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**Paper title:** Micro-solid phase extraction of some polycyclic aromatic hydrocarbons from environmental water samples using magnetic  $\beta$ -cyclodextrin-carbon nano-tube composite as a sorbent

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### **Abstract**

Polycyclic aromatic hydrocarbons are ubiquitous and widely spread in the environment whose in the atmosphere and in aquatic systems. These compounds pose a significant risk for human health because of their carcinogenic properties. Therefore, numerous analytical methods are proposed to identify and quantify them. In this framework, a magnetic solid phase microextraction with  $\beta$ -cyclodextrin-carbon nano-tube sorbent coupled to a GC-FID was assessed for water samples, on the level of its validation and performances. Regarding validation, all parameters were not or properly evaluated with a global lack of precision for the linearity and accuracy, especially for statistic parameters. Besides, matrix effects were not considered neither for the calibration nor the validation. Furthermore, according to European directive (2013/39/EC), the analytical system was not sensitive enough for fluoranthene and benzo[a]pyrene. Concerning the performances, the LOD and LOQ were respectively in the range from 0.6-3.0  $\mu\text{g/L}$  and 2.0-10.0  $\mu\text{g/L}$  (linear range 2.0-500  $\mu\text{g/L}$ ) against 2.0-8.5  $\text{ng/L}$  (LOD) and a linear range of 20-500  $\text{ng/L}$  for multi walled carbon nano tubes (MWCNTs) GC-MS. Thus, to increase sensitivity, selectivity as well as resolution, a program temperature vaporization injector with large volume of injection could be implemented with a (50%-phenyl)-methylpolysiloxane column and a mass spectrometer as detector.

### **Analyte(s):**

Polycyclic aromatic hydrocarbons (PAH) are organic compounds ubiquitous in the environment, composed of two or more fused aromatic rings<sup>1</sup>. These contaminants are formed either by natural processes (volcano eruptions, forest fires...) or anthropogenic activities with the incomplete combustion (pyrolysis) of fossil fuels, coal, organic matter... On the level of

their general characteristics, they have a high boiling point, a low vapour pressure and a low solubility in water, which decreases with the increase of their molecular mass. Besides, as PAHs is a very broad class, numerous isomers are broadly present in environmental samples<sup>2</sup>. These contaminants can widely spread and distribute in different environmental compartments such as the atmosphere, in run off waters, in aquatic systems<sup>3</sup> and in soil<sup>4</sup>. Indeed, they pose a significant hazard to environment because of their ability to bioaccumulation and persistency as well as to the human health with their high toxicity, mutagenicity, carcinogenicity and propension to cause endocrine disruptions. Therefore, according to the US environmental protection agency, a list of sixteen priority organic pollutants was created with a tolerance limits between of 100-300 ng/L in water. Besides, numerous PAHs are listed by European Union as priority substances (2013/39/EC)<sup>8</sup>. The analytes studied in this article are listed in annex 2.

Finally, nowadays, benzo[a]pyrene is widely used as a biomarker in assessment of air pollution, risks, food and health damages with particular dna adducts<sup>5</sup>.

### **Matrice(s):**

PAHs are present in a diversity of ecological compartments such as the atmosphere which allows their transportation and deposition, in soil (bonded with humic compounds...) and in aquatic systems<sup>5</sup>. Besides, due to their general characteristics, there are spread either in gas phase with lower molecular weight (mw) PAHs or adsorbed onto solid phase through small particles for higher mw PAHs<sup>8</sup>. Then, thanks to the atmosphere, these micropollutants contaminate the ecological systems due to rain, dry deposition (sediments) and gas absorption. More precisely, regarding aquatic systems, PAHs are more likely to be adsorbed on suspended particles (ssp) because of their hydrophobicity. However, it also depends on their nature, concentration and the size and surface area of the particules<sup>6</sup>. Furthermore, PAHs (especially light ones) are water soluble and thus, are mainly detected in streams and groundwaters<sup>5</sup>.

PAHs are also found in food through vegetables, crops, mussels, seashells... contaminated by the deposition of particles and their growth in contaminated soil or contaminated water. Besides, they can be produced due to the processing of food such as barbecuing, roasting, baking<sup>10</sup>...

### **Description of the technique**

#### ***Sample preparation method:***

In order to extract the target PAH analytes from the sample medium, a micro-solid phase extraction using a sorbent constituted of a magnetic  $\beta$ -cyclodextrin-carbon nano-tube composite was implemented (Annex 1).

The extraction process began with the preparation of 10 mL of sample, containing 20% w/v of sodium chloride, in vials of 15 mL with conical bottom. Then, 15 mg of sorbent was added to the sample and the vial was stirred through a vortex during 20 minutes, at a rate of 2000 rpm. Afterwards, the sorbent was isolated from the solution thanks to a magnet. Once the supernatant was completely decanted, the sorbent was mixed with 200  $\mu$ L of toluene and again vortexed for 10 minutes at 2000 rpm. The same step of isolation of the sorbent was performed and finally, 300  $\mu$ g of chrysene, the internal standard, was joined to the medium to obtain a concentration of 30  $\mu$ g/mL.

#### ***Instrumental method:***

The GC analysis was carried out using a Variant CP-3800 system equipped with a FID detector. This instrument was equipped with a capillary column (HP-5, 30 m  $\times$  0.32 mm  $\times$  0.25  $\mu$ m, Agilent, USA) with a 5%-Phenylmethylpolysiloxane stationary phase. In addition, the analysis was performed through a temperature program with an oven initial temperature of 120°C for 1 min, then with an increment rate of 7°C/min until 300°C. Besides, the injection volume was of 1.0  $\mu$ L and the injector was on mode splitless, at 280°C for 1 min and after, a split ratio of 7:1 was set while the FID temperature was at 300°C. Finally, the carrier gas was ultra-pure nitrogen (>99.999%) at a constant pressure of 6.0 psi.

#### **Method performance evaluated:**

First of all, the method validation was based on a single laboratory approach, meaning without assessment of the robustness. Besides, it was not based on any validation method guideline.

As the precise origin of the samples was not clear, it was decided to use EU water quality norms as reference. Moreover, the choice of target analytes did not fit with the 16 toxic PAHs of the US EPA.

Firstly, the linearity of the different models (for all contaminants) was only presented through the correlation coefficient ( $R^2$ ). If it was the only considered parameter, this would be erroneous because it only expresses the fraction of y ordinate variation explicated by the linear regression. Besides, the number of different concentrations used for the calibration curve was not specified as well as the concentration of these points in order to check the entire coverage of the linear

range. Therefore, it was not possible to assess the quality of this curve. Moreover, given maximal allowed concentrations of some PAHs established by the water quality norm (2013/39/EU), they were extremely close from the lower limit of the linear range of the paper, which was not adequate to precise quantification. Ultimately, the internal standard used was chrysene, a PAH composed of four fused aromatic rings whereas naphthalene has two, acenaphthene three, and benzo[a]pyrene, five. Thus, as the volatility between these compounds was different, quantitative errors could still happen, including during the split mode. Besides, as chrysene is really common, it was more likely that samples already contained some.

Secondly, the accuracy was composed of two components, trueness and precision, assessed with respectively relative recovery and repeatability of working solutions (mix of reference materials) in pure water. Thus, concerning the trueness, the bias was determined without taking into account the matrix effect of the different medium samples (ssp). Besides, there was not any precision about the realisation of repetition or calculation with different levels of concentration to strengthen these values. Subsequently, no study of the reproducibility was performed. Otherwise, the precision, expressed with the relative standard deviation and compared to other analytical methods, was only evaluated with one concentration of analyte, at 200 ng/mL, whereas it is generally dependant on analyte concentration. Therefore, to obtain a reliable precision value, this parameter has to be determined across the range of interest (validated range) including the lower and upper extremes. Besides, the tested materials should be representative of test samples on the level of their matrix (matrix with ssp and humic acids) and concentration<sup>12</sup>. Nevertheless, intra and inter day variations were presented through relative standard deviation, which allowed a better vision of the result quality.

Regarding the sensitivity, LOD and LOQ concentrations were determined with a signal to noise ratio respectively of 3 and 10. On the one hand, their determination was also realised without taking into account the matrix effect. Thus, it would be more pertinent to use spiking samples of the different mediums. On the other hand, it was quite strange that all values for the linear range of 2.0-500 µg/L had the same LOQ and LOD, as well as for this of 10.0-1000 µg/L. A hypothesis could be that the author rounded all target analyte LOQs at the same value for analyte with the same linear range. Moreover, according to (2013/39/EU), maximal values allowed in water for fluoranthene and benzo[a]pyrene are respectively 0.12 µg/L and 0.27 µg/L. Nevertheless, in this paper, LOQ of both compounds was about 10 µg/L. Hence, this meant that the method was not sensitive enough for the quantification of these PAHs. Furthermore, no assessment regarding the sensitivity between the analytical signal and analyte

concentration was realised. Regarding the specificity, only partially erroneous (cf. above) relative recoveries permitted to evaluate it whereas no identification test was performed to confirm the presence of the target analyte at its retention time peak.

Finally, the selectivity of this analytical method was only assessed compared to one different composition of sorbent but none analysis was performed regarding the coextraction of impurities with SPME that could possibly lead to interferences in the GC-FID.

### **Critical evaluation:**

Firstly, microextraction techniques enable the reduction of solvent use compared to liquid liquid and solid phase extraction<sup>13</sup>. Regarding the sample preparation, magnetic nanosorbents (NPs) have interesting properties thanks to their important surface area and their sorption abilities, as well as their higher selectivity for PAHs<sup>2</sup>. Regarding the sorbent composite, graphene has higher adsorption abilities for phenantrene than multi walled carbon nano tubes<sup>11</sup>. Furthermore, functionalization of NPs surface with organic compounds allows to reach higher selectivity towards PAHs. Thus, concerning immobilization of  $\beta$  cyclodextrin ( $\beta$ -CD) in this paper,  $\beta$ -CD allowed to increase the extraction efficiency of naphthalene about 20%, whereas extraction of heavier molecules was about 6 to 15%<sup>20</sup>. Nevertheless, carbon NPs have a high tendency to aggregate in aqueous solutions due to the hydrophobicity of their surface<sup>2</sup>. Indeed, this reduces the surface area of the sorbent, as well as its diffusion in the entire sample solution. Currently, trials are performed to prevent this aggregation through functionalization with hydrophilic compounds in order to solvate CNPs. In addition, magnetic NPs permitted ( $\text{Fe}_3\text{O}_4$  deposit), through an external magnetic field, to improve the extraction of the sorbent from the samples solutions<sup>4</sup>. Otherwise, NPs extraction efficiency, and more precisely its sorption abilities, are broadly influenced by matrix<sup>2</sup> through the presence of humic acids, salt... Besides, no analyse was performed in order to assess the sorbent ability to desorb PAHs from ssp or other binding compounds. Ultimately, this technique requires a desorption phase, which leads inevitably to an additional work and a loss of target analytes, whereas system with fiber system can be directly thermally desorbed in the GC injector.

All in all, NPs show interesting proprieties for the extraction of PAHs despite a LOD of 0.6-3.0  $\mu\text{g/L}$ , partially due to losses of sensitivity of the analytical system (cf. below). Nevertheless, it appeared that it is currently complicated to reach high selectivity for all PAHs in a mixture with different feature PAHs. Besides, recurrent issues of aggregation, leading to decreasing efficiency, are still ongoing in aqueous solutions. Hence, functionalization has to be optimised to solve these issues. Nevertheless, in order to decrease time, labour, target analytes loss and

the lack of automatisation, SPME with fiber seems to be very appealing. In spite of the absence of description of the sample nature to choose the right SPME, PAL (Prep and load solution) SPME Arrow in direct immersion, using polydimethylsiloxane sorbent, allowed a LOQ of 0.1-0.8 ng/L with GC-MS in the frame of groundwater samples, thanks to an enlarged sorption phase<sup>8</sup>.

Regarding the instrumental technique, the choice of a suitable injector mode is important for PAHs analysis because of the occurrence of high discrimination for high molecular weight (mw) compounds compared to small and medium ones, due to different volatilities<sup>9</sup>. Ultimately, this issue leads to an increase of limit of detection<sup>9</sup>. Thus, given target analyte mw, it can be assumed that discrimination occurred and the type of mode injector was not the most appropriated. Besides, split mode is not adapted for trace analytes. Therefore, to reduce split-splitless injection discrimination and increase sensitivity, program temperature vaporization can be performed in combination with large volume injection (LVI). Besides, no precision was made about the liner, even if glass wool can cause heavy PAH analyte loss due to their sorption on its active sites. Hence, the most appropriated device was a multi-baffle or a packed liner. For LVI, however, toluene has a high boiling point, it permits to dissolve heavy PAHs<sup>14</sup>.

On the level of the column, the use of a mid-polar (50%-phenyl)-methylpolysiloxane column (10mx0.1mmx0.1µm) with precise conditions is better because it separates all PAHs, including isomers<sup>15</sup>, and reduces the discrimination (strong interaction with stationary phase) in term of peak height for heavier PAHs<sup>9</sup>. Besides, a constant flow rate allows a faster run time and sharper peaks<sup>15</sup>. The temperature program has also to be optimised to reach the best resolution<sup>9</sup>.

For this experiment, a flame ionization detector was inappropriate because of its nonselectivity. Therefore, mass spectrometry (MS) can be used because of its ability to reduce coeluting interferences through its natural selectivity, provides structural information (TIC) and a higher sensitivity (sim mode) for quantification<sup>8</sup> and also, does not require fluorescence of molecules<sup>10</sup>. Hence, this detector permits to analyse complex samples, except those with potential coelution compounds producing same fragment(s) than the target analyte. Analysis of water samples with a SPME with graphite MWCNTs sorbent coupled to a GC-MS (sim mode) reached a LOD of 2.0-8.5 ng/L<sup>8</sup>. Nevertheless, MS detector is more expensive than FID.

Finally, ultra-high performance liquid chromatography with a fluorescence detector is also a solid alternative thanks to its very good selectivity, sensitivity, its larger capacity to separate isomers compared to GC<sup>10</sup> and the shorter time of analysis<sup>5</sup>.

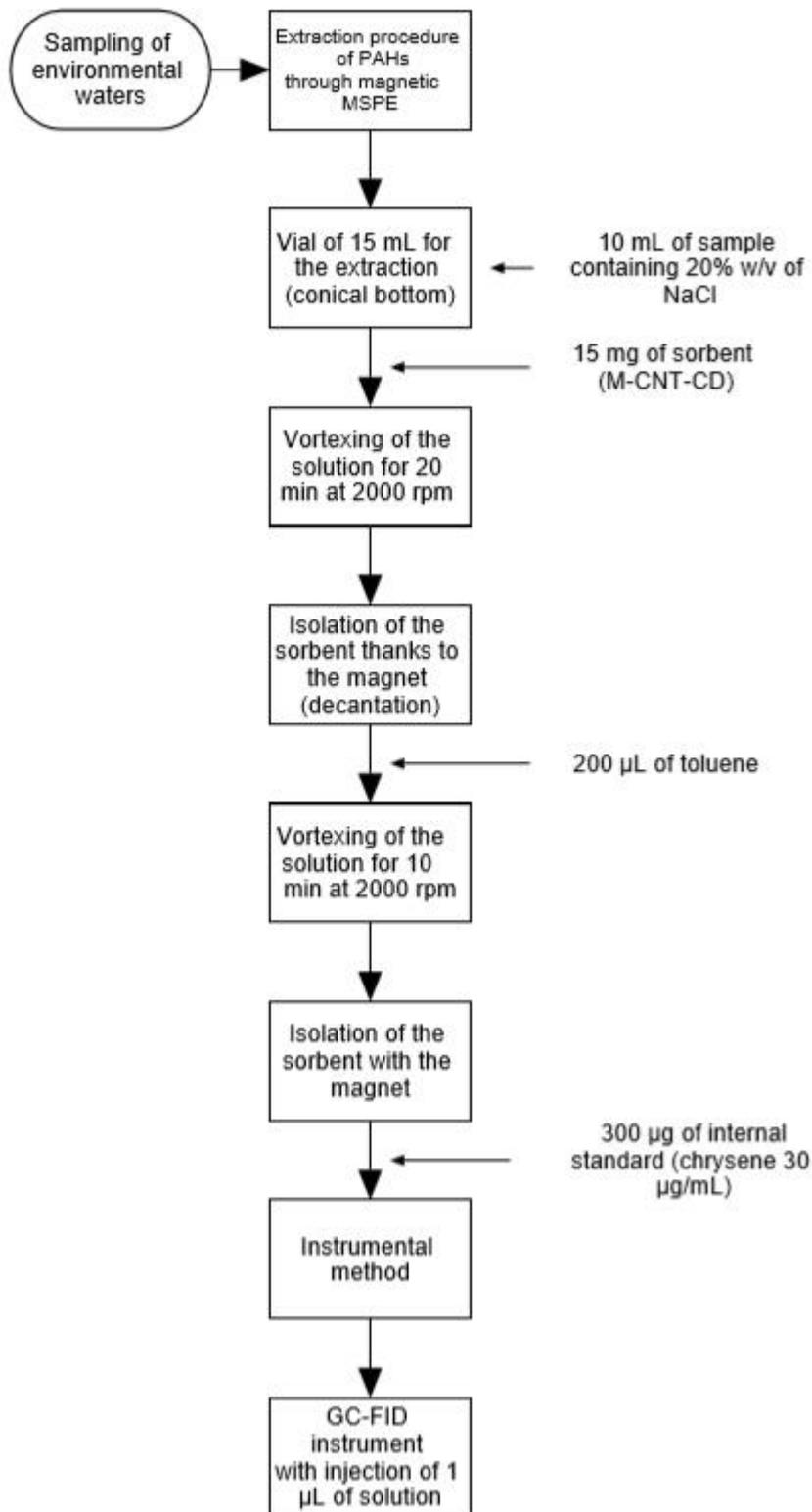
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## Annex

### Annex 1



## Annex 2

Compounds	Abbreviation	mw	Formula
<i>EPA PRIORITY</i>			
Naphthalene	Na	128	
Acenaphthene	Ac	154	
Fluorene	F	166	
Phenanthrene	Pa	178	
Fluoranthene	Fl	202	
Pyrene	P	202	
Benzo[a]pyrene	BaP	252	