Determination of Chemical Contaminants in Marine Shellfish using the Agilent 7000 Triple Quadrupole GC/MS System

Application note

Food Safety

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Abstract

A sample preparation method based on a modified QuEChERS extraction has been developed along with a GC/MS/MS method for the determination of selected Organo-chlorine pesticides, Polyaromatic hydrocarbons and Polychlorinated biphenyl congeners. The analytical method meets the detection limit requirements for the organic chemical contaminants in marine shellfish tissue (mussel) stipulated in the United Kingdom's Clean Seas Environmental Monitoring Program.

Agilent Technologies

Introduction

The Clean Seas Environmental Monitoring Program (CSEMP) is an initiative designed to monitor the levels of chemical contamination in the UK's coastal and estuarine areas. The major drivers for this program are

- To meet the mandatory monitoring requirements under Oslo and Paris Convention (OSPAR) Joint Assessment and Monitoring Program (JAMP).
- Compliance with EC Directives.

The EC dangerous substance directive (76/464/EEC) requires the analysis of sediment or biota to determine the trend in the substances discharged. Organic compounds in Shellfish are also monitored to meet some requirements of Shellfish Water Directive (79/923/EEC), the Shellfish Hygiene Directive (91/492/EEC) and as amended by 97/61/EC, and Fisheries Products Directive (91/493/EEC) [1].

The program specifies 16 organo-chlorine compounds (OCPs), 28 polyaromatic hydrocarbons (PAHs) and 7 polychlorinated biphenyl congeners (PCBs). The Limit of Detection (LoD) requirements are 0.1 µg/Kg for OCPs and PCBs, and 0.5 – 1.0 µg/Kg for PAHs.

An extraction method for these organic contaminants in marine shellfish tissue (mussel), based on a modified QuEChERS [2], [3] extraction method, has been developed and the extracts from which were analysed by gas chromatography coupled to a triple quadrupole mass spectrometer (GC-QQQ). The chromatographic method includes a post-column pressure controlled tee which facilitates post-column, post-run backflush in order to remove high boiling matrix components that would otherwise remain in the column between analyses and subsequently cause degradation of chromatographic performance and contamination of the mass spectrometer ion source. The effectiveness of post-column back flush has been demonstrated in a previously published Agilent application note [4].

Experimental

Calibration Standards

Calibration mixtures of native PAHs and isotope labelled PAH internal standards were obtained from SPEX Certiprep and Cambridge Isotopes, respectively. Custom made mixture for OCPs and PCB congeners were procured from LGC Promochem. PCB 155 and isotope labelled OCP internal standards were obtained from QMX and CDN Isotopes.

Sample Preparation

2 g amounts of homogenized mussel tissue samples were extracted using a modified QuEChERS extraction method. The extraction and clean up workflow is shown in Figure 1.

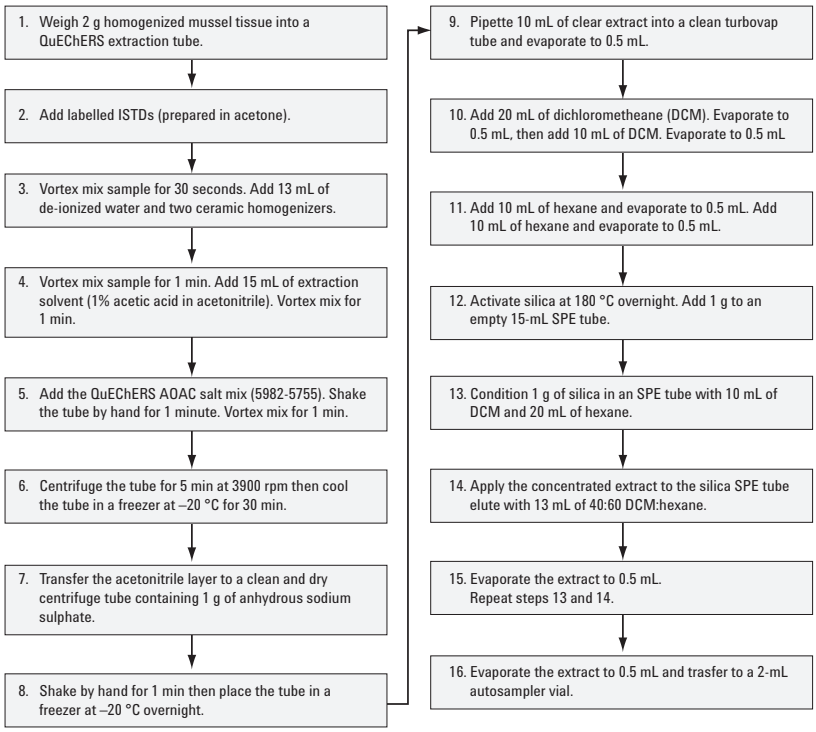

Figure 1. Flow diagram of the sample extraction and clean-up procedure.

GC/MS/MS Analysis

The analyses were performed on an Agilent 7890 GC / 7000 Triple Quadrupole GC/MS system. The 7890 Series GC was configured with a carbon dioxide cooled Multimode Inlet (MMI) and a 15 m × 0.25 mm id, 0.25 µm DB-5MSUI capillary column coupled to a 0.65 mm id × 0.15 mm id, 0.15 µm DB-5MSUI restrictor to the mass spectrometer via a capillary flow pressure controlled tee. A schematic diagram of the GC/MS/MS system configuration is shown in Figure 2.

The analytical column was operated in constant flow mode and the chromatography was retention time locked using PCB 118 as the locking compound at a retention time of 12.370 minutes. The pressure controlled tee was operated in constant pressure mode with helium controlled by a pneumatics control module (PCM).

An Agilent 7693A auto-liquid sampler with was employed and either 1 µL cold splitless injections using a 10 µL syringe (during GC/MS/MS method optimization) or 10 µL solvent vent injections made using a 25 µL syringe (for instrument calibration and sample analyses).

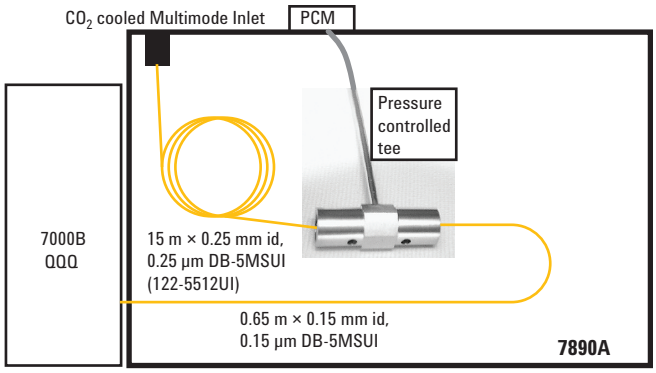

Figure 2. Schematic diagram of GC/MS/MS system configuration.

The GC instrument conditions are listed in Table 1.

The mass spectrometer was operated in electron impact ionization (EI) MS/MS mode using multiple reaction monitoring (MRM) for all the analytes and their associated internal standards. Mass spectrometer operating conditions are given in Table 2 and the full list of analytes with their respective retention times, monitoring ion transitions, collision energies and, dwell times are shown in Table 3.

Table 1. GC Analysis Conditions

Column (1)	15 m × 0.25 mm id, 0.25 µm DB-5MSUI (122-5512UI)
Column (2)	0.65 m × 0.15 mm id, 0.15 µm DB-5MSUI (cut from 165-6626)
Injection mode (1)	1 µL cold pulsed splitless using CO_2 cooled Multimode Inlet (MMI) and a 10 µL syringe
Inlet temperature program	50 °C (0.05 min), 600 °C/min to 325 °C
Inlet pressure pulse	13.0 psig for 0.75 min
Purge Flow to Split Vent	50 mL/min at 1.0 min
Injection port liner	2 mm id, multi-baffled (5190-2296)
Injection mode (2)	10 µL solvent vent using CO_2 cooled Multimode Inlet (MMI) and a 25 µL syringe
Inlet temperature program	40 °C (0.31 min), 600 °C/min to 325 °C
Inlet Vent pressure	5.0 psig
Inlet vent flow	100 mL/min
Inlet vent time	0.31 min
Outlet pressure	0 psig
Injection speed	100 µL/min
Purge Flow to Split Vent	50 mL/min at 1.0 min
Injection port liner	2 mm id, multi-baffled (5190-2296)
Carrier Gas	Helium, constant flow 1.2 mL/min
Oven temp program	50 °C (1) – 20 – 200 °C/min (0) – 10 °C/min – 300 °C (1.5)
RTL Compound	PCB 118, locked at 12.370 min
Pressure controlled tee	G3186B, operated at 2.0 psig constant pressure
Back flush conditions	Inlet pressure 1.0 psig, PCM pressure 60 psig, time 2.0 min

Table 2. Mass Spectrometer Operating Conditions

MS Transfer line temp	325 °C
MS Source	300 °C
MS Quad 1 , 2 temp	150 °C , 150 °C
Collision cell gases	Nitrogen 1.5 mL/min, Helium 2.25 mL/min
MS1 / MS2 Resolution	Wide/wide
MRM settings	See Table 3
Electron energy	-70 eV
Ionization mode	Electron impact (EI)
EI Autotune	Gain normalized
Gain factor	5

Table 3. MS/MS Settings for OCPs, PAHs, PCB Congeners and Labelled Internal Standards

TS	Time	Analyte	RT (min)	Precursor	Product	Dwell (ms)	CE(V)	Precursor	Product	Dwell (ms)	CE(V)
1	4.0	d3-135-TCB	5.050	182.9	147.9	25	35	182.9	110.9	25	35
		135-TCB	5.068	179.9	144.9	25	35	179.9	108.9	25	35
		d8-Napthalene	5.479	136.0	108.0	25	25				
		Naphthalene	5.504	128.0	102.0	25	22	128.0	127.0	25	20
		HCBD	5.658	224.9	189.9	25	22	224.9	187.9	25	22
2	6.8	d8-Acenapthylene	7.308	160.0	132.0	25	30	160.0	108.0	25	30
		Acenaphthylene	7.321	152.0	151.0	25	40	152.0	150.0	25	40
		d10-Acenapthene	7.494	164.0	162.0	25	30	164.0	160.0	25	30
		Acenaphthene	7.525	154.0	152.0	25	40	153.0	152.0	25	40
3	7.8	d10-Fluorene	8.099	176.0	174.0	15	30				
		Fluorene	8.131	166.0	165.0	15	30				
		d6-HCH - alpha	8.699	224.0	187.0	15	15	224.0	150.0	15	15
		HCH - alpha	8.730	181.0	145.0	15	15	181.0	109.0	15	30
		HCB	8.770	283.9	248.8	15	25	283.9	213.9	15	35
		HCH- beta	8.990	181.0	145.0	15	15	181.0	109.0	15	30
		d6-HCH- gamma	9.077	224.0	187.0	15	15	224.0	150.0	15	15
		HCH - gamma	9.107	218.8	183.0	15	5	181.0	109.0	15	30
		Dibenzothiophene	9.110	184.0	152.0	15	40	184.0	139.0	15	40
		d10-Phenanthrene	9.274	188.0	184.0	15	40	188.0	160.0	15	40
		Phenanthrene	9.299	178.0	176.0	15	34				
		Anthracene	9.367	178.0	176.0	15	34				
		HCH - delta	9.428	181.0	145.0	15	15	181.0	109.0	15	30
4	9.6	PCB 28	9.820	256.0	186.0	20	26	258.0	186.0	20	26
		PCB 52	10.250	289.9	220.0	20	28	291.9	222.0	20	28
		Aldrin	10.480	298.0	263.0	20	8	263.0	191.0	20	30
		Isodrin	10.880	262.9	193.0	20	35	262.9	191.0	20	35
5	11.0	d10-Fluoranthene	11.103	212.0	210.0	15	45	212.0	208.0	15	45
		Fluoranthene	11.128	202.0	201.0	15	30	202.0	200.0	15	50
		PCB 155	11.280	357.8	287.9	15	28	359.8	289.9	15	28
		op-DDE	11.375	248.0	176.0	15	30	246.0	211.0	15	20
		PCB 101	11.437	323.9	253.9	15	28	325.9	255.9	15	28
		d10-Pyrene	11.486	212.0	210.0	15	45	212.0	208.0	15	45
		Pyrene	11.512	202.0	201.0	15	30	202.0	200.0	15	45

Table 3. MS/MS Settings for OCPs, PAHs, PCB Congeners and Labelled Internal Standards (Continued)

TS	Time	Analyte	RT (min)	Precursor	Product	Dwell (ms)	CE(V)	Precursor	Product	Dwell (ms)	CE(V)
5		pp-DDE	11.857	248.0	176.0	15	30	246.0	211.0	15	20
		C13-Dieldrin	11.933	269.8	200.0	15	40	269.8	198.0	15	40
		Dieldrin	11.940	262.8	193.0	15	30	262.8	191.0	15	30
		op-DDD	11.956	237.0	165.0	15	20	235.0	200.0	15	8
6	12.15	Endrin	12.265	281.0	245.0	25	20	263.0	193.0	25	35
		PCB 118 (RTL compound)	12.370	323.9	253.9	25	28	325.9	255.9	25	28
		pp-DDD	12.500	237.0	165.0	25	20	235.0	199.1	25	8
		op-DDT	12.543	237.0	165.0	25	20	235.0	199.1	25	20
		PCB 153	12.698	357.8	287.9	25	28	359.8	289.9	25	28
		C13-pp-DDT	13.091	247.0	177.0	25	20	247.0	211.0	25	20
		pp-DDT	13.099	237.0	165.0	25	20	235.0	199.1	25	20
		PCB 138	13.112	357.8	287.9	25	28	359.8	289.9	25	28
7	13.5	Benzo[a]anthracene	13.897	228.0	226.0	40	38				
		d12-Chrysene	13.915	240.0	236.0	40	35				
		Chrysene / Triphenylene	13.965	228.0	226.0	40	38				
		PCB 180	14.175	393.8	323.9	40	30	395.8	325.9	40	30
8	15.0	Benzo[b+j]fluoranthene	16.06	252.0	250.0	75	42	250.0	248.0	75	40
		d12-Benzo[k]fluoranthene	16.084	264.0	260.0	75	40				
		Benzo[k]fluoranthene	16.116	252.0	250.0	75	42	250.0	248.0	75	40
		Benzo[e]pyrene	16.561	252.0	250.0	75	42	250.0	248.0	75	40
		d12-Benzo[a]pyrene	16.616	264.0	260.0	75	40				
		Benzo[a]pyrene	16.654	252.0	250.0	75	42	250.0	248.0	75	40
		Perylene	16.814	252.0	250.0	75	42	250.0	248.0	75	40
9	18.0	d12-Indeno[123-cd]pyrene	18.600	288.0	284.0	75	50				
		Indeno(123-cd)pyrene	18.631	276.0	274.0	75	42				
		d14-Dibenz[a,h]anthracene	18.662	292.0	288.0	75	50				
		Dibenz[a,h]anthracene	18.712	278.0	276.0	75	38				
		d12-Benzo[g,h,i]perylene	19.020	288.0	284.0	75	45				
		Benzo[ghi]perylene	19.064	276.0	274.0	75	38				

Results and Discussion

Chromatography

The total ion chromatogram (TIC) for all MRM transitions of all analytes is shown in Figure 3. For additional clarity, labelled TIC MRM chromatograms of the OCPs, PAHs and PCB congeners are shown in Figures 4, 5, and 6, respectively.

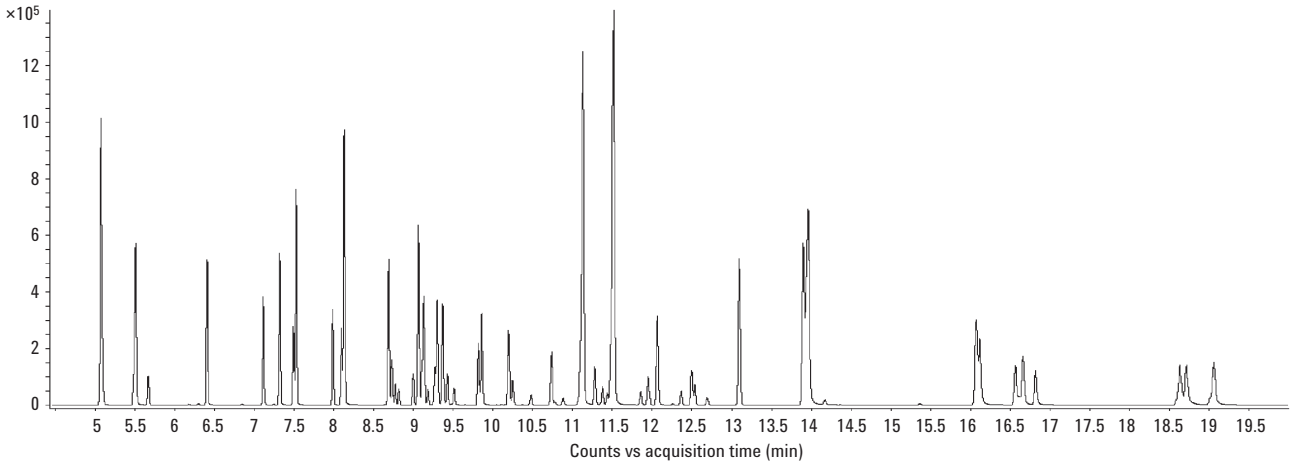

Figure 3. TIC MRM Chromatogram for a calibration standard.

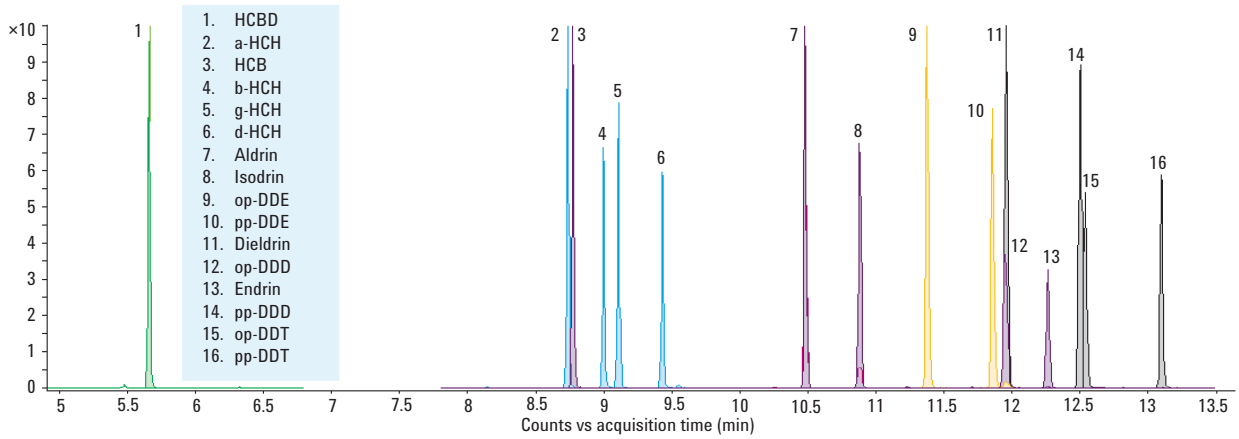

Figure 4. TIC MRM Chromatogram for OCP analytes.

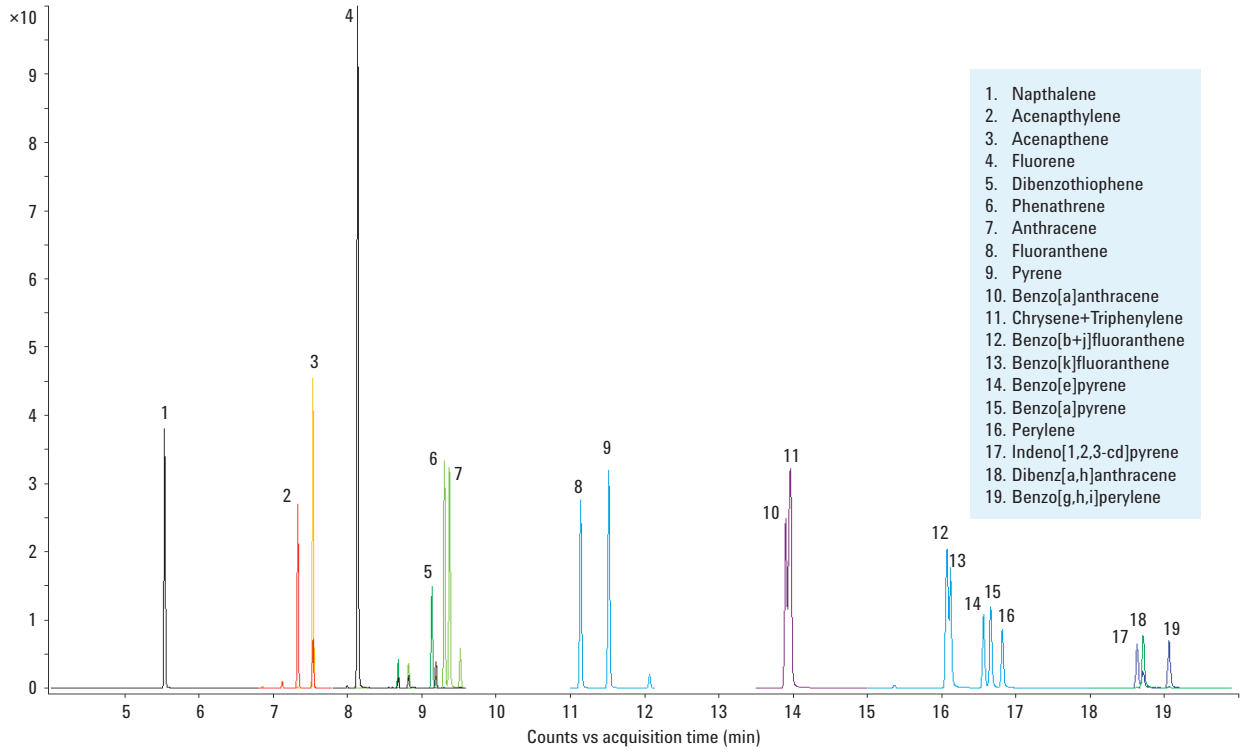

Figure 5. TIC MRM Chromatogram for PAH analytes.

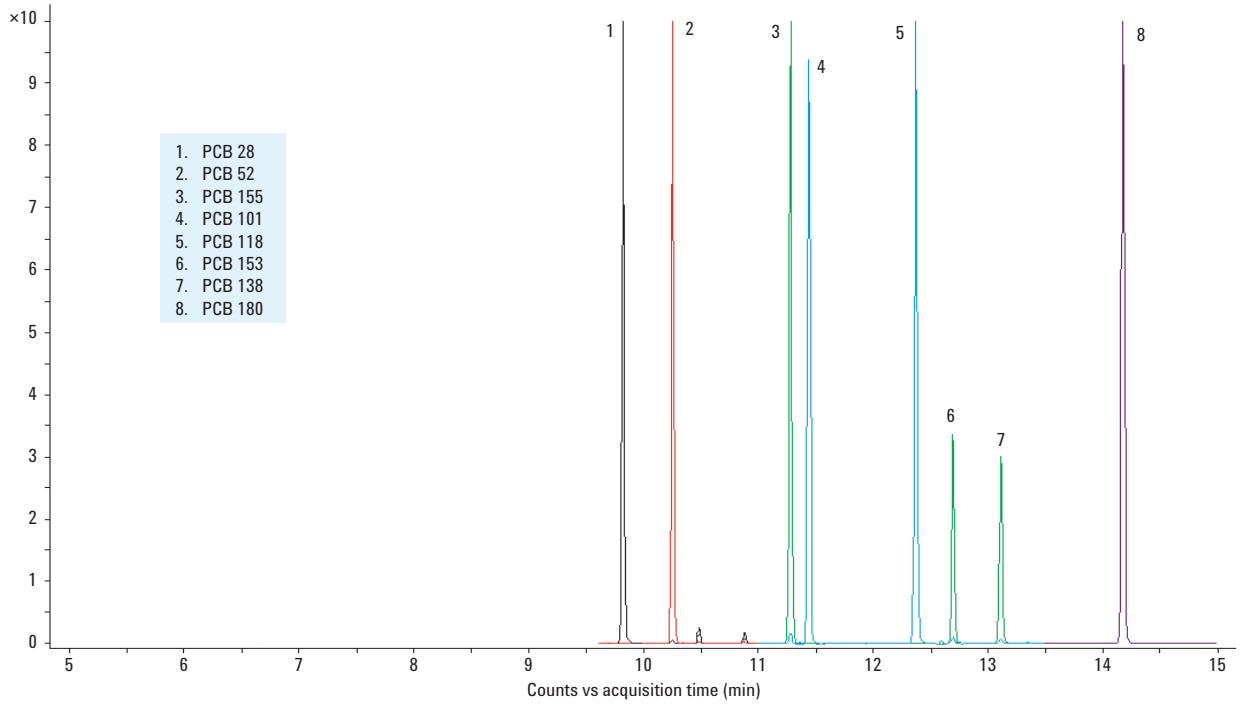

Figure 6. TIC MRM Chromatogram for PCB congeners.

Analyte calibration curves

The GC-MS/MS system was calibrated using a five-point internal standard (ISTD) calibration. The calibration standards for target analytes were prepared in hexane at concentrations of 0.4, 2.0, 8.0, 80.0, and 200.0 pg/µL. All ISTDs were added at 80.0 pg/µL. Calibration curves were created using 10 µL solvent vent mode injections. The calibration curves for all analytes gave correlation coefficients greater than 0.999. Table 4 shows curve fit types and correlation coefficient values.

Table 4. Curve Fits and Correlation Coefficients for ISTD Calibration Curves

Analyte	Curve fit	R^2
HCBD	Quadratic	0.9994
a-HCH	Linear	0.9996
HCB	Linear	0.9998
b-HCH	Linear	0.9995
g-HCH	Linear	0.9999
d-HCH	Linear	0.9991
Aldrin	Quadratic	0.9999
Isodrin	Quadratic	0.9999
op-DDE	Linear	0.9998
p,p-DDE	Linear	0.9993
Dieldrin	Quadratic	0.9992
op-DDD	Quadratic	0.9999
Endrin	Linear	0.9997
pp-DDD	Linear	0.9997
o,p-DDT	Linear	0.9992
p,p-DDT	Linear	0.9995
Napthalene	Linear	0.9997
Acenapthylene	Linear	0.9997
Acenapthene	Linear	0.9999
Fluorene	Linear	0.9997
Dibenzothiophene	Quadratic	0.9999
Phenanthrene	Linear	0.9999
Anthracene	Linear	0.9997
Fluoranthene	Linear	0.9992
Pyrene	Linear	0.9996
Benzo[a]anthracene	Linear	0.9998
Chrysene+Triphenylene	Quadratic	0.9999
Benzo[b+j]fluoranthene	Linear	0.9998
Benzo[k]fluoranthene	Quadratic	0.9997
Benzo[e]pyrene	Linear	0.9996
Benzo[a]pyrene	Linear	0.9998
Perylene	Linear	0.9999
Indeno[123-cd]pyrene	Quadratic	0.9996
Dibenz[a,h]anthracene	Quadratic	0.9999
Benzo[g,h,i]perylene	Quadratic	0.9997
PCB 28	Linear	0.9998
PCB 52	Linear	0.9998
PCB 101	Linear	0.9999
PCB 118	Linear	0.9996
PCB 153	Linear	0.9998
PCB 138	Linear	0.9998
PCB 180	Linear	0.9994

Example calibration graphs over the range of interest for g-HCH, PCB 118 and Benzo[a]pyrene are shown in Figure 7.

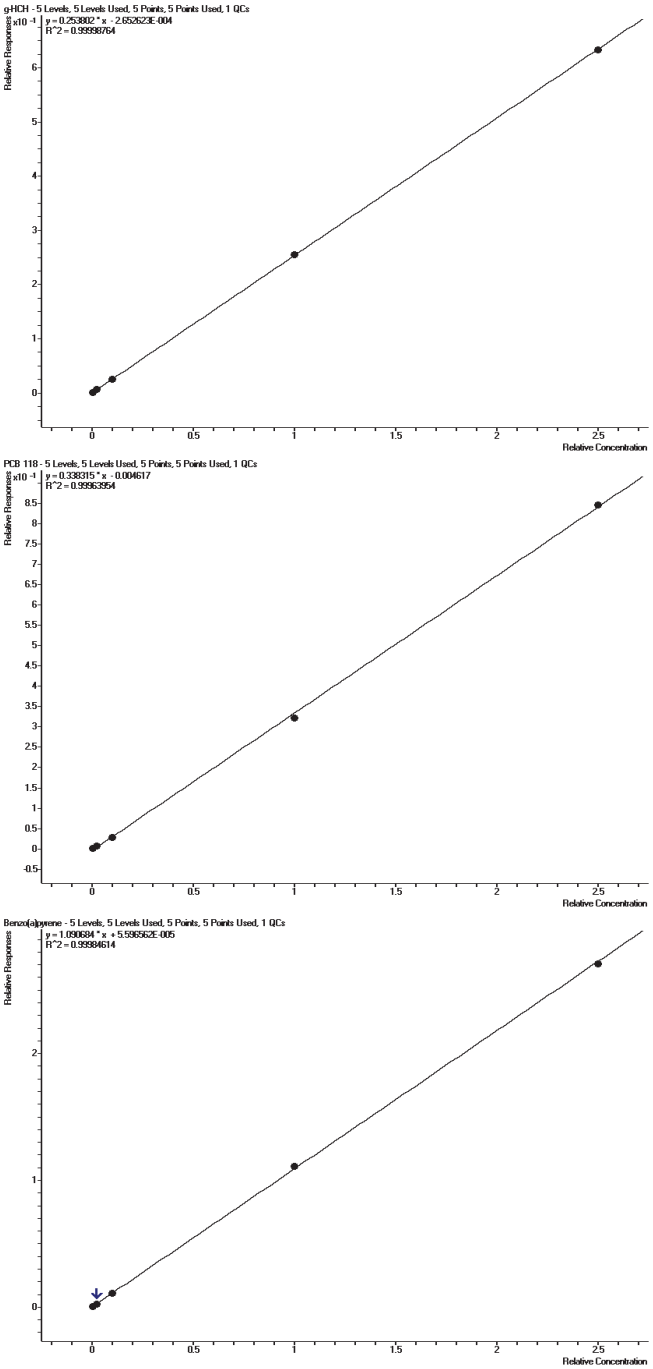

Figure 7. Five point ISTD calibration curves for g-HCH (Top), PCB 118 (middle) and Benzo[a]pyrene (bottom).

Recovery of target analytes and Quantitative Reproducibility

Five sample aliquots (2g) from homogenized mussel tissue were weighed into QuEChERS extraction tubes. The samples were spiked with an acetone solution of target analytes at a level equivalent to 4 µg/Kg (8 ng/2g sample) and internal standards. The extraction tubes were then vortex mixed for 1 minute and the samples were extracted using the procedure given in Figure 1.

Relative percent standard deviations (RSD%) and Spike Recoveries (Recovery %) were calculated for each of the target analytes as given below;

$$RSD\% = \frac{SD}{Mean} \times 100$$

$$\text{Recovery } \% = \frac{Mean(spiked)}{[Mean(unspiked) + Spike]} \times 100$$

where SD is the Standard Deviation.

The list of target analytes, grouped by chemical class (OCPs, PAHs and PCBs) plus their associated internal standards, quantitative reproducibility values and percentage recovery values are shown in Table 5. Percent recovery values for the OCPs, PAHs and PCB congeners from spiked mussel tissue are also shown graphically in Figure 8, (a), (b) and (c), respectively.

Table 5. Target Analytes, Their Associated ISTDs, RSD% Values for Quantitative Reproducibility and Recovery% Values

Analyte	ISTD	RSD% [n=5]	Recovery%
HCBD	d3-135-TCB	11.1	85.4
a-HCH	d6-g-HCH	3.9	115.1
HCB	d6-a-HCH	13.3	92.0
b-HCH	PCB-155	7.0	116.8
g-HCH	d6-g-HCH	2.3	114.1
d-HCH	PCB-155	6.7	123.9
Aldrin	PCB-155	15.8	108.9
Isodrin	PCB-155	13.9	108.7
op-DDE	PCB-155	3.5	120.4
p,p-DDE	PCB-155	4.8	121.5
Dieldrin	^{13}C-Dieldrin	4.0	93.4
op-DDD	PCB-155	4.0	119.9
Endrin	^{13}C-Dieldrin	7.7	112.7
pp-DDD	^{13}C-pp-DDT	6.1	101.6
o,p-DDT	^{13}C-pp-DDT	3.5	104.1
p,p-DDT	^{13}C-pp-DDT	1.1	100.0
Napthalene	d8-Napthalene	3.7	107.7
Acenapthylene	d8-Acenapthylene	7.4	98.5
Acenapthene	d10-Acenapthene	5.0	102.5
Fluorene	d10-Fluorene	8.4	100.9
Dibenzothiophene	d10-Fluorene	8.5	105.6
Phenanthrene	d10-Phenanthrene	7.8	102.6
Anthracene	d10-Phenanthrene	5.6	100.2
Fluoranthene	d10-Fluoranthene	0.9	101.0
Pyrene	d10-Pyrene	7.0	92.3
Benzo[a]anthracene	d12-Chrysene	4.1	103.5
Chrysene+Triphenylene	d12-Chrysene	1.1	104.5
Benzo[b+j]fluoranthene	d12-Benzo[k]fluoranthene	24.0	107.7
Benzo[k]fluoranthene	d12-Benzo[k]fluoranthene	5.4	104.1
Benzo[e]pyrene	d12-Benzo[a]pyrene	1.6	105.0
Benzo[a]pyrene	d12-Benzo[a]pyrene	3.3	102.9
Perylene	d12-Benzo[a]pyrene	1.1	106.4
Indeno[123-cd]pyrene	d14-Dibenz[a,h]anthracene	2.8	94.9
Dibenz[a,h]anthracene	d14-Dibenz[a,h]anthracene	2.4	103.0
Benzo[g,h,i]perylene	d14-Dibenz[a,h]anthracene	4.8	103.1
PCB 28	PCB-155	3.9	105.5
PCB 52	PCB-155	3.0	105.8
PCB 101	PCB-155	3.5	112.3
PCB 118	PCB-155	6.1	107.0
PCB 153	PCB-155	3.6	107.6
PCB 138	PCB-155	4.5	109.9
PCB 180	PCB-155	4.8	110.1

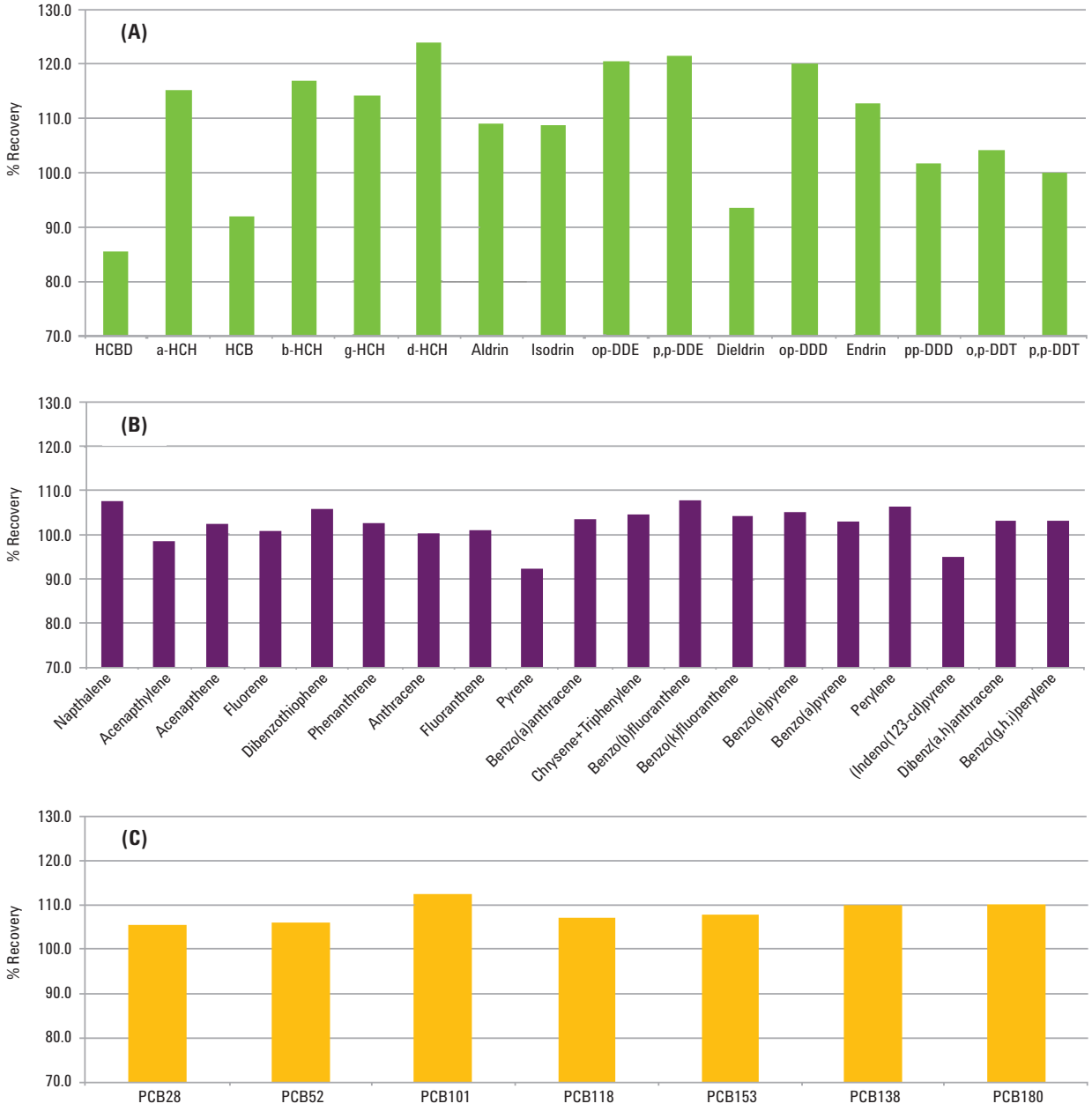

Figure 8. Graphical representation of analyte percent recovery values for (a) OCPs, (b) PAHs and (c) PCB congeners in spiked mussel tissue.

Sample Analysis

Marine mussel samples were sourced from local commercial shell fish suppliers, homogenized, extracted and analysed using the sample preparation and GC/MS/MS conditions as described. MRM chromatograms for the incurred HCH isomers quantified in a mussel sample are shown in Figure 9, the incurred Fluoranthene and Pyrene PAHs in Figure 10 and, the incurred PCB 180 congener in Figure 11, respectively.

Conclusion

A sample preparation method based on a modified QuEChERS extraction and clean up regime has been developed and applied to the extraction of OCPs, PAHs and PCB congeners from marine mussel tissue. The quantitative GC/MS/MS method demonstrated good reproducibility and recoveries for all analytes were in the range of 85.4% – 123.9% in spiked mussel tissue.

The Agilent 7000 Triple Quadrupole GC/MS system provided reproducible and sensitive detection of OCPs, PAHs and PCB congeners in mussel tissue down to concentration levels of 0.1 µg/Kg. The performance of the extraction/clean-up and analysis by GC/MS/MS meets the requirements of the CSEMP legislation.

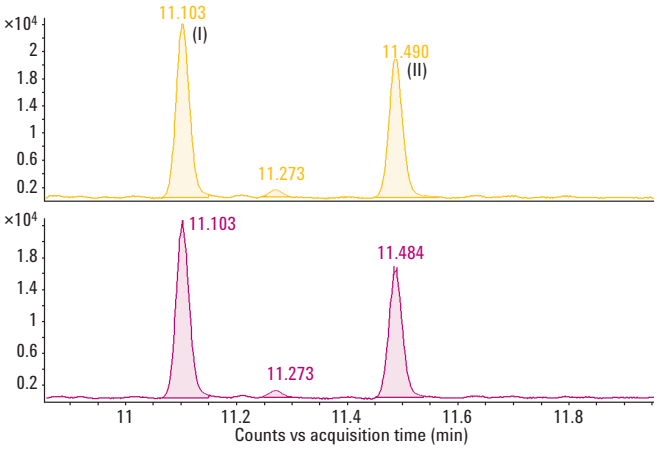

Figure 10. MRM Chromatograms for (i) incurred Fluoranthene and (ii) incurred Pyrene in mussel sample, Concentrations 8.64 and 5.83 µg/Kg, respectively.

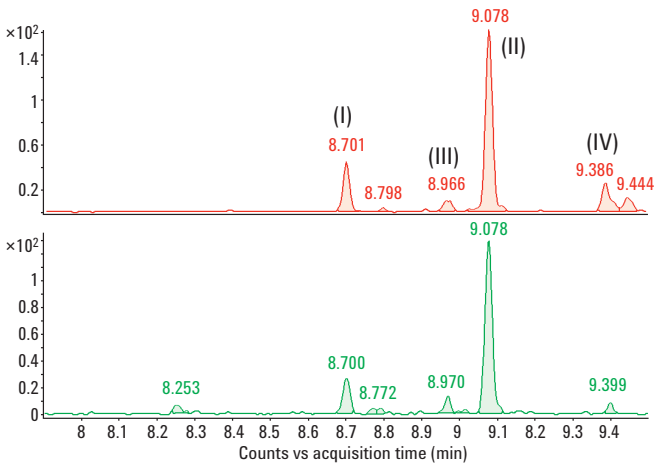

Figure 9. MRM Chromatograms for (i) incurred a-HCH and (ii) incurred g-HCH in mussel sample, Concentrations 0.06 and 0.30 µg/Kg, respectively. Peaks (iii) and (iv) are traces of incurred b-HCH and d-HCH, respectively.

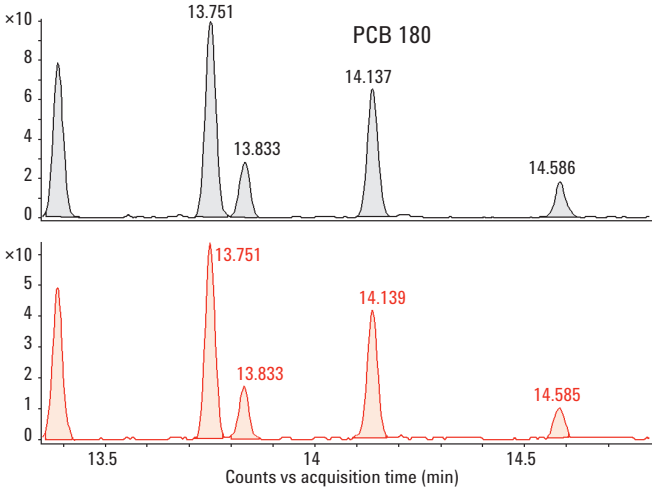

Figure 11. MRM Chromatograms for incurred PCB 180 in mussel sample, Concentration 0.14 µg/Kg.

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