ENVIRO

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.

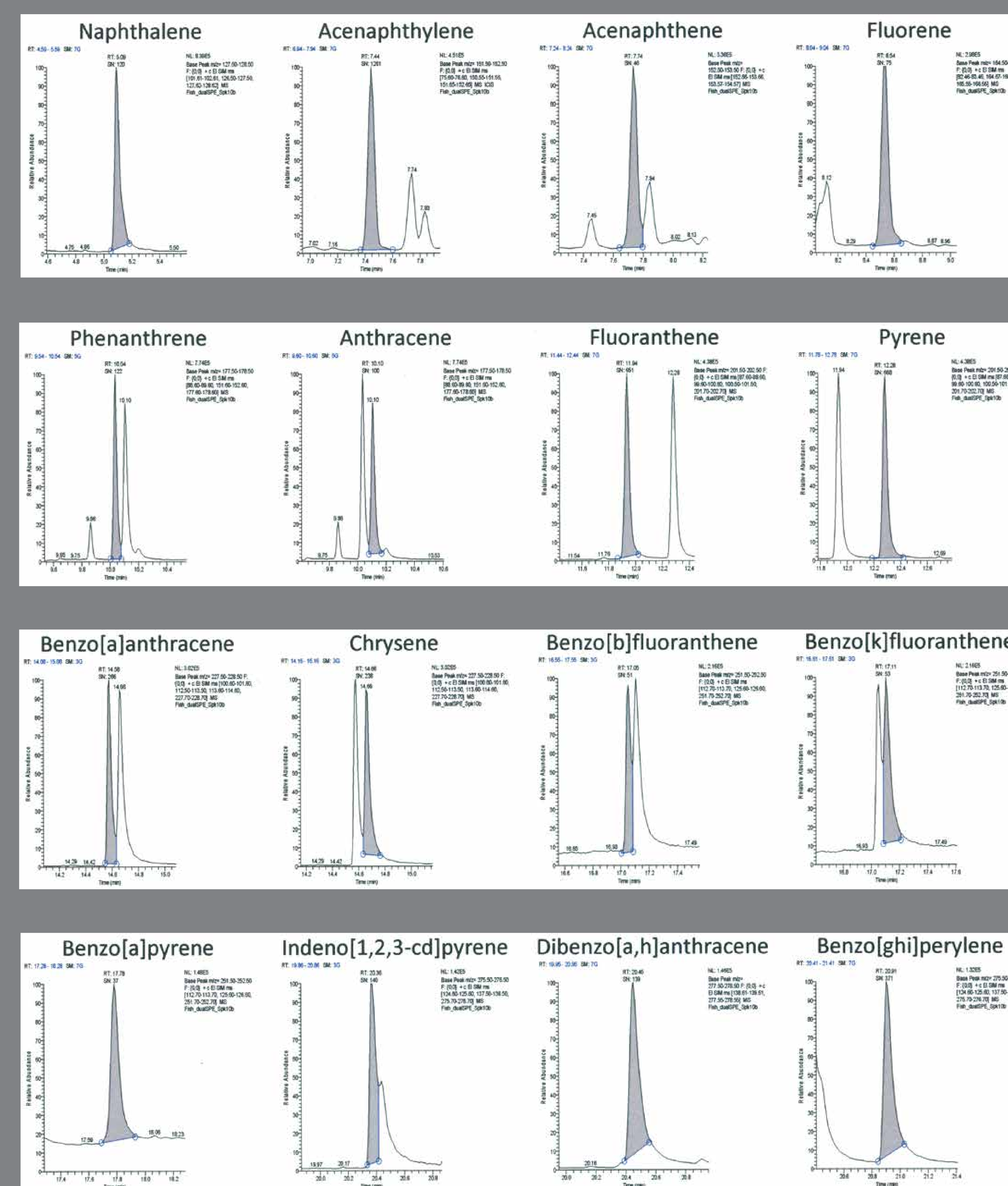

Figure 1. Chromatogram of fish sample fortified with 10 ng/g of PAHs

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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