Food and Environmental

The Power of Precision

Analysis of Pesticides in Food in Compliance with EU Regulations Using the SCIEX X500R QTOF System

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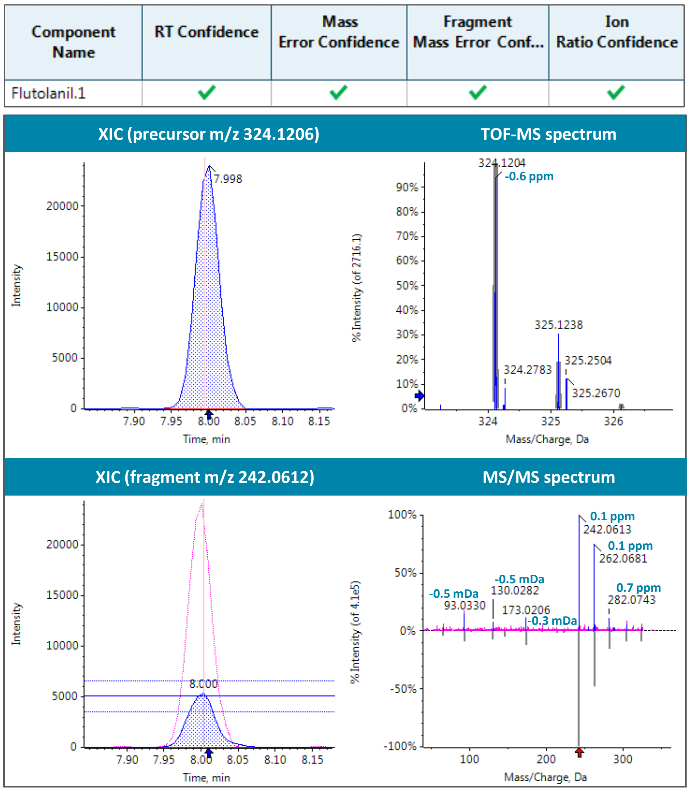
Pesticides are widely used in agriculture, growing of fruit and vegetables, and garden plots to protect against pests and to control the growth of unwanted plants, both to minimize crop loss. The use of pesticides is strongly regulated to ensure that any hazards for humans, animals, and the environment are minimized. Regarding maximum residue limits in food, approved pesticides either have specific limits, or they need to fulfill a general maximum residue quantity of 0.01 mg/kg.[1]



Component Name	RT Confidence	Mass Error Confidence	Fragment Mass Error Conf...	Ion Ratio Confidence
Flutolanil.1	✓	✓	✓	✓

Figure 1: Flutolanil in Mandarin spiked at 0.1 mg/kg as shown in SCIEX OS. Green checkmarks in the upper results table confirm positive identification with respect to the four key criteria: retention time, precursor mass error, fragment mass error, and ion ratio. (Top left) Extracted ion chromatogram (precursor). (Top right) TOF-MS spectrum (experimental spectrum in blue, theoretic isotopic pattern superposed in grey). (Bottom left) Extracted ion chromatogram: fragment in blue, precursor in pink. Solid blue line indicates expected ion ratio, dotted lines show tolerances for ion ratio (± 30 %). (Bottom right) MS/MS spectrum. Pink trace shows MS/MS fragments from SWATH® acquisition spectrum, blue signals correspond to deconvoluted fragment signals of flutolanil.

To monitor pesticide residues in food, LC-MS/MS in combination with QuEChERS sample preparation is the method of choice for the majority of relevant pesticides.[2] With the *SANTE/11813/2017* document, the European Commission published specific guidelines on quality control and validation procedures for analytical methods for pesticides in food and feed.[3] It specifically defines identification criteria valid for high resolution mass spectrometers, including retention time, mass accuracy and ion ratio requirements.

Previous work has demonstrated the capability of the SCIEX X500R QTOF system for the analysis of pesticides in baby food.[4] Here it is demonstrated that the X500R QTOF system together with SCIEX OS software can be used for the analysis of pesticides in food extracts in compliance with EU regulations. All identification requirements for HR-MS including retention time, mass accuracy of two ions and their specific ion ratio can be easily reviewed in SCIEX OS (Figure 1). In addition, the X500R system provides excellent mass accuracy with mass errors < 5 ppm, respectively < 1 mDa for ions below m/z 200.

Materials and Methods

Sample Preparation: Acetonitrile (10 mL) was added to homogenized leek (10 g) and homogenized mandarin (10 g). Samples were mixed for 1 minute, followed by centrifugation for 10 minutes at 5000 rpm. The supernatants were tenfold diluted with water. A calibration curve was prepared in 10% acetonitrile in water from a stock solution of the iD*Quant*™ standards kit, with final concentrations at 0.1, 0.5, 1, 5, 10, 50, and 100 ng/mL.

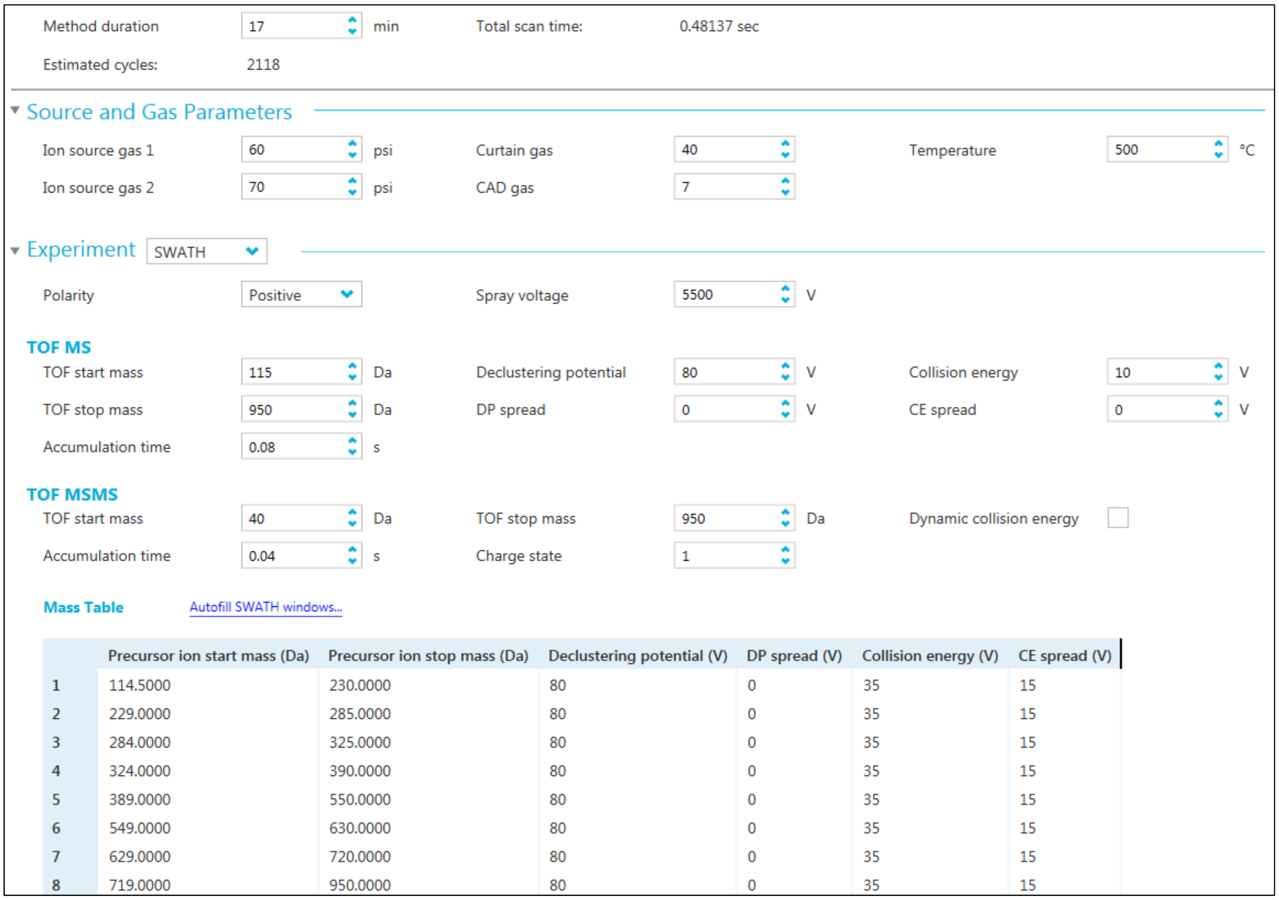
Chromatography: Pesticides were chromatographically separated on a Phenomenex Luna® Omega Polar UHPLC column (100 x 2.1 mm, 2.6 µm), using the SCIEX ExionLC™ AD UHPLC system. Mobile phase was 2 mM ammonium formate and 0.1 % formic acid in water (eluent A) and in methanol (eluent B), respectively. The LC gradient is shown in the table below. Oven temperature was set at 50 °C. Injection volume was 5 µL.

Mass Spectrometry: Mass spectrometric analysis was performed on the SCIEX X500R QTOF system in positive mode, operated with the electrospray Turbo V™ Source. Data acquisition was performed using SWATH® Acquisition, where a TOF MS scan was looped with eight MS/MS experiments. Figure 2 shows the MS method as displayed in SCIEX OS.

Table 1. LC Gradient.

	A [%]	B [%]	Flow [mL/min]
0.0 min	98	2	0.6
0.5 min	98	2	0.6
3.5 min	60	40	0.6
12.0 min	5	95	0.6
14.0 min	5	95	0.6
14.1 min	98	2	0.6
17.0 min	98	2	0.6

Data Processing: Data processing was done in SCIEX OS Software version 1.3.



Method duration 17 min
Total scan time: 0.48137 sec
Estimated cycles: 2118

Source and Gas Parameters

Ion source gas 1: 60 psi
Curtain gas: 40
Temperature: 500 °C
Ion source gas 2: 70 psi
CAD gas: 7

Experiment: SWATH

Polarity: Positive
Spray voltage: 5500 V

TOF MS

TOF start mass: 115 Da
Declustering potential: 80 V
Collision energy: 10 V
TOF stop mass: 950 Da
DP spread: 0 V
CE spread: 0 V
Accumulation time: 0.08 s

TOF MSMS

TOF start mass: 40 Da
TOF stop mass: 950 Da
Dynamic collision energy: ☐
Accumulation time: 0.04 s
Charge state: 1

Mass Table

	Precursor ion start mass (Da)	Precursor ion stop mass (Da)	Declustering potential (V)	DP spread (V)	Collision energy (V)	CE spread (V)
1	114.5000	230.0000	80	0	35	15
2	229.0000	285.0000	80	0	35	15
3	284.0000	325.0000	80	0	35	15
4	324.0000	390.0000	80	0	35	15
5	389.0000	550.0000	80	0	35	15
6	549.0000	630.0000	80	0	35	15
7	629.0000	720.0000	80	0	35	15
8	719.0000	950.0000	80	0	35	15

Figure 1: MS Method in SCIEX OS Software.

SWATH® Acquisition

In SWATH acquisition mode, MS/MS spectra of all precursors are acquired by using a wider Q1 isolation window and stepping this across the entire mass range of interest. At each step, all precursors in that mass range at that retention time are transported through the Q1 into the collision cell, where ions are fragmented then analyzed in the TOF analyzer.[5] In this study, variable Q1 windows were used, and the Q1 window widths were determined from the average ion density observed across the m/z range for the matrix samples.

From the acquired data, each analyte can be quantified using the TOF MS across the samples. The MS/MS data collected in the same data file is used to give additional identification confidence using the fragment mass and its ion ratio. Furthermore, if the TOF MS trace shows interferences in complex matrices, quantitation may also be performed using MS/MS fragment ions.

Mass Accuracy

For SWATH acquisition type data collection modes, EU regulations require as identification criterion a mass accuracy of ≤ 5 ppm for two ions, including at least one fragment ion. For ions < 200 m/z, the tolerance is 1 mDa. Previously, QTOF systems were known to show mass errors >1mDa for small fragments (m/z < 150 Da) due to effects in the TOF causing non-linearity of the mass calibration equation. The X500R QTOF system overcomes these limitations using a patented homogenized pulsed electric field in a ring stack ion accelerator yielding excellent mass accuracy also for small ions.[6]

Figure 3 shows the mass accuracies calculated for all precursors in a standard solution at 5 ng/mL, as well as in leek and mandarin extracts spiked at 0.1 mg/kg. All precursors were measured with a mass accuracy within the limit set in the SANTE guideline. Requirements in terms of mass accuracy were easily fulfilled for all tested analytes (Figures 4).

It should be noted that in case of any matrix interferences which could hamper the mass accuracy, the user can switch to another fragment as qualifier, since in SWATH acquisition full scan MS/MS is collected over the entire mass range and thus available for quantitation or identification.

Table 2: Calculated Ion Ratios.

	# of Pesticides Passing Ion Ratio Error Requirement < 30%
Solvent (5 ng/mL)	100%
Mandarin extracts (0.01 mg/kg)	100%
Leek extracts (0.01 mg/kg)	100%

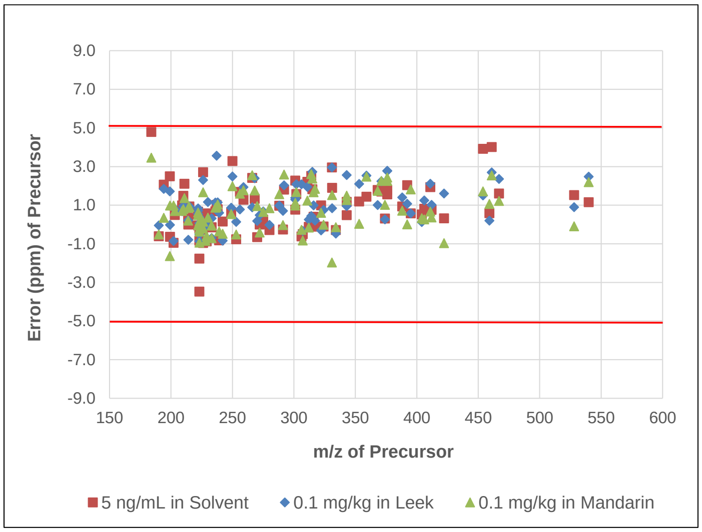


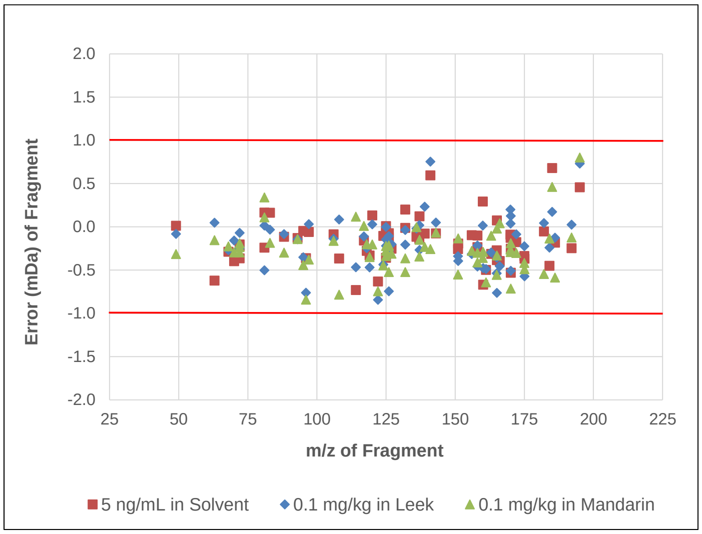
Figure 3: Experimental Mass Errors of Precursors. Red lines show accepted tolerances of max. ± 5 ppm.

Ion Ratios

The ratio of two ions, e.g. the ratio of the precursor and one of its fragments or of two fragments, may serve as further, facultative identification criterion. The SANTE guideline recommends a maximum deviation from the expected ion ratio (calculated from standard solutions) of 30 % for unknown samples for definite identification. As summarized in Table 2, 100% of the tested analytes of the iD*Quant* standard fulfilled the requirements. As already mentioned, the user can select a different fragment ion to be used based on ion ratio, in case of any matrix interferences in complex samples.

Confident Display in SCIEX OS

In routine analysis, it is important for the user to easily and quickly review large sample sets and to clearly identify positive samples. For this, SCIEX OS provides a convenient traffic light system, which displays green checkmarks (within the user-defined tolerances), yellow triangles (needs review by the user) and red dots (non-concordant) for easy filtering large data sets (Figure 1). Furthermore, the sample view in SCIEX OS puts together the extracted ion chromatograms of the precursor and the fragment, including the display of the expected ion ratio as solid line as well as the tolerances. Finally, the TOF MS spectrum with its theoretical isotopic pattern and the MS/MS spectrum with the library spectrum in mirror view can be displayed (Figure 1).



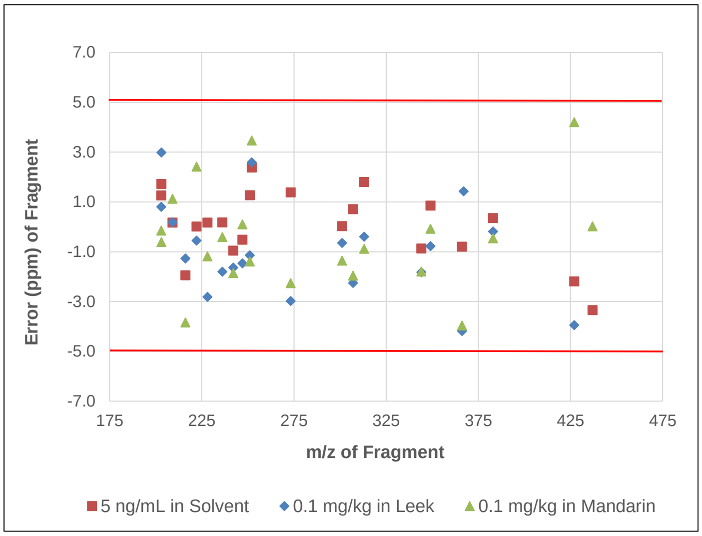


Figure 4: Experimental Mass Errors of MS/MS Fragments. (Top) Mass accuracy on fragments with m/z <200 Da. Red lines show accepted tolerances of max. ± 1 mDa. (Bottom) Mass accuracy for fragments with m/z >200 Da. Red lines show accepted tolerances of max. ± 5 ppm.

Conclusion

The SCIEX X500R QTOF system with SWATH acquisition provides MS and MS/MS spectra for the quantitative and qualitative analysis of pesticides in food. Retention time, mass accuracy of precursor and fragment, and the ion ratio serve as identification criteria according EU regulations. Due to the stability of the system and design of the TOF Analyzer, very high mass accuracy can be obtained on the MS/MS, including the for small ions with m/z < 200 Da.

References

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