Food Residue Screening using Liquid Chromatography Coupled to High Resolution MS/MS (LC-HR-MS/MS) - Multi-Pesticide Identification and Quantitation

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ABSTRACT

Here we present results of using a novel approach of multi-target screening to identify and quantify chemical residues in food. Fruit and vegetable samples were extracted using a QuEChERS procedure and analyzed with core-shell particle reversed phase LC. High resolution and accurate mass MS and MS/MS information was collected in a single run using information dependent acquisition on the new SCIEX X500R QTOF system. Qualitative and quantitative data was processed using in the new SCIEX OS software.

INTRODUCTION

LC-MS/MS using Electrospray Ionization (ESI) is a powerful analytical tool for the analysis of a wide molecular weight range of polar, semi-volatile and thermally labile compounds. Especially triple quadrupole based mass analyzers are popular for targeted quantitation of hundreds of food contaminants in a single analysis because of their extra degree of selectivity and sensitivity when operated in Multiple Reaction Monitoring (MRM) mode. Advancements in LC-MS/MS technology, including hybrid systems like triple quadrupole linear ion trap (QTRAP®) and quadrupole-quadrupole Time-of-Flight (QTOF), now provide the ability to perform targeted and non-targeted screening on a routine basis. However, full scan chromatograms are very rich in information and contain easily thousands of ions from both any compounds present in the sample as well as from the sample matrix itself. Thus, powerful software tools are needed to explore the high resolution and accurate mass data generated.

Here we present residue results of using a novel approach of multi-target screening using QuEChERS extraction and LC separation with core-shell particles followed by high resolution and accurate mass MS/MS detection. TOF-MS and MS/MS data were acquired using the SCIEX X500R QTOF system.

TOF-MS information was used to screen for and identify targeted food contaminants. Quantitative information was achieved by performing multi level calibration. Identification was based on matching retention time, mass accuracy and isotope pattern of the quasi-molecular ion, isotopic pattern and MS/MS fragmentation pattern (library searching). The molecular fingerprint saved into MS/MS spectra allowed to differentiate isomeric compounds and greatly reduced the number of potential false positive results. The new SCIEX OS software allows quick processing and easy result review and reporting capabilities.

MATERIALS AND METHODS

- Fruit and vegetable samples from a local supermarket
- Quantitation using a pesticide standard provided by the EURL
- QuEChERS extraction using Phenomenex roQ QuEChERS kit buffer-salt mix and dSPE kits following the European standard method 15662
- 10-20x dilution of sample extracts to minimize possible matrix effects
- UHPLC using a SCIEX ExionLC™ AC system with a Phenomenex Kinetex Biphenyl column (50 x 2.1 mm, 2.6 µm)
- Gradient of water and methanol with 5 mM ammonium formate
- Flow rate of 0.5 mL/min
- Column oven temperature of 40ºC
- Injection volume of 5 µL
- Detection using a SCIEX X500R 500 QTOF system with Turbo V™ source operated in ESI mode
- Continuous recalibration between injections using the Calibrant Delivery System (CDS) using a TwinSpray setup (dual ESI needle)
- Information Dependent Acquisition (IDA):
  - TOF-MS survey scan 100-1000 Da (200 ms) using Declustering Potential (DP) of 80 V
  - 10 dependent TOF-MS/MS scans 50-1000 Da (50 ms) using Collision Energy (CE) of 35 V with Collision Energy Spread (CES) of ± 15 V
  - Ion source parameters: CUR = 30 psi, GS1 = 50 psi, GS2 = 70 psi, IS = 5500 V, TEM = 450ºC
- Dynamic background subtraction (DBS) was activated for best IDA coverage, no inclusion list was used to allow retrospective unknown identification without the need for a second injection to acquire MS/MS data

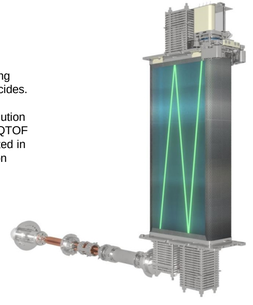
- Data acquisition and qualitative-quantitative data processing using SCIEX OS software

RESULTS

SCIEX X500R QTOF System Performance Characteristics

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).



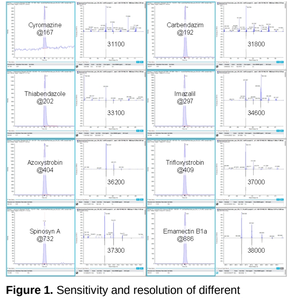


Figure 1. Sensitivity and resolution of different pesticides, left: XIC of the molecular ion of each compound ± 5 mDa at 1 ng/mL (Emamectin at 10 ng/mL), right: TOF-MS spectrum of molecular ion with achieved resolution (average of seven X500R QTOF systems)

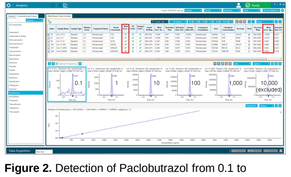


Figure 2. Detection of Paclobutrazol from 0.1 to 10,000 ng/mL with good linearity (0.1 to 1,000 ng/mL) and mass errors of < 1 ppm even at the highest concentration above the upper limit of quantitation

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 (r2 = 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.

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.

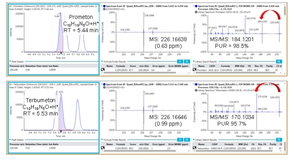


Figure 3. Confident identification of isomers Prometon and Terbumeton using characteristic MS/MS fragment ions and MS/MS library searching, the two compounds have unique and characteristic fragment ions, $C_7H_{14}N_5O^+$ and $C_8H_{12}N_5O^+$, respectively, which can be used for identification. Molecular and fragment ions have been measured with good mass accuracy of < 5 ppm and less < 1 mDa, respectively

Targeted Data Analysis Workflow

XIC are generated based on user input (formula and expected retention time for all target analytes) – MS and MS/MS information is automatically evaluated if signal exceeds user defined intensity threshold or S/N, confidence in identification is ranked based on retention time matching, mass accuracy, isotope fit, and MS/MS library searching, calibration lines are automatically generated based on peak areas of XIC and used to calculate concentrations in unknown samples (Figure 4).

Figure 4. Targeted data processing workflow

Figure 5. Easy review of quantitative and quantitative results, concentrations exceeding reporting levels are automatically flagged and identification confidence is displayed using 'traffic lights'

Results of EU Proficiency Test Samples

Two samples of an EU proficiency test (EUPT) for pesticides and fruits and vegetables were extracted and analyzed for pesticides. Results are listed in Table 1. 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 iDQuant™ standards kit for pesticide analysis.

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 2.

Table 1. Pesticides identified and quantified in two EUPT samples

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

Pesticide	RT error (min)	Mass error (ppm)	Isotope ratio error	MS/MS FIT (%)
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

Table . In store-bought samples

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

CONCLUSIONS

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

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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

TRADEMARKS/LICENSING

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