Short communication

Simultaneous Determination of Nitrophenols and Poly-Aromatic Hydrocarbons in Aquatic Samples by Solid Phase Extraction and HPLC Analysis

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Abstract

A method for the simultaneous extraction and HPLC analysis of nitrophenols and poly-aromatic hydrocarbons (PAHs) in aquatic samples is presented. The efficiency of the solvent extraction and the solid phase extraction (SPE) is compared. The results showed that the polymeric SPE sorbent is the best compromise for the simultaneous extraction of the tested compounds taking into consideration the lipophilicity of PAHs and the polarity of nitrophenols. The best results were obtained on polymeric Strata X sorbent, elution with dichloromethane followed by elution with methanol. Silica gel C18 sorbent is improper for nitrophenols due to the strong interactions with the residual hydroxyl groups of silica gel. The developed SPE-HPLC method was successfully applied for the analysis of these pollutants in different water samples.

Keywords: Nitrophenols, poly-aromatic hydrocarbons, solid phase extraction, high performance liquid chromatography, aquatic sample

1. Introduction

Nitrophenols and poly-aromatic hydrocarbons (PAHs) belong to the most potent environmental pollutants which occur in all environmental compartments. PAHs are formed during fuel combustion and from different industrial and domestic sources,[1] while nitrophenols are a result of car emissions and the photochemical reactions of nitrogen oxides with hydrocarbons.

These compounds have been identified as having toxic and hazardous properties, some of them, like benzo(a)pyrene, indeno(l,2,3-c,d)pyrene and benzo(b)fluoranthene, being carcinogenic both to animals and humans.[2] Different studies, have been proved that nitrophenols are toxic to algae and fish and can interrupt human metabolism at levels of 10 mM,[3,4] while their presence in the rain leaded to the continuous degradation of forests in Central Europe.[5] Moreover, due to the high toxicity, some nitrophenols and PAHs, are included in the European Union REACH SIN List,[6] and have to be monitored in all environmental compartments.

The poly-aromatic hydrocarbons are neutral compounds of high lipophilicity (log K_{ow} of tested compounds between 3.3 and 5.99) while the nitrophenols are acidic compounds with a high polarity due to the presence in their molecule of hydroxyl and nitro groups (log K_{ow} between 1.37 and 2.00). Usually, due to their different physicochemical properties, these two classes of organic compounds are separately analyzed using different extraction methods and chromatographic techniques.[4,6–25]

The most used techniques for the extraction of these compounds from aquatic matrices involve solvent extraction and solid phase extraction. The classical solvent extraction needs specific protocols for each class of compounds. PAHs are extracted with nonpolar (hexane, chloroform) or polar aprotic (dichloromethane) solvents,[14] while nitrophenols are extracted with polar solvents such as diethyl carbonate.[15]

Recently new solvent micro extraction methods such as dispersive liquid-liquid extraction (DLLM) dispersive liquid–liquid microextraction method based on solidification of a floating organic droplet (DLLME-SFO) or ultrasound-assisted emulsification-microextraction (USAEME) etc. have been developed for the extraction of PAHs[16–18] and nitrophenols,[19] form aquatic matrices. These methods have the main advantages in speed and simplicity of operation, low sample volume, high enrichment factor and low cost, but unfortunately the protocols of extraction are optimized for the extraction of the compounds with similar physicochemical properties.

Regarding the SPE of these compounds from aquatic matrices, different protocols which involve SPE,[7–11,13,20] solid phase micro extraction[21–23] or stir bar sorbtive extraction[24,25] have been developed. In all the cases, the conditions of extraction and the solvents used for elution are different, and depend on the classes of the compounds which were retained on the sorbent. Thus, SPE of PAHs involves non polar solvent (hexane) or polar aprotic (dichloromethane) solvents for their elution from the sorbent,[7,9,13] while SPE of nitrophenols needs polar solvents (methanol, acetone or ethyl acetate) for elution.[8,10,11,20] Even in the case of thermal desorbtion[21–25] these compounds cannot be analyzed together due to the selectivity of the sorbent used for extraction.

The aim of this study was to develop a method for the simultaneous extraction and HPLC analysis of some nitrophenols and PAHs from aquatic samples. To find the most suitable method for the extraction of the target compounds, the solvent extraction and the solid phase extraction were compared. The developed method was applied for the analysis of these compounds in different water samples collected from the Cluj-Napoca area, Romania.

2. Experimental

2. 1. Materials and Methods

Acetonitrile, methanol, dichloromethane, ethyl acetate, glacial acetic acid and hydrochloric acid, all in high purity where purchased from Supelco, USA.

For the optimization of the extraction and analysis methods a standard solution (MIX13) containing eight PAHs (naphthalene, Naph; acenaphthene, Ace; fluorene, Flu; phenanthrene, Phen; anthracene, Ant; pyrene, Pyr; benzo(a)anthracene, BaA; benzo(a)pyrene, BaP) each in concentration of 5 µg mL^{-1}, 25 µg mL^{-1} of 2- nitrophenol (2-NP), 3-nitrophenol (3-NP), 2,6-dinitrophenol (2,6-DNP) respectively and 50 µg mL^{-1} of phenol (Ph) and 4-nitrophenol (4-NP) respectively purchased from Merck and Sigma Aldrich was prepared in a mixture of water:acetonitrile 30:70 (*v/v*).

The calibration curves were built at five concentration levels of MIX13, as follows: 1, 2.5, 5, 10, and 20 µg mL–1 of each PAH 5, 10, 25, 50, 100 µg mL^{-1} of 2-NP, 3-NP and 2,6-DNP respectively and 10, 25, 50, 100, 200 µg mL^{-1} of Ph and 4-NP respectively. The linearity of the method was expressed by the regression coefficient (*r*) of each calibration curve constructed using pick area versus concentration of tested compounds. The limit of detection (LOD) and the limit of quantification (LOQ) of tested compounds were determined using the standard deviation of the regression line (s) and the slope (S) of each calibration curve according to the equations[26]: $LOD = 3\sigma/S$ and $LOQ = 10\sigma/S$.

SPE. Two types of sorbents purchased from Phenomenex, Strata C18-U based on silica gel and Strata X based on polymer support (functionalized styrene-divinylbenzene) were tested. For this purpose, 100 mL of MilliQ water sample acidified at pH 3 with 0.1 M HCl in water, was spiked with 1 mL of MIX13 containing 5 µg of each PAH 25 µg of 2-NP, 3-NP and 2,6-DNP respectively and 50 µg of Ph and 4-NP respectively. Before extraction, the SPE cartridges were activated by washing with 5 mL methanol and 5 mL MilliQ water. The spiked water samples were passed through the cartridges with a flow rate of 2 mL min^{-1} and the retained compounds were eluted with 3 mL of three different solvents (methanol, dichloromethane and methanol–dichloromethane mixture 1:1 (*v/v*)). After elution, the extract was evaporated to dryness under nitrogen. The residue was dissolved in 1.0 mL mixture of MilliQ water–acetonitrile 1:1 (*v/v*) and analyzed by HPLC.

Liquid-liquid extraction. The extraction efficiency of two solvents, dichloromethane and ethyl acetate, was compared. For this purpose, a volume of 100 mL of the spiked water sample was extracted three times with 15 mL of each organic solvent. In the case of each solvent, the three resulted organic phases were collected, reunified in a vial and evaporated to dryness with a rotary evaporator system. Each residue was dissolved in 1.0 mL of mixture of water–acetonitrile 1:1 (*v/v*) and analyzed by HPLC.

HPLC. For the experiments a HPLC system, ABL&E Jasco model 980 equipped with PU-980 pump, column thermostat model CO 2060 Plus and UV-Vis detector model UV-975 was used. The separation of compounds was performed on C18 silica gel column, Luna C18 10A (5 µm, 250 × 4.6 mm) purchased from Phenomenex, and the detection was done at 254 nm. The column was operated in gradient mode using a mobile phase containing a mixture of acetonitrile (ACN), and water acidified 1% (*v/v*) with glacial acetic acid at a flow rate of 1 mL min^{-1}. The most suitable gradient found was the following: start with 30% ACN for 2 min then increase at 100% ACN in 20 min, keep 3 min at 100% ACN and return to the initial conditions in 2 min.

To analyze the nitrophenols and PAHs from different real water samples, 400 mL of water sample, acidified at pH 3 with a solution of 0.1 M HCl, were passed through a Strata X cartridge. The retained compounds were eluted with 3 mL of dichloromethane followed by 3 mL of methanol. The obtained extract was evaporated to dryness under nitrogen, dissolved in 1 mL mixture of water–acetonitrile 1:1 (*v/v*) and then submitted to the HPLC analysis. Different water samples (rainwater, river water, and wastewater) collected from the Cluj-Napoca area (Romania) were analyzed. The rainwater sample was collected during one month (July, 2011) using a Hallman Pluviometer, while the river and wastewater samples were collected in a polyethylene flask. All collected samples were acidified at pH = 3 with a solution of 0.1M HCl and stored at 8 °C prior to the analysis. Prior SPE extraction all water samples were filtered through 0.45 µm PTFE membrane filters (Millipore).

3. Results and Discussion

3. 1. HPLC Separation and Quantification

The obtained chromatogram of the best HPLC separation by gradient elution is shown in Figure 1 and the retention times are given in Table 1. Even if this HPLC method does not provide the baseline separation of all compounds, it can be considered satisfactory for the analysis of studied compounds in a single run, taking into consideration the obtained values for the resolution between these compounds that was calculated using the peak width at half-height[27,28] ($R_{2,6\text{-DNP}/2\text{-NP}} = 1.63$).

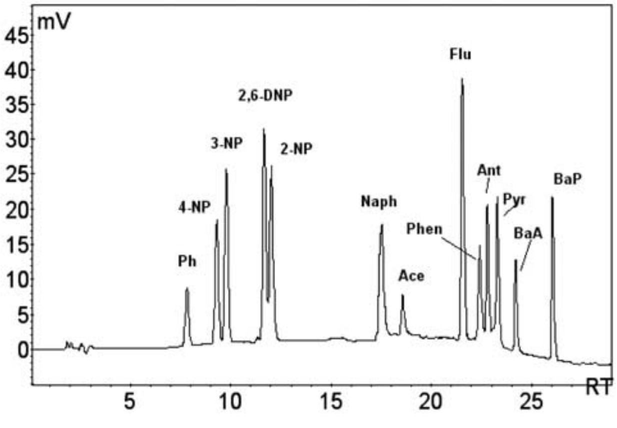

Figure 1. The HPLC chromatogram of MIX13

Regarding the performance of the developed method it demonstrated a good linearity for all studied compounds having the regression coefficients over 0.99 in the range of 1–20 µg mL^{-1} for each PAH; 5–100 µg mL^{-1} 2-NP, 3-NP, 2,6-DNP respectively and 10–200 µg mL^{-1} for Ph and 4-NP respectively. The limits of detection and quantification calculated from the calibration curves are low enough to permit the quantification of the compounds at ppb level. The lowest values of LOD and LOQ were obtained for benzo(a)pyrene and the highest for phenol (Table 1).

Table 1. Retention times, regression equations, regression coefficients, LODs and LOQs of the tested compounds

Compound	Retention time, [min]	Regression equation	Regression coefficient (*r*)	SD[a)]	LOD [µg L^{-1}]	LOQ [µg L^{-1}]
Ph	7.82	$y = 0.498x - 0.091$	0.9972	0.16	0.96	3.21
2-NP	12.03	$y = 0.291x - 0.345$	0.9987	0.06	0.62	2.06
3-NP	9.79	$y = 0.321x - 0.140$	0.9978	0.07	0.65	2.18
4-NP	9.31	$y = 0.512x - 0.094$	0.9976	0.17	1.00	3.32
2,6-DNP	11.66	$y = 0.351x - 0.324$	0.9988	0.07	0.60	1.99
Naph	17.53	$y = 1.482x - 2.545$	0.9933	0.09	0.18	0.61
Ace	18.57	$y = 1.523x - 0.226$	0.9976	0.08	0.16	0.53
Flu	21.55	$y = 1.551x - 3.241$	0.9971	0.06	0.12	0.39
Phen	22.42	$y = 1.231x - 0.900$	0.9976	0.08	0.19	0.65
Ant	22.80	$y = 1.341x - 0.268$	0.9944	0.06	0.13	0.45
Pyr	23.30	$y = 1.846x - 0.354$	0.9932	0.08	0.13	0.43
BaA	24.21	$y = 1.268x - 0.309$	0.9980	0.06	0.14	0.47
BaP	26.05	$y = 1.215x - 0.330$	0.9966	0.05	0.12	0.41

[a)] Standard deviation

3. 2. Simultaneous Extraction of Nitrophenols and PAHs

The efficiency of the extraction of nitrophenols and PAHs from aquatic samples by liquid-liquid extraction and solid phase extraction were compared. All experiments were repeated three times and the standard deviation (SD) of each compound was calculated.

Regarding the SPE efficiency of two tested sorbents, Strata C18-U and Strata X, the best results were obtained on Strata X cartridge both for nitrophenols and PAHs. As it can be seen in Table 2 the extraction efficiency depends on the type of sorbent, the properties of compounds as well as the solvent used for elution.

Thus, on Strata X cartridge we obtained recoveries over 80% with elution with dichloromethane (3 mL) followed by elution with methanol (3 mL). The PAHs, compounds of high lipophilicity and high values for log K_{ow}, were eluted with dichloromethane, while the nitrophenols, hydrophilic compounds with low values for log K_{ow}, were eluted with methanol. For Strata C18-U, the obtained recoveries were lower (64.53% for acenaphthene and 91.77% for 3-nitrophenol) compared with those obtained on polymeric Strata X cartridge (80.04% for acenaphthene and 95.13% for 3-nitrophenol). The better recoveries for nitrophenols, polar compounds with low molecular mass, were obtained on the polymeric Strata X sorbent and can be explained by the fact that porous polymer base sorbent has higher cavity term compared with octadecyl silica gel sorbent which is preferred for the nonpolar and weakly polar compounds.[29]

In the case of dichloromethane–methanol 1:1 (*v/v*) mixture, good recoveries were obtained for 3-NP and 4-NP nitrophenols (over 80%) while for the other com-

Table 2. The recovery of studied compounds (%) obtained by SPE and L-L extraction respectively (mean value of three extractions)

Compound	**Solid phase extraction [%]**				**Solvent extraction [%]**	
	Strata C18-U		**Strata X**		**CH_2Cl_2**	**Ethyl acetate**
	MeOH–CH_2Cl_2 1:1 (*v/v*)	**CH_2Cl_2 followed by MeOH[a)]**	**MeOH–CH_2Cl_2 1:1 (*v/v*)**	**CH_2Cl_2 followed by MeOH[a,b)]**		
Ph	64.03 ± 1.44	75.67 ± 1.65	67.37 ± 0.95	85.22 ± 0.98	51.14 ± 2.96	15.63 ± 4.25
2-NP	65.23 ± 1.05	77.03 ± 2.00	71.60 ± 1.20	84.61 ± 1.02	71.30 ± 3.30	25.29 ± 4.88
3-NP	81.43 ± 1.15	91.77 ± 1.75	83.13 ± 1.05	95.13 ± 1.04	72.10 ± 3.90	26.20 ± 4.01
4-NP	81.57 ± 0.95	90.07 ± 1.01	82.80 ± 1.00	93.23 ± 0.99	56.43 ± 5.05	26.41 ± 6.26
2,6-DNP	68.37 ± 0.86	76.27 ± 0.75	69.10 ± 0.70	87.51 ± 0.64	55.50 ± 4.50	19.23 ± 3.00
Naph	62.33 ± 1.26	89.10 ± 1.10	73.20 ± 0.80	97.14 ± 2.50	70.59 ± 2.59	44.63 ± 4.14
Ace	45.27 ± 1.25	64.53 ± 0.65	64.12 ± 0.88	80.04 ± 2.17	68.40 ± 2.80	30.43 ± 4.77
Flu	53.53 ± 1.37	70.10 ± 1.10	65.23 ± 0.75	90.49 ± 1.07	84.37 ± 2.45	39.23 ± 3.64
Phen	64.17 ± 1.80	80.25 ± 0.75	75.10 ± 1.10	92.04 ± 1.23	72.80 ± 3.20	36.56 ± 3.58
Ant	67.07 ± 2.00	84.17 ± 0.80	77.90 ± 0.85	94.24 ± 1.78	85.50 ± 2.70	38.34 ± 4.20
Pyr	63.33 ± 2.25	84.43 ± 1.05	74.22 ± 0.83	94.42 ± 1.65	82.70 ± 4.10	31.83 ± 7.73
BaA	64.80 ± 1.25	84.77 ± 0.85	75.75 ± 1.13	95.09 ± 2.01	86.23 ± 2.09	29.67 ± 4.70
BaP	63.57 ± 0.95	84.50 ± 1.10	77.24 ± 0.86	92.53 ± 2.26	88.57 ± 2.71	28.10 ± 5.14

[a)] 5 mL CH_2Cl_2 followed by 5 mL MeOH; [b)] The best recovery of tested compounds.

pounds the recoveries were unsatisfying,[30] less than 80% for the both Strata sorbents.

In the case of liquid-liquid extraction the results showed that this technique is not adequate for the simultaneous extraction of these two classes of compounds due to their large difference of polarity. The obtained results showed that, for dichloromethane the recovery of the compounds was generally increasing with the increasing of their log K_{ow}. Thus, the best recovery with this solvent was obtained for benzo(a)pyrene (88.57%; log K_{ow} 5.99) and the lowest recovery for phenol (51.14%; log K_{ow} 1.46). In the case of ethyl acetate all recoveries of tested compounds were situated under 50%.

3. 3. Analysis of Nitrophenols and PAHs in Different Water Samples

The developed method was applied for the analysis of nitrophenols and PAHs in different water samples (rainwater, river water, and wastewater) collected from The Cluj-Napoca area (Romania). The extraction of the target compounds from the water samples was performed using Strata X cartridges following the protocol described previously.

The results showed that the developed method can be applied for the simultaneous analysis of nitrophenols and PAHs in water samples, having a good selectivity for all studied compounds and being a suitable method for the sample enrichment and sample clean-up (Figure 2).

The amounts (μg L^{-1}) of identified nitrophenols and PAHs in analyzed water samples are presented in Table 3.

As can be seen in Table 3, phenols were identified only in rain water and wastewater samples, while PAHs were identified in all samples. The amount of identified PAHs varied from 5.65 μg L^{-1} in river water to 11.41 μg L^{-1} in wastewater being in agreement with the data from the literature [31,32].

Table 3. The concentration of the identified compounds in rainwater, river water and wastewater samples

Compound	**Concentration (μg L^{-1})**		
	Rainwater	**River water**	**Wastewater**
Ph	Nd*	Nd	9.25
2-NP	Nd	Nd	Nd
3-NP	Nd	Nd	Nd
4-NP	4.04	Nd	3.44
2,6-DNP	Nd	Nd	Nd
Naph	1.21	0.81	0.94
Ace	Nd	Nd	Nd
Flu	1.93	1.04	2.25
Phen	4.2	3.5	5.5
Ant	Nd	Nd	Nd
Pyr	0.91	0.30	0.81
BaA	0.36	Nd	Nd
BaP	0.57	Nd	1.91
Total PAHs	9.18	5.65	11.41

* Nd – not detected

4. Conclusion

The isolation of compounds having large different polarities from the aquatic samples could be performed with good recovery by SPE method using polymeric sorbent such as Strata X polymer and methanol for the elution of compounds with low log K_{ow} and dichloromethane for the elution of compounds with high log K_{ow} values.

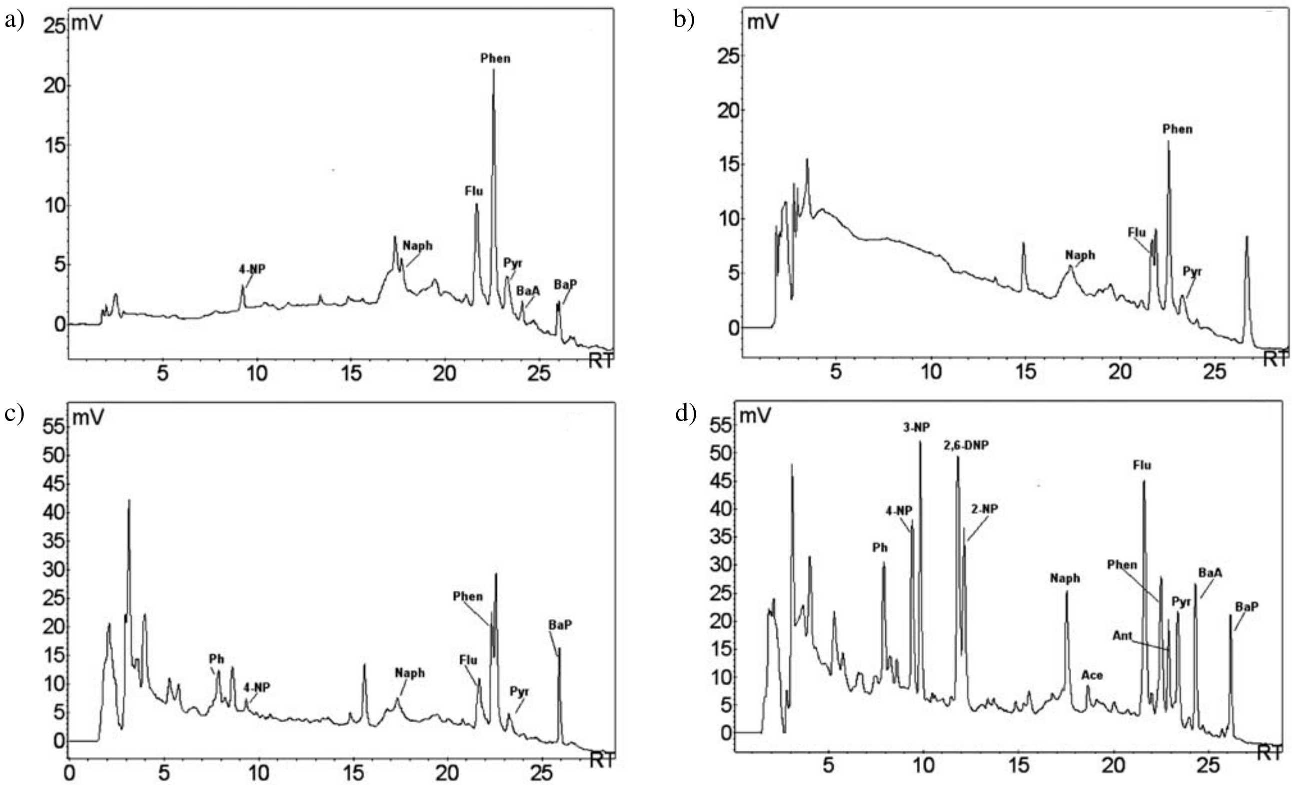

Figure 2. The HPLC chromatograms of rainwater (a), river water (b), wastewater (c) and spiked wastewater (d) samples

Thus, a successive elution with dichloromethane and methanol provides a suitable recovery for both clases of compounds.

The application of the developed method for analysis of real water samples proved that this method could be used for the simultaneous analysis of nitrophenols and PAHs in water samples at ppb level being a good alternative to the classical methods which involve separated analyses for each class of compounds.

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Povzetek

Razviti postopek ekstrakcije in HPLC metoda omogočata istočasno določanje nitrofenolov in poliaromatskih ogljikovodikov (PAH) v vodnih vzorcih. Primerjava različnih tipov ekstrakcije je pokazala, da je zaradi lipofilnosti poliaromatskih ogljikovodikov in polarnosti nitrofenolov ekstrakcija na polimernem SPE sorbentu najboljši kompromis za istočasno ekstrakcijo testiranih spojin. Najboljši rezultati so bili dobljeni z uporabo polimernega sorbenta Strata X in elucije z diklorometanom ter dodatne elucije z metanolom. Silikagelski C18 sorbent ni primeren za nitrofenole. Razvita SPE-HPLC metoda je bila uspešno uporabljena za analizo teh onesnaževal v različnih vzorcih vode.