology, including hybrid systems like quadrupole-quadrupole Time-of-Flight (QTOF), now provide the ability to perform targeted and non-targeted screening in food samples on a routine basis.[3]

The SCIEX X500R QTOF system is a robust, high performance high resolution MS/MS system designed for routine use providing:

- Sensitivity to easily detect compounds at maximum residue levels
- Resolving power to remove interference from complex food matrices
- Linearity to quantify over up to 3 orders of magnitude
- Mass accuracy to identify compounds following regulatory guidelines
- Confident identification using MS/MS spectra and ion ratios
- Industry leading robustness of Turbo V™ source and Curtain Gas™ interface

Full scan chromatograms are very rich in information and easily contain thousands of ions from any residue present in the sample, including the food matrix itself. Powerful software is needed to explore the high resolution MS/MS spectra generated to get answers and results from these complex data.

The SCIEX OS software is a single platform for MS control, data processing, and reporting and provides:

- Simple software workflows that deliver reliable results
- Simultaneous identification and quantitation
- Quick data review and reporting utilizing customizable flagging and filtering of results

Experimental

Standards

A standard mix of 200 pesticides was used to prepare serial dilutions for quantitative analysis.

Sample preparation

EU proficiency test samples and food samples from a local supermarket were extracted using a QuEChERS procedure following guideline EN 15662/2007. Sample extracts were diluted 10x to minimize possible matrix effects.

LC Separation

LC separation was performed using a SCIEX ExionLC™ AC system with 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). The injection volume was 5 µL.

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

MS/MS Detection

The SCIEX X500R QTOF system with Turbo V™ source and Electrospray Ionization (ESI) was used.

Mass calibration was achieved using the integrated calibrant delivery system (CDS) with the TwinSprayer probe (dual ESI needle).

High resolution data were acquired using an IDA method consisting of a TOF-MS survey (100-1000 Da for 100 msec) and up to 20 dependent MS/MS scans (50-1000 Da for 35 msec). MS/MS fragmentation was achieved using CE of 35 V with a collision energy spread (CES) of ±15 V.

Dynamic background subtraction (DBS) was activated for best MS/MS coverage, and no inclusion list was used to also allow retrospective unknown identification without the need for a second injection to acquire MS/MS data.

Data Acquisition and Processing

All data were acquired and processed using SCIEX OS software version 1.0, which showcases a thoughtfully designed user interface that is fast to learn and delivers improved lab productivity.

Results and Discussion

X500R Performance Characteristics

Resolution > 20,000 (at full width half height) and mass accuracy <5 ppm are often sufficient to separate the analytes of interest from interfering matrices and, thus, are identified as the set requirements for compound identification in various guidelines.[1, 2]

The X500R QTOF system utilizes N-optics design to maximize resolution while maintaining benchtop design and a minimized footprint. Its resolving power increases with mass range providing ~30000 to 40000 for the typical molecular weight range of pesticides.

The 4 mm orifice leading into the TOF accelerator delivers resolution without compromise in sensitivity. The sensitivity of the X500R QTOF system is comparable to a SCIEX QTRAP® 5500 system operated in MRM mode, allowing extract dilution to minimize ion suppression while detecting easily at 10 µg/kg levels (Figure 1).

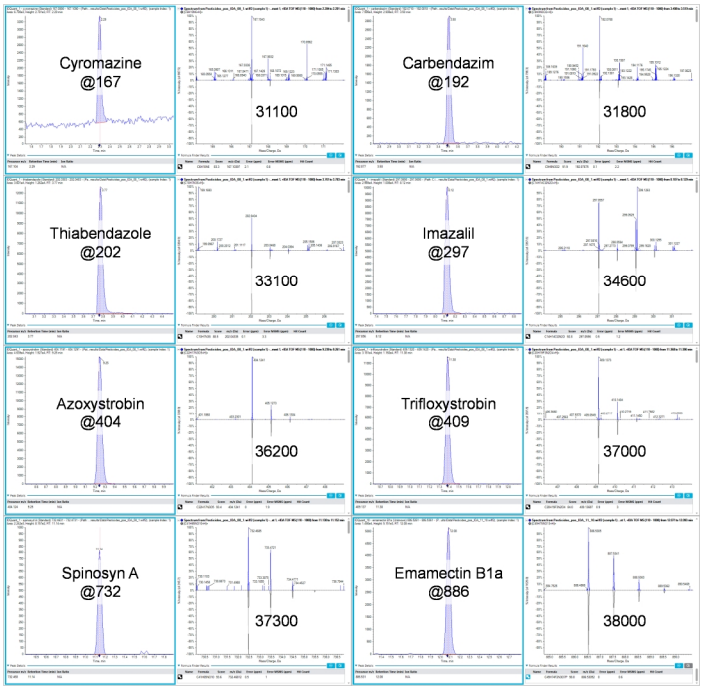

Figure 1. Sensitivity and resolution of different pesticides, left: XIC of the molecular ion of each compound ± 5 mDa at 1 ng/mL (Emamectin at 10 ng/mL), right: TOF-MS spectrum of molecular ion with achieved resolution value (average of seven X500R QTOF systems)

The X500R QTOF system achieves stable mass accuracy of less than 2 ppm by using a heated TOF configuration, with 6 heater drones throughout the TOF path to maintain mass accuracy and robustness. In addition, the integrated CDS with the TwinSprayer probe provides an independent calibrant delivery path for reliable auto-calibration. The CDS setup maintains mass accuracy over long periods of time by automatically calibrating in batch mode (it is recommended to infuse a calibrant standard every hour or two).

Furthermore, the X500R QTOF's mass accuracy is supplemented by legendary dynamic transmission control and dynamic background calibration, introduced in 2010 with the TripleTOF® system and optimized over time.

Figure 2 shows an example of mass accuracy for a selected pesticide detected over a wide concentration range. Paclobutrazol was quantified from 0.1 to 1,000 ng/mL with good linearity (r^2 = 0.9993). Excellent mass accuracy was achieved (-0.2 to 0.91 ppm) at all levels, even at the highest concentration of 10,000 ng/mL which was above the upper limit of quantitation for this analyte.

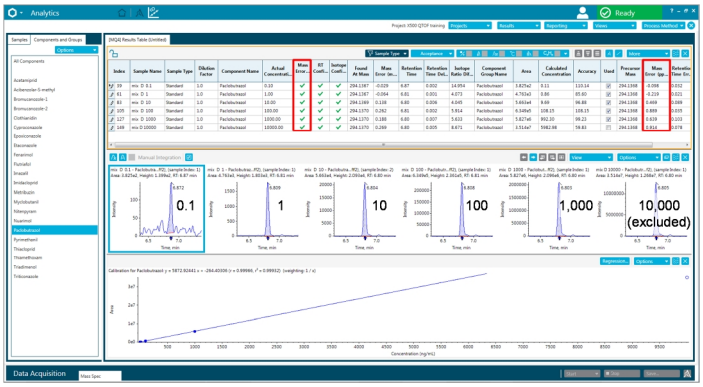

Figure 2. Detection of Paclobutrazol from 0.1 to 10,000 ng/mL with good linearity (0.1 to 1,000 ng/mL) and mass errors of < 1 ppm even at the highest concentration above the upper limit of quantitation

Despite the high selectivity of high resolution MS detection, there is a risk of false positive findings due to interfering isomers and matrix signals. As a result food testing guidelines require the detection of the "molecular ion" and "at least one fragment ion", and for "a higher degree of confidence in identification, further evidence may be gained from additional mass spectrometric information. For example, evaluation of full scan spectra, isotope pattern, adduct ions, additional accurate mass fragment ions… (in MS/MS)".[2]

The example shown in Figure 3 highlights the need of fragment ion detection to confidently differentiate between isomers.

The pesticides Prometon and Terbumeton have identical molecular formulae ($C_{10}H_{19}N_5O$) and as a result the identical molecular ion and isotope pattern. The retention time difference of less than 0.1 min, due to highly similar structures, is not sufficient to differentiate both pesticides.

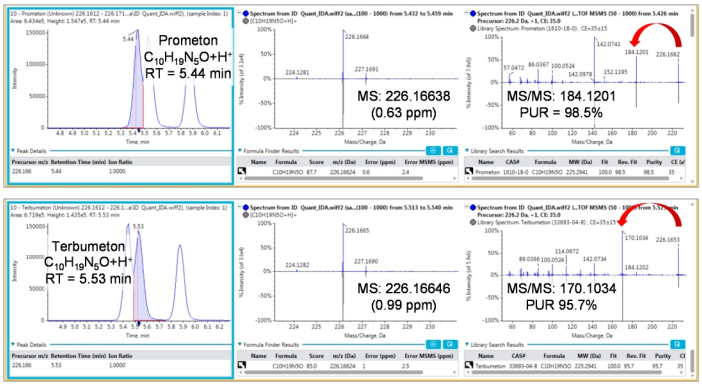

Figure 3. Confident identification of isomers Prometon and Terbumeton using characteristic MS/MS fragment ions and MS/MS library searching

However, the two compounds have unique and characteristic fragment ions, $C_7H_{14}N_5O^+$ and $C_6H_{12}N_5O^+$, respectively, which can be used for identification. Molecular and fragment ions have been measured with good mass accuracy of < 5 ppm and less < 1 mDa, respectively.

Processing Workflow for Targeted Identification and Quantitation in SCIEX OS Software

Extracted Ion Chromatograms (XIC) of all target analytes are generated based on user input (chemical formula and expected retention time). MS and MS/MS information is automatically evaluated if an XIC signal is detected and compounds are identified by matching retention time, accurate mass and isotope pattern of the molecular ion and MS/MS library searching. Qualitative rules are defined in the processing method and can be used for results review and filtering (Figures 4a and b).

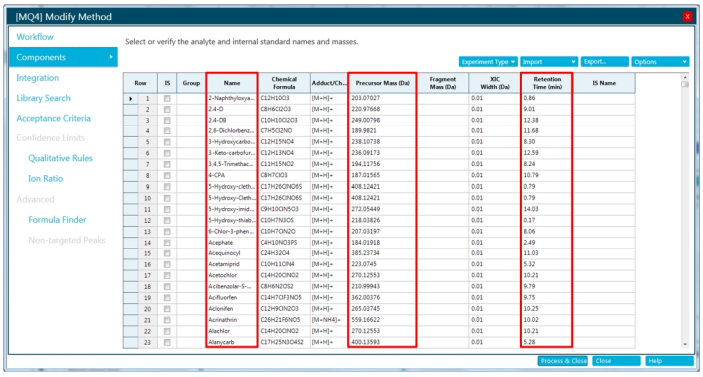

Figure 4a. Method editor in SCIEX OS software, user input for target compounds including chemical formula to calculate precursor ion mass and expected retention time

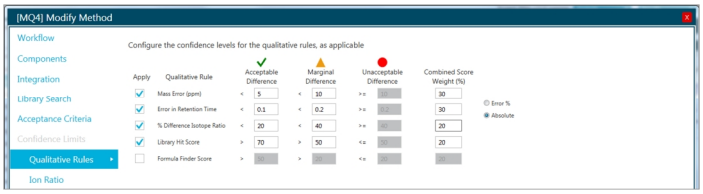

Figure 4b. Method editor in SCIEX OS software, user input for qualitative rules (traffic lights) to enable easy results review and filtering

In the same data processing step standard calibration lines are generated to automatically calculate concentrations in unknown samples (Figure 2).

Results of EU Proficiency Test Samples

Two samples of an EU proficiency test for pesticides and fruits and vegetables were extracted and analyzed for pesticides. Results are listed in Table 2. Retention time errors were less than 0.1 min and mass errors were between -1.20 and 1.17 ppm and were well below the required 5 ppm (SANTE/11945/2015).

Concentrations were assigned for pesticides present in the SCIEX iD*Quant*™ standards kit for pesticide analysis.

Table 2. Pesticides identified and quantified in two EU proficiency test (EUPT) samples based on matching retention time (RT), accurate mass and isotope pattern and MS/MS library searching

Pesticide	RT error (min)	Mass error (ppm)	Isotope ratio error	MS/MS FIT (%)	Conc. (µg/kg)
EUPT 1					
Acetamiprid	0.00	0.09	2.2	100.0	449
Acrinathrin	0.00	0.61	1.0	98.9	-
Buprofezin	0.01	0.32	1.1	100.0	204
Chlorpyrifos	0.00	-0.78	3.3	95.2	-
Cypermethrin	0.01	-0.27	4.9	99.2	-
Cyprodinil	0.01	-0.17	1.1	100.0	374
Diazinon	0.00	-0.20	1.7	100.0	-
Difenoconazole	0.00	0.22	1.8	100.0	1092
Fenamiphos	0.00	-1.74	1.3	99.9	-
Fenamiphos-sulfone	0.00	-0.26	1.7	100.0	-
Fenamiphos-sulfoxide	0.00	-0.94	1.3	97.1	-
Fenhexamid	0.02	0.16	0.6	100.0	871
Fludioxonil (-)	0.01	-0.69	0.8	99.6	236
lambda-Cyhalothrin	0.00	0.42	2.4	99.0	-
Methoxyfenozide	0.02	0.63	12.2	100.0	94.0
Pirimicarb	0.02	-0.37	0.3	100.0	478
Pyridaben	0.01	0.41	3.1	100.0	1063
Spinosyn A	0.01	-0.24	3.3	100.0	366
Spinosyn D	0.01	1.17	13.3	N/A	57.4
Tetraconazole	0.01	-0.36	9.3	100.0	111

Table 2. cont. (sample 2)

Pesticide	RT error (min)	Mass error (ppm)	Isotope ratio error	MS/MS FIT (%)	Conc. (µg/kg)
EUPT 2					
Atrazine	0.00	0.12	7.3	100.0	
Cadusafos	0.00	-1.20	2.3	99.2	
Carbetamide	0.02	-1.02	16.3	100.0	
Demeton-S-methyl-sulfone	0.00	0.21	0.4	99.7	
Ethoprophos	0.00	-0.47	1.7	98.7	
Fenpropidin	0.00	-0.34	2.2	100.0	
Fipronil (-)	0.00	0.20	7.3	100.0	
Flubendiamide (-)	0.00	0.11	8.9	0.0	
Fluometuron	0.01	-0.03	0.9	99.9	
Fuberidazole	0.02	-0.56	1.3	99.7	
Furathiocarb	0.01	-0.31	2.3	100.0	
Metosulam	0.00	-0.42	1.7	100.0	
Prosulfocarb	0.00	-0.54	1.2	100.0	
Secbumeton	0.00	0.06	1.6	100.0	
Spiromesifen	0.01	-0.84	5.9	99.0	

(-): identified in negative polarity

Figures 5a and 5b show screenshots of the result table used for pesticide identification in proficiency test samples.

Figure 5a. Pesticides identified in proficiency test sample 1 in positive polarity based on matching retention time, accurate mass, isotope pattern and MS/MS library searching (note: Fludioxonil was identified in negative polarity)

Figure 5b. Pesticides identified in proficiency test sample 2 in positive polarity based on matching retention time, accurate mass, isotope pattern and MS/MS library searching (note: Fipronil and Flubendiamide were identified in negative polarity)

No false positive results were reported. MS/MS data and mass spectral library searching were crucial to differentiate and correctly identify structural isomers. Library searching results were reported as FIT and in all cases were above 90%.

The pesticide Flubendiamide was not present in our MS/MS libraries. Here the built-in 'Fragments Tool' of SCIEX OS was used to compare the structure of the suspected compound with the high resolution MS/MS spectrum. All measured fragment ions matched the theoretical fragmentation pathway, resulting in a tentative identification of Flubendiamide.

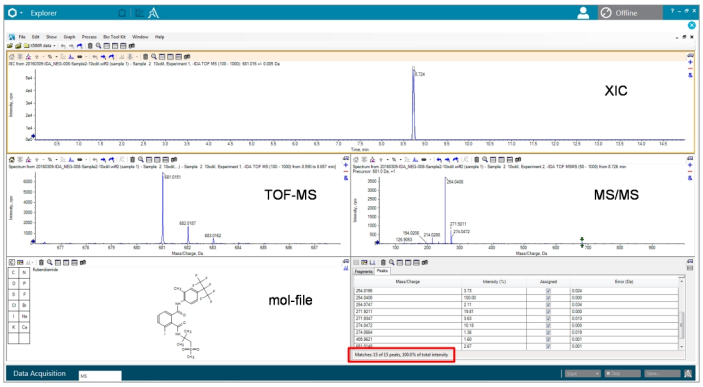

Figure 6. Tentative identification of Flubendiamide based on a comparison of the HR-MS/MS spectrum with the theoretical fragmentation pathway

Results of Store-bought Samples

Fruit and vegetable samples obtained from a local supermarket were extracted and tested for pesticide residues. Results above 10 µg/kg are listed in Table 3.

Table 3. Pesticides identified and quantified in store-bought fruit and vegetable samples based on matching retention time (RT), accurate mass and isotope pattern and MS/MS library searching

Sample / Pesticide	RT error (min)	Mass error (ppm)	Isotope ratio error	MS/MS FIT (%)	Conc. (µg/kg)
Banana					
Buprofezin	0.01	0.32	3.5	100.0	341
Imazalil	0.02	0.79	15.1	91.5	565
Thiabendazole	0.01	-1.51	13.9	97.6	444
Blueberry			n.d.		
Carrot			n.d.		
Grapes					
Boscalid	0.01	-0.80	8.8	97.2	115
Buprofezin	0.01	0.22	7.3	99.6	17.3
Cyprodinil	0.01	-0.87	3.3	94.8	412
Imidacloprid	0.01	-0.58	14.6	96.1	82.5
Pyraclostrobin	0.00	-1.31	4.8	100.0	46.7
Lemon					
Imazalil	0.02	0.74	7.3	94.7	1080
Pyrimethanil	0.01	-0.77	1.0	99.2	164
Pyriproxyfen	0.01	0.43	11.4	95.3	31.6
Organic banana					
Spinosyn D	0.00	2.33	19.8	100.0	12.6
Organic strawberry					
Spinosyn A	0.01	0.55	9.1	100.0	13.9
Spinosyn D	0.01	1.63	6.0	99.4	33.3
Spinach			n.d.		
Strawberry					
Acetamiprid	0.08	-0.35	6.5	98.7	19.2
Boscalid	0.00	-0.49	4.9	99.3	161
Myclobutanil	0.00	-0.31	13.9	100.0	85.0
Pyraclostrobin	0.00	1.33	16.3	99.0	40.5
Pyrimethanil	0.00	0.32	4.7	97.3	391
Tomato *(n.d.)*			n.d.		

n.d.: no pesticide detected

Summary

A new method to identify and quantify pesticide residues in food samples was developed using the SCIEX X500R QTOF system. Qualitative and quantitative data processing was performed in SCIEX OS software.

The method was successfully applied to EU proficiency test samples and store-bought fruit and vegetable samples. Samples were extracted using a QuEChERS procedure and analyzed using LC-HR-MS/MS. Limits of quantitation of 10 µg/kg were achieved for all compounds after 10x dilution the extracts to minimize possible matrix effects.

Pesticides were automatically identified by matching retention time, accurate mass and isotope pattern of the molecular ion and MS/MS library searching using SCIEX OS software. In the same data processing step compounds were quantified and unknown samples were flagged when a user-defined reporting level was exceeded.

Acknowledgement

The authors thank Amadeo Fernandez-Alba (EURL) Almeria, Spain for providing EUPT samples.

References

1 EU Commission Decision ‘concerning the performance of analytical methods and the interpretation of results’ #2002/657/EC

2 EU Commission Guidance Document: ‘on analytical quality control and method validation procedures for pesticides residues analysis in food and feed’ #SANTE/11945/2015

3 André Schreiber et al.: ‘Using the X500R QTOF System and SCIEX OS Software to Quickly Identify Unknowns in Food Samples’ Application Note SCIEX (2016) # RUO-MKT-02-3761-A

Publication number: RUO-MKT-02-3760-A

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