thermo scientific

GC-MS for food safety analysis

Applications compendium

ThermoFisher SCIENTIFIC

Introduction

In order to protect consumers and the environment, monitoring the food supply to ensure levels of chemical residues and contaminants are compliant with statutory levels set by regulatory bodies is imperative. Because regulations differ in different parts of the world, analytical food testing laboratories and food manufacturers must first navigate the complexity of regulatory frameworks before considering the analysis. Detecting and quantifying many thousands of residues and contaminants from different chemical classes at potentially extremely low levels in diverse food commodities and products is very challenging. This challenge is further complicated when we consider that food products are traded in complex global supply chains, for which details of the history of products, such as cultivation, treatment, storage and processing, are often unknown. For example, the use of pesticides to protect crops from pests during cultivation, storage and transport will often leave detectable residues in food, while persistent organic pollutants (POPs) in the soil, water or in the air can contaminate crops. Additionally, chemicals in food packaging materials can leach into the food. Biocides used in food preparation facilities can also lead to contamination of food. These are just a few examples of many sources of contamination. It is easy to see why the comprehensive analysis of individual samples often requires multiple analyses assessments by a range of analytical techniques, such as liquid chromatography, ion chromatography and gas chromatography in combination with selective detectors and or mass spectrometers, as well as spectroscopic techniques. Thermo Fisher Scientific can offer the complete portfolio of instruments needed for comprehensive, targeted and non-targeted analysis. This compendium focuses on gas chromatographic solutions applicable to testing laboratories involved in food-related analyses.

This compendium incorporates selected application examples to highlight the use of Thermo Fisher Scientific GC-MS portfolio solutions for food analysis. One of the tasks for the analyst is to choose appropriate instrumentation based on the method requirements. The first application example is based on the use of the Thermo Scientific™ TRACE™ 1310 Gas Chromatograph equipped with a flame ionization detector (FID) for the analysis of fatty-acid methyl ester (FAMES) in the profiling of fatty foods. A unique feature of the Thermo Scientific GC systems is modularity, which allows instant-connect injectors and detectors to be exchanged in minutes without tools, enabling a single GC system to provide a high level of flexibility. The use of head space sampling coupled with GC-FID/MS is demonstrated for the analysis of residual solvents in food packaging materials. Additional applications show the use of the Thermo Scientific™ ISQ™ 7000 single quadrupole GC-MS system for the analysis of phthalates, a ubiquitous contaminant class in plastics, and the quantitation of acrylamide, a process contaminant formed by the Maillard reaction between sugar and amino acid molecules when heated.

The Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system which provides higher selectivity than the ISQ system, is highlighted in combination with automated online micro-SPE food extract cleanup for the analysis of pesticides. Automated micro-SPE is based on the Thermo Scientific™ TriPlus™ RSH robotic autosampler to automate the removal of matrix co-extractives online with GC injection, increasing method robustness and instrument uptime for an ultimate increase in productivity. Applications showing the TSQ 9000, equipped with an Advanced Electron Ionization (AEI) source for unparalleled ultra-high sensitivity, are included to demonstrate the ultra-trace targeted analysis of pesticides, dioxins and polychlorinated biphenyls (PCBs). An upgrade path from the single quadrupole system to any of the triple quadrupole systems, enables laboratories not only to adapt to analytical developments, but also to future proof their investment.

The final applications listed in this compendium focus on Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system which uses full-scan, high-resolution/accurate-mass non-targeted acquisition with unprecedented resolving power, sub-ppm accurate mass and ppt level sensitivity. The system can be used for targeted analysis of a pre-defined list of chemicals, or non-targeted analysis of unknown chemicals as demonstrated by the accurate and precise quantitation of pesticides, POPs and the profiling of food packaging materials.

More information on these technologies is available here.

Contents

Next page

APPLICATION NOTE

No. 21557

Separation of 37 Fatty Acid Methyl Esters Utilizing a High-Efficiency 10 m Capillary GC Column with Optimization in Three Carrier Gases

Aaron L. Lamb
Thermo Fisher Scientific, Runcorn, UK

Key Words
TR-FAME, fatty acid methyl esters, FAMEs, GC, GC-MS, carrier gas

Goal
To demonstrate the separation of 37 fatty acid methyl esters (FAMEs) on the highly efficient 10 m Thermo Scientific™ TRACE™ TR-FAME GC column, and to show increased sample throughput of up to 400% relative to a 100 m column by optimizing the separation for efficiency and speed using three commonly available carrier gases: nitrogen, hydrogen, and helium.

Introduction

Fats are a major constituent of many foodstuffs including edible oils, meat, fish, grain, and dairy products. They consist of triacylglycerides, which are species that contain glycerol sub-units esterified with aliphatic fatty acid groups (Figure 1).

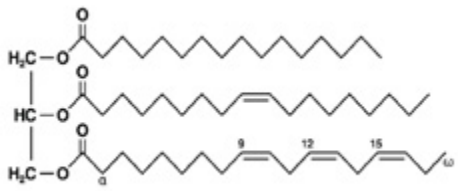


Figure 1. A general triacylglyceride.

The aliphatic chain can vary in carbon length, degree of unsaturation, and isomerization around double bonds giving *cis* and *trans* forms of the fatty acids. *Trans* and hydrogenated fats are important food components that are regularly measured.

ThermoFisher SCIENTIFIC

Gas chromatography (GC) is a common method for determining identity and concentration of fatty acids. In order for the fatty acids to be analyzed by GC, the fats in any given matrix require a three-step preparation that includes:

- Extraction from the matrix with a non-polar solvent for clean-up
- Saponification, rendering the free fatty acids
- Derivatization to FAMEs for more amenable analysis

Derivatization of the saponified fatty acids via methylation leads to the formation of the corresponding fatty acid methyl esters (FAMEs), which are the preferred derivatives due to their volatility and high thermal stability. However, separation of the 37 common FAMEs can be difficult to achieve as many differ only slightly in their physical and chemical properties.

Generally, high polarity cyanopropyl or biscyanopropyl chemistries are employed for GC separation to provide the necessary selectivity and resolve all components. In these instances, 100 m columns are often used to provide the required resolution; however, they are expensive, analysis times are extended, and sample throughput is low. This can result in a very high cost of analysis per sample.

TRACE TR-FAME columns have a high polarity phase optimized for FAME analysis. The 70% cyanopropyl polysilphenylene-siloxane phase utilized has a higher operating temperature compared to some other columns and gives extremely low bleed, making it amenable to detection by mass spectrometry.

Here, the advantages of utilizing shorter, high-efficiency FAME columns for this complex analysis are investigated. Higher throughput and potential cost savings for the customer can be realized if the shorter columns provide similar performance and reduced analysis time when compared to commonly used 100 m columns. Additionally, the effects of different carrier gases on the chromatography were investigated to tune the separation for speed or efficiency.

Carrier gas choice has a significant effect on the chromatography. Helium is the most common carrier gas for GC as it is widely available within laboratories, inert, and amenable to MS detection. However, there are instances where hydrogen or nitrogen can be successfully employed to improve a separation.

The modified Golay plot (Figure 2) shows this graphically. The three common carrier gasses (helium, hydrogen, and nitrogen) can be compared by plotting carrier gas linear velocity against the height equivalent of a theoretical plate (HETP). An understanding of the relationship between carrier gas linear velocity and optimum efficiency can then be achieved. The modified Golay plot highlights some key qualities of each carrier gas.

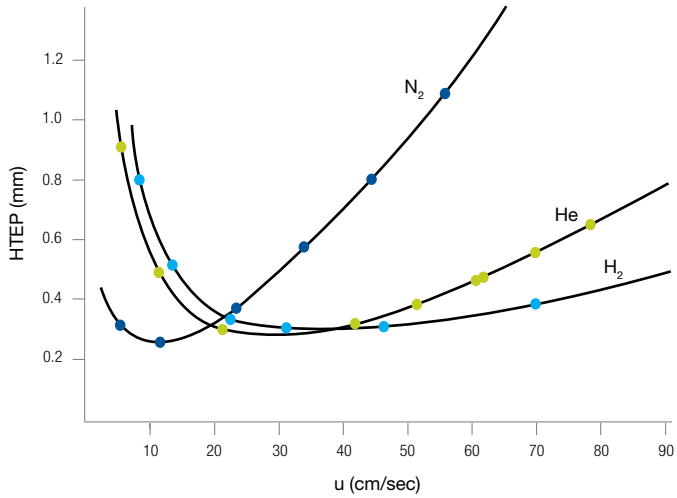


Figure 2. Golay plot of carrier gas HETP vs. linear velocity for helium, hydrogen, and nitrogen.

When comparing the modified Golay plot of helium (the most common carrier gas) to hydrogen, it can be seen that the highest efficiency separations (the minima in the plots) occur at similar linear velocities. However, as velocity increases, the increase in HETP, and therefore the corresponding drop in efficiency, is less pronounced with hydrogen. This property allows high linear velocity separations without a significant loss in resolution, making very fast analysis possible.

When comparing the modified Golay plot of helium to nitrogen, it can be seen that the highest efficiency separations (the plot minima) occur with nitrogen. This means that for a given column, the highest resolution of critical pairs in a chromatographic separation can be achieved with nitrogen. However, since the optimal linear velocity of nitrogen is significantly lower than helium and occurs over a very narrow range which drops off sharply, these high efficiency separations occur at the expense of analysis speed.

Instrument choice can also affect the analysis. The experiments performed here used the Thermo Scientific™ TRACE™ 1300 Series Gas Chromatograph, which is the latest technology to simplify workflow and increase analytical performance. The TRACE 1300 Series GC offers the most versatile GC platform in the market, with unique "Instant Connect" modularity for ground-breaking ease of use and performance, setting a new era in GC technology.

Detection was carried out on a Thermo Scientific™ Instant Connect Flame Ionization Detector (FID) and data capture and analysis using Thermo Scientific™ Chromeleon™ 7.2 SR3 Chromatography Data System.

Experimental

Consumables

Column

- TRACE TR-FAME, 10 m × 0.1 mm × 0.2 µm (P/N 260M096P)

Injection septum

- Thermo Scientific™ BTO, 11 mm (P/N 31303233-BP)

Injection liner

- Thermo Scientific™ LinerGOLD™, Split/Splitless liner with glass wool (P/N 453A2265-UI)

Column ferrules

- 15% Graphite/85% Vespel® 0.1–0.25 mm (P/N 290VA191)

Injection syringe

- 10 µL fixed needle syringe for Thermo Scientific™ TriPlus™ RSH Autosampler (P/N 365D0291)

Vials and closures

- Thermo Scientific™ National™ SureStop™ MS Certified 9 mm screw vials with Blue Silicone/PTFE AVCS closure (P/N MSCERT5000-34W)

Compounds

A mixture containing the most common 37 FAMEs was used. Contents are detailed in Table 1.

Table 1. Summary table of components present within the 37 FAME standard.

Peak Name	Component*
Methyl butyrate	1
Methyl hexanoate	2
Methyl octanoate	3
Methyl decanoate	4
Methyl undecanoate	5
Methyl laurate	6
Methyl tridecanoate	7
Methyl myristate	8
Methyl myristoleate	9
Methyl pentadecanoate	10
Methyl *cis*-10-pentadecenoate	11
Methyl palmitate	12
Methyl palmitoleate	13
Methyl heptadecanoate	14
cis-10-Heptadecanoic acid methyl ester	15
Methyl stearate	16
trans-9-Elaidic acid methyl ester	17
cis-9-Oleic acid methyl ester	18
Methyl linolelaidate	19
Methyl linoleate	20
Methyl arachidate	21
Methyl γ-linolenate	22
Methyl *cis*-11-eicosenoate	23
Methyl linolenate	24
Methyl heneicosanoate	25
cis-11,14-Eicosadienoic acid methyl ester	26
Methyl behenate	27
cis-8,11,14-Eicosatrienoic acid methyl ester	28
Methyl erucate	29
cis-11,14,17-Eicosatrienoic acid methyl ester	30
cis-5,8,11,14-Eicosatetraenoic acid methyl ester	31
Methyl tricosanoate	32
cis-13,16-Docosadienoic acid methyl ester	33
Methyl lignocerate	34
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	35
Methyl nervonate	36
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	37

*Peaks were not identified by MS and were therefore only tentatively assigned.

Sample Pre-treatment

The test mix was injected as supplied without any dilution.

Method Optimization

Three carrier gases were investigated using the same instrumentation and column.

Instrumentation

- TRACE 1310 GC (P/N 14800302)
- TriPlus RSH Autosampler (P/N 1R77010-0100)
- Instant Connect Electron Flame Ionization Detector (FID) (P/N 19070001FS)

Separation Conditions

Experiment 1 (Helium)

Carrier Gas	Helium
Split Flow	88.0 mL/min
Split Ratio	251:1
Column Flow	0.35 mL/min
Oven Temperature	40 °C (1 min hold), 80 °C/min to 150 °C (0 min hold), 8 °C/min to 240 °C (1 min hold)
Injector Type	Split/Splitless
Injector Mode	Split, constant flow
Injector Temperature	220 °C
Detector Type	Flame ionization detector (FID)
Detector Temperature	250 °C
Detector Air Flow	350 mL/min
Detector Hydrogen Flow	35 mL/min
Detector Nitrogen Flow	40 mL/min

Experiment 2 (Hydrogen)

Carrier Gas	Hydrogen
Split Flow	75.0 mL/min
Split Ratio	250:1
Column Flow	0.30 mL/min
Oven Temperature	40 °C (0.83 min hold), 96 °C/min to 150 °C (0 min hold), 9.6 °C/min to 240 °C (0.2 min hold)
Injector Type	Split/Splitless
Injector Mode	Split, constant flow
Injector Temperature	220 °C
Detector Type	Flame ionization detector (FID)
Detector Temperature	250 °C
Detector Air Flow	350 mL/min
Detector Hydrogen Flow	35 mL/min
Detector Nitrogen Flow	40 mL/min

Experiment 3 (Nitrogen)

Carrier Gas	Nitrogen
Split Flow	28.0 ml/min
Split Ratio	255:1
Column Flow	0.11 mL/min
Oven Temperature	40 °C (2.07 min hold), 38.57 °C/min to 150 °C (0 min hold), 3.86 °C/min to 240 °C (0.62 min hold)
Injector Type	Split/Splitless
Injector Mode	Split, constant flow
Injector Temperature	220 °C
Detector Type	Flame ionization detector (FID)
Detector Temperature	250 °C
Detector Air Flow	350 mL/min
Detector Hydrogen Flow	35 mL/min
Detector Nitrogen Flow	40 mL/min

Data Processing

Software

Chromeleon 7.2 SR3 Chromatography Data System.

Results and Discussion

Typically, methods for FAME analysis have been carried out using a 100 m × 0.25 mm × 0.2 µm biscyanopropyl column with helium carrier gas. This required analysis times of around an hour to obtain the necessary resolution of the major components.

The equivalent separation on the 10 m length column with a narrower, 0.1 mm ID diameter is shown below (Figures 3a–c). By changing the column dimensions, the analysis time was reduced to approximately 12 minutes while maintaining resolution and efficiency.

In previously published methods, the components 25–32 were least resolved. Maintaining good separation of critical pairs in this region of the chromatogram was a key objective for this updated method. By using the 10 m column, the separation of critical pairs 25–26 and 28–29 was significantly improved compared to the 100 m column (Figures 3a–c). This is largely due to the increased efficiency of the narrower ID column.

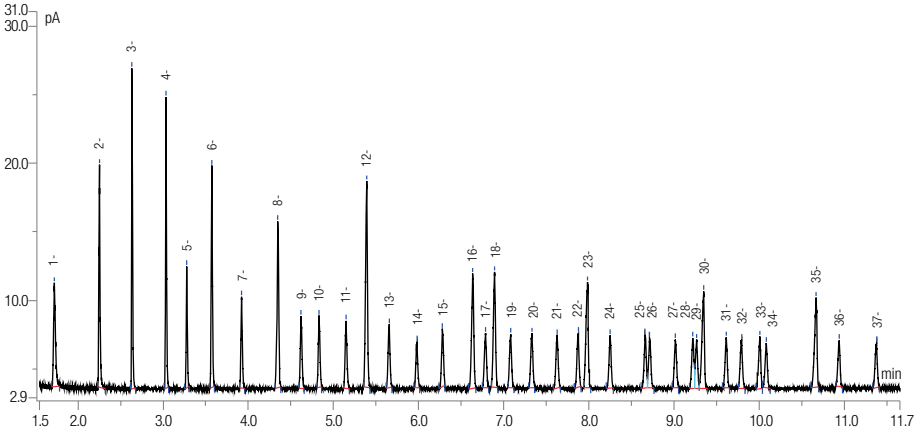


Figure 3a. Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with helium carrier gas. (Experiment 1)

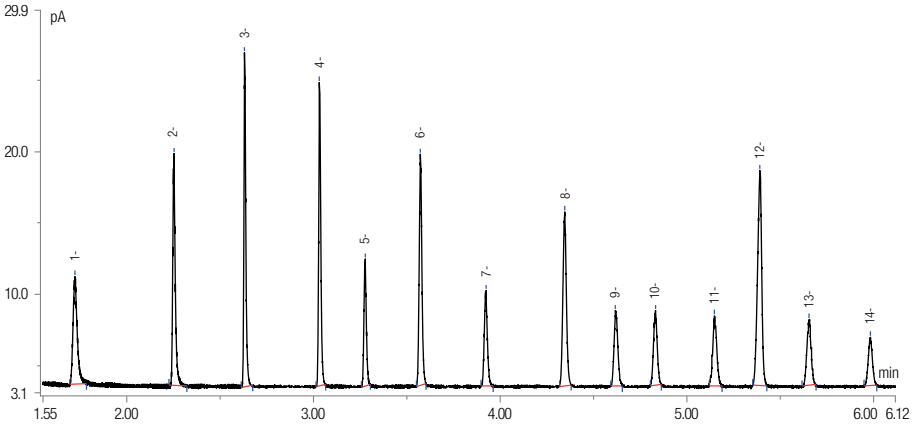


Figure 3b (peaks 1–14). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with helium carrier gas. (Experiment 1)

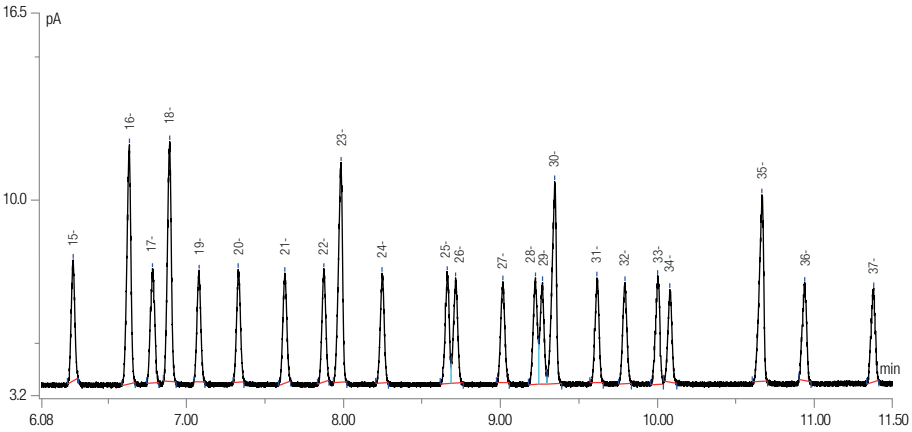


Figure 3c (peaks 15–37). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with helium carrier gas. (Experiment 1)

Conditions for the helium carrier gas separation were fully optimized and further improvements in speed or efficiency could only be achieved with this column using alternative carrier gasses. The next sets of experiments were conducted using hydrogen to attempt improvements in speed of analysis.

Hydrogen was able to give a faster separation than helium with all 37 components eluting in less than 9.3 minutes. There was, however, an impact on resolution of critical pairs (Figures 4a–c). While resolution was reduced, it was still possible to successfully integrate all peaks and for the majority, the resolution was still > 1.5 (Table 2).

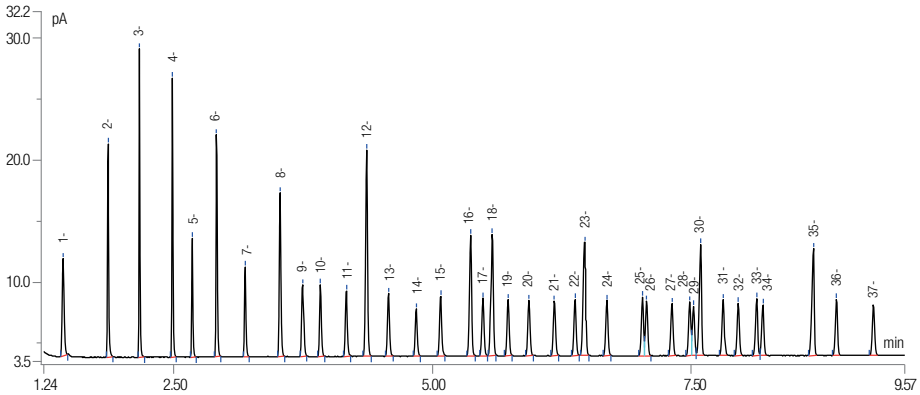


Figure 4a (full chromatogram hydrogen). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with Hydrogen carrier gas. (Experiment 2)

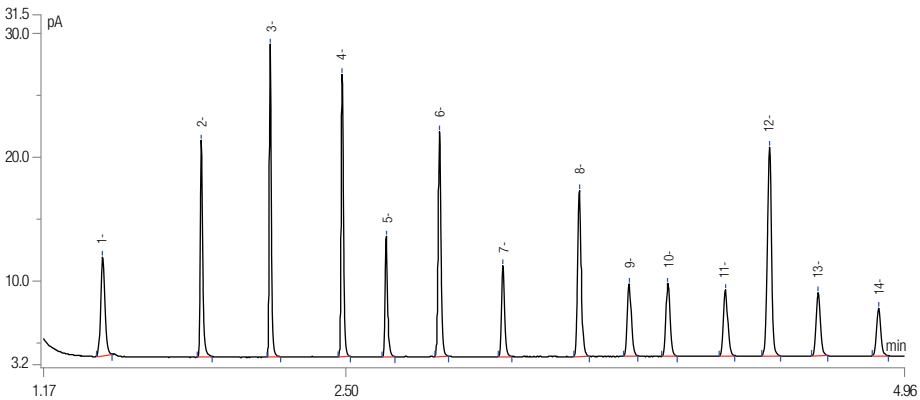


Figure 4b (peaks 1–14). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with hydrogen carrier gas. (Experiment 2)

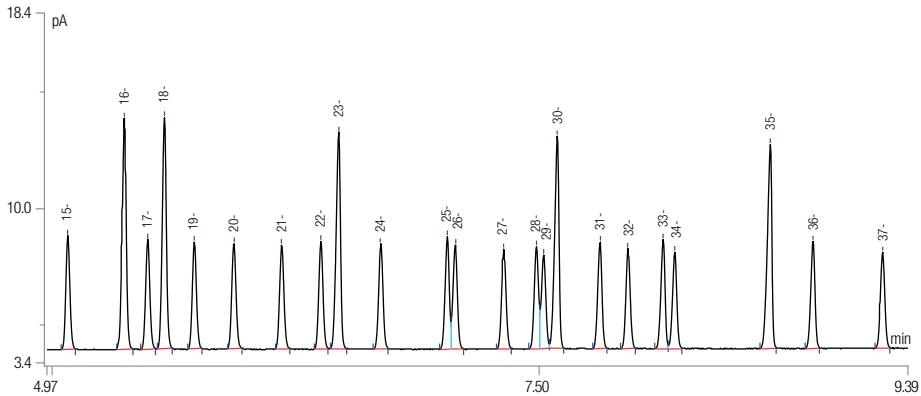


Figure 4c (peaks 15–37). Fast analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm with hydrogen carrier gas. (Experiment 2)

Table 2. Resolution for all components.

Peak Name	Component*	Helium Resolution (EP)	Hydrogen Resolution (EP)	Nitrogen Resolution (EP)
Methyl butyrate	1	17.82	16.18	21.69
Methyl hexanoate	2	19.37	17.74	22.53
Methyl octanoate	3	21.46	19.83	24.28
Methyl decanoate	4	11.5	10.68	13.08
Methyl undecanoate	5	12.15	11.45	13.88
Methyl laurate	6	12.85	12.03	14.54
Methyl tridecanoate	7	13.49	12.57	15.27
Methyl myristate	8	7.75	7.28	8.92
Methyl myristoleate	9	5.94	5.41	6.59
Methyl pentadecanoate	10	8.62	7.73	9.47
Methyl *cis*-10-pentadecenoate	11	6.23	5.66	6.76
Methyl palmitate	12	6.52	6.07	7.31
Methyl palmitoleate	13	8.08	7.42	8.83
Methyl heptadecanoate	14	7.16	6.47	7.69
cis-10-Heptadecanoic acid methyl ester	15	8.04	7.54	8.82
Methyl stearate	16	3.18	3.08	3.67
trans-9-Elaidic acid methyl ester	17	2.33	2.2	2.62
cis-9-Oleic acid methyl ester	18	4.15	4.02	4.7
Methyl linolelaidate	19	5.52	5.13	6.15
Methyl linoleate	20	6.44	5.99	7.06
Methyl arachidate	21	5.41	4.99	5.78
Methyl γ-linolenate	22	2.33	2.29	2.48
Methyl *cis*-11-eicosenoate	23	5.61	5.26	6.15
Methyl linolenate	24	9.08	8.62	9.74
Methyl heneicosanoate	25	1.16	0.99	1.27
cis-11,14-Eicosadienoic acid methyl ester	26	6.38	5.87	7.07
Methyl behenate	27	4.19	3.94	4.71
cis-8,11,14-Eicosatrienoic acid methyl ester	28	0.92	0.85	1
Methyl erucate	29	1.63	1.66	1.81
cis-11,14,17-Eicosatrienoic acid methyl ester	30	5.52	5.43	6.09
cis-5,8,11,14-Eicosatetraenoic acid methyl ester	31	3.76	3.52	4.07
Methyl tricosanoate	32	4.49	4.48	4.86
cis-13,16-Docosadienoic acid methyl ester	33	1.66	1.54	1.79
Methyl lignocerate	34	11.87	11.88	13.1
cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester	35	5.51	5.33	6.03
Methyl nervonate	36	9.32	8.76	10.05
cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	37	"	"	"

*Peaks were not identified by MS and were therefore only tentatively assigned.

The throughput of separations based on all three carrier gasses run times (Table 3) with 6-minute recycling time is given below (Figure 5). Published methods on 100 m columns using helium carrier gas could practically analyze up to 24 samples per day. Moving to a 10 m column increases throughput to a maximum of 80 samples per day. Even the use of a shorter column with nitrogen carrier gas increases throughput to 48 samples per day. If the carrier gas is then changed to hydrogen this further increases as high as 100 samples per day, a 400% increase.

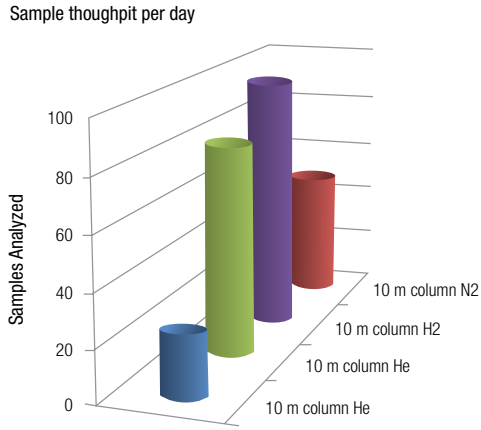


Figure 5. Sample throughput when comparing a 100 m column to a 10 m column using helium, hydrogen, and nitrogen as carrier gases.

Table 3. Experiment run times.

Experiment	Carrier gas	Run time (min)
1	Helium	11.9
2	Hydrogen	9.5
3	Nitrogen	23.7

Further experiments were then conducted using nitrogen in an attempt to increase separation efficiency and gain improvements in resolution. Figures 6a–c show the separation achieved.

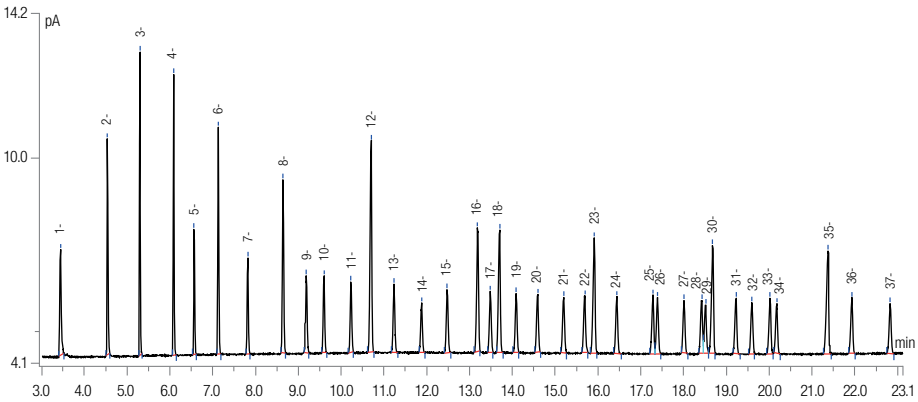


Figure 6a (full chromatogram nitrogen). Analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm cyanopropyl phase using nitrogen carrier gas. (Experiment 3)

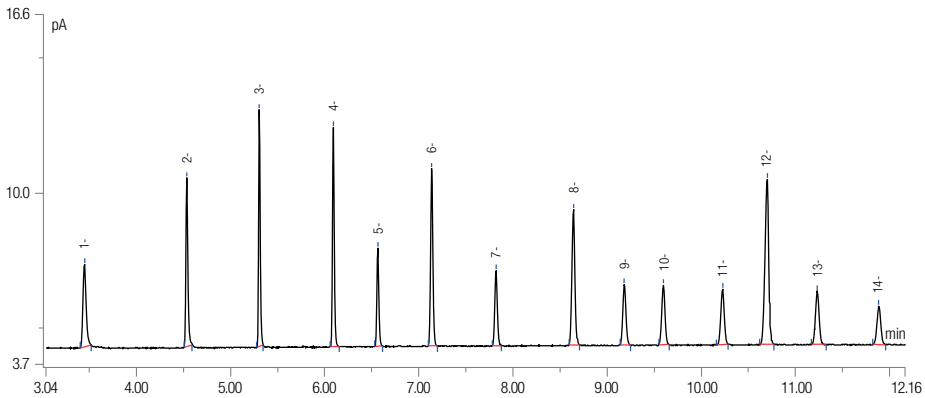


Figure 6b (peaks 1–14). Analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm cyanopropyl phase using nitrogen carrier gas. (Experiment 3)

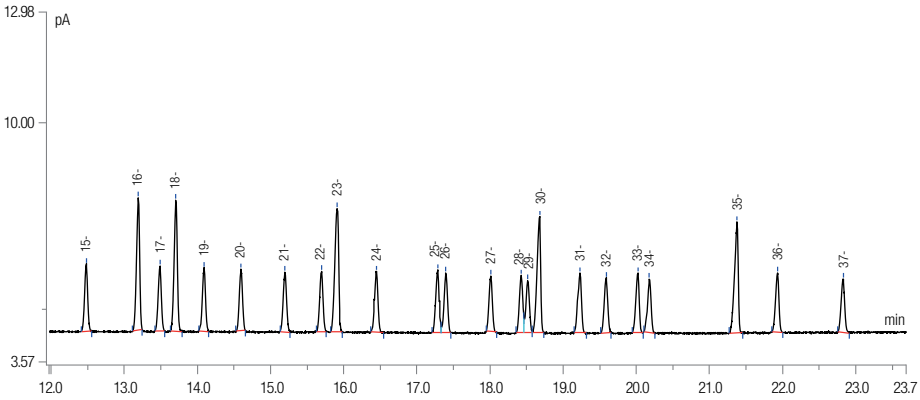


Figure 6c (peaks 15–37). Analysis on a TRACE TR-FAME GC column 10 m × 0.10 mm × 0.2 µm cyanopropyl phase using nitrogen carrier gas. (Experiment 3)

The differences in resolution for all components using each carrier gas are displayed graphically (Figure 7), while individual resolution values are tabulated (Table 2).

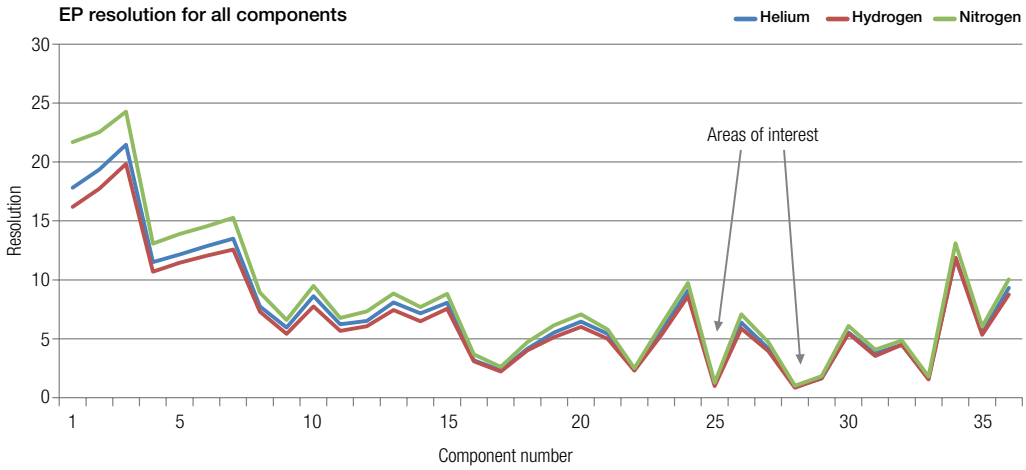
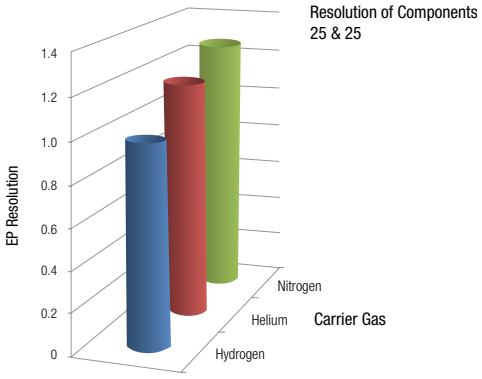
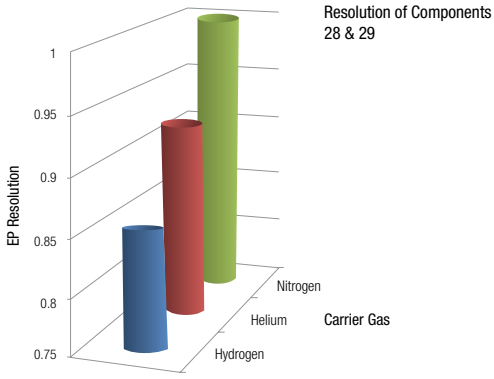


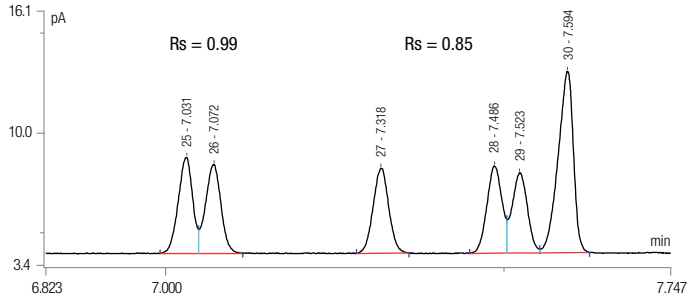
Figure 7. Graphs to show differences in carrier gas resolution for all components when comparing helium, hydrogen, and nitrogen.

In this graph it can be seen that resolution is greatest for nitrogen for all components, with the green line tracking highest across the range. For most peaks, there is significant resolution and the use of nitrogen as a carrier gas is not required; however, in the highlighted region, 25–26 and 28–29, it becomes crucial. The regions for these critical peaks were expanded to look closer at resolution differences between the different carrier gasses (Figure 8).

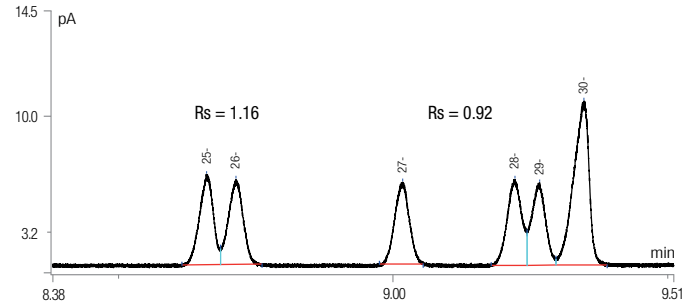




Hydrogen Carrier Gas



Helium Carrier Gas



Nitrogen Carrier Gas

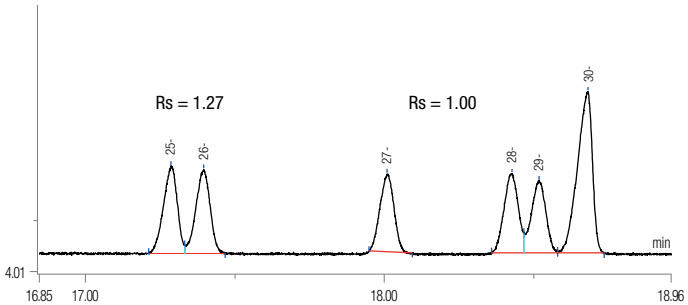


Figure 8. Graphs and chromatograms to show differences in carrier gas resolution for critical pairs when comparing hydrogen, helium, and nitrogen.

As seen above, the separation of the critical pairs is better with the nitrogen carrier gas. The resolution of critical pairs 25–26 and 28–29 was significantly improved compared with separations using helium and hydrogen. Resolution for peaks 25–26 for the nitrogen carrier gas was found to be 22% greater than hydrogen and 9% greater than helium. Similarly comparing the resolution for peaks 28–29 using nitrogen carrier gas was found to be 15% greater than hydrogen and 8% greater than helium.

Due to the increased efficiency of nitrogen as a carrier gas, the critical components could be better resolved. The benefit of an increase in resolution includes improvement in quantitation as peak assignment and integration are both easier to achieve. This translates to improved confidence in the results and the achievement of lower detection levels.

An analysis of the increased resolution found for nitrogen revealed increased peak efficiencies of the critical pairs, compared to the other carrier gasses. EP plate count (a standard measure of efficiency) was used to determine this (Table 4).

Nitrogen was found to be 18% more efficient than helium and 39% more efficient than hydrogen under these conditions. The efficiency gain meant that peak resolution was significantly improved to the hydrogen.

Table 4. Efficiencies of different carrier gases.

Peak Number	Peak Name	Helium	Hydrogen	Nitrogen	Efficiency Increase %	
		Plates			N_2 compared to HE	N_2 compared to H
25	Component 25	573023	505099	642339	12	27
26	Component 26	530880	451019	639285	20	42
27	Component 27	536955	472868	659995	23	40
28	Component 28	596524	470381	690580	16	47
				Mean %	18	39

Conclusions

- The separation of 37 fatty acid methyl esters (FAMEs) on the highly efficient 10 m TR-FAME GC column was significantly improved compared to the analysis on a 100 m FAMEs column, demonstrating greater resolution and increased sample throughput of up to 400%.

- By using different carrier gases, the separation of FAMEs can be optimized for reduced analysis time, resolution of critical pairs, and efficiency.

Find out more at **thermofisher.com/columnsforgc**

ThermoFisher SCIENTIFIC

For Research Use Only. Not for use in diagnostic procedures. © 2016 Thermo Fisher Scientific Inc. All rights reserved. Vespel is a registered trademark of E. I. du Pont de Nemours and Company. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.
AN21557-EN 0916S

APPLICATION NOTE 10689

Rapid qualitative and quantitative analysis of residual solvents in food packaging by static headspace coupled to GC-FID/MS

Authors
Giulia Riccardino and Cristian Cojocariu

Thermo Fisher Scientific, Runcorn, UK

Keywords
Residual solvents, flexible food packaging, food safety, valve and loop, headspace-gas chromatography, HS-GC, multiple headspace extraction, MHE, flame ionization detector, FID, mass spectrometer detector, MS, single quadrupole GC-MS, ISQ 7000, TriPlus 500 HS

Goal

The aim of this application note is to demonstrate the qualitative and quantitative performance of the Thermo Scientific™ TriPlus™ 500 Gas Chromatography Headspace Autosampler coupled to a dual-detector GC-FID/MS for the determination of residual solvents in food packaging according to the European Standard EN 13628-1 method[1] and to highlight a highly efficient workflow through extended automation from sampling to data reporting.

Introduction

Packaging materials are essential for maintaining food integrity and to ensure safe handling, transportation, and storage. Common food packaging materials are polymer-based thin films or paper-based coatings often layered or imprinted on the outside with inks, dyes, and paints intended to address the consumer appeal and convenience. The chemical components of such food packaging (especially from polymers, dyes, and inks) can migrate into the food products, modifying the organoleptic properties and the composition of the food and posing health risks to the consumer. As a consequence, regulatory measures are in place to make sure that food contact materials do not transfer any components to the packed foodstuff in quantities that could affect human health, change the composition, or modify the organoleptic

ThermoFisher SCIENTIFIC

properties of the product.[2] In the United States a migration limit of 50 ppm is applicable for residual solvents and non-volatile food additives.[3] In addition, precise quantification of residual solvents in flexible packaging is also regulated through set methods such as EN 13628-1:2002.

Analysis of volatile impurities in solid polymers is challenging, especially with regard to sampling and extraction techniques. Liquid injections of such samples require dissolution of packaging polymers into a suitable solvent prior to gas chromatography (GC) injection. This can result in high viscosity solutions containing non-volatile, long chain polymers that can potentially contaminate the GC injector ports. This, in turn, will require frequent inlet liner replacement and system maintenance that will increase the cost of analysis.

An alternative to liquid injections is headspace sampling: a fast and simple technique that enables the extraction of volatile and semi-volatile compounds from food packaging samples without the need for time-consuming sample preparation. In particular, static headspace with multiple headspace extraction (MHE)[4] can be used for absolute quantitative analysis of volatiles in solid matrices. This technique is particularly useful when matrix-matched calibration reference materials are not available.

In this study, the quantitative results for residual solvent analysis in food packaging materials, obtained with the TriPlus 500 Headspace (HS) autosampler, are reported. A dual detector FID/MS configuration allowed for the detection, identification (flame ionization detection), and confirmation (mass spectrometry detection) of unknown impurities. The experiments also focused on assessing method linearity[1] according to EN 13628:1:2002 and precision, as well as the overall quantitative performance of the analytical setup for routine analysis of residual solvents in food packaging.

Experimental

In all experiments, a TriPlus 500 HS autosampler was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph equipped with a Thermo Scientific™ Instant Connect Split/Splitless SSL Injector. A Thermo Scientific™ Dual Detector Microfluidics device (P/N 19071030) was used to split 1:1 the carrier gas flow from the analytical column between a Thermo Scientific™ Instant Connect Flame Ionization Detector (FID) and a Thermo Scientific™ ISQ™ 7000 Single Quadrupole GC-MS system.

Chromatographic separation was achieved on a Thermo Scientific™ TraceGOLD™ TG-1MS GC capillary column, 30 m × 0.32 mm × 3.0 µm (P/N 26099-4840). Additional HS-GC-FID/MS conditions are given in Table 1. The GC oven temperature program was optimized to decrease the analysis time and improve sample throughput; all peaks of interest are eluting in <7 minutes with adequate peak chromatographic resolution (Rs > 1). An incubation time of 40 minutes per MHE step was optimized to cover the majority of food packaging material types. According to the EN 13628-1:2002 method, linearity was assessed on n = 4 headspace extraction cycles.

Table 1 (part 1). HS-GC-FID and ISQ 7000 mass spectrometer operating conditions for residual solvent determination

TRACE 1310 GC	
Inlet Module and Mode:	SSL, split
Split Ratio:	20:1
Septum Purge Mode, Flow (mL/min):	Constant, 5
Carrier Gas, Carrier Mode, Pressure (kPa):	He, constant pressure, 110
Oven Temperature Program	
Temperature 1 (°C):	50
Hold Time (min):	1
Temperature 2 (°C):	110
Rate (°C/min):	30
Temperature 2 (°C):	250
Rate 2 (°C/min):	20
FID	
Temperature (°C):	250
Air Flow (mL/min):	350
H_2 Flow (mL/min):	35
N_2 Flow (mL/min):	40
Acquisition Rate (Hz):	25
ISQ 7000 Single Quadrupole GC-MS system	
Ion Source:	ExtractaBrite
Transfer Line Temp. (°C):	250
Source Temperature (°C):	250
Ionization Mode:	EI
Electron Energy (eV):	70
Acquisition Mode:	Full-scan (*m/z* 25-350)

Table 1 (part 2). HS-GC-FID and ISQ 7000 mass spectrometer operating conditions used for residual solvent determination

TriPlus 500 HS Autosampler Parameters (MHE)	
Incubation Temp. (°C):	120
Incubation Time (min):	40
Vial Shaking:	Medium
Vial Pressurization Mode:	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen):	55
Vial Pressure Equilibration Time (min):	1
Loop Size (mL):	1
Loop/Sample Path Temp. (°C):	120
Loop Filling Pressure (kPa):	34
Loop Equilibration Time (min):	1
Extraction Cycles:	4
Needle Purge Flow Level:	4
Injection Mode:	MHE
Injection Time (min):	1

Table 1 (part 3). HS-GC-FID and ISQ 7000 mass spectrometer operating conditions for residual solvent determination

TriPlus 500 HS Autosampler Parameters (total vaporization)	
Incubation Temp. (°C):	120
Incubation Time (min):	40
Vial Shaking:	Medium
Vial Pressurization Mode:	Pressure
Vial Pressure (kPa) (Auxiliary Gas Nitrogen):	55
Vial Pressure Equilibration Time (min):	1
Loop Size (mL):	1
Loop/Sample Path Temp. (°C):	120
Loop Filling Pressure (kPa):	34
Loop Equilibration Time (min):	1
Needle Purge Flow Level:	4
Injection Mode:	Standard
Injection Time (min):	1

Data acquisition, processing and reporting

The data was acquired, processed, and reported using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2. Integrated instrument control ensures full automation from instrument set-up to raw data processing, reporting, and storage. Simplified e-workflows deliver effective data management ensuring ease of use, sample integrity, and traceability. Chromeleon CDS also offers the option to scale up the data handling process in the laboratory from a single workstation to an enterprise environment to further improve productivity.[5]

Standard and sample preparation

Two standard mixtures, each containing different residual solvents that can be found in packaging materials (mixture 1 and mixture 2 at 7.14% v/v and 9.09% v/v, respectively), were purchased from Sigma Aldrich® (P/N 48994-U and 48995-U). A volume (1 µL) of each standard solution (corresponding to 71.4 µg and 90.9 µg of mixture 1 and 2, respectively) was spiked into the same 10 mL empty sealed headspace glass vial and used as retention time reference for compound identification as well as for MHE linearity assessment with total vaporization. A complete list of analyzed compounds is reported in Table 2.

Samples of packaged foods (pizza, cookies, bread, salad, and salami) were purchased locally and the packaging (cling film, wraps, and trays) was separated from the food and analyzed following the EN 13628-1:2002 method. A sample surface of 40 cm^2 (2 × 20 cm) was cut, coiled, and sealed into a 10 mL crimp cap headspace vial (vials P/N 10CV, caps P/N 20-MCBC-ST3). As specified in the EN 13628-1:2002 method, the ratio between the sample area (in cm^2) and the vial volume (in mL) was maintained between 3 and 5.

Table 2. Correlation coefficients (R^2) calculated using the full-scan EI traces. For all compounds in the reference standard $R^2 \geq 0.997$. Correlation coefficients for FID data were 1.000 for all components, hence data are not shown.

MHE Linearity		
Component Name	RT (min)	Correlation Coefficient (R^2)
Methanol	1.72	0.997
Ethanol	2.11	0.997
Acetone	2.37	0.998
2-Propanol	2.44	0.999
Methyl acetate	2.73	0.999
1-Propanol	2.98	0.998
2-Butanone	3.33	0.999
2-Butanol	3.42	1.000
Ethyl acetate	3.53	0.999
2-Methyl-1-propanol	3.68	0.999
2-Methoxyethanol	3.74	0.997
Tetrahydrofuran	3.80	0.999
Isopropyl acetate	4.04	0.998
1-Methoxy-2-propanol	4.20	0.997
Cyclohexane	4.34	0.998
Propylacetate	4.57	0.999
4-Methyl-2-pentanone	4.89	0.998
Isobutyl acetate	5.22	0.999
Toluene	5.38	0.997
Butyl acetate	5.63	0.999
2-Methoxyethyl acetate	5.74	0.997
2-Etoxyethyl acetate	6.47	0.998
Cyclohexanone	6.66	0.999

Results and discussion

MHE linearity assessment according to EN 13628-1:2002 method

A reference solvent standard mix was prepared as described in the standard and sample preparation section and analyzed using the total vaporization technique[4] applying the MHE conditions reported in Table 1. MHE allows the extrapolation of the total content of analytes in a liquid or solid matrix through multiple headspace cycles. The amount of analyte present in the sample is calculated by direct comparison of the peak area responses to external standards previously analyzed in a similar way but without matrix.

MHE linearity was assessed by plotting the natural logarithm of the peak areas in the standard solution versus the number of headspace cycles (n = 4). Chromeleon CDS interactive charts and reprocessing features allowed for fast MHE calibration plots and correlation coefficient calculations without the need of external calculation tools, as shown in Figure 1. For all the investigated compounds, the calculated correlation coefficients (R^2) were 1.000 for FID data and ≥0.997 for EI full-scan MS traces (Table 2). In both cases calculated correlation coefficients met the method requirement ($R^2 \geq 0.98$) confirming an excellent linearity.

Quantification of residual solvent in food packaging materials using MHE

The packaging materials were prepared as described and analyzed using the MHE conditions reported in Table 1. The microfluidic device allowed for splitting the gas flow 1:1 to the FID and the ISQ single quadrupole mass spectrometer, ensuring a minimal effect on the retention times (max RT shifts 0.04 min) by choosing either the FID or MS chromatogram as reference. The sample and the standard solution FID chromatograms were compared to verify the presence of known residual solvents. Several residual solvents such methanol (RT = 1.72 min) and ethylacetate (RT = 3.53 min) were detected in the sliced salami lid (D) and plastic tray (E), whereas ethanol (RT = 2.11 min) and acetone (RT = 2.37 min) were present in salad wrap (C) (Figure 3).

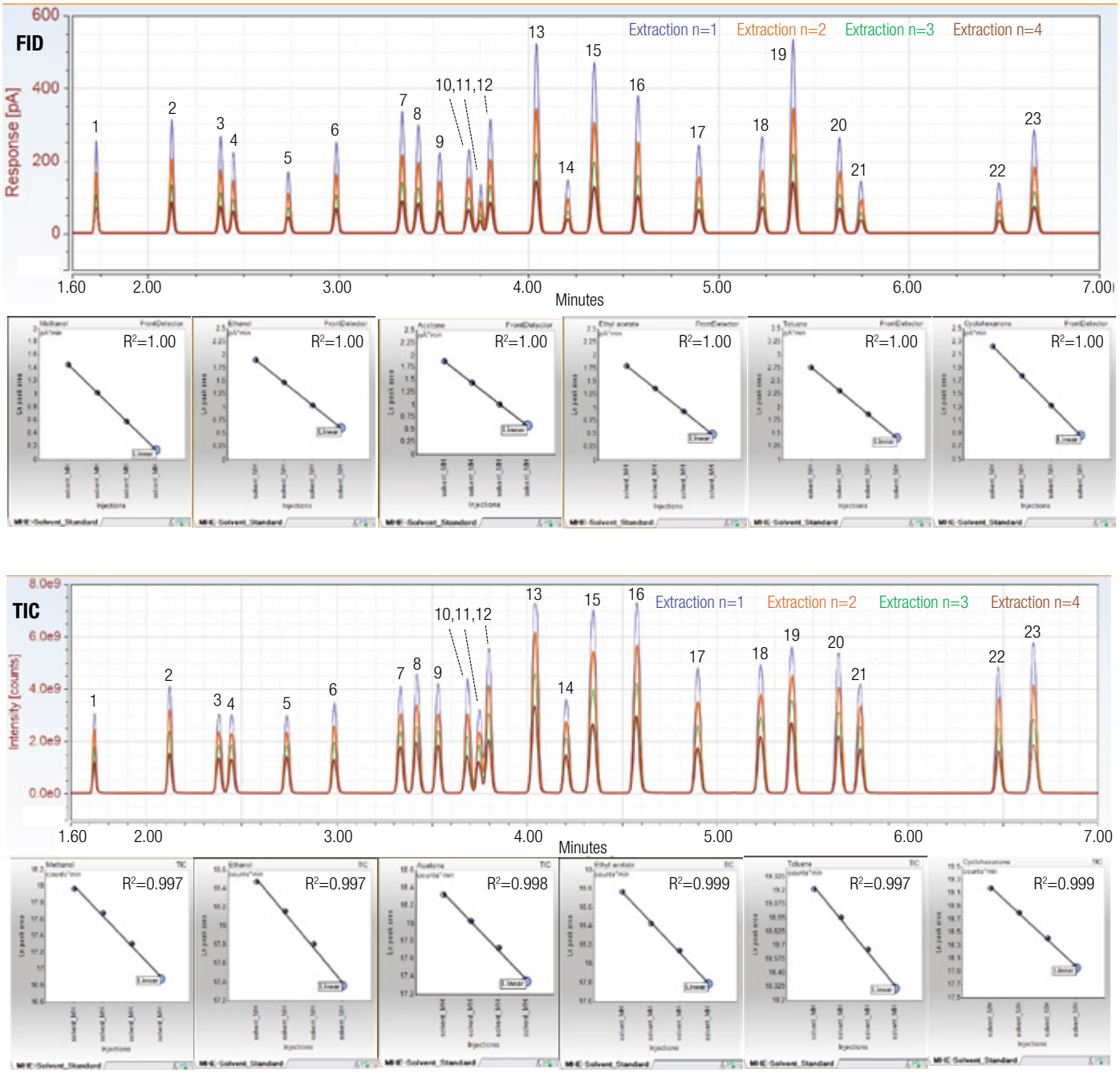


Figure 1. FID and TIC (full-scan, EI at 70 eV) traces for reference standard and corresponding MHE calibration curves for selected compounds (left to right: methanol, ethanol, acetone, ethyl acetate, toluene, and cyclohexanone) as examples. Calibration curves were obtained by plotting the natural logarithm of peak area responses (total vaporization MHE) versus the corresponding MHE extraction step.

Full-scan data were used to putatively confirm the identity of detected solvent impurities, increasing the confidence in compound indentification. When searching the mass spectrum of the peak eluting at RT = 1.72 min against NIST17 library, the best library match was acetaldehyde (not included in the standard mixtures) with a SI score of 953 (sliced salami tray:E) and 729 (sliced salami lid:D) (Figure 3). Acetaldehyde is usually present in meat and meat products.[6] Using the same approach, ethanol and acetone in salad wrap (C) and ethyl acetate in sliced salami (lid:D and tray:E) were also putatively confirmed with a SI score of 929, 913, 874, and 950, respectively. These chemicals are actually released by the packaging since they are typically used in solvent-based inks imprinted on the external layer of flexible packages.[7] Additional unknown compounds (*) detected in the samples were confirmed using spectral library comparison against NIST17 library (Figure 2).

Peaks (A):

1. Methanol
2. Ethanol
3. Acetone
4. 2-Propanol
5. Methyl acetate
6. 1-Propanol
7. 2-Butanone
8. 2-Butanol
9. Ethyl acetate
10. 2-Methyl-1-propanol
11. 2-Methoxyethanol
12. Tetrahydrofuran,
13. Isopropyl acetate
14. 1-Methoxy-2-propanol,
15. Cyclohexane
16. Propyl acetate
17. 4-Methyl-2-pentanone
18. Isobutyl acetate
19. Toluene
20. Butyl acetate
21. 2-Methoxyethyl-acetate
22. 2-Ethoxyethyl acetate,
23. Cyclohexanone

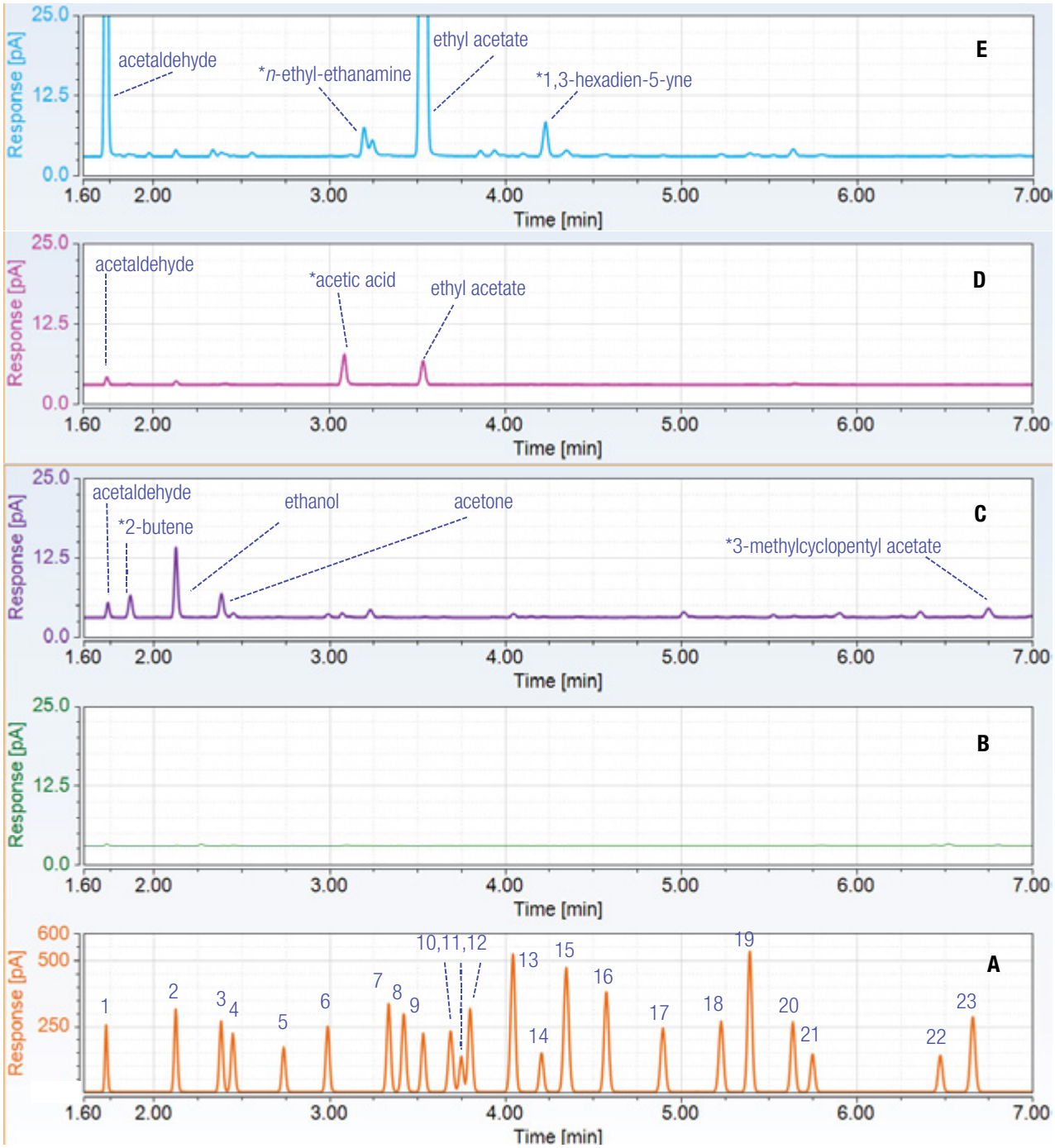


Figure 2. FID chromatograms showing a comparison between the residual solvents in the reference standard solution (A), empty blank vial (B), salad wrap (C), sliced salami wrap: lid (D) and tray (E). Based solely on retention time comparison, methanol and ethyl acetate were detected in both sliced salami samples (lid:D, tray:E). Ethanol and acetone were found in salad wraps (C). FID signal responses (y-axis) are normalized for the empty vial (B) and samples (C,D,E). Unknown peaks (*) in the samples were confirmed comparing their mass spectra (full-scan, EI traces) against the NIST17 library and are reported as an example. Peaks not annotated were below the integration threshold of 0.04 pA * min.

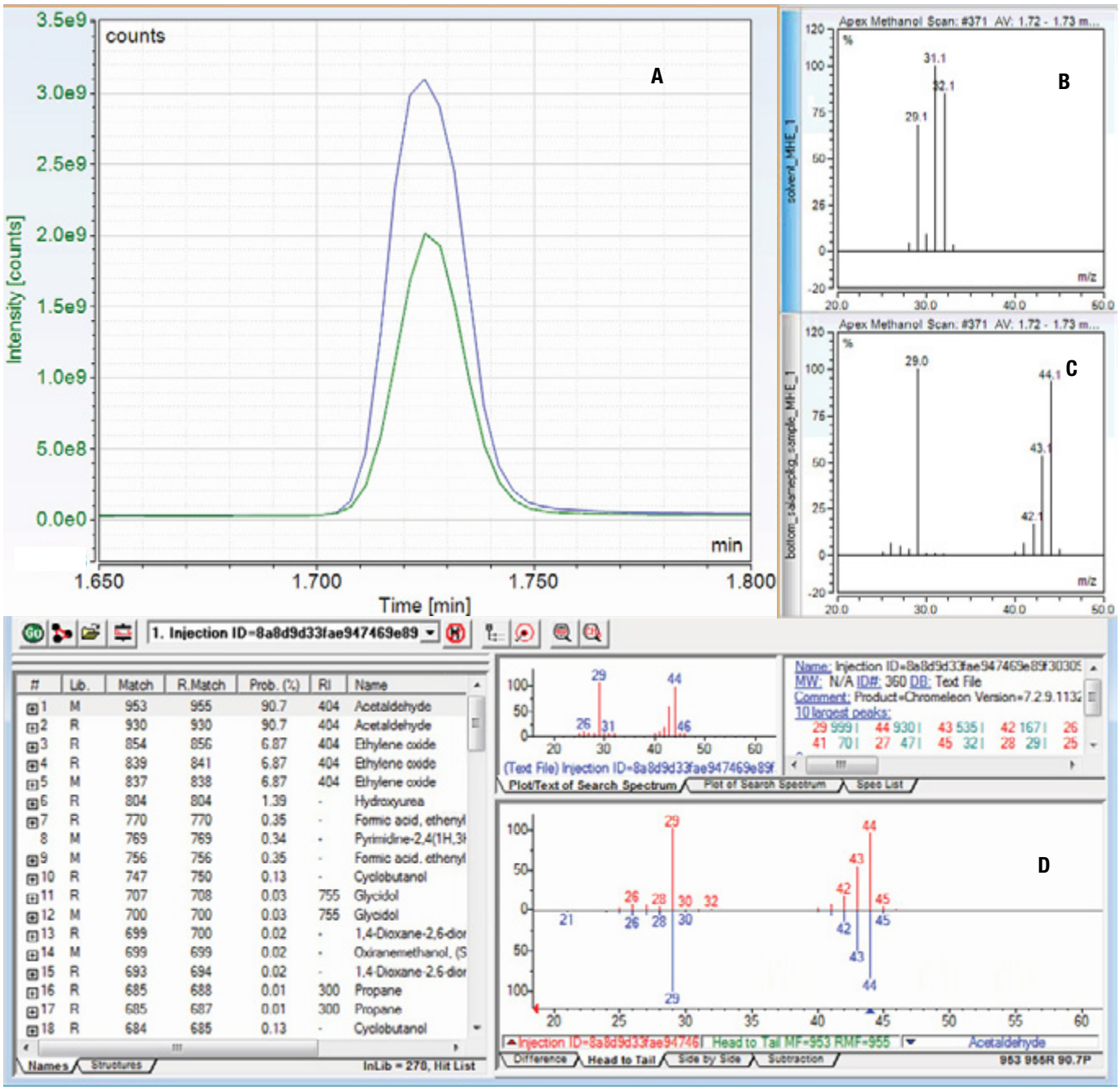


Figure 3. Identification of residual solvent peak eluting at RT=1.72 min in salami tray sample. Comparison of TIC chromatograms (full-scan, EI at 70 eV) showing retention time comparison of peak eluting at RT=1.72 min in solvent standard (blue) and salami tray (green) (A). Background subtracted EI mass spectra for this peak in solvent standard (B) and in the sliced salami tray (C) did not confirm methanol. NIST library result (D) putatively identified this compound as acetaldehyde with a SI score of 953 and a probability of 91%.

Obtaining good ($R^2 \geq 0.98$) MHE linearity is fundamental to achieve accurate quantitation of residual solvents in solid food packaging materials. MHE linearity in the samples was assessed as previously described. The correlation coefficients (R^2) were 0.998 and 0.995 for ethyl acetate in sliced salami (lid and tray, respectively). R^2 for ethanol and acetone in salad wrap were 0.996 and 0.998, respectively (Figure 4).

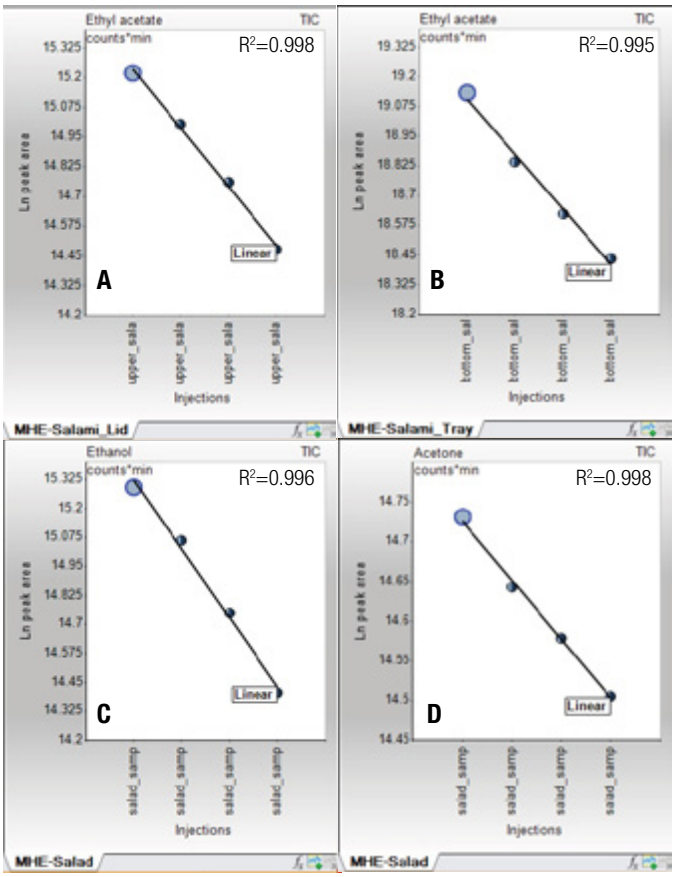


Figure 4. MHE linearity for ethyl acetate in sliced salami lid (A) and sliced salami tray (B), ethanol (C), and acetone (D) in salad wrap. The resulting correlation coefficients (R^2) were 0.998 and 0.995 for sliced salami (lid and tray, respectively) and 0.996 and 0.998 for ethanol and acetone, respectively, in salad wrap.

The concentration (in mg/m^2) of residual solvents detected in the samples was calculated using the FID data applying the formula reported in paragraph 9.2.10.1 of the EN method. No residual solvents were found in the majority of samples. Traces of ethyl acetate were found in the sliced salami wrap (lid: 0.76 mg/m^2, tray: 29 mg/m^2). Ethanol (0.97 mg/m^2) and acetone (1.9 mg/m^2) were also present in salad wrap. All levels were within the safety limits reported for residual solvent and non-volatile food additives.[3]

Conclusions

The results obtained with the TriPlus 500 HS autosampler are compliant with the EN 13628-1:2002 standard method requirements.

- The MHE capability allows for absolute quantitative analysis of residual solvent impurities in solid samples, overcoming the matrix effect and eliminating the need of sample preparation. Using the MHE mode, excellent linearity with correlation coefficient $R^2 \geq 0.995$ was obtained for all analytes in both solvent standard and samples, meeting the minimum required value of $R^2 \geq 0.98$, thus confirming excellent instrument performance for MHE quantitative analysis.
- Traces of residual solvents were found in three of the six analyzed food packaging samples. Acetone and ethanol were detected at 1.9 and 0.97 mg/m^2 in salad wrap samples, respectively, and ethyl acetate was found in sliced salami tray at 29 mg/m^2 and lid at 0.76 mg/m^2. No residual solvents were present in pizza cling film, cookies, and bread wraps.
- The dual detector configuration FID/MS increases the confidence in compound identification, allowing for the detection of possible analyte co-elution, otherwise difficult to assess in the absence of MS data. Moreover several unknown peaks in the samples have been putatively confirmed (using spectral library match score thresholds of >950 SI) through comparison with NIST17 spectral library.
- The low bleed and superior inertness of the TraceGOLD column allowed for highly reliable results. The high analytical column efficiency allowed for fast GC oven ramp with adequate chromatographic separation (Rs ≥ 1.0) for all the analyzed compounds, reducing analysis time. Moreover, up to 240 sample vials can be accommodated into the trays for unattended 24-hour operation.

- The automated cycle time optimization allows for continuous sample processing ensuring the overlapping between the MHE cycles of the same sample. The overlapping capability is maintained between the final injection of one sample and the incubation of the next one increasing the sample throughput.
- Chromeleon CDS software ensures data integrity, traceability, and effective data management from instrument control to the final report. The integrated charts and the advanced report capability allowed for easy and integrated MHE data reprocessing, thus eliminating the need for external calculation tools.

Overall the results obtained show that the TriPlus 500 HS autosampler coupled to the TRACE 1310 GC and the ISQ 7000 single quadrupole GC-MS system represents a robust analytical configuration for routine laboratories delivering outstanding reliability for MHE quantitative analysis of residual solvents in food packaging.

References

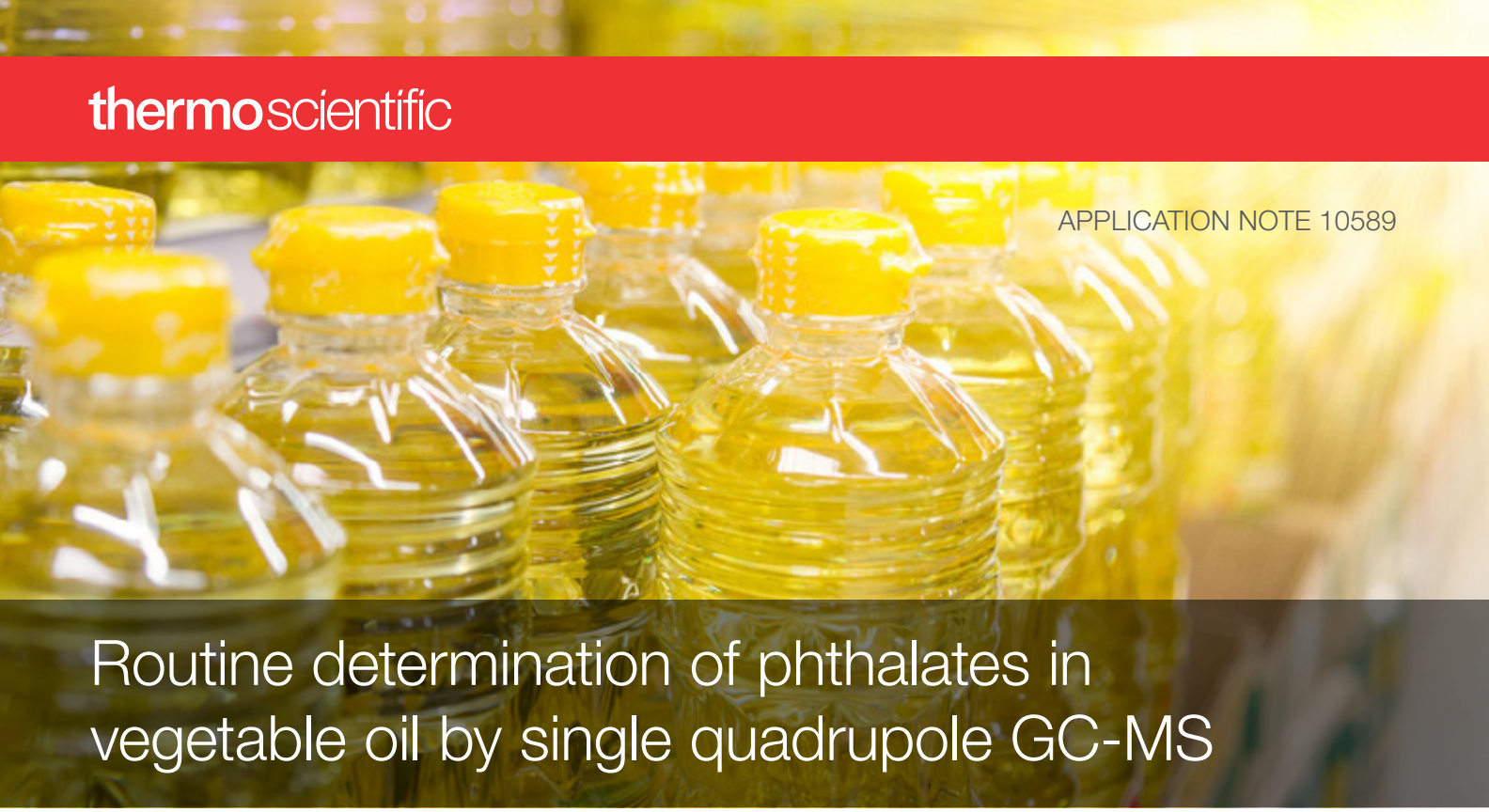
1. EN 13628-1:2002 Packaging- Flexible Packaging Material – Determination of residual solvents by static headspace gas chromatography – Part 1.; Absolute methods.
2. Food Contact Materials - Regulation (EC) No 1935/2004 – European Implementation Assessment Study, May 2016.
3. Title 21, Code of Federal Regulation, Direct Additive Part, 170.3, Indirect Additive, Part 174–179.
4. Kolb, B., Ettre, l., Static headspace-gas chromatography Theory and Practice, Second Edition Wiley, 2006.
5. Thermo Fisher Scientific, Chromeleon CDS Enterprise – Compliance, Connectivity, Confidence, BR72617-EN0718S.
6. Kosowska, M., Majcher, M.A., Fortuna, T., Volatile compounds in meat and meat products, Food Science and Technology, Campinas, vol. 37 issue 1, Jan-March 2017, 1–7.
7. Packaging Materials - Printing inks for food packaging composition and properties of printing inks, International Life Science Institute ILSI Europe, December 2011.

Find out more at **thermofisher.com/TriPlus500**

©2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Sigma-Aldrich is a registered trademark of Sigma-Aldrich Co., LLC. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details.

AN10689-EN 0319S

ThermoFisher SCIENTIFIC



APPLICATION NOTE 10589

Routine determination of phthalates in vegetable oil by single quadrupole GC-MS

Authors
Aaron Lamb, Dominic Roberts, and Cristian Cojocariu
Thermo Fisher Scientific, Runcorn, UK

Keywords
Food safety, phthalates, trace analysis, gas chromatography, ISQ 7000, single quadrupole mass spectrometry, selected ion monitoring, vegetable oil, sensitivity, advanced electron ionization, AEI

Goal

To evaluate the suitability of the new Thermo Scientific™ ISQ™ 7000 GC-MS system, configured with the highly sensitive Advanced Electron Ionization (AEI) source, for the analysis of phthalates. Method selectivity, linearity, recoveries, and robustness were assessed using a challenging vegetable oil matrix.

Introduction

Phthalates (phthalate acid esters, PAEs) are a class of chemicals that are used mainly as plasticizers in various industries. Plasticizers are not chemically bound to their native polymer and therefore can leach into food from packaging materials in significant amounts.[1] Due to their lipophilic nature, phthalates are highly likely to be found in fat containing foods including cooking oils. The most important congener is di-(2-ethylhexyl)-phthalate (DEHP), which accounts for about 50% of the world production of phthalates (Figure 1).[1]

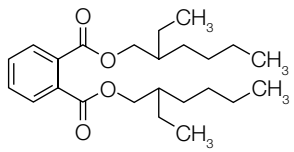


Figure 1. Chemical structure of the most prevalent phthalate, di-(2-ethylhexyl)-phthalate).

ThermoFisher SCIENTIFIC

Previously, phthalates were believed to be non-toxic to humans, but now are classified as endocrine disruptors with associated adverse health effects and with links to autism in children.[2,3] Recent cases of food contamination include the discovery that DEHP was intentionally added to sports drinks, fruit juice, tea beverages, and other food products as a clouding agent.[4] Vegetable oils in the US and EU consumer markets have been found to contain phthalates.[5] As a result, the European Food Safety Authority (EFSA) panel on food additives, processing aids, flavorings, and materials in contact with food has undertaken evaluations of the safety of food contact materials (FCM), as well as assessments on other substances used in food. In 2012 the EFSA set limits for phthalates in FCMs at 0.1%. Also, China and Taiwan have set limits in food products at 1 part per million (ppm), corresponding to 1000 µg/kg.

Sensitive and robust methods for the analysis of phthalates in food are clearly needed to protect the end consumer from food adulteration and phthalate migration from FCMs. One of the major challenges for laboratories that will be required to test for phthalates in food commodities is the analysis of fatty matrices such as cooking oils. These are complex mixtures of triacyl glycerides that are difficult to chromatograph and present a challenge to the selectivity, sensitivity, and robustness for GC-MS analysis.

In this work, the analytical performance of a new single quadrupole GC-MS system using the Advanced Electron Ionization (AEI) source was tested.

Experimental

Calibration standard preparation

Vegetable oil was purchased from a local store. To test the limit of detection (LOD) / limit of quantification (LOQ) and assess the linearity, individual phthalate solvent standard solutions (LGC Ltd, UK) were prepared by spiking GC-grade n-hexane with calibration solutions prepared at 100-fold increased concentration in n-hexane. Nine calibration levels for 13 phthalate compounds were prepared: 0.5, 1.0, 2.5, 5.0, 10, 25, 50, 100, and 250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil).

Sample preparation

Samples of vegetable oil were spiked prior to extraction at three concentration levels: 5, 25, and 50 µg/kg (Figure 2). GC and MS system parameters are listed in Tables 1 and 2. Consumables are listed in the Appendix.

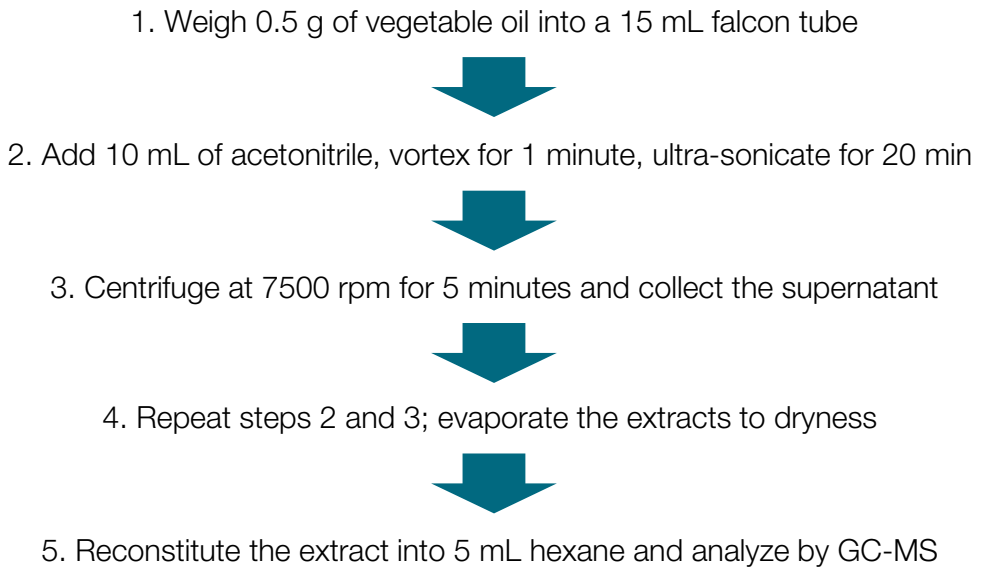


Figure 2. Vegetable oil sample preparation.

Table 1. Thermo Scientific™ AS 1310 autosampler and Thermo Scientific™ TRACE™ 1310 GC oven parameters.

Instrument conditions		
Autosampler parameters		
Fill strokes	10	
Air volume	1.0 µL	
Sample wash	2	
GC inlet parameters		
Injection volume	1 µL	
Injection mode	Splitless	
Temperature	300 °C	
Split flow	80.0 mL/min	
Splitless time	1.0 min	
Purge flow	5.0 mL/min	
Flow mode	Constant flow (1.0 mL/min)	
Carrier gas	Helium	
GC oven settings		
Ramp rate (°C)	Target value (°C)	Hold time (min)
0	100	1.0
20	190	0.0
10	280	5.0
30	320	10.0

See Appendix for consumables used.

Table 2. ISQ 7000 GC-MS system parameters.

MS conditions	
Transfer line temperature	300 °C
Ion source temperature	350 °C
Acquisition mode	Timed (SIM)
Ionization mode	EI (45 eV)
Emission current	10 µA
Minimum peak width	3 s
Minimum scans/peak	12

Name	RT (min)	(SIM) *m/z*		
		Quant	Qual 1	Qual 2
DMP	5.8	163	194	77
DEP	6.7	149	177	121
DAP	7.8	149	41	132
DIBP	8.8	149	205	223
DBP	9.6	149	223	205
DPP	11.1	149	237	219
DHXP	12.6	251	149	104
BBP	12.7	149	91	206
DCHP	14.0	149	167	249
DEHP	14.1	149	167	279
DNOP	15.6	149	279	167
DINP	16.1	293	149	167
DIDP	17.7	307	149	167

Results and discussion

To assess the selectivity, sensitivity, linearity, and robustness of the ISQ 7000 GC-MS system configured with the AEI source, a complex vegetable oil matrix was selected. An example of the complexity of the total ion current (TIC) chromatography for full scan (FS) data of vegetable oil is shown in Figure 3.

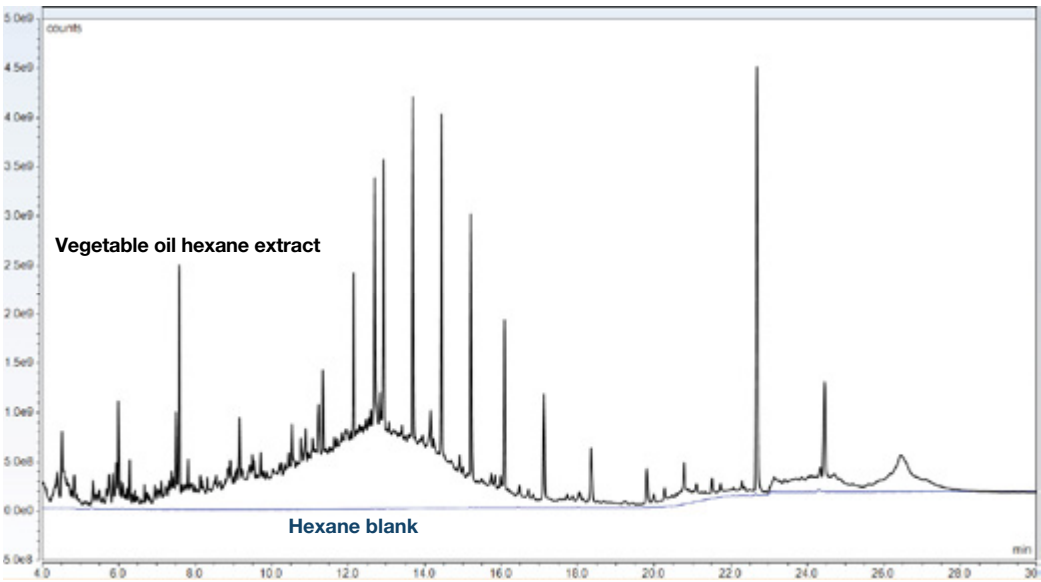


Figure 3. Example showing the cleanliness of the n-hexane blank and the complexity of the vegetable oil n-hexane extract overlays.

When using full scan acquisition, it is difficult to selectively detect phthalates such as DEHP from the background ions. In contrast, by using selective ion monitoring mode (SIM), a significant selectivity and sensitivity improvement is obtained (Figure 4).

Given the complexity of the chromatogram, analysis of phthalates in vegetable oil was carried out using timed-SIM. Timed-SIM mode is an excellent choice for quantitative GC-MS analysis because it allows the detection of analytes with increased sensitivity. In SIM mode, data are gathered only for masses of interest rather than a full mass range, and the optimization of both scan rate and dwell time can be performed automatically using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software by inputting the desired number of points across the narrowest peak of interest and its peak width in seconds. This leads to greatly increased sensitivity and lower limits of quantification.

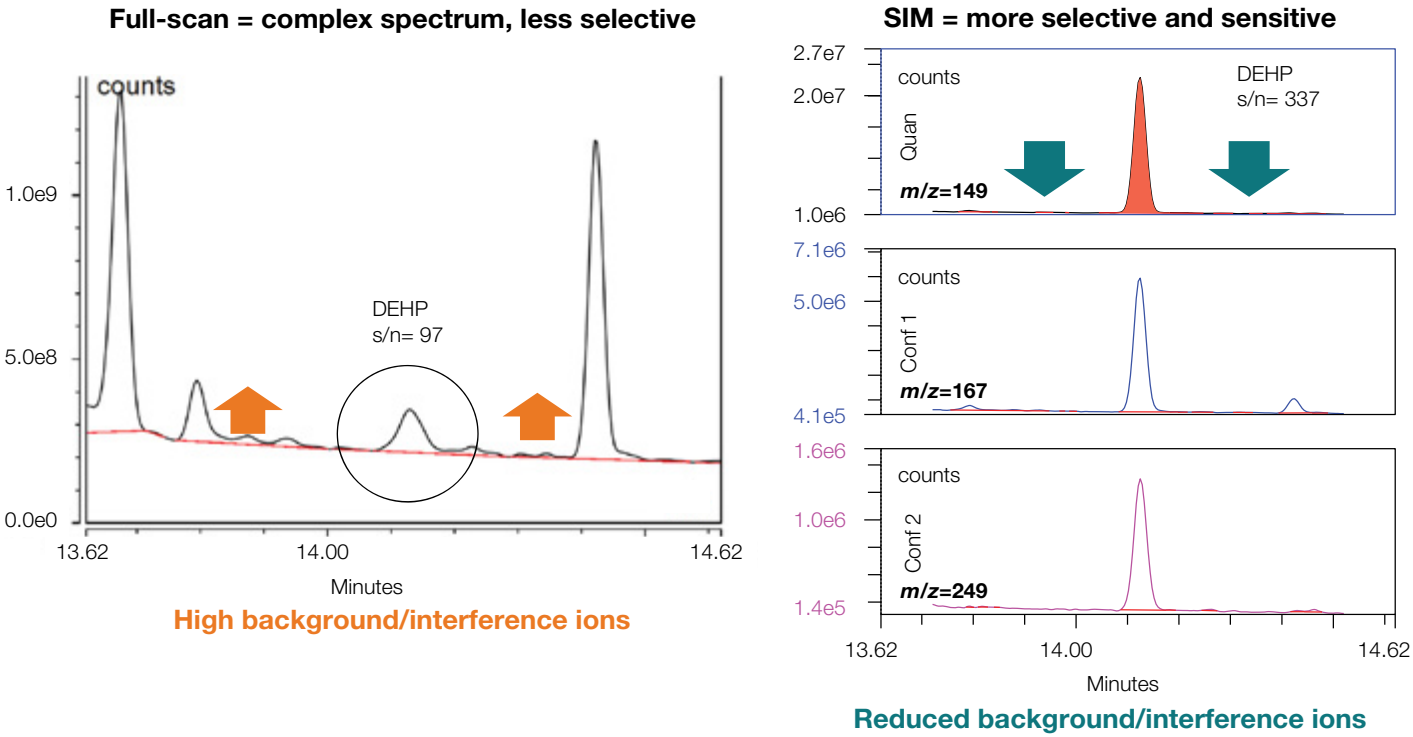


Figure 4. Example of selectivity and sensitivity obtained for DEHP when using SIM and FS for a vegetable oil hexane extract.

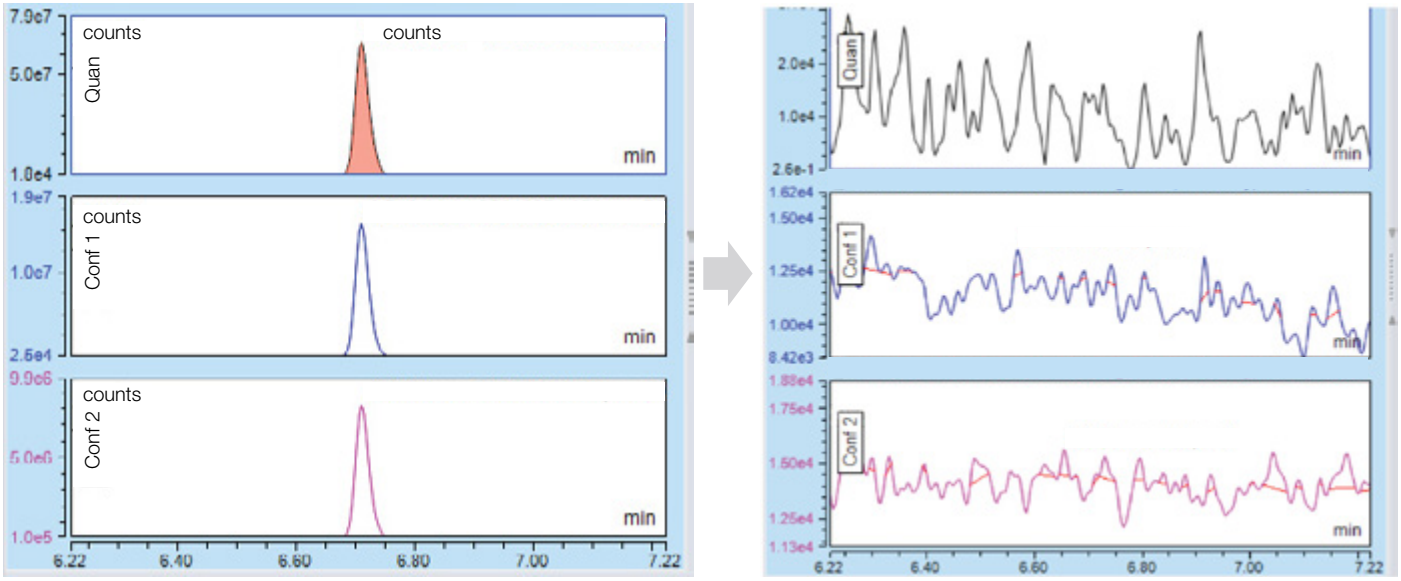


Figure 5. Example of DEP analyzed in a 50 ng/mL solvent standard and in a consecutive n-hexane blank injection showing no compound carry-over.

Overcoming contamination in phthalates analysis

As phthalates are ubiquitous compounds, during routine GC-MS analysis many sources of contamination can arise, such as potential plastic contact materials (polyethylene terephthalate). This problem is exacerbated by the high vapor pressures and chemical properties of phthalates increasing their persistence in the inlet, transfer line, and ion source if instrumental conditions are non-optimized. To avoid such contamination and to reduce potential carry-over from injection to injection, optimal consumable choice and method parameters are critical. This includes using PTFE/siloxane vial closures and bleed temperature optimized (BTO) inlet septa, as well as using optimized wash, inlet, and MS conditions (Figure 5).

Enhanced selectivity using SIM

Using SIM acquisition mode, selective and sensitive detection of phthalates in the food matrices was achieved. An example of SIM chromatograms including a stacked chromatogram (quantitation ion and 2x confirmation ions) at 0.5 ng/mL (5 µg/kg) level are shown in Figure 6 for the vegetable oil sample.

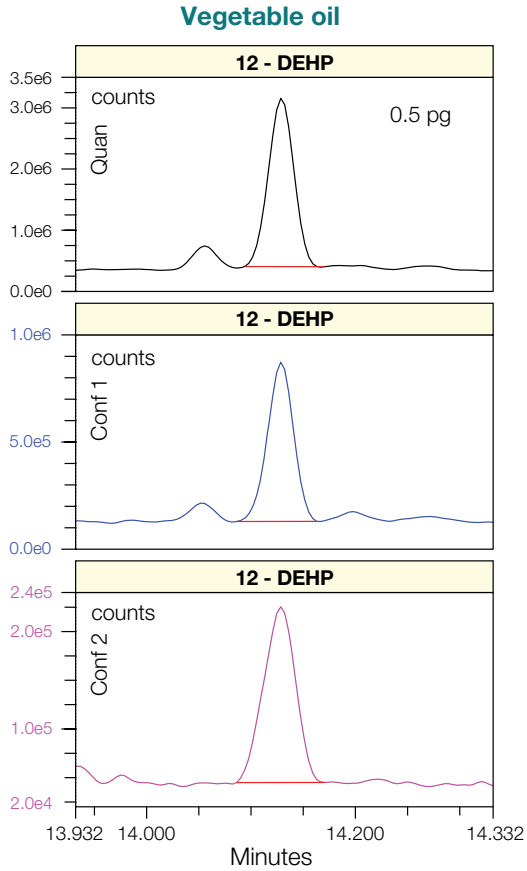


Figure 6. Example of SIM chromatograms for DEHP spiked at 0.5 ng/mL (5 µg/kg) in a vegetable oil n-hexane extract showing excellent sensitivity. On-column amount is also annotated.

Determination of LOD and LOQ

Phthalate residues in food products currently have no regulatory limits in the EU. However in this application levels of 5–25 µg/kg were achieved.

To practically assess the method's limit of detection, 18 replicate injections (of standards around the LOQ for each component) were performed. The instrumental detection limit (IDL) for each individual compound was then calculated by taking into account the injected amount, % RSD, and t-score of 2.567, corresponding to 17 degrees of freedom at 99% confidence (Table 3).

In addition to this, the LOQ was determined as the lowest concentration level of phthalates with a peak area repeatability of < 15% RSD and ion ratios within < ±15% of the expected values calculated as an average across a calibration curve ranging from 0.5 to 250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil). Based on these criteria, the estimated LOQs for compounds ranged from 5 to 25 µg/kg. An example of LOQ determination for the most difficult matrix is shown in Table 4.

Table 3. Estimated IDLs and absolute peak area repeatability (as % RSD) for phthalates determined from n=18 injections of a lowest concentrated standard where the peak area % RSD was lower than 15%.

Estimated IDL levels			
Component	**Level injected (pg OC*)**	**% RSD**	**IDL (pg OC*)**
DMP	0.1	4.1%	0.01
DEP	0.1	11%	0.03
DAP	0.1	7.8%	0.02
DIBP	0.1	2.7%	0.01
DBP	0.1	3.2%	0.01
DPP	0.1	5.7%	0.01
DXHP	1.0	9.2%	0.24
BBP	0.1	14%	0.04
DCHP	25	4.5%	3.0
DEHP	0.1	5.8%	0.01
DNOP	0.1	7.6%	0.02
DINP	25	2.4%	1.6
DIDP	25	3.0%	1.9

* OC = on column

Table 4. LOQ, absolute peak area, and ion ratio stability for targeted phthalates in vegetable oil (n=3 injections) at 5.0 µg/kg and at 25 µg/kg.

Estimated LOQ levels					
Compound name	**LOQ (µg/kg)**	**Peak area % RSD 5 µg/kg**	**Ion ratio % RSD 5 µg/kg**	**Peak area % RSD 25 µg/kg**	**Ion ratio % RSD 25 µg/kg**
DMP	5.0	1.0	2.2	0.9	2.9
DEP	5.0	0.3	4.6	1.2	3.2
DAP	5.0	7.3	7.7	1.3	2.4
DIBP	5.0	0.9	6.8	1.0	2.5
DBP	5.0	3.1	4.1	1.2	0.9
DPP	5.0	11	7.7	1.5	11
DXHP	5.0	7.9	1.7	0.6	2.7
BBP	5.0	0.4	6.5	1.5	0.4
DCHP	25	NA	NA	2.3	2.8
DEHP	5.0	6.9	1.6	1.1	1.9
DNOP	5.0	5.5	4.2	1.9	2.2
DINP	25	NA	NA	2.5	5.4
DIDP	25	NA	NA	2.8	4.7

With the innovative design of the new AEI source, less frequent source cleaning is required as the improved source geometry leads to increased ionization efficiency and a narrower ion beam. This means the source filament can be operated at a reduced emission current, which in turn means less ionization of complex matrices in the source. Additionally, the highly focused ion beam significantly reduces the risk of source contamination. These features make the AEI source extremely robust, extending the time before maintenance is required. The enhanced sensitivity of the new source also means that the sample matrix can be diluted more or the split ratio can be increased, further reducing the amount of potential contamination to the GC flow path.

Linearity

Linearity was determined using n-hexane solvent phthalate standards at concentrations of 0.5–250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil extracts). All compounds showed excellent linear response with coefficient of determination $R^2 > 0.998$, and average response factor values across this calibration range were all below 10% (Figure 7).

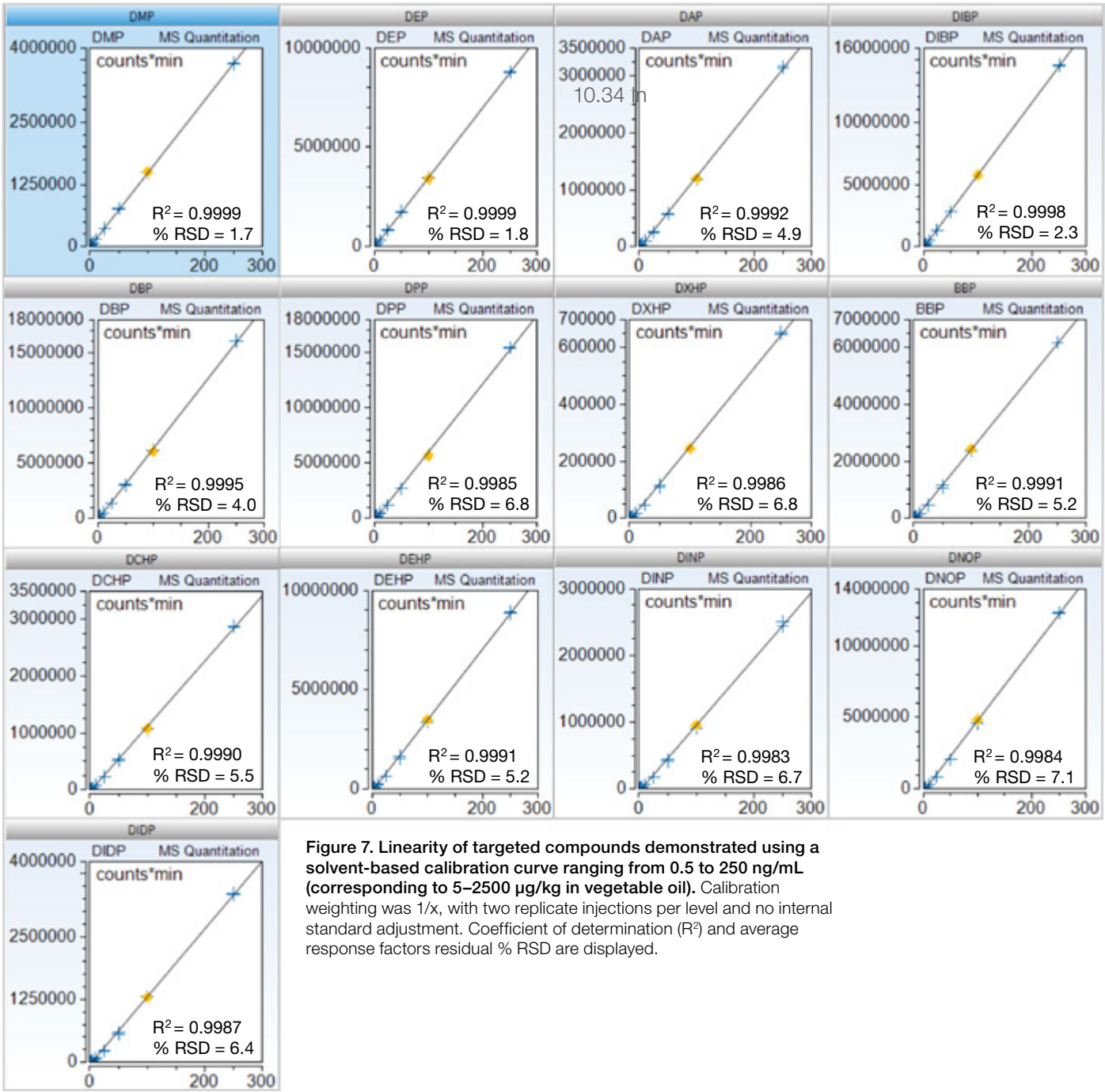


Figure 7. Linearity of targeted compounds demonstrated using a solvent-based calibration curve ranging from 0.5 to 250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil). Calibration weighting was 1/x, with two replicate injections per level and no internal standard adjustment. Coefficient of determination (R^2) and average response factors residual % RSD are displayed.

Method performance

The performance of the method was assessed by evaluating the recoveries in the pre- and post-spiked vegetable oil samples with a mixed phthalates standard at 5, 25, and 50 µg/kg. Three injections (technical replicates) per level were used and the results show average recovery values between 80% and 102% (Table 5).

Table 5. Recoveries (%) calculated for mixed phthalates pre-spiked in vegetable oil at three different concentration levels (5, 25, and 50 µg/kg) from n=3 injections. Recovery % RSD (n=3) is also shown.

Compound name	% Recovery 5.0 µg/kg spike level	% RSD (n=3)	% Recovery 25 µg/kg spike level	% RSD (n=3)	% Recovery 50 µg/kg spike level	% RSD (n=3)
DMP	101	1.7	98	1.9	104	5.2
DEP	102	1.8	98	4.8	100	3.7
DAP	97	1.7	95	0.8	99	3.0
DIBP	101	4.1	97	2.3	99	4.3
DBP	100	3.0	97	1.2	100	3.2
DPP	97	1.4	96	2.2	97	1.4
DXHP	97	5.9	91	0.5	95	0.6
BBP	92	3.4	93	0.3	91	2.0
DCHP*	NA	NA	91	1.3	84	4.8
DEHP	96	2.2	91	6.7	93	5.5
DNOP	93	3.9	93	2.6	97	0.5
DINP*	NA	NA	96	2.0	101	0.1
DIDP*	NA	NA	92	2.0	84	0.4

* The % recoveries were not calculated at the 5 µg/kg level as this was below the LOQ.

Conclusion

- The new innovative Thermo Scientific AEI source exhibits excellent sensitivity with unrivaled instrument detection limits of phthalate esters down to low ppt levels (0.01 ng/mL).
- Outstanding linearity for 13 phthalates analyzed was demonstrated over a range of 0.5 to 250 ng/mL (corresponding to 5–2500 µg/kg in vegetable oil). All compounds showed linear responses with coefficient of determinations $R^2 > 0.998$ average response factor RSDs < 10%.
- Compound recoveries demonstrated across three separate spiking levels were between 80% and 102%, well within the required method performance limits.

The ISQ 7000 GC-MS system configured with the AEI source provides unrivaled levels of sensitivity and robustness due to improved source geometry resulting in enhanced ionization efficiency and a narrower ion beam. This allows the user the flexibility to dilute their sample more, inject less, or use split methods while still being able to achieve the required limits of detection. Reduced matrix load on the GC-MS system means reduced frequency of costly preventive instrument maintenance, such as consumable replacement and source cleaning, increasing the profitability and laboratory productivity.

References

1. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Official Journal of the European Union.
2. Kavlock, R.J.; Daston, G.P. DeRosa, C.; Fenner-Crisp, P.; Gray, L.E.; Kaattari, S., et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S.EPA-sponsoredworkshop. *Environ. Health Perspect.* **1996**, Aug;104 Suppl 4, 715–40. doi: 10. 1289/ehp.96104s4715.
3. Clausen, P.A.; Hansen, V.; Gunnarsen, L.; Afshari, A.; Wolkoff, P. Emission of di-2-ethylhexyl phthalate from PVC flooring into air and uptake in dust: emission and sorption experiments in FLEC and CLIMPAQ. *Environ. Sci. Technol.* **2004**, *38*(9), 2531–7. doi: 10.1021/es0347944.
4. Yanga, J.; Hausera, R.; Goldman, R.H.; Taiwan food scandal: The illegal use of phthalates as a clouding agent and their contribution to maternal exposure, *Food and Chemical Toxicology.* **2013**; *58*, 362–368, doi.org/10.1016/j.fct.2013.05.010.
5. Serrano, S.E.; Braun, J.; Trasande, L.; Dills, R.; Sathyanarayana, S. Phthalates and diet: a review of the food monitoring and epidemiology data. *Environ. Health.* **2014**, *13*, 43.

Appendix A. Consumables list.

Consumable	Part Number
Column: Thermo Scientific™ TraceGOLD™ TG-5MS, 30 m × 0.25 mm x 0.25 µm	26098-1420
Injection septum: Thermo Scientific™ BTO, 11 mm	31303215-BP
Injection liner: Thermo Scientific™ LinerGOLD™, Single taper liner with quartz wool	453A2922-UI
Column inlet ferrules: Thermo Scientific™ 15% Graphite/85% Vespel 0.1–0.25 mm ID	290VA191
Column MS ferrules: Thermo Scientific™ 15% Graphite/85% Vespel 0.1–0.25 mm ID	290VT221
Spring loaded transfer line nut: Thermo Scientific™	1R120434-0010
Inlet base seal: Thermo Scientific™ 0.8 mm ID single column gold seal	290GA081
Injection syringe: Thermo Scientific™ 10 µL fixed needle syringe	365D0291
Solvent: Fisher Chemical™ Optima™ LC/MS Grade acetonitrile	Fisher Scientific A955-1
Solvent: Alfa Aesar™ Environmental Grade GC, >95%, n-hexane	Fisher Scientific AA42100K7
Conical centrifuge tubes: Corning™ Falcon™, 15 mL	Fisher Scientific 10136120
Conical centrifuge tubes: Corning™ Falcon™, 50 mL	Fisher Scientific 10788561
Vial: Thermo Scientific™ Virtuoso™, clear 2 mL kit with septa and cap	60180-VT402
Vial Identification System: Thermo Scientific™ Virtuoso™	60180-VT100

Find out more at **thermofisher.com/ISQ7000**

©2018 Thermo Fisher Scientific Inc. All rights reserved. Corning and Falcon are trademarks of Corning Life Sciences. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details.

AN10589-EN 0318S

thermo scientific

APPLICATION NOTE 73241

Simple and cost-effective determination of acrylamide in food products and coffee using gas chromatography-mass spectrometry

Author
Jane Cooper

Thermo Fisher Scientific,
Runcorn, UK

Keywords
Acrylamide, gas chromatography, mass spectrometry, GC-MS, single quadrupole mass spectrometry, ISQ 7000, vacuum probe interlock, VPI, ExtractaBrite, silylation, derivatization, MSTFA

Goal

To demonstrate a simple, cost-effective analytical solution for the routine determination of low level acrylamide in food and coffee samples, from sample extraction to detection and quantification, using a Thermo Scientific™ ISQ™ 7000 GC-MS system coupled with a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software.

Introduction

Acrylamide (2-propeneamide) is a chemical that has been found in certain cooked foods, including fried and baked starchy foods, such as potato crisps (potato chips) and chips (French fries), roasted coffee, breads, peanuts, and cigarette smoke.[1,2] In baked and fried foods, acrylamide is formed as a by-product of the Maillard reaction, occurring between asparagine and reducing sugars (fructose, glucose, etc.) or reactive carbonyls at temperatures above 120 °C.[1,3,4,5]

Acrylamide is highly toxic; can cause neurotoxicity, genotoxicity, and reproductive harm; and is a likely human carcinogen.[6] The Food Standards Agency (FSA) regulation 2017/2158 provides legislation concerning acrylamide levels in food, guidance for food business operators, and benchmark levels of acrylamide in different food categories.[7]

ThermoFisher SCIENTIFIC

Current sample preparation and analytical technologies used for the analysis of acrylamide involve extraction methods such as Soxhlet extraction, liquid-liquid extraction, and solid phase extraction (SPE), which are time-consuming and require large amounts of organic solvents, which are costly to dispose of. They are followed by either liquid chromatography/tandem mass spectrometry (LC-MS/MS) or gas chromatography (GC) coupled to electron capture detection (ECD), flame ionization detection (FID), or mass spectrometry (MS). Due to its high-water solubility, aqueous extraction followed by LC-MS/MS has emerged as the main method for the determination of acrylamide in food matrices. Since water will also extract high molecular weight compounds, including proteins, a time-consuming sample clean-up is often required.[8] Current GC-MS methods mainly involve derivatization via bromination,[9] which is labor-intensive, and the brominated acrylamide may break down at high temperature in the GC injector or column.

This work aims to overcome the analytical limitations of current methods applied for acrylamide analysis in food by considering a cost-effective, robust, and selective approach, by the use of acetonitrile as the extraction solvent and derivatization using silylation, followed by GC-MS for the analysis of food and coffee samples.

Experimental

Sample preparation

Various food and coffee samples were purchased locally for targeted quantitative analysis of acrylamide, using splitless injection.

Five milliliters of acetonitrile were added to a ground sample (1 g). The sample was extracted in an ultrasonic bath (10 min) and vortexed (20 s). An aliquot (~1 mL) was centrifuged (5752 g for 5 min). Then, 500 µL of the supernatant was transferred to a crimp-top GC vial and 100 µL of the silylation reagent MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide) with 1% TMCS (2,2,2-trifluoro-*N*-methyl-N-(trimethylsilyl)-acetamide, chlorotrimethylsilane) as catalyst (P/N TS48915) was added. The solution was mixed and heated at 70°C for 60 min. After cooling naturally to room temperature, the sample extract was ready for analysis. The analytical workflow for the analysis of acrylamide is illustrated in Figure 1.

To assess acrylamide linearity and instrument performance, working calibration solvent standards were prepared in acetonitrile and subjected to the derivatization steps described previously (ranging from 1 ppb to 1000 ppb, equivalent to 5–5000 µg/kg in the sample). Standard addition calibrations were used for quantification, samples unspiked and spiked at 1000 µg/kg and 2000 µg/kg, and subjected to derivatization.

Instrument and method setup

An ISQ 7000 GC-MS system was used in all experiments. The MS was configured with the vacuum probe interlock (VPI) and the ExtractaBrite source, and was operated in timed selected ion monitoring (t-SIM) using electron ionization (EI). A TRACE 1310 Gas Chromatograph was equipped with a Thermo Scientific™ Instant Connect split/splitless (SSL) injector, and configured with a Thermo Scientific™ TriPlus™ RSH™ autosampler.

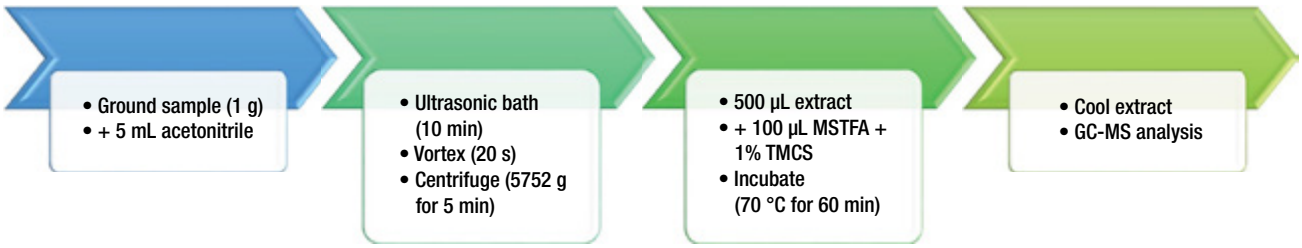


Figure 1. Acrylamide analytical workflow, highlighting the main steps of the process in which a low sample amount (1 g) is derivatized using silylation reagent prior to GC-MS analysis

Compound separation was achieved using a Thermo Scientific™ TraceGOLD™ TG-WaxMS 30 m × 0.25 mm i.d. × 0.25 µm film capillary column (P/N 26088-1420).

Additional details on instrument parameters are listed in Tables 1 and 2.

Table 1. GC and injector conditions

TRACE 1310 GC system parameters	
Liner:	Splitless liner, single taper, 4.0 mm × 6.5 mm × 78.5 mm
Inlet temperature (°C):	250
Carrier gas, mL/min, mode:	He, 1.2, constant flow
Inlet module and mode:	SSL, splitless
Split flow (mL/min):	100
Splitless time (min):	2
Septum purge flow (mL/min):	5
Column:	TraceGOLD TG-WaxMS 30 m × 0.25 mm i.d. × 0.25 µm
Injection volume (µL):	1.0

Oven temperature program

	RT (min)	Rate (°C/min)	Target Temp (°C)	Hold Time (min)
Initial	0	-	50	2.0
Stage 1	2.0	3	100	0.0
Final	18.7	25	250	5.0
Run time	30	-	-	-

Table 2. Mass spectrometer conditions

Transfer line (°C):	250
Ionization mode:	EI (ExtractaBrite)
Ion source (°C):	250
Electron energy (eV):	70
Acquisition mode:	Timed selected ion monitoring (t-SIM)
SIM ions:	*m/z* 128 (quantification ion) and *m/z* 85 (confirming ion)

Data processing

Data were acquired, processed, and reported using Chromeleon CDS software, version 7.2. Chromeleon CDS software allows the analyst to set up acquisition, processing, and reporting methods with easy data reviewing and flexible data reporting.

Results and discussion

The object of this study was to evaluate the utility of a simplified approach that uses GC-MS to analyze acrylamide in food. For this, MSTFA was employed to derivatize acrylamide. In-depth investigation of the derivatization parameters, including derivatization volume, temperature, and time was performed. The analytical method was tested by considering various analytical parameters, including selected ion monitoring (SIM) conditions, chromatographic resolution, linearity, sensitivity, repeatability, and robustness in matrix, and selectivity.

Chromatography

Using the GC conditions described in Table 1, the peak shapes obtained are shown in the extracted ion chromatograms (EIC, *m/z* 128) for acrylamide in solvent standards, samples containing incurred residues, and spiked samples (Figures 2A, 2B, and 2C, accordingly). Peak asymmetry values for acrylamide, with tailing factors (T_f) between 0.91 and 1.01 (indicating almost perfect Gaussian peak shapes), and narrow peak widths of ~4 s were observed, measured at 10% peak height (Figure 2).

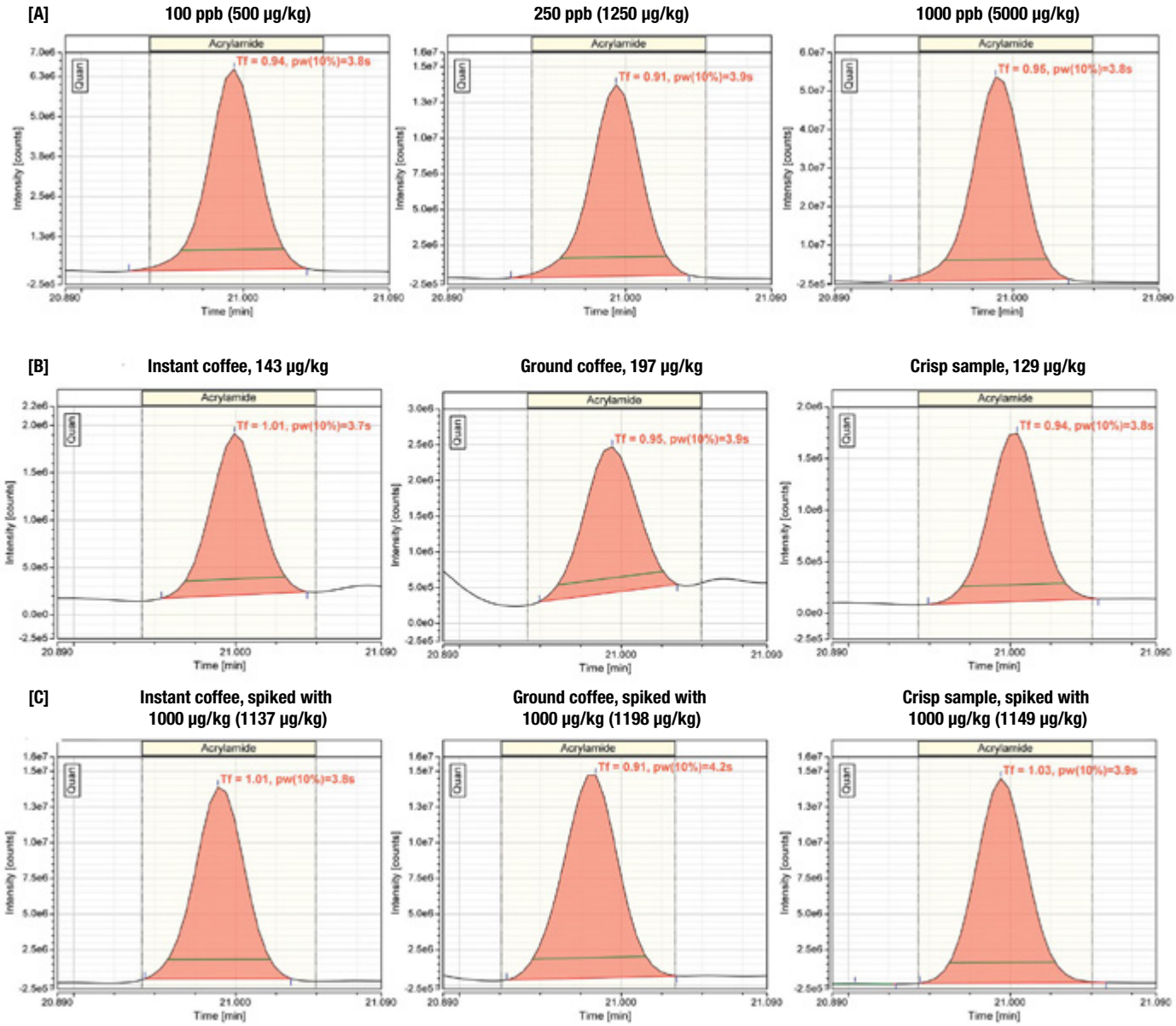


Figure 2. Example of chromatographic separation of acrylamide in A: derivatized calibration solvent standards at 100, 250, and 1000 ppb, B: derivatized samples, instant coffee and crisps, and C: spiked samples, instant coffee and ground coffee. Annotated with tailing factor (Tf) and peak width, measured at 10% pk ht (green line). Samples and spiked sample results quoted using standard addition calibration.

Linearity of response

External standard calibration

Solvent standards were used to assess linearity and instrument performance. Linearity of external calibration was assessed using eight calibration levels (1 to 1000 ppb) prepared in solvent (equivalent to between 5 and 5000 µg/kg in the analyzed samples) using a 1/× weighting factor. Excellent linearity was demonstrated for acrylamide, with a coefficient of determination (R^2) of 0.9993 and an average residual %RSD (AvCF %RSD) of 4.8. An example calibration curve for acrylamide is shown in Figure 3 where both the R^2 value and the AvCF %RSD are annotated.

Standard addition calibration

Standard addition calibration was used for quantification, to compensate for matrix effects. Potato crisps, instant coffee, and ground coffee samples, unspiked and spiked at 1000 µg/kg and 2000 µg/kg (three replicates at each level), were quantified using a 1/× weighting factor. Excellent linearity was demonstrated for acrylamide, with an R^2 value of ≥0.9987 and an AvCF %RSD of ≤4.0 achieved for crisps, instant coffee, and ground coffee standard addition calibration curves; see Figure 4 where both R^2 value and the residual %RSD are annotated.

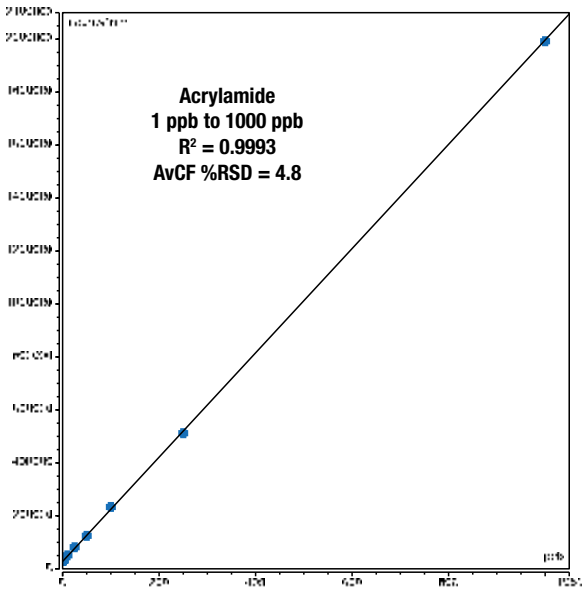


Figure 3. Example solvent calibration curve for acrylamide, illustrating the linearity obtained, over eight calibration levels ranging from 1 to 1000 ppb (equivalent to 5–5000 µg/kg in food samples). Annotated with coefficient of determination (R^2) and the average calibration factor (AvCF) (as %RSD).

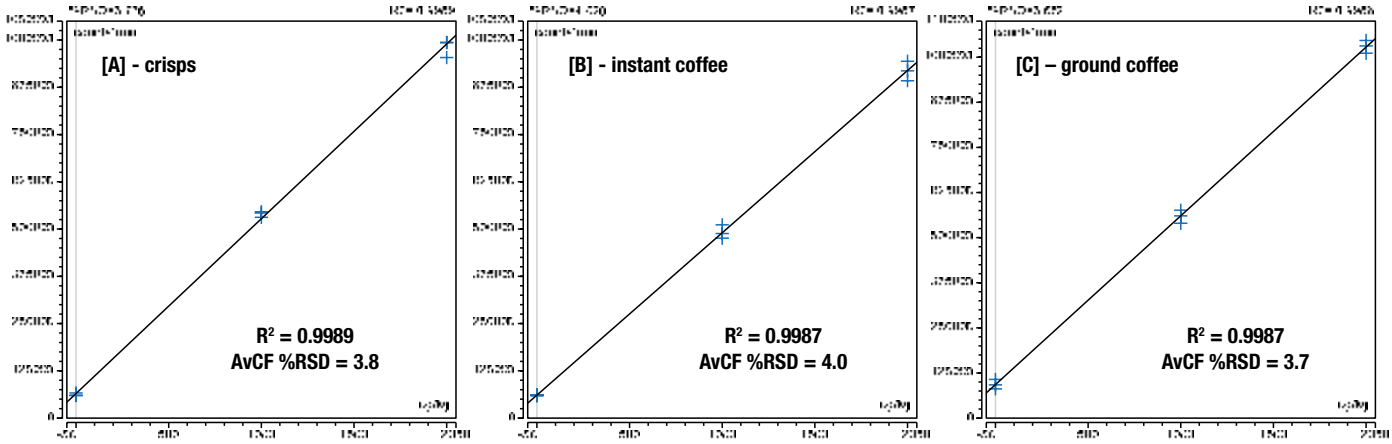


Figure 4. Standard addition calibration curve used for quantification for A: crisps, B: instant coffee, and C: ground coffee, unspiked, and spiked at two levels (1000 µg/kg and 2000 µg/kg), three replicates at each level. Annotated with coefficient of determination (R^2) and the average calibration factor (AvCF) (as %RSD).

Sensitivity

A limit of identification (LOI) of 1 ppb (equivalent to 5 µg/kg in the analyzed samples) was achieved using the detailed method (Figure 5). LOI is a measure of method sensitivity and was determined based on the criteria for identification of pesticide residue in food and feed (as outlined in the SANTE/11813/2017 guidelines) considering the lowest concentration of acrylamide solvent standard passing the criteria: Ion ratios within ±30% of the expected values calculated as an average across the calibration range 1 to 1000 ppb (equivalent to between 5 and 5000 µg/kg in the analyzed samples) and ion co-elution within ±0.01 minutes.

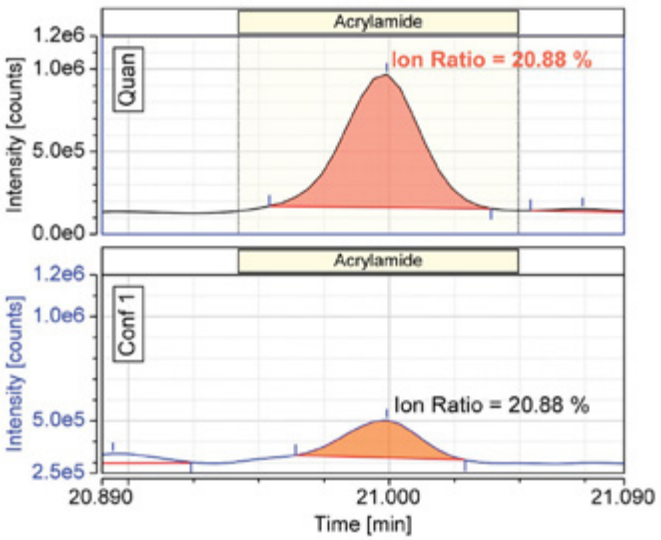


Figure 5. Extracted ion chromatograms for the quantification ion (*m/z* 128, upper) and the confirming ion (*m/z* 85, lower) at 1 ppb (LOI) for acrylamide. Ion ratio value achieved within ±30% of expected ion ratio (calculated as an average across the calibration range).

Peak area repeatability and robustness in matrix

Repeatability and robustness of acrylamide responses in matrix were assessed by carrying out repeated injections (n=16) of a QC ground coffee sample, spiked with 200 ppb acrylamide (equivalent to 1000 µg/kg) prior to extraction, as part of a 99-injection analytical sequence, containing derivatized blanks, calibration standards, crisp, instant coffee, and ground coffee samples. Three QC injections were mid sequence (lines 46–48), with the additional 13 injections analyzed near the end of the sequence (lines 79–92). Excellent repeatability is illustrated in Figure 6, with a peak area %RSD of 2.9 for the acrylamide absolute peak area for all 16 injections, and robustness highlighted with peak area %RSD of 1.3 comparing the spiked samples injection mid-sequence to those injected at the end of the analytical sequence. No inlet, column, MS maintenance, or MS tuning were performed over the injection sequence.

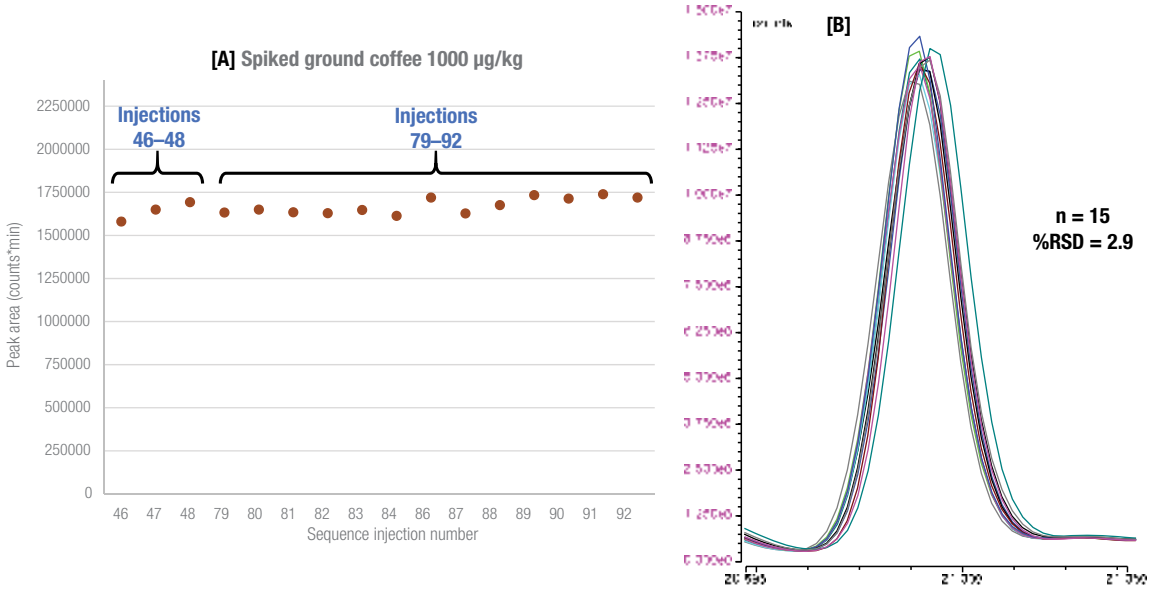


Figure 6. [A] Robustness data shown as consistent peak area counts for acrylamide determined in QC ground coffee samples spiked at 200 ppb (equivalent to 1000 µg/kg), analyzed mid (inj. no. 46–48), and end (inj. no. 79–92) of a 99-injection analytical sequence, containing derivatized blanks, calibration standards, crisp, instant coffee, and ground coffee samples. [B] overlaid EIC (*m/z* 128) of the QC ground coffee sample (n=16 injections) analyzed across the whole analytical sequence. For all QC ground coffee samples containing acrylamide at the 200 ppb level across the analytical sequence of 99 injections the calculated %RSD absolute peak area counts was 2.9.

Selectivity in matrix

By using MSTFA as the derivatization reagent, sensitivity and selectivity for the analysis of acrylamide is enhanced (when compared to non-derivatized). Using acetonitrile instead of water as the extraction solvent avoids the extraction of proteins and other high molecular weight compounds that could interfere chromatographically and compete for the silylation reagent. Derivatized acrylamide, compared to the free acrylamide, has both greater chemical and thermal stability, which makes it more applicable to GC-MS analysis.

Compared to detection of free acrylamide (without derivatization), co-extracts of low *m/z* ions, which can interfere with acrylamide, which in matrix can markedly affect the detection limits and lead to erroneous detection and inaccurate results. This is demonstrated in Figure 7, which illustrates the chromatographic separation and example results achieved for the same samples and standards, prepared as detailed, but with and without derivatization. For the non-derivatized analysis, the same calibration solvent standards were analyzed, acquiring *m/z* 55 (quantification ion) and *m/z* 71 (confirming ion) and resulting in linearity with R^2=0.9989 and residual %RSD of 6.0. Figure 7 shows that for the same sample extract, the non-derivatized chromatogram resulted in closely eluting peaks, which makes the integration and associated result achieved questionable. For the derivatized samples there was a significant increase in signal response and improvements in selectivity.

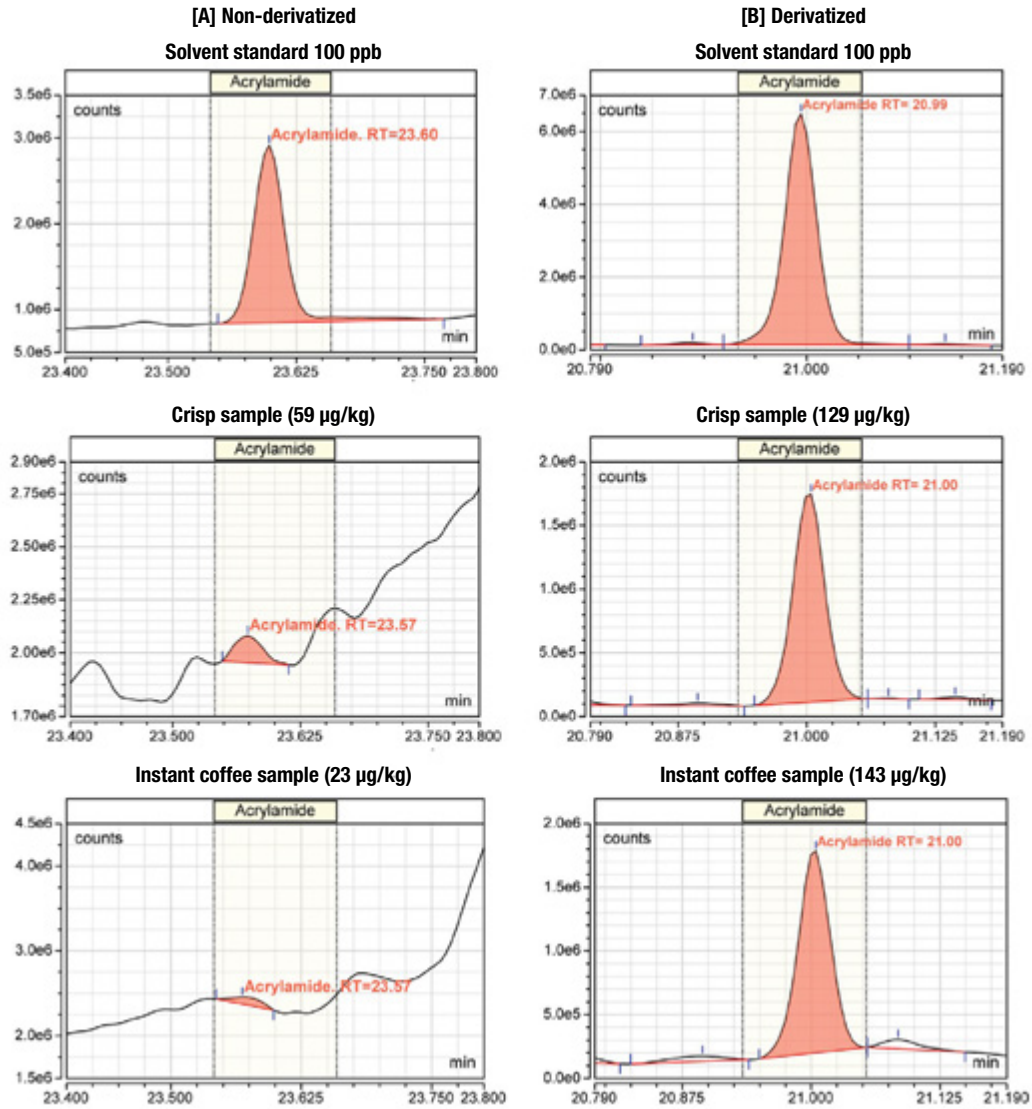


Figure 7. Examples of chromatographic selectivity of the same acrylamide calibration working standard (100 ppb), crisp and instant coffee samples, A: non-derivatized (m/z 55), and B: derivatized with MSTFA + 1% TMCS (*m/z* 128). Sample results quoted using standard addition calibration.

Quantification of acrylamide in food samples

Samples of potato crisps and coffee (instant and ground) were prepared and analyzed in triplicate using the derivatization protocol. Samples were analyzed before spiking, to determine the acrylamide content, and spiked at two levels (1000 and 2000 µg/kg) to assess recovery and method precision. Acrylamide quantification was performed using a standard addition calibration for each matrix, which eliminated the need for an expensive ^{13}C-labeled internal standard. A summary of results for potato crisps, instant and ground coffee samples is shown in Figure 8.

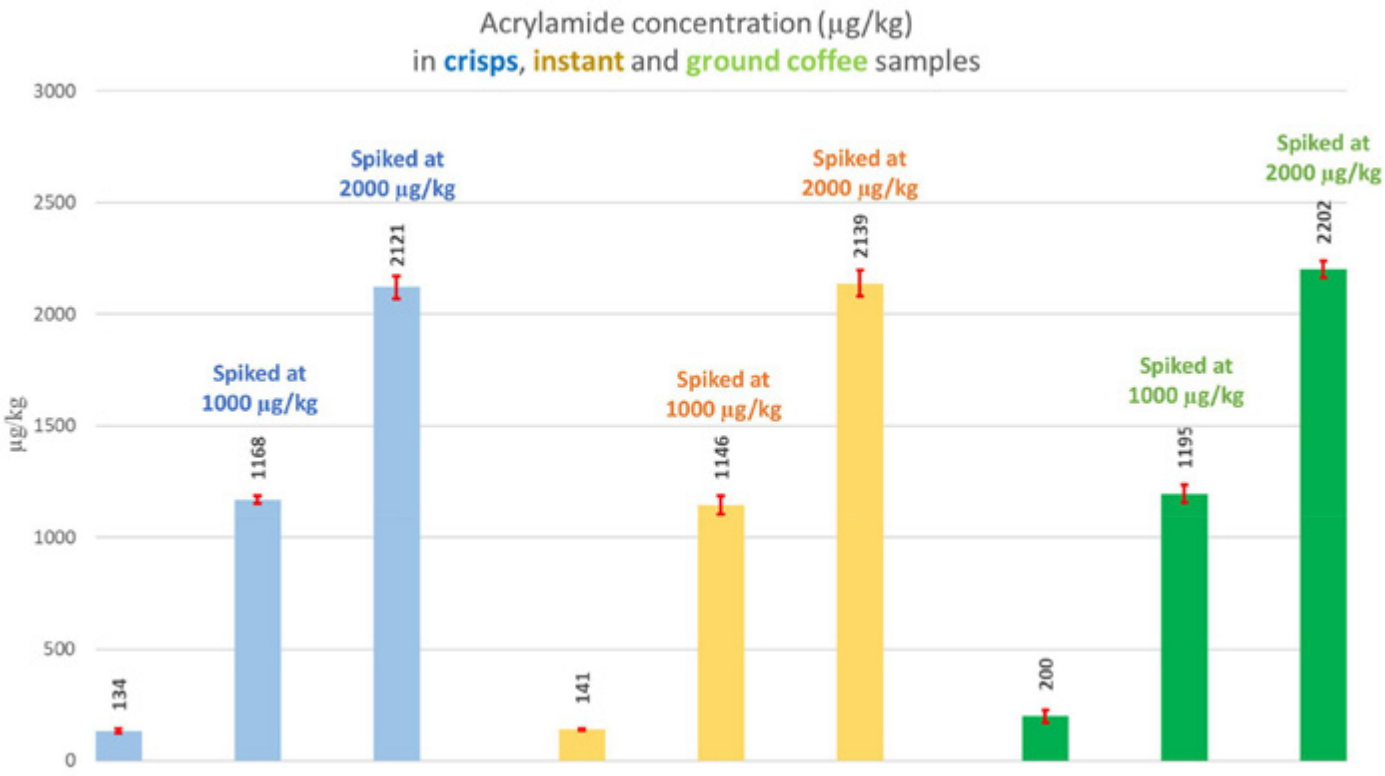


Figure 8. Average concentration of acrylamide (n=3) using standard addition calibration determined for unspiked and spiked (1000 and 2000 µg/kg) potato crisp, and instant and ground coffee samples, showing consistency at low and high levels. Standard deviation calculated from the three replicates is annotated, demonstrating the repeatability of the method.

Conclusion

The results obtained clearly demonstrate that the ISQ 7000 GC-MS system with a TRACE 1310 Gas Chromatograph, in combination with the TriPlus RSH autosampler and the Chromeleon CDS software, offers a viable alternative to laboratories that analyze low level contaminants such as acrylamide in food commodities. This statement is based on the following findings:

- Good chromatographic resolution with excellent peak asymmetry values (tailing factors between 0.91 and 1.01), and peak width (+10%) ≤4 s was achieved.
- Compound linearity obtained for derivatized acrylamide over a calibration range of 1 to 1000 ppb resulted in an average coefficient of determination R^2 of 0.9993 and average residual %RSD of 4.8.
- Excellent linearity was also demonstrated using standard addition calibration for acrylamide, to compensated for matrix effects, samples unspiked, and samples spiked at 1000 µg/kg and 2000 µg/kg, with R^2 value of ≥0.9987 and average residual %RSD of ≤4.0 achieved for potato crisps, instant coffee, and ground coffee samples.
- The sensitivity of the method, defined as the limit of identification (LOI), of 1 ppb (equivalent to 5 µg/kg in the analyzed samples) was achieved using the detailed method.
- Excellent repeatability was achieved for the analysis of spiked ground coffee samples, 1000 µg/kg (n=16) achieving a %RSD of 2.9.

- Robustness of acrylamide responses in matrix was assessed by analyzing spiked ground coffee samples, mid and late during the sequence (n=13) with %RSD of 1.3 when comparing average peak areas of mid to late sequence injected spiked samples. In addition, no inlet, column, MS maintenance, or MS tuning were performed over the injection sequence.
- Acylamide quantification using standard addition calibration eliminated the need for an expensive ^{13}C-labeled internal standard. The results illustrated consistency at low to high levels.
- Silylation of food and coffee samples extracted with acetonitrile, quantified in t-SIM mode, maximizes sensitivity and selectivity for the analysis of acrylamide. The enhanced chemical and thermal stability of the silylated product compared to non-derivatized acrylamide analysis makes the analysis using silylation more applicable to GC-MS analysis.
- Chromeleon CDS software simplifies the workflow with user-friendly data acquisition and data processing suitable for high-throughput analysis, with intuitive data reviewing and flexible data reporting.

References

1. Friedman, M.; Chemistry, Biochemistry, and Safety of Acrylamide. A Review. *J. Agric. Food Chem.* **2003,** *51,* 4504.
2. Swedish National Food Administration: WHO to hold urgent expert consultation on acrylamide in food after findings of Swedish National Food Administration. April 2002. [Online] https://www.who.int/mediacentre/news/releases/release32/en/ (accessed 3rd July, 2019).
3. Mottram, D.S.; Wedzicha, B.L.; Dodson, A.T. Acrylamide is formed in the Maillard reaction. *Nature* **2002,** *419,* 448–449.
4. Stradler, R.H; Blank, I; Varga, N; Hau, J.; Guy, P.; Robert, M.C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002,** *419,* 449–450.
5. Rydberg, P.; Eriksson, S.; Tareke, E. Karlsson, P.; Ehrenberg, L.; Tornqvist, M. Investigations of Factors That Influence the Acrylamide Content of Heated Foodstuffs. *J. Agric. Food Chemistry* **2003,** *51,* 7012.
6. Smith, E.A.; Oehme, F.W. Acrylamide and polyacrylamide: a review of production, use, environmental fate and neurotoxicity. *Rev. Environmental Health* **1991,** *9,* 215.
7. Acrylamide legislation: Information on the measures concerning acrylamide levels in food, guidance for food business operators and benchmark levels for monitoring acrylamide levels in different food categories. Food Standards Agency. [Online] https://www.food.gov.uk/business-guidance/acrylamide-legislation (accessed 3rd July, 2019).
8. Reiediker, S.; Stadler, R.H. Analysis of acrylamide in food by isotope-dilution liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *J. Chromatography* **2003,** *1020,* 121–130.
9. Andrawes, F.; Greenhouse, S.; Draney, D. Chemistry of acrylamide bromination for trace analysis by gas chromatography and gas chromatography-mass spectrometry. *J. Chromatography* **1987,** *399,* 269–275.

Find out more at **thermofisher.com**

ThermoFisher SCIENTIFIC

©2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all locations. Please consult your local sales representatives for details. **AN73241-EN 1219S**

Automated micro-SPE clean-up for GC-MS/MS analysis of pesticide residues in cereals

Authors: Sarvendra Pratap Singh, Subodh Kumar Budakoti, and Dasharath Oulkar

Customer Solution Center, Thermo Fisher Scientific, Ghaziabad, India

Keywords: Pesticide residues, cereals, QuEChERS, micro-solid phase extraction (µSPE), GC-MS/MS, advanced electron ionization (AEI), targeted quantitation, TSQ 9000, Chromeleon Chromatography Data System

Goal

To assess the suitability of an automated micro-solid phase extraction (µSPE) clean-up of QuEChERS extracts for the determination of pesticide residues in cereal samples by gas chromatography coupled to triple quadrupole mass spectrometry.

Introduction

Worldwide food demand is set to increase substantially in the next few decades[1], and consequently, food safety concerns are also growing quickly[2,3]. To meet the demand for food, pesticides are used to control pests and ensure high crop yields, but there are some concerns that banned pesticides are still used illegally. If used incorrectly, pesticides can affect consumer's health, hence the importance regulatory bodies place on screening food samples for the presence of pesticide residues.

Given the large number and types of food samples that need to be tested, any delays in the analysis could ultimately impact the timely import/export of food products, which is crucial for perishable products. The extraction of pesticides from food matrices is typically carried out using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) acetonitrile method. Many versions of QuEChERS have been published but one of the most widely used versions is AOAC 2007.01[4]. This method includes a manual dispersive solid-phase extraction clean-up step (dSPE) of the initial non-cleaned extract. This clean-up procedure can be time-consuming and can result in limited removal of matrix co-extractives. By replacing this manual clean-up step with an automated µSPE clean-up approach, laboratories can save time, achieve more effective removal of co-extractives, and thus improve the consistency of the results. A miniaturized SPE method, consisting of sorbents

contained in a miniaturized cartridge, was first introduced by Morris and Schriner[5] for LC-MSMS analysis. Lehotay *et al*[6] and Goon *et al*[7] have since published workflows based on the use of miniaturized SPE clean-up of QuEChERS extracts before GC-MS/MS analysis.

This work was aimed at assessing the suitability of an automated µSPE clean-up approach of QuEChERS extracts of rice and wheat samples for the multi-class determination of a large number of pesticides. The cleaned-up extracts were analyzed using a Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system equipped with the advanced electron ionization source (AEI). The sample introduction and automated clean-up were performed using a Thermo Scientific™ TriPlus™ RSH robotic autosampler configured with a liquid injection tool as well as with the dedicated µSPE tool and cartridges tray for automated clean-up. Data acquisition and processing were carried out using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2.

Experimental

GC-MS/MS analysis

A gas chromatograph (Thermo Scientific™ TRACE™ 1310 GC) was coupled to a TSQ 9000 triple quadrupole GC-MS/MS. The GC conditions are given in Table 1a, the MS parameters are detailed in Table 1b, and the autosampler parameters in Table 1c.

Glassware, reagents, and chemicals

- Anhydrous $MgSO_4$, Thermo Scientific™ (P/N 80020-415-500)
- Acetonitrile, Optima™ LC/MS Grade, Fisher Scientific™ (P/N A955-4)
- Acetic acid glacial (certified ACS), Fisher Scientific™ (P/N A38S-500)
- QuEChERS Salts (2007.01) mylar pouch 6 g magnesium sulfate (anhydrous),1.5 g sodium acetate, Thermo Scientific™ HyperSep™ (P/N 60105-341)
- µSPE GC cartridges 45 mg: 20 mg MgSO4, 12 mg PSA, 12 mg C18 and 1 mg CarbonX (P/N 60101-45GC)
- 2 mL screw vial kit, clear glass vials with caps, Thermo Scientific™ (P/N 60180-599)
- Screw caps with PTFE starburst slitted septum (LEAP PAL Parts + Consumables™, CAP-ND9-ST-SP10SB-100)
- Mixer grinder (Maharaja™ Whiteline, Delhi, India)

Table 1a. GC instrument conditions[8]

TRACE 1310 gas chromatograph parameters	
Column	Thermo Scientific™ TraceGOLD™ TG-5SILMS with 5 m SafeGuard, 30 m × 0.25 mm ID × 0.25 µm (P/N 26096-1425)
Injector	Split/Splitless (SSL)
Liner	SSL Splitless liner, single taper (P/N 453A1925UI)
Injector mode	Splitless with surge
Splitless time	0.3 min
Surge pressure and time	250 kPa for 1 min
Injection volume	1.0 µL
Injector temperature	250 °C
Column flow	1.20 mL/min
Carrier gas and purity	Helium (99.999%)
Purge flow	5.00 mL/min
Split flow	50.00 mL/min; Gas Saver Flow 10 mL/min after 10 min
Total run time	33.4 min
GC oven program	90 °C, 3 min 25 °C/min to 180 °C 5 °C/min to 280 °C 10 °C/min to 300 °C, 5 min

Table 1b. Mass spectrometer parameters

TSQ 9000 mass spectrometer parameters	
Acquisition mode	Timed selected reaction monitoring (t-SRM)
MS transfer line temp.	300 °C
Ion source temp.	320 °C
Ion source	AEI (Advanced Electron Ionization)
Electron energy	70 eV
Ionization	Electron Ionization (EI)
Collision gas and pressure	Argon at 70 psi
Peak width	0.7 Da (both Q1 and Q3)
Tune	AEI SmartTune

Table 1c. Autosampler parameters[6]

TriPlus RSH autosampler parameters	
µSPE sample load volume	300 µL
µSPE sample fill speed	20 µL/s
µSPE sample load speed	2 µL/s
µSPE sample vial penetration depth	30 mm
Mixing cycles	5
Mixing speed	20 µL/s
Mixing volume	250 µL
Pre-wash solvent	Acetonitrile
Pre-washing cycles	2
Post-wash solvent	Acetonitrile:Methanol:Water (1:1:1)
Post-wash cycles	5
Injection mode	Air plug

Sample preparation

Rice and wheat were purchased locally. Both types of samples were ground and homogenized separately to achieve a consistent particle size of approximately 200–500 µm. Rice has a high content of carbohydrate (80%), protein (7%), fat (2%), and fiber (11%); whereas, wheat has fewer carbohydrates (71%), more protein (12.6%), and similar fat (1.5%) and fiber (12%) amounts. Sub-samples (5 g) of the homogenized sample were weighed into a centrifuge tube and then spiked with pesticides at the concentration of 0.01 mg/kg. Water (10 mL) was added to rehydrate the sample to ensure the moisture content is enough for effective liquid-liquid partitioning on the addition of 1% acetic acid in acetonitrile (15 mL). After extraction and centrifugation, the extract supernatant was frozen at -20 °C to freeze out lipid co-extractives. The samples were centrifuged at -5 °C and an aliquot of supernatant cleaned up using µSPE, as outlined in Figure 1.

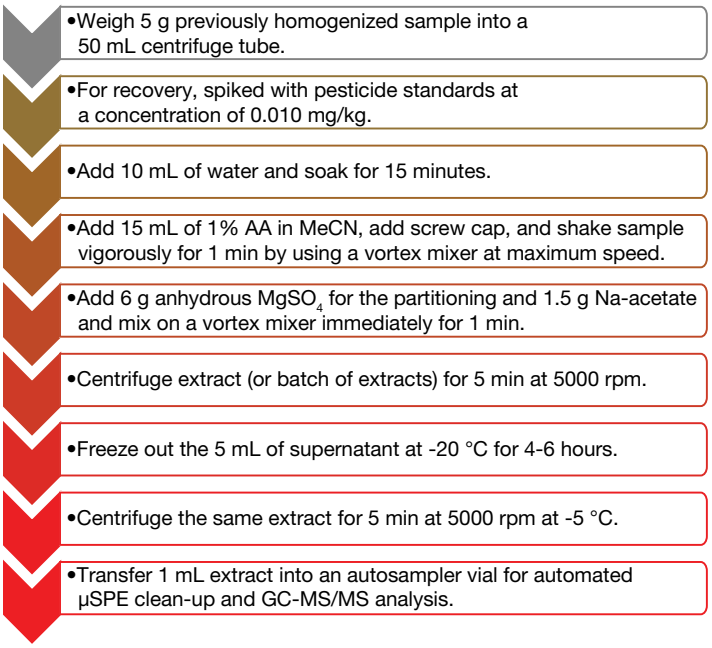


Figure 1. Sample preparation workflow before automated µSPE clean-up

Automated µSPE clean-up

Automated µSPE was performed using a TriPlus RSH autosampler fitted with the Thermo Scientific µSPE GC QuEChERS clean-up kit (P/N 1R77010-1160), including 1 mL volume syringe for solvent/sample dispensing and dedicated aluminum trays for cartridges and clean extract collection. The µSPE cartridge (P/N 60101-45GC) containing a total of 45 mg of an optimized sorbent blend (20 mg $MgSO_4$, 12 mg PSA, 12 mg C18, and 1 mg CarbonX) were used for clean-up. The µSPE clean-up reduces the number of steps and requirement for manual input as shown in Figure 2. The µSPE cartridges and the sample extracts (2 mL in each glass vial) were placed in the allocated positions on the corresponding TriPlus RSH autosampler trays. A volume of 300 µL of the sample extract was aspirated by the syringe first, and then the cartridge was transferred to the dedicated tray, where the cartridge was inserted into 2 mL glass vials with pre-split septa. The sample was loaded onto the cartridge for the clean-up. The sample extract was collected in a collecting vial and mixed with five cycles of mixing (pumping) with a 1 mL syringe. Then using a 10 µL syringe, 1 µL of the cleaned-up extract was injected into the GC-MS/MS. One advantage of µSPE vs manual SPE is that the solvent evaporation step is not needed. Further details are given in Table 2 and Figure 3. Preconditioning, loading, transfer, and elution of µSPE cartridges are performed automatically by the robotic autosampler during the analysis of the previous sample, with no increase in analysis cycle time (Figure 2).

Preparation of calibration standards

- Solvent standard calibration: The solvent standard calibration was prepared at 0.0025, 0.005, 0.01, 0.025, 0.05, and 0.1 mg/L.
- Matrix-matched calibration standards (µSPE): Aliquots of blank matrix extract were spiked after the initial extraction and before clean-up. The matrix-matched calibration was prepared at 0.0025, 0.005, 0.01, 0.025, 0.05, and 0.1 mg/kg as per the procedure given in the Thermo Scientific Application Note[9]. The non-cleaned matrix-matched calibration solutions were placed in vials and then loaded onto the autosampler tray for clean-up and GC-MS analysis.
- Sample extracts, as well as a matrix-matched standards, blank, and recovery spiked extracts were analyzed by GC-MS/MS.

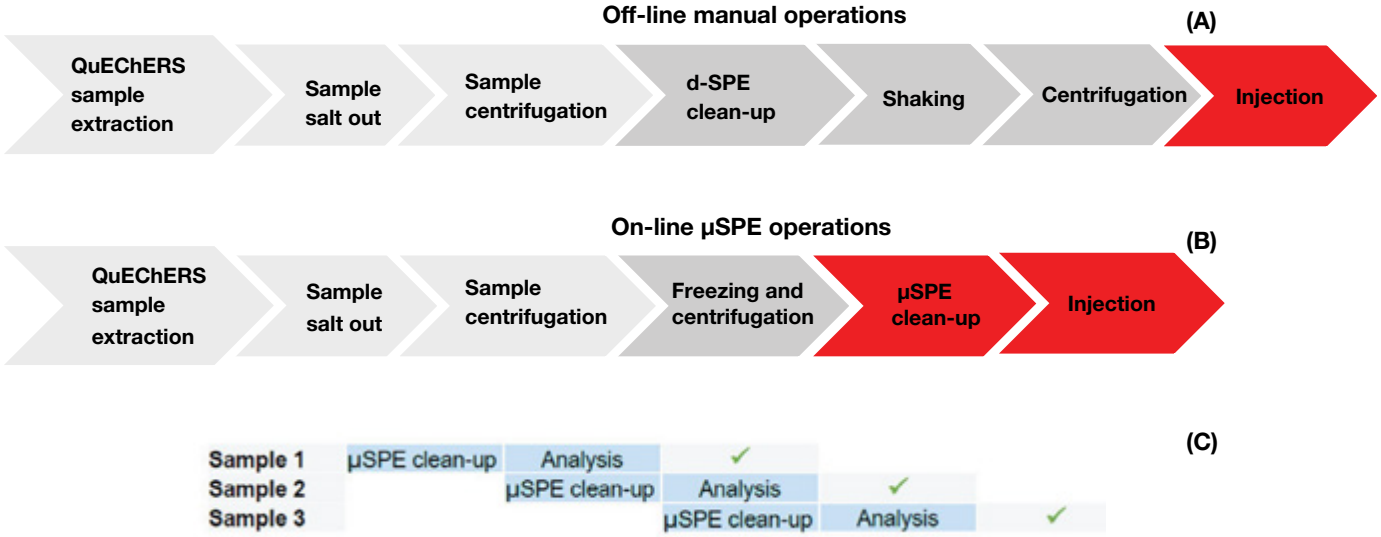


Figure 2. Sample extraction procedure as per the AOAC 2007.01; A) Manual clean-up with dSPE; B) Automated sample clean-up with µSPE; C) Script for the sample overlaid analysis and µSPE clean-up

Table 2. TriPlus RSH autosampler cycle used for automated µSPE clean-up

No.	Step description	Time (s)
1	Parking of 10 µL syringe to the home position	40
2	Pick up of 1 mL syringe and move to the home position	20
3	Fast wash the 1 mL syringe with MeCN (2 pumps of 1 mL each)	60
4	Mixing the extract with 1 mL syringe (2 pumps of 0.25 mL each)	30
5	Load 300 µL extract from vial in Tray 1 into 1 mL syringe	40
6	Place mini-cartridge above collection vial (with glass insert) in Tray 2	10
7	Elute extract through mini-cartridge at 2 µL s^{-1}	150
8	Discard mini-cartridge into a waste receptacle	10
9	Mix the eluate with 1 mL syringe (5 pumps of 250 µL each)	100
10	Wash the 1 mL syringe with 1/1/1 MeCN/MeOH/water (2 pumps of 0.5 mL each)	30
11	Wash the 1 mL syringe with MeCN (4 pumps of 0.5 mL each)	45
12	Switch to 10 µL syringe and wash with MeCN (2 pumps of 5 µL each)	80
13	Wash the 10 µL syringe with 1/1/1 MeCN/MeOH/water (5 pumps of 5 µL each)	40
14	Wash the 10 µL syringe with extract (3 pumps of 3 µL each)	30
15	Mixing the extract with 10 µL syringe (2 pumps of 3 µL each)	15
16	Injection of 1 µL of cleaned extract to GC-MS/MS	10

Data acquisition and processing

The data acquisition and processing were carried out using the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, which allows instrument control, method development, quantitative analysis, and customizable reporting all within one package. The target list of analytes with their selected reaction monitoring (SRM) parameters is given in Appendix 2. The data were acquired in t-SRM mode, which includes a minimum of two or more transitions per analyte. For data processing, the ion ratio (± 30%), retention time (± 0.1 min), linearity (R^2 > 0.995 with residuals < ± 20%), recovery (70–120%) and precision (± 20%) were set as user-defined criteria as per the SANTE guidelines[10].

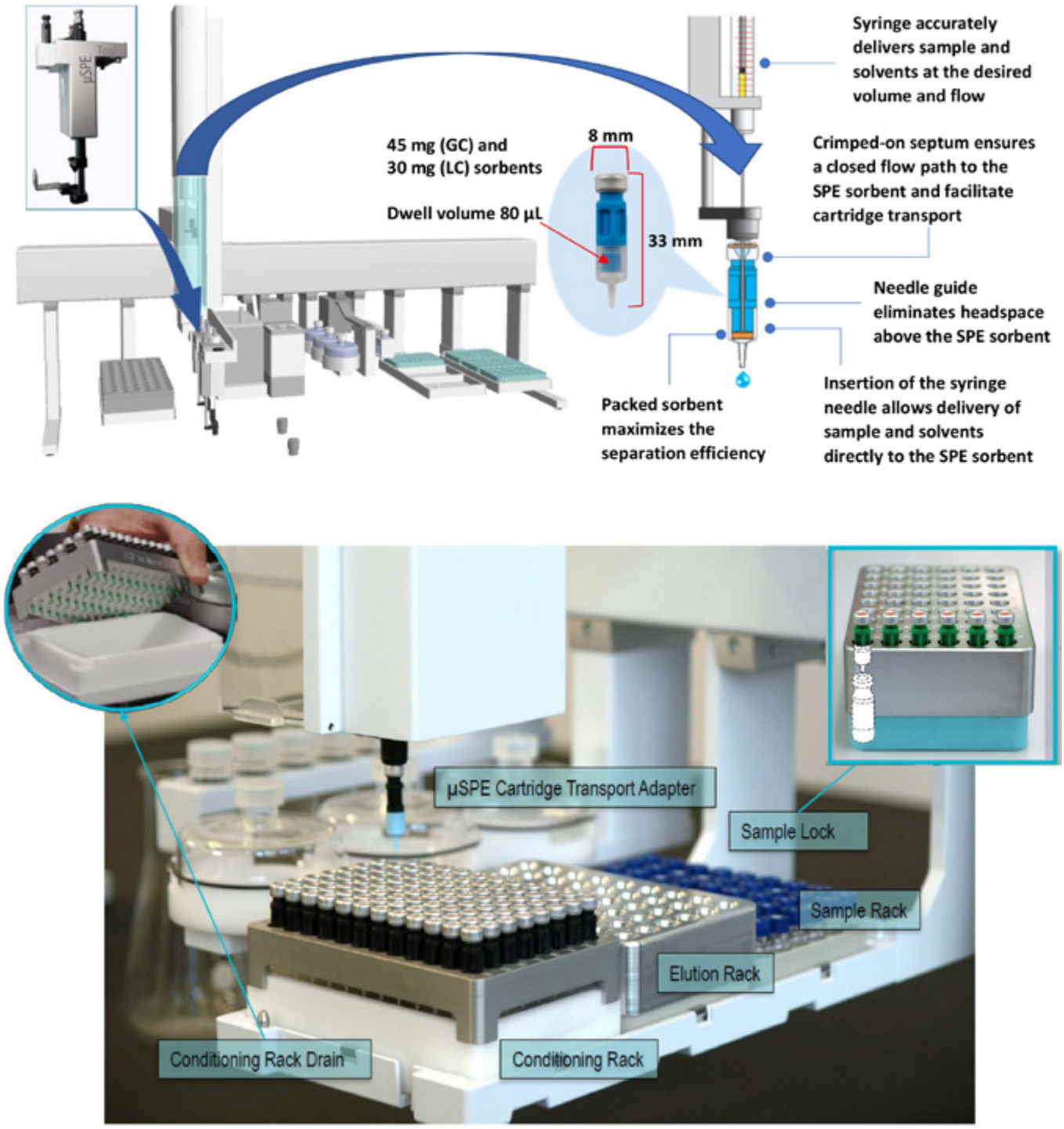


Figure 3. TriPlus RSH autosampler, µSPE tool operation with cartridge, and µSPE clean-up tray module

Results and discussion

Since the matrix-matched standards were subjected to the µSPE clean-up, the results are effectively corrected for any analyte losses on the cartridge but not losses during extraction. Nevertheless, this calibration approach improves accuracy and precision and is permitted by the EU SANTE guidelines. For identification, two SRM transitions per analyte were considered for all the target analytes at 0.01 mg/kg in rice and wheat with the retention time stability (± 0.1 min) and ion ratios (± 30%). The ion ratio (%) for ethalfluralin is represented in Figure 4.

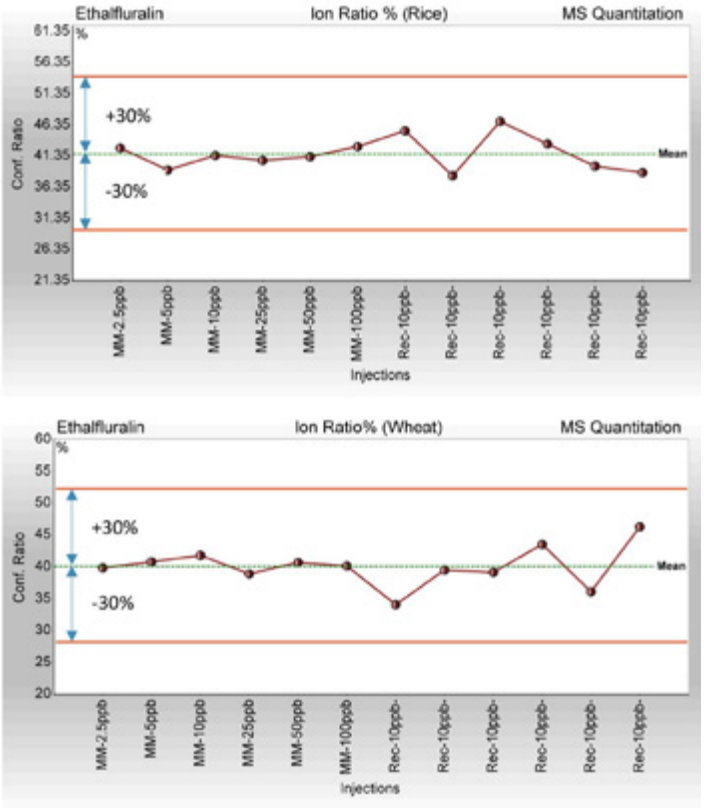


Figure 4. Ion ratio % for ethalfluralin in rice (top) and wheat (bottom) matrix-matched standards and recovery samples (n=6) pre-spiked at 0.01 mg/kg and subjected to µSPE clean-up. The EU SANTE ion ratio tolerance (%) is represented by red lines.

For confident quantification, maintaining peak symmetry with enough data points per peak is critical to achieving satisfactory repeatability. For example, the matrix-matched calibration standard of propachlor is shown in Figure 5 at 0.025 mg/kg. Also, propachlor provided excellent recovery and repeatability (RSD ≤ 4%, n=6) at 0.01 mg/kg (Appendix 1).

The matrix-matched calibration standards (rice and wheat) were linear over the concentration range of 0.0025 to 0.1 mg/kg. The coefficient of determination R^2 were mostly >0.995 with residual values (as % average calibration factors) of <20% for all target analytes. An example of linearity is shown for propachlor in rice and wheat (Figure 6).

The recoveries at 0.01 mg/kg in rice were in the range of 78 to 119% (n=6) with less than 20% RSD for all target analytes (Figure 7), except chlorothalonil and tolylfluanid, which gave low responses. These pesticides are known to be susceptible to instability during analysis. At 0.01 mg/kg, the recoveries (n=6) of pesticides in wheat were between 75 and 104%, with associated %RSD < 13% for 203 of 209 pesticides as shown in Figure 8.

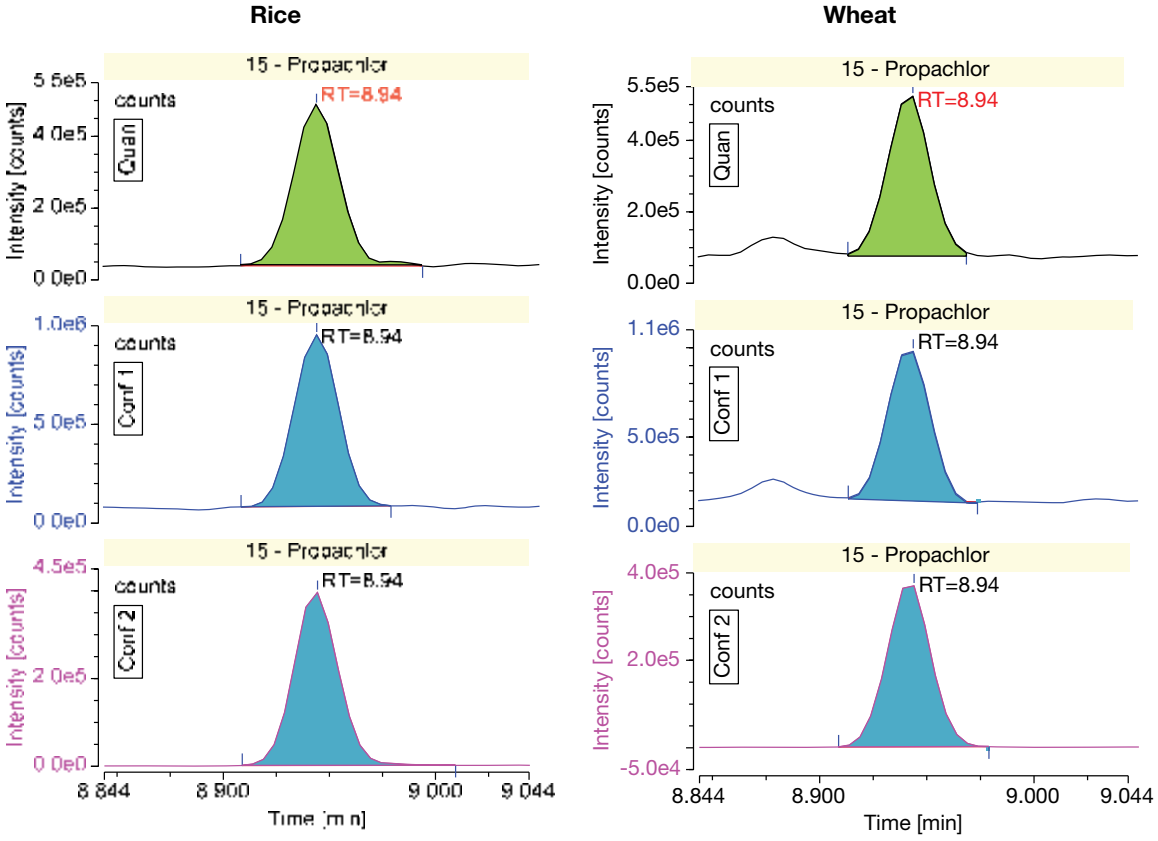


Figure 5. Response observed for propachlor (quantitative and confirmatory ions) in rice (left) and wheat (right) matrix-matched standards prepared with the µSPE clean-up at a concentration of 0.025 mg/kg

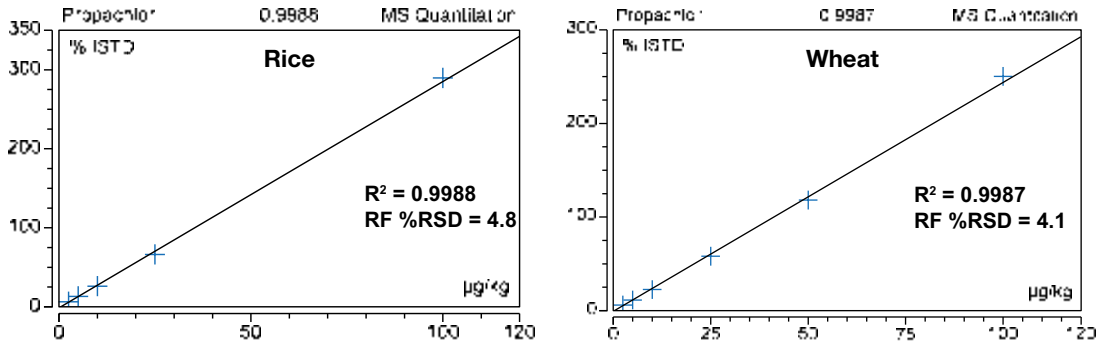


Figure 6. Calibration curve for propachlor analysis in rice and wheat matrix-matched standards with the µSPE clean-up over a concentration range of 0.0025 to 0.1 mg/kg

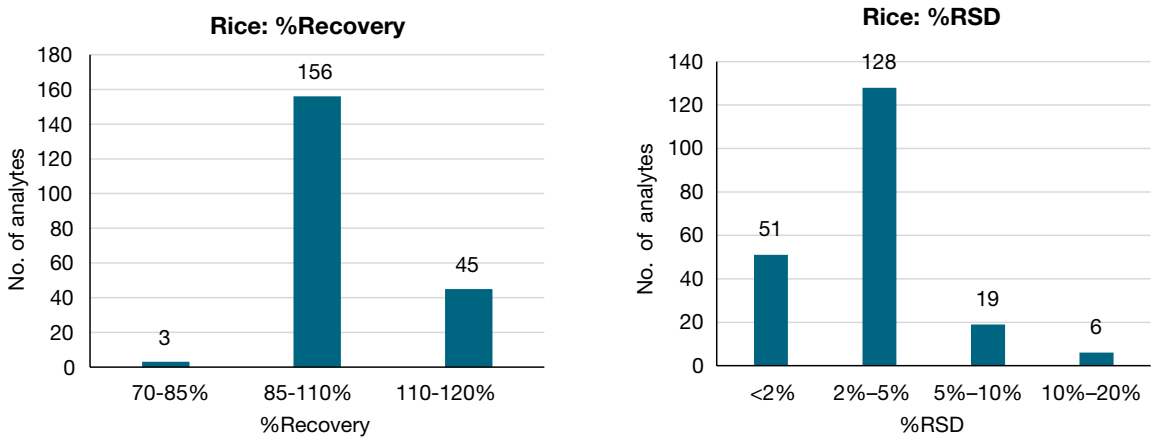


Figure 7. Recovery (%) and RSD (%) for rice matrix at 0.01 mg/kg followed by the µSPE clean-up

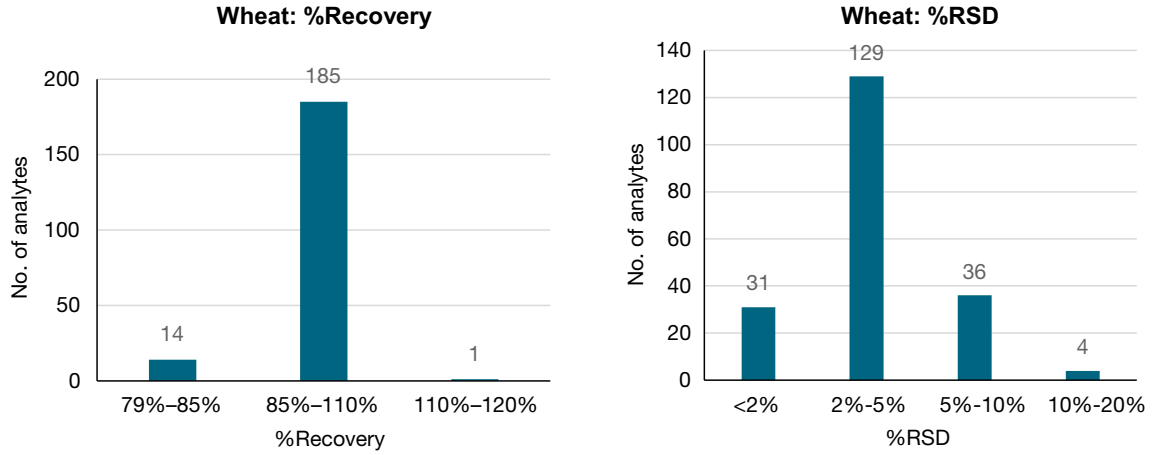


Figure 8. Recovery (%) and RSD (%) for wheat matrix pre-spiked at 0.01 mg/kg followed by the clean-up with µSPE

The vast majority of %RSDs at 0.01 mg/kg in rice and wheat were <5%. Information for individual pesticide-matrix combinations is given in Appendix 1. Overall, excellent recovery and precision values were obtained, which confirmed that the µSPE can be used as a replacement for the labor-intensive, more time-consuming dSPE manual clean-up method.

Conclusion

The experiments demonstrate that automated µSPE compared to dSPE with weighing the sorbents can significantly reduce the sample preparation time by 40 to 50% and increase sample throughput in routine laboratories by more than 1.5 times considering a batch of 10 samples. The miniaturized SPE cartridge features an optimized sorbent amount and composition, which acts with the optimum and controlled elution rate to provide high selectivity and high clean-up efficiency.

- Replacing the manual d-SPE procedures with µSPE delivers optimum recovery and precision while reducing the risk of human errors.
- The automated on-line clean-up workflow allows labor and time savings during sample preparation and increases unattended sample throughput in the laboratory.
- One cartridge type removes pigments, lipids, etc., so it is suitable for a large number of different sample types. Since it is not necessary to match the sample type to a specific blend of sorbents, the laboratory workflow is simplified.
- Excellent linearity was obtained using matrix-matched calibration standards over a concentration range of 0.0025 to 0.1 mg/kg with R^2 values mostly >0.995 and %RSD of residuals <5%.
- The performance has been checked with six replicates of pre-spiked samples at 0.01 mg/kg. The results (%recovery and %RSD) were in the range of 70 to 120% and <20%, respectively, and thus in compliance with the EU SANTE guideline criteria.

References

1. World Trade Organization, International trade statistics, 2014.
2. European Food Safety Authority, The 2013 European Union report on pesticide residues in food, *EFSA Journal,* **2015,** *13,* 1–169.
3. USDA Agricultural Marketing Service, Pesticide data program annual summary, calendar year 2014. https://www.ams.usda.gov/sites/default/files/media/2014%20PDP%20Annual%20Summary.pdf
4. AOAC Official Method 2007.01 Pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate. 2007 AOAC international.
5. Morris, B.D.; Schriner, R.B. Development of an automated column solid-phase extraction cleanup of QuEChERS extracts, using a zirconia-based sorbent, for pesticide residue analyses by LC-MS/MS. *J. Agric. Food Chem.* **2015,** *63,* 5107–5119.
6. Lehotay, S.J.; Han, L.; Sapozhnikova, Y. Automated mini-column solid-phase extraction cleanup for high-throughput analysis of chemical contaminants in foods by low-pressure gas chromatography—tandem mass spectrometry. *Chromatographia,* **2016,** *79,* 1113–1130.
7. Goon, *et al.:* Journal of AOAC International Vol. 103, No. x, **2019.**
8. Budakoti, S.K.; Singh, S.P.; Oulkar, D. Thermo Scientific Application Note 65609: A sensitive and robust analytical solution for pesticide residues analysis in apple using GC-(AEI)-MS/MS, **2019.** https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-65609-gc-aei-ms-pesticides-apple-an65609-en.pdf
9. Singh, S.P.; Budakoti, S.K.; Oulkar, D. Thermo Scientific Application Note 73039: Large-scale screening and quantitation of pesticide residues in milk using GC-(EI)-MS/MS, **2019.** https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-73039-gc-ei-ms-pesticides-milk-an73039-en.pdf
10. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed (2019) SANTE/12682/2019, 01/01/2019.

Appendix 1. List of pesticides analyzed in rice and wheat results (retention time, linearity, recovery, and precision at 0.01 mg/kg) with the µSPE clean-up

Sr. No.	Compound Name	RT	Rice (0.01 mg/kg)			Wheat (0.01 mg/kg)		
			R^2	%Rec.	%RSD	R^2	%Rec.	%RSD
1	2,3,5,6-Tetrachloroaniline	9.19	0.9983	97	3.1	0.9980	92	2.9
2	2,4'-Methoxychlor	18.91	0.9960	108	1.3	0.9989	94	1.2
3	3,4-Dichloroaniline	7.55	0.9966	97	2.1	0.9988	79	2.9
4	4,4'-Methoxychlor olefin	18.32	0.9983	94	2.5	0.9987	94	1.4
5	Acetochlor	11.86	0.9940	111	6.7	0.9984	87	5.9
6	Acrinathrin	22.59	0.9977	105	1.9	0.9967	104	4.6
7	Alachlor	12.09	0.9955	111	2.7	0.9987	93	2.7
8	Aldrin	13.32	0.9993	87	2.2	0.9979	91	4.9
9	Allidochlor	6.47	0.9968	106	2.0	0.9990	96	2.3
10	Anthraquinone	13.32	0.9980	101	5.2	0.9941	89	3.2
11	Atrazine	10.31	0.9966	108	6.3	0.9990	91	4.6
12	Azinphos-ethyl	22.67	0.9988	113	2.8	0.9975	93	2.3
13	Azinphos-methyl	21.53	0.9956	115	2.3	0.9947	92	3.7
14	Benfluralin	9.21	0.9949	113	2.7	0.9963	91	2.8
15	BHC, Alpha	9.91	0.9973	101	1.0	0.9979	92	1.7
16	BHC, Beta	10.37	0.9971	99	2.6	0.9983	93	5.6
17	BHC, delta	11.16	0.9979	100	1.2	0.9981	93	3.2
18	BHC, gamma	10.60	0.9979	100	2.3	0.9963	90	3.4
19	Bifenthrin	20.36	0.9992	100	1.2	0.9984	96	2.2
20	Bromfenvinphos	15.58	0.9972	111	2.5	0.9988	99	4.6
21	Bromfenvinphos-methyl	14.38	0.9955	104	3.8	0.9987	100	5.5
22	Bromophos-ethyl	14.98	0.9978	107	3.0	0.9984	92	2.0
23	Bromophos-methyl (Bromophos)	13.76	0.9965	104	1.5	0.9985	93	4.0
24	Bromopropylate	20.38	0.9965	106	1.8	0.9985	93	1.7
25	Bupirimate	16.35	0.9971	103	2.1	NA	NA	NA
26	Carbophenothion	18.31	0.9928	108	2.2	0.9990	93	3.9
27	Carfentrazon-ethyl	18.23	0.9957	111	1.8	0.9989	91	2.7
28	Chlorbenside	15.02	0.9989	97	2.0	0.9981	83	3.0
29	Chlordane alpha-Cis	15.48	0.9973	110	5.6	0.9982	91	4.3
30	Chlordane Gamma-trans	15.07	0.9944	101	1.7	0.9978	94	2.8
31	Chlorfenapyr	16.64	0.9977	99	4.4	0.9983	88	10.3
32	Chlorfenson	15.75	0.9978	93	2.2	0.9981	94	2.8
33	Chlorfenvinphos	14.36	0.9928	109	2.6	0.9981	96	3.5
34	Chlorobenzilate	17.17	0.9980	101	3.8	0.9983	89	3.1
35	Chloroneb	8.07	0.9962	92	3.1	0.9977	95	2.4
36	Chlorpropham	9.35	0.9986	102	4.1	0.9986	84	4.1
37	Chlorpyrifos-ethyl	13.13	0.9972	104	2.0	0.9986	99	2.9
38	Chlorpyrifos-methyl	11.90	0.9920	108	3.0	0.9972	91	4.2
39	Chlorthal-dimethyl (Dacthal)	13.30	0.9972	95	2.7	0.9990	96	2.6
40	Chlorothalonil	10.95	NA	NA	NA	0.9952	100	6.0
41	Chlorthiophos	17.54	0.9985	100	1.0	0.9982	90	1.4
42	Chlozolinate	14.27	0.9954	99	3.6	0.9989	96	4.1

<LOQ= Below limit of quantification (0.01 mg/kg)

Appendix 1 (continued). List of pesticides analyzed in rice and wheat results (retention time, linearity, recovery, and precision at 0.01 mg/kg) with the µSPE clean-up

			Rice (0.01 mg/kg)			Wheat (0.01 mg/kg)		
Sr. No.	Compound Name	RT	R^2	%Rec.	%RSD	R^2	%Rec.	%RSD
43	Clomazone	10.42	0.9980	104	2.0	0.9988	92	2.1
44	Coumaphos	23.84	0.9965	107	1.4	0.9982	94	3.1
45	Cycloate	9.22	0.9973	88	4.9	0.9940	103	6.2
46	Cyfluthrin peak 1	24.71	0.9984	109	3.1	0.9981	95	3.0
47	Cyfluthrin peak 2	24.91	0.9979	113	2.3	0.9982	98	2.5
48	Cyfluthrin peak 3	25.02	0.9988	108	2.9	0.9974	97	4.1
49	Cyfluthrin peak 4	25.11	0.9985	107	3.6	0.9978	94	2.0
50	Cyhalothrin I (lambda)	22.20	0.9969	108	2.4	0.9988	95	5.8
51	Cypermethrin peak 1	25.31	0.9984	104	4.4	0.9980	94	3.1
52	Cypermethrin peak 2	25.52	0.9978	109	3.9	0.9984	97	4.2
53	Cypermethrin peak 3	25.62	0.9985	109	2.9	0.9979	95	4.0
54	Cypermethrin peak 4	25.71	0.9978	111	4.2	0.9969	96	4.8
55	Cyprodinil	14.11	0.9988	102	2.3	0.9959	92	2.0
56	DDD p,p	17.45	0.9983	103	2.0	0.9991	96	3.0
57	DDD, o, p	16.34	0.9983	97	2.3	0.9988	95	2.5
58	DDE o,p	15.14	0.9982	93	1.9	0.9984	93	2.2
59	DDE p, p	16.13	0.9978	88	1.7	0.9983	92	2.6
60	DDT o,p	17.55	0.9972	97	2.4	0.9980	91	2.4
61	DDT p,p	18.70	0.9971	98	3.2	0.9982	90	2.7
62	Deltamethrin	28.25	0.9962	112	3.6	0.9968	99	4.8
63	Diallate-cis	9.95	0.9981	103	4.2	0.9989	93	4.6
64	Diallate-trans	9.74	0.9974	104	2.3	0.9984	93	4.0
65	Diazinon	10.71	0.9970	110	3.8	0.9981	94	3.9
66	Dichlobenil	6.90	0.9981	91	1.6	0.9989	94	1.4
67	Dichlorobenzophenone, 4, 4	13.56	0.9981	96	2.0	0.9983	92	0.9
68	Dicloran (Bortran)	10.15	0.9989	112	6.5	0.9958	94	1.8
69	Dieldrin	16.31	0.9965	94	4.8	0.9968	100	5.6
70	Dimethachlor	11.75	0.9956	116	1.5	0.9988	93	2.9
71	Diphenamid	13.75	0.9971	103	2.0	0.9992	90	2.9
72	Diphenylamine	9.13	0.9982	100	8.1	0.9982	78	5.6
73	Disulfoton	11.01	0.9942	111	1.5	0.9981	78	3.8
74	Edifenphos	18.38	0.9990	104	3.7	0.9961	104	3.9
75	Endosulfan ether	11.67	0.9978	97	2.0	0.9987	93	2.3
76	Endosulfan peak 1	15.48	0.9926	113	8.2	0.9977	93	8.5
77	Endosulfan peak 2	17.27	0.9966	93	5.0	0.9992	91	3.3
78	Endosulfan sulfate	18.51	0.9962	96	2.5	0.9985	104	4.3
79	Endrin	16.95	0.9971	106	2.4	0.9985	93	6.9
80	Endrin Aldehyde	17.75	NA	NA	NA	0.9935	100	6.7
81	Endrin-Ketone	20.05	0.9981	92	4.4	0.9979	92	6.4
82	EPN	20.31	0.9962	106	1.8	0.9972	96	2.6
83	Esfenvalerate	27.35	0.9992	104	5.5	0.9979	92	3.1
84	Ethalfluralin	9.23	0.9983	89	11.4	0.9986	92	7.5
85	Ethion	17.45	0.9943	107	1.9	0.9985	92	2.1

<LOQ= Below limit of quantification (0.01 mg/kg)

Appendix 1 (continued). List of pesticides analyzed in rice and wheat results (retention time, linearity, recovery, and precision at 0.01 mg/kg) with the µSPE clean-up

Sr. No.	Compound Name	RT	Rice (0.01 mg/kg)			Wheat (0.01 mg/kg)		
			R^2	%Rec.	%RSD	R^2	%Rec.	%RSD
86	Etofenprox	25.91	0.9979	104	1.5	0.9978	91	4.1
87	Etridiazole (Terrazole)	7.66	0.9967	108	2.7	0.9986	91	1.3
88	Fenamiphos	15.69	0.9949	110	5.1	NA	NA	NA
89	Fenarimol	22.44	0.9979	111	2.1	0.9985	90	2.4
90	Fenchlorfos	12.35	0.9976	109	2.0	0.9983	91	2.8
91	Fenitrothion	12.68	0.9932	107	2.6	0.9973	90	2.1
92	Fenpropathrin	20.67	0.9981	104	2.7	0.9986	99	3.3
93	Fenson	13.71	0.9975	99	2.0	0.9983	93	1.9
94	Fenthion	13.23	0.9964	100	2.2	0.9982	70	1.3
95	Fenvalerate	26.98	0.9947	114	1.4	0.9972	98	2.7
96	Fipronil	14.17	0.9968	115	4.8	0.9971	93	2.7
97	Fluazifop-P-butyl	16.90	0.9983	102	2.5	0.9990	96	2.7
98	Fluchloralin	10.75	0.9909	113	4.4	0.9951	94	3.9
99	Flucythrinate peak 1	25.65	0.9969	108	1.4	0.9983	99	1.7
100	Flucythrinate peak 2	26.03	0.9988	113	4.2	0.9980	99	2.0
101	Fludioxonil	15.80	0.9983	93	3.2	0.9956	95	7.2
102	Fluquinconazole	23.86	0.9976	105	3.5	0.9986	95	2.2
103	Fluridone	26.27	0.9931	113	2.1	0.9963	92	2.1
104	Flusilazole	16.32	0.9973	109	2.6	1.0000	92	13.1
105	Flutolanil	15.71	0.9975	95	3.4	0.9995	94	9.1
106	Flutriafol	15.55	0.9981	105	2.8	0.9975	98	3.2
107	Fluvalinate peak 1	27.25	0.9977	114	2.1	0.9972	102	3.9
108	Fluvalinate peak 2	27.38	0.9934	117	3.1	0.9954	103	3.3
109	Fonofos	10.75	0.9957	115	4.5	0.9970	76	8.8
110	Heptachlor	12.35	0.9975	108	2.2	0.9984	93	3.0
111	Heptachlor epoxide	14.38	0.9969	103	3.7	0.9989	94	5.5
112	Hexachlorobenzene	10.03	0.9978	80	17.8	0.9968	99	10.3
113	Hexazinone	18.81	0.9970	107	2.4	0.9984	89	1.4
114	Iodofenfos	15.76	0.9931	104	2.3	0.9975	93	3.8
115	Iprodione	20.02	0.9929	104	7.0	NA	NA	NA
116	Isazophos	11.00	0.9932	118	4.4	0.9970	90	4.9
117	Isodrin	14.12	0.9984	86	5.2	0.9975	90	3.2
118	Isopropalin	13.87	0.9929	109	2.0	0.9957	92	3.7
119	Lenacil	18.50	0.9973	100	4.4	0.9984	90	1.5
120	Leptophos	21.50	0.9991	99	2.4	0.9965	92	2.3
121	Linuron	12.88	NA	NA	NA	0.9985	108	10.8
122	Malathion	12.90	0.9954	109	1.8	0.9980	84	4.5
123	Metalaxyl	12.24	0.9958	113	3.2	0.9979	94	4.3
124	Metazachlor	14.12	0.9976	115	3.4	0.9976	94	3.3
125	Methacrifos	7.95	0.9969	106	1.4	0.9986	93	2.9
126	Methoxychlor	20.56	0.9959	107	3.0	0.9984	93	1.8
127	Metolachlor	13.09	0.9962	102	2.3	0.9987	93	2.1
128	Mevinphos	7.42	0.9975	96	4.0	0.9993	74	2.2

<LOQ= Below limit of quantification (0.01 mg/kg)

Appendix 1 (continued). List of pesticides analyzed in rice and wheat results (retention time, linearity, recovery, and precision at 0.01 mg/kg) with the µSPE clean-up

Sr. No.	Compound Name	RT	Rice (0.01 mg/kg)			Wheat (0.01 mg/kg)		
			R^2	%Rec.	%RSD	R^2	%Rec.	%RSD
129	MGK-264 A	13.80	0.9983	96	3.8	0.9983	92	7.2
130	MGK-264 B	14.16	0.9975	99	3.9	0.9989	97	6.7
131	Mirex	22.20	0.9996	86	7.0	0.9957	81	5.7
132	Myclobutanil	16.23	0.9973	109	2.5	NA	NA	NA
133	N-(2,4-Dimethylphenyl)formamide	7.89	0.9963	112	7.1	0.9983	78	5.3
134	Nitralin	19.45	0.9944	107	4.0	0.9925	100	8.4
135	Nitrofen	16.87	0.9954	103	3.9	0.9978	92	1.9
136	Nonachlor-cis	17.47	0.9976	99	3.1	0.9980	92	2.5
137	Nonachlor-trans	15.58	0.9949	105	4.4	0.9963	89	4.6
138	Norflurazon	18.35	0.9971	107	1.5	0.9981	91	0.9
139	Ortho-phenylphenol	8.23	0.9994	96	1.6	0.9984	75	3.5
140	Oxadiazon	16.14	0.9972	93	2.6	0.9986	96	3.1
141	Oxyfluorfen	16.30	0.9929	110	4.3	0.9966	94	4.5
142	Paclobutrazol	15.21	0.9951	113	4.6	0.9979	89	3.5
143	Parathion (ethyl)	13.33	0.9959	108	3.4	0.9973	91	3.3
144	Parathion-methyl	12.04	0.9920	119	1.4	0.9982	90	3.5
145	Pebulate	7.71	0.9964	115	7.9	0.9989	103	2.5
146	Penconazole	14.25	0.9979	108	2.9	0.9986	92	1.3
147	Pendimethalin	14.08	0.9901	113	3.0	0.9951	91	5.8
148	Pentachloroaniline	11.64	0.9986	109	7.7	0.9974	101	4.6
149	Pentachloroanisole	10.12	0.9984	95	1.4	0.9987	90	3.6
150	Pentachlorobenzene	8.27	0.9977	88	11.0	0.9985	103	7.0
151	Pentachlorobenzonitrile	10.60	0.9958	104	3.1	0.9973	92	3.0
152	Pentachlorothioanisole	12.91	0.9951	87	4.2	0.9965	88	5.4
153	Permethrin peak 1	23.66	0.9983	78	7.4	0.9988	100	3.3
154	Permethrin peak 2	23.91	0.9980	110	5.0	0.9982	90	2.2
155	Perthane (Ethylan)	16.92	0.9975	103	2.1	0.9992	94	1.8
156	Phenothrin	21.38	0.9990	101	12.9	0.9985	105	11.3
157	Phorate	9.75	0.9975	117	2.9	0.9979	85	3.4
158	Phosalone	21.45	0.9905	108	1.2	0.9981	95	3.2
159	Phosmet	20.17	0.9958	101	2.3	0.9987	90	2.3
160	Phthalimide	7.80	0.9972	106	13.0	0.9989	87	5.6
161	Piperonyl butoxide	19.45	0.9945	107	2.8	0.9982	93	3.1
162	Pirimiphos-ethyl	13.71	0.9978	108	2.8	0.9984	94	1.9
163	Pirimiphos-methyl	12.60	0.9979	109	1.2	0.9991	93	5.6
164	Pretilachlor	15.93	0.9976	97	2.0	0.9982	93	4.2
165	Prochloraz	24.00	0.9936	112	4.2	0.9967	91	3.2
166	Procymidone	14.65	0.9968	104	3.6	0.9985	91	1.8
167	Prodiamine	12.64	0.9921	111	4.2	0.9972	92	1.7
168	Profenofos	15.99	0.9947	102	4.1	0.9973	103	6.1
169	Profluralin	10.49	0.9917	107	2.7	0.9987	89	4.4

<LOQ= Below limit of quantification (0.01 mg/kg)

Appendix 1 (continued). List of pesticides analyzed in rice and wheat results (retention time, linearity, recovery, and precision at 0.01 mg/kg) with the µSPE clean-up

Sr. No.	Compound Name	RT	Rice (0.01 mg/kg)			Wheat (0.01 mg/kg)		
			R^2	%Rec.	%RSD	R^2	%Rec.	%RSD
170	Propachlor	8.94	0.9987	104	1.9	0.9990	96	3.2
171	Propanil	11.79	0.9945	114	7.4	0.9977	90	5.4
172	Propargite	19.26	0.9978	102	4.3	0.9991	92	9.2
173	Propisochlor	12.19	0.9958	112	2.5	0.9993	94	2.5
174	Propyzamide	10.70	0.9973	109	1.5	0.9987	91	2.6
175	Prothiofos	15.86	0.9982	93	3.4	0.9947	90	9.7
176	Pyraclofos	22.99	0.9946	97	4.1	0.9969	99	3.1
177	Pyrazophos	22.50	0.9931	117	2.0	0.9979	93	2.9
178	Pyridaben	23.91	0.9965	108	1.8	0.9979	96	2.2
179	Pyridaphenthion	19.97	0.9927	110	1.7	0.9987	93	2.9
180	Pyrimethanil	10.88	0.9985	110	4.0	0.9975	92	2.5
181	Pyriproxyfen	21.80	0.9981	105	1.7	0.9981	90	3.0
182	Quinalphos	14.54	0.9931	104	2.5	0.9969	91	7.5
183	Quintozene	10.52	0.9965	101	3.9	0.9983	89	3.1
184	Resmethrin peak 2	19.54	0.9982	98	3.2	0.9988	101	3.9
185	Sulfotep	9.48	0.9948	112	3.1	0.9974	93	1.4
186	Sulprofos	17.97	0.9982	101	2.5	0.9987	76	2.1
187	Tebuconazole	19.13	0.9933	109	3.1	0.9981	89	2.4
188	Tebufenpyrad	20.85	0.9992	101	1.2	0.9987	96	1.6
189	Tecnazene	8.85	0.9994	98	3.2	0.9990	89	4.5
190	Tefluthrin	11.01	0.9960	104	2.1	0.9985	92	3.2
191	Terbacil	11.00	0.9995	106	4.9	0.9974	87	5.0
192	Terbufos	10.62	0.9907	115	1.2	0.9975	88	1.7
193	Terbuthylazine	10.61	0.9984	103	2.9	0.9991	92	4.4
194	Tetrachlorvinphos	15.16	0.9910	101	3.3	0.9984	105	6.4
195	Tetradifon	21.24	0.9984	93	3.2	0.9985	93	1.2
196	Tetrahydrophthalimide (THPI)	7.97	0.9991	105	5.3	0.9986	97	2.9
197	Tetramethrin peak 1	20.13	0.9965	85	10.7	0.9966	115	9.1
198	Tetramethrin peak 2	20.42	0.9968	110	3.8	0.9986	96	2.6
199	Tolclofos-methyl	12.08	0.9968	110	2.9	0.9983	91	2.0
200	Tolyfluanid	14.34	NA	NA	NA	0.9991	69	3.9
201	Transfluthrin	12.10	0.9965	108	3.7	0.9979	96	3.5
202	Triadimefon	13.42	0.9967	107	4.0	0.9987	92	5.7
203	Triadimenol	14.65	0.9980	106	2.6	0.9978	98	6.8
204	Triallate	11.20	0.9966	108	2.3	0.9985	94	3.0
205	Triazophos	17.91	0.9915	117	1.7	0.9988	94	5.2
206	Triflumizole	14.71	0.9976	116	7.4	0.9988	91	9.4
207	Trifluralin	9.37	0.9987	116	1.5	0.9975	91	3.0
208	Vinclozolin	11.98	0.9952	103	2.8	0.9971	97	3.3
209	Tricyclazole	15.97	0.9964	116	8.5	NA	NA	NA
210	Triphenylphosphate (IS)	19.28	NA	NA	NA	NA	NA	NA

<LOQ= Below limit of quantification (0.01 mg/kg)

Appendix 2. Target list of analytes with their SRM parameters

Compound Name	RT (min)	Q1	Q3	CE
2,3,5,6-Tetrachloroaniline	9.19	230.8	157.9	18
2,3,5,6-Tetrachloroaniline	9.19	230.8	159.8	18
2,3,5,6-Tetrachloroaniline	9.19	230.8	194.8	10
2,4'-Methoxychlor	18.91	227.1	121.1	10
2,4'-Methoxychlor	18.91	228.1	122.1	16
2,4'-Methoxychlor	18.91	152.0	126.1	24
2,4'-Methoxychlor	18.91	152.0	151.1	16
3,4-Dichloroaniline	7.56	160.9	99.0	20
3,4-Dichloroaniline	7.56	160.9	90.0	18
3,4-Dichloroaniline	7.56	160.9	126.0	10
4,4'-Methoxychlor olefin	18.32	238.1	152.1	34
4,4'-Methoxychlor olefin	18.32	238.1	223.1	10
4,4'-Methoxychlor olefin	18.32	308.0	238.2	12
Acetochlor	11.86	146.1	130.0	24
Acetochlor	11.86	146.1	131.0	12
Acetochlor	11.86	174.1	146.1	10
Acetochlor	11.86	223.1	132.0	20
Acetochlor	11.86	131.8	117.0	14
Acetochlor	11.86	146.0	117.7	8
Acrinathrin	22.59	208.1	180.9	8
Acrinathrin	22.59	289.0	93.1	8
Acrinathrin	22.59	181.0	152.0	22
Alachlor	12.09	188.1	130.0	32
Alachlor	12.09	188.1	132.0	14
Alachlor	12.09	188.1	160.1	8
Alachlor	12.09	160.1	131.7	10
Aldrin	13.32	254.9	219.9	20
Aldrin	13.32	262.7	191.0	30
Aldrin	13.32	262.7	192.9	32
Aldrin	13.32	330.0	298.9	10
Allidochlor	6.48	132.0	56.1	8
Allidochlor	6.48	134.0	56.0	8
Allidochlor	6.48	132.0	49.0	26
Allidochlor	6.48	138.1	95.9	6
Anthraquinone	13.33	180.1	152.0	12
Anthraquinone	13.33	208.1	152.0	22
Anthraquinone	13.33	208.1	180.1	10
Atrazine	10.32	200.1	122.0	8
Atrazine	10.32	200.1	132.0	8
Atrazine	10.32	215.1	58.1	10
Azinphos-ethyl	22.67	132.0	77.0	12
Azinphos-ethyl	22.67	132.0	51.0	26
Azinphos-ethyl	22.67	160.0	77.0	16
Azinphos-methyl	21.52	132.0	77.0	12
Azinphos-methyl	21.52	160.0	50.9	34
Azinphos-methyl	21.52	160.0	77.0	16
Benfluralin	9.42	292.0	264.0	8
Benfluralin	9.42	292.0	160.0	20
Benfluralin	9.42	292.0	206.0	12
BHC, Alpha	9.91	180.9	144.9	14
BHC, Alpha	9.91	216.9	181.0	8
BHC, Alpha	9.91	218.9	183.0	8
BHC, Alpha	9.91	182.8	146.7	12
BHC, Alpha	9.91	218.8	146.6	20
BHC, Beta	10.38	180.9	145.0	14
BHC, Beta	10.38	216.9	180.9	8
BHC, Beta	10.38	218.9	183.0	8
BHC, Beta	10.38	218.7	146.6	18
BHC, delta	11.16	180.9	144.9	14
BHC, delta	11.16	182.9	147.0	14
BHC, delta	11.16	218.9	182.9	8
BHC, delta	11.16	218.8	146.5	20
BHC, gamma	10.60	180.9	144.9	12
BHC, gamma	10.60	216.9	180.9	8
BHC, gamma	10.60	218.9	183.0	8
BHC, gamma	10.60	180.9	109.0	26
Bifenthrin	20.37	181.0	165.9	10
Bifenthrin	20.37	181.0	179.0	12
Bifenthrin	20.37	165.1	163.6	24
Bromfenvinphos	15.60	266.9	159.0	14
Bromfenvinphos	15.60	268.9	161.1	14
Bromfenvinphos	15.60	323.1	266.9	10
Bromfenvinphos	15.60	266.9	203.0	10
Bromfenvinphos-methyl	14.38	294.9	109.0	16
Bromfenvinphos-methyl	14.38	294.9	79.1	30
Bromfenvinphos-methyl	14.38	109.0	79.0	6
Bromophos-ethyl	14.99	358.8	302.8	14
Bromophos-ethyl	14.99	302.8	284.8	14
Bromophos-ethyl	14.99	302.8	210.9	30
Bromophos-ethyl	14.99	96.9	65.0	16
Bromophos-ethyl	14.99	96.9	78.9	12
Bromophos-methyl (Bromophos)	13.77	330.8	315.8	14
Bromophos-methyl (Bromophos)	13.77	328.9	313.8	14
Bromophos-methyl (Bromophos)	13.77	330.8	93.0	24
Bromophos-methyl (Bromophos)	13.77	125.0	79.0	6
Bromopropylate	20.39	340.8	185.0	14

Appendix 2 (continued). Target list of analytes with their SRM parameters

Compound Name	RT (min)	Q1	Q3	CE
Bromopropylate	20.39	184.9	156.9	12
Bromopropylate	20.39	184.9	75.5	30
Bupirimate	16.36	273.1	193.2	8
Bupirimate	16.36	273.1	108.0	14
Bupirimate	16.36	316.2	208.1	10
Bupirimate	16.36	208.1	140.1	12
Bupirimate	16.36	208.1	165.0	12
Captafol	19.38	150.1	79.0	6
Captafol	19.38	151.1	79.1	18
Captafol	19.38	183.1	79.1	8
Captafol	19.38	150.1	77.2	24
Captan	14.58	149.0	70.0	20
Captan	14.58	117.0	82.0	30
Captan	14.58	151.0	79.0	14
Captan	14.58	151.0	80.0	6
Captan	14.58	149.0	78.8	14
Captan	14.58	149.0	105.0	6
Carbophenothion	18.31	342.0	157.0	10
Carbophenothion	18.31	157.0	45.0	12
Carbophenothion	18.31	199.0	142.9	10
Carfentrazon-ethyl	18.23	340.1	312.1	10
Carfentrazon-ethyl	18.23	290.0	99.9	36
Carfentrazon-ethyl	18.23	311.9	150.7	18
Chlorbenside	15.03	125.0	89.0	16
Chlorbenside	15.03	125.0	99.0	16
Chlorbenside	15.03	268.0	125.0	10
Chlorbenside	15.03	125.0	62.8	28
Chlordane alpha-Cis	15.49	372.8	265.9	14
Chlordane alpha-Cis	15.49	271.8	236.8	12
Chlordane alpha-Cis	15.49	271.8	236.8	14
Chlordane alpha-Cis	15.49	372.8	265.8	20
Chlordane alpha-Cis	15.49	374.7	265.8	20
Chlordane alpha-Cis	15.49	376.6	268.0	20
Chlordane Gamma-trans	15.08	372.8	265.9	20
Chlordane Gamma-trans	15.08	374.8	265.9	20
Chlordane Gamma-trans	15.08	271.9	236.9	14
Chlordane Gamma-trans	15.08	372.7	263.7	20
Chlorfenapyr	16.64	136.9	102.0	12
Chlorfenapyr	16.64	248.9	112.0	24
Chlorfenapyr	16.64	248.9	137.1	18
Chlorfenapyr	16.64	327.9	246.9	14
Chlorfenson	15.76	175.0	111.0	10
Chlorfenson	15.76	111.0	75.0	14
Chlorfenson	15.76	175.0	75.0	28
Chlorfenvinphos	14.37	266.9	159.0	16
Chlorfenvinphos	14.37	268.9	161.0	14
Chlorfenvinphos	14.37	323.0	266.9	14
Chlorfenvinphos	14.37	266.9	203.0	10
Chlorobenzilate	17.18	139.0	111.0	12
Chlorobenzilate	17.18	251.0	111.0	34
Chlorobenzilate	17.18	251.0	139.0	14
Chlorobenzilate	17.18	111.0	75.1	14
Chlorobenzilate	17.18	139.0	74.9	26
Chlorobenzilate	17.18	139.0	111.0	12
Chloroneb	8.00	190.9	113.0	14
Chloroneb	8.00	193.0	53.1	32
Chloroneb	8.00	193.0	115.0	14
Chloroneb	8.00	190.9	141.0	10
Chloroneb	8.00	206.0	190.9	12
Chlorothalonil	10.95	263.9	132.9	40
Chlorothalonil	10.95	265.9	133.0	36
Chlorothalonil	10.95	265.9	170.0	24
Chlorothalonil	10.95	228.8	168.0	8
Chlorpropham	9.35	127.0	65.0	20
Chlorpropham	9.35	171.0	127.0	8
Chlorpropham	9.35	213.0	127.0	14
Chlorpropham	9.35	213.0	171.0	6
Chlorpyrifos-ethyl	13.14	198.9	171.0	14
Chlorpyrifos-ethyl	13.14	196.9	168.9	12
Chlorpyrifos-ethyl	13.14	313.9	257.9	12
Chlorpyrifos-ethyl	13.14	196.7	107.0	36
Chlorpyrifos-methyl	11.91	285.9	270.9	14
Chlorpyrifos-methyl	11.91	285.9	92.9	20
Chlorpyrifos-methyl	11.91	287.9	92.9	20
Chlorpyrifos-methyl	11.91	287.9	272.9	14
Chlorpyrifos-methyl	11.91	125.0	47.0	12
Chlorpyrifos-methyl	11.91	125.0	79.0	6
Chlorthal-dimethyl (Dacthal)	13.30	300.9	272.9	12
Chlorthal-dimethyl (Dacthal)	13.30	300.9	222.9	22
Chlorthal-dimethyl (Dacthal)	13.30	222.9	166.9	20
Chlorthiophos	17.55	324.9	268.9	12
Chlorthiophos	17.55	268.9	205.0	14
Chlorthiophos	17.55	296.9	268.9	8
Chlozolinate	14.28	186.0	145.0	14
Chlozolinate	14.28	188.0	147.0	14
Chlozolinate	14.28	259.0	187.9	12

Appendix 2 (continued). Target list of analytes with their SRM parameters

Compound Name	RT (min)	Q1	Q3	CE
Chlozolinate	14.28	259.0	152.9	26
Chlozolinate	14.28	331.0	259.0	8
Clomazone	10.42	125.0	89.0	16
Clomazone	10.42	125.0	99.0	16
Clomazone	10.42	204.0	107.0	18
Clomazone	10.42	138.0	74.9	24
Clomazone	10.42	138.0	111.0	12
Coumaphos	23.85	226.0	163.0	18
Coumaphos	23.85	362.0	226.0	10
Coumaphos	23.85	209.9	119.0	22
Coumaphos	23.85	209.9	182.0	10
Cycloate	9.22	154.1	83.1	8
Cycloate	9.22	154.1	55.1	18
Cycloate	9.22	215.1	154.1	6
Cycloate	9.22	83.1	55.1	6
Cyfluthrin peak 1	24.70	163.0	127.1	6
Cyfluthrin peak 1	24.70	226.0	206.1	12
Cyfluthrin peak 1	24.70	163.0	91.1	12
Cyfluthrin peak 1	24.70	163.0	65.1	26
Cyfluthrin peak 2	24.91	163.0	127.0	6
Cyfluthrin peak 2	24.91	226.0	206.1	12
Cyfluthrin peak 2	24.91	163.0	91.1	12
Cyfluthrin peak 2	24.91	206.0	151.1	18
Cyfluthrin peak 3	25.02	163.0	127.0	6
Cyfluthrin peak 3	25.02	226.0	206.1	12
Cyfluthrin peak 3	25.02	163.0	91.1	12
Cyfluthrin peak 4	25.11	163.0	127.0	6
Cyfluthrin peak 4	25.11	226.0	206.1	10
Cyfluthrin peak 4	25.11	163.0	91.1	12
Cyhalothrin I (lambda)	22.21	180.9	152.0	22
Cyhalothrin I (lambda)	22.21	197.1	141.1	10
Cyhalothrin I (lambda)	22.21	207.9	180.9	8
Cypermethrin peak 1	25.32	163.0	127.1	6
Cypermethrin peak 1	25.32	165.0	127.1	5
Cypermethrin peak 1	25.32	163.0	91.1	12
Cypermethrin peak 1	25.32	180.9	152.1	20
Cypermethrin peak 2	25.53	163.0	127.0	6
Cypermethrin peak 2	25.53	165.0	127.1	5
Cypermethrin peak 2	25.53	163.0	91.1	12
Cypermethrin peak 2	25.53	180.9	151.9	18
Cypermethrin peak 3	25.61	163.0	127.0	6
Cypermethrin peak 3	25.61	165.0	127.1	5
Cypermethrin peak 3	25.61	163.0	91.0	12

Compound Name	RT (min)	Q1	Q3	CE
Cypermethrin peak 3	25.61	180.9	152.2	20
Cypermethrin peak 4	25.72	163.0	127.1	6
Cypermethrin peak 4	25.72	165.0	127.1	5
Cypermethrin peak 4	25.72	163.0	91.1	12
Cypermethrin peak 4	25.72	180.9	152.2	20
Cyprodinil	14.12	224.1	208.1	18
Cyprodinil	14.12	224.1	197.1	20
Cyprodinil	14.12	225.1	210.1	16
DDD p,p	17.46	235.0	165.0	20
DDD p,p	17.46	235.0	199.0	14
DDD p,p	17.46	237.0	165.0	20
DDD, o, p	16.34	235.0	165.0	20
DDD, o, p	16.34	235.0	199.0	14
DDD, o, p	16.34	237.0	165.0	20
DDE o,p	15.14	246.0	176.1	28
DDE o,p	15.14	248.0	176.1	30
DDE o,p	15.14	317.9	248.0	18
DDE o,p	15.14	317.8	246.0	20
DDE p, p	16.14	246.0	176.1	28
DDE p, p	16.14	315.9	246.0	14
DDE p, p	16.14	317.9	246.0	20
DDE p, p	16.14	317.9	248.0	18
DDT o,p	17.56	235.0	165.1	22
DDT o,p	17.56	235.0	199.1	10
DDT o,p	17.56	237.0	165.1	22
DDT p,p	18.70	235.0	165.1	22
DDT p,p	18.70	236.8	165.0	22
DDT p,p	18.70	235.0	199.5	10
Deltamethrin	28.25	252.8	92.9	16
Deltamethrin	28.25	181.0	152.1	22
Deltamethrin	28.25	252.8	172.0	8
Diallate-cis	9.94	234.1	150.0	18
Diallate-cis	9.94	235.8	152.0	18
Diallate-cis	9.94	235.8	194.0	12
Diallate-trans	9.75	234.1	150.0	18
Diallate-trans	9.75	234.1	192.0	12
Diallate-trans	9.75	235.8	152.0	18
Diallate-trans	9.75	235.8	194.0	12
Diazinon	10.72	137.1	84.1	12
Diazinon	10.72	137.1	54.1	20
Diazinon	10.72	199.0	92.9	14
Diazinon	10.72	179.1	121.5	26
Dichlobenil	6.90	170.9	99.9	24

Appendix 2 (continued). Target list of analytes with their SRM parameters

Compound Name	RT (min)	Q1	Q3	CE
Dichlobenil	6.90	170.9	136.0	12
Dichlobenil	6.90	172.8	99.8	24
Dichlorobenzophenone, 4, 4	13.57	139.0	111.0	12
Dichlorobenzophenone, 4, 4	13.57	139.0	74.9	26
Dichlorobenzophenone, 4, 4	13.57	141.0	113.0	10
Dichlorobenzophenone, 4, 4	13.57	111.0	74.9	12
Dicloran (Bortran)	10.16	206.0	176.0	10
Dicloran (Bortran)	10.16	160.0	124.1	8
Dicloran (Bortran)	10.16	176.0	148.0	12
Dieldrin	16.31	262.9	192.9	30
Dieldrin	16.31	262.9	190.9	30
Dieldrin	16.31	262.9	227.8	16
Dieldrin	16.31	276.9	240.8	6
Dimethachlor	11.76	197.1	148.0	10
Dimethachlor	11.76	134.0	77.0	24
Dimethachlor	11.76	134.0	105.1	12
Diphenamid	13.75	167.1	152.1	16
Diphenamid	13.75	167.1	165.1	20
Diphenamid	13.75	239.1	167.1	8
Diphenamid	13.75	239.1	72.1	10
Diphenylamine	9.14	168.1	167.1	14
Diphenylamine	9.14	169.1	167.1	24
Diphenylamine	9.14	169.1	168.1	12
Diphenylamine	9.14	168.1	139.0	38
Disulfoton	11.01	88.0	59.8	6
Disulfoton	11.01	88.0	45.0	18
Disulfoton	11.01	142.0	81.0	10
Disulfoton	11.01	185.9	96.9	16
Edifenphos	18.39	172.9	109.0	8
Edifenphos	18.39	310.0	109.0	26
Edifenphos	18.39	172.9	65.1	30
Endosulfan ether	11.68	238.9	204.0	12
Endosulfan ether	11.68	240.9	206.0	14
Endosulfan peak 1	15.49	240.8	205.8	14
Endosulfan peak 1	15.49	262.8	192.9	30
Endosulfan peak 1	15.49	194.9	160.0	8
Endosulfan peak 1	15.49	194.7	125.0	22
Endosulfan peak 1	15.49	194.7	159.4	8
Endosulfan peak 2	17.28	158.9	123.0	12
Endosulfan peak 2	17.28	240.6	205.8	12
Endosulfan peak 2	17.28	194.9	159.0	8
Endosulfan peak 2	17.28	236.8	118.9	30
Endosulfan sulfate	18.52	271.7	236.8	12
Endosulfan sulfate	18.52	238.7	203.9	12
Endosulfan sulfate	18.52	271.7	234.9	12
Endrin	16.97	262.8	192.9	30
Endrin	16.97	244.9	173.0	22
Endrin	16.97	280.8	244.9	8
Endrin Aldehyde	17.75	173.0	138.1	16
Endrin Aldehyde	17.75	249.8	214.9	24
Endrin Aldehyde	17.75	278.9	242.9	10
Endrin Aldehyde	17.75	344.9	281.0	8
Endrin-Ketone	20.06	316.8	281.0	10
Endrin-Ketone	20.06	316.8	208.9	28
Endrin-Ketone	20.06	209.2	138.4	30
EPN	20.31	169.0	77.0	22
EPN	20.31	157.0	77.0	22
EPN	20.31	169.0	141.0	8
Esfenvalerate	27.36	167.0	125.0	10
Esfenvalerate	27.36	125.0	89.0	18
Esfenvalerate	27.36	167.0	89.0	32
Esfenvalerate	27.36	225.1	119.1	18
Ethalfluralin	9.24	276.0	202.0	14
Ethalfluralin	9.24	276.0	248.1	8
Ethalfluralin	9.24	315.9	276.1	8
Ethalfluralin	9.24	292.0	264.0	8
Ethion	17.45	230.9	128.9	22
Ethion	17.45	230.9	174.9	12
Ethion	17.45	153.0	97.0	10
Ethion	17.45	120.9	65.0	10
Etofenprox	25.92	163.1	107.1	16
Etofenprox	25.92	163.1	135.1	10
Etofenprox	25.92	163.1	77.1	32
Etridiazole (Terrazole)	7.67	182.8	139.9	14
Etridiazole (Terrazole)	7.67	211.0	139.9	18
Etridiazole (Terrazole)	7.67	211.0	182.9	10
Fenamiphos	15.60	303.1	195.0	8
Fenamiphos	15.60	154.0	139.0	10
Fenamiphos	15.60	217.0	202.0	12
Fenarimol	22.45	139.0	111.0	14
Fenarimol	22.45	139.0	74.9	26
Fenarimol	22.45	219.0	107.0	10
Fenchlorfos	12.35	284.9	269.9	14
Fenchlorfos	12.35	284.9	93.0	24
Fenchlorfos	12.35	286.9	271.9	14
Fenchlorfos	12.35	124.9	47.0	12

Appendix 2 (continued). Target list of analytes with their SRM parameters

Compound Name	RT (min)	Q1	Q3	CE
Fenchlorfos	12.35	124.9	79.0	6
Fenchlorfos	12.35	169.0	110.4	6
Fenitrothion	12.69	277.0	260.0	6
Fenitrothion	12.69	277.0	109.0	16
Fenitrothion	12.69	277.0	109.0	14
Fenitrothion	12.69	125.0	79.0	8
Fenpropathrin	20.67	181.0	151.9	22
Fenpropathrin	20.67	181.0	126.8	28
Fenpropathrin	20.67	97.1	55.1	6
Fenson	13.71	141.0	77.0	8
Fenson	13.71	141.0	50.9	30
Fenson	13.71	268.0	77.0	20
Fenson	13.71	77.0	51.0	14
Fenthion	13.24	278.0	109.0	18
Fenthion	13.24	278.0	169.0	14
Fenthion	13.24	278.0	125.0	14
Fenthion	13.24	245.3	125.0	12
Fenvalerate	26.98	167.0	125.0	10
Fenvalerate	26.98	125.0	89.0	18
Fenvalerate	26.98	167.0	89.0	32
Fipronil	14.18	366.9	212.9	28
Fipronil	14.18	366.9	244.9	20
Fipronil	14.18	368.9	214.9	30
Fluazifop-P-butyl	16.91	282.1	91.1	18
Fluazifop-P-butyl	16.91	282.1	238.1	16
Fluazifop-P-butyl	16.91	383.1	282.1	14
Fluchloralin	10.76	306.0	264.0	8
Fluchloralin	10.76	264.0	206.0	8
Fluchloralin	10.76	326.0	63.0	12
Fluchloralin	10.76	264.0	159.5	14
Flucythrinate peak 1	25.65	157.0	107.1	12
Flucythrinate peak 1	25.65	199.1	107.1	22
Flucythrinate peak 1	25.65	199.1	157.1	8
Flucythrinate peak 2	26.03	157.0	107.0	12
Flucythrinate peak 2	26.03	199.0	107.0	22
Flucythrinate peak 2	26.03	199.0	157.1	8
Fludioxonil	15.82	248.0	127.0	26
Fludioxonil	15.82	248.0	154.0	18
Fludioxonil	15.82	248.0	182.0	10
Fludioxonil	15.82	153.7	127.0	8
Fluquinconazole	23.87	340.0	298.0	16
Fluquinconazole	23.87	340.0	108.1	36
Fluquinconazole	23.87	340.0	313.0	14
Fluridone	26.31	328.1	189.1	38
Fluridone	26.31	328.1	258.8	24
Fluridone	26.31	329.1	328.5	12
Flusilazole	16.32	233.1	164.9	16
Flusilazole	16.32	233.1	151.9	14
Flusilazole	16.32	315.1	233.1	10
Flusilazole	16.32	206.0	151.3	14
Flutolanil	15.73	173.0	95.0	28
Flutolanil	15.73	281.0	173.0	10
Flutolanil	15.73	173.0	145.0	14
Flutriafol	15.57	123.0	75.0	24
Flutriafol	15.57	219.1	95.0	34
Flutriafol	15.57	219.1	123.0	12
Flutriafol	15.57	123.0	95.0	12
Fluvalinate peak 1	27.25	250.0	55.1	16
Fluvalinate peak 1	27.25	250.0	199.9	18
Fluvalinate peak 1	27.25	180.8	152.1	22
Fluvalinate peak 2	27.38	250.0	55.1	16
Fluvalinate peak 2	27.38	250.0	200.0	16
Fluvalinate peak 2	27.38	180.8	152.1	20
Folpet	14.77	261.9	130.0	14
Folpet	14.77	259.9	130.0	14
Folpet	14.77	104.0	76.0	10
Folpet	14.77	130.0	102.0	12
Fonofos	10.75	137.0	109.0	6
Fonofos	10.75	109.0	62.9	10
Fonofos	10.75	246.0	109.0	14
Fonofos	10.75	246.0	137.0	6
Heptachlor	12.35	271.8	236.8	12
Heptachlor	12.35	273.8	238.8	14
Heptachlor	12.35	273.8	236.8	14
Heptachlor	12.35	99.8	39.0	26
Heptachlor	12.35	99.8	65.0	12
Heptachlor epoxide	14.39	352.8	262.9	16
Heptachlor epoxide	14.39	354.7	264.9	12
Heptachlor epoxide	14.39	262.9	192.9	30
Hexachlorobenzene	10.03	281.8	211.8	28
Hexachlorobenzene	10.03	283.8	213.8	30
Hexachlorobenzene	10.03	283.8	248.8	16
Hexachlorobenzene	10.03	285.8	250.8	18
Hexazinone	18.82	171.1	71.1	14
Hexazinone	18.82	171.1	85.1	12
Hexazinone	18.82	127.7	83.0	10

Appendix 2 (continued). Target list of analytes with their SRM parameters

Compound Name	RT (min)	Q1	Q3	CE
Iodofenfos	15.76	376.8	361.8	16
Iodofenfos	15.76	378.8	363.8	14
Iodofenfos	15.76	125.0	47.0	12
Iodofenfos	15.76	125.0	79.0	6
Iodofenfos	15.76	376.8	361.8	16
Iprodione	20.03	314.0	245.0	10
Iprodione	20.03	315.7	247.0	10
Iprodione	20.03	315.7	273.0	8
Isazophos	11.00	160.9	119.0	8
Isazophos	11.00	118.9	76.0	18
Isazophos	11.00	256.9	161.9	4
Isazophos	11.00	161.0	146.0	6
Isodrin	14.13	192.9	157.0	20
Isodrin	14.13	146.9	111.1	10
Isodrin	14.13	192.9	123.0	28
Isopropalin	13.87	280.1	238.2	8
Isopropalin	13.87	280.1	180.2	10
Isopropalin	13.87	264.1	222.1	6
Isopropalin	13.87	280.1	117.8	26
Lenacil	18.52	153.0	135.6	12
Lenacil	18.52	153.0	82.1	16
Lenacil	18.52	153.0	110.0	14
Leptophos	21.51	171.0	77.1	18
Leptophos	21.51	171.0	51.0	38
Leptophos	21.51	171.0	124.3	10
Linuron	12.88	248.0	61.1	8
Linuron	12.88	159.8	133.0	12
Linuron	12.88	187.0	124.0	20
Malathion	12.90	158.0	125.0	6
Malathion	12.90	173.1	99.0	12
Malathion	12.90	127.0	99.0	6
Malathion	12.90	92.8	63.0	8
Malathion	12.90	125.0	79.0	8
Metalaxyl	12.25	234.1	146.1	20
Metalaxyl	12.25	249.1	190.1	6
Metalaxyl	12.25	234.1	174.1	10
Metazachlor	14.13	209.0	132.1	16
Metazachlor	14.13	133.1	132.1	12
Metazachlor	14.13	132.1	117.1	14
Metazachlor	14.13	133.1	117.3	22
Methacrifos	7.96	207.9	180.1	6
Methacrifos	7.96	124.9	47.1	12
Methacrifos	7.96	125.0	79.0	8
Methacrifos	7.96	180.0	93.0	10
Methacrifos	7.96	240.0	180.0	10
Methoxychlor	20.56	227.1	141.1	32
Methoxychlor	20.56	227.1	169.1	22
Methoxychlor	20.56	227.1	212.1	12
Metolachlor	13.10	238.1	162.1	10
Metolachlor	13.10	238.1	133.1	26
Metolachlor	13.10	162.1	133.1	14
Mevinphos	7.43	127.0	109.0	10
Mevinphos	7.43	127.0	95.0	14
Mevinphos	7.43	192.0	127.0	10
MGK-264 A	13.80	164.1	98.1	10
MGK-264 A	13.80	164.1	80.1	24
MGK-264 A	13.80	164.1	93.1	10
MGK-264 B	14.17	164.1	98.1	12
MGK-264 B	14.17	164.1	67.1	6
MGK-264 B	14.17	164.1	80.1	22
Mirex	22.22	272.0	236.8	14
Mirex	22.22	273.8	238.8	14
Mirex	22.22	236.8	142.9	26
Myclobutanil	16.23	179.0	125.0	14
Myclobutanil	16.23	179.0	90.0	28
Myclobutanil	16.23	150.0	123.0	14
Myclobutanil	16.23	179.0	151.9	8
N-(2,4-Dimethylphenyl)formamide	7.90	149.1	106.1	16
N-(2,4-Dimethylphenyl)formamide	7.90	149.1	120.1	14
N-(2,4-Dimethylphenyl)formamide	7.90	149.1	121.1	6
Nitralin	19.46	316.2	274.0	8
Nitralin	19.46	274.0	216.0	8
Nitralin	19.46	274.0	169.0	12
Nitrofen	16.88	202.0	139.0	24
Nitrofen	16.88	283.0	162.0	20
Nitrofen	16.88	283.0	253.0	10
Nonachlor-cis	17.48	408.8	299.9	18
Nonachlor-cis	17.48	406.8	299.9	14
Nonachlor-cis	17.48	262.9	192.9	28
Nonachlor-cis	17.48	410.8	301.8	14
Nonachlor-cis	17.48	236.7	142.9	24
Nonachlor-trans	15.59	408.8	299.8	18
Nonachlor-trans	15.59	406.8	299.8	14
Nonachlor-trans	15.59	271.8	236.8	14
Nonachlor-trans	15.59	408.8	301.8	14
Nonachlor-trans	15.59	236.8	142.9	24

Appendix 2 (continued). Target list of analytes with their SRM parameters

Compound Name	RT (min)	Q1	Q3	CE
Nonachlor-trans	15.59	262.8	192.9	28
Norflurazon	18.36	303.0	145.0	20
Norflurazon	18.36	145.0	95.0	16
Norflurazon	18.36	145.0	74.7	28
Ortho-phenylphenol	8.24	170.1	141.1	22
Ortho-phenylphenol	8.24	141.1	115.1	14
Ortho-phenylphenol	8.24	170.1	115.0	34
Oxadiazon	16.14	175.0	112.0	12
Oxadiazon	16.14	175.0	76.0	28
Oxadiazon	16.14	258.0	175.0	6
Oxadiazon	16.14	174.9	147.2	6
Oxyfluorfen	16.30	300.0	223.0	14
Oxyfluorfen	16.30	252.0	146.0	30
Oxyfluorfen	16.30	252.0	169.8	28
Paclobutrazol	15.22	236.0	125.0	12
Paclobutrazol	15.22	236.0	167.0	10
Paclobutrazol	15.22	138.0	103.1	14
Paclobutrazol	15.22	125.0	89.0	18
Parathion (ethyl)	13.33	291.0	109.0	12
Parathion (ethyl)	13.33	138.9	109.0	6
Parathion (ethyl)	13.33	154.9	125.0	6
Parathion (ethyl)	13.33	109.0	81.0	10
Leptophos	21.51	171.0	77.1	18
Leptophos	21.51	171.0	51.0	38
Leptophos	21.51	171.0	124.3	10
Linuron	12.88	248.0	61.1	8
Linuron	12.88	159.8	133.0	12
Linuron	12.88	187.0	124.0	20
Parathion (ethyl)	13.33	124.9	97.0	6
Parathion-methyl	12.04	263.0	109.0	12
Parathion-methyl	12.04	233.0	109.0	10
Parathion-methyl	12.04	263.0	79.0	30
Parathion-methyl	12.04	124.9	47.0	12
Parathion-methyl	12.04	124.9	79.0	6
Pebulate	7.71	128.1	57.1	8
Pebulate	7.71	128.0	72.0	6
Pebulate	7.71	161.0	128.1	6
Pebulate	7.71	108.1	77.1	24
Pebulate	7.71	108.1	79.0	14
Penconazole	14.26	248.1	157.0	22
Penconazole	14.26	248.1	192.0	12
Penconazole	14.26	159.0	123.0	20
Penconazole	14.26	158.9	89.0	28
Pendimethalin	14.04	252.1	162.1	8
Pendimethalin	14.04	252.1	161.1	14
Pendimethalin	14.04	252.1	191.3	8
Pentachloroaniline	11.64	262.9	191.9	20
Pentachloroaniline	11.64	264.9	193.6	18
Pentachloroaniline	11.64	266.9	193.9	20
Pentachloroaniline	11.64	264.8	202.8	20
Pentachloroaniline	11.64	264.8	229.3	12
Pentachloroanisole	10.12	264.8	236.9	10
Pentachloroanisole	10.12	266.8	238.9	10
Pentachloroanisole	10.12	279.9	236.8	22
Pentachlorobenzene	8.28	249.8	214.8	16
Pentachlorobenzene	8.28	247.9	212.9	18
Pentachlorobenzene	8.28	248.0	142.0	42
Pentachlorobenzene	8.28	249.8	143.6	38
Pentachlorobenzene	8.28	249.8	178.5	24
Pentachlorobenzonitrile	10.60	272.9	237.9	16
Pentachlorobenzonitrile	10.60	274.8	204.9	28
Pentachlorobenzonitrile	10.60	274.8	239.9	18
Pentachlorothioanisole	12.91	295.7	262.9	12
Pentachlorothioanisole	12.91	262.7	192.9	28
Pentachlorothioanisole	12.91	295.7	245.9	30
Permethrin peak 1	23.65	183.1	153.0	12
Permethrin peak 1	23.65	183.1	168.0	12
Permethrin peak 1	23.65	163.0	91.1	12
Permethrin peak 2	23.91	183.0	165.1	10
Permethrin peak 2	23.91	183.0	153.0	14
Permethrin peak 2	23.91	183.0	168.1	10
Perthane (Ethylan)	16.93	223.1	167.0	12
Perthane (Ethylan)	16.93	223.1	179.0	20
Perthane (Ethylan)	16.93	223.1	193.0	28
Phenothrin	21.38	123.1	81.1	8
Phenothrin	21.38	123.1	41.1	24
Phenothrin	21.38	123.1	79.1	14
Phorate	9.75	260.0	75.0	8
Phorate	9.75	121.0	65.0	10
Phorate	9.75	75.0	47.0	8
Phosalone	21.47	182.0	74.8	30
Phosalone	21.47	121.1	65.0	10
Phosalone	21.47	182.0	111.0	14
Phosmet	20.17	160.0	76.9	22
Phosmet	20.17	160.0	133.0	10
Phosmet	20.17	160.0	50.9	38
Phthalimide	7.80	147.0	76.0	25
Phthalimide	7.80	147.0	103.3	10
Phthalimide	7.80	103.7	76.0	10
Piperonyl butoxide	19.46	176.1	103.1	22
Piperonyl butoxide	19.46	176.1	117.0	18
Piperonyl butoxide	19.46	176.1	131.1	12
Pirimiphos-ethyl	13.72	304.1	168.1	12
Pirimiphos-ethyl	13.72	318.1	166.1	12
Pirimiphos-ethyl	13.72	318.1	182.1	10
Pirimiphos-methyl	12.61	233.0	151.1	6
Pirimiphos-methyl	12.61	290.1	125.0	20
Pirimiphos-methyl	12.61	290.1	233.0	8

Appendix 2 (continued). Target list of analytes with their SRM parameters

Compound Name	RT (min)	Q1	Q3	CE
Pirimiphos-methyl	12.61	305.1	180.1	8
Pretilachlor	15.94	162.0	132.1	20
Pretilachlor	15.94	262.1	202.1	6
Pretilachlor	15.94	176.1	147.1	14
Pretilachlor	15.94	202.1	174.2	8
Pretilachlor	15.94	202.1	145.5	14
Pretilachlor	15.94	238.1	146.1	10
Prochloraz	24.01	308.0	70.0	12
Prochloraz	24.01	180.0	69.0	14
Prochloraz	24.01	180.1	138.1	12
Prochloraz	24.01	69.9	42.0	8
Prochloraz	24.01	308.0	147.1	12
Procymedone	14.66	283.0	96.1	8
Procymedone	14.66	283.0	68.1	24
Procymedone	14.66	285.0	96.1	10
Procymedone	14.66	95.9	53.0	16
Procymedone	14.66	95.9	67.1	8
Prodiamine	12.64	321.1	279.1	6
Prodiamine	12.64	275.1	255.1	8
Prodiamine	12.64	321.1	203.0	10
Prodiamine	12.64	279.0	203.1	6
Profenofos	16.00	338.9	268.9	14
Profenofos	16.00	336.9	266.9	12
Profenofos	16.00	296.9	268.9	10
Profenofos	16.00	336.9	308.9	8
Profluralin	10.49	318.1	199.1	12
Profluralin	10.49	318.1	55.0	12
Profluralin	10.49	347.1	330.1	6
Profluralin	10.49	318.1	284.1	10
Profluralin	10.49	330.2	69.1	20
Propachlor	8.94	120.0	50.9	32
Propachlor	8.94	120.0	77.0	16
Propachlor	8.94	176.1	57.1	8
Propanil	11.79	217.0	161.0	8
Propanil	11.79	161.0	90.0	24
Propanil	11.79	161.0	99.0	24
Propanil	11.79	160.9	125.7	16
Propargite	19.26	135.1	107.1	12
Propargite	19.26	135.1	77.1	26
Propargite	19.26	150.1	135.1	8
Propisochlor	12.20	162.1	144.1	8
Propisochlor	12.20	162.1	91.1	30
Propisochlor	12.20	162.1	120.1	12
Propisochlor	12.20	162.1	147.1	12
Propyzamide	10.70	172.9	109.0	24
Propyzamide	10.70	172.9	145.0	14
Propyzamide	10.70	174.9	147.0	14
Propyzamide	10.70	172.9	74.0	38
Prothiofos	15.87	266.9	220.9	18
Prothiofos	15.87	266.9	238.9	8
Pentachlorothioanisole	12.91	295.7	262.9	12
Pentachlorothioanisole	12.91	262.7	192.9	28
Pentachlorothioanisole	12.91	295.7	245.9	30
Permethrin peak 1	23.65	183.1	153.0	12
Permethrin peak 1	23.65	183.1	168.0	12
Permethrin peak 1	23.65	163.0	91.1	12
Prothiofos	15.87	309.0	238.9	14
Pyraclofos	23.00	194.0	138.0	18
Pyraclofos	23.00	360.0	194.1	12
Pyraclofos	23.00	139.2	96.9	6
Pyrazophos	22.48	221.0	193.1	8
Pyrazophos	22.48	231.9	204.1	10
Pyrazophos	22.48	221.0	148.7	14
Pyridaben	23.90	147.1	117.1	20
Pyridaben	23.90	147.1	132.1	12
Pyridaben	23.90	147.1	119.1	8
Pyridaphenthion	19.97	340.0	199.1	8
Pyridaphenthion	19.97	199.0	77.1	24
Pyridaphenthion	19.97	199.0	92.1	14
Pyrimethanil	10.89	198.1	118.0	32
Pyrimethanil	10.89	198.1	158.1	18
Pyrimethanil	10.89	198.1	183.1	14
Pyriproxyfen	21.81	136.1	78.0	20
Pyriproxyfen	21.81	136.1	96.0	10
Pyriproxyfen	21.81	226.1	186.1	12
Quinalphos	14.55	146.0	118.1	10
Quinalphos	14.55	157.1	102.0	22
Quinalphos	14.55	157.1	129.0	14
Quintozene	10.53	294.8	236.9	14
Quintozene	10.53	213.8	178.9	14
Quintozene	10.53	213.8	141.9	28
Resmethrin peak 1	19.34	171.0	127.9	14
Resmethrin peak 1	19.34	143.0	128.1	10
Resmethrin peak 1	19.34	123.1	81.1	8
Resmethrin peak 2	19.54	171.0	127.9	14
Resmethrin peak 2	19.54	143.0	128.0	10
Resmethrin peak 2	19.54	123.1	81.1	8
Sulfotep	9.48	202.0	145.9	10
Sulfotep	9.48	237.9	145.9	12
Sulfotep	9.48	322.0	145.9	22
Sulfotep	9.48	265.9	145.9	15
Sulprofos	17.97	322.0	156.1	10
Sulprofos	17.97	156.0	108.0	30
Sulprofos	17.97	156.0	141.0	14
Tebuconazole	19.13	250.0	125.0	20
Tebuconazole	19.13	125.0	89.0	16
Tebuconazole	19.13	125.0	99.0	16
Tebufenpyrad	20.85	276.1	171.0	10

Appendix 2 (continued). Target list of analytes with their SRM parameters

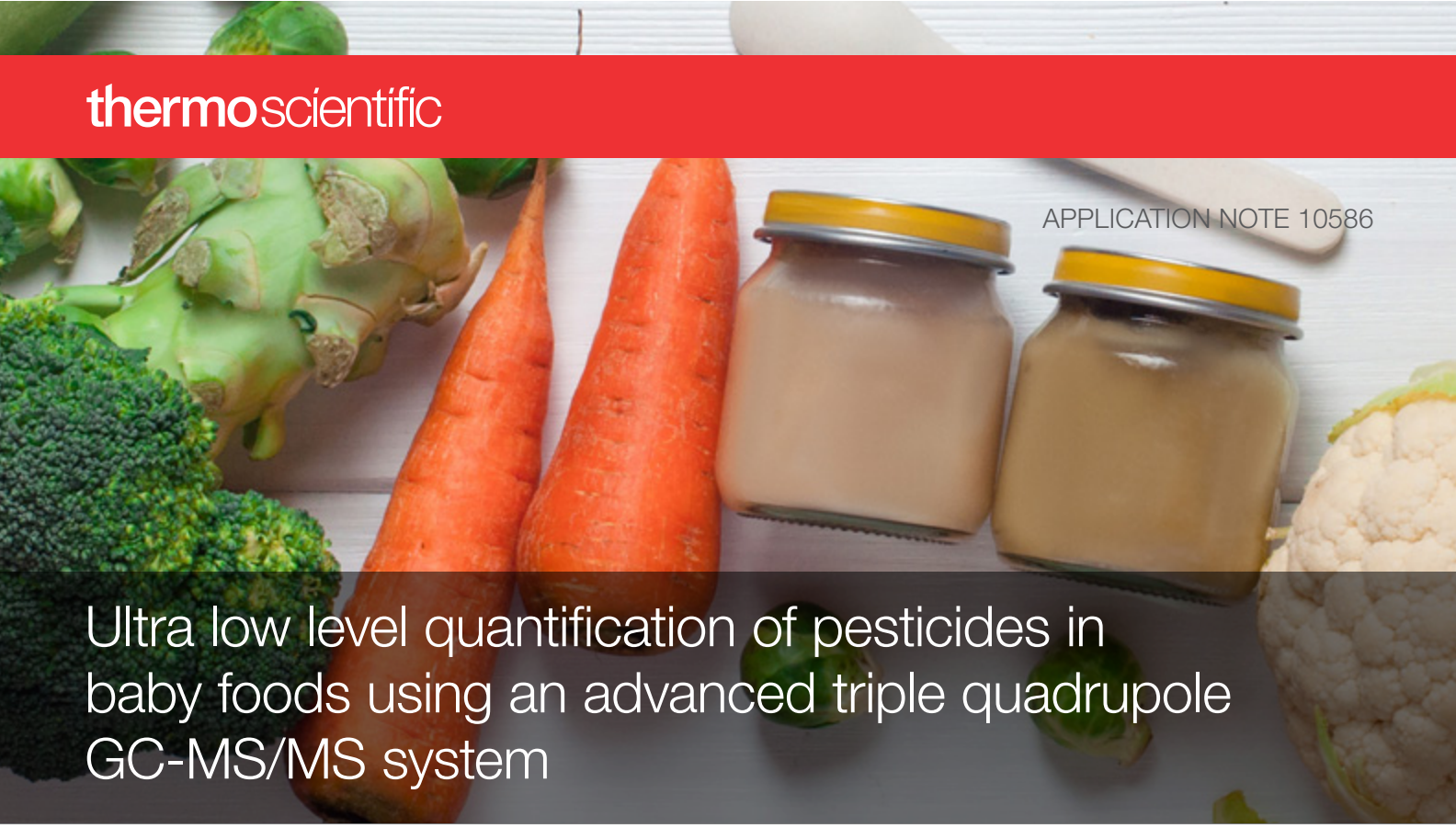
Compound Name	RT (min)	Q1	Q3	CE
Tebufenpyrad	20.85	318.1	131.1	14
Tebufenpyrad	20.85	318.1	145.1	14
Tecnazene	8.85	258.9	201.0	12
Tecnazene	8.85	202.9	142.9	18
Tecnazene	8.85	214.8	178.9	8
Tecnazene	8.85	214.8	143.6	20
Tecnazene	8.85	214.8	179.9	15
Tefluthrin	11.01	177.0	127.0	14
Tefluthrin	11.01	177.0	137.0	16
Tefluthrin	11.01	197.0	141.0	10
Terbacil	11.00	161.0	144.0	12
Terbacil	11.00	160.0	117.0	8
Terbacil	11.00	160.0	76.0	12
Terbufos	10.63	231.0	128.9	20
Terbufos	10.63	231.0	175.0	10
Terbufos	10.63	231.0	203.0	8
Terbuthylazine	10.62	214.1	104.1	16
Terbuthylazine	10.62	214.1	132.1	10
Terbuthylazine	10.62	229.1	173.1	10
Tetrachlorvinphos	15.17	328.9	109.0	18
Tetrachlorvinphos	15.17	330.9	109.0	18
Tetrachlorvinphos	15.17	332.9	109.0	14
Tetrachlorvinphos	15.17	109.0	79.0	6
Tetradifon	21.23	159.0	131.0	10
Tetrahydrophthalimide (THPI)	7.97	151.0	79.9	6
Tetrahydrophthalimide (THPI)	7.97	151.0	77.1	32
Tetrahydrophthalimide (THPI)	7.97	151.0	122.1	8
Tetramethrin peak 1	20.12	164.0	107.1	12
Tetramethrin peak 1	20.12	164.0	77.1	24
Tetramethrin peak 1	20.12	164.0	135.1	8
Tetramethrin peak 2	20.43	164.0	107.1	12
Tetramethrin peak 2	20.43	164.0	77.1	22
Tetramethrin peak 2	20.43	164.0	135.1	8
Tolclofos-methyl	12.09	265.0	250.0	12
Tolclofos-methyl	12.09	265.0	219.9	20

Compound Name	RT (min)	Q1	Q3	CE
Tolclofos-methyl	12.09	266.9	252.0	12
Tolylfluanid	14.34	238.0	137.0	10
Tolylfluanid	14.34	137.0	65.1	28
Tolylfluanid	14.34	137.0	91.1	18
Tolylfluanid	14.34	238.0	91.0	40
Tolylfluanid	14.34	240.0	137.0	14
Transfluthrin	12.10	163.0	143.0	14
Transfluthrin	12.10	127.0	91.1	8
Transfluthrin	12.10	163.0	91.1	12
Triadimefon	13.43	208.0	111.0	20
Triadimefon	13.43	208.0	126.7	12
Triadimefon	13.43	208.0	180.8	8
Triadimenol	14.66	168.1	70.0	10
Triadimenol	14.66	128.0	65.0	18
Triadimenol	14.66	112.0	58.0	8
Triadimenol	14.66	128.0	100.0	10
Triallate	11.21	268.0	183.9	18
Triallate	11.21	86.1	43.3	6
Triallate	11.21	268.0	226.0	12
Triazophos	17.92	161.1	134.1	8
Triazophos	17.92	257.0	162.1	6
Triazophos	17.92	161.1	106.1	12
Triazophos	17.92	162.1	119.1	12
Triflumizole	14.72	206.0	179.0	14
Triflumizole	14.72	179.0	144.0	14
Triflumizole	14.72	206.0	186.0	8
Trifluralin	9.37	306.1	264.1	8
Trifluralin	9.37	264.0	160.0	14
Trifluralin	9.37	306.1	206.0	10
Triphenylphosphate	19.28	215.0	168.1	16
Triphenylphosphate	19.28	326.1	325.1	10
Triphenylphosphate	19.28	326.1	169.1	28
Vinclozolin	11.98	186.8	124.0	18
Vinclozolin	11.98	197.9	145.0	14
Vinclozolin	11.98	212.0	172.0	14

Find out more at **thermofisher.com**

© 2020 Thermo Fisher Scientific Inc. All rights reserved. LEAP PAL Parts + Consumables is a trademark of LEAP PAL Parts + Consumables, LLC. Maharaja is a trademark of Groupe SEB India. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details. **AN65906-EN 0920S**

thermo scientific



APPLICATION NOTE 10586

Ultra low level quantification of pesticides in baby foods using an advanced triple quadrupole GC-MS/MS system

Authors

Richard Law, Aaron Lamb, Paul Silcock, and Cristian Cojocariu

Thermo Fisher Scientific, Runcorn, UK

Keywords

TSQ 9000, advanced electron ionization, AEI, baby food, sensitivity, robustness, pesticide residue, QuEChERS, triple quadrupole mass spectrometry, GC-MS/MS, Programmable Temperature Vaporization, food safety

Goal

The aim of the study was to assess the quantitative performance of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system fitted with the Advanced Electron Ionization (AEI) source for the analysis of pesticide residues at ultra low levels in baby food.

Introduction

The detection and subsequent quantification of pesticides, contaminants, and other chemical residues are of paramount importance, especially when the food stuff is intended to be consumed by infants or young children. The maximum residue level (MRL) for the majority of pesticide-commodity combinations is set at the default level of 10 µg/kg.[1–3] However, the European Union (EU) has established LOD MRLs between 3–8 µg/kg for specific pesticides prohibited in baby foods.[4] These pesticides and their metabolites may cause infants and young children (under worst-case intake conditions) to exceed the acceptable daily intake (ADI) values. The high sensitivity and selectivity of GC-MS/MS enables the detection and identification of residues of prohibited compounds, in compliance with the residue definitions, even when dealing with the diverse composition of multi-ingredient baby foods.

ThermoFisher SCIENTIFIC

Also, the increased levels of selectivity and sensitivity provided by triple quadrupole instruments compared to single quadrupole instruments enabled analysts to adopt faster, less specific sample extraction procedures such as QuEChERS (quick, easy, cheap effective, rugged and safe).

The QuEChERS procedure has become the standard approach for sample preparation in many laboratories because of improvement in productivity.[5] The method usually involves extraction with acetonitrile in the presence of various salts followed by dispersive solid phase extraction (dSPE) clean-up with a combination of PSA, C18, and carbon sorbents. The efficiency of the dSPE clean-up is limited so high concentrations of matrix-coextractives can remain in the final extract and cause system contamination. Also, use of acetonitrile (which has a high coefficient of expansion) limits the injection volume and hence the sensitivity of the method.

Taking all of these considerations together, it is evident that an ultra-sensitive, selective, reliable, and robust GC-MS/MS system is needed to address the challenge of routine high-throughput determination of pesticide residues at trace concentrations in baby foods. In this study, the quantitative performance of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system was assessed for the analysis of more than 200 pesticides in baby food at ultra low concentrations (as low as 0.025 µg/kg). A complete evaluation of method performance included sample preparation, overall method suitability measured from pesticides recoveries, selectivity, sensitivity, linearity, and long-term robustness.

Experimental

Sample preparation

Samples of carrot/potato and apple/pear/banana baby food samples were extracted using the citrate-buffered QuEChERS protocol using Thermo Scientific™ HyperSep™ dispersive solid phase extraction (dSPE) products.

Homogenized sample (10 g) was extracted with acetonitrile (10 mL) followed by the addition of $MgSO_4$ (4 g), NaCl (1.0 g), disodium hydrogen citrate sesquihydrate (0.5 g), and trisodium citrate dihydrate (1.0 g). Dispersive solid phase extraction (dSPE) [$MgSO_4$ (150 mg), PSA (25 mg) and GCB (25 mg) per 1 mL of extract for carrot/potato and $MgSO_4$ (150 mg) and PSA (25 mg) for apple/pear/banana] was used for sample clean-up.

Preparation of matrix-matched calibrations

Immediately after dSPE clean-up, the final extracts (1 g sample/mL of acetonitrile) were acidified with 5% formic acid in acetonitrile and were spiked with a mixture of 211 pesticides at 14 concentrations spanning a range of 0.025–250 µg/kg. Robustness was tested using repeat injections of samples (carrot/potato) spiked at the 10 µg/kg level.

For method evaluation, samples of carrot/potato and apple/pear/banana baby food samples were each spiked at 1.0, 2.5, and 10.0 µg/kg (n = 6 for each concentration) before extraction, clean-up, and acidification were carried out as described above.

GC-MS/MS analysis

A TSQ 9000 triple quadrupole GC-MS/MS system equipped with a Thermo Scientific™ Advanced Electron Ionization (AEI) source and coupled with a Thermo Scientific™ TRACE™ 1310 GC system was used. The AEI source provides a highly efficient electron ionization of analytes and a more tightly focused ion beam that provides an unparalleled level of sensitivity.

Liquid injections of the sample extracts were performed using a Thermo Scientific™ TriPlus™ RSH™ autosampler, and chromatographic separation was achieved by a Thermo Scientific™ TraceGOLD™ TG-5SilMS 30 m × 0.25 mm I.D. × 0.25 µm film capillary column with 5 m integrated SafeGuard. Additional details of instrument parameters are displayed in Table 1.

Table 1. Gas chromatograph and mass spectrometer parameters.

TRACE 1310 GC System Parameters				
Injection Volume (µL)	1			
Liner	Siltek™ six baffle PTV liner (P/N 453T2120)			
Inlet (°C)	70			
Carrier Gas, (mL/min)	He, 1.2			
Inlet Mode	Splitless (split flow 50 mL/min after 2 min)			
Column	TraceGOLD TG-5SilMS with SafeGuard (30 m × 0.25 mm, 0.25 µm with 5 m integrated guard column (P/N 26096-1425)			
PTV Parameters	**Rate (°C/s)**	**Temp. (°C)**	**Time (min)**	**Flow (mL/min)**
Injection	-	70	0.10	-
Transfer	5.0	300	2.00	-
Cleaning	14.5	320	5.00	75.0
Oven Temperature Program				
Ramp	**RT (min)**	**Rate (°C/min)**	**Target Temp. (°C)**	**Hold Time (min)**
Initial	0	-	40	1.50
1	1.5	25.0	90	1.50
2	5.0	25.0	180	0.00
3	8.6	5.0	280	0.00
Final	28.6	10.0	300	5.00
Run time	35.6	-	-	-
TSQ 9000 Mass Spectrometer Parameters				
Transfer Line (°C)	250			
Ionization Type	EI			
Ion Source (°C)	320			
Acquisition Mode	timed-SRM			
Tuning parameters	AEI SmartTune			
Collision gas and pressure (psi)	Argon at 70			
Peak Width (Da)	0.7 (both Q1 and Q3)			

Data processing

Data were acquired, processed, and reported using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, which allows instrument control, method development, quantitative/qualitative analysis, and customizable reporting all within one package.

Data review is highly customizable, allowing the user to display the information required on screen in real time. Furthermore, the flexibility of Chromeleon CDS software ensures that SANTE[3] compliance criteria can easily by flagged, tracked, and reported to the user's individual requirements.

Results and discussion

Compliance with EU SANTE criteria

The method performance was tested in accordance to the SANTE/11813/2017 guidance document, which requires that the following criteria are satisfied for identification of pesticide residues:

I. A minimum of two product ions are detected for each pesticide with peak S/N >3 (or, in case noise is absent, a signal should be present in at least five subsequent scans) and with the mass resolution for precursor-ion isolation equal to or better than unit mass resolution.

II. Retention time tolerance of ± 0.1 minutes compared with standards in the same sequence.

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

Wherever SANTE compliance is referenced in this study, all three criteria have been met fully.

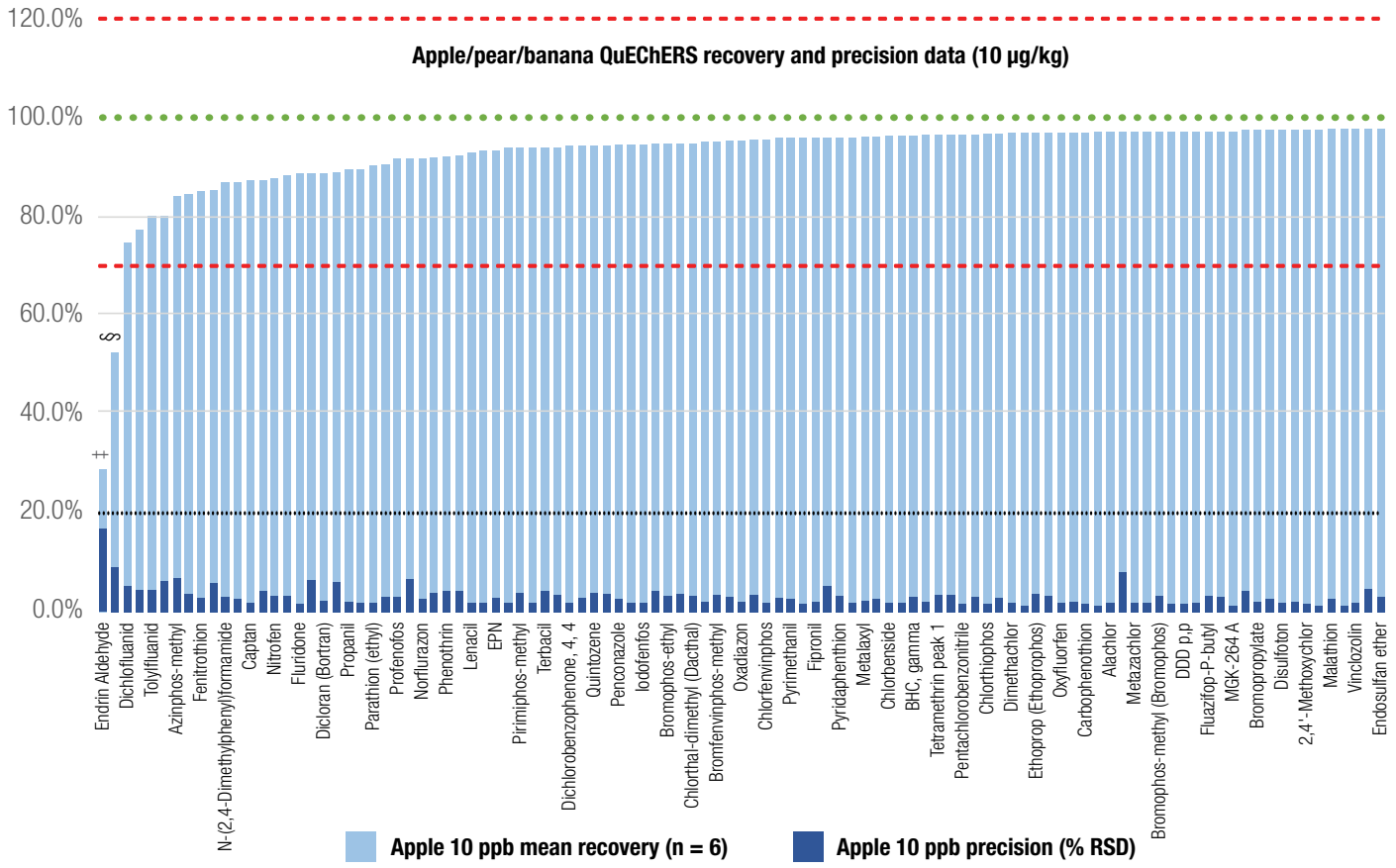
Recoveries

Pesticide recoveries were obtained from the QuEChERS extractions performed on the samples spiked before extraction. All detected compounds, at the three spiking levels in both matrices satisfied all SANTE requirements. More than 97% of the target pesticide residues at 1 µg/kg had recoveries between 70% and 120%. An example of the recovery and precision data for the apple/pear/banana matrix spiked at the default MRL (10 µg/kg) is displayed in Figure 1. A full table of results can be found in Appendix B.

Chromatography and selectivity

Analysis of a large number of pesticides in a single injection requires careful optimization of parameters, especially when injecting acetonitrile. As acetonitrile is a low molecular weight low polarity solvent, it has a relatively high expansion volume and is insoluble in the low polarity phases normally used for routine pesticide analysis (this makes solvent focusing in a standard splitless type injection incredibly difficult). These issues can be addressed by using an optimized programmable temperature vaporisation (PTV) injection. Figure 2 shows an example of three pesticides eluting in the beginning (A–dichlobenil – 0.025 µg/kg), middle (B–dieldrin 0.5 µg/kg), and end (C–deltamethrin 0.05 µg/kg) of the chromatographic run in the lowest detectable standard in carrot and potato matrix, levels at which all compounds detected meet the SANTE requirements. Peak shapes were Gaussian and coefficient of determination (R^2) was >0.990 for all three compounds indicating good chromatography and excellent linear response.

Identification of all 210 component peaks was made using an in-house, commercially available Thermo Scientific SRM pesticide compound database (cdb). In addition to this, retention time alignment of target compounds can be easily performed using the Thermo Scientific RTA tool,[8] eliminating the need for manual correction of compound retention times whenever column maintenance is performed. The cdb database contains >1000 compounds with >3700 unique SRM transitions. Due to the fast scanning speed of the EvoCell technology and the intelligent scheduling of the timed-SRM,[9] it is possible to acquire data with several transitions per compound with minimal loss in sensitivity. This makes it simple to select the most optimal transitions in differing matrices to perform quantitation and qualification on, removing the need to develop matrix-matched SRM compound databases (Figure 3).



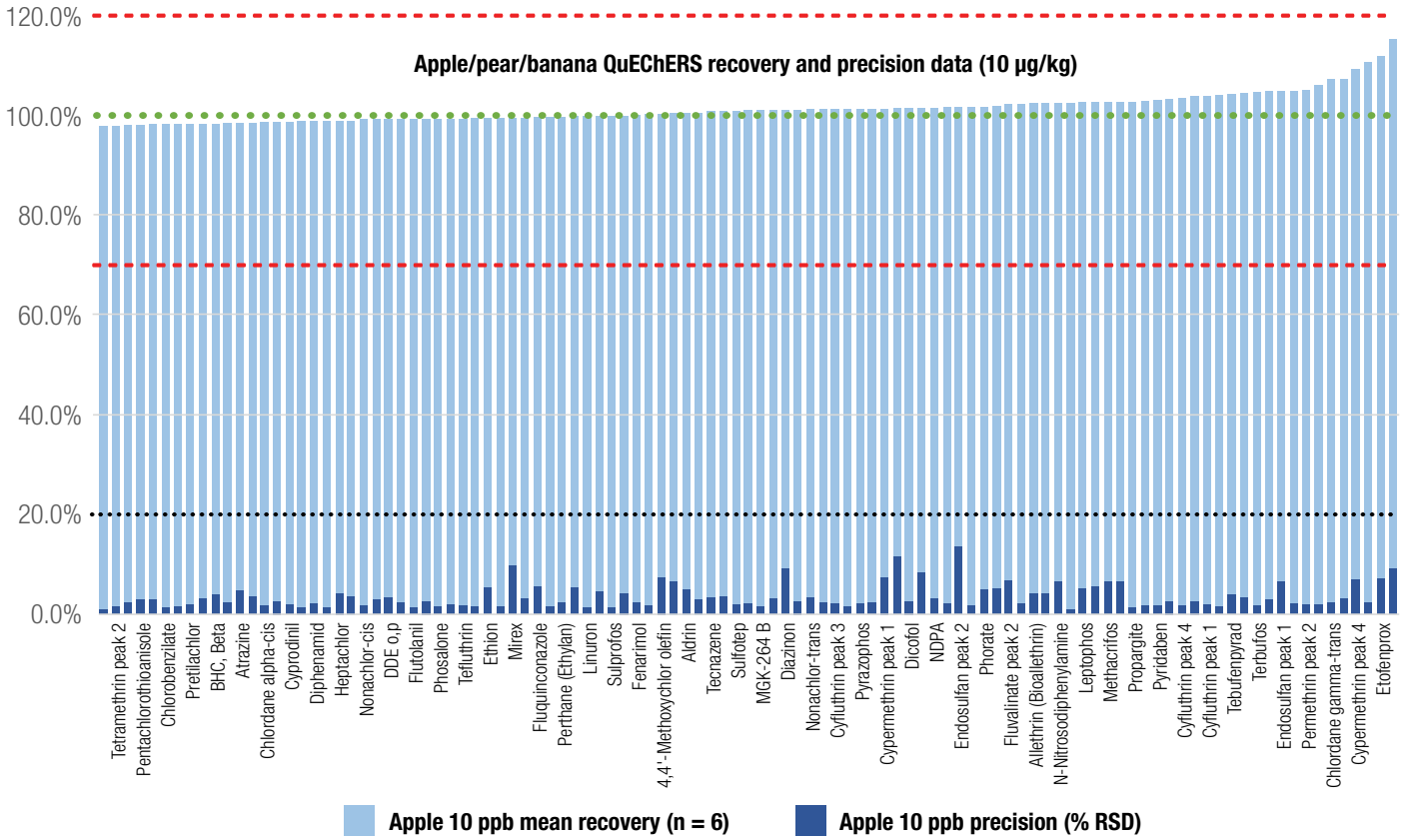


Figure 1. Recovery and precision data for apple/pear/banana extractions (n=6) at a concentration of 10 µg/kg. ‡ Endrin aldehyde recoveries were low, potentially due to reaction with PSA. § Recoveries of chlorothalonil, known to be problematic in QuEChERS extractions,[7] were low.

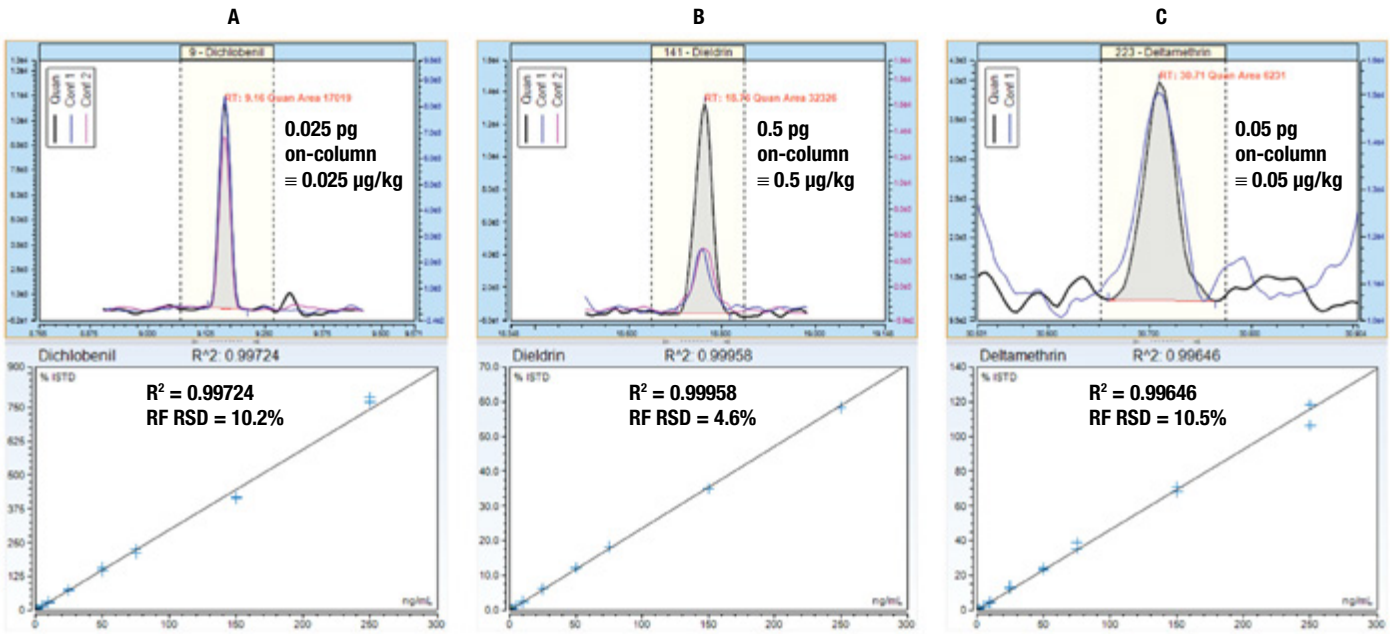


Figure 2. Example (A - Dichlobenil, B - dieldrin and C – deltamethrin) chromatographic peaks showing the lowest detectable matrix matched standard which meets SANTE requirements. The MRLs are 10 µg/kg, 3 µg/kg* and 10 µg/kg respectively. Calibration curves show duplicate injection at 14 discrete levels ranging from 0.025 pg to 250 pg on column. * Dieldrin is classed as a prohibited pesticide and 3 µg/kg considered to be the current limit of quantification, but is subject to regular review.[4]

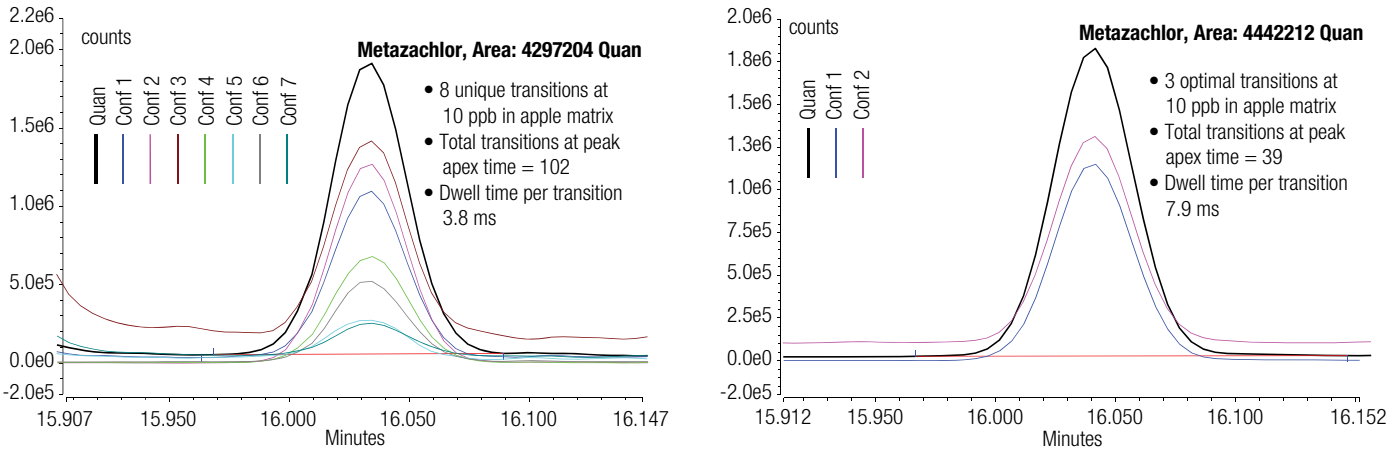


Figure 3. Comparison of Metazachlor SRM chromatographic peaks acquired using an injection containing 1317 unique transitions (left, 8 transitions) and an injection containing 663 (right, 3 transitions). No significant difference in the peak area for quantitation transition is observed indicating no loss in sensitivity.

Sensitivity and linearity

The TSQ 9000 AEI system easily met SANTE criteria (ion ratios ±30%, etc.) at the default MRL of 10 µg/kg for all pesticides targeted. Moreover, over 90% of pesticides detected at < 0.5 µg/kg meet the SANTE requirements and 10% of them meet SANTE criteria even at 0.025 µg/kg level (Figure 4). Resolution settings of 0.7 Daltons for Q1 and Q3 were used, ensuring the optimum combination of selectivity and sensitivity.

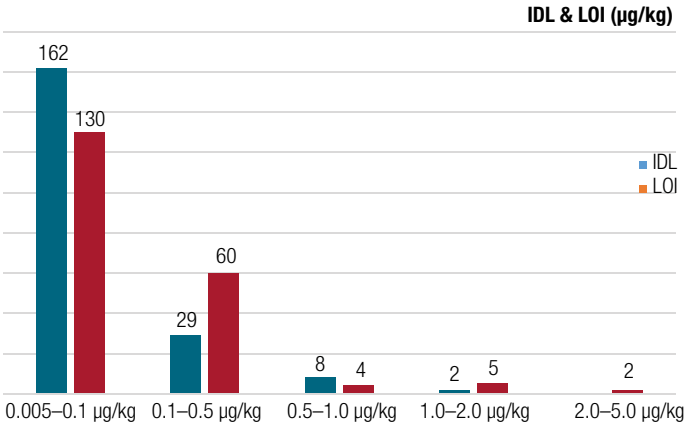


Figure 4. Number of target compounds satisfying the SANTE requirements, with over 90% below 0.5 µg/kg, and over 60% below 0.1µg/kg – 100 times lower than the default MRL [sample matrix – carrot/potato].

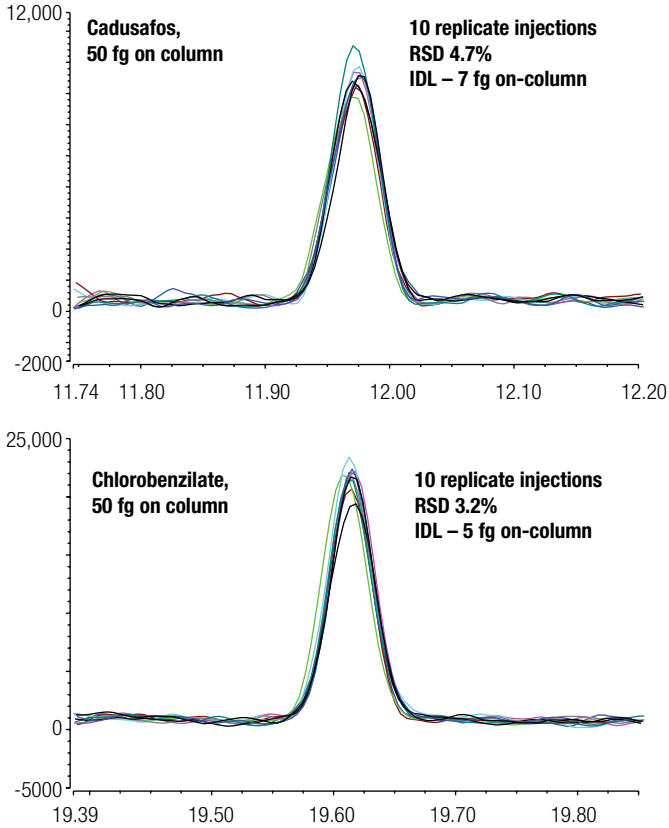


Figure 5. Example quantification SRM overlays of cadusafos and chlorbenzilate injected at the lowest level that met all SANTE criteria. Annotated are on column concentration, %RSD derived from absolute peak area response and calculated IDLs.

Over 90% of the target compounds had a Limit of Identification (LOI) (satisfying all SANTE requirements) below 0.5 µg/kg, and over 60% below 0.1 µg/kg.

System sensitivity, defined as instrumental detection limits (IDLs), was determined experimentally for each compound by performing n=10 replicate injections of the lowest matrix-matched standard of carrot and potato that met all SANTE criteria. Calculations of IDLs were then made using one-tailed student *t*-test at the 99% confidence interval for the corresponding degrees of freedom and taking into account the concentration and absolute peak area %RSD for each compound (Figures 5 and 6).

Fipronil and fipronil-desulfinyl, expressed as fipronil, have a multi-component MRL specified at 4 µg/kg. Therefore, to satisfy the current regulations, each component must be identified at 2 µg/kg. Figure 7 shows fipronil and fipronil-desulfinyl at concentrations of 0.2 µg/kg, ten times lower than the requisite MRL, with back-calculated concentrations versus the linear calibration annotated.

Compound linearity was assessed by injecting matrix-matched standards in the range of 0.025 to 250 µg/kg in duplicate for both carrot/potato and apple/pear/banana. Both sets of linearity data showed $R^2 > 0.990$ and response factor (RF) % RSDs of <20% for over 96% of component peaks indicating excellent linear response. Examples of linearity are shown in Figure 2 and in a comprehensive table provided in Appendix A.

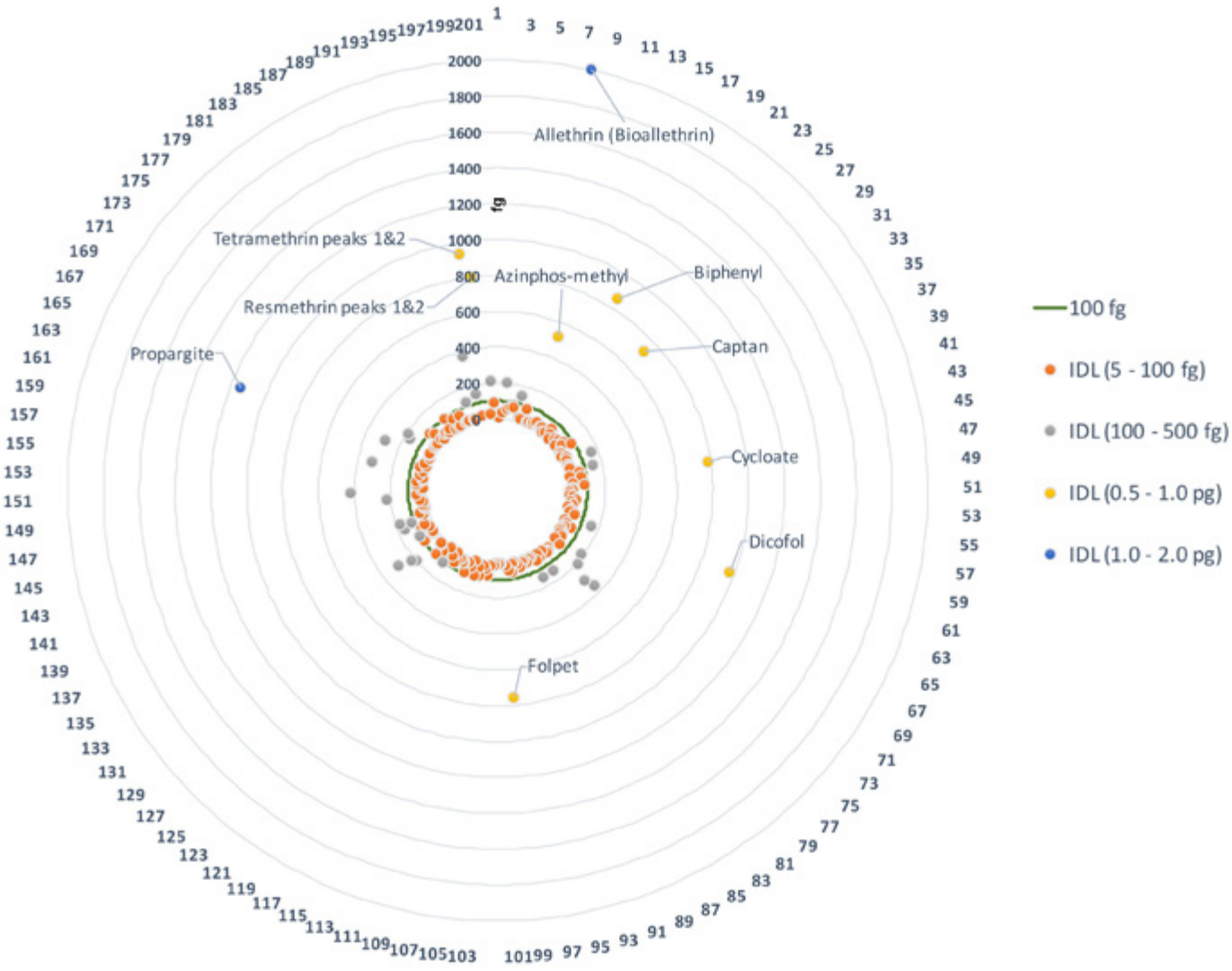


Figure 6. Plot showing the calculated IDLs for all pesticides. IDLs ranged from ~5 fg (chlorobenzilate) to ~2.0 pg (bioallethrin) with >95% of compounds showing an IDL of less than 500 fg on column (equivalent to 0.5 µg/kg in sample extract). See Appendix A for tabulated data.

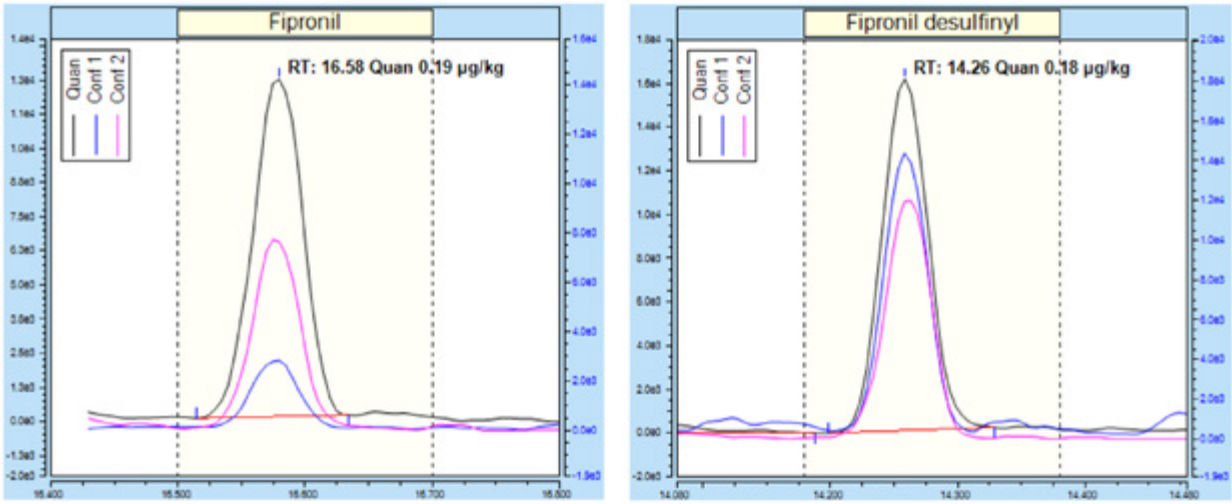


Figure 7. Fipronil and fiproni desulfinyl, at a concentration of 0.2 µg/kg equating to 0.4 µg/kg fipronil (sum), with SANTE compliance throughout.

AEI source robustness

The TSQ 9000 AEI system was set up as described in Table 1. After an initial source cleaning, repeat injections of a QuEChERS sample extract (1 g/mL carrot and potato) spiked at the default MRL (10 µg/kg) were made (Figure 8). Extracts resulting from the QuEChERS methodology contain many undesirable matrix co-extracted components which can easily contaminate the GC inlet, the chromatographic column and the MS ion source. To test the robustness of the AEI ion source only (as far as reasonably practicable), after every 100 sample injections, the PTV liner was replaced along with the injector septum, approximately 10 cm was trimmed from the head of the guard column followed by automatic tuning of the system using the SmartTune feature. SmartTune uses the MS parameters established during the initial tuning on a clean source and intelligently assess the performance of the system, only re-tuning when MS performance has been compromised. No additional maintenance was performed.

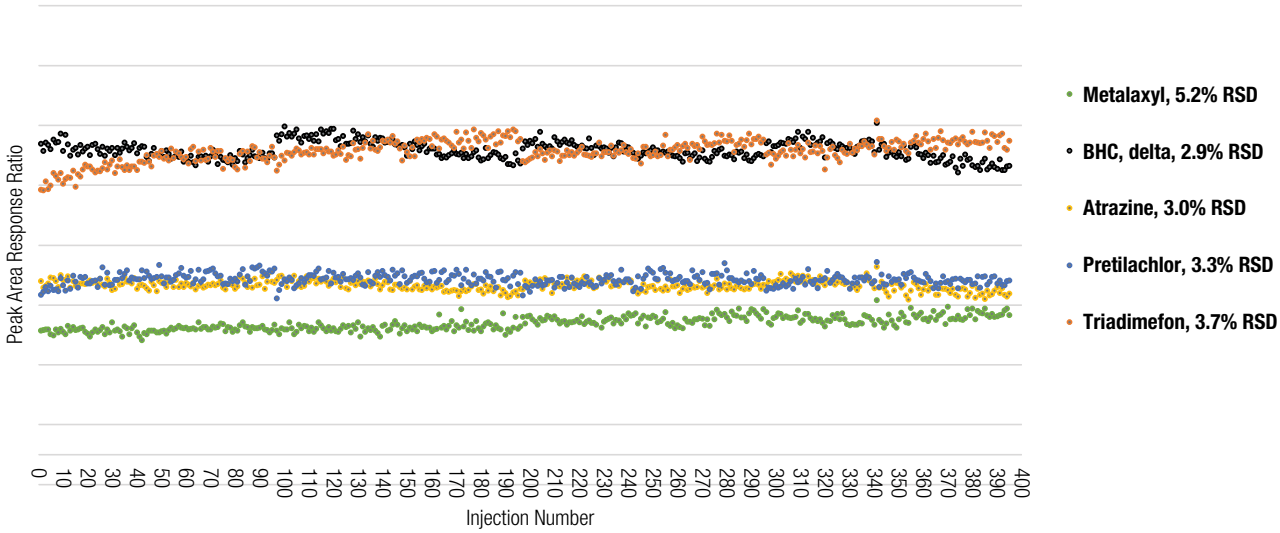


Figure 8A. Example of normalised peak area response for selected compounds across ~400 consecutive injections at the default MRL (10 µg/kg) in carrot/potato matrix.

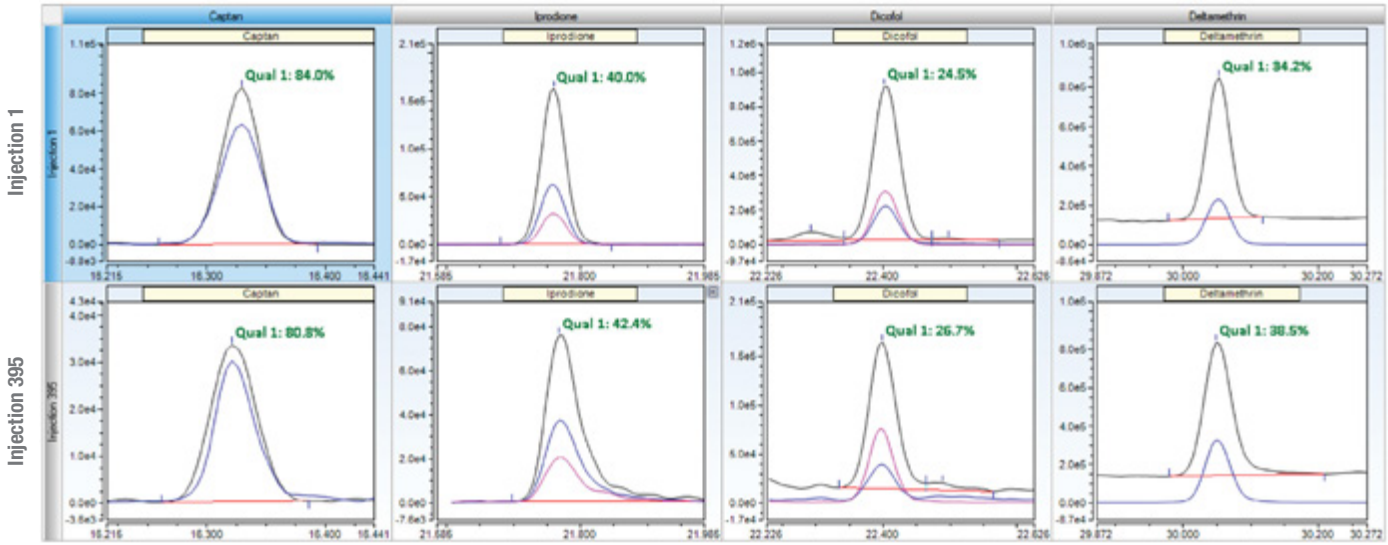


Figure 8B. Peak shapes, intensities and ion ratios of the primary qualifier ion for injection 1 (top row) and injection 395 (bottom row) for captan, iprodione, dicofol and deltamethrin.

Ion ratios at the default MRL were stable, Figure 9 shows pretilachlor ion qualifier ratios 1 and 2 in the first and last batches of injections. Ratios were well within the ±30% SANTE identification criteria.

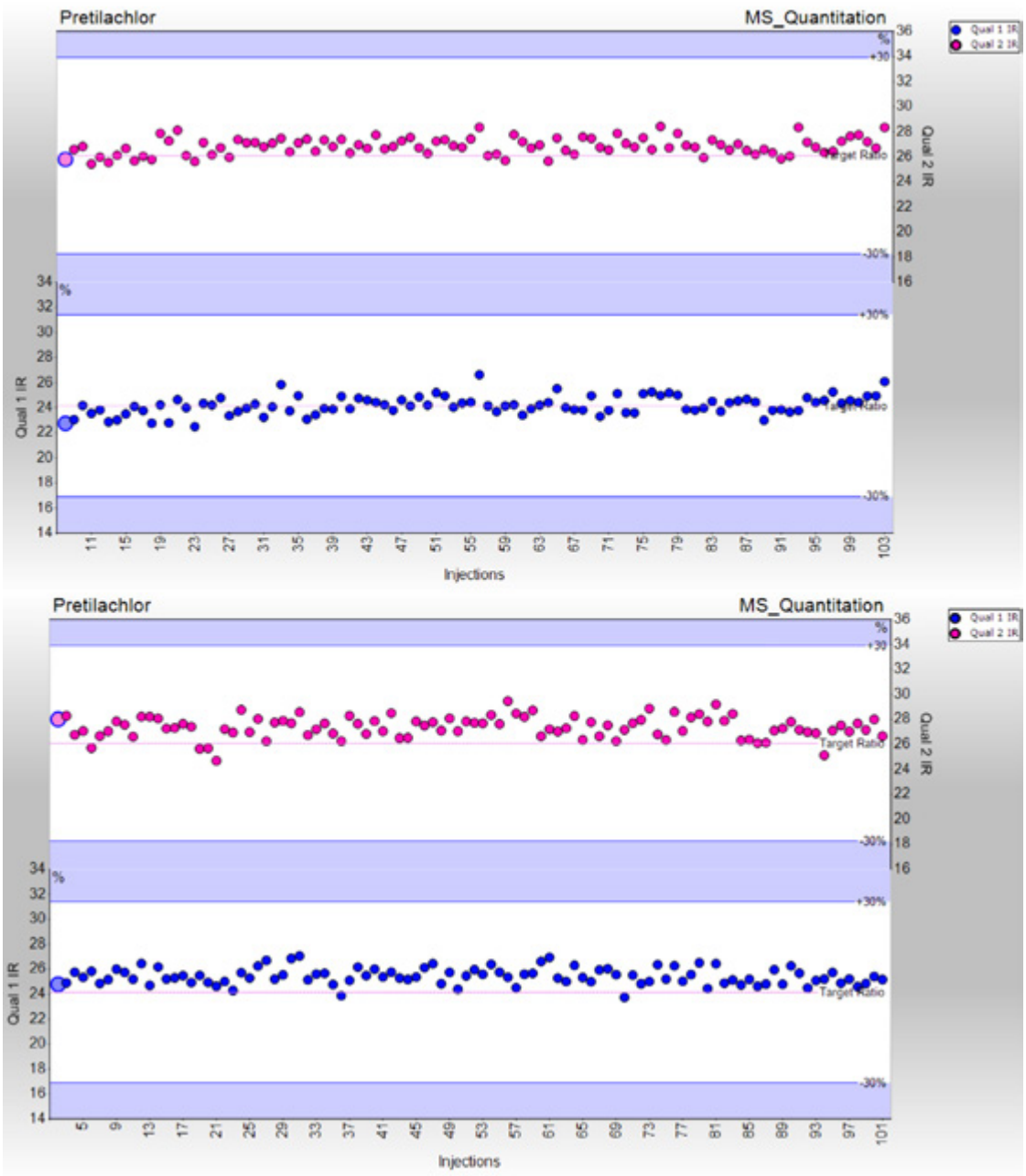


Figure 9. Pretilachlor Ion ratios of robustness injections 1-95 (top) and 295-395 (bottom).

Conclusions

In this work it has been demonstrated that by using QuEChERS with Thermo Scientific HyperSep dSPE products and a direct injection of acetonitrile extracts, the TSQ 9000 AEI system delivers outstanding quantitative performance for low-level pesticide residue analysis in baby food.

- QuEChERS extraction and subsequent clean-up of over 200 pesticides from replicate analysis (n=6 each at three concentrations) of each of two sample matrices, demonstrating excellent accuracy (recovery) and precision.
- Accurate, quantitative analysis of over 200 pesticides over up to five orders of magnitude (0.025–250 µg/kg), showing outstanding LODs and linear response.
- Robustness displayed over approximately 400 consecutive injections of sample matrix (1 g/mL), with SANTE compliance at the default MRL throughout.
- High sensitivity providing the real possibility to dilute the sample extract, thus limiting matrix contamination and system maintenance, leading to an increase in laboratory productivity.

The results of this study establish the TSQ 9000 triple quadrupole GC-MS/MS system, in combination with Chromeleon CDS software and HyperSep dSPE products, as the ideal solution for the routine analysis of pesticides in baby food, providing unprecedented sensitivity, robustness, ease of use, cost effectiveness, and reliability.

References

1. https://ec.europa.eu/food/plant/pesticides/max_residue_levels/guidelines_en
2. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=URISERV:l13002a
3. SANTE/11813/2017. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Supersedes SANTE/11945/2015. Implemented by 01/01/2018. https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf
4. http://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A32006L0125
5. http://quechers.cvua-stuttgart.de/
6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4890451/
7. Belmonte Valles, N.; Retamal, M.; Martinez-Uroz, M.A.; Mezcua, M.; Fernandez-Alba, A.R.; de Kok, A. Determination of chlorothalonil in difficult-to-analyse vegetable matrices using various multiresidue methods. Analyst, 2012, 137(10), 2513–2520.
8. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-10580-gc-ms-ms-ptv-pesticides-water-an10580-en.pdf
9. http://tools.thermofisher.com/content/sfs/posters/PN-10394-Fast-SRM-Transition-PN10394-EN.pdf

Appendix A – Linearity data sets

Appendix A (Part 1). Linearity data sets.

Chart Number	Compound Name	Apple/Pear/Banana Linearity			Carrot/Potato Linearity			IDL_{99}	
		R^2	RF RSD(%)	Range (ppb)	R^2	RF RSD(%)	Range (ppb)	pg on Column	IDL (fg)
1	2,3,5,6-Tetrachloroaniline	0.99931	6.80	0.1–250	0.99955	7.70	0.05–250	0.05	20
2	2,4'-Methoxychlor	0.99987	3.50	0.1–250	0.99950	7.30	0.025–250	0.05	8
3	4,4'-Methoxychlor olefin	0.99976	5.80	0.1–250	0.99932	4.20	0.05–250	0.05	31
4	Acetochlor	0.99972	3.60	0.2–250	0.99962	3.60	0.2–250	0.20	201
5	Acrinathrin	0.99963	2.70	0.2–250	0.99955	3.90	0.2–250	0.20	57
6	Alachlor	0.99965	5.50	0.1–250	0.99955	5.10	0.2–250	0.20	71
7	Aldrin	0.99959	8.00	0.1–250	0.99983	4.60	0.1–250	0.20	75
8	Allethrin (Bioallethrin)	0.99826	19.40	10–250	0.99888	7.50	5–250	5.00	2007
9	Allidochlor	0.99926	15.20	0.1–250	0.99631	6.30	0.2–250	0.20	145
10	Anthraquinone	0.99966	9.80	0.2–250	0.99988	17.60	0.025–250	0.05	27
11	Atrazine	0.99963	7.40	0.1–250	0.99990	6.70	0.05–250	0.05	19
12	Azinphos-ethyl	0.99465	9.40	0.2–250	0.99935	4.50	0.2–250	0.20	82
13	Azinphos-methyl	0.98165	16.90	1–250	0.99758	19.60	0.5–250	1.00	521
14	BHC, Alpha	0.99981	5.30	0.025–250	0.99949	6.60	0.025–250	0.05	15
15	BHC, Beta	0.99967	6.20	0.05–250	0.99985	8.80	0.025–250	0.05	15
16	BHC, delta	0.99971	4.10	0.05–250	0.99992	7.60	0.025–250	0.05	20
17	BHC, gamma	0.99970	7.30	0.05–250	0.99971	6.70	0.05–250	0.05	31
18	Bifenthrin	0.99989	4.20	0.5–250	0.99976	2.40	0.5–250	1.00	42
19	Biphenyl	0.99822	19.50	2–250	0.99573	14.50	5–250	5.00	865
20	Bromfenvinphos	0.99963	5.10	0.05–250	0.99960	7.60	0.025–250	0.05	31
21	Bromfenvinphos-methyl	0.99917	3.20	0.5–250	0.99971	3.60	0.1–250	0.20	33
22	Bromophos-ethyl	0.99946	3.30	0.1–250	0.99523	5.90	0.05–250	0.05	12
23	Bromophos-methyl (Bromophos)	0.99957	5.60	0.05–250	0.99848	5.90	0.05–250	0.05	24
24	Bromopropylate	0.99960	4.80	0.1–250	0.99806	5.40	0.1–250	0.20	61
25	Bupirimate	0.99947	10.10	0.05–250	0.99961	5.50	0.05–250	0.05	33
26	Cadusafos	0.99982	3.80	0.1–250	0.99952	6.40	0.025–250	0.05	7
27	Captan	0.98233	23.80	1–250	0.98303	16.60	0.5–250	1.00	733
28	Carbophenothion	0.99968	3.50	0.2–250	0.99970	4.40	0.1–250	0.20	30
29	Carfentrazon-ethyl	0.99929	6.10	0.2–250	0.99575	7.50	0.1–250	0.20	41
30	Chlorbenside	0.99981	5.50	0.025–250	0.99984	3.20	0.025–250	0.05	11
31	Chlordane alpha-cis	0.99875	8.10	0.05–250	0.98923	8.10	0.1–250	0.20	61
32	Chlordane gamma-trans	0.99949	6.50	0.05–250	0.99956	7.30	0.025–250	0.05	38
33	Chlorfenapyr	0.99979	6.10	0.2–250	0.99983	3.30	0.2–250	0.20	90
34	Chlorfenson	0.99986	3.60	0.025–250	0.99979	2.30	0.025–250	0.05	10
35	Chlorfenvinphos	0.99966	8.60	0.05–250	0.99987	5.70	0.025–250	0.05	16
36	Chlorobenzilate	0.99990	3.10	0.025–250	0.99976	3.70	0.025–250	0.05	5
37	Chloroneb	0.99962	4.70	0.5–250	0.99907	10.90	0.1–250	0.20	28
38	Chlorothalonil	0.99752	7.40	0.1–250	0.99635	18.40	0.05–250	0.05	24
39	Chlorpropham	0.99985	13.90	0.5–250	0.99981	12.60	2–250	5.00	166
40	Chlorpyrifos-ethyl	0.99948	15.00	0.1–250	0.99916	5.50	0.05–250	0.05	22

Appendix A (Part 2). Linearity data sets.

Chart Number	Compound Name	Apple/Pear/Banana Linearity			Carrot/Potato Linearity			IDL_{99}	
		R^2	RF RSD(%)	Range (ppb)	R^2	RF RSD(%)	Range (ppb)	pg on Column	IDL (fg)
41	Chlorpyrifos-methyl	0.99969	12.30	0.1–250	0.99960	15.70	0.025–250	0.05	24
42	Chlorthal-dimethyl	0.99917	6.20	0.1–250	0.99912	5.80	0.025–250	0.05	20
43	Chlorthiophos	0.99976	7.20	0.1–250	0.99971	3.00	0.2–250	0.20	153
44	Chlozolinate	0.99981	7.90	0.2–250	0.99988	4.30	0.2–250	0.20	67
45	Clomazone	0.99983	4.30	0.025–250	0.99993	6.40	0.025–250	0.05	12
46	Coumaphos	0.99817	4.80	0.5–250	0.99966	8.50	0.2–250	0.20	78
47	Cycloate	0.99815	13.10	2–250	0.99890	4.80	2–250	5.00	789
48	Cyhalothrin I (lambda)	0.99966	7.50	0.2–250	0.99959	2.90	0.2–250	0.20	28
49	Cyprodinil	0.99983	5.00	0.1–250	0.99993	3.90	0.1–250	0.20	86
50	DDD p,p	0.99985	4.30	0.05–250	0.99992	4.00	0.025–250	0.05	22
51	DDD, o, p	0.99988	3.00	0.05–250	0.99985	7.50	0.05–250	0.05	20
52	DDE o,p	0.99974	3.80	0.025–250	0.99976	4.30	0.025–250	0.05	8
53	DDE p, p	0.99957	2.60	0.05–250	0.99989	4.20	0.05–250	0.05	9
54	DDT o,p	0.99988	5.70	0.05–250	0.99960	9.20	0.05–250	0.05	25
55	DDT p,p	0.99962	8.40	0.2–250	0.99937	14.40	0.1–250	0.20	41
56	Deltamethrin	0.99983	6.70	0.05–250	0.99646	11.00	0.05–250	0.05	22
57	Diazinon	0.99949	5.20	0.1–250	0.99906	5.00	0.1–250	0.20	33
58	Dichlobenil	0.99866	5.10	0.025–250	0.99724	10.20	0.025–250	0.05	5
59	Dichlofluanid	0.99949	6.40	0.2–250	0.99966	4.40	0.1–250	0.20	28
60	Dichlorobenzophenone, 4, 4*	0.99979	2.80	0.05–250	0.99957	7.50	0.025–250	0.05	17
61	Dicloran (Bortran)	0.99908	7.10	0.2–250	0.99801	7.00	0.2–250	0.20	58
62	Dicofol*	0.99272	13.50	0.5–250	0.98598	14.70	0.5–250	1.00	973
63	Dieldrin	0.99909	7.20	0.5–250	0.99958	4.60	0.5–250	1.00	162
64	Dimethachlor	0.99964	5.10	0.025–250	0.99968	4.60	0.025–250	0.05	10
65	Dimethoate	0.99903	6.30	0.2–250	0.99973	10.40	0.1–250	0.20	30
66	Diphenamid	0.99974	7.30	0.2–250	0.99974	5.20	0.2–250	0.20	62
67	Diphenylamine	0.99981	9.50	0.2–250	0.99931	17.00	0.1–250	0.20	28
68	Disulfoton	0.99982	6.80	0.2–250	0.99943	4.30	0.2–250	0.20	19
69	Edifenphos	0.99908	4.00	0.05–250	0.99967	11.50	0.025–250	0.05	10
70	Endosulfan ether	0.99983	5.70	0.05–250	0.99982	12.20	0.025–250	0.05	21
71	Endosulfan peak 1	0.99963	4.50	0.2–250	0.99989	4.50	0.2–250	0.20	42
72	Endosulfan peak 2	0.99982	5.20	0.5–250	0.99988	4.00	0.5–250	1.00	190
73	Endosulfan sulfate	0.99981	3.10	0.1–250	0.99980	3.60	0.05–250	0.05	20
74	Endrin	0.99981	3.70	0.5–250	0.99975	4.80	0.2–250	0.20	59
75	Endrin Aldehyde	0.99893	7.30	0.5–250	0.99786	9.30	0.5–250	1.00	209
76	Endrin-Ketone	0.99920	6.00	0.5–250	0.99872	5.80	0.5–250	1.00	353
77	EPN	0.99591	7.10	1–250	0.99334	14.00	1–250	1.00	302
78	Ethion	0.99981	3.60	0.1–250	0.99987	3.90	0.05–250	0.05	29
79	Ethoprop (Ethoprophos)	0.99975	6.10	0.05–250	0.99923	6.40	0.1–250	0.20	39
80	Etofenprox	0.99978	7.40	0.2–250	0.99992	3.70	0.2–250	0.20	42

* - 4,4-dichlorobenzophenone is a breakdown product of dicofol and therefore may be overestimated.

Appendix A (Part 3). Linearity data sets.

Chart Number	Compound Name	Apple/Pear/Banana Linearity			Carrot/Potato Linearity			IDL_{99}	
		R^2	RF RSD(%)	Range (ppb)	R^2	RF RSD(%)	Range (ppb)	pg on Column	IDL (fg)
81	Etridiazole (Terrazole)	0.99954	5.50	0.1–250	0.99599	16.30	0.025–250	0.05	18
82	Fenamiphos	0.99958	3.00	0.5–250	0.99848	3.70	0.5–250	1.00	147
83	Fenarimol	0.99984	3.20	0.2–250	0.99990	2.60	0.2–250	0.20	45
84	Fenchlorfos	0.99964	3.90	0.05–250	0.99956	4.80	0.05–250	0.05	17
85	Fenitrothion	0.99241	13.50	0.1–250	0.99559	13.30	0.1–250	0.20	52
86	Fenpropathrin	0.99969	3.70	0.5–250	0.99976	5.00	1–250	1.00	147
87	Fenson	0.99989	4.40	0.05–250	0.99995	5.60	0.025–250	0.05	11
88	Fenthion	0.99959	10.20	0.05–250	0.99970	10.60	0.05–250	0.05	18
89	Fenvalerate	0.99992	3.10	0.1–250	0.99974	5.80	0.1–250	0.20	36
90	Fipronil	0.99923	5.10	0.1–250	0.99405	8.90	0.05–250	0.05	20
91	Fipronil desulfinyl	0.99826	5.60	0.05–250	0.98489	10.00	0.05–250	0.05	27
92	Fluazifop-P-butyl	0.99971	7.40	0.1–250	0.99976	10.50	0.1–250	0.20	45
93	Fludioxonil	0.99980	9.90	0.05–250	0.99951	8.00	0.025–250	0.05	29
94	Fluquinconazole	0.99953	7.70	0.05–250	0.99609	7.40	0.025–250	0.05	15
95	Fluridone	0.99890	13.60	0.2–250	0.99593	7.10	0.025–250	0.05	22
96	Flusilazole	0.99969	11.20	0.1–250	0.99982	9.00	0.1–250	0.20	66
97	Flutolanil	0.99966	5.80	0.05–250	0.99982	9.30	0.025–250	0.05	51
98	Flutriafol	0.99960	7.20	0.1–250	0.99989	9.50	0.025–250	0.05	24
99	Folpet	0.97866	23.90	0.5–250	0.98874	14.20	0.2–250	0.20	757
100	Fonofos	0.99970	4.80	0.05–250	0.99986	4.20	0.05–250	0.05	14
101	Heptachlor	0.99963	4.20	0.025–250	0.99976	6.40	0.025–250	0.05	7
102	Hexachlorobenzene	0.99862	7.80	0.025–250	0.99939	5.60	0.025–250	0.05	11
103	Hexazinone	0.99971	7.20	0.1–250	0.99983	5.50	0.05–250	0.05	18
104	Iodofenfos	0.99859	6.10	0.05–250	0.99012	11.60	0.05–250	0.05	19
105	Iprodione	0.99976	7.70	0.2–250	0.99536	20.80	0.1–250	0.20	80
106	Isazophos	0.99934	6.60	0.1–250	0.99945	12.10	0.1–250	0.20	46
107	Isodrin	0.99983	5.90	0.1–250	0.99992	6.20	0.1–250	0.20	26
108	Lenacil	0.99928	7.90	0.2–250	0.99971	5.30	0.1–250	0.20	83
109	Leptophos	0.99947	3.30	0.2–250	0.99909	3.80	0.2–250	0.20	36
110	Linuron	0.99913	8.00	0.5–250	0.99831	8.70	0.2–250	0.20	92
111	Malathion	0.99989	5.70	0.05–250	0.99972	6.10	0.025–250	0.05	12
112	Metalaxyl	0.99947	4.70	0.2–250	0.99985	21.80	0.1–250	0.20	54
113	Metazachlor	0.99958	3.80	0.1–250	0.99978	8.10	0.025–250	0.05	32
114	Methacrifos	0.99977	4.50	0.2–250	0.99819	4.80	0.2–250	0.20	91
115	Methoxychlor	0.99918	4.50	0.1–250	0.99921	5.40	0.1–250	0.20	38
116	Metolachlor	0.99978	4.20	0.05–250	0.99992	4.20	0.025–250	0.05	49
117	Mevinphos	0.99985	3.80	0.05–250	0.99937	4.60	0.1–250	0.20	31
118	MGK-264 A	0.99986	5.00	0.2–250	0.99966	4.00	0.2–250	0.20	51
119	MGK-264 B	0.99984	4.50	0.2–250	0.99974	4.40	0.2–250	0.20	65
120	Mirex	0.99980	4.60	0.025–250	0.99981	3.00	0.025–250	0.05	8

Appendix A (Part 4). Linearity data sets.

Chart Number	Compound Name	Apple/Pear/Banana Linearity			Carrot/Potato Linearity			IDL_{99}	
		R^2	RF RSD(%)	Range (ppb)	R^2	RF RSD(%)	Range (ppb)	pg on Column	IDL (fg)
121	Myclobutanil	0.99978	1.90	0.1–250	0.99986	3.50	0.1–250	0.20	40
122	N-(2,4-Dimethylphenyl) formamide	0.99953	4.80	1–250	0.99982	8.30	1–250	1.00	106
123	NDBA	0.99866	18.00	0.5–250	0.99414	21.60	0.05–250	0.05	12
124	NDEA	0.99826	6.90	0.1–250	0.98989	9.80	0.2–250	0.20	74
125	NDPA	0.99865	8.70	0.5–250	0.99133	8.80	0.1–250	0.20	49
126	NEMA	0.99657	8.00	1–250	0.98500	12.80	0.2–250	0.20	87
127	Nitrofen	0.99590	13.50	0.05–250	0.99512	15.00	0.2–250	0.20	30
128	*N*-Nitrosodiphenylamine	0.99971	12.70	0.2–250	0.99931	17.00	0.1–250	0.20	28
129	*N*-Nitrosomorpholine	0.99759	18.50	0.5–250	0.99342	20.00	0.5–250	1.00	198
130	*N*-Nitrosopiperidine	0.99738	7.90	0.5–250	0.99368	14.20	0.5–250	1.00	217
131	*N*-Nitrosopyrrolidine	0.99830	14.70	1–250	0.99359	8.00	0.5–250	1.00	289
132	Nonachlor-cis	0.99591	7.20	0.2–250	0.97664	11.80	0.1–250	0.20	90
133	Nonachlor-trans	0.99924	4.70	0.1–250	0.99968	6.70	0.1–250	0.20	28
134	Norflurazon	0.99886	7.60	0.2–250	0.99937	5.10	0.05–250	0.05	23
135	Ortho-phenylphenol	0.99979	20.20	0.5–250	0.99957	14.70	0.5–250	1.00	102
136	Oxadiazon	0.99964	5.80	0.025–250	0.99970	14.10	0.1–250	0.20	22
137	Oxyfluorfen	0.99610	9.30	0.5–250	0.99445	18.80	0.5–250	1.00	73
138	Paclobutrazol	0.99977	5.40	0.05–250	0.99991	6.70	0.05–250	0.05	49
139	Parathion (ethyl)	0.99534	11.00	0.5–250	0.99395	19.40	0.5–250	1.00	158
140	Parathion-methyl	0.99478	12.00	0.05–250	0.99736	8.20	0.2–250	0.20	111
141	Pebulate	0.99885	14.30	0.5–250	0.99691	13.30	0.5–250	1.00	171
142	Penconazole	0.99985	6.30	0.05–250	0.99992	7.90	0.05–250	0.05	37
143	Pentachloroaniline	0.99973	6.10	0.1–250	0.99961	4.60	0.05–250	0.05	52
144	Pentachloroanisole	0.99939	5.30	0.05–250	0.99956	11.30	0.025–250	0.05	18
145	Pentachlorobenzene	0.99665	8.50	0.025–250	0.99765	11.40	0.025–250	0.05	12
146	Pentachlorobenzonitrile	0.99984	5.10	0.05–250	0.99973	8.40	0.025–250	0.05	23
147	Pentachlorothioanisole	0.99951	8.40	0.025–250	0.99973	6.00	0.05–250	0.05	22
148	Perthane (Ethylan)	0.99994	8.00	0.05–250	0.99982	4.50	0.1–250	0.20	30
149	Permethrin peak 1	0.99971	9.90	1–250	0.99979	12.90	0.2–250	0.20	219
150	Permethrin peak 2	0.99970	6.10	0.5–250	0.99979	5.80	0.5–250	1.00	48
151	Phenothrin	0.99950	17.90	1–250	0.99972	7.40	2–250	5.00	413
152	Phorate	0.99964	3.10	0.5–250	0.99910	13.50	0.025–250	0.05	18
153	Phosalone	0.99862	8.80	0.05–250	0.99982	8.90	0.05–250	0.05	18
154	Phosmet	0.99738	7.00	0.5–250	0.99916	24.50	0.2–250	0.20	54
155	Piperonyl butoxide	0.99977	6.50	0.1–250	0.99990	4.90	0.1–250	0.20	51
156	Pirimiphos-ethyl	0.99964	3.00	0.05–250	0.99967	5.30	0.025–250	0.05	21
157	Pirimiphos-methyl	0.99949	5.50	0.05–250	0.99949	4.80	0.025–250	0.05	18
158	Pretilachlor	0.99984	3.60	0.2–250	0.99989	2.40	0.2–250	0.20	44
159	Prochloraz (parent)	0.99749	14.30	1–250	0.99920	7.80	0.5–250	1.00	320
160	Procymidone	0.99991	4.00	0.1–250	0.99969	7.50	0.05–250	0.05	26

Appendix A (Part 5). Linearity data sets.

Chart Number	Compound Name	Apple/Pear/Banana Linearity			Carrot/Potato Linearity			IDL_{99}	
		R^2	RF RSD(%)	Range (ppb)	R^2	RF RSD(%)	Range (ppb)	pg on Column	IDL (fg)
161	Profenofos	0.99938	9.40	0.1–250	0.99654	10.40	0.1–250	0.20	53
162	Propachlor	0.99977	1.60	1–250	0.99926	15.30	0.025–250	0.05	13
163	Propanil	0.99925	12.30	0.025–250	0.99974	3.70	0.1–250	0.20	32
164	Propargite	0.99970	3.30	2–250	0.99881	19.40	2–250	5.00	1143
165	Propisochlor	0.99972	21.10	0.2–250	0.99953	5.80	0.5–250	1.00	284
166	Propyzamide	0.99966	4.50	0.1–250	0.99985	4.20	0.025–250	0.05	15
167	Prothiofos	0.99881	10.50	0.1–250	0.99842	5.90	0.1–250	0.20	28
168	Pyraclofos	0.99656	15.00	0.1–250	0.99920	20.60	0.05–250	0.05	28
169	Pyrazophos	0.99911	13.30	0.5–250	0.99979	16.60	0.2–250	0.20	163
170	Pyridaben	0.99987	3.10	0.2–250	0.99988	2.50	0.2–250	0.20	186
171	Pyridaphenthion	0.99941	3.80	0.2–250	0.99699	8.90	0.1–250	0.20	24
172	Pyrimethanil	0.99985	19.10	0.1–250	0.99971	8.40	0.05–250	0.05	23
173	Pyriproxyfen	0.99979	9.20	0.1–250	0.99990	2.30	0.2–250	0.20	24
174	Quinalphos	0.99926	10.80	0.5–250	0.99925	4.60	0.5–250	1.00	88
175	Quintozene	0.99912	10.20	0.2–250	0.99774	15.00	0.2–250	0.20	72
176	Sulfotep	0.99970	9.80	0.025–250	0.99962	9.40	0.025–250	0.05	26
177	Sulprofos	0.99986	3.30	0.05–250	0.99850	5.20	0.025–250	0.05	12
178	Tebuconazole	0.99983	9.10	0.5–250	0.99994	4.70	0.025–250	0.05	30
179	Tebufenpyrad	0.99980	4.20	0.05–250	0.99976	4.10	0.05–250	0.05	37
180	Tecnazene	0.99958	8.90	0.05–250	0.99815	13.90	0.025–250	0.05	15
181	Tefluthrin	0.99982	12.50	0.025–250	0.99944	6.60	0.025–250	0.05	34
182	Terbacil	0.99929	7.30	0.2–250	0.99974	5.40	0.1–250	0.20	95
183	Terbufos	0.99973	4.90	0.1–250	0.99978	5.00	0.05–250	0.05	13
184	Terbuthylazine	0.99967	8.60	0.2–250	0.99982	6.10	0.1–250	0.20	72
185	Tetrachlorvinphos	0.99941	8.20	0.05–250	0.99651	10.10	0.025–250	0.05	13
186	Tetradifon	0.99988	3.80	0.2–250	0.99990	17.50	0.025–250	0.05	17
187	Tetrahydrophthalimide (THPI)	0.99744	10.40	0.5–250	0.99985	6.00	0.5–250	1.00	67
188	Tolclofos-methyl	0.99985	4.70	0.05–250	0.99986	10.50	0.05–250	0.05	18
189	Tolylfluanid	0.99911	7.30	0.1–250	0.99952	7.90	0.1–250	0.20	27
190	Triadimefon	0.99965	8.80	0.05–250	0.99973	7.50	0.05–250	0.05	18
191	Triadimenol	0.99983	15.90	0.5–250	0.99973	11.20	0.5–250	1.00	116
192	Triallate	0.99983	2.20	0.1–250	0.99984	6.10	0.025–250	0.05	18
193	Triazophos	0.99937	6.30	0.1–250	0.99983	5.60	0.05–250	0.05	16
194	Tricyclazole	0.99883	9.50	2–250	0.99947	4.50	0.5–250	1.00	367
195	Triflumizole	0.99976	6.70	0.2–250	0.99978	6.00	0.5–250	1.00	147
196	Vinclozolin	0.99967	10.10	0.05–250	0.99969	5.50	0.05–250	0.05	22
197	Tetramethrin peaks 1&2	N/A	N/A	0.5–250	N/A	N/A	0.5–250	5.00	929
198	Resmethrin peaks 1&2	N/A	N/A	2–250	N/A	N/A	2–250	5.00	797
199	Fluvalinate peaks 1&2	N/A	N/A	0.1–250	N/A	N/A	0.1–250	0.20	32
200	Cypermethrin peaks I-IV	N/A	N/A	1–250	N/A	N/A	1–250	1.00	214

Appendix A (Part 6). Linearity data sets.

Chart Number	Compound Name	Apple/Pear/Banana Linearity			Carrot/Potato Linearity			IDL_{99}	
		R^2	RF RSD(%)	Range (ppb)	R^2	RF RSD(%)	Range (ppb)	pg on Column	IDL (fg)
201	Cyfluthrin peaks I-IV	N/A	N/A	0.5–250	N/A	N/A	0.5–250	1.00	91
N/A	Tetramethrin peak 1	0.99965	14.70	N/A	0.99968	16.50	N/A	N/A	N/A
N/A	Tetramethrin peak 2	0.99974	18.80	N/A	0.99984	20.80	N/A	N/A	N/A
N/A	Resmethrin peak 1	0.99950	13.60	N/A	0.99976	19.70	N/A	N/A	N/A
N/A	Resmethrin peak 2	0.99983	6.60	N/A	0.99967	10.50	N/A	N/A	N/A
N/A	Fluvalinate peak 1	0.99946	6.90	N/A	0.99936	5.50	N/A	N/A	N/A
N/A	Fluvalinate peak 2	0.99933	10.20	N/A	0.99886	4.40	N/A	N/A	N/A
N/A	Cypermethrin peak 1	0.99971	4.30	N/A	0.99989	13.40	N/A	N/A	N/A
N/A	Cypermethrin peak 2	0.99987	2.40	N/A	0.99988	5.10	N/A	N/A	N/A
N/A	Cypermethrin peak 3	0.99982	2.80	N/A	0.99975	3.10	N/A	N/A	N/A
N/A	Cypermethrin peak 4	0.99981	2.70	N/A	0.99991	2.90	N/A	N/A	N/A
N/A	Cyfluthrin peak 1	0.99967	2.50	N/A	0.99988	3.90	N/A	N/A	N/A
N/A	Cyfluthrin peak 2	0.99905	4.40	N/A	0.99972	3.00	N/A	N/A	N/A
N/A	Cyfluthrin peak 3	0.99837	10.70	N/A	0.99979	3.30	N/A	N/A	N/A
N/A	Cyfluthrin peak 4	0.99723	8.10	N/A	0.99981	10.50	N/A	N/A	N/A

Appendix B – QuEChERS Recovery data

Appendix B (Part 1). QuEChERS Recovery data.

Component Name	Carrot 1 µg/kg (n=6)		Apple 1 µg/kg (n=6)		Carrot 2.5 µg/kg (n=6)		Apple 2.5 µg/kg (n=3)		Carrot 10 µg/kg (n=6)		Apple 10 µg/kg (n=6)	
	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)
2,3,5,6-Tetrachloroaniline	100.9%	6.2%	97.8%	10.2%	94.7%	5.1%	99.2%	3.4%	87.6%	3.4%	99.4%	5.5%
2,4'-Methoxychlor	98.1%	0.9%	98.4%	1.2%	96.4%	1.0%	98.1%	1.2%	100.1%	1.2%	97.5%	1.1%
4,4'-Methoxychlor olefin	99.9%	2.8%	106.7%	3.5%	98.8%	2.6%	99.4%	1.6%	101.0%	1.1%	100.2%	1.6%
Acetochlor	89.9%	8.2%	90.9%	9.1%	79.1%	8.4%	110.5%	3.4%	97.0%	2.2%	98.2%	3.1%
Acrinathrin	96.5%	4.2%	92.3%	4.5%	112.3%	3.2%	96.4%	4.4%	109.2%	2.0%	97.4%	4.2%
Alachlor	107.8%	6.8%	97.5%	3.4%	96.9%	2.4%	102.0%	1.4%	100.9%	1.7%	97.2%	2.0%
Aldrin	109.8%	7.7%	105.3%	7.6%	93.5%	2.4%	107.7%	2.0%	98.7%	1.6%	100.4%	2.8%
Allethrin (Bioallethrin)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	105.7%	1.2%	102.1%	2.2%
Allidochlor	113.1%	10.1%	93.4%	12.6%	104.7%	12.9%	99.6%	1.9%	88.9%	5.3%	100.4%	4.8%
Anthraquinone	36.3%	27.8%	102.4%	14.5%	39.6%	6.5%	94.6%	3.5%	34.1%	47.7%	95.8%	2.7%
Atrazine	111.1%	4.1%	94.4%	6.0%	90.9%	4.0%	98.7%	5.0%	98.7%	0.9%	98.4%	2.4%
Azinphos-ethyl	95.2%	6.4%	86.1%	2.8%	96.3%	3.3%	92.4%	2.0%	100.9%	3.3%	93.3%	1.9%
Azinphos-methyl	84.5%	4.3%	87.3%	11.4%	87.1%	7.1%	100.2%	0.5%	94.3%	6.3%	84.1%	6.9%
BHC, Alpha	102.0%	3.6%	95.0%	5.6%	94.3%	3.6%	104.2%	3.2%	94.1%	1.9%	99.8%	4.2%
BHC, Beta	98.3%	2.3%	96.3%	7.7%	94.8%	0.9%	96.7%	6.3%	97.7%	1.0%	98.2%	1.9%
BHC, delta	98.5%	1.8%	93.2%	3.2%	95.1%	2.8%	101.1%	0.8%	96.5%	1.5%	97.2%	1.8%
BHC, gamma	99.5%	4.2%	96.3%	5.2%	90.7%	2.9%	109.3%	3.1%	97.4%	2.0%	96.4%	3.2%
Bifenthrin	106.1%	2.2%	106.5%	2.5%	99.9%	1.5%	101.8%	0.9%	99.5%	0.3%	102.6%	1.3%
Biphenyl	<LOQ	<LOQ	<LOQ	<LOQ	86.4%	10.5%	118.1%	3.0%	79.5%	7.7%	111.6%	7.1%
Bromfenvinphos	111.2%	3.2%	97.0%	2.7%	91.8%	1.9%	103.6%	2.8%	97.3%	1.7%	94.6%	2.0%
Bromfenvinphos-methyl	110.5%	3.4%	87.8%	5.1%	92.3%	2.5%	106.4%	2.3%	98.4%	0.9%	95.2%	3.6%
Bromophos-ethyl	113.2%	2.4%	87.2%	6.8%	93.7%	7.1%	113.1%	3.6%	97.2%	3.0%	94.8%	3.4%
Bromophos-methyl (Bromophos)	104.0%	4.3%	90.0%	7.6%	93.2%	2.9%	114.8%	2.5%	95.9%	2.6%	97.3%	3.2%
Bromopropylate	101.2%	14.2%	97.2%	5.3%	96.1%	2.0%	111.1%	2.6%	97.1%	2.3%	97.4%	2.3%
Bupirimate	111.7%	11.6%	98.6%	5.9%	99.7%	2.4%	101.4%	0.7%	100.9%	2.0%	97.1%	2.1%
Cadusafos	101.9%	3.4%	101.4%	3.8%	96.4%	3.8%	101.9%	1.8%	97.6%	1.7%	101.4%	3.1%
Captan	85.0%	16.5%	80.4%	9.1%	96.7%	11.9%	78.8%	2.4%	97.6%	5.6%	87.5%	4.2%
Carbophenothion	103.2%	4.3%	99.3%	3.6%	95.6%	1.5%	96.6%	1.9%	99.2%	1.0%	97.1%	1.5%
Carfentrazon-ethyl	103.9%	11.0%	104.7%	7.6%	94.2%	4.3%	109.0%	3.8%	99.3%	2.7%	94.8%	3.3%
Chlorbenside	88.8%	4.8%	91.1%	4.0%	88.1%	0.8%	94.0%	2.5%	88.0%	6.6%	96.3%	1.8%
Chlordane alpha-cis	110.5%	4.8%	96.1%	2.9%	95.4%	3.0%	100.1%	4.1%	99.3%	3.1%	98.5%	1.7%
Chlordane gamma-trans	112.4%	4.5%	109.7%	5.3%	95.9%	1.9%	116.2%	2.9%	97.3%	1.9%	105.9%	1.8%
Chlorfenapyr	96.9%	6.2%	96.5%	6.0%	97.2%	3.2%	95.4%	4.0%	99.7%	2.3%	99.9%	2.4%
Chlorfenson	97.9%	2.5%	95.3%	3.5%	92.9%	1.8%	94.8%	0.4%	93.1%	2.2%	94.4%	3.7%
Chlorfenvinphos	105.9%	4.3%	92.1%	3.7%	99.5%	3.3%	104.5%	1.6%	100.6%	0.7%	95.7%	2.0%
Chlorobenzilate	102.5%	2.3%	95.7%	4.2%	95.5%	0.9%	95.0%	2.3%	97.4%	0.8%	98.1%	1.5%
Chloroneb	102.6%	4.3%	97.6%	5.5%	99.9%	6.3%	105.6%	4.8%	89.0%	4.6%	102.0%	6.6%
Chlorothalonil	34.1%	8.8%	30.7%	16.9%	35.8%	29.2%	36.4%	19.1%	38.7%	8.0%	52.5%	9.1%
Chlorpropham	92.2%	4.9%	99.7%	4.3%	92.3%	3.7%	92.0%	3.6%	93.1%	2.5%	94.3%	2.7%
Chlorpyrifos-ethyl	100.1%	5.6%	99.5%	6.2%	94.1%	2.1%	101.7%	1.2%	96.7%	1.2%	97.1%	1.2%
Chlorpyrifos-methyl	113.9%	2.8%	88.1%	5.5%	93.3%	5.8%	104.9%	2.5%	95.3%	1.9%	98.0%	2.7%
Chlorthal-dimethyl (Dacthal)	117.1%	4.7%	96.9%	4.0%	93.1%	1.5%	109.9%	5.4%	98.0%	1.4%	94.8%	3.8%
Chlorthiophos	110.4%	3.5%	102.1%	7.0%	96.4%	3.3%	103.8%	0.9%	100.4%	2.0%	96.7%	1.5%
Chlozolinate	117.5%	8.4%	99.7%	8.6%	94.6%	4.1%	98.4%	1.9%	100.6%	1.8%	97.3%	3.2%
Clomazone	99.1%	3.6%	98.3%	4.0%	95.0%	2.8%	96.8%	0.8%	97.8%	0.9%	99.0%	2.9%
Coumaphos	98.6%	6.7%	93.0%	3.5%	88.5%	5.6%	91.4%	2.3%	94.9%	3.7%	92.5%	4.2%
Cycloate	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	114.7%	6.3%	115.2%	9.0%

Appendix B (Part 2). QuEChERS Recovery data.

Component Name	Carrot 1 µg/kg (n=6)		Apple 1 µg/kg (n=6)		Carrot 2.5 µg/kg (n=6)		Apple 2.5 µg/kg (n=3)		Carrot 10 µg/kg (n=6)		Apple 10 µg/kg (n=6)	
	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)
Cyfluthrin peak 1	109.7%	3.2%	103.2%	7.3%	106.0%	3.1%	96.1%	16.7%	106.3%	2.0%	103.6%	2.6%
Cyfluthrin peak 2	95.5%	3.5%	98.5%	2.5%	104.1%	1.7%	103.0%	1.4%	105.7%	2.6%	101.2%	2.3%
Cyfluthrin peak 3	107.8%	4.8%	105.7%	7.2%	103.3%	3.5%	97.5%	2.5%	104.7%	2.2%	101.1%	2.1%
Cyfluthrin peak 4	110.3%	4.9%	98.3%	6.4%	100.5%	2.4%	99.9%	1.8%	105.3%	2.6%	103.3%	2.7%
Cyhalothrin I (lambda)	106.2%	4.5%	100.3%	3.6%	102.8%	3.4%	99.5%	2.4%	104.3%	1.8%	104.5%	1.8%
Cypermethrin peak 1	80.2%	3.6%	94.8%	2.4%	94.8%	4.0%	101.6%	1.2%	101.1%	2.8%	101.2%	2.2%
Cypermethrin peak 2	87.0%	10.2%	80.2%	6.7%	104.7%	2.8%	98.0%	0.8%	106.7%	4.1%	105.1%	1.9%
Cypermethrin peak 3	102.0%	5.6%	99.0%	4.9%	109.1%	1.3%	101.9%	2.7%	105.6%	3.6%	107.0%	2.4%
Cypermethrin peak 4	102.1%	2.9%	102.8%	5.9%	102.2%	4.0%	101.8%	0.5%	107.5%	3.2%	107.1%	3.1%
Cyprodinil	103.5%	3.2%	93.5%	2.1%	96.3%	2.8%	99.2%	2.6%	92.8%	4.8%	98.7%	2.7%
DDD p,p	101.1%	1.9%	102.0%	2.4%	96.6%	1.2%	99.2%	0.8%	99.1%	1.3%	97.3%	1.5%
DDD, o, p	104.1%	2.2%	104.0%	4.1%	95.5%	1.8%	101.4%	1.3%	98.5%	1.3%	96.3%	1.6%
DDE o,p	109.1%	1.7%	97.2%	2.5%	96.9%	0.9%	103.9%	2.9%	99.7%	1.1%	99.0%	1.7%
DDE p, p	106.9%	1.7%	99.3%	4.0%	96.2%	1.5%	97.8%	2.4%	98.8%	1.1%	97.5%	1.5%
DDT o,p	101.4%	2.8%	100.2%	4.5%	95.1%	3.3%	96.4%	2.9%	100.7%	0.8%	97.4%	1.8%
DDT p,p	97.9%	2.6%	97.0%	0.8%	95.4%	2.0%	97.3%	2.1%	100.7%	0.8%	96.6%	1.5%
Deltamethrin	77.2%	5.2%	95.2%	7.0%	114.9%	6.0%	100.0%	8.5%	110.6%	2.3%	101.2%	7.4%
Diazinon	102.0%	3.5%	103.9%	4.4%	102.3%	2.9%	97.4%	4.0%	99.0%	2.1%	100.9%	3.1%
Dichlobenil	97.2%	6.4%	94.6%	10.5%	98.9%	7.3%	103.4%	5.6%	86.1%	5.6%	102.5%	6.5%
Dichlofluanid	58.8%	4.1%	56.8%	4.9%	50.7%	13.9%	62.5%	7.6%	57.5%	3.0%	74.9%	5.3%
Dichlorobenzophenone, 4, 4	115.3%	2.7%	99.1%	4.1%	97.3%	1.8%	95.2%	1.4%	98.0%	2.7%	94.2%	1.7%
Dicloran (Bortran)	86.6%	6.0%	84.9%	6.8%	86.6%	3.8%	91.7%	4.1%	86.4%	3.7%	88.8%	2.4%
Dicofol	34.9%	15.0%	69.0%	12.1%	113.2%	5.2%	92.3%	9.6%	105.9%	15.6%	101.3%	11.5%
Dieldrin	104.9%	17.3%	98.9%	13.4%	103.0%	6.4%	105.5%	3.2%	96.6%	3.2%	95.4%	3.0%
Dimethachlor	102.2%	3.2%	94.3%	1.8%	94.3%	1.7%	98.5%	3.2%	98.6%	2.4%	96.9%	1.2%
Dimethoate	97.7%	10.4%	71.9%	8.5%	87.0%	10.1%	77.3%	1.3%	94.9%	1.8%	85.3%	5.8%
Diphenamid	102.6%	6.1%	82.9%	4.4%	100.8%	1.9%	104.7%	2.3%	99.8%	1.8%	98.8%	1.2%
Diphenylamine	98.1%	2.1%	113.2%	7.1%	97.4%	4.2%	107.7%	4.5%	94.3%	2.0%	102.3%	4.3%
Disulfoton	96.5%	4.0%	94.3%	8.8%	95.8%	1.5%	100.1%	5.1%	98.3%	1.6%	97.4%	2.5%
Edifenphos	96.1%	3.3%	93.5%	2.6%	96.6%	1.5%	92.5%	1.8%	100.3%	2.2%	96.9%	2.0%
Endosulfan ether	105.3%	5.6%	98.8%	3.0%	95.7%	2.6%	101.5%	3.0%	94.5%	1.5%	97.8%	3.0%
Endosulfan peak 1	101.7%	6.6%	107.1%	6.2%	94.8%	2.8%	116.7%	4.6%	99.0%	2.1%	104.7%	2.8%
Endosulfan peak 2	97.9%	6.1%	95.6%	5.4%	100.7%	5.3%	98.2%	2.9%	98.0%	1.8%	101.6%	2.1%
Endosulfan sulfate	101.2%	4.9%	106.0%	8.3%	100.0%	2.3%	108.2%	0.7%	100.5%	1.6%	100.8%	1.5%
Endrin	105.6%	3.5%	93.4%	10.7%	99.3%	2.1%	99.7%	1.2%	98.0%	1.5%	96.8%	2.8%
Endrin Aldehyde	55.6%	14.3%	27.6%	26.3%	40.0%	18.9%	27.2%	28.2%	37.6%	13.2%	29.0%	16.7%
Endrin-Ketone	91.5%	17.1%	105.2%	10.9%	84.3%	16.2%	116.0%	4.9%	96.0%	2.3%	97.3%	3.0%
EPN	116.0%	9.0%	96.9%	7.3%	92.6%	2.2%	87.9%	6.2%	95.5%	2.7%	93.4%	2.8%
Ethion	107.0%	3.7%	98.3%	2.3%	96.8%	1.4%	97.8%	1.4%	100.0%	1.2%	99.3%	1.5%
Ethoprop (Ethoprophos)	97.2%	4.3%	91.4%	4.9%	96.5%	2.6%	96.0%	3.8%	95.0%	3.0%	97.0%	3.8%
Etofenprox	107.0%	4.6%	109.1%	4.3%	106.7%	2.2%	96.3%	5.0%	104.9%	1.6%	110.6%	2.3%
Etridiazole (Terrazole)	100.0%	7.6%	91.9%	11.5%	100.5%	7.2%	102.8%	6.3%	87.1%	6.6%	100.3%	7.4%
Fenamiphos	101.5%	8.3%	87.9%	10.1%	92.1%	4.7%	71.8%	8.3%	97.9%	1.4%	84.7%	3.7%
Fenarimol	107.9%	3.7%	101.3%	1.6%	104.5%	1.4%	102.5%	1.7%	100.3%	0.8%	99.8%	1.1%
Fenchlorfos	112.6%	3.0%	97.7%	3.8%	95.3%	2.6%	103.0%	3.2%	98.1%	2.1%	101.1%	2.3%
Fenitrothion	101.7%	9.5%	79.5%	9.1%	87.0%	6.1%	97.9%	2.8%	91.9%	1.0%	85.0%	2.8%
Fenpropathrin	97.2%	6.0%	80.4%	5.8%	102.5%	6.0%	97.5%	2.7%	98.1%	1.3%	103.4%	1.6%

Appendix B (Part 3). QuEChERS Recovery data.

Component Name	Carrot 1 µg/kg (n=6)		Apple 1 µg/kg (n=6)		Carrot 2.5 µg/kg (n=6)		Apple 2.5 µg/kg (n=3)		Carrot 10 µg/kg (n=6)		Apple 10 µg/kg (n=6)	
	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)
Fenson	98.9%	2.5%	95.5%	3.8%	96.5%	2.2%	96.0%	2.0%	98.8%	1.1%	97.0%	2.0%
Fenthion	109.0%	4.5%	92.3%	2.3%	100.4%	2.7%	108.7%	1.9%	99.6%	2.2%	95.1%	2.2%
Fenvalerate	101.3%	2.9%	102.6%	4.2%	110.1%	2.6%	104.6%	2.6%	110.3%	1.8%	104.2%	4.0%
Fipronil	103.5%	7.8%	83.8%	8.6%	94.9%	3.7%	110.6%	4.4%	100.5%	2.8%	95.9%	3.3%
Fipronil desulfinyl	101.5%	8.4%	92.2%	8.3%	95.2%	5.4%	108.4%	2.1%	99.5%	1.8%	98.5%	3.6%
Fluazifop-P-butyl	111.0%	3.9%	96.2%	6.6%	95.8%	2.2%	100.8%	2.4%	99.7%	2.3%	97.3%	1.4%
Fludioxonil	105.1%	3.2%	93.7%	3.7%	89.3%	4.5%	93.9%	5.0%	92.3%	3.3%	89.6%	1.6%
Fluquinconazole	103.1%	16.6%	102.9%	7.0%	94.3%	3.0%	91.1%	3.9%	98.2%	2.3%	99.4%	3.0%
Fluridone	100.7%	17.2%	85.6%	11.3%	92.1%	4.2%	107.2%	0.4%	100.9%	2.3%	88.7%	1.5%
Flusilazole	95.2%	5.4%	98.4%	9.7%	96.9%	1.9%	92.0%	2.1%	95.6%	1.7%	98.8%	2.1%
Flutolanil	99.0%	2.7%	95.1%	1.9%	95.0%	0.9%	96.7%	0.6%	97.0%	0.8%	99.1%	1.6%
Flutriafol	96.9%	2.5%	99.4%	5.4%	93.3%	2.2%	99.8%	2.4%	95.6%	1.4%	97.8%	1.0%
Fluvalinate peak 1	100.3%	5.9%	115.1%	18.5%	118.9%	3.6%	98.5%	3.5%	120.0%	2.4%	100.6%	3.2%
Fluvalinate peak 2	93.7%	17.3%	108.4%	18.8%	117.6%	5.8%	104.1%	8.2%	113.6%	3.9%	101.9%	5.2%
Folpet	90.2%	11.4%	72.2%	4.1%	86.6%	10.8%	70.7%	7.1%	97.0%	4.2%	77.3%	4.4%
Fonofos	105.9%	3.9%	99.9%	4.6%	99.5%	2.1%	105.4%	4.8%	97.1%	2.7%	101.3%	2.5%
Heptachlor	108.7%	5.4%	97.1%	5.1%	95.9%	3.4%	105.2%	3.6%	94.8%	0.9%	98.9%	3.5%
Hexachlorobenzene	95.1%	7.1%	94.6%	8.3%	86.7%	2.9%	109.1%	4.6%	75.4%	9.7%	99.3%	5.4%
Hexazinone	89.1%	1.9%	87.7%	2.1%	93.5%	1.0%	91.6%	0.9%	95.0%	1.7%	93.9%	2.0%
Iodofenfos	97.6%	4.6%	96.9%	3.2%	83.3%	6.7%	96.7%	0.8%	93.5%	4.2%	94.6%	4.3%
Iprodione	95.2%	16.3%	99.6%	7.6%	97.3%	5.5%	108.6%	1.2%	107.3%	3.8%	90.5%	2.9%
Isazophos	111.1%	9.4%	72.2%	5.0%	105.2%	4.0%	101.5%	2.1%	98.7%	1.5%	99.1%	2.5%
Isodrin	103.3%	4.9%	98.0%	4.6%	96.0%	1.9%	100.4%	4.8%	97.2%	0.8%	98.7%	2.0%
Lenacil	90.0%	10.8%	92.5%	8.2%	93.8%	6.0%	88.3%	4.6%	94.0%	5.3%	92.8%	2.0%
Leptophos	98.9%	6.8%	101.1%	1.8%	97.9%	1.3%	95.0%	1.1%	97.8%	4.0%	102.4%	1.1%
Linuron	104.4%	9.9%	82.4%	3.2%	92.9%	4.7%	97.2%	7.8%	90.9%	3.6%	99.7%	4.5%
Malathion	103.0%	2.1%	82.6%	3.7%	91.5%	2.1%	91.4%	5.1%	94.3%	1.4%	97.6%	1.3%
Metalaxyl	106.0%	6.0%	99.8%	12.5%	96.2%	4.8%	111.5%	1.8%	98.5%	1.9%	96.0%	2.3%
Metazachlor	98.5%	5.0%	99.8%	4.9%	97.4%	1.3%	100.4%	0.2%	98.7%	0.9%	97.2%	1.6%
Methacrifos	95.6%	4.0%	95.7%	5.5%	101.9%	6.6%	103.1%	4.0%	92.6%	3.8%	102.5%	6.6%
Methoxychlor	95.5%	2.3%	97.0%	5.3%	98.2%	2.0%	102.6%	1.7%	102.0%	0.9%	98.9%	1.2%
Metolachlor	98.9%	2.9%	93.3%	2.4%	94.2%	1.1%	96.7%	3.1%	97.8%	1.5%	97.3%	1.2%
Mevinphos	94.2%	4.6%	89.4%	5.4%	99.3%	7.7%	94.4%	4.3%	104.1%	3.6%	95.9%	5.3%
MGK-264 A	109.5%	16.6%	96.3%	10.5%	102.3%	6.8%	94.6%	0.6%	100.0%	2.4%	97.3%	1.9%
MGK-264 B	105.9%	4.6%	100.4%	6.1%	96.7%	2.9%	105.4%	3.4%	97.1%	1.5%	100.8%	2.2%
Mirex	97.6%	4.0%	103.6%	2.2%	93.9%	1.8%	102.0%	1.0%	102.5%	1.7%	99.3%	1.4%
Myclobutanil	100.6%	4.5%	97.6%	5.4%	96.7%	2.7%	101.5%	2.8%	98.1%	2.0%	96.5%	2.1%
N-(2,4-Dimethylphenyl) formamide	86.6%	5.6%	85.0%	8.4%	90.0%	7.6%	77.6%	3.0%	89.8%	5.0%	86.8%	3.0%
NDBA	110.7%	7.6%	110.9%	10.8%	105.2%	9.0%	116.5%	7.8%	90.0%	7.3%	104.8%	6.4%
NDEA	94.9%	7.2%	91.1%	11.1%	97.3%	10.2%	105.3%	5.2%	97.0%	12.2%	101.6%	13.7%
NDPA	97.2%	8.1%	74.8%	13.5%	97.3%	7.7%	90.5%	11.7%	85.4%	9.4%	101.3%	8.4%
NEMA	87.5%	4.1%	85.2%	19.4%	91.7%	15.3%	104.7%	15.3%	83.1%	12.4%	97.2%	8.2%
Nitrofen	109.8%	9.3%	97.4%	2.2%	90.9%	3.3%	93.7%	1.7%	92.8%	1.5%	87.9%	3.2%
N-Nitrosodiphenylamine	98.1%	2.1%	113.2%	7.1%	97.4%	4.2%	107.7%	4.5%	94.3%	2.0%	102.3%	4.3%
N-Nitrosomorpholine	84.9%	8.2%	80.7%	10.3%	85.1%	8.4%	80.5%	3.5%	81.8%	6.9%	91.8%	6.8%
N-Nitrosopiperidine	95.5%	7.2%	81.0%	19.8%	105.0%	11.2%	98.9%	10.5%	86.5%	8.2%	102.3%	6.6%
N-Nitrosopyrrolidine	95.4%	11.0%	114.0%	13.8%	101.6%	6.4%	91.4%	14.2%	82.5%	6.1%	99.4%	9.7%

Appendix B (Part 4). QuEChERS Recovery data.

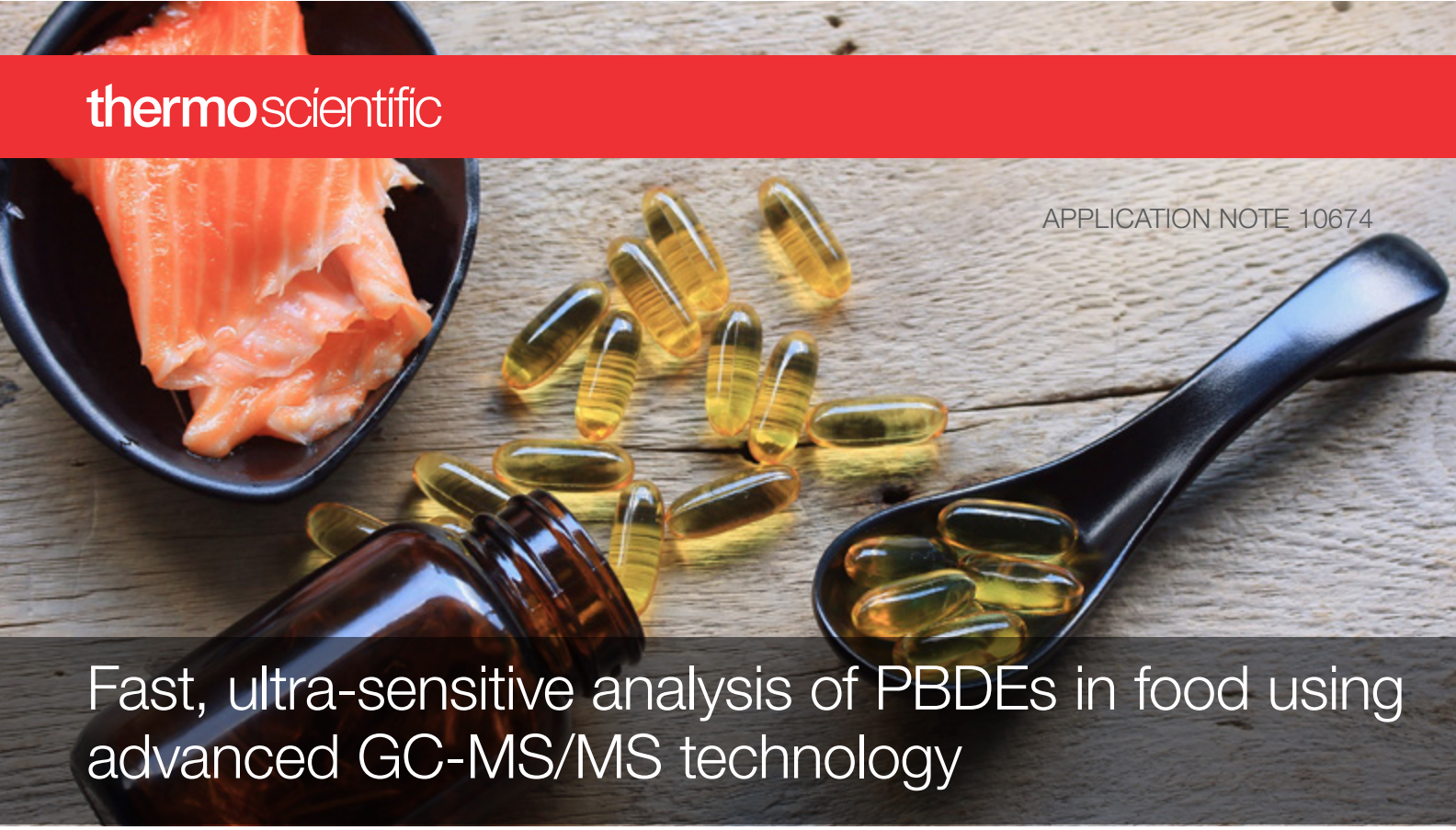
Component Name	Carrot 1 µg/kg (n=6)		Apple 1 µg/kg (n=6)		Carrot 2.5 µg/kg (n=6)		Apple 2.5 µg/kg (n=3)		Carrot 10 µg/kg (n=6)		Apple 10 µg/kg (n=6)	
	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)
N-Nitrosopyrrolidine	95.4%	11.0%	114.0%	13.8%	101.6%	6.4%	91.4%	14.2%	82.5%	6.1%	99.4%	9.7%
Nonachlor-cis	109.4%	15.3%	114.4%	8.8%	94.6%	10.4%	113.1%	7.2%	100.5%	2.5%	98.9%	4.1%
Nonachlor-trans	107.8%	3.7%	103.7%	8.7%	96.1%	5.1%	107.3%	2.2%	99.4%	2.1%	101.0%	2.6%
Norflurazon	100.4%	7.8%	83.9%	17.3%	93.7%	3.5%	96.7%	1.8%	95.5%	1.8%	91.8%	2.7%
Ortho-phenylphenol	90.6%	5.1%	115.5%	3.5%	82.3%	6.2%	102.6%	2.1%	90.9%	3.2%	99.7%	5.4%
Oxadiazon	100.3%	4.3%	97.7%	4.6%	95.2%	1.9%	92.9%	1.1%	96.9%	2.1%	95.5%	3.6%
Oxyfluorfen	102.4%	15.7%	113.2%	8.9%	97.7%	7.1%	101.6%	4.5%	95.6%	2.0%	97.0%	3.2%
Paclobutrazol	111.2%	2.9%	93.8%	4.4%	98.3%	1.9%	97.4%	2.0%	95.1%	3.9%	94.6%	1.9%
Parathion (ethyl)	118.4%	7.4%	111.1%	5.7%	96.0%	4.0%	111.5%	4.5%	92.0%	3.4%	90.4%	1.8%
Parathion-methyl	119.6%	6.7%	83.7%	4.1%	94.0%	2.5%	95.8%	1.1%	91.0%	3.3%	86.9%	2.7%
Pebulate	112.9%	4.6%	118.8%	5.6%	113.9%	7.6%	119.3%	0.3%	93.4%	5.4%	109.2%	6.9%
Penconazole	100.3%	4.7%	96.3%	3.5%	94.4%	1.7%	100.1%	5.3%	98.1%	0.9%	94.4%	2.7%
Pentachloroaniline	94.9%	2.8%	91.8%	4.3%	85.1%	4.0%	104.1%	0.3%	84.6%	8.2%	96.1%	2.7%
Pentachloroanisole	104.3%	4.1%	92.8%	4.9%	94.8%	4.3%	104.6%	3.7%	90.6%	2.4%	102.5%	5.6%
Pentachlorobenzene	100.1%	4.0%	94.5%	6.3%	95.8%	5.6%	117.9%	6.9%	83.2%	4.4%	100.9%	8.9%
Pentachlorobenzonitrile	102.5%	5.7%	91.4%	3.3%	95.2%	6.4%	103.3%	1.9%	87.7%	2.7%	96.6%	3.5%
Pentachlorothioanisole	106.7%	6.3%	97.4%	3.2%	86.8%	3.4%	106.5%	0.2%	82.8%	9.7%	98.0%	2.3%
Permethrin peak 1	91.6%	13.6%	81.8%	6.2%	82.8%	8.1%	98.9%	2.0%	89.4%	2.3%	97.4%	2.1%
Permethrin peak 2	97.6%	7.0%	98.7%	3.2%	96.2%	2.6%	98.9%	0.3%	100.4%	2.5%	104.8%	2.0%
Perthane (Ethylan)	109.8%	3.1%	101.1%	2.4%	96.6%	0.7%	103.1%	0.8%	101.0%	0.8%	99.4%	1.4%
Phenothrin	<LOQ	<LOQ	<LOQ	<LOQ	89.7%	14.9%	81.7%	13.3%	108.4%	11.3%	92.2%	4.2%
Phorate	116.6%	15.9%	103.1%	7.0%	102.5%	4.8%	103.3%	3.0%	98.9%	3.0%	101.7%	4.8%
Phosalone	100.2%	3.7%	97.2%	4.6%	98.4%	1.8%	70.4%	13.3%	100.4%	2.1%	99.1%	1.2%
Phosmet	85.2%	3.8%	89.6%	6.9%	89.3%	3.6%	89.7%	2.2%	95.3%	3.3%	88.7%	6.4%
Piperonyl butoxide	105.0%	6.8%	99.7%	2.4%	103.1%	1.3%	99.9%	0.5%	102.2%	1.2%	103.8%	1.8%
Pirimiphos-ethyl	104.9%	4.3%	107.5%	4.0%	100.2%	2.0%	102.9%	4.2%	100.0%	2.5%	98.1%	2.8%
Pirimiphos-methyl	117.2%	5.1%	97.0%	4.8%	93.6%	1.9%	110.7%	4.2%	99.1%	1.6%	94.0%	4.0%
Pretilachlor	103.3%	4.1%	95.3%	5.0%	94.6%	2.0%	98.4%	5.2%	99.4%	1.0%	98.1%	1.2%
Prochloraz	104.5%	6.2%	110.6%	12.3%	115.0%	7.9%	98.9%	2.3%	99.3%	4.1%	92.0%	3.9%
Procymidone	115.0%	2.8%	100.8%	5.4%	96.4%	3.0%	103.3%	1.5%	98.7%	1.5%	99.0%	3.2%
Profenofos	114.4%	7.1%	99.0%	6.8%	86.5%	4.3%	99.9%	4.2%	95.7%	3.5%	91.6%	3.0%
Propachlor	94.4%	8.4%	104.0%	2.1%	99.3%	7.2%	99.9%	2.8%	92.7%	1.3%	97.7%	4.6%
Propanil	89.6%	7.5%	78.8%	10.2%	91.3%	5.6%	91.6%	2.3%	95.1%	4.2%	89.5%	2.1%
Propargite	<LOQ	<LOQ	<LOQ	<LOQ	89.1%	7.3%	101.7%	4.9%	103.2%	11.1%	102.5%	5.2%
Propisochlor	97.5%	7.0%	99.2%	3.6%	101.7%	3.2%	99.2%	1.5%	101.3%	3.0%	99.1%	1.6%
Propyzamide	99.8%	6.1%	102.1%	8.9%	100.7%	1.5%	99.5%	2.7%	101.6%	1.3%	99.6%	2.4%
Prothiofos	115.8%	4.8%	92.3%	2.1%	92.1%	3.5%	105.4%	4.7%	96.0%	1.2%	98.2%	4.0%
Pyraclofos	96.9%	8.3%	89.6%	7.2%	92.3%	3.6%	87.6%	1.8%	94.1%	6.6%	94.1%	4.1%
Pyrazophos	110.6%	2.6%	96.0%	3.0%	93.7%	3.8%	97.5%	1.9%	101.9%	3.1%	101.1%	1.4%
Pyridaben	106.8%	2.7%	100.0%	2.7%	98.9%	1.7%	119.5%	1.0%	100.1%	2.1%	102.8%	1.8%
Pyridaphenthion	94.2%	18.0%	96.3%	8.6%	96.3%	3.3%	113.7%	2.6%	100.4%	1.9%	95.9%	2.2%
Pyrimethanil	118.4%	7.6%	80.4%	13.3%	117.0%	9.1%	104.7%	9.1%	101.3%	2.7%	95.8%	2.8%
Pyriproxyfen	103.9%	4.5%	105.7%	1.4%	99.5%	2.9%	80.0%	7.3%	101.2%	1.3%	102.9%	1.6%
Quinalphos	95.2%	4.3%	86.3%	11.5%	78.9%	3.7%	85.1%	4.9%	100.0%	2.3%	95.5%	2.1%
Quintozene	107.3%	8.7%	101.9%	6.1%	97.6%	5.6%	94.6%	2.2%	88.9%	1.6%	94.3%	4.0%
Resmethrin peak 1	<LOQ	<LOQ	<LOQ	<LOQ	94.7%	2.9%	104.0%	7.1%	96.3%	3.7%	87.5%	2.0%
Resmethrin peak 2	<LOQ	<LOQ	<LOQ	<LOQ	92.9%	7.7%	95.4%	4.3%	94.4%	2.6%	89.0%	6.0%

Appendix B (Part 5). QuEChERS Recovery data.

Component Name	Carrot 1 µg/kg (n=6)		Apple 1 µg/kg (n=6)		Carrot 2.5 µg/kg (n=6)		Apple 2.5 µg/kg (n=3)		Carrot 10 µg/kg (n=6)		Apple 10 µg/kg (n=6)	
	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)	Mean Recovery	Precision RSD(%)
Sulfotep	103.2%	2.1%	101.1%	5.1%	96.7%	3.6%	101.8%	0.9%	96.7%	2.5%	100.6%	3.6%
Sulprofos	105.8%	2.6%	98.4%	4.3%	94.2%	1.4%	96.3%	3.0%	101.8%	1.3%	99.7%	1.1%
Tebuconazole	99.4%	3.2%	94.7%	4.5%	94.7%	1.2%	96.6%	1.8%	96.6%	0.6%	95.8%	1.5%
Tebufenpyrad	101.5%	4.7%	105.1%	2.2%	98.2%	1.5%	108.3%	0.7%	104.7%	2.0%	103.8%	1.4%
Tecnazene	100.9%	7.5%	94.3%	9.4%	94.2%	5.0%	104.3%	1.3%	86.9%	3.7%	100.4%	6.5%
Tefluthrin	103.2%	2.2%	101.7%	4.5%	99.0%	2.4%	104.5%	1.5%	100.1%	1.1%	99.1%	2.5%
Terbacil	98.7%	4.7%	89.0%	2.5%	94.6%	2.1%	89.2%	2.9%	95.1%	2.6%	94.1%	3.6%
Terbufos	109.8%	5.4%	97.9%	5.7%	98.0%	2.5%	107.3%	1.4%	98.8%	1.9%	104.3%	3.4%
Terbuthylazine	107.3%	6.2%	99.8%	4.3%	92.7%	4.0%	96.4%	4.9%	101.2%	3.9%	96.6%	3.0%
Tetrachlorvinphos	104.6%	4.4%	86.0%	5.3%	96.5%	3.5%	117.6%	1.7%	102.2%	4.7%	94.1%	1.6%
Tetradifon	92.1%	4.9%	105.0%	9.5%	94.4%	2.2%	99.1%	3.9%	97.5%	2.2%	100.8%	1.9%
Tetrahydrophthalimide (THPI)	88.8%	4.7%	87.6%	4.5%	98.6%	3.8%	89.3%	4.2%	93.8%	1.1%	88.3%	3.3%
Tetramethrin peak 1	<LOQ	<LOQ	<LOQ	<LOQ	108.2%	15.9%	79.5%	12.3%	95.1%	2.7%	96.5%	3.4%
Tetramethrin peak 2	93.4%	6.4%	118.7%	11.5%	96.9%	2.7%	96.1%	3.0%	100.7%	0.8%	97.8%	1.5%
Tolclofos-methyl	111.6%	3.3%	103.2%	5.3%	97.3%	2.5%	106.7%	2.2%	98.1%	1.8%	99.1%	1.9%
Tolylfluanid	70.7%	3.5%	66.5%	5.9%	64.9%	8.7%	70.0%	3.6%	71.7%	2.7%	79.9%	4.4%
Triadimefon	108.2%	5.2%	94.9%	5.8%	97.3%	2.7%	99.0%	0.3%	96.7%	0.7%	97.6%	2.0%
Triadimenol	109.3%	5.6%	105.8%	4.3%	95.9%	2.4%	98.5%	1.8%	99.1%	1.1%	98.4%	4.6%
Triallate	110.7%	2.5%	98.8%	4.6%	97.6%	3.1%	103.0%	3.0%	96.6%	0.6%	101.0%	3.4%
Triazophos	99.1%	4.9%	92.7%	4.1%	97.4%	2.2%	91.5%	1.4%	98.1%	1.6%	95.9%	1.7%
Tricyclazole	88.2%	17.8%	71.2%	5.0%	81.3%	9.1%	78.4%	6.3%	78.1%	6.0%	80.0%	6.3%
Triflumizole	102.5%	8.0%	89.7%	6.8%	96.0%	4.6%	94.4%	4.7%	100.4%	3.1%	101.7%	1.8%
Vinclozolin	106.6%	7.2%	100.3%	6.7%	99.1%	2.3%	98.5%	6.4%	98.2%	1.6%	97.6%	2.5%

Find out more at **thermofisher.com/TSQ9000**

©2018 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details. **AN10586-EN 0218S**



APPLICATION NOTE 10674

Fast, ultra-sensitive analysis of PBDEs in food using advanced GC-MS/MS technology

Authors

Aaron Lamb[1], Cristian Cojocariu[1], and Sean Panton[2]

[1] Thermo Fisher Scientific, Runcorn, UK

[2] Fera Science, Ltd., York, UK

Keywords

Food safety, polybrominated diphenyl ethers (PBDEs), trace analysis, gas chromatography, triple quadrupole mass spectrometry, selective reaction monitoring (SRM), sensitivity, routine, TSQ 9000, advanced electron ionization (AEI), Chromeleon, isotope dilution

Goal

The aim of the study was to assess the quantitative performance of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system equipped with the advanced electron ionization (AEI) source for the analysis of polybrominated diphenyl ethers (PBDEs) in food at low concentrations.

Introduction

PBDEs are a class of brominated hydrocarbons with a basic structure containing two phenyl rings linked by an oxygen atom. There are 209 possible PBDE congeners that differ in the number and location of bromine atoms in the phenyl rings.

PBDEs are used as additive flame retardants in different materials such as plastics, textiles, upholstery, and circuitry that can leach into the environment where they persist and bioaccumulate.[1] As a consequence, the use of certain toxic PBDEs with links to cancer (including penta, tetra, and deca BDE) have been banned, and are currently listed in the Stockholm Convention inventory of persistent organic pollutants.[2]

ThermoFisher SCIENTIFIC

The major challenges for PBDE analysis are sensitivity and selectivity in complex matrices, chromatographic resolution of critical pairs, degradation of higher brominated compounds, and the cost per sample. Gas chromatography-high resolution mass spectrometry (GC-HRMS) is the analytical technique of choice for PBDE determination in food, and triple quadrupole GC-MS/MS instrumentation in particular has recently become popular for this application due to its high selectivity and sensitivity provided through selective reaction monitoring (SRM) acquisition mode. High selectivity and sensitivity are required to (i) reduce interferences from matrix and background chemical ions that can result in false positive detection and erroneous quantification of PBDEs and (ii) detect ultra-trace levels of these toxic compounds in complex matrices.

The chromatographic resolution of the critical pair (BDE-49 and BDE-71) in PBDE analysis is essential because many of the congeners are isobaric and share common SRM transitions, meaning chromatographic separation is a necessity. For this reason reported methods have low sample throughput with analysis times of up to 45 minutes on costly capillary columns typically of 60 m in length.[3] In this study a new high efficiency, high selectivity 15 m Thermo Scientific™ TraceGOLD™ TG-PBDE capillary column was evaluated for increased sample throughput and reduced cost per sample.

The aim of this project was to evaluate the analytical performance of the TSQ 9000 GC-MS/MS system using the AEI source for the ultra-trace analysis and separation of PBDEs in food matrices. This was attempted using a fast, sensitive, selective method on the high efficiency TraceGOLD TG-PBDE capillary column. The following analytical performance criteria were evaluated: sensitivity, repeatability, linearity, limit of quantification, and the accuracy of measurements in matrix.

The assessment of system robustness and suitability for routine PBDE GC-MS/MS analysis, which was outside the scope of this application note, can be found in a supporting 2018 technical note.

Experimental

Preparation of solvent calibration curve, instrument detection limit (IDL), and limit of quantification (LOQ) standards

Calibration standards containing 27 native PBDE congeners at five concentration levels (Table 1, Appendix), and 16 (^{13}C labeled) PBDEs internal standards (Table 2, Appendix), were acquired from Wellington Laboratories, Inc. (Ontario, Canada).

For the calculation of IDLs and LOQs for individual BDE congeners, the lowest concentration standard was serially diluted with *n*-nonane to 0.5, 0.25, 0.1, 0.075, and 0.05 pg/µL for BDE-209 ready for repeat injections.

Preparation of samples

Sample preparation was performed according to that described in a scientific paper by A. Fernandes et al.[3] The procedure involves sample homogenization/freeze drying, fortification of 10 g of homogenized/freeze-dried sample in 200 mL of *n*-hexane with isotopically labeled ^{13}C PBDE internal standards followed by loading onto a multi-packed silica column containing acidified silica, basified silica, and activated charcoal. PBDEs were then eluted from the multi-packed silica/activated carbon column using 100 mL of *n*-hexane and 400 mL of *n*-hexane/dichloromethane (60:40, v:v) then evaporated to dryness and reconstituted to 0.5 mL in *n*-hexane. The extracts were cleaned further using a silica alumina column and 20 mL of DCM/*n*-hexane (30:70) followed by the addition of ^{13}C-labeled PBDE syringe standards, evaporation, and reconstitution to 25 µL with *n*-nonane prior to analysis.

GC-MS/MS analysis

A TSQ 9000 triple quadrupole GC-MS/MS instrument equipped with an AEI source and coupled with a Thermo Scientific™ TRACE™ 1310 gas chromatograph was used. The AEI source provides highly efficient electron ionization of analytes and a more tightly focused ion beam that leads to an unparalleled level of sensitivity.

Liquid injections of the sample extracts were performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was achieved using a TraceGOLD TG-PBDE 15 m × 0.25 mm I.D. × 0.10 µm film capillary column (P/N 26061-0350). Additional details of the instrument parameters are displayed in Tables 3 and 4. Full details of all consumables used are available from the Thermo Scientific™ AppsLab™ Library of Analytical Applications.

Table 3. Gas chromatograph and injector conditions. The full list of consumables and instrument conditions, including SRM transitions, are available from the AppsLab library.

TRACE 1310 GC Parameters	
Injection volume:	2.0 µL
Liner:	PTV 6 baffle liner 2.0 mm × 2.75 mm × 120 mm (Thermo Scientific™ LinerGOLD™ GC Liner) (P/N 453T2845-UI)
Inlet:	65 °C
Inlet module and mode:	PTV, cold splitless
Transfer delay:	0.2 min
Injection time:	0.1 min
Transfer rate:	5.0 °C/s
Transfer temp.:	330 °C
Transfer time:	5 min
Cleaning rate:	14.5 °C/s
Cleaning temp:	330 °C
Carrier gas:	He, 1.5 mL/min
Oven Temperature Program	
Temperature 1:	100 °C
Hold time:	2.0 min
Temperature 2:	340 °C
Rate:	30 °C/min
Hold time:	3 min
Total GC run time:	13 min

Table 4. Mass spectrometer conditions

TSQ 9000 AEI Mass Spectrometer Parameters	
Transfer line:	300 °C
Ion source (Ionization type):	AEI (EI)
Ion source:	300 °C
Electron energy:	50 eV
Emission current:	50 µA
Gain amplification:	×7
Acquisition modes:	Timed-SRM
Q1 & Q3 resolution:	mono-hepta BDE normal (0.7 amu) octa-deca BDE wide (1.2 amu)
Tuning parameters:	AEI SmartTune
Collision gas, pressure:	Argon, 70 psi

Data processing

Data were acquired using timed-SRM mode, processed and reported using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2, which allows instrument control, method development, quantitative/qualitative analysis, and customizable reporting all within one platform (Figure 1, Appendix).[4] This application highlights use of isotope dilution software processing features implemented Chromeleon CDS from version 7.2.9 onwards.

Results and discussion

PBDE chromatography, selectivity, sensitivity in terms of IDLs, LOQs in sample, and linearity were evaluated using solvent-based standards. Extracted food samples were obtained from Fera Science, Ltd., York and were used in the experiments described below.

Chromatography

All target congeners were separated in under 11 minutes including excellent separation of the critical pair BDE-49 and BDE-71 (Figure 2). Resolution of these compounds was 0.6% based on valley height relative to the height of the shortest peak, which is well within the EPA 1614 requirement of less than 40%.[5] Compared with existing GC-HRMS methods (~45 min run times), this will allow for the analysis of up to 100 samples per day (compared to 30 in a published paper) giving an increase in sample throughput of 3× and a significant reduction in cost per sample.[3] Using the TG-PBDE capillary column, good chromatographic peak shape was obtained for all compounds (Figure 2), even for BDE-209, which is particularly challenging for this analysis due to susceptibility to breakdown and peak tailing.

Selectivity

Due to the diversity of matrices with various degrees of complexity, selectivity can be challenging in routine GC-MS analysis. An example of sample complexity is shown in Figure 3 as an overlay of the TIC of fish containing incurred residues (top chromatogram) and of timed SRM (bottom chromatogram) showing target PBDEs.

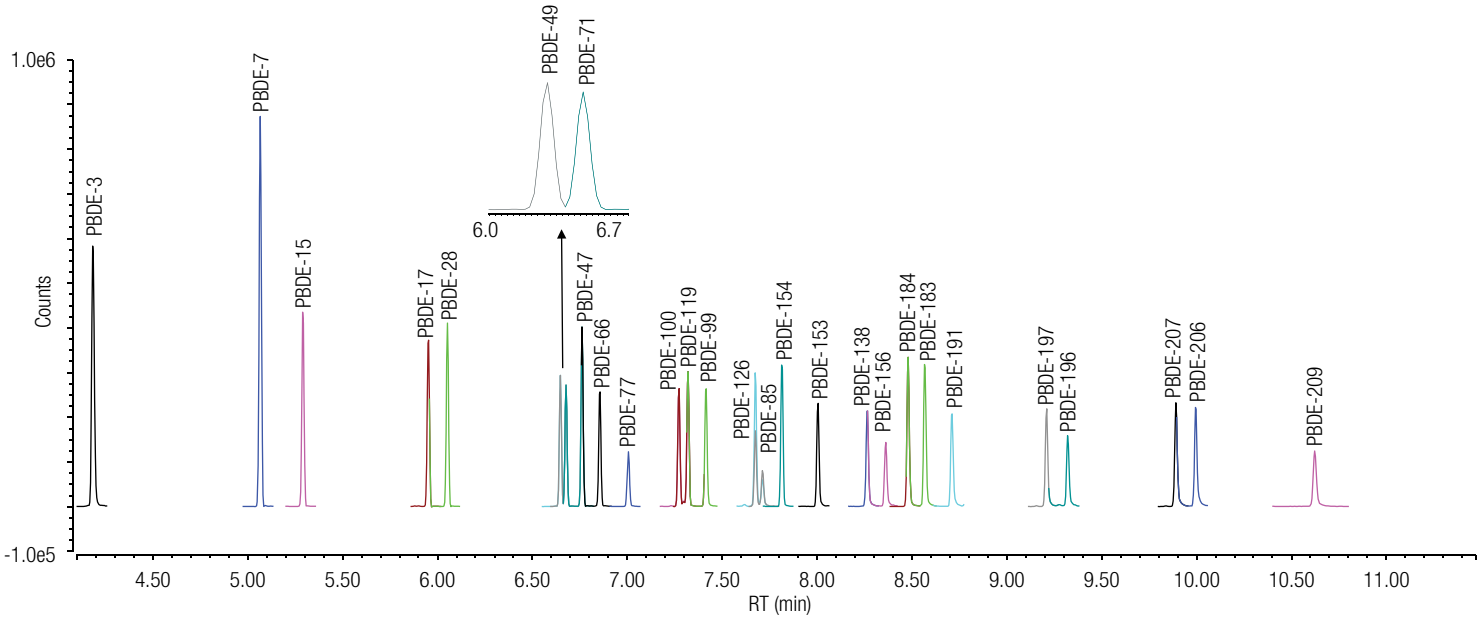


Figure 2. Chromatogram showing the SRM transition (quant ion) for PBDEs in a 1–5 pg/µL solvent standard (CS-1) (equivalent to 2.5–12.5 ng/kg in sample) with excellent chromatographic peak shapes for all compounds. ^{13}C-labeled internal and syringe standards were not displayed to show native peak shapes clearly.

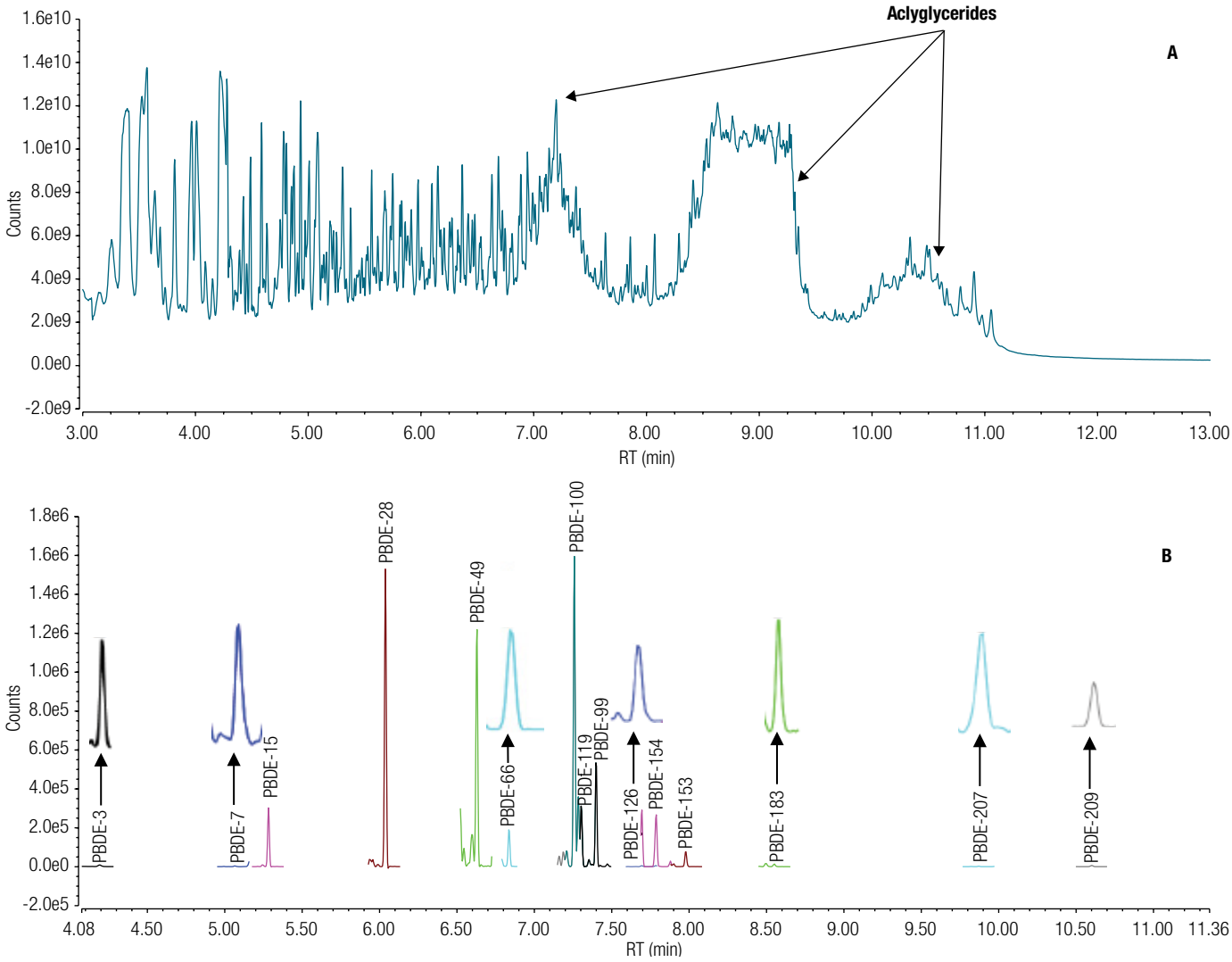


Figure 3. (A) Extracted fish matrix TIC acquired in full-scan containing late eluting acylglycerides (top chromatogram) and (B) corresponding timed-SRM for quant ion of targeted PBDEs (bottom chromatogram)

Carryover assessment

Carryover can be a problem for this application; however, it was found that using a mixed needle wash solvent of dichloromethane/toluene/*n*-nonane (50:25:25) eliminated this potential problem. In Figure 4 an example SRM transition of the highest concentration injected standard for BDE-209 (2000 pg/µL, 4 ng on column (oc)) (top chromatogram) and the consecutive *n*-nonane blank (bottom chromatogram) demonstrates that there is no carryover.

Sensitivity: determination of IDLs

The enhanced sensitivity of the new AEI source is demonstrated for the most challenging compound analyzed, BDE-209 (Figure 5). Here a 250 fg/µL (500 fg oc) solvent standard shows excellent signal precision with peak area repeatability <10% RSD at low ppt levels (equivalent to 0.6 ng/kg) in sample extracts). Excellent peak shape was also observed for this high molecular weight compound (MW = 959.2), which is due to the thin film and excellent surface deactivation of the TG-PBDE column coupled with the highly uniform heating profile of the newly designed TSQ 9000 GC-MS/MS system transfer line. These factors result in less peak tailing for low volatility, high boiling compounds such as higher brominated PBDEs and make accurate integration possible.

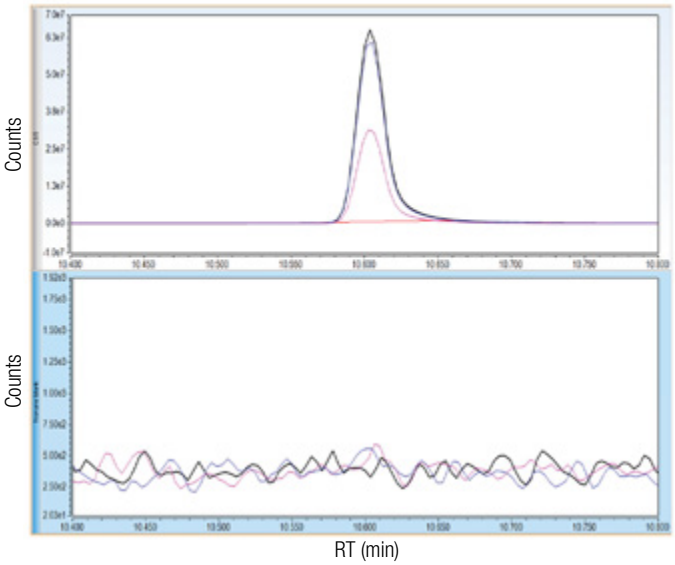


Figure 4. BDE 209 overlaid quantification ion and qualification ions for the highest standard in *n*-nonane/ toluene 2000 pg/µL corresponding to 4 ng on-column (OC) (top chromatogram) and a consecutive *n*-nonane blank (bottom chromatogram). Data is unsmoothed and was acquired in timed-SRM mode.

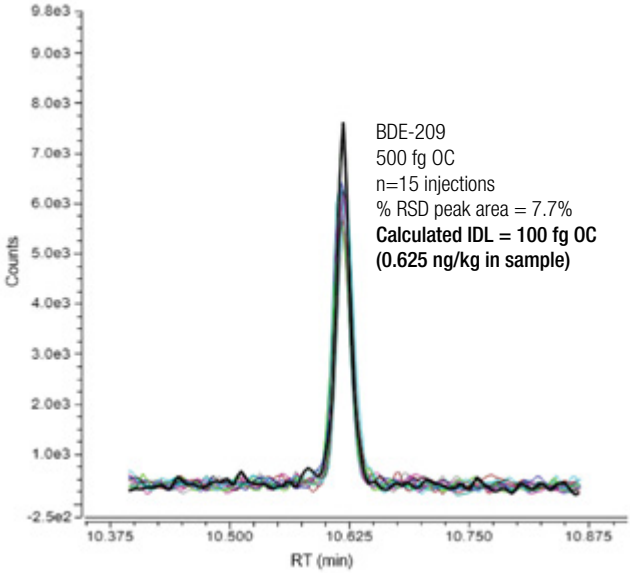


Figure 5. Overlaid quantification SRM transitions (797.3→637.3 *m/z*) from n=15 consecutive injections of a 250 fg/µL BDE-209 solvent standard (corresponding to 0.625 ng/kg in sample). No data smoothing was used and data was acquired in timed-SRM mode.

To practically assess the IDLs n=15 replicate injections of the lowest serially diluted solvent standard with a peak area % RSD of <15% was used. IDLs were then calculated by taking into account the injected amount, peak area % RSD, and t-score of 2.624, corresponding to n=14 degrees of freedom at 99% confidence level (Figure 6). The IDL values calculated ranged from 2 to 100 fg OC (corresponding to 0.003–0.125 ng/kg in sample).

Sensitivity: determination of limit of quantitation (LOQ)

Method LOQs were calculated using serially diluted calibration standards described in the IDL section. Fifteen replicate injections of each of the diluted standards ranging between 0.02 pg/µL and 0.25 pg/µL were performed (equivalent to 0.05–0.63 ng/kg in sample (Table 6).

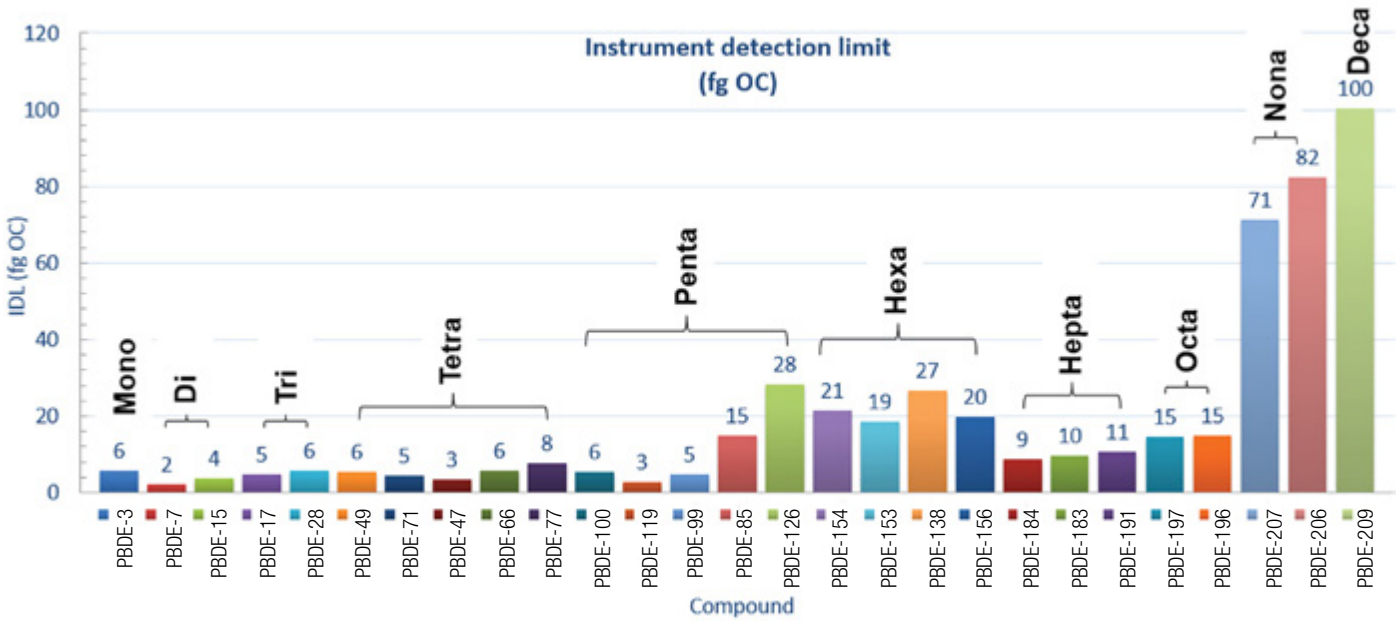


Figure 6. Graph showing individual IDLs in fg on column for 27 native PBDEs calculated from n=15 replicate injections of the lowest serially diluted standards

Table 6. Calculated equivalent LOQ concentration in sample from fifteen injections of solvent standards

BDE Number	Bromination No*	Quantification Transition	Amount Injected (pg OC)	Target Ion Ratio %	Mean Measured Ion Ratio (Average) %	Peak Area % RSD	LOQ (pg OC)	LOQ (ng/kg)
BDE-3	Mono	250→115	0.04	60	62	5.6%	0.04	0.05
BDE-7	Di	326→139	0.04	15	15	3.6%	0.04	0.05
BDE-15	Di	326→139	0.10	48	49	3.6%	0.10	0.13
BDE-17	Tri	406→139	0.04	95	93	5.9%	0.04	0.05
BDE-28	Tri	406→139	0.04	79	78	6.4%	0.04	0.05
BDE-49	Tetra	484→217	0.10	34	34	3.3%	0.10	0.13
BDE-71	Tetra	484→217	0.10	33	34	4.1%	0.10	0.13
BDE-47	Tetra	484→217	0.10	36	36	4.3%	0.10	0.13
BDE-66	Tetra	484→217	0.10	30	31	5.0%	0.10	0.13
BDE-77	Tetra	484→217	0.10	115	112	7.0%	0.10	0.13
BDE-100	Penta	564→404	0.10	85	74	4.1%	0.10	0.13
BDE-119	Penta	564→404	0.10	48	48	3.8%	0.10	0.13
BDE-99	Penta	564→404	0.10	62	57	3.0%	0.10	0.13
BDE-85	Penta	564→404	0.10	60	56	5.7%	0.10	0.13
BDE-126	Penta	564→404	0.20	122	131	7.1%	0.20	0.25
BDE-154	Hexa	642→482	0.20	66	64	8.1%	0.20	0.25
BDE-153	Hexa	642→482	0.20	65	57	7.3%	0.20	0.25
BDE-138	Hexa	642→482	0.20	68	63	8.0%	0.20	0.25
BDE-156	Hexa	642→482	0.20	70	74	8.7%	0.20	0.25
BDE-184	Hepta	721→564	0.20	46	46	6.3%	0.20	0.25
BDE-183	Hepta	721→564	0.20	47	49	4.3%	0.20	0.25
BDE-191	Hepta	721→564	0.20	48	47	5.0%	0.20	0.25
BDE-197	Octa	642→482	0.20	48	48	4.0%	0.20	0.25
BDE-196	Octa	642→482	0.20	61	64	5.8%	0.20	0.25
BDE-207	Nona	879→721	0.50	52	53	5.4%	0.50	0.63
BDE-206	Nona	879→721	0.50	60	49	6.3%	0.50	0.63
BDE-209	Deca	797→637	0.50	100	96	7.7%	0.50	0.63

The criteria used to assess individual PBDE LOQs were:

- Ion ratios within ±30 % of the expected values calculated as an average across a calibration curve ranging from 5 to 2000 pg/µL (BDE-209) (corresponding to 12.5–5000 ng/kg in extracted fat)
- Peak area repeatability of <15 % RSD
- Relative response factor (RRF) within ± 30% of that calculated from the average of the calibration

Linearity

Linearity was determined using solvent standards at concentrations 1–400 pg/µL for mono-penta PBDEs, 2–800 pg/µL for hexa-octa PBDEs, and 5–2000 pg/µL nona-deca PBDEs. The calibration of each PBDE was performed using average calibration factor (AvCF) and isotopic dilution functions in Chromeleon CDS with triplicate injections at each concentration (Figure 8).

All compounds show excellent linear responses with coefficients of determination R^2 >0.98, and average RRF % RSD across the calibration range being <10% (Table 7).

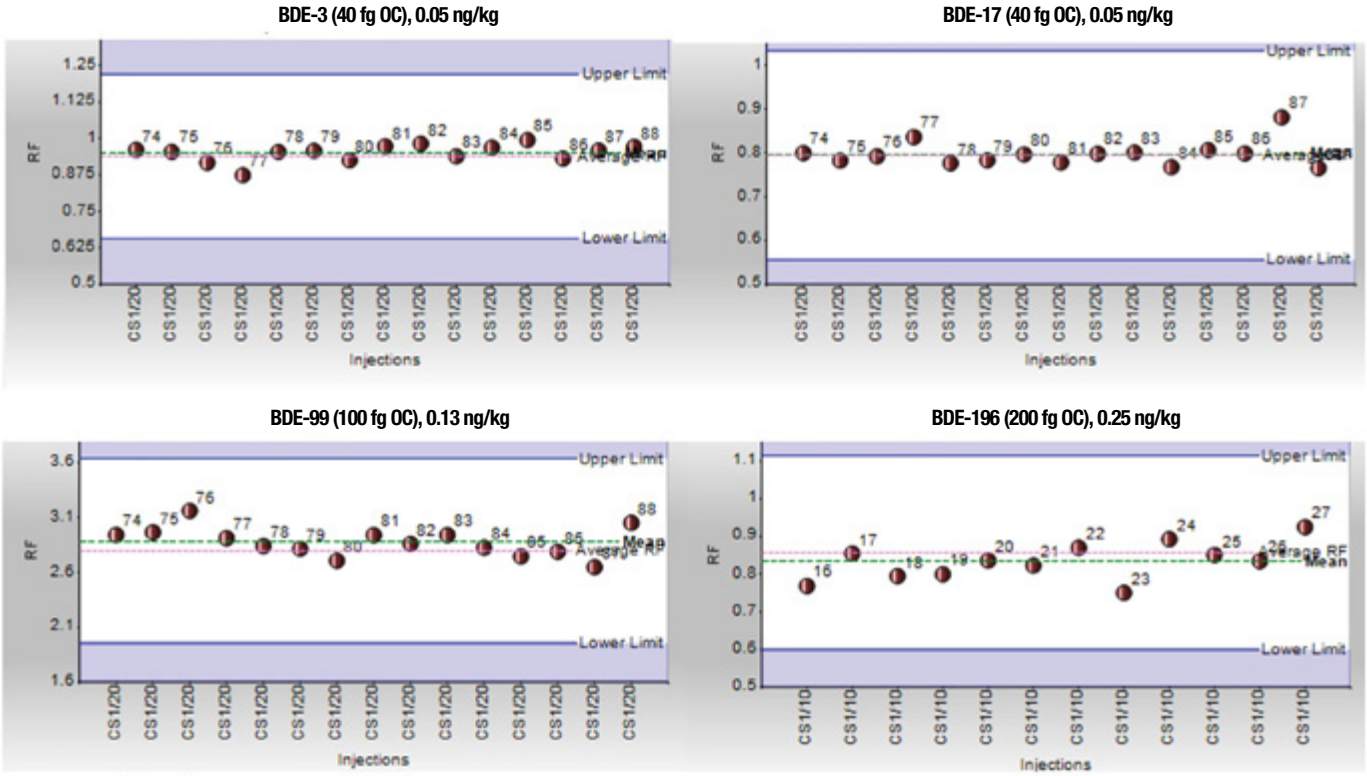
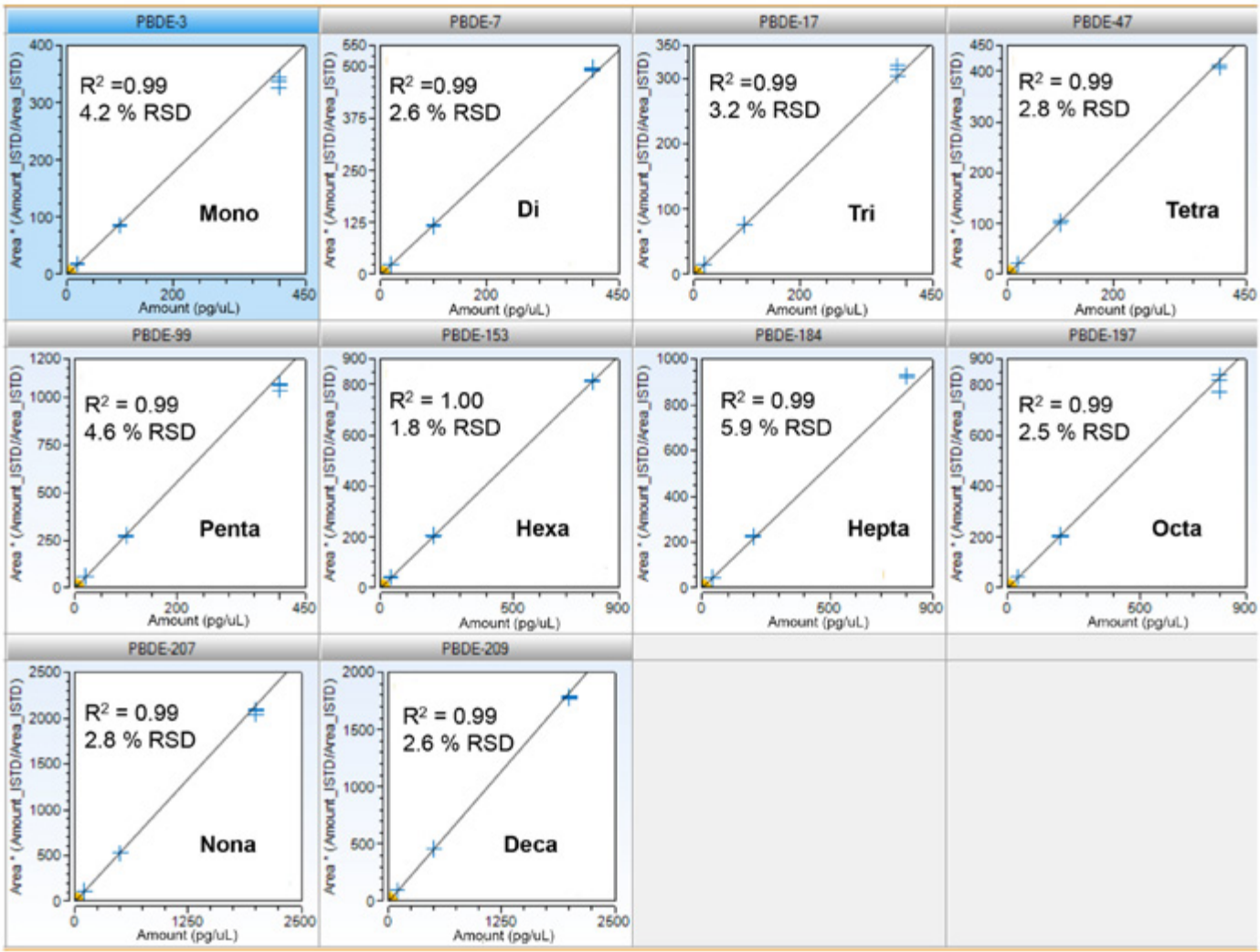


Figure 7. Graphs showing RRF consistency for selected PBDEs over n=15 replicate injections at the LOQ level. The average RRF calculated from the calibration range is displayed as a pink dotted line in the center. The ±30% upper and lower RRF tolerance windows are also defined, and for all PBDEs the RRFs for injections were within specification. This also illustrates how using Chromeleon CDS interactive charts allows the user to easily handle and interpret MS data

A



B

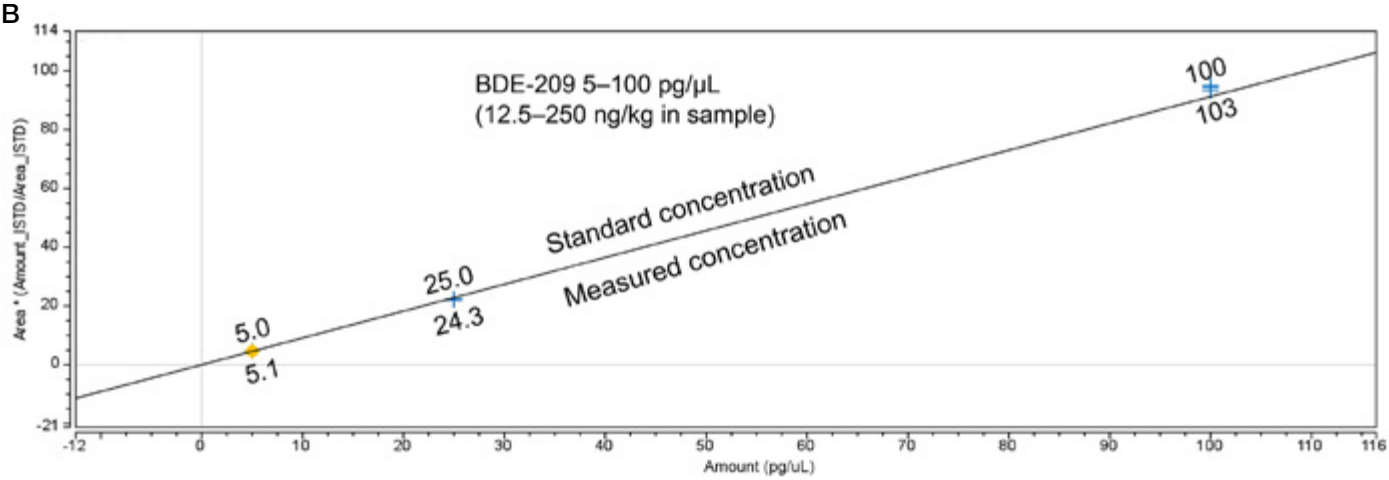


Figure 8. (A) Linearity of PBDEs demonstrated using a solvent-based calibration curve ranging from 1.0 to 2000 pg/µL in the case of BDE-209 (corresponding to 2.5–5000 ng/kg in food). Average calibration factor (AvCF) function was used in Chromeleon CDS with three replicate injections at each concentration and internal standard adjustment was conducted. Coefficient of determination (R^2) and average RRF % RSD are displayed. (B) Expanded region of calibration for BDE-209 from 5 to 100 pg/µL is shown (corresponding to 12.5–250 ng/kg in extracted fat) demonstrating excellent accuracy and precision for triplicate injections per point.

Table 7. Coefficient of determination (R^2) and RRF % RSD

Compound	R^2	RRF % RSD	Compound	R^2	RRF % RSD
BDE-3	0.9941	4.2	BDE-126	0.9980	2.8
BDE-7	0.9987	2.6	BDE-154	0.9923	5.1
BDE-15	0.9987	2.9	BDE-153	1.0000	1.8
BDE-17	0.9980	3.2	BDE-138	0.9976	2.7
BDE-28	0.9999	1.5	BDE-156	0.9795	9.9
BDE-49	0.9995	3.8	BDE-184	0.9927	5.9
BDE-71	0.9953	4.2	BDE-183	0.9992	1.8
BDE-47	0.9991	2.8	BDE-191	0.9879	7.6
BDE-66	0.9970	4.4	BDE-197	0.9976	2.5
BDE-77	0.9959	6.6	BDE-196	0.9823	9.3
BDE-100	0.9994	1.7	BDE-207	0.9982	2.8
BDE-119	0.9921	5.1	BDE-206	0.9988	2.6
BDE-99	0.9952	4.6	BDE-209	0.9991	2.6
BDE-85	0.9975	7.3			

PBDE quantification in food samples and comparison to GC-HRMS data

Several food samples were tested for the PBDE content and examples of sensitivity, selectivity, and accuracy of measurements are highlighted below (Figures 9 and 10).

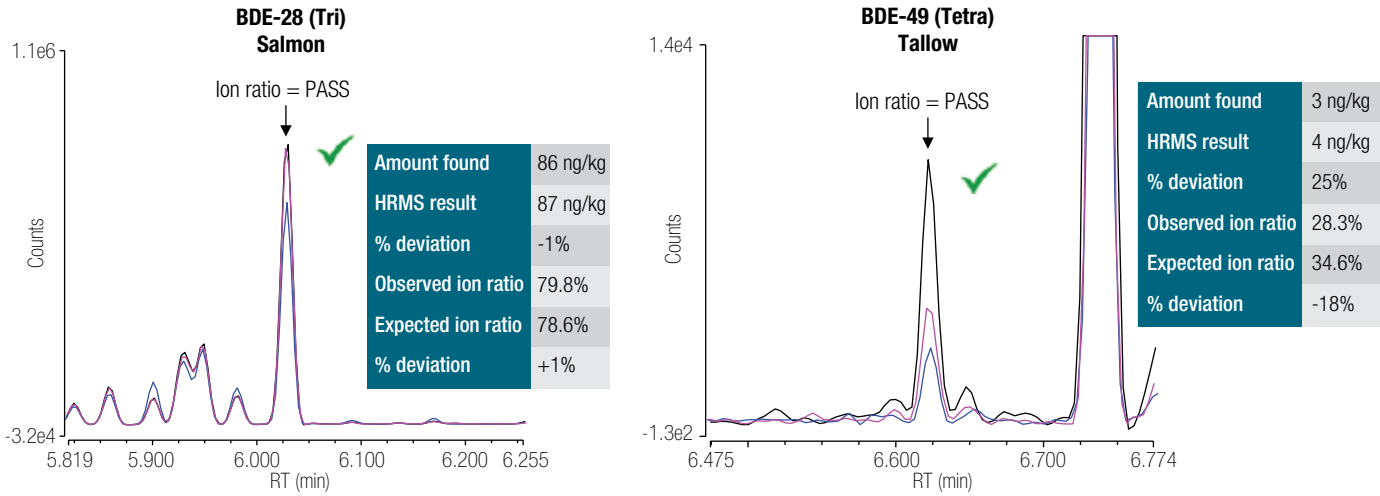


Figure 9. Examples of SRMs chromatograms (quantification in black, and confirmation ions in pink and blue) for BDE-28 in salmon (left chromatogram) and BDE-49 in tallow (right chromatogram); visit the AppsLab Library for full SRM transitions. Below each of the chromatograms the following is shown: (i) amount found in sample in ng/kg, (ii) HRMS (magnetic sector data) result provided from Fera Science, Ltd. in ng/kg, (iii) % deviation from Fera Science, Ltd. result, (iv) observed ion ratio between quantification and primary confirmation ion, (v) expected ion ratio calculated from the average of the calibration, and (vi) % deviation of observed ion ratio versus the expected ion ratio.

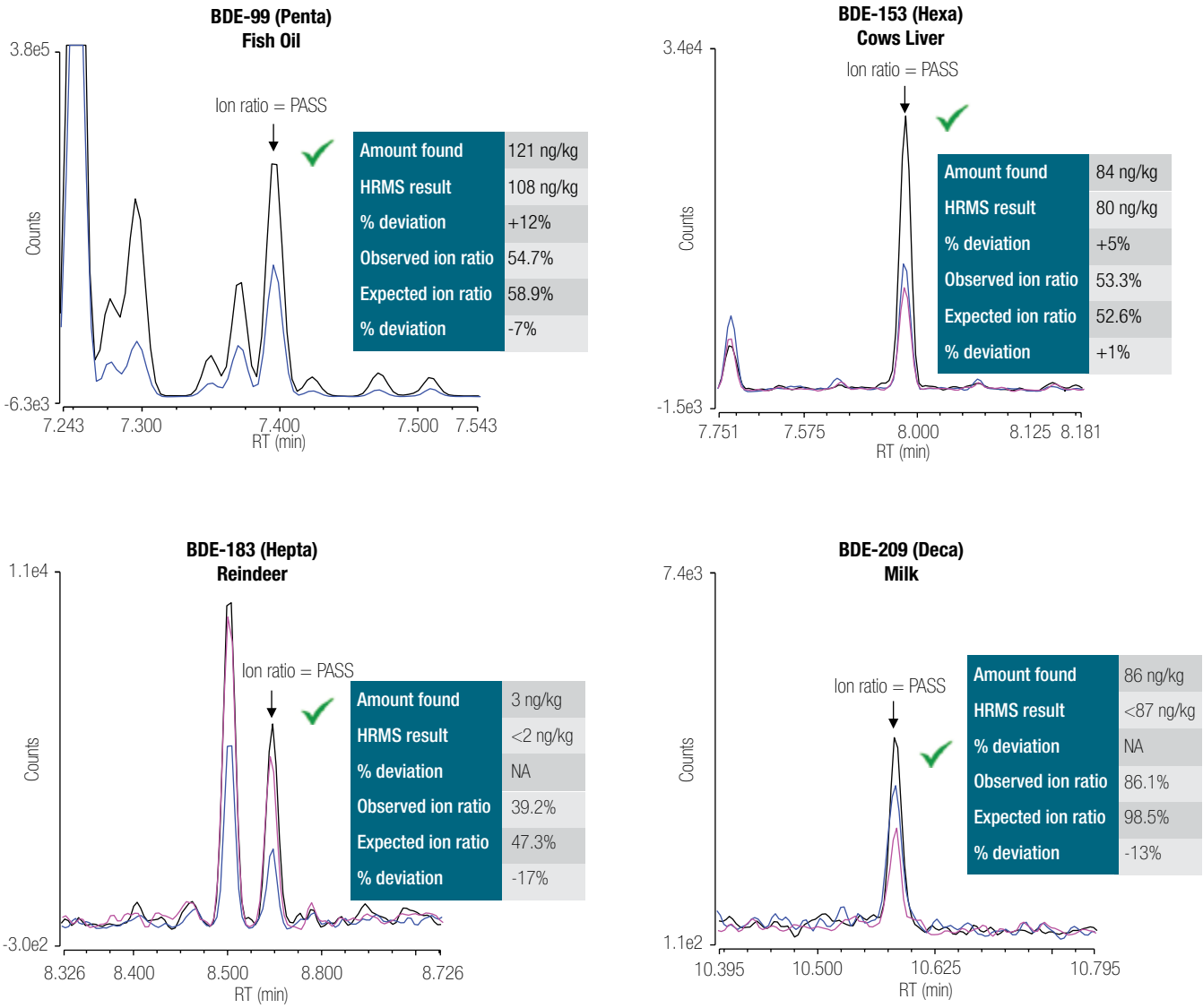


Figure 10. The two chromatograms show the overlaid selective reaction monitoring transitions (quantification in black, and confirmation ions in pink and blue) for BDE-99 in fish oil (top left chromatogram), BDE-153 in cows liver (top right chromatogram), BDE-183 in reindeer (bottom left chromatogram), and BDE-209 in milk (bottom right chromatogram).

In summary, the results comparison for triple quadrupole technology versus GC-HRMS shows very close agreement. The low limits of quantification that are achievable using triple quadrupole technology are clearly demonstrated in the case of BDE-49 in tallow and BDE-183 in reindeer; in both cases low ppt (ng/kg in extracted fat) results were reported with ion confirmation within the ±30% tolerance of the averaged ion ratio across the calibration. In the case of the complex fish oil matrix, the power of the mass spectrometer and TG-PBDE column combination become apparent in their ability to selectively resolve complex matrix interferences and congeners with the deviation in measured ion ratio versus the calibration of only 7%.

Conclusions

The purpose of these experiments was to assess the quantitative performance of the TSQ 9000 GC-MS/MS system for increased sample throughput. Additionally, the results obtained were compared with those from GC-HRMS to assess the measurement accuracy.

- All 27 native BDE congeners were chromatographically separated in <11 min, allowing an increase in sample throughput of 3X compared to existing GC-MS methods.[3]
- The levels of sensitivity obtained allowed IDLs ranging from 2 to 100 fg OC (corresponding to 0.003 to 0.125 ng/kg in extracted fat).
- LOQs (derived from solvent standards) were equivalent to 0.05 to 0.63 ng/kg in extracted fat calculated from n=15 repeat injections of the lowest serially diluted standard that satisfied the acceptance criteria defined below:
  - Ion ratios within ±30% of the expected values calculated as an average across a calibration curve ranging from 5 to 2000 pg/µL (corresponding to 10–5000 ng/kg in extracted fat).
  - Peak area repeatability of <15% RSD.
  - RRFs were within ±30% of that calculated from the average of the calibration.
- Excellent linearity was achieved across a calibration of 1–2000 pg/µL (corresponding to 2.5–5000 ng/kg in extracted fat) with all RRF % RSDs <10% and R^2 values >0.98.
- The overall quantitative performance of the TSQ 9000 GC-MS/MS system was demonstrated by the ability to easily detect and confirm (using ion ratio values) low levels of PBDEs even in most critical sample types such as fish oil.
- The results obtained from the TSQ 9000 GC-MS/MS system experiments were in close agreement with HRMS (magnetic sector) data provided from Fera Science, Ltd., York was achieved even at very low concentrations.

Taken together these results demonstrate that the TSQ 9000 GC-MS/MS system configured with the AEI source provides unparalleled levels of quantitative performance making it an ideal analytical tool for routine food safety testing laboratories.

References

1. Hites, R.A. Polybrominated Diphenyl Ethers in the Environment and in People: A Meta-Analysis of Concentrations. *Environ. Sci. Technology* **2004,** *38,* 945.
2. Guidance for the inventory of polybrominated diphenyl ethers (PBDEs) listed under the Stockholm Convention on POPs. [Online] http://chm.pops.int/Implementation/NationalImplementationPlans/Guidance/GuidancefortheinventoryofPBDEs/tabid/3171/Default.aspx (accessed May 8, 2018).
3. Fernandes, A.; White, S.; D'Silva, K.; Rose. M. Simultaneous determination of PCDDs, PCDFs, PCBs and PBDEs in food. *Talanta* **2004,** *63,* 1147–1155.
4. Thermo Fisher Scientific (2009) Timed SRM technical note: https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/AN51797-TimedSRM-Multitarget-Compound-Analysis.pdf
5. United States Environmental Protection Agency, Method 1614A Brominated Diphenyl Ethers in Water, Soil, Sediment, and Tissue by HRGC/HRMS, May 2010. https://www.epa.gov/sites/production/files/2015-08/documents/method_1614a_2010.pdf

Appendix

Table 1. Details of 27 native PBDE congeners analyzed, including BDE number, chemical formula, CAS number, and calibration range

BDE Number	Native BDEs	Chemical Formula	CAS Number	Calibration Range (ng/mL)
3	4-Bromodiphenyl ether	$C_{12}H_9BrO$	101-55-3	1.0 to 400
7	2,4-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	171977-44-9	1.0 to 400
15	4,4'-Dibromodiphenyl ether	$C_{12}H_8Br_2O$	2050-47-7	1.0 to 400
17	2,2',4-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	147217-75-2	0.96 to 384
28	2,4,4'-Tribromodiphenyl ether	$C_{12}H_7Br_3O$	41318-75-6	1.0 to 400
47	2,2',4,4'-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	5436-43-1	1.0 to 400
49	2,2',4,5'-Tetrabromodiphenyl ether	$C_{12}H6Br_4O$	243982-82-3	1.0 to 400
66	2,3',4,4'-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	189084-61-5	1.0 to 400
71	2,3',4',6-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	189084-62-6	1.0 to 400
77	3,3',4,4'-Tetrabromodiphenyl ether	$C_{12}H_6Br_4O$	93703-48-1	1.0 to 400
85	2,2',3,4,4'-Pentabromodiphenyl ether	$C_{12}H_6Br_5O$	182346-21-0	1.0 to 400
99	2,2',4,4',5-Pentabromodiphenyl ether	$C_{12}H_6Br_5O$	32534-81-9	1.0 to 400
100	2,2',4,4',6-Pentabromodiphenyl ether	$C_{12}H_6Br_5O$	189084-64-8	1.0 to 400
119	2,3',4,4',6-Pentabromodiphenyl ether	$C_{12}H_6Br_5O$	189084-66-0	1.0 to 400
126	3,3',4,4',5-Pentabromodiphenyl ether	$C_{12}H_6Br_5O$	366791-32-4	1.0 to 400
138	2,2',3,4,4',5-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	446254-95-1	2.0 to 800
153	2,2',4,4',5,5'-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	68631-49-2	2.0 to 800
154	2,2',4,4',5,6'-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	207122-15-4	2.0 to 800
156	2,3,3',4,4',5-Hexabromodiphenyl ether	$C_{12}H_4Br_6O$	405237-85-6	2.0 to 800
183	2,2',3,4,4',5',6-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	207122-16-5	2.0 to 800
184	2,2',3,4,4',6,6'-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	117948-63-7	2.0 to 800
191	2,3,3',4,4',5',6-Heptabromodiphenyl ether	$C_{12}H_3Br_7O$	446255-30-7	2.0 to 800
196	2,2',3,3',4,4',5,6'-Octabromodiphenyl ether	$C_{12}H_2Br_8O$	446255-39-6	2.0 to 800
197	2,2',3,3',4,4',6,6'-Octabromodiphenyl ether	$C_{12}H_2Br_8O$	117964-21-3	2.0 to 800
206	2,2',3,3',4,4',5,5',6-Nonabromodiphenyl ether	$C_{12}HBr_9O$	63936-56-1	5.0 to 2000
207	2,2',3,3',4,4',5,6,6'-Nonabromodiphenyl ether	$C_{12}HBr_9O$	437701-79-6	5.0 to 2000
209	Decabromodiphenyl ether	$C_{12}Br_{10}O$	1163-19-5	5.0 to 2000

Table 2. Details of 16 ^{13}C-labeled PBDEs internal standards, including BDE isomer number, chemical formula, CAS number, and concentration (suffix "L" indicates mass-labeled)

BDE isomer number	^{13}C-labeled PBDEs	Chemical Formula	Concentration (ng/mL)
3L	4-Bromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_9BrO$	100
15L	4,4'-Dibromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_8Br_2O$	100
28L	2,4,4'-Tribromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_7Br_3O$	100
47L	2,2',4,4'-Tetrabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_6Br_4O$	100
79L	3,3',4,5'-Tetrabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_6Br_4O$	100
99L	2,2',4,4',5-Pentabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_5Br_5O$	100
100L	2,2',4,4',6-Pentabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_5Br_5O$	100
126L	3,3',4,4',5-Pentabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_5Br_5O$	100
138L	2,2',3,4,4',5-Hexabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_4Br_6O$	200
153L	2,2',4,4',5,5'-Hexabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_4Br_6O$	200
154L	2,2',4,4',5,6'-Hexabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_4Br_6O$	200
183L	2,2',3,4,4',5',6-Heptabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_3Br_7O$	200
197L	2,2',3,3',4,4',6,6'-Octabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}H_2Br_8O$	200
206L	2,2',3,3',4,4',5,5',6-Nonabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}HBr_9O$	500
207L	2,2',3,3',4,4',5,6,6'-Nonabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}HBr_9O$	500
209L	Decabromo[$^{13}C_{12}$]diphenyl ether	$^{13}C_{12}Br_{10}O$	500

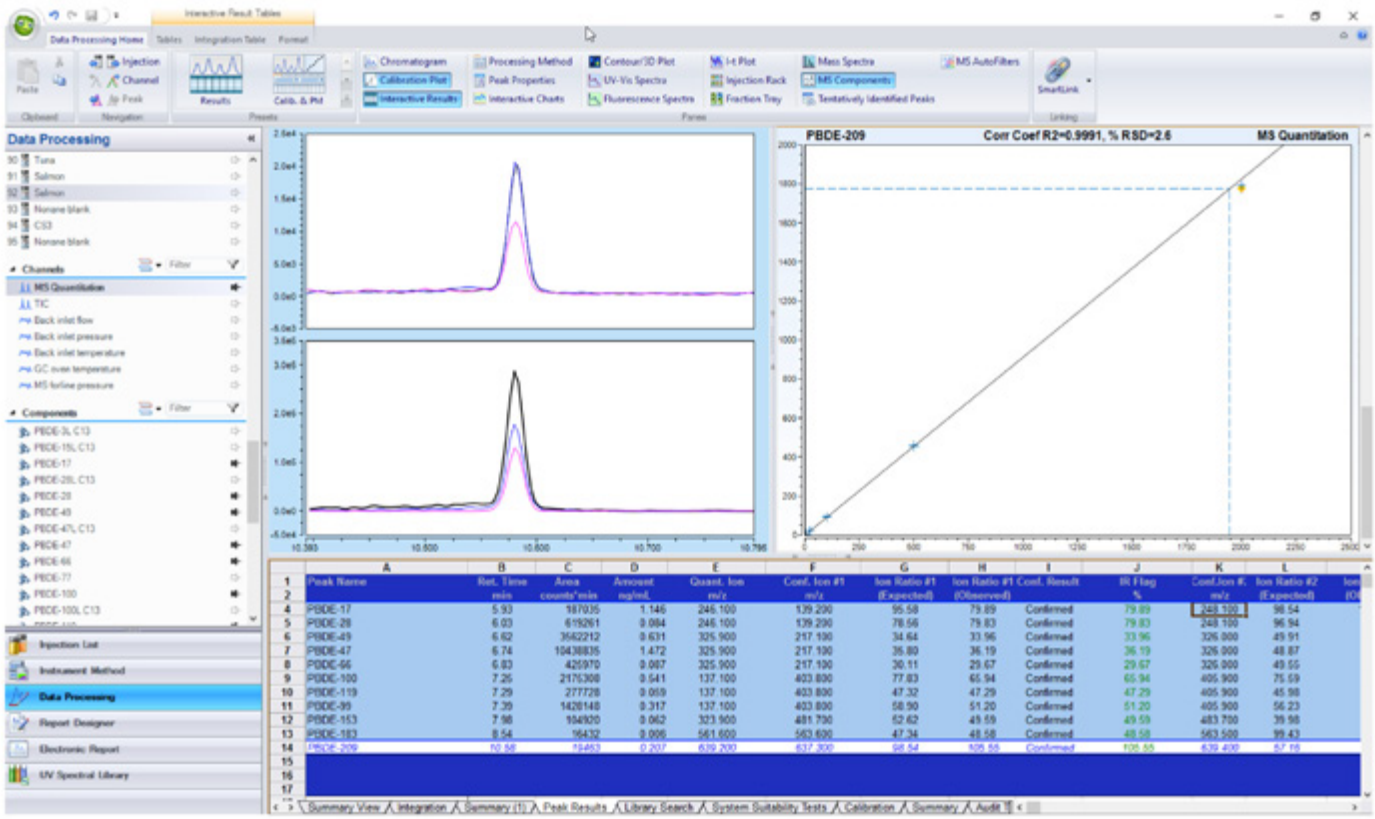


Figure 1. Example of the Chromeleon CDS interactive results browser used to assess and interrogate sample data in real time

Find out more at **thermofisher.com/TSQ9000**

©2018 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

AN10674-EN 1018S

thermo scientific

APPLICATION NOTE 10703

Routine, regulatory analysis of dioxins and dioxin-like compounds in food and feed samples

Authors

Richard Law[1], Alexander Schaechtele[2], Amit Gujar[3], Jiangtao Xing[4], and Cristian Cojocariu[1]

[1]Thermo Fisher Scientific, Runcorn, UK

[2]European Union Reference Laboratory (EURL) for Halogenated POPs in Feed and Food, Freiburg, Germany

[3]Thermo Fisher Scientific, Austin, Texas

[4]Thermo Fisher Scientific, Beijing, China

Keywords

Triple quadrupole GC-MS/MS, persistent organic pollutants, POPs, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzo-*p*-furans, PCDD/Fs, dioxins, polychlorinated biphenyls, PCBs, confirmatory analysis, TSQ 9000, advanced electron ionization, AEI

Goal

To demonstrate the utility of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software for the routine and regulatory compliant analysis of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzo-*p*-furans (PCDD/Fs), dioxin-like polychlorinated biphenyls (PCBs), and indicator PCBs in food and feed samples.

Introduction

Dioxins and dioxin-like compounds are highly toxic substances classed as persistent organic pollutants (POPs). Due to their high fat-solubility, dioxins accumulate in the fatty tissues of animals. As a result, more than 90% of human exposure to dioxins is through food, especially meat, dairy, fish, etc. Therefore, accurate monitoring of food and feed is essential to control dioxin uptake from the food chain.[1]

In 2014 a change in European Commission regulations[2,3] permitted gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) to be used as an alternative to gas chromatography-high resolution mass spectrometry (GC-HRMS) for confirmatory analysis and for the control of

ThermoFisher SCIENTIFIC

maximum levels (MLs) and action levels (ALs) in certain food and feed samples. Even though the utility of GC-MS/MS for this application has been demonstrated in principle,[4] there is a lack of robust data to validate the suitability of GC-MS/MS, especially for the long-term routine analysis of hundreds of samples. This is further confused by the absence of a clear protocol regarding the setting of appropriate limit of quantification (LOQ) values for GC-MS/MS analysis, with both signal-to-noise (S:N) and calibration-based approaches being used in some validations.

In addition to the deficiencies in validation data, there is a need for software packages to deal with the complexities of the calculations required to process and report data using isotopic dilution. As a consequence many laboratories adopt external software tools to manipulate the data. This practice is not only time-consuming, but can lead to errors in transcription and rounding, and also to an uncontrolled data trail. It is preferable to have the capability to acquire data, process data, and perform calculations and report the required results on a single, compliant software platform.

In this study, the performance of the TSQ 9000 triple quadrupole GC-MS/MS system equipped with an advanced electron ionization (AEI) source was evaluated. Data was acquired on two different TSQ 9000 AEI systems located in two different laboratories and operated by different chemists (UK and USA). Commercially available solvent standards, food/feedstuff, and proficiency test (PT) samples were used to evaluate the performance of each system for the analysis of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzo-*p*-furans (PCDFs), dioxin-like polychlorinated biphenyls (PCBs), and non-dioxin-like (indicator) PCBs. Guidance from the European Union Reference Laboratories (EURL) on the use of a calibration approach was followed to set suitable LOQs:[5] essentially, to demonstrate sufficient sensitivity to enable reporting at 1/5th of the maximum level (ML) upper bound sum toxic equivalences (TEQs).

To demonstrate the robustness required to operate in a routine environment an experiment involving continuous analysis of extracts over a period of two weeks was carried out.

Experimental

Instrumental and method setup

In the experiments described here, a TSQ 9000 AEI triple quadrupole mass spectrometer was coupled to a Thermo Scientific™ TRACE™ 1310 Gas Chromatograph. Injection of liquid samples was performed automatically using a Thermo Scientific™ TriPlus™ RSH autosampler. See Appendix 2 for a list of the consumables used. Mass spectrometer operation was as per AN10590[4] unless otherwise specified. Importantly, acquisition, processing, and reporting of the data were all performed on a single platform using Chromeleon CDS software, version 7.2. Two separate GC-MS/MS methods were used: one for the analysis of non-ortho PCBs and PCDD/Fs (Table 1), and one to capture other dioxin and non dioxin-like compounds such as mono-ortho, di-ortho, and indicator PCBs fraction (Table 2). See Tables 1 and 2 in Appendix 1.

Samples, extraction, and clean-up

Food and feedstuff samples (including PT samples) were provided by the EURL for Halogenated POPs in Feed and Food, Freiburg, Germany. A nominal sample intake weight of 2 grams (fat) was used for the samples unless indicated otherwise (Table 3). European method EN:1948 standard solutions; EN-1948CVS, WM48-CVS (calibration and quantitation), EN-1948ES, EN-1948IS, P48-W-ES, P48-M-ES, and P48-RS (extraction) were utilized for the extraction, calibration, and quantitation of PCDD/Fs, dioxin-like PCBs, and indicator PCBs. All standards were obtained from Wellington Laboratories Inc., Canada.

Extraction (where required) was performed by Twisselmann hot extraction (comparable with Soxhlet extraction) or pressurized liquid extraction. Automated clean-up of extracts was performed using a three column (multi-layered acidic silica, alumina, and carbon columns) setup on the DEXTech™ Plus system (LCTech GmbH). Two extract fractions were provided per sample, the first containing the non-ortho PCBs and PCDD/Fs (final volume 20 µL nonane) and the second containing the mono-ortho and di-ortho PCBs and indicator PCBs (final volume 100 µL nonane). Due to the absence of a non-ortho syringe standard in the calibration and extraction solutions, recoveries were not calculated for the four ^{13}C-labeled non-ortho PCBs. As all the non-ortho PCBs were found in all samples at values greater than the LOQ this does not impact the validity of the results obtained.

Table 3. Sample types and nominal intake weight

Sample type	Matrix	Nominal weight taken (g)	Number of replicates	Basis
PT	Pork sausage	2	2	Fat
PT	Whole egg	2	2	Fat
PT	Milk powder	2	2	Fat
PT	Halibut fillet	13	2	Wet weight
PT	Sugar beet pulp	20	2	Product
QK1	Mixed fat	2	6	Fat
Food	Meat	2	5 (individual)	Fat
Food	Milk	2	4 (individual)	Fat
Food	Fish	25 and 34	2 (individual)	Wet weight
Food	Fish oil	2	2 (individual)	Fat
Food	Eggs	2	5 (individual)	Fat
Feed	Fish meal	12	1	Product
Feed	Grass meal	20	1	Product
Feed	Sepiolite	20	1	Product
Feed	Palm fatty acid distillate (PFAD)	2	1	Product
Feed	Feed fat	2	1	Product

Results and discussion

Chromatography

The proprietary phase of the Thermo Scientific™ TraceGOLD™ TG-Dioxin capillary GC column (P/N 26066-1540) provided excellent separation of all 17 toxic PCDD/F and 18 dioxin-like and non dioxin-like PCB congeners in under 45 minutes, particularly the tetra (Figure 1) and penta-substituted PCDD/Fs.

By contrast, using a 5% phenyl type column, the 2,3,4,7,8-pentachlorodibenzofuran (PeCDF) congener (a major contributor to the WHO-PCDD/F-TEQ) can sometimes co-elute with some of the other non-toxic PeCDF congeners,[6] resulting in an overestimation of the concentration of this important congener. This could ultimately lead to a false TEQ being reported and, in a worst case scenario, false exceedance of MLs. All chromatographic criteria stated in regulation were met using the TG-Dioxin capillary GC column in this study.[2-4]

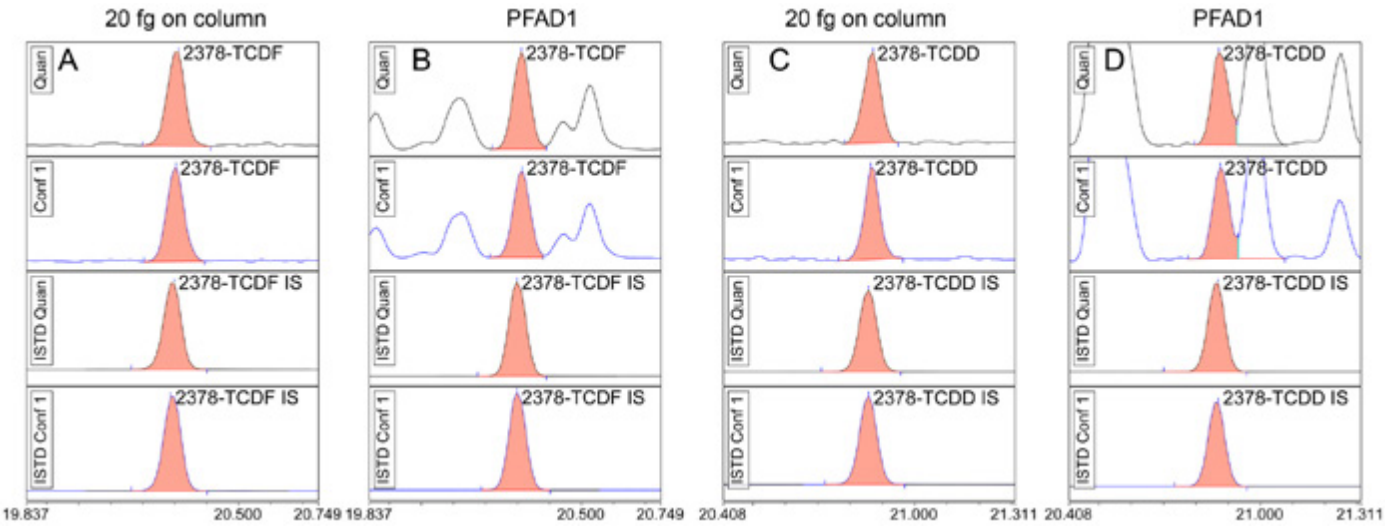


Figure 1. TCDD/F congener separation in solvent standard and palm fatty acid distillate (PFAD) PT sample. Associated ^{13}C-labeled congeners are displayed. (A) 20 fg on-column 2,3,7,8-tetrachlorodibenzofuran (TCDF); solvent standard, (B) ~180 fg on column 2,3,7,8-TCDF; PFAD sample, (C) 20 fg on-column 2,3,7,8-tetrachlorodibenzodioxin (TCDD); solvent standard and, (D) ~55 fg on column 2,3,7,8-TCDD; PFAD sample. All quantification and confirmation ions are labeled.

Determination of limits of quantitation (LOQs)

As previously described (AN10590), calculation of LOQs based on signal-to-noise ratios obtained using GC-MS/MS systems is unreliable; hence, it is more appropriate to use a calibration-based approach.[4,5] Employing calibration standards at the LOQ, and subsequent check standards at this level, allows the user to demonstrate continual method performance throughout the analytical sequence (Figures 3 and 4). It also allows for a simple calculation to determine the LOQ, which will be achieved for PCDD/Fs using a fixed sample weight (Formula 1):

where

Min $Conc_n$	is the lowest calibration concentration point of congener *n*;
Sample volume	is the final sample volume;
Sample weight	is the sample intake weight;
Recovery I	is the recovery of the associated ^{13}C-labeled congener *I*.

$$\text{Sample LOQ (pg/g)} = \sum_{n=\text{PCDD/F}}^{17} \text{Min Conc}_n\ (\text{pg/µL}) * \left(\frac{\text{Sample volume (µL)}}{\text{Sample weight (g)} * \text{Recovery}_I\ (\%)} \right)$$

Formula 1. Calculation to determine the LOQ for PCDD/Fs

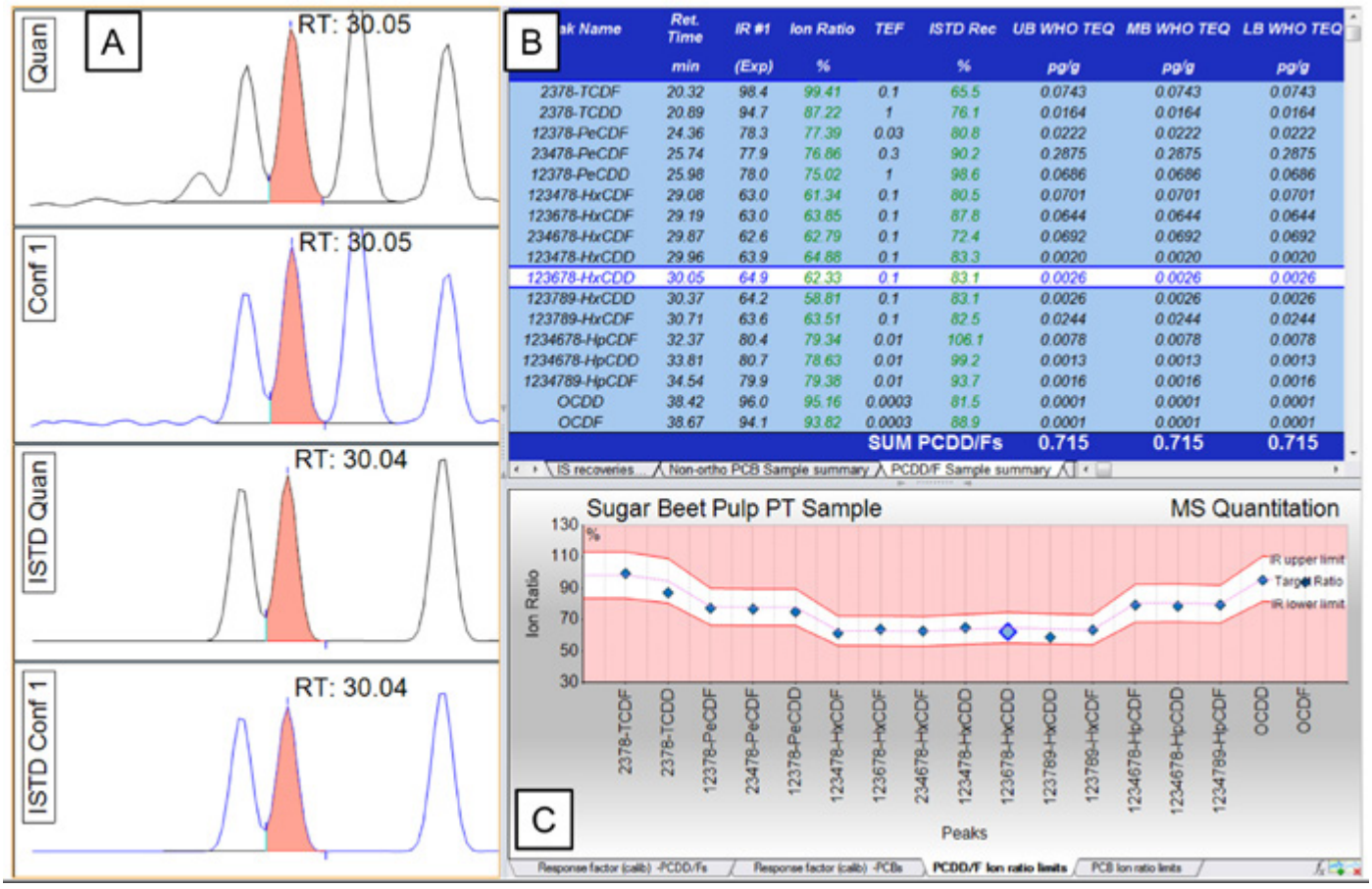


Figure 2. Typical Chromeleon processing browser showing (A) native quantification and confirmation peak with associated ^{13}C-labeled quantification and confirmation peaks, (B) interactive sample results browser showing upper-, middle- and lower-bound, WHO-PCDD/F-TEQ values, flagged ion ratios (IRs) and ^{13}C-labeled congener recovery, (C) IRs and LOQs visual display to easily check if the IR is outside the allowable range and if the peak amount is below the LOQ. Similar displays are available for PCBs. Sugar beet pulp PT sample shown; WHO-PCDD/F-TEQ 0.715 pg/g.

Assuming equal injection volume for standards and samples. This formula can also be applied to sum the total 29 PCDD/Fs and dioxin-like PCBs. Individual congener LOQs calculated in this way can be applied to upper-bound, middle-bound, and lower-bound TEQ results by simply replacing the result of any congeners that fall below the lowest calibration point with this value multiplied by the toxic equivalence factor (TEF) of the congener. Figure 2 shows an example of a real-time updated Chromeleon view including upper-, middle- and lower-bound sum values.

To assess the response factor (RF) deviation throughout the analytical sequences, regular standards at the specified LOQ were analyzed at the beginning, during (after every nine sample extracts injections), and end of the sequence. Chromeleon CDS interactive results panes with real-time updates including pass/fail for IR and RF deviation (calculated as deviation from the average calibration factor) are shown in Figure 3.

Using a nominal weight of 2 g and the lowest calibration level to establish the LOQ, a minimum upper-bound value of 0.152 pg/g WHO-PCDD/F-TEQ can be achieved (assuming 100% ^{13}C-labeled standard recovery and all natives are less than the LOQ in sample). This level is sufficient to demonstrate 1/5th ML compliance for all food and feed stuffs with a nominal intake of 2 g with the exception of food *for infants and young children and liver of terrestrial animals,* both with legal limits on fresh weight basis.[6,8] In which case, either a larger sample intake would be required or a magnetic sector instrument, such as the Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS system, should be the technique of choice.

Calibration

Calibration standards (eight levels for PCDD/Fs and seven levels for PCBs) were analyzed for four analytical sequences (PCDD/Fs and non-ortho PCBs and di- and mono-ortho PCBs and indicator PCBs), over the two systems with duplicate injection per level. The results of

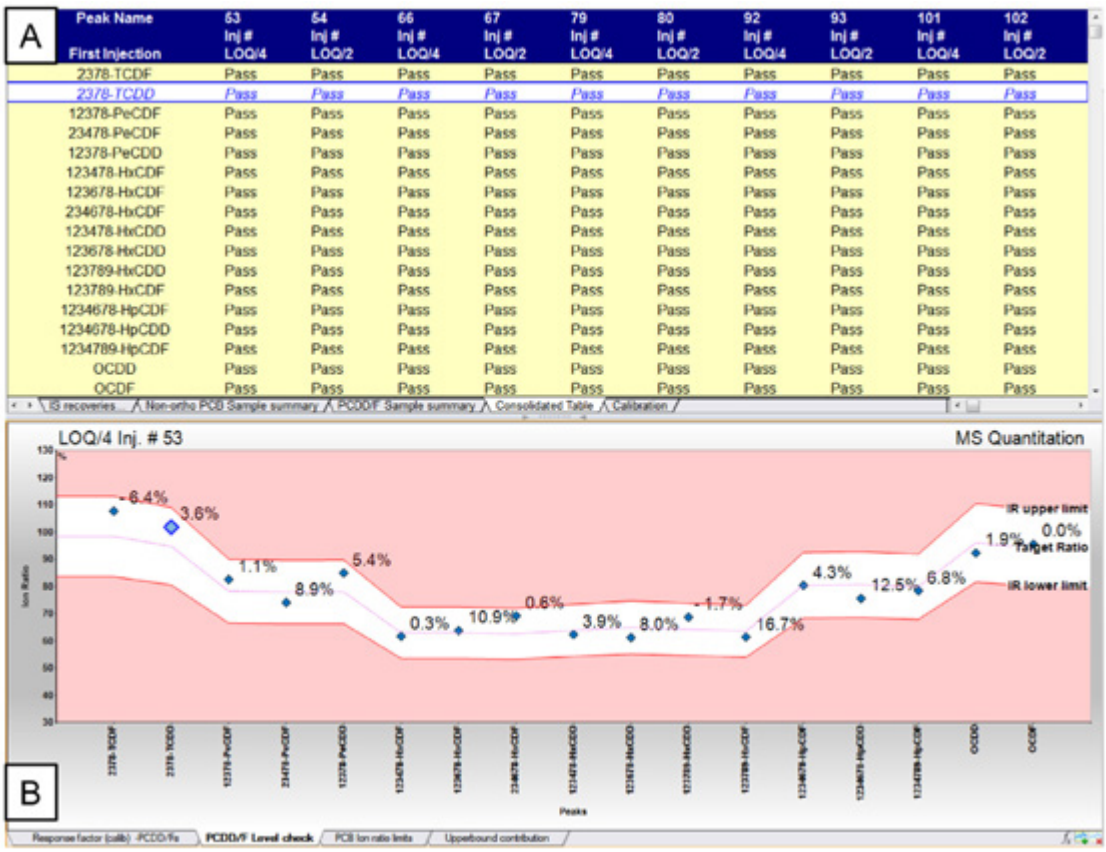


Figure 3. Chromeleon results browser showing (A) interactive results display with real-time updated Pass/Fail statements for each check standard, and (B) IR and RF deviation visual display to easily check if the IR is out of the allowable range (±15%) and if the congener has an RF within acceptable deviation (<30% from calibration average – indicated by the data label). Similar displays are available for PCBs.

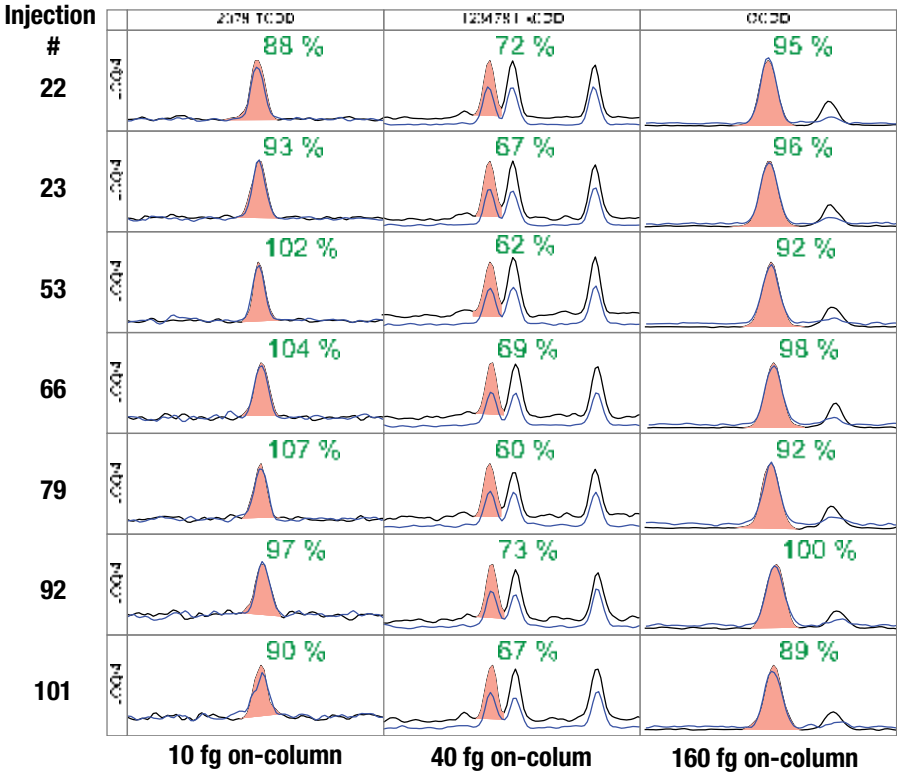


Figure 4. LOQ repeatability during the UK-based PCDD/F and non-ortho PCB sequence. Overlaid extracted ion chromatograms (XICs) are displayed (quantification and confirmation ions) for selected TCDD, 1,2,3,6,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD) and 1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin (OCDD) congeners, all IRs (as displayed in green) and RFs were within the allowable tolerances (IR ±15% from theoretical or average value; RF <30% deviation from average value) as defined by EURL guidance[4] throughout the sequence.

all four calibration sequences demonstrated RF %RSDs well within the EU regulations.[2,3] Table 4 shows examples of the data obtained for the UK-based dioxin-like PCBs and PCDD/Fs. Calibration ranges displayed are absolute amount on-column (pg).

Quantification and confirmation of PCDD/Fs, dioxin-like PCBs, and indicator PCBs in food and feed samples

A total of 29 different samples were analyzed [39 separate sample extractions, with two fractions for each (see Table 3)], over two sites, on two separate TSQ 9000 AEI GC-MS/MS systems for non-ortho PCBs and PCDD/Fs and di-, mono-ortho PCBs and indicator PCBs. To demonstrate the efficacy of the TSQ 9000 AEI GC-MS/MS systems, six replicate extractions of a mixed fat quality control sample (QK1 – reference value: 0.87 pg sum WHO-PCDD/F-TEQ) were prepared. These were split between the sites and analyzed at regular intervals throughout the analytical sequences (14 injections in total over the two non-ortho PCBs and PCDD/Fs sequences). An example of the chromatography achieved for a selection of congeners in the non-ortho PCBs and PCDD/Fs fraction is shown in Figure 5.

The measured WHO-PCDD/F-TEQ (pg/g) value for each congener was in excellent agreement with the reference value provided by the EURL (Figure 6), with the upper-bound WHO-PCDD/F-TEQ (pg/g) not deviating by more than 6% from the reference value over all 14 measurements. Furthermore, the deviation between the upper-bound and lower-bound WHO-PCDD/F-TEQ (pg/g) for each measurement was consistently less than 1.2%, well below the maximum 20% deviation required for samples that exceed the ML as specified in EU regulation (Figure 7).[6]

Table 4. Native dioxin-like PCBs and PCDD/Fs calibration data for the UK sequences (as average calibration response factors)

Peak Name	Ret.Time (min)	Number of Points	RF RSD (%)	Coeff. of Determination (R^2)	Average RF (Slope)	Range (pg)
PCB 81	16.38	14	1.49	0.9997	1.06	0.04 – 160
PCB 77	16.86	14	1.08	0.9997	1.00	0.04 – 160
PCB 123	17.40	14	2.66	0.9998	0.92	0.02 – 200
PCB 118	17.64	14	1.46	0.9999	0.96	0.1 – 1000
PCB 114	18.18	14	3.02	0.9989	1.04	0.02 – 200
PCB 105	18.96	14	5.95	0.9947	0.96	0.02 – 200
2378-TCDF	20.30	16	3.87	0.9995	0.96	0.01 – 64
2378-TCDD	20.86	16	4.72	0.9996	1.04	0.01 – 64
PCB 126	20.90	14	5.69	0.9985	0.95	0.04 – 160
PCB 167	21.52	14	1.74	0.9998	1.15	0.02 – 200
PCB 156	22.91	14	1.97	0.9998	1.14	0.02 – 200
PCB 157	23.12	14	2.41	0.9999	1.11	0.02 – 200
12378-PeCDF	24.34	16	1.66	0.9999	0.93	0.02 – 128
PCB 169	25.48	14	4.00	0.9999	1.08	0.04 – 160
23478-PeCDF	25.71	16	5.36	0.9977	1.03	0.02 – 128
12378-PeCDD	25.96	16	3.60	0.9999	1.05	0.02 – 128
PCB 189	27.28	14	1.96	0.9989	0.99	0.02 – 200
123478-HxCDF	29.06	16	2.98	0.9996	1.02	0.02 – 128
123678-HxCDF	29.17	16	1.95	0.9998	1.00	0.02 – 128
234678-HxCDF	29.86	16	2.83	0.9993	1.02	0.02 – 128
123478-HxCDD	29.94	16	2.49	0.9990	1.12	0.04 – 128
123678-HxCDD	30.04	16	2.01	0.9991	1.12	0.04 – 128
123789-HxCDD	30.35	16	3.82	0.9987	1.09	0.04 – 128
123789-HxCDF	30.71	16	3.52	0.9997	0.95	0.02 – 128
1234678-HpCDF	32.35	16	1.78	0.9999	1.03	0.04 – 256
1234678-HpCDD	33.78	16	5.99	0.9968	1.09	0.04 – 256
1234789-HpCDF	34.52	16	1.88	0.9998	1.04	0.04 – 256
OCDD	38.39	16	1.64	1.0000	1.12	0.16 – 256
OCDF	38.64	16	1.34	0.9997	0.94	0.16 - 256
		Max	**5.99**	**1.0000**		
		Min	**1.08**	**0.9947**		

Peak Name	Ion Ratio	ISTD Rec	<LOQ?	UB WHO TEQ	MB WHO TEQ	LB WHO TEQ
	%	%		pg/g	pg/g	pg/g
2378-TCDF	96.37	79.2		0.0493	0.0493	0.0493
2378-TCDD	*105.26*	*89.2*		*0.0683*	*0.0683*	*0.0683*
12378-PeCDF	88.79	94.2		0.0041	0.0041	0.0041
23478-PeCDF	78.57	94.8		0.0686	0.0686	0.0686
12378-PeCDD	75.45	99.7		0.3410	0.3410	0.3410
123478-HxCDF	55.86	81.2		0.0113	0.0113	0.0113
123678-HxCDF	59.95	87.3		0.0113	0.0113	0.0113
234678-HxCDF	68.85	76.8		0.0112	0.0112	0.0112
123478-HxCDD	65.58	88.4		0.0477	0.0477	0.0477
123678-HxCDD	65.59	96.0		0.1020	0.1020	0.1020
123789-HxCDD	65.90	96.0		0.0557	0.0557	0.0557
123789-HxCDF	IR High	71.2	<LOQ	0.0071	0.0035	0.0000
1234678-HpCDF	85.31	60.7	<LOQ	0.0017	0.0008	0.0000
1234678-HpCDD	80.13	94.2		0.0688	0.0688	0.0688
1234789-HpCDF	78.94	81.8	<LOQ	0.0012	0.0006	0.0000
OCDD	94.97	91.3		0.0023	0.0023	0.0023
OCDF	102.53	83.0	<LOQ	0.0001	0.0001	0.0000
		SUM PCDD/Fs		0.852	0.847	0.842

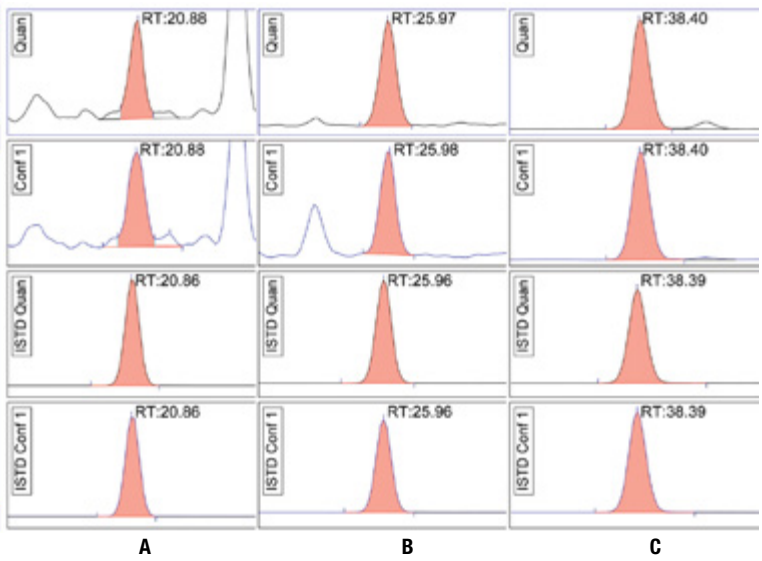


Figure 5. QK1 mixed fat quality control sample example chromatography where (A) 2,3,7,8-TCDD [0.03 pg on-column], (B) 1,2,3,7,8-PeCDD [0.14 pg on-column] and (C) OCDD [3.1 pg on-column]. The Chromeleon interactive results pane (left) displays IRs and internal standard recoveries, as well as real-time updated WHO-PCDD/F-TEQ (pg/g) values.

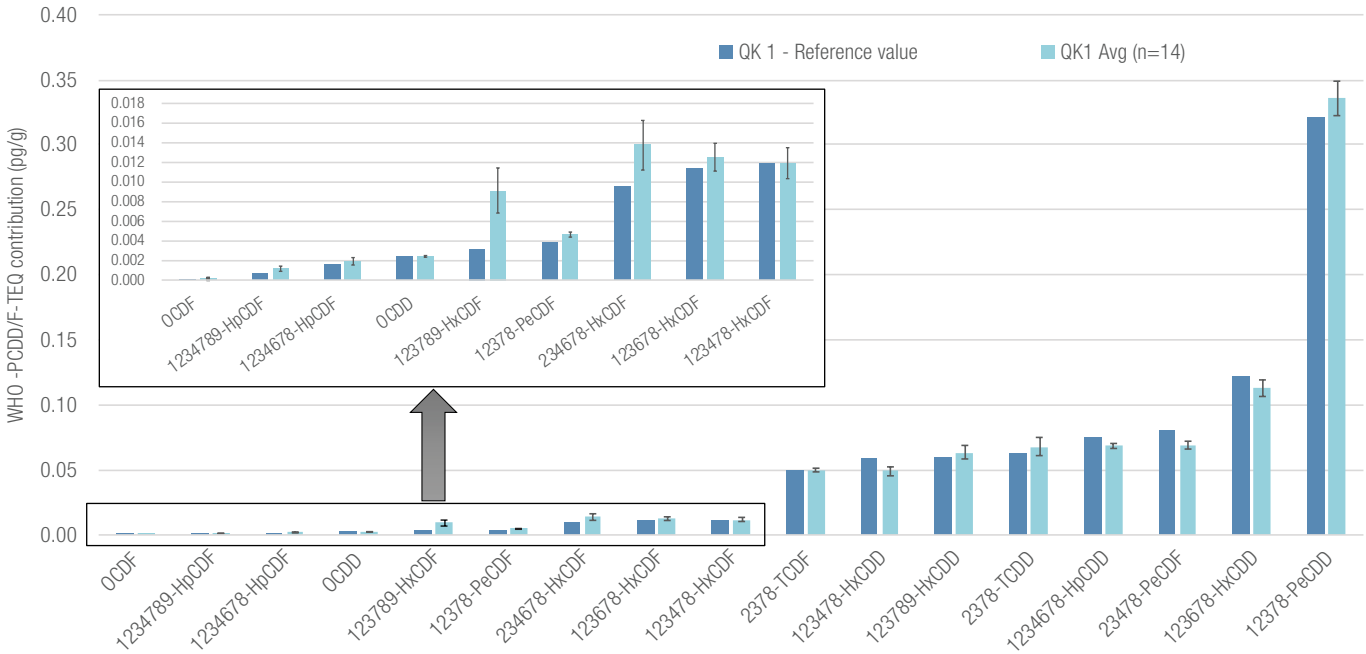


Figure 6. Congener contribution to the WHO-PCDD/F-TEQ (pg/g) for the mixed animal fat quality control sample. Congeners are ranked from left to right in order of contribution. Error bars show ±1σ standard deviation.

The remainder of the samples were analyzed routinely, with eight sample injections bracketed by blanks, LOQ check standards, and quality control samples (QK1). Figures 8A, 8B, and 8C show the correlation of the results obtained on the TSQ 9000 AEI systems with the reference value obtained by the EURL for PCDD/Fs, dioxin-like PCBs, and indicator PCBs, respectively. Where the reference value was below the minimum reportable TSQ 9000 AEI upper-bound WHO-PCDD/F-TEQ (pg/g) value, the samples have been circled with a broken blue line (Figure 8A). These samples all had upper-bound WHO-PCDD/F-TEQ (pg/g) values of less than 0.3 pg/g, which is below 1/5th MLs for these sample types (meat ×2, eggs ×2, and milk).[6] Pearson correlation coefficients were; 0.9902 for PCDD/Fs (Figure 8A), 0.9998 for dioxin-like PCBs (Figure 8B), and 0.9992 for indicator PCBs (Figure 8C), where a value of 1 is total positive linear correlation.

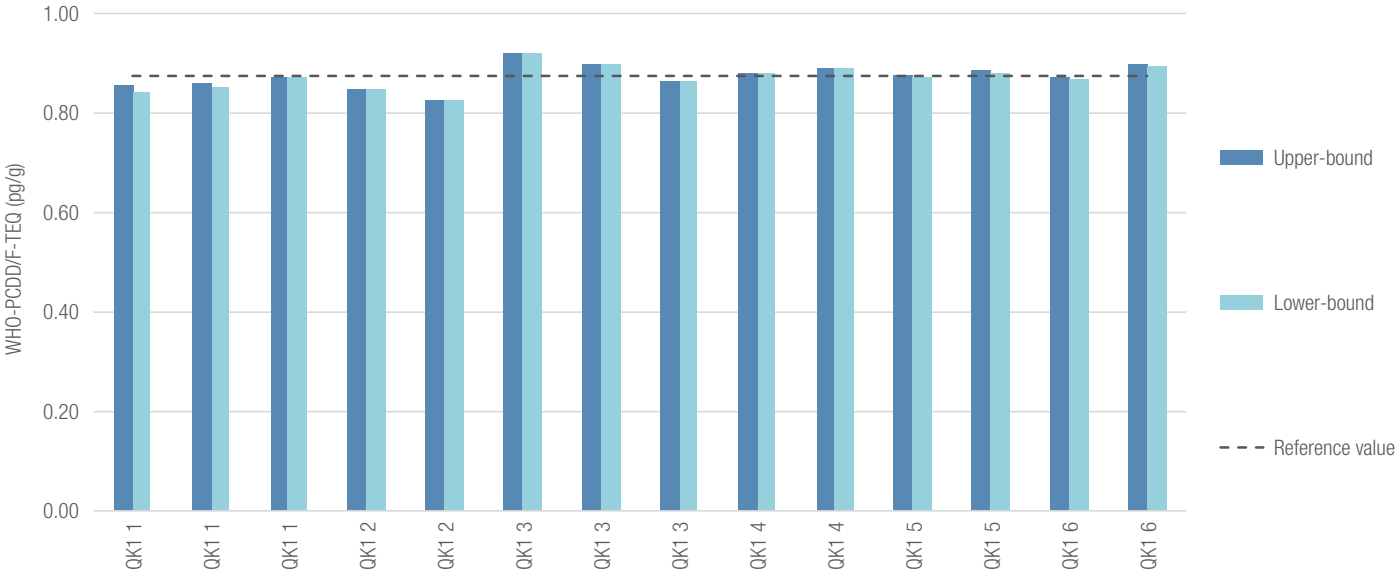


Figure 7. Upper-bound and lower-bound WHO-PCDD/F-TEQ (pg/g) results for all 14 measurements of the QK1 mixed animal fat quality control sample (six replicate extractions)

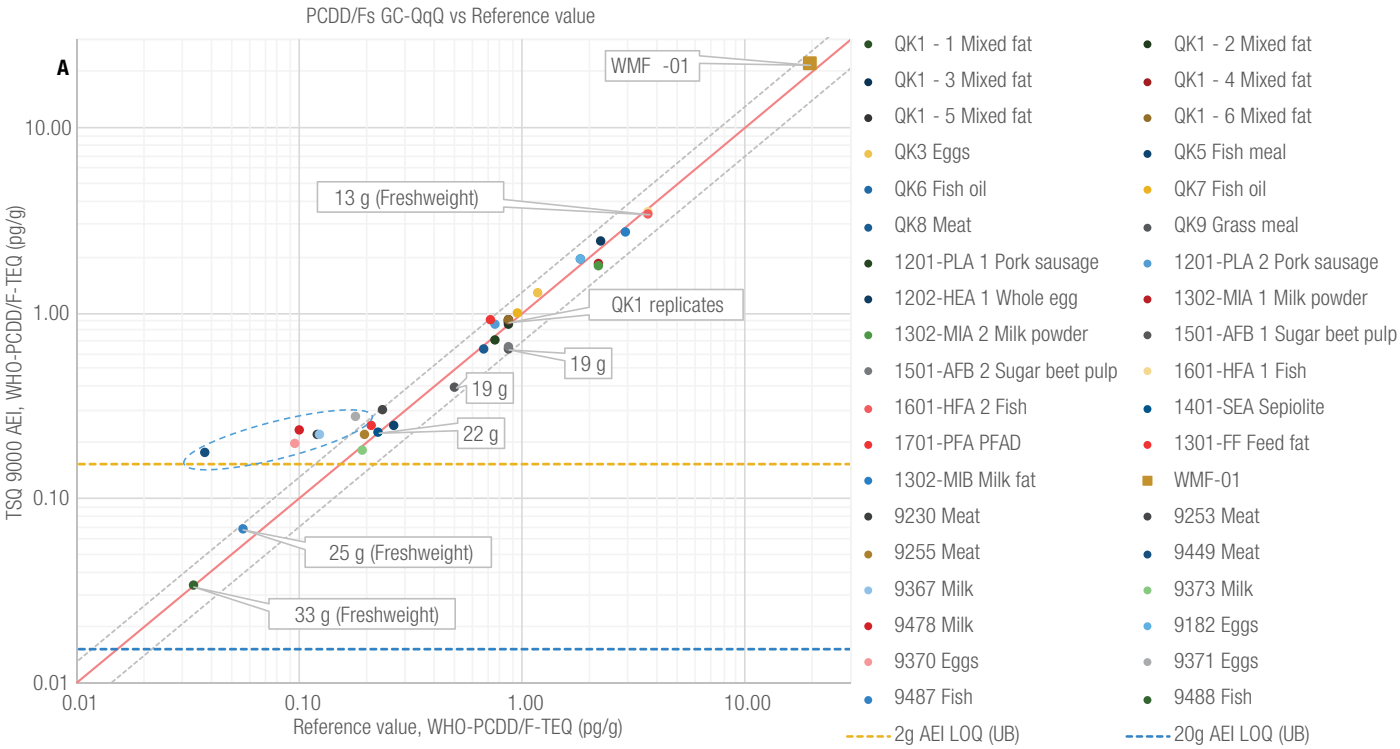


Figure 8 (A). Comparison of data [(A) PCDD/Fs] obtained on the GC-MS/MS with the EURL reference values. The center red line represents 100% agreement with the value and the upper and lower greyed lines represent a ±30% deviation from this value. Unless specified, sample intake weight was 2 g, amount scales are logarithmic to aid comparison.

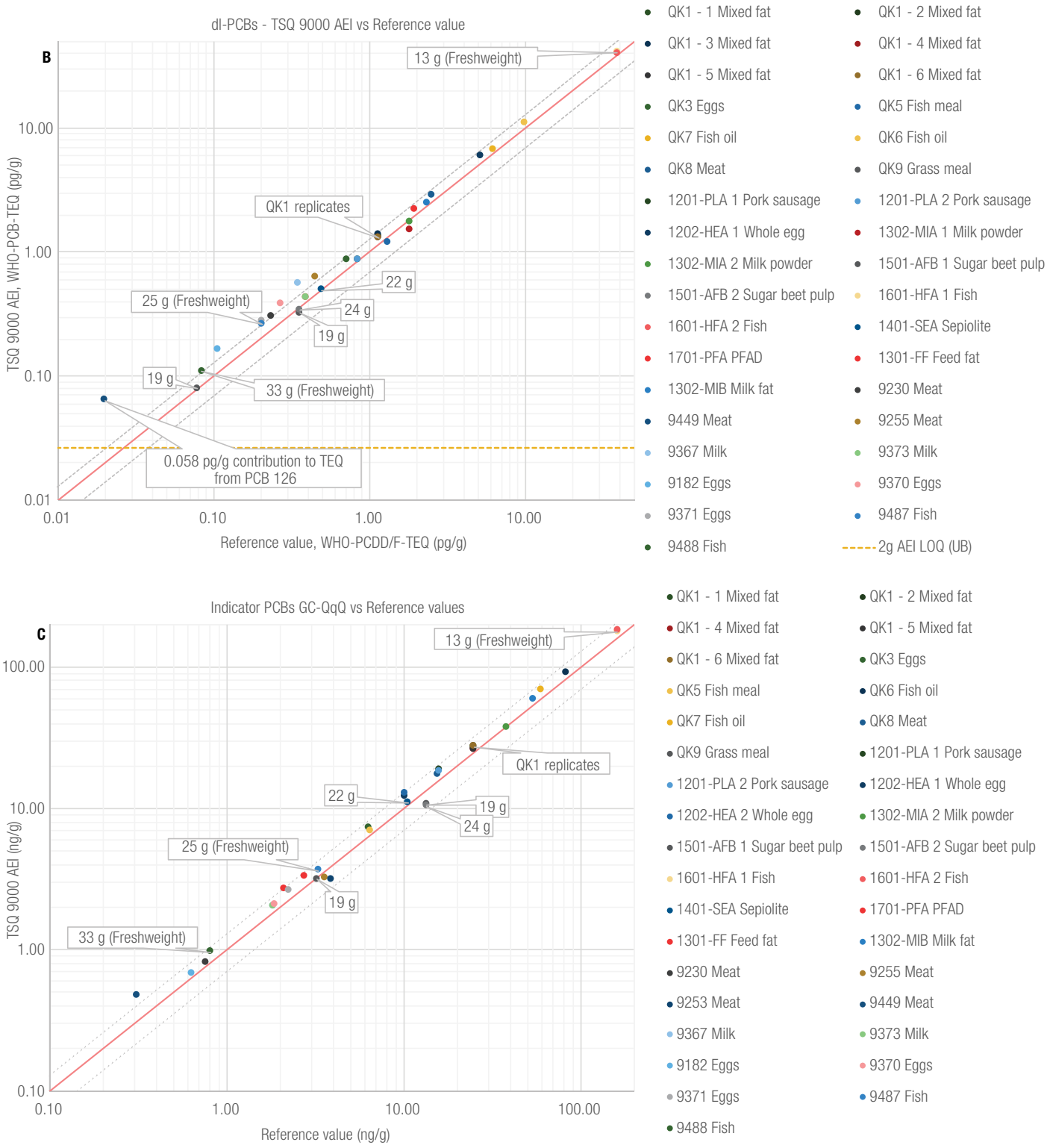


Figure 8 (B and C). Comparison of data [(B) dl-PCBs, and (C) indicator-PCBs] obtained on the GC-MS/MS with the EURL reference values. The center red line represents 100% agreement with the value and the upper and lower greyed lines represent a ±30% deviation from this value. Unless specified, sample intake weight was 2 g, amount scales are logarithmic to aid comparison.

To provide further validation data, an additional certified reference material (CRM) was extracted and analyzed on a PTV TSQ 9000 AEI system in Beijing, China. One gram of CRM WMF-01 (Wellington Laboratories Inc., Canada) was extracted and analyzed in triplicate (modified oven ramp, 5 µL PTV injection). The results obtained were excellent agreement with the reference values published, with all congeners within the specified tolerance (Figure 9). The calculated SUM WHO-PCDD/F-TEQ (pg/g) for the measurements versus the calculated reference SUM WHO-PCDD/F-TEQ (pg/g) is also displayed in Figure 8A.

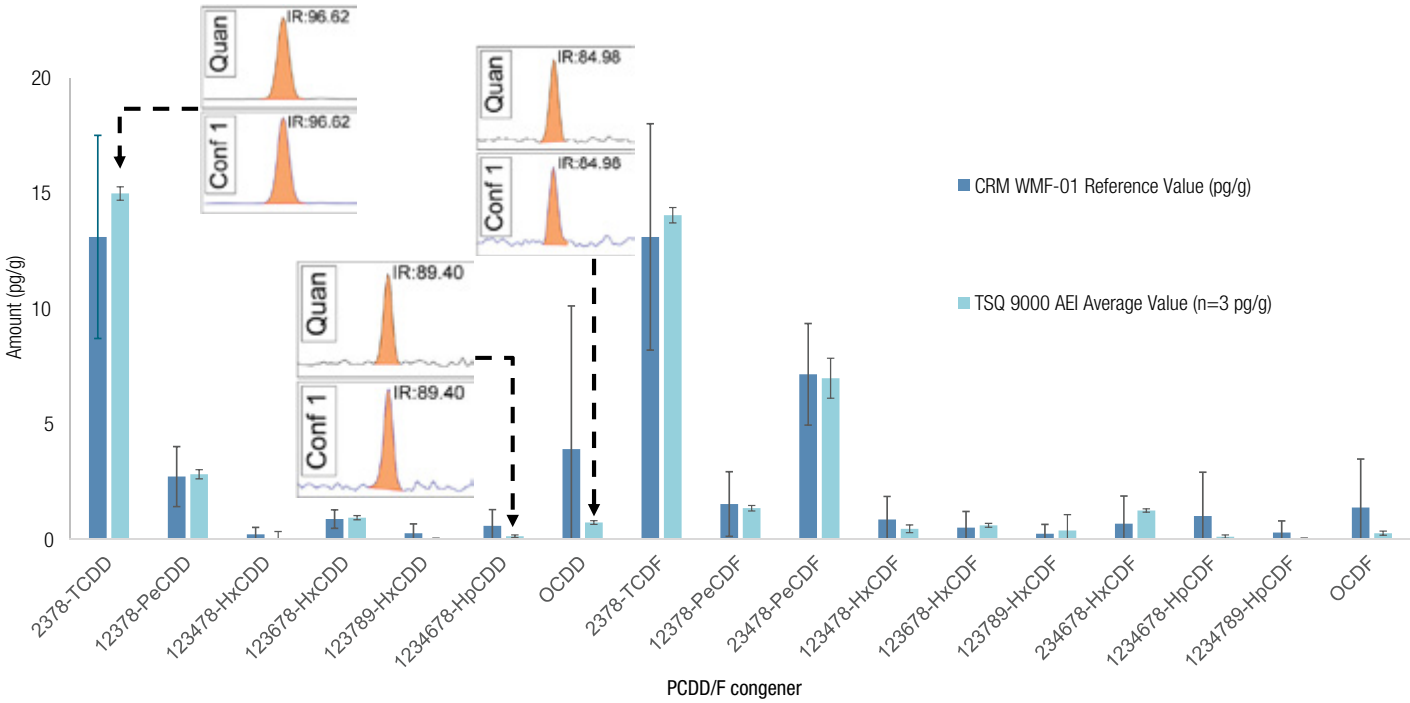


Figure 9. WMF-01 CRM reference value (pg/g) shown in dark blue, average (n = 3) TSQ 9000 AEI value for the WMF-01 CRM (pg/g) shown in light blue. Example XICs for quantification and confirmation ion are inlayed for 2,3,7,8-TCDD (15.13 pg/g), 1,2,3,4,6,7,8-heptachlorodibenzo-P-dioxin (HpCDD) (0.36 pg/g), and OCDD (2.01 pg/g). Error bars show the allowable deviation from the reference value and standard deviation of the TSQ 9000 AEI result.

Robustness

To further assess the robustness of the analytical system, the remaining extracts from the non-ortho PCBs and PCDD/Fs samples were pooled together into mixed matrix extract. This pooled matrix sample was then analyzed alongside nonane blank and LOQ standard injections. The injection sequence was set up as follows: four injections (LOQ, blank, pooled matrix, blank) were followed by a four-hour hold at the initial oven temperature and repeated, resulting in a total of 161 injection sequence containing n = 40 matrix injections and n = 40 LOQ standards, run over ~2 weeks period. The system maintained sensitivity throughout delivering excellent robustness, even considering the high matrix complexity and load on column (Figures 10A and B). No maintenance (such as source cleaning, liner replacement, tuning, or analytical column trimming) was performed during the sequence.

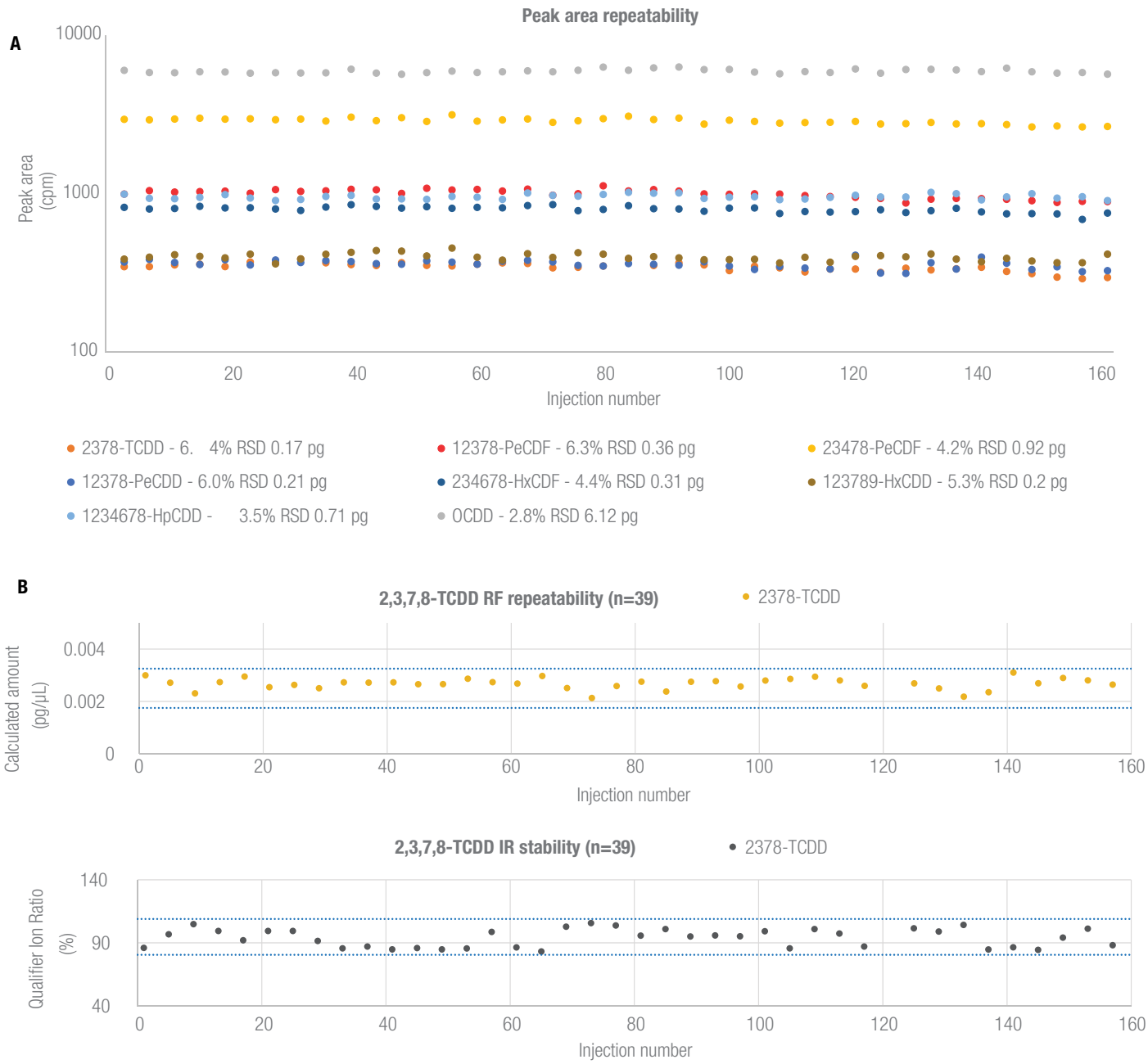


Figure 10. (A) Absolute peak area repeatability over two weeks of analysis, for selected PCDD/F congeners in pooled matrix sample. Relative standard deviations and amounts on-column (pg) are annotated for each selected congener, (B) LOQ RF deviation (upper plot, calculated as deviation from target amount) and IR (lower plot) for the 10 fg on-column 2,3,7,8-TCDD congener (2.5 fg/µL, 4 µL injection).

Conclusions

The results of these comprehensive experiments demonstrate that the TSQ 9000 GC-MS/MS system, configured with the AEI source and controlled using Chromeleon CDS software, can deliver routine-grade performance for the quantification and confirmation of PCDD/Fs, dioxin-like PCBs, and indicator PCBs in food and feedstuffs.

- Successful validation of method performance criteria (LOQ, precision, accuracy, and calibration) was carried out on two separate TSQ 9000 AEI systems, in two geo-locations.
- The sensitivity achieved with the TSQ 9000 AEI system allowed for upper-bound WHO-PCDD/F-TEQ (pg/g) values as low as 0.15 (for a 2 g sample intake weight), meeting the 1/5th maximum level requirements for all but the most challenging matrices.
- The outstanding linear range and accurate quantitative performance generated excellent comparative data to the EURL reference data supplied, with calibration data showing RF %RSD of <6 over more than 4 orders of magnitude for many congeners.
- Minimizing user intervention has been demonstrated by running over two weeks with no maintenance (such as source cleaning, liner replacement, tuning, or analytical column trimming), allowing maximum uptime and sample throughput.
- Chromeleon CDS software, version 7.2, provides an integrated platform, with the ability to automatically setup, easily acquire, process and report compliant data in a fully regulated environment, eliminating the need for using external spreadsheet programs. Chromeleon eWorkflows, available from Thermo Scientific™ AppsLab Library of Analytical Applications, also provide error-free execution of each analysis to meet standard operating procedure (SOP) requirements, further simplifying the user experience.

Acknowledgement

Thermo Fisher Scientific would like to acknowledge Wellington Laboratories Inc., Canada for the production and supply of the LOQ standards used in this validation.

References

1. World Health Organization, Dioxins and their effects on human health. https://www.who.int/news-room/fact-sheets/detail/dioxins-and-their-effects-on-human-health (assessed Jan 28, 2019).
2. European Commission, Commission Regulation (EU) 2017/644, Off. J. Eur. Union, L 92 9-34, 2017.
3. European Commission, Commission Regulation (EU) 2017/771, Off. J. Eur. Union, L 115 22-42, 2017.
4. Low level quantification of PCDD/Fs in animal feed using the TSQ 9000 triple quadrupole GC-MS/MS system with AEI source. https://www.thermofisher.com/uk/en/home/global/forms/industrial/low-level-quantification-pcdd-fs-animal-feed-tsq-9000-tq-gcms-ms.html (assessed Jan 28, 2019)
5. Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food. https://ec.europa.eu/jrc/en/publication/guidance-document-estimation-lod-and-loq-measurements-field-contaminants-feed-and-food (assessed Jan 28, 2019)
6. Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed, Off. J. Eur. Communities, L 140 10-21, 2002, in current amendment http://data.europa.eu/eli/dir/2002/32/2015-02-27 (assessed Jan 28, 2019)
7. US EPA Method 1613: Tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS (Revision B), 1994.
8. European Commission COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Off. J. Eur. Union, L 364 5-24, 2006, in current amendment

Appendix 1. Conditions

Table 1. PCDD/Fs and non-ortho PCBs; Injector/Autosampler and GC-MS/MS conditions

TRACE 1310 GC PTV Parameters					
Operating Mode:	Large Volume				
Injection Volume (µL):	4				
Initial Inlet Temperature (°C):	75				
Carrier Gas, Flow (mL/min):	Helium, 1.2				
Splitless Time (min):	1				
Split Flow (mL/min):	100				
Septum Purge (mL/min):	5 (constant)				
PTV Ramp Settings					
	Pressure (Psi)	*Rate (°C/s)*	*Temp. (°C)*	*Time (min)*	*Flow (mL/min)*
Injection:	-	-	-	0.2	100.0
Transfer:	-	5	300.0	1.0	-
Cleaning:	-	14.5	330.0	5.0	200.0
Autosampler Settings					
Injection Depth (mm):	45				
Penetration Speed (mm/s):	100				
Injection Speed (µL/s):	1				
TRACE 1310 GC Parameters					
Oven Temperature Program					
Temperature 1 (°C):	120 (initial)				
Hold Time (min):	2				
Temperature 2 (°C):	250				
Rate (°C/min):	25				
Hold Time (min):	0				
Temperature 3 (°C):	260				
Rate (°C/min):	2.5				
Hold Time (min):	5				
Temperature 4 (°C):	285				
Rate (°C/min):	2.5				
Hold Time (min):	0				
Temperature 5 (°C):	320				
Rate (°C/min):	10				
Hold Time (min):	15				
Total Run Time (min):	44.7				
TSQ 9000 AEI Mass Spectrometer Parameters					
Transfer Line (°C):	300				
Ionization Type (Source type):	EI with the Advanced EI source				
Ion Source (°C):	350				
Electron Energy (eV):	50				
Acquisition Mode:	Timed SRM with Dwell Time Prioritization (×10 – natives HIGH, labeled LOW)				
Tuning Parameters:	AEI Smart Tune				
Collision Gas:	Argon – 70 PSI				

Table 2. Mono-ortho, di-ortho, and indicator PCBs; Injector/ Autosampler and GC-MS/MS conditions

TRACE 1310 GC PTV Parameters					
Operating Mode:	Splitless				
Injection Volume (µL):	1				
Initial Inlet Temperature (°C):	75				
Carrier Gas, Flow (mL/min):	Helium, 1.2				
Splitless Time (min):	1				
Split Flow (mL/min):	100				
Septum Purge (mL/min):	5 (constant)				
PTV Ramp Settings					
	Pressure (Psi)	*Rate (°C/s)*	*Temp. (°C)*	*Time (min)*	*Flow (mL/min)*
Injection:	-	-	-	0.2	
Transfer:	-	5	300.0	1.0	-
Cleaning:	-	14.5	330.0	5.0	200.0
Autosampler Settings					
Injection Depth (mm):	45				
Penetration Speed (mm/s):	100				
Injection Speed (µL/s):	1				
TRACE 1310 GC Parameters					
Oven Temperature Program					
Temperature 1 (°C):	120 (initial)				
Hold Time (min):	2				
Temperature 2 (°C):	250				
Rate (°C/min):	25				
Hold Time (min):	0				
Temperature 3 (°C):	260				
Rate (°C/min):	2.5				
Hold Time (min):	5				
Temperature 4 (°C):	285				
Rate (°C/min):	2.5				
Hold Time (min):	0				
Temperature 5 (°C):	320				
Rate (°C/min):	10				
Hold Time (min):	15				
Total Run Time (min):	44.7				
TSQ 9000 AEI Mass Spectrometer Parameters					
Transfer Line (°C):	300				
Ionization Type (Source type):	EI with the Advanced EI source				
Ion Source (°C):	350				
Electron Energy (eV):	50				
Acquisition Mode:	Timed SRM with Dwell Time Prioritization (×10 – natives HIGH, labeled LOW)				
Tuning Parameters:	AEI Smart Tune				
Collision Gas:	Argon – 70 PSI				

Appendix 2. List of consumables used

Part number	Description
	Autosampler
365D0291	10 µL fixed needle syringe, 57 mm, 26s gauge, cone tip
	PTV
453T2845-UI	Thermo Scientific™ LinerGOLD™ PTV Concentric Baffle
29053488	Graphite ferrule for inlet
31303233-BP	11 mm BTO septa
29001318	Liner sealing ring for PTV
290VA191	Graphite/Vespel ferrule for MS
07-CPV (A)	0.7 mL crimp top tapered vial – amber
8-AC-ST101	8 mm aluminum crimp cap silicone/ptfe liner
	Column
26066-1540	GC Column, TG-Dioxin 60 m × 0.25 mm, 0.25 µm

Find out more at **thermofisher.com/POPsinFood**

ThermoFisher SCIENTIFIC

©2019 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. DeXTech is a trademark of DeXTech Limited. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details. **AN10703-EN 0219S**

APPLICATION NOTE

Characterizing unknowns in food packaging using GC Orbitrap Mass Spectrometry

Authors: Dominic Roberts[1], Jesus Varela[2], Yves-Alexis Hammel[2] and Paul Silcock[1]

[1]Thermo Fisher Scientific, Runcorn, UK

[2]Nestle, Lausanne, Switzerland

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.

ThermoFisher SCIENTIFIC

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.

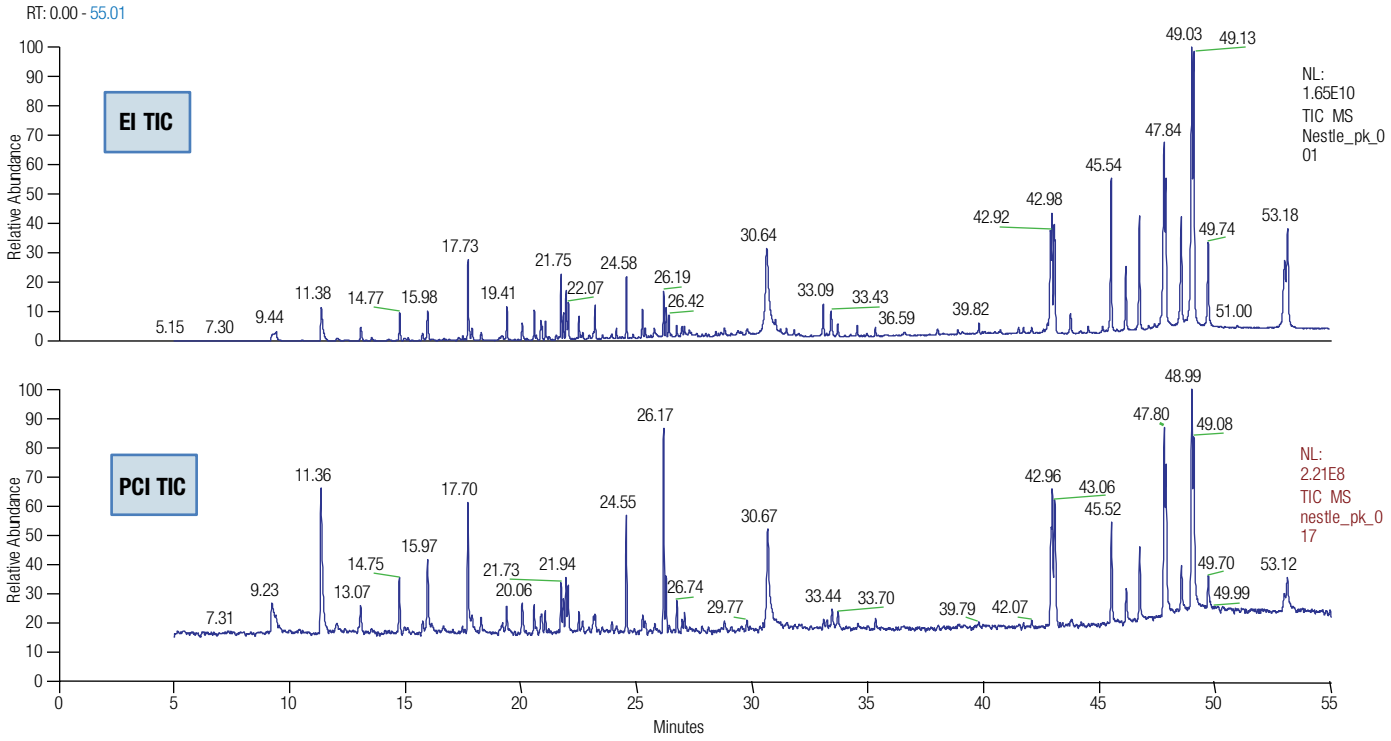


Figure 1. GC-MS electron ionization (EI) and positive chemical ionization (PCI) total ion chromatograms (TIC) of the packaging sample.

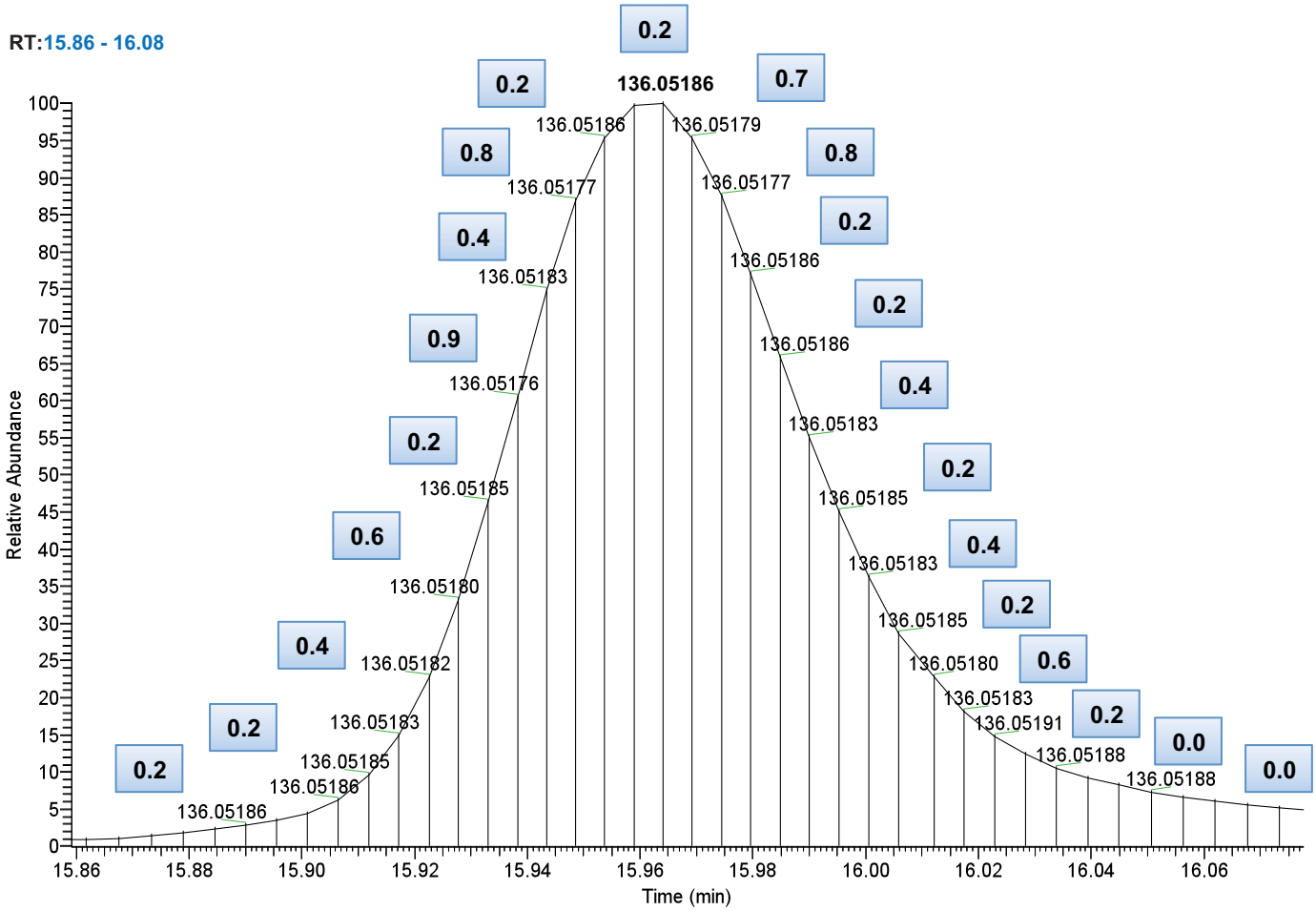


Figure 2. Extracted ion chromatogram for compound 2-Hydroxy-5-methyl-1,3-benzenedicarboxaldehyde fragment (m/z 136.05188 ±5 ppm mass window) in packaging sample 34 scans/peak. Data acquired in full-scan at 120,000 FWHM resolving power. Excellent accurate mass stability is shown for each individual scan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

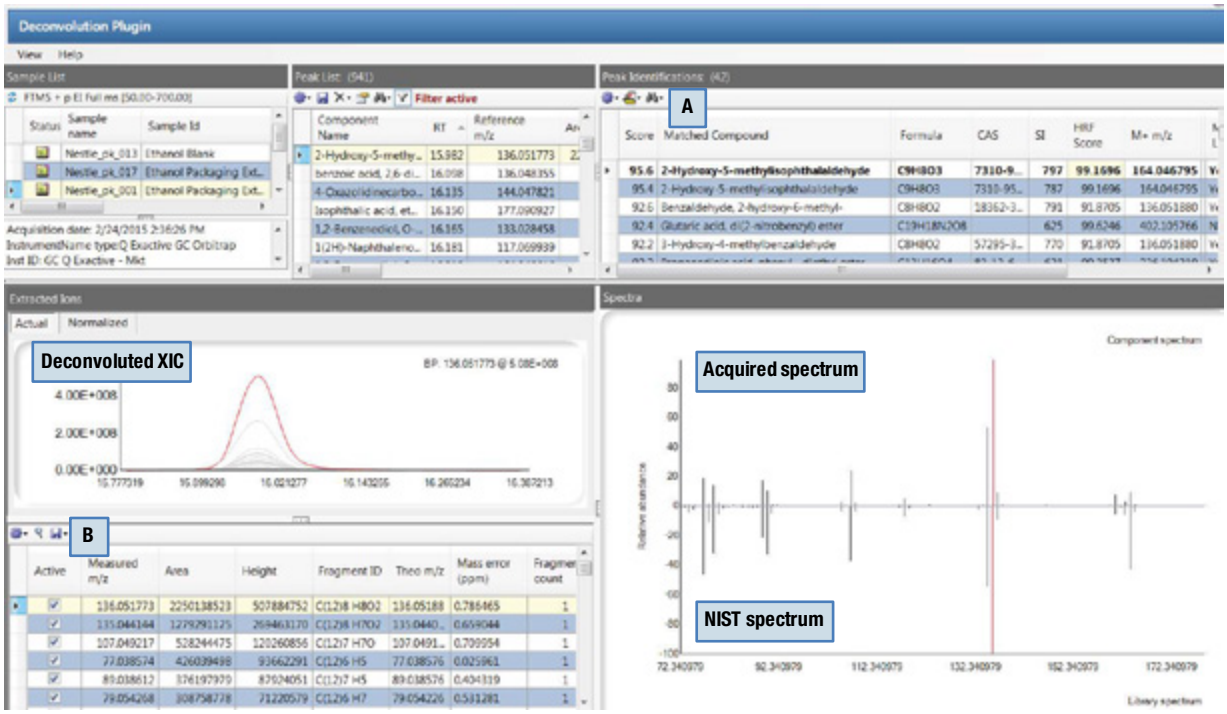


Figure 3. Identification of peak at 15.98 minutes as 2-hydroxy-5-methyl-1,3-benzenedicarboxaldehyde. Screenshot of the deconvoluted data and library match in TraceFinder. (A) List of library hits sorted by score (combination of SI and HRF). (B) List of fragment ions from EI spectrum and elemental composition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

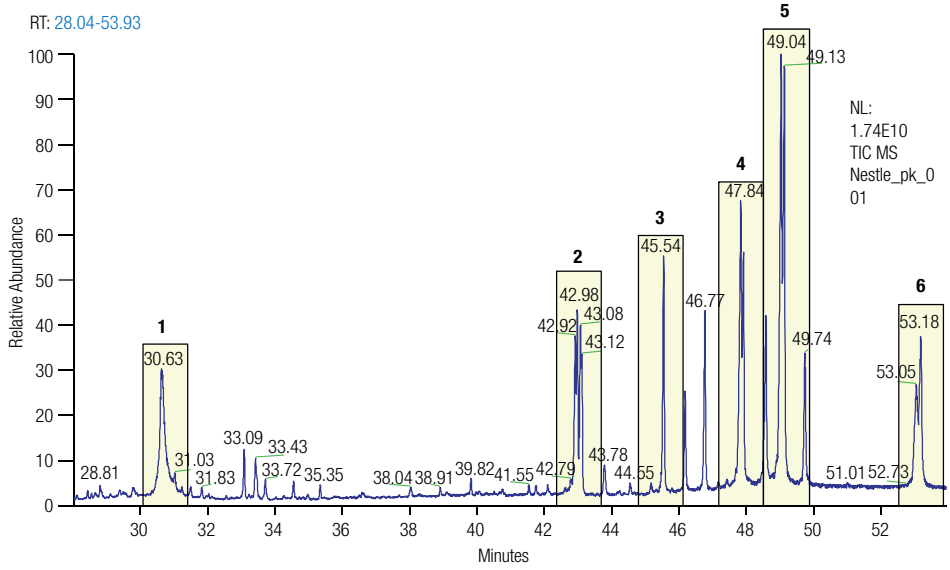


Figure 4. Zoomed region showing the six peaks of interest in the electron impact (EI) total ion chromatogram of the packaging sample.

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.

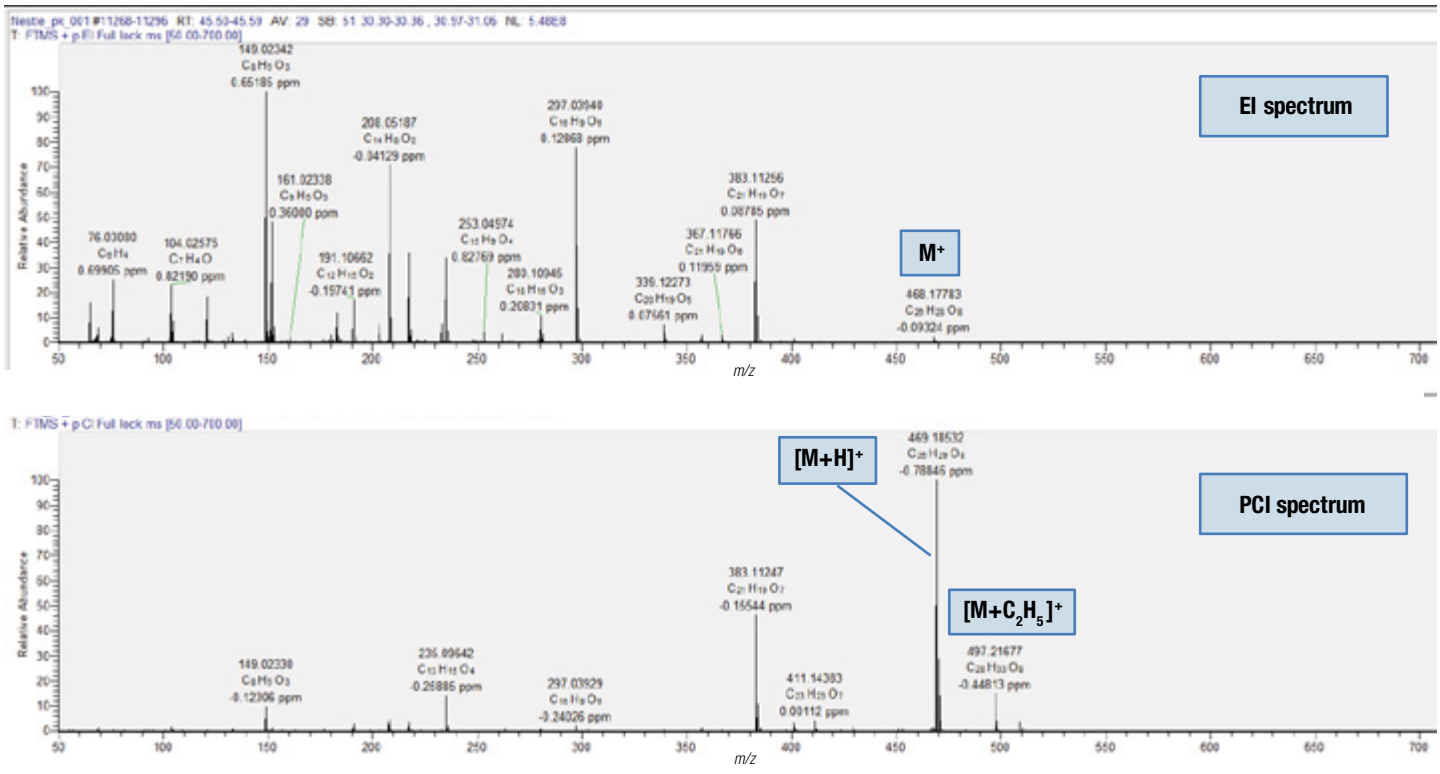


Figure 5. EI and PCI spectra at 45.5 minutes in packaging sample proposing a chemical formula of $C_{26}H_{28}O_8$. Peaks are annotated with chemical formula and mass difference in ppm. PCI data supports identification of parent ion with formula with sub 1 ppm mass accuracy.

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.

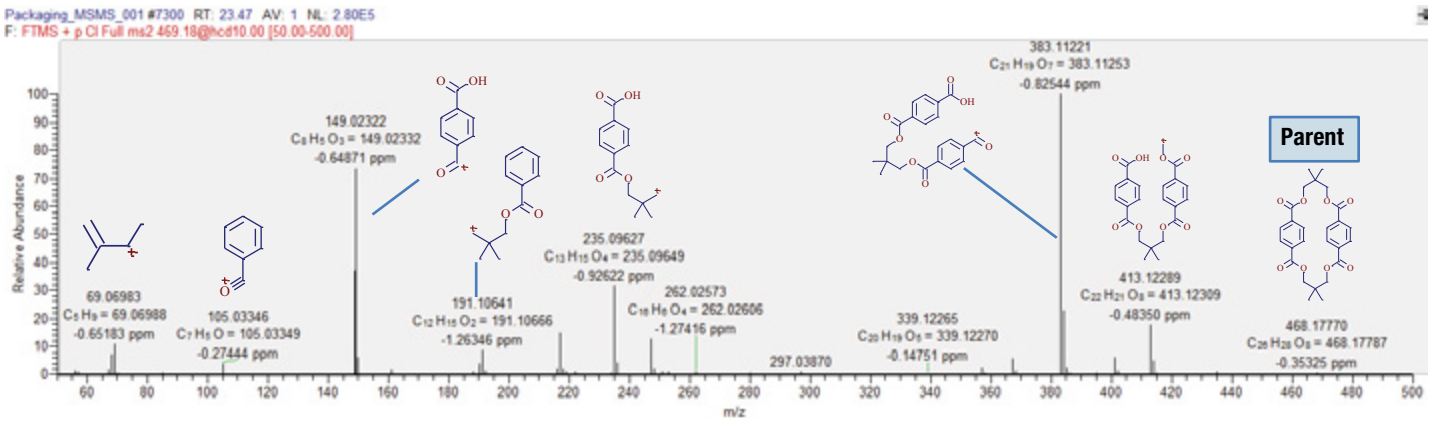


Figure 7. MS/MS spectrum of PCI ion *m/z* 469.18 selected in the quadrupole and fragmented in the HCD cell. MassFrontier used to explain the fragments observed within 3 ppm mass accuracy window.

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.

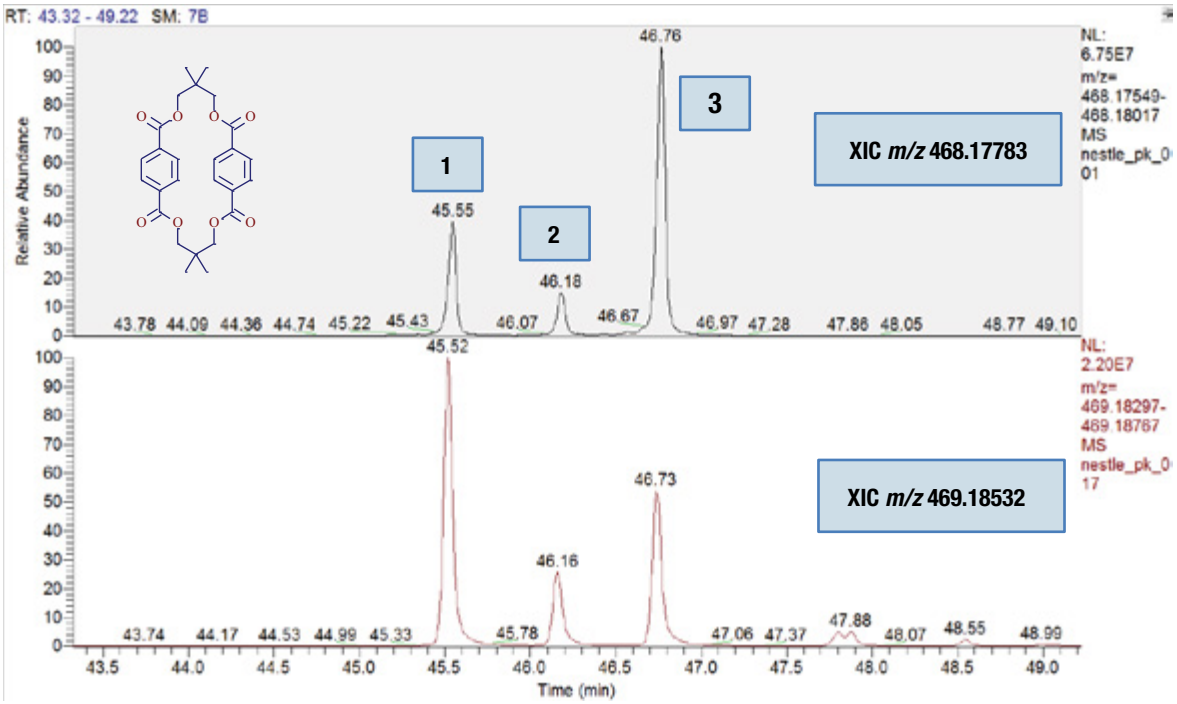


Figure 8. XIC *m/z* 468.17783 from the full-scan EI data and *m/z* 469.18532 from the full-scan PCI data in packaging sample shows 3 isomers of the same parent mass. Inset proposed chemical structure of compound.

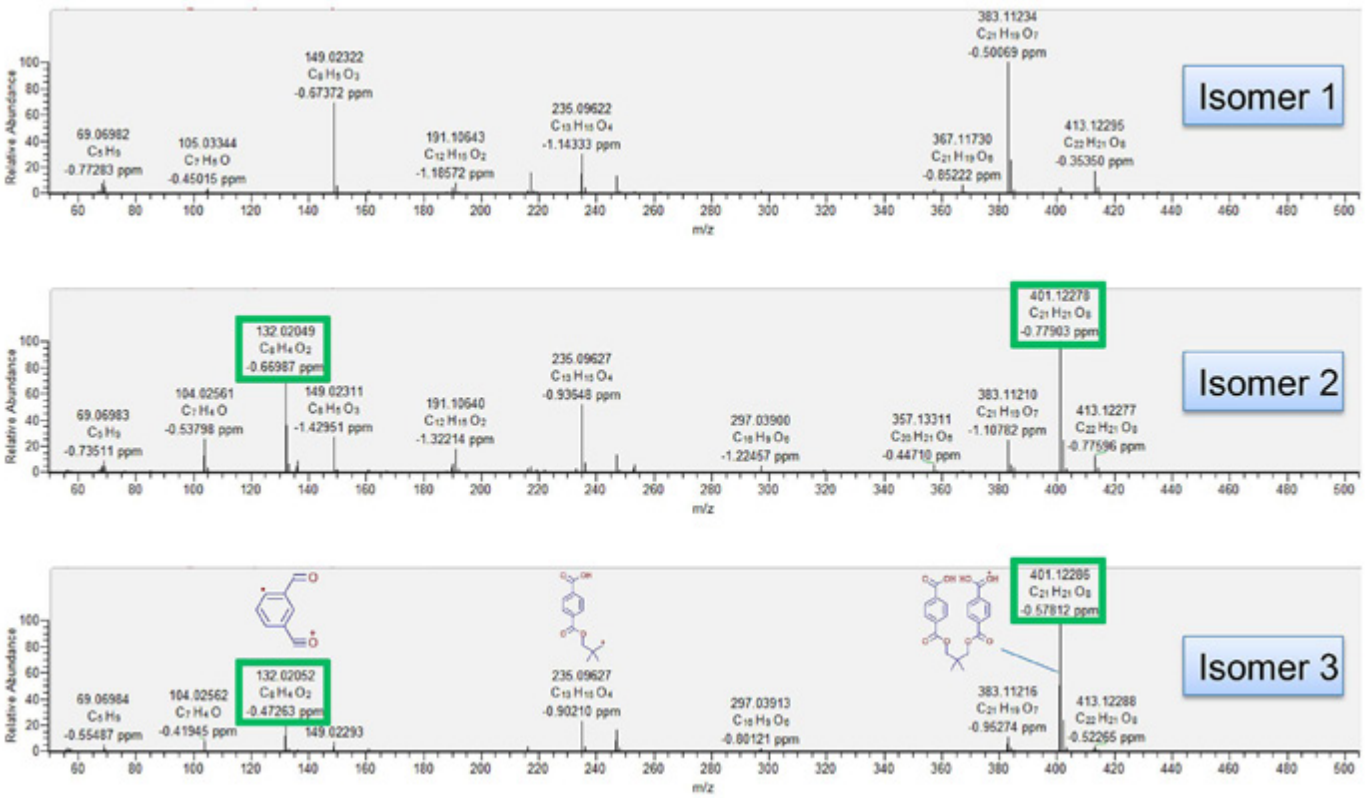


Figure 9. MS/MS spectra of m/z 469.18 of the three isomers reveals different fragmentation patterns for isomers 2 and 3. Of particular note, the base peak is 401.12286 and the presence of m/z 132.02049 ion.

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.

References

1. Migration from Food Contact Materials. L. L. Katan (editor), Blackie Academic and Professional, Glasgow, UK, 1996.
2. Barnes, K.A., Sinclair, C.R. & Watson, D.H. (2006) Chemical Migration and Food Contact Materials. Woodhead publishing.
3. Thermo Fisher Scientific AN10492. Chemical profiling and differential analysis of whiskies using Orbitrap GC-MS. Runcorn, United Kingdom.

Find out more at **www.thermofisher.com/QExactiveGC**

©2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details.

AN10527 1016

thermo scientific

APPLICATION NOTE 10541

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

Authors

Dominic Roberts,[1] Mette Erecius Poulsen,[2] and Paul Silcock[1]

[1]Thermo Fisher Scientific, Runcorn, United Kingdom

[2]European Union Reference Laboratory for Pesticide Residues in Cereals and Feedstuffs, Technical University of Denmark, Denmark

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.

ThermoFisher SCIENTIFIC

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.

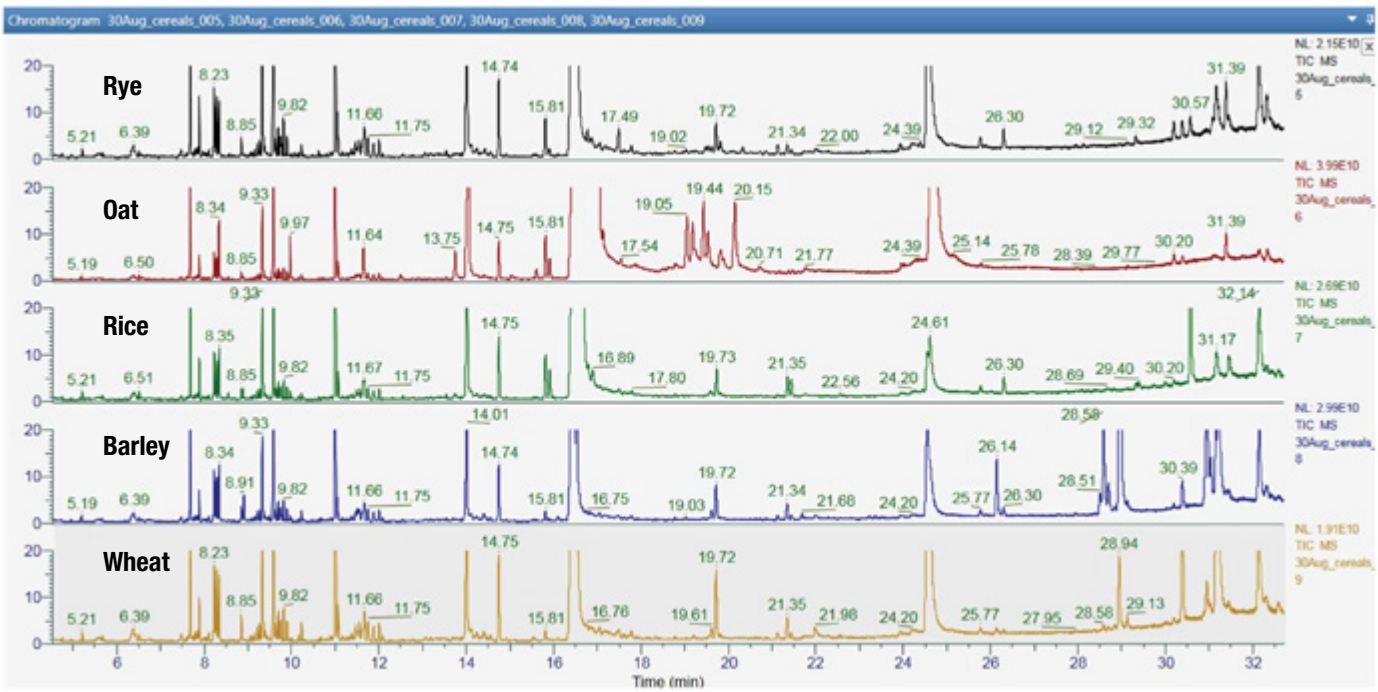


Figure 1. Full-scan total ion chromatogram (TIC) with zoomed Y axis of cereal extracts showing the complexity of the sample matrices used in this study.

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

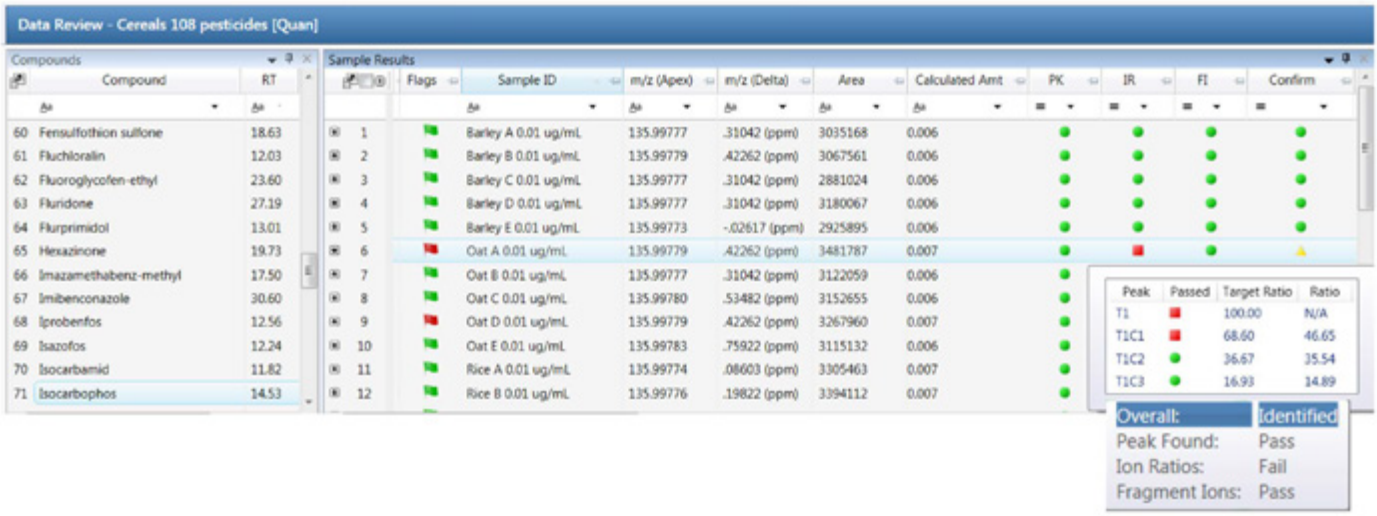


Figure 2. TraceFinder software browser enables fast data review and confirmation. The software quickly points the analyst to the data that supports a positive identification using a traffic light system along with real data values. More importantly, it will flag when a parameter is outside of tolerance, 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.

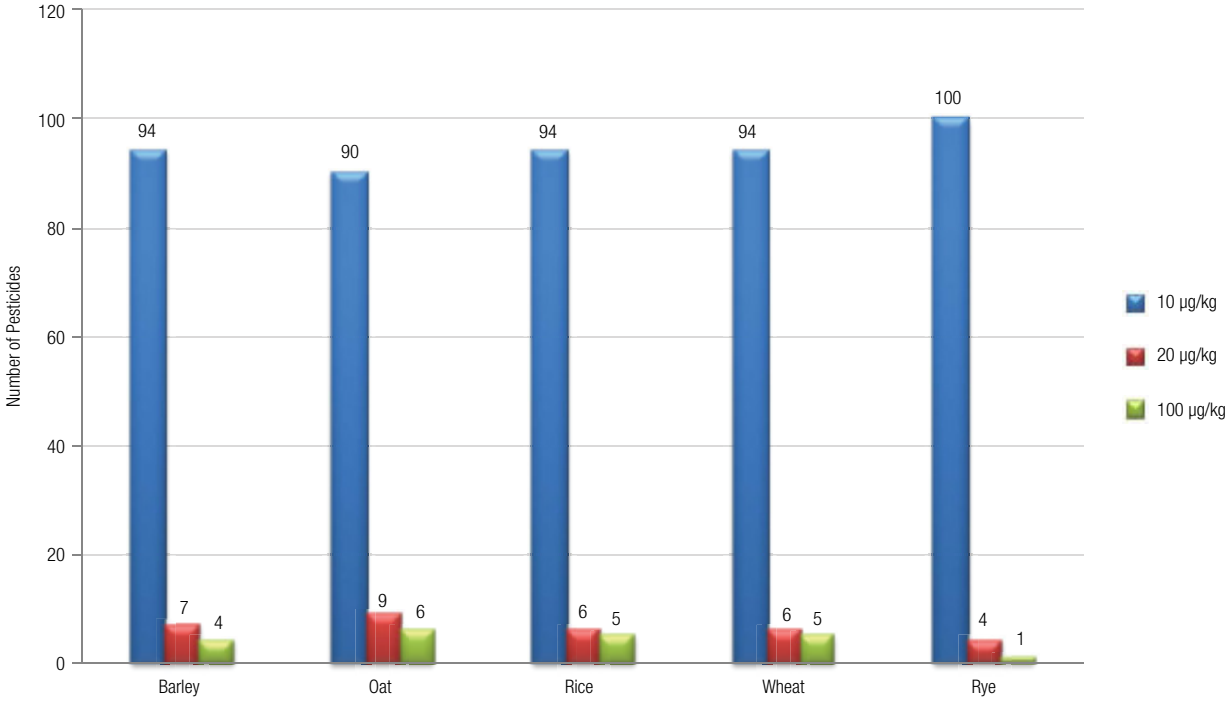


Figure 3. The lowest concentration confirmed (two ions within 5 ppm, ion ratios within ±30%) for each pesticide in each of the five sample matrices. The total number of pesticides is 105.

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.

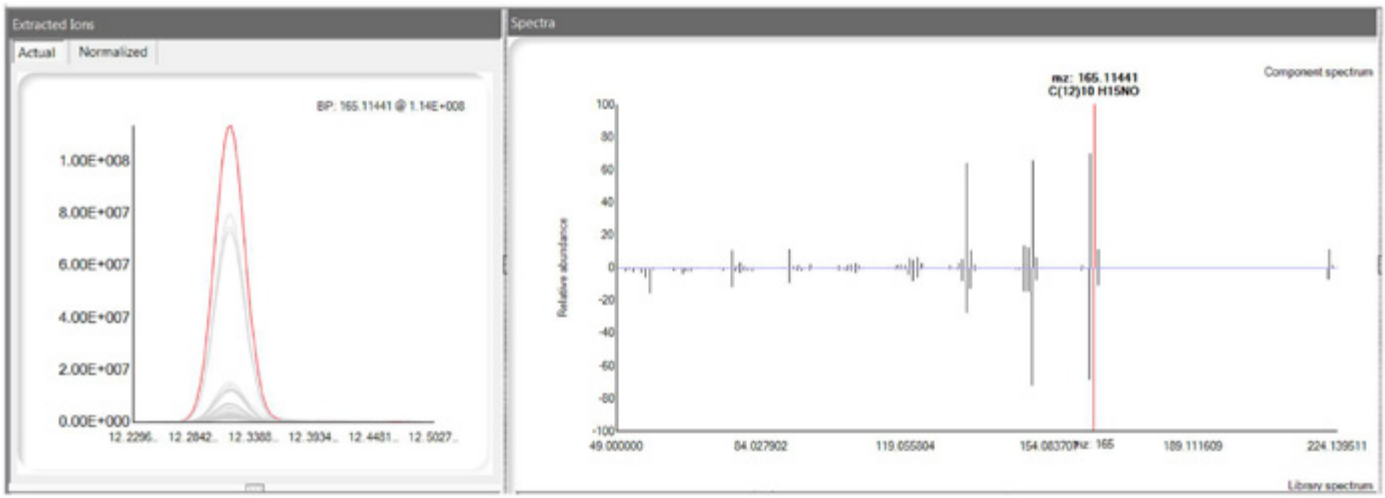


Figure 4. TraceFinder software deconvoluted peaks (left). Acquired spectrum and library spectrum (right) for mexacarbate with search index score of 905.

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.

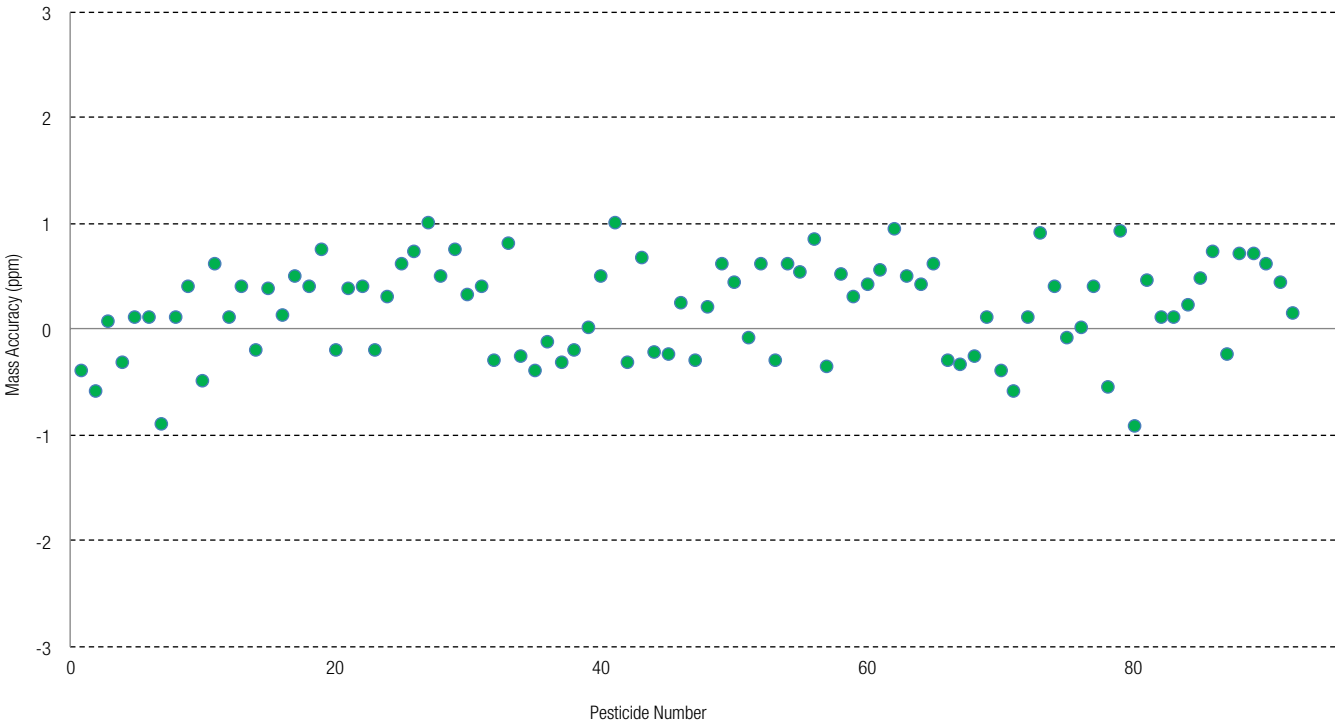


Figure 5. Mass difference measurements at 10 µg/kg for each pesticide in wheat.

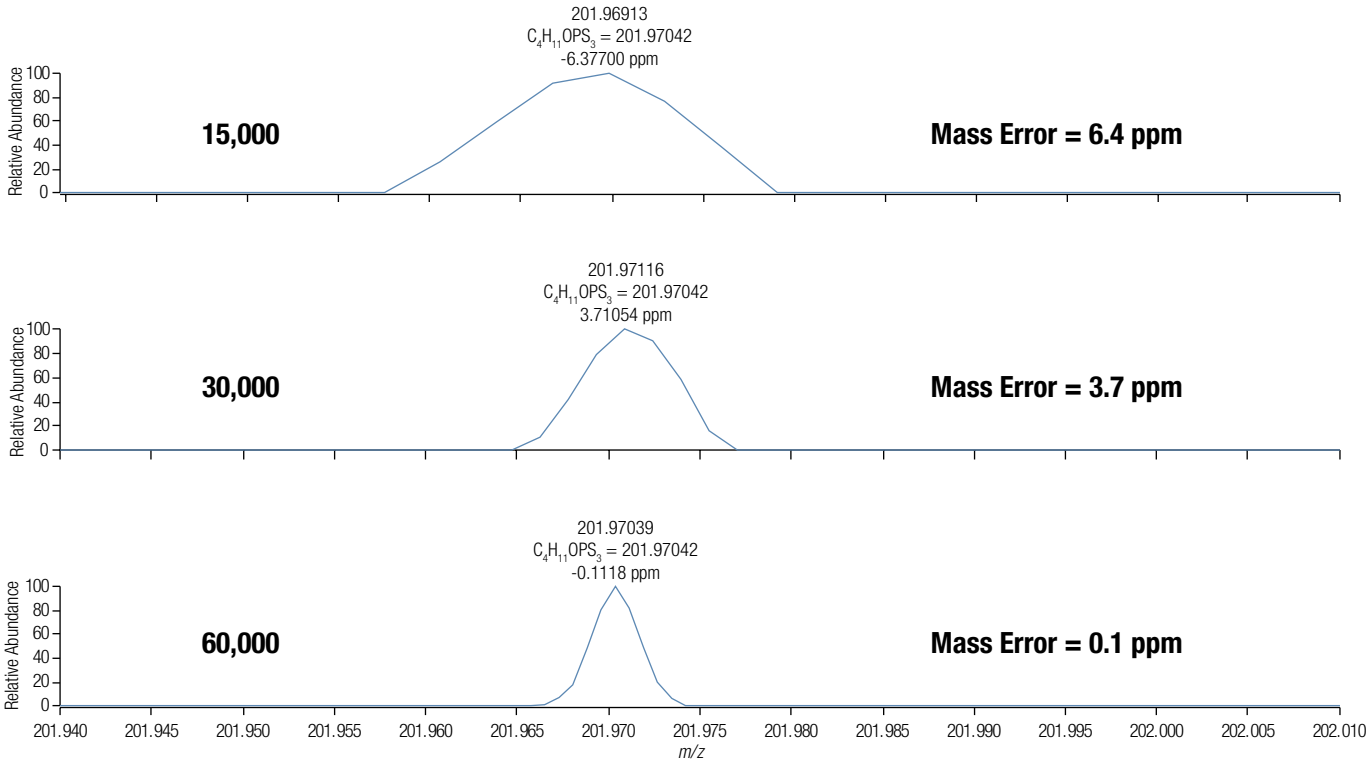


Figure 6. Effect of resolving power on mass accuracy of diagnostic ion (*m/z* 201.97042) tribufos at 20 µg/kg in oat acquired at different resolutions of 15K, 30K, and 60K.

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.

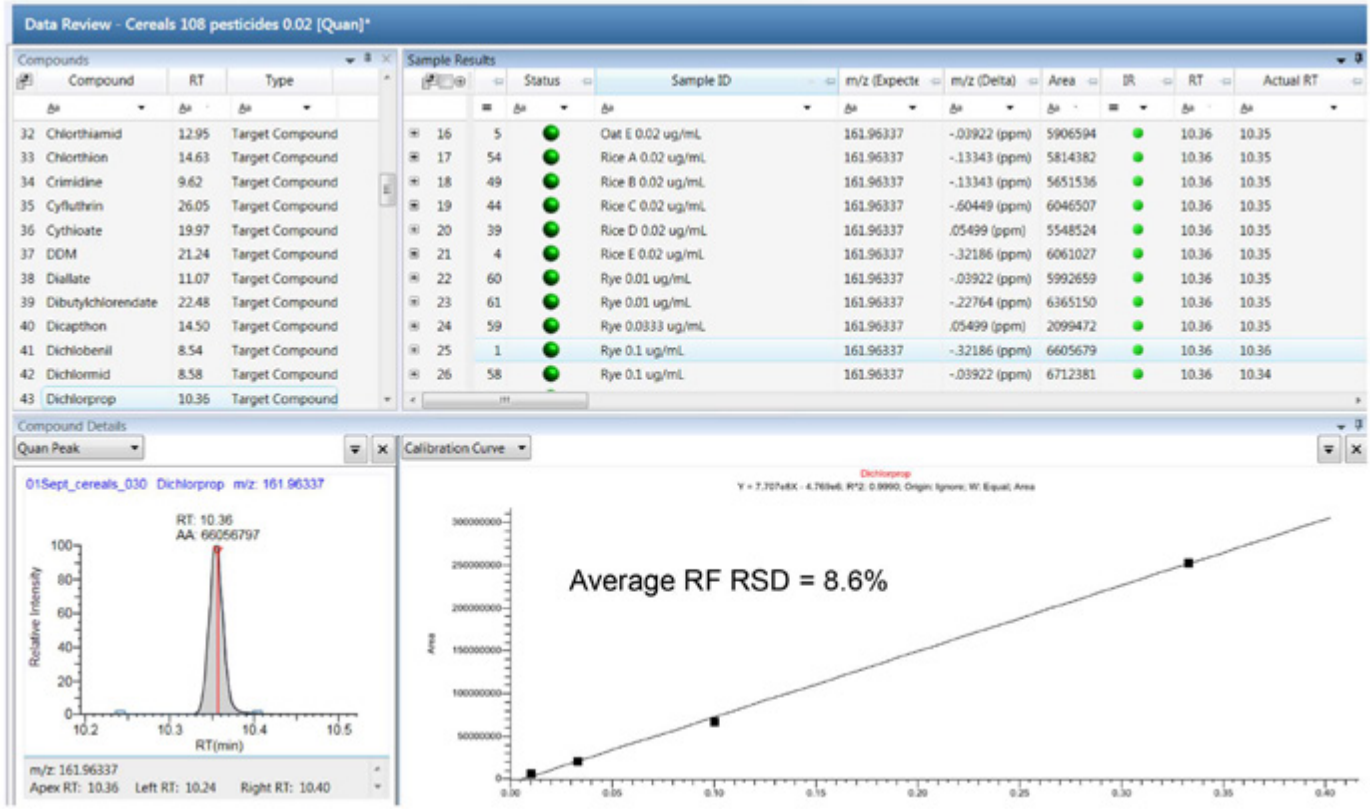


Figure 7. TraceFinder software browser showing positively identified pesticides, extracted ion chromatogram, and calibration graph (dichlorprop methyl ester as an example). Sub-ppm mass accuracy for dichlorprop across the calibration range and in replicates of 20 mg/kg. Identification criteria information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

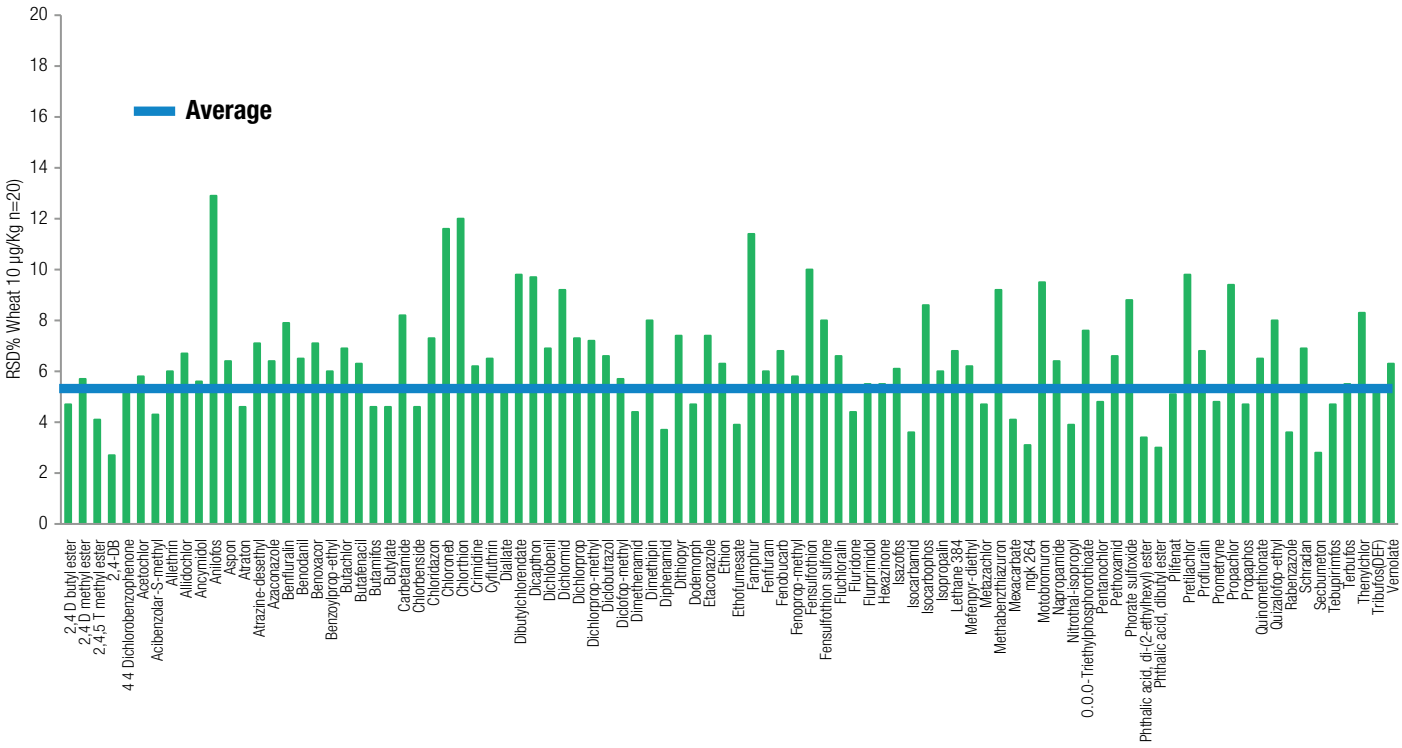


Figure 8. Repeatability (%RSD) for 10 µg/kg (n=20) for each pesticide in wheat.

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.

References

1. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=URISERV:l13002a
2. Mol, H.G.; Tienstra, M.; Zomer, P. Evaluation of gas chromatography - electron ionization - full scan high resolution Orbitrap mass spectrometry for pesticide residue analysis, *Analytica Chimica Acta,* **2016,** doi: 10.1016/j.aca.2016.06.017.
3. Thermo Fisher Scientific Application Note: 10509. Routine quantitative method of analysis for pesticides using GC-Orbitrap mass spectrometry in accordance with SANTE/11945/2015 guidance.
4. SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Supersedes SANCO/12571/2013. Implemented by 01/01/2016.

Find out more at **thermofisher.com/ExactiveGC**

©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details. **AN10541-EN 0317S**

thermo scientific

APPLICATION NOTE 10584

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

Authors

Dominic Roberts,[1] Jim Garvey,[2] Richard Law,[1] and Paul Silcock[1]

[1]Thermo Fisher Scientific, Runcorn, United Kingdom

[2]Department of Agriculture, Food and the Marine, County Kildare, Ireland

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.

ThermoFisher SCIENTIFIC

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

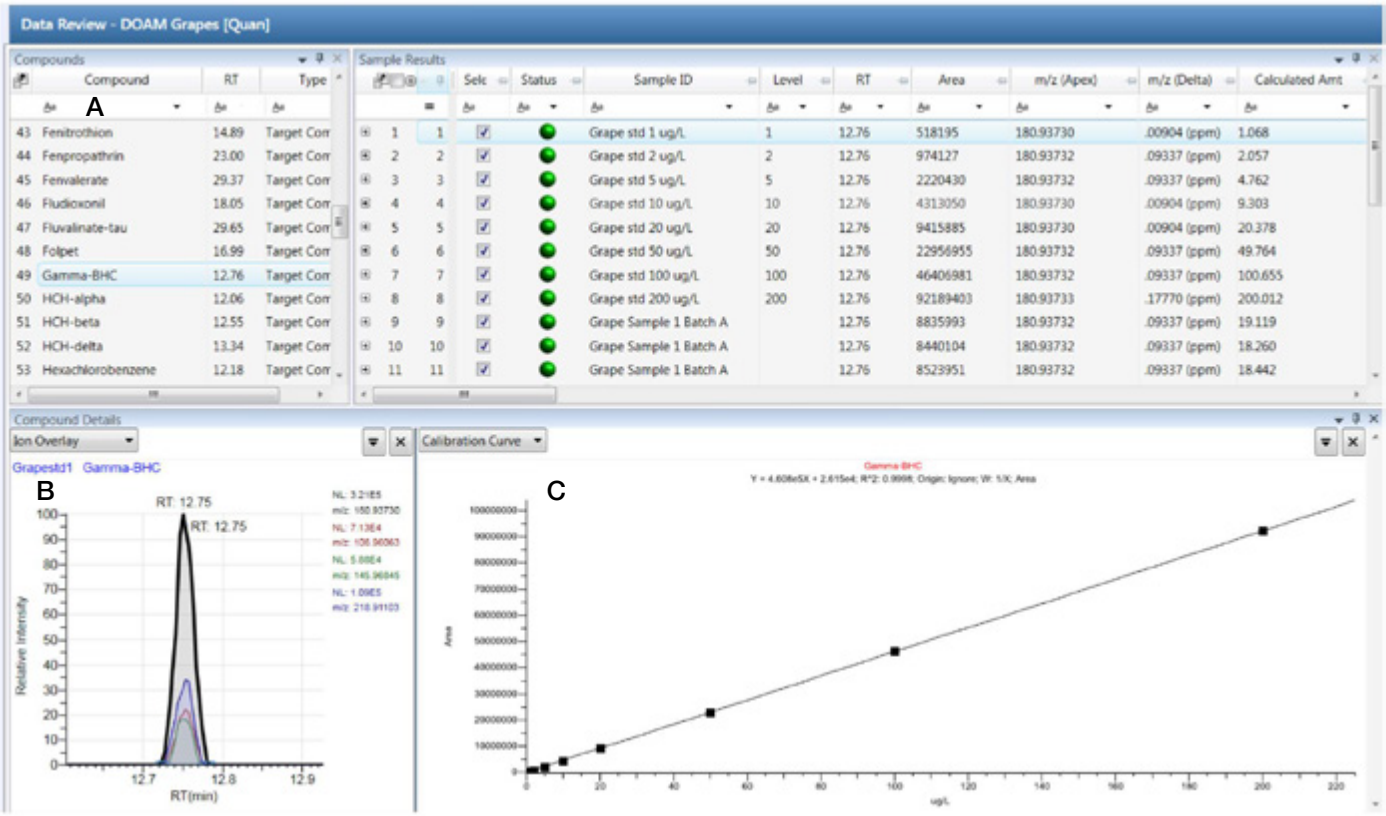


Figure 1. TraceFinder browser showing identified pesticides (A), overlay of extracted ion chromatograms (B), and linear response (C) (HCH-gamma as an example). Linearity R^2 = 0.9998, average response factor RSD% = 5.7.

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.

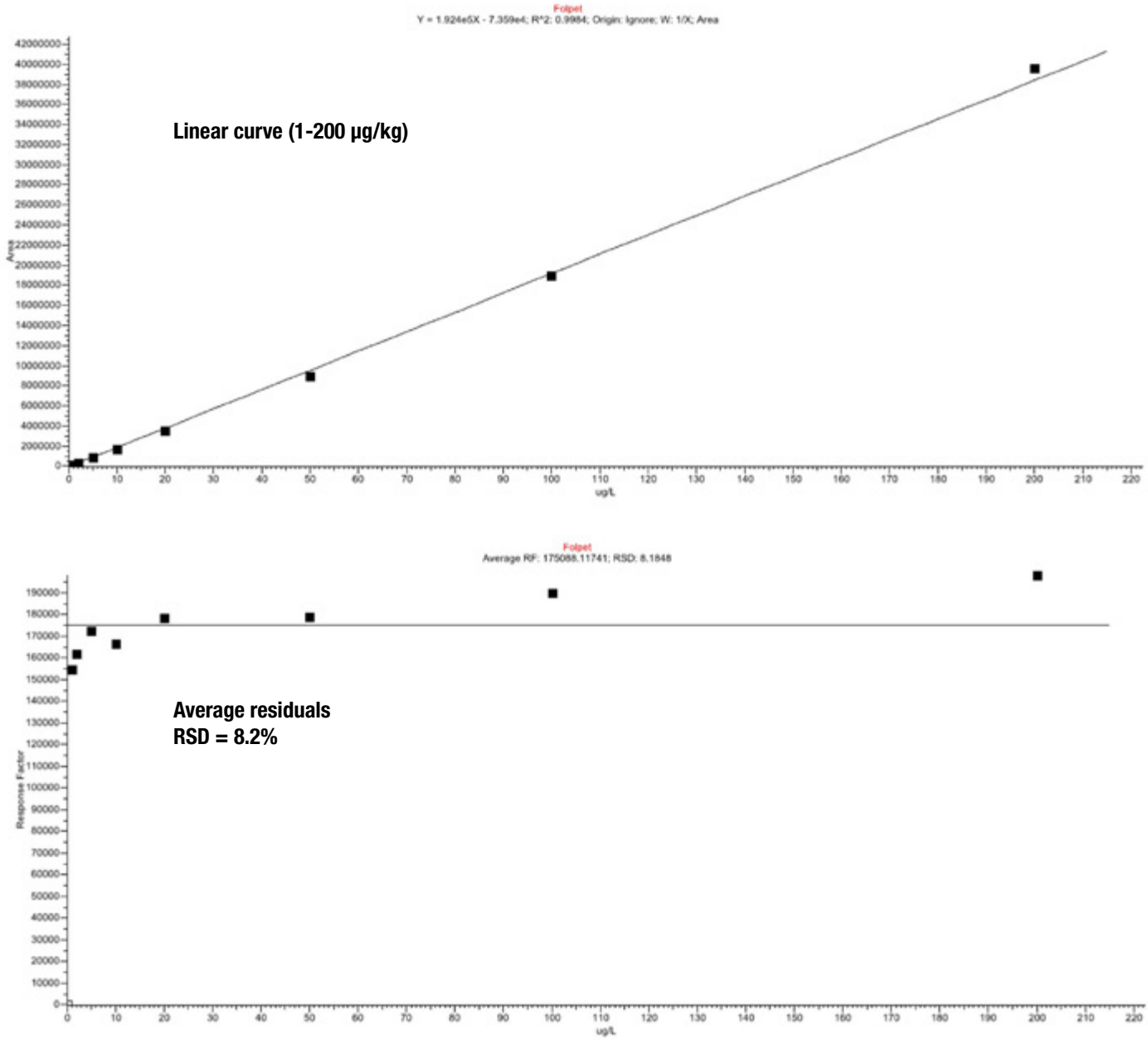


Figure 2. Calibration data for folpet in onion matrix.

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.

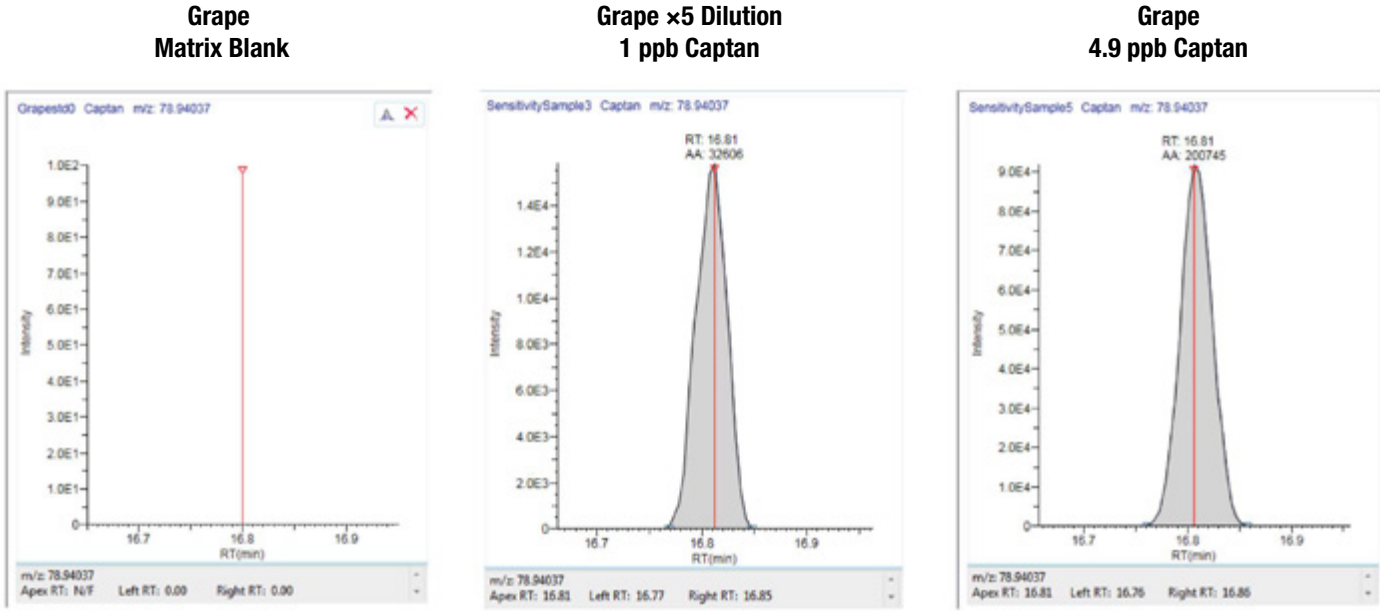


Figure 3. Extracted ion chromatogram and calculated concentration for captan in grape blank, 5× dilution and grape sample.

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.

References

1. http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc_11945_en.pdf
2. http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=URISERV:l13002a
3. SANTE/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. Supersedes SANCO/12571/2013. Implemented by 01/01/2016.
4. Mol, H.; Tienstra, M.; Zomer, P. Evaluation of gas chromatography electron ionization full scan high resolution Orbitrap mass spectrometry for pesticide residue analysis? *Analytica Chimica Acta,* **2016,** *935,* 161–172.
5. Ucles, S.; Lozano, A.; Martinez Bueno, M.J.; Fernandez-Alba, A.R. Shifting the paradigm in gas chromatography mass spectrometry pesticide analysis using high resolution accurate mass spectrometry. *Journal of Chromatography A,* **2017,** *1501,* 107–116.
6. Thermo Fisher Scientific, Application Note 10448. High efficiency, broad scope screening of pesticides using gas chromatography high resolution Orbitrap mass spectrometry, 2016. [online] https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/AN-10448-GC-MS-Orbitrap-Pesticides-AN10448-EN.pdf
7. Thermo Fisher Scientific, Application Note 10509. Routine quantitative method of analysis for pesticides using GC-Orbitrap mass spectrometry in accordance with SANTE/11945/2015 guidelines, 2016. [online] https://tools.thermofisher.com/content/sfs/brochures/AN-10509-GC-MS-Pescitides-SANTE-11945-2015-AN10509-EN.pdf
8. Thermo Fisher Scientific, Application Note 10541. Multi-residue pesticide screening in cereals using GC-Orbitrap mass spectrometry, 2017. [online] https://tools.thermofisher.com/content/sfs/brochures/AN-10541-GC-MS-Pesticides-Cereal-AN10541-EN.pdf
9. Lozano, A.; Kiedrowsk, B.; Scholten, J.; de Kroon, M.; de Kok, A.; Fernández-Alba, A.R. Miniaturisation and optimisation of the Dutch mini-Luke extraction method for implementation in the routine multi-residue analysis of pesticides in fruits and vegetables. *Food Chemistry.* **2016,** *192,* 668–681.

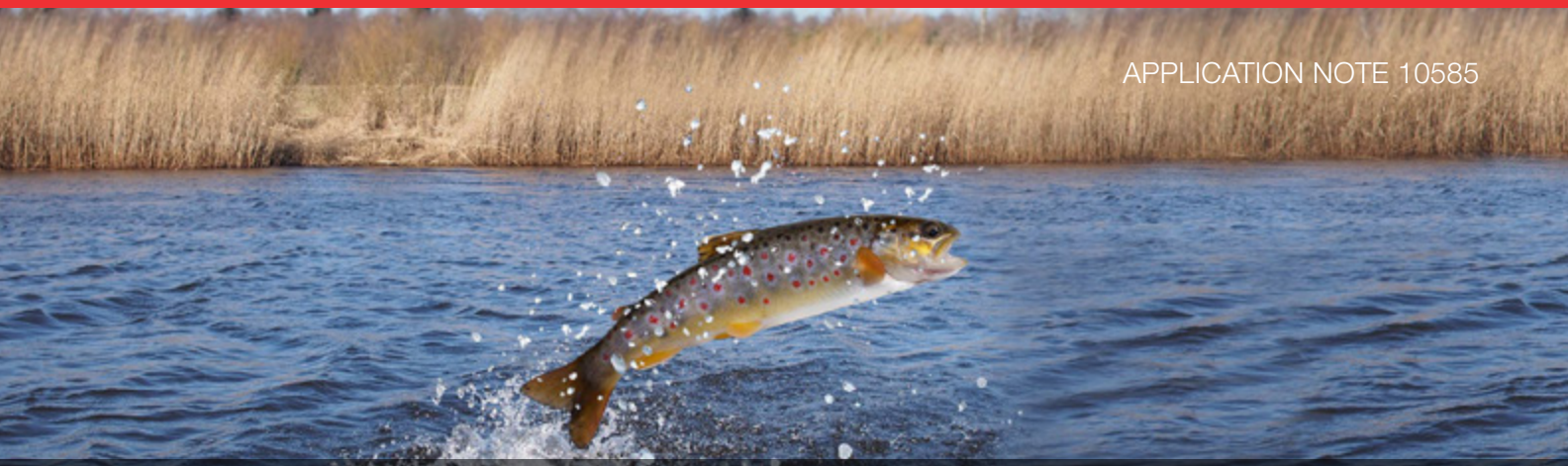
Find out more at **thermofisher.com/OrbitrapGCMS**

©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. ULTRA-TURRAX is a registered trademark of IKA - Werke GMBH & Co. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details.

AN10584-EN 1117S

thermo scientific

APPLICATION NOTE 10585



Determination of short- and medium-chained chlorinated paraffins in salmon samples using GC Orbitrap-MS

Authors

Kerstin Krätschmer,[1] Cristian Cojocariu,[2] Alexander Schächtele,[1] Paul Silcock,[2] and Rainer Malisch[1]

[1]European Union Reference Laboratory (EURL) for Dioxins and PCBs in Feed and Food, Freiburg, Germany

[2]Thermo Fisher Scientific, Runcorn, UK

Keywords

Chlorinated paraffins, polychlorinated biphenyls, PCBs, Orbitrap, mass spectrometry, persistent organic pollutants, quantification, chemical ionization

Goal

To demonstrate the quantitative performance of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ mass spectrometer for the analysis of short- and medium-chained chlorinated paraffins in salmon samples.

Introduction

The coupling of gas chromatography (GC) to high-resolution mass spectrometry (HRMS) using Orbitrap™ technology opens a broad spectrum of possible applications in environmental and food/feed analysis. Although known for several decades and widely used as plasticizers or flame retardants,[1] short-chain chlorinated paraffins (SCCPs) have been only recently added to Annex A of the Stockholm Convention list of persistent organic pollutants (POPs).[2]

ThermoFisher SCIENTIFIC

Previous efforts to ban SCCPs caused the production of medium-chain CPs (MCCPs) to increase to replace SCCPs.[3] As SCCPs alone consist of several thousand congeners with only four different carbon chain lengths to choose from, quantification of SCCPs and MCCPs in samples is a highly complex problem. In addition, other halogenated POPs like polychlorinated biphenyls (PCBs) are known to co-elute and add to the complexity of any analysis.

With this in mind, experiments focusing on linear dynamic range, sensitivity, and selectivity were performed using full-scan acquisition and negative chemical ionization (NCI) at 60,000 and 120,000 resolution (FWHM, *m/z* 200). In this study, mixtures of different CP and PCB standards were examined as well as food samples that were prepared with and without separation of co-eluting POPs during sample clean-up.

Experimental

Chemicals and standards

Two standard solutions resembling technical mixtures of SCCP (100 mg/L in cyclohexane, C_{10}–C_{13} 55.5% Cl) and MCCP (100 mg/L in cyclohexane, C_{14}–C_{17} 42% Cl) were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). As internal standards, 1,5,5,6,6,10-^{13}C-Hexachloro-decane (100 mg/L in nonane) was purchased from Cambridge Isotope Laboratories, Inc. (Andover, USA) and ε-HCH (100 mg/L in cyclohexane) was purchased from Dr. Ehrenstorfer GmbH.

For calibration, solutions of SCCP and MCCP with concentrations of 0.1 ppm, 0.5 ppm, 1 ppm, 5 ppm, 10 ppm, and 15 ppm and the addition of 0.1 ppm 1,5,5,6,6,10-^{13}C-Hexachlorodecane and 0.05 ppm ε-HCH were prepared in cyclohexane.

For extraction and clean-up of the samples, silica gel 60 (230–400 mesh) and Florisil® PR (60–100 mesh) for pesticide analysis were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany). Acetone, n-hexane, and methanol in residue analysis grade (LCG Standards GmbH, Wesel, Germany) were used as solvents.

Samples acquisition and preparation

The samples were acquired from supermarkets and vendors in Baden-Württemberg as part of the food control. Part of the homogenized sample was extracted using accelerated solvent extraction (ASE) at 150 °C with acetone/n-hexane 1:3 (v/v), followed by elution through a sulfuric acid primed silica gel column. Further clean-up was done using a Florisil column and eluting the PCB fraction with n-hexane and the CP fraction with dichloromethane. Some of the samples were additionally prepared without the clean-up on a Florisil column.

GC-MS analysis

Experiments were performed using a Q Exactive GC Orbitrap GC-MS/MS system coupled to a Thermo Scientific™ TRACE™ 1310 gas chromatograph equipped with a Thermo Scientific™ TraceGOLD™ TG5-SilMS 15 m × 0.25 mm × 0.25 µm column (P/N 26096-1300). Automatic tuning of the Q Exactive GC mass spectrometer was made using FC-43 as the tuning reagent and methane as the ionization gas. Full scans of the standards and samples were obtained using a mass range of *m/z* 50–650. Further details regarding the analytical system are given in Table 1.

Data processing

Data were acquired and processed using Thermo Scientific™ TraceFinder™ software.

Table 1. Parameters of the Q Exactive GC Orbitrap GC-MS/MS system used in this project.

TRACE 1310 GC System Parameters	
Injection volume:	1.5 µL
Liner:	Single gooseneck (P/N:4530924-UI)
Inlet:	280 °C
Inlet module and mode:	Splitless/Surge (9 psi for 1 min)
Splitless time:	1.2 min
Split flow:	50 mL/min
Column flow:	1.4 mL/min
Oven Temperature Program:	
Temperature 1:	60 °C
Hold time:	2 min
Temperature 2:	300 °C
Rate:	50 °C/min
Hold time:	5 min

Q Exactive GC Orbitrap GC-MS/MS System Parameters	
Transfer line:	280 °C
Ionization type:	NCI (methane)
Ion source:	180 °C
Electron energy:	70 eV
Acquisition mode:	Full-scan
C-Trap energy:	2 V
Mass range:	50-650 *m/z*
Mass resolution (FWHM at *m/z* 200):	60k and 120k

Results and discussion

Linearity and dynamic range

The linearity and dynamic range was assessed for both SCCP and MCCP technical mixtures (55.5% and 42% chlorine, respectively) using a dilution series in cyclohexane that resembles the usual analytical range of CP sample analysis in food. The coefficient of determination (R^2) of over 0.99 indicates good linearity beyond this concentration range for almost all chosen congeners when assigned the concentration of the technical mixture. Taking their percentage of the technical mixtures in account, an LOQ of 1.5 ppb (MCCP) and 0.1 ppb (SCCP) could be achieved for some congeners, with the corresponding LOD being as low as 0.3 ppb (MCCP) and estimated below 0.05 ppb (SCCP).

Selectivity

One of the biggest challenges of CP analysis is the high complexity of the compound mixtures found in both samples and standards (Figure 1). In addition to a high degree of overlapping of the different CP homologues, other persistent organic pollutants such as PCBs are known to co-elute, thus further complicating the analysis. To investigate possible influences, a mixture of the SCCP and MCCP technical mixtures spiked with a high concentration of PCBs was analyzed. The mixture showed no significant influence on peak shapes in comparison with the separate technical mixtures (Figure 2), even with the PCB congeners clearly dominating the total ion chromatogram (TIC) and degrading the CP chromatographic hump to mere baseline disturbance. Therefore, the high resolution of the Q Exactive GC Orbitrap-MS system allows for the quantification of both SCCP and MCCP even in the presence of significant amounts of PCBs in samples without congener groups being overestimated due to mass overlaps. This was further verified by the sum concentrations of SCCP and MCCP, which were determined both in the standards and the standard mix. The twelve homologues chosen to serve as examples gave in ten cases relative standard deviations of < 10% from the sum concentration determined in the single standards. The slightly elevated concentrations of both groups of CPs shown in Table 2 most likely stem from the known impurities of both standards; a small amount of SCCPs could be found in the MCCP standard, and it has been commented on in literature that SCCP standards seem to contain MCCPs.[4]

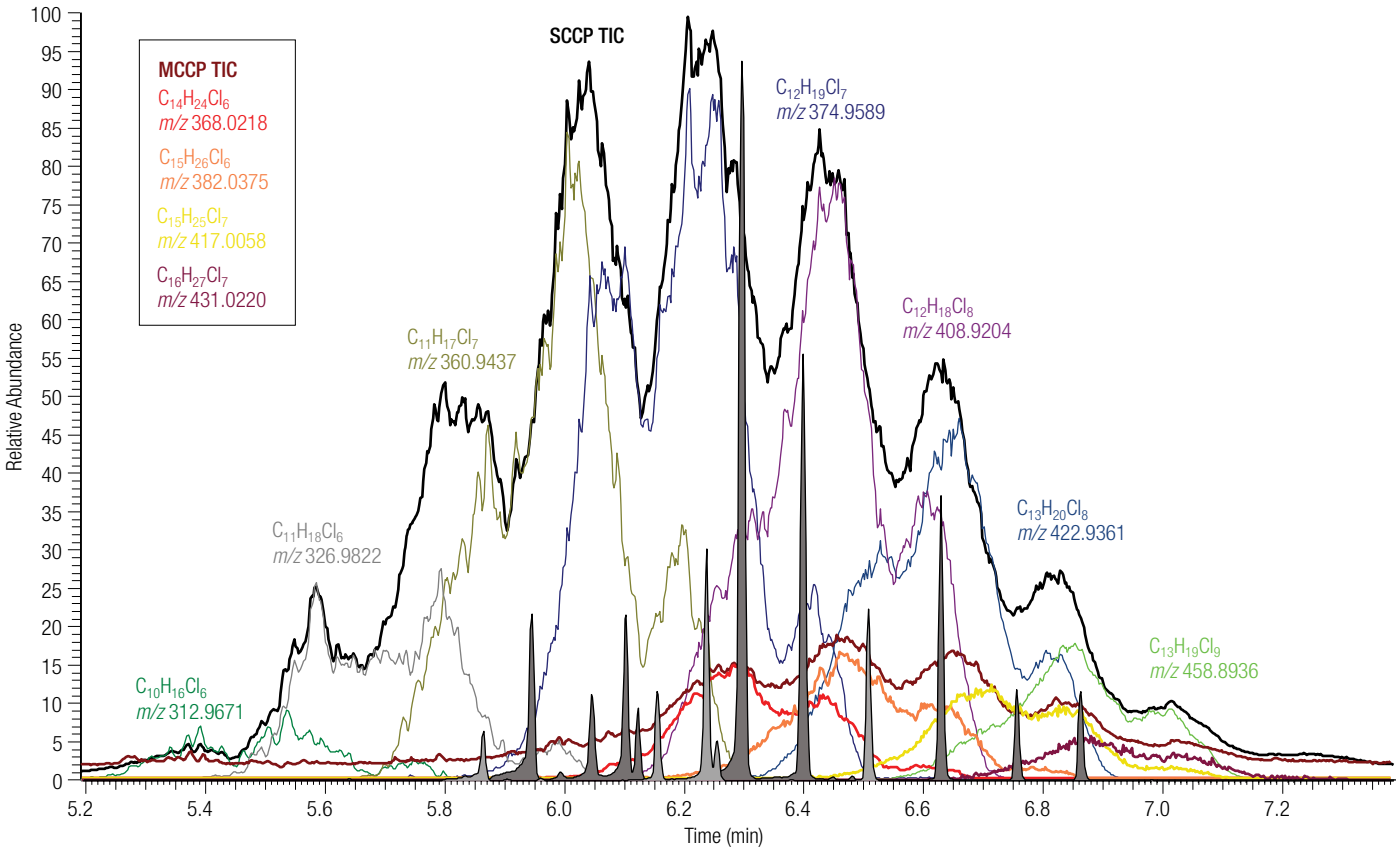


Figure 1. Overlaid chromatograms of SCCP, MCCP, and PCB standards with added extracted ion chromatograms of selected CP homologues measured with the Q Exactive GC Orbitrap GC-MS/MS system.

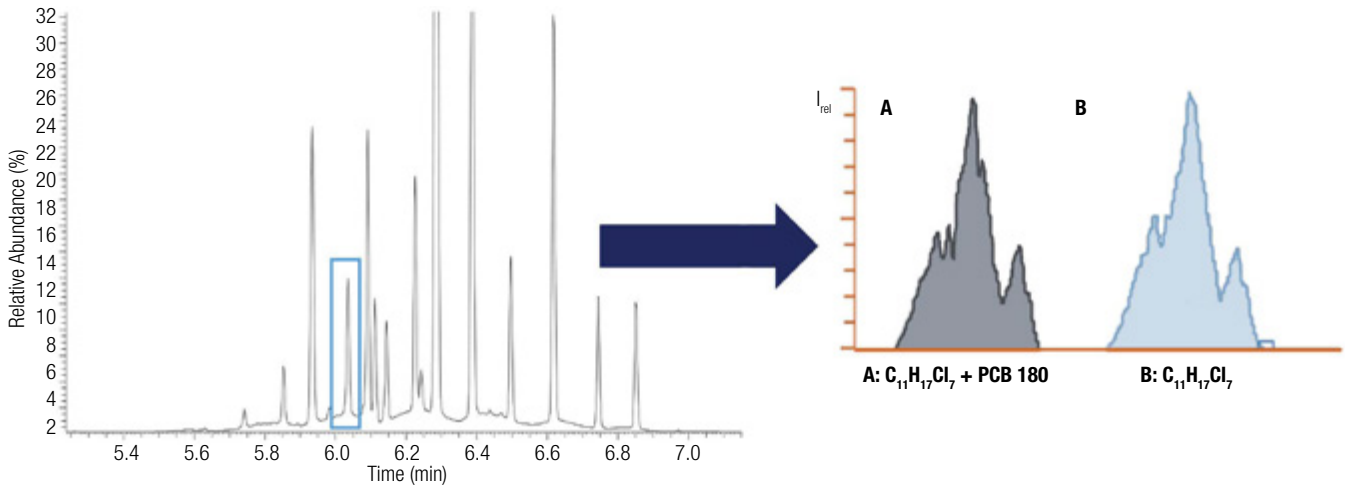


Figure 2. TIC of a mixture of SCCP, MCCP, and PCBs with extracted ion chromatograms of a CP homologue that is isobar with PCB 180 in its nominal mass and therefore eluting simultaneously. No significant influence on the peak shape and peak area could be observed.

Table 2. Concentrations of the sum of SCCPs and MCCPs in the mix of SCCPs, MCCPs, and PCBs as well as the single standards determined using different homologues (1-12).

∑SCCP Concentration [ppm]				∑MCCP Concentration [ppm]			
	SCCP Standard	Standard Mix	$s_{x,rel}$		MCCP Standard	Standard Mix	$s_{x,rel}$
1	4.04	4.49	8%	7	4.87	5.28	6%
2	3.99	4.49	9%	8	4.86	6.38	22%
3	4.22	4.45	4%	9	4.83	4.95	2%
4	4.29	4.64	6%	10	4.81	5,50	10%
5	4.22	4.45	4%	11	4.82	5.03	3%
6	4.25	4.56	5%	12	4.85	10.82	87%

Application to salmon samples

To explore the performance of the Orbitrap-MS system with real-life matrices, different samples of salmon prepared with two different clean-up methods were analyzed. The sum concentration of SCCPs and MCCPs respectively was additionally obtained beforehand by GC-EI-LRMS/MS. As seen in Figure 3, samples that were not subjected to a Florisil clean-up step show many additional, overlapping compounds (gray chromatogram) in comparison to the regularly prepared sample (blue chromatogram). The identified additional compounds in the gray chromatogram included several PCBs, dieldrin, DDT, and DDD as well as several toxaphenes. In the present experiment, a deviation of the CP pattern in comparison to cleansed samples could be observed. Although even between the two cleaned samples a slight deviation is visible, the differences to the uncleaned sample are more pronounced, in particular looking at the relation between selected homologues. Especially, indication of certain SCCPs being held back during Florisil clean-up should be investigated further. The comparison of sum SCCP and MCCP concentration, as determined by the Q Exactive GC Orbitrap GC-MS system and as determined by GC-EI-LRMS/MS, showed good agreement between the differently cleaned samples for the Orbitrap measurements with less than 10% deviation. On the other hand, a reliable determination of CP concentrations using low-resolution mass spectrometry was almost impossible in the uncleaned sample, leading to more than 50% deviation from the results obtained using the clean sample due to significant overestimation. The comparison of regular samples with samples from the same fish that were not cleaned using a Florisil column is therefore only possible because of the high selectivity of the Orbitrap-MS system, as other methods are too affected by the sheer number of different, overlaying compounds in the chromatographic window.

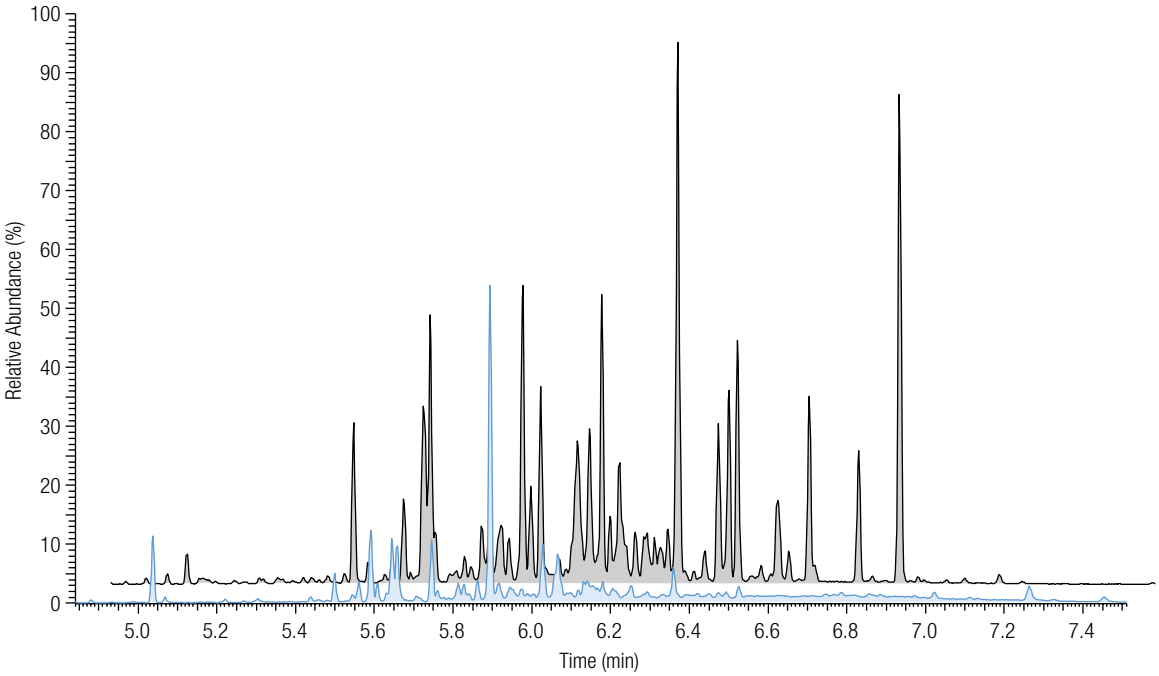


Figure 3. Q Exactive GC Orbitrap-MS full-scan TIC chromatograms of a regularly prepared salmon sample (blue) and of the same sample prepared without further clean-up (gray).

Conclusions

- The results of this study demonstrate very good linearity at concentrations of < 2 ppb. Determination of both CPs and PCBs in the same sample in one run is possible, suggesting the same for other halogenated compounds.
- TraceFinder software is an intuitive tool for processing data from full-scan analyses, allowing fast quantification and unprecedented insights into the pattern and content of CPs.
- A shortened sample preparation without separation of co-eluting compounds showed no influence on analysis results, while other instrumental setups struggled with the high number of compounds.
- Furthermore, the high selectivity of the Q Exactive GC Orbitrap GC-MS/MS system showed that possibly some CPs are held back during clean-up procedures, therefore influencing quantitative and qualitative results.
- Taken together, the Q Exactive GC mass spectrometer is a powerful analytical tool with simple setup and full-scan high-resolution experiments at a high selectivity, representing a potential for shorter sample preparation and quicker analyses of several types of POPs in one run, which is crucial considering the ever-growing list of compounds to be monitored in food and feed.

References

1. Tomy, G.T. (2010) *The Handbook of Environmental Chemistry,* Vol. 10, Springer, London.
2. Stockholm Convention on Persistent Organic Pollutants, UNEP-POPS-COP.8/14, final report not yet published as of 24.July.2017.
3. Gluege, J.; Wang, Z. et al. *Science of the Total Environment,* **2016,** 573, 1132–1146.
4. Reth, M. et al. *Analytical and Bioanalytical Chemistry,* **2004,** *378,* 1741–1747

Find out more at **thermofisher.com/OrbitrapGCMS**

©2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. FLORISIL is a trademark of U.S. SILICA COMPANY. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details. **AN10585-EN 1117S**

thermo scientific

Thermo Scientific gas chromatography mass spectrometry systems provide complete solutions to the most challenging applications. Combined with productivity enhancing software, our advanced GC-MS instruments empower you to meet your application's most stringent requirements for performance, reliability, and value.

Find out more at **thermofisher.com/gcms**

ThermoFisher SCIENTIFIC

For Research Use Only. Not for use in diagnostic procedures. © 2020 Thermo Fisher Scientific Inc. All rights reserved.
All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. **AI73859-EN 1120M**