Multi-Compound and Multi-Class Identification and Quantification using High Resolution LC-MS/MS

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OVERVIEW

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 quadruple 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 MasterView™ software allows quick processing and easy result review and reporting capabilities.

EXPERIMENTAL

- Fruit and vegetable samples from a local supermarket
- Quantitation using a 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
- Injection volume of 5 µL

- Detection using a SCIEX X500R 500 QTOF system with Turbo V™ source operated in Electrospray Ionization (ESI)
- Continuous recalibration between injections using the Calibrant Delivery System (CDS) using a TwinSpray setup
- Information Dependent Acquisition (IDA):
  - TOF-MS survey scan 100-1000 Da (100 ms)
  - 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
- 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
- Qualitative and quantitative data processing using SCIEX OS software

RESULTS

SCIEX X500R QTOF System Performance Characteristics
Resolution > 20,000 (at full width half height) and mass accuracy <5 ppm is often sufficient to separate the analytes of interest from interfering matrices and, thus, is a requirement for compound identification in various guidelines.[1,2] The X500R QTOF system, using an N-optic design and a 4 mm orifice leading into the accelerator, provides resolving power of 25000 to 35000 for small molecular weight compounds and mass accuracy <2 ppm. The sensitivity and linear dynamic range of the X500R QTOF system is comparable to a QTRAP® 5500 system operated in MRM mode, allowing extract dilution to minimize ion suppression while detecting easily at 10 µg/kg levels (Figures 1 and 2).

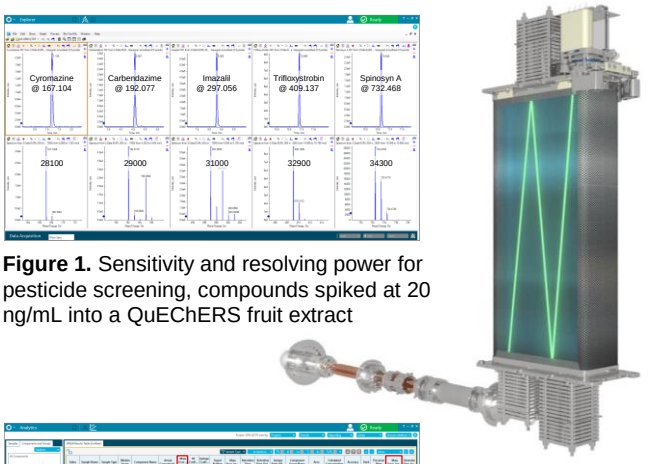


Figure 1. Sensitivity and resolving power for pesticide screening, compounds spiked at 20 ng/mL into a QuEChERS fruit extract

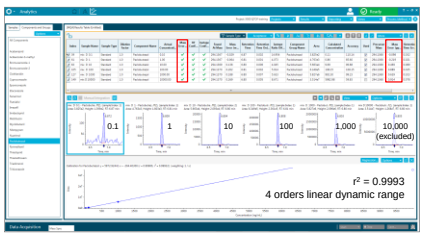


Figure 2. Linearity and mass accuracy for Paclobutrazol (0.1 to 10,000 ng/mL), linearity was achieved over 4 orders of magnitude, mass accuracy of less than 1 ppm was maintained even above the upper limit of quantitation ULOQ

Targeted Data Analysis Workflow

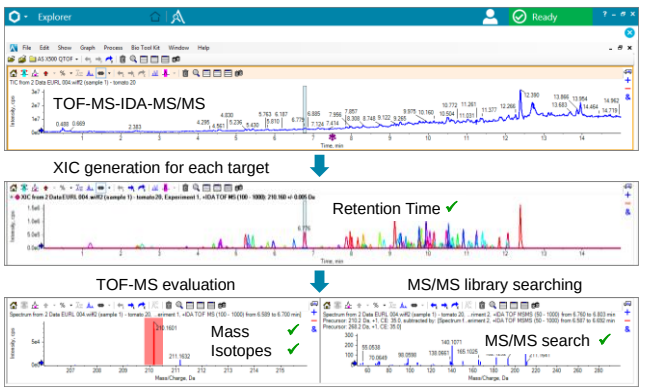
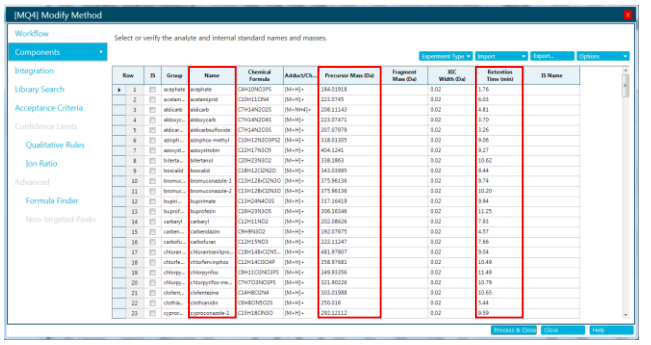


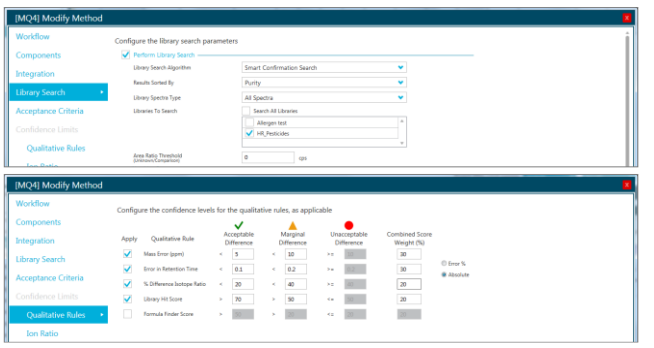
Figure 3. XIC are generated based on user input (formula and expected retention time for all target analytes) – MS and MS/MS information is automatically evaluated if signal exceeds user defined intensity threshold or S/N, confidence in identification is ranked based on retention time matching, mass 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

Data Analysis Workflow in SCIEX OS Software

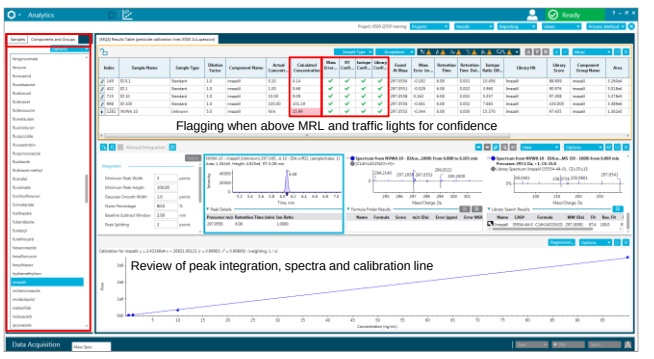
1) Define retention time and accurate mass for each target analyte



2) Define identification criteria and confidence settings



3) Easy review of quantitative and quantitative results, concentrations exceeding reporting levels are automatically flagged and identification confidence is displayed using ‘traffic lights’



Application of the Developed Method to Samples of Food

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

SUMMARY

In this poster we presented a novel approach of multi-target screening to identify and quantify pesticide residues in food samples. Fruit and vegetable samples were extracted using a QuEChERS procedure and analyzed utilizing 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 SCIEX X500R QTOF system. Data was processed using the new SCIEX OS software. Compound identification was achieved with high confidence based on automatic evaluation of retention time, mass accuracy, isotope pattern, and MS/MS library searching. A ‘traffic lights’ display is utilized for easy data review and reporting. Quantitative results were obtained in the same data processing step using multi level calibration lines.

REFERENCES

1 EU Commission Decision ‘concerning the performance of analytical methods and the interpretation of results’ #2002/657/EC
2 SANCO Document: ‘Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed’ #SANCO/12495/2011

TRADEMARKS/LICENSING