Gas Chromatography

Applying high resolution GC-Orbitrap mass spectrometry for the quantitative analysis of environmental contaminants in food

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Abstract

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 evel 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. 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.

Materials and methods

Sample Preparation

Depending on the matrix, the extraction for all samples was performed by accelerated solvent extraction (ASE) or Soxhlet extraction with addition of 13C-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.

Test method

Automatic sample injection was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was performed using a Thermo Scientific™ TRACE™ 1610 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 parameters

Thermo Scientific™TRACE™ 1610 GC Parameters	
Injector	
Injector type	iConnect™ Programmable Temperature Vaporizing (PTV) injector
Operating mode	Splitless
Splitless time [min]	1.5
Split flow [mL/min]	50
Vacuum compensation	On
Temperature [˚C]	40
PTV Ramp Settings	
Injection Time [min]	0.1
Transfer Rate [˚C/s]	14.5
Transfer Temperature [˚C]	330
Transfer Time [min]	5
Cleaning Rate [˚C/s]	14.5
Cleaning Temperature [˚C]	330
Cleaning Time [min]	5
Oven	
Column	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).
Carrier gas	He
Carrier gas flow [ml/min]	1
Oven temperature program	
Temperature 1 [˚C]	80
Hold [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 [min]	5

Table 2. MS parameters

Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS Parameters	
Transfer line temperature [˚C]	280
Ion source temperature [˚C]	280
Electron energy [eV]	70
Emission current [µA]	50
Acquisition mode	Full scan
Mass range (m/z)	50-600
Mass resolution	60,000 (FWHM @ m/z 200,scan speed 7.4 Hz)
AGC target	1E+06

Data were acquired and processed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software, which allows instrument control, method development, and quantitation capabilities.

Results

Linearity and sensitivity

A wide linear dynamic range is essential, especially when the samples analyzed contain a complex chemical background (Figure 1) 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.

All the evaluated PCBs had a coefficient of correlation (R2) equal or higher than 0.9999. The other investigated analytes were characterized by the R2 > 0.9950, except trans-heptachlorepoxide, octaBDE (BDE-197), and benzo(a)pyrene, which were slightly below that value. The R2 values for OCPs, PCBs, BFRs, and PAHs can be found in Tables 3, 4, 5, and 6, respectively.

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	R2	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

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	R2	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

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	R2	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

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
PCB 28	0.362	0.7	0.6
PCB 52	1.29	0.6	0.7
PCB 101	4.8	0.9	0.7
PCB 138	10.5	1.0	0.8
PCB 153	15.9	1.8	2.3
PCB 180	5.57	0.9	1.0
PCB 105	1300	0.5	0.8
PCB 114	77.5	0.4	1.1
PCB 118	5500	1.4	0.5
PCB 123	66.8	-1.0	-0.9
PCB 156	907	0.4	0.7
PCB 157	156	0.5	0.4
PCB 167	525	0.1	-0.1
PCB 189	92.4	-0.1	0.3
PCB 77	28.4	0.2	1.0
PCB 126	15.3	-0.2	0.7
PCB 169	1.77	-0.3	-1.2

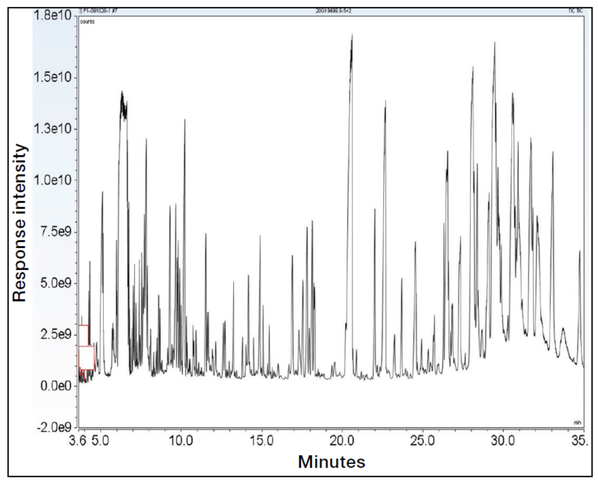

Figure 1. Full scan total ion current chromatogram of the fish fillet extract demonstrating high sample complexity

Comparison of the Orbitrap Exploris GC MS 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 MS, 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| ≤2.0 acceptable, 2.0 < |z| <3.0 questionable, |z| ≥3.0 unacceptable. As seen in Tables 7 and 8, both systems provided very good, consistent results in terms of quantification. Figure 2 shows results of a fish sample analyzed by both techniques.

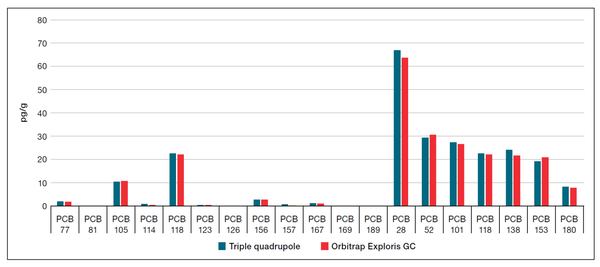

Figure 2. Comparison of the results obtained with a triple quadrupole mass spectrometer and an Orbitrap Exploris GC MS in the analysis of a real sample (fish matrix)

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
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

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 [µg/kg]	Reported result [µg/kg]		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
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

Conclusions

- This study demonstrated the suitability of the Orbitrap Exploris GC mass spectrometer for the analysis of organic contaminants in food.
- The method provided very good sensitivity and linearity for a broad spectrum of contaminants (organochlorine pesticides, polychlorinated biphenyls, polyaromatic hydrocarbons, and brominated flame retardants).
- The results obtained in the proficiency test revealed that Orbitrap Exploris GC MS provides quantitative results as good as the triple quadrupole instruments, which are commonly considered as the gold standard 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.

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Learn more at thermofisher.com/OrbitrapExplorisGC