



# Determination of PAH in ground, surface and drinking water using Symbiosis™ Environ

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## INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAH), a group of chemically related compounds, are known for their potential carcinogenic properties. In the environment these compounds are formed during pyrolysis and incomplete combustion processes. They are found throughout the environment in air, water and soil. The (by law enforced) maximum acceptable concentration level of PAH in water is approximately 10 ppt for each individual compound.

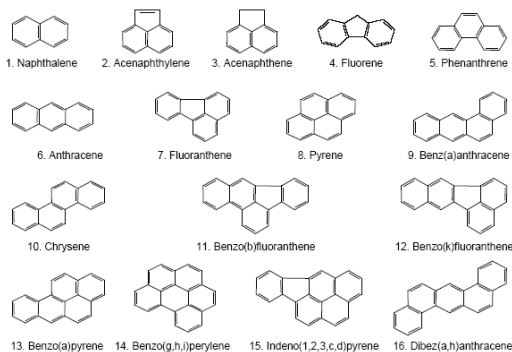


Figure 1: Polycyclic Aromatic Hydrocarbons.

Symbiosis™ Pico (see figure 2) is Spark Holland's unique solution for integrated online SPE-HPLC in environmental analyses. Water samples up to 1 L are pre-concentrated on a Solid Phase Extraction cartridge and eluted online (and fully automated) to an HPLC.

This Application note demonstrates the capability of the Symbiosis™ Pico in conjunction with a fluorescence detector to analyse PAH in water at ppt levels using only 10 mL of water sample. The Symbiosis™ method is compared to a traditional offline method (NEN-EN-ISO 17993, "Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction").



Figure 2: Symbiosis™ Pico

## EXPERIMENTAL

### SAMPLE PRETREATMENT

To minimize adsorption of PAH to the glass wall of the sample container, each amber borosilicate sample container is pretreated by three rinses: isopropanol, 20% nitric acid; isopropanol. After rinsing each container is filled with a fixed volume of isopropanol. The water samples are added to the isopropanol and mixed to obtain a final concentration of 25 % isopropanol. All samples are stored in the dark at 4°C.

### AUTOSAMPLER CONDITIONS

10 mL sample is introduced by connecting the HPD of the Symbiosis™ Pico to the injector valve of the Alias (figure 2).

### SPE CONDITIONS

SPE cartridge	Focus, L=10 mm I.D.=2 mm (Varian part number:12221301)
Solvation 1	6 mL acetonitrile 6 mL/min.
Solvation 2	6 mL methanol 6 mL/min.
Equilibration	3 mL water 4 mL/min.
Sample load	10 mL sample 2 mL/min.
Washing	2 mL 10% methanol 2 mL/min.
Elution	5 min. with acetonitrile (mixed with water to form the LC gradient)

### HPLC CONDITIONS

Figure 3 shows the total system configuration; The hydrophobic SPE cartridge is eluted with one of the Symbiosis high pressure LC pumps by eluting the SPE cartridge with 100% acetonitrile, ensuring a quick desorption. The eluent is mixed with water from a second high pressure LC pump using a high pressure and dynamic mixing chamber. In the proper mixing ratio the solvent is applied to an analytical column for separation and detection of PAH.

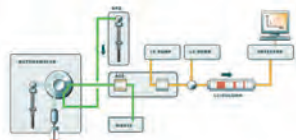


Figure 3: Schematic representation of flow path

Analytical Column	100 mm*4.6 mm*3 µm, Varian Chrompack (pn: CP29424)
Mobile phase A:	water
Mobile phase B:	acetonitrile
Temperature:	25°C
UV-Detection	254 nm
Fluorescence Detection, see table 1.	
Gradient program, see table 2.	

Compound	Excitation	Emission
Naphthalene	275	325
1-Methylnaphthalene	275	325
Acenaphthene	275	325
Fluorene	275	325
Phenanthrene	255	375
Anthracene	255	375
Fluoranthene	270	420
Pyrene	270	420
Triphenylene	270	420
Benzo(a)anthracene	275	383
Chrysene	275	383
Benzo(b)fluoranthene	295	410
Benzo(k)fluoranthene	295	410
Benzo(a)pyrene	295	410
di benzo(a,h)anthracene	301	420
Benzo(g,h,i)perylene	301	420
Indeno(1,2,3-c,d)pyrene	304	501

Time mm:ss	Flow mL/min	A (%)	B (%)
00:01	1.3	80	20
02:30	1.3	80	20
05:00	1.3	50	50
15:00	1.3	0	100
28:00	1.3	0	100
30:00	1.3	80	20
32:00	1.3	80	20

## RESULTS

The following samples are prepared using triphenylene as Internal Standard (IS).

- PAH standard solutions: See table 3.
- Ground water samples:
  - low concentration level of PAH
  - high concentration level of PAH

PAH standards (µg/L)	blank	1	2	3	4	5	6	7	8	9	10
Naphthalene	0.000	0.030	0.060	0.120	0.240	0.600	1.200	2.400	4.800	9.600	30.000
Acenaphthene	0.000	0.010	0.020	0.040	0.080	0.200	0.400	0.800	1.600	3.200	
Fluorene	0.000	0.010	0.020	0.040	0.080	0.200	0.400	0.800	1.600	3.200	
Fenanthrene	0.000	0.013	0.025	0.050	0.100	0.250	0.500	1.000	2.000	4.000	
Anthracene	0.000	0.005	0.010	0.020	0.040	0.100	0.200	0.400	0.800	1.600	
Fluoranthene	0.000	0.043	0.085	0.170	0.340	0.850	1.700	3.400	6.800	13.600	
Pyrene	0.000	0.030	0.060	0.120	0.240	0.600	1.200	2.400	4.800	9.600	
Benzo(a)anthracene	0.000	0.008	0.015	0.030	0.060	0.150	0.300	0.600	1.200	2.400	
Chrysene	0.000	0.010	0.020	0.040	0.080	0.200	0.400	0.800	1.600	3.200	
Benzo(b)fluoranthene	0.000	0.030	0.060	0.120	0.240	0.600	1.200	2.400	4.800	9.600	
Benzo(k)fluoranthene	0.000	0.003	0.006	0.012	0.024	0.060	0.120	0.240	0.480	0.960	
Benzo(a)pyrene	0.000	0.006	0.012	0.024	0.048	0.120	0.240	0.480	0.960	1.920	
di benzo(a,h)	0.000	0.015	0.030	0.060	0.120	0.300	0.600	1.200	2.400	4.800	
Benzo(ghi)	0.000	0.030	0.060	0.120	0.240	0.600	1.200	2.400	4.800		
Indeno(1,2,3-c,d)	0.000	0.120	0.240	0.480	0.960	2.400	4.800	9.600	19.200		

The PAH standard solutions were processed using the newly developed XLC method and plotted against the NEN-EN-ISO 17993 method (see figures 4).

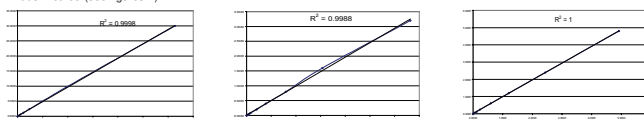


Figure 4: Correlation curve XLC-method vs. NEN-method using UV detection of Naphthalene, Acenaphthene and Benzo(ghi)perylene.

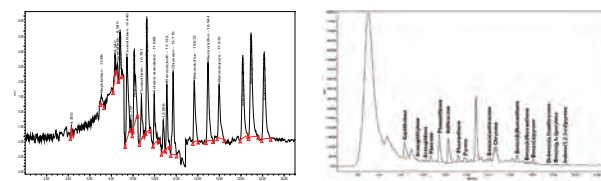


Figure 5: Chromatograms of ground water samples containing a low and high concentration level, respectively, of PAH using UV detection. The resolution of the individual compounds is presented in table 5.

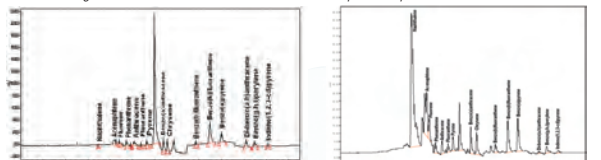


Figure 6: Chromatograms of ground water samples containing a low and high concentration level, respectively, of PAH using fluorescence detection.

The detection limits are lower (as low as 0.5 ppt) using fluorescence detection for all PAH compounds except acenaphthalene that can only be detected by UV detection (all individual detection limits are presented in table 4).

The XLC Method was validated by determining the Coefficient of Variation (CV) of eight injections containing a low concentration range of PAH and eight injections containing a high concentration range of PAH. The CV of the individual PAH are presented in table 4.

Components	Correlation (R)	Detection limit (ng/L)		vCr low level (n=8)		vCr high level (n=8)		Recovery low level (%)		Recovery high level (%)		Resolution	
		Flu	UV	Flu	UV	Flu	UV	Flu	UV	Flu	UV		
Naphthalene	0.9993	0.9998	5	9	4.2	7.8	2.3	4.2	98	96	96	93	3.6
Acenaphthalene	0.9997	--	--	7	6.7	--	3.3	--	92	--	109	5.0	
Acenaphthene	0.9988	0.9999	3	4	4.8	10.6	2.5	4.9	99	106	95	95	1.5
Fluorene	0.9999	0.9997	2	6	4.2	4.3	1.2	2.9	97	95	93	98	3.3
Fenanthrene	0.9995	0.9992	3	5	5.2	7.8	1.1	4.0	99	96	92	93	3.7
Anthracene	0.9999	0.9999	2	10	4.4	13.8	1.6	7.8	99	103	100	87	3.8
Fluoranthene	0.9989	0.9999	4	6	2.7	3.4	0.7	2.9	98	96	95	98	3.3
Pyrene	1.0000	0.9997	4	7	2.5	2.5	2.0	4.1	101	98	97	106	2.9
Benzo(a)anthracene	0.9999	0.9994	2	5	2.3	6.6	2.0	6.3	101	97	96	104	3.4
Chrysene	1.0000	1.0000	2	5	4.3	3.1	0.7	3.0	99	98	97	105	2.5
Benzo(b)fluoranthene	1.0000	1.0000	2	7	1.2	2.3	1.0	3.0	97	96	97	83	10.8
Benzo(k)fluoranthene	0.9999	0.9994	0.5	7	2.0	13.1	1.0	13.6	97	98	97	95	4.8
Benzo(a)pyrene	1.0000	0.9999	5	5	6.2	11.8	12.1	11.4	91	104	107	104	4.4
Dibenzo(a,h)anthracene	1.0000	0.9999	1	9	4.3	13.1	6.1	3.3	94	96	100	83	6.7
Benzo(ghi)perylene	1.0000	0.9994	3	7	10.7	10.0	4.7	3.0	110	105	103	101	4.3
Indeno(1,2,3-c,d)pyrene	0.9993	0.9881	5	6	3.8	3.4	2.8	2.9	95	95	97	105	2.7

## CONCLUSIONS

Polycyclic Aromatic Hydrocarbons (PAH) are in general difficult to analyze, especially when these compounds are present in groundwater or surface water.

From this study it is concluded that using the Symbiosis™ system, careful pretreatment of the glass sample containers, with isopropanol as a modifier and UV- or fluorescence detection, PAH can be analyzed fully automated with excellent recoveries (92 to 107 %) and acceptable reproducibility (CV = 0.7 to 13.8 %) in groundwater matrix. The pre-concentration of 10 (up to 100) mL of sample volume, consisting of 25 % isopropanol and 75 % water, enables the determination of analyte concentrations as low as 2 ng/L with fluorescence detection. The developed method shows excellent correlation (r=0.9881-1.0000) with the NEN-EN-ISO-17993 and proved to be an excellent alternative for the normally used liquid-liquid extraction method.

## REFERENCES

NEN-EN-ISO 17993: Water quality - Determination of 15 polycyclic aromatic hydrocarbons (PAH) in water by HPLC with fluorescence detection after liquid-liquid extraction. This International Standard specifies a method using liquid-liquid extraction and high performance liquid chromatography (HPLC) with fluorescence detection for the determination of 15 selected PAH (see table 1) in drinking water and ground water in concentrations above 0,005 µg/L (for each single compound) and surface waters in concentrations above 0,01 µg/L. This method is, with minor modifications, also suitable for the analysis of waste water. The method may be applicable to other PAH, but needs to be validated for each additional compound.