

Research Article

Quantification of Polynuclear Aromatic Hydrocarbons in Retention Pond Waters Using Gas Chromatography-Tandem Mass Spectrometry

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Abstract

The individual quantification of 16 Polynuclear Aromatic Hydrocarbons (PAHs) in French retention pond water samples is reported. The full analytical protocol including Solid Phase Extraction (SPE) and tandem mass spectrometry detection allowed the detection limit in the range of 0.5–5 ng L⁻¹. PAHs quantification is achieved using a seven point calibration plot with internal calibration. Real sample analysis proved that the procedure is convenient for PAHs determination in environmental water. Due to the high levels of PAHs in retention pond waters care must be taken when considering the reuse of such samples.

Keywords: Triple quadrupole mass spectrometry; PAHs; Water; Retention ponds

Introduction

During the last decades, tremendous improvements in analytical instrumentation have allowed a better detection of pollutant in diluted systems such as water. However monitoring of pollution of ecosystems is still an ongoing challenge. Due to their mutagenic and carcinogenic properties [1,2], PAHs are included in the US Environmental Protection Agency (EPA) priority pollutant list [3]. PAHs are derived from natural or anthropogenic sources. Natural sources include forest and prairie fires [4] or post-depositional transformations of biogenic precursors [5]. Anthropogenic sources include combustion of fossil-fuel [6], long-range atmospheric transport of PAHs [7,8]; urban runoff [5] and spillage of petroleum and its refined products [9]. These organic pollutants are ubiquitous. They are found, for example, in food [10], water [11], soils, sediments [12] and air particulates [13]. In recent years, they have received considerable attention as, in addition to their toxicity, they are highly persistent and can accumulate in environmental aqueous systems. Presence of PAHs in water is now part of environmental concern.

Over the past few decades, retention ponds have been built alongside highways and motorways with the initial role of controlling water flow during rainstorms. Urban and road runoff waters contribute to pollution of receiving ponds, streams and lakes. Previous studies show that PAHs are highly concentrated in sludge of some retention ponds, i.e. 400-900 times the Dutch threshold value for polluted soils [14,15]. Thus, concentrations of such compounds in water retention ponds must be evaluated.

Many analytical techniques have been developed for the determination of organic compounds in water samples. PAHs can be extracted from aqueous matrices by various methods such as liquid/liquid extraction [11-16], Solid Phase Extraction (SPE) [17,18], Solid-Phase Micro Extraction (SPME) [19,20] or Stir Bar Sorptive Extraction (SBSE) [21,22]. Detection is performed by Gas Chromatography (GC)

or High Performance Liquid Chromatography (HPLC). Detection limits for the EPA-PAHs depend on the selected concentration method and the detection. SPE coupled to HPLC / fluorescence is the most common procedure to quantify PAH traces in water with a detection limit in the range of ngL⁻¹. Compared to single stage MS modes, tandem Mass Spectrometry (MS-MS) offers a higher degree of sensitivity. MS-MS enables the analysis of organic molecule trace levels in the presence of interfering compounds without losing identification capability due to a drastic reduction of the background signal [23,24]. Triple quad technology directly enables the recovery of three different mass spectra (precursor ions, product ions and neutral fragments).

This paper presents GC-MS-MS as a sensitive alternative to HPLC for the analyses of PAHs in environmental samples. Validation parameters such as linearity, reproducibility, limits of detection and quantification are determined. Finally, the procedure is applied to the quantification of PAHs from two French retention pond waters.

Material and Methods

Chemicals and reagents

HPLC grade solvents (acetonitrile, dichloromethane and methanol) are purchased from Merck (Darmstadt, Germany) and are re-distilled before use. Water is purified on a Milli-Q SP reagent water system (Millipore, Bedford, MA, USA). Standard mixture of the 16 priority PAHs, at 10 mgL⁻¹ in acetonitrile, is purchased from Sigma-Aldrich. The surrogate internal standard is a mixture containing perpetuated PAHs (²H₈] naphthalene (Np-d₈), [²H₁₀] acenaphthene (Ace-d₁₀), [²H₁₀] phenanthrene (Ph-d₁₀), [²H₁₂] chrysene (Chry-d₁₂), [²H₁₂] perylene (Pe-d₁₂) purchased from Sigma-Aldrich.

SPE is performed onto Waters Oasis HLB Sorbent cartridges (6cc/200mg) purchased from Waters. Stir bars (Twisters), 10 mm long, coated with a 0.5 mm film thickness layer (24 L) of PDMS, are from Gerstel (Mulheim, Germany).

Table 1: GC-MS-MS segment program with fragment ions chosen for each PAH.

Segment time (min)	Compound	Retention time (min)	Precursor ion (m/z)	Channel	Product ion	CID voltage (eV)
0-12	Naphthalene	9.62	128	3	127	10
				2	102	20
				1	78	20
12-15	Acenaphthylene	13.63	152	1	151	10
12-15	Acenaphthene	14.09	154	2	153	10
15-16	Fluorene	15.45	166	1	165	10
16-20	Phenanthrene	17.96	178	2	177	10
				1	152	20
16-20	Anthracene	18.10	178	2	177	10
				1	152	20
20-24	Fluoranthene	21.11	202	2	201	10
				1	200	35
20-24	Pyrene	21.70	202	2	201	10
				1	200	35
				3	227	10
24-26.50	Benz[a]anthracene	24.89	228	2	226	35
				1	202	35
				3	227	10
24-26.50	Chrysene	24.99	228	2	226	35
				1	202	20
				2	250	35
26.50-30	Benzo[b]fluoranthene	27.55	252	1	226	35
				2	250	35
26.50-30	Benzo[k]fluoranthene	27.61	252	1	226	35
				2	250	35
26.50-30	Benzo[a]pyrene	28.29	252	1	226	35
				2	250	35
30-33	Indeno[1,2,3-cd]pyrene	30.79	276	1	275	10
30-33	Dibenzo[a,h]anthracene	30.85	278	4	277	35
				3	276	10
30-33	Benzo[ghi]perylene	31.47	276	1	275	10

Sample description

The Wissous pond was created in 1999. Located in the industrial area of Villemilan (France), it drains the A6 motorway area. The Saint Joseph pond is located at the North of Nantes (on the French West coast). It was created in the mid of the 20th century; it is of urban type, draining the St Joseph district.

Solid phase extraction

SPE cartridges are conditioned with 5mL dichloromethane and 5mL methanol. Before loading on cartridges acetonitrile is added to avoid PAHs adsorption upon glassware. Standard solutions and water samples (50mL) are loaded at a flow rate of 1 mL.min⁻¹. Then the cartridge is rinsed with 5 mL Milli-Q water. Organics are extracted using 8 mL dichloromethane, evaporated and reconstituted in 200 µL dichloromethane. Pure dichloromethane processed through SPE units is used as the procedural blank.

Stir bar sorptive extraction

Stir bars extraction is performed according to Garcia Falcon et al. (2004) on PDMS coated bars. PAHs desorption is performed with an ultrasonic device (Sonorex Digital 10P, Bandelin GmbH, Germany) for 10 minutes in 200 µL acetonitrile.

Gas chromatography-mass spectrometry

GC-MS-MS analyses are carried out on a Varian 3800 GC gas chromatograph coupled to a Varian 1200 L triple Quadrupole mass spectrometer (Varian, Les Ulis, France). The GC system is equipped with a PTV injector with a SGE Liner. All analysis is carried out in split less mode at the temperature of 300 °C. The split less valve is closed for 0.75 min, and then operated in the split mode at 20 mL/

min. All injections volumes are 1µL.

Separation is achieved on a 30 m x 0.25 mm i.d.VF-5ms (Factor Four, Varian, France) coated with 5% phenyl, 95 % dimethylpolysiloxane (film thickness 0.25µm). The temperature program is 1 min at 50 °C, 10°C/min up to 100 °C, 9°C/min up to 310 °C and temperature is finally hold for 4 minutes at 310 °C. The carrier gas is Helium and the column head pressure is 15 psi to reach a constant flow rate of 1.2 ml/min.

The Quadrupole mass spectrometer is operated under electron impact ionization (70eV). The filament emission current is 500µA. The source and transfer line temperatures are at 250°C and 310°C, respectively. The electron multiplier is set at 1880 V. The acquisition mode chosen for mass spectrometry analysis is MRM mode (Multiple Reaction Monitoring). The scantime is 0.3 s/scan. Peak detection and integration are carried out using Varian Workstation version 6.3. Precursor ions are isolated using 0.7 amu isolation window and subjected to Collision Induced Dissociation (CID) in the second Quadrupole (Q2). The dissociation is induced by collisions with neutral target species of the collision gas: Argon. In the third step, ions are transmitted in the third Quadrupole (Q3) for separation. In MRM mode the fragment ions are also selected in the third quadrupole. Different collision energies and multiple transitions are studied for each compound.

PAHs quantization is achieved using a seven point calibration plot with internal calibration over the linearity GCMS-MS range (1-100 µg/L) established with 40 µg/L internal perpetuated standards and 1,5,10,25, 50, 75 and 100µg/L PAH solutions. Limit of Detection (LOD) and Limit of Quantification (LOQ) are estimated at the lowest

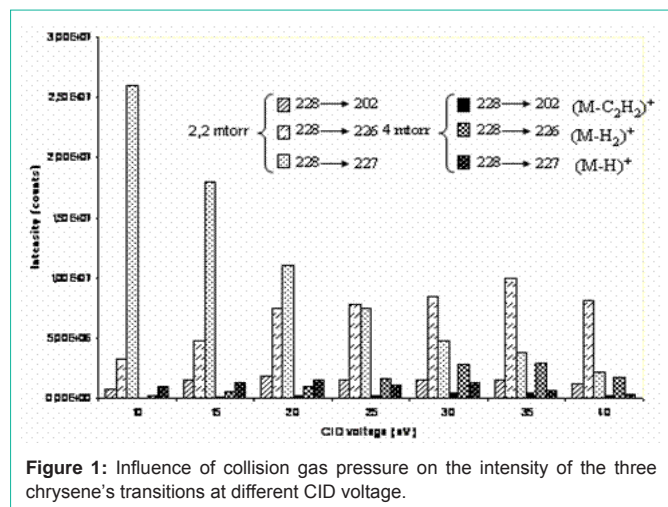


Figure 1: Influence of collision gas pressure on the intensity of the three chrysene's transitions at different CID voltage.

concentration of analytes having clear discerned peaks with Signal to Noise ratio (S/N) of 3 and 10 respectively.

Results and Discussion

Mass spectrometry and quantification

In order to improve sensitivity and selectivity, the MRM acquisition mode is used. In a triple quadrupole instrument, the ions generated in the EI source are transmitted to the first Quadrupole (Q1) where precursor ions are selected. A preliminary study is carried out in MS-MS mode under full scan conditions on Q3 in order to determine the fragment ions to be used for further MRM experiments. For each PAH from one to three ions are observed. In most cases these ions are molecular ions (M^+), ($M-H^+$), ($M-H_2^+$) or ($M-C_2H_2^+$) fragment ions. The collision energy is selected for each PAH to obtain the maximum signal dissociation of the product ions

(table 1). The optimization of the CID voltage of each transition is performed using multiple scan function in different channel for each retention time window (segment). The influence of CID voltage is studied from 10V to 40 V at 5V steps. The example for chrysene is given in Figure 1: 10eV for ($M-H^+$) (227), 35eV for ($M-H_2^+$) (226) and 20 eV for ($M-C_2H_2^+$) (202). The argon collision gas pressure is set at 2.2 mtorr. The higher pressure of 4 mtorr is discarded because of the lower sensibility obtained at each transition (Figure 1).

LOD and LOQ of GCMSMS analysis are in the range of HPLC/Fluorescence limits and are better than those obtained with ion trap tandem mass spectrometry [25].

SPE and SBSE extractions

To study environmental samples from urban and motorway retention ponds we first evaluate the extraction technique on a wide concentration range. SPE and SBSE are compared by spiking 50 mL Milli-Q water samples with the standard PAH solution in order to study concentrations ranging from 5 to 100 ng/L. Stir Bar Sorptive Extraction is a technique which has been introduced by Baltussen et al. [21], using stir bars (magnetic stirring rod incorporated in a glass jacket) coated with polydimethylsiloxane (PDMS). This phase is previously used for Solid Phase Micro Extraction (SPME) but due to a larger amount of PDMS relative to the SPME fiber, SBSE enables to increase recovery of analytes. Temperatures (room temperature, 40°C, 60°C, 80°C and 100°C) and adsorption time (from 1 to 10 hours) are studied (Figure 2). Compared to SPE, SBSE avoids the evaporation step which could explain the better recoveries (40%) for the lower molecular weight compounds at the lower concentration. For 5 ngL⁻¹ SPE does not allow a recovery higher than 15% whereas 45% are recovered at 100 ngL⁻¹. However the best conditions achieved after optimization are time and energy consuming steps (60°C and 6 hours). For the high molecular weight compounds SBSE recoveries

Table 2: Validation parameters of the SPE-GC-MS-MS method.

Compound	SPE	SBSE	Detection limit including SPE	Quantitation limit including SPE	Calibration curves $y=ax+b$	R ²
	Recovery %	Recovery %	(ngL ⁻¹)	(ngL ⁻¹)		
Naphthalene	30	40	1.40	3.50	10.3558x	0,997
Acenaphthylene	30	40	1.40	6.60	4.0393x	0,999
Acenaphthene	30	40	1.40	6.60	2.7364x+0.0688	0,995
Fluorene	27	53	1.40	3.70	0.8649x	0,998
Phenanthrene	72	63	1.50	1.50	6.3735x+0.3451	0,999
Anthracene	75	62	0.50	5.50	4.7588x+0.4	0,999
Fluoranthene	82	75	1.40	2.50	1.9916x+0.32	0,972
Pyrene	85	75	0.50	2.50	2.2094x	0,973
Benzo[a]anthracene	94	55	1.00	4.20	1.1434x	0,993
Chrysene	95	47	1.00	4.20	1.3334x	0,996
Benzo[b]fluoranthene	91	53	3.00	5.50	2.3022x	0,983
Benzo[k]fluoranthene	94	48	3.00	5.50	2.6742x	0,997
Benzo[a]pyrene	91	45	4.00	6.60	2.4684x	0,995
Indeno[1,2,3-cd]pyrene	90	44	3.00	5.80	4.5376x	0,996
Dibenzo[a,h]anthracene	94	37	4.00	6.40	1.3937x	0,987
Benzo[ghi]perylene	91	32	2.00	4.40	7.3390x	0,998

Mean recovery obtained for three samples and for three concentrations 5, 25 and 100 ngL⁻¹

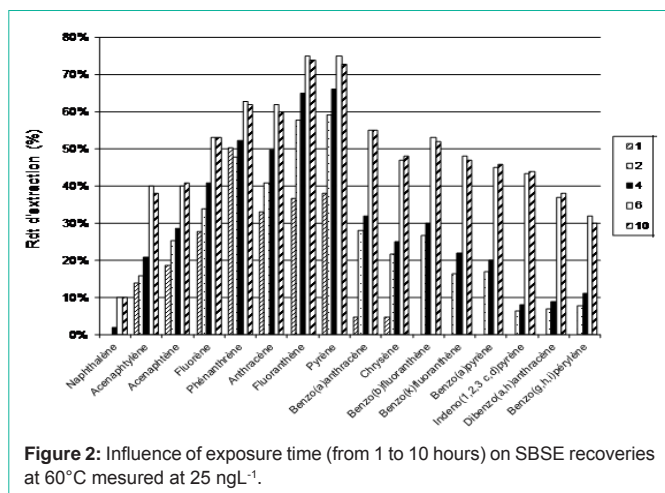


Figure 2: Influence of exposure time (from 1 to 10 hours) on SBSE recoveries at 60°C measured at 25 ngL⁻¹.

are similar to those previously described in literature (from 40 to 50% with RSD (n=9) <10%) [22]. But are far lower than those achieved in present SPE experiments (average recovery by SPE 90%, Table 2). Regarding the diagnostic values of high molecular weight compounds we choose the SPE concentration method.

For the study of natural samples, the SPE concentration procedure thus is performed. For natural waters, including SPE concentration step, LOD ranges between 0.50 and 4.00 ng/L (Table 2) which is ten times better than SPE GC FID validated method for similar water samples [26]. All standard solutions are injected 5 times to estimate intra-day (RDS<4%) and inter-day (RSD <6%) precisions of the SPE-GC-MS-MS method (Table 2).

Analysis of samples from water retention ponds

In water retention ponds, PAHs may be present as very complex mixture with many isomers. The use of a sensitive and selective method is then needed. The Wissous and St Joseph water samples are analyzed by the present SPE-GC-MS-MS method. Results are presented in Table 3 and illustrated on Figure 3. Low molecular weight PAHs are not detected in the two studied samples. Overall PAH concentration (sum of the 16 EPA-HAPs) is 3 times higher in Wissous sample than in Saint Joseph one (0.31 and 0.98 µg/L respectively). Ratios listed in table 3 indicate a pyrogenic origin for Saint Joseph PAHs whereas some petroleum origin is inferred for the Wissous ones [9]. Thus, chrysene (Chry) and benz [a] anthracene (B[a] A), often looked upon as the most toxic of the PAHs, are present in both samples. Regarding the value of the ratio Bz[a]A / (Bz[a]A + Chry) the dominant source of these pyrogenic PAHs is combustion (burning of wood or coal, or natural forest fires) (0.79>0.5) in St Joseph sample but is more debatable in Wissous one (0.56) [27]. The traffic pollution is however confirmed by the B[a]Pyr / B[ghi]Pe ratio close to the 0.3-0.44 range, characteristic of traffic emission [27]. This pollution is already described in Wissous sludge [14]. Part of the PAHs found in Wissous water samples could come from the two motorways located in the neighborhood. Indeed, in such retention ponds, urban run-off could contain PAHs from gasoline and oil drips or spills exhaust products, tyre particles, and bitumen's from road surfaces [5]. This result implies that heavy metal pollution linked to traffic could also be assumed. Furthermore, high concentration of B[a]Pyr (strongly carcinogen) in Wissous could rise serious ecological and human health risks.

Table 3: PAHs distribution of Saint Joseph and Wissous water samples.

Compound	Saint Joseph ngL ⁻¹	Wissous ngL ⁻¹
Naphtalene	*ND	ND
Acenaphthylene	ND	ND
Acenaphthene	ND	ND
Fluorene	58	60
Phenanthrene	12	ND
Anthracene	ND	ND
Fluoranthene	14	18
Pyrene	ND	ND
Benzo(a)anthracene	22	94
Chrysene	6	74
Benzo(b)fluoranthene	22	110
Benzo(k)fluoranthene	28	130
Benzo(a)pyrene	22	96
Indeno(1,2,3 c,d)pyrene	64	134
Dibenzo(a,h)anthracene	ND	104
Benzo(g,h,i)perylene	66	158
Σ 16 PAH	314	978
Bz[a]A / (Bz[a]A + Chry)	0.79	0.56
B[a]Pyr / B[ghi]Pery	0.33	0.61
ΣB[b]Fl, B[k]Fl, In and B[g,h,i]Pery	0.18	0.53

*ND: not detected

As streams and rivers, ponds are frequently used for potable water supply, contamination of watercourses, where water re-use is practiced, is particularly undesirable. European community directive, dealing with drinking water, has stated a maximum level for benz[a] pyrene (B[a]Py, strongly carcinogen) at 10 ng/L. In addition to be classified as drinking water, the sum of benz[b]fluoranthene (B[b] Fl), benz[k]fluoranthene (B[k]Fl), benzo[g,h,i] perylene (B[g,h,i]Pe) and indeno[1,2,3-cd] pyrene (In), must not reach 100 ngL⁻¹ [28]. For Wissous and Saint Joseph the threshold values are by far exceeded. Thus, care must be taken if waters of the two samples have to be re-used.

Conclusion

As PAHs are persistent organic pollutants with highly toxic properties, monitoring their concentration in water samples is important for environment and of course for human health care. So, to analyze PAHs in such media a very selective and sensitive method has to be developed. Usually done by HPLC, in this paper, quantification is performed by gas chromatography coupled with tandem mass spectrometry. Before analysis water samples need to be concentrated by SPE which is the most efficient phase extraction to recover PAHs in the present working conditions. The GC-MS-MS methods enable to obtain low detection and quantization limits due to the ability to follow exclusively parents and fragments ions. The global method is successfully applied to water from retention ponds. The two studied samples exhibit high concentrations of natural and anthropogenic PAHs.

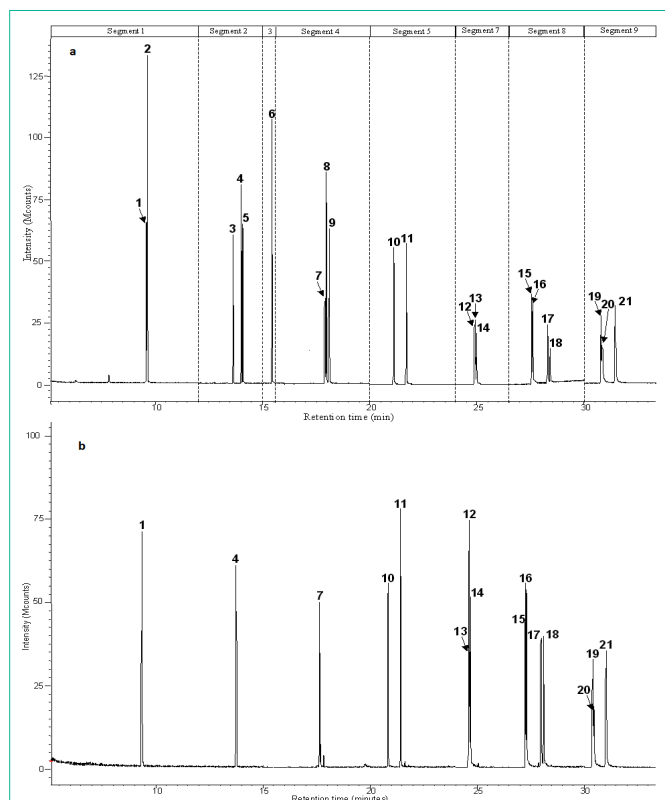


Figure 3: Chromatogram of the standards SEPARATION and b Chromatogram of PAH from water of Wissou sample. Time segment program illustration. 1 Np-d₈, 2 Np, 3 Acy, 4 Ace-d₁₀, 5 Ace, 6 F, 7 Ph-d₁₀, 8 Ph, 9 An, 10 Fl, 11 Py, 12 B[a]An, 13 Chry-d₁₂, 14 Chry, 15 B[b]Fl, 16 B[k]Fl, 17 B[a]Py, 18 Pe-d₁₂, 19 In, 20 Db[a,h]An and 21 B[g,h,i]Pe.

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