Development and applications of a pesticide multiresidue analysis turn-key system utilizing UHPLC-Orbitrap mass spectrometry and post data processing

James Chang[1], Charles Yang[1], Serei Thach[2] and Paul Yang[2], Kristi Akervik[1], Maciej Bromirski[1], Dipankar Ghosh[1]
1. ThermoFisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134-1706, USA; 2. Ontario Ministry of the Environment, 125 Resources Road, Etobicoke, Ontario, M9P3V6, Canada

Overview

Goals of this work was to evaluate the sensitivity and ability of a targeted qualitative and quantitative turn-key system in the analysis of pesticides. The turn-key system consists of an ultra-high performance liquid chromatography-Orbitrap mass spectrometry system (UHPLC-Orbitrap) and post data processing software. The ability of this turn-key system for automated detection of pesticides in environmental and food matrices was demonstrated at sub-ppb level.

Introduction

Multresidue methods are employed to monitor chemical contaminants in environmental and food samples and to ensure that concentrations present do not exceed the tolerance levels established by government regulations. Many of these methods have been developed for polar organic pollutants analysis employing UHPLC-tandem mass (UHPLC-MS/MS) in targeted analysis mode. These methods are selective, sensitive, cost-effective and can be optimized to analyze many target analytes in a single injection. However, optimizing MS/MS parameters is a very time consuming process; and requires re-optimization of the duty cycle, scanning efficiency and multiple reaction monitoring (MRM) transitions whenever new pollutants are added. Unexpected pesticides of concern that are not targeted in the LC-MS/MS method will not be revealed from the MRM analysis and became undetected if they happen to be in the sample. The Orbitrap Mass Spectrometer can provide full scan mass spectrometric data for non-targeted, retrospective data analysis without the intensive works required to maintain MS/MS-based methods; making UHPLC-Orbitrap-mass spectrometric and cheminformatic-based multiresidue methods an attractive alternative.

Methods

Sample Preparation

Analytical standards consists of 281 pesticides were prepared at six concentration levels and used in the method development works. Individual pesticide standards were obtained from the United States Environmental Protection Agency Pesticide Repository (Ft. Meade, MD), Fluka/Sigma Aldrich (St. Louis, MO), EQ Laboratories (Atlanta, GA) and Wako Chemicals USA (Richmond, VA). Two deuterium (^2H) isotope labeled internal standards, i.e. diazinon-d10 (diethyl-d10) and dimethoate-d6 (O, O-dimethyl-d6) were purchased from CDN-Isotopes (Montreal, Quebec, Canada). Analytical standards caffeine, MRFA tetrapeptide (Met-Arg-Phe-Ala Acetate were purchased from Sigma Aldrich (St. Louis, MO) and used to prepare MSCAL5 in-house for the Orbitrap sensitivity tuning and mass axis calibration. Vegetation and food samples consist of orange and rasin were collected or purchased from commercially available sources. Samples were prepared using QuEChERS (Quick, **E**ffective, **Ch**eap, **E**asy-to-use, **R**ugged and **S**afe) [1] and suspended solid phase extraction cleanup kits were purchased purchased from United Chemical Technologies (UCT, Bristol, PA).

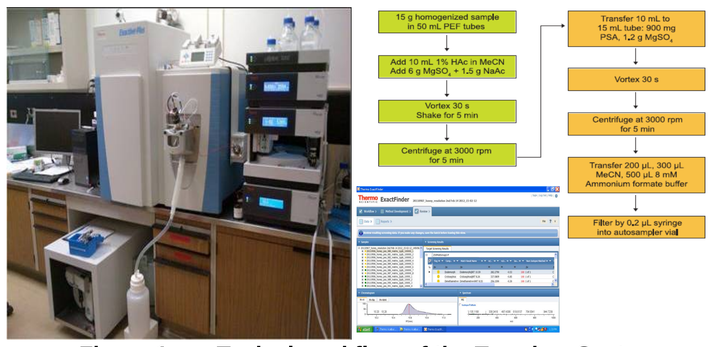

Figure 1. Typical workflow of the Turn-key System

UHPLC-HRMS Analysis

Two UHPLC-HRMS were used in this study. The first LC-orbitrap used consisted of an Accela High Speed LC (1250 binary pump, 18000 PSI) coupled to an Exactive® Orbitrap while the second system consisted of an UltiMate 3000 rapid separation LC (15000 PSI) coupled to an Exactive® Plus Orbitrap (Thermo Fisher Scientific, Inc., Bremen, Germany). Both Orbitrap MS systems were tuned (for optimized sensitivity) and calibrated (for accurate mass measurement) in positive mode by infusion of standard mixtures of MSCAL5. This procedure was done on a weekly basis. High purity nitrogen (> 99%) obtained from a nitrogen generator was used in the electrospray ionization source and to carry out higher energy collisional dissociation experiment. Mass spectrometric data were acquired without using lock mass(es). Separation was achieved using a Thermo Hypersil aQ C18 column (3 µm and 1.9 µm, 2.1x100 mm). The injection volume used was 10 and 5 mL for the Exactive® and Exactive® Plus Orbitrap detector based system, with UHPLC parameters listed in Table 1, typical chromatographic full-width-at-half-maximum (FWHM) were about <5 and <3 seconds. Mass spectrometric data were collected at four orbitrap resolution settings (R_{FWHM}) from 10000 to 140000 and scanning rate of 1 to 12 scans/second, using Auto-Gain-Ranging 0f $1x10^6$ and a maximal C-trap injection time of 50 ms to ensure optimized performance for low and high energy limited analysis.

Column Oven Temperature	35°C		
Mobile Phase	A: 95:5/H_2O:MeOH, 0.5 mM $HCOONH_4$ & 0.1% HCOOH B: 5:95/H_2O:MeOH, 0.5 mM $HCOONH_4$ & 0.1% HCOOH		
Flow Rate	375 µL/min		
Gradient	**Time (min)**	**% A**	**%B**
	0	85	15
	1.8	40	60
	6	2	98
	12	2	98
	12.2	85	15
	15	85	15

Table 1. UHPLC Parameters

Data Analysis

Analytical data collected were processed offline using Thermo Scientific Xcalibur and Thermo Scientific ExactFinder data processing packages depending on needs. Xcalibur™ was used for process mass spectral data for graphic presentation. The ExactFinder™ software system was used to identify targeted pesticides according to parameters shown in Table 2. Analytical results were exported to Microsoft Excel® with which analytical data were compiled and tabulated for presentation.

Table 2. Typical input/output parameters and values of ExactFinder

ExactFinder Input	
In-house compound library	Name, chemical formula, possible adducts (H^+, NH_4^+ or Na^+) and optional retention time of 565 target pesticides
Criteria for identification	Accurate mass deviation: 5 ppm Area threshold: 5,000 XIC signal-to-noise (SNR) threshold: 5
Isotopic pattern fit (Confirmation)	Threshold: 80% Mass deviation: 3 ppm Intensity deviation: 20%
ExactFinder Output	
Target name, Identified, Confirmed, Accurate mass deviation (ppm), Retention time, Isotopic pattern fit scores, target compound area counts (quantitative analysis), adduct type (auto-select via the largest area of given adducts), and SNR of XIC.	

Results

Mass resolving power and Signal-to-noise ratio of XIC

The term mass-resolving power (RP) is used to specify the ability of the orbitrap doing high resolution mass analysis and is defined as the ratio of the mass *M* and the full-width-at-half-maximum (FWHM, RP_{FWHM}) of that mass spectral peak. The FWHM of a mass spectral peak decreases with increasing RP_{FWHM}, allowing the use of a mass extraction window (MEW) proportional to the FWHM of each spectral peak to derive extracted ion chromatogram (XIC) for quantitative analysis. A narrower MEW results in less noise in the XIC. As the signal (peak intensity) remained constant; thus, the signal-to-noise ratio (SNR) of reconstructed XICs are expected to improve with increasing RP_{FWHM}. This is shown in Figure 2 using dimethylvinphos data obtained from the Exactive® Plus as an example. With the RP_{FWHM} decreases from 140000 (XIC trace A) to 17500 (XIC trace D), the noise level increases at the baseline of the XIC, as indicated by the blue arrows in the four XICs.

Simulated spectrum of dimethylvinphos (trace E) and accurate masses of the two chlorine isotopic peaks are listed in the insert ed table, along with that measured at the four RP_{FWHM} settings, with mass accuracy < 5 ppm. This available mass accuracy allowed the use of a smaller MEW according to the FWHM and allows for improved accuracy in the integrated areas by excluding interference peaks that may exist in the sample. Molecular ion in mass spectrum collected at the lowest RP_{FWHM} of 17500 (trace I, red arrow) was overlapped with another peak and had to be integrated together, resulting the compound at retention time (RT) 4.10 min. There were traces of this peak even at RP_{FWHM} of 70000 (XIC B) and will require a RP_{FWHM} for the complete removal of the RT 4.10 peak.

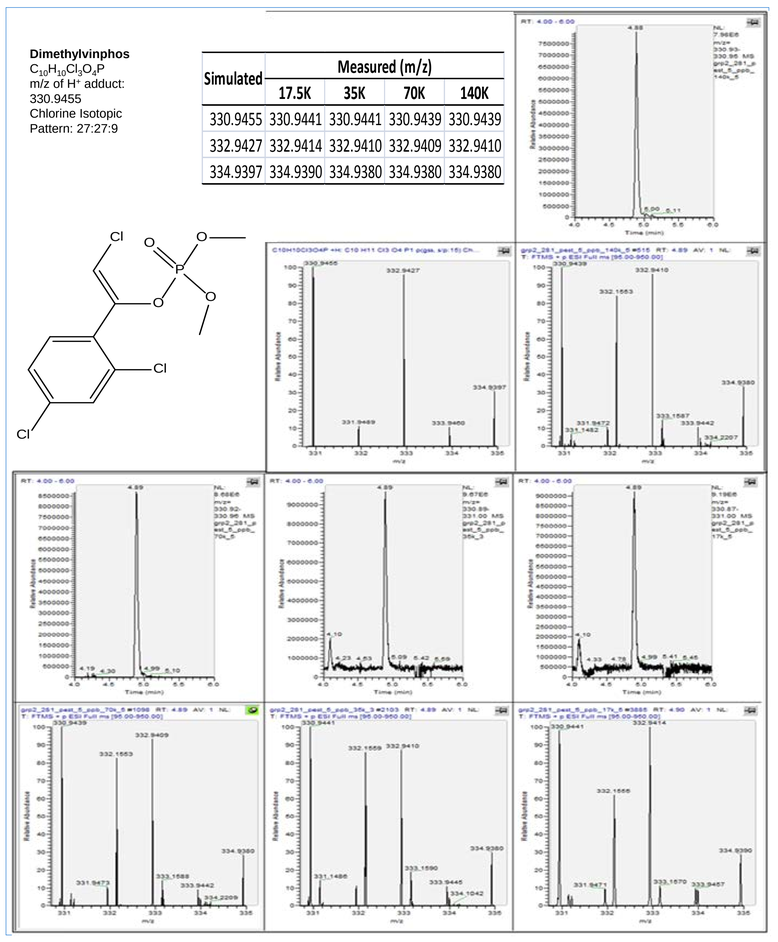

Figure 2. Typical workflow of the modified QuEChERS procedure

Expected sensitivity of Exactive Plus

Using calculated column loadings, SNR of XICs from three pesticides with molecular ions 233.02429 (Diuron, H^+ adduct), dimethylvinphos and 886.53111 (EmamectinB1A, H^+ adduct) representing high, medium and low ionization efficiency were evaluated. Show in Figure 3 are results obtained from diuron and dimethylvinphos. Both compounds can be measured at column loadings of 0.78, 3.12 and 12.48 pg and a RSD < 3% (N=5) for area count. Note the relative intensity changes of the analyte and interference in the bottom row. It is not possible to separate these two peaks with a unit mass system and the need of high resolution analysis to avoid false-positive identification

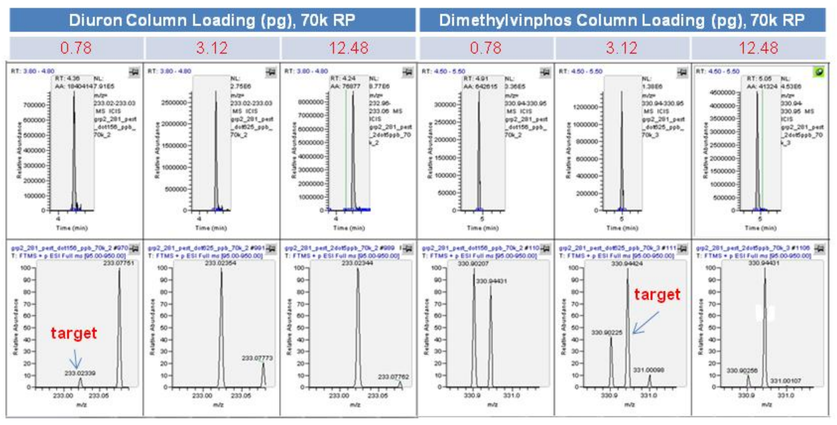

Figure 3. Mass spectra and XIC of diuron and dimethylvinphos at three column loadings

Figure 4 to the left showed the performance of the low ionization compound emamectinB1A. Unlike in the LC-Tandem mass analysis where the mectins were a challenge even at concentrations of low µg/mL (ppm), we were able to measure this compound at a column loading as low as 0.78 pg, with the caveat that XICs show for the 0.78 pg column loading was smoothed by five data points for presentation. There is more room to reduce the colmun loading futher.

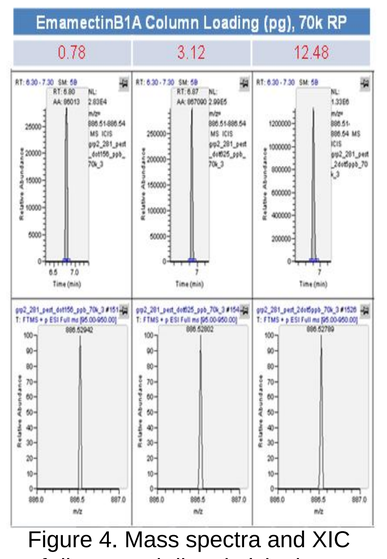

Figure 4. Mass spectra and XIC of diuron and dimethylvinphos

ExactFinder based targeted analysis

We evaluated the Exactive and ExactFinder based turn-key system by analyzing the 281 pesticides analytical standard at seven levels of concentration, four RP settings and in replicate of eight.

At 16 min/analysis, this rigorous test took > 60 hours to complete for each sample matrix. Analytical data obtained from the three matrices (solvent, orange and raisin) were processed offline using the ExactFinder against a compound database consist of chemical formula, retention time (not used in this study) and three possible adducts (H^+, NH_4^+ and Na^+). The ExactFinder processed data from each analysis and look for the three adducts of each target analytes listed in the database. A target pesticide is reported as "Identified" if a specific adduct located within a 5 ppm window was found. If more than one adducts were found, then the adduct with the highest area counts is reported. Confirmation of a specific adduct is done by using the isotopic pattern with relative intensity and mass accuracy set at 10% and 3 ppm, respectively. Number of total pesticides identified and corresponding relative standard deviation (N=8) at each level of concentration and RF setting were listed in Table 3.

Of the 281 pesticides analyzed, 16 of them could not be detected due to their chemical nature (need be analyzed in negative mode). Within the same sample matrix group, number of detectable pesticides was expected to increase with increasing concentration. It was shown that SNR of XIC would increase with increasing RP and therefore, within the same concentration group, number of detectable pesticides was observed to increase with increasing RP. This number of detectable pesticides also correlated well with matrix effects exerted with solvent (minimal matrix effects) has the highest number of detectable pesticides and the lowest RSD; followed by raisin and as reported earlier, orange would have contributed a high level of matrix effects[1] and have the lowest number of detectable pesticides and high RSD values.

Table 3. Average and RSD of detectable pesticides in various matrices and RP settings

	0.5 ppb, 10000		0.5 ppb, 25000		0.5 ppb, 50000		0.5 ppb, 100000		1 ppb, 10000		1 ppb, 25000		1 ppb, 50000	
	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD
Solvent	-	-	-	-	-	-	-	-	228	0.7%	230	0.6%	236	0.7%
Orange	159	2.2%	187	2.3%	200	0.7%	218	0.8%	178	2.5%	202	1.8%	212	1.4%
Raisin	211	1.2%	222	1.1%	231	0.8%	233	0.5%	220	1.3%	227	0.6%	216	28.0%
	1 ppb, 100000		2 ppb, 10000		2 ppb, 25000		2 ppb, 50000		2 ppb, 100000		5 ppb, 10000		5 ppb, 25000	
	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD
Solvent	248	0.7%	230	0.8%	240	0.7%	246	0.4%	255	0.8%	243	0.6%	251	0.6%
Orange	228	1.0%	191	1.9%	219	0.7%	223	0.9%	237	0.9%	210	1.0%	228	1.2%
Raisin	240	0.9%	228	1.0%	238	0.8%	245	0.6%	248	1.1%	238	1.1%	249	0.7%
	5 ppb, 50000		5 ppb, 100000		10 ppb, 10000		10 ppb, 25000		10 ppb, 50000		10 ppb, 100000		100 ppb, 10000	
	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD
Solvent	255	0.3%	260	0.8%	249	0.7%	256	0.3%	257	0.2%	260	0.8%	256	0.7%
Orange	237	0.7%	245	0.8%	223	1.8%	237	1.0%	243	0.6%	251	0.8%	240	1.2%
Raisin	252	0.6%	256	0.4%	247	0.8%	250	0.6%	254	0.3%	258	0.4%	251	0.9%
	100 ppb, 25000		100 ppb, 50000		100 ppb, 100000		1000 ppb, 10000		1000 ppb, 25000		1000 ppb, 50000		1000 ppb, 100000	
	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD	Average	RSD
Solvent	259	0.5%	259	0.5%	265	0.5%	255	1.0%	234	30.0%	260	0.4%	265	0.3%
Orange	251	0.6%	253	0.5%	261	0.7%	248	1.3%	253	0.6%	258	0.3%	260	0.6%
Raisin	255	0.5%	260	0.5%	263	0.6%	252	0.4%	258	0.7%	260	0.3%	263	0.4%

Conclusion

It is demonstrated that the Orbitrap and ExactFinder based turn-key system can be a powerful tool performing automated qualitative analysis with enhanced data quality. Consistency in area counts at high fg column loading showed the ability of system doing quantitative analysis. We hope to proceed current work in areas include but not limited to:

- We recommend the use of RPFWHM of 140,000 for initial screening of target compounds.
- To expand current compound database for common environmental pollutants including pharmaceutically active compounds, steroids and hormones, antibiotics, veterinary drugs, water treatment by-products to make the best use of targeted analysis and reduce the need for unknown analysis;
- To improve the ability of current turn-key system in unknown analysis

Referrences

1. Zhang, K*.; Wong, J. W.*; Yang, P.*; Tech, K.; DiBenedetto, A. L.; Lee, N. S.; Hayward, D. G.; Makovi, C. M.; Krynitsky, A. J.; Banerjee, K.; Jao, L.; Dasgupta, S.; Smoker, M. S.; Simonds, R.; Schreiber, A. J. Agric. Food Chem. 2011, 59(14), 7636-7646.

Acknowledgements

We would like to thank Dr. Jon Wong and Dr. Kai Zhang for providing required analytical standards and carry out data acquisition works for data verification purposes.