Ultra-Sensitive Detection of PCBs and PAHs in seafood by GC-MS/MS following the European Union regulations

Abstract

A GC-MS/MS method has been developed for the simultaneous analysis of non-dioxin-like polychlorinated biphenyls (NDL-PCBs) and polycyclic aromatic hydrocarbons (PAHs) in seafood samples using the Bruker gas chromatography triple quadrupole mass spectrometry system EVOQ™ GC-TQ Premium. The outstanding sensitivity, selectivity and robustness of the system enabled the detection of sub-ppb amounts with high confidence while injecting only 1 µL of sample. The calibration and reporting limits have

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been set at 0.5 µg/kg, after achieving a limit of detection (LOD) < 0.1 µg/kg in routine assays. This ultra-sensitivity permits the analysis of more dilute samples, which can reduce instrument cleaning and maintenance. This method exceeds the sensitivity, selectivity and specificity requirements demanded in European Union Regulations [1-6].

Table 1: Maximum level for the sum of the six targeted PCBs and limit of quantitation per congener in seafood

Foodstuff	Maximum level	Limit of Quantitation
	Sum of PCB 28, 52, 101, 138, 153, 180	
Muscle meat of fish, shellfish, and products thereof	75 ng/g wet weight	1 ng/g per congener

Introduction

Polychlorinated Biphenyls (PCBs) encompass a class of chlorinated compounds with more than 200 variations, or congeners, with different physical and chemical characteristics. PCBs can be released into the general environment via several sources, e.g., from poorly maintained toxic waste sites, by illegal or improper dumping of PCB wastes, such as transformer fluids, through leaks or fugitive emissions from electrical transformers whose oil often contains PCBs, and by disposal of PCB-containing consumer products in municipal landfills. Chronic exposure of PCBs to animals can lead to hormone balance disruptions, reproductive failures, or cancer. Foods can be a major source of human PCB exposure, typically from fish and animal fat. PCBs are lipophilic, and they preferentially separate from water and adsorb onto sediment at the bottoms of lakes and rivers. Bottom feeders and other aquatic organisms then ingest and accumulate PCBs, resulting in a bio-concentration effect which migrates upward in the food chain.

The European Commission Regulations EU 252/2012 [1] and 1259/2011 [2] distinguish between dioxin-like (DL-PCB) and non-dioxin-like PCBs (NDL-PCB) based on their structural characteristics and toxicity, consequently leading to different methodologies and maximum levels for these two groups. Six marker NDL-PCBs are included in the presented method: PCB 28, 52, 101, 138, 153 and 180.

These PCBs comprise about half of the total amount of NDL-PCBs present in foodstuffs and their sum is considered as an appropriate marker for occurrence and human exposure to NDL-PCBs and is therefore set as the maximum level [2]. Performance criteria for analysis of NDL-PCBs by GC-MS/MS are detailed within the EU 589/2014 regulations [4].

Polycyclic aromatic hydrocarbons (PAHs) are potent atmospheric pollutants and are of concern because some have been identified as carcinogenic, mutagenic or teratogenic. As with PCBs, PAHs are lipophilic and generally have a very poor aqueous solubility. Therefore, they can accumulate in lipid tissues of plants and animals. Foods can be contaminated by PAHs that are present in air (by deposition), soil (by transfer) or water (deposition and transfer). Some PAHs are semi-volatile, but most tend to adsorb on organic particulate matter. Heavier PAHs preferentially associate with particulate matter, thus atmospheric fall out is a principal route of contamination. When particulates fall out onto a water surface, they are transported in suspension, eventually ending in fresh water or marine sediments. PAHs become strongly bound to these sediments, effectively creating a potential pollution reservoir for subsequent PAH release. Sediment-dwelling and filtering organisms are most susceptible to contamination. Most organisms have

Table 2: Maximum levels for benzo(a)pyrene and the sum of the four PAHs and limits of detection and quantitation

Foodstuff	Maximum level (µg/kg)		Limit of Detection	Limit of Quantitation
	Benzo(a)pyrene	Sum of 4 PAHs		
Smoked sprats and canned smoked sprats, bivalve mollusks (fresh, chilled or frozen), heat treated meat and heat treated meat products sold to the final consumer	5.0	30.0	≤0.30 µg/kg for each of the four substances	≤0.0 µg/kg for each of the four substances
Bivalve mollusks (smoked)	6.0	35.0		

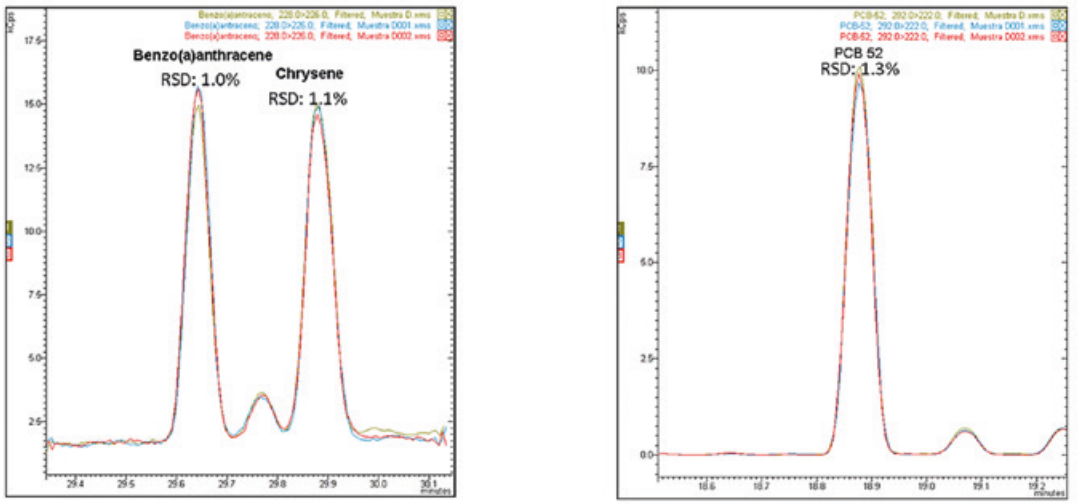

Figure 8: Three replicate injections of a mussel extract spiked with 0.8 µg/kg of PAHs and PCBs

mation ion 1 and 7.8% for confirmation ion 2. Accepted tolerances are ±20% and ±50%, respectively [4].

The robustness of the method was proven by analyzing replicates of bivalve mollusks spiked with PAHs and PCBs; specifically wedge and hard clams (in Spain known as berberechos and almejas, respectively).

Figures 10 and 11 show wedge clam extracts spiked at 0.8 µg/kg with PAHs and PCBs respectively. As shown, all analytes are perfectly identified, showing excellent response for the quantitation ion, as well as the confirmation ions for an unequivocal identification thus avoiding any false positive identification and reporting.

Minimizing the amount of matrix content injected is a valuable criterion for evaluation, because it often leads to a reduction of the maintenance required to maintain sensitivity and robustness. To evaluate the performance of the instrument with a more diluted sample, hard clam extracts spiked at slightly lower than the required limit of quantitation (LOQ) for PAHs/PCBs (0.8 µg/kg) was diluted two fold.

The required LODs are still exceeded after diluting the samples by two fold, as shown in Figure 12. This supports an increase in both method and instrument robustness according the European regulations for a routine 24/7 operation.

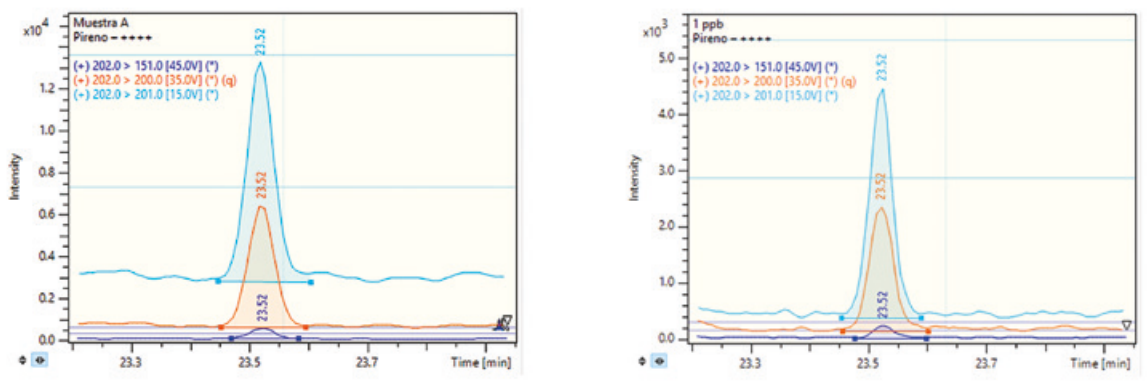

Figure 9: Analysis of a wedge clam extract (left) in comparison to a pyrene standard solution (right). Orange: Quantitation ion, Sky Blue: Confirmation ion 1, Dark Blue. Confirmation ion 2

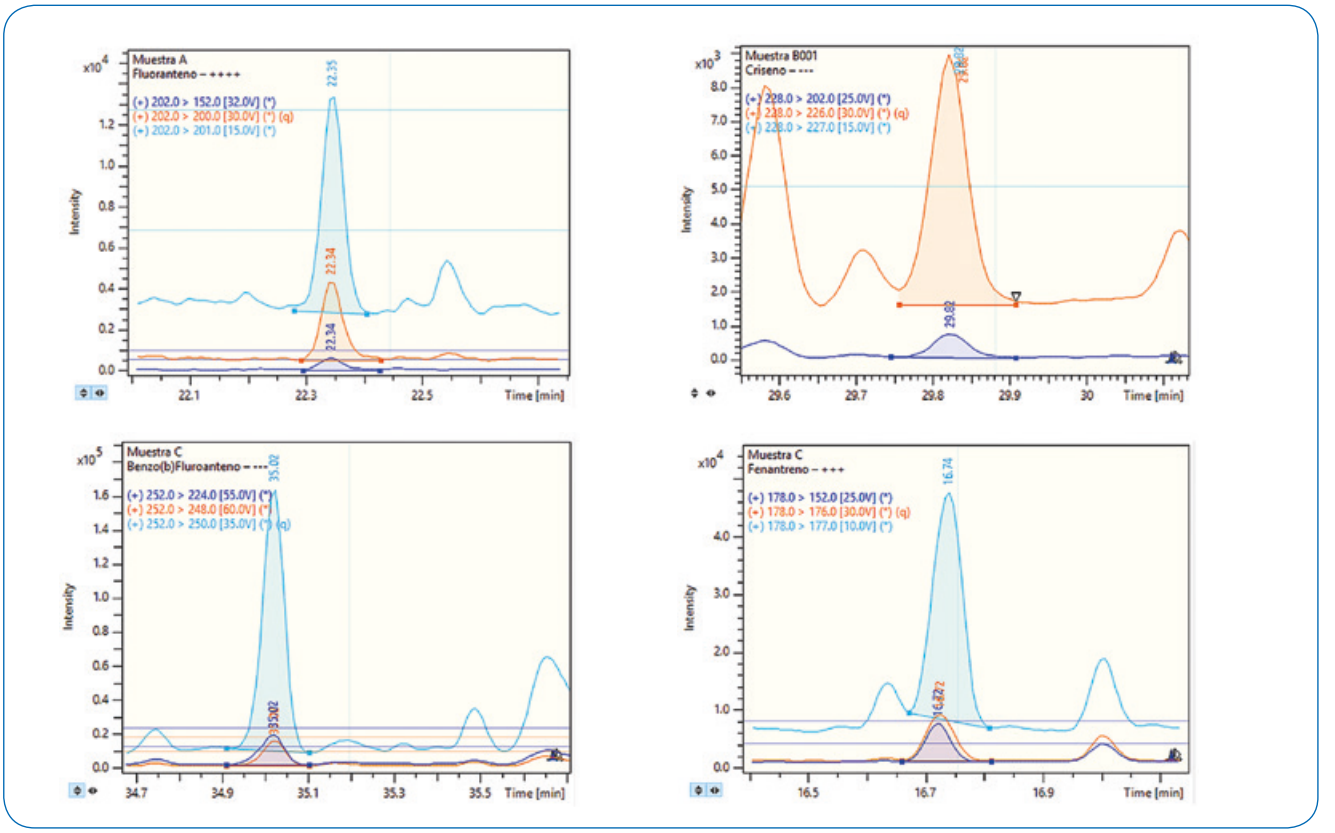

Figure 10: Analysis of different wedge clam extracts spiked at 0.8 µg/kg PAHs. Each time window shows the MRM transitions used for each compound.

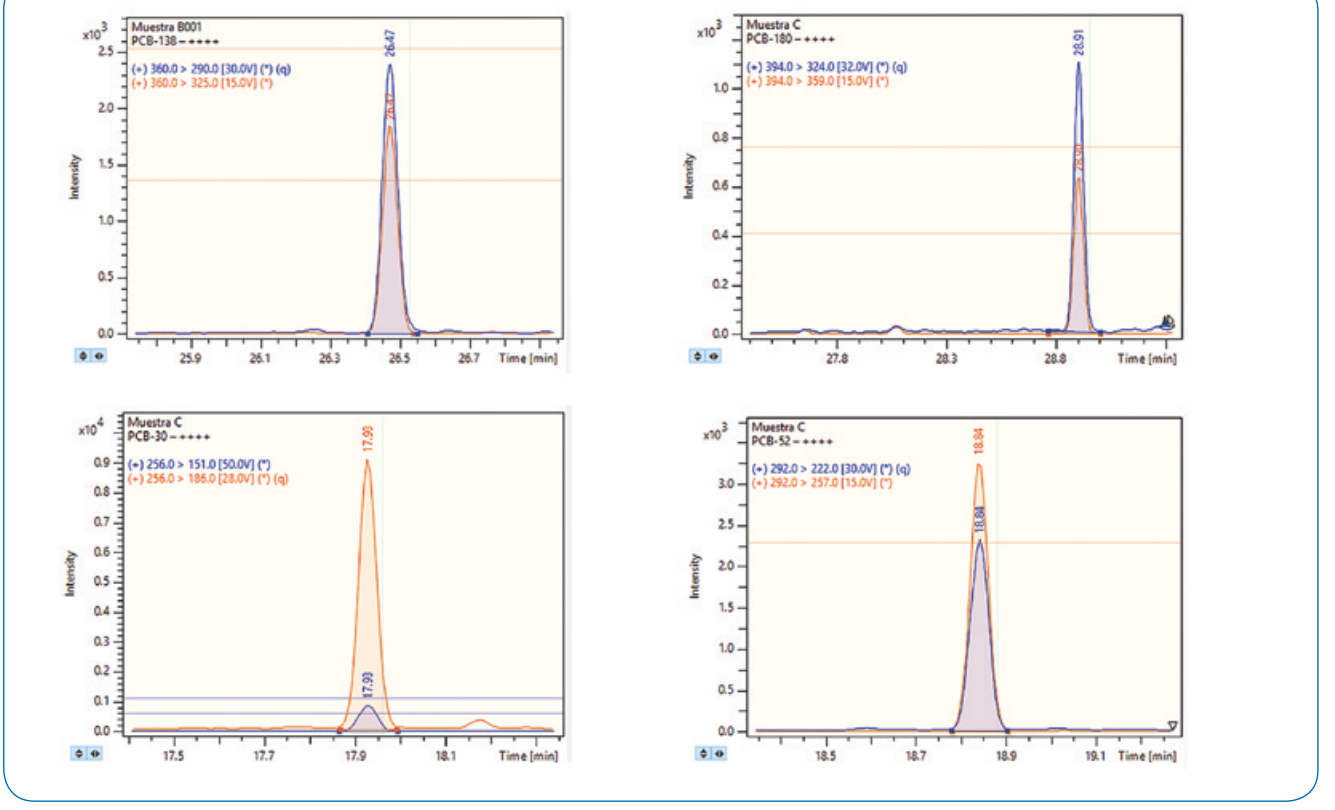

Figure 11: Analysis of different wedge clam extracts spiked at 0.8 µg/kg PCBs. Each time window shows the MRM transitions used for each compound.

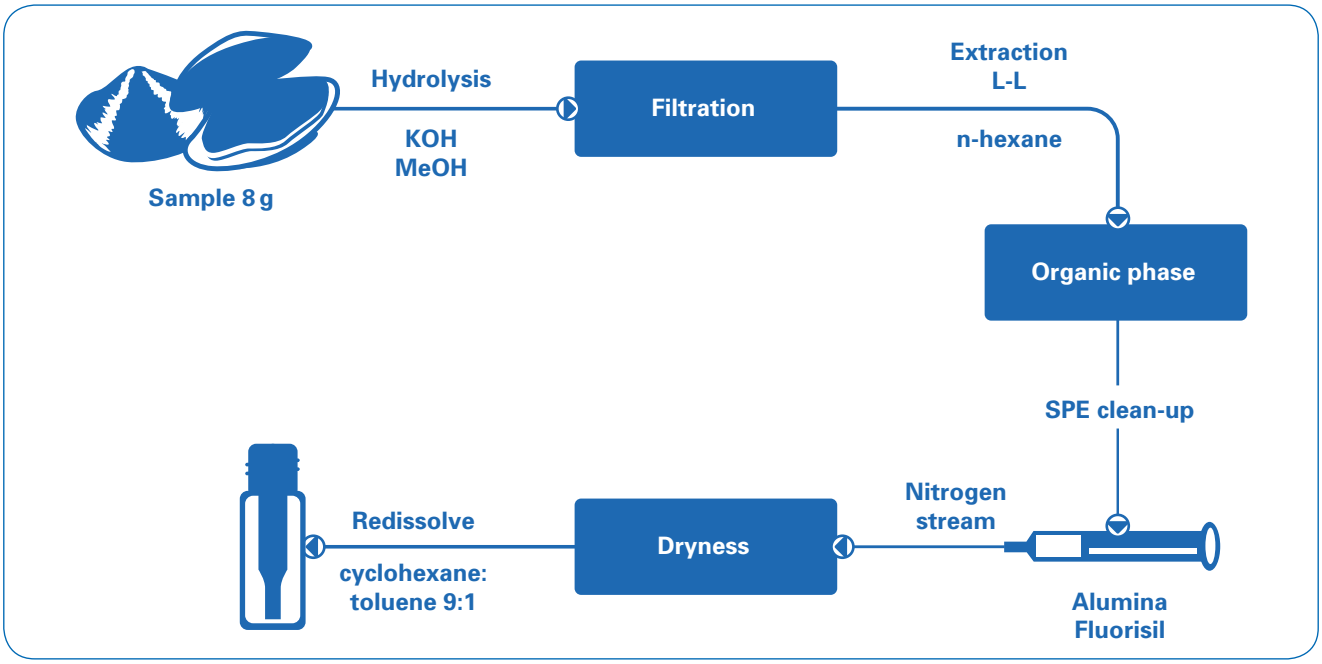

Figure 1: Sample preparation workflow

a high bio-transformation potential for PAHs, resulting in no significant bio-magnification in the aquatic food chain. However, filter-feeding bivalves (e.g., mussels and oysters) may accumulate PAHs as they filter large volumes of water and have a low metabolic capacity for these compounds.

Until 2008, benzo(a)pyrene was used as a marker for the occurrence of PAHs in foods. But, in 2008, the Scientific Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA) concluded that benzo(a)pyrene alone was not a suitable marker for the occurrence of PAHs in foods and that a system of four specific substances (benzo(a) pyrene, benzo(a)anthracene, benzo(b) fluoranthene and chrysene) would be more suitable markers [4]. Consequently, Commission Regulation EU 835/2011 [5] amended Regulation (EC) 1881/2006 in order to set maximum levels in specific foodstuffs for the sum of these four PAHs, while their LODs and limits of quantitation (LOQs) were set by Commission Regulation EU 836/2011 [6], as shown in Table 2.

A method has been developed for the simultaneous analysis of the six markers NDL-PCBs, and 16 PAHs (including the four specifically regulated PAHs) in bivalve mollusk samples using the Bruker EVOQ GC-TQ Premium MS/MS system.

Experimental

Sample Preparation

Clam and mussel samples were collected from the southern coast of Spain. Samples (8 g) were hydrolyzed with KOH and MeOH, filtered and extracted with n-hexane. Extracts were purified by loading into a cartridge containing alumina and florisil [7-9]. The purified extracts were evaporated to dryness under a nitrogen stream and reconstituted in 2 mL of cyclohexane:toluene (9:1). Figure 1 shows the sample preparation workflow.

PCBs and PAHs standards were obtained from Dr. Ehrenstorfer GmbH (Augsburg – Germany) and spiked samples prepared.

Methodology

A total number of 41 compounds were analyzed: 16 PAHs, 16 deuterated PAHs used as internal standards (IS) and 9 PCBs.

Where possible, up to three MRM transitions per compound were utilized in order to increase specificity. A complete list of MRM transitions is shown in Table 4.

Results and discussion

The procedures and analytical requirements to monitor the levels of PAHs and NDL-PCBs in foodstuffs within the EU [1-6] are very strict, and are required to meet performance criteria regarding accuracy, linearity and precision, amongst other criteria. In accordance with the provisions of the EU regulations, laboratories shall be accredited following ISO 17025 stan-

Table 3: Mass Spectrometry Method Conditions

Mass Spectrometer	**Bruker EVOQ GC-TQ MS system**
MS Conditions	
Ionization	EI, 70 eV
Emission Current	40 µA
Active Focusing Q0	135 °C with Helium
Transfer Line Temperature	300 °C
Source Temperature	300 °C
CID Gas	Ar, 2.0 mTorr
Detector Mode	EDR
Scan Mode	MRM, 0.6 sec/scan
Gas Chromatograph	**Bruker 436 GC**
GC Conditions	
Injector	1177 Split/splitless
Sample Volume/Injection Mode	1 µL, splitless
Injector Insert	4 mm single taper splitless with deact. wool (p/n: SG092003)
GC Oven Temperature	70 °C (1.7 min) → 30 °C/min → 180 °C (0´) → 5 °C/min → 320 °C (17´)
GC Column	Bruker BR-PCB, 40 m x 0.18 mm, 0.18 micron (p/n: BR58697)
Carrier Gas	Helium, 0.8 mL/min constant flow
Total Run Time	50 min
Autosampler	Bruker 8400 autosampler
Software	Bruker MSWS 8.2.1/TASQ 1.4 processing software

dard by a recognized body operating as per ISO Guide 58 to ensure the analytical quality assurance. The results shown below include all the analytical quality criteria required by the European regulations.

GC separation

The GC operating conditions were optimized to obtain an optimal peak shape without any tailing effects, in particular for the late eluting PAHs as shown in Figure 2. Additionally, this optimized separation provided high sensitivity.

Using a narrow bore capillary column (40 m x 0.18 mm), an outstanding chromatographic separation for the more critical pairs of compounds could be achieved (as shown in Figure 3) thus avoiding peak co-elution that could potentially mask some peaks and produce erroneous results.

Linearity

The linearity of response of this method has been evaluated from the reporting limits upward. Nine different solutions of increasing concentrations were prepared: 0.5 ppb, 1 ppb, 2.5 ppb, 5 ppb, 12.5 ppb, 25 ppb, 50 ppb, 75 ppb and 100 ppb, and spiked with the same amount of deuterated standards. Each standard solution was analyzed in triplicate.

Selected calibration curves for PAHs and PCBs are shown in Figures 4 and 5, respectively.
Table 5 shows a summary of the calibration results showing the linearity of the method with regression coefficients $R^2 > 0.995$ and relative standard deviation (RSD) < 15%.

Sensitivity and detection limits

To validate the sensitivity of the method, one standard solution with a concentration of 0.1 ppb (100 femtogram on-column) was injected three times. Signal-to-noise (S/N) > 40 was achieved for all compounds and replicates. Therefore, the LOD for all analytes is < 0.1 ppb. MRM chromatograms for selected PAHs and PCBs are shown in Figures 6 and 7, respectively.

Precision and repeatability

Table 4: MRM conditions for the PCBs and PAHs monitored

Compound Name	RT (min.)	Precursor Ion	Quan Ion	CE	Confirm Ion 1	CE	Confirm Ion 2	CE
Naphthalene-d8	6.61	136	134		132	-25	-	-
Naphthalene	6.70	128	102		126	-20	127	-5
Acenaphthalene-d8	10.75	160	158		156	-25	-	-
Acenaphthalene	10.80	152	150		151	-15	126	-28
Acenaphthene-d10	11.14	164	160		162	-18	-	-
Acenaphthene	11.24	153	127		151	-25	152	-20
Fluorene-d10	12.84	174	172		170	-30	-	-
Fluorene	12.95	165	164		163	-30	139	-30
Phenanthrene-d10	16.67	188	184		186	-20	-	-
Phenanthrene	16.79	178	176		177	-10	152	-25
Anthracene-d10	16.98	188	184		186	-20	-	-
Anthracene	17.08	178	176		152	-25	177	-10
PCB-28	15.30	256	186		151	-50	-	-
PCB-30	18.01	256	186		151	-50	-	-
PCB-52	18.94	292	222		257	-15	-	-
PCB-101	22.21	326	256		291	-15	-	-
Fluoranthene-d10	22.34	212	208		210	-15	-	-
Fluoranthene	22.44	202	200		201	-15	152	-32
Pyrene-d10	23.53	212	208		210	-15	-	-
Pyrene	23.63	202	200		201	-15	151	-45
PCB-153	25.43	360	290		325	-15	-	-
PCB-138	26.61	360	290		325	-15	-	-
PCB-183	27.11	394	324		359	-15	-	-
PCB-180	29.04	394	324		359	-15	-	-
Benzo(a)anthracene-d12	29.60	240	236		238	-20	-	-
Benzo(a)anthracene	29.73	228	226		202	-30	227	-18
Chrysene-d12	29.82	240	236		238	-20	-	-
Chrysene	29.96	228	226		202	-25	227	-18
PCB-170	30.22	394	324	32	359	-15	-	-
Benzo(b)fluoranthene-d12	35.06	264	260		262	-30	-	-
Benzo(b)fluoranthene	35.21	252	250		248	-60	224	-55
Benzo(k)fluoranthene-d12	35.16	264	260		262	-30	-	-
Benzo(k)fluoranthene	35.30	252	250		248	-60	224	-55
Benzo(a)pyrene-d12	37.06	264	260		262	-30	-	-
Benzo(a)pyrene	37.24	252	250		248	-60	224	-55
Dibenzo(a,h)anthracene-d14	45.10	292	288		290	-20	-	-
Dibenzo(a,h)anthracene	45.50	278	276		250	-50	277	-20
Indene(1,2,3-c,d)pyrene-d12	45.45	288	284		286	-20	-	-
Indene(1,2,3-c,d)pyrene	45.75	276	272		273	-45	274	-40
Benzo(g,h,i)perylene-d12	48.16	288	286		284	-40		
Benzo(g,h,i)perylene	48.47	276	272		273	-45	274	-40

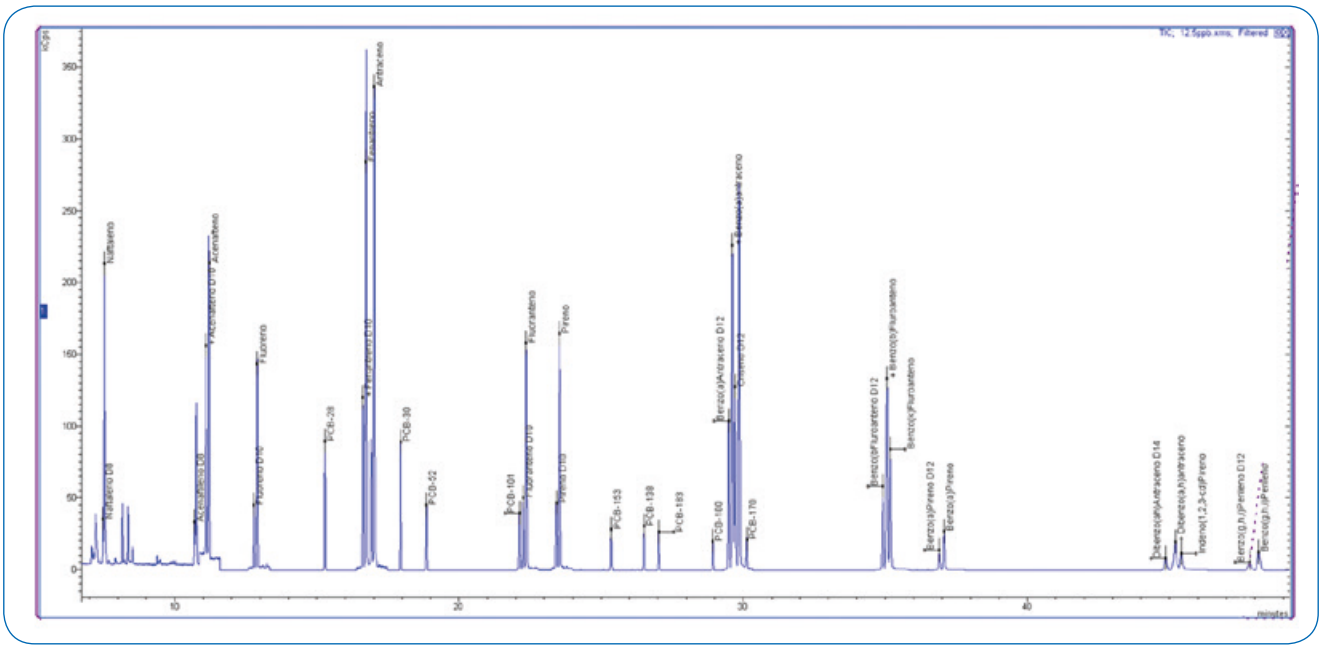

Figure 2: Total Ion Chromatogram (TIC) of 12.5 ppb standard mix (PAHs and PCBs)

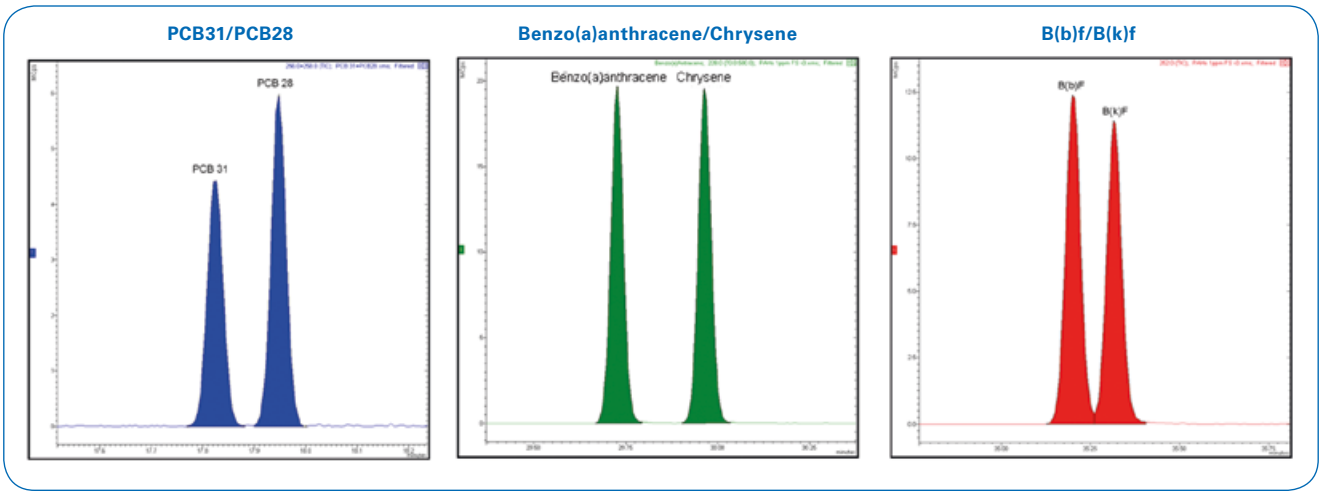

Figure 3: Total Ion Chromatogram (TIC) of 12.5 ppb standard mix (PAHs and PCBs) expanded in indicated areas

The precision, expressed as repeatability, was calculated on the results achieved from three replicate analyses of a mussel extract spiked with PAHs and PCBs at 0.8 µg/kg. Note this level is slightly below the LOQ (0.9 µg/kg) required for PAHs (see Table 2).

An example of repeatability for selected PAHs and PCBs is shown in Figure 8.
Excellent relative standard deviation below 4% was obtained for all analytes in mussel extract spiked at 0.8 µg/kg as shown in Table 6.

Selectivity, ion ratios stability and robustness

Selectivity was tested by comparing the response of analytes in spiked mussel samples with those of spiked standards. No interferences or co-elution effects were found in this study. Further, no deviation in retention times between samples and standard chromatograms was found. Stability of the ion ratio is also very important for an unequivocal identification when using triple quadrupole instruments, as it helps to avoid any false positive reporting. Figure 9 shows a comparison of pyrene analysis in a wedge clam extract and standard solution. The relative retention time (RRT) difference for pyrene in wedge clam extract and standard is -0.03%, where the tolerance allowed is ±0.25% [4]. The ion ratios differences are 1.1% for confir-

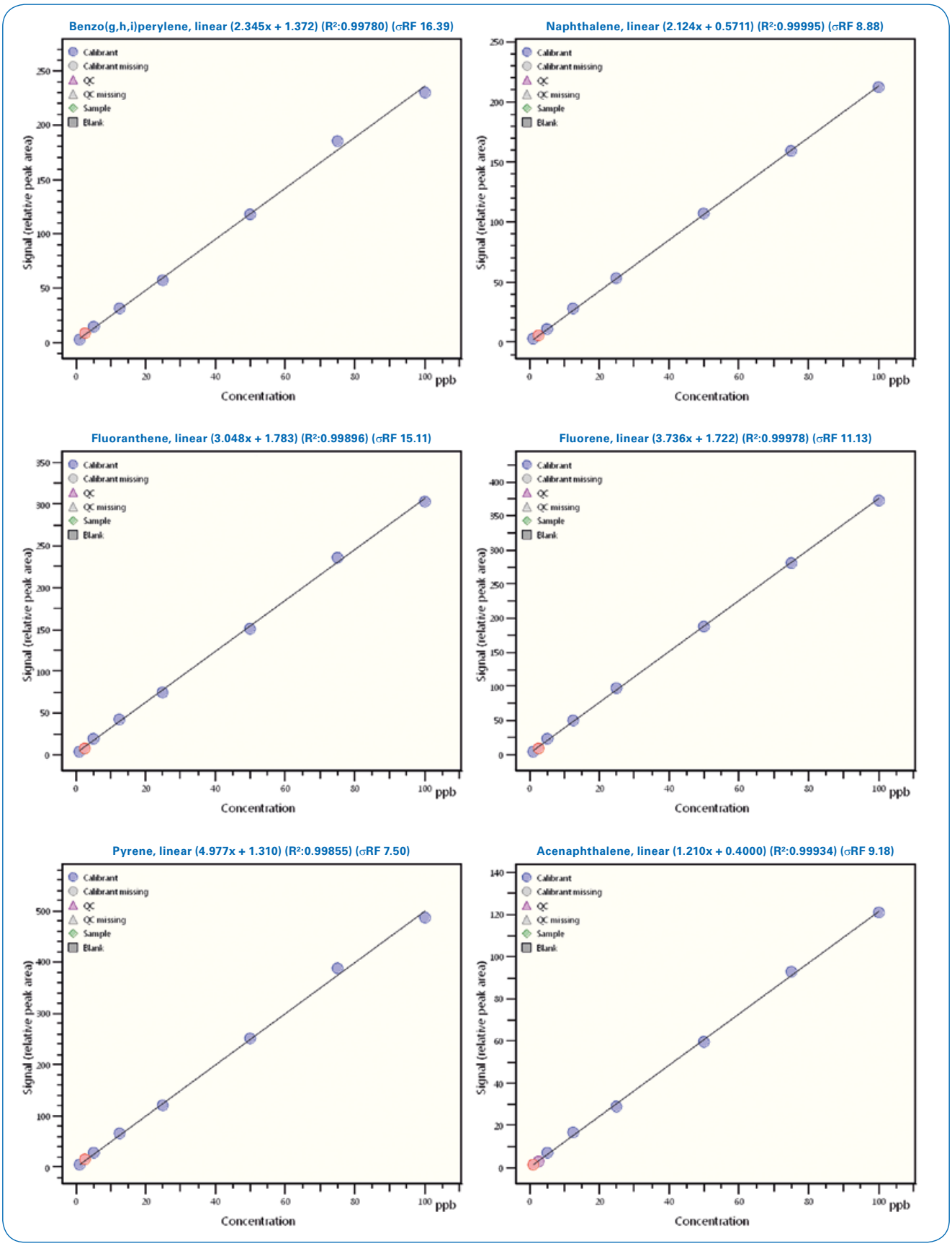

Figure 4: Calibration curves for selected PAHs from 0.5 ppb to 100 ppb

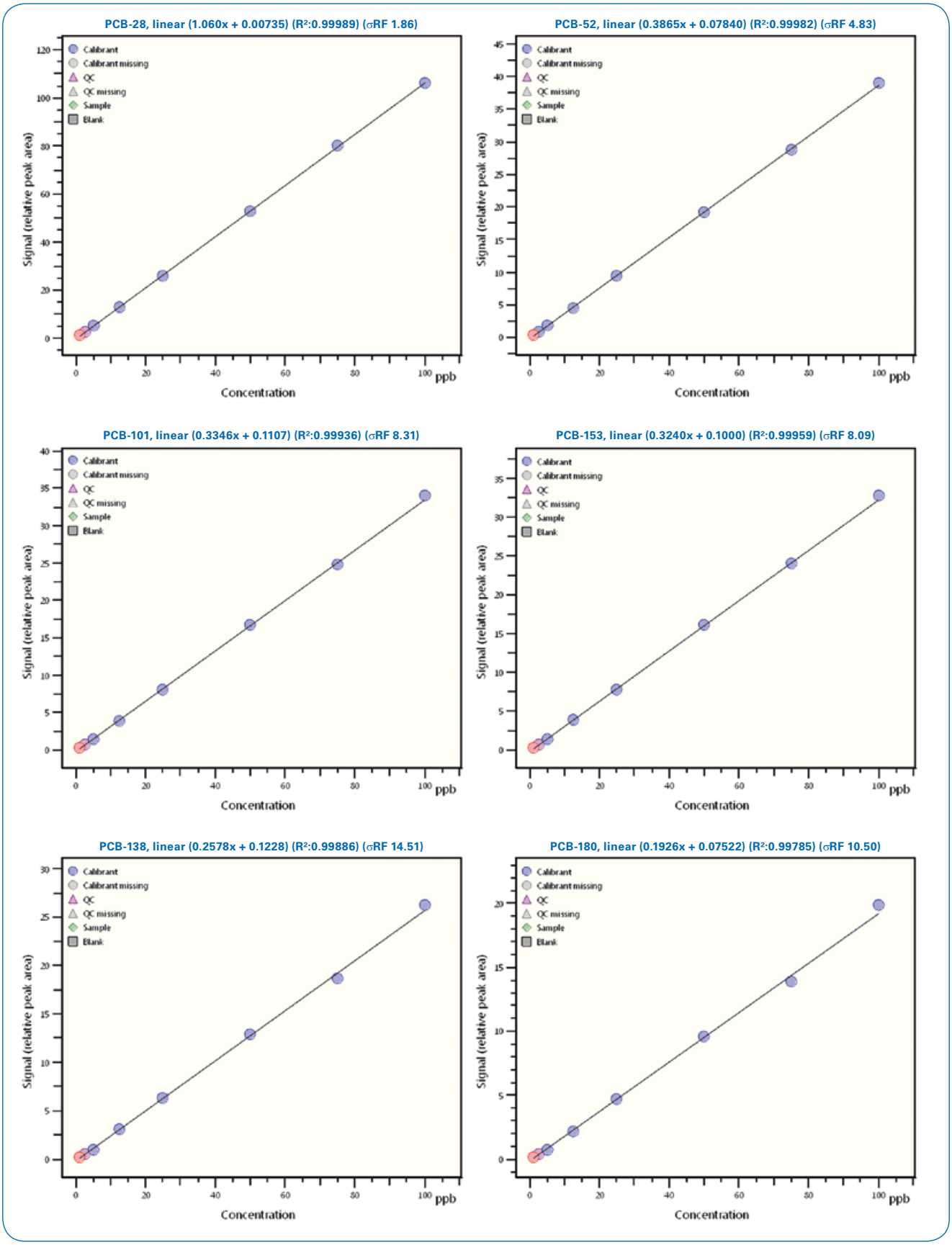

Figure 5: Calibration curves for selected PCBs from 0.5 ppb to 100 ppb

Table 5: Summary of calibration results, with nine calibration levels from 0.5 to 100 ppb

Compound name	R^2	RSD (%)	Compound name	R^2	RSD (%)
Naphthalene	0.99995	8.88	PCB-138	0.99886	14.51
Acenaphthalene	0.99934	9.18	PCB-183	0.99558	14.51
Acenaphthene	0.99620	11.42	PCB-180	0.99785	10.50
Fluorene	0.99978	11.13	Benzo(a)anthracene	0.99956	12.7
Phenanthrene	0.99886	6.32	Chrysene	0.99959	12.06
Anthracene	0.99845	12.03	PCB-170	0.99613	14.94
PCB-28	0.99989	1.86	Benzo(b)fluoranthene	0.99854	7.90
PCB-30	0.99931	8.57	Benzo(k)fluoranthene	0.99674	11.12
PCB-52	0.99982	4.83	Benzo(a)pyrene	0.99721	13.78
PCB-101	0.99936	8.31	Dibenzo(a,h)anthracene	0.99613	14.40
Fluoranthene	0.99896	15.11	Indene(1,2,3-c,d)pyrene	0.99752	12.88
Pyrene	0.99855	7.50	Benzo(g,h,i)perylene	0.99780	16.39
PCB-153	0.99959	8.09	-	-	-

Table 6: Summary of area repeatability for selected PCBs and PAHs in a mussel extract spiked at 0.8 µg/kg

Compound name	Replicate 1 (Area)	Replicate 2 (Area)	Replicate 3 (Area)	Average (Area)	RSD (%)
Acenaphthene	50042	50822	50083	50316	0.7
Acenaphthalene	10673	10596	10669	10646	0.3
Anthracene	44029	43066	43873	43656	1.0
Benzo(a)pyrene	23148	23450	23986	23528	1.5
Benzo(a)anthracene	43193	43780	44175	43716	0.9
Benzo(b)fluoranthene	261459	266883	261520	263287	1.0
Benzo(g,h,i)perylene	12876	12977	13155	13003	0.9
Benzo(k)fluoranthene	29562	29539	29145	29415	0.7
Chrysene	42581	43704	43343	43209	1.1
Dibenzo(ah)anthracene	13906	13937	14000	13948	0.3
Phenanthrene	47378	46220	47637	47078	1.3
Fluoranthene	46342	45928	45007	45759	1.2
Fluorene	41185	40994	42628	41602	1.8
Indene(1,2,3-c,d)pyrene	11494	11184	11072	11250	1.6
Naphthalene	21826	22009	22823	22219	2.0
PCB 101	53189	49522	53118	51943	3.3
PCB 138	40578	40392	40627	40532	0.2
PCB 153	141830	137724	135958	138504	1.8
PCB 180	47887	46108	47638	47211	1.7
PCB 28	33700	33934	32112	33249	2.4
PCB 52	32109	31240	31233	31527	1.3
Pyrene	74599	73822	73394	73938	0.7

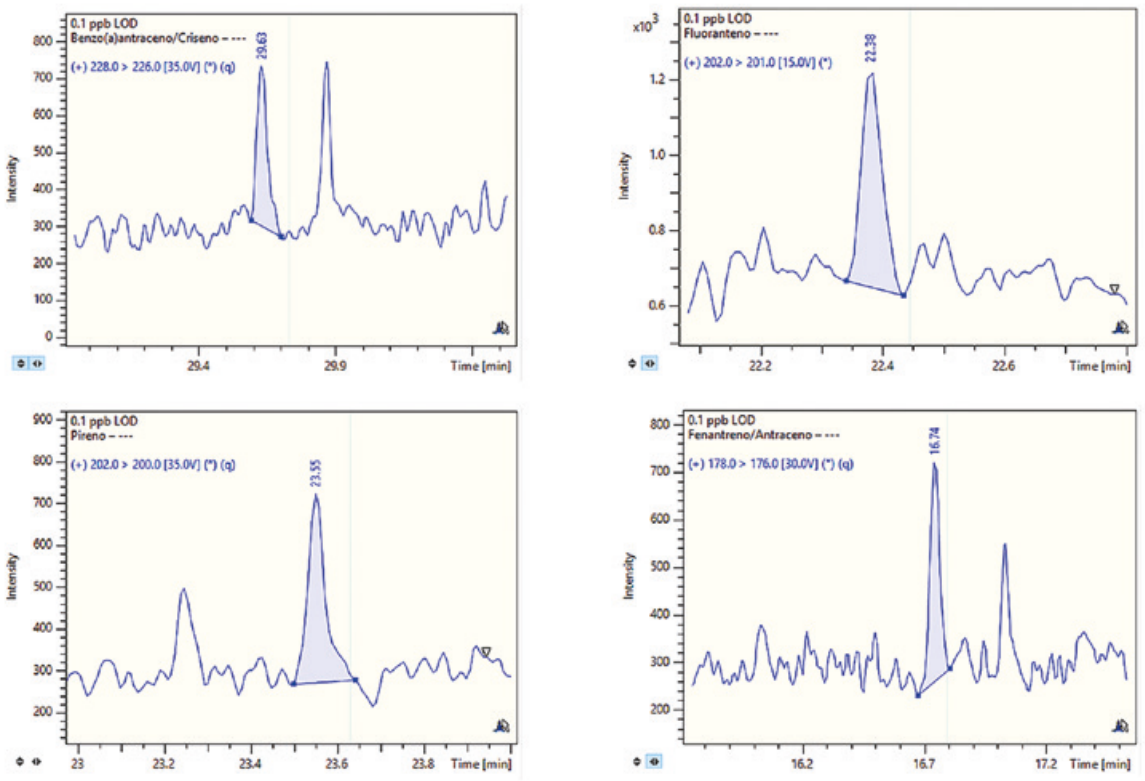

Figure 6: MRM chromatograms for selected PAHs at 0.1 ppb level (100 femtogram on-column)

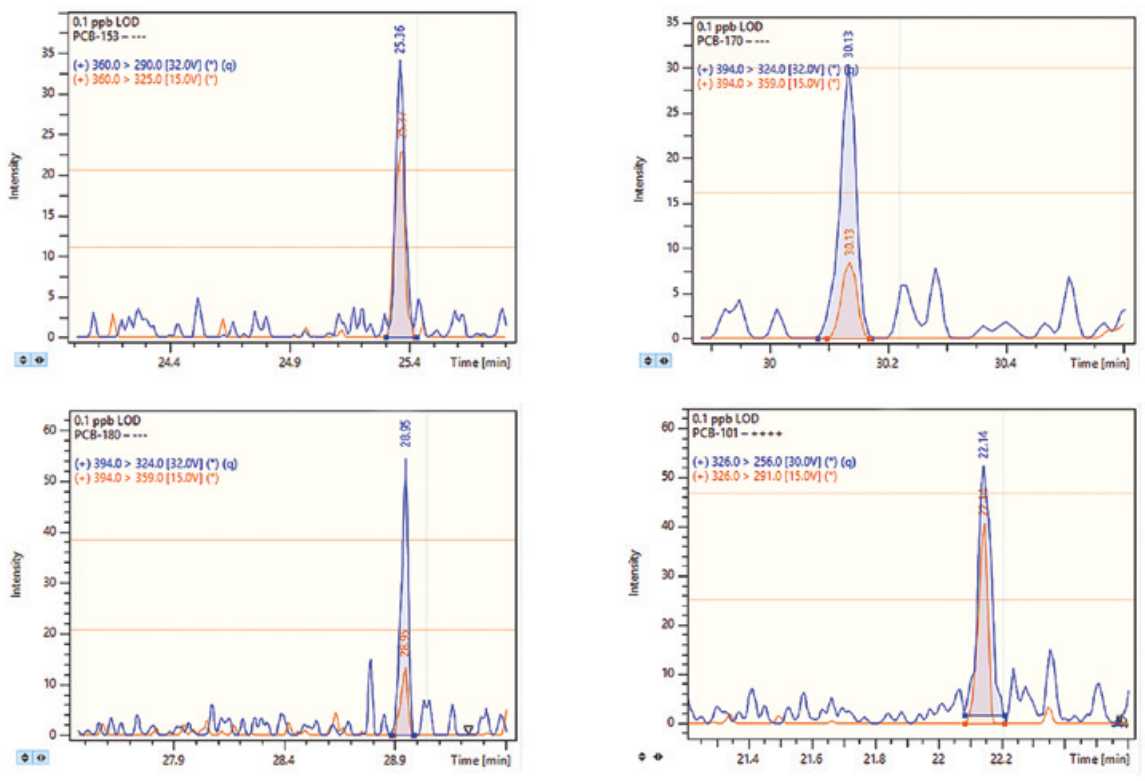

Figure 7: MRM chromatograms for selected PCBs at 0.1 ppb level (100 femtogram on-column)

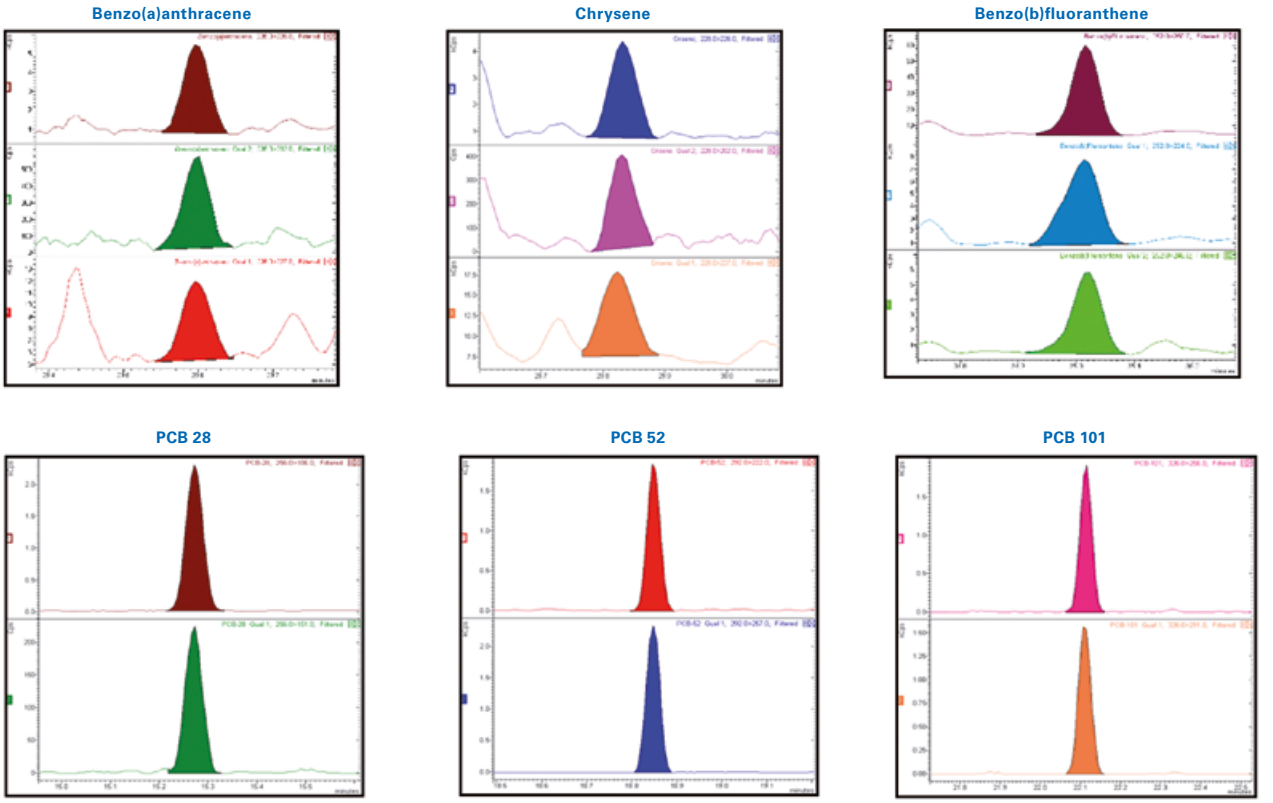

Figure 12: Analysis of a hard clam extract spiked at 0.8 µg/kg with PAHs/PCBs and diluted two fold (400 femtogram on-column). Each time window shows the MRM transitions used for each compound.

Conclusion

A method for the analysis of 16 PAHs and 6 markers NDL-PCBs by GC-MS/MS in bivalve mollusks has been developed according to the European Regulations. The outstanding sensitivity, selectivity and robustness of the Bruker EVOQ GC-TQ Premium MS system enables limits of detection < 0.1 µg/kg while injecting only 1 µL of sample. With this sensitivity, it is possible to work with diluted samples, which may prolong the instrument cleaning and maintenance cycles. The fast 40 m x 0.18 mm GC column demonstrates good resolution for compounds that often co-elute (e.g., PCB28/PCB31, B(b)F/B(k)F, B(a)A/Chrysene/Triphenylene). The run time is also reduced considerably in comparison with 60 m columns. A wide linear calibration range (from 0.5 ppb to 100 ppb) with $R^2 > 0.99$ and RSD < 15% was obtained for all the analyzed compounds. The remarkable reproducibility and performance of the Bruker EVOQ™ GC-TQ MS produced RSD (%) lower than 4% at the limit of quantitation for all the compounds analyzed in the seafood samples. This method has been validated for routine 24/7 operation, if required.

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