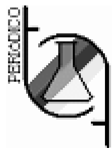

DETERMINAÇÃO DE HIDROCARBONETOS AROMÁTICOS POLICÍCLICOS (PAHS) EM AMOSTRAS AQUOSAS POR EXTRAÇÃO LIQUIDA-LÍQUIDA DE HPLC-FLD

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN AQUEOUS SAMPLES BY HPLC-FLD WITH SALTING-OUT LIQUID-LIQUID EXTRACTION

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RESUMO

Os hidrocarbonetos aromáticos policíclicos (PAHs) são um grupo de mais de 100 substâncias químicas, que contêm dois ou mais anéis aromáticos fundidos, a maioria deles tóxicos ou cancerígenos. Neste estudo, as condições de extração do Quechers foram adaptadas para realizar a extração de amostras aquosas.O método quechers tem-se tornado em uma técnica padrão para a análise de pesticidas e outras substâncias. O baixo custo e simplicidade deste método têm contribuído a sua popularidade em laboratórios no mundo inteiro para o processamento de amostras como as frutas e legumes. O sucesso deste método em amostras sólidas contrasta com os avanços na extração das amostras aquosas, onde etapas tediosas de extração, concentração e troca de solventes ainda devem ser realizadas antes da análise instrumental da amostra. Neste estudo, as condições de extração do método Quechers foram adaptadas para realizar a extração das amostras aquosas. Uma metodologia foi desenvolvida para a extração líquido-líquido (água / acetonitrila) de 15 hidrocarbonetos aromáticos policíclicos (PAHs) em matrizes aquosas. A mistura foi saturada com sulfato de sódio anidro para garantir a partição e analisou-se o extrato por Cromatografia Líquida de Alta Resolução com Detecção de Fluorescência (HPLC-FLD). As recuperações de analitos alcançadas (61-114%) foram comparáveis às esperadas usando técnicas tradicionais de extração e com moderada incerteza. Os resultados foram consistentes com as expectativas em termos das faixas analíticas e de concentração requeridas.

Palavras-chave: Extração, PAHs, HPLC-FLD, amostras aquosas, partição líquido-líquido.

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a group of more than 100 different chemical substances, which contain two, or more fused aromatic rings most of them toxic or carcinogenic. In this study, the Quechers extraction conditions were adapted to perform the extraction of aqueous samples. Quechers has become a standard technique for the analysis of pesticides and other organic substances. Its simplicity and low cost have made it popular in laboratories around the world for processing samples such as fruits and vegetables. The success of this method on solid samples contrasts with the advances in the extraction of aqueous samples where tedious steps of extraction, concentration and solvent changes must be carried out prior to the instrumental analysis. A methodology for the liquid-liquid extraction (water/acetonitrile) of 15 PAHs in aqueous matrices was developed. The mixture was saturated with anhydrous sodium sulfate to cause the partitioning and the extract was analyzed by High-Resolution Liquid Chromatography with Fluorescence Detection (HPLC-FLD). The recoveries of analytes achieved (61-114%) were comparable to those expected using traditional extraction techniques, and with moderate uncertainty. The results were consistent with the expectations for of the required analytical and concentration ranges.

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Keywords: Extraction, HAPs, HPLC-FLD, Aqueous samples, Liquid-liquid partition.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of more than 100 different chemical substances (US ATSDR, 1996) (Baird and Cann, 2012), which contain two or more fused aromatic rings (Nollet and Toldra, 2012). They are formed naturally in fuels such as coal, oil, gasoline, gas, or as products of their combustion in low-oxygen atmospheres between 500 to 900 °C, or in the pyrolysis of other organic materials such as wood, garbage and in cigarette smoke. The PAHs generated from these sources are complex mixtures of compounds. High-temperature cooking forms PAHs in food (US HSS, CDC, 2013). Natural sources of PAHs are forest fires and volcanoes (Nollet and Toldra, 2012).

PAHs are toxic and carcinogenic organic compounds (Food safety authority of Ireland, 2015). It is considered that PAHs attached to particles are very dangerous for human health (WHO EU, 2010) (Baird and Cann, 2012). PAHs are also important water pollutants that enter the aquatic environment due to offshore oil spills. The larger PAHs bioaccumulates in the fatty tissues of some marine organisms, which has been linked to liver injuries and tumors in some fish. It is thought that PAHs, polychlorinated biphenyls and Mirex have devastated populations of beluga whales. In drinking water, PAHs are not a representative source of these compounds for humans (Baird and Cann, 2012). The major source of contamination of PAHs in drinking water in developing countries is coal tar, which is used as protection against corrosion in iron pipes. The consumption and exposure to PAHs caused by this source can equal or exceed the amount produced through other routes such as food. Despite this, fluoranthene, which is the compound most frequently associated with this type of contamination, is not regulated because the concentrations found in drinking water are below limits detrimental to human health. The levels of PAHs in uncontaminated underground water are usually in the range of 0-5ng/l; Concentrations in contaminated underground water can exceed 10μg/l; and the typical concentration range for the sum of PAHs in drinking water is approximately 1ng /l - 11μg/l (WHO WS, 2003).

In the last 40 years, a large number of techniques for the determination of PAHs have been published; they are also cited as PNAs (Polynuclear Aromatics), and within other chemicals families like neutral bases and semi-volatile compounds. The methods commonly involve extraction, clean-up and quantification by chromatography techniques (Nollet and Toldra, 2012). Table 1 shows a summary of the instrumental techniques of some standardized methods and table 2 shows the most commonly used extraction methods.

In the GC analysis, there is possibility of total or partial coelution of compounds. In the EPA 8100 and SM 6440 method, four coelutions are listed: anthracene-phenanthrene; chrysene-benzo(a)anthracene; benzo(b)fluoranthene-benzo(k)fluoranthene; dibenzo(a,h)anthracene-indeno(1,2,3-cd)pyrene. The critical group are the 3 benzo (b,j,k) fluoranthenes.

The HPLC method is a reversed phase separation based on a water-acetonitrile gradient with the C18 column, this separation resolves completely the coelutions (US EPA 8310, 1986) (APHA, 2012). The detection is made by UV absorption at 254 nm or fluorescence. Most PAHs are natural fluorophores, therefore, detection by FLD is direct without derivatization (APHA, 2012). Acenaphthylene and Cyclopenta(cd)pyrene do not exhibit fluorescence. Comparatively, detection by FLD is more sensitive and selective than UV (Nollet and Toldra, 2012). The HPLC methods show some difficulties because is necessary to make steps like KD concentration and solvent changes normally from dichloromethane to acetonitrile or water-miscible solvents, Steps that are complex, time-consuming and that could be error sources.

Other immunoassay techniques like the method US EPA 4035 have also been developed, the PAHs present reacts with antibodies in the ELISA kit giving color; the concentration is determined at 450nm. These techniques are more sensitive to three-ring and four-ring PAHs; but also react with the majority of compounds with five and six rings. Those kits are calibrated with and report the existing species as phenanthrene or benzo(a)pyrene without differentiating them (US EPA 4035, 1996).

The determination of PAHs in the laboratory an inherent risk due to the potential danger that the analytes represent, but also by

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the analysis itself due to the use of dichloromethane as extraction solvent (NIOSH, 2016). Despite the analytical importance of this family of chemicals, no fundamental changes have been observed in standardized methods since the eighties. The QuEChERS technique (Quick, Easy, Cheap, Effective, Rugged and Safe) and similar ones such as US EPA 8330 (2007) make use of the partition phenomenon that is observed in certain homogeneous mixtures of solvents after saturating the mixture with a salt. The use of this property allows the liquid-liquid extractions to change the traditional non-polar solvents immiscible with water such as hexane and dichloromethane for others more polar and partitionable by the action of salts such as acetonitrile, isopropanol and acetone (Lehotay, Anastassiades and Majors, 2010). This type of extraction called SALLE Salting-out Liquid-Liquid Extraction (Majors, 2009) has been used successfully in several analytes including PAHs in soil and food matrices (Pule 2012; Gratz 2010).

The Quechers is 2-step procedure; first is the extraction where the sample is homogenized in water, added acetonitrile, and then the mixture is separated by adding a high amount of anhydrous magnesium sulfate. In the AOAC variant, the mixture is buffered with acetate and 1% acetic acid and in the CEN (European Standardization Committee) variant this function is carried out with citrate and sodium chloride. The buffer effect at pH 5 improves the recovery of specific pesticides. The second step is a clean-up phase this is called dispersive SPE (dSPE) where anhydrous magnesium sulfate is used with, PSA and C18 (Lehotay, Anastassiades and Majors, 2010; Waters, 2012).

In this study modifications were made to the Quechers technique for the direct extraction of the water sample with acetonitrile, the partitioning was done with magnesium sulfate. The analytes were 15 PAHs fluorophores: Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Dibenz[a,h]anthracene, Benzo[ghi]perylene, Indeno[1,2,3-cd]pyrene. The evaluated matrices were drinking water, natural water and wastewater. This work encompassed the optimization of the instrumental conditions, the establishment of the extraction, and the method validation for the listed matrices

MATERIALS AND METHODS

2.1. Standards and reagents

The solutions used for calculating the instrumental response factor and accuracy were made with dilutions of 2 different standards PAH Mixture # 4-550 of Chemservice (used for verification purposes) and PAH Mix M-8310-QC-ATI from Accustandard (used for calibration and spiking). The calibration was performed using between 4 and 6 concentration levels for each compound.

Recovery and uncertainty was tested in 4 concentration levels, 3 of these levels were spiked water matrices these are: drinking water (from laboratory water supply), natural water (untreated water from the supply of a drinking water treatment plant in Quito) and wastewater (from a dairy plant in Quito) these are real samples received at the laboratory. For the fourth concentration level was used a Certified Reference Material from a Proficiency Testing (PT) round organized in April 2016 by Asequality in Quito.

For the partitioning, previously muffled magnesium sulfate at more than 400 °C for at least 6 hours was used. The sodium acetate was also treated in an oven at a temperature of 150 ° C for at least 6 hours

The extractions, standards and dilutions were carried out with acetonitrile. 2 different brands were tested, Fisher and Merck. The best results were obtained with Merck LiCrosolv 2.2. Instrumental

Samples and standards were analyzed by high-performance chromatography liquid with a fluorescence detector (HPLC-FLD) on an HPLC Agilent infinity 1260 with an Agilent ZORBAX Eclipse column PAH C18 4.6 x 50mm 1.8um. The signal processing was performed in the software OpenLab CDS EZChrom, the signal subtraction ability is required.

The chromatographic separation was achieved by adapting the conditions of Henderson (2008). The flow rate of 2 ml/min causes a high back pressure that exceeds the maximum pressure of the used column guard cartridge, for this reason, the flow rate was reduced to 1 ml/min.

The chromatographic separation was performed in a 13 minute in gradient mode with

Water (A) and Acetonitrile (B) as mobile phase (0 min 45% B, 7 min 100% B, 11.30 min 100% B, 11.90 45%B). The injection volume was 5 uL.

With this method all peaks were resolved in less than 12 min. figure 1, shows chromatograms examples obtained through the detailed conditions. The 4 peak coelution formed by 1 and 2 methyl-naphthalenes, acenaphthene and fluorene, presented no problems for identification and quantification.

The FLD instrumental response optimization was tested trying to get the highest signal possible, initially were tested different bibliographic emission and excitation wavelengths, but because the substantial differences obtained, and starting from the precept that on different equipment and with different software, you can obtain results remarkably different (Agilent, 2012). Was decided to determine individually the wavelengths to obtain the maximum sensitivity

The excitation wavelengths optimization was performed using bibliographic information because the HPLC does not have DAD. The emission wavelengths were optimized by controlled peak elution to the detector, multi-channel detection and using the fluorescence spectra tool in the FLD. Table 3 shows the wavelengths selected for each peak and the retention times. And figure 2 shows an obtained fluorescence spectra. The setting of the PMT gain (detector´s signal gain factor) was set on the basis of the signals in the lowest standards. This manner the factor 18, which is the maximum gain, was chosen, under this conditions the noise level and baseline disturbances do not increase significantly

2.2. Preparation of the sample

To define the extraction to be used, the US EPA 8330 and the AOAC2007.01 standardized extraction methods were selected initially, since these are based on mixtures of water and acetonitrile and uses easily obtained salts. The extraction based on EPA 8330, was discarded and spiking tests were not performed because the acetonitrile recovered after the partitioning was around 6,3% of the original used volume and sodium chloride dissolving was difficult.

The method AOAC 2007.01 is a standardized method for fruits and vegetables. However, in the notes section, details the use of 13 ml of reagent-grade water like extraction blank. Based on this, a proportion of 0.5 g of anhydrous magnesium sulfate per gram of water (sample) was used (AOAC, 2007). Two water: solvent ratios were tested. One of them was 10 ml of water with 10 ml of acetonitrile (10:10), the partitioning carried out with 6 g of anhydrous magnesium sulfate. The second one was 25 ml of water with 5 ml of acetonitrile (25:5) and 12 g of anhydrous magnesium sulfate for partitioning, this in order to achieve a concentration factor in the extracting solvent.

The recovered acetonitrile in the 10:10 ratio extraction was about 9.5 ml equivalent to 95% of the added solvent amount. In the 25:5 ratio extraction, was recovered about 4.5 ml equivalent to 90%. In both cases the salt solvatation were exothermic but the dissolution is easily performed. Hence, both extractions were used for evaluation. The 25:5 ratio was used in the low and medium-low levels of the spiked concentrations. The 10:10 ratio was used for the two higher concentration levels.

The 25:5 extracts were filtered directly in a vial by PTFE syringe filter and injected to the HPLC by this extraction were processed the drinking and natural waters. The 10:10 extracts were clean up by dSPE and them filtered and analyzed like the other one

In the original AOAC 2007.01 method acetonitrile with 1% acetic acid is used as an extraction solvent to give a buffer effect with sodium acetate that improves the extraction of certain pesticides. However, in this study acetic acid was not use, in order to simplify the extraction and based on the results obtained by Pule (2012), it is not necessary for PAHs extraction.

Additional tests were performed with anhydrous sodium acetate and with Quechers extraction packets from Agilent Technologies (Part.No 5982-5755). The clean-up was performed with, dSPE kits: 2 types were tested (Part.No 5982-5158) from Agilent Technologies and (Part.No QUDISAOFK2) from Scharlau Science Group.gradient HPLC (1,00030.4000).

RESULTS AND DISCUSSION:

3.1 Method optimization

In the evaluation of the use of anhydrous

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sodium acetate, it was observed that its addition increases the recovery of the analytes between 5 to 12% with an average of 8%. However, the use of sodium acetate causes disturbances in the baseline, thus difficulting the detection of analytes at low concentrations. An attempt was made to eliminate this effect through oven heat treatment at 150 ° C given the impossibility of subjecting the acetate to higher temperatures due to the risk of decomposition, however, the troubles in the baseline were not reduced, which is why sodium acetate was not used at low and medium-low levels.

For the blank tests performed with the Quechers extraction kit (Part.No 5982-5755) from Agilent Technologies two different lots were used, in both, it was found that in the extracts obtained by this way it shows discrete peaks in or near of the retention times of the naphthalene acenaphthene, fluorene, phenanthrene. In one of the, both tested lots an extra peak was observed between the retention time of dibenzo (a,h) anthracene and benzo(g, h, i)perylene. The number of discrete peaks and their size was variable between the evaluated lots. Some of these peaks were larger than the signal produced by the analytes, for this reason, the possibility of using this product in the evaluation was ruled out.

The results showed that the effect of the use of the tube for clean-up by dSPE is also beneficial for the recovery of the analytes, with an increase in the recovery from 3 to 10% with an average of 7% for the tubes that were extracted with sodium acetate. And from 11 to 30% with an average of 14% for the analytes extracted without sodium acetate.

In the tested dSPE tubes, as the same manner than in the Agilent extraction kit, discrete peaks were found in the chromatogram, in the retention times of the naphthalene, acenaphthene, fluorene, and phenanthrene. But in concentrated samples, their effect on the chromatogram has lesser importance and decreases when the extracts are diluted. Comparatively the signal from the Agilent´s dSPE tube was slightly lower than the Scharlau´s tube. Due to these considerations and to the detriment of the recovery of some analytes, and based on the studies of (Gratz, et al., 2010) the clean-up phase was not used for clean samples, these correspond to the matrices of drinking and natural water. For residual water matrices, (the 2 highest levels) Agilent´s dSPE was used because these extracts required dilutions and additionally in order to avoid that contaminants from wasted samples pollute the column and the HPLC system.

The multiple wavelength changes in the FLD and the gradient that is used causes different changes in the baseline. This effect is increased in the blanks and sample injections because of matrix effects, reagents, vials and containers. To improve the signal processing and for reducing some of these troubles to all chromatograms of samples were subtracted the chromatogram of the matrix blank both at the same dilution. This allowed to extend the working ranges in the lowest concentrations. In other tests were found that the main source of the noise and discrete peaks could be the plastic tubes used in the extraction, the better results were got with 50ml tubes (Part.No CLS430291) from Corning cooled with water after the salt addition. The chromatograms of the calibration solutions were integrated as it, without subtraction.

As the basis of quality goals, for calibration and verification only concentration levels from standards prepared throughout the entire calibration range with average areas that meet the RSD ≤15% were used (Standard Methods, 6440B, 2012). The limits for the slope of the calibration curve was set to ± 20% of the slope obtained from standards with a different origin (US EPA 8000, 2014).

3.2 Recovery tests and validation data

Table 4 shows the recovery tests in the four concentration levels performed for all the compounds. The percentage presented is the average of the 9 repetitions carried out per compound and per level according to the statistical plan proposed. The origin of these four levels must be specified to interpret the results obtained. The 3 levels low, medium-low and high, were spiked samples. For these samples not characterized by any external source of certification, the assigned value was the theoretical concentration resulting from the calculated fortification. This means that in these samples the real value (“made to” or gravimetric) and assigned value (certified) is the same which in practice does not occur in the certification of interlaboratory materials and tests.

The certified value is the average result obtained from various sources or techniques or laboratories and this is a value that is commonly

lower than the real value at which the sample was fortified since the result is affected by the recovery of the methods used to characterize the material. According to Standard Methods and the FoPT of TNI-Nelac. In PAHs techniques, it is expected to have recoveries of around 70 and 80% and even lower recoveries in the standardized methods. This fact causes that in the case of the MRC some recoveries exceeded 100% reason why it is deduced that the extraction tested is more efficient in certain compounds. With this considerations, in the 4 levels tested in a general way recovery between 61 to 114% were obtained.

In the case of the MRC Asequality, in the medium-high level, the highest recovery was obtained in naphthalene with 114%, confirming what is described in the literature that this technique has greater recovery than traditional techniques in more polar and volatile compounds. The advantage can come from performing the extraction in a closed container and the omission of the steps like KD concentration and solvent change. Opposite effect to that observed in compounds of higher molecular weights such as dibenzo(a,h)anthracene, and benzo(g,h,i) perylene, suggestively, the high polarity of acetonitrile could be one of the causes.

In relation to the fortified materials, in almost all the analyzed compounds a reduction of the recovery is observed while the concentration increases, this is attributed to the contribution in the total volume of acetonitrile caused by the fortification. This extra volume of acetonitrile was not considered in the total extraction volume. In the highest range it is 417 ul equivalent to 4.2% and in this group the lowest recovery occurred in the case of the Indeno(1,2,3-cd)pyrene with 61%. In the low and medium-low level, the amount of solvent provided is 93.4 ul and 333 ul respectively. This translates into a dilution of the extract of 1.9% for the low range and 6.7% for the low middle range.

If this additional volume is considered and the recovery percentages are corrected, the values go up and begin to approach each other. But the same tendency is retained due to recovery being lower at higher concentrations. No possible cause was found for this phenomenon. Saturation is ruled out since PAHs are very soluble in acetonitrile and the standards even have higher concentrations (without magnesium sulfate) and can only exhibit that behavior at low temperatures, conditions that were not employed in this case.

Table 5 shows the uncertainty data obtained for each compound, a very similar behavior is observed in all compounds, and in the different levels. The average uncertainty is 13.5% with a maximum uncertainty of 23.1% which corresponds to acenaphthene in the Asequality MRC. This increase is caused by the reading level in the curve since this compound has the smallest peak in relation to the other compounds in the reference material see Figure 1.

The uncertainty distribution in function to the concentration is very homogeneous and the values are very close in the fortified samples. This must happen because, at the time of making the dilutions from the extracts of the fortified samples, the final concentration was adjusted to enter a narrow range of the curve between 3 standards where all the peaks are in the calibration range so as not to make several dilutions and injections for the same concentration level. Thus, the influence of uncertainty due to the curve was minimized.).

However, the samples undergo an extraction process in different concentrations from the matrix, maintain different sample and solvent relationships and are subject to dilution, which are factors that also contribute to the data dispersion. These contributions are also moderate since the homogeneity of the uncertainties is maintained, which indicates that homogeneity is maintained in the processing of the samples. Additionally, this point would not apply to the MRC Asequality since it has a distinct origin and concentrations of the compounds different from the fortifications but despite this, the homogeneity is maintained in all cases except for the acenaphthene that was already discussed.

In general, the realization of the method followed the guidelines presented in the reference methods and achieved recoveries of analytes at low levels comparable to those expected using traditional extraction techniques, and moreover with moderate uncertainty. Having fulfilled the expectations of the laboratory for the analytical requirement and in the concentration ranges useful for the most frequent samples in the laboratory, the method was declared valid.

The analites recovery raises when sodium acetate is used and after the clean up by dSPE due to this fact, the recovery of some compounds may exceed the expected ranges in the standard

methods and can fall out of the approval window in proficiency testing programs. This is a factor to decide when this technique is implemented.

CONCLUSIONS:

Comparing the traditional extraction carried out with dichloromethane or other non-polar solvents with SALLE extraction, for the determination of PAHs in water by HPLC, it was determined that it presents the following advantages and disadvantages:

Advantage

It requires common laboratory instruments, closed containers, vortex and centrifuge. No distillation systems or large or permanent spaces are required for performing this extraction. This makes easy the development of this technique in any laboratory without large investments. A large amount of sample is not required which allows for reducing the number of bottles that arrive at the laboratory per sample resulting in the decongestion of the lab spaces, fridges and storage sites. It reduces logistics and shipping costs easing the workload of sampling personnel

Between 5 and 10 ml of acetonitrile is required per sample which reduces the cost of extraction, facilitates the processing of the samples and reduces wastes and the risk of accidents when handling large volumes of solvent. A solvent change procedure is not necessary since the extraction solvent is acetonitrile and can be injected directly into the HPLC. This reduces the time and improves the recovery of volatile analytes such as Naphthalene. It allows for the quick extraction of several samples simultaneously which reduces labor costs and increases the processing capacity of the laboratory. In the tests carried out, it was proven that a single analyst can process up to 8 samples in less than one hour.

The risk is reduced since acetonitrile is a less dangerous and toxic compound than dichloromethane and is not very volatile, making it difficult to reach high concentrations in the environment. It does not mean that acetonitrile is not dangerous, but their toxicity is lower and to date, no security agency has classified it as a carcinogen.

Low-cost disposable materials can be used, which reduce the risk of cross-contamination or carryover thus, eliminating the risk to the laboratory personnel by washing contaminated materials with residues from samples or fortifications.

Since acetonitrile is a more polar compound than dichloromethane (dipolar moment of 3.92 D vs 1.6 D of dichloromethane), a wider range of substances with medium to high polarity is dissolved, thus improving the recovery of a wide range of analytes which could be used in the development of other methods.

Disadvantages

SALLE, when using low amounts of sample and solvent, does not easily allow for use of concentration techniques to reach lower limits, therefore, the instrument used must be more sensitive than traditional techniques.

Acetonitrile in addition to the analytes of interest can extract interferers other than those extracted with dichloromethane. This requires that the instrumental technique be very selective and robust to distinguish the matrix signal from the analytes.

SALLE extraction in an almost exclusive way makes use of the clean-up by dSPE, which does not have such a high effectiveness. To carry out a traditional clean-up operation, solvent change or concentration procedures may be performed where analytes could be lost. The amount of salt used is large in relation to the amount of sample. So, the price of this reagent is important in the cost of the analysis and limits the possibility of using larger amounts of sample to improve quantification limits. In addition, their wastes require treatment.

Despite the disadvantages exposed, there are numerous benefits insolvent savings, risk reduction, time-saving, ease of extraction and the great applicability that has been seen of this technique for HAPs and other analytes in more complex matrices.

Recommendations

The working ranges in calibration and in sample spiking were strongly limited because was difficult to adjust the dilutions of the standards in premixed form with the different peak sensitivities. None of the concentrations premixed like EPA 8310, 550 or isotonic

standards are easily adjusted to the working ranges achieved in the HPLC-FLD. In addition, isotonic standards are generally used for GC and they are prepared in incompatible solvents for reverse phase. Could be more beneficial to design a specific concentration mix to improve the working ranges

The analyst must be scrupulous in the selection of work materials, i.e., vials, filters, syringes, solvents; even the same variability between reagent lots from the same manufacturer could give distortions on the baseline and discrete peaks. Some problems were solved with the subtraction by the software of the blank signals in the samples and by the heat treatment of the salts, however, it is always more valuable to have the chromatogram free of these problems.

The personnel performing the extraction must be very familiar with the technique and have sufficient dexterity, since salinization, being an exothermic reaction, is one of the greatest sources of error due to evaporation of the solvent and can be the cause of accidents. The cleaning of the material must be carried out according to exposure in the Standard Methods and the material must not be used for other analyses (dedicated material). Even the sharing of automatic pipettes with disposable tips often leads to problems in all types of instrumental chromatography.

As with all HPLC techniques, a record of the pressure variation in the column must be maintained throughout its shelf life which eventually affects the retention times in a few seconds. This does not affect the integration windows, but it does affect the wavelength-time change programmed in the detector, with drifts in the response function which may impair quantification.

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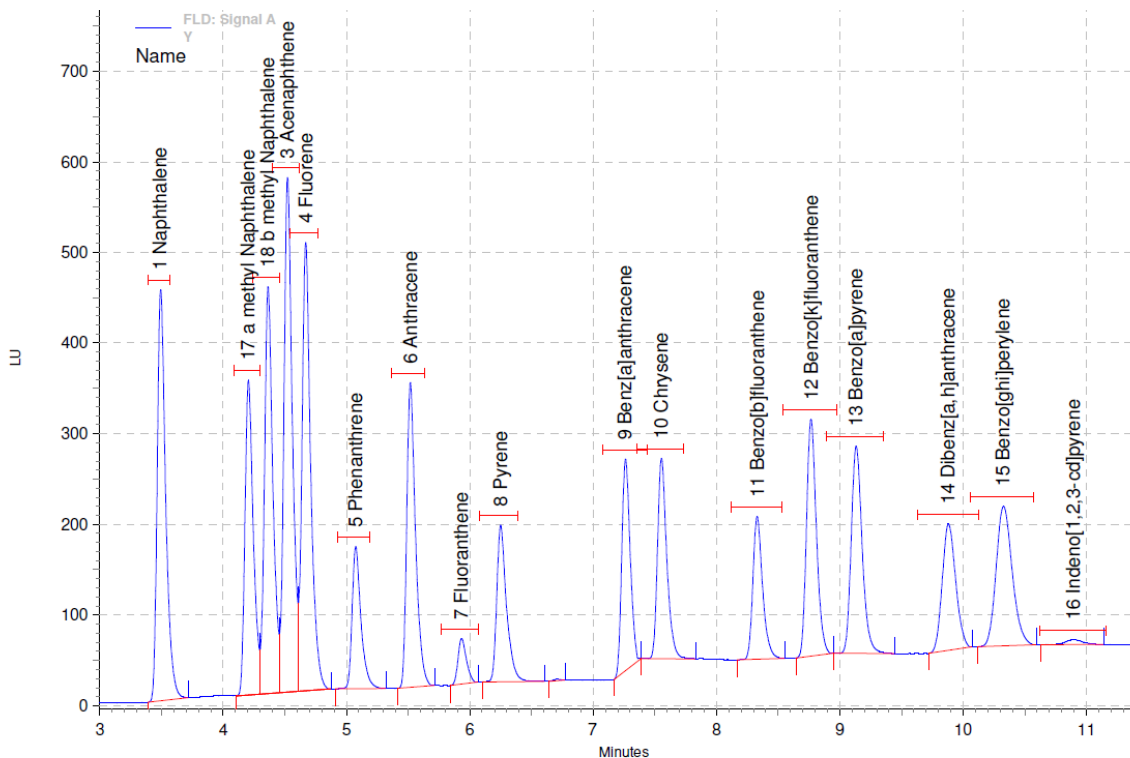

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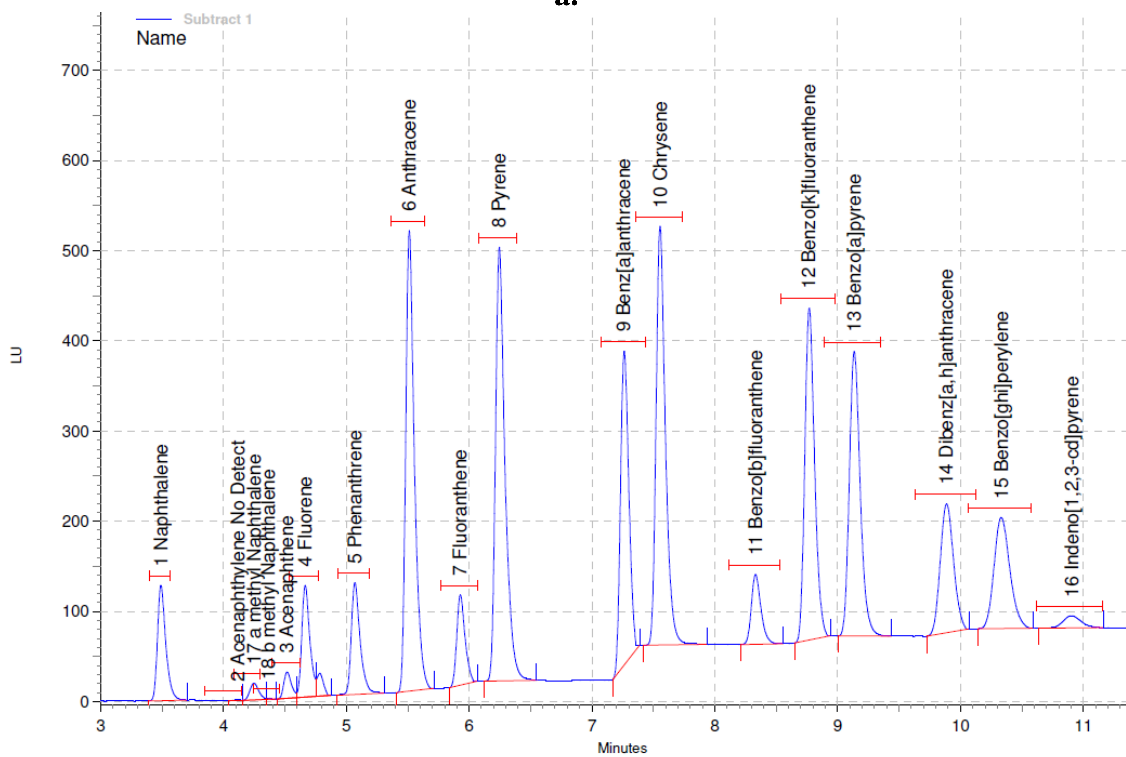

Figure 1. *PAHs chromatograms examples (a. Midpoint calibration solution. b. MRC sample signal subtracted the sample blank signal).*

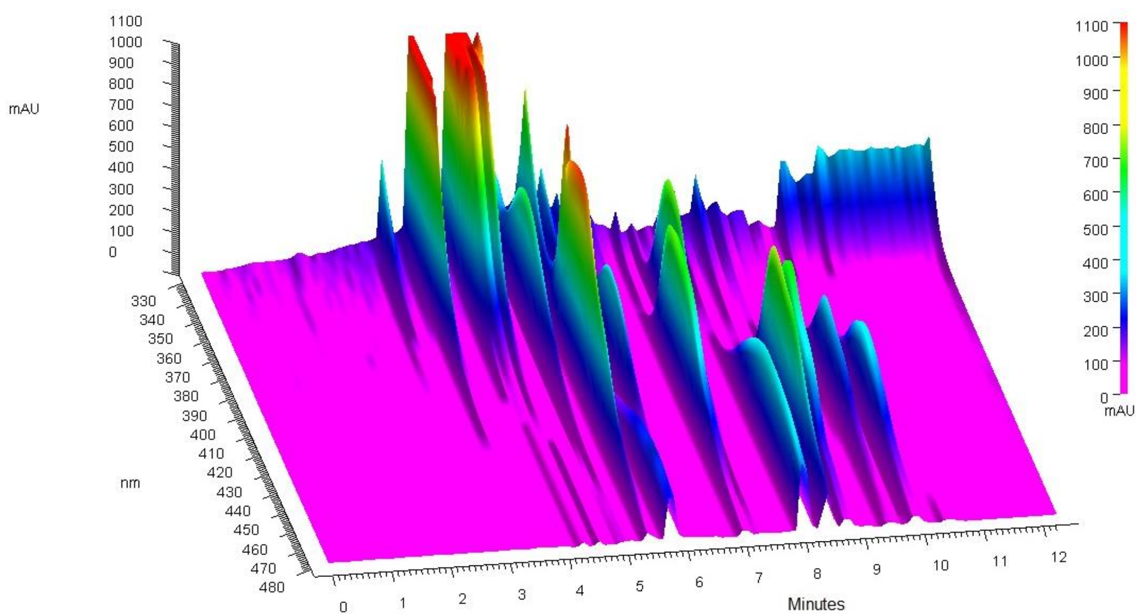

***Figure 2**. Fluorescence spectrum obtained during signals optimization.*

Table 1. Chromatographic techniques for PAH (Summary)

Analytic Technique	Standardized Methods
GC-FID Gas chromatography with Flame ionization detector	US EPA 8100 de 1986 US EPA 610 de 1984 NIOSH 5515 de 1994
GC-MSD Gas chromatography with Mass spectrometric detector	US EPA 525.2 de 1995 US EPA 625 de 1984 US EPA 8270 de 1998
HPLC-UV/FLD High-performance liquid chromatography with Ultraviolet Detector / Fluorescence Detector	US EPA 550 de 1990 US EPA 610 de 1984 US EPA 8310 de 1986 Standard Methods 6440b Ed22 del 2012 US FDA 4475 del 2010 NIOSH 5506 de 1998

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Table 2. *PAH standardized extraction methods (Summary)*

Extraction type	Used Solvents	Sample injection form to GC	Sample injection form to HPLC	Required Laboratory Apparatus or Wares
Liquid – liquid Sample size 1L	Dichloromethane	Dichloromethane Direct Injection	Solvent change to Acetonitrile	Separatory funnel KD Concentrator
SPE disks or Cartridges SDB-XC C 18 Sample size 1L	Methanol Acetone Ethyl Acetate Dichloromethane	Dichloromethane Direct Injection	Solvent change to Acetonitrile	SPE disks or Cartridges Manifold Vacuum pump KD Concentrator

Table 3. *Retention times and wavelengths used in FLD*

Analytes	Retention time	Excitation nm	Emission nm
Naphthalene	3,503	224	335
Acenaphthene	4,529	269	327
Fluorene	4,676	269	327
Phenanthrene	5,087	250	364
Anthracene	5,53	252	399
Fluoranthene	5,944	234	468
Pyrene	6,264	265	380
Benz[a]anthracene	7,279	280	397
Chrysene	7,574	265	375
Benzo[b]fluoranthene	8,356	260	439
Benzo[k]fluoranthene	8,802	255	419
Benzo[a]pyrene	9,173	260	411
Dibenz[a,h]anthracene	9,947	280	403
Benzo[ghi]perylene	10,404	290	420
Indeno[1,2,3-cd]pyrene	11,009	293	485

__Table 4__. Results by concentration level

Analytes	Conc Level ug/l	%Sr Repeatability	%SR Reproducibility	% Recovery	%U (k=2)
Naphthalene	6,58	2,97	4,65	81,8	12,57
	23,47	5,87	7,97	63,2	12,28
	84,35	4,22	4,22	114,5	14,46
	1836,6	7,65	7,65	73,6	13,39
Acenaphthene	6,53	2,28	3,90	88,4	11,88
	23,28	4,24	4,24	76,2	11,43
	18,63	4,85	6,63	98,3	23,06
	1822	7,4	7,4	76,7	14,92
Fluorene	1,33	1,99	3,51	89,3	12,16
	4,76	4,73	4,73	79	12,9
	19,89	4,68	4,68	87,5	14,5
	372,3	7,34	7,34	77,4	15,67
Phenanthrene	0,65	2,99	3,73	91,1	12,21
	2,33	4,48	4,48	81,6	11,41
	29	4,22	4,22	94,7	15,28
	182,7	8,06	8,06	78,4	15,52
Anthracene	0,66	1,79	2,88	90,9	11,55
	2,36	4,68	4,68	82,1	12,95
	49,35	3,93	4,10	106,8	14,31
	184,8	7,55	7,55	78,2	15,93
Fluoranthene	1,32	2,14	3,66	83,2	15,45
	4,71	4,60	4,60	75,2	9,31
	132,6	3,83	3,99	91,4	10,85
	368,8	8,17	8,17	71,9	15,57
Pyrene	0,65	2,16	3,93	89,1	12,12
	2,33	4,63	4,7	80,9	12,03
	103,7	3,67	5,82	88,7	14,72
	182	8,08	8,08	76,2	15,61
Benz[a] anthracene	0,67	2	6,23	77,4	13,68
	2,38	4,73	4,86	72,3	9,78
	54,46	4,05	6,74	77,6	14,12
	186,1	7,98	7,98	70	13,18
Chrysene	0,67	1,79	4,26	91,9	12,65
	2,38	4,58	4,58	82,4	12,43
	72,55	3,73	4,79	95,5	11,74
	186,2	7,35	7,35	75,4	15,03
Benzo[b] fluoranthene	1,33	1,9	3,46	90,2	12,02
	4,76	4,57	4,57	80,8	12,1

	31,35	3,71	4,37	100,9	11,42
	372,3	7,03	7,03	72,3	14,14
Benzo[k] fluoranthene	0,66	1,83	4,36	86,6	12,25
	2,37	4,25	4,25	79	11,59
	50,86	3,23	4,98	84,1	13,05
	185,3	7,33	7,33	70,7	12,49
Benzo[a] pyrene	0,66	1,66	3,65	87,7	12,02
	2,34	4,31	4,31	81,6	12,48
	46,45	3,14	5,46	93,4	15,6
	183,1	6,59	6,59	70,2	11,82
Dibenz[a,h] anthracene	1,32	1,57	8,05	76,2	15,66
	4,7	5,21	6,06	73,5	13,26
	72,4	3,41	9,6	71,8	17,07
	367,9	7,36	7,36	65,6	11,94
Benzo[ghi] perylene	1,3	1,53	5,48	82	13,16
	4,64	4,55	4,76	77,6	11,92
	60,66	2,82	8,15	71,1	16,49
	363,3	6,87	6,87	62,5	11,04
Indeno[1,2,3-cd] pyrene	0,66	3,51	3,51	80	17,13
	2,36	5,4	6,22	74,3	14,69
	82,25	2,76	7,31	83,1	14,24
	184,9	5,09	5,09	60,7	13,17

Table 5. *Uncertainty comparison at tested levels vs PT study*

	PT Study S	**Drinking Water**	**Natural Water**	**PT water sample**	**Waste Water**
Naphthalene	22,0%	12,6%	12,3%	14,5%	13,4%
Acenaphthene	18,2%	11,9%	11,4%	23,1%	14,9%
Fluorene	17,6%	12,2%	12,9%	14,5%	15,7%
Phenanthrene	15,8%	12,2%	11,4%	15,3%	15,5%
Anthracene	16,3%	11,6%	13,0%	14,3%	15,9%
Fluoranthene	15,4%	15,5%	9,3%	10,9%	15,6%
Pyrene	17,5%	12,1%	12,0%	14,7%	15,6%
Benz[a]anthracene	14,8%	13,7%	9,8%	14,1%	13,2%
Chrysene	16,3%	12,7%	12,4%	11,7%	15,0%
Benzo[b]fluoranthene	18,2%	12,0%	12,1%	11,4%	14,1%
Benzo[k]fluoranthene	23,9%	12,3%	11,6%	13,1%	12,5%
Benzo[a]pyrene	19,1%	12,0%	12,5%	15,6%	11,8%
Dibenz[a,h]anthracene	19,2%	15,7%	13,3%	17,1%	11,9%
Benzo[ghi]perylene	18,4%	13,2%	11,9%	16,5%	11,0%
Indeno[1,2,3-cd]pyrene	20,6%	17,1%	14,7%	14,2%	13,2%

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