Accurate Quantitation of Pesticides and PCB's in Grape and Onion Extracts using High Resolution GC-Orbitrap Mass Spectrometry

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ABSTRACT

Purpose: Quantitative pesticide residue and polychlorinated biphenyl (PCB) testing in fruits and vegetables is a challenging analysis. It involves the accurate and reliable detection of hundreds of compounds present at trace levels in a wide range of sample commodities with various degrees of matrix complexity. When using high-resolution mass spectrometry, the default acquisition mode is untargeted (full-scan) meaning that all the ions are acquired at the same time across a specified mass range, making it simple to manage and giving the analyst the flexibility to decide post-acquisition which pesticides and ions to measure. The quantitative performance of this technology, compared to regulatory guidelines, for routine testing was the focus of this study.

Methods: Grape and onion samples were obtained from the market and extracted using the miniLuke procedure. A calibration series containing 88 pesticides and 7 PCB's were prepared in grape and onion at concentrations equivalent to 1, 2, 5, 10, 20, 50, 100 and 200 µg/Kg. In addition to the calibration series, a grape and onion sample were post extraction spiked with different compounds at varying concentrations and analysed blind to replicate a real life sample. A Thermo Scientific[™] Exactive[™] GC Orbitrap[™] GC-MS system was used for accurate mass measurements in full-scan at 60,000 mass resolution (FWHM *m/z* 200). Data were acquired and processed using Thermo Scientific[™] TraceFinder[™] software.

Results: The objective of this study was to evaluate the quantitative performance of the Exactive GC Orbitrap system for the analysis of pesticides and PCB's in two sample matrices of varying complexity. The high resolution capability in combination with low limits of detection increases the scope of the analysis without the need for optimization of individual compound acquisition parameters. The first aim of the study was to establish the limit of detection (LOD) of the main quantifier ion for the 95 compounds in both the grape and onion samples. All of the compounds had a LOD ≤ 2 ng/mL with the exception of binapacryl, captafol and propargite (LOD = 5 ng/mL) in both grape and onion samples. Quantitative linearity in matrix was assessed across a concentration of 1-200 µg/Kg. In all cases, the coefficient of determination (R2) was >0.99 and the average response factor RSD% was < 20% for each analyte from its LOD to 200 μ g/Kg in both the grape and onion matrices. To assess the accuracy of quantitation, a grape and onion sample were analysed blind after being post-spiked with compounds at concentrations varying from 0.5-100 µg/Kg. The concentrations were extrapolated from the matrix matched calibration curves. The results showed good agreement between the spiked and calculated concentrations.

INTRODUCTION

Pesticide residue and PCB testing in fruits and vegetables is a challenging analysis as it involves the accurate and reliable detection of hundreds of compounds present at trace levels in a wide range of sample commodities with various degrees of matrix complexity. The sensitivity requirements for both groups of compounds are also demanding; for pesticides the default maximum residue level (MRL) is set by the European Union for most pesticides at 10 µg/Kg.¹⁻³ Further to this, stringent confirmation and quantitative performance criteria are set so that detections are both robust and standardized across member states. The low levels of detection required means that sensitive and selective instrumentation is needed. For pesticides and PCB's, this has meant analysis by the coupling of gas chromatography to triple stage quadrupole mass spectrometric systems (GC-MS/MS). These systems can detect a wide range of regulated compounds with the required sensitivity and selectivity. However, the analyst needs to decide upfront which compounds to measure, as targeted methods are limited to only detecting those measured at the time of acquisition. These targeted methods also require additional time to set-up, as they often use selected reaction monitoring (SRM) transitions which require constant attention to ensure that the acquisition windows remain viable for the compounds of interest and in the matrices assessed. The introduction of high resolution Orbitrap mass spectrometry has provided a valuable alternative to MS/MS techniques with additional analytical advantages.⁴ ⁸ With high resolution mass spectrometry, the default acquisition mode is untargeted (full-scan) meaning that all the ions are acquired at the same time across a specified mass range, making it simple to manage and giving the analyst the flexibility to decide post-acquisition which pesticides and ions to measure. This can extend into retrospective analysis to evaluate the presence of other compounds not necessarily of interest at the time of acquisition.

In this study, the quantitative performance of the Exactive GC Orbitrap mass spectrometer was demonstrated for the analysis of GC-amenable pesticides and PCB's in grape and onion samples. The primary focus was on the quantitative performance of the Exactive GC Orbitrap system including system sensitivity, linearity in terms of correlation coefficient and average response factors, precision and accuracy of measurement.

MATERIALS AND METHODS

Sample Preparation

Grape and onion samples were obtained from the market and extracted using the miniLuke procedure. Acetone (30ml) was added to 15g of cryogenically homogenised sample in a PTFE centrifuge tube. The sample was blended using an Ultra-Turrax® blender (IKA Works). Dichloromethane (30 ml), petroleum ether (40-60 °C) and sodium sulphate were added and the sample re-blended using the Ultra-Turrax blender. The sample was centrifuged at 3500rpm for 5 min and 60 ml of the supernatant taken (equivalent to 1g/ml sample). The sample volume was reduced by rotary evaporation and a solvent exchange into ethyl acetate (EtAc) was performed. The sample was transferred to a 10ml volumetric flask and made up to volume with EA.

A calibration series containing 88 pesticides and 7 PCB's were prepared in grape and onion at concentrations equivalent to 1, 2, 5, 10, 20, 50, 100 and 200 µg/Kg. In addition to the calibration series a grape and onion sample were post extraction spiked with different compounds at varying concentrations and analyzed blind to replicate a real life sample.

Test Method(s)

Automatic sample injection was performed using a Thermo Scientific[™] TriPlus[™] RSH autosampler, and chromatographic separation was obtained with a Thermo Scientific[™] TRACE[™] 1310 gas chromatograph and a Thermo Scientific[™] TraceGOLD[™] TG-5SilMS 30 m x 0.25 mm l.D. x 0.25 µm film capillary column with a 5 m integrated guard (P/N: 26096-1425). The integrated guard is beneficial for routine analysis as there are no column connections necessary and column maintenance can be performed without impacting on analyte retention time. Finally, an Exactive GC Orbitrap mass spectrometer was used for accurate mass measurements in full-scan at 60,000 mass resolution (FWHM m/z 200). Additional details of instrument parameters are displayed in Table 1

Data Analysis

Data were acquired and processed using TraceFinder software, which allows easy instrument control, method development and quantitation capabilities. For target analysis a compound database for the 95 compounds was prepared containing compound name, quantification ion and confirming ion accurate masses, retention times and elemental compositions of parent and fragment masses. To generate the extracted ion chromatograms an extraction mass window of 5 ppm was used, meaning that only ions with a mass accuracy <5 ppm are extracted.

Table 1. GC and MS experimental parameters.

TRACE 1310 GC system Parameters

Injection Volume:	1 μL
Liner:	PTV six baffle liner (Siltek) P/N 453T2120
Inlet:	70 °C
Transfer Rate:	5 °C
Final Temperature:	300 °C
Transfer Time:	2 min.
Inlet Module and Mode:	PTV, splitless
Carrier Gas, mL/min:	He, 1.2 mL/min.
Oven Temperature Program:	
Temperature 1:	40 °C
Hold Time:	1.5 min.
Temperature 2:	90 °C
Rate:	25 °C/min.
Hold Time:	1.5 min.
Temperature 3:	280 °C
Rate:	5 °C/min.
Hold Time:	0 min.
Temperature 3:	300 °C
Rate:	10 °C/min.
Hold Time:	5 min.

RESULTS

The objective of this study was to evaluate the quantitative performance of the Exactive GC Orbitrap GC-MS system for the analysis of pesticides and PCB's in two food matrices with varying complexity.

Sensitivity and Linearity

The sensitivity of target compounds in matrix is a key parameter when assessing the suitability of a quantitative analytical technique. Therefore, the first aim of the study was to establish the limit of detection (LOD) using the main quantifier ion for the 95 compounds in both the grape and onion samples. This assessment was made by evaluating the matrix-matched calibration series, and the LOD was defined as the presence of a peak with S/N (peak to peak) > 3:1, and with > 8 scans/peak in the extracted ion chromatogram (EIC with ± 5 ppm window) of the main quantifier ion. Table 3 summarizes the quantitative performance criteria for the 95 pesticides and PCB's in the grape and onion matrices.

All compounds had an LOD $\leq 2 \mu g/kg$ except for binapacryl, captafol, and propargite (LOD = 5 $\mu g/kg$) in both grape and onion samples. These values are below the MRL and therefore exceed the detection requirements required for residue monitoring. An example of compound sensitivity is shown in Figure 2 for HCH-gamma in grape. Here, the overlay of the diagnostic ions at 1 µg/kg and the linear response for this compound are shown (R2 = 0.9998, Average response factor (RF) %RSD = 5.7). The customizable views in TraceFinder software allow the user to quickly review the key detection criteria and any parameters outside of specified tolerances will be flagged automatically.

Quantitative evaluation of linearity was made in matrix across a concentration of 1–200 µg/kg. In all cases, the coefficient of determination was > 0.99 and the average response factor RSD% was < 20 for each analyte from its LOD to 200 μ g/kg in both the grape and onion samples (Table 3). When the average response factor RSD% is less than 20%, the linear model is appropriate over the range of standard concentrations analyzed.

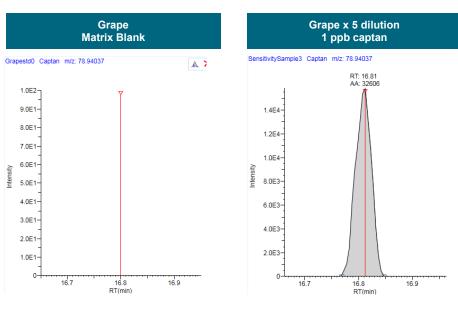
Exactive GC Orbitrap Mass	Spectrometer Parameters
Transfer Line:	250 °C
Ionization Type:	Electron Ion (EI)
Ion Source:	250 °C
Electron Energy:	70 eV
Acquisition Mode:	Full-scan
Mass Range:	50-700 Da
Resolving Power:	60,000 FWHM at <i>m/z</i> 200
Lockmass, Column Bleed:	207.03235 <i>m/z</i>

Accurate quantitation

To assess the accuracy of quantitation a grape and onion sample was analyzed blind after being postspiked with compounds at concentrations varying from 0.5-100 µg/Kg. The concentrations were extrapolated from the matrix matched calibration curves. Table 2 summarizes these results which show good agreement between the spiked and calculated concentrations. Furthermore, the grape sample was diluted by a factor of 5 and an example EIC for captan (1 µg/Kg) is shown in Figure 1 along with a blank and the original grape sample (4.9 μ g/Kg). This demonstrates the level of sensitivity that the Exactive GC Orbitrap GC-MS system can deliver even for complex matrices and for difficult pesticides.

Table 2. Summary of spiked and calculated concentrations of pesticides and PCB's in grape and onion.

Compound		Grape			Onion			
	Spiked concentration (µg/Kg)	Calculated concentration (µg/Kg)	Agreement	Spiked concentration (µg/Kg)	Calculated concentration (µg/Kg)	Agreement		
Azoxystrobin	17	14	82%	50	49.9	100%		
Boscalid	-	-	-	34	32	94%		
Captan	5	4.9	98%	-	-	-		
Chlordane-trans	-	-	-	53	56	106%		
Chlorothalonil	15.8	15.5	98%	95	108	114%		
Chlorpropham	22	18	82%	-	-	-		
Cyfluthrin	4.3	3.9	91%	58	56	97%		
Cypermethrin	17	17	100%	-	-	-		
Cyproconazole	44	37	84%	-	-	-		
Deltamethrin	-	-	-	45	44.1	98%		
Diazinon	1.2	1.1	92%	58	61	105%		
Dimethoate	29	30	103%	58	56	97%		
Endosulfan beta	88	85	97%	-	-	-		
Fenbuconazole	-	-	-	47	50	106%		
Fludioxonil	24	32	133%	63	54	86%		
Folpet	0.96	0.97	101%	-	-	-		
HCB	1.1	1.1	100%	58	49	84%		
Hexaconazole	5.9	5.1	86%	-	-	-		
Iprodione	13	10.1	78%	52	49.5	95%		
o,p-DDE	5.2	5.1	98%	59	66	112%		
p,p-DDD	0.5	0.6	120%	-	-	-		
Omethoate	45	39.1	87%	75	71	95%		
PCB 180	1.01	1.2	119%	34	32	94%		
PCB 153	17	20	118%	-	-	-		
Permethrin	62	50	81%	-	-	-		
Phosmet	45	36	80%	-	-	-		
Propargite	6.3	5.7	90%	95	97	102%		
Triadimenol	73	68	93%	-	-	-		



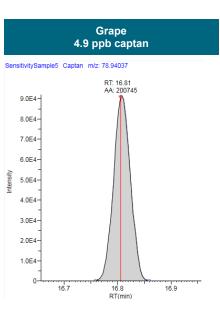


Figure 1. Extracted ion chromatogram and calculated concentration for captan in grape blank, 5 x grape sample dilution and undiluted grape sample.

Table 3. Summary of quantitative performance for 95 pesticides and PCB's in grape and onion.

Compound	Grape LOD (ng/mL)	Grape Linearity (R²)	Grape Average Response Factor (RSD%)	Onion LOD (ng/mL)	Onion Linearity (R²)	Onion Average Response Factor (RSD%)
Acephate	2	0.9990	2.1	1	0.9991	12.4
Acrinathrin	2	0.9983	12.6	1	0.9963	15.1
Aldrin Anthraquinone	1	0.9996	11.9 3.8	1	0.9992	10.6 7.2
Azinphos-methyl	2	0.9997	4.2	2	0.9970	9.6
Azoxystrobin	1	0.9994	15.0	1	0.9974	9.0
Bifenthrin	1	0.9999	2.9	1	0.9989	4.2
Binapacryl Biphenyl	5	0.9975	15.1 3.5	5	0.9967	17.9 5.4
Bitertanol	1	0.9988	11.4	1	0.9974	7.6
Boscalid	1	0.9972	16.0	1	0.9982	5.6
Bromopropylate	1	0.9992	5.8	1	0.9984	5.2
Captafol Captan	<u> </u>	0.9977	16.1 6.2	5	0.9994	8.0 14.6
Chlordane-cis	1	0.9985	6.5	2	0.9994	8.9
Chlordane-trans	1	0.9994	2.6	1	0.9967	8.8
Chlorfenapyr	2	0.9999	7.7	2	0.9994	10.2
Chlorothalonil Chlorpropham	<u> </u>	0.9998	6.4	1	0.9988	4.3
Chlorpyrifos-methyl	1	0.9956	6.4	1	0.9998	4.2
Chlorthal-dimethyl	1	0.9996	7.0	1	0.9984	8.1
Cyfluthrin	2	0.9993	16.0	1	0.9984	13.7
Cyhalothrin lambda	1	0.9991	16.6	1	0.9986	18.0
Cypermethrin Cyproconazole	<u> </u>	0.9994	2.3	1	0.9975	14.7 7.1
DDD- p.p'	1	0.9999	3.3	1	0.9993	4.0
DDD-o,p'	1	0.9997	4.0	1	0.9987	5.0
DDE- o,p'	1	0.9996	8.0	1	0.9992	4.3
DDE- p,p' DDT- o,p'	1	0.9999	10.4	1	0.9994	4.6
DDT- p.p'	1	0.9995	5.2	1	0.9990	5.9
Deltamethrin	2	0.9995	6.5	2	0.9965	11.6
Diazinone	1	0.9999	2.1	1	0.9996	5.5
ichlorobenzophenone-	1	0.9999	1.8	1	0.9997	2.1
Dicofol Dieldrin	2	0.9910	9.3 3.9	1	0.9981	4.7 5.2
Dimethoate	1	0.9996	4.2	1	0.9993	7.9
Diphenylamine	1	0.9996	4.7	1	0.9988	3.7
Endosulfan alpha	1	0.9997	7.0	2	0.9998	15.0
Endosulfan beta	1	0.9998	14.4	1	0.9992	10.0
Endosulfan ether Endosulfan lacton	1	0.9996	8.9	1	0.9994	8.5 6.2
Endosulfan sulfate	1	0.9993	9.8	1	0.9986	13.6
Endrin	1	0.9974	11.3	1	0.9992	9.3
Ethoprophos	1	0.9995	6.1	1	0.9986	3.8
Etoxazole Fenarimol	2	0.9991	10.4	2	0.9991	10.1 8.3
Fenazaguin	2	0.9998	17.0	2	0.9986	8.1
Fenbuconazole	1	0.9999	9.3	1	0.9971	10.1
Fenitrothion	1	0.9989	9.8	1	0.9983	8.9
Fenpropathrin	1	0.9995	5.4	1	0.9987	4.6
Fenvalerate Fludioxonil	2	0.9998	3.1 2.6	1 2	0.9975	18.0 11.9
Fluvalinate-tau	1	0.9996	17.3	1	0.9976	13.6
Folpet	1	0.9988	10.4	1	0.9984	8.2
HCH-alpha	1	0.9994	6.4	1	0.9999	4.1
HCH-beta HCH-delta	1	0.9999	4.0	1	0.9996	5.5 3.1
HCH-gamma	1	0.9998	5.7	1	0.9999	5.2
Hexachlorobenzene	1	0.9995	5.9	1	0.9999	2.5
Hexaconazole	1	0.9998	8.7	1	0.9987	6.1
Iprodione	<u> </u>	0.9998	7.2 5.3	1	0.9972	14.5
Iprovalicarb Lenacil	1	0.9999	4.0	1	0.9994	4.3
MCPA Methyl ester	1	0.9985	7.9	1	0.9992	2.8
Methamidiphos	1	0.9995	11.4	2	0.9994	18.8
Molinate	2	0.9988	12.0	1	0.9994	5.3
o-Hydroxybiphenyl Omethoate	<u> </u>	0.9997	4.8	1	0.9991	2.8
Oxy-Chlordane	1	0.9999	11.6	1	0.9995	6.4
PCB 101	1	0.9990	6.3	1	0.9990	7.0
PCB 118	1	0.9994	2.3	1	0.9988	3.8
PCB 138	2	0.9997	13.8	1	0.9995	17.5
PCB 153 PCB 180	1	0.9996	8.9 18.8	1 2	0.9993	5.0 11.5
PCB 28	1	0.9998	4.0	1	0.9990	7.0
PCB 52	1	0.9974	11.8	1	0.9997	12.7
Pendimethalin	1	0.9952	16.6	1	0.9964	12.2
Permethrin	1	0.9999	1.8 2.5	1	0.9986	10.0 3.7
Phosmet Prochloraz	2	0.9999	19.0	1	0.9991	<u> </u>
Profenofos	1	0.9998	10.4	1	0.9995	16.0
Propargite	5	0.9956	18.0	5	0.9965	14.4
Propiconazole	1	0.9999	6.3	1	0.9988	9.5
Prothiofos	1	0.9999	7.7	1	0.9983	11.5
Pyridaben Resmethrin	2	0.9999 0.9997	12.7 2.0	2	0.9983	12.5 8.1
Spirodiclofen	1	0.9995	11.7	1	0.9985	16.4
Tefluthrin	1	0.9998	3.1	1	0.9999	2.7
Tetraconazole	1	0.9997	6.6	1	0.9989	7.6
Tetramethrin	1	0.9995	4.8	1	0.9983	4.7
Tolclofos-methyl Triadimefon	<u> </u>	0.9996	4.9	1	0.9987	4.8 13.0
Triadimeton	1	0.9997	7.4	1	0.9984	13.0
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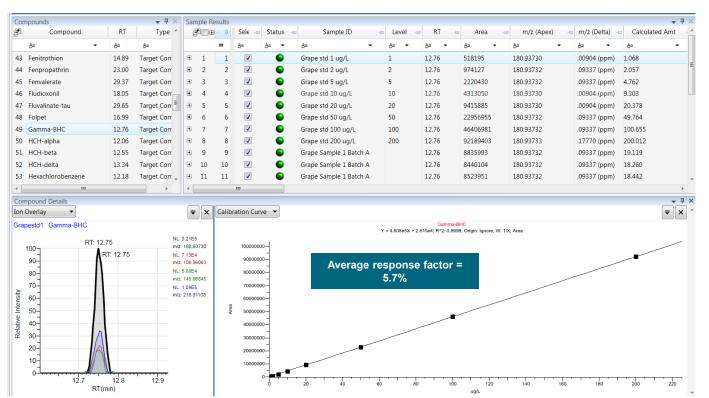


Figure 2. TraceFinder browser showing identified pesticides (A), overlay of extracted ion chromatograms (B), and linear response (C) (HCH-gamma as an example). Linearity R2 = 0.9998, average response factor RSD% = 5.7.

CONCLUSIONS

The results of this study demonstrate that the Exactive GC Orbitrap high resolution mass spectrometer, in combination with TraceFinder software, delivers sensitive quantitative performance for pesticide analysis in fruits and vegetables.

- Sensitive and robust full-scan analysis allows for easy and flexible the data acquisition and processing.
- All of the 95 compounds were detected at levels below the MRL with calculated limits of detection of $< 2 \mu g/kg$ for the majority of compounds (92 of the 95 compounds).
- Excellent linearity was demonstrated with R2 >0.99 and average response factors RSD% < 20 across the 8 point (1-200 µg/kg) matrix matched calibration series which ensures accurate quantitation. No internal standards were used to correct the response
- Analysis of a grape and onion samples showed confident detection and accurate quantitation of 15 spiked compounds.

REFERENCES

- 1. http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc_11945_en.pdf
- 2. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=URISERV:I13002a
- 3. SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Supersedes SANCO/12571/2013. Implemented by 01/01/2016.
- 4. Mol, H., Tienstra, M & Zomer, P. (2016) Evaluation of gas chromatography electron ionization full scan high resolution Orbitrap mass spectrometry for pesticide residue analysis? Analytica Chimica Acta, 935, 161-172.
- 5. Ucles, S., Lozano, A., Martinez Bueno, M.J., Fernandez-Alba, A.R. (2017) Shifting the paradigm in gas chromatography mass spectrometry pesticide analysis using high resolution accurate mass spectrometry. Journal of Chromatography A.
- 6. Thermo Fisher Scientific, Application note, 10448. (2016) High efficiency, broad scope screening of pesticides using gas chromatography high resolution Orbitrap mass spectrometry.
- 7. Thermo Fisher Scientific, Application note, 10509. (2016) Routine guantitative method of analysis for pesticides using GC-Orbitrap mass spectrometry in accordance with SANTE/11945/2015 guidelines.
- 8. Thermo Fisher Scientific, Application note, 10541. (2017) Multi-residue pesticide screening in cereals using GC-Orbitrap mass spectrometry.

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