EPA Method 8310: Solid Phase Extraction of Polynuclear Aromatic Hydrocarbons (PAHs) in Water

UCT Part Numbers

ECUNIPAH
ENVIRO-CLEAN® Unendcapped C18
2000mg/Universal Cartridge

ECUNIMSS
ENVIRO-CLEAN®
Muffled sodium sulfate
20g/Universal Cartridge

ECUCTADP
ENVIRO-CLEAN®
Glass Cartridge Adaptor

ECUNIBHD
ENVIRO-CLEAN®
White Bottle Holder

ECUCTVAC6
ENVIRO-CLEAN®
6 Station Vacuum Manifold Stainless Steel System

Summary:

Polynuclear aromatic hydrocarbons (PAHs) are consisted of a large group of organic compounds with two or more fused aromatic rings. Hundreds of different PAHs may be generated from incomplete combustion or pyrolysis of organic materials, smoked or grilled food, vehicle exhaust and cigarettes [1]. PAHs may also be emitted from natural activities, such as forest fire, volcanoes and hydrothermal processes [2]. Some PAHs undergo metabolic activation to diol epoxides which may bind to DNA, resulting with errors in DNA replication and mutations that start the carcinogenic process in mammals [3]. EPA method 8310 determines 16 PAHs including acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, chrysene, dibenzo[a,h]anthracene, fluoranthene, fluorene, indeno[1,2,3-c,d]pyrene, naphthalene, phenanthrene and pyrene [4], among which benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, and indeno[1,2,3-c,d]pyrene are probable human carcinogens. EPA method 8310 is a performance based hazardous waste test method (SW-846) regulated under the Resource Conservation and Recovery Act (RCRA), which allows analysts modify sampling and analytical approaches flexibly to meet the measurement requirements [5].

This application note outlines a solid phase extraction (SPE) procedure using C18 SPE universal cartridges to extract 16 PAHs in water. The target PAHs are retained on the C18 sorbent, and are later eluted with acetone and dichloromethane (DCM). Sodium sulfate drying cartridges are used to remove any residual water in the SPE eluates. The dried eluates are concentrated, exchanged to acetonitrile, and analyzed by HPLC with ultraviolet (UV) or fluorescence detectors (FLD).

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SPE Procedure:

1. **Cartridge Conditioning**
   a) Place the glass adapters (**ECUCTADP**) on the 6-station manifold (**ECUCTVAC6**), attach the SPE cartridges (**ECUNIPAH**) to the glass adapters, then the bottle holders (**ECUNIBHD**) to the top of the SPE cartridges.
   b) Add 10 mL DCM to the SPE cartridges by rinsing the bottle holders and the SPE cartridges, let DCM wet and soak the SPE sorbent for 1 min before drawing to waste. Leave full vacuum on for 1 min.
   c) Add 10 mL methanol (MeOH) to the SPE cartridges, let MeOH wet and soak the SPE sorbent for 2 min, then pull MeOH through (or by gravity) leaving a thin layer above the frit.
   d) Add 20 mL reagent water to the SPE cartridges, pull water through leaving a water layer of about 1 cm above the frit.

2. **Sample Extraction**
   a) Dechlorinate the samples with 50 mg/L sodium sulfite if free chlorine present.
   b) Add surrogate and target analyte spiking solutions prepared in water miscible solvents, such as MeOH.
   c) Add 5 mL MeOH to the sample bottles and mix well.
   d) Load the sample bottles to the bottle holders, adjust vacuum for a fast dropwise flow (about 30 mL/min).

3. **Cartridge Drying**
   a) Dry the SPE cartridges under full vacuum for 10 min. Move the cartridges from the manifold during drying, and shake the cartridges to remove excess water from the bottom of the SPE cartridges.

4. **Analyte Elution**
   a) Insert glass vials (40 or 50 mL) into the SPE manifold to collect the SPE eluates.
   b) Rinse the sample bottles with 5 mL of acetone, add the rinsates to the SPE cartridges, let elution solvents wet and soak the sorbent for 1 min before drawing slowly to the collection vials. Leave full vacuum on for 1 min.
   c) Repeat the above step with 2 x 10 mL DCM.

5. **Eluate Drying**
   a) Remove the SPE cartridges and bottle holders from the glass adapters, and remove the collection vials from the manifold.
   b) Place the drying cartridges (**ECUNIMSS**) on the glass adapters, rinse with 10 mL of DCM.
   c) Insert new glass vials into the manifold, pass the eluates through the drying cartridges and collect.
   d) Rinse the collection vials with 10 mL DCM, pass the rinsates through and collect.

6. **Eluate Evaporation**
   a) Add 2 mL acetonitrile to the dried extracts and evaporate to 0.7 - 0.9 mL using TurboVap under a gentle stream of nitrogen (7 - 8 psi) at 40°C.
   b) Add internal standard and adjust the final volume to 1 mL using a small amount of acetonitrile.
   c) Transfer the concentrated extracts to 2-mL autosampler vials, and analyze by HPLC-UV or HPLC-FLD.

*Note: UCT's ENVIRO-CLEAN® Universal Cartridges can be used on Horizon SPE-DEX® 4790 Automated Extraction System directly, or on J.T. Baker® BAKERBOND Speedisk™ Expanded Extraction Station with the use of universal cartridge adapters (**ECBMADP**) and bottle holders (**ECUNIBHD**).*

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Results:

Average Recoveries of 4 Laboratory Fortified Blanks (LFB)
Compared to the QC Acceptance Criteria of EPA Method 8310

Compound Name	EPA Method 8310 Recovery QC*	Recovery% of UCT Method	
		LFB at 1 µg/L	LFB at 10 µg/L
Acenaphthene	D - 124	102.2	98.4
Acenaphthylene	D - 139	62.1	96.2
Anthracene	D - 126	92.3	98.3
Benz[a]anthracene	12 - 135	99.6	100.1
Benzo[a]pyrene	D - 128	100.2	89.8
Benzo[b]fluoranthene	6 - 150	87.4	106.7
Benzo[ghi]perylene	D - 116	104.0	89.3
Benzo[k]fluoranthene	D - 159	99.1	85.2
Chrysene	D - 199	123.5	82.3
Dibenz[a,h]anthracene	D - 110	102.5	90.3
Fluoranthene	14 - 123	114.7	101.8
Fluorene	D - 142	102.4	100.8
Indeno[1,2,3-cd]pyrene	D - 116	81.0	101.7
Naphthalene	D - 122	113.4	99.7
Phenanthrene	D - 155	109.1	96.3
Pyrene	D - 140	104.3	99.3

*: adopted from Table 3 in EPA method 8310

Conclusion:

A simple and efficient SPE method has been demonstrated for the extraction of 16 PAHs in water by EPA method 8310, a performance based method allowing for modifications with the QC acceptance criteria met. Excellent recoveries and RSD% (< 10%, n = 4) have been obtained using this SPE method, all parameters have passed the QC limits required by EPA method 8310, offering environmental testing labs a successful alternate extraction method that is more selective, consumes less organic solvents, and yields no emulsion which is a bottleneck when using liquid-liquid extraction for real world samples.

References:

[1] Polycyclic Aromatic Hydrocarbons in Food, Scientific Opinion of the Panel on Contaminants in the Food Chain, EFSA Journal 724 (2008) 1.
[2] D.L. Poster, M.M. Schantz, L.C. Sander, S.A. Wise, Anal. Bioanal. Chem. 386 (2006) 859.
[3] International Agency for Research on Cancer, Polycyclic Aromatic Compounds. Part I. Chemicals, Environment and Experimental Data, IARC Monographs on the Evaluation of Carcinogen Risk of Chemicals to Humans, 32 (1983) 453.
[4] https://www.epa.gov/sites/production/files/2015-12/documents/8310.pdf
[5]https://waste.zendesk.com/hc/en-us/articles/217452058-How-does-EPA-s-Performance-Based-Measurement-System-PBMS-approach-affect-SW-846-

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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

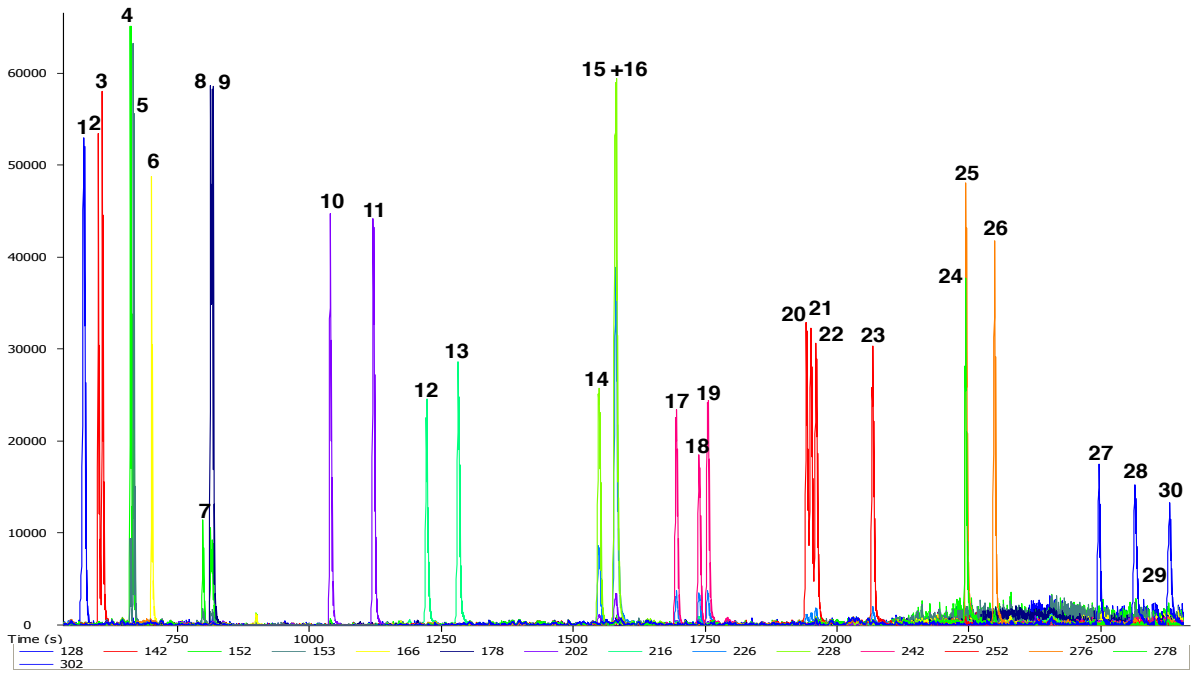
MS Ions (*m/z*) for Quantification and Identification of Target PAHs for Single-stage MS Instruments

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.

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DETERMINATION OF PAHS IN FISH BY QUECHERS EXTRACTION AND DUAL LAYER SPE CLEANUP

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INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that contain two or more fused aromatic rings, generated from incomplete combustion or pyrolysis of organic materials, smoked or grilled food, vehicle exhaust and cigarettes [1]. PAHs may also be emitted from natural activities, such as forest fires, volcanoes and hydrothermal processes [2]. Some PAHs undergo metabolic activation to diol epoxides which may bind to DNA, resulting in errors in DNA replication and mutations that start the carcinogenic process in mammals [3]. The US Environmental Protection Agency (EPA) has classified benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, and indeno[1,2,3-c,d]pyrene as probable human carcinogens. The European Union (EU) has set up the maximal level (ML) for benzo[a]pyrenein various foods, such as 2 µg/kg in fish and 5 µg/kg in smoked fish [4].

The analysis of PAHs in food is important and challenging due to the matrix complexity and the low detection limits that are required. QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged, and Safe) is a promising technique first reported in 2003 by scientists in the US Department of Agriculture (USDA) to determine pesticide residues in vegetables and fruits [5]. Since then QuEChERS is widely applied for the analysis of pesticides and other emerging compounds from various food matrices such as oil, milk, meat, and seafood.

The aim of this study is to develop a simple and sensitive method using a basic analytical instrument to determine the sixteen US EPA priority PAHs in fish. Methodology based on QuEChERS extraction and dual layer SPE cleanup with primary secondary amine (PSA) and endcapped C18 was developed. PSA is capable of removing sugars, fatty acids, organic acids and some pigments; while endcapped C18 can remove long chain fatty compounds, sterol and other non-polar matrix interferences. In this study a dual layer SPE cartridge containing 500 mg PSA and 500 mg endcapped C18 was utilized for the very first time to cleanup fish samples. The method is sensitive enough to report PAHs at the EU regulation ML of 2 µg/kg of benzo[a]pyrene in fish using GC/MS with normal splitless injector. The newly developed method is an attractive alternative for environmental and food safety laboratories without investing in large volume injection techniques for their key instrument (GC/MS), or for more sensitive analytical instruments, such as LC/FLD, GC/MS/MS, GC/TOF/MS or LC/MS/MS.

EXPERIMENTAL

Materials:
50 mL polypropylene centrifuge tube for PAH analysis (UCT Part#: ECPAHFR50CT)

Mylar Pouch containing 4000 mg MgSO4 and 2000 mg NaCl (UCT Part#: ECQUUS2-MP)

Dual layer SPE cartridge with 500 mg of PSA and 500 mg of endcapped C18 in a 6 ml cartridge (UCT Part#: ECPSAC1856)

Sodium sulfate, anhydrous, ACS, Granular 60 Mesh (UCT Part#: ECSS05K)

Sample Preparation:
About 500g fresh tilapia tissue sample was bought from a local supermarket. The fish sample was homogenized using a food processor and stored at -20 °C in a plastic container until use.

QuEChERS Extraction:
Weigh 5 ± 0.1 g homogenized fish sample into 50 mL centrifuge tubes and spike with 100 ng/g benzo[a]pyrene d12 (IS), spike with 10 ng/g and 200 ng/g of PAHs for fortified samples. Vortex for 30 sec and equilibrate for 15 min.
Add 5 mL of DI water and 10 mL of MeCN, shake for 1 min.
Add 4 g MgSO4 and 2 g NaCl packed in Mylar pouch, shake vigorously for 1 min.
Centrifuge at 4000 rpm for 5 min, the upper layer extract is ready for cleanup.

Dual layer SPE cleanup:
Attach dual layer SPE cartridges topped with 2 g of anhydrous Na2SO4 to 24-port vacuum manifold, rinse with 2 x 3 mL of MeCN, turn full vacuum on for 1 min. Insert collection tubes into the manifold, load 5 mL of the upper layer fish extract to the cartridge, apply about 3" Hg vacuum and collect the filtrate. Rinse the cartridge with 4 mL of MeCN and collect the rinse, combine the filtrate and rinse.
Add 0.5 mL toluene as a keeper and exchanging solvent, concentrate to 0.5 mL with a gentle stream of dry nitrogen at 35 °C. Transfer the concentrated extract into a 2 mL amber vial, the extract is ready for GC/MS analysis.

QuEChERS Extraction Kit

Cleanup of fish samples with dual layer SPE cartridges

INSTRUMENTAL

GC/MS: Thermo Scientific Trace GC Ultra coupled with ISQ single quadrupole MS and TriPlus Autosampler; Xcalibur (Version: 2.1) software for data acquisition and analysis.
Column: Restek Rtx®-5MS (30m*0.25mm*0.25um) integrated with 10 m guard column
Injection: 2 µL splitless injection at 220 °C, 50 mL/min split vent at 1 min.
Liner: Splitless Straight Liner 5 x 8 x 105mm (I.D. x O.D. x L) with deactivated glass wool.
Temperature program: Initial oven temperature of 65 °C, hold for 0.5 min; ramp at 15 °C/min to 240 °C; ramp at 7 °C/min to 310 °C and hold for 2.83 min. Total run time is 25 min. Begin data acquisition at 4 min.
Carrier gas: Ultra high purity Helium at a constant flow of 1.2 mL/min.
MSD condition: Transfer line temperature: 280 °C; Ion source: 250 °C.
Full scan: 50-400 amu.

Table 1: Retention times and SIM parameters of the 16 US EPA priority PAHs

Analytes	Rt (min)	Group #	Start (min)	Quantitfy ion	Qualifier ion 1	Qualifier ion 2
Naphthalene	5.10	1	4.0	128.12	102.11	127.00
Acenaphthylene	7.46	2	6.5	152.15	76.10	151.05
Acenaphthene	7.75			153.16	76.09	154.07
Fluorene	8.54			165.17	82.96	166.05
Phenanthrene	10.05	3	9.4	178.14	89.14	152.06
Anthracene	10.12	4		178.14	89.17	152.15
Fluoranthene	11.94		11.0	202.18	100.09	88.10
Pyrene	12.29	5	13.5	202.14	100.99	88.10
Benzo[a]anthracene	14.58			228.17	114.11	101.13
Chrysene	14.68	6	16.0	228.16	112.97	101.10
Benzo[b]fluoranthene	17.06			252.17	126.13	113.29
Benzo[k]fluoranthene	17.12			252.16	125.98	113.27
Benzo[a]pyrene d_{12}	17.76			264.23	131.99	
Benzo[a]pyrene	17.80	7	19.0	252.17	126.13	113.10
Indeno[1,2,3-cd]pyrene	20.38			276.18	138.33	125.07
Dibenzo[a,h]anthracene	20.46			278.05	139.11	124.99
Benzo[ghi]perylene	20.93			276.17	137.98	125.09

Dwell time is 0.1 s for all the ions monitored.

RESULTS AND DISCUSSIONS

Elution profile of dual layer SPE cartridge:
After QuEChERS extraction, the upper layer fish extract was cleaned up by passing through the dual layer cartridge with PSA and C18, on which the matrix interferences of fish sample were retained. PAHs with high molecular weights,such as indeno[1,2,3-cd]pyrene and benzo[ghi]perylene were observed with poor recoveries. Rinses with 4 mL of MeCN were carried out to release the PAHs. The recoveries of the filtrate and the first and second rinse with 4 mL of MeCN are listed in Table 2. Up to 62.8% of the PAHs were recovered by the first rinse, while less than 1.1% of the PAHs were recovered by the second rinse, which was negligible and thus was omitted from the procedure.

Table 2: Elution effect on PAHs recoveries

Compound	Filtrate	1st Rinse	2nd Rinse
Naphthalene	83.7	15.2	1.1
Acenaphthylene	83.4	16.0	0.6
Acenaphthene	80.1	19.1	0.8
Fluorene	81.0	18.2	0.8
Phenanthrene	80.3	19.2	0.5
Anthracene	78.3	21.1	0.6
Fluoranthene	73.9	25.7	0.4
Pyrene	70.6	28.9	0.5
Benzo[a]anthracene	68.9	30.8	0.3
Chrysene	68.7	30.7	0.6
Benzo[b]fluoranthene	60.8	38.9	0.3
Benzo[k]fluoranthene	59.2	40.0	0.7
Benzo[a]pyrene	53.5	45.9	0.5
Indeno[1,2,3-cd]pyrene	36.4	62.7	0.9
Dibenzo[a,h]anthracene	54.3	44.7	1.0
Benzo[ghi]perylene	36.5	62.8	0.7

Matrix matched calibration:
Matrix matched calibration curves were generated by analyzing matrix matched standards to compensate for matrix effects. The matrix matched standards were prepared by spiking appropriate amounts of PAHs standard and IS into the blank fish extracts to generate six calibration levels at concentrations of 2, 10, 20,100, 500 and 1000 ng/g. Dynamic linearity ranges, regression equations and correlation coefficients (R2) are listed in Table 3. The limit of quantification (LOQ) of this method is 2 ng/g, at which the signal-to-noise ratio is greater than 10.

Table 3: Linearity data of matrix matched calibration curve

Compound	Linearity Range (ng/g)	Regression Equation	R^2
Naphthalene	2-1000	Y = 0.197459+0.0406831*X	0.9955
Acenaphthylene	2-1000	Y = 0.0449732+0.0379289*X	0.9919
Acenaphthene	2-1000	Y = 0.0506444+0.0277214*X	0.9898
Fluorene	2-1000	Y = 0.0492769+0.0276983*X	0.9910
Phenanthrene	2-1000	Y = 0.0506201+0.0343744*X	0.9942
Anthracene	2-1000	Y = 0.0169946+0.0352951*X	0.9945
Fluoranthene	2-1000	Y = 0.0281097+0.0297256*X	0.9970
Pyrene	2-1000	Y = 0.0332384+0.0310519*X	0.9971
Benzo[a]anthracene	2-1000	Y = 0.000329639+0.02107*X	0.9982
Chrysene	2-1000	Y = 0.0175941+0.0196721*X	0.9988
Benzo[b]fluoranthene	2-1000	Y = 0.00991758+0.0143426*X	0.9992
Benzo[k]fluoranthene	2-1000	Y = 0.0385193+0.0157281*X	0.9990
Benzo[a]pyrene	2-1000	Y = 0.0157183+0.0142253*X	0.9994
Indeno[1,2,3-cd]pyrene	2-1000	Y = -0.000115949+0.0102454*X	0.9985
Dibenzo[a,h]anthracene	2-1000	Y = 0.0129756+0.0120726*X	0.9992
Benzo[ghi]perylene	2-1000	Y = 0.00734013+0.011293*X	0.9993

Recoveries of PAHs from fortified fish samples:
PAHs were not found in the fish sample with a reporting limit of 2 ng/g. The recovery study was carried out by spiking the negative fish sample with 10 and 200 ng/g PAHs. The results based on four replicates are listed in Table 4.

Table 4: Recovery and reproducibility data

Compound	Fortified at 10 ng/g		Fortified at 200 ng/g	
	Recovery%	RSD% (n=4)	Recovery	RSD% (n=4)
Naphthalene	98.5	2.7	101.2	1.1
Acenaphthylene	102.3	2.6	94.5	4.1
Acenaphthene	102.1	2.7	95.5	3.4
Fluorene	101.8	1.0	94.0	4.1
Phenanthrene	105.4	1.8	93.2	1.9
Anthracene	103.7	1.3	92.4	2.1
Fluoranthene	104.4	1.5	93.8	2.2
Pyrene	104.3	1.3	90.6	2.6
Benzo[a]anthracene	110.9	3.6	97.0	1.5
Chrysene	105.7	2.0	96.8	2.3
Benzo[b]fluoranthene	104.2	2.6	93.7	3.0
Benzo[k]fluoranthene	98.3	4.0	95.5	1.4
Benzo[a]pyrene	100.5	1.3	91.2	0.8
Indeno[1,2,3-cd]pyrene	96.1	1.4	82.6	1.2
Dibenzo[a,h]anthracene	96.0	3.0	91.6	2.0
Benzo[ghi]perylene	95.9	2.0	83.1	0.6

Satisfactory recoveries ranging from 82.6 to 110.9% with an overall recovery of 97.4% were obtained. Relative standard deviations (RSDs) were less than 4.1%. The data indicated that this method is suitable to determine PAHs in fish samples.

CHROMATOGRAMS

Chromatograms of fish samples fortified with 10 ng/g of PAHs are shown in Figure 1. The quantifying ions are free of interferences and are easily quantified.

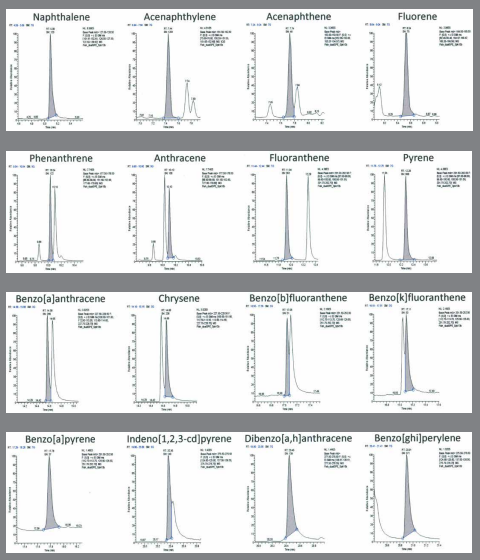

Figure 1. Chromatogram of fish sample fortified with 10 ng/g of PAHs

CONCLUSIONS

A simple, novel and effective method has been developed for the determination of the sixteen US EPA priority PAHs in fish. Fish samples were extracted by QuEChERS, cleaned up with dual layer SPE cartridge containing PSA and endcapped C18, and detected by GC/MS in SIM mode. To our knowledge, this is the very first study utilizing dual layer SPE with PSA and endcapped C18 to cleanup fish samples. Excellent recoveries ranging from 82.6 to 110.9% with RSD less than 4.1 % were achieved with this newly developed method.

References:
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Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Solid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection*

UCT Products:
ECUNIPAH (2000 mg unendcapped C18, 83 mL cartridge)
ECSS25K (Anhydrous Sodium Sulfate)

EPA Method 550.1

Procedure

1) Cartridge Preparation

a. Wash with 4 x 10 mL aliquots of methylene chloride ($MeCl_2$)
b. Wash with 4 x 10 mL aliquots of methanol (MeOH)
c. Wash with 2 x 10 mL aliquots of reagent water

Do not let the cartridge dry out after step 1) c. otherwise repeat starting at 1) b.

2) Sample Extraction

a. Adjust the vacuum setting for a flow rate of 10-15 mL per minute
b. Add the 1 liter sample to the cartridge
c. Rinse sample bottle with reagent water, add to cartridge and draw through
d. Dry cartridge by drawing full vacuum for 10 minutes

3) Sample Elution and Drying

a. Elute the cartridge dropwise by using 2 x 5 mL aliquots of $MeCl_2$ and collect
b. Rinse sample container with 5 mL of $MeCl_2$, add to cartridge and draw through
c. Prepare a drying column/funnel containing 10-20 g sodium sulfate by rinsing with 10 mL of $MeCl_2$ and discard
d. Add the eluate to the drying column, draw through and collect
e. Rinse the eluate vial and drying column with a 2 x 5 mL aliquot of $MeCl_2$ and collect

4) Sample Evaporation

a. Evaporate the extract using a gentle stream of N_2 with a water bath or heating block temperature of 40°C. Evaporate to about 1.0 mL

b. Add 3.0 mL of acetonitrile (ACN)

c. Concentrate to a final volume of 0.5 mL

5) Sample Analysis

a. Inject 5 - 100 µL into an HPLC

*See "Determination of Polycyclic Aromatic Hydrocarbons in Drinking Water by Liquid-Solid Extraction and HPLC with Coupled Ultraviolet and Fluorescence Detection", W. J. Bashe & T.V. Baker (Technology Applications, Inc, Environmental Monitoring Systems Laboratory, US Environmental Protection Agency, Cincinnati, OH

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