

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

Dominic Roberts<sup>1</sup>, Jim Garvey<sup>2</sup>, Richard Law<sup>1</sup> and Paul Silcock<sup>1</sup>

<sup>1</sup>Thermo Fisher Scientific, Runcorn, United Kingdom; <sup>2</sup>Department of Agriculture, Food and the Marine, County Kildare, Ireland

## ABSTRACT

Pesticide residue and polychlorinated biphenyl (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.<sup>1-3</sup> 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 PCBs, this has meant analysis by the coupling of gas chromatography (GC) to triple 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 setup, 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.<sup>4-8</sup> 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 search for. This can extend into retrospective analysis to evaluate the presence of other compounds not necessarily of interest at the time of acquisition.

## INTRODUCTION

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.

In this study, the quantitative performance of the Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer was demonstrated for the routine analysis of GC-amenable pesticides and PCBs in grape and onion samples. 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.

## MATERIALS AND METHODS

Grape and onion samples were obtained from the market and extracted using the miniLuke procedure.

A calibration series containing 88 pesticides and 7 PCBs 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. An Exactive GC Orbitrap GC-MS system was used for accurate mass measurements in full-scan at 60,000 mass resolution (FWHM *m/z* 200). Details of the GC and MS parameters used in the experiments are given in Table 1. Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software.

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
Temperature 3:	280 °C
Rate:	5 °C/min.
Hold Time:	0 min.
Temperature 3:	300 °C
Rate:	10 °C/min.
Hold Time:	5 min.

## 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>

## RESULTS

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 2 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<sup>2</sup> = 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 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.

Table 2. Summary of quantitative performance for 95 pesticides and PCBs in grape and onion LOD.

Compound	Grape LOD (ng/mL)	Grape Linearity (R <sup>2</sup> )	Grape Average Response Factor (RSD%)	Onion Lowest Standard (ng/mL)	Onion Linearity (R <sup>2</sup> )	Onion Average Response Factor (RSD%)
Acephate	2	0.999	2.1	1	0.9991	12.4
Acinathrin	2	0.9983	12.6	1	0.9963	15.1
Aldrin	1	0.9996	11.9	1	0.9992	10.6
Anthracinone	1	0.9998	3.8	1	0.9984	7.2
Azinphos-methyl	2	0.9997	4.2	2	0.997	9.6
Azoxystrobin	1	0.9994	15	1	0.9974	9
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
Bifenox	1	0.9998	11.4	1	0.9974	7.6
Boscalid	1	0.9972	16	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
Captan	1	0.9998	6.2	1	0.9998	14.6
Chloridane-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
Chlorothalnil	1	0.9998	6.4	1	0.9998	4.3
Chlorpyrifam	1	0.9998	3.6	1	0.9999	2.2
Chlorpyrifos-methyl	1	0.9998	6.1	1	0.9998	4.2
Chlorthal-dimethyl	1	0.9996	7	1	0.9984	8.1
Cyfluthrin	2	0.9993	16	1	0.9984	13.7
Cyhalothrin lambda	1	0.9991	16.6	1	0.9986	18
Cypermethrin	1	0.9997	2.3	1	0.9975	14.7
Cyproconazole	1	0.9996	4	1	0.9992	7.1
DDD-p'	1	0.9999	3.3	1	0.9993	4
DDD-o,p'	1	0.9997	4	1	0.9987	5
DDE-o,p'	1	0.9996	8	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	0.9988	5.9
DDT-p,p'	1	0.9995	5.2	1	0.999	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-	1	0.9999	1.8	1	0.9997	2.1
Disofol	2	0.9991	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	2	0.9996	19
Endosulfan beta	1	0.9996	14.4	1	0.9992	10
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	0.9994	11.3	1	0.9992	9.3
Ethionphos	1	0.9995	6.1	1	0.9998	3.8
Etoxazole	2	0.9991	10.4	2	0.9991	10.1
Fenarimol	1	0.9998	4.2	1	0.9984	8.3
Fenazacrin	2	0.9996	17	2	0.9996	8.1
Fenbuconazole	1	0.9999	3	1	0.9991	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
Fludioxonil	1	0.9999	2.6	2	0.9983	11.9
Fluralaner-lau	1	0.9999	17.3	1	0.9996	13.6
Folpet	1	0.9998	10.4	1	0.9994	8.2
HCH-alpha	1	0.9994	6.4	1	0.9999	4.1
HCH-beta	1	0.9999	4	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
Iprovalcarb	1	0.9999	5.3	1	0.9994	2.7
Imidacloprid	1	0.9999	4.3	1	0.9999	4.3
MCPA Methyl ester	1	0.9985	7.9	1	0.9952	2.8
Methamidophos	1	0.9995	11.4	2	0.9994	18.8
Molinate	2	0.9988	12	1	0.9994	5.3
o-Hydroxybiphenyl	1	0.9997	4.8	1	0.9991	2.8
Omethoate	1	0.9998	5.1	1	0.9995	7.6
Oxy-Chlordane	1	0.9999	11.6	1	0.9999	6.4
PCB 101	1	0.999	6.3	1	0.999	7
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	0.9999	8.9	1	0.9993	5
PCB 180	1	0.9998	18.8	2	0.999	11.5
PCB 28	1	0.9985	4	1	0.9994	7
PCB 52	1	0.9974	11.8	1	0.9997	12.7
Pendimethalin	1	0.9952	16.6	1	0.9994	12.2
Permethrin	1	0.9999	1.8	1	0.9996	10
Phosmet	1	0.9999	2.5	1	0.9991	3.7
Prochloraz	2	0.9941	19	1	0.9914	19
Profenofos	1	0.9998	10.4	1	0.9995	16
Propargite	5	0.9956	18	5	0.9965	14.4
Propiconazole	1	0.9999	6.3	1	0.9998	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	1	0.9982	8.1
Spirodiclofen	1	0.9995	11	1	0.9985	16.4
Tefluthrin	1	0.9999	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	0.9994	13
Triadimenol	1	0.9999	7.4	1	0.999	18.6
Trifluralin	2	0.9989	15.5	1	0.9965	8.1

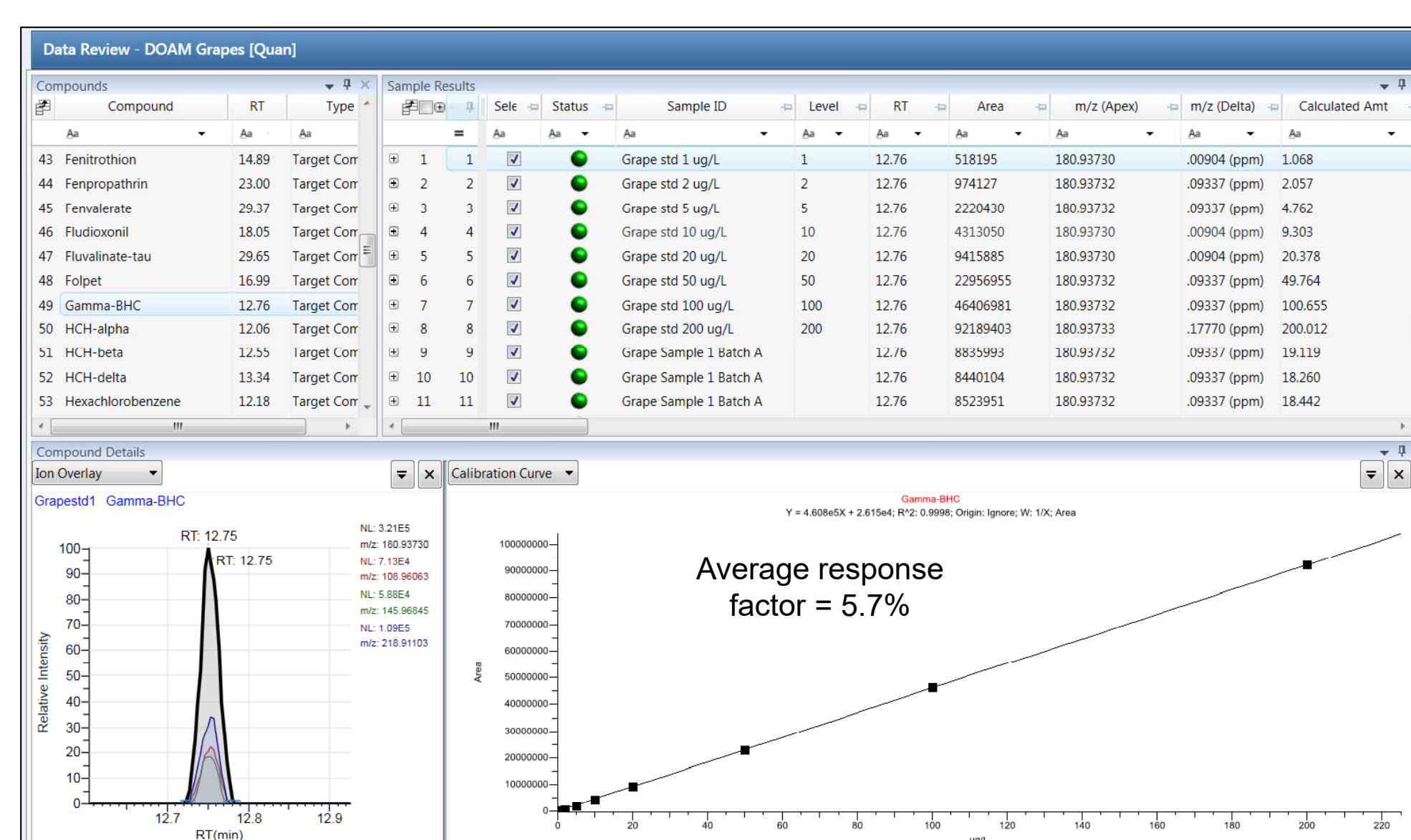


Figure 1. TraceFinder browser showing identified pesticides (A), overlay of extracted ion chromatograms (B), and linear response (C) (HCH-gamma as an example). Linearity R<sup>2</sup> = 0.9998, average response factor RSD% = 5.7.

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<sup>2</sup>). Figure 2 shows the linear response and the average response factor calibration for one of the most challenging pesticides, folpet, in onion matrix.

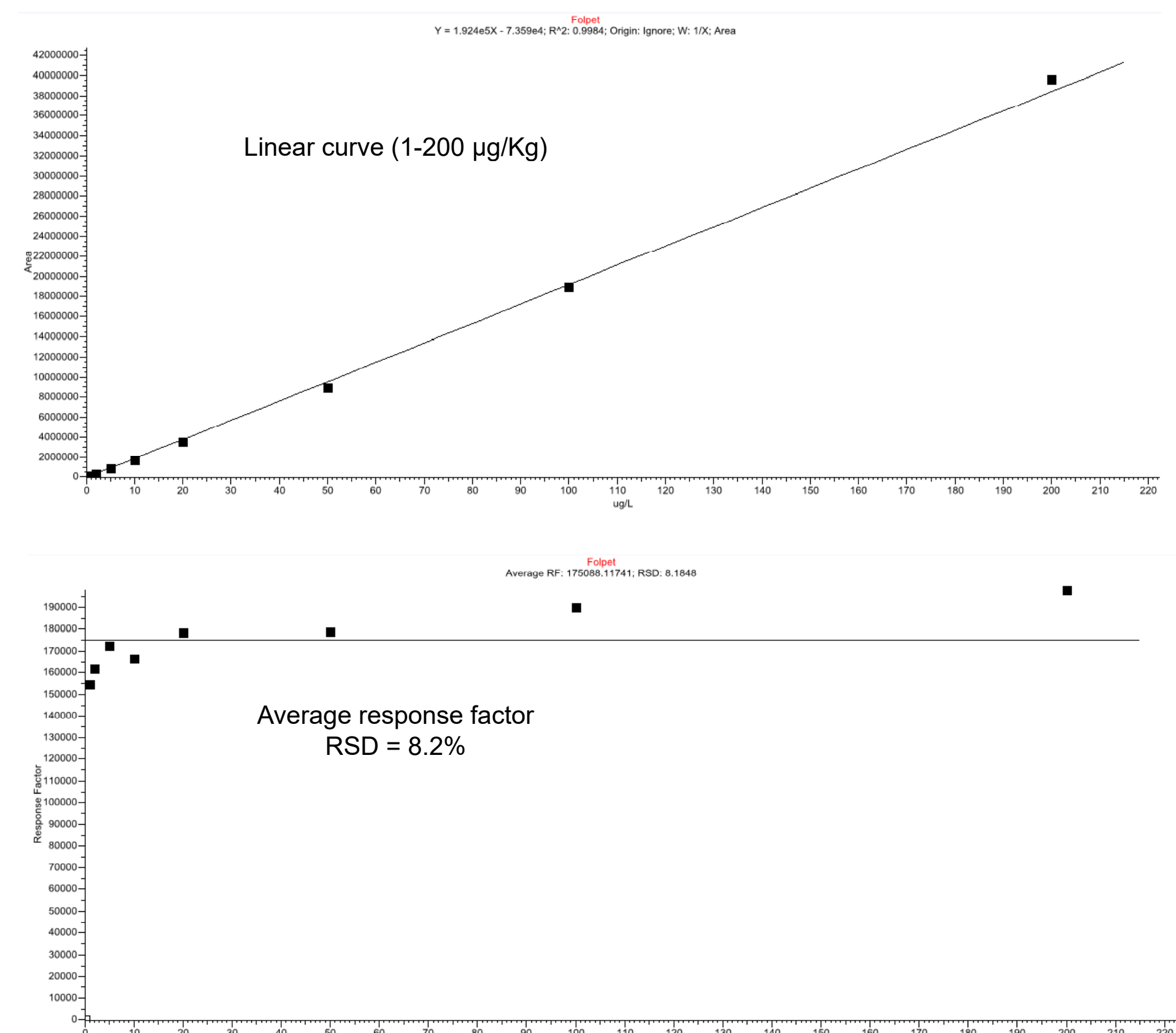


Figure 2. Calibration data for folpet in onion matrix.

## 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 3 summarizes these results, which show good agreement between the spiked and calculated concentrations.

Table 3. 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 Onion Concentration (µg/Kg)
Azoxystrobin	17	14	50	49.9
Boscalid	-	-	34	32
Captan	5	4.9	-	-
Chlordane-trans	-	-	53	56
Chlorothalonil	15.8	15.5	95	108
Cyfluthrin	4.3	3.9	58	56
Deltamethrin	-	-	45	44.1
Diazinon	1.2	1.1	58	61
Dimethoate	29	30	58	56
Fenbuconazole	-	-	47	50
Folpet	0.96	0.97	-	-
HCB	1.1	1.1	58	49
Hexaconazole	5.9	5.1	-	-
Iprodione	13	10.1	52	49.5
o,p-DDE	5.2	5.1	59	66
p,p-DDD	0.5	0.6	-	-
Omethoate	45	39.1	75	71
PCB 180	1.01	1.2	34	32
Propargite	6.3	5.7	95	97

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.

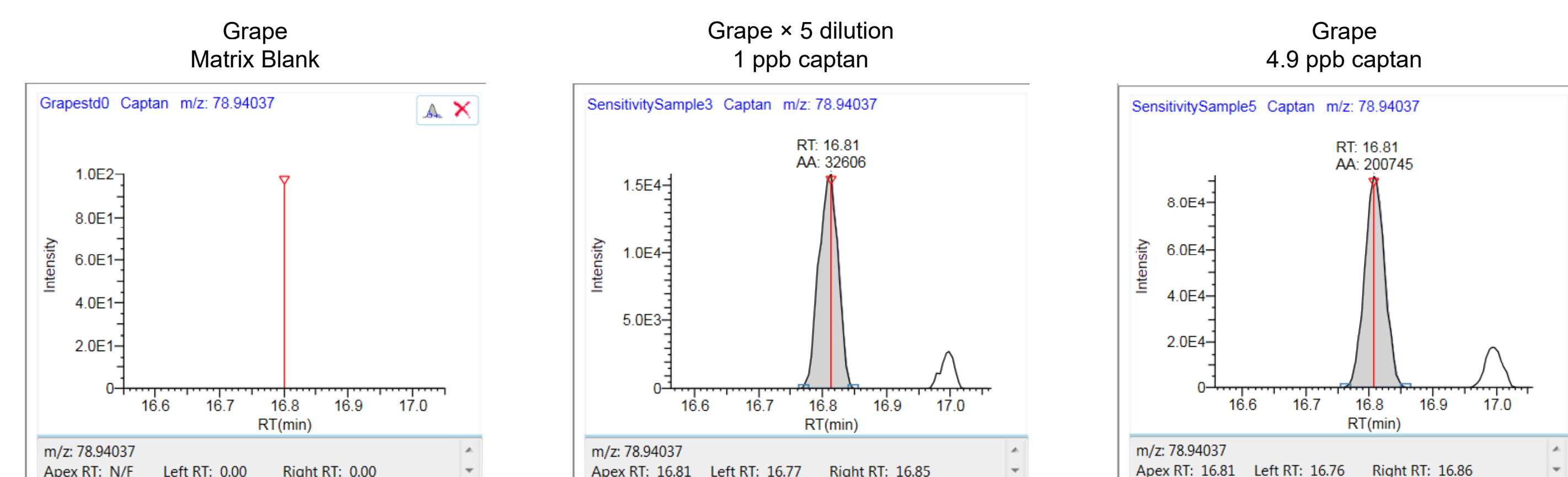


Figure 3. Extracted ion chromatogram and calculated concentration for captan in grape blank, 5x dilution and grape sample.

## 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<sup>2</sup> > 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.



## REFERENCES

1. [http://ec.europa.eu/food/plant/docs/plant\\_pesticides\\_mrl\\_guidelines\\_wrkdco\\_11945\\_en.pdf](http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdco_11945_en.pdf)
2. <http://eur-lex.europa.eu/legal-Content/EN/TXT/?uri=URISERV:113002a>
3. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. SANTE/11945/2015 implemented by 01/01/2016 (supersedes SANCO/12571/2013).
4. Mol, H.; Tienstra, M.; Zomer, P. Evaluation of gas chromatography electron ionization full scan high resolution Orbitrap mass spectrometry for pesticide residue analysis? *Anal. Chim. Acta* **2016**, *935*, 161–172.
5. Ucles, S.; Lozano, A.; Martinez Bueno, M. J.; Fernandez-Alba, A. R. Shifting the paradigm in gas chromatography mass spectrometry pesticide analysis using high resolution accurate mass spectrometry. *J. Chromatogr., A* **2017**.
6. High efficiency, broad scope screening of pesticides using gas chromatography high resolution Orbitrap mass spectrometry. Thermo Fisher Scientific Application Note 10448, 2016.
7. Routine quantitative method of analysis for pesticides using GC-Orbitrap mass spectrometry in accordance with SANTE/11945/2015 guidelines. Thermo Fisher Scientific Application Note 10509, 2016.
8. Multi-residue pesticide screening in cereals using GC-Orbitrap mass spectrometry. Thermo Fisher Scientific Application Note 10541, 2017.

## TRADEMARKS/LICENSING

© 2018 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is not intended to encourage use of these products in any manner that