APPLICATION NOTE 10584

The quantitative power of high-resolution GC-Orbitrap mass spectrometry for the analysis of pesticides and PCBs in food

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Keywords
Pesticides, fruits and vegetables, GC Orbitrap mass spectrometry, quantitation, accurate mass, TraceFinder software

Goal

To demonstrate the quantitative performance of the Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer for the analysis of GC-amenable pesticides and PCBs in grape and onion samples.

Introduction

The accurate and reliable determination of pesticide residues and polychlorinated biphenyls (PCBs) in food is challenging because of the large number of compounds and diversity of sample types involved. The sensitivity requirements for these compounds are also demanding. In the European Union (EU), the default maximum residue level (MRL) for thousands of pesticide-commodity combinations is set at 10 μg/kg.[1-3] Further to this, stringent confirmation and quantitative performance criteria are set so that residue results are equivalent across member states.

The low levels of detection require MS instruments that provide high sensitivity and high selectivity as well as fragmentation for confirmation. For pesticides and PCBs, gas chromatography coupled to triple quadruple mass spectrometers (GC-MS/MS) have been the systems of choice. Although these systems can detect a wide range of compounds with the required sensitivity, selectivity, and precision, the scope is limited to the target compounds programmed into the acquisition method. In other words, the analyst has to select the compounds in advance. 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 coupling of high-resolution Orbitrap mass spectrometry with gas chromatography is a valuable alternative to triple quadrupole techniques but with additional analytical advantages.[4–8] With high-resolution, accurate-mass (HRAM) mass spectrometry, the default acquisition mode is untargeted (full-scan) meaning that all the ions are acquired with high selectivity at the same time across a specified mass range, making the acquisition simple to manage and giving the analyst the flexibility to decide which pesticides to search for and to quantify. 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 Thermo Scientific Exactive GC Orbitrap mass spectrometer was demonstrated for the analysis of GC-amenable pesticides and PCBs in grape and onion samples. The identification performance to regulatory standards is covered in previous work.[4–8] The primary focus was on the quantitative performance of the Exactive GC-MS system including system sensitivity, linearity in terms of correlation coefficient and average response factors, precision, and accuracy of measurement.

Experimental

Sample preparation

Grape and onion samples were obtained from the market and extracted using the mini-Luke procedure[9]. Acetone (30 mL) was added to 15 g of cryogenically homogenized sample in a PTFE centrifuge tube. The sample was blended using an ULTRA-TURRAX®. Dichloromethane (30 mL) and petroleum ether, 40–60 °C, and sodium sulfate were added and the sample re-blended using the ULTRA-TURRAX blender. The sample was centrifuged at 3500 rpm for 5 min and 60 mL of the supernatant taken (equivalent to 1 g/mL sample). The sample volume was reduced by rotary evaporation and a solvent exchange into ethyl acetate (EA) was performed. The sample was transferred to a 10 mL volumetric flask and made up to volume with EA.

A series of matrix-matched calibration standards containing 88 pesticides and 7 PCBs, equivalent to 1, 2, 5, 10, 20, 50, 100, and 200 µg/kg, were prepared by spiking grape and onion extracts (Table 3A). In addition to the calibration series, grape and onion extracts were spiked with different combinations of the compounds at varying concentrations and analyzed blind to replicate real-life samples.

Instrument and method setup

Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was performed using a Thermo Scientific™ TRACE™ 1310 GC system fitted with a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.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 analyte retention time. Finally, a Thermo Scientific Exactive GC Orbitrap mass spectrometer was used for accurate mass measurements in full-scan mode at 60,000 mass resolution (FWHM *m/z* 200). Additional details of instrument parameters are displayed in Table 1 and Table 2.

Table 1. GC and injector conditions.

TRACE 1310 GC System Parameters	
Injection volume (µL):	1
Liner:	Siltek six baffle PTV liner (P/N 453T2120)
Inlet (°C):	70
Transfer rate (°C):	5
Final temperature (°C):	300
Transfer time (min):	2
Inlet module and mode:	PTV, splitless
Carrier gas, (mL/min):	He, 1.2
Oven Temperature Program:	
Temperature 1 (°C):	40
Hold time (min):	1.5
Temperature 2 (°C):	90
Rate (°C/min):	25
Hold time (min):	1.5
Temperature 3 (°C):	180
Rate (°C/min):	25
Hold time (min):	0
Temperature 3 (°C):	280
Rate (°C/min):	5
Hold time (min):	0
Temperature 4 (°C)	300
Rate (°C/min)	10
Hold time (min)	5

Table 2. Mass spectrometer conditions.

Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	250
Ionization type:	EI
Ion source (°C):	250
Electron energy (eV):	70
Acquisition mode:	Full-scan
Mass range (Da):	50–700
Resolving power (FWHM at *m/z* 200):	60,000
Lockmass, column bleed (*m/z*):	207.03235

Data processing

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

Results and discussion

The objective of this study was to evaluate the quantitative performance of the Exactive GC system for the analysis of pesticides and PCBs in two food matrices with varying complexity.

Sensitivity and linearity

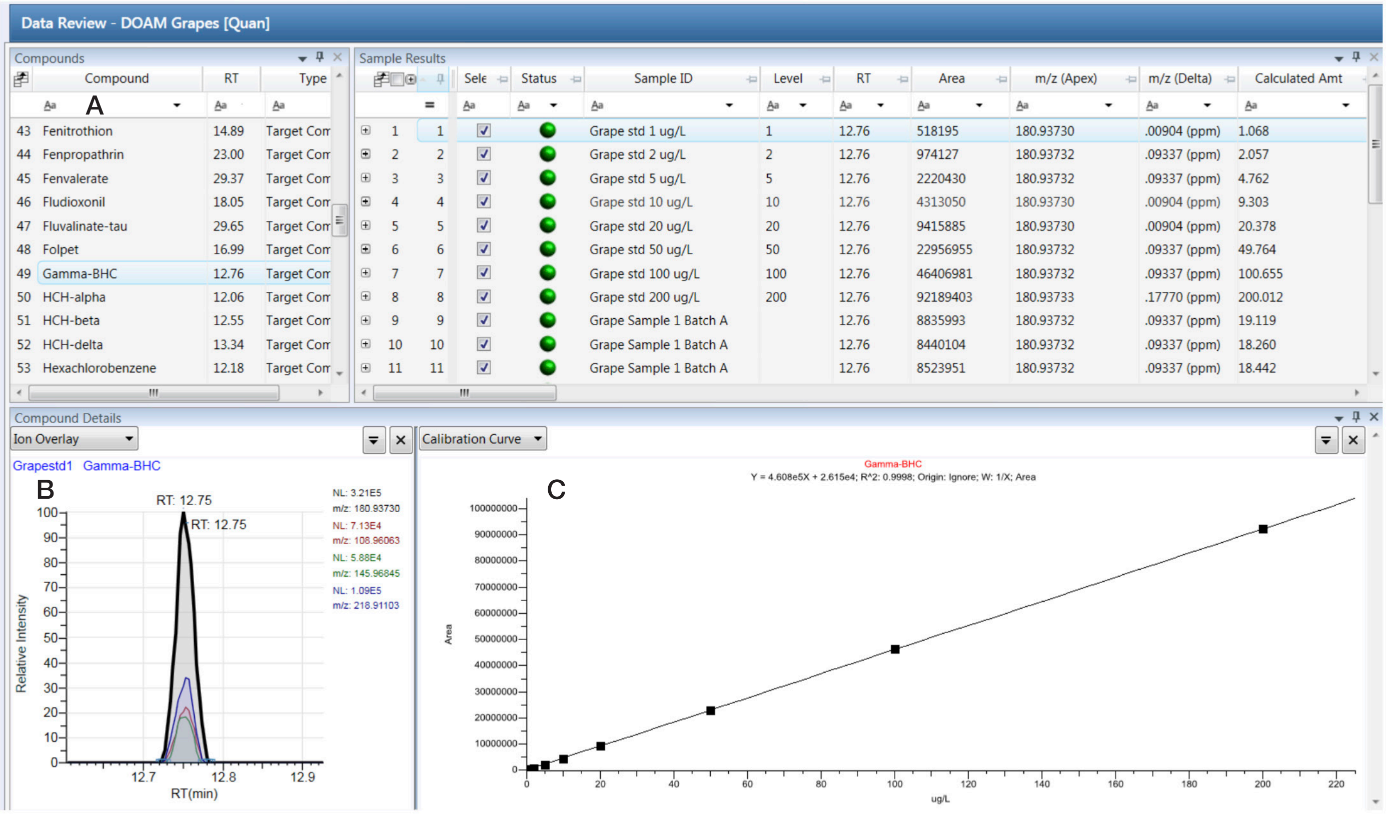
The sensitivity of target compounds in matrix is a key parameter when assessing the suitability of a quantitative analytical technique. Therefore, the first aim of the study was to establish the limit of detection (LOD) using the main quantifier ion for the 95 compounds in both the grape and onion samples. 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 PCBs in the grape and onion matrices. All compounds had an LOD ≤ 2 µg/kg except for binapacryl, captafol, and propargite (LOD = 5 µg/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 1 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 ($R^2 = 0.9998$, 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.

Table 3A. Summary of quantitative performance for 95 pesticides and PCBs in grape and onion LOD.

Compound	Grape LOD (µg/kg)	Grape Linearity (R^2)	Grape Average RF (RSD%)	Onion LOD (µg/kg)	Onion Linearity (R^2)	Onion Average RF (RSD%)
Acephate	2	0.9990	2.1	1	0.9991	12.4
Acrinathrin	2	0.9983	12.6	1	0.9963	15.1
Aldrin	1	0.9996	11.9	1	0.9992	10.6
Anthraquinone	1	0.9998	3.8	1	0.9984	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	5	0.9975	15.1	5	0.9967	17.9
Biphenyl	1	0.9993	3.5	1	0.9992	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	5	0.9977	16.1	5	0.9994	8.0
Captan	1	0.9998	6.2	1	0.9998	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	1	0.9998	6.4	1	0.9988	4.3
Chlorpropham	1	0.9998	3.6	1	0.9999	2.2
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	1	0.9994	2.3	1	0.9975	14.7
Cyproconazole	1	0.9996	4.0	1	0.9993	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'	1	0.9999	10.4	1	0.9994	4.6
DDT- o,p'	1	0.9998	2.9	1	0.9998	5.9
DDT- p.p'	1	0.9995	5.2	1	0.9990	5.4
Deltamethrin	2	0.9995	6.5	2	0.9965	11.6
Diazinone	1	0.9999	2.1	1	0.9996	5.5
Dichlorobenzophenone-4,4	1	0.9999	1.8	1	0.9997	2.1
Dicofol	2	0.9910	9.3	1	0.9981	4.7
Dieldrin	1	0.9996	3.9	1	0.9991	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	1	0.9996	8.9	1	0.9994	8.5
Endosulfan lacton	1	0.9993	4.7	1	0.9994	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	2	0.9991	10.4	2	0.9991	10.1
Fenarimol	1	0.9998	4.2	1	0.9984	8.3
Fenazaquin	2	0.9986	17.0	2	0.9986	8.1

Table 3B. Summary of quantitative performance for 95 pesticides and PCBs in grape and onion LOD.

Compound	Grape LOD (µg/kg)	Grape Linearity (R^2)	Grape Average RF (RSD%)	Onion LOD (µg/kg)	Onion Linearity (R^2)	Onion Average RF (RSD%)
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	2	0.9998	3.1	1	0.9975	18.0
Fludioxonil	1	0.9999	2.6	2	0.9983	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	1	0.9999	4.0	1	0.9996	5.5
HCH-delta	1	0.9999	6.5	1	0.9996	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	1	0.9998	7.2	1	0.9972	14.5
Iprovalicarb	1	0.9999	5.3	1	0.9994	2.7
Lenacil	1	0.9999	4.0	1	0.9989	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	1	0.9997	4.8	1	0.9991	2.8
Omethoate	1	0.9988	5.1	1	0.9995	7.6
Oxy-Chlordane	1	0.9999	11.6	1	0.9999	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	1	0.9996	8.9	1	0.9993	5.0
PCB 180	1	0.9998	18.8	2	0.9990	11.5
PCB 28	1	0.9985	4.0	1	0.9994	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	1	0.9986	10.0
Phosmet	1	0.9999	2.5	1	0.9991	3.7
Prochloraz	2	0.9941	19.0	1	0.9914	19.0
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	2	0.9999	12.7	2	0.9983	12.5
Resmethrin	1	0.9997	2.0	1	0.9982	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	1	0.9996	4.9	1	0.9987	4.8
Triadimefon	1	0.9997	14.2	1	0.9984	13.0
Triadimenol	1	0.9999	7.4	1	0.9990	18.6
Trifluralin	2	0.9989	15.5	1	0.9985	8.1



Data Review - DOAM Grapes [Quan]

Compounds

A

	Compound	RT	Type
43	Fenitrothion	14.89	Target Com
44	Fenpropathrin	23.00	Target Com
45	Fenvalerate	29.37	Target Com
46	Fludioxonil	18.05	Target Com
47	Fluvalinate-tau	29.65	Target Com
48	Folpet	16.99	Target Com
49	Gamma-BHC	12.76	Target Com
50	HCH-alpha	12.06	Target Com
51	HCH-beta	12.55	Target Com
52	HCH-delta	13.34	Target Com
53	Hexachlorobenzene	12.18	Target Com

Sample Results

		Sele	Status	Sample ID	Level	RT	Area	m/z (Apex)	m/z (Delta)	Calculated Amt
1	1	☑	●	Grape std 1 ug/L	1	12.76	518195	180.93730	.00904 (ppm)	1.068
2	2	☑	●	Grape std 2 ug/L	2	12.76	974127	180.93732	.09337 (ppm)	2.057
3	3	☑	●	Grape std 5 ug/L	5	12.76	2220430	180.93732	.09337 (ppm)	4.762
4	4	☑	●	Grape std 10 ug/L	10	12.76	4313050	180.93730	.00904 (ppm)	9.303
5	5	☑	●	Grape std 20 ug/L	20	12.76	9415885	180.93730	.00904 (ppm)	20.378
6	6	☑	●	Grape std 50 ug/L	50	12.76	22956955	180.93732	.09337 (ppm)	49.764
7	7	☑	●	Grape std 100 ug/L	100	12.76	46406981	180.93732	.09337 (ppm)	100.655
8	8	☑	●	Grape std 200 ug/L	200	12.76	92189403	180.93733	.17770 (ppm)	200.012
9	9	☑	●	Grape Sample 1 Batch A		12.76	8835993	180.93732	.09337 (ppm)	19.119
10	10	☑	●	Grape Sample 1 Batch A		12.76	8440104	180.93732	.09337 (ppm)	18.260
11	11	☑	●	Grape Sample 1 Batch A		12.76	8523951	180.93732	.09337 (ppm)	18.442

Compound Details

Figure 1. TraceFinder browser showing identified pesticides (A), overlay of extracted ion chromatograms (B), and linear response (C) (HCH-gamma as an example). Linearity $R^2 = 0.9998$, average response factor RSD% = 5.7.

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 µg/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. The combination of linear response and the average response factor provides a more complete assessment of the system linearity and variability across the concentration range than only using the coefficient of determination (R^2). Figure 2 shows the linear response and the average response factor calibration for one of the most challenging pesticides, folpet, in onion matrix.

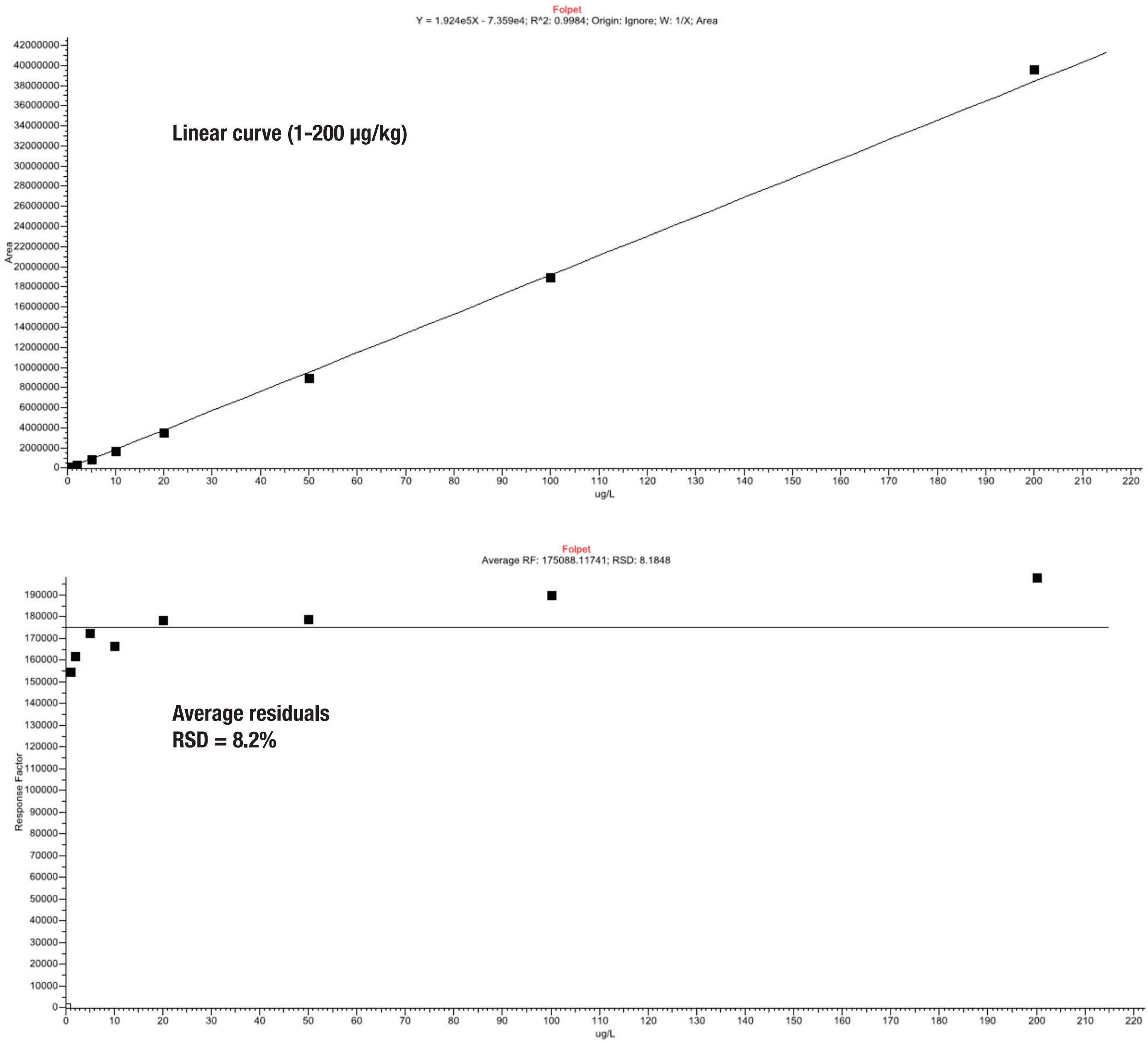


Figure 2. Calibration data for folpet in onion matrix.

Accurate quantitation

To assess the detectability and accuracy of quantitation, grape and onion samples were analyzed blind (the number and concentration of spiked compounds from a list of 97 were unknown to the analyst) after being post-spiked with compounds at concentrations varying from 0.5 to 100 µg/kg. The concentrations were calculated from the matrix-matched calibration curves. Table 4 summarizes these results, which show good agreement between the spiked and calculated concentrations.

Table 4. Summary of spiked and calculated concentrations of pesticides and PCBs in grape and onion.

Compound	Spiked Grape Concentration (µg/kg)	Calculated Grape Concentration (µg/kg)	Spiked Onion Concentration (µg/kg)	Calculated in Onion Concentration (µg/kg)
Azoxystrobin	17.0	14.0	50	50
Boscalid	-	-	34	32
Captan	5.0	4.9	-	-
Chlordane-trans	-	-	53	56
Chlorothalonil	15.8	15.5	95	108
Chlorpropham	22.0	18.0	-	-
Cyfluthrin	4.3	3.9	58	56
Cypermethrin	17.0	17.0	-	-
Cyproconazole	44.0	37.0	-	-
Deltamethrin	-	-	45	44
Diazinon	1.2	1.1	58	61
Dimethoate	29.0	30.0	58	56
Endosulfan beta	88.0	85.0		
Fenbuconazole	-	-	47	50
Fludioxonil	24.0	32.0	63	54
Folpet	0.96	0.97	-	-
HCB	1.1	1.1	58	49
Hexaconazole	5.9	5.1	-	-
Iprodione	13.0	10.1	52	50
o,p-DDE	5.2	5.1	59	66
p,p-DDD	0.5	0.6	-	-
Omethoate	45.0	39.1	75	71
PCB 180	1.0	1.2	34	32
PCB 153	17.0	20.0	-	-
Permethrin	62.0	50.0	-	-
Phosmet	45.0	36.0	-	-
Propargite	6.3	5.7	95	97
Triadimenol	73.0	68.0		

Furthermore, the grape sample was diluted by a factor of 5, and an example EIC for captan (1 µg/kg) is shown in Figure 3 along with a blank and the original grape sample (4.9 µg/kg). This demonstrates the level of sensitivity that the Exactive GC Orbitrap mass spectrometer can deliver, even for complex matrices and for difficult pesticides.

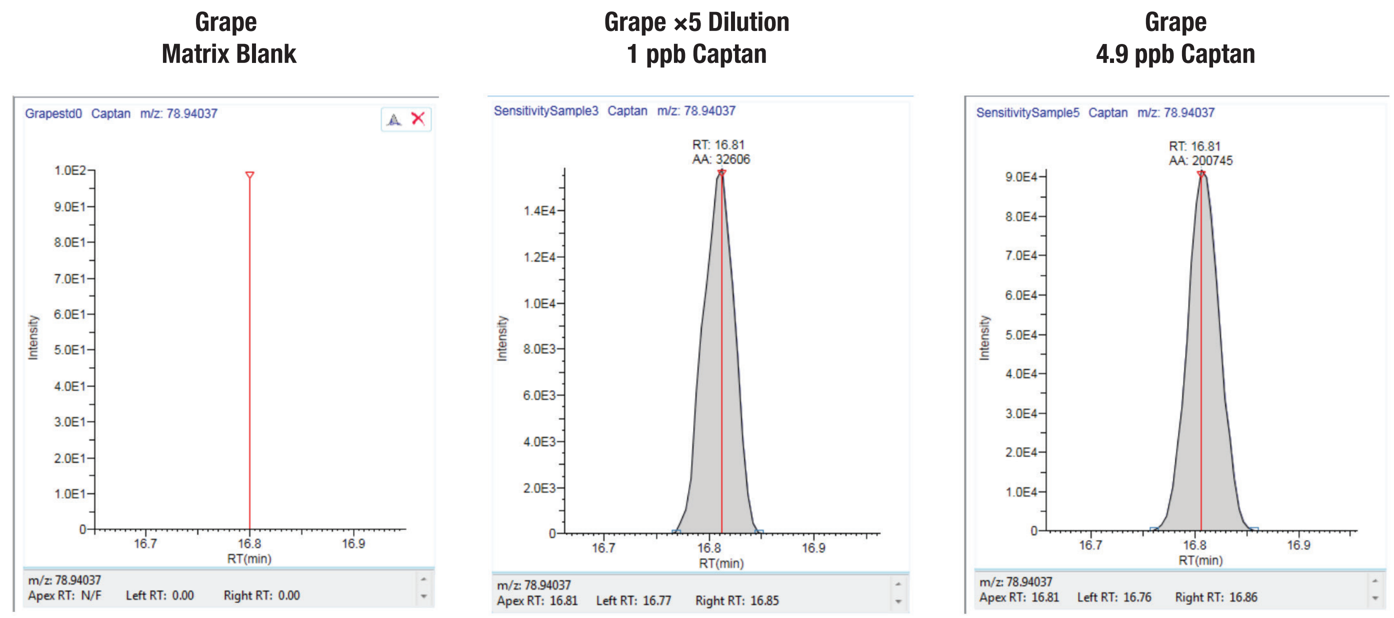


Figure 3. Extracted ion chromatogram and calculated concentration for captan in grape blank, 5× dilution and grape sample.

Conclusions

The results of this study demonstrate that the Exactive GC Orbitrap HRAM mass spectrometer, in combination with TraceFinder software, offers an excellent solution that simplifies the analysis of pesticides in food commodities and delivers sensitive quantitative performance for pesticide analysis in fruits and vegetables.

- Sensitive and robust full-scan analysis allows for easy and flexible data acquisition and processing.
- All 95 compounds were detected at levels below the MRL, with calculated limits of detection of < 2 µg/kg for most compounds (92 of the 95 compounds).
- Excellent linearity was demonstrated with $R^2 > 0.99$ 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.
- Blind analysis of a grape and onion sample showed reliable detection and accurate quantitation of spiked compounds.

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