

# Optimization of parameters affecting the recovery in the analysis of polycyclic aromatic hydrocarbons in natural waters by hplc-fluorescence.

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*Optimización de parámetros que afectan la recuperación en el análisis de hidrocarburos aromáticos policíclicos en aguas naturales por hplc-fluorescencia.*

*Optimització de paràmetres que afecten la recuperació en l'anàlisi d'hidrocarburs aromàtics policíclics en aigües naturals per hplc-fluorescència*

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## ABSTRACT

To improve the recovery percentages in the analysis of PAH (polycyclic aromatic hydrocarbons) in natural waters, parameters such as standard stability, filtration, storage time and matrix effect were optimized for five PAH by HPLC-FD. For aqueous standards, with the addition of 10% acetonitrile, good linearity was found for naphthalene, acenaphthene, anthracene, pyrene, and chrysene curves up to the second day of standards preparation. In filtration, the best results were obtained using the AP10 and Nylon filters with 10% acetonitrile, with recovery ranges of 98-72% and 93-56%, respectively. In contrast, lower values were obtained with cellulose filters in a polyethylene holder without the addition of 10% acetonitrile with recoveries of 52-0%. A 2<sup>3</sup> factorial design established, for natural water, that the most important factors were: storage time for naphthalene and acenaphthene, with area losses at 48 hours and the use of organic solvent for anthracene, pyrene and chrysene, with area gain for 20 % acetonitrile. For liquid-liquid extraction in standards, recoveries of 62-45%

were found in aqueous matrix without acetonitrile and 98-35% with 20% acetonitrile + 2.5% NaCl, at 24 hours of preparation.

**Key words:** natural water, matrix effect, polycyclic aromatic hydrocarbons, HPLC, percentage recovery.

## RESUMEN

Para mejorar los porcentajes de recuperación en el análisis de HAP (hidrocarburos aromáticos policíclicos) en aguas naturales, fueron optimizados parámetros como: estabilidad de patrones, filtración, tiempo de almacenamiento y efecto matriz, de cinco HAP por HPLC-FD. Para patrones acuosos, con agregado del 10% de acetonitrilo, se encontró una buena linealidad en las curvas de naftaleno, acenafteno, antraceno, pireno y criseno hasta el segundo día de preparación de patrones. En la filtración, los mejores resultados se obtuvieron usando los filtros AP10 y Nylon con 10% de acetonitrilo, con rangos de recuperación de 98-72% y de 93-56%, respectivamente. En cambio, bajos valores



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fueron obtenidos con los filtros de celulosa en soporte de polietileno sin el agregado de 10% de acetonitrilo con recuperaciones del 52-0%. Con un diseño factorial 2<sup>3</sup> se estableció, para agua natural, que los factores más importantes fueron: el tiempo de almacenamiento para naftaleno y acenafteno, con pérdidas de áreas a las 48 horas y el uso de solvente orgánico para antraceno, pireno y criseno, con ganancia de área para 20% de acetonitrilo. Para la extracción líquido-líquido en patrones, se encontraron recuperaciones de 62-45% en matriz acuosa sin acetonitrilo y 98-35% con 20% de acetonitrilo + 2.5% NaCl, a las 24 horas de preparación.

**Palabras claves:** agua natural, efecto matriz, hidrocarburos aromáticos policíclicos, HPLC, porcentaje de recuperación.

## RESUM

Per millorar els percentatges de recuperació a l'anàlisi de HAP (hidrocarburs aromàtics policíclics) en aigües naturals, van ser optimitzats paràmetres com: estabilitat de patrons, filtració, temps d'emmagatzematge i efecte matriu, de cinc HAP per HPLC-FD. Per a patrons aquosos, amb agregat del 10% d'acetonitril, es va trobar una bona linealitat als revolts de naftalè, acenaftè, antracè, pirè i crisè fins al segon dia de preparació de patrons. A la filtració, els millors resultats es van obtenir usant els filtres AP10 i Nylon amb 10% d'acetonitril, amb rangs de recuperació de 98-72% i de 93-56%, respectivament. En canvi, baixos valors van ser obtinguts amb els filtres de cel·lulosa en suport de polietilè sense l'agregat de 10% d'acetonitril amb recuperacions del 52-0%. Amb un disseny factorial 2<sup>3</sup> es va establir, per a aigua natural, que els factors més importants van ser: el temps d'emmagatzematge per a naftalè i acenaftè, amb pèrdues d'àrees a les 48 hores i l'ús de solvent orgànic per a antracè, pirè i crisè, amb guany d'àrea per a 20% d'acetonitril. Per a l'extracció líquid-líquid en patrons, es van trobar recuperacions de 62-45% en matriu aquosa sense acetonitril i 98-35% amb 20% d'acetonitril + 2.5% NaCl, a les 24 hores de preparació.

**Paraules clau:** aigua natural, efecte matriu, hidrocarburs aromàtics policíclics, HPLC, percentatge de recuperació.

## INTRODUCTION

In recent years the damage caused to ecosystems worldwide by persistent organic compounds (POPs)<sup>1</sup> has been evaluated, determining the concentration, origin and distribution in water reserves, making a great effort to control their quality, mainly in areas that have been exposed to contamination by different industrial, domestic and urban activities and even forest fires, affecting the formation and accumulation of these toxic compounds in natural systems<sup>2</sup>.

Among these substances are Polycyclic Aromatic Hydrocarbons (PAH) and their derivatives. PAHs are generated by the incomplete combustion of organic matter, being in great danger because they can remain in the environment for long periods without altering their toxic properties. This property classifies them as potentially carcinogenic and mutagenic agents for both man and fauna<sup>3</sup>. These characteristics are why there are strict regulations by entities such as the Environmental Protection Agency of the United States of America (EPA USA) that have established 16 PAHs as priority pollutants<sup>4</sup>.

PAHs are lipophilic compounds that, due to their non-polar nature and high molecular weight, have low solubility in water<sup>5</sup>. Despite these properties, it has been shown that their concentration in natural waters is much higher than expected. Some authors have attributed this behavior to the water matrix, since this matrix can be composed of a heterogeneous mixture of carbohydrates, lignins, organic acids and humic substances, all of which are grouped in the Dissolved Organic Matter (DOM). It should be noted that humic substances can comprise between 50 and 80% of the DOM, increasing the solubility of PAHs in natural waters<sup>5</sup>.

The analysis of PAHs in the aqueous phase requires a series of steps ranging from sample filtration and pre-concentration, for the enrichment of the analytes of interest, to instrumental analysis either by gas chromatography with flame ionization or mass selective detector<sup>6</sup> or by High Performance Liquid Chromatography (HPLC) with a fluorescence detector (FD) or ultraviolet (UV)<sup>4</sup>. EPA methods for the analysis of these pollutants<sup>7-9</sup>, which are reproduced by some authors<sup>10</sup>, indicate a sample preservation time of seven days before extraction and forty days after extraction with an organic solvent. The stability of PAHs in organic solvents is well defined in the literature<sup>6-9</sup>, but a decrease of PAH signal in aqueous matrices has been reported with time, before the extraction process<sup>11-13</sup> and with different procedures during the experimental methodology such as, for example, filtration<sup>11,14</sup>. However, EPA methods for PAH analysis in waters with different matrices only state the possible causes of PAH lose to photolytic decomposition and recommend using amber glass bottles together with sample refrigeration to avoid these drawbacks<sup>7-9</sup>. Despite this, more studies are needed to resolve the differences found.

Based on the previously exposed data, this research aims to optimize some processes used for the analysis of PAHs in natural waters, such as preservation and filtration of samples, among others, using high-performance liquid chromatography with fluorescence detector as an analysis system, in order to improve analytical practices and obtain higher recovery percentages.

## MATERIALS AND METHODS

### Materials and reagents.

PAH standards were prepared from the solid reagents provided by Sigma-Aldrich (purity 98%). NaCl and

NaSO<sub>4</sub> salts were purchased from Aldrich Chemical Co. (purity 99%). The solvents used were acetonitrile (ACN) Lichrosolv® (HPLC grade at 99.8%, Merck), chloroform and methanol (HPLC grade); these solvents also from Aldrich Chemical, purity > 99%. Glacial acetic acid (purity 100 %, Merck) was used for pH adjustment. The 18 MΩ water was obtained with the Barnstead NANO pure ultra-purifying water system.

## Methodology.

### Selection of HAP

Four low molecular weight polycyclic aromatic hydrocarbons (Naphthalene, Acenaphthene, Anthracene and Pyrene) and one of medium molecular weight (Chrysene) were selected to ensure their solubility in deionized water matrix at low concentrations. This selection also took into account the PAHs considered a priority by the EPA and which have been reported in natural waters<sup>11,15</sup>.

### Preparation of standards

From the solid standards of the different PAHs selected, each was dissolved in acetonitrile by weighing and obtaining the stock solutions (Table 1, solution 1). To prepare the intermediate solutions, each PAH was diluted again in acetonitrile by weighing (Table 1, solution 2). Then a multistandard solution (dilution 3) was prepared by taking an aliquot of the intermediate solutions of each PAH and diluting it in deionized water. The concentrations of the standards used for the calibration curves were prepared from the multi-standard solution (dilution 3) by making different dilutions in deionized water to obtain the working concentrations in each multi-standard of 10, 20, 30, 40 and 50 µg/L. The final concentration of acetonitrile in the standards was < 0.5%.

### HPLC-FD system and operating conditions

The analysis was conducted on a Hewlett-Packard (now Agilent Technologies) 1100 series high-performance liquid chromatography instrument equipped with a G1311A quaternary pump, a G1322A vacuum degasser, a G1313A ALS autosampler and a 1260 Infinity G1321B fluorescence detector (FD). The column used was Merck Purospher® STAR RP-18e (150x4.6 mm, 5 µm).

The chromatographic separation was accomplished under the optimized experimental parameters for the 5 PAHs evaluated, the optimal working conditions

being: isocratic mobile phase methanol/water 85:15 % v/v, working temperature 25° C, mobile phase flow rate 1.6 mL/min and injection volume 20 µL.

Following the recommendations of Thermo Scientific in their application note 70923<sup>16</sup>, the programming of the fluorescence detection system was performed as follows: first 4.9 min  $\lambda_{ex}/\lambda_{em}$ : 220/325 nm to favor the signal of Naphthalene and Acenaphthene, from 4.9 to 9.0 min  $\lambda_{ex}/\lambda_{em}$  275/420 nm to favor the signal of Anthracene and Pyrene and after 9.0 min,  $\lambda_{ex}/\lambda_{em}$  270/385 nm to favor the signal of Chrysene.

Each PAH was identified by comparing the retention times of the individually injected standards under optimal working conditions.

### Stability of PAH standards with and without added organic solvent

To evaluate the behavior of the calibration curves of the selected PAH at different preparation time intervals (1, 2, and 3 days) with and without adding 10% acetonitrile, the peak area of each PAH was plotted as a function of the concentration. The equations of the straight lines and correlation coefficient R<sup>2</sup> were determined for the different work days with the prepared standards, considering the study carried out by Vera et al.<sup>11</sup>. In this study, the standards were kept refrigerated and protected from light during the days of analysis. Additionally, the standards were not filtered and three replicates injections of each standard were performed.

### Sample filtration

When a routine analysis is fulfilled for natural water samples, filtration is recommended<sup>17</sup>, usually with cellulose filters, to obtain water free of impurities, thus avoiding the growth of bacteria and possible clogging of the column or damage to the HPLC pump pistons. For analytical columns, filters of 0.45 µm pore diameter are usually recommended. However, some authors recommend using nylon filters and conditioning them with acetonitrile to avoid PAHs losses<sup>11</sup>.

In search of the best working conditions, the influence of the filtration process on PAH recovery was determined with four (4) filters on different supports, and as in the previous point, the effect of adding or not adding acetonitrile to the standards was evaluated. The percentage of acetonitrile used was 10% v/v and the concentration of each PAH was 30 µg/L.

To perform this evaluation, the areas of three (3) sample replicate with three (3) filters of the same char-

**Table 1.** Prepared standards of polycyclic aromatic hydrocarbons (PAHs)

PAH	Stock standards solution in acetonitrile dilution 1 (mg/Kg)	Intermediate standard in acetonitrile. dilution 2 (mg/Kg)	Multi-standard in water dilution 3 (µg/L)
Naphthalene	130.2 ± 0.1	12.692 ± 0.008	99.7 ± 0.2
Acenaphthene	130.1 ± 0.1	12.725 ± 0.008	100.0 ± 0.2
Anthracene	135.6 ± 0.1	12.723 ± 0.007	100.0 ± 0.2
Pyrene	135.2 ± 0.1	12.723 ± 0.007	100.0 ± 0.2
Chrysene	143 ± 1	12.68 ± 0.07	100.0 ± 0.7

acteristic were determined and calculations of percent (%) recovery and relative standard deviation (RSD) were performed for each study.

The types of filters and filtration characteristics used were: 1) AP 10 filter with a pore diameter ranging from 0.2 to 0.6  $\mu\text{m}$  in a stainless steel holder and a glass sample syringe, 2) Cellulose filter with a pore diameter of 0.45  $\mu\text{m}$  in a stainless steel holder and a glass sample syringe, 3) Cellulose filter with a pore diameter of 0.45  $\mu\text{m}$  in a polyethylene holder and a polyethylene syringe for sample, and 4) Nylon filter with a pore diameter of 0.45  $\mu\text{m}$  in a polyethylene holder and a polyethylene syringe for sample.

### Natural water samples

For the tests of the matrix effect in natural waters, a sample was collected from the water server of Avenida Boyacá in the La Castellana distributor of the Warairarepano National Park (coordinates: 10°32'25"-10°37'35" north latitude and 66°40' 08" - 66°59'12" west longitude) located in Venezuelan territory in the mountainous area between the Caribbean Sea and the valley of Caracas.

A one-liter amber glass bottle was used for sampling, filled to the top (leaving no air space), sealed and immediately transferred to the laboratory in a refrigerated cellar with ice<sup>11</sup>.

### Matrix effect

It was already mentioned that the sample matrix influences PAH solubility<sup>2</sup>; due to this and in search of the best conditions for storage and analysis of PAH in natural water samples, a statistical design 2<sup>3</sup> was applied to evaluate the effect of the variables: storage time (-) 0 and (+) 48 h, % acetonitrile (-) 10 and (+) 20 % v/v and pH (-) 4 (adjusted with HAC) and (+) 7 (natural pH of the sample). For this study, natural water samples were spiked with 30  $\mu\text{g/L}$  of each of the PAHs evaluated, filtered with a 0.45  $\mu\text{m}$  nylon filter and the design conditions established for each experiment were applied. The samples were refrigerated and protected from light in those experiments that required 48 hours for analysis. Due to the unknown presence or absence of PAH in these samples, the area of the peaks was selected as the response for the statistical design.

### Liquid-liquid extraction method.

Some authors have recommended using acetonitrile to avoid PAH loss in water samples over time<sup>11-14</sup>. The amount of acetonitrile added by these authors was different since it depended on the extraction and pre-concentration methods used.

Considering the above, the recovery of PAHs was evaluated with the liquid-liquid extraction method bearing in mind the addition of organic solvent in the samples. For this purpose, three samples of one liter of distilled water were prepared (one with water only and the other with 20% v/v acetonitrile). Each sample was spiked with a multi-standard of PAHs in sufficient concentration so that after the liquid-liquid extraction process and regeneration of the extract in an organic solvent, the final sample contained 30  $\mu\text{g/L}$  of each of the PAHs evaluated.

The liquid-liquid extraction was performed following the guidelines of the method proposed by Reategui and Leal<sup>18</sup>, for the extracting of POPs in water samples, using 200 mL of sample and 60 mL of chloroform, the latter divided into three portions. To eliminate water residue in the organic phase, activated sodium sulfate was added and filtered. The organic extract was collected in a balloon and rota-evaporated in a water bath at 60 °C. The residue was reconstituted with a water bath at 60 °C. The sample was then filtered and the organic extract was collected in a balloon and rota-evaporated in a water bath at 60 °C. The residue was reconstituted with 2 mL of acetonitrile for subsequent analysis by HPLC-FD.

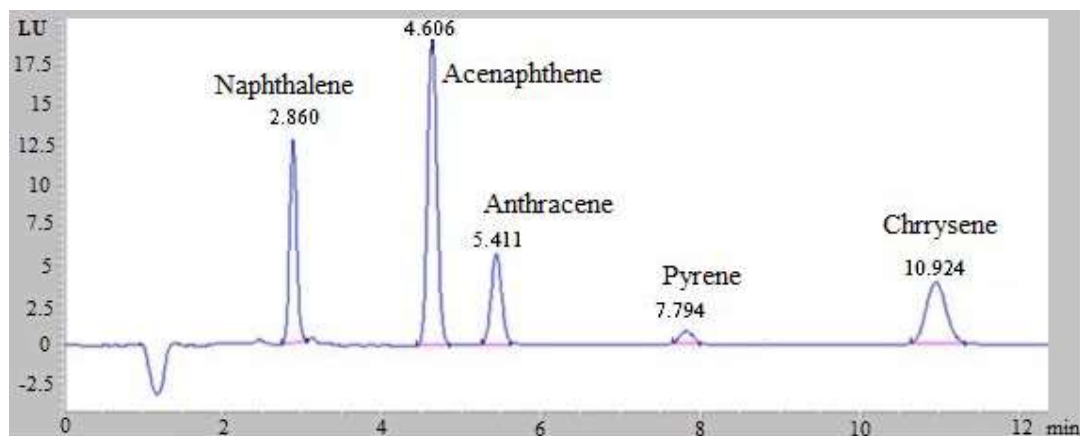
Liquid-liquid extraction was performed 24 hours after sample preparation; during this time, the samples were refrigerated and protected from light.

## RESULTS AND DISCUSSIONS

An aliquot of 20  $\mu\text{L}$  of a standard solution, composed of the five PAHs under study with concentrations of 60  $\mu\text{g/L}$  for each, were injected to check the chromatographic separation conditions. Figure 1 shows a chromatogram where a good separation of the five PAHs evaluated can be seen with a run time of 12 minutes.

**Table 2.** PAHs calibration curves for the different days elapsed of the preparation of standards in deionized water.

PAHs	1st Day	2nd Day	3th Day
Naphthalene	0.8136x + 0.0683 R <sup>2</sup> = 0.9979	0.2214x + 0.0683 R <sup>2</sup> = 0.9833	0.1381x + 1.5889 R <sup>2</sup> = 0.9665
Acenaphthene	1.4671x + 0.2967 R <sup>2</sup> = 0.9998	0.5846x + 4.624 R <sup>2</sup> = 0.9916	0.3456x + 1.9568 R <sup>2</sup> = 0.9841
Anthracene	0.445x + 1.3667 R <sup>2</sup> = 0.9932	0.2936x + 1.3418 R <sup>2</sup> = 0.9924	0.2093x + 0.8387 R <sup>2</sup> = 0.9933
Pyrene	0.0777x + 0.6619 R <sup>2</sup> = 0.9843	0.0835x + 0.2383 R <sup>2</sup> = 0.9696	0.068x + 0.1993 R <sup>2</sup> = 0.9553
Chrysene	1.5398x - 5.0443 R <sup>2</sup> = 0.9965	1.186x - 2.4976 R <sup>2</sup> = 0.9986	1.1245x - 4.4587 R <sup>2</sup> = 0.9988



**Figure 1** Chromatogram obtained for a multicomponent standard of 5 PAHs analyzed in the system HPLC-FD under working condition.

### Stability evaluation of standards with and without the addition of organic solvent

Table 2 shows the results obtained for the calibration curves of the different PAHs as a function of the days that elapsed from preparing the standards in deionized water. It can be seen that there is an appreciable loss of sensitivity (slope of the straight line) in the calibration curves at 24 hours (day 2) of the preparation of the standards for naphthalene, acenaphthene, anthracene and chrysene, while for pyrene there is no appreciable change. On day 3 (48 hours), the drops in sensitivity were significant for all 5 PAHs studied. A good linear correlation of the calibration curves for days 1, and 2 is also evident for the different PAHs evaluated, with a range of 0.9833-0.9998, except for pyrene, the compound with the lowest detection sensitivity, with a drop in correlation from 0.9843 to 0.9696. By day 3, only anthracene and chrysene maintain their linear correlation in ranges of 0.9924 to 0.9988, while naphthalene, acenaphthene and pyrene drop to ranges of 0.9555 and 0.9665.

Table 3 summarizes the line equations and correlation coefficient obtained for the calibration curves of the different PAHs studied as a function of the days

elapsed from preparing the standards in deionized water with 10% v/v acetonitrile. In this case it is found that for day 2 concerning day 1 there is no appreciable loss of sensitivity for 4 of the 5 PAHs evaluated. On day 3, the drops in sensitivity are evident for 4 of the 5 PAHs. As for the correlation coefficients, an excellent linear correlation of the calibration curves for days 1 and 2 of the different PAHs is visualized with a range of 0.9855-0.9994, while for day 3, the linear correlations fall for naphthalene, acenaphthene and pyrene with a range of 0.8961 to 0.9841, while anthracene and chrysene are maintained.

These results show that even with the addition of 10% v/v acetonitrile in the standards, stability in the concentration of the PAHs evaluated in the aqueous matrix cannot be assured after 24 hours of preparation.

### Study of the effect of filters

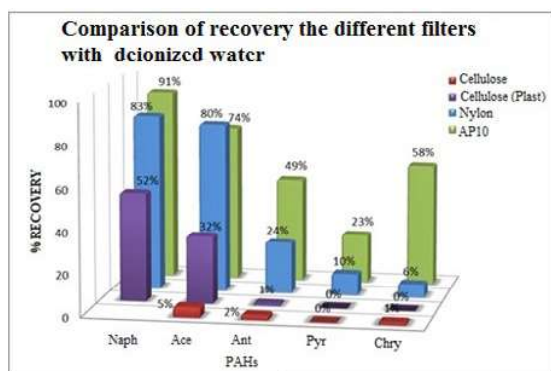
Figure 2 shows the bar graphs with the results obtained for recovering the different PAHs prepared in a) deionized water and b) with 10 % v/v acetonitrile, according to the filters used.

These results show that the best filters are the AP10 and nylon filters; furthermore, using 10% v/v acetonitrile

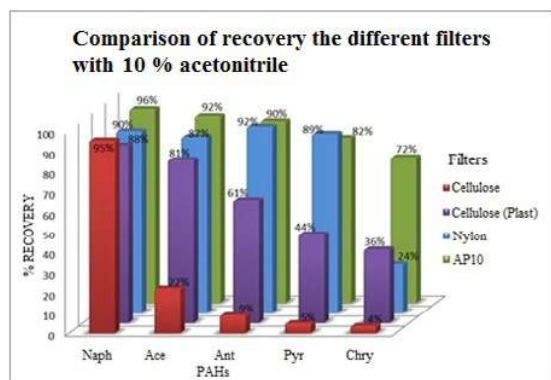
**Table 3.** PAHs calibration curves for the different days elapsed of the preparation of standards in deionized water with 10% of acetonitrile.

PAHs	1st Day	2nd Day	3th Day
Naphthalene	0.894x + 2.8668 R <sup>2</sup> = 0.9988	0.983x - 4.0809 R <sup>2</sup> = 0.991	0.5742x - 2.5325 R <sup>2</sup> = 0.8961
Acenaphthene	1.6014x + 2.9749 R <sup>2</sup> = 0.9987	1.5953x - 2.2619 R <sup>2</sup> = 0.9994	1.2533x - 3.457 R <sup>2</sup> = 0.9551
Anthracene	0.5221x + 5.5284 R <sup>2</sup> = 0.9989	0.463x + 5.7733 R <sup>2</sup> = 0.9927	0.413x + 6.0721 R <sup>2</sup> = 0.9869
Pyrene	0.1512x + 0.1809 R <sup>2</sup> = 0.996	0.1503x + 0.1995 R <sup>2</sup> = 0.9955	0.1449x + 0.157 R <sup>2</sup> = 0.9553
Chrysene	1.1769x + 5.1982 R <sup>2</sup> = 0.9928	0.9814x + 6.7656 R <sup>2</sup> = 0.9855	1.1245x - 4.4587 R <sup>2</sup> = 0.9988

in the standards significantly improves the % recoveries of all PAHs. Chrysene (medium molecular weight PAH) has the lowest % recoveries even with the use of 10% acetonitrile, with a value of 73% for the AP10 filter and only 24% with the nylon filter. Improvements are also observed in the RSDs going from ranges of 2 to 51% for the AP10 filter and 8 to 66% for the nylon filter in aqueous standards to ranges of 4 to 26% for the AP10 filter and 1 to 9% for the nylon filter in aqueous standards with 10% v/v acetonitrile. The most remarkable results are the low recoveries obtained for the cellulose filters regardless of the support used and the use of acetonitrile, in some cases with total losses of some PAHs.



(a)



(b)

**Figure 2.** Block diagram for compare the recovery effects of each filter with standards of naphthalene (Naph), acenaphthene (Ace), Anthracene (Ant), Pyrene (Pyr) y Chrysene (Chry) with: a) water and b) 10% of acetonitrile.

It is also worth mentioning that the AP10 filter has a less uniform pore diameter (0.2-0.6  $\mu\text{m}$ ) than the nylon filter (0.45  $\mu\text{m}$ ) and is often used for pre-filtration of water samples before storage. Likewise, when samples are extracted with organic solvent and analyzed by gas chromatography, the second filtration step<sup>3</sup> can be omitted before sampling injection into the equipment. However, when samples HPLC are analyzed, a filtration step with 0.45  $\mu\text{m}$  filters is required to protect the columns. From the above, it is recommended to filter the samples with 0.45  $\mu\text{m}$  nylon filters directly, if they

are to be analyzed by HPLC, to avoid the double loss of PAH due to double filtration.

As in this research, Chen et al.<sup>19</sup>, optimize the use of different filters, in this case, for compounds of pharmaceutical interest. They evaluate acetone, nylon, cellulose, and fiberglass, finding differences according to the groups of compounds; nylon and cellulose are the most commonly used. However, for pharmaceutical compounds, fiberglass was more suitable. On the other hand, Karlsson et al.<sup>20</sup>, optimized the use of cellulose and fiberglass filters for the removal of organic acids from water. The results showed that fiberglass filters performed poorly when compared to cellulose filters. It is important to note that the selection of filters for organic compounds in the aqueous phase depends on the analytes studied and the matrix in which they are found. Therefore, using a single filter cannot be generalized for all cases. These studies are crucial in determining the appropriate filter to use.

Additionally, the recommendations of Vera et al.<sup>7</sup> to condition the nylon filter by immersing it for one hour in acetonitrile and then at the end of filtration, 1 mL of this solvent was used to recover the PAHs from the filter, with this additional step improved the recoveries from 91 to 93% of naphthalene to pyrene and 56% for chrysene. Likewise, the results of this study allowed us to confirm the author's proposal to use acetonitrile as a solvent to stabilize the filtered sample.

### Matrix effect study

Table 4 summarizes the area values obtained for each PAH analyzed in the different experiments of the 2<sup>3</sup> factorial designs applied.

In these 2<sup>n</sup> designs, analysis of variance is used to estimate the effect of each factor and its interactions. To contrast whether each of the sources of variation is significant, these sources are compared with the residual mean square, which can be calculated by the existing variation between replicates or between the variations for the interactions of the 3<sup>rd</sup> order. The critical F or significance limit<sup>21</sup> must be calculated to determine whether the variances of each effect evaluated with respect to the reference variance are significant. In this study, the calculations were performed with the statistical program STARGRAPHICS Plus for WINDOWS version 1.4.

A Pareto chart can be used to evaluate the effect of the variables on the system response, where the values obtained from  $F_{\text{experimental}}$  are represented concerning  $F_{\text{critical}}$ ; the latter was calculated considering 95 % confidence and a one-tailed F giving a value of 4.207.

Figure 3 shows the Pareto diagrams for the different PAHs evaluated. In the case of naphthalene, the most crucial variable is time (A) with a negative sign, which indicates loss of response when moving towards the positive value of this variable, which would be the 48 hours of storage. For acenaphthene the most important variables were: time (A) with a negative sign which implies loss of area at 48 hours and % acetonitrile (B) with a positive sign, indicating improvement in the area towards the value of 20 %. While anthracene, pyrene,

**Table 4.** Factorial design matrix 2<sup>3</sup>. Response: Peak area

Exp	A Time (h)	B Acetonitrile (% v/v)	C pH	Naphthalene	Acenaphthene	Anthracene	Pyrene	Chrysene
1	-(0)	-(10)	-(4)	44.7	52.9	11.4	1.4	4.0
2	-(0)	-(10)	+(7)	37.9	56.3	12.3	2.0	6.8
3	-(0)	+(20)	-(4)	46.3	60.9	18.0	3.8	13.4
4	-(0)	+(20)	+(7)	39.0	59.9	17.2	3.4	12.3
5	+(48)	-(10)	-(4)	10.7	21.7	9.2	1.2	3.5
6	+(48)	-(10)	+(7)	12.8	23.3	9.1	1.6	6.5
7	+(48)	+(20)	-(4)	22.2	44.9	17.4	3.8	13.3
8	+(48)	+(20)	+(7)	19.1	41.0	16.5	3.3	12.1

**Table 5.** Extraction percentage and relative standard deviation of the different PAHs evaluated for liquid-liquid extraction with different sample matrix.

Type of sample	Naphthalene		Acenaphthene		Anthracene		Pyrene		Chrysene	
	Percentage of extraction	RSD	Percentage of extraction	RSD	Percentage of extraction	RSD	Percentage of extraction	RSD	Percentage of extraction	RSD
Water	57	6	62	3	45	2	47	1	58	8
Water with 20% ACN	92	26	86	27	N.C	N.C	56	4	15	9
Water with 20% ACN + NaCl	86	8	98	13	38	6	35	3	51	7

N.C: not quantified; ACN: acetonitrile; RSD: relative standard deviation.

and chrysene have the same behavior, the essential variable being for them the % of acetonitrile (B) with a positive sign, favoring the zone towards a value of 20%. The variable C (pH) did not affect the area of the different PAHs studied.

According to these results, all PAHs have a higher area signal if 20% v/v acetonitrile is used in the natural water samples at time 0 (Table 5, experiments 3 and 4). However, at 48 hours, there is a decrease in the area of 2 of the 5 PAHs studied, even in the presence of 20 % acetonitrile (Table 5, experiments 7 and 8).

Additionally, these results show the need to perform the analysis of natural water samples within 24 hours of collection or at least to perform the process of separation of PAHs from the aqueous matrix within 24 hours, provided that the samples have between 10 to 20 % v/v acetonitrile, to avoid loss of PAH signal during this time and to avoid losses in the filtration process.

#### Evaluation of the effect of organic solvent addition on the liquid-liquid extraction method.

Table 5 shows the results obtained for the recovery percentages and RSD of the PAHs evaluated for the studied extraction conditions.

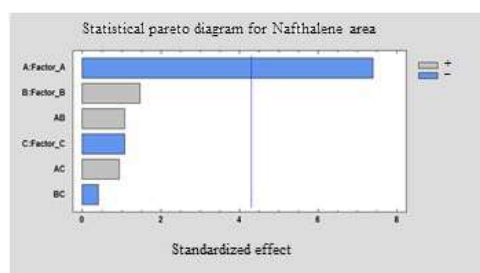
It can be observed for the standards with water only, that PAH recoveries were from 45 to 62 % with RSD 1 to 8 %. When 20 % acetonitrile is used, the recovery of naphthalene, acenaphthene and pyrene is improved concerning to water only, but for chrysene, the recovery drops and for anthracene the signal is completely lost; also, the RSD increases to ranges from 4 to 27 %,

which implies a greater dispersion of the results for water only. When 2.5% NaCl is added to improve the partitioning of these compounds to the organic phase, better recoveries are achieved to water only with values from 98 to 35 % and RSD from 6 to 13 %.

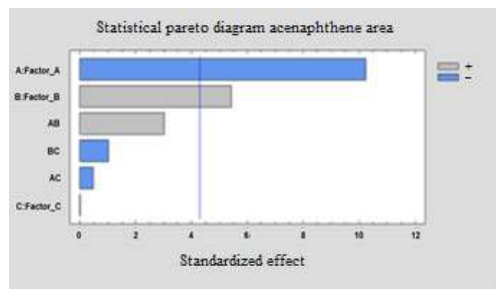
These results indicate that the extraction conditions need to be optimized if an organic solvent is to be used to avoid PAH signal losses in aqueous matrices within 24 hours of preparation (or sampling), since the addition of organic solvent can interfere with the extraction process of the analytes of interest from the sample.

## CONCLUSIONS

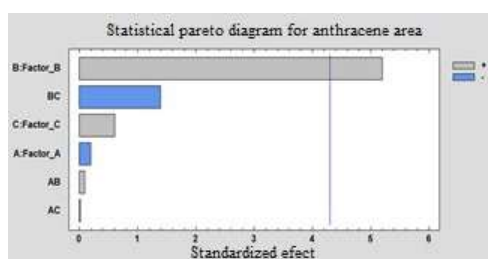
The studies showed good stability and linearity of the naphthalene, acenaphthene, anthracene and pyrene standards at 24 hours of preparation when 10% v/v acetonitrile was added, in contrast to the standards without the addition of acetonitrile, where part of the signal of most of the PAHs was lost at 24 hours of preparation. It was found that another variable that produces loss of PAH signal is the filtration process, obtaining the worst recoveries with cellulose filters and the best recoveries with AP10 and nylon filters with standards containing 10% acetonitrile. Similarly, in the natural water matrix, the effect of PAH area drops was found within 48 hours even with 20% acetonitrile for naphthalene and acenaphthene, while for anthracene, pyrene and chrysene the peak's area was improved when 20% acetonitrile was added during the 48 hours. The liquid-liquid extraction results showed



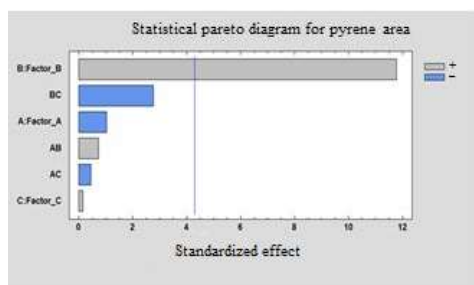
(a)



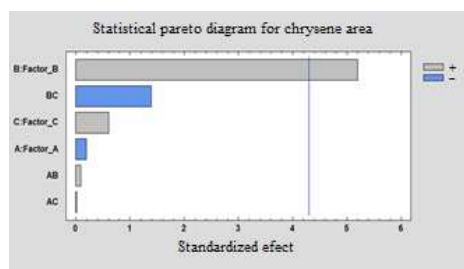
(b)



(c)



(d)



(e)

**Figure 3.** Pareto diagram for the standardized effects on peak area for: a) naphthalene, b) acenaphthene, c) anthracene, d) pyrene y e) chrysene.

the need to optimize the extraction method if 20% acetonitrile is added to the sample to avoid PAH signal losses over time. Finally, the analysis or extraction of PAHs in natural waters on the same day of collection is recommended; if this is not possible, 10 to 20 % acetonitrile can be added to avoid PAH losses within 24 h of collection but the extraction method should be optimized for pre-concentration and separation of PAHs from the sample matrix.

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