Application note | 000469

Food safety

Quantitative analysis of environmental contaminants using Orbitrap Exploris GC

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Keywords

Orbitrap Exploris GC, Orbitrap technology, high resolution, accurate mass, sensitivity, robustness, gas chromatography, analytical testing, pesticides, PCBs, quantitation, Chromeleon CDS

Goal

To demonstrate the benefits of the Thermo Scientific™ Orbitrap Exploris™ GC mass spectrometer system for the analysis of trace level contaminants, such as pesticides, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and brominated flame retardants (BFRs), in a high-throughput testing laboratory.

Introduction

Analytical testing laboratories are faced with the challenge of delivering results for ever-growing lists of target compounds with faster turnaround times and at competitive cost. Essentially it comes down to the efficiency of operations to increase sample throughput and minimize instrument downtime.

In a high-throughput environment, robust streamlined analytical and data processing workflows are key requirements for the accurate and reliable determination of trace level contaminants in food or environmental samples. These methods must overcome the challenges of an ever-growing list of compounds and diversity of sample matrices, in addition to ever-more demanding sensitivity and identification requirements. Typically, gas chromatography coupled to a low-resolution, nominal mass triple quadruple mass spectrometer (GC-MS/MS) has been the system of choice for the sensitive and selective detection of a wide range of target compounds.

A GC-MS/MS acquisition method requires at least two precursor ions for product selected reaction monitoring (SRM) transitions to be optimized for selectivity and sensitivity for each analyte. The development of additional hyphenated GC-MS analytical systems such as high-resolution, accurate mass (HRAM) Orbitrap mass spectrometry coupled to GC has proved to be a valuable alternative to triple quadrupole GC-MS.[1-11] With HRAM mass spectrometry, the default acquisition mode is untargeted (full-scan), meaning all the ions are acquired with high selectivity across a specified mass range. This makes the method setup and data acquisition simple to manage and gives the analyst the flexibility to decide on which compounds to focus. This can extend into retrospective analysis of data to evaluate for the presence/absence of other contaminants not necessarily of interest at the time of acquisition.

In the experiments described below, the analytical performance and suitability of a benchtop HRAM Orbitrap GC-MS for analytical laboratories was assessed. System setup simplicity as well as typical method performance parameters including sensitivity, linearity, and quantitation were evaluated. Proficiency test samples were used to demonstrate accuracy of results compared to assigned values and results from GC-MS/MS.

Experimental

Sample and standard preparation

Depending on the matrix, the extraction for all samples was performed by accelerated solvent extraction (ASE) or Soxhlet extraction with addition of ^{13}C-labeled or deuterated internal standards. The raw extract was cleaned using a deactivated Florisil™-silica column with a fat capacity of 0.4–0.6 g/sample, SPE silica or sulfuric acid silica, depending on matrix and scope to clean even fish oils for measurement procedure. Final solvent of the injected extract was toluene.

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**). Finally, an Orbitrap Exploris GC mass spectrometer was used for accurate mass measurements in full-scan mode at 60,000 mass resolution (FWHM at *m/z* 200).

Table 1. GC and injector conditions

TRACE 1310 GC parameters	
Injection volume (µL)	2
Liner	PTV Siltek baffled liner **P/N 453T2120**
Inlet (˚C)	40
Inlet module and mode	PTV, Splitless
Splitless time (min)	1.5
Split flow (mL/min)	50
Septum purge flow (mL/min)	5
Carrier gas, flow rate (mL/min)	He, 1
PTV parameters (injection)	
Injection time (min)	0.1
PTV parameters (transfer)	
Rate (˚C/s)	14.5
Temp (˚C)	330
Time (min)	5
Flow (mL/min)	-
PTV parameters (cleaning)	
Rate (˚C/s)	14.5
Temp (˚C)	330
Time (min)	5
Split flow (mL/min)	50
Oven temperature program	
Temperature 1 (˚C)	80
Hold time (min)	1
Temperature 2 (˚C)	230
Rate (˚C/min)	10
Temperature 3 (˚C)	280
Rate (˚C/min)	3
Temperature 4 (˚C)	330
Rate (˚C/min)	20
Hold time (min)	5
Total GC run time (min)	40

Table 2. Mass spectrometer condition

Orbitrap Exploris GC EI GC-MS parameters	
Transfer line (˚C)	280
Ion source (ionization type)	ExtractaBrite™ (EI)
Ion source (˚C)	280
Electron energy (eV)	70
Emission current (µA)	50
Acquisition mode	Full scan (FS)
Mass range (*m/z*)	50–600
Mass resolution	60,000 (FWHM @ *m/z* 200, scan speed 7.4 Hz)
AGC target	1E+06

Data processing

The Orbitrap Exploris GC-MS can be tuned and calibrated very quickly (~1.5 min) and efficiently using a next-generation tune software designed for ease of use while offering maximum functionality (Figure 1).

The method editor is simple and intuitive and features ready-to-use, pre-optimized method templates for a wide range of typical application, including control of food safety (Figure 2).

Data were acquired and processed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, which allows instrument control, method development, and quantitation capabilities. Ready-to-go templates for instrument and processing method setup allow walk up and use capability.

For targeted analysis, a compound database was prepared containing compound name, accurate masses for quantification and confirming ions, retention times, and elemental compositions of precursor and product masses. To generate the extracted ion chromatograms (EIC), a mass window of ±5 ppm was used, meaning only ions with a mass accuracy ≤5 ppm are extracted.

Results and discussion

The objective of this study was to evaluate the quantitative performance and ease of use of the Orbitrap Exploris GC system for the analysis of persistent organic pollutants (POPs) in food matrices. Amongst the evaluated contaminants were organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs), and polyaromatic hydrocarbons (PAHs). The evaluation also covered a comparison with a triple-stage quadrupole instrument in terms of real sample analysis.

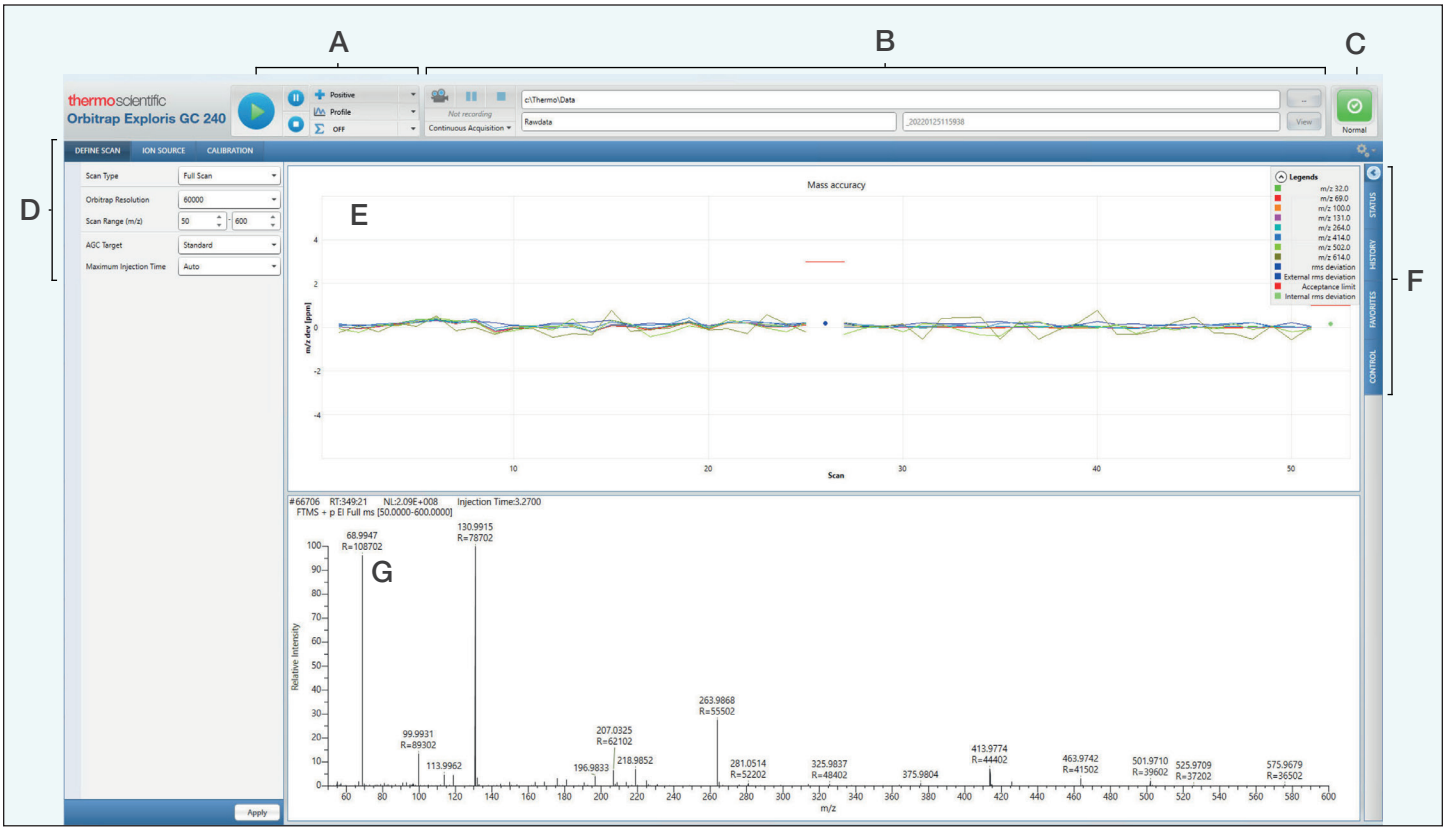

Figure 1. Orbitrap Exploris GC-MS tune page user interface developed for simplicity of use while offering maximum functionality to enable fast and efficient system set up. System calibration, tuning, and general checks (e.g., leak checks) take ~1.5 minutes and are only required to be performed weekly. The tune page includes: [A] power and instrument icons; [B] data acquisition buttons; [C] Instrument status icon; [D] scan, ion source and calibration panes; [E] plot view; [F] status panes; and [G] spectrum view.

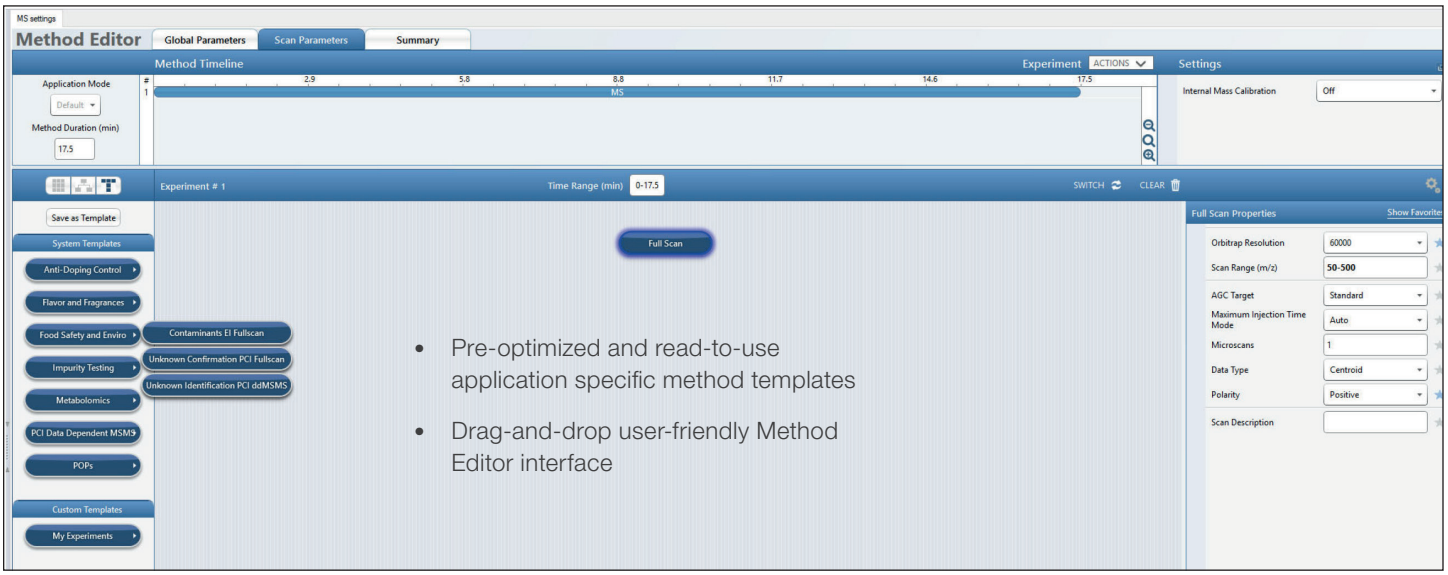

Figure 2. Orbitrap Exploris GC method editor system templates, enabling user friendly method set up using pre-optimized application specific method templates

Linearity and sensitivity

A wide linear dynamic range is essential, especially when the samples analyzed contain a complex chemical background (Figure 3) that could potentially interfere with the analytes of interest. Linearity was determined using solvent standards at concentrations 0.1–2,000 pg/µL. The calibration of each compound was performed using the linear/average calibration factor function in Chromeleon CDS (AvCF) over three injections at each concentration level. To determine the instrument LOQ, each standard was injected six times for standard deviation information.

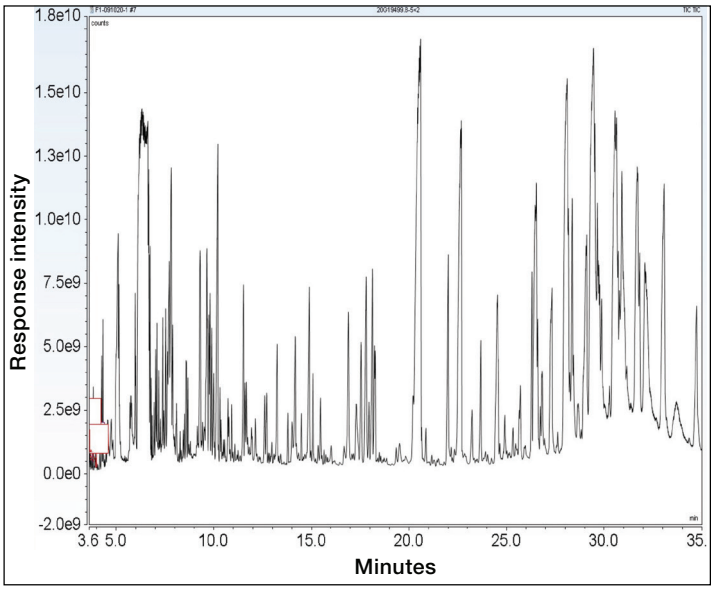

Figure 3. Full scan total ion current chromatogram of the fish fillet extract demonstrating high sample complexity

All the evaluated PCBs had a coefficient of correlation (R^2) equal or higher than 0.9999. The other investigated analytes were characterized by the $R^2 > 0.9950$, except trans-heptachlorepoxide, octaBDE (BDE-197), and benzo(*a*)pyrene, which were slightly below that value. The R^2 values for OCPs, PCBs, BFRs, and PAHs can be found in Tables 3, 4, 5, and 6, respectively, whereas Figures 4, 5, and 6 depict examples of calibration curves.

Sensitivity is one of the crucial parameters of an analytical method. A sensitive instrument is necessary to detect and quantify analytes present in the sample at low concentration levels as well as the analytes characterized by a low response.

In this study, the calibration curves were used to evaluate limits of detection and limits of quantitation. As shown in Tables 3-5, OCPs and PCBs had LOQs below 0.100 pg/µL, whereas BFRs showed LOQ <1 pg/µL.

In the case of PAHs, a different approach was applied. Instead of evaluating LODs and LOQs, the precision at 1 pg/µL was calculated. As can be seen in Table 6, the precision was better than 10% for all PAH compounds evaluated. A correction with the internal standard provided further improvement of the results.

Table 3. Coefficients of determination, limits of detection, and limits of quantitation for the OCPs evaluated based on the standards in the range of 0.1 to 1 pg/µL. LOQ value is based on signal-to-noise calculation of calibration in lowest applied concentration range.

Compound	R^2	LOD [pg/µL]	LOQ [pg/µL]
Pentachlorbenzol	0.9990	0.016	0.047
Hexachlorbenzol	0.9998	0.010	0.029
alpha-HCH	0.9997	0.014	0.041
beta-HCH	0.9991	0.004	0.013
gamma-HCH	0.9996	0.015	0.045
delta-HCH	0.9995	0.016	0.047
epsilon-HCH	0.9995	0.016	0.049
2,4´-DDT	0.9999	0.022	0.066
4,4´-DDT	0.9995	0.016	0.048
2,4´-DDE	0.9994	0.018	0.053
4,4´-DDE	0.9996	0.016	0.047
2,4´-DDD	0.9994	0.017	0.052
4,4´-DDD	0.9995	0.017	0.050
Aldrin	0.9990	0.023	0.070
Dieldrin	0.9991	0.022	0.065
Endrin	0.9987	0.026	0.079
alpha-Endosulfan	0.9994	0.018	0.053
beta-Endosulfan	0.9993	0.019	0.057
Endosulfan-Sulfat	0.9989	0.025	0.074
Toxaphene Parlar 26	0.9990	0.023	0.070
Toxaphene Parlar 50	0.9995	0.016	0.048
Toxaphene Parlar 62	0.9982	0.032	0.094
Mirex	0.9965	0.044	0.130
alpha-Chlordan	0.9978	0.035	0.105
gamma-Chlordan	0.9985	0.029	0.086
Oxychlordane	0.9981	0.032	0.096
trans-Nonachlor	0.9986	0.027	0.082
Heptachlor	0.9987	0.027	0.081
cis-Heptachlorepoxide	0.9987	0.026	0.079
trans-Heptachlorepoxide	0.9945	0.055	0.164
Hexachlorbutadien	0.9989	0.024	0.072
Octachlorstyrol	0.9988	0.026	0.078

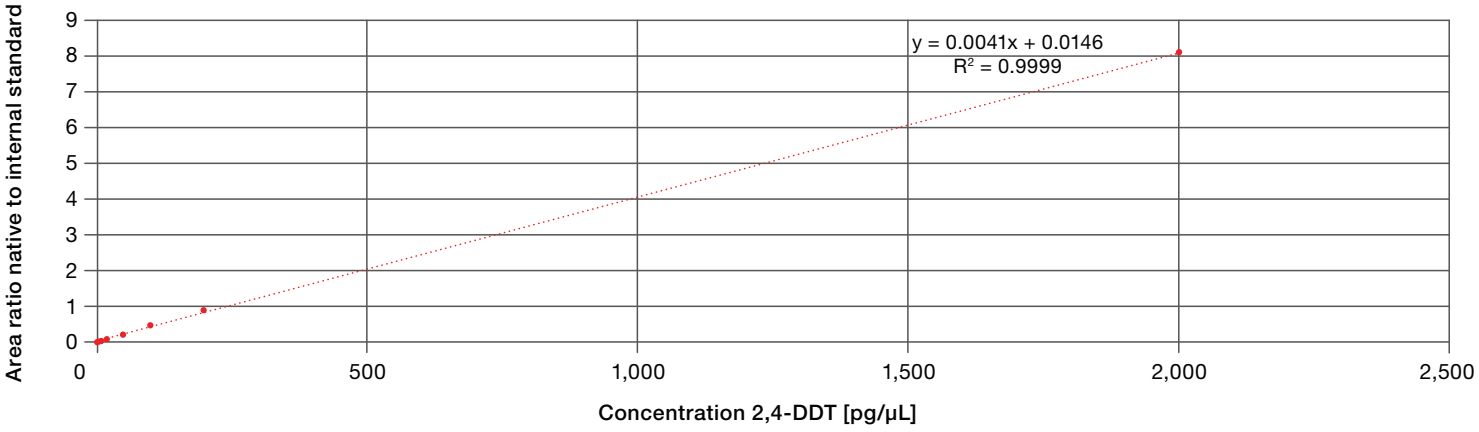

Figure 4. Linearity of 2'4-DDT 2–2,000 pg/µL

Table 4. Coefficients of determination, limits of detection, and limits of quantitation for the PCBs evaluated based on the calibration curve in the range of 0.005 to 11 pg/µL (substance specific). LOQ value is based on signal-to-noise calculation of calibration in lowest applied concentration range.

Compound	R^2	LOD [pg/µL]	LOQ [pg/µL]
PCB 77	1.0000	0.004	0.011
PCB 81	0.9999	0.007	0.020
PCB 105	1.0000	0.003	0.009
PCB 114	1.0000	0.004	0.013
PCB 118	1.0000	0.009	0.027
PCB 123	1.0000	0.004	0.011
PCB 126	1.0000	0.004	0.012
PCB 156	0.9999	0.009	0.027
PCB 157	0.9999	0.007	0.020
PCB 167	0.9999	0.007	0.021
PCB 169	1.0000	0.006	0.017
PCB 189	1.0000	0.003	0.008
PCB 28	1.0000	0.005	0.014
PCB 52	0.9999	0.009	0.027
PCB 101	0.9999	0.008	0.024
PCB 138	1.0000	0.007	0.021
PCB 153	1.0000	0.006	0.018
PCB 180	1.0000	0.005	0.016

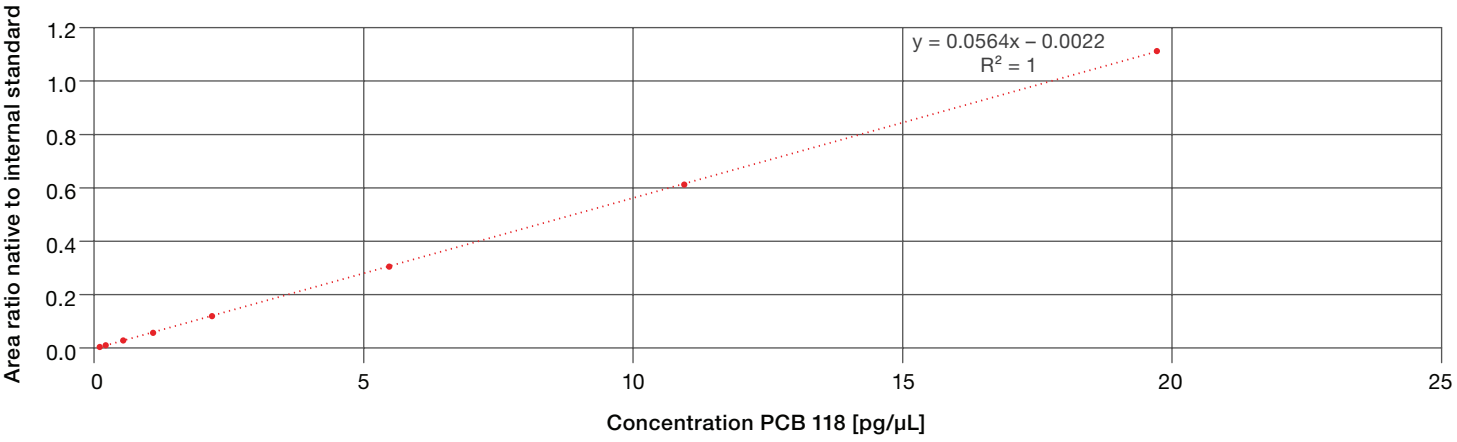

Figure 5. Linearity of PCB 118 0.11–19.7 pg/µL

Table 5. Coefficients of determination, limits of detection, and limits of quantitation for the BFRs evaluated based on the calibration curve in the range of 0.2 to 2 pg/µL. LOQ value is based on signal-to-noise calculation of calibration in lowest applied concentration range.

Compound	R^2	LOD [pg/µL]	LOQ [pg/µL]
TriBDE (BDE-17)	0.9987	0.053	0.159
TriBDE (BDE-28)	0.9990	0.048	0.143
TetraBDE (BDE-47)	0.9991	0.043	0.130
TetraBDE (BDE-49)	0.9951	0.104	0.313
TetraBDE (BDE-66)	0.9981	0.065	0.194
TetraBDE (BDE-71)	0.9943	0.112	0.335
TetraBDE (BDE-77)	0.9982	0.063	0.187
PentaBDE (BDE-85)	0.9985	0.569	0.171
PentaBDE (BDE-99)	0.9988	0.051	0.154
PentaBDE (BDE-100)	0.9992	0.042	0.125
PentaBDE (BDE-119)	0.9994	0.037	0.111
PentaBDE (BDE-126)	0.9981	0.643	0.193
HexaBDE (BDE-138)	0.9992	0.085	0.255
HexaBDE (BDE-153)	0.9987	0.149	0.315
HexaBDE (BDE-154)	0.9989	0.100	0.301
HexaBDE (BDE-156)	0.9978	0.139	0.417
HeptaBDE (BDE-183)	0.9975	0.148	0.445
HeptaBDE (BDE-184)	0.9972	0.157	0.470
HeptaBDE (BDE-191)	0.9971	0.158	0.475
OctaBDE (BDE-196)	0.9944	0.221	0.663
OctaBDE (BDE-197)	0.9897	0.302	0.905

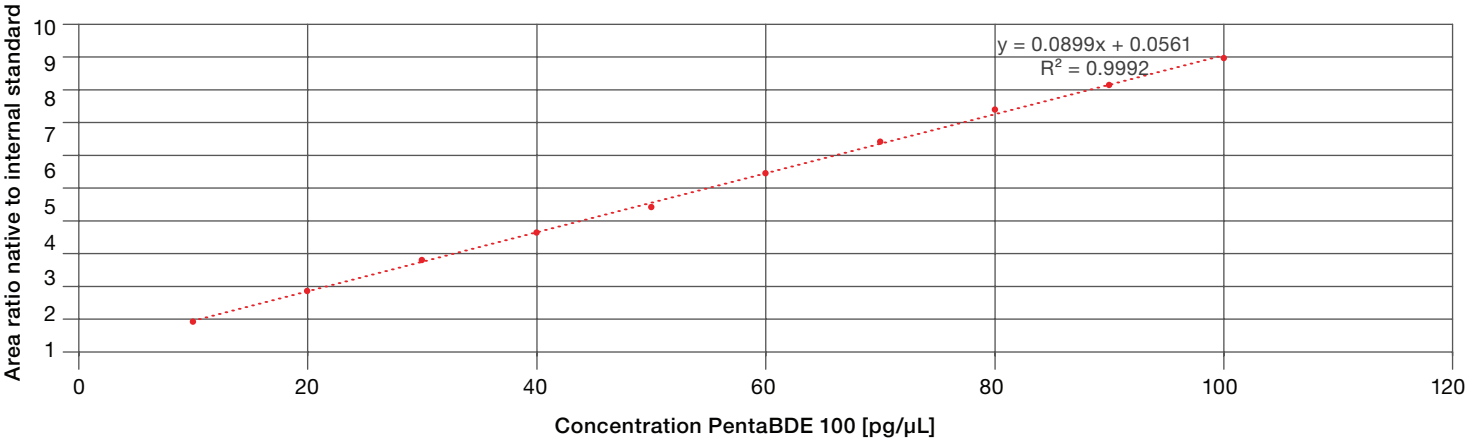

Figure 6. Linearity of PentaBDE 100 10–100 pg/µL

Table 6. Coefficients of determination and precision for the PAHs. The R^2 was evaluated in the range 0.2–2,000 pg/µL, whereas precision was tested at the 1 pg/µL level.

Compound	R^2	Precision (Relative Standard Deviation [%])	
		Uncorrected	Corrected
Naphthalin	0.9998	4.0	4.2
Acenaphthylen	0.9996	5.6	5.5
Acenaphthen	0.9991	9.0	6.3
Fluoren	0.9998	3.1	2.9
Phenanthren	0.9997	7.1	1.9
Anthracen	0.9999	8.1	6.6
Fluoranthen	0.9999	5.0	4.0
Pyren	0.9997	6.4	4.0
Benzo(*b*)naphtho(2,1-*d*)thiophen	0.9994	4.3	1.9
Benzo(*ghi*)fluoranthen	0.9995	5.9	3.8
Cyclopenta(*cd*)pyren	0.9973	6.1	4.1
Benz(*a*)anthracen	0.9982	4.7	2.9
Chrysen	0.9992	4.9	2.6
Benzo(*b*)fluoranthen	0.9973	2.4	1.7
Benzo(*j*)fluoranthen	0.9990	4.0	3.3
Benzo(*k*)fluoranthen	0.9988	4.5	3.8
Benzo(*e*)pyren	0.9995	2.3	1.9
Benzo(*a*)pyren	0.9943	1.5	1.2
Perylen	0.9966	1.8	1.4
Dibenz(*a,h*)anthracen	0.9980	2.5	1.7
Indeno(1,2,3-*cd*)pyren	0.9984	6.8	3.7
Benzo(*ghi*)perylen	0.9980	6.8	3.6
Anthanthren	0.9975	7.2	4.1

Separation of PBDEs

The chromatographic separation of isobaric PBDEs is crucial to obtain a reliable quantitative analysis as these compounds show the same elemental composition and therefore they cannot be identified based on their molecular composition. Thus, they were indistinguishable for the mass spectrometer. A complete chromatographic separation of those pairs was crucial for the selectivity of the analysis. Figure 7 shows the separation of two critical pairs of the BFRs.

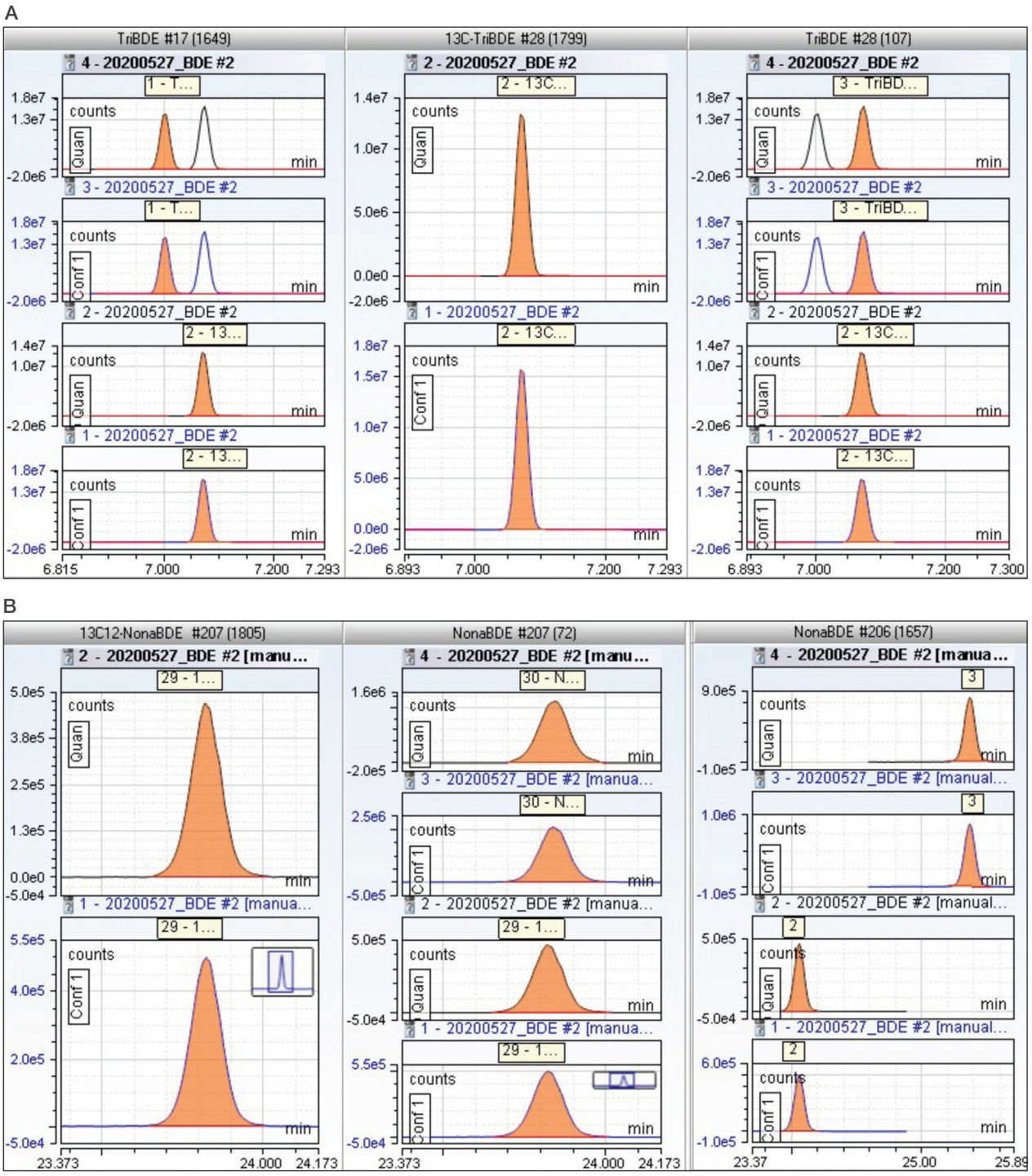

Figure 7. A) Chromatographic separation of TriBDE 17 and TriBDE 28, B) chromatographic separation of NonaBDE 207 and NonaBDE 206

Comparison of the Orbitrap Exploris GC to a triple quadrupole mass spectrometer

Triple quadrupole mass spectrometry is considered an excellent tool for quantitative analysis because of their high sensitivity, selectivity, and very good precision. To check the performance of the Orbitrap Exploris GC, a comparison with a triple quadrupole mass spectrometer was done. Two proficiency test samples and a set of real samples were injected on both instruments. The results were compared with the assigned values and the z-scores were calculated. Depending on the z-score, the results can be categorized as follows: $|z| \leq 2.0$ acceptable, $2.0 < |z| < 3.0$ questionable, $|z| \geq 3.0$ unacceptable. As seen in Tables 7 and 8, both systems provided very good, consistent results in terms of quantification. The same observation was made after the analysis of the real samples. Figure 8 shows results of a fish sample analyzed by both techniques.

Table 7. EU priority PAHs in olive oil. Fapas Food Chemistry proficiency test 0690.

Compound	Assigned value [µg/kg]	Reported result [µg/kg]		z-Score	
		Triple quadrupole	Orbitrap Exploris GC	Triple quadrupole	Orbitrap Exploris GC
Benzo[*a*]anthracene	3.80	4.33	4.03	0.6	0.3
Cyclopenta[*c,d*]pyrene	2.42	not measured	2.37	not measured	-0.1
Chrysene	4.43	4.7	4.68	0.3	0.3
5-Methylchrysene	1.33	not measured	not measured	not measured	not measured
Benzo[*b*]fluoranthen	1.59	1.31	1.79	-0.8	0.6
Benzo[*j*]fluoranthen	1.98	1.82	2.46	-0.4	1.1
Benzo[*k*]fluoranthen	1.98	2.54	2.18	1.3	0.5
Benzo[*a*]pyren	1.65	1.82	1.85	0.5	0.6
Indeno[1,2,3-*cd*]pyren	1.51	1.32	1.71	-0.6	0.6
Dibenz[*a,h*]anthracen	1.41	1.15	1.56	-0.8	0.5
Benzo[*g,h,i*]perylene	1.65	1.53	1.59	-0.3	-0.2
PAH 4 [sum]	11.30	11.9	14.8	0.2	1.4

Table 8. EURL proficiency test on the determination of PCDD/Fs, PCBs, BFRs, PFASs, and CPs in fish fillet (EURL-PT-POP_2001-FI)

Compound	Assigned value [ng/g]	Reported result [ng/g]		z-Score	
		Triple quadrupole	Orbitrap Exploris GC	Triple quadrupole	Orbitrap Exploris GC
PCB 28	0.362	0.41	0.40	0.7	0.6
PCB 52	1.29	1.44	1.46	0.6	0.7
PCB 101	4.8	5.71	5.54	0.9	0.7
PCB 138	10.5	12.55	12.15	1.0	0.8
PCB 153	15.9	21.66	23.26	1.8	2.3
PCB 180	5.57	6.62	6.77	0.9	1.0

Compound	Assigned value [pg/g]	Reported result [pg/g]		z-Score	
		Triple quadrupole	Orbitrap Exploris GC	Triple quadrupole	Orbitrap Exploris GC
PCB 105	1300	1444.5	1531.7	0.5	0.8
PCB 114	77.5	83.6	93.8	0.4	1.1
PCB 118	5500	7071.9	6116.8	1.4	0.5
PCB 123	66.8	53.3	54.9	-1.0	-0.9
PCB 156	907	972.9	1023.9	0.4	0.7
PCB 157	156	172.5	170.2	0.5	0.4
PCB 167	525	537.3	513.2	0.1	-0.1
PCB 189	92.4	90.5	97.7	-0.1	0.3
PCB 77	28.4	29.3	33	0.2	1.0
PCB 126	15.3	14.8	17	-0.2	0.7
PCB 169	1.77	1.7	1.5	-0.3	-1.2

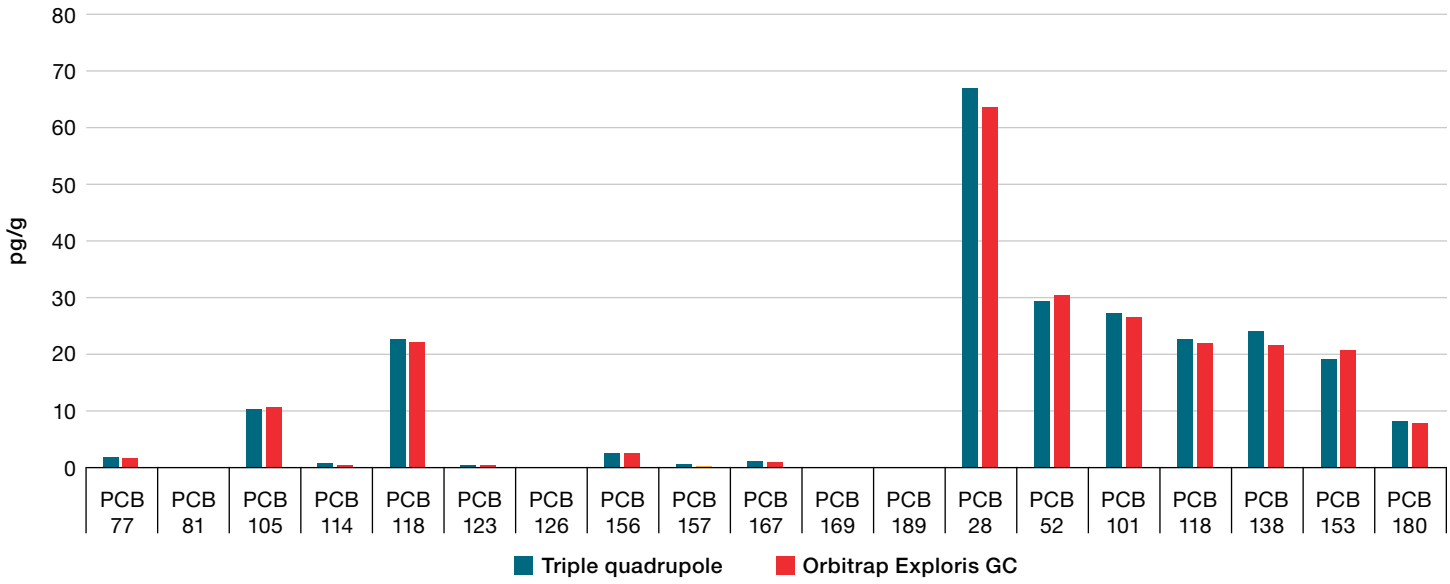

Figure 8. Comparison of the results obtained with a triple quadrupole mass spectrometer and an Orbitrap Exploris GC in the analysis of a real sample (fish matrix)

Conclusion

This study demonstrated the suitability of the Orbitrap Exploris GC mass spectrometer coupled to the TRACE 1310 GC for the analysis of organic contaminants in food. The instrument provided very good sensitivity and linearity for a broad spectrum of contaminants (organochlorine pesticides, polychlorinated biphenyls, polyaromatic hydrocarbons, and brominated flame retardants). The direct comparison with a triple-stage quadrupole proved that the Orbitrap mass spectrometry is not only a qualitative technique, but it is also an extremely accurate tool for quantitative analysis. The advantages of high-resolution MS are easy widening of the scope of analysis, consolidation of compound class methods, simple full scan acquisition, and additional points of identification. The results obtained in the proficiency test revealed that Orbitrap Exploris GC provides quantitative results as good as the triple quadrupole instruments, which are commonly considered as the gold standard for quantitative analysis.

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