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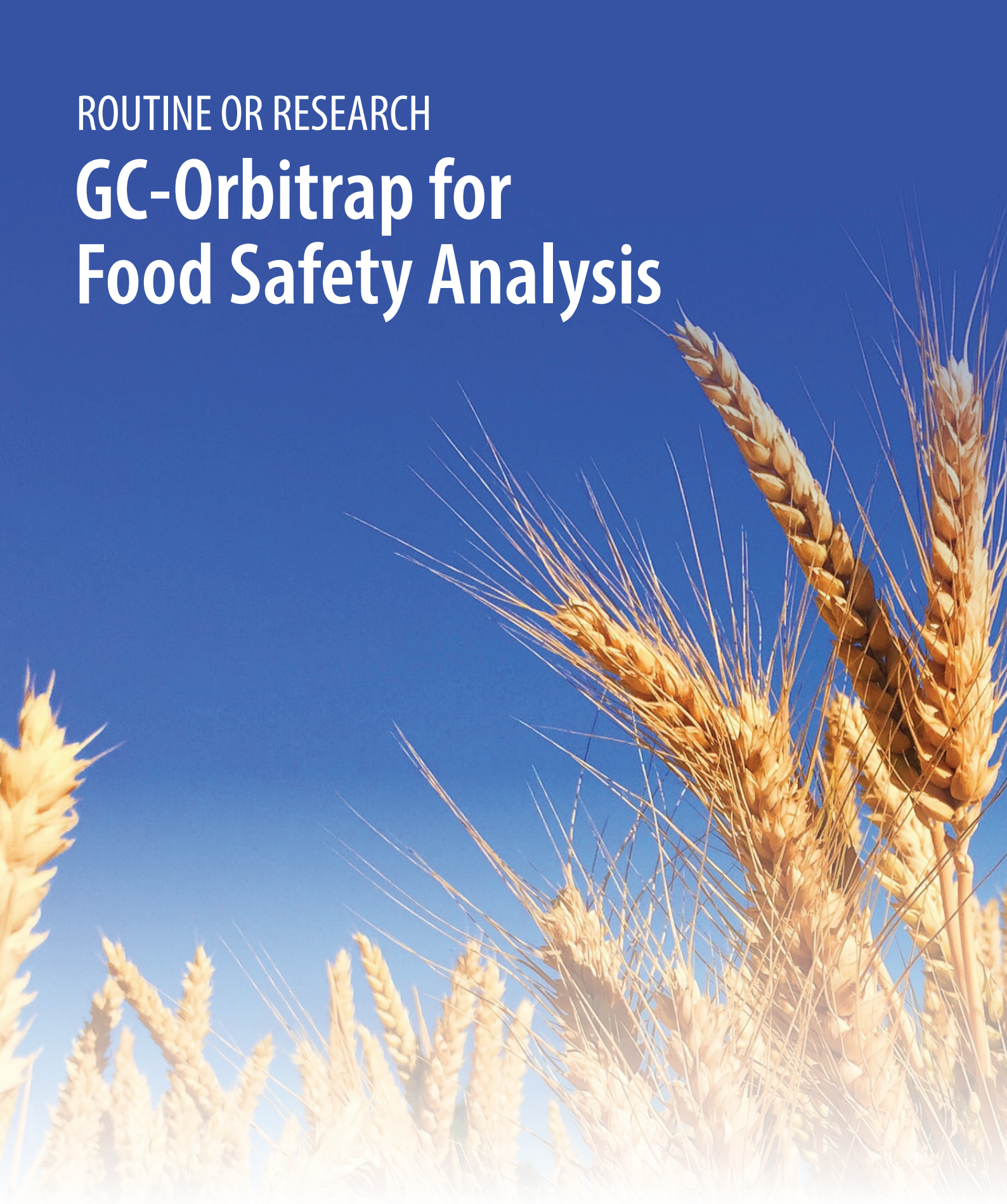
GC-Orbitrap for Food Safety Analysis



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GC-Orbitrap for Food Safety Analysis

Foreword

The Quantitative Power of High-resolution GC-Orbitrap Mass Spectrometry for the Analysis of Pesticides and PCBs in Foods
In this study, 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 is demonstrated.

Routine Quantitative Method of Analysis for Pesticides using GC Orbitrap MS in accordance with SANTE/11945/2015 Guidelines
In this study, the quantitative performance of the Thermo Scientific™ Exactive GC Orbitrap™ mass spectrometer was evaluated for the routine analysis of GC-amenable pesticides in fruits and vegetables following SANTE/11945/2015 guidelines using full scan acquisition.

Multi-residue Pesticide Screening in Cereals using GC-Orbitrap Mass Spectrometry
The goal of this study was to demonstrate the performance of the Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer for the routine analysis of GC-amenable pesticides in cereals (wheat, barley, oat, rye and rice).

High Efficiency, Broad Scope Screening of Pesticides Using Gas Chromatography High Resolution Orbitrap Mass Spectrometry
In this study, we evaluate the performance of the Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer (MS) for the accurate screening of GC-amenable pesticides.

Streamlining the Identification of Unknowns in Food Packaging using GC Orbitrap Mass Spectrometry
This study focuses on the utilization of a new GC-MS system with high mass resolution performance and high mass accuracy for fast and confident identification of unknown compounds in food packaging.

Chemical Profiling and Differential Analysis of Whiskies Using Orbitrap GC-MS
In this proof-ofconcept study, we seek to take advantage of the performance of the Thermo Scientific™ Q Exactive™ GC Hybrid Quadrupole-Orbitrap™ Mass Spectrometer for the profiling of whisky of different origins, ages and types.

Fast Screening, Identification, and Quantification of Pesticide Residues in Baby Food Using GC Orbitrap MS Technology
In this work, we demonstrate the use of GC coupled with Orbitrap™ MS technology for fast, high throughput pesticide residues analysis in baby food samples, with an almost unlimited scope in the analysis through full scan acquisition.

Case study: GC Orbitrap MS/MS Technology Adds Extra Certainty to Food Safety and Environmental Testing
This case study outlines how by using the Thermo Scientific Q Exactive GC Orbitrap GC-MS/MS system, the Laboratori de l'Agència de Salut Pública de Barcelona, realizes the additional certainty provided by high specificity and sensitivity, substantially reducing the chance of false negative and false positive results.

Chemometric Assessment of Volatile Fraction of Pesto by SPME Arrow GC Orbitrap Mass Spectrometry
In this study headspace solid phase micro-extraction (SPME) with Arrow technology coupled with gas-chromatography (GC) and Thermo Scientific™ Orbitrap™ high resolution mass spectrometry (HRMS) was used to determine the volatile profile of various pesto samples that were produced using various technological methods.

A Comprehensive Strategy for Confident Detection of Oregano Adulteration by GC-Orbitrap Mass Spectrometry
In this study the Orbitrap technology coupled with SPME Arrow extraction was used to assess the volatile profile of oregano.

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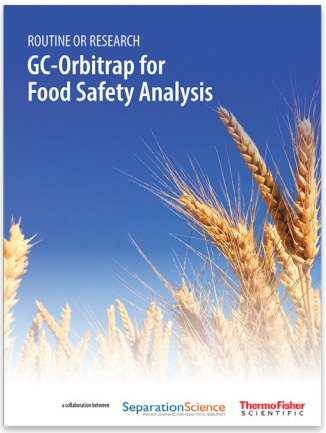
GC-Orbitrap for Food Safety Analysis

Foreword



Ensuring the safety of the world's food supply is critical, but there are concerns about the safety of global food supply chains because food can be grown and processed in widely differing environments under a variety of regulatory frameworks. At any point in the process, products may become contaminated and unfit for consumption. Whether you are ensuring regulatory compliance or involved in product development you will face the demand for ever changing regulations, increasing the scope of analysis and needing to be right first time. These challenges call for sophisticated analytical techniques such as Orbitrap-based high-resolution mass spectrometry that provides distinct advantages.

Since the launches of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS in 2015 and the Thermo Scientific™ Exactive™ GC Orbitrap™ GC-MS System in 2016, food safety scientists and analysts have had the ability to obtain an unprecedented depth in analysis of their samples. Whether their goals have been targeted routine analysis of contaminants, profiling for authenticity or research into emerging compounds, the power of high-resolution full-scan analysis has opened up new possibilities.

For routine food safety analysis GC-Orbitrap offers distinct benefits, including consolidation of multiple methods onto a single system, increasing the scope of analysis to keep pace with changing regulations and the ability to retrospectively detect compounds. For research applications, the power to confidently and accurately identify unknown compounds is critical to understand contaminant levels and broader food profiling to detect adulteration. Once detected, accurate and precise quantification is delivered with ease through the wide dynamic range provided by Orbitrap technology.

In this new eBook we bring together some of the key application notes written in collaboration with scientists from around the world that demonstrate how GC-Orbitrap makes a real difference to food safety.

thermo scientific

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.

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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

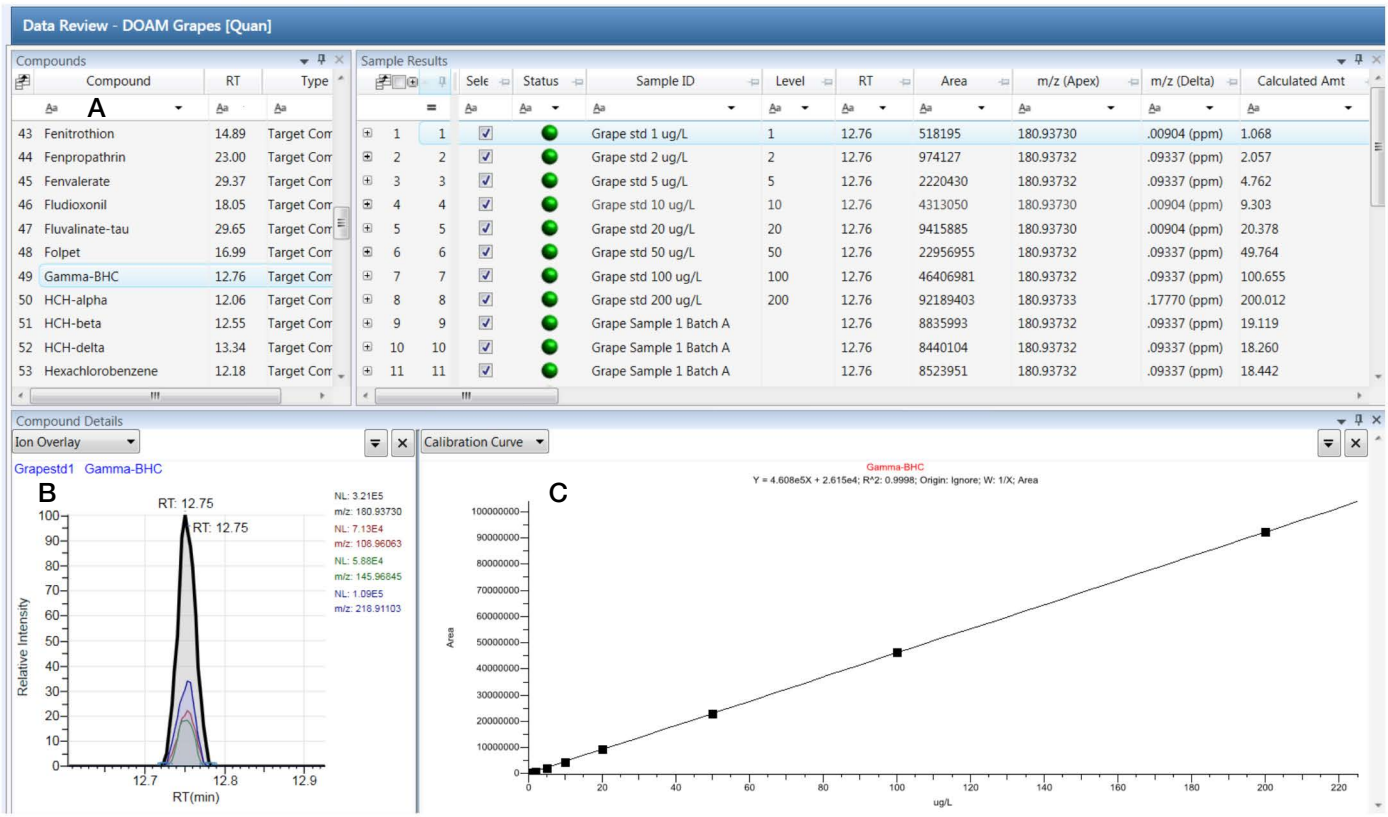

Figure 1. TraceFinder browser showing identified pesticides (A), overlay of extracted ion chromatograms (B), and linear response (C) (HCH-gamma as an example). Linearity R^2 = 0.9998, average response factor RSD% = 5.7.

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.

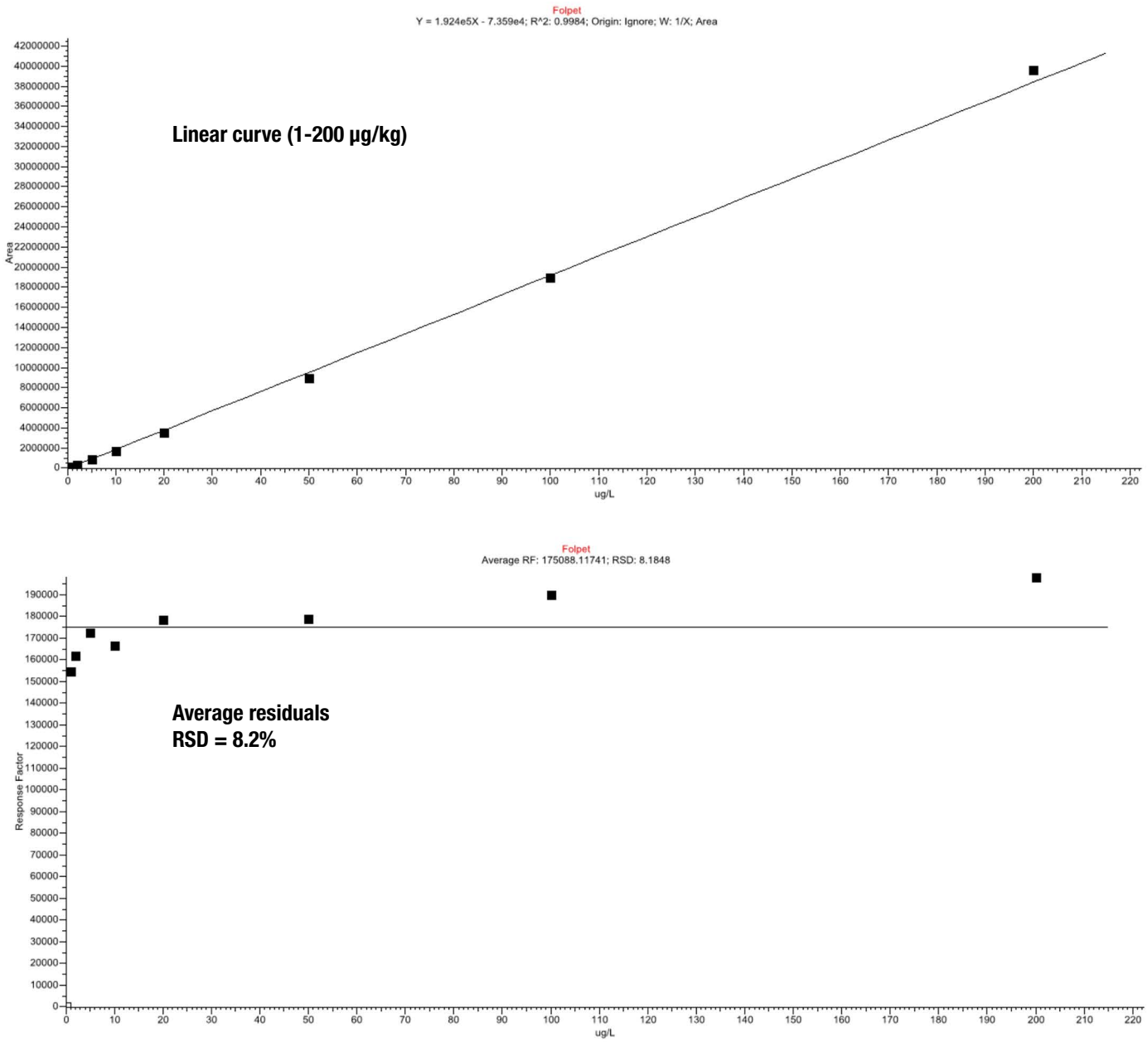

Figure 2. Calibration data for folpet in onion matrix.

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.

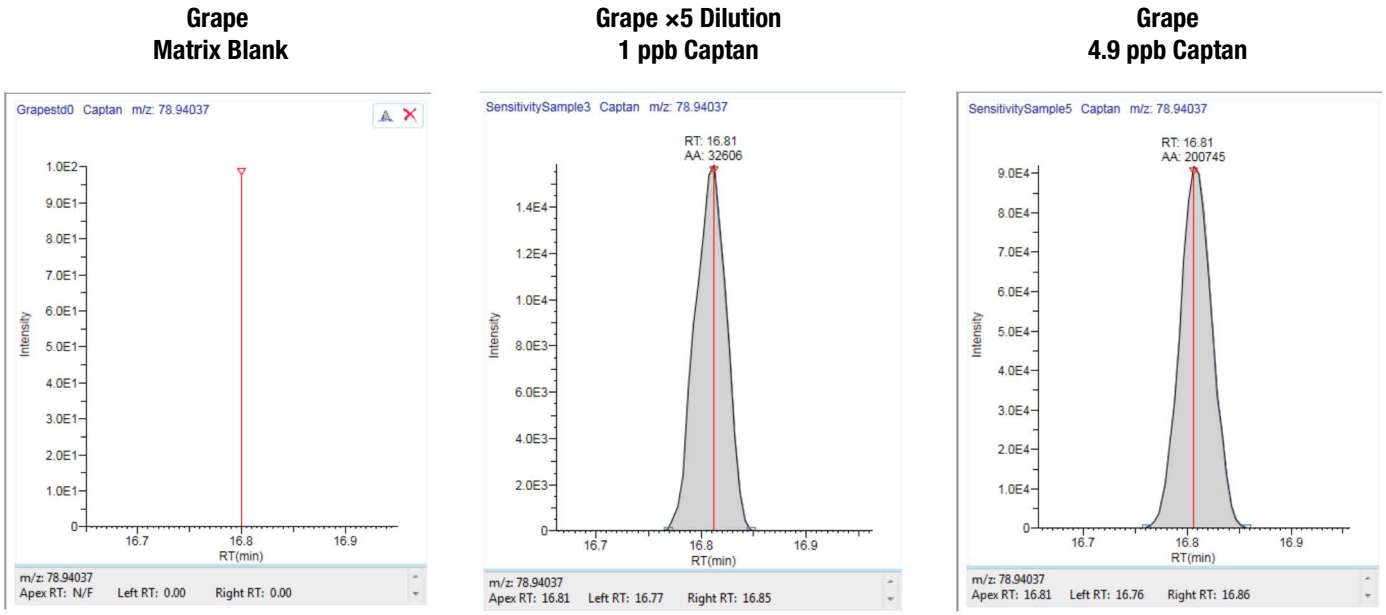

Figure 3. Extracted ion chromatogram and calculated concentration for captan in grape blank, 5× dilution and grape sample.

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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APPLICATION NOTE

No. 10509

Routine Quantitative Method of Analysis for Pesticides using GC Orbitrap Mass Spectrometry in accordance with SANTE/11945/2015 Guidelines

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Keywords: Pesticides, QuEChERS, Complex matrices, GC Orbitrap Mass Spectrometry, Quantitation, Accurate Mass, TraceFinder

Introduction

The international trade in food commodities has enabled a wide variety of fruits and vegetables to be made available year round. However, this also creates a challenge for food safety regulators who seek to ensure a safe food supply chain, particularly with regard to the potentially hundreds of different pesticides in use across the globe. The European Union (EU) has some of the most stringent pesticide residue regulations. In 2008, it implemented regulation EC No. 396/20051, which sets default maximum residue levels (MRLs) at 10 µg/Kg for all pesticide/commodity combinations for which no substantive MRL had been set. Further to this, in 2009, the pesticide safety review EU 91/414/EEC2 led to the approval of approximately 250 pesticides and effectively set the permissible level for all other pesticides to the default limit (10 µg/Kg). Recently, at the beginning of 2016, the latest version of the SANTE/11945/2015 guidance document on analytical quality control and validation procedures for pesticide residues in food and feed took effect.[3] This document describes the method validation and analytical quality control (AQC) requirements to support the validity of data reported within the framework of official controls on pesticide residues and used for checking compliance with maximum residue levels (MRLs), enforcement actions, or assessment of consumer exposure. It is intended for use by Official control laboratories in Europe, but in practice it is used by pesticide laboratories worldwide. Implementation of the stringent requirements present a major challenge to testing laboratories who seek to provide an accurate and cost competitive services.

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Pesticide residue testing requires detection using both liquid and gas chromatographic techniques typically coupled with triple quadrupole mass spectrometers. These analytical techniques can cover the range of compounds that need to be monitored with the required sensitivity and selectivity. However, they are limited to detecting pesticides that are measured at the time of acquisition and require careful method optimization and management to ensure selected ion monitoring windows remain viable. In recent years, high-resolution Orbitrap mass spectrometry has provided an alternative to MS/MS techniques with additional analytical advantages.[4] With high-resolution mass spectrometry (HRMS), the default acquisition mode is untargeted (full-scan) making it simple to manage and potentially allows for an unlimited number of pesticides to be monitored in a single injection. In addition to this, full-scan data analysis provides access to supplementary identification points such as spectral matching and enables retrospective interrogation of samples to additionally search for emerging pesticides or other contaminants that were not considered at the time of acquisition.

In this study, the quantitative performance of the Thermo Scientific™ Exactive GC Orbitrap™ mass spectrometer was evaluated for the routine analysis of GC-amenable pesticides in fruits and vegetables following SANTE/11945/2015 guidelines using full scan acquisition. The Exactive GC-MS system provides routine high-mass resolving power up to 60,000 (*m/z* 200) full width at half maximum (FWHM) with scan speeds suitable for GC peaks to facilitate the detection of trace compounds in the presence of high matrix components.

Experimental Conditions

Sample Preparation

Tomato, leek and orange were purchased from a local supermarket and extracted following a citrate buffered QuEChERS procedure. Briefly, 10 mL of acetonitrile was added to 10 g of homogenized sample and shaken for 4 minutes. A mixture of salts was added and the centrifuge tube shaken for 4 minutes and centrifuged for 5 minutes at 3700 rpm. Supernatant (5 mL) was transferred to a 15 mL PTFE centrifuge tube containing magnesium sulphate and 125 mg of PSA. The extract was shaken in a vortex mixer and centrifuged as above. The final acetonitrile extracts (1g/mL) were used as blank matrix. The calibration series was prepared by taking 100 µl of acetronitrile blank matrix and drying under a stream of nitrogen to complete dryness. The sample was reconstituted in 100 µl ethyl acetate containing the appropriate concentration of pesticides.

Three calibration series of 51 pesticides were prepared in tomato, leek and orange at concentrations equivalent to 0.5, 1, 2, 5, 10, 20, 50, 100, 200 and 500 µg/Kg. The 51 pesticides included in the study cover a wide range of chemical classes and, with the three matrices, it generated a total of 153 pesticide/matrix combinations. To assess compound linearity, the matrix matched calibration series were analyzed first, followed by ten replicate injections of the 10 µg/Kg sample for each matrix. To assess repeatability over an extended period of time, the 10 µg/Kg tomato standard was further injected 100 times from the same vial.

Instrument and Method Setup

In all experiments, an Exactive™ GC Orbitrap™ mass spectrometer was used. Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was obtained using a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m x 0.25 mm I.D. x 0.25 µm film capillary column with a 5 m integrated guard (P/N:26096-1425). Additional details of instrument parameters are given in Table 1 and Table 2.

Table 1. GC and Split/Splitless injector conditions.

TRACE 1310 GC Parameters	
Injection Volume (µL):	**1**
Liner:	LinerGOLD™ single taper (P/N: 453A1345-UI)
Inlet (°C):	280
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):	280
Rate (°C/min):	5
Hold Time (min):	0
Temperature 3 (°C):	300
Rate (°C/min):	10
Hold Time (min):	5

Table 2. Mass spectrometer conditions

Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	**280**
Ionization type:	EI
Ion source(°C):	250
Electron energy (eV):	70
Acquisition Mode:	Full-scan
Mass range (Da):	50-550
Resolving power (FWHM at *m/z* 200):	60,000
Lockmass, column bleed (*m/z*):	207.03235

Data Processing

Data were acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. For target analysis a compound database for the 51 pesticides was prepared using the Thermo Scientific™ Orbitrap GC-MS Contaminants Library containing compound name, quantification ion and identification ions, accurate masses, retention times and elemental compositions of molecular ion and fragment masses. For the generation of extracted ion chromatograms an mass extraction window of 5 ppm was used.

Results and Discussion

The objective of this study was to evaluate the analytical performance of the Exactive GC system for the routine analysis of pesticides in three different sample matrices following SANTE requirements. The sample types chosen (tomato, leek and orange) provided both easy and difficult matrices that are typically encountered in routine testing. To illustrate, the varying sample complexity total ion chromatograms with fixed Y-axis are shown in Figure 1. The leek matrix is clearly the most complex matrix and this is where high-mass resolution is required to extract target analytes from background chemical noise. The QuEChERS generic sample extraction technique employed in routine testing produces complex extracts containing high and variable concentrations of matrix components depending on the sample type. The lack of selectivity during sample preparation needs to be compensated for by a selective instrumental analysis. This was achieved using high-mass resolving power of the Exactive GC system (60k @*m/z* 200). This capability in combination with a full-scan acquisition increases the scope of the analysis without the need for optimization of acquisition parameters, as is the case with targeted analyses.

For routine pesticide screening, the HRMS processing software needs to be fast, accurate and customizable. TraceFinder meets all of these requirements and was used to process each batch of calibration standards and ten replicates in less than five minutes. In TraceFinder, the results are presented to the user in a table format and data flags are used to quickly identify which pesticides are positive and which criteria are satisfied. Flexible reporting options means that data can be either exported to other software packages or reported directly from within TraceFinder.

Identification to Guideline Requirements

One aim of the analysis was to determine the limit of detection (LOD), limit of identification (LOI), linearity and peak area repeatability for all of the pesticides in all three matrices. Although the LOD is not discussed in the SANTE guidelines, it is useful to know the limit of detection of the quantifier ion as it is used in forming the calibration series that will ultimately be used in determining the concentration of a detected pesticide in a sample. This assessment was made by evaluating the matrix matched calibration series and the repeat injections at 10 µg/Kg for each matrix. The LOD was defined as the presence of a peak with S/N (peak to peak) >3 in the extracted ion chromatogram (XIC) of the main quantifier ion of a pesticide. For the determination of the LOI the SANTE/11945/2015 guidance document was followed. This requires that the following criteria are satisfied for a positive identification:

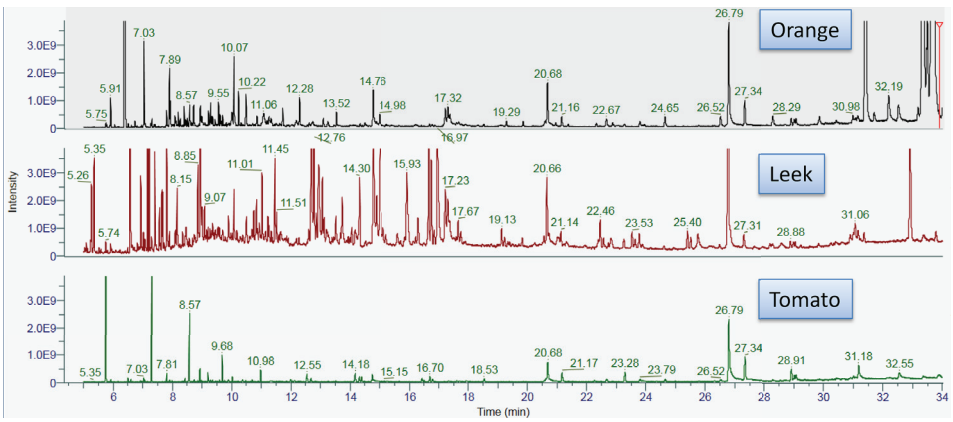

Figure 1. Full scan Total Ion Chromatogram (TIC) of orange, leek and tomato extracts with y axis fixed at4.0 e9 showing the complexity of the sample matrices used in this study.

(i) Two ions are detected for each pesticide with mass accuracy <5 ppm and peak S/N > 3

(ii) Retention time tolerance of ± 0.1 minutes compared with standards in the same sequence

(iii) Ion ratio within ± 30% of the average of calibration standards from the same sequence

(iv) Optional: For higher confidence in identification additional criteria can be used such as full-scan spectra, isotope pattern matching and additional fragment ions

All of the pesticides were identified following the regulatory criteria (LOI) in all of the matrices at or below 5 µg/Kg (Tables 3-5) with the exception of chlorothalonil in leek, which is known to suffer losses due to interaction with sulphur compounds in the leek matrix.5 The majority of the 153 pesticide/matrix combinations (79%) had an LOI ≤2 µg/Kg. The calculated LODs are summarized in Figure 2 which shows that the LOD for 93% of the pesticide/matrix combinations was ≤1 µg/Kg. Having multiple identification points and limits of detection well below the MRL increases the confidence in identifications and minimizes false negative and positive results. Using highly efficient electron ionisation (EI) in combination with full-scan acquisition provides the opportunity to use multiple diagnostic ions for the identification of pesticides. The

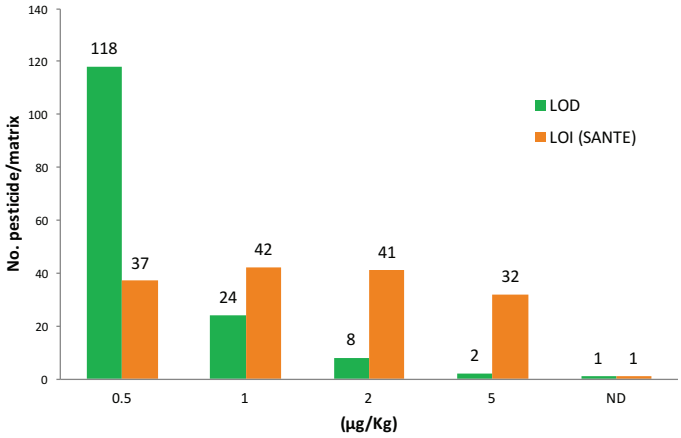

Figure 2. The limit of detection (LOD) and limit of identification (LOI) for pesticides/matrix combinations.

Exactive GC system generates standard EI spectra that are highly reproducible and library searchable (nominal or high resolution MS libraries). This facilitates detection and identification of pesticides based on spectral matching. Additional compounds can be quickly added to the compound database as chemical formulas can be easily assigned to accurate mass fragment ions due to the high mass accuracy of the Orbitrap analyzer.

Reliable Quantitation

Quantitative linearity was assessed using matrix matched standards across a concentration of 0.5-500 µg/Kg. In all cases, the coefficient of determination (R^2) was >0.99 for each pesticide from its LOD to 500 µg/Kg in the three matrices, an example of the TraceFinder browser showing propazine is given in Figure 3. One exception to this, possibly due to analyte adsorption, was fenpropidin which was linear up to 200 µg/Kg. Accurate quantitation is reliant upon a number of factors, one of which is an acquisition speed fast enough to provide at least 12 points across chromatographic peak. At a resolution of 60,000 the Exactive GC system has a scan speed of approximately 7 Hz. An example is shown in Figure 4 for the peak of chlorobenzilate which has 38 points across the 6 second peak.

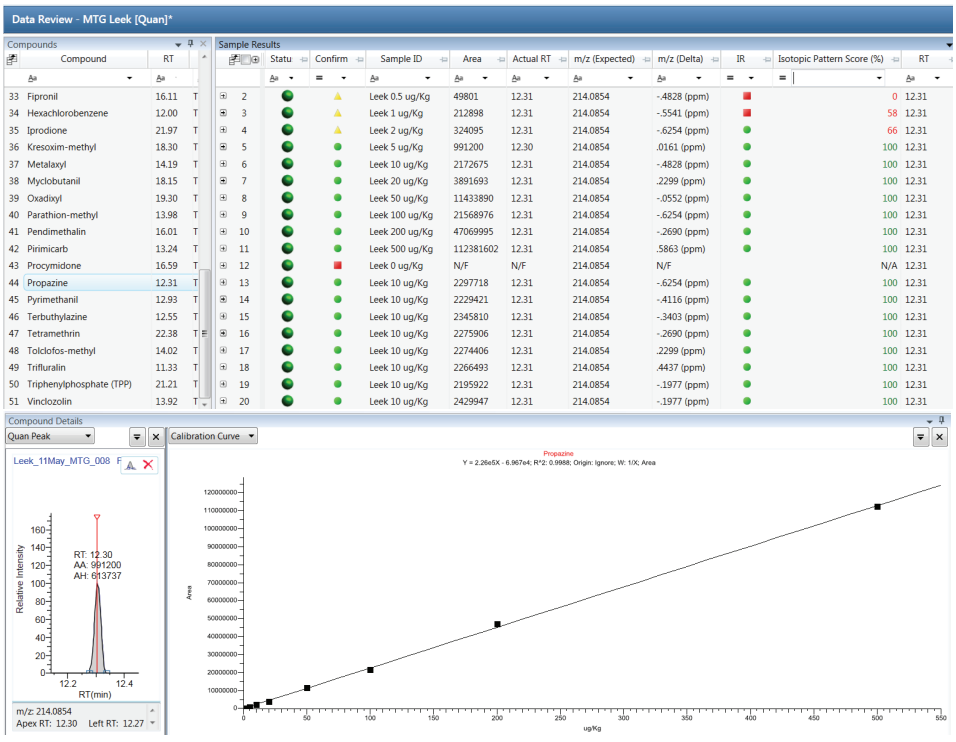

Figure 3. TraceFinder browser showing positively identified pesticides, extracted ion chromatogram and calibration graph (propazine as an example). Sub-ppm mass accuracy for propazine across the calibration range and in replicates of 10 µg/Kg. Identification criteria information is available and flagged when out of tolerance.

Table 3. Summary of method performance results for pesticides in leek. * Chlorothalonil known to degrade in leek.

Pesticide	LOD (µg/Kg)	LOI (µg/Kg)	R^2 LOD-500 (µg/Kg)	Mass Accuracy at LOI (ppm)	Leek 10 µg/Kg (%RSD) n=10
2-phenylphenol	0.5	1	0.9986	-0.53	2.5
Acrinathrin	2	5	0.9975	-0.68	6
Azoxystrobin	1	5	0.9961	0.1	6.3
BHC, Alpha	0.5	1	0.9993	-0.6	4.4
BHC, beta	0.5	1	0.9992	0.8	4.4
BHC, gamma	0.5	2	0.9986	-0.8	4.5
Bifenthrin	0.5	0.5	0.9989	-0.5	4
Biphenyl	0.5	0.5	0.9986	-0.9	3.3
Bromopropylate	0.5	1	0.9973	0.3	6.4
Bupirimate	0.5	1	0.9979	-0.4	5.1
Chlorobenzilate	0.5	2	0.9979	1.04	3.8
Chlorothalonil	ND*	ND*	-	-	-
Chlorpropham	0.5	2	0.9991	0.7	2.9
Chlorpyrifos	1	5	0.999	0.1	4.6
Chlorpyrifos-methyl	0.5	2	0.9988	0.5	4.1
Cyhalothrin	1	2	0.9954	-0.6	6.9
Cypermethrin I-IV	5	5	0.9962	0.5	7.9
DDD p,p'	0.5	2	0.9982	0.7	4.7
DDE p,p'	0.5	1	0.9988	0.41	3.5
DDT o,p	0.5	2	0.9982	0.7	4.4
DDT p,p'	0.5	5	0.9962	0.1	4.2
Diazinon	1	2	0.9983	-0.34	3.5
Dichlorvos	0.5	1	0.9991	-0.5	4.1
Dieldrin	2	5	0.992	0.3	3.6
Endosulfan sulfate	1	5	0.999	-0.2	5.9
Endosulphan alpha	2	5	0.994	-0.2	9.1
Endosulphan beta	2	5	0.9982	-0.4	7.5
Etofenprox	2	5	0.9978	-0.1	6.2
Fenitrothion	2	2	0.9968	0.1	6.6
Fenpropidin	0.5	5	0.9986	-0.3	4.1
Fenpropimorph	0.5	5	0.9977	-1.1	2.8
Fenvalerate SS,RR	0.5	2	0.9954	0.6	6.5
Fipronil	0.5	1	0.9979	0.2	6.3
Hexachlorobenzene	0.5	1	0.9985	1.1	3
Iprodione	0.5	5	0.9975	0.4	7.5
Kresoxim-methyl	0.5	2	0.9989	0.36	4.3
Metalaxyl	2	5	0.9989	-0.91	4.9
Myclobutanil	0.5	5	0.9987	-0.96	5
Oxadixyl	1	2	0.9983	0.34	6
Parathion-methyl	1	5	0.9985	0.61	4.8
Pendimethalin	2	5	0.9989	0.98	6.5
Pirimicarb	0.5	2	0.9991	-0.28	3.1
Procymidone	1	1	0.9988	0.26	5.9
Propazine	0.5	2	0.9988	-0.62	2.9
Pyrimethanil	0.5	1	0.9984	-0.31	3.6
Terbuthylazine	0.5	1	0.9985	-0.19	4
Tetramethrin	1	5	0.9991	-0.23	5.4
Tolclofos-methyl	0.5	1	0.9991	0.55	2.5
Trifluralin	1	1	0.9963	-0.52	3.9
Triphenylphosphate	1	2	0.9979	0	6
Vinclozolin	0.5	2	0.9987	-0.6	4.6

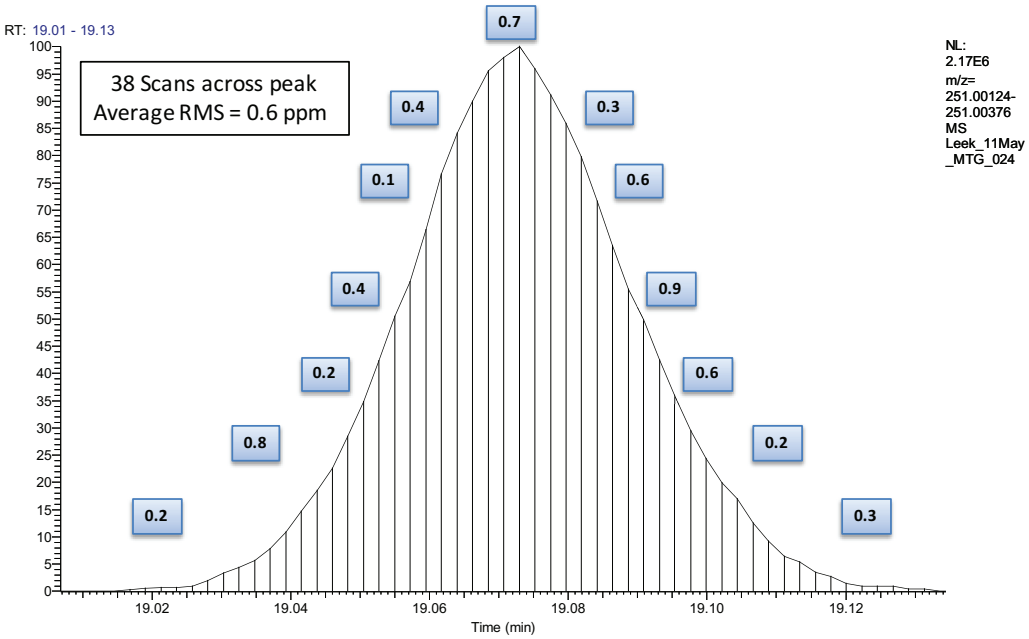

Figure 4. Extracted ion chromatogram of chlorobenzilate (m/z 251.0025 ±5 ppm mass window) acquired at 60,000 resolution (FWHM at m/z 200) in leek spiked at 10 µg/Kg showing ~38 scans/peak (peak width 6 sec). Sub 1 ppm accurate mass is achieved for each individual scan (every third scan labelled). Average RMS mass difference of 0.6 ppm across the peak.

The results of the 10 replicate injections at 10 µg/Kg in all three matrices are presented in Figure 5. All detected pesticides had RSD% of less than 10%, well below the 20% threshold requirement in the SANTE guidance document. This shows that the Exactive GC system operated in full-scan at 60k resolution has the selectivity and sensitivity required to analyse pesticides in a robust manner well below the respective MRLs.

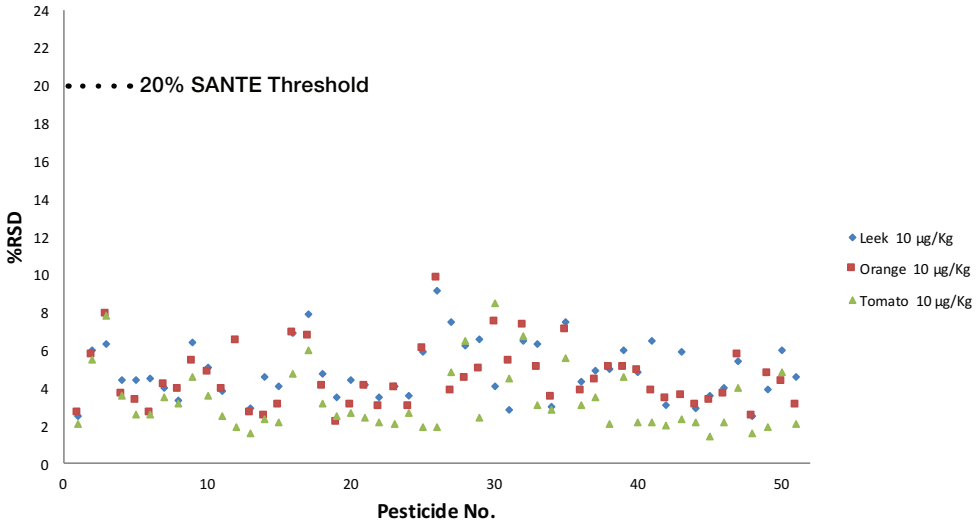

Figure 5. Peak area repeatability (%RSD) for 10 µg/Kg (n=10) for each pesticide in the three matrices studied. SANTE guideline of 20% threshold shown is also indicated.

Table 4. Summary of method performance results for pesticides in orange. *LOD-200 µg/Kg

Pesticide	LOD (µg/Kg)	LOI (µg/Kg)	R^2 LOD-500 (µg/Kg)	Mass Accuracy at LOI (ppm)	Leek 10 µg/Kg (%RSD) n=10
2-phenylphenol	0.5	0.5	0.997	-0.1	2.7
Acrinathrin	1	5	0.9956	-0.42	5.7
Azoxystrobin	1	5	0.9977	-0.1	7.9
BHC, Alpha	0.5	0.5	0.9984	-0.6	3.7
BHC, beta	0.5	1	0.9985	-0.6	3.3
BHC, gamma	0.5	0.5	0.9989	-0.21	2.7
Bifenthrin	0.5	0.5	0.9972	-0.7	4.2
Biphenyl	0.5	0.5	0.998	-0.37	3.9
Bromopropylate	0.5	1	0.9985	-0.16	5.4
Bupirimate	0.5	0.5	0.9987	0.36	4.8
Chlorobenzilate	0.5	0.5	0.9982	0.37	3.9
Chlorothalonil	0.5	0.5	0.9987	0.42	6.5
Chlorpropham	0.5	2	0.9981	-0.13	2.7
Chlorpyrifos	0.5	1	0.9982	0.1	2.5
Chlorpyrifos-methyl	0.5	1	0.9989	0.38	3.1
Cyhalothrin	1	5	0.9963	-0.6	6.9
Cypermethrin I-IV	5	5	0.9986	-0.5	6.7
DDD p,p'	0.5	2	0.9986	-0.1	4.1
DDE p,p'	0.5	0.5	0.9989	0	2.2
DDT o,p	0.5	2	0.9988	0.14	3.1
DDT p,p'	0.5	5	0.9967	-0.11	4.1
Diazinon	0.5	0.5	0.999	0.51	3
Dichlorvos	0.5	0.5	0.9983	0.29	4
Dieldrin	0.5	2	0.9989	0.5	3
Endosulfan sulfate	1	2	0.9986	1.2	6.1
Endosulphan alpha	1	5	0.9987	-1.2	9.8
Endosulphan beta	1	2	0.9988	0.4	3.8
Etofenprox	0.5	2	0.9937	0.4	4.5
Fenitrothion	0.5	2	0.998	0.1	5
Fenpropidin	1	5	0.993*	1	7.5
Fenpropimorph	0.5	2	0.9924	-0.44	5.4
Fenvalerate SS,RR	0.5	2	0.9919	0.37	7.3
Fipronil	0.5	0.5	0.9983	-0.8	5.1
Hexachlorobenzene	0.5	1	0.999	-0.17	3.5
Iprodione	0.5	1	0.9983	-0.5	7.1
Kresoxim-methyl	0.5	1	0.9984	0.43	3.8
Metalaxyl	0.5	1	0.9991	-0.8	4.4
Myclobutanil	0.5	2	0.9977	-0.2	5.1
Oxadixyl	0.5	2	0.9983	0.46	5.1
Parathion-methyl	0.5	2	0.9988	-0.3	4.9
Pendimethalin	0.5	2	0.9978	1	3.8
Pirimicarb	0.5	1	0.9976	-0.65	3.4
Procymidone	0.5	2	0.9977	0.1	3.6
Propazine	0.5	1	0.9981	0.3	0.3
Pyrimethanil	0.5	1	0.9935	-0.3	3.3
Terbuthylazine	0.5	1	0.999	-0.2	3.7
Tetramethrin	0.5	5	0.9979	-0.41	5.7
Tolclofos-methyl	0.5	1	0.9986	0.78	2.5
Trifluralin	0.5	1	0.9974	0.56	4.7
Tri-phenylphosphate	0.5	1	0.9977	0.28	4.3
Vinclozolin	0.5	1	0.999	0.5	3.1

Robust Mass Accuracy

Acquiring reliable accurate mass measurements is critical when detecting low level pesticides in complex sample matrices. Low mass errors, allow selectivity to be maintained through the use of narrow mass extraction windows during data processing and help ensure positive detections are robust. The low mass errors observed with the Exactive GC system are enabled through the high-mass resolving power that is able to discriminate between matrix interferences and target analyte ions. When the resolution is insufficient, the mass profile of two ions overlap, which results in the incorrect assignment of the mass of the target compound. This is demonstrated in Figure 6 where the leek 10 µg/Kg matrix standard was analysed at resolving powers of 15K, 30K and 60K. The zoomed mass spectra show the quantifier ion for pyrimethanil and a matrix ion of a similar mass causing interference. At 15K and 30K, the pyrimethanil ion is not resolved resulting in poor mass accuracy of 10.1 and 6.3 ppm respectively. However, the ions are sufficiently resolved at 60K resulting in the expected sub 1 ppm mass accuracy. Without this level of mass resolution this pesticide would have failed the SANTE identification criteria of <5 ppm and would have been a false negative (reported as not detected). This supports previous a report that a resolving power of 60k (at 200 *m/z*) is required in some cases to ensure the highest selectivity.[6]

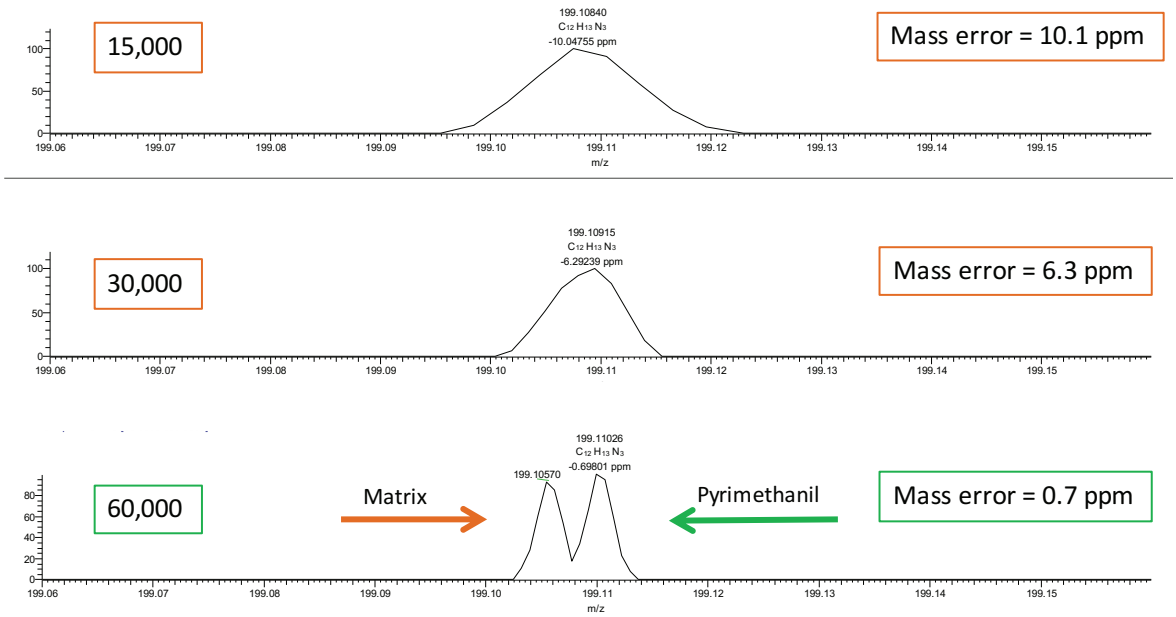

Figure 6. Effect of resolving power on mass accuracy of the diagnostic ion of Pyrimethanil at 10 µg/Kg in leek acquired at different resolutions of 15K, 30K and 60K. At 15K and 30K the Pyrimethanil ion is not resolved from the interfering matrix ion resulting in poor mass accuracy assignment.

Table 5. Summary of method performance results for pesticides in tomato.

Pesticide	LOD (µg/Kg)	LOI (µg/Kg)	R^2 LOD-500 (µg/Kg)	Mass Accuracy at LOI (ppm)	Leek 10 µg/Kg (%RSD) n=10
2-phenylphenol	0.5	0.5	0.9999	-0.71	2.1
Acrinathrin	1	5	0.9915	-0.34	5.5
Azoxystrobin	1	2	0.9938	-0.1	7.8
BHC, Alpha	0.5	0.5	0.9984	-0.46	3.6
BHC, beta	0.5	0.5	0.9984	-0.21	2.6
BHC, gamma	0.5	0.5	0.9984	-0.63	2.6
Bifenthrin	0.5	0.5	0.9981	-0.75	3.5
Biphenyl	0.5	0.5	0.9977	-0.37	3.2
Bromopropylate	0.5	1	0.9939	0.37	4.6
Bupirimate	0.5	0.5	0.9969	-0.51	3.6
Chlorobenzilate	0.5	0.5	0.9982	0.43	2.5
Chlorothalonil	0.5	0.5	0.9985	1	1.9
Chlorpropham	0.5	0.5	0.999	0.7	1.6
Chlorpyrifos	0.5	0.5	0.999	0.14	2.3
Chlorpyrifos-methyl	0.5	0.5	0.999	0.81	2.2
Cyhalothrin	0.5	1	0.999	-0.76	4.7
Cypermethrin I-IV	5	5	0.997	-0.5	6
DDD p,p'	0.5	1	0.9974	0.1	3.2
DDE p,p'	0.5	0.5	0.9995	0.35	2.5
DDT o,p	0.5	1	0.997	0.34	2.7
DDT p,p'	0.5	5	0.9923	-0.17	2.4
Diazinon	0.5	0.5	0.9991	-0.68	2.2
Dichlorvos	0.5	0.5	0.9987	-0.11	2.1
Dieldrin	0.5	2	0.9988	0.21	2.7
Endosulfan sulfate	1	2	0.9975	0.15	1.9
Endosulphan alpha	1	2	0.9993	0.19	1.9
Endosulphan beta	1	2	0.9981	-0.64	4.8
Etofenprox	1	5	0.9982	-0.37	6.5
Fenitrothion	0.5	2	0.9943	0.49	2.4
Fenpropidin	0.5	2	0.999	0.36	8.5
Fenpropimorph	0.5	5	0.999	0.51	4.5
Fenvalerate SS,RR	0.5	2	0.991	0.31	6.7
Fipronil	0.5	0.5	0.9949	0.36	3.1
Hexachlorobenzene	0.5	1	0.9993	0.54	2.8
Iprodione	0.5	1	0.9964	0.39	5.6
Kresoxim-methyl	0.5	0.5	0.9984	0.36	3.1
Metalaxyl	0.5	1	0.9993	-0.53	3.5
Myclobutanil	0.5	2	0.9984	0.4	2.1
Oxadixyl	0.5	1	0.9985	0.46	4.6
Parathion-methyl	0.5	1	0.9974	0.73	2.2
Pendimethalin	0.5	2	0.9936	0.62	2.2
Pirimicarb	0.5	0.5	0.9992	-0.37	2
Procymidone	0.5	1	0.9984	0.58	2.3
Propazine	0.5	0.5	0.9989	-0.12	2.2
Pyrimethanil	0.5	1	0.998	0.13	1.4
Terbuthylazine	0.5	0.5	0.9989	-0.12	2.2
Tetramethrin	0.5	5	0.9948	-0.41	4
Tolclofos-methyl	0.5	1	0.9992	0.78	1.6
Trifluralin	0.5	0.5	0.9947	0.76	1.9
Tri-phenylphosphate	0.5	0.5	0.9968	-0.1	4.8
Vinclozolin	0.5	0.5	0.9991	0.9	2.1

The mass accuracy was assessed for all 51 pesticides at their LOI and the results are shown graphically in Figure 7. The mass error values did not exceed 1.2 ppm for any of the analytes, well below the guideline limit of 5 ppm delivering the highest confidence in accurate and selective detection.

In pesticide analysis, it is also essential that the instrument is able to maintain mass accuracy across the complete range of possible analyte concentrations encountered. It would not be acceptable if a high concentration pesticide violation was missed due to detector saturation. On the Exactive GC system, the Orbitrap analyzer is protected from saturation through the use of automatic gain control (AGC) which regulates the number of ions entering. This ensures that, no matter what concentration is encountered, the mass accuracy performance is preserved. This is demonstrated in Figure 8 that shows the mass accuracy for four pesticides at concentrations ranging from 0.5 to 500 µg/Kg in leek matrix is always < 1 ppm.

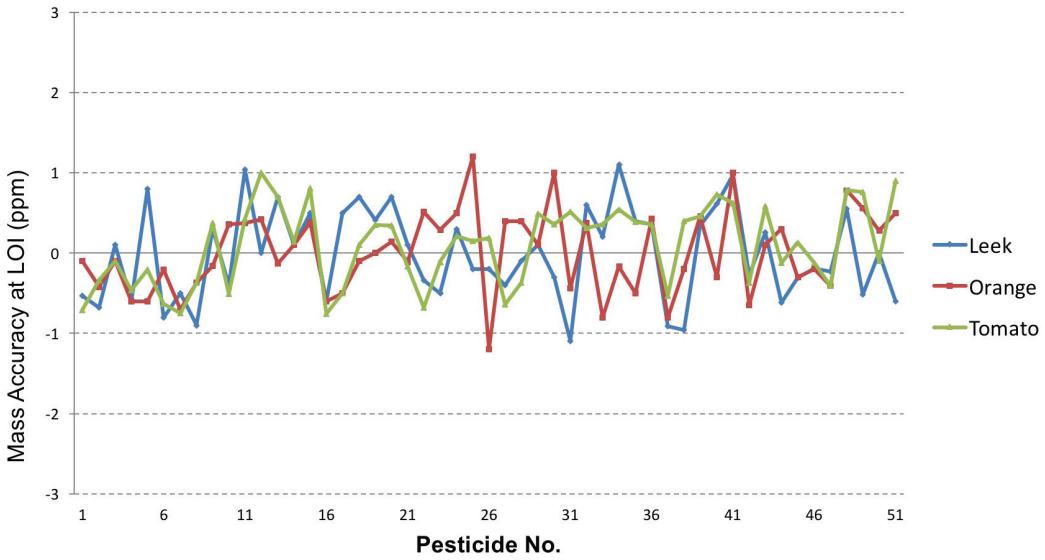

Figure 7. Mass difference measurements at the LOI level for each pesticide across the three matrices.

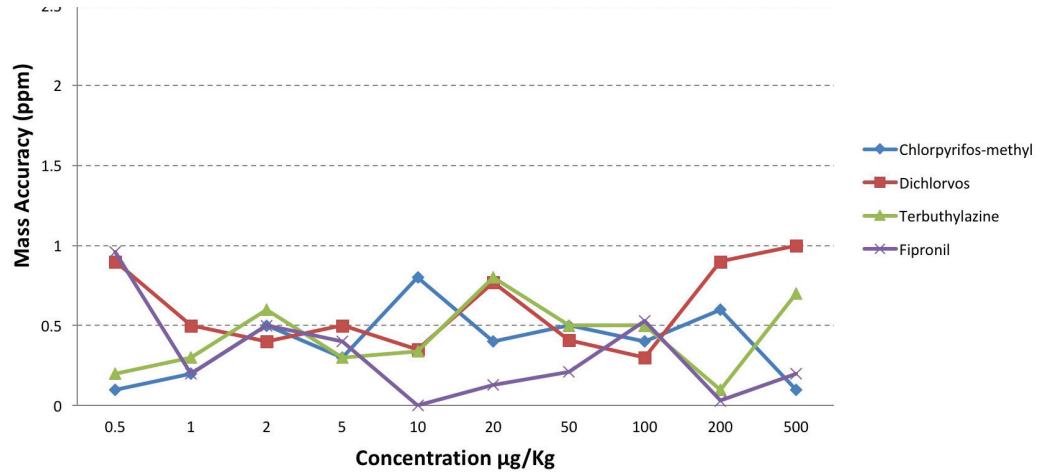

Figure 8. Mass accuracy measurements across the concentration range (0.5-500 µg/mL) for four pesticides in leek. Mass accuracy is maintained at sub 1 ppm level.

Real World Performance

In a high-throughput routine pesticide analysis laboratory, mass spectrometry instruments are in near constant operation and it is essential that they provide the same level of performance over an extended period of time. To evaluate the performance of the Exactive GC system over a longer period, a tomato extract at 10 µg/Kg was repeatedly injected (n=100) from a single vial. Prior to commencing analysis, a new injector liner was installed, the source tuned and the MS calibrated. No further interventions were made during the 66 hours of continual operation. The results showed that the system, from injector to MS, provided outstanding performance. Figure 9 shows the peak area response of hexachlorobenzene, vinclozolin and trifluralin at 10 µg/Kg in tomato over the 100 injections, with RSD% of 5.3, 4.6 and 3.8%, respectively. Furthermore, the mass accuracy stability remained <1.2 ppm (99% ≤1ppm) for the duration of the analysis without further mass calibration (Figure 10).

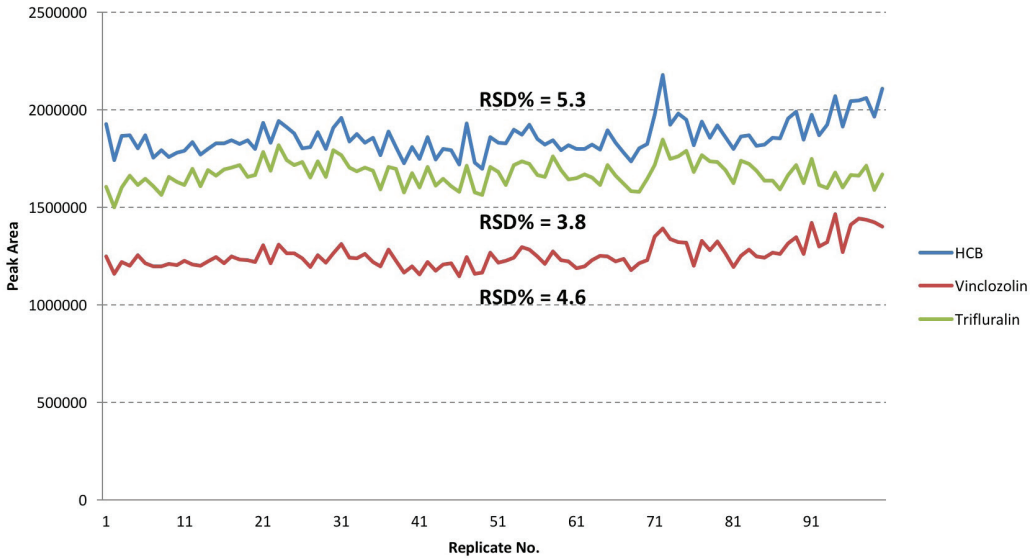

Figure 9. Repeat injections (n=100) of a tomato extract spiked at 10 µg/Kg showing that the sensitivity is maintained over the 66 hours of continual operation.

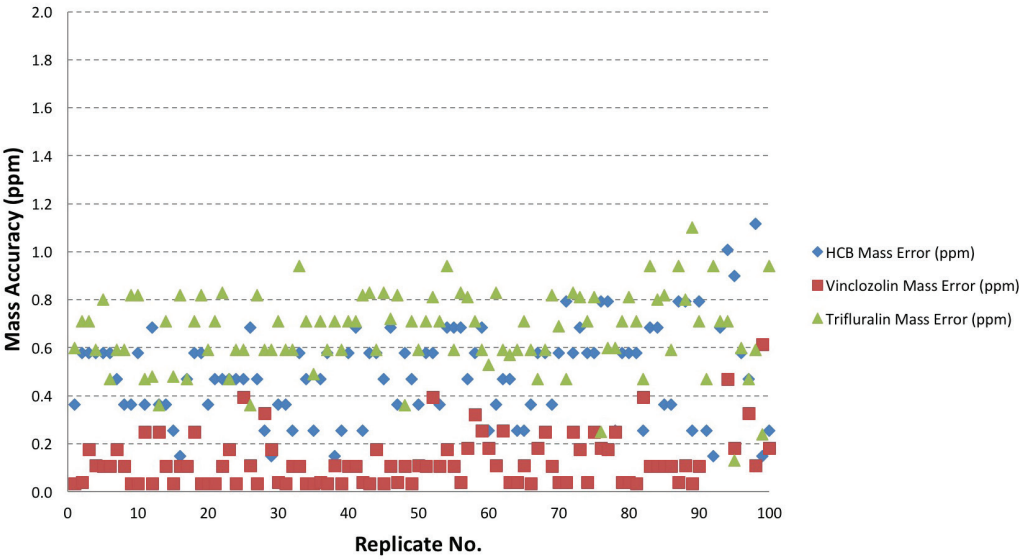

Figure 10. Mass accuracy (ppm) over 100 injections for hexachlorobenzene, vinclozolin and trifluralin in tomato extract at 10 µg/Kg. Data was acquired with same liner and without further calibration of the mass spectrometer or tuning of the source.

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Conclusions

The results of this study demonstrate that the Thermo Scientific Exactive GC Orbitrap high-resolution mass spectrometer, in combination with TraceFinder software, is a high performance analytical system that delivers robust and sensitive performance for routine pesticide analysis in fruits and vegetables in complete accordance with SANTE guidance document.

- 99.3% of the pesticide/matrix combinations were detected below the MRL with excellent linearity and meeting the required performance criteria. Importantly, the scope of the analysis is increased by acquisition in full-scan with targeted data processing with a compound database.
- Acquisition at 60,000 FWHM resolution dramatically reduces matrix interferences and increases confidence in results when screening for pesticides in complex sample matrices. Consistent sub ppm mass accuracy was achieved for all compounds over a wide concentration range ensuring that compounds are detected with confidence at low and high concentration levels.
- Repeated injections of a tomato matrix at 10 µg/Kg showed that the system is able to maintain a consistent level of performance over an extended period of time as is demanded by a routine testing laboratory.

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APPLICATION NOTE 10541

Multi-residue pesticide screening in cereals using GC-Orbitrap mass spectrometry

Authors

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Keywords

Pesticides, QuEChERS, Cereals, GC Orbitrap Mass Spectrometry, Screening, Quantitation, Accurate Mass, High Resolution, TraceFinder

Goal

To demonstrate the performance of the Thermo Scientific™ Exactive™ GC Orbitrap™ mass spectrometer for the routine analysis of GC-amenable pesticides in cereals (wheat, barley, oat, rye and rice).

Introduction

Pesticides are used to improve cereal crop yields and to minimize degradation during storage and processing. However, the widespread use of pesticides and the potential for residues to remain on the final product is of concern to consumers and to governments whose responsibility it is to ensure a safe food supply. Consequently, legislation has been introduced to protect consumers from exposure to contaminated foods.[1] Pesticide application to cereal crops is regulated by international organizations, and maximum residue levels (MRLs) are set for each pesticide/commodity combination. In the EU, if no substantive MRL has been set, a default MRL value of 0.01 mg/kg is usually applied.

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For complete coverage of the hundreds of pesticides in use, routine residue testing requires both liquid and gas chromatographic (GC) techniques coupled with mass spectrometers. Triple quadrupole mass spectrometers can provide the required sensitivity and selectivity to ensure that residue limits are not exceeded and the regulations are enforced. However, such targeted MS methods are limited to only detecting pesticides that are measured at the time of data acquisition and require careful method optimization and management to ensure selected reaction monitoring (SRM) windows remain viable. The alternative technique of high-resolution Orbitrap mass spectrometry provides distinct advantages over low-resolution MS/MS techniques and can substantially increase the scope of the analysis. With high-resolution mass spectrometry (HRMS), the default acquisition mode is untargeted (full-scan), making it simple to manage methods and allowing for a potentially unlimited number of pesticides to be monitored in a single injection. Unlike SRM acquisition on a triple quadrupole MS, high-resolution, full-scan data acquisition provides increased selectivity and enables retrospective interrogation of samples to search for emerging pesticides or other contaminants that were not screened for at the time of acquisition.[2, 3]

In this study, the performance of the Thermo Scientific Exactive GC Orbitrap mass spectrometer was evaluated for the routine analysis of GC-amenable pesticides in cereals (wheat, barley, oat, rye, and rice). The Exactive GC-MS system is routinely operated at a resolving power of 60,000 (measured at *m/z* 200 as full width at half maximum) for the detection of trace compounds against a complex chemical background as encountered in cereal sample extracts.

Experimental conditions

Sample preparation

Cereal samples (barley, oat, rice, rye, and wheat) were ground (or milled) to flour and then extracted using a citrate buffered QuEChERS procedure. The final acetonitrile extracts were acidified with 5% formic acid and diluted 1:1 with acetonitrile so that the standards and samples had the same level of matrix.

Each cereal type was spiked with 105 pesticides prior to extraction at a concentration of 100 µg/kg with five replicate extractions performed. Further dilutions of this extract were made to 10 and 20 µg/kg. These concentrations were equivalent to 5, 10, and 50 µg/L in the vial after the 1:1 dilution. For the assessment of compound linearity, a calibration series in rye matrix was prepared over the range from 10 to 300 µg/kg. The 105 pesticides included in the study cover a wide range of chemical classes and, with the five matrices, a total of 525 pesticide/matrix combinations were generated. The pesticides chosen in this study are not usually found as part of routine screening, therefore, their performance on the system was tested. The performance of more routine pesticides has been studied previously.[2,3]

Instrument and method setup

In all experiments, an Exactive GC Orbitrap mass spectrometer was used. Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column with a 5 m integrated guard (P/N 26096-1425). 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 splitless
Liner:	Siltek 1, splitless six baffle PTV liner (P/N: 453T2120)
Inlet (°C):	70
Split Flow (mL/min):	50
Transfer Rate (°C):	2.5
Final Temperature (°C):	300
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):	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):	280
Ionization type:	EI
Ion Source (°C):	250
Electron Energy (eV):	70
Acquisition Mode:	Full-scan
Mass Range (Da):	50–600
Resolving Power (FWHM at *m/z* 200):	60,000
Lockmass, Column Bleed (*m/z*):	207.03235

Data processing

Data were acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. For targeted analysis, a customised compound database contained the 105 compound names, accurate masses for quantification and identification ions, retention times, and elemental compositions of fragment masses. For the generation of extracted ion chromatograms, an extraction mass window of ±5 ppm was used.

Results and discussion

The objective of this study was to screen for 105 pesticides in five replicate extractions of different cereal matrices with a high degree of confidence. The lowest concentration at which each pesticide could be detected was to be determined. Further assessments of mass accuracy, linearity in matrix, and repeatability are also reported.

The five sample types chosen provided both typical and difficult matrices that are encountered in routine cereals testing. The full-scan total ion chromatograms shown in Figure 1 illustrate the high complexity and diversity of the different cereal samples. This is one reason why high-resolution, accurate-mass mass spectrometry is required to selectively extract target analytes from background chemical noise. In comparison to most fruit and vegetable samples, cereals have a high fat content that results in heterogeneous extracts when generic extraction techniques are used. The low selectivity of the QuEChERS sample extraction approach needs to be compensated for by selective instrumental analysis. On the Exactive GC, this is achieved using high mass resolving power. This capability, in combination with a full-scan acquisition, increases the scope of the analysis without the need for optimization of acquisition parameters, as is the case with targeted analyses.

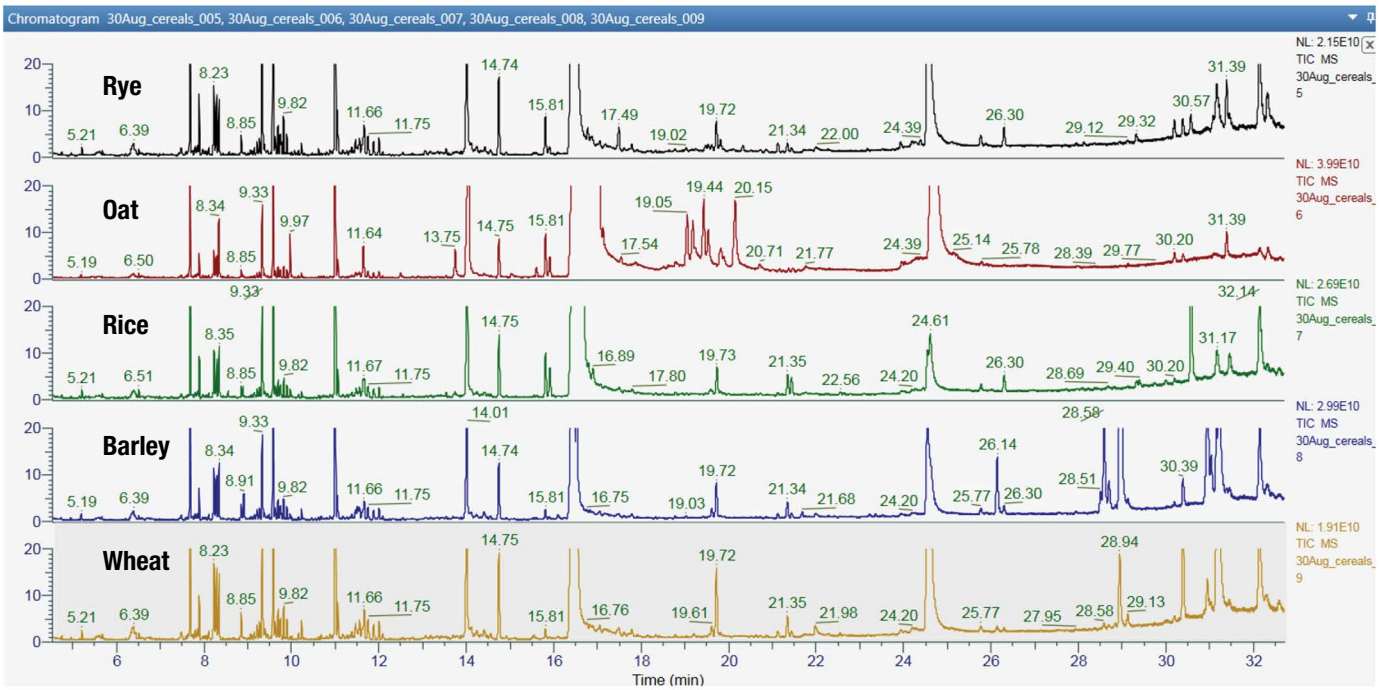

Figure 1. Full-scan total ion chromatogram (TIC) with zoomed Y axis of cereal extracts showing the complexity of the sample matrices used in this study.

The primary aim of the analysis was to determine how many of the fortified pesticides could be detected at each of the concentration levels (10, 20, and 100 µg/kg). For a positive detection, the following criteria based on SANTE guidelines[4] had to be satisfied:

1. Two ions detected for each pesticide with mass accuracy < 5 ppm and peak S/N > 3.
2. Retention time tolerance of ± 0.1 minutes compared with standards in the same sequence.
3. Ion ratio within ±30% of the average of calibration standards from the same sequence.

Intelligent data processing

TraceFinder software provides automated data acquisition and processing that quickly extracts and displays the identification information for all 105 spiked pesticides in approximately 20 seconds per sample file (0.75 GB). The software enables the analyst to rapidly review the data and to confidently confirm the presence of a pesticide. As Figure 2 shows, the analyst is presented with a traffic light system alongside raw data to show which identification criteria have been satisfied. More importantly, it will also flag when a parameter is outside of expected tolerance and alert the analyst to carefully review all of the available information before making the final decision to confirm a positive identification. In the example in Figure 2, the ion ratio of one of the fragment ions of isocarbophos in oat sample A (46.7%) is just outside the allowable ratio window of 48–89% due to peak integration. This is flagged to the analyst by a red square in the ion ratio (IR) column. By hovering over this square, further details are displayed. In this case, isocarbophos can be confirmed despite this flag as the other criteria are met and alternative fragment ion ratios are within the 30% tolerance. The multiple identification points provided by full-scan analysis along with user friendly software enables a faster time to result, which is vital in routine pesticide analysis.

Following the criteria listed previously, the lowest concentration level at which each pesticide was detected and confirmed in each of the five matrices is summarized in Figure 3. Of the 525 pesticide/matrix combinations, 90% were confirmed at ≤ 10 µg/kg and 96% at ≤ 20 µg/kg. Having multiple identification points and

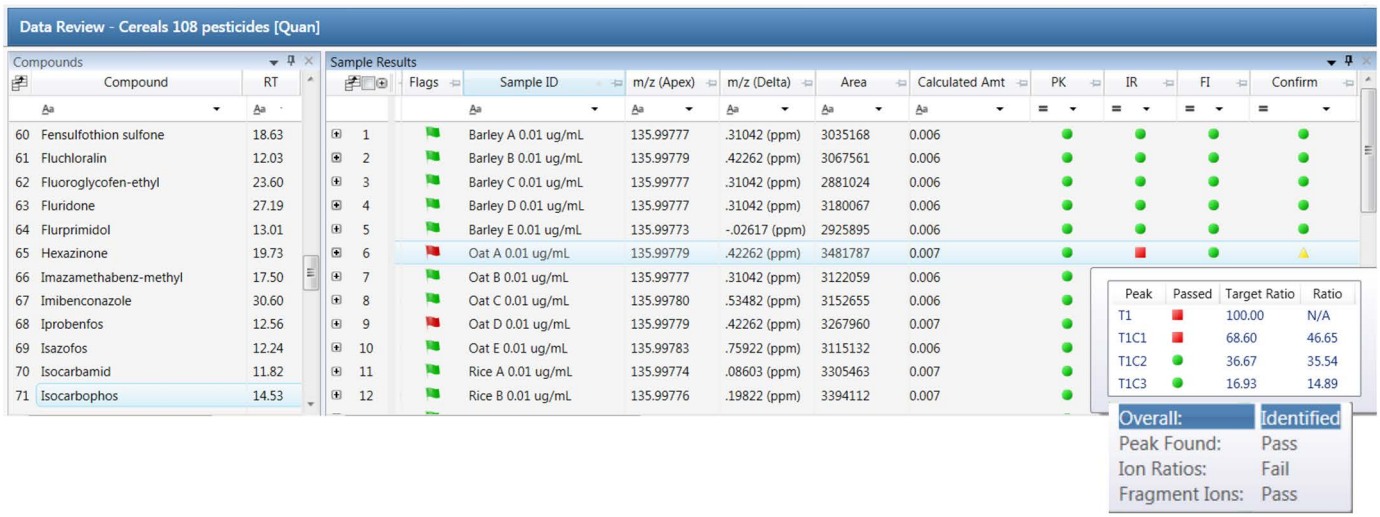

Figure 2. TraceFinder software browser enables fast data review and confirmation. The software quickly points the analyst to the data that supports a positive identification using a traffic light system along with real data values. More importantly, it will flag when a parameter is outside of tolerance, and by what value, and allow the analyst to make the final decision to confirm an identification. Hovering above the red square (below) brings up further details.

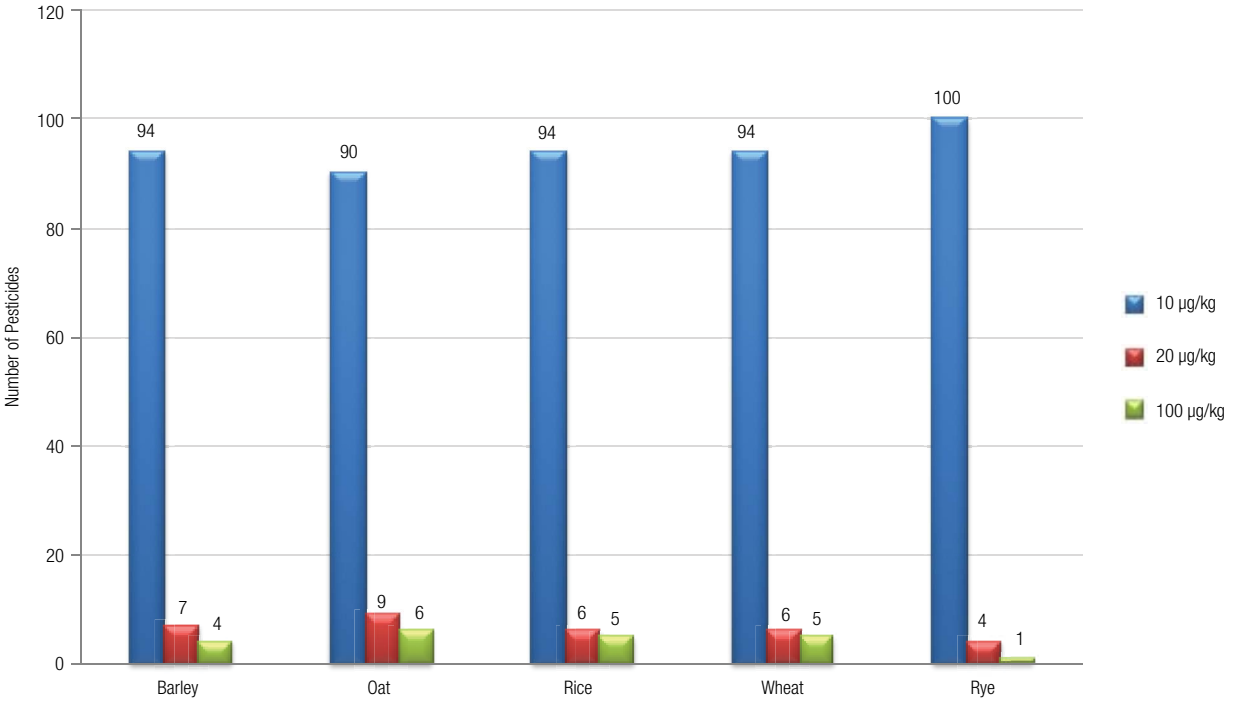

Figure 3. The lowest concentration confirmed (two ions within 5 ppm, ion ratios within ±30%) for each pesticide in each of the five sample matrices. The total number of pesticides is 105.

limits of detection below the MRL increases the confidence in positive detections. This also minimizes the risk of false negative results and ensures that the limits of false positive detects are at a manageable level within a routine environment. All 105 pesticides were detected at concentrations lower than 10 µg/kg (5 µg/L in vial) if screened based on retention time and the main quantifier ion. The limiting factor for confirmed identification in the case of a few analytes was the sensitivity of additional ions that were much lower in intensity compared to the main ion. As the criteria applied here has shown, using electron ionization (EI) in combination with full-scan acquisition provides the opportunity to use multiple diagnostic ions for the identification of pesticides. In addition to individual ions, compound spectra can be used to confirm identifications. The Exactive GC generates standard EI spectra that are highly reproducible and library searchable (using nominal- or high-resolution MS libraries commercially available or custom made). An example of spectral matching with NIST 2014 for the pesticide mexacarbate (SI 905) is shown in Figure 4.

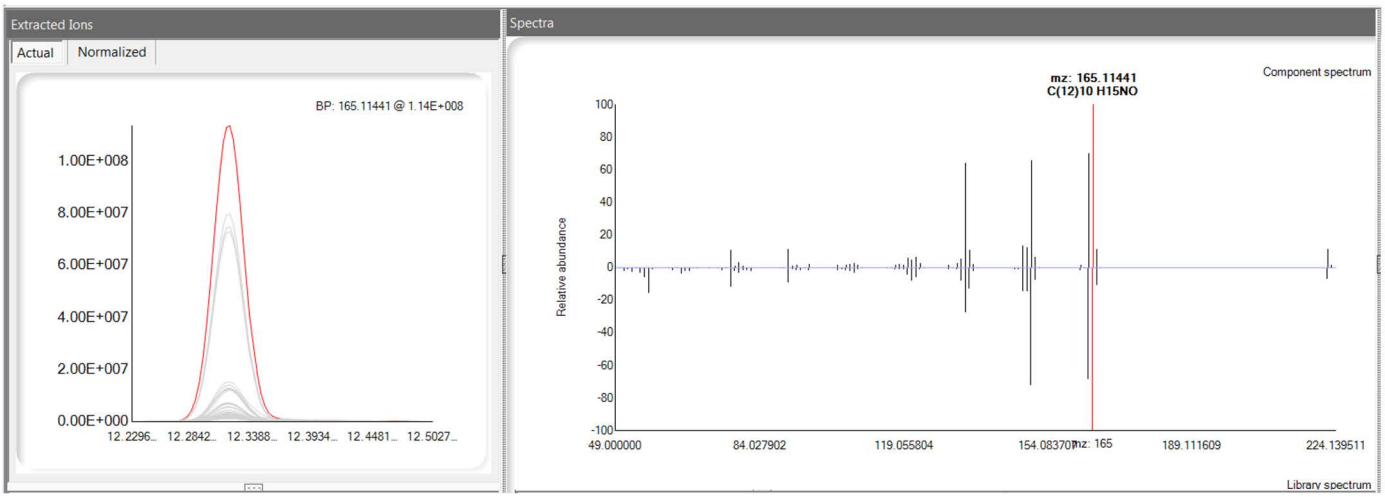

Figure 4. TraceFinder software deconvoluted peaks (left). Acquired spectrum and library spectrum (right) for mexacarbate with search index score of 905.

True mass accuracy

Acquiring reliable accurate mass measurements is critical when detecting pesticide residues at low concentrations in complex sample matrices. Low mass errors ensure that compound selectivity is high and that detection and indentification are robust. The low mass errors (ppm) observed with the Exactive GC are achieved through the high mass resolving power that can discriminate between matrix interferences and target analyte ions. Internal mass correction enables mass accuracies of ≤ 1 ppm to be consistently achieved regardless of analyte concentration or matrix complexity. As an example, the mass accuracy of all detected pesticides in wheat at 10 µg/kg is shown in Figure 5. All pesticides are detected with sub-1 ppm mass accuracy, well below the guideline limit of 5 ppm (< 1 mDa for *m/z* < 200), delivering the highest confidence in accurate and selective detection. The low mass accuracy also allows for tighter tolerances to be applied for extracted ion chromatograms, which will result in fewer false positive detects thus increasing efficiency by reducing the need for manual review.

When the mass resolution is insufficient, it can result in target ions that have a mass accuracy outside of the required identification criteria. This is demonstrated in Figure 6 where the oat 20 µg/kg matrix sample was analyzed at resolving powers of 15K, 30K, and 60K. The zoomed mass spectra show the quantifier ion for tribufos. At 15K and 30K, the *m/z* 201.97042 ion demonstrates poor mass resolution resulting in mass accuracies of 6.4 and 3.7 ppm, respectively. However, the ion is well resolved at 60K resulting in the expected sub-1 ppm mass accuracy. At 15K this pesticide would have failed the identification criteria of < 5ppm and would have been reported as not detected.

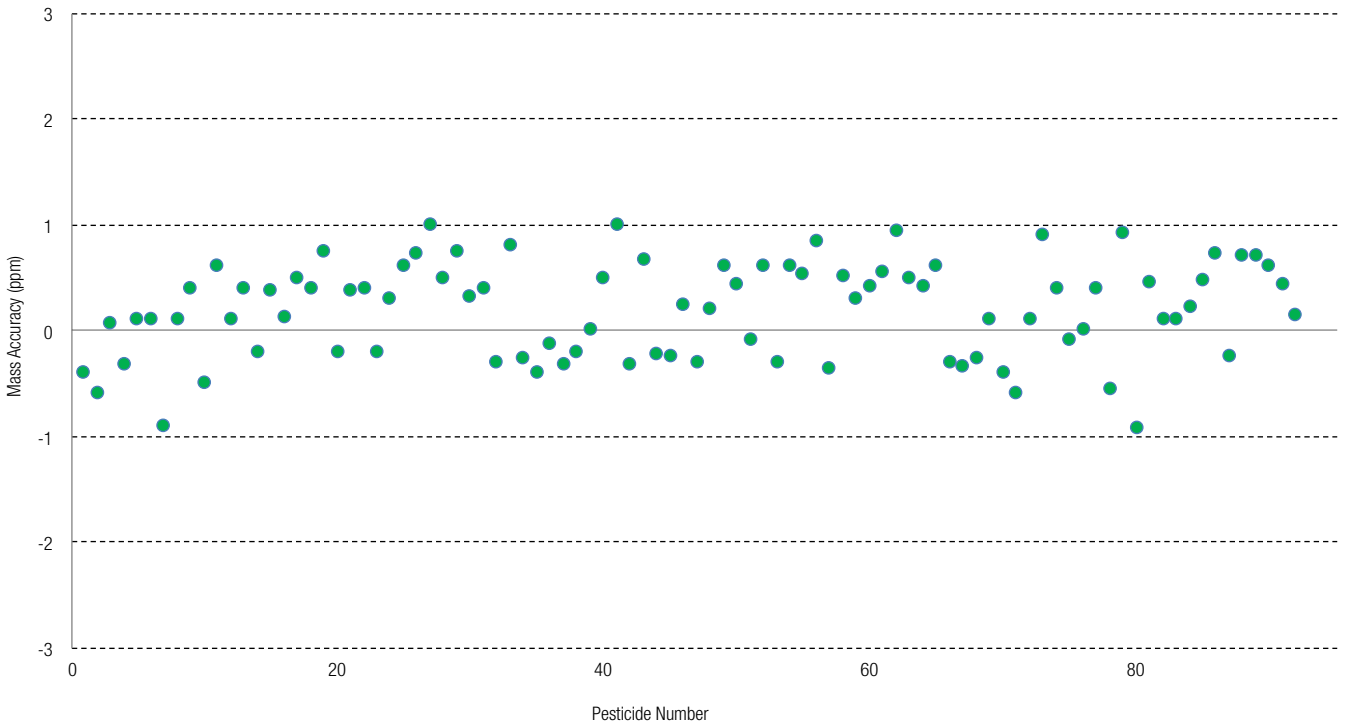

Figure 5. Mass difference measurements at 10 µg/kg for each pesticide in wheat.

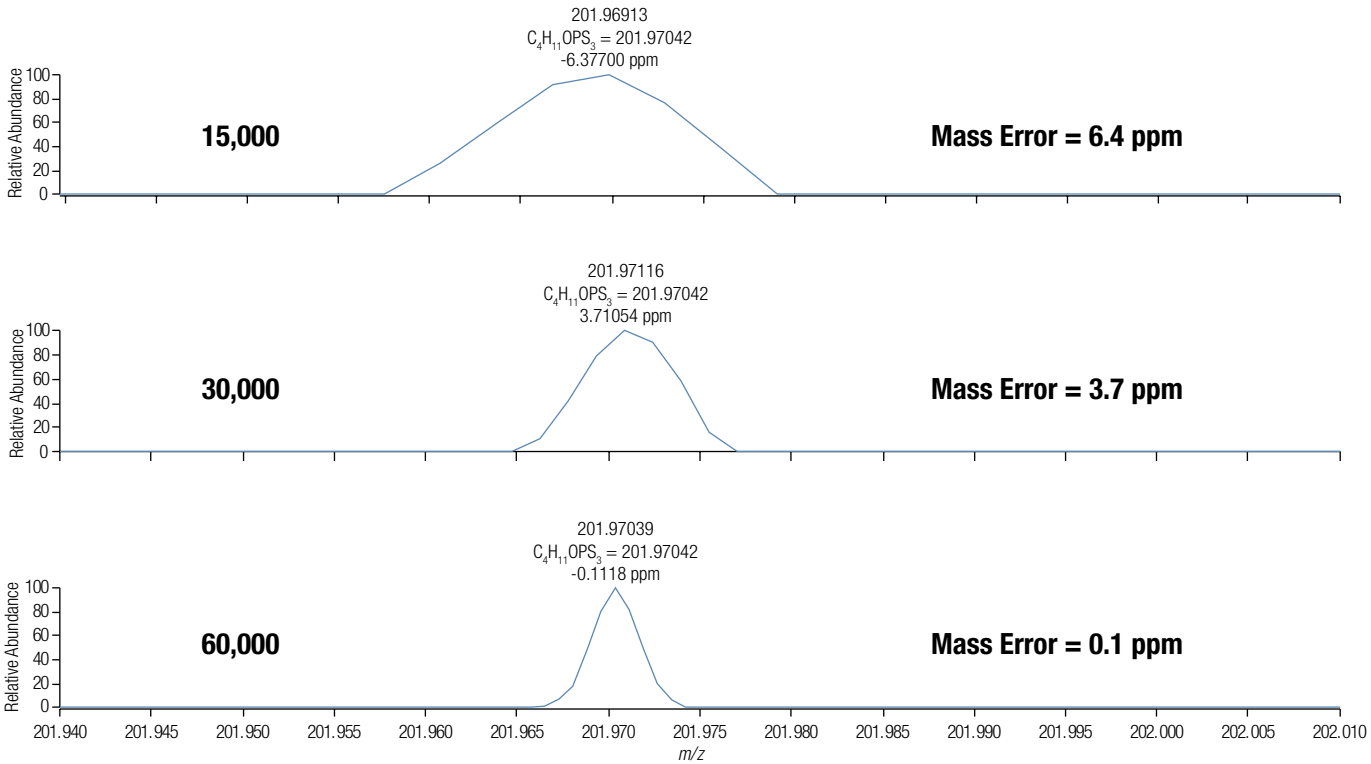

Figure 6. Effect of resolving power on mass accuracy of diagnostic ion (*m/z* 201.97042) tribufos at 20 µg/kg in oat acquired at different resolutions of 15K, 30K, and 60K.

Robust quantitative performance

Having reliably identified a pesticide in a sample, the final stage is to determine its concentration. The Exactive GC quantitative linearity was assessed using matrix matched standards in rye across a concentration of 10–300 µg/kg. In all cases, the coefficient of determination (R^2) was > 0.99 for each pesticide from its LOD value to 300 µg/kg. An example of the TraceFinder software quantification results browser showing dichlorprop methyl ester is given in Figure 7.

A final assessment was made of the peak area repeatability at low analyte level by running n = 20 replicate injections at 10 µg/kg in wheat. All detected pesticides had RSD% of less than 13%, (Figure 8). This shows that the Exactive GC operated in full-scan at 60k resolution has the selectivity and sensitivity required for robust and reliable routine anlysis of pesticides residues at or below the MRLs in a range of different types of cereal samples.

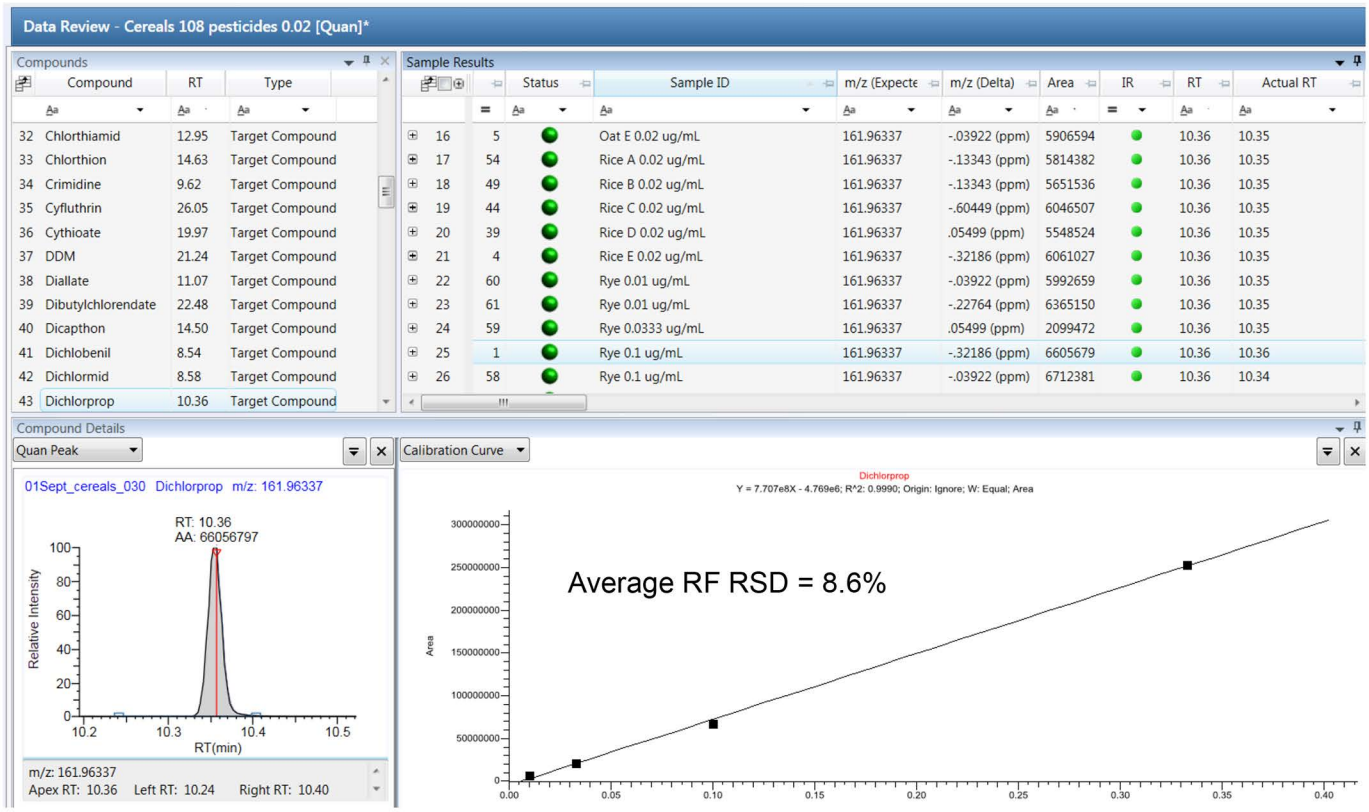

Figure 7. TraceFinder software browser showing positively identified pesticides, extracted ion chromatogram, and calibration graph (dichlorprop methyl ester as an example). Sub-ppm mass accuracy for dichlorprop across the calibration range and in replicates of 20 mg/kg. Identification criteria information is available and flagged when out of tolerance for quick data review.

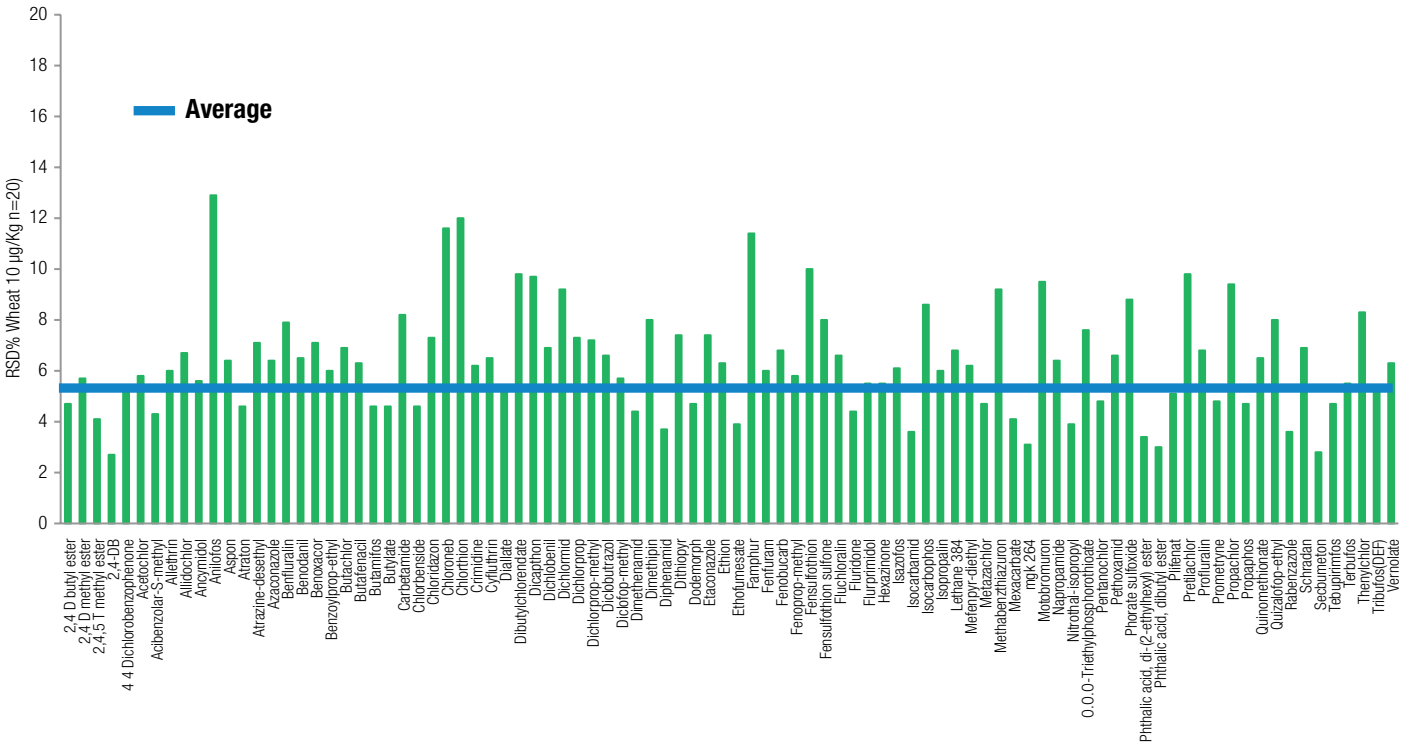

Figure 8. Repeatability (%RSD) for 10 µg/kg (n=20) for each pesticide in wheat.

Conclusions

The results of this study demonstrate that the Exactive GC Orbitrap high-resolution mass spectrometer, in combination with TraceFinder software, delivers robust and sensitive performance for routine pesticide analysis in cereals to regulatory standards.

- All 105 pesticides were detected at 10 µg/kg (5 µg/L in vial). 96% of the 525 pesticide/matrix combinations were confirmed at < 20 µg/kg (< 10 µg/L in vial) with excellent linearity, and in full compliance with the EU SANTE method performance criteria.
- The full scan acquisition permits efficient targeted data processing by use of a compound database and has the capability to easily add further analytes into the method scope.
- Intelligent software allows for results to be reviewed and detections confirmed in an efficient manner.
- Consistent sub-ppm mass accuracy was achieved for all compounds over a wide concentration range, ensuring that compounds are detected with high confidence at low and high concentration levels.
- Repeated injections of a wheat matrix at 10 µg/kg showed that the system is able to maintain a consistent level of performance over an extended period of time as is demanded by a routine testing laboratory.

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High Efficiency, Broad Scope Screening of Pesticides Using Gas Chromatography High Resolution Orbitrap Mass Spectrometry

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Application Note 10448

Keywords
Accurate Mass, Complex Matrices, GC Orbitrap Mass Spectrometry, Pesticide Analysis, QuEChERS, Screening, TraceFinder Software

Introduction

Pesticides are used globally to improve the production and yields of agricultural crops and their use is essential to ensure a sufficient global food supply. However, this widespread use of pesticides and the potential for them to remain in the final product is of significant concern to consumers and to governments whose responsibility it is to ensure a safe food supply. Consequently, legislation exists to protect consumers from exposure to contaminated foods. This legislation requires that foods are monitored for both the type and quantity of the pesticide present, with each pesticide given a maximum residue limit (MRL) in a particular sample commodity. The list of compound and sample combinations is extensive, creating a challenge for accurate and reliable routine monitoring.

Laboratories are under ever-increasing pressure to screen samples for pesticides in a single analysis, with a fast turnaround time and at a competitive cost. Most existing laboratories rely on targeted analytical approaches using both gas chromatography and liquid chromatography coupled to mass spectrometry instrumentation. These techniques cover the wide range of chemical classes that need to be monitored and at the required levels of sensitivity and selectivity. However, they are limited to only those compounds in the target list, which are usually selected based on the residue definition and legislation requirements to demonstrate that the food is fit for consumption. These techniques require careful optimization of acquisition parameters for each compound and the monitoring of acquisition time windows to ensure detection of the analyte.

To increase the scope of the analysis, chemical screening methods using high-resolution, full-scan mass spectrometry have received significant attention in recent years. These methods use non-targeted acquisition, in which a generic full scan acquisition is run, followed by targeted data processing of a list of compounds within a database.

Although data interrogation is performed against a list of target compounds, retrospective data analysis is possible in order to identify new compounds that were not screened for at the time of acquisition. For this approach to be used in routine analysis, screening data processing software needs to be fast and accurate enough to detect residues at low concentrations with an acceptably low level of false negative results, as described in the European Union guidelines.[1] There is no recommendation for the number of false positives, but it is necessary for routine laboratories to keep this number as low as possible to minimize the time required for additional investigation. The majority of samples that pass through a laboratory are compliant with the legislation. Therefore, it is efficient to quickly screen compliant samples from those that are suspected to be contaminated. Following an initial screen, the suspect positive samples are reanalyzed using a second confirmatory method (e.g., GC-MS/MS) to confirm suspect positives and to accurately determine the concentration of the pesticide present. The confirmatory analysis contains a complete calibration series in an appropriate matrix that is not included in the screening analysis.

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In this study, we evaluate the performance of the Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer (MS) for the accurate screening of GC-amenable pesticides. The Q Exactive GC Orbitrap MS provides high mass resolving power up to 120,000 (*m/z* 200) full width half maxima (FWHM) to facilitate highly accurate mass measurements and to enable confident discrimination of co-eluting and isobaric compounds in complex samples. Fast scan speeds and a high intrascan dynamic range (>5000) facilitate the detection of trace compounds in the presence of high matrix components.

Experimental Conditions

Sample Preparation

Food and feed samples were extracted following an acetate buffered QuEChERS-based approach. Briefly, 10 mL of acidified (1% acetic acid) acetonitrile was added to 5 g (cereals/feed) or 10 g (fruit/vegetables) of homogenized sample. A mixture of salts was added and the centrifuge tube shaken and spun. The final acetonitrile extracts (0.5 or 1 g/mL in acetonitrile) were fortified with a mixture of 55 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb). A variety of difficult sample matrices were studied including wheat, leek, and horse feed.

Instrument and Method Setup

In all experiments, a Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler and chromatographic separation was obtained using a Thermo Scientific™ TRACE™ 1310 gas chromatograph (GC) and a Thermo Scientific™ TraceGOLD TG-5SilMS™ 15 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N: 26096-1301).

Additional details of instrument parameters are displayed.

GC and Injector Conditions	
TRACE 1310 GC Parameters	
Injection Volume (µL):	1
Liner:	Asymmetric baffled (P/N: 45352062)
Inlet (°C):	75
Inlet Module and Mode:	PTV, cold splitless
PTV Transfer delay (min):	1
Injection time (min):	0.1
Transfer rate (°C/sec):	2.5
Transfer temperature (°C):	300
PTV Transfer time (min)	3
Cleaning rate (°C/sec):	330
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	180
Rate (°C/min)	25
Temperature 3 (°C):	300
Rate (°C/min)	100
Hold Time (min):	3

Mass Spectrometer Conditions	
Q Exactive Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source(°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (Da):	50–500
Resolving power (FWHM):	60,000
Lockmass (*m/z*):	207.03235

The Q Exactive GC system was operated in EI full scan mode using 60,000 (FWHM *m/z* 200) resolving power. Additional experiments were run at different resolution modes of 15K, 30K, and 120K. Chromatographic data was acquired with a minimum of 11 points/peak to ensure consistent peak integration.

Data Processing

Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software. This single software package integrates instrument control, method development functionality, and qualitative-screening and quantitation-focused workflows.

Results and Discussion

The objective of this study was to screen for a wide range of pesticides in different sample matrices with the highest level of confidence. The aim of the analysis was to determine if a pesticide is present in a sample above the lowest MRL, which is typically 10 ppb. This assessment was made by screening fortified wheat, horse feed, and leek extracts spiked at different concentrations to determine their limits of detection for screening under the conditions described. These matrices were selected because they are known to be highly complex and challenging matrices for pesticide analysis, as is shown in the total ion chromatograms in Figure 1.

The sample extraction techniques used in routine pesticide analysis are very generic (e.g., QuEChERS) and produce highly complex and variable solutions. The lack of selectivity in sample preparation stages has to be made up for by selectivity in the instrumental analysis. This selectivity can be achieved using high mass resolving power and high mass accuracy. As sample types increase in complexity, the resolving power of the mass spectrometer becomes a key factor in reliable pesticide detection. This resolving power has already been demonstrated for the analysis of LC-amenable pesticides.[2] Furthermore, high-resolution, full-scan analysis increases the scope of the analysis without the need for optimization of the acquisition parameters.

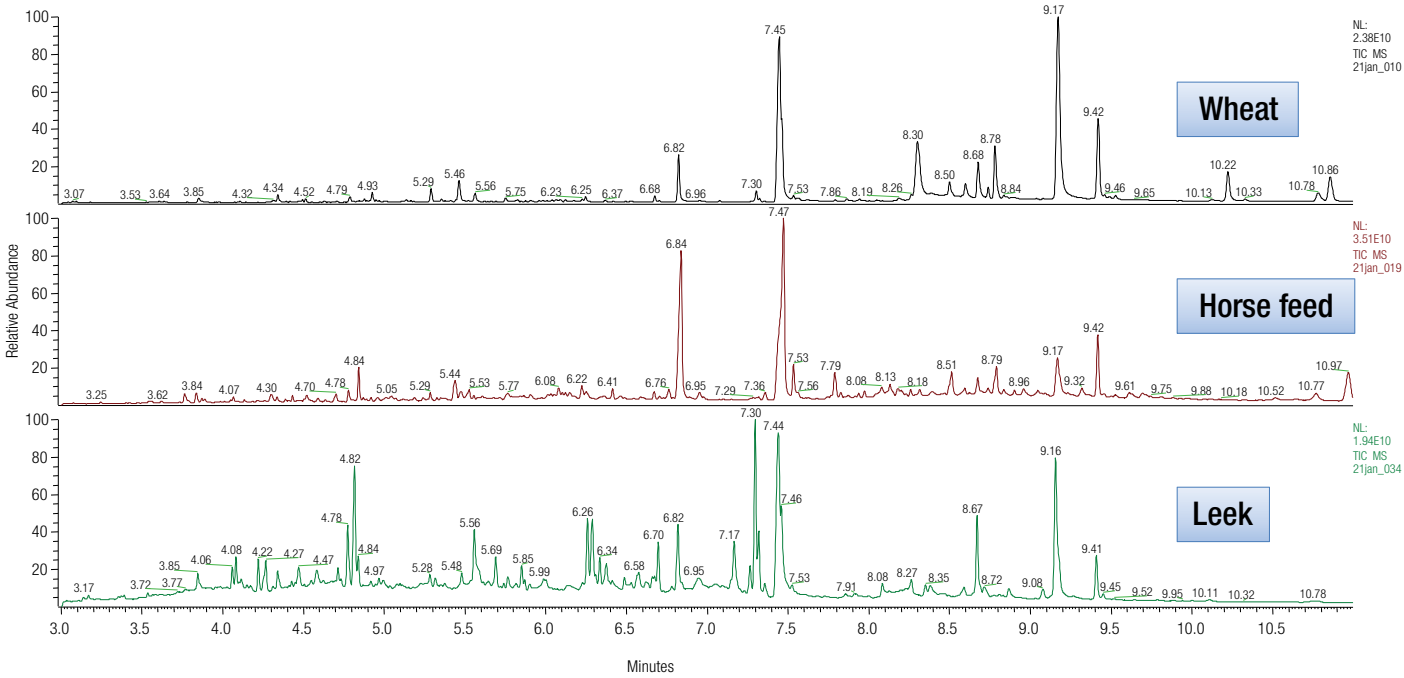

Figure 1. Full scan total ion chromatogram (TIC) for 55 pesticides spiked into wheat, horse feed, and leek extracts showing the complexity of the samples.

Sample Throughput

Sample throughput is a key consideration in pesticide analysis. As such, a fast chromatographic method was used to test the system under typical conditions. This method resulted in a complete analysis within 17 minutes (injection to injection), enabling up to 84 analyses to be performed within a 24 hour period. Although this is a fast GC method, the scan speed of the mass spectrometer provided a minimum of at least 11 points/peak. Figure 2 shows the peak for diazinon with 11 points across the 1.8 second peak.

Screening

Following full scan analysis at a mass resolution of 60,000, TraceFinder software was used to process the data. An in-house database of 183 pesticides, containing information for formula, accurate mass, retention time, isotopic pattern (via formula of diagnostic ion), and fragments was used to screen the samples. Although all parameters can be used for identification, the criterion used by the software for a positive identification was that a peak must be observed in the extracted ion chromatogram (XIC) of the main diagnostic ion at the expected retention time within ± 20 seconds, and the exact mass of the ion should be within ± 2 ppm of the theoretical value.

Pesticide detection can be confirmed by assessing the retention time and mass accuracy of the fragment ions as well as the isotopic pattern fit. The inclusion of these parameters increases the confidence in the detection and reduces the number of false positives.

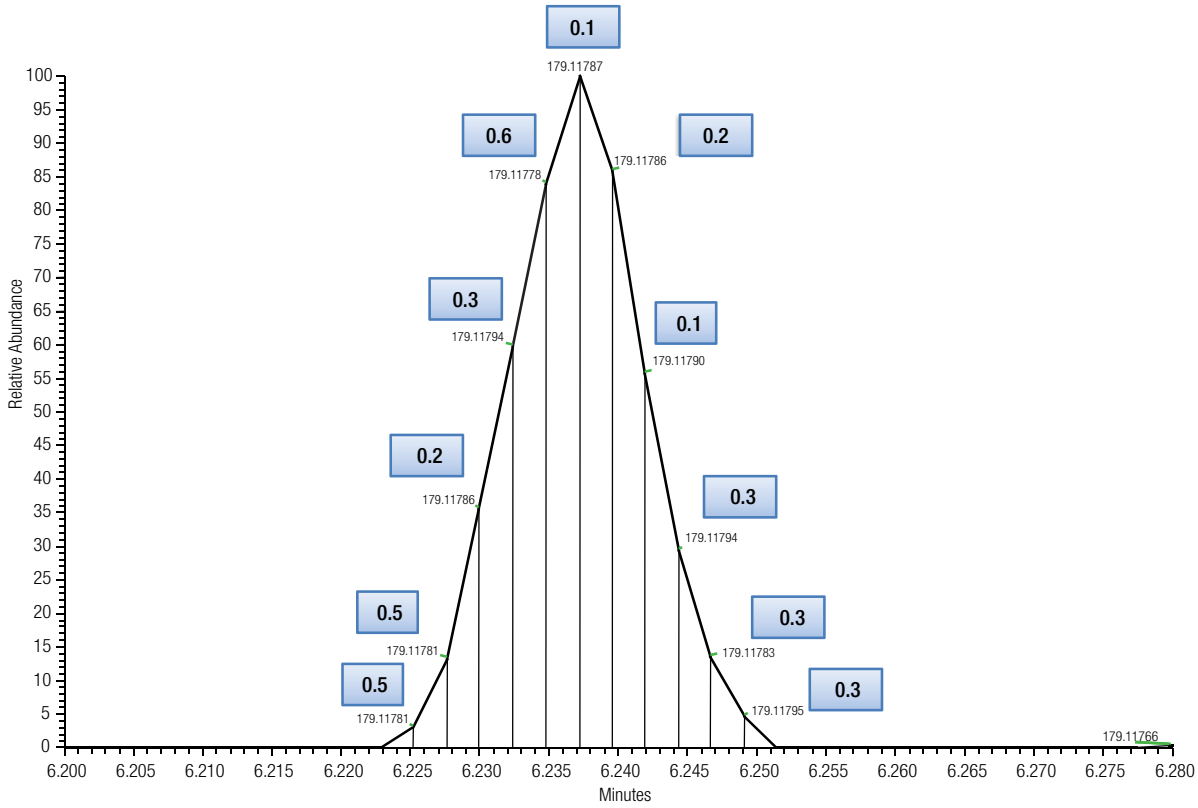

Figure 2. Extracted ion chromatogram (XIC) of diazinon (*m/z* 179.11789 ± 5 ppm mass window) in wheat spiked at 10 ng/mL showing ~11 scans/peak (peak width 1.8 sec). Data acquired in full scan at 60,000 FWHM at *m/z* 200 resolving power. Excellent accurate mass is shown for each individual scan as well as mass difference (in ppm). Average mass difference of 0.3 ppm across the peak.

Screening Software

The processing software is critical to the successful implementation of routine screening. TraceFinder software was used to quickly screen the data for the presence of the target pesticides. A target compound database was used to detect and report the pesticides found and to indicate which criteria were satisfied. Figure 3 shows an example TraceFinder browser window for some of the detected pesticides in wheat spiked with 10 ng/mL. The pesticide p,p'-DDT, which has been detected and confirmed based on retention time, accurate mass (0.21 ppm), fragment, and isotopic match is highlighted in the summary window. The data is displayed to the user in a traffic light system that enables quick review of the data. More detailed information is available in the summary columns and in the window panes, showing in this example the XIC and the measured and theoretical isotopic pattern for p,p'-DDT. The exceptional accurate mass provided by this system, even in complex matrices, enables compounds to be detected with a high degree of confidence. All pesticides are screened at < 2ppm and, as shown in Figure 3, the accurate mass is typically sub ppm. This specificity of accurate mass for both the main diagnostic ions and fragments enables the false detects to be screened out automatically or quickly assessed by the user.

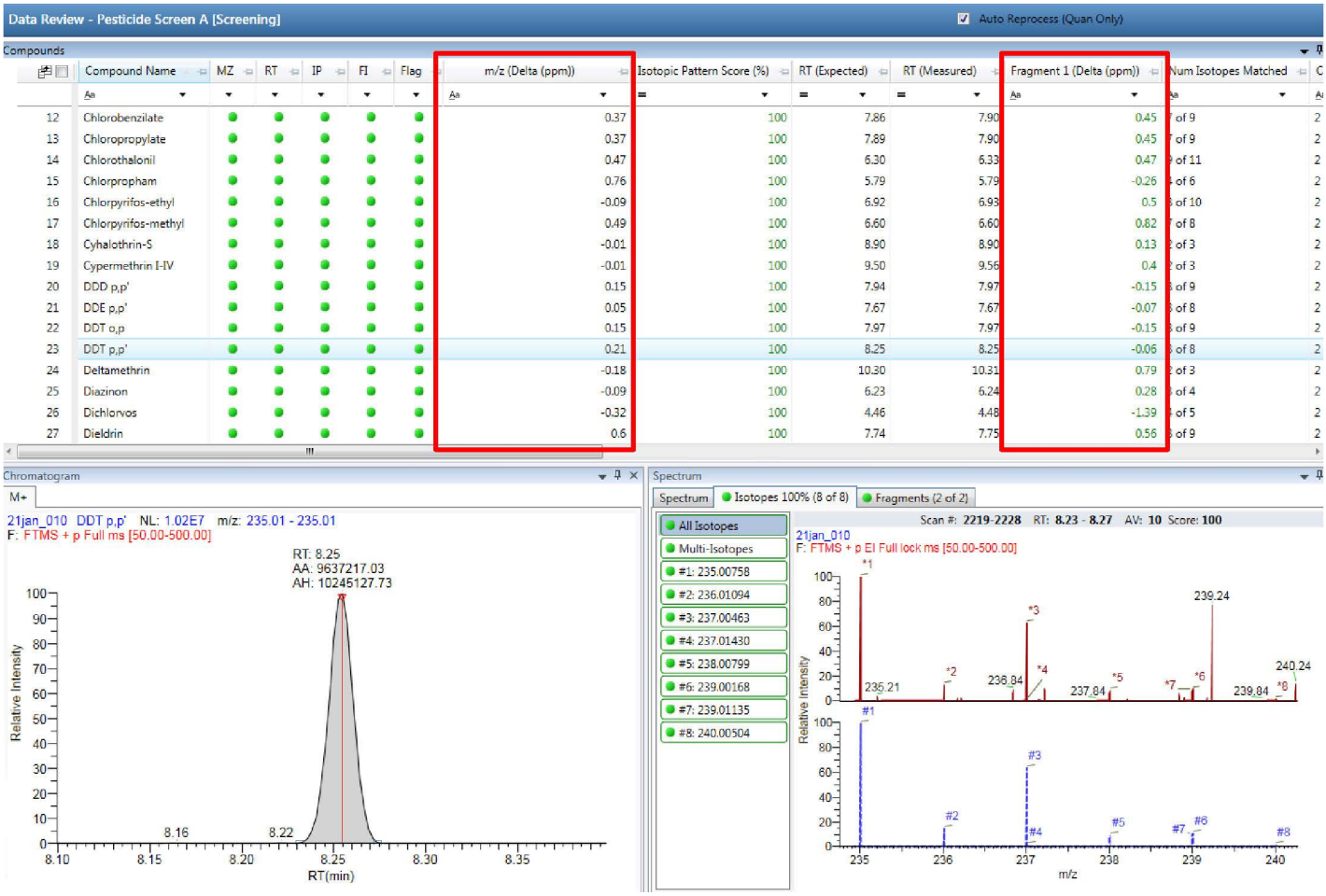

Figure 3. TraceFinder screening browser showing positively identified pesticides (p,p'-DDT as an example) in wheat spiked at 10 ng/mL, based on accurate mass confirmation (± 2 ppm mass window), retention time (RT), isotopic pattern (IP), fragment ions (FI). Sub-ppm mass accuracy for both main and confirmatory ions is highlighted in red boxes.

Screening Below MRL

In this study, all 55 pesticides were detected in the wheat, horse feed, and leek samples when spiked with 10 ng/mL. The majority of pesticides were detected at much lower concentrations. As shown in Figures 4 and 5, 53 pesticides were detected at a concentration of < 2.5 ng/mL in wheat matrix with 47 detected in the 0.5 ng/mL spiked extract. This excellent sensitivity in complex matrices makes confident screening at, or even below, the MRL a unique feature of the Q Exactive GC system.

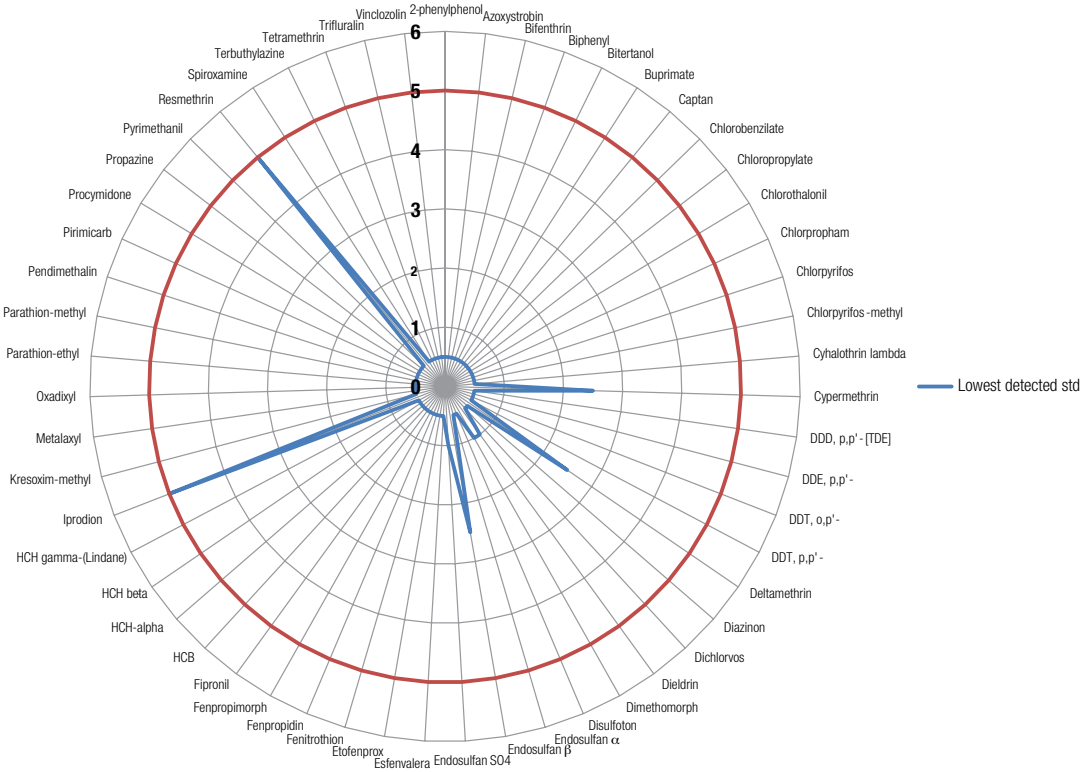

Figure 4. Graph showing the lowest detected standard for 55 pesticides in wheat. Identification based on accurate mass < 2ppm and retention time ± 20 seconds. 5 ng/mL level displayed.

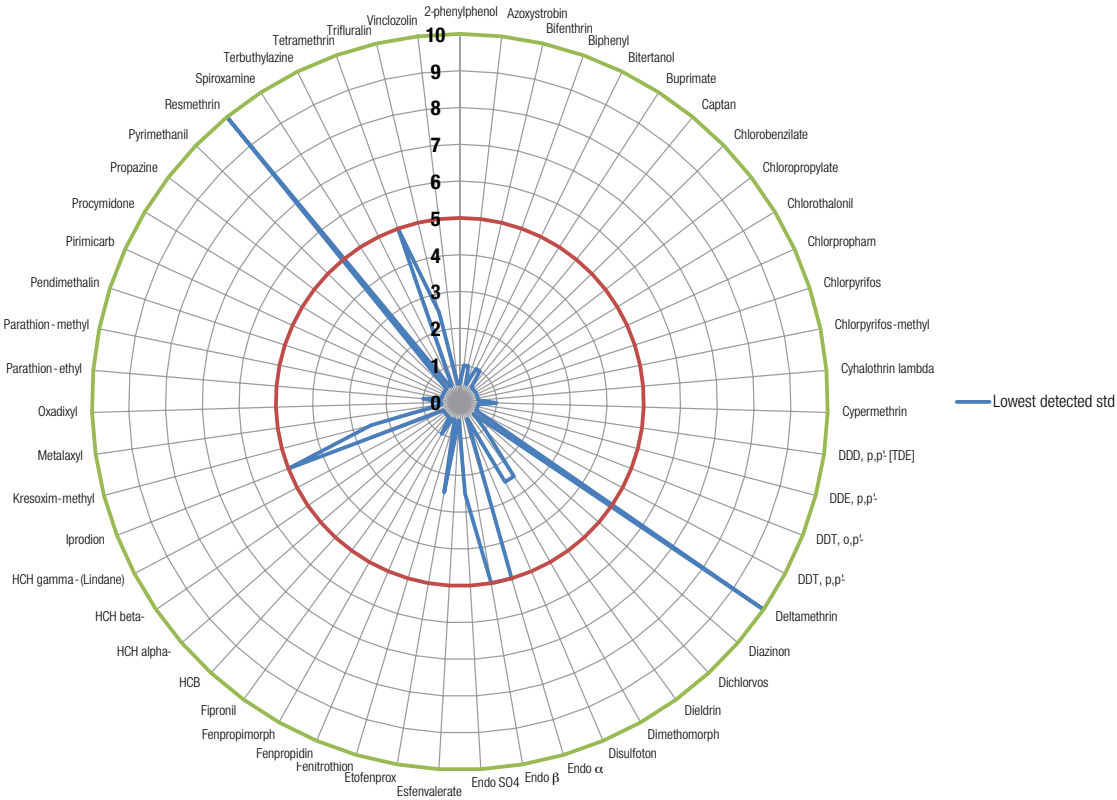

Figure 5. Graph showing the lowest detected standard for 55 pesticides in horse feed. Identification based on accurate mass < 2ppm and retention time ± 20 seconds. 5 ng/mL and 10 ng/mL levels highlighted.

Avoiding False Negatives Using Resolving Power

The use of a narrow mass accuracy tolerance is possible only when the resolving power is sufficient to isolate target compounds from matrix interferences or other target compounds. When two mass profiles overlap, the measured mass profile is the sum of the two individual profiles. This summed profile results in the incorrect assignment of the mass of the target compound. This phenomenon is demonstrated in Figure 6, where the leek sample was analyzed four times at resolving powers of 15K, 30K, 60K, and 120K. The mass spectra show a diagnostic ion of chlorpropham and a background matrix ion at a similar mass, resulting in interference. The expected mass accuracy was achieved at 60K and 120K with near baseline resolution. However, at 15K and 30K, chlorpropham was not resolved from the interference, resulting in poorer mass accuracy. At 15K, the mass accuracy is significantly affected with a value of 18.4 ppm mass difference. Under the screening criteria used in this study, and even under a wider tolerance of 10 ppm, this peak would have resulted in a false negative for chlorpropham. This result shows that a minimum resolving power is needed. The required minimum resolving power depends on the complexity of the sample being analyzed and the concentration of both target analytes and interferences.

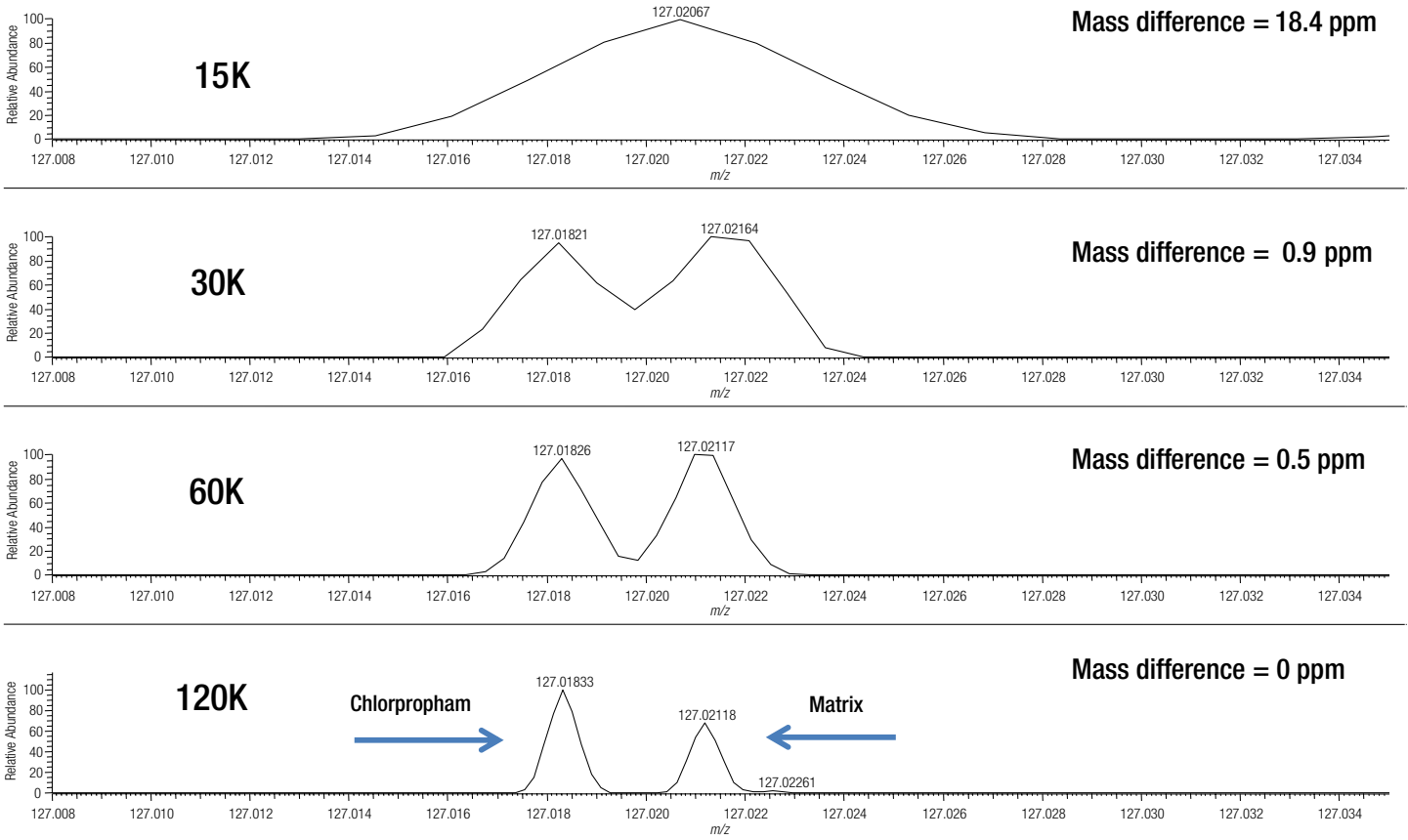

Figure 6. Effect of resolving power on mass accuracy of an analyte in matrix. Mass profiles of a diagnostic ion of chlorpropham at 10 ng/mL in leek, acquired at different resolutions of 15K, 30K, 60K, and 120K. At 15K and 30K the chlorpropham ion is not resolved from matrix interference resulting in poorer mass accuracy. At 15K, under screening criteria applied in this study, this pesticide would have been missed (false negative).

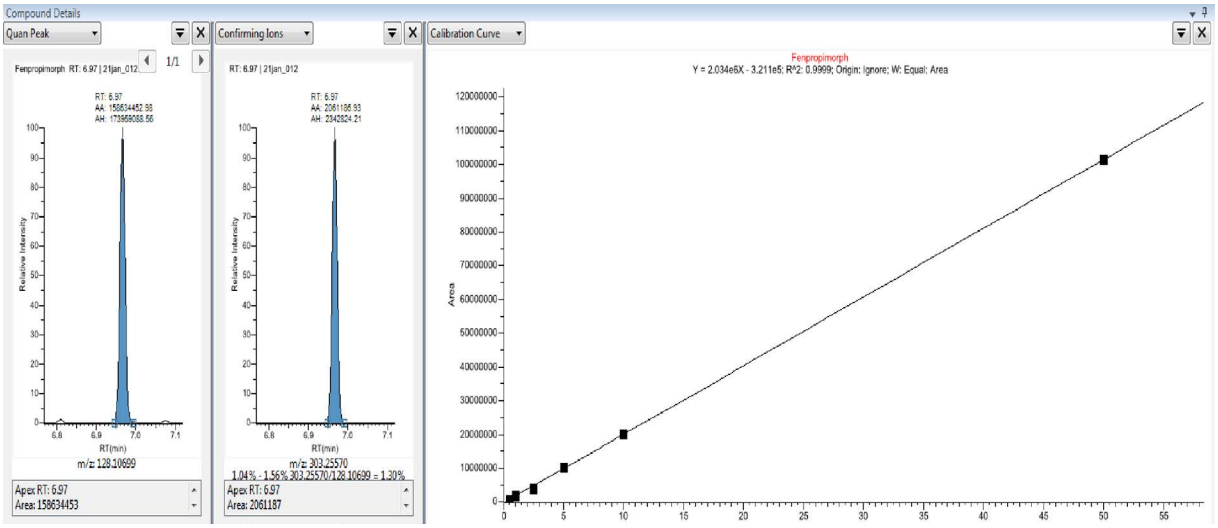

Figure 7. TraceFinder software view of the extracted ion chromatograms and calibration curve for fenpropimorph in leek. Triplicate injections of the calibration series were performed with good linearity.

Quantitative Pesticide Performance

The next step in routine analysis is to determine the concentration of the pesticide detected in the sample. Pesticide linearity was assessed across a concentration range of 0.5–50 ng/mL using matrix-matched standards and using triplicate injections of each calibration standard. In all cases, the coefficient of determination (R^2) was >0.99 with an average value of $R^2 = 0.997$ and with residual values from the regression line of <25%. An example of compound linearity for fenpropimorph is shown in Figure 7. Full quantitation of detected compounds was not in the scope of this study, but is reported in more detail for pesticides in Thermo Scientific Application Note 10449.[3]

Conclusions

The results of this evaluation demonstrate that the Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, in combination with TraceFinder software, is an extremely effective tool for the routine screening of pesticides in food and feed samples. The Orbitrap mass spectrometer delivers excellent resolving power, mass accuracy, and sensitivity.

- Screening using full-scan, high-resolution mass spectrometry is an effective way to increase the scope of an analysis. This technology allows for more compounds to be analyzed from a single injection without prior optimization of the acquisition parameters.
- Fast GC analysis and acquisition speeds allow for increased laboratory productivity and sample throughput. The outstanding mass accuracy, in combination with excellent sensitivity, makes confident routine pesticide screening possible.
- Routine resolving power of 60,000 FWHM eliminates matrix interferences, increasing confidence in results when screening pesticides in complex matrices. Consistent sub-ppm mass accuracy achieved for all compounds ensures confident compound identification.

References

1. SANCO/12571/2013. Method validation and quality control procedures for pesticides residue analysis in food and feed.
2. Kellmann, M., Muenster, H., Zomer, P. & Mol, H. (2009) Full Scan MS in Comprehensive Qualitative and Quantitative Residue Analysis in Food and Feed Matrices: How Much Resolving Power is Required? *J Am Soc Mass Spectrom*, 20, 1464-1476.
3. Thermo Scientific Application Note 10449: Fast screening, identification, and quantification of pesticide residues in baby food using GC Orbitrap MS technology. Runcorn, UK.

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APPLICATION NOTE

No. 10527

Characterizing unknowns in food packaging using GC Orbitrap Mass Spectrometry

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Key Words

Food packaging, Q Exactive GC, Orbitrap mass spectrometry, unknown identification, structural elucidation, food safety

Introduction

Packaging is an essential element of a safe food supply chain, with its main purpose to preserve the food it covers and to maintain its quality over the course of the products shelf life. Without an adequate barrier, food producers and manufacturers risk potentially serious microbial and chemical food safety incidents that may result in serious health risks over the short or long term. However, it is also well known that the chemical components used in the packaging can migrate into the food and present an even greater threat.[1] Food and beverages can interact strongly with any surface that they come into contact with and can potentially impact the quality of the product.[2] They can be corrosive or cause other physical breakdown of the packaging that will, in turn, leach chemicals into the product. Unfortunately, no packaging material is entirely inert; glass, paper, plastics and ceramics can all leach chemicals into the food at significant concentrations. For these reasons, it is important that regulators and manufacturers monitor and understand the health risk associated with packaging and take steps to minimize the risk to the consumer.

Gas Chromatography-Mass Spectrometry (GC-MS) is a popular analytical technique and has been widely used in food packaging studies as it provides analytical advantages of chromatographic resolution, reproducibility, peak capacity and, importantly, extensive spectral libraries to aid in identification. The analytes of interest are either volatile or semi-volatile (<1000 Da) in nature, and are therefore well-suited to analysis by GC-MS. The primary materials, such as monomers, additives and solvents used in the food packaging are usually well understood. However, these materials can also contain non-intentionally added substances (NIAS) such as impurities, reaction intermediates, breakdown products of polymer/additives, and contaminants from recycling.

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When investigating NIAS in food packaging, the analysis is challenging because there is very little information of the potential chemicals involved. Therefore, the approach taken needs to be as non-selective as possible so that the maximum chemical information is captured. To achieve this, the sample extraction technique is generic and often involves simple liquid extraction and concentration. This is followed by analysis in full-scan to obtain wide coverage of a sample. When using nominal mass GC-MS instruments for unknown analysis the procedure can be complex, time consuming, and expensive as it takes longer to interpret the mass spectrum and the confidence in any proposed assignment is low. Furthermore, there is a need for improved sensitivity because currently there can be extensive sample preparation and pre-treatment to isolate and concentrate samples which adversely impacts on the time to result.

This study focused on the utilization of a new GC-MS system with high mass resolution performance and high mass accuracy for fast and confident identification of unknown compounds in food packaging. Prior to this work, some of the unknown compounds were initially detected using nominal mass instrumentation (single quadrupole GC-MS), but this proved limited in the ability to assign an elemental formula, structure, and confident compound identification. Full-scan and MS/MS high mass resolution experiments are important to achieve the selectivity and mass accuracy needed for confident elemental composition proposals, structural elucidation and discrimination of co-eluting compounds. These features, in combination with novel software algorithms for automated spectral deconvolution and compound ID, create a powerful solution for fast, confident and comprehensive chemical characterization of food packaging samples.

Experimental conditions

Sample preparation

The sample investigated in this study was a tin can with an internal coating. The internal coating was extracted using a 300 mL solution of hexane: acetone (1:1) held at room temperature for 16 hours. The 300 mL was then evaporated to approximately 1 mL before being transferred to a crimp cap amber GC vial for analysis.

Instrument and method setup

In all experiments a Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC system and a Thermo Scientific™ TraceGOLD TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column with a 10 m guard (P/N 26096-1421). 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	Single gooseneck P/N 453A0344-UI
Inlet (°C)	SSL 280
Carrier Gas, (mL/min)	He, 1.3
Oven Temperature Program	
Temperature 1 (°C)	40
Hold Time (min)	0.5
Temperature 2 (°C)	325
Rate (°C/min)	5.5
Hold Time (min)	12

Table 2. Mass spectrometer conditions.

Q Exactive GC Mass Spectrometer Parameters	
Transfer line (°C)	280
Ionization type	EI/PCI
Ion source (°C)	230 EI / 190 CI
Electron energy (eV)	70
Acquisition mode	Full-scan
Mass range (Da)	50–700
Resolving power (FWHM at *m/z* 200)	120,000
Lockmass, column bleed *(m/z)*	207.03235

The Q Exactive GC system was operated in EI full-scan mode using 120,000 (FWHM at *m/z* 200) resolving power. Additional experiments were run using positive chemical ionization (PCI) with methane as reagent gas at a flow of 1.5 mL/min to obtain information on the molecular ions and to support the identification of unknown component peaks.

Data processing

Data were acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. TraceFinder also contains accurate mass spectral deconvolution and spectral matching functionality. Thermo Scientific™ MassFrontier™ spectral interpretation software was used for structural elucidation.

Results and discussion

The objective of this study was to analyze the packaging sample using a non-target full-scan data acquisition using electron ionization (EI) and positive chemical ionization (PCI), and to identify the most intense peaks. In addition, the aim was to provide structural information for the peaks detected using nominal mass GC-MS, where confirmation of the identity was not possible.

Extracting key features

Full-scan chromatograms were obtained for the sample and the total ion chromatograms (TICs) are shown in Figure 1. The Q Exactive GC system acquires accurate mass data with a wide dynamic range. This is very powerful when the objective is to identify unknown peaks in a complex sample, such as a food packaging extract with a high degree of confidence. The first step in this analysis was to isolate the peaks of interest and although peaks can be seen visually in the TICs, it is essential that all features are extracted from the data.

This was achieved with TraceFinder which first performs a high resolution accurate mass deconvolution of the data with the aim of detecting all of the peaks above a signal to noise threshold of 100:1. The deconvolution ensures that only ions that maximize at the same retention time remain for library matching. Using these thresholds, 961 features (peak clusters) were detected in the packaging sample. An example peak for 2-Hydroxy-5-methyl-1,3-benzenedicarboxaldehyde is shown in Figure 2, along with the number of scans across the peak, the accurate mass and ppm difference.

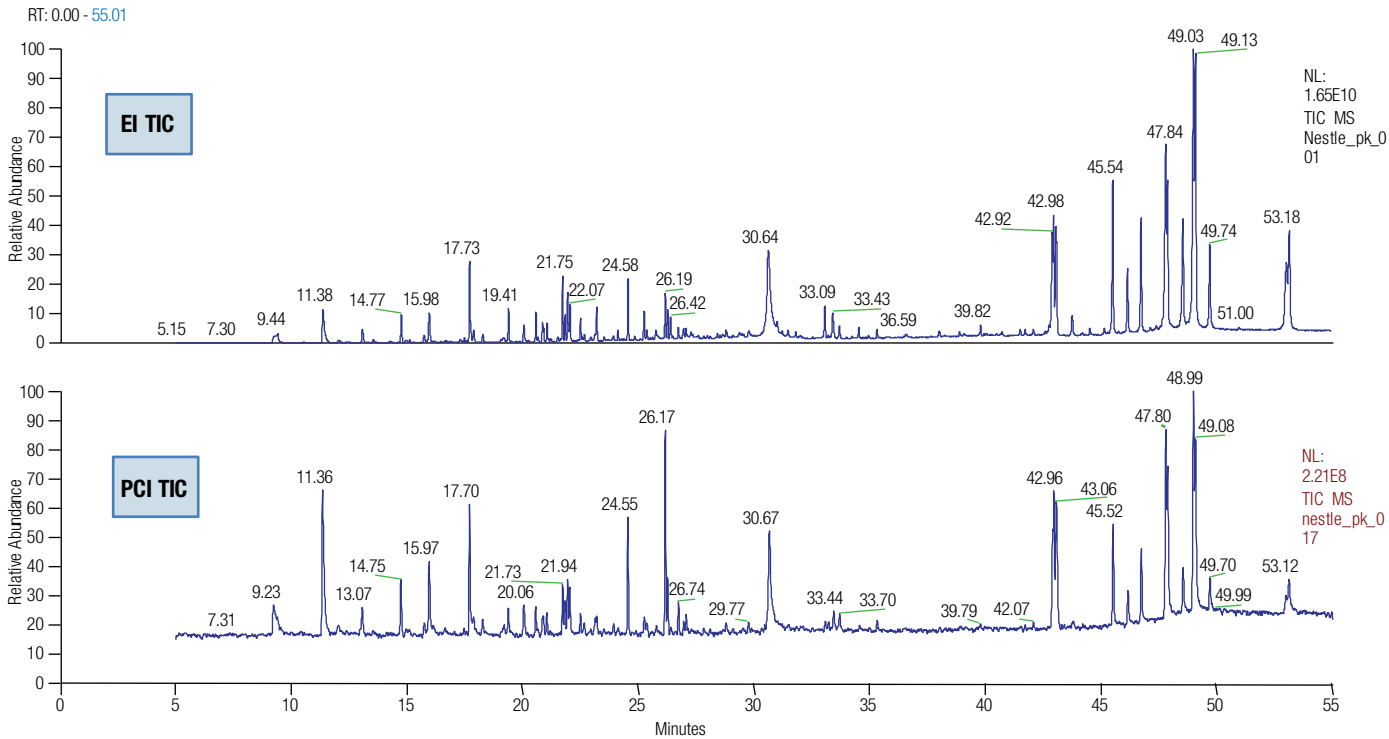

Figure 1. GC-MS electron ionization (EI) and positive chemical ionization (PCI) total ion chromatograms (TIC) of the packaging sample.

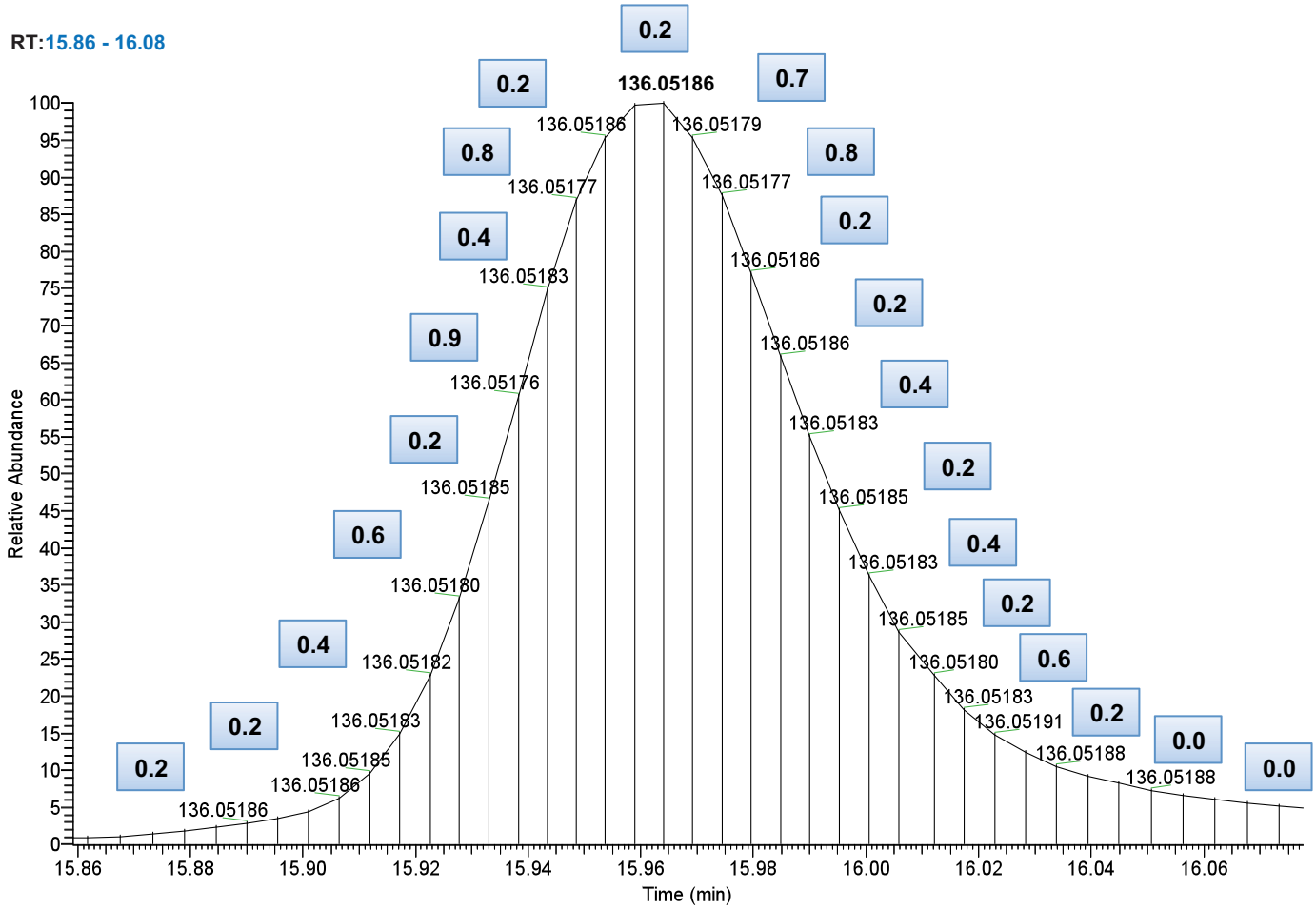

Figure 2. Extracted ion chromatogram for compound 2-Hydroxy-5-methyl-1,3-benzenedicarboxaldehyde fragment (m/z 136.05188 ±5 ppm mass window) in packaging sample 34 scans/peak. Data acquired in full-scan at 120,000 FWHM resolving power. Excellent accurate mass stability is shown for each individual scan as well as mass difference labelled (in ppm).

Accelerate known compound identification

Having performed a peak extraction, the deconvoluted spectrum was first searched against a commercially available nominal mass spectral library (NIST 2014). If available, the data could also be searched against an in-house nominal or accurate mass spectral library. The lists of hits were scored based on a combination of the search index (SI) score and high resolution filtering (HRF) value. The HRF value is the percentage of the mass spectrum that can be explained by the chemical formula in the library search.[3]

The combination of accurate mass and percentage of explained ions observed in the spectrum provides a fast and confident route to the identification of compounds. The utilization of accurate mass information speeds up the identification process as the user is no longer faced with long lists of spectral library matched compounds that are difficult to confirm or eliminate. For example, the top hit for the peak at 15.98 minutes was for the compound 2-Hydroxy-5-methyl-1,3-benzenedicarboxaldehyde, where 99.2% of the spectrum can be explained based on accurate mass (Figure 3). The fragments observed are matched to the elements in the proposed compound with sub 1 ppm mass accuracy which adds confidence in the identification. If only spectral matching was used, it would be difficult to confirm the identification.

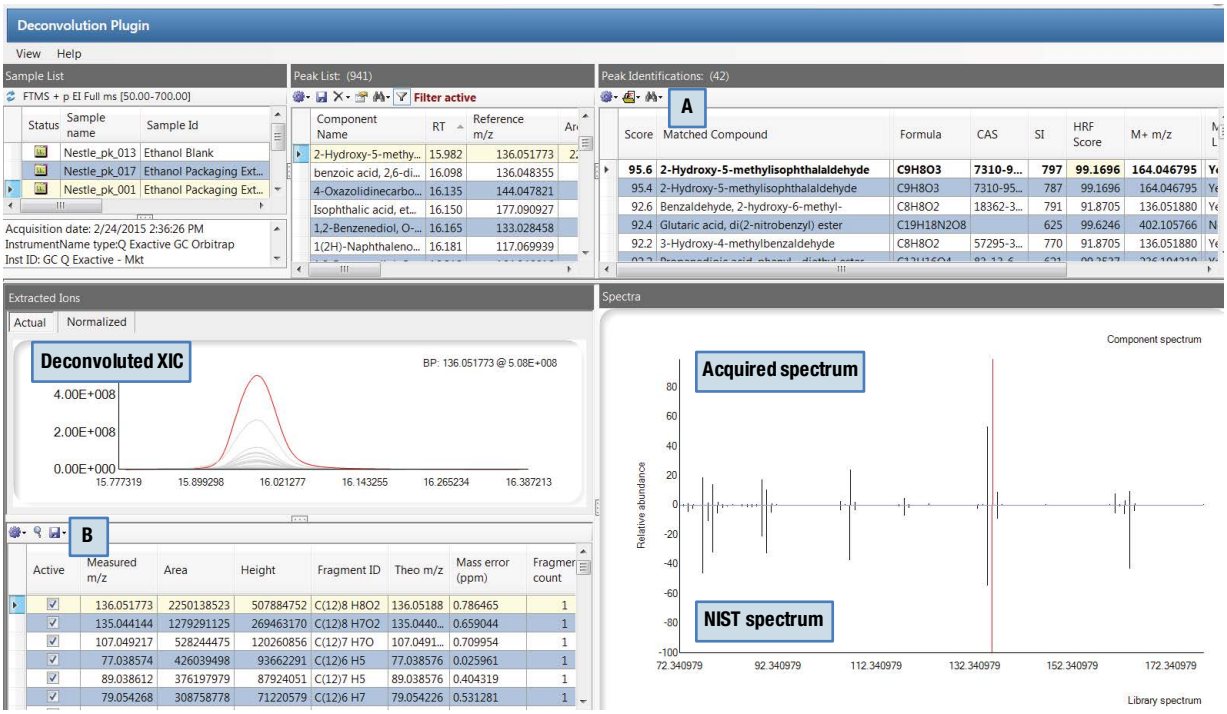

Figure 3. Identification of peak at 15.98 minutes as 2-hydroxy-5-methyl-1,3-benzenedicarboxaldehyde. Screenshot of the deconvoluted data and library match in TraceFinder. (A) List of library hits sorted by score (combination of SI and HRF). (B) List of fragment ions from EI spectrum and elemental composition based on elements in top hit.

Encountering unknowns

In a previous study, the same food packaging sample was analyzed using nominal mass GC-MS and a group of peaks were identified as being of interest, and they are also intense peaks in the high resolution MS TIC. These peaks eluted at RT: 30.6, 42.9, 45.5, 47.8, 49.1, and 53.2 minutes and are highlighted in Figure 4. As they are among the most intense peaks in the TIC, it is essential from a food safety view point to determine what they are as a first step to deciding whether they present any health risk.

Importantly, none of these peaks had a match in NIST 2014. With no spectral match it becomes extremely difficult using nominal mass to derive an acceptable degree of confident chemical compositional information about these compounds.

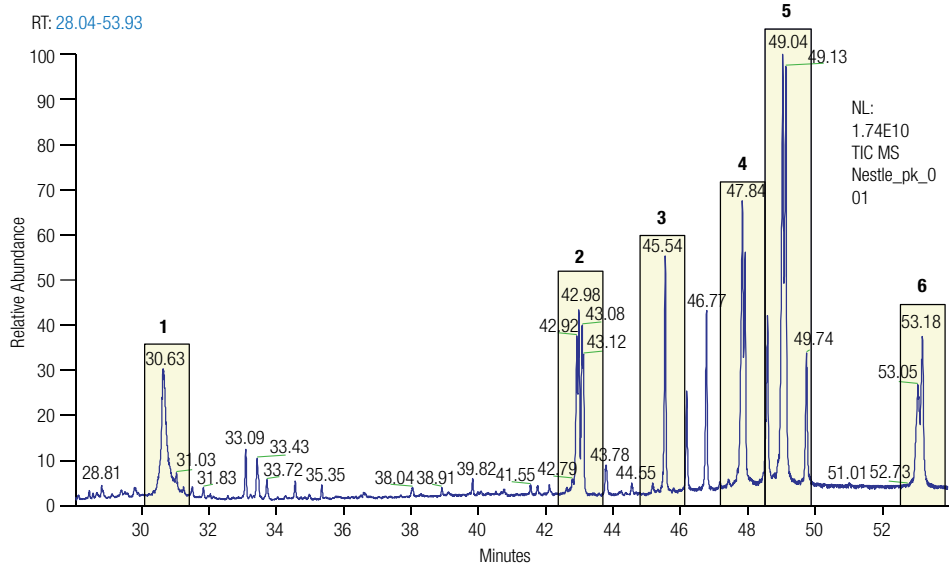

Figure 4. Zoomed region showing the six peaks of interest in the electron impact (EI) total ion chromatogram of the packaging sample.

When the spectral library match from the EI spectrum is inconclusive, then the PCI data can be used to establish the molecular ion, and to propose an elemental composition. When CI data is acquired using methane as the reagent gas, three adducts are typically observed: $[M+H]^+$, $[M+C_2H_5]^+$ and $[M+C_3H_5]^+$. Figure 5 shows the EI and PCI spectra for the peak at 45.5 minutes. The PCI spectrum shows the adducts $[M+H]^+$ (-0.8 ppm) for ion *m/z* 469.18532, $[M+C_2H_5]^+$ (-0.5 ppm) for ion *m/z* 497.21677. The presence of these adducts indicated that the *m/z* 468.17783 was the molecular ion. Without the PCI adducts it would not be possible to determine if the *m/z* 468.17783 was a fragment or the molecular ion. From this ion, an elemental composition of the parent molecule can be proposed.

Elemental composition assignment is a critical stage in the compound identification process and it is where excellent mass accuracy and isotopic pattern can be used to limit the number of possible chemical formulae. An elemental composition calculator was used to propose a formula for the $[M+H]^+$ ion (Figure 6). The software assigns formulae by using an isotopic pattern matching algorithm that accounts for isotope accurate mass and intensity ratios. The algorithm uses a single mass to calculate all possible elemental compositions that lie within a tolerance window and then calculates the theoretical isotopic pattern for each suggestion. It then gives a score between 0 and 100 percent, where 0 is completely different and 100 an exact isotopic match. For example, when a 5 ppm mass accuracy window is used 12 possible formulae are proposed for the $[M+H]^+$ ion using the elements Carbon (1–30), Hydrogen (1–60), Nitrogen (1–5), Oxygen (1–10), Phosphorus (1) and Sulphur (1). This is compared to 1 ppm mass accuracy window that suggests three possible formulae. Only one of these suggestions has a 100 percent match with the theoretical isotopic pattern: $C_{26}H_{29}O_8$. This level of mass accuracy significantly reduces the number of formulae that need to be investigated, which speeds up the analysis, and also increases the confidence in any proposed assignment.

One final stage to support the proposed formula and to derive structural information is to use the accurate mass fragments. To achieve this, either the fragments in the EI spectrum can be used or an additional MS/MS experiment can be performed to be confident that the fragments are indeed from the molecular ion. The $[M+H]^+$ (PCI) *m/z* 469.18 was isolated in the quadrupole and fragmentation induced in the HCD cell using 15V energy.

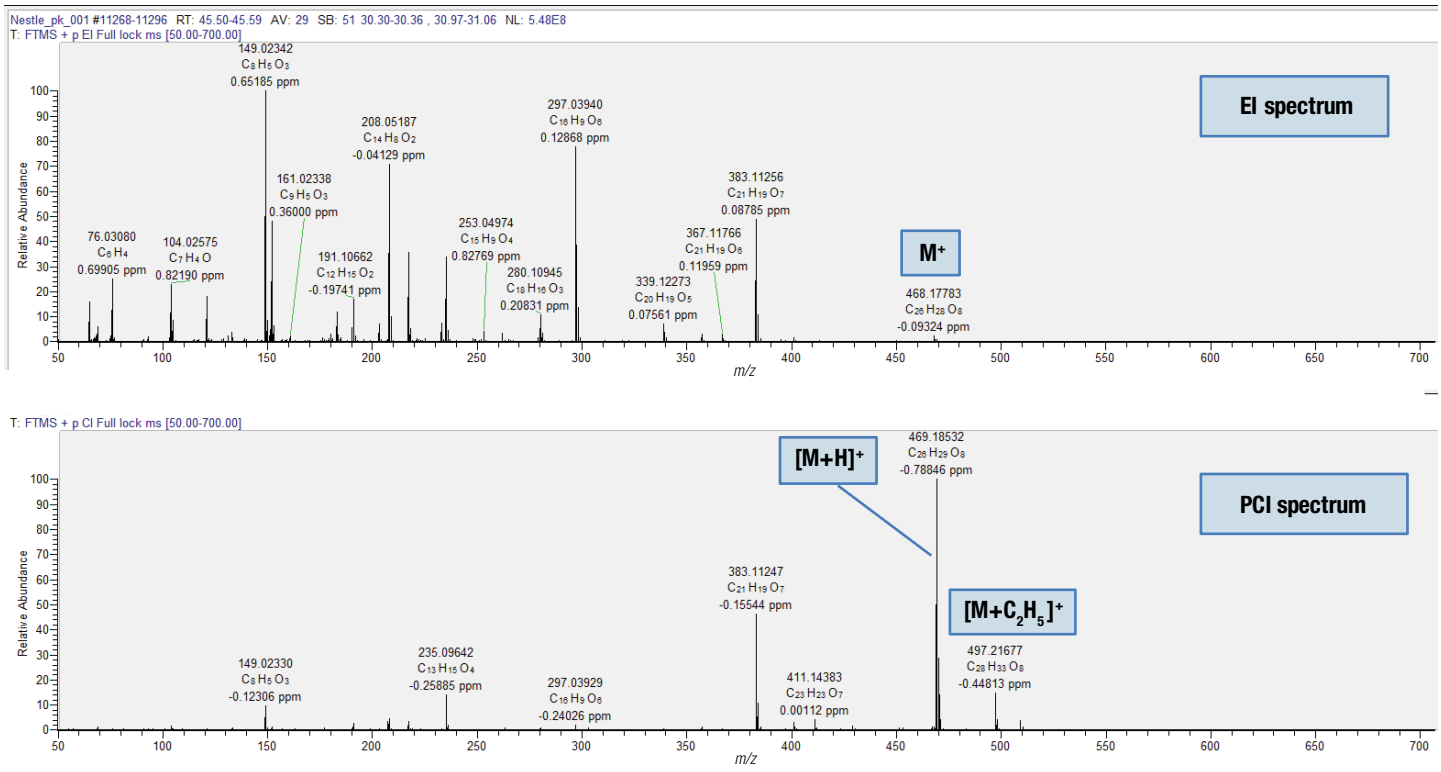

Figure 5. EI and PCI spectra at 45.5 minutes in packaging sample proposing a chemical formula of $C_{26}H_{28}O_8$. Peaks are annotated with chemical formula and mass difference in ppm. PCI data supports identification of parent ion with formula with sub 1 ppm mass accuracy.

Figure 7 shows the resulting MS/MS spectrum for *m/z* 469.18. The fragments measured contain the elements in the proposed parent and all with good mass accuracy. Based on this information, a proposed structure of the compound was made and is shown inset in Figure 7. MassFrontier was used to theoretically fragment the proposed chemical structure and match these to the measured fragments in the MS/MS spectrum. Therefore, even if at this stage a compound name cannot be confidently assigned, enough information can be obtained with respect to the chemical formula of the unknown compound.

Each of the six peaks were evaluated using the same workflow, and the results are summarized in Table 3. The mass accuracy obtained (<1 ppm) enabled confident elemental compositions to be assigned and these are supported by accurate mass fragments in the EI spectra. It was noted that all of the peaks contained a *m/z* 149.02332 ion and shared a common structure.

Figure 6. Elemental composition calculator screen in FreeStyle for the peak at 45.5 minutes in packaging sample proposing a chemical formula of $C_{26}H_{29}O_8$ for the $[M+H]^+$ ion based on accurate mass and isotope pattern. The three candidates are all within 1 ppm, but the top hit has a 100% isotopic match with the theoretical pattern.

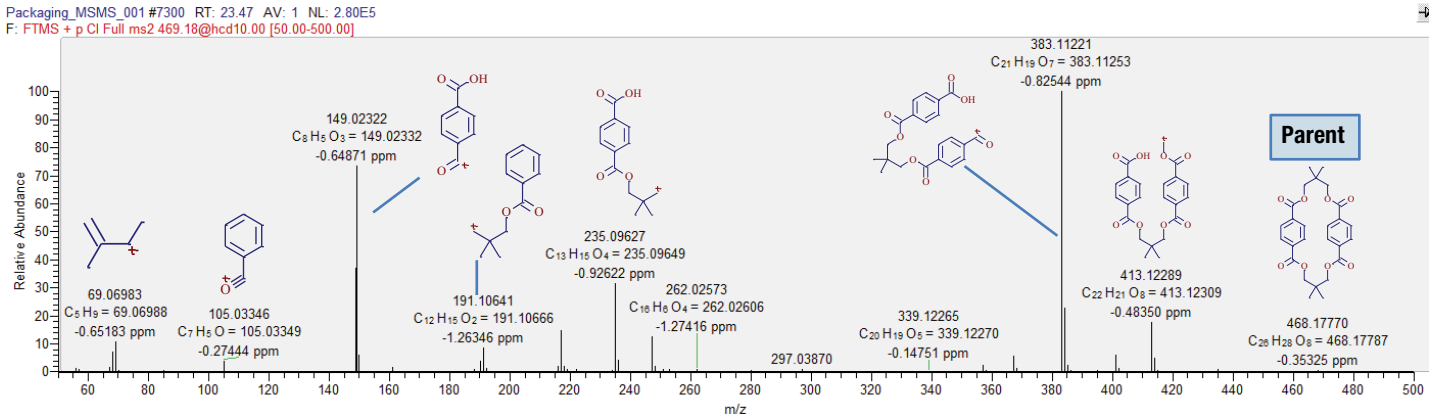

Figure 7. MS/MS spectrum of PCI ion *m/z* 469.18 selected in the quadrupole and fragmented in the HCD cell. MassFrontier used to explain the fragments observed within 3 ppm mass accuracy window.

Table 3. Summary of the peaks and the tentative identification of the elemental composition of the compounds. Excellent mass accuracy (<1 ppm) for all quasi-molecular ions adds confidence to the proposed identities.

Peak No.	Retention Time (min)	Formula	$[M+H]^+$ *m/z*	Mass Error of $[M+H]^+$ (ppm)	Mass Error of $[M+C_2H_5]^+$ (ppm)	Mass Error of $[M+C_3H_5]^+$ (ppm)
1	30.6	$C_{14}H_{18}O_6$	283.11762	0.0	0.5	0.1
2	42.98	$C_{22}H_{20}O_8$	413.12303	-0.2	-0.3	0.0
3	45.5	$C_{26}H_{28}O_8$	469.18532	0.7	-0.4	0.0
4	47.5	$C_{24}H_{24}O_8$	441.15424	-0.4	-0.4	-0.3
5	49.1	$C_{27}H_{30}O_8$	483.20112	-0.5	-0.1	0.3
6	52.0	$C_{28}H_{32}O_8$	497.21684	-0.3	0.1	0.3

Unlocking structural information

Further investigation of the full-scan EI and PCI data showed that when the parent mass for $C_{26}H_{28}O_8$ was extracted there were three peaks in the chromatogram (Figure 8). The capability to perform accurate mass MS/MS experiments provides valuable structural information that may be vital in determining what the compound is and if it is a safety concern. The MS/MS spectra for the three isomers (Figure 9) shows both similarities and differences between the isomers. Isomers 2 and 3 have a base peak at *m/z* 401.12309 ($C_{21}H_{21}O_8$) and an additional ion *m/z* 132.02058 ($C_8H_4O_2$). The base peak in isomer 1 is *m/z* 383.11253 ($C_{21}H_{19}O_7$) and the *m/z* 132.02058 is absent. The capacity to confidently assign elemental compositions to these ions is highly beneficial and provides the analyst with a complete picture. The *m/z* 401.12309 corresponds to a loss of C_5H_7 from the parent and *m/z* 383.11253 a loss of $C_5H_{10}O$. MassFrontier was used to explain how these ions can be derived from the proposed chemical structure. From this information, the flexibility to perform MS/MS experiments with accurate mass information allows for detailed structural information to be determined.

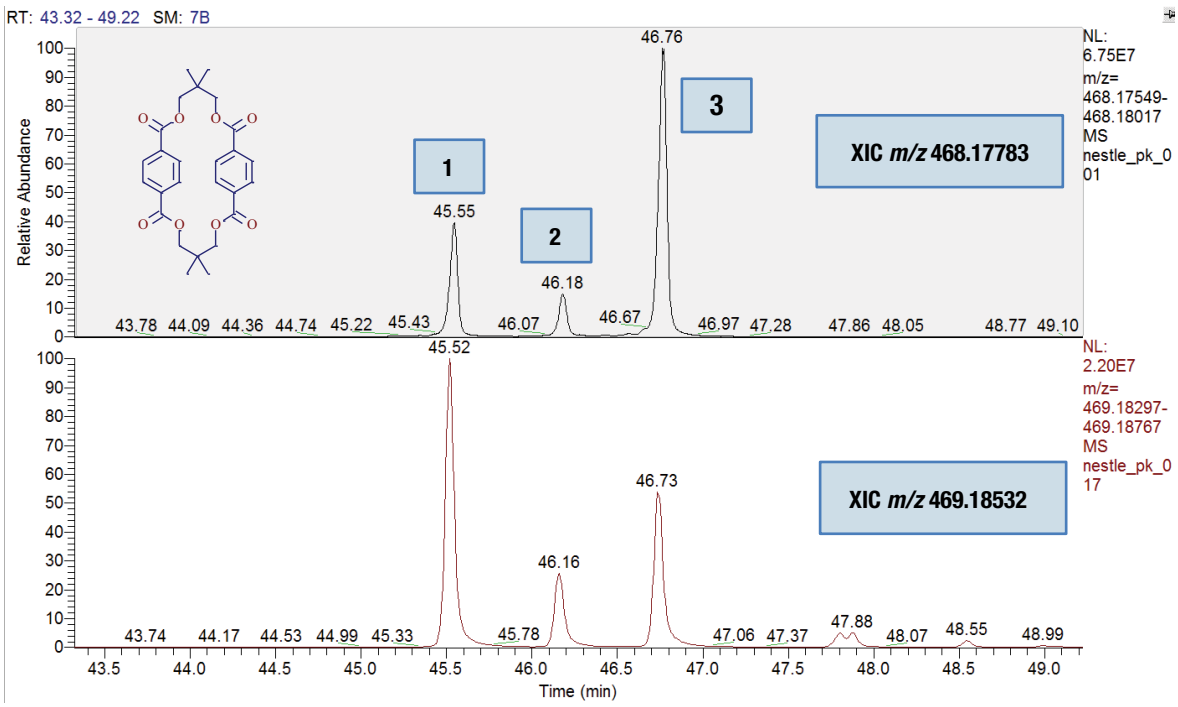

Figure 8. XIC *m/z* 468.17783 from the full-scan EI data and *m/z* 469.18532 from the full-scan PCI data in packaging sample shows 3 isomers of the same parent mass. Inset proposed chemical structure of compound.

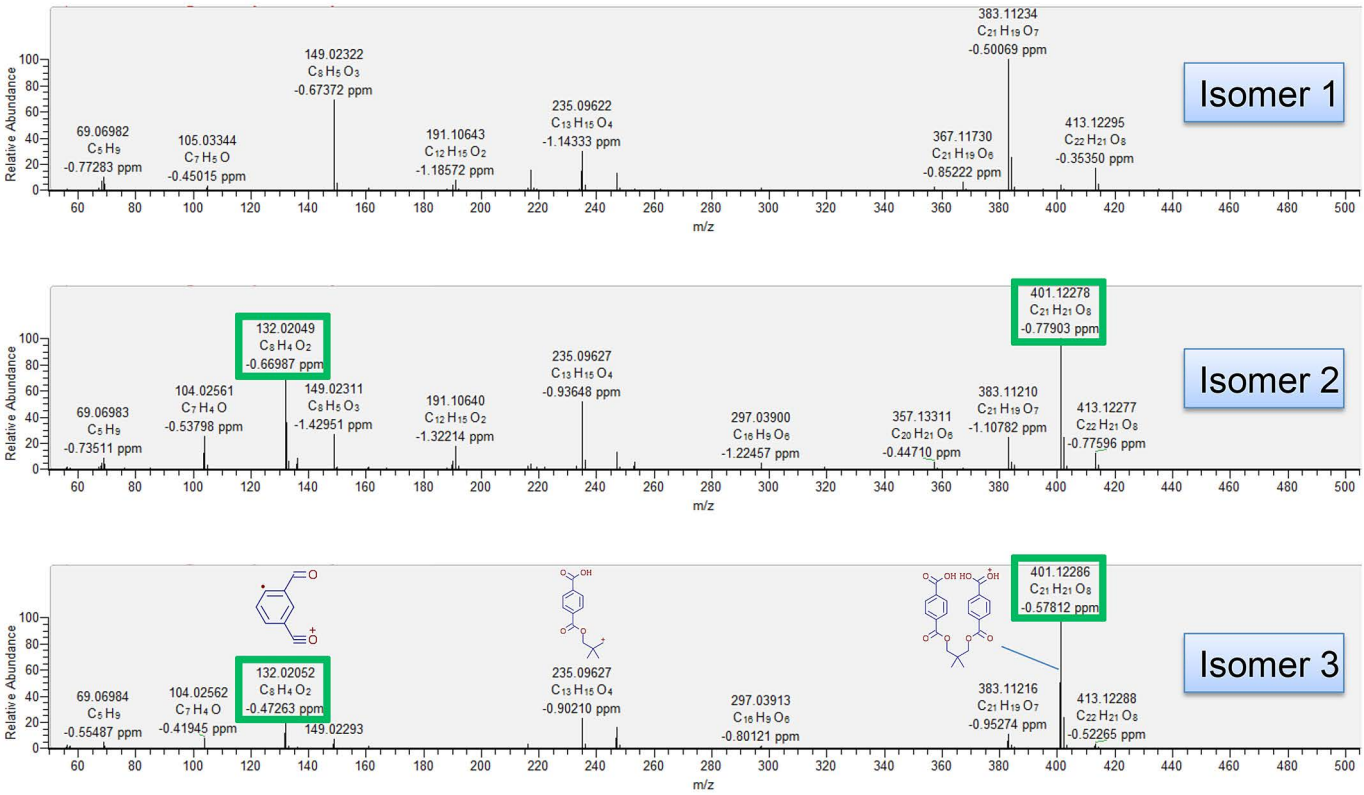

Figure 9. MS/MS spectra of m/z 469.18 of the three isomers reveals different fragmentation patterns for isomers 2 and 3. Of particular note, the base peak is 401.12286 and the presence of m/z 132.02049 ion.

Conclusions

The results of this study demonstrate that the Thermo Scientific Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, in combination with easy-to-use software tools, is a powerful tool for the profiling of complex samples and for the identification of unknown chemicals. The Orbitrap mass spectrometer delivers excellent resolution and mass accuracy which leads to fast and confident characterization of samples regardless of the concentration. A food packaging sample was quickly screened for known compounds using spectral matching and rationalisation using accurate mass. EI and PCI information leads to confident chemical formulas to be proposed for molecular ions and fragments for compounds with no library match. Furthermore, the ability to perform high resolution, accurate mass MS/MS experiments completes the unknown identification workflow and allows for an even higher level of confidence and provides important structural information.

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Chemical Profiling and Differential Analysis of Whiskies Using Orbitrap GC-MS

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Key Words
Chemical profiling, whisky, Q Exactive GC, Orbitrap mass spectrometry, differential and statistical analysis, identification, accurate mass

Introduction

Whisky is a premium spirit beverage that is distilled by following long established methods which involves a complex aging process. It is produced by the mixing of various grains with water to form a mash that is fermented with yeast, distilled to generate an alcoholic distillate, and finally matured in wooden barrels or casks.[1] This is a complex and traditional process that results in a beverage that has both a high value and high degree of variability. For example, whiskies produced on the West coast of Scotland often have a very smoky flavor, while those from the Speyside region can have flavors characteristic of honey, vanilla and fruit.[2] The production technology plays a significant role in the chemical composition and hence the flavor characteristics of the final whisky product.

As a result of these distinguishing features and the rising global demand, whisky has become an economically important commodity in many regions of the world. The entire whisky industry is a major source of both employment and tax revenues in these regions. For example, the whisky market is worth ~£5 billion to the UK economy,[3] and in the USA, distilled spirits are collectively worth $120 billion.[4] As whisky has a high retail price, counterfeiting and/or adulteration is commonplace and is a threat to the integrity of the industry. The adulteration can take many forms and can occur on both small and large scales. For example, one of the most extensive forms of adulteration is to add the main chemical constituents of whisky to an alternative cheaper spirit to create an “artificial” whisky. This is of particular concern as there is no safety control over which chemicals are being added, their quality or concentration. Another form of adulteration includes the labeling of bottles with more expensive brands and falsely claiming the age for which the whisky was matured in the barrel. The latter type of adulteration can be performed either by the addition of artificial colors or by heating during the aging process to speed up the coloration to mimic the aging process.The bottles are then mis-labeled claiming that the whisky was aged for an extended period in the barrel, which then justifies a higher price. Both processes can appear to achieve in a few months or days what otherwise would have taken many years.

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Therefore, it is essential that whisky producers use the available analytical technology to accurately and comprehensively characterize their products so that adulteration can be confidently identified and action taken to protect their product and brands. It may also be beneficial for whisky producers to chemically profile their products as part of their quality control procedures to enable comparison of different production batches and detection of any changes in the production process over time. This will help ensure that the whisky they bottle consistently contains the particular signature flavors and characteristics expected by the consumer.

Gas chromatography mass spectrometry (GC-MS) has been widely used to characterize whisky as it provides analytical advantages of chromatographic resolution, reproducibility, peak capacity, and, importantly, extensive spectral libraries to aid in identification of volatile and semi-volatile chemical constituents. In this proof-of-concept study, we seek to take advantage of the performance of the Thermo Scientific™ Q Exactive™ GC Hybrid Quadrupole-Orbitrap™ Mass Spectrometer for the profiling of whisky of different origins, ages and types. Another aim is to evaluate the application of a complete untargeted chemometric workflow using the Q Exactive GC system to detect and identify chemical components in whisky. It will also show the process of identifying chemical differences in whiskies of different origins. Samples were analyzed using a full scan non-targeted acquisition and high mass resolving power to obtain accurate mass measurements. This is important to enable elucidation of the elemental composition and discrimination of co-eluting and isobaric compounds. Fast scan speeds in combination with a high in-scan dynamic range and high sensitivity facilitates the detection of both low and high intensity components. These features in combination with unique software algorithms for automated deconvolution and sample comparison create a powerful solution for comprehensive characterization, quality control, and product brand protection.

Experimental Conditions

Sample Preparation

A total of nine whisky samples were included in the study, the details of each sample are shown in Table 1. The samples were prepared for GC analysis using the following procedure: 3 mL of whisky sample was mixed with 10 mL of distilled water and shaken with 15 mL of ethyl acetate. The organic layer was filtered through 3 g of sodium sulfate. The ethyl acetate extract was carefully evaporated under a gentle stream of nitrogen at room temperature. The evaporated extract was re-dissolved in 0.5 mL of ethyl acetate and transferred into the GC vial. For statistical analysis a pooled, or composite, sample was prepared by pipetting 50 µL of each whisky extract into a single GC vial. A blank ethyl acetate was analyzed to eliminate background systematic peaks. Each sample, including the pool, was injected 4 times and analyzed in a random order.

Table 1. Details of whisky samples included in the study.

Sample ID	Type	Age	Country of Origin	Region
2263	Single	12	Scotland	Lowlands
2264	Single	18	Scotland	Lowlands
2265*	Single	NAS	Scotland	Lowlands
2281	Single	10	Scotland	Campbeltown
2282	Single	15	Scotland	Campbeltown
2283	Single	15	Scotland	Campbeltown
2284	Single	12	Scotland	Highland
2285	Single	18	Scotland	Highland
2295	Bourbon	—	USA	Kentucky

NAS – No age statement. * Aged in three different casks.

Instrument and Method Setup

In all experiments, a Q Exactive GC system was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH Autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column with a 10 m integrated guard. (P/N 26096-1425). Additional details of instrument parameters are displayed in Tables 2 and 3.

Table 2. GC and injector conditions.

TRACE 1310 GC Parameters	
Injection Volume (µL):	1 Splitless
Liner:	Single gooseneck
Inlet (°C):	250
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	45
Hold Time (min):	1
Temperature 2 (°C):	330
Rate (°C/min):	10
Hold Time (min):	5

Table 3. Mass spectrometer conditions.

Q Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (Da):	50–600
Resolving power (FWHM):	60,000 (*m/z* 200)
Lockmass, column bleed (*m/z*):	207.03235

The Q Exactive GC system was operated in EI full scan mode using 60,000 (FWHM at *m/z* 200) resolving power. Additional experiments were run using positive chemical ionization (PCI) with methane as reagent gas (1.5 mL/min) to obtain information on the molecular ions and to support the identification of unknown component peaks.

Data Processing

Data was acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. TraceFinder also contains spectral deconvolution and spectral matching functionality. Statistical analysis was performed using SIEVE™ 2.2 and Mass Frontier™ 7.0 was used for structural elucidation.

Results and Discussion

The objective of this proof-of-concept study was to analyze the whisky samples using a non-target full scan data acquisition and to identify, using statistical tools, if there were differences between the samples and to propose an identity to any differences observed. In addition, the aim was to also quickly characterize which compounds are present in an individual sample using accurate mass deconvolution and spectral matching. The workflow used to achieve these objectives is summarized in Figure 1.

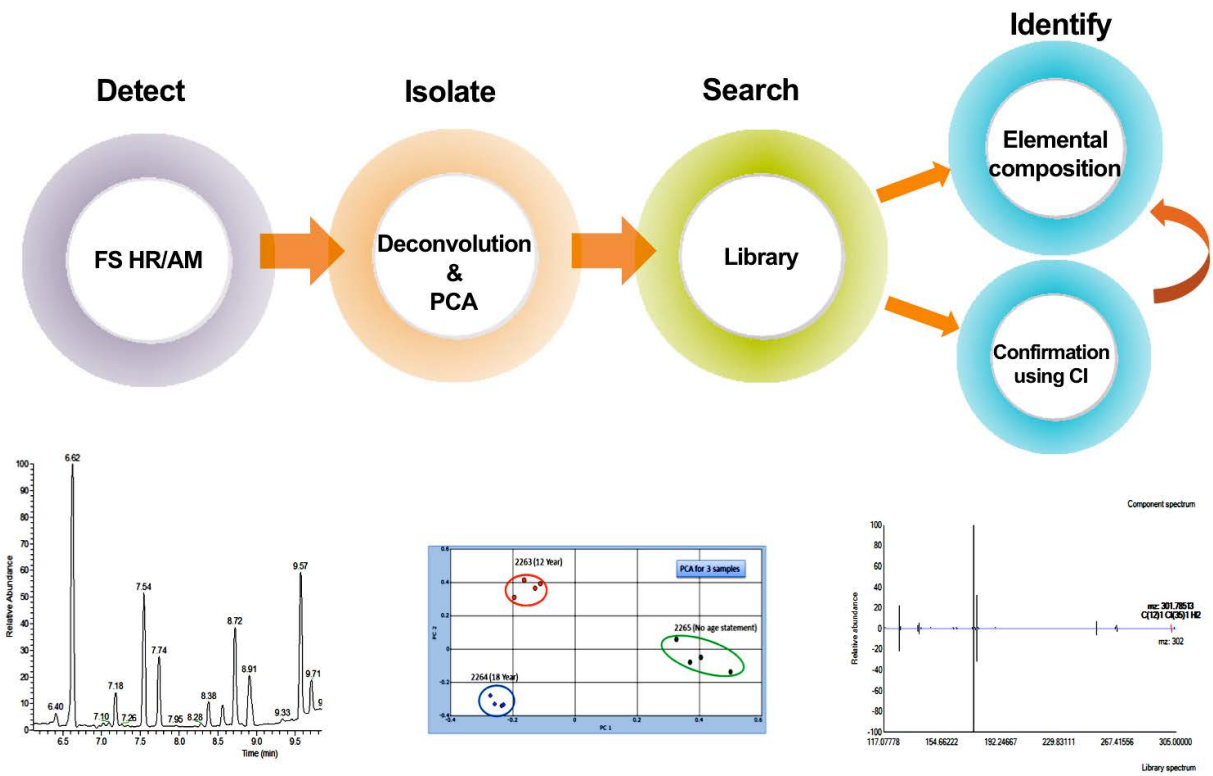

Figure 1. Workflow for the Q Exactive GC system for chemical profiling and marker identification.

Discovering Differences Between Samples

Four replicate full scan chromatograms were obtained for each sample and an example of the total ion chromatograms (TICs) are shown in Figure 2 for a single malt and a bourbon whisky. The TICs show a large number of peaks that are present at varying intensities and retention times. Importantly, for chemical profiling, the Q Exactive GC system provides a wide dynamic range to accurately capture components that are present at both trace and very high concentrations. The complete data set, including all nine samples, pooled sample and replicates, was processed in SIEVE 2.2 for component extraction and statistical analysis. This software initially performed a peak alignment to correct for any retention time variation across the batch, followed by peak detection and finally statistical analysis. The results of this are shown in Figure 3, which shows a principal component analysis (PCA) of all the samples and replicates. From visual analysis of the PCA, it is clear that all of the whisky technical replicates cluster together, and as expected, the pooled sample lies towards the center of the PCA plot.

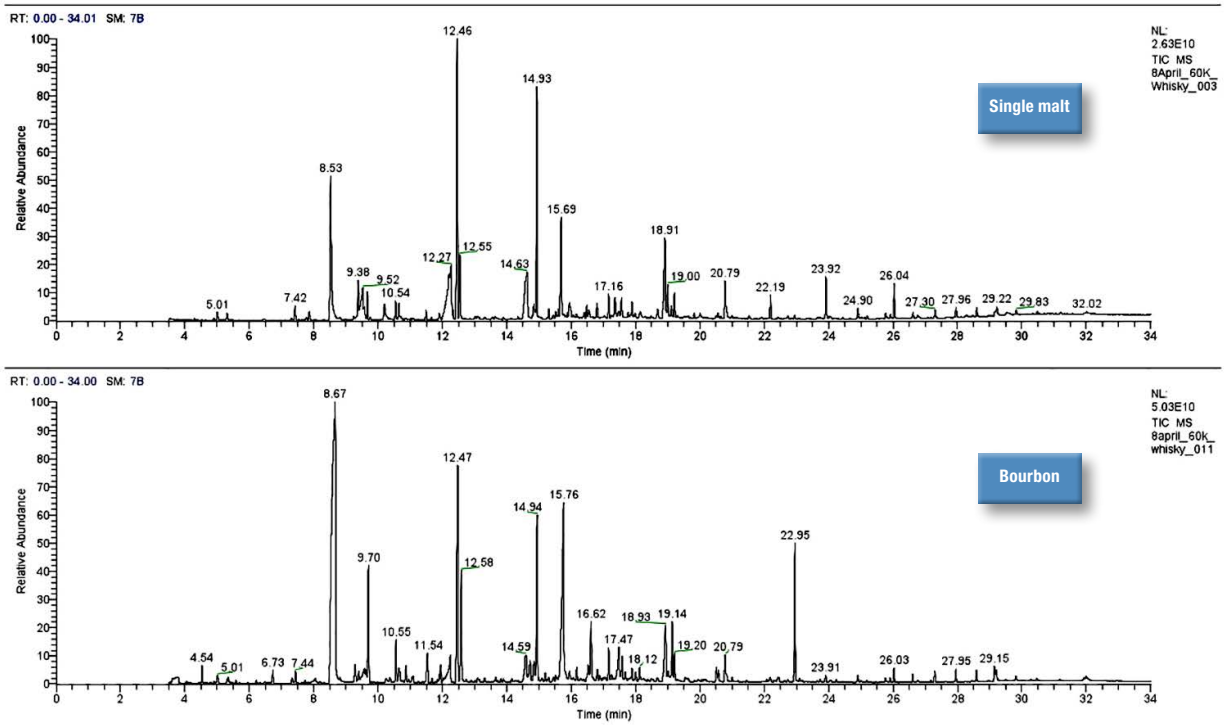

Figure 2. GC-MS total ion chromatograms of a single malt whisky (sample 2265) and a bourbon whisky (sample 2295).

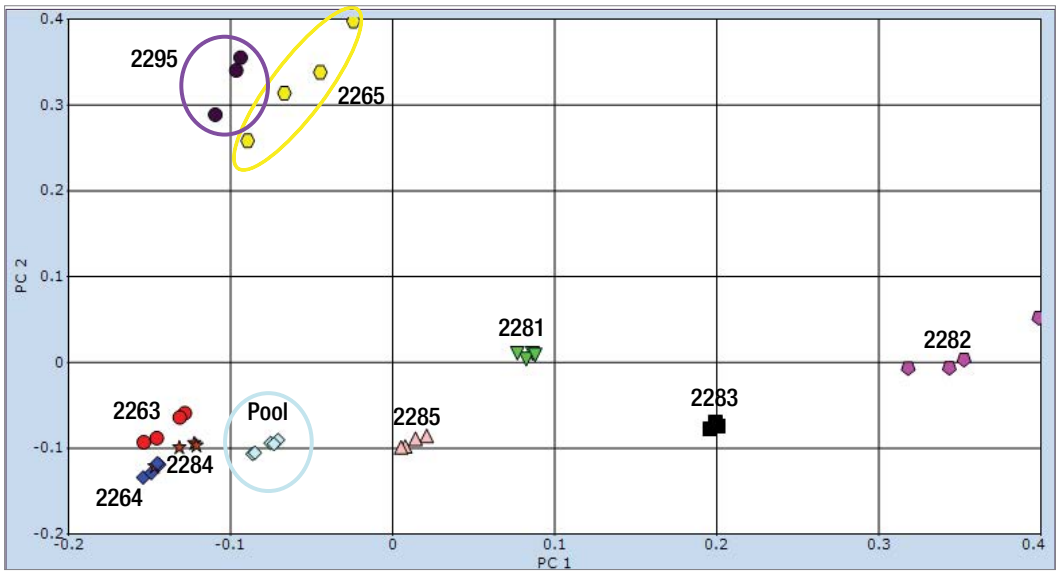

Figure 3. Principal component model, from SIEVE, of the 9 whisky samples with 4 replicate injections of each. Note that the replicates cluster appropriately. Whiskies 2295 (bourbon) and 2265 (aged in three barrels) are different to the others, but show some similarities to each other.

Isolating Unique Components

From the PCA and the list of detected peaks presented in SIEVE 2.2 it was possible to investigate which peaks contributed significantly to the differences observed between the different sample types. One observation from the PCA was that samples 2295 and 2265 were significantly different from the other whiskies. The 4841 component list (containing retention time and exact mass pairs) was sorted. This was achieved by ordering the list based on the p value (<0.05) for statistical significance. This revealed a number of component peaks were either unique or elevated in intensity in sample 2295 and were consistent across the technical replicates. A summary of the top five statistically different peaks were investigated and the results are shown in Table 4.

Taking a look at this process in more detail, and as indicated by the trend intensity graph from SIEVE 2.2 (Figure 4), a peak at retention time 13.6 minutes was significantly elevated in sample 2295 as compared to all other samples. The next step is to propose an elemental composition using the accurate mass to attempt to identify the chemical structure of the component. This can be a very difficult process and to successfully achieve this it is helpful to use both the EI spectrum and the PCI spectrum. The EI spectrum can be used to search against commercially available spectral libraries, such as NIST, and to propose a tentative compound identification. The accurate mass information can then be used to support this identification. In cases where there is no library match from the EI spectrum, the PCI data can be used to deduce the possible elemental composition of the molecular ion. Excellent mass accuracy is essential to limit the list of possible chemical formulae and to increase confidence when a proposed identification is made.

Table 4. Summary of five peaks identified as being elevated in sample 2295 and their tentative identification.

No.	Retention Time (min)	Base Peak *(m/z)*	Compound Name	Formula	Mass Accuracy Base Peak (ppm)	Mass Accuracy Molecular ion (ppm)
1	13.6	177.12736	Trans β ionone	$C_{13}H_{20}O$	0.84	0.31
2	11.54	139.11180	Furanone	$C_9H_{16}O_2$	0.22	0.12
3	10.87	137.05974	Phenol, 4 ethyl -2 methoxy	$C_9H_{12}O_2$	0.29	0.08
4	16.16	194.09037	2,3-dimethoxy-4-phenol	$C_{11}H_{14}O_3$	0.15	0.15
5	18.00	167.07028	Furan propanoic acid	$C_{12}H_{16}O_5$	0.23	0.13

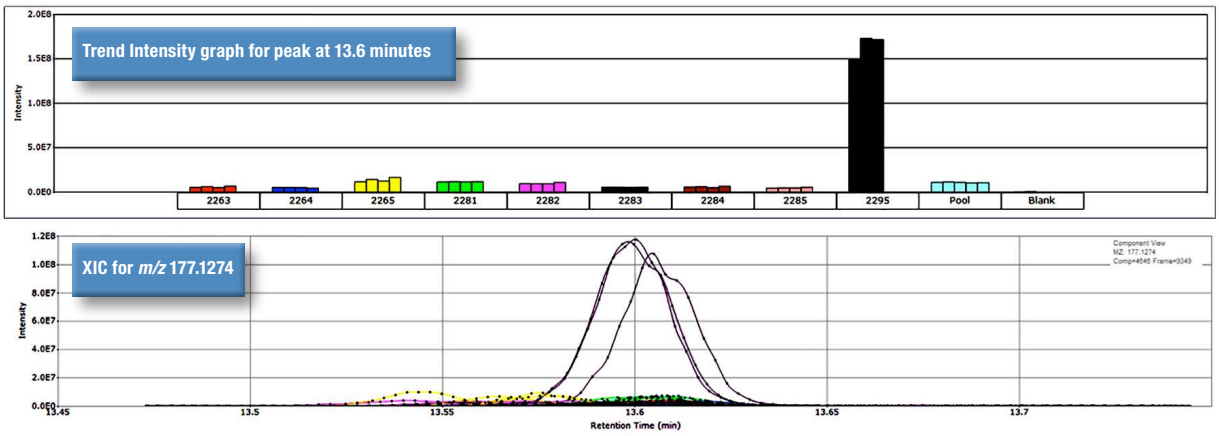

Figure 4. Trend intensity bar graph and extracted ion chromatogram for *m/z* 177.1274 of peak number 4646 at retention time 13.6 minutes across all of the whisky samples and replicate injections. This peak is elevated in sample 2295.

To simplify the identification of the peak at 13.6 minutes the raw data was deconvoluted in TraceFinder to provide a cleaned spectrum that was then matched against NIST14 (Figure 5). The subsequent hits from the NIST library are scored based on a combination of the search index (SI) score and high-resolution filtering (HRF) value. The HRF value is the percentage of the spectrum that can be explained by the chemical formula in the library search. For the top hit, trans β ionone, 98.4% of the spectrum can be explained based on accurate mass of the ions in the spectrum. The fragments observed can also be matched to the elements in the proposed compound to add confidence in the identification and to explain how the fragments can form based on the hit proposed. This gives a combined SI and HRF score of 75.3%. Figure 6 shows the fragments in the EI spectrum labeled with the proposed formulas and mass errors. All of the fragments are measured with sub-1 ppm mass accuracy providing increased confidence in the assignment. Elevated levels of trans β ionone, a breakdown product of carotene, were found in sample 2295 which was a bourbon whisky. Significantly, one of the differences between bourbon and Scotch whisky is that bourbon is brewed from corn rather than barley. Corn is naturally higher in beta-carotene than barley (53 µg/200g compared to 7 µg/200g for barley,[5] which would explain the elevated levels observed.

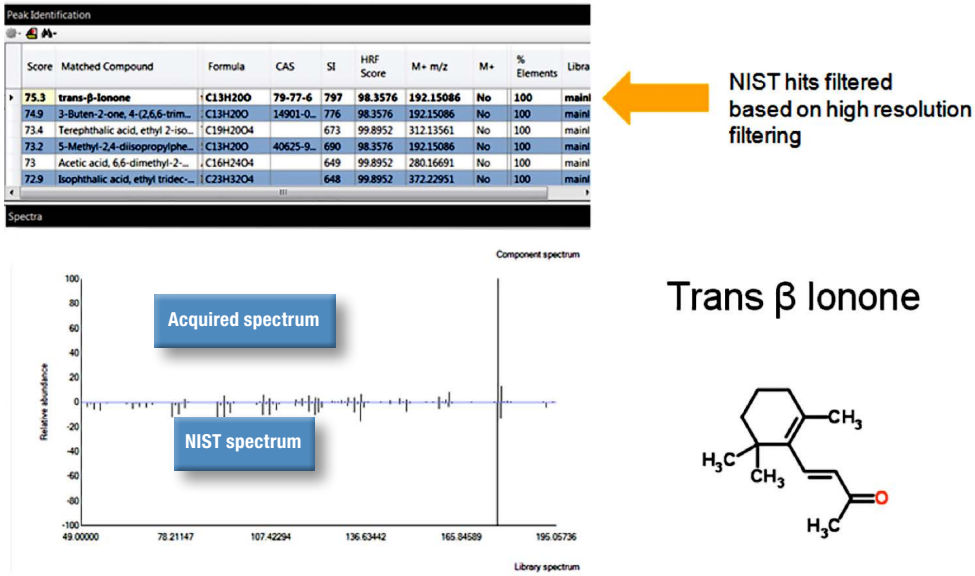

Figure 5. Identification of peak at 13.6 minutes as Trans β Ionone. Screenshot of the deconvoluted data and library match in TraceFinder 3.3. List of library hits, top hit 75.3% of spectrum explained for trans β ionone (upper). Acquired and library spectra mirror image (lower).

Fragment Ion (*m/z*)	Formula	Mass Accuracy (ppm)
192.15092	$C_{13}H_{20}O$	0.25
177.12736	$C_{12}H_{17}O$	0.16
162.10384	$C_{11}H_{14}O$	0.45
135.08040	$C_9H_{11}O$	0.34
105.06997	C_8H_9	0.89
91.05424	C_7H_7	0.10
69.03350	C_4H_5O	0.18

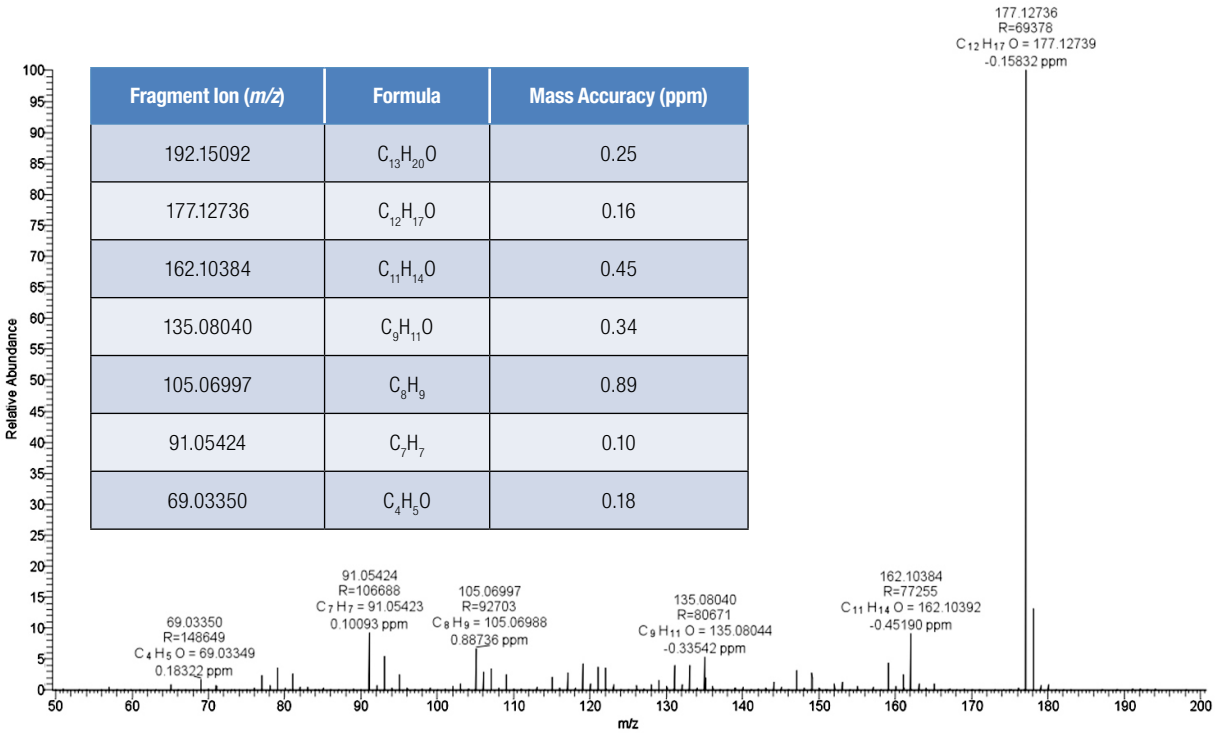

Figure 6. EI spectrum labeled with formula and mass error (ppm) for the fragments of trans β ionone. The molecular ion and the 6 most intense fragments support identification with < 1 ppm mass accuracy. R = Resolution.

Identifying Compounds with No Spectral Library Match

When there is no match using spectral libraries the process of identification is more complicated. For example, the PCA showed elevated levels of the peak at 18 minutes in sample 2295. The EI and PCI spectra (Figure 7) were used to isolate the molecular ion and propose an elemental formula. The $[M+H]^+$ and the $[M+C_2H_5]^+$ adducts were identified in the PCI spectrum and from this an elemental composition of the parent molecule could be proposed. This is a critical stage in the process and it is where excellent mass accuracy will substantially limit the number of possible chemical formulae. For example, when a 10 ppm mass accuracy window was used, nine possible formulae were proposed for the $[M+H]^+$ ion of *m/z* 241.10699 using the elements Carbon (1-50), Hydrogen (1-100), Nitrogen (1-5), Oxygen (1-10) and Chlorine (1-10). By contrast, the use of a 1 ppm mass accuracy window resulted in only one possible formula, $C_{12}H_{17}O_5$. This level of mass accuracy reduces the number of formulae that need to be investigated and also increases the confidence in any proposed assignment. The identification is further supported by the mass accuracy and elemental formula for the molecular ion *m/z* 240.09924 seen in the EI spectrum (0.1 ppm mass error).

The proposed chemical formula for the compound $C_{12}H_{16}O_5$ was searched using the online chemical database ChemSpider™. The results were investigated and the fragment information was used to either support or exclude possible suggestions. Mass Frontier 7.0 was used to theoretically fragment proposed compounds and match these to the measured fragments in the EI spectrum (Figure 7). The fifth compound hit suggested by ChemSpider, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid, was the only compound that could explain the fragments measured in the EI spectrum. The sub-1 ppm mass accuracy allows compounds to be quickly excluded or included and adds confidence in assignments.

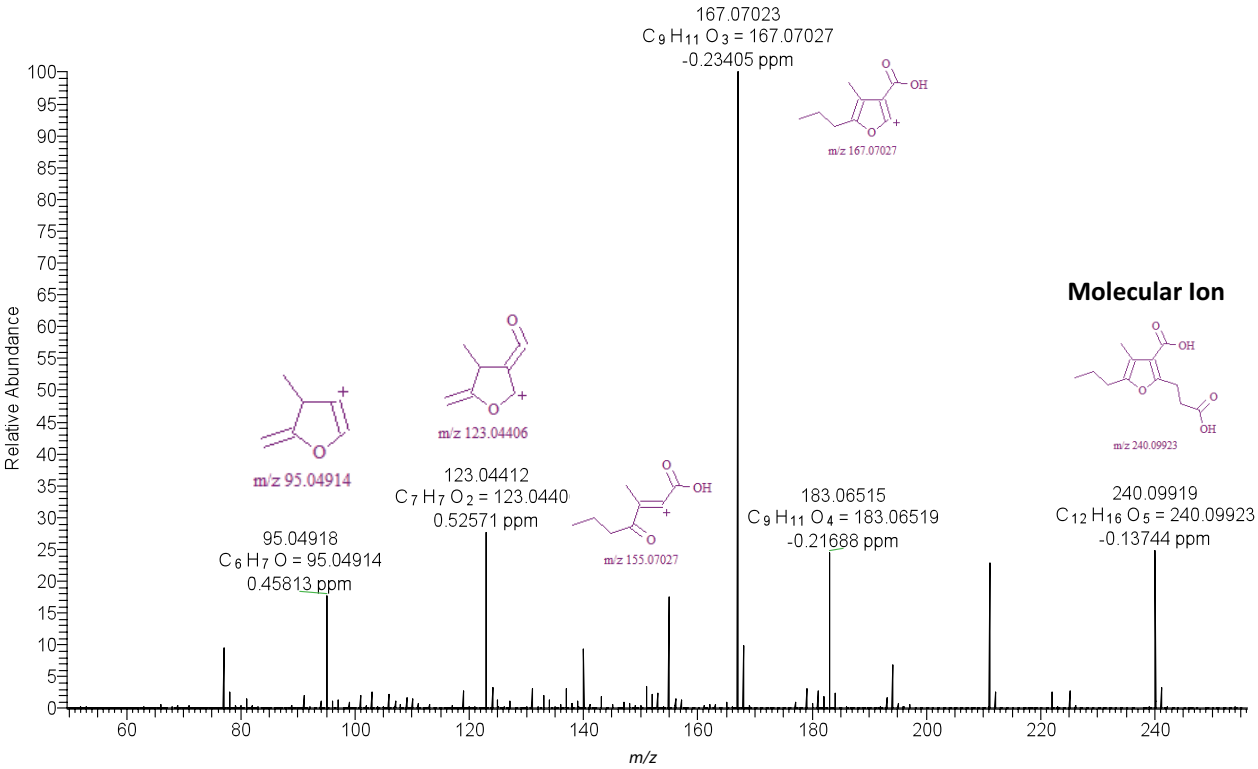

Figure 7. EI spectrum for peak at 18.00 minutes where no library match was made. Peaks are labeled with structure, formula and mass error in ppm. The sub 1ppm mass errors provide high confidence in the proposed identification of 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid. Peaks are annotated with structures identified in Mass Frontier 7.0.

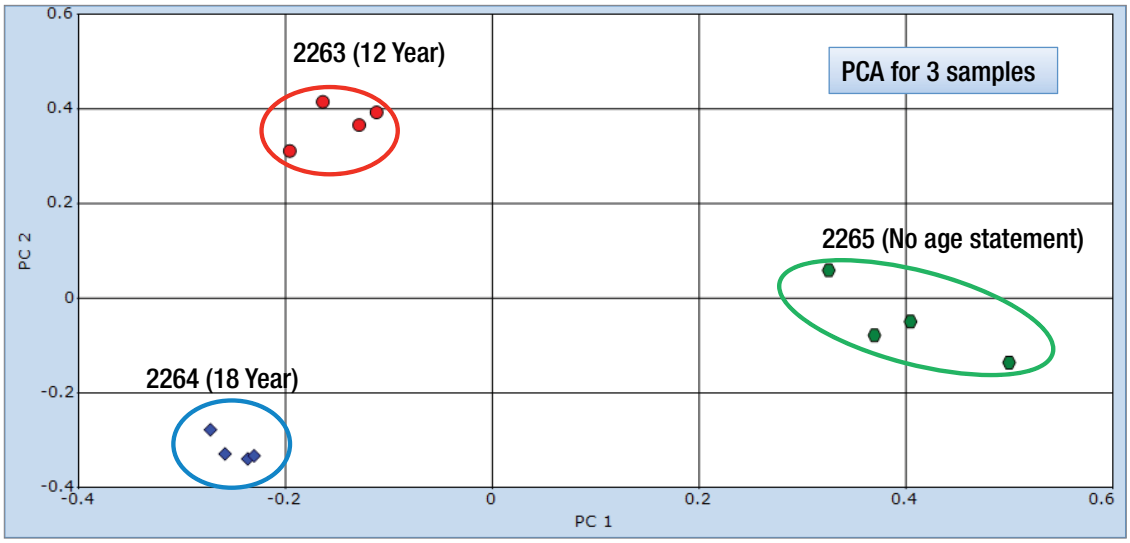

Figure 8. PCA chart generated with SIEVE for whiskies from the same distillery but with a different age declaration. Technical replicates agree.

Differences Between Whiskies from the Same Distillery

A further investigation of the dataset was to look at differences between whiskies from a single distillery, but with either a different age statement or different aging process. The replicate data for samples 2263 (12 years), 2264 (18 years) and 2265 (no age statement, aged in three barrels) was processed using SIEVE. The PCA showed that clear differences between these three samples existed and that the replicate injections were in agreement (Figure 8). The compounds that contribute to these differences were quickly isolated in TraceFinder. The deconvoluted peak list is presented as a heat map (Figure 9) to show how the intensities of peaks vary between the samples, thereby quickly isolating the peaks of interest.

A proposed identification of hydroxymethyl furfural was made using TraceFinder (Figure 10) which used spectral matching and accurate mass to sort the list of suggested compounds. This was based on the search index (>600) and scoring the proportion of the spectrum that could be explained by the suggested compound based on accurate mass. The presence of hydroxymethyl furfural (HMF) in whisky is not unusual as it is formed during the dehydration of sugars.[6,7] The elevated levels seen in sample 2265, which was aged in three different barrels including high sugar containing ex sherry and bourbon barrels, could possibly be explained by the interaction of the whisky with the wood surfaces. In particular, the heat charring of oak bourbon barrels is known to generate high levels of HMF.

Data Review - Whisky Lowlands [Unknown]

Heat Map

	Retention Time	M/Z	8April_60K_Whisky_003 MS Area	8April_60K_Whisky_004 MS Area	8April_60K_Whisky_005 MS Area
12	10.43	97.03	369,099	302,490	6,641,407,475
13	15.69	182.06	5,724,487,496	5,798,208,026	5,693,394,891
14	14.64	87.04	3,961,360,796	4,556,568,200	5,684,275,725
15	14.94	73.03	4,094,135,138	5,884,332,870	5,122,210,007

Figure 9. TraceFinder heat map showing peak area differences between the three aged whiskies. The peak at 10.43 minutes with a base peak of *m/z* 97.03 has a significantly higher response in the no age declared whisky compared to the 12 and 18 year old.

Peak Identification

Score	Matched Compound	Formula	CAS	SI	HRF Score	M+ m/z	M+	% Elements
94.2	**5-Hydroxymethylfurfural**	**C6H6O3**	**67-47-0**	**717**	**99.6216**	**126.03114**	**Yes**	**100**
69.4	4-Ethyl-2-hydroxycyclopent-...	C7H10O2	28017-6...	708	88.0651	126.06753	No	100
68.5	2-Butyn-1-al diethyl acetal	C8H14O2	2806-97...	662	88.0651	142.09883	No	100
67.9	Cyclopentanecarboxylic acid...	C13H16O2	55229-4...	630	88.1169	204.11448	No	100
67.3	Cyclopentanecarboxylic acid...	C13H22O2		602	88.1169	210.16143	No	100
67.2	Cyclopentanecarboxylic acid...	C12H13NO4		612	99.7754	235.0839	No	75
45.3	4-Hepten-3-one, 4-methyl-	C8H14O	22319-3...	699	28.2184	126.10391	No	100
44.5	4-Hexen-3-one, 4,5-dimethyl-	C8H14O	17325-9...	658	28.2184	126.10391	No	100
44.3	Furan, 2,3-dihydro-4-(1-met...	C8H14O	34379-5...	650	28.2184	126.10391	No	100

Hydroxymethyl furfural

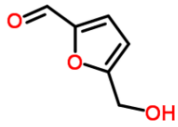

Figure 10. Identification of peak at 10.43 minutes as hydroxymethyl furfural. Screenshot of library match in TraceFinder. List of library hits are sorted based on a combination score (column 1) of spectral matching and high resolution filtering. Other acceptable spectral hits can be quickly eliminated based on accurate mass.

Conclusions

The results of this proof-of-concept study demonstrate that the Thermo Scientific Q Exactive GC Hybrid Quadrupole-Orbitrap Mass Spectrometer in combination with TraceFinder and SIEVE 2.2 software is an extremely effective tool for the chemical profiling of complex samples. The Orbitrap mass spectrometer delivers excellent mass accuracy for all components in a sample that leads to fast and confident characterization of samples regardless of the concentration of the component.

- Reliable and robust chromatographic separation in combination with fast data acquisition speeds make the Q Exactive GC system an ideal platform for chemical profiling of complex samples.
- Routine resolving power of 60,000 FWHM and a wide dynamic range eliminates isobaric interferences, combined with consistent sub-1 ppm mass accuracy and excellent sensitivity, increases confidence in the identification of compounds, especially in complex matrices.
- SIEVE 2.2 and TraceFinder software facilitated fast and comprehensive characterization of the whisky samples, isolating and identifying compounds with confidence.
- The EI and PCI data obtained was used for tentative compound identification against commercial libraries. Where no library match was made, the mass accuracy allowed for elemental compositions to be proposed with a high degree of confidence. Proposed identifications can be quickly confirmed or eliminated based on accurate mass of fragments using Mass Frontier 7.0.

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Fast Screening, Identification, and Quantification of Pesticide Residues in Baby Food Using GC Orbitrap MS Technology

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Application Note 10449

Keywords
Baby Food, Exact Mass, Screening, Food Safety, GC Orbitrap, High Resolution GC-MS, Pesticide Analysis, Quantification, TraceFinder

Introduction

Pesticides are chemicals widely used to control a variety of pests, such as insects, plant pathogens, weeds, etc. The use of pesticides may result in residues in crops, therefore, strict regulations are in place to control the use of these chemicals and to ensure that concentrations do not exceed statutory maximum residue levels (MRLs).[1]

Pesticides are measured almost exclusively by liquid chromatography (LC) and gas chromatography (GC) analytical methodologies. GC coupled to a mass spectrometer (MS) as a detector is widely used in many pesticide residue laboratories, because many pesticides are not amenable to LC-MS or ionize poorly under soft ionization techniques. GC offers good separation efficiency and a choice of MS detectors, including single or triple quadrupoles. Quadrupole mass analyzers are selective, sensitive, and cost-effective instruments that operate at nominal mass resolution. When using quadrupole MS, the selectivity required to separate target pesticides from chemical background is achieved by by the use of either selected ion monitoring (SIM) or selected reaction monitoring (SRM). Both SIM and SRM are used in targeted experiments in which the mass spectrometer is pre-programmed using a list of preselected pesticides. However, targeting specific compounds during acquisition limits the scope of analysis and can result in false negative results (non-detection) for both unknown and untargeted compounds, which may be of concern with respect to food safety.

This limitation has led to increased interest in developing methods using MS analyzers that can operate in full scan with a higher mass resolving power than triple quadrupoles, but provide similar levels of selectivity and quantitative performance. Until now, high-resolution, accurate-mass GC-MS instruments have not gained wide acceptance due to their limited ability to provide full scan selectivity and quantitative performance comparable to triple quadrupole instruments operated in SRM.

In this work, we demonstrate the use of GC coupled with Orbitrap™ MS technology for fast, high throughput pesticide residues analysis in baby food samples, with an almost unlimited scope in the analysis through full scan acquisition. Quantitative performance comparable to triple quadrupoles and compliance with SANCO® guidelines[2] will also be demonstrated.

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Sample Preparation

Baby food samples were extracted using the a citrate buffered QuEChERS protocol, described previously.[4] The final extracts (1 g/mL in acetonitrile) were spiked with a mixture of 132 pesticides at concentrations corresponding to 0.5–100 ng/g (ppb) for the majority of analytes and 1.0–200 for some analytes.

Instrument and Method Setup

In all experiments, a Thermo Scientific™ Q Exactive™ GC hybrid quadrupole-Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH Autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SilMS 15 m × 0.25 mm I.D. × 0.25 µm film capillary column (P/N: 26096-1301). Additional details of instrument parameters are shown.

GC and Injector Conditions

TRACE 1310 GC Parameters	
Injection Volume (µL):	1.0
Liner:	asymmetric baffled (P/N: 45352062)
Inlet (°C):	75
Inlet Module and Mode:	PTV, cold splitless
Injection time (min):	0.1
Transfer rate (°C/sec):	2.5
Transfer temperature (°C):	300
Transfer time (min):	3
Cleaning rate (°C/sec):	330
Carrier Gas, (mL/min):	He, 1.2
Oven Temperature Program	
Temperature 1 (°C):	40
Hold Time (min):	1.5
Temperature 2 (°C):	180
Rate (°C/min)	25
Temperature 3 (°C):	300
Rate (°C/min)	100
Hold Time (min):	3

The Q Exactive GC system was tuned and calibrated using peaks of known mass from a calibration solution (FC 43, CAS 311-89-7) to achieve mass accuracy of < 0.5 ppm RMS. The system was operated in electron ionization mode (EI) using full scan and 60,000 mass resolution (Full Width at Half Maxima, measured at *m/z* 200), meeting the recommended SANCO resolution criteria[2] for high resolution analytical instrumentation. Chromatographic data was acquired with a minimum of 12 points/peak to ensure consistent peak integration.

Mass Spectrometer Conditions

Q Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	280
Ionization type:	EI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (*m/z*):	50–500
Mass resolution (FWHM at *m/z* 200):	60,000
Lockmass (*m/z*):	207.03235

Data Processing

Data was acquired and processed using Thermo Scientific™ TraceFinder™ software. TraceFinder software allows the analyst to build acquisition and processing methods for high throughput screening and quantitative analysis and incorporates library searching capabilities as well as easy data reviewing and data reporting.

Results and Discussion

The objective of this study was to evaluate the utility of Orbitrap-based GC-MS technology for fast pesticides screening and quantification to increase sample throughput and laboratory productivity. Various analytical parameters were assessed and the results of these experiments are described.

Chromatography

Good chromatographic separation was obtained using the GC conditions described. An example of chromatography for the matrix-matched standard (corresponding to 100/200 ng/g) is given in Figure 1. The total ion chromatogram, as well as the extracted ion chromatograms (XIC, ±2 ppm extraction mass window) of the first (dichlorvos, *m/z* 184.97650, RT = 4.46 min) and last (deltamethrin, *m/z* 252.90451, RT = 10.33 min) eluting pesticides, are shown. The fast separation allowed for a high sample throughput as described elsewhere.[4]

MS Acquisition Speed

When using short GC run times, the analyte chromatographic peak widths are narrow, typically 2.5 seconds. This narrow peak width necessitates fast MS acquisition rates in order to obtain enough scans/chromatographic peak. When the number of points per peak is not sufficient to define a Gaussian shape, the peaks of interest can be integrated inaccurately, which in turn affects the reproducibility, peak integration, and ultimately, the accuracy of target compound quantification. An example of typical number of scans acquired using the Q Exactive GC system operated at 60,000 resolution for EPTC in baby food is shown in Figure 2. Aside from producing an adequate number of scans/peak (17), excellent mass accuracy (0.5 ppm RMS) was obtained for every scan across the peak.

Pesticides Targeted Screening

A simple, targeted screening experiment was set up as a first test to screen for pesticides that were spiked into the baby food matrix. This was performed using the TraceFinder software against an in-house compound database containing 183 pesticides. The database contains the compound name, theoretical exact masses for at least three fragment ions, and expected retention time information for the GC conditions used for the sample analysis.

Compound detection and identification was based on retention time (±0.1 min window), accurate mass information (±2 ppm window), isotopic pattern similarity (measured versus theoretical), and library search hit (NIST14). Using these criteria, all 132 pesticides were positively detected and confirmed in the 10/20 ng/g baby food sample.

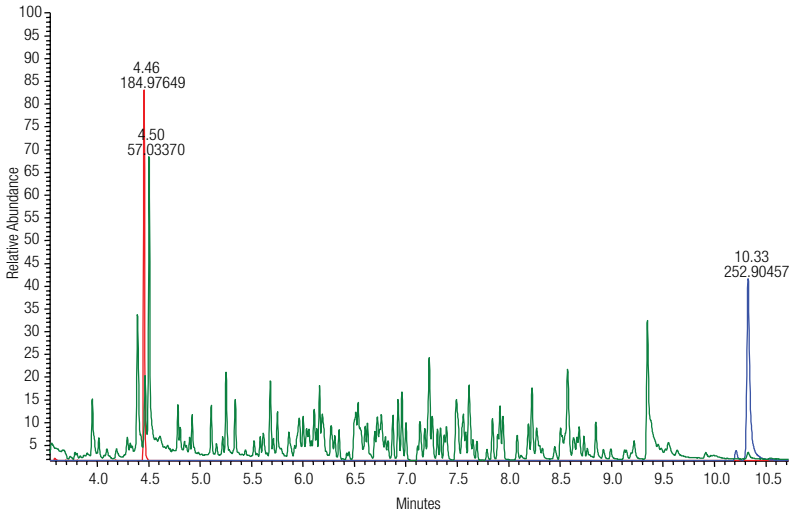

Figure 1. Overlay of the total ion chromatogram (EI full scan) and the extracted ion chromatograms (XIC) of the first (dichlorvos, RT = 4.46 min) and the last (deltamethrin, RT = 10.33 min) eluting pesticides. Relative abundance (Y axis) adjusted to emphasize XIC for dichlorvos and deltamethrin.

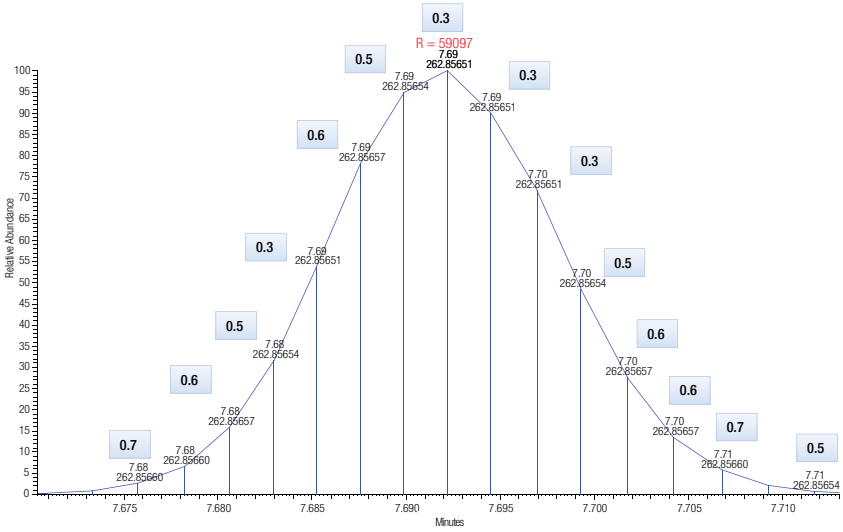

Figure 2. Extracted ion chromatogram (XIC) of dieldrin (*m/z* 262.85642, ±2 ppm mass window) showing 17 scans/peak (peak width 2.4 sec). Data acquired in full scan at 60,000 FWHM resolution (the exact resolution used is annotated in red). Measured accurate mass for each scan is shown as well as mass difference (ppm).

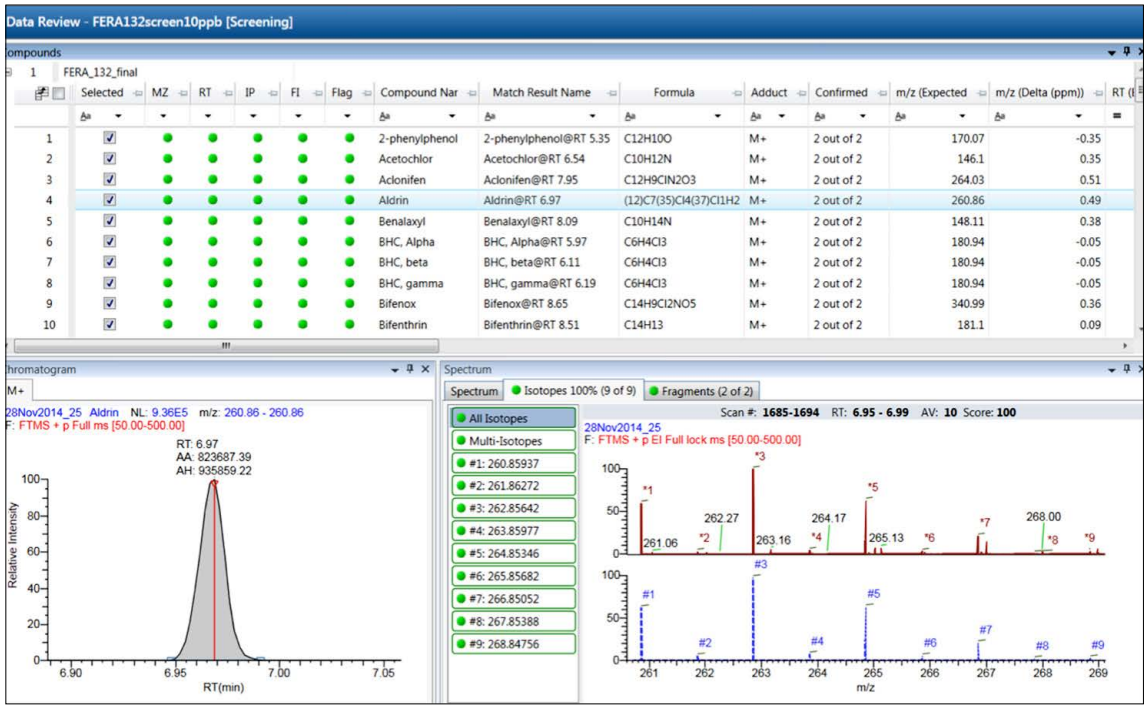

Figure 3. TraceFinder software screening result browser showing positively identified pesticides in the 10 ng/g sample. Compound identification and confirmation (aldrin showed as an example) was based on accurate mass identification (±2 ppm mass window), retention time (RT), isotopic pattern (IP), and fragment ions (FI). Measured and theoretical isotopic clusters are shown.

An example of the compound detection and identification workflow for aldrin is shown in Figure 3. Data acquired in full scan is deconvoluted and retention time and accurate mass information are then used to identify the compound. Aldrin was identified based on the RT, and the presence of an accurate mass quantification ion (<0.5 ppm mass error) and the characteristic fragment ions. Moreover, the elemental composition of the quantification ion ($C_7C_{15}H_2$) was used to check the isotopic pattern fit against the measured isotopic pattern. As shown in Figure 3, a 100% isotopic fit was obtained for aldrin, adding to the confidence in compound identification.

Pesticide Residue Quantification

The quantitative performance of the Q Exactive GC system for compound quantification was tested for all 132 pesticides. To assess quantitative performance, a matrix-match calibration curve was constructed over a concentration range of 0.5–100 ng/g (or 1.0–200 ng/g). System sensitivity, linearity, and peak area reproducibility were evaluated. Additionally, mass accuracy of the target pesticides was assessed across the concentration levels.

Sensitivity

Almost all pesticides (95%) were detected in the lowest calibration matrix-matched standard 0.5 (or 1.0) ng/g. Examples of chromatography at this concentration level are shown in Figure 4. At the 5 ng/g level, all of the compounds detected had ion ratios valued within a 15% limit of the average ion ratio values derived from the calibration curve across all concentrations.

Estimation of Instrument Detection Limit (IDL) and Peak Area Repeatability

System sensitivity was assessed by calculating the IDL for each pesticide. The IDL of the target pesticides represents the smallest signal above background noise that an instrument can consistently and reliably detect. This signal was determined empirically by repeatedly injecting (n=10) the 5 ng/g (and 10 ng/g) matrix-matched standard and taking into account the Student's-*t* critical values for the corresponding degrees of freedom (99% confidence). The results of this experiment showed an average %RSD for the peak area reproducibility of 6 % (Figure 5).

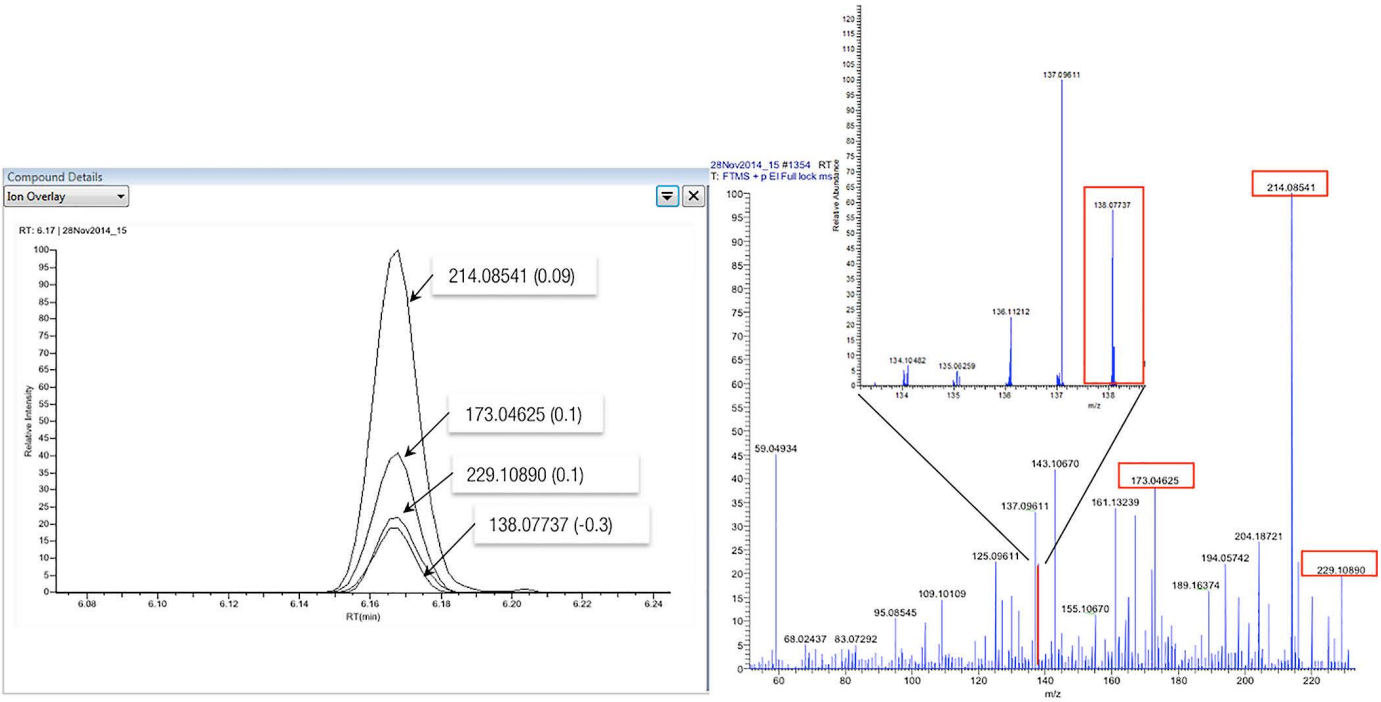

Figure 4. Terbuthylazine at 0.5 pg (on column concentration) showing an XIC overlay for the quantification ion and three additional confirmation fragment ions (left). The measured mass for each ion and mass error (in ppm) are annotated. Mass spectrum (right) highlighting the ions used for quantification and confirmation; the zoomed area shows the least intense fragment (*m/z* 138.07737) measured with a mass accuracy of 0.3 ppm.

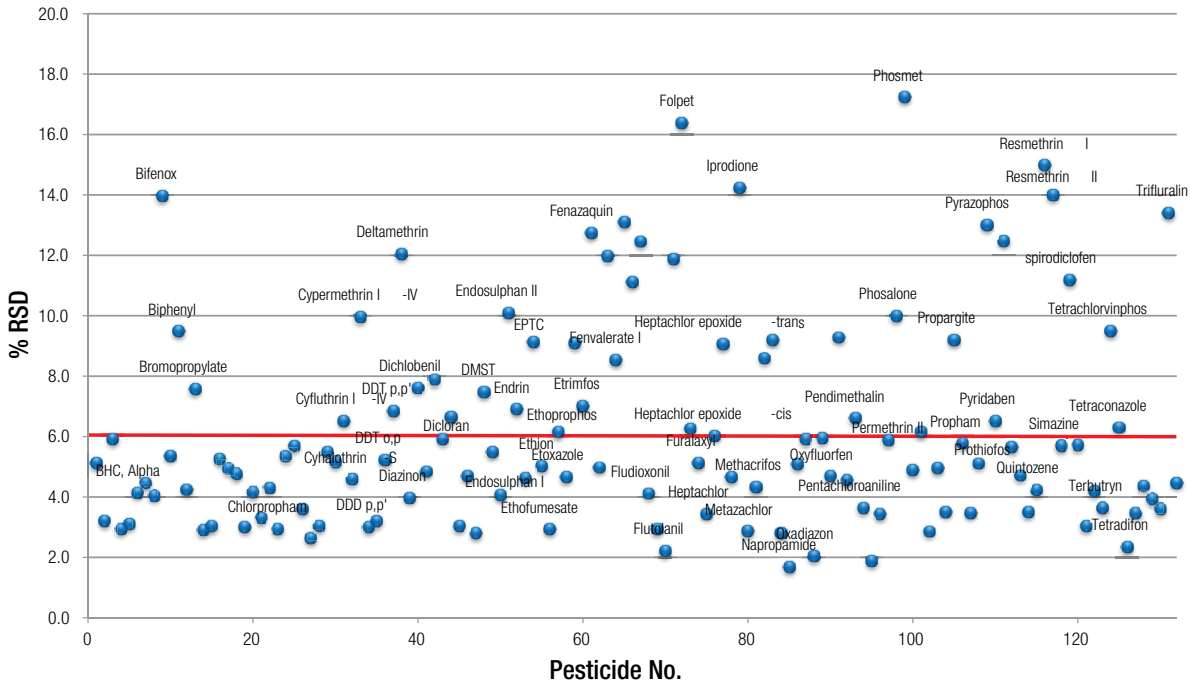

Figure 5. Absolute peak area repeatability (% RSD, n=10) at 5 or 10 pg injected on column for all 132 pesticides measured. The average %RSD value (solid line) is shown.

All the IDLs derived from the Q Exactive GC system data were lower than the typical MRLs established by the European Union for baby food samples. For most pesticides, these MRLs are currently set at <0.01 mg/kg (10 ng/g).[3] Calculated IDLs were compared to the IDL values obtained for the same pesticides using the Thermo Scientific™ TSQ™ 8000 Evo Triple Quadrupole GC-MS/MS system.[4] The results of this experiment demonstrated that the sensitivity of the Q Exactive GC system is comparable to that of the TSQ 8000 Evo GC-MS/MS system, with 91% of pesticides having an IDL < 2 ng/g (Figure 6).

Mass Accuracy

Obtaining accurate mass information in a consistent manner is critical for determining the identity of a pesticide as well as maintaining a high degree of discrimination through the resolving power of the instrument, against matrix interference.[5] The mass accuracy for all 132 pesticides was assessed at the 5 ng/g (or 10 ng/g, depending on compound) level from a series of n = 10 repeat injections. The mass deviation values did not exceed 1 ppm for any of the analytes and the overall mass accuracy average value was 0.4 ppm, providing the highest confidence in accurate and selective detection (Figure 7).

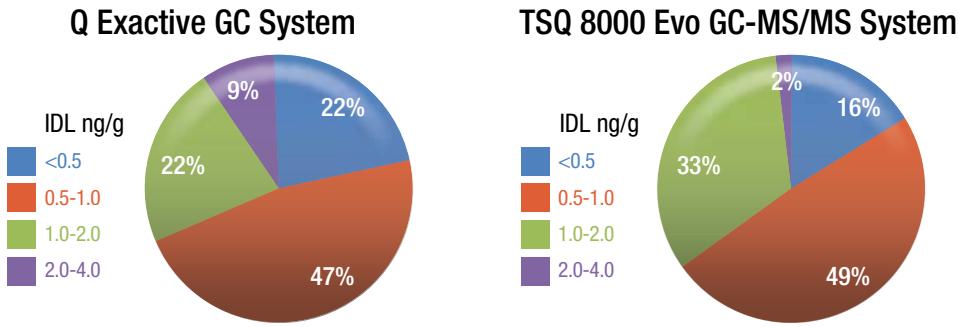

Figure 6. Comparison of the IDL99 (ng/g) calculated for 132 pesticides from a 5 ng/g matrix-matched standard from the Q Exactive GC System (left) and TSQ 8000 Evo GC-MS/MS system (right). The percentage of pesticides and corresponding IDL interval, relative to the total number of target compounds (132), is indicated.

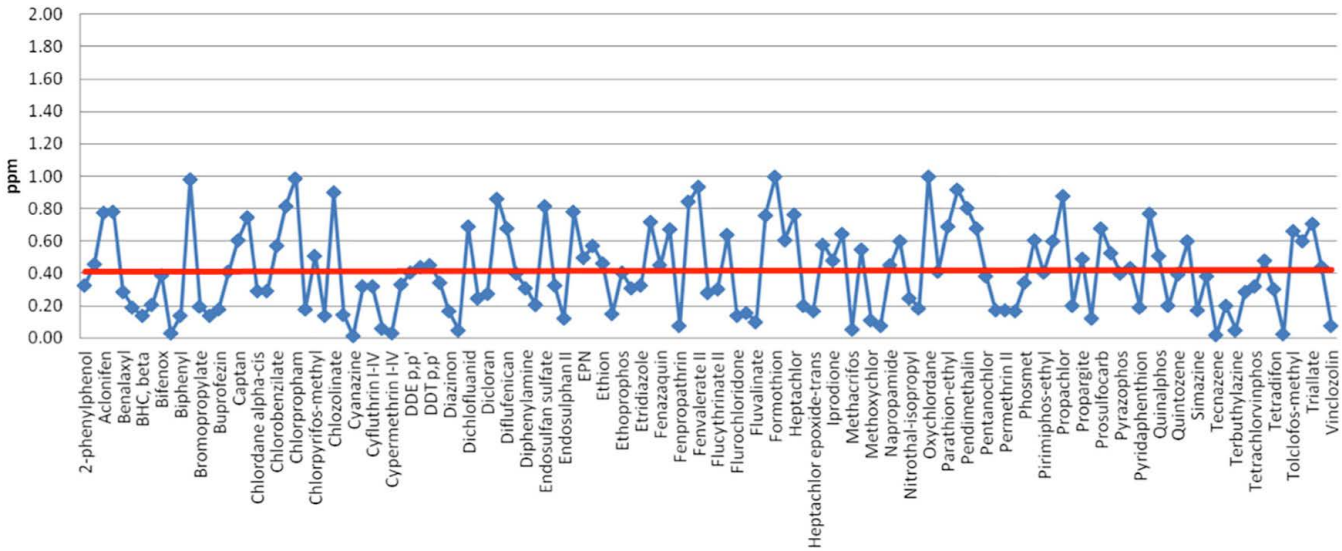

Figure 7. Accurate mass measurements (average value of n = 10) for the pesticides identified in the baby food sample at the 5 (or 10) ng/g level.

Linearity of Response

Quantitative linearity was assessed across a concentration range of 0.5–100 ng/g (or 1–200 ng/g for some analytes) using matrix-matched calibration standards injected in triplicate at each level. In all cases, the coefficient of determination (R^2) was >0.99 with an average value of R^2 = 0.997 and with residual values from the regression line of <25%. Examples of compound linearity are shown in Figure 8.

Conclusions

- The Q Exactive GC system provides high performance quantitative analysis in full scan for broad-scope pesticide residue testing, even with fast GC separations.
- The fast scan speed, high resolution, and outstanding mass accuracy, together with full scan sensitivity allow reproducible and accurate pesticide quantification at very low levels.
- Acquisition with a routine mass resolution of 60,000 FWHM at *m/z* 200 eliminates isobaric interferences, increasing confidence in results when screening pesticides in complex matrices. The consistent sub-ppm mass accuracy achieved for all compounds ensures confident compound identification.
- The Q Exactive GC system provides quantitative performance that is highly comparable to that of GC triple quadrupole MS instruments.
- Thermo Scientific TraceFinder software enables analysts to develop high throughput screening and quantitative analyses quickly and accurately.

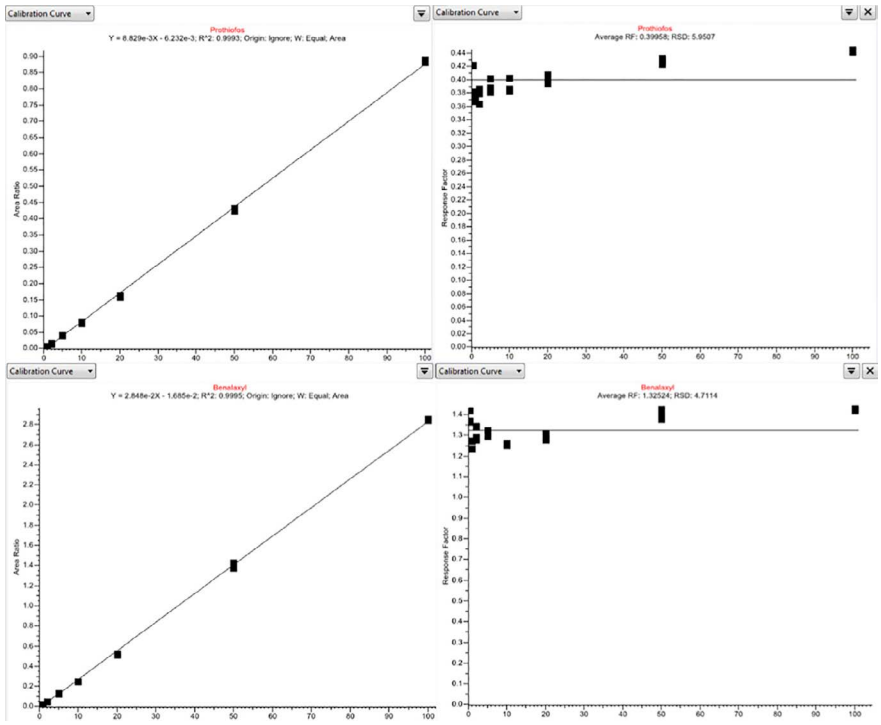

Figure 8. Coefficient of determination (left) and residuals values (%RSD) for prothiofos and benalaxyl calculated for a linear range of 0.5–100 ng/g.

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CASE STUDY
Laboratori de l'Agència de Salut Pública de Barcelona

GC Orbitrap MS/MS technology adds extra certainty to food safety and environmental testing

Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS System

"Confidence in analytical results is extremely important because they have legal and financial consequences. We need to eliminate chances of false positive or false negatives. Though we routinely use triple quadrupole instruments, if we get any inconclusive results, we go to GC Orbitrap MS/MS technology to add additional specificity and sensitivity to our workflows."

— Dr. Nuria Cortés-Francisco, Laboratori de l'Agència de Salut Pública de Barcelona

CSB Consorci Sanitari de Barcelona

Agència de Salut Pública

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"Having another technology to confirm results is making our work easier by ensuring there are no errors due to matrix interferences. And, with flexible scope accreditation, we have to accept any analytical request, set up a method, and provide results as soon as possible."

— Dr. Nuria Cortés-Francisco

Food safety and environmental testing labs face two important obstacles when using traditional triple quadrupole GC-MS methods. First, it is difficult to reach very low limits of quantitation (LOQs) for some emerging compounds of concern, such as polybrominated diphenyl ethers (PBDEs), in complex sample matrices without time-consuming sample concentration prior to analysis. High molecular weight compounds such as BDE-209 pose an additional challenge, because the sensitivity of triple quadrupole systems drops off at higher molecular weights. Achieving certainty in results using a practical confirmatory method or alternative technology is extremely important when triple quadrupole methods produce ambiguous results.

These are among the challenges faced by the Laboratori de l'Agència de Salut Pública de Barcelona, the laboratory chartered with supporting food safety and environmental surveillance programs for Barcelona, Spain. With ISO/IEC 17025: 2005, accreditation, including flexible scope accreditation by the National Entity of Accreditation (ENAC), and frequent new EU alerts about potential contamination, the laboratory must also rapidly respond to emerging food safety and environmental matters.

Using the Thermo Scientific Q Exactive GC Orbitrap GC-MS/MS system, the laboratory realizes the additional certainly provided high specificity and sensitivity, substantially reducing the chance false negative and false positive results.

GC Orbitrap MS/MS technology solves specificity and sensitivity challenges

Food safety and environmental testing methods often rely on triple quadrupole GC and LC-MS methods. These methods usually provide the sensitivity needed to meet regulatory requirements, but when they don't because of matrix interferences, or when a confirming method is needed, orthogonal technology with enhanced specificity and sensitivity is desirable. This is why the Laboratori de l'Agència de Salut Pública de Barcelona adopted a Q Exactive GC Orbitrap GC-MS/MS approach to confirming suspect triple quadrupole GC-MS results.

According to Dr. Nuria Cortés-Francisco, "the concern we had with our triple quadrupole methods was matrix interferences, where in some cases we were not confident in our results because the confirming ion ratio requirements weren't met. Based on triple quadrupole method requirements, you would say it's a negative, but with an obvious peak we were concerned that the negative was false. In these cases, we confirm our triple quadrupole results using the Q Exactive GC Orbitrap GC-MS/MS system. With a triple quadrupole system you only obtain the nominal mass so you can't be sure if it's the target compound or matrix interferences."

To develop the protocol, the laboratory built a HRAM database that includes the retention times, exact masses, and confirmatory ions for target compounds of interest. Using the database, the laboratory can quickly set up a method for a confirmatory analysis. The confirmatory method uses three ions—one for quantification and two for confirmation to compare two ion ratios—exceeding the SANTE guidelines that require two ions measured by HR MS and one ion ratio.

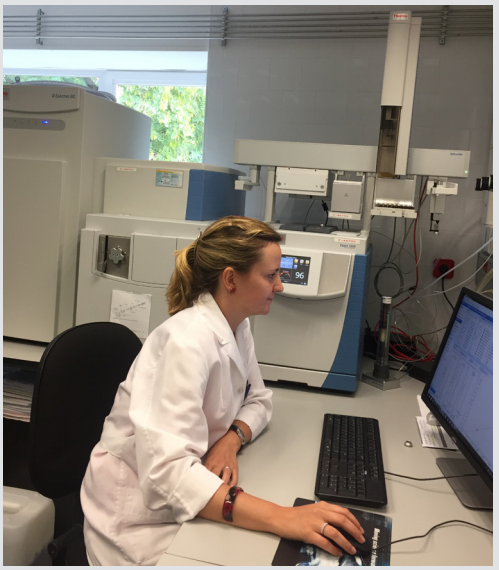

Photo courtesy of Laboratori de l'Agència de Salut Pública de Barcelona

"All suspicious results from low-resolution GC-MS triple quadrupole instruments are now confirmed by the Q Exactive GC Orbitrap GC-MS/MS system. Using both full-scan and SIM methods, and our HRAM compound database, confirmation of results is extremely fast and effective."

— Dr. Nuria Cortés-Francisco

Confirming suspect results for propargite

In 2016, Dr. Cortés-Francisco applied the Q Exactive GC Orbitrap GC-MS/MS system protocol to address an alert for propargite in oranges. When analyzed using traditional QuEChErs sample preparation and triple quadrupole GC-MS, most of the samples tested negative for propargite. However, some results were inconclusive. All of the samples had some signal for both target ion transitions, but the confirming ion ratio criteria were only met in some cases. Using the database, the laboratory promptly set up a Q Exactive GC Orbitrap GC-MS/MS method, reanalyzed the samples, and confidently determined one sample positive.

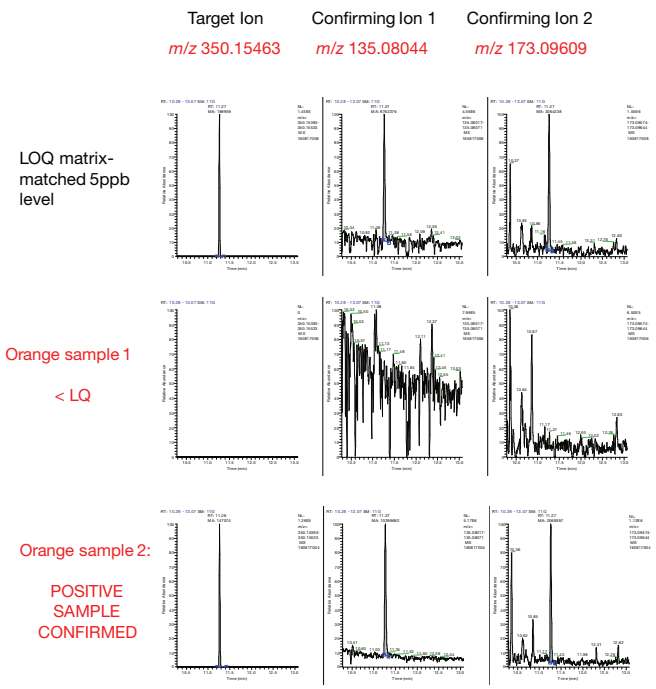

Figure 1. Q Exactive GC Orbitrap GC-MS/MS system analysis of orange samples for propargite (MRL for propargite is 0.01 mg/kg.) When some triple quadrupole results were inconclusive, the samples were reanalyzed using the Q Exactive GC Orbitrap GC-MS/MS system, which was subsequently able to distinguish between the matrix and pesticide signals, confirming the positive findings.

"The Q Exactive GC system provides high selectivity as we can use very high resolution at scan speeds amenable to GC, and its sensitivity is comparable to the best triple quadrupole systems."

— Dr. Nuria Cortés-Francisco

Achieving low detection limits for difficult PBDEs

Though brominated compounds have been analyzed in environmental applications for years, in 2014 the EU published a recommendation that these compounds should also be analyzed in foods down 0.01 ng/g. Of the brominated compounds targeted, one of the largest with ten bromines—BDE-209—is difficult to analyze using triple quadrupole GC-MS due to its limited sensitivity for higher molecular weight compounds in matrices. For this reason, some labs have chosen to perform the analysis using magnetic sector methods.

"Because we needed to achieve very low LOQs for PBDEs in many kinds of difficult matrices—seafood, vegetables, meat, eggs, olive oil, etc.—we applied the Q Exactive GC Orbitrap GC-MS/MS system," noted Dr. Cortés-Francisco. "With a simple method that uses selected ion monitoring (SIM) windows, we monitor not only the main peak but also the isotopic pattern, allowing us to be sure of a positive hit."

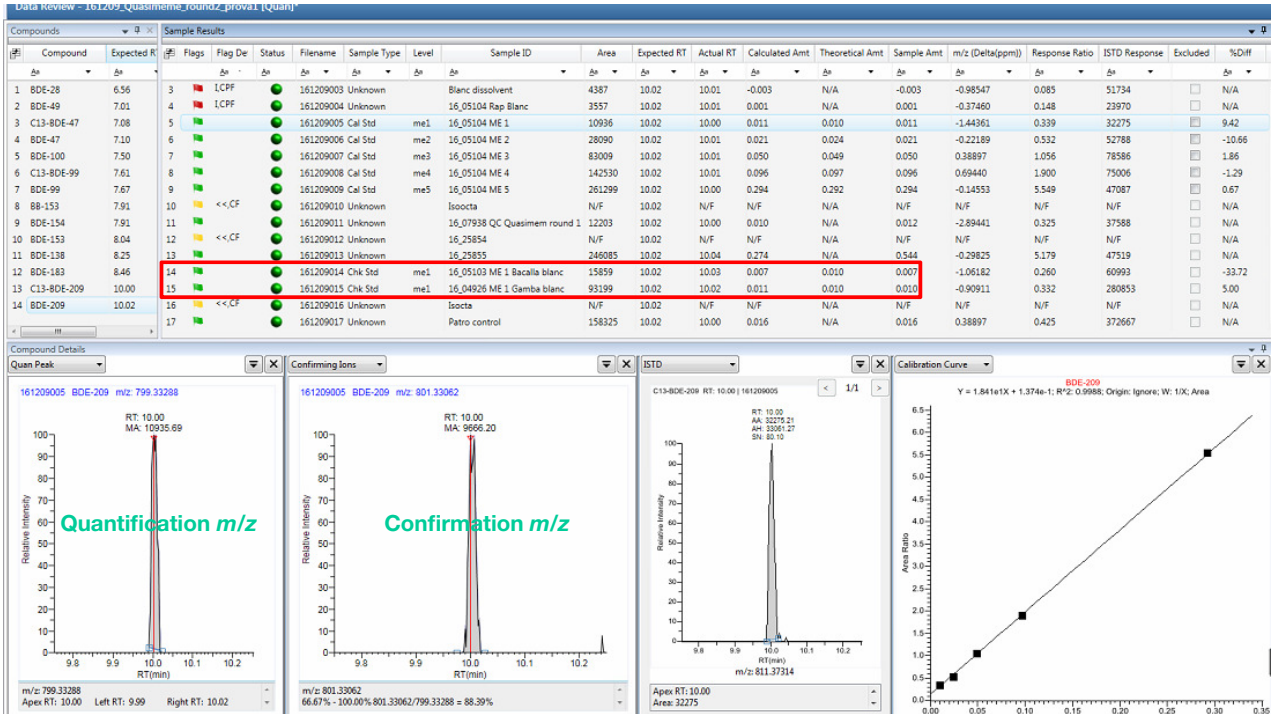

Figure 2. Q Exactive GC Orbitrap GC-MS/MS method results. Matrix-matched calibration curve for monkfish sample spiked with BDE-209 from 0.01 to 0.3 ng/g. Courtesy of Laboratori de l'Agència de Salut Pública de Barcelona.

"We're easily reaching the LOQs for PBDEs, because the Q Exactive GC Orbitrap GC-MS/MS system has the necessary sensitivity even at higher masses, and its high resolution allows us resolve matrix interferences. There are not many labs analyzing PBDE compounds at 0.01 ng/g levels because its nearly impossible, but we are able to do it thanks to the Q Exactive GC Orbitrap GC-MS/MS system."

— Dr. Nuria Cortés-Francisco

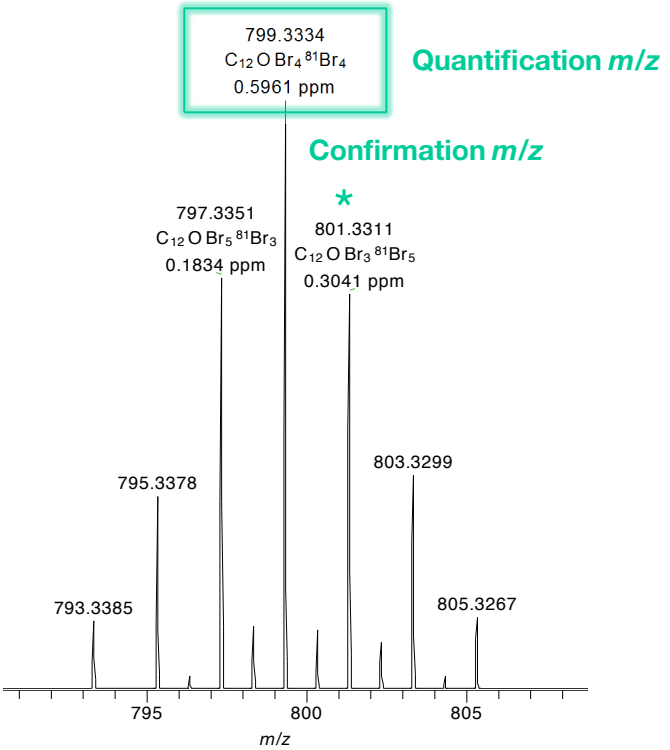

Figure 3. The Q Exactive GC Orbitrap GC-MS/MS system can acquire a full isotopic pattern for difficult compounds such as BDE-209 (shown here) without sensitivity loss, providing more confidence in results. Courtesy of Laboratori de l'Agència de Salut Pública de Barcelona.

Conclusion

Confidence in the results of food safety and environmental testing is of utmost importance. The Q Exactive GC Orbitrap GC-MS/MS system brings together the power of high-resolution GC and HRAM Orbitrap MS to provide high-specificity, high-sensitivity target compound analyses when GC-MS triple quadrupole methods produce inconclusive results or cannot achieve required detection limits. When a confirming method is needed, the Q Exactive GC Orbitrap GC-MS/MS system is the orthogonal technology to apply.

Photo courtesy of Laboratori de l'Agència de Salut Pública de Barcelona

About Nuria Cortés-Francisco

Dr. Nuria Cortés-Francisco received her PhD in Analytical Chemistry from the University of Barcelona. She developed her career at the Spanish Council for Scientific Research, where she mainly worked on the use and study of high-resolution mas spectrometry for the analysis of organic pollutants in environmental and food samples. Since 2014, she has been the Emerging Contaminants and Mass Spectrometry Specialist at the Laboratori de l'Agència de Salut Pública de Barcelona.

About the Laboratori de l'Agència de Salut Pública de Barcelona

The Laboratori de l'Agència de Salut Pública de Barcelona (Laboratory of the Public Health Agency of Barcelona) was created in 1887. Since then, it has evolved to meet the needs of public health in the city, and in 2003 it underwent a transformation with the integration of the human and material resources of the Laboratory of Public Health of the Generalitat de Catalunya in Barcelona.

The laboratory contributes to the identification and control of public health programs in the community. This is done by supporting analytical monitoring programs for food safety and environmental surveillance and involves screening of over 35000 samples per year. The laboratory has more than 1,000 analytical parameters of reference covered by the accreditation ISO 17025: 2005, granted by the National Entity of Accreditation (ENAC). Modern instrumentation and having the scope of flexible accreditation enables the laboratory to rapidly respond to emerging problems in the areas of food safety. The work of the laboratory is based on collaboration with clients, adaptation to new needs and demands, and the continuous expension of services and competences. Everyone can access the services offered by the laboratory; both public administrations and companies and individuals that want to ensure the quality and safety of products in accordance with current food legislation.

Photo courtesy of Laboratori de l'Agència de Salut Pública de Barcelona

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GC-Orbitrap for Food Safety Analysis

Posters

Chemometric Assessment of Volatile Fraction of Pesto by SPME Arrow GC Orbitrap Mass Spectrometry


Chemometric Assessment of Volatile Fraction of Pesto by SPME Arrow GC Orbitrap Mass Spectrometry

'Pesto genovese' is a well-known pasta sauce. Pesto is a basil-based sauce characterized by unique organoleptic features associated with its ingredients, consisting mainly of crushed basil leaves, cheese (parmesan or pecorino), pine nuts and garlic blended with extra-virgin olive oil. The production of pesto for wide distribution requires the use of additional ingredients and various technologies such as pasteurization and sterilization to extend the product shelf-life ensuring freshness for consumers. The preservation processes usually require high temperatures that can lead to changes in pesto composition affecting its taste and aroma.

In this study headspace solid phase micro-extraction (SPME) with Arrow technology coupled with gas-chromatography (GC) and Thermo Scientific™ Orbitrap™ high resolution mass spectrometry (HRMS) was used to determine the volatile profile of various pesto samples that were produced using various technological methods.

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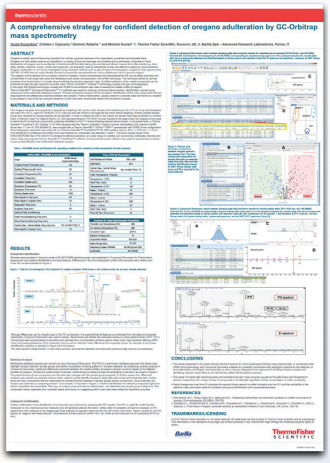
A comprehensive strategy for confident detection of oregano adulteration by GC-Orbitrap mass spectrometry

A Comprehensive Strategy for Confident Detection of Oregano Adulteration by GC-Orbitrap Mass Spectrometry

The oregano aroma derives from a complex mixture of volatiles, mainly monoterpenes and sesquiterpenes that can be easily extracted and concentrated in one single step using the headspace solid phase microextraction (HS-SPME) technique. This technique allows for sample extraction and concentration in a single step simplifying the sample preparation step. Confident detection of the volatile compounds can be achieved through the high resolution accurate mass Thermo Scientific™ Orbitrap™ technology coupled with gas chromatography.

In this study the Orbitrap technology coupled with SPME Arrow extraction was used to assess the volatile profile of oregano. Thermo Scientific™ Compound Discoverer™ 3.1 software was used for un-known compound deconvolution, identification, sample group assessment and multivariate statistical analysis.

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