Trading Rules in Targeted/Unknown Multiresidue Pesticides Analysis Using UHPLC-Orbitrap MS and Data Mining Technologies

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Overview

The purpose of this work was to characterize operational parameters of ultra-high performance liquid chromatography with Thermo Scientific Orbitrap mass spectrometry systems (UHPLC-Orbitrap) to make the best use of the system for qualitative and quantitative analysis. Inter-relationship between the mass accuracy/precision, resolution and area counts precision of the extracted ion chromatogram (XIC) was studied. "Trading rules" on the use of UPLC-Orbitrap to acquire good quality data were described.

Introduction

There has been tremendous momentum in the development and application of UHPLC-HRMS based analytical methods performing multiresidue analysis in food and environmental samples since the mid-2000s. Several publications have discussed the advantages and trade-offs in the use of HRMS, including Orbitrap™ technology, to obtain analytical data with good quality. The actual quantitative evaluation of resolution, measurement time and precision/accuracy and signal-to-noise ratio (SNR) of the XIC peaks (thus precision in area counts to deliver reliable quantitative data has not been discussed in full. A full understanding in the inter-relationship of these parameters is imperative to make the best use of various UHPLC-HRMS systems. Using an UHPLC-Orbitrap mass spectrometer system, "trading rules" of these parameters are developed, evaluated and discussed

Methods

Sample Preparation

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 (^{2}H) 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). Orbitrap calibration standards, MSCAL5 (caffeine, MRFA tetrapeptide (Met-Arg-Phe-Ala Acetate), and Ultramark 1621) and MSCAL6 (sodium dodecyl sulfate, taurocholic acid sodium salt, and Ultramark 1621) were purchased from Sigma Aldrich (St. Louis, MO). Fresh produce consisting of orange and spinach and other food products such as hazelnut, were purchased as organic or conventional products from commercially available sources. Incurred produce samples were collected in the fields. Samples were prepared using QuEChERS (Quick, effective, cheap, easy-to-use, rugged and safe) [1] with cleanup done by suspended solid phase extraction.

UHPLC-HRMS Analysis

Two UHPLC-HRMS were used in this study. The first instrument used was a Thermo Scientific Accela High Speed LC system (1250 binary pump) coupled to a Thermo Scientific Exactive Orbitrap mass spectrometer while the second system consists of an Thermo Scientific UltiMate 3000 rapid separation LC system coupled to a Thermo Scientific Exactive Plus Orbitrap mass spectrometer. The Orbitrap MS systems were tuned and calibrated in positive and negative modes by infusion of standard mixtures of MSCAL5 and MSCAL6, respectively. High purity nitrogen (> 99%) was used in the electrospray ionization source and to carry out higher energy collisional dissociation experiment. Separation was achieved using either a Thermo Scientific Hypersil GOLD or a Hypersil GOLD™ aQ C18 column (3 μm and 1.9 μm, 2.1x100 mm). The injection volume used was 10 µL. The mobile phase consisted of a linear gradient from 5 mM ammonium formate/0.1% formic acid/water: methanol (95:5) with a 1.0 min hold to 5 mM ammonium formate/0.1% formic acid/methanol:water (5:95) at 8.0 min and held for an additional 4.0 minutes, at a flow rate of 300-450 μL/min, resulting chromatographic full-width-at-half-maximum (FWHM) of 3-5 seconds. The initial mobile phase was returned in 1.0 min and the column was allowed to equilibrate for an additional 3.5 min. The total run and column equilibration time was 16.5 minutes. Analytical data were collected at resolution (R_{FWHM}) from 10,000 to 140,000 and scanning rate 1 to 12 scans/second.

Data Analysis

Analytical data collected were processed offline using Thermo Scientific Xcalibur and Thermo Scientific ExactFinder data processing packages depending on needs. Xcalibur™ software was used to process mass spectral data for graphic presentation. ExactFinder™ software was used to derive mass accuracy data, area counts and exported to Microsoft Excel® with which analytical data were compiled and statistical analysis performed and tabulated.

Results

Mass Resolving Power and Mass Resolution

The term mass-resolving power (RP)^2 has been used to specify the ability of mass analyzers performing high resolution mass analysis and is defined and/or calculated by using the mass M and the FWHM (RP_{FWHM})of a mass spectral peak:

$$RP = M / FWHM \qquad (1)$$

The term mass resolution^2 is used to quantify the separation of two measured mass spectral peaks with mass M_1 and M_2, such that the valley between the two peaks will be at 10% height of the smaller peak ($R_{10\%}$) :

$$R_{10\%} = M_2 / (M_2 - M_1) = M_2 / (\delta M_{2\text{-}1}) \qquad (2)$$

Both RP_{FWHM} and $R_{10\%}$ are used in association with a M.

In the Orbitrap mass spectrometer, RP_{FWHM} decreases proportionally to $(1/M)^{1/2}$. Therefore, RP_{FWHM} specification of 25000 and100000 @ m/z 200 for Orbitrap, respectively would have become 100000 x $(200/314)^{1/2}$ ≈19930 and 100000 x $(200/314)^{1/2}$ ≈ 79800.

Minimal Mass-resolving Power Required to Separate Isobaric Pesticides

We used four isobaric pesticides within differing masses of adjacent pesticides as small as 0.0099 Dalton as shown in the Table below.

	CAS #	M.W.	MH+	δM	$R_{10\%}$
Isazophos (M_1)	42509-80-8	313.04168	314.04895	-	-
Isoxathion (M_2)	18854-01-8	313.053769	314.06104	0.01209 ($\delta M_{2\text{-}1}$)	25976.92639
Triazophos (M_3)	24017-47-8	313.065002	314.07225	0.01121 ($\delta M_{3\text{-}2}$)	28017.14987
Hexaconazole (M_4)	79983-71-4	313.074868	314.08214	0.00988 ($\delta M_{4\text{-}3}$)	31757.54702

From the Table above, one can calculate the $R_{10\%}$ between triazophos and hexaconazole as 313.07 / $\delta M_{4\text{-}3}$ ≈ 31757. As can be seen in the Figure below, a RP_{FWHM} of at least 79800 is required to achieve the $R_{10\%}$ value of 31757.

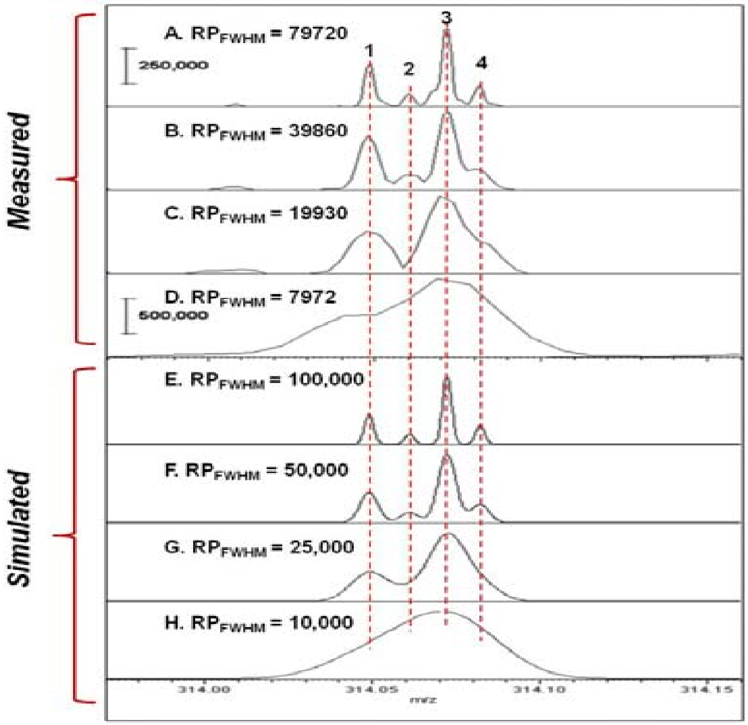
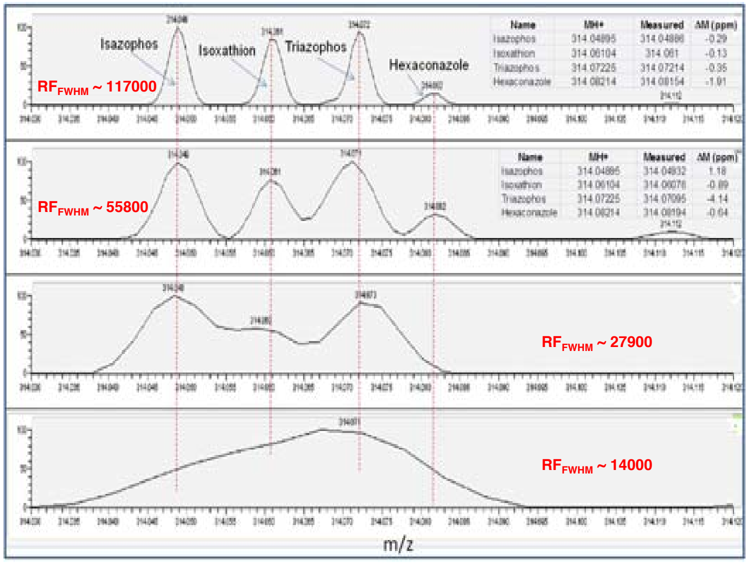


Figure 1. Relationship between required RF_{FWHM} , measured $R_{10\%}$ and mass spectra of isobaric pesticides at various instrument settings

From these discussions, we conclude that RP_{FWHM} value needs to be at least 2.5 folds larger than the mass resolution of two adjacent mass spectral peaks to achieve required separation for identification and quantitation purposes.

The Figure below showed the same four isobaric pesticides measured at an estimated RP_{FWHM} of 11700, 55800, 27900 and 14000 @ m/z 314. Clearly demonstrated that the highest RP_{FWHM} possible should be used for the unambiguous identification of isobaric compounds. Mass accuracy of the four pesticides were calculated and expressed in ΔM in part-per-million (ppm). Note that ΔM for triazophos at RF_{FWHM} ~ 55800 was affected by the two overlapping peaks from both sides and became abnormally high.



Name	MH+	Measured	ΔM (ppm)
Isazophos	314.04895	314.04886	-0.29
Isoxathion	314.06104	314.061	-0.13
Triazophos	314.07225	314.07214	-0.35
Hexaconazole	314.08214	314.08154	-1.91

Name	MH+	Measured	ΔM (ppm)
Isazophos	314.04895	314.04932	1.18
Isoxathion	314.06104	314.06078	-0.89
Triazophos	314.07225	314.07095	-4.14
Hexaconazole	314.08214	314.08194	-0.64

Figure 2. Mass spectra of isobaric pesticides and mass accuracy can be achieved at various Exactive Plus RF_{FWHM} settings.

RP_{FWHM}, Mass Extraction Window and Signal-to-Noise Ratio of XIC

To achieve good XIC selectivity, avoid missing data points and do good quantitative analysis, the mass extraction window (MEW) used to reconstruct XIC from UHPLC-HRMS data should be optimized according to RP_{FWHM} [3]. Using a TOF-MS of RF_{FWHM} = 20000, the MEW was determined to be ≤ ± 20 ppm, approximately the mass accuracy can be achieved by state-of-the-art TOF-MS system. Mass accuracy of the Orbitrap is similar to Fourier transform ion cyclotron reasonance mass spectrometry and is expected to be <5 and <2 ppm, respectively with external and internal calibration.

Table 2 showed typical mass accuracy obtained from representative pesticides with masses from 166 to 743 Da, measured in replicates of eight, at the four different RP_{FWHM} settings over a period of >50 hours. Due to ionization efficiency and co-elutions, 67 of the 281 pesticides studied were having more than five "Non-Detects" and were not used in the statistical summaries.

Table 2. Average (Avg.) and standard deviation (Stdev) of mass accuracy data

Name		Diphenylamine	Picaridin	Chlorpyrifos	Dithiopyr	MilbemycinA	Azadirachtin	Avg ± Stdev (ppm) N = 214
M.W.		170.09643	252.15701	349.93356	402.06154	551.29792	743.25216	
Conc.	RF_{FWHM}	Mass Accuracy, ΔM, Avg. ± Stdev., ppm (N=8)						
1 ppb	10000	0.31 ± 2.79	0.89 ± 1.44	0.57 ± 0.24	0.15 ± 0.33	1.77 ± 0.51	1.40 ± 0.30	0.25 ± 1.19
	25000	0.50 ± 0.91	1.87 ± 2.02	-0.24 ± 0.20	-0.69 ± 0.12	-0.24 ± 0.24	1.02 ± 0.23	-0.23 ± 1.03
	50000	-0.18 ± 0.26	-0.07 ± 0.29	-0.54 ± 0.27	-0.67 ± 0.20	-0.02 ± 0.24	0.83 ± 0.24	-0.16 ± 0.84
	100000	-0.29 ± 0.25	-0.33 ± 0.26	-0.54 ± 0.29	-0.98 ± 0.24	-0.24 ± 0.23	0.46 ± 0.17	-0.42 ± 0.86
2 ppb	10000	0.38 ± 2.70	-0.79 ± 2.66	-0.07 ± 0.18	-0.37 ± 0.30	0.64 ± 0.20	1.32 ± 0.16	-0.12 ± 1.04
	25000	-0.58 ± 0.37	1.75 ± 1.06	-0.91 ± 0.20	-0.93 ± 0.30	-0.72 ± 0.19	0.57 ± 0.20	-0.68 ± 0.94
	50000	-0.81 ± 0.16	-1.53 ± 0.81	-1.07 ± 0.08	-1.29 ± 0.20	-0.68 ± 0.19	0.03 ± 0.18	-0.92 ± 0.88
	100000	-1.12 ± 0.10	-1.33 ± 0.60	-1.20 ± 0.10	-1.56 ± 0.12	-0.73 ± 0.16	-0.29 ± 0.13	-1.11 ± 0.87
5 ppb	10000	0.48 ± 3.46	0.06 ± 1.78	-0.68 ± 0.17	-0.88 ± 0.18	-0.40 ± 0.18	0.93 ± 0.13	-0.49 ± 1.05
	25000	-0.46 ± 0.42	1.40 ± 1.76	-0.89 ± 0.25	-1.06 ± 0.18	-0.65 ± 0.25	0.48 ± 0.11	-0.70 ± 0.86
	50000	-0.51 ± 0.21	-1.11 ± 0.76	-0.96 ± 0.17	-1.04 ± 0.20	-0.54 ± 0.16	0.29 ± 0.21	-0.77 ± 0.89
	100000	-0.66 ± 0.26	-1.08 ± 0.38	-0.96 ± 0.27	-1.21 ± 0.25	-0.57 ± 0.24	-0.03 ± 0.30	-0.94 ± 0.80
10 ppb	10000	-0.54 ± 3.19	0.81 ± 0.95	-0.67 ± 0.15	-0.99 ± 0.11	-0.60 ± 0.18	0.72 ± 0.19	-0.47 ± 0.90
	25000	0.56 ± 2.56	0.39 ± 1.66	-1.29 ± 0.20	-1.40 ± 0.24	-1.07 ± 0.18	-0.15 ± 0.15	-1.12 ± 0.80
	50000	-1.29 ± 0.20	-2.43 ± 0.51	-1.70 ± 0.24	-2.02 ± 0.30	-1.63 ± 0.14	-0.81 ± 0.13	-1.71 ± 0.79
	100000	-1.95 ± 0.23	-2.26 ± 0.35	-2.04 ± 0.28	-2.25 ± 0.22	-1.72 ± 0.28	-1.25 ± 0.24	-2.08 ± 0.77
100 ppb	10000	-2.43 ± 0.95	-1.24 ± 1.84	-1.86 ± 0.26	-2.44 ± 0.15	-1.92 ± 0.12	-0.77 ± 0.28	-1.68 ± 0.79
	25000	-1.73 ± 0.72	-0.55 ± 1.97	-2.03 ± 0.12	-1.88 ± 0.28	-1.68 ± 0.18	-1.13 ± 0.23	-1.77 ± 0.91
	50000	-1.86 ± 0.39	-2.71 ± 0.64	-1.99 ± 0.21	-2.00 ± 0.34	-1.81 ± 0.18	-1.61 ± 0.19	-2.01 ± 0.75
	100000	-1.78 ± 0.35	-1.85 ± 0.29	-1.94 ± 0.19	-2.28 ± 0.29	-1.77 ± 0.17	-1.64 ± 0.15	-1.98 ± 0.91
1000 ppb	10000	-0.78 ± 1.14	-0.87 ± 3.84	-1.71 ± 0.21	-1.90 ± 1.32	-1.69 ± 0.18	-0.66 ± 0.24	-1.21 ± 0.94
	25000	-0.30 ± 1.30	-0.19 ± 2.27	-1.66 ± 0.45	-0.61 ± 1.80	-1.39 ± 0.07	-0.84 ± 0.14	-1.37 ± 0.78
	50000	-1.11 ± 0.18	-1.99 ± 1.02	-1.69 ± 0.07	-2.44 ± 1.22	-1.45 ± 0.21	-1.40 ± 0.25	-1.63 ± 0.95
	100000	-1.22 ± 0.24	-1.82 ± 0.67	-1.63 ± 0.23	-2.05 ± 0.44	-1.44 ± 0.24	-1.32 ± 0.25	-1.64 ± 0.81

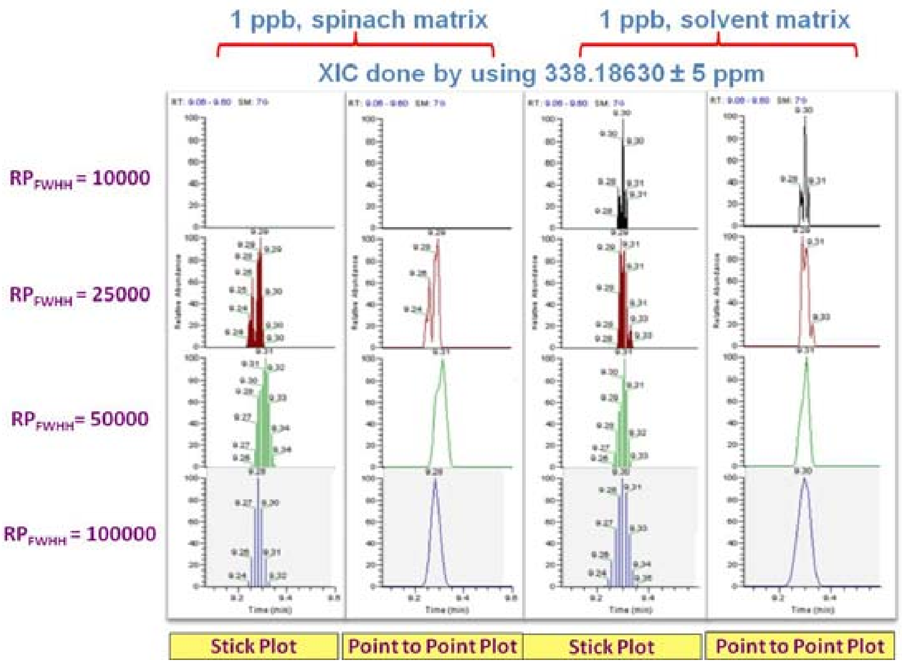


Figure 3. XIC's of bitertanol obtained using a ppm MEW at four different RF_{FWHM} settings

Table 2 showed that with external calibration, the orbitrap used in this study had performed well with the average mass accuracy remained < 2 ppm for all RP_{FWHM} and concentration combinations except for the RP_{FWHM} 100000 and 50000 at concentrations 10 and 100 ppb. This allowed the use of an universal MEW of ± 5 ppm to do the XIC for selectivity and quantitation purposes. A typical result is shown in Figure 3 using a pesticide bitertanol ($C_{20}H_{23}N_3O_2$) with XIC's obtained by using m/z 338.1863 ± 5 ppm using data collected at four different RP_{FWHM} settings.

From Figure 3, one could observe that the use of a MEW of ± 5 ppm to achieve good selectivity would cause missing data points in the XIC's in solvent matrix (right column, point-to-point plot) at RP_{FWHM} of 25000 and 50000. With the existence of the matrix effects (i.e., spinach, left column, point to point plot), the situation became worse. With increasing RP_{FWHM} and from both the stick and point-to-point plots, line shape of XIC's obtained from both sample matrices improved and assumed a Gaussian line shape at RP_{FWHM} of 100000. The stick plots also demonstrated that no smoothing was applied.

Table 3. Average area counts and relative standard deviation (RSD, %) obtained from representative compounds

Name		Diphenylamine	Picaridin	Chlorpyrifos	Dithiopyr	MilbemycinA	Azadirachtin
M.W.		170.09643	252.15701	349.93356	402.06154	551.29792	743.25216
Conc.	RF_{FWHM}	Mass Accuracy, ΔM, Avg. ± Stdev., ppm (N=8)					
1 ppb	10000	3.9E+05 ± 4.5%	7.8E+05 ± 3.3%	7.4E+04 ± 19.1%	1.2E+05 ± 3.4%	1.2E+06 ± 4.2%	3.0E+05 ± 92.0%
	25000	4.4E+05 ± 11.4%	8.0E+05 ± 4.1%	8.5E+04 ± 2.1%	1.3E+05 ± 4.7%	1.3E+06 ± 2.7%	3.5E+05 ± 33.8%
	50000	4.0E+05 ± 4.1%	7.8E+05 ± 2.0%	8.5E+04 ± 4.7%	1.2E+05 ± 5.6%	1.3E+06 ± 2.7%	7.6E+05 ± 4.4%
	100000	3.7E+05 ± 2.3%	7.4E+05 ± 2.8%	7.1E+04 ± 5.7%	1.1E+05 ± 8.1%	1.2E+06 ± 5.2%	6.8E+05 ± 7.6%
2 ppb	10000	6.8E+05 ± 6.9%	1.4E+06 ± 3.1%	1.3E+05 ± 5.5%	2.3E+05 ± 5.0%	2.2E+06 ± 2.4%	1.1E+06 ± 47.1%
	25000	7.3E+05 ± 8.0%	1.5E+06 ± 4.6%	1.5E+05 ± 3.3%	2.4E+05 ± 5.4%	2.4E+06 ± 1.9%	1.1E+06 ± 13.3%
	50000	7.2E+05 ± 5.2%	1.5E+06 ± 2.5%	1.5E+05 ± 5.7%	2.4E+05 ± 4.0%	2.4E+06 ± 4.2%	1.5E+06 ± 3.5%
	100000	7.1E+05 ± 4.4%	1.5E+06 ± 4.1%	1.4E+05 ± 5.7%	2.2E+05 ± 4.8%	2.4E+06 ± 5.3%	1.3E+06 ± 4.3%
5 ppb	10000	2.2E+06 ± 7.8%	4.7E+06 ± 3.2%	4.7E+05 ± 5.8%	7.3E+05 ± 3.5%	7.7E+06 ± 2.7%	4.6E+06 ± 16.2%
	25000	2.1E+06 ± 7.3%	4.6E+06 ± 2.5%	4.4E+05 ± 6.9%	7.1E+05 ± 4.4%	7.7E+06 ± 3.4%	4.4E+06 ± 6.9%
	50000	1.8E+06 ± 2.3%	4.3E+06 ± 1.0%	3.9E+05 ± 2.9%	6.7E+05 ± 3.0%	7.0E+06 ± 2.3%	3.9E+06 ± 1.7%
	100000	1.6E+06 ± 4.3%	3.8E+06 ± 1.5%	3.3E+05 ± 6.6%	5.8E+05 ± 4.3%	6.1E+06 ± 3.6%	3.5E+06 ± 3.1%
10 ppb	10000	3.9E+06 ± 5.8%	9.8E+06 ± 6.5%	7.8E+05 ± 5.0%	1.3E+06 ± 20.4%	1.6E+07 ± 4.1%	8.6E+06 ± 5.6%
	25000	3.7E+06 ± 3.4%	9.1E+06 ± 2.6%	8.2E+05 ± 7.6%	1.4E+06 ± 3.1%	1.5E+07 ± 3.0%	8.6E+06 ± 4.8%
	50000	3.5E+06 ± 1.9%	8.5E+06 ± 1.2%	7.4E+05 ± 3.0%	1.3E+06 ± 1.9%	1.4E+07 ± 1.4%	7.4E+06 ± 5.8%
	100000	3.0E+06 ± 4.5%	7.7E+06 ± 2.3%	6.5E+05 ± 3.7%	1.1E+06 ± 3.6%	1.3E+07 ± 3.7%	7.1E+06 ± 9.4%
100 ppb	10000	2.4E+07 ± 1.5%	7.0E+07 ± 5.7%	3.2E+06 ± 24.4%	9.8E+06 ± 3.5%	1.3E+08 ± 3.8%	6.2E+07 ± 24.1%
	25000	2.4E+07 ± 4.1%	6.9E+07 ± 4.1%	3.9E+06 ± 9.2%	1.0E+07 ± 3.8%	1.3E+08 ± 4.7%	6.7E+07 ± 11.6%
	50000	2.2E+07 ± 3.0%	6.4E+07 ± 1.1%	3.6E+06 ± 4.4%	9.0E+06 ± 3.4%	1.2E+08 ± 1.7%	6.2E+07 ± 2.8%
	100000	1.9E+07 ± 4.0%	5.5E+07 ± 2.2%	3.0E+06 ± 4.2%	7.9E+06 ± 3.4%	1.1E+08 ± 2.7%	5.7E+07 ± 2.0%
1000 ppb	10000	7.7E+07 ± 6.8%	2.1E+08 ± 3.3%	1.4E+07 ± 33.3%	6.2E+07 ± 3.2%	4.0E+08 ± 7.7%	3.0E+08 ± 2.3%
	25000	7.1E+07 ± 41.2%	1.8E+08 ± 40.5%	1.4E+07 ± 41.5%	5.3E+07 ± 40.6%	3.3E+08 ± 40.9%	2.6E+08 ± 40.6%
	50000	7.1E+07 ± 1.4%	2.0E+08 ± 1.4%	1.5E+07 ± 2.0%	5.7E+07 ± 1.9%	3.5E+08 ± 2.3%	2.8E+08 ± 1.1%
	100000	6.3E+07 ± 2.0%	1.8E+08 ± 2.7%	1.2E+07 ± 2.4%	4.9E+07 ± 3.8%	3.2E+08 ± 2.3%	2.5E+08 ± 0.5%

Data Points Required to Define XIC, Scanning Rate and Consistent Area Counts

Results discussed above showed a RP_{FWHM} of ~ 80000 can be ideal to achieve good identification and quantification for the analysis. One of the concerns of using high RP_{FWHM} in data acquisition has to do with available data points can be used to define a XIC peak. The general consensus is at least five data points across the FWHM are required to define a Gaussian line for consistent area counts and thus, quantitative analysis. Experiments carried out in this study used reliable UHPLC parameters that will allow the effective use of state-of-the-art filtration technologies to suite the need of environmental and food samples. Typical FWHM is around 3-5 seconds depending on column flow rate and gradient of elution. The 2009 Exactive vintage used can do one full scan at RP_{FWHM} of 100000 and may be considered insufficient as <6 data points were used to define the XIC. Table 3 showed average (Avg.) and relative standard deviation (RSD, %) data obtained from the six pesticides listed in Table 2. At a column loading of 10 pg (10-µL of 1 ppb injection) and due to ionization efficiency, RSD of area counts may be high at the lower RP_{FWHM} but reached a norm of < 5% in most of the higher RP_{FWHM} settings.

Conclusion

It is demonstrated that the Exactive™ mass spectrometer can be a powerful tool improving data quality. Cautions must take to avoid collecting analytical data of inferior quality. These include:

- Mass resolving power (RP_{FWHM}) is different from the mass resolution ($R_{10\%}$). RP_{FWHM} must be set at least 2.5 folds higher than calculated $R_{10\%}$ to obtain data for quantitation purposes.
- To achieve unambiguous identification of pesticides, including isobaric ones, an RP_{FWHM} of > 80000 is required.
- A n one Hz data acquisition rate is sufficient for XIC with FWHH of 3 seconds (or <6 seconds in the baseline).

References

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.
2. Alan G. Marshall and Christopher L. Hendrickson, Annu. Rev. Anal. Chem. 2008. 1:579–99
3. Yuan-Qing Xia, Jim Lau, Timothy Olah and Mohammed Jemal, Rapid Commun. Mass Spectrom. 2011, 25, 2863–2878

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