Using UHPLC and Orbitrap Mass Spectrometry for Fast Pesticides Analysis in Food Matrices and Environmental Samples

Leo Jinyuan Wang, Charles Yang, Guifeng Jiang
Thermo Fisher Scientific, San Jose, CA, USA

Overview

Purpose: To develop a fast and sensitive screening and quantitative method for multi-residue (>300) pesticides analysis using ultra-high performance liquid chromatography and high resolution/accurate mass (UHPLC-HR/AM) platform. And to evaluate its performance for pesticides screening and quantitation in food and environmental matrices.

Methods: Chromatographic separation was achieved on an Thermo Scientific Dionex UltiMate 3000 RSLC UHPLC system with a Thermo Scientific Accucore aQ column. A Thermo Scientific Exactive Plus HR/AM mass spectrometer was operated in full scan or full scan/all ion fragmentation (AIF) scan mode for data acquisition.

Results: More than 300 pesticides were sufficiently retained and separated within 16 minutes. Screening for target pesticides was performed using Thermo Scientific ExactFinder data processing software with retention time used for identification, isotopic pattern and/or fragment ions used for confirmation. Quantitation was performed using Thermo Scientific TraceFinder data processing software. For selected representative pesticides, excellent quantitation performance was achieved with r^2 >0.99 for most compounds, and accuracy ranges from 90.7% to 116% at 10 ppb with %RSD in the range 0.6% to 11%. The limit of quantitation (LOQ) was observed from 50 ppt to 1 ppb for most pesticides.

Introduction

For the fast analysis of large number of pesticides residue, current methodologies usually involve chromatographic separation and mass spectrometric (MS) detection. The chromatographic run time usually increases with increasing number of target analytes due to the increased instrumental analytical cycle time.

Here an UHPLC-MS method is presented for fast screening and quantitative analysis of more than 300 pesticides in food and environmental water samples featuring optimized chromatography on an Accucore^{TM} column with solid core particles and Exactive^{TM} Plus bench-top Orbitrap^{TM} HR/AM mass spectrometer with superior mass resolution, speed of analysis and sensitivity.

Methods

Sample Preparation

Extracted food matrix blanks (onion, peach, bell pepper, and orange) were obtained from California Department of Food and Agriculture laboratory and were prepared following QuEChERS sample preparation protocol. Pesticides standard mixtures were kindly supplied by U.S. FDA. Wine samples were diluted in water (1 to 10) and injected directly for analysis.

Environmental and drinking water samples were injected directly for analysis.

Matrix matched calibration standards were prepared in the range from 0.05 ppb to 100 ppb at 9 levels. QC standards were prepared at 1, 10, and 100 ppb in extracted onion matrix.

Ultra-High Performance Liquid Chromatography

Chromatography was performed on an UltiMate® 3000 RSLC system featuring a high pressure mixing binary pump module with a 35 µL gradient mixing kit, ultra-low carry-over split-loop thermostated autosampler with 25 µL injection loop, and a column oven. Separation was achieved on an Accucore aQ reverse-phase column (2.1×150mm, 2.6µm) with an aQ guard column (2.1×10mm, 2.6µm). A binary gradient was applied with mobile phase component A being water with 2% methanol, and component B being methanol with 2% water. Both components were buffered with 0.1% formic acid and 4mM ammonium formate. The column was equilibrated for 3 minutes before injection. And gradient started from 0% to 20% B from 0 to 4 minutes; 20% to 60% B from 4 to 5.5 minutes; then 60% to 100% B from 5.5 to 10 minutes and hold at 100% for 2.9 minutes and then return to initial condition in 0.1 minutes. Column oven was set at 25 ºC and autosampler tray was set at 10 ºC. Injection volume was 5 µL.

Mass Spectrometry

An Exactive Plus bench-top Orbitrap mass spectrometer was used in this study and operated in full scan or full scan/AIF mode with scan range from 80 to 1200 m/z. The mass resolution was set at 70,000 (FWHM) at 200 m/z. The AGC target was set at 1e6 and maximum inject time was set at 250 milliseconds. A heated electrospray source with HESI II probe was used and the source conditions were set as: heater temperature at 295 ºC, sheath gas flow rate at 33 and aux gas flow rate at 7 arbitrary units, spray voltage at 2.2 kV, capillary temperature at 280 ºC and S-lens RF level at 60.

Data Analysis

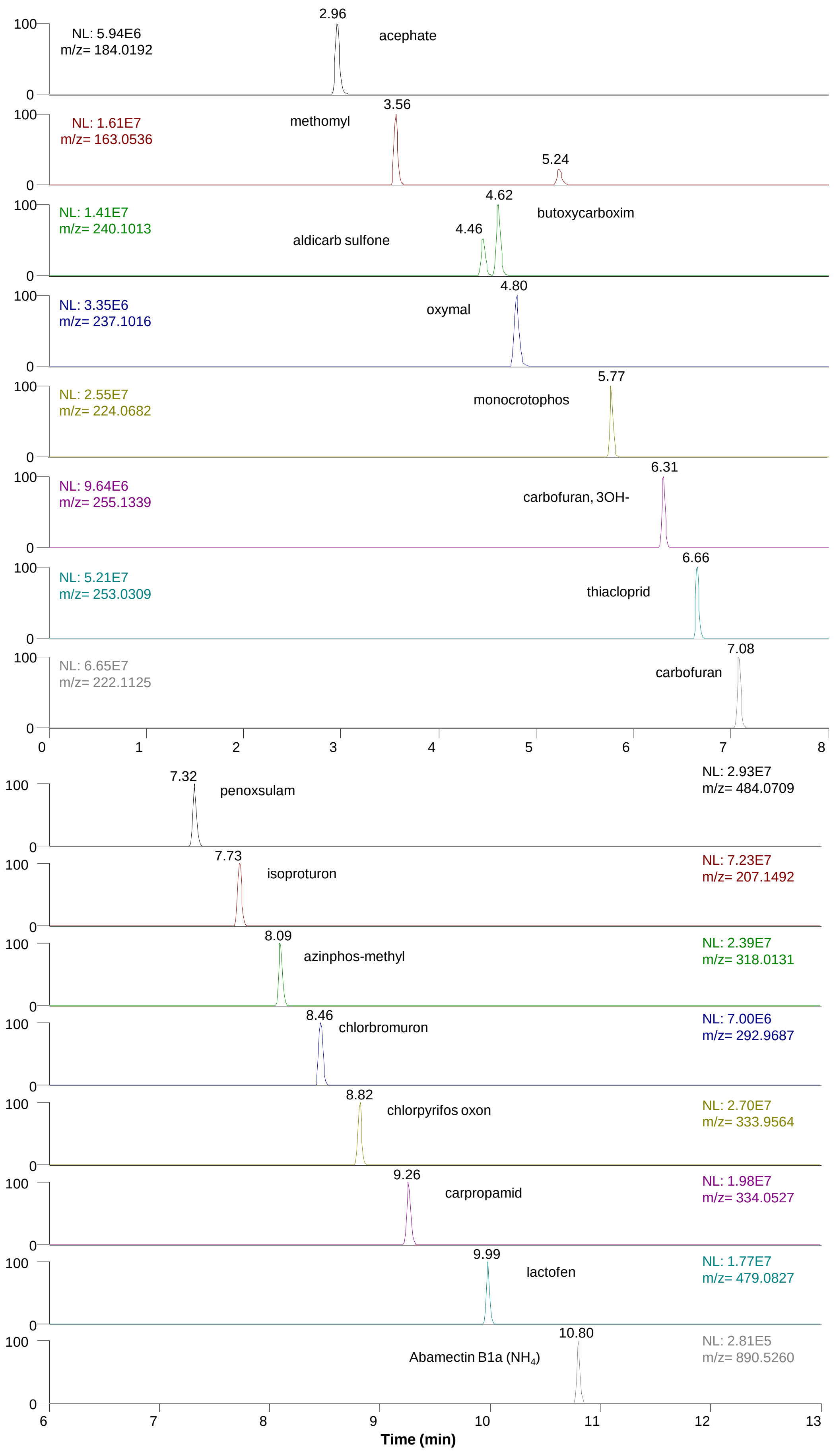
ExactFinder data processing software (version 2.0) was used for screening analysis.

TraceFinder data processing software (version 2.1) was used for quantitation analysis.

Results

Extracted ion chromatograms (XIC) for randomly selected pesticides is shown in Figure 1. Under the optimized conditions, most analytes were sufficiently retained with the minimum retention factor (k') greater than 2. With the combined selectivites from chromatographic separation and HR/AM measurement, all targeted pesticides can be differentiated.

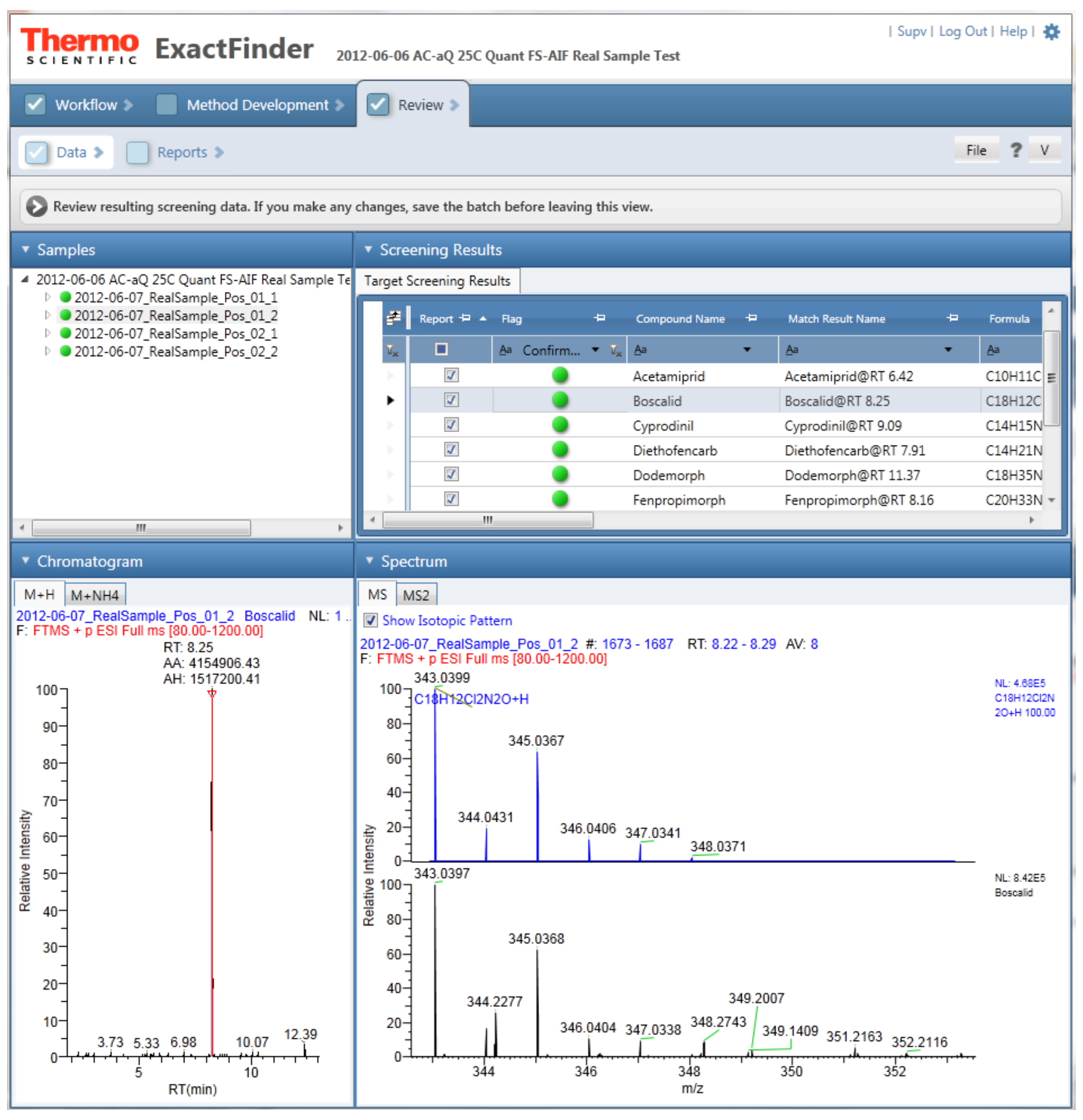
FIGURE 1. Exacted Ion Chromatograms of Selected Pesticides in Onion Matrix



Targeted/Unknown Pesticides Screening in Smoothies

In this study, targeted and unknown screening was performed using ExactFinder^{TM} software. To evaluate the screening performance of this methodology. Two smoothie samples were prepared (1 to 10 diluted in water/ACN (1/9), centrifuged for 15 mintutes at 5000 rpm) and analyzed in duplicates. As shown in Figure 2, 12 pesticides were identified and confirmed with isotopic pattern and fragment match in sample 1, and 13 pesticides in sample 2. The details of isotopic pattern match and fragments match are demonstrated in Figure 3.

FIGURE 2. Screening Analysis Using ExactFinder



2012-06-06 AC-aQ 25C Quant FS-AIF Real Sample Test

Review resulting screening data. If you make any changes, save the batch before leaving this view.

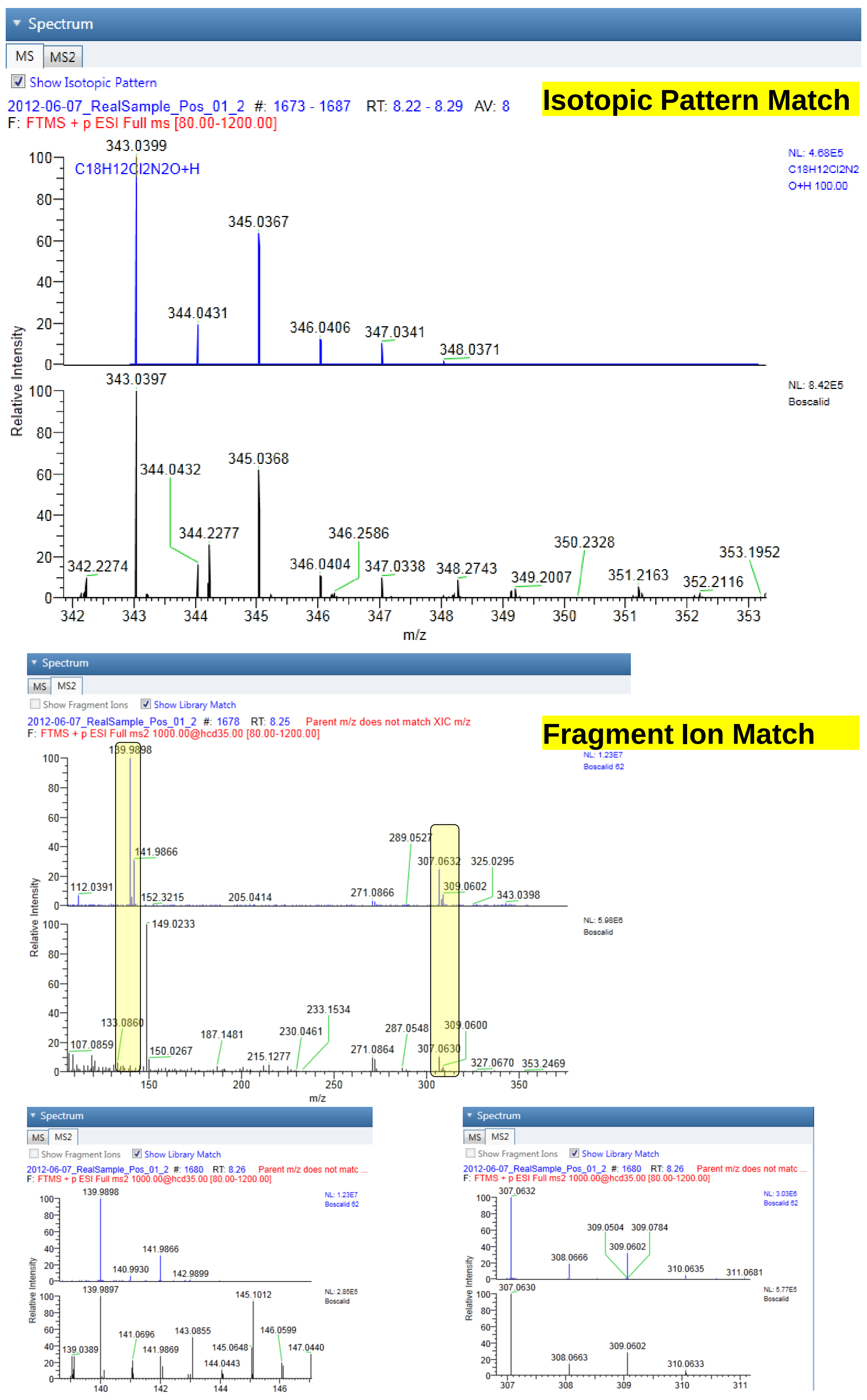
2012-06-06 AC-aQ 25C Quant FS-AIF Real Sample Te
- 2012-06-07_RealSample_Pos_01_1
- 2012-06-07_RealSample_Pos_01_2
- 2012-06-07_RealSample_Pos_02_1
- 2012-06-07_RealSample_Pos_02_2

Report	Flag	Compound Name	Match Result Name	Formula
☑	●	Acetamiprid	Acetamiprid@RT 6.42	C10H11C
☑	●	Boscalid	Boscalid@RT 8.25	C18H12C
☑	●	Cyprodinil	Cyprodinil@RT 9.09	C14H15N
☑	●	Diethofencarb	Diethofencarb@RT 7.91	C14H21N
☑	●	Dodemorph	Dodemorph@RT 11.37	C18H35N
☑	●	Fenpropimorph	Fenpropimorph@RT 8.16	C20H33N

2012-06-07_RealSample_Pos_01_2 Boscalid NL: 1 ..
F: FTMS + p ESI Full ms [80.00-1200.00]
RT: 8.25
AA: 4154906.43
AH: 1517200.41

☑ Show Isotopic Pattern
2012-06-07_RealSample_Pos_01_2 #: 1673 - 1687 RT: 8.22 - 8.29 AV: 8
F: FTMS + p ESI Full ms [80.00-1200.00]

FIGURE 3. Isotopic Pattern Match and Fragment Match Using ExactFinder



☑ Show Isotopic Pattern
2012-06-07_RealSample_Pos_01_2 #: 1673 - 1687 RT: 8.22 - 8.29 AV: 8
F: FTMS + p ESI Full ms [80.00-1200.00]

☐ Show Fragment Ions ☑ Show Library Match
2012-06-07_RealSample_Pos_01_2 #: 1678 RT: 8.25 Parent m/z does not match XIC m/z
F: FTMS + p ESI Full ms2 1000.00@hcd35.00 [80.00-1200.00]

☐ Show Fragment Ions ☑ Show Library Match
2012-06-07_RealSample_Pos_01_2 #: 1680 RT: 8.26 Parent m/z does not matc ...
F: FTMS + p ESI Full ms2 1000.00@hcd35.00 [80.00-1200.00]

☐ Show Fragment Ions ☑ Show Library Match
2012-06-07_RealSample_Pos_01_2 #: 1680 RT: 8.26 Parent m/z does not matc ...
F: FTMS + p ESI Full ms2 1000.00@hcd35.00 [80.00-1200.00]

Quantitation of Target Analytes in Onion Matrix

Randomly selected pesticides were evaluated for quantitation performance. For most pesticides, coefficient of determination (r^2) was achieved greater than 0.99, and LOQ was observed in the range 0.05 to 0.5 ppb. Precision and Accuracy were evaluated at 1, 10 and 100 ppb with excellent result. The details of quantitation performance are summarized in Table 1 for selected pesticides.

This method has been successfully applied for the quantitation of targeted pesticides in various food and environmental samples and the results will be presented elsewhere.

Table 1. Quantitation Performance of Selected Pesticides in Onion Matrix

Name	R.T.	r^2	LOQ (ppb)	1 ppb (n=7)		10 ppb (n=7)		100 ppb High (n=7)	
				%Accuracy	%RSD	%Accuracy	%RSD	%Accuracy	%RSD
Methomyl	3.55	0.9998	0.05	91.5%	3.1%	99.1%	1.1%	96.0%	1.7%
Propamocarb	3.99	0.9995	0.10	89.3%	2.1%	98.6%	1.5%	96.1%	1.2%
Dinotefuran	4.20	0.9998	0.05	93.6%	1.3%	101%	1.5%	96.3%	1.4%
Oxamyl	4.74	0.9998	0.05	93.3%	4.4%	98.6%	2.1%	96.1%	2.5%
Flonicamid	5.25	0.9996	1.00	99.1%	3.4%	103%	1.8%	96.0%	0.9%
Monocrotophos	5.69	0.9995	0.05	89.0%	2.4%	98.2%	1.3%	97.9%	2.4%
Thiabendazole	6.12	0.9998	0.05	90.4%	2.3%	97.2%	0.9%	95.6%	1.5%
Acetamiprid	6.34	0.9996	0.05	91.9%	2.6%	99.8%	1.3%	94.4%	2.4%
Pirimicarb	6.67	0.9996	0.05	89.2%	3.2%	98.9%	1.6%	97.5%	1.2%
Carbofuran	7.00	0.9997	0.05	99.1%	2.4%	102%	0.9%	98.9%	1.3%
Tebuthiuron	7.08	0.9997	0.05	96.6%	2.3%	102%	1.0%	95.8%	2.1%
Phorate Sulfoxide	7.40	0.9998	0.05	92.6%	2.4%	101%	1.5%	97.2%	1.7%
Imazalil	7.55	0.9997	0.05	89.0%	4.2%	97.8%	1.2%	97.1%	1.2%
Diuron	7.84	0.9997	0.10	96.0%	3.7%	102%	1.4%	96.8%	1.3%
Pyrimethanil	8.08	0.9998	0.05	89.6%	1.6%	98.7%	1.4%	96.1%	1.9%
Methoxyfenozide	8.32	0.9997	1.00	96.7%	3.5%	103%	0.6%	97.0%	1.4%
Fenbuconazole	8.77	0.9993	0.05	94.9%	2.7%	103%	1.4%	97.7%	6.1%
Propiconazole	9.14	0.9999	0.10	88.8%	2.6%	100%	1.1%	96.4%	1.4%
Trifloxystrobin	9.55	0.9994	0.05	88.7%	2.7%	98.3%	1.3%	90.7%	2.4%
Propargite	10.38	0.9977	0.05	87.3%	4.5%	93.0%	1.7%	87.6%	3.4%

Conclusion

This paper describes an UHPLC-MS method for fast screening and quantitative analysis of more than 300 pesticides base on UltiMate UHPLC system and HR/AM Exactive Plus bench-top orbitrap mass spectrometer. Here we demonstrate:

- Workflow for screening based on retention time, isotopic pattern and fragment ions;
- Excellent quantitation performance

Acknowledgements

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