Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood Using GC/MS

UCT Part Numbers:

ECQUUS2-MP (4 g of muffled anh. $MgSO_4$ and 2 g of NaCl)

ECPAHFR50CT (50 mL centrifuge tubes, PAHs removed)

EUSILMSSM26 (6 mL, 1g silica gel cartridge with 200 mg of muffled anhydrous sodium sulfate on top)

Background

This method is used for the determination of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in fish and seafood--oyster, shrimp, and mussel. Benzo[*a*]pyrene is the main analyte of interest. GC/MS instrumentation is used for analysis.

PAH Analytes Covered in this Method

PAH	Abbreviation	PAH	Abbreviation
Anthracene	Ant	**Indeno[1,2,3-*cd*]pyrene**	IcdP
Benz[*a*]anthracene	BaA	**Naphthalene**	Naph
Benzo[*a*] pyrene	BaP	**Phenanthrene**	Phe
Benzo[*b*]fluoranthene	BpF	**Pyrene**	Pyr
Benzo[*k*]fluoranthene	BkF	**3-Methylchrysene**	3-MC
Benzo[*g,h,i*]perylene	BghiP	**1-Methylnaphthalene**	1-MN
Chrysene	Chr	**1-Methylphenanthrene**	1-MP
Dibenz[*a,h*]anthracene	DBahA	**2,6 Dimethylnaphthalene**	2,6-DMN
Fluoranthene	Flt	**1,7-Dimethylphenanthrene**	1,7-DMP
Fluorene	Fln		

Procedure

1) **Extraction**

   a) To the 50-mL polypropylene centrifuge tube add 10 ± 0.1 g of homogenized seafood sample

   b) Add 50 µL of 1 µg/mL ^{13}C-PAHs solution

   c) Vortex sample for 15 sec and then equilibrate for 15 min

   d) Add 5 mL of reagent water and 10 mL of ethyl acetate (EtOAC)

   e) Shake for 1 min

2) Partition

   a) Add the contents of pouch **ECQUUS2-MP.** Tightly seal the tube to ensure that salts do not get into the screw threads

   b) Shake for 1 min

   c) Centrifuge at > 1,500 rcf for 10 min

   d) Remove 5-mL aliquot of the upper ethyl acetate layer, add 50 µL of isooctane as a keeper

   e) Evaporate all ethyl acetate until only isooctane and co-extracted sample fat remain

   f) Reconstitute with 1 mL of hexane

3) Clean-Up

   a. Condition a silica SPE column **EUSILMSSM26** (**Note 1**) (1 g of silica gel with approx. 0.2 g of muffled anh. sodium sulfate on the top) with 6 mL of hexane:dichloromethane (3:1 v/v) and 4 mL of hexane

   b) Apply the reconstituted extract to the silica SPE cartridge (**Note 2**)

   c) Elute with 10 mL of hexane:dichloromethane (3:1 v/v) and collect the eluent

   d) Add 0.5 mL isooctane to the eluent as a keeper and evaporate to 0.5 mL to remove hexane and dichloromethane from the final extract

   e) Transfer the final extract into an autosampler vial for GC/MS analysis

Notes:

1. The fat capacity of the 1-g silica gel SPE column is approx. 0.1 g. If the ethyl acetate extract aliquot contains more than 0.1 g of fat, use a smaller aliquot to avoid sample breakthrough

2. Ethyl acetate should not be present in the extract applied to the silica cartridge as it affects extract polarity and potential retention of fat and analytes on the silica gel.

GC Conditions for the Analysis of PAHs

Column	BPX-50 (30 m x 0.25 mm i.d. x 0.25 µm film thickness)
Oven Temperature Program	80°C (hold for 4.3 min), 30°C/min to 220°C, 2°C/min to 240°C, and 10°C/min to 360°C (hold for 17 min)
He Flow Rate	1.3 mL/min (hold for 19 min), then 50 mL/min to 2 mL/min (hold for 16 min)
Injection Technique	PTV solvent vent
Injection Volume	1 x 8 µL
Vent Time	2.3 min
Vent Flow	50 mL/min
Vent Pressure	50 psi
Inlet Temperature Program	50°C (hold for 2.3 min), then 400°C/min. to 300°C

MS Conditions

Any GC-MS instrument (single quadrupole, triple quadrupole, time-of-flight or ion trap) with electron ionization (EI) may be used

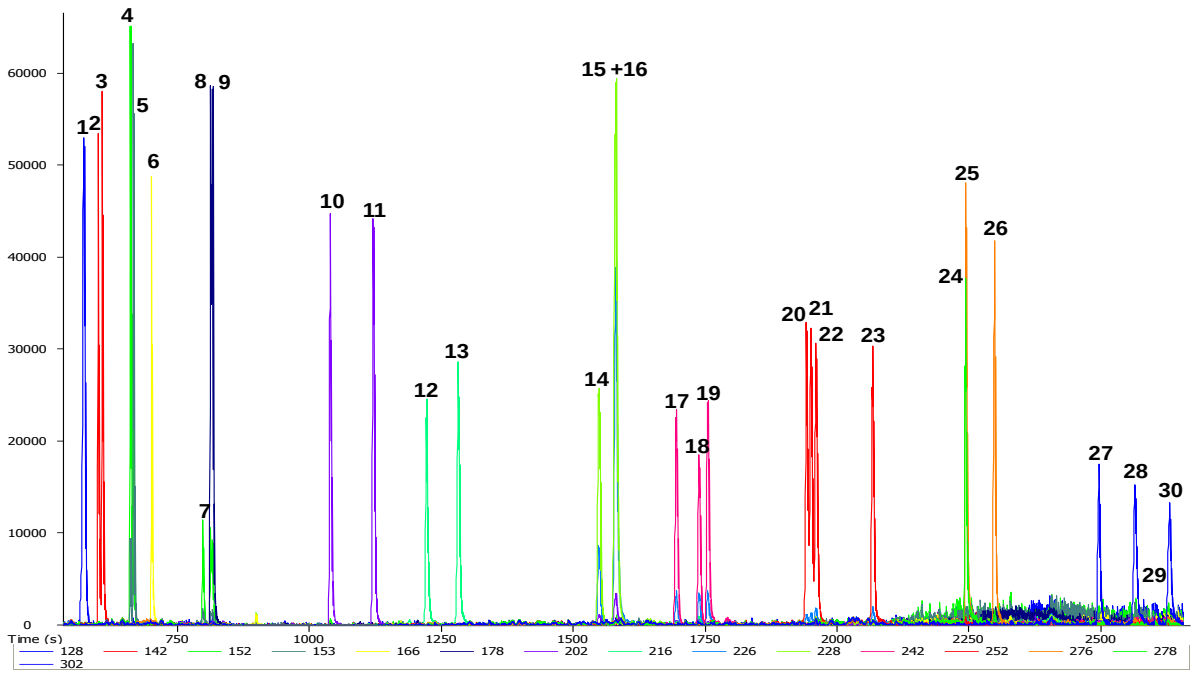
MS Ions (*m/z*) for Quantification and Identification of Target PAHs for Single-stage MS Instruments

Analyte PAH's	Abbreviation	Confirmation Ions *(m/z)*	Quantification Ions *(m/z)*
Anthracene	Ant	177	178
Benz[*a*]anthracene	BaA	226	228
Benzo[*a*] pyrene	BaP	253	252
Benzo[*b*]fluoranthene	BpF	253	252
Benzo[*k*]fluoranthene	BkF	253	252
Benzo[*g,h,i*]perylene	BghiP	277	276
Chrysene	Chr	226	228
Dibenz[*a,h*]anthracene	DBahA	276	278
Fluoranthene	Flt	200	202
Fluorene	Fln	165	166
Indeno[1,2,3-cd]pyrene	IcdP	277	276
Naphthalene	Naph	127	128
Phenanthrene	Phe	177	178
Pyrene	Pyr	200	202
3-Methylchrysene	3-MC	241	242
1-Methylnaphthalene	1-MN	115	142
1-Methylphenanthrene	1-MP	189	192
2,6 Dimethylnaphthalene	2,6-DMN	141	192156
1,7-Dimethylphenanthrene	1,7-DMP	191	156206

MS Ions (*m/z*) for Quantification and Identification of Target ^{13}C-PAHs for Single-stage MS Instruments

Analyte PAH's	Abbreviation	Confirmation Ions *(m/z)*	Quantification Ions *(m/z)*
Naphthalene ($^{13}C_6$)	Naph- $^{13}C_6$	133	134
Fluorene ($^{13}C_6$)	Fln- $^{13}C_6$	171	172
Phenanthrene ($^{13}C_6$)	Phe- $^{13}C_6$	183	184
Anthracene ($^{13}C_6$)	Ant- $^{13}C_6$	183	184
Fluoranthene ($^{13}C_6$)	Flt-$^{13}C_6$	205	208
Pyrene ($^{13}C_6$)	Pyr-$^{13}C_6$	208	205
Benz[*a*]anthracene ($^{13}C_6$)	BaA- $^{13}C_6$	232	234
Chrysene ($^{13}C_6$)	Chr- $^{13}C_6$	232	234
Benzo[*b*]fluoranthene ($^{13}C_6$)	BbF- $^{13}C_6$	259	258
Benzo[*k*]fluoranthene ($^{13}C_6$)	BkF- $^{13}C_6$	259	258
Benzo[*a*]pyrene ($^{13}C_4$)	BaP- $^{13}C_4$	257	256
Indeno[1,2,3-*cd*]pyrene ($^{13}C_6$)	IcdP- $^{13}C_6$	283	282
Dibenz[*a,h*]anthracene ($^{13}C_6$)	DBahA- $^{13}C_6$	282	284
Benzo[*g,h,i*]perylene ($^{13}C_{12}$)	BghiP- $^{13}C_{12}$	289	288

An example chromatogram of a GC separation of PAHs and their alkyl homologues in a standard solution mixture at 25 ng/mL in isooctane



1 – naphthalene, 2 – 2-methylnaphthalene, 3 – 1-methylnaphthalene, 4 – acenaphtylene,
5 – acenaphthene, 6 – fluorene, 7 – dibenzothiophene, 8 – phenanthrene, 9 – anthracene,
10 – fluoranthene, 11 – pyrene, 12 – 1-methylpyrene, 13 – benzo[*c*]fluorene,
14 – benz[*a*]anthracene, 15 – cyclopenta[*c,d*]pyrene, 16 – chrysene, 17 – 1-methylchrysene,
18 – 5-methylchrysene, 19 – 3-methylchrysene, 20 – benzo[*b*]fluoranthene,
21 – benzo[*k*]fluoranthene, 22 – benzo[*j*]fluoranthene, 23 – benzo[*a*]pyrene,
24 – dibenz[*a,h*]anthracene, 25 – indeno[1,2,3-*cd*]pyrene, 26 – benzo[*g,h,i*]perylene,
27 – dibenzo[*a,l*]pyrene, 28 – dibenzo[*a,e*]pyrene, 29 – dibenzo[*a,h*]pyrene,
30 – dibenzo[*a,i*]pyrene

*The analyst should refer to Katerina Mastovska, Wendy R. Sorenson, Covance Laboratories Inc
Jana Hajslova, Institute of Chemical Technology, Prague "Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Seafood using Gas Chromatography-Mass Spectrometry: A Collaborative Study"

References

Lucie Drabova, Kamila Kalachova, Jana Pulkrabova, Tomas Cajka, Vladimir Kocourek and Jana Hajslova. "Rapid Method for Simultaneous Determination of Polycyclic Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs) and Polybrominated Diphenyl Ethers (PBDEs) in Fish and Sea Food Using GC-TOFM," ICT document, Prague, Czech Republic, 2010.

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255 • www.unitedchem.com • Email: methods@unitedchem.com