Analysis of Polycyclic Aromatic Hydrocarbons in Fish with Agilent SampliQ QuEChERS AOAC Kit and HPLC-FLD

Application Note

Food

Authors

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Abstract

An HPLC-Florescence Detection (FLD) method was developed and validated for the determination of sixteen polycyclic aromatic hydrocarbons (PAHs) in fish fillets. The analyzed compounds included naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr), 1,2-benza[a]anthracene (BaA), chrysene (Chr), benzo[e]pyrene (BeP), benzo[e]acenaphthylene (BeA), benzo[k]fluoranthene (BkF), dibenzo[a,h]anthracene (DahA), benzo[g,h,i]perylene (Bghi)P and indeno[1,2,3-cd]pyrene (InP). The method employs a quick, easy, cheap, effective, rugged and safe (QuEChERS) multiresidue sample preparation procedure adopted from the Association of Analytical Communities (AOAC) Official method 2007.01 for extraction and cleanup. The analytes were separated on an Agilent ZORBAX Eclipse PAH HPLC column (4.6 mm × 50 mm, 1.8 µm) by gradient elution with a binary system of acetonitrile - water and subsequent fluorescence detection set at appropriate excitation and emission wavelengths. The analyte recoveries ranged from 83.4% to 101% with relative standard deviations ranging from 0.6 to 1.9% at three different fortification levels. The limits of detection and quantification ranged from 0.04 to 0.84 and 0.1 to 2.80 ng/g, respectively.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds included in the European Union and US Environmental Protection Agency (US EPA) priority pollutant list because of their mutagenic and carcinogenic properties [1]. Excluding smokers and occupationally vulnerable populations, most individuals are exposed to PAHs predominantly from dietary sources [2]. In the marine environment, PAHs are bioavailable to marine species via the food chain, as waterborne compounds, and contaminated sediments. As lipophilic compounds they can easily cross lipid membranes and have the potential to bioaccumulate in aquatic organisms. Although for most people, fish and seafood represents only a small part of the total diet, the contribution of this food group to the daily intake of PAHs in some individuals may be comparatively important [3].

The AOAC QuEChERS method has been widely applied in the analysis of pesticides in food since it was introduced by USDA scientists [4-5]. In general, there are two major steps: extraction and dispersive SPE cleanup. The method uses a single step buffered acetonitrile extraction while simultaneously salting out water from the aqueous sample using anhydrous magnesium sulfate ($MgSO_4$) to induce liquid-liquid partitioning. After removing an aliquot from an organic layer, for further cleanup, a dispersive solid phase extraction (dSPE) step is conducted using a combination of primary secondary amine (PSA) sorbent to remove organic acids from other components and anhydrous $MgSO_4$ to reduce the remaining water in the extract. Other sorbents, such as graphitized carbon black (GCB), may be added to remove pigments and sterol, or C18 to remove lipids and waxes.

This application note presents a method for the analysis of PAHs at trace levels in fish tissue with HPLC-FLD. The HPLC methods are useful for PAH analysis since UV and fluorescence detection offer enhanced selectivity over other techniques such as GC with flame ionization detection [6]. The method includes sample preparation with SampliQ AOAC Buffered Extraction kit (p/n 5982-5755) and SampliQ AOAC Fatty Dispersive SPE 15 mL kit (p/n 5982-5158). Chemical structures of the PAHs in this study are shown in Figure 1.

Experimental

Reagents and Chemicals

All reagents were analytical or HPLC grade. Acetonitrile (CH_3CN) and PAHs were purchased from Sigma-Aldrich (St. Louis, MO, USA). The water used was from a MilliQ system (Milford, Mass, USA). The mobile phase was filtered through a Whatman membrane filter (47 mm diameter and 2 µm pore size).

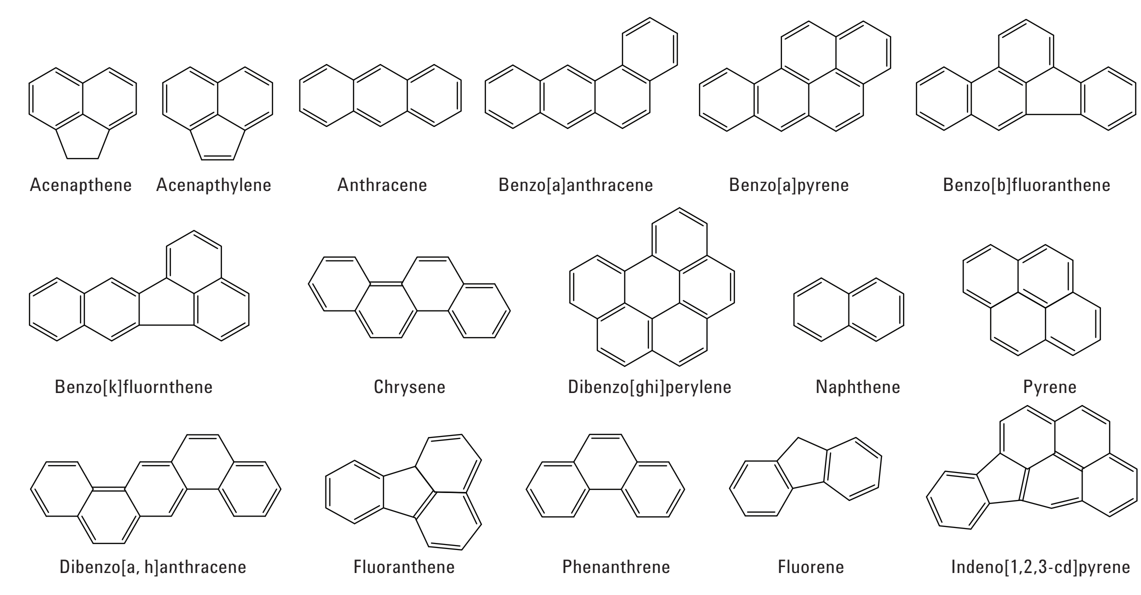

Figure 1. Chemical structures for the polycyclic aromatic hydrocarbons used in the study.

Standard Solutions

Standard stock solutions (1 mg/mL) were prepared by dissolving 10 mg of the desired PAH in 10 ml CH_3CN and stored at –20 °C. All working solutions were prepared fresh daily by serial dilution with CH_3CN.

Equipment and Material

The analysis was performed on an Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a binary pump and a fluorescence detector (FLD) set at varying excitation and emission wavelengths (Table 1). The selection of the excitation and emission wavelengths for detection was based on the optimum responses for the various PAHs. Separation of the compounds was achieved on an Agilent ZORBAX Eclipse PAH column (4.6 mm × 50 mm, 1.8 µm), p/n 959941-918. The data was processed by HPLC 2D Chemstation software.

Extraction and cleanup were achieved with Agilent SampliQ Buffered QuEChERS AOAC Extraction kit, p/n 5982-5755 and SampliQ QuEChERS AOAC Dispersive SPE kit, p/n 5982-5158, (Agilent Technologies).

A Kenwood Grinder (obtained from a local appliance store) was employed for homogenizing the fish sample.

Instrument conditions

HPLC conditions

Table 1. HPLC Conditions used for Separation of PAHs

Column	Agilent ZORBAX Eclipse PAH C18 4.6 × 50 mm, 1.8 µm	
Flow rate	0.8 mL/min	
Column temperature	18 °C	
Injection volume	5 µL	
Mobile phase	A = Deionized H_2O	B = CH_3CN
Gradient	T (min)	% B
	0	60
	1.5	60
	7	90
	13	100
Detection	UV at 230 nm (Acy) and varying fluorescence excitation (Ex) and emission (Em) wavelengths	
Wavelengths:		
Time (min)	**Ex/Em wavelengths (nm)**	**PAH detected**
0 – 5 (dark blue)	260/352	Nap, Ace, Flu, Phe, Chr
0 – 14 (red)	260/420	Ant, Pyr, BeP, DahA, BghiP
0 – 14 (light blue)	260/460	Fln, 1,2-BaA,BeA, BkF, InP

Sample preparation

The fish fillets were purchased from a local food store, minced, and deep frozen until analysis.

Extraction

A 5.0 g sample of fish homogenate was placed into a 50 mL centrifuge tube from the SampliQ QuEChERS AOAC Extraction kit and the tube was centrifuged for 20 s. Samples were then spiked with appropriate spiking solutions to yield appropriate working solutions for recoveries and reproducibility studies. A 2000 µL volume of spiking solution was added to all samples except the blank, and the tubes were shaken vigorously for 1 min. Next, 8 mL of CH_3CN, then an Agilent SampliQ QuEChERS AOAC extraction salt packet (p/n 5082-5755) containing 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaOAc were added to the tubes. The sample tubes were hand shaken vigorously for 1 min, then further centrifuged at 4000 rpm for 5 min.

Dispersive SPE Cleanup

A 6.0 mL aliquot of the upper CH_3CN layer was transferred into a SampliQ QuEChERS AOAC Dispersive SPE 15 mL tube. This SPE tube contained 400 mg of PSA, 400 mg of C18EC, and 1200 mg of anhydrous $MgSO_4$. After one minute of shaking, the tubes were centrifuged at 4000 rpm for 5 min. A 4 mL aliquot of the extract was filtered through a 0.45 µm PVDF syringe filter, then 1000 µL of the extract was placed in an autosampler vial for HPLC-FLD analysis.

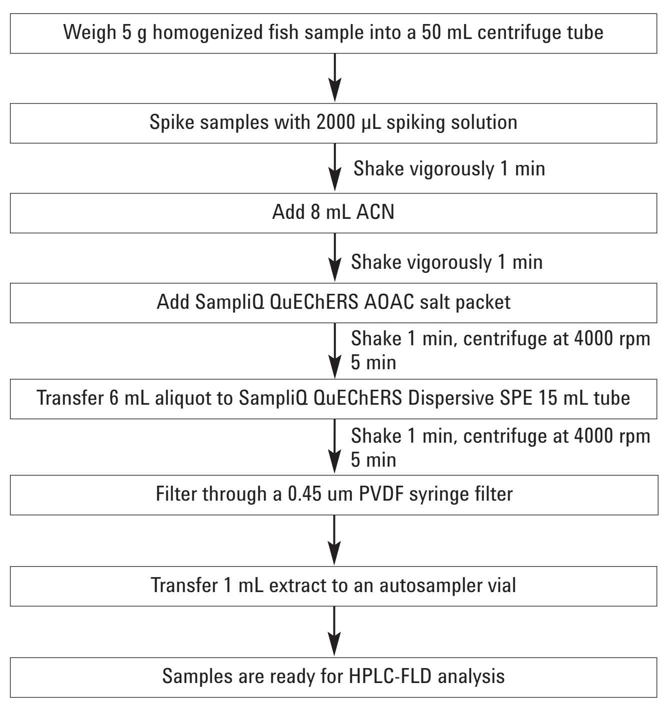

Figure 2. Flow chart of QuEChERS AOAC sample preparation procedure.

Results and Discussion

Chromatographic results

Figure 3 shows an overlay of color-coded chromatograms at various fluorescence conditions (Table 1) of the standard mixture of the 16 PAHs. A chromatogram of the blank fish extract is presented in Figure 4. Overlay chromatograms of the spiked fish sample at spiking level 1 are shown in Figure 5.

QuEChERS extraction

The use of CH_3CN as an extracting solvent in a salting-out condition, without the need to add co-solvents, attained high extraction yields as shown by the recoveries in Table 4. The CH_3CN solvent is compatible with the HPLC – FLD procedure in this application note. Therefore no evaporation or reconstitution solvent was required. This is particularly important for the PAHs since some of these compounds (naphthalene, acenaphthene and fluorene) are extremely volatile and may be lost during an evaporation step [1].

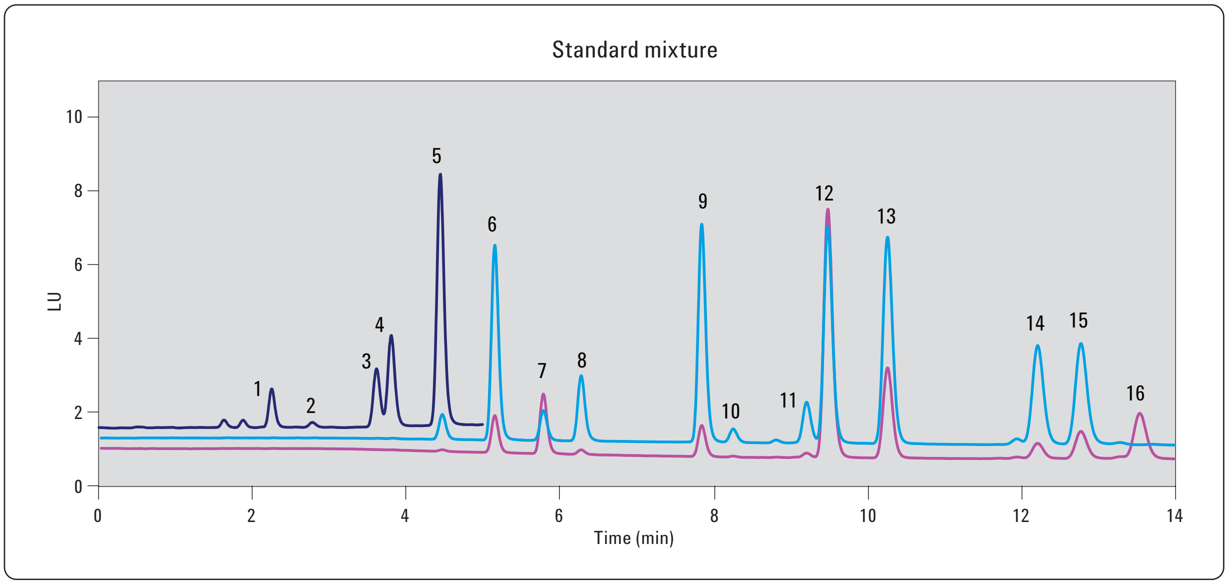

Figure 3. Overlay HPLC – FLD chromatograms of the standard mixture containing: 1. Nap 2. Acy 3. Ace 4. Flu 5. Phe 6. Ant 7. Fln 8. Pyr 9. BaA 10. Chr 11. BeP 12. BeA 13. BkF 14. DahA 15. BghiP 16. InP . The concentration of the PAHs was 1 mg/mL. The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion 260-nm/420-nm; the light blue-portion: 260-nm/440-nm. For acenaphthylene, UV detection at 230-nm was used. Chromatographic conditions are shown in Table 1.

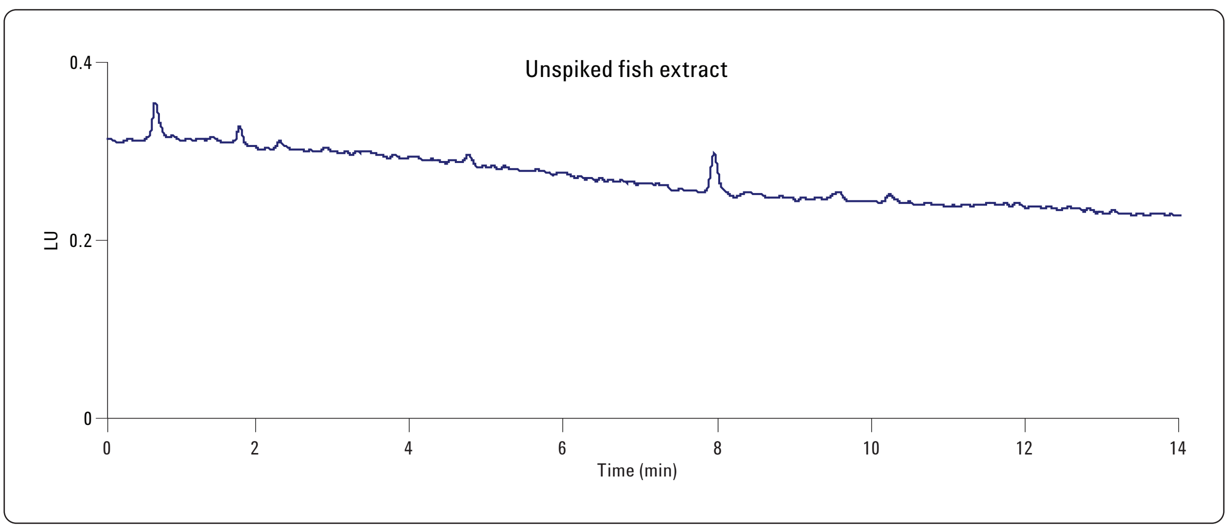

Figure 4. Chromatogram of the blank fish extract. Chromatographic conditions are shown in Table 1. The baseline chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm. The other excitation/emission conditions showed no other interferences.

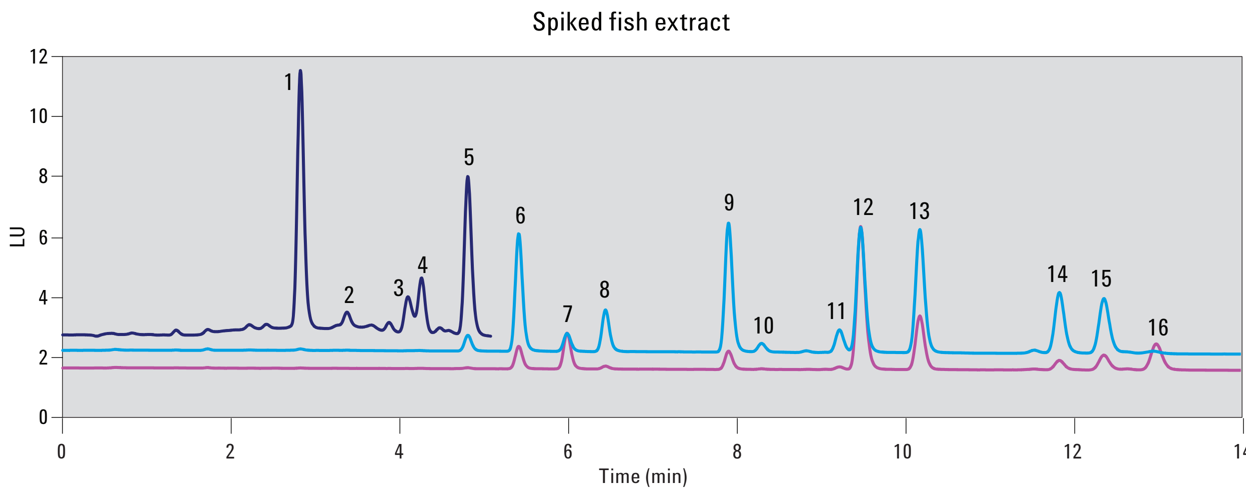

Figure 5. Overlay HPLC – FLD chromatograms of the spiked fish sample containing: 1. Nap 2. Acy 3. Ace 4. Flu 5. Phe 6. Ant 7. Fln 8. Pyr 9. BaA 10. Chr 11. BeP 12. BeA 13. BkF 14. DahA 15. BghiP 16. InP. The spiking level for this sample was level 1 (see Table 3). The blue portion of the chromatogram used the following excitation/emission wavelengths: 260-nm/352-nm; the red portion 260-nm/420-nm; the light blue portion: 260-nm/440-nm. For acenaphthylene, UV detection at 230-nm was used. Chromatographic conditions are shown in Table 1.

Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

Linearity

The linear calibration curves were obtained by plotting the peak area for each analyte versus its concentration. Curves were generated by spiking the sample blanks at a concentration range of 0 – 300 ng/g.

Limits of Detection and Quantification

The limits of detection and quantification were estimated from the concentration of sulfonamides required to give a signal-to-noise ratio of 3 and 10 respectively. Table 2 shows the regression equation, correlation coefficients, and very acceptable limits of detection and quantification.

Table 2. Linearity, LOD and LOQ for the Sixteen Polycyclic Aromatic Hydrocarbons

PAH	Regression equation	R^2	LOD	LOQ
Naphthalene	Y = 0.0222x + 0.1366	0.9991	0.62	2.07
*Acenaphthylene	Y = 0.0544x – 0.0130	0.9993	0.25	0.83
Acenaphthene	Y = 0.0184 x – 0.0204	0.9998	0.56	1.87
Fluorene	Y = 0.0323x – 0.1717	0.9990	0.12	0.40
Phenanthrene	Y = 0.0950x + 0.0086	0.9995	0.18	0.60
Anthracene	Y = 0.0838x – 0.1265	0.9991	0.24	0.80
Fluoranthene	Y = 0.0247x – 0.0237	0.9994	0.04	0.16
Pyrene	Y = 0.0218x – 0.0432	0.9998	0.09	0.30
1,2-Benzanthracene	Y = 0.0120x – 0.0103	0.9994	0.03	0.10
Chrysene	Y = 0.0052x + 0.0086	0.9990	0.28	0.93
Benzo[e]pyrene	Y = 0.0144x – 0.0037	0.9997	0.04	0.16
Benz[e]acenaphthylene	Y = 0.1186x – 0.032	0.9995	0.07	0.23
Benzo[k]fluoranthene	Y = 0.0464x + 0.0969	0.9997	0.05	0.16
Dibenzo[a,h]anthracene	Y = 0.0531x + 0.0001	0.9990	0.84	2.80
Benzo[g,h,i]perylene	Y = 0.0440x + 0.0722	0.9993	0.11	0.36
Indeno[1,2,3-cd]pyrene	Y = 0.0324x – 0.0912	0.9993	0.05	0.18

* UV detection at 230 nm

Recovery and Reproducibility

The recovery and reproducibility (RSD) were evaluated on spiked samples at three different levels (Table 3). The analysis was performed in replicates of six (n = 6) at each level. Table 4 shows the very good to excellent recoveries, and excellent RSD values for the sixteen polycyclic aromatic hydrocarbons.

Conclusions

A simple and fast mulitiresidue method based on SampliQ QuEChERS AOAC and HPLC-FLD has been developed for the simultaneous determination of sixteen polycyclic aromatic hydrocarbons at parts-per-billion (ppb) levels in fish tissue. High recoveries with excellent RSD were attained, therefore the method should be applied for quality control of PAHs in real samples.

Table 3. PAHs Spiking Levels

PAH	Spiking level (ng/g)		
	1	2	3
Naphthalene	20	100	200
*Acenaphthylene	20	100	200
Acenaphthene	10	50	100
Fluorene	10	50	100
Phenanthrene	10	50	100
Anthracene	10	50	100
Fluoranthene	10	50	100
Pyrene	10	50	100
1,2-Benzanthracene	5	20	50
Chrysene	10	50	100
Benzo[e]pyrene	5	20	50
Benz[e]acenaphthylene	5	20	50
Benzo[k]fluoranthene	5	20	50
Dibenzo[a,h]anthracene	5	20	50
Benzo[g,h,i]perylene	5	20	50
Indeno[1,2,3-cd]pyrene	5	20	50

* UV detection at 230 nm

Table 4. Recoveries and RSDs for the Sixteen Polycyclic Aromatic Hydrocarbons in Fish Sample (n = 6)

PAH	Level of spiking (ng/g) (n = 6)					
	1		2		3	
	%Recovery	%RSD	%Recovery	%RSD	%Recovery	%RSD
Naphthalene	94.7	1.4	97.9	1.1	93.8	1.4
*Acenaphthylene	87.8	1.7	96.3	1.2	85.6	0.8
Acenaphthene	92.1	1.5	93.0	1.8	96.7	0.8
Fluorene	98.1	1.5	89.9	1.0	97.2	0.9
Phenanthrene	90.6	0.9	93.8	0.8	83.1	1.7
Anthracene	96.7	1.0	87.6	0.8	92.1	0.6
Fluoranthene	83.4	1.3	93.9	1.5	95.9	1.2
Pyrene	93.5	1.8	86.1	1.3	95.0	1.4
1,2-Benzanthracene	94.5	1.3	89.6	1.6	94.9	1.0
Chrysene	101.0	1.4	97.8	1.7	87.2	1.6
Benzo[e]pyrene	88.8	1.5	85.2	1.9	95.0	1.4
Benz[e]acenaphthylene	95.5	0.7	92.7	0.7	89.2	0.9
Benzo[k]fluoranthene	93.5	0.8	94.6	0.9	98.9	0.8
Dibenzo[a,h]anthracene	88.2	0.9	97.3	1.1	97.1	0.6
Benzo[g,h,i]perylene	98.4	0.8	95.5	1.6	98.2	0.7
Indeno[1,2,3-cd]pyrene	91.5	1.5	97.9	0.9	94.3	0.7

* UV detection at 230 nm

References

1. M. J. Ramalhosa, P. Paiga, S. Morais, C. Delerue-Matos, M. B. P. P. Oliveira,"Analysis of Polycyclic Aromatic Hydrocarbons in Fish: Evaluation of a Quick, Easy, Cheap, Effective, Rugged and Safe Extraction Method," *J. Sep. Sci.* 2009 32 3529 – 3538.
2. L. R. Bordajandi, M. Dabrio, F. Ulberth, H. Emons, "Optimisation of the GC – MS Conditions for the Determination of IS EU Foodstuff Priority Polycyclic Aromatic Hydrocarbons," *J. Sep. Sci*. 2008 31 1769 – 1778.
3. J. L. Domingo,"Health Risks Arising From the Dietary Intake of Chemical Contaminants: a Case Study of the Consumption of Edible Marine Species in Catalonia," Contrbutions to Science, 3 (4): 459–468 (2007).
4. L. Zhao, J. Stevens, "Analysis of Pesticides Residues in Spinach Using Agilent SampliQ QuEChERS AOAC kits by LC/MS/MS," Agilent Technologies publication, 5990-4248EN.
5. M. Anastassiades, S. Lehotay, "Fast and Easy Multiresidue Method Employment Acetonitrile Extraction/Partitioning and 'Dispersive Solid-Phase Extraction' for the Determination of Pesticide Residues in Produce," 2003, 86, 412 – 431.
6. J. W. Henderson, Jr., W. Biazzo, W. Long, "Polycyclic Aromatic Hydrocarbons (PAH) Separation Using ZORBAX Eclipse PAH Columns – Analyses from Six to 24 PAHs," Agilent Technologies publication, 5989-7968EN.

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