

Determination of 24 PAHs in Drinking Water

Application Note

Food Testing and Agriculture

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants formed at high temperature and under pyrolytic conditions during the incomplete combustion of organic matter. They are found throughout the environment in air, water, soil, and food. Due to carcinogenic and mutagenic properties shown for certain PAHs, systematic monitoring is applied in most countries world wide. Recommended analytical procedures are documented or proposed in several US and EU guidelines. The number of PAHs and the maximum levels for PAHs documented in these guidelines were increased in the past and will be extended again in future. Today, most laboratories determine up to 16 PAHs in water but as discussions continue and, if in the future additional PAHs should be identified, it was our goal to develop an analytical method to detect a wide range of PAHs.

We present a validation method for the determination of 24 PAHs in which we use an automated solid phase extraction (SPE) method to concentrate the analyte in an 800 mL water sample, with a further analysis by HPLC with fluorescence detector (FLD) and diode-array detector (DAD).



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Method and Results

HPLC analysis

The first step was to optimize a chromatographic separation for PAHs, using fast HPLC.

Chromatographic conditions and FLD parameters

Time program for fluorescence detection

RT	λ exc	λ em	PMT-gain	PAHs
0.0	269	327	12	Naphthalene
9.5	250	328	12	Acenaphthene, fluorene
11.6	250	375	12	Phenanthrene, anthracene
13.8	237	440	12	Fluoranthene
14.6	270	376	12	Pyrene, benzo[c]fluorene
17.2	265	380	12	Benzo[a]anthracene, chrysene, methylchrysene
19.3	240	505	12	Benzo[j]fluoranthene
19.9	290	440	12	Benzo[b]fluoranthene, Benzo[k]fluoranthene, benzo[a]pyrene
22.5	293	485	15	Dibenzo[a,h]anthracene, dibenzo[a,l]pyrene, benzo[g,h,i]perylene, indeno[1,2,3-c,d]pyrene
24.5	280	404	15	Dibenzo[a,e]pyrene
26.0	292	440	15	Dibenzo[a,i]pyrene
27.5	260	456	15	Dibenzo[a,h]pyrene

HPLC column Agilent Pursuit PAH,
100 × 4.6 mm, 3 μ m
(p/n A7001100X046)

Column temperature 30 °C

Injection volume 5 μ L

HPLC gradient profile

RT(min)	H ₂ O (%)	ACN (%)	Flow (mL/min)
0	60	40	1.8
4	60	40	1.8
23	10	90	1.8
23.5	10	90	2.0
24	5	95	2.0
24.5	0	100	3.0
25	0	100	3.5
29	0	100	3.5
29.5	60	40	1.8
34	60	40	1.8

The optimized chromatogram on a Agilent Pursuit 3 PAH column led to a separation of 24 PAHs in 27 minutes. This is a definite improvement as traditional HPLC methods need around 45 minutes per run to separate a similar amount of analytes. Figure 1 shows the FLD chromatogram of the 22 fluorescent analytes spiked at the quantification limit of 0.02 μ g/L.

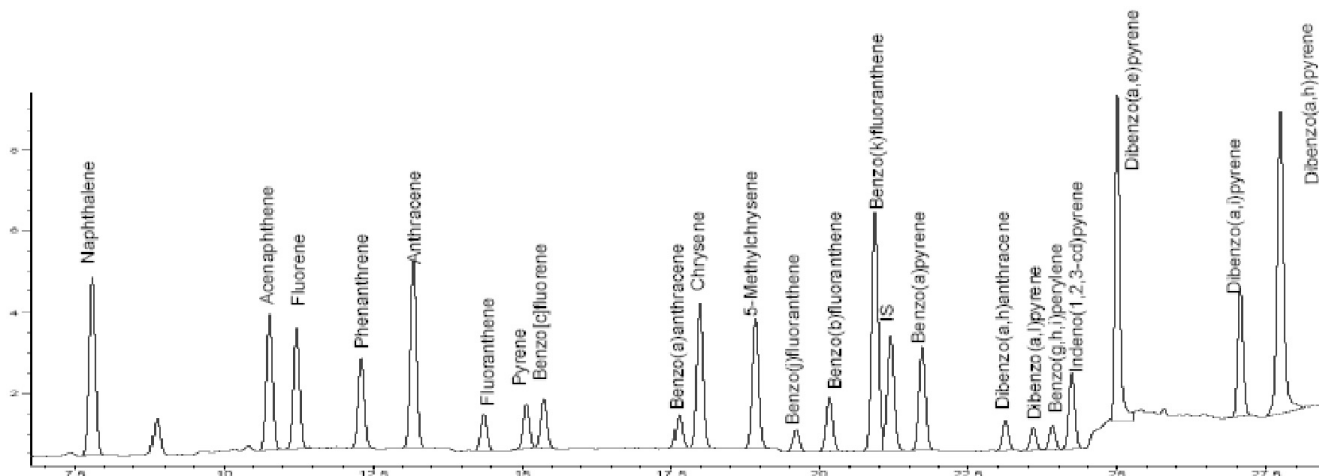


Figure 1. HPLC/FLD Chromatogram of a 5 µL injection of the 20 ppt PAH standard solution on the Agilent Pursuit 3 PAH column.

The two PAHs, acenaphthylene and cyclopentapyrene, that are not revealed by FLD are detected by UV at 254 nm. Figure 2 shows the UV chromatograms of acenaphthylene and cyclopentapyrene, both at a concentration of 50 ppt.

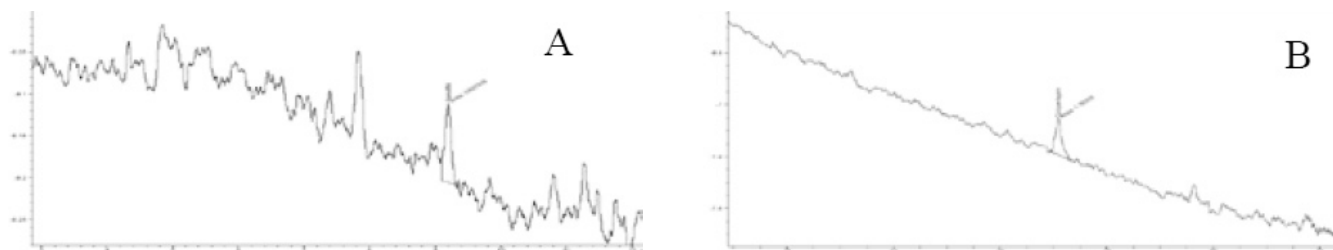


Figure 2. HPLC/DAD Chromatogram of acenaphthylene (A) and cyclopentapyrene (B), both at a concentration of 50 ppt, separated on the Agilent Pursuit 3 PAH column.

Sample preparation

The second step was the optimization of the SPE method. We tested different polymer- and silica-based sorbents, drying time, and pH adjustment, and optimized the recoveries by adding isopropanol to provide a final concentration of 5% in the water sample. The internal standard benzo[a]pyrene-d¹² was added to the 800 mL sample prior to the SPE step.

Best results and most robust application were achieved on the polymer-based sorbent Bond Elut Plexa. Bond Elut Plexa is a polymeric SPE product, designed for improved analytical performance and ease-of-use. Its uniqueness lies in the novel hydroxylated exterior and advanced polymeric architecture. Due to this structure, analytes from a large polarity scale are extracted with high recoveries and very good standard deviation.

SPE method

The optimized SPE conditions are:

800 mL water sample + 5% isopropanol + internal standard (BaP-d¹²)

SPE Sorbent, Bond-Elut Plexa 200 mg (p/n 12109610)

Condition with 4 mL ethyl acetate /
4 mL dichloromethane / 4 mL methanol / 4 mL water

Load sample

Dry for 30 minutes

Elute with 4 mL ethyl acetate / 4 mL dichloromethane

Take to 10 mL with ethyl acetate / dichloromethane (1:1)

Evaporation of 4 mL with N₂ (avoid dryness)

Redissolve in 500 µL acetonitrile

The process for the 800 mL water sample was automated on the Auto Trace Workstation (Zymark). The method has been fully validated and results are presented in Table 1. Linearity was excellent across the range studied, giving values of 0.99 or greater in all cases with very high precision.

Table 1. Validation Results and Guidelines

PAH Name	RT	Signal	LOQ (ng/mL)	Precision (% RSD)	Accuracy	Linearity
Naphthalene	7.8	FLD	0.20	7.97	73.88	0.9999
Acenaphthylene	9.2	DAD	0.50	5.39	78.09	0.9749
Acenaphthene	10.8	FLD	0.20	6.57	75.54	0.9980
Fluorene	11.3	FLD	0.02	5.82	76.71	0.9972
Phenanthrene	12.3	FLD	0.02	6.34	87.17	0.9944
Anthracene	13.2	FLD	0.02	4.78	79.19	0.9961
Fluoranthene	14.4	FLD	0.02	7.81	91.55	0.9961
Pyrene	15.1	FLD	0.02	16.69	95.85	0.9948
Benzo[c]fluorene	15.4	FLD	0.02	6.76	92.22	0.9985
Cyclopentapyrene	17.0	DAD	2.50	2.72	88.88	0.9874
Benzo[a]anthracene	17.7	FLD	0.02	8.37	90.01	0.9979
Chrysene	18.0	FLD	0.02	8.32	91.38	0.9977
Methylchrysene	18.9	FLD	0.02	8.34	90.52	0.9980
Benzo[j]fluorene	19.6	FLD	1.00	5.23	93.36	0.9974
Benzo[b]fluorene	20.2	FLD	0.02	8.85	90.60	0.9974
Benzo[k]fluorene	20.9	FLD	0.02	9.18	88.67	0.9977
Benzo[a]pyrene	21.7	FLD	0.01	3.06	61.20	0.9964
Dibenzo[a,h]anthracene	23.1	FLD	0.02	9.91	80.30	0.9985
Dibenzo[a,l]pyrene	23.6	FLD	0.02	3.87	64.17	0.9974
Benzo[g,h,i]perylene	23.9	FLD	0.02	12.84	82.25	0.9918
Indeno(1,2,3-cd)pyrene	24.2	FLD	0.02	11.37	79.18	0.9956
Dibenzo[a,e]pyrene	25.0	FLD	0.02	10.02	63.22	0.9922
Dibenzo[a,i]pyrene	27.1	FLD	0.02	13.36	48.18	0.9902
Dibenzo[a,h]pyrene	27.7	FLD	0.02	14.36	45.28	0.9910

Conclusion

The combination of the Agilent Pursuit PAH 3 μ m HPLC column and automated SPE on Bond Elut Plexa provide a perfect and fast solution for the analysis of 24 PAHs from a large volume of water. The HPLC analysis time of 28 minutes was a definite improvement as traditional HPLC methods need around 45 minutes per run to separate a similar number of analytes. The method showed excellent reproducibility and led to high recoveries for all 24 PAHs under investigation.

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Printed in the USA
August 14, 2012
5990-7686EN



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