Perfect Balance to Elevate your Lab's Performance

Using the X500R QTOF System and SCIEX OS Software to Quickly Identify Unknowns in Food Samples

André Schreiber[1], Yuji Aita[2], and Jianru Stahl-Zeng[3]
[1]SCIEX Concord, Ontario (Canada), [2]SCIEX Tokyo (Japan), [3]SCIEX Darmstadt (Germany)

Overview

Here we present results using a new method to identify unexpected chemical residues and contaminants in food using the SCIEX X500R QTOF system. Samples were extracted using a QuEChERS method and analyzed by LC-HR-MS/MS.

Unknown compounds were automatically identified by using a non-target peak finding algorithm followed by sample-control-comparison to separate matrix and sample specific signals from true contaminations. TOF-MS and MS/MS data for ions of interest were automatically processed using formula finding and searched against mass spectral libraries and online databases, such as ChemSpider, for identification. The SCIEX OS software offers an easy to use and intuitive workflow to tentatively identify unexpected chemicals in food.

Introduction

Hybrid LC-MS/MS systems like quadrupole-quadrupole Time-of-Flight (QTOF) provide the ability to perform targeted and non-targeted screening in food samples on a routine basis.

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 relevant concentrations
- Resolving power to remove interference from complex food matrices
- Linearity over up to 3 orders of magnitude to identify compounds at different concentration levels
- 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 chemical 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
- Automated identification of unknowns
- Quick data review and reporting utilizing customizable flagging and filtering of results

Experimental

Sample preparation

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 unknown screening

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 (Figure 1). Its resolving power increases with mass range providing ~30000 to 40000 for the typical molecular weight range of pesticides.[3]

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.[3]
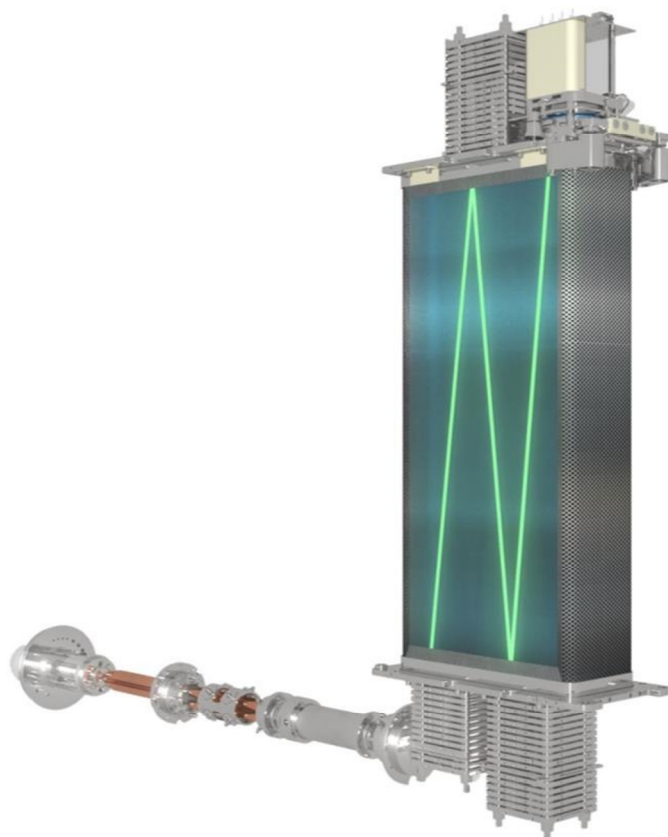

Figure 1. N-optics design of the X500R QTOF system to maximize resolution while maintaining benchtop design and a minimized footprint, 6 heater drones are integrated into the TOF path to maintain mass accuracy and robustness

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.

While accurate mass measurement of the molecular ion is important for empirical formula finding, this is not the only information available. Combining all available accurate mass MS and MS/MS information is crucial to minimize the list of potential formulae. Figures 2, 3 and Table 2 illustrate that the number of formulae can be reduced from over 200 to a single match by not only using the accurate mass of the molecular ion but also including the isotope pattern and MS/MS matching in the formula-finding algorithm.

Using the combined scoring of MS and MS/MS matches, SCIEX OS lists the most likely chemical formula at the top of results table. Also, SCIEX OS downloads a ChemSpider hit count for each calculated formula which further assists in identifying the correct result (Figure 2).
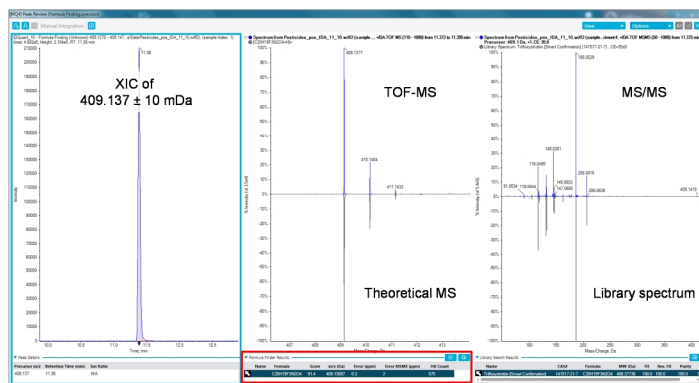

Figure 2. TOF-MS and MS/MS spectra used for empirical formula finding, results are ranked by a combined score using MS and MS/MS information, and when combined with the ChemSpider hit count, can be used to quickly find the correct match
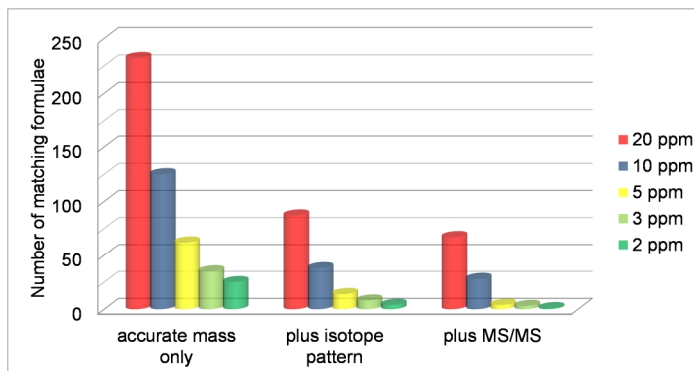

Figure 3. Number of matching molecular formulae depending on the information and mass accuracy used for empirical formula finding (elements allowed $C_{49}H_{75}Br_3Cl_5F_3I_3N_{10}O_{10}PS_3$)

Table 2. Ranking of matching formulae using MS and MS/MS information collected for Trifloxystrobin, the MS rank combines mass accuracy and isotope pattern matching and the MS/MS rank combines mass accuracy and number of ions (n)

Hit	Formula	MS Rank	ppm	MS/MS Rank	ppm (n=11)
1	$C_{20}H_{19}F_3N_2O_4$	2	0.3	2	2.0
2	$C_{21}H_{15}F_3N_6$	9	-2.9	4	3.0
3	$C_{18}H_{16}N_8O_4$	4	0.9	6	4.8
4	$C_{15}H_{17}FN_8O_5$	11	-1.9	5	4.8
5	$C_{16}H_{13}FN_{12}O$	7	-5.2	10	9.0
6	$C_{14}H_{20}F_3N_6O_3P$	22	2.8	1	2.0
7	$C_{16}H_{21}N_6O_5P$	7	-3.1	11	9.4
8	$C_{23}H_{18}F_2N_2O_3$	9	3.1	14	9.4
9	$C_{21}H_{23}F_2O_4P$	1	-0.9	24	22.1
10	$C_{19}H_{21}FN_2O_7$	16	-8.4	12	9.4

In addition to more efficient formula finding, MS/MS spectra are also needed for structural elucidation. Without MS/MS spectra it is impossible to conclude a correct structure from a molecular formula alone.

The example shown in Figure 4 highlights the need of fragment ion detection to confidently differentiate between isomers Prometon and Terbumeton.
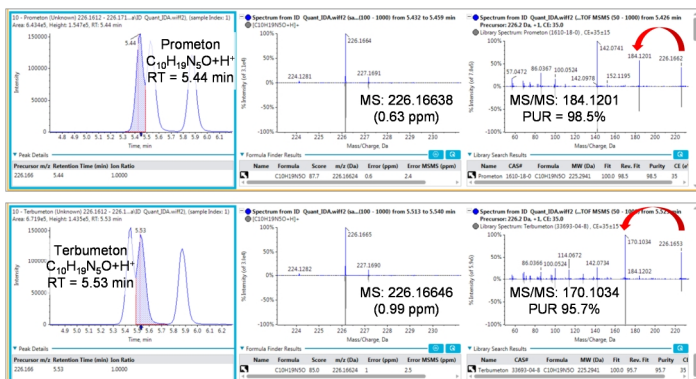
Figure 4. Confident identification of isomers Prometon and Terbumeton using characteristic MS/MS fragment ions and MS/MS library searching

Processing Workflow for Unknown Identification in SCIEX OS Software

Extracted Ion Chromatograms (XIC) are generated using a non-target peak finding algorithm. No masses or retention times are provided to find chromatographic features. Sample-control-comparison is used to separate matrix and sample-specific signals from true contaminations.

High resolution TOF-MS and MS/MS data of ions of interest are automatically processed using:

- MS/MS library searching to identify compounds already present in existing libraries
- Empirical formula finding based on TOF-MS and MS/MS
- ChemSpider searching
- Comparison of structures retrieved from ChemSpider against the acquired HR-MS/MS spectra

The method editor in SCIEX OS software to setup parameters and criteria for unknown identification is shown in Figure 5a-c.

Figure 5a. Method editor in SCIEX OS software for unknown identification, selection of sample and control-sample for non-target peak finding
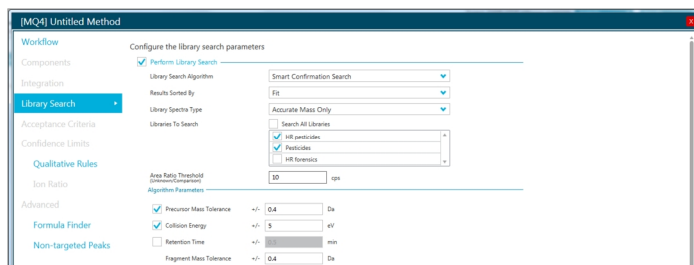

Figure 5b. Method editor in SCIEX OS software for unknown identification, configuration of library search parameters
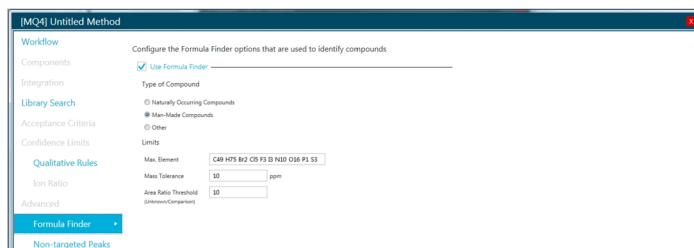

Figure 5b. Method editor in SCIEX OS software for unknown identification, configuration of formula finding options

SCIEX offers true HR-MS/MS spectral libraries for over 2500 compounds, including pesticides, veterinary drugs, toxins, fluorochemicals, pharmaceuticals, and illicit drugs.

Results of Unknown Identification

Two samples of bell pepper, including an organic pepper, were extracted and analyzed using the developed LC-HR-MS/MS method in positive and negative polarity. Both samples were processed using the described non-target workflow.

A total of 2358 (positive polarity) and 1563 (negative polarity) chromatographic features were identified using the non-target peak finding algorithm. Less than 50 features were found to be characteristic for the contaminated bell pepper after sample-control-comparison using an area ratio of 10.

Results can be sorted and filtered for easy data review after performing sample-control-comparison. Library searching and formula finding results and scores are listed in the result table. More details and a visual display of XIC, TOF-MS and MS/MS for both samples can be found in peak review (Figure 6).
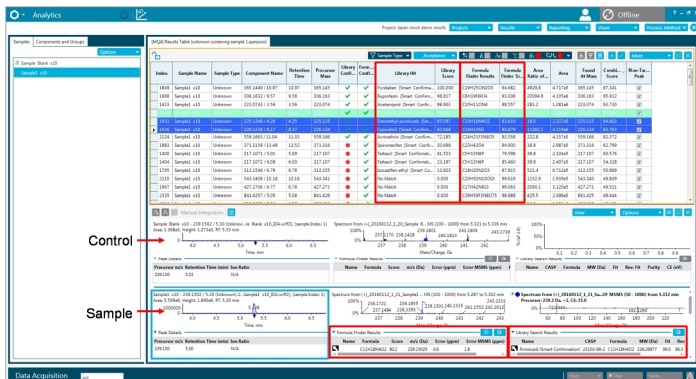
Figure 6. Results display after non-target screening, library searching and formula finding results are displayed in the table (top) and chromatograms and spectra with result details can be reviewed (bottom)

Formula finding results are displayed below the TOF-MS spectrum in the peak review window. Results are automatically ranked by mass accuracy (MS and MS/MS) and the matching of the isotope pattern. In addition the ChemSpider hit count is listed to quickly identify the correct match. The formulae can be searched against ChemSpider. Structural information from ChemSpider will be automatically compared against the acquired MS/MS spectrum to provide feedback for a quick identification.

Examples of tentatively identified pesticides in the bell pepper sample are shown in Figures 7, 8 and 9.
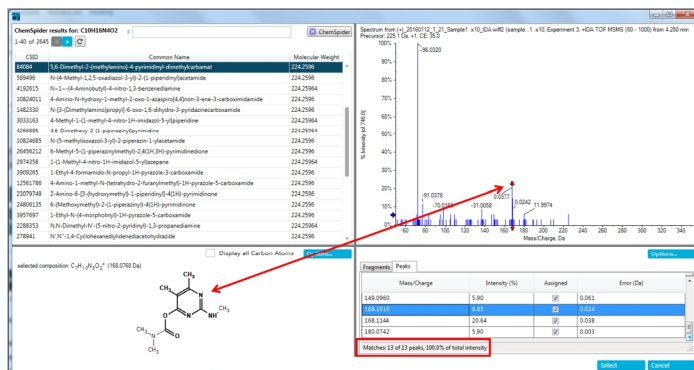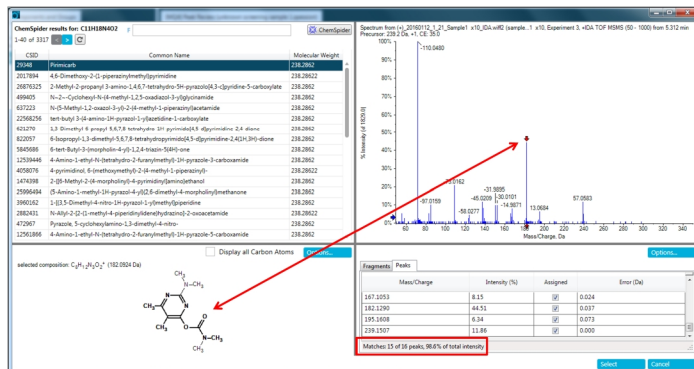

Figure 7b. The ChemSpider search and automatic elucidation of the MS/MS spectrum led to the tentative identification of Pirimicarb (top) and also of its metabolite Desmethyl-pirimicarb (bottom), both compounds were confirmed by MS/MS library searching

Figure 7a. Review of XIC of m/z 239.150 at RT 5.3 min and spectra with a found formula of $C_{11}H_{18}N_4O_2$
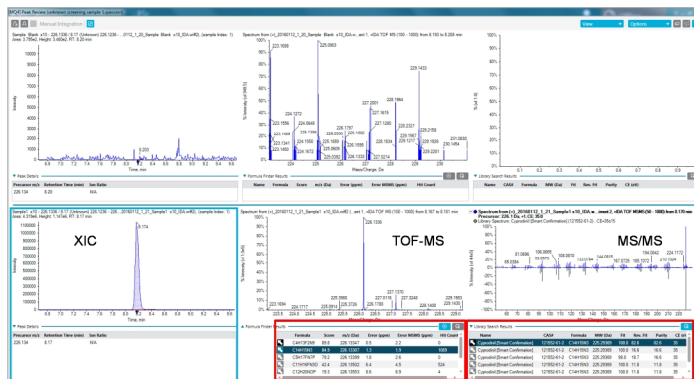

Figure 8a. Review of XIC of m/z 226.134 at RT 8.2 min and spectra with a found formula of $C_{14}H_{15}N_3$, although ranked second based on mass accuracy the high ChemSpider hit count revealed the correct match
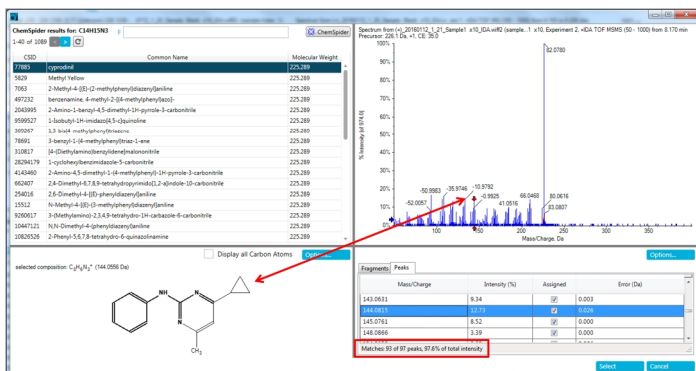
Figure 8b. The ChemSpider search and automatic elucidation of the MS/MS spectrum led to the tentative identification of Cyprodinil, this compound was confirmed by MS/MS library searching

Figure 9a. Results display after non-target screening of the negative polarity data, review of XIC of m/z 367.203 at RT 6.7 min and spectra with a found formula of $C_{22}H_{28}N_2O_3$
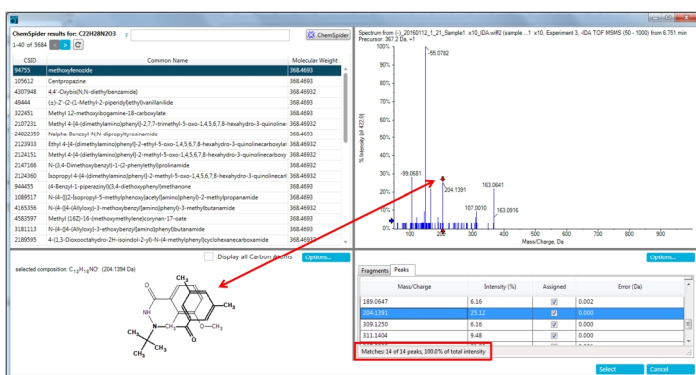
Figure 9b. The ChemSpider search and automatic elucidation of the MS/MS spectrum led to the tentative identification of Methoxyfenozide

Summary

A new method to identify unexpected chemical residues and contaminants in food samples was developed using the SCIEX X500R QTOF system. Store-bought food samples were extracted using a QuEChERS procedure and analyzed by LC-HR-MS/MS.

Data processing was performed in SCIEX OS software. The processing workflow consists of peak finding using a non-target algorithm (no masses or retention times were provided to find chromatographic features). Automatic sample-control-comparison was used to separate matrix and sample specific signals from true contaminations. In a final step, tools such as empirical formula finding, MS/MS library searching and online database searching was used for identification.

The method was successfully applied to tentatively identify pesticide residues in vegetable 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 Identify and Quantify Food Residues' Application Not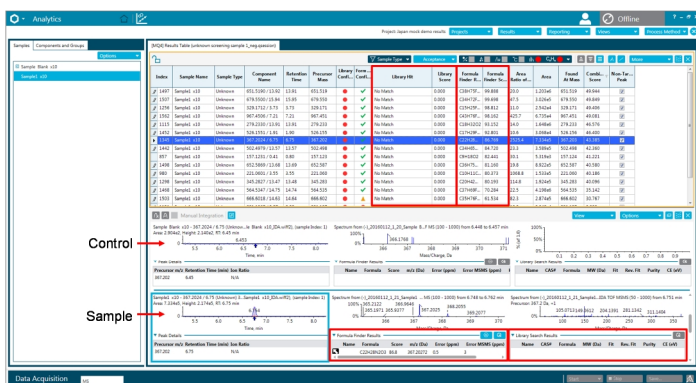e SCIEX (2016) # RUO-MKT-02-3760-A