Master Your Data to Get to the Answer, View Results with Ease and Confidence

Using TripleTOF® Technology and MasterView™ Software to Identify and Quantify Food Residues

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Overview

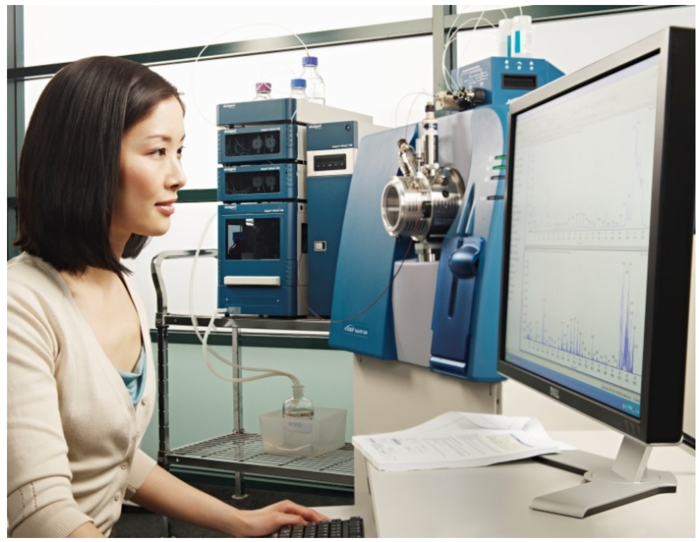
Here we present results of using a novel data processing approach of comparative multi-target screening to rapidly and accurately identify and quantify chemical residues in food. Fruit and vegetable samples were extracted using a QuEChERS procedure and analyzed with reversed phase LC. High resolution and accurate mass MS and MS/MS information was collected in a single injection on AB SCIEX TripleTOF® 4600 and 5600 systems. Data were processed using the new MasterView™ software.

Introduction

LC-MS/MS is a powerful analytical tool for the analysis of food residues and contaminants. In particular, 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 (TripleTOF®), now provide the ability to perform both targeted and non-targeted screening in food samples on a routine basis. However, full scan chromatograms are very rich in information and contain easily thousands of ions from both chemical 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 to get answers and results from these complex data.

TOF-MS and MS/MS information was used to identify and quantify targeted food residues. Quantitation was achieved by performing single concentration standard addition at the level of the Maximum Residue Limit (MRL). Identification was based on retention time, accurate mass of the quasi-molecular ion, isotopic pattern and MS/MS library searching. The molecular fingerprint saved into MS/MS spectra allow us to differentiate isomeric compounds and greatly reduce the number of potential false positive results.



The new MasterView™ software allows quick processing and easy results review and reporting capabilities.

Experimental

- Fruit and vegetables samples from a local supermarket and samples from a EU proficiency tests for pesticides and fruits and vegetables
- Quantitation using the Sciex iD*Quant*™ standards kit for pesticide analysis
- QuEChERS extraction following guideline EN 15662/2007 using commercial QuEChERS kits
- 5-20x dilution of sample extracts to minimize possible matrix effects
- UHPLC using a Shimadzu $UFLC_{XR}$ system with Restek Ultra Aqueous C18 (100 x 2.1 mm) 3 µm column
- Gradient of water and methanol with 10 mM ammonium formate at a flow rate of 0.5 mL/min and injection volume of 10 µL

- AB SCIEX TripleTOF® 4600 and 5600 system with DuoSpray™ source operated in ESI mode
- Continuous recalibration between injections using the Calibrant Delivery System (CDS)
- Information Dependent Acquisition (IDA) using a TOF-MS survey scan 100-1000 Da (100 ms) and up to 10 or 20 dependent TOF-MS/MS scans 50-1000 Da (100 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

LC-MS/MS data were processed using the new MasterView™ software version 1.0.

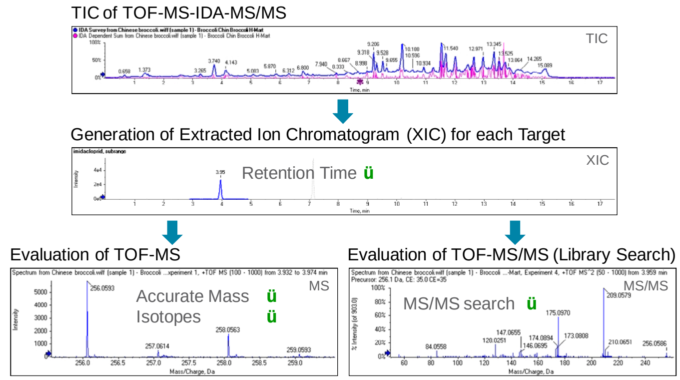


Figure 1. Data analysis workflow for targeted screening in MasterView™ software, identification with high confidence is based on retention time matching, mass accuracy, isotope fit, and MS/MS library searching

Results and Discussion

TripleTOF® System Performance Characteristics

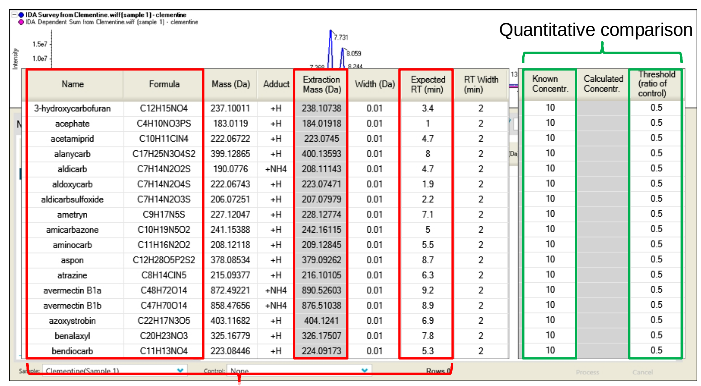
Resolution > 20,000 (at full width half height) and mass accuracy <5 ppm are often sufficient to separate the analytes of interest from interfering matrices and, thus, are identified as the set requirements for compound identification in various guidelines.[1, 2] The sensitivity of TripleTOF® 4600 and 5600 system is comparable to the AB SCIEX QTRAP® 4500 and 5500, respectively, operated in MRM mode, allowing extract dilution to minimize ion suppression while detecting easily at 10 µg/kg levels.[3, 4]

Data Analysis Workflow for Targeted Screening

Extracted Ion Chromatograms (XIC) are generated based on user input (formula and expected retention time of all target analytes). MS and MS/MS information is automatically evaluated if the detected signal exceeds the user defined intensity threshold or S/N. Data processing results are ranked based on 4 selectivity criteria: (1) retention time matching, (2) mass accuracy, (3) isotope pattern fit, and (4) MS/MS library searching, to provide a high degree of confidence in assigning compound identifications to detected compounds. In addition, the intensity is compared to a standard sample of known concentration to obtain quantitative information.

Targeted Compound Identification in MasterView™ Software

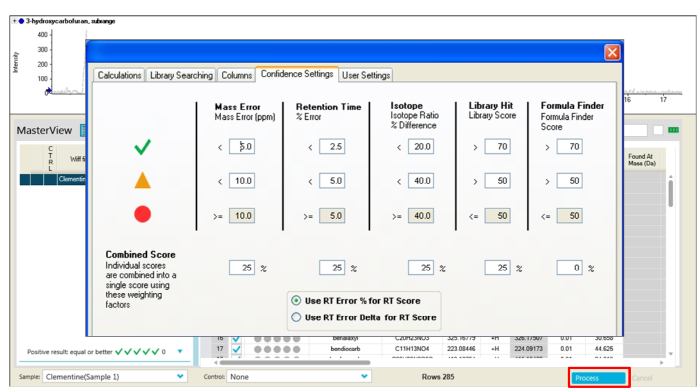
1. Open data file(s), build or open target XIC list, and define concentrations and threshold (ratio of unknown sample(s) and control) for quantitative comparison.



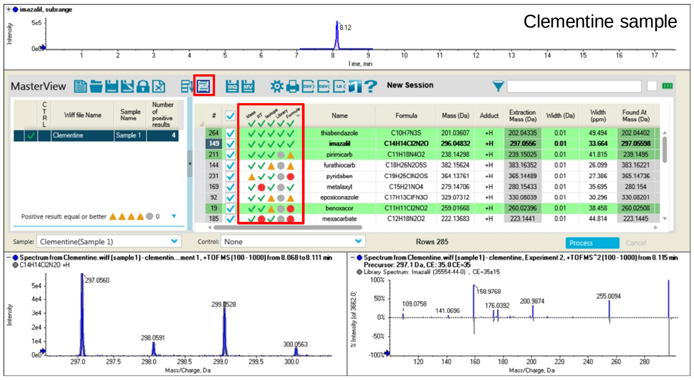
Name	Formula	Mass (Da)	Adduct	Extraction Mass (Da)	Width (Da)	Expected RT (min)	RT Width (min)	Known Concentr.	Calculated Concentr.	Threshold (ratio of control)
3-hydroxycarbofuran	C12H15NO4	237.10011	+H	238.10738	0.01	3.4	2	10		0.5
acephate	C4H10NO3PS	183.0119	+H	184.01918	0.01	1	2	10		0.5
acetamiprid	C10H11ClN4	222.06722	+H	223.0745	0.01	4.7	2	10		0.5
alanycarb	C17H25N3O4S2	399.12865	+H	400.13593	0.01	8	2	10		0.5
aldicarb	C7H14N2O2S	190.0776	+NH4	208.11143	0.01	4.7	2	10		0.5
aldoxycarb	C7H14N2O4S	222.06743	+H	223.07471	0.01	1.9	2	10		0.5
aldicarbsulfoxide	C7H14N2O3S	206.07251	+H	207.07979	0.01	2.2	2	10		0.5
ametryn	C9H17N5S	227.12047	+H	228.12774	0.01	7.1	2	10		0.5
amicarbazone	C10H19N5O2	241.15388	+H	242.16115	0.01	5	2	10		0.5
aminocarb	C11H16N2O2	208.12118	+H	209.12845	0.01	5.5	2	10		0.5
aspon	C12H28O5P2S2	378.08534	+H	379.09262	0.01	8.7	2	10		0.5
atrazine	C8H14ClN5	215.09377	+H	216.10105	0.01	6.3	2	10		0.5
avermectin B1a	C48H72O14	872.49221	+NH4	890.52603	0.01	9.2	2	10		0.5
avermectin B1b	C47H70O14	858.47656	+NH4	876.51038	0.01	8.9	2	10		0.5
azoxystrobin	C22H17N3O5	403.11682	+H	404.1241	0.01	6.9	2	10		0.5
benalaxyl	C20H23NO3	325.16779	+H	326.17507	0.01	7.8	2	10		0.5
bendiocarb	C11H13NO4	223.08446	+H	224.09173	0.01	5.3	2	10		0.5

Screening using extracted ion chromatograms (XIC)

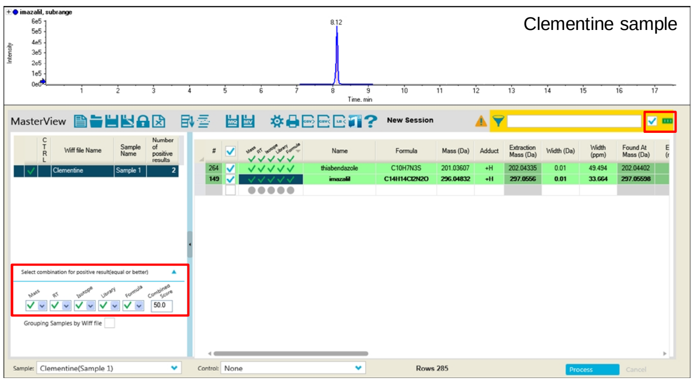
2. Set confidence settings and start processing by clicking ‘Process’. XIC lists and settings can be saved and opened for immediate data processing.



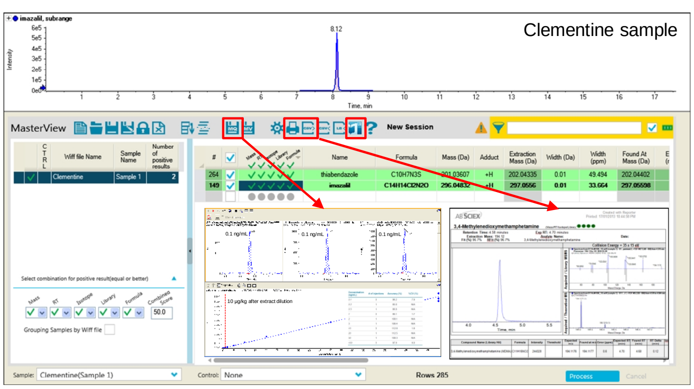
3. Review results using ‘traffic lights’ and display of MS and MS/MS spectra



4. Filter results based on identification criteria



5. Select results for:
   - Reporting
   - Export into a LIMS system
   - Further processing in MultiQuant™ software
   - Further processing in LibraryView™ software



Quantitation in MasterView™ Software

The quantitative comparison in MasterView™ software allows users to quickly identify compounds above a concentration of interest, such as the Maximum Residue Limit (MRL).

Load samples into MasterView™ software and specify the control sample. Load the target XIC table and click the ‘Process’ button to start data processing. Compounds identified with high confidence are indicated using green check symbols. Sort the table by the ‘traffic light’ column and/or filter by ‘identification criteria’ for easy result review and select compounds for report.

The batch of 20 samples shown in Figure 2 (containing one standard and 19 food extracts) was processed on a Windows 7 64-bit computer (8 core, 16 GB memory) in only 1:30 minutes.

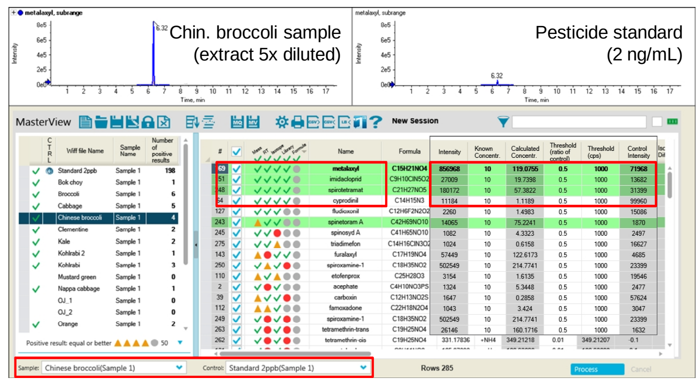


Figure 2. Quantitative comparison in MasterView™ software: four pesticides were identified in the broccoli with high confidence and three of them were present at a concentration above 10 µg/kg in the sample (metalaxyl, imidacloprid, and spirotetramat)

Results of Samples of the EU Proficiency Test

Two samples from the EU proficiency test for pesticides and fruits and vegetables were extracted and analyzed for pesticides (Figures 3 and 4).

Retention times errors were between 0 and 2.4% and mass errors were between -1.7 and 2.1 ppm (positive polarity) and -0.7 and 2.4 ppm (negative polarity) and were well below the required 5 ppm (SANCO/12495/2011). The results summary is listed in Tables 1 and 2.

Table 1. Result summary of the leek sample of the EU proficiency test

In Sample	Identified	RT Error (%)	Mass Error (mDa)	Isotope Ration Difference (%)	MS/MS PUR (%)	Comment
Carbofuran	Carbofuran	0.1	0.2	1.7	99.2	
Cyromazine	Cyromazine	1.1	-0.1	0.3	99.4	
DEET	DEET	0.1	0.1	0.2	100	
Diuron	Diuron	0.1	-0.2	2.8	100	also identified in negative polarity
Ethoxyquin	Ethoxyquin	2.4	-0.1	16.2	45.7	low QuEChERS recovery – low MS/MS fit
Fenpropidin	Fenpropidin	0.1	0.4	0.4	99.1	
Fenpyroximate	Fenpyroximate	0.1	-0.5	8.4	96.8	
Furathiocarb	Furathiocarb	0.2	-0.1	2.3	98.4	
Isofenphos-methyl	Isofenphos-methyl	0.1	-0.3	1.9	100	
Isoprocarb	Isoprocarb	0.1	0.2	0.4	100	
Mecarbam	Mecarbam	0.2	-0.2	2.8	92.4	
Metolachlor	Metolachlor	0.3	0.1	1.1	97.8	
Metribuzin	Metribuzin	0.3	-0.2	5.2	97.7	
Mevinphos	Mevinphos	0.3	-0.1	0.5	100	
Phorate	Phorate	0.3	-0.5	14.7	77.4	also Phorate-sulfoxide identified
Picolinafen	Picolinafen	0.1	-0.3	0.3	99.5	also identified in negative polarity
Prometryn	Prometryn	0.3	0.0	0.8	99.7	isomer Terbutryn with different MS/MS
Propazine	Propazine	0.3	-0.2	2.4	100	isomers sec- and tert-Butylazine with different MS/MS
Propoxur	Propoxur	0.1	0.1	0.5	99.7	
Pyraclostrobin	Pyraclostrobin	0.3	0.2	2.4	98.4	
Quinoclamine	Quinoclamine	0.1	0.1	1.9	100	also identified in negative polarity
Simazine	Simazine	0.1	0.3	3.3	99.8	
minimum		0.1	-0.5	0.2	45.7	
maximum		2.4	0.4	16.2	100.0	
average		0.3	-0.1	3.2	95.5	

Table 2. Result summary of the pear sample of the EU proficiency test

In Sample	Identified	RT Error (%)	Mass Error (mDa)	Isotope Ration Difference (%)	MS/MS PUR (%)	Comment
Bromacil	Bromacil	0.0	0.1	10.3	100.0	also identified in negative polarity
Bromoxinyl	Bromoxinyl (negative)	0.1	0.3	13.7	99.0	
Cadusafos	Cadusafos	0.2	0.5	1.1	99.8	
Diflubenzuron	Diflubenzuron	0.3	0.4	2.7	99.9	also identified in negative polarity
Diniconazole	Diniconazole	0.3	0.7	6.5	100.0	
Ethoxyquin						low QuEChERS recovery – not detected
Etrimfos	Etrimfos	0.1	0.4	1.2	99.3	
Fenpropidin	Fenpropidin	0.1	0.2	0.1	99.8	
Flufenacet	Flufenacet	0.1	0.3	2.0	100.0	
Flutolanil	Flutolanil	0.3	0.3	0.5	98.0	also identified in negative polarity
Imazapyr	Imazapyr	1.3	0.3	0.5	99.5	also identified in negative polarity
Ioxynil	Ioxynil (negative)	0.2	0.2	0.5	99.7	
Isoproturon	Isoproturon	0.3	0.1	0.3	98.6	
Metazachlor	Metazachlor	0.2	0.2	16.9	100.0	interference on 13C isotope
Napropamide	Napropamide	0.1	0.2	1.5	100.0	
Prometryn	Prometryn	0.2	0.2	1.7	100.0	isomer Terbutryn with different MS/MS
Propaquizafop	Propaquizafop	0.3	-0.3	1.8	99.4	
Pyrifenox	Pyrifenox	0.1	0.1	7.3	100.0	
Spinosad	Spinosyn A + D	0.2	-0.9	1.8	100.0	
Terbacil	Terbacil (negative)	0.3	0.5	3.8	99.2	
Terbumeton	Terbumeton, Secbumeton	0.0	0.4	1.0	97.3, 99.6	no LC separation of Terbumeton and Secbumeton, isomer Prometon with different MS/MS
Vamidothion	Vamidothion	0.3	0.4	0.5	99.8	
minimum		0.0	-0.9	0.1	97.3	
maximum		1.3	0.7	16.9	100.0	
average		0.2	0.2	3.6	99.5	

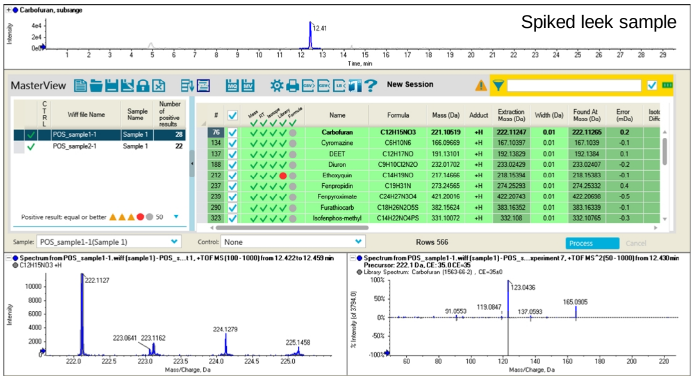


Figure 3. 22 (out of 22) pesticides were identified in the leek sample

Figure 4. 21 (out of 22) pesticides were identified in the pear sample (positive polarity top and negative polarity bottom)

43 out of 44 pesticides were identified in the leek and pear sample. The one false negative result was due to low QuEChERS recovery of Ethoxyquin.[5]

No false positive results were reported. MS/MS data and mass spectral library searching was found crucial to differentiate and correctly identify structural isomers (Figure 5).

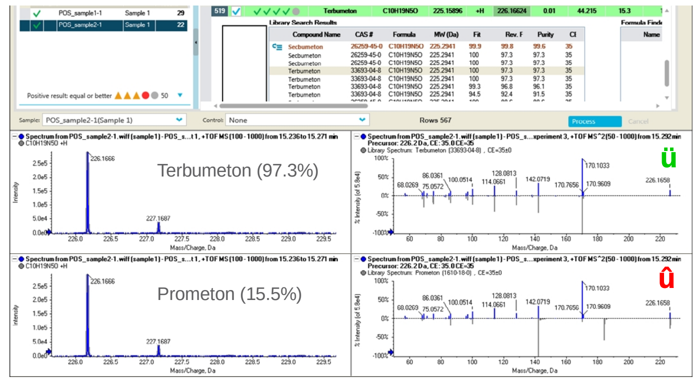


Figure 5. Correct identification Terbumeton present in the pear sample using MS/MS library searching.

Summary

A novel approach of comparative multi-target screening to identify and quantify pesticide residues in food samples was developed and successfully applied to EU proficiency test samples. Fruit and vegetable samples were extracted using a QuEChERS procedure and analyzed with reversed phase LC. High resolution and accurate mass MS and MS/MS information was collected in a single run using information dependent acquisition on AB SCIEX TripleTOF® 4600 and 5600 systems. Data was processed using MasterView™ 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 light' display and filter is utilized for easy data review and reporting.

Acknowledgement

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

References

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Screen for targeted compounds in your unknown samples

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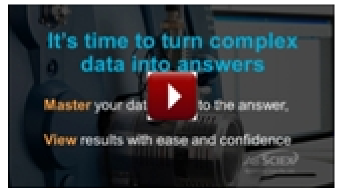

Video 2:

Easy set-up of data processing and data review parameters

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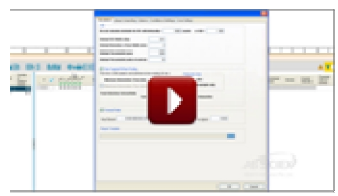

Video 3:

Screen for non-targeted compounds in your unknown samples

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Publication number: 7830413-01

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