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DETERMINATION OF PAH IN THE BLACK SEA WATER BY GC/MS FOLLOWING PRECONCENTRATION WITH SOLID-PHASE EXTRACTION

OZNACZANIE WWA W WODACH MORZA CZARNEGO METODĄ GC/MS Z ZATEŻANIEM POPRAZ EKSTRAKCJĘ DO FAZY STAŁEJ

Abstract: Determination of PAHs in marine coastal waters has to be carried out with great care to avoid serious losses occurring during the sampling and storage stage. This is due to the hydrophobic properties of PAHs, and their tendency to be adsorbed to surfaces they are in contact with, including suspended particulate matter. The best technique to separate PAHs is gas chromatography with mass spectrometer. Because of low concentration levels to be quantified in water samples, an enrichment step is needed before chromatographic analysis. For PAHs, several preconcentration techniques have been used, but the most concentration technique is solid-phase extraction (SPE). The main objective of this work is to adapt and implement a relatively simple and rapid method for gas chromatographic determination of individual PAHs in samples of coastal and running waters. The present work analyses the yield and precision of extraction of PAHs by solid phase extraction with using different sorbents for SPE (Bond Elut: C18 and Plexa SPE discs), the two most common methods used for aqueous samples.

Keywords: polycyclic aromatic hydrocarbons (PAHs), solid-phase extraction (SPE), different sorbents (Bond Elut: C18 and Plexa SPE discs), analysis of coastal waters

The monitoring of *polycyclic aromatic hydrocarbons* (PAHs) is an important problem due to their well-known mutagenic and carcinogenic effects [1, 2]. These substances can be found in various matrices, both biological and environmental. PAHs are typical non-polar compounds and have excellent retention on a reversed-phase adsorbent such as C18 bonded silica. Although their solubility in water is very low, concentrations in the $\mu\text{g dm}^{-3}$ level are commonly encountered in the environment. Since these compounds are considered toxic at this level, their presence needs to be monitored.

The Water Framework Directive [1] imposes on the EU Member States the obligation of monitoring natural water quality, including the marine coastal waters. Coastal seawater is a very specific matrix, quite different from inland waters, due to salinity and high particulate

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matter content. Shore waters are often under strong anthropogenic stress, which results amongst others in eutrophication, intensifying their turbidity in comparison with the open sea or ocean waters. Papers concerning PAHs in seawater are scarce, due to their very low concentrations in that matrix, in comparison with the concentrations in wastes, sewage, contaminated soils or marine sediments and even other aqueous matrices like rain water [3].

Determination of PAHs in marine coastal waters has to be carried out with great care to avoid serious losses occurring during the sampling and storage stage. This is due to the hydrophobicity of these compounds, and their tendency to be adsorbed to surfaces they are in contact with, including suspended particulate matter. The best technique to separate PAHs is gas chromatography with mass spectrometer. Because of low concentration levels to be quantified in water samples, an enrichment step is needed before chromatographic analysis.

In recent decades, the development of preconcentration steps in order to be implemented prior to analytical determinations of trace level compounds has been explored in considerable depth. With a view to eliminating or at least minimizing the use of organic solvents employed in conventional *liquid-liquid extraction* (LLE) [4-6], which has been used by the *environmental protection agency* (EPA), other methods have been developed. Among them, membrane extraction [7, 8], *solid-phase extraction* (SPE) [9, 10] or *solid-phase microextraction* (SPME) [11, 12] are the most common ones.

For PAHs, several preconcentration techniques have been used, but the most concentration technique is *solid-phase extraction* (SPE). SPE is a technique that is becoming increasingly popular, because unlike *liquid-liquid extraction* (LLE) it does not require large volumes of toxic organic solvents, analysis time can be decreased significantly and on-line and/or automated procedures are easily designed. Another advantage of SPE over LLE is the wide variety of extraction conditions which may be used to achieve the desired separation and concentration. The great variety of types of sorbents commercially available has increased the use of SPE.

The main objective of this work is to adapt and implement relatively simple and rapid method for gas chromatographic determination of individual PAHs in samples of coastal and transitional waters. The present work analyses the yield and precision of extraction of PAHs by solid phase extraction with using different sorbents for SPE (**Bond Elut: C18** and **Plexa** SPE discs), the two most common used for aqueous samples.

Experimental

Reagents and materials

Standards

Standard 1: Standard mixtures of 16 PAHs $2000 \mu\text{g}/\text{cm}^3$ each in methylene chloride (CH_2Cl_2) was used, SV Calibration Mix 16 RESTEK. For spiking, 1 cm^3 from standard 1 was diluted with methylene chloride to 50 cm^3 . Additionally 0.02 mm^3 (μL) of standard 1 was added to the seawater samples before SPE as an internal standard.

Sampling

Samples were collected in June 2010 from the harbour of town of Sozopol, SW Black Sea coast, Bulgaria to assess the spatial PAHs distribution. Seawater was collected from the surface in 2.5 dm³ bottles. After collection the samples were immediately transferred to the laboratory and stored under proper conditions. Seawater was kept at 2±4°C. We make analysis of 4 samples, all taking in area of town Sozopol.

Analyses

Solid-phase extraction (SPE) procedure

Pretreatment of seawater samples:

Pretreatment was performed at natural pH ~8.00. Four subsamples of seawater, 500 cm³ each were filtered through glass-fibre filter Whatman GF/C (pore size 9.0 μm). In all 500 cm³ seawater samples 50 cm³ isopropyl alcohol was added and mixed thoroughly.

Steps of solid-phase extraction (SPE) procedure for determination PAHs in seawater

Conditioning: Add 5 cm³ CH₂Cl₂ to the cartridge. Apply vacuum and discard the eluent. Repeat with 5 cm³ methanol (MeOH) then 5 cm³ water. Do not allow the sorbent to dry at any point during this step.

Samples loading: Attach a 50 cm³ sample reservoir to the top of the cartridge. Add to the reservoir water sample in portions avoiding sorbent drying. Apply the vacuum and discard the eluent. The flow rate should be no greater than 10 cm³/min. The literature listed different speeds to cause the sample to the solid-phase column. Most manufacturers recommend 5±15 cm³·min⁻¹. We decided to work with speed of 10 cm³·min⁻¹ for two sorbents. Higher speeds lead to low retention volume. This can lead to losses in the extraction of these compounds that have retention volume close to the sample size.

Washing: Add 3 cm³ of acetonitrile/water (50/50) to the cartridge. Apply vacuum and discard the eluent. Leave the vacuum on for 30 seconds and after that repeat procedure for washed of samples.

Elution: Place a collection tube beneath the cartridge. Add 3 cm³ methylene chloride to the cartridge. Apply vacuum and collect the eluent. Concentrate to 50±200 mm³ (μL) under a stream of dry nitrogen. Stop concentrating as close to 200 mm³ (μL) as possible. We not heat the samples during concentration - this will result in the substantial loss of many of the smaller ring size PAHs. Add methylene chloride to bring the final volume to 1000 mm³ (μL). Inject 1 mm³ (μL) into the GC/MS.

SPE cartridges: Agilent SampliQ C18 6 cm³, 50 cm³ sample reservoir (PN 5982-1365); Bond Elut Plexa VARIAN 6 cm³, 50 cm³ sample reservoir (P/N 5982-9305); coupling fitting; vacuum manifold (12-port).

Reagents: water (deionization water); methanol; isopropyl alcohol; methylene chloride.

Instrumentation

Gas Chromatographic - Mass Spectrometric analyses (GC-MS) were carried out on a DSQ II MSD coupled to a FOCUS GC (Thermo Scientific). One mm³ (μL) samples were injected in splitless mode. The injector was heated at 290°C. Separations were

achieved using a capillary column coated with ZB-5MS (Zebron, 20 m, 0.18 mm i.d., 0.18 μm film thickness) Phenomenex. The column temperature was programmed from 60 to 325°C at 8°C min^{-1} and held at 300°C for 15 min. Helium was used as carrier gas with constant velocity. Data acquisition was done at EI of 70 eV and *selected ion monitoring* (SIM) mode was used. Two characteristic ions were selected for each compound to allow a better identification. The standard PAH mixture was also analyzed in SCAN mode in the mass range of 50÷400 Da. Before analysis, relevant standards (reference mixture of PAHs) were run to check column performance, peak heights and resolution. With each set of samples to be analyzed, a solvent blank and a standard mixture were run in sequence to check for contamination, peak identification and quantification.

Results and discussion

We present a validation method for the determination of 16 PAHs in which we use a semi-automated solid phase extraction (SPE) method, with different sorbents in cartridges - Agilent SampliQ C18 and Bond Elut Plexa VARIAN, to concentrate the analyzed in a 500 mL water sample, with a further analysis by GC/MS technique.

The mass spectrometer is tuned daily meet the relative ion abundance requirements:

- A minimum of five concentration levels of each analyte are prepared to establish calibration factors with < 20% variance over the linear working range of the calibration curve. The linear calibration curves were obtained by plotting the peak area of each compound versus its concentration. The limits of detection and quantification were evaluated from the concentration of sulphonamides required to give a signal-to-noise ratio of 3 to 10 - respectively. Table 1 show the correlation coefficient and retention time for all PAHs.

Table 1

Calibration table for PAHs

PAHs	Molecular Ion (from experiment)	Retention time (from experiment)	Linearity R^2
1. Naphthalene	128.10	6.06	0.9991
2. Acenaphthylene	152.13	9.55	0.9988
3. Acenaphthene	152.16; 154	9.97	0.9991
4. Fluorene	166; 165	11.18	0.9990
5. Phenanthrene	178; 176	13.38	0.9991
6. Anthracene	178; 176	13.50	0.9991
7. Fluoranthene	202.11; 200	16.28	0.9996
8. Pyrene	202; 200	16.68	0.9998
9. Benz[a]anthracene	228; 226	19.73	0.9988
10. Chrysene	228	19.82	0.9975
11. Benzo[b]fluoranthene	252; 250	22.16	0.9972
12. Benzo[k]fluoranthene	252	22.21	0.9988
13. Benzo[a]fluoranthene	252.06	22.84	0.9990
14. Dibenzo[a,h]anthracene	276.12; 274	26.03	0.9984
15. Indeno[1,2,3-cd]pyrene	278	26.20	0.9996
16. Benzo[ghi]perylene	276	26.95	0.9991

- The initial calibration curve is verified each working day by the measurement of a mid-scale standard. The predicted response must not vary by more than $\pm 20\%$.

- Additional validation of the GC/MS performance is determined by surrogate standard recovery. The recovery of the surrogate standard should deviate from 100% (C18) by less than 20% (Plexa).
- The abundance ratio of qualifying to quantitation ions was within $\pm 20\%$ of target levels. (Example: for an ion with abundance relative to the quantization ion of 50% in the standard spectrum, the corresponding sample ion abundance must be between 30 and 70% of the quantization ion abundance).

The first step was to optimize a chromatographic separation for PAHs, using a capillary column coated with ZB-5MS (Zebron, 20 m, 0.18 mm i.d., 0.18 μm film thickness) working in isothermal program. This type column give as better separation and identification of PAHs in seawater samples (Figure 1 and Table 1). The results are obtained operating in SIM mode for the standard solution.

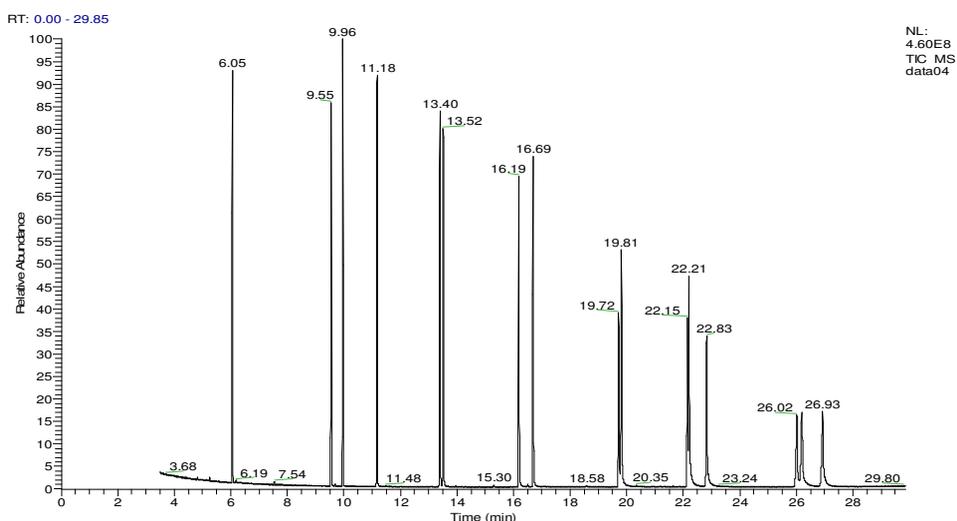


Fig. 1. GC analysis of Standard PAH Mix 16 (Agilent). Identification of compounds with retention times are presented in Table 1

The second step in our work was the optimization of the SPE method. We tested polymer- and silica-based sorbents, drying time and optimized the recoveries by adding iso-propanol to provide a final concentration of 5% in the water sample.

Pretreatment of samples make in identifiable conditions with using of different sorbents. Specific characteristic of two types of cartridge sorbents are presented in Table 2.

The Agilent SampliQ C18 sorbent is based on octadecylsilane-bonded, irregular silica gel (silica) particles. This non-polar, non-end capped sorbent provides reversed-phase binding of hydrophobic compounds. In addition, polar interactions are associated with the surface silanol groups.

Agilent SampliQ C18 is recommended as a general purpose SPE phase for both polar and non-polar analyses. The SampliQ C18 sorbent provides highly reproducible recoveries for a wide range of compounds following a simple protocol. C18 is generally regarded as the least selective silica-based sorbent, since it retains most organic analytes from aqueous

matrices. When analyzing small to intermediate molecules, C18 can be used for desalting aqueous matrices prior to ion exchange, as salts pass through the sorbent unretained.

Table 2

Sorbent specifications

Sorbent phase	Category	Bonded functional group/ Base material	Format	Typical carbon loading [%]	Surface area [m ² /g]	Particle size [µM] and shape	Mean pore size [Å]
Agilent SampliQ C18	Non-polar	Trifunctional octadecyl/silica based	Packed bed	17.4	500	40 and 120, irregular	60
Bond Elut Plexa VARIAN	Polar enhanced	Hydrophilic styrene divinylbenzene	Packed bed	-	550	45, spherical monodisperse	100

Bond Elut Plexa is a new generation of polymeric SPE products, designed for simplicity, improved analytical performance and ease-of-use. Its uniqueness lies in the novel hydroxylated exterior, hydrophobic interior and advanced polymeric architecture. Bond Elut Plexa, with an internal polar surface, is universally applicable and the best choices for the extraction of a wide range of acidic, neutral and basic analyses from different matrices. Bond Elut Plexa PCX is a cation exchanger with mixed mode sorbent characteristics and therefore suitable for the extraction and clean up of bio-matrix. Bond Elut Plexa PCX demonstrates the same excellent particle size distribution and integrity as Bond Elut Plexa.

M.C. Díaz Ramos and al [13] showed that the combination of the Agilent Pursuit PAH 3 µm HPLC column and automated SPE on Bond Elut Plexa provide a perfect and fast solution for the analysis of 24 PAHs from a large volume of water. The results of explained measurements [13] determine our decision to tested Bond Elut Plexa in process of SPE of coastal and running sea-water.

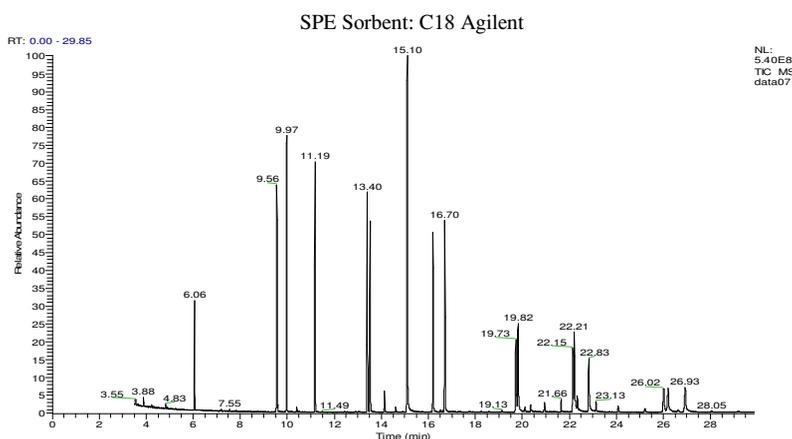
We make analysis of 4 samples, all taking in area of town Sozopol. Samples were collected in June 2010 from the harbour of town, SW Black Sea coast, Bulgaria to assess the spatial PAHs distribution. Pretreatment was performed at natural pH ~8.00. Four subsamples of seawater, 500 cm³ each were filtered and in all samples 50 cm³ isopropyl alcohol was added and mixed thoroughly. Procedure of solid-phase extraction (SPE) for determination PAHs for all samples is identical. The literature listed at different speeds to move the sample to the solid-phase column. Cited rates vary quite a wide range: from 2÷5 cm³·min⁻¹, 10÷30 cm³·min⁻¹ or even up to 100 cm³·min⁻¹. Most manufacturers recommend 5÷15 cm³·min⁻¹. You decided to work with specimen is 10 cm³·min⁻¹. Higher speeds lead to low retention volume. After elution of samples from different sorbents cartridges injected 1 mm³ (µL) into the GC/MS for analysis.

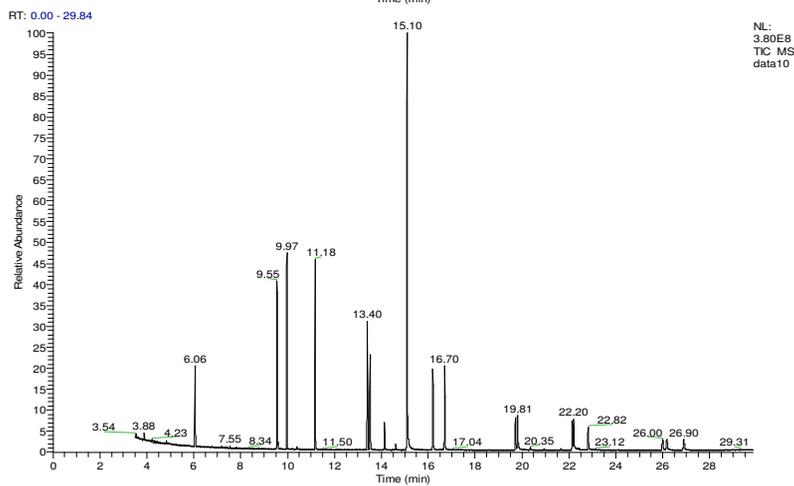
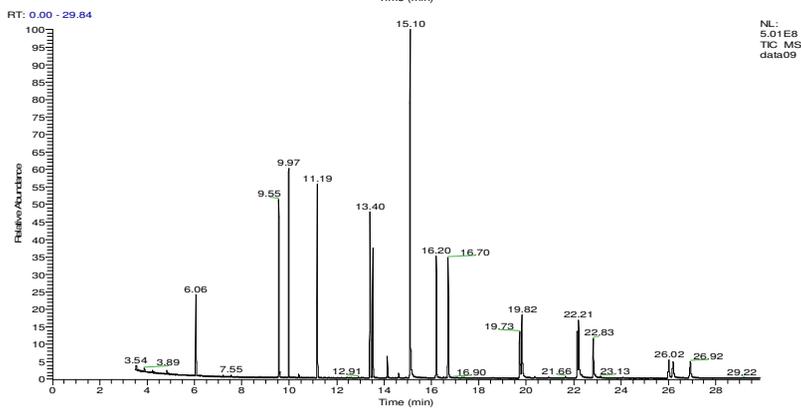
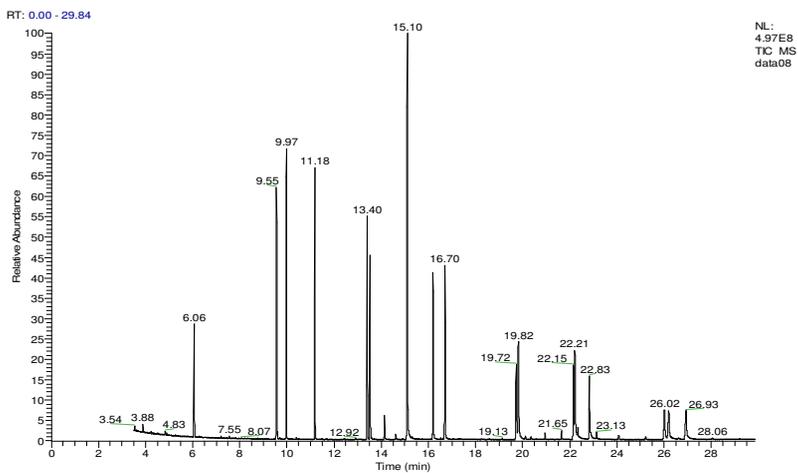
When comparing results after GC/MS analysis of samples, concentrated by two different sorbents, results are very different. The results show that the effect of sorbents type for extraction efficiency varies greatly among different PAH species. Compared with C18 and Plex extracted water samples, the efficiencies for all 15 PAHs without of naphthalene are higher for C18. Parameters that affect the efficiency of SPE with C18 columns are: the salt, organic content, flow rate through the sample columns for SPE, the method of drying the column after loading the sample, elution of PAHs and pH of samples. In principal the

salt content reduces the extraction efficiency for all the PAHs except naphathene [14]. On the other hand, the presence of excessive salt in water could lead to a change of the physical configuration of the chemical functionalities on the surface coating, which would then depresses the extraction efficiency.

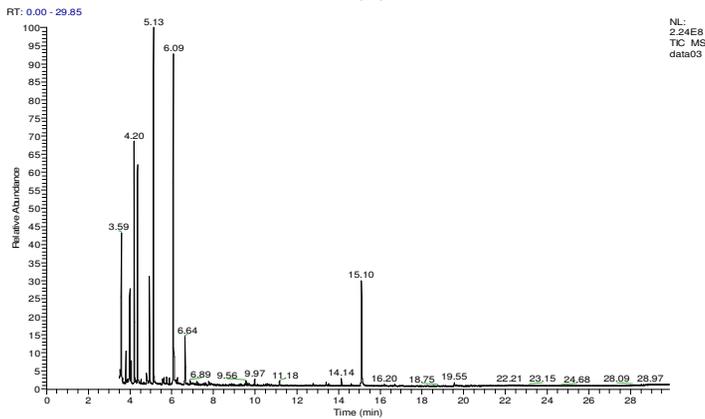
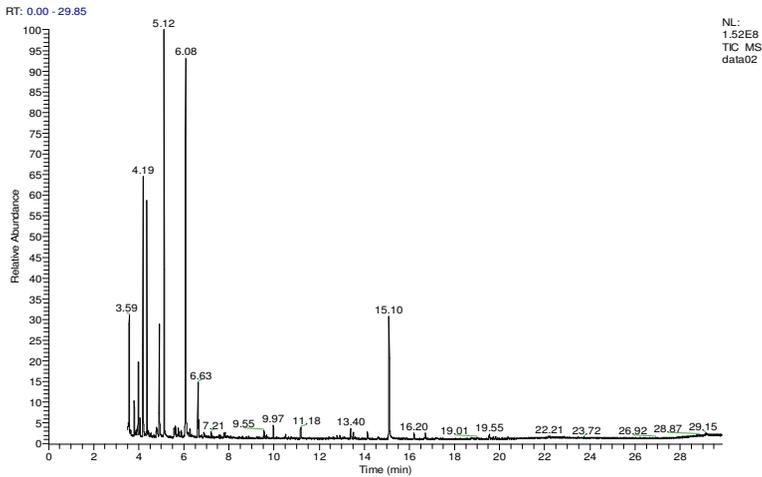
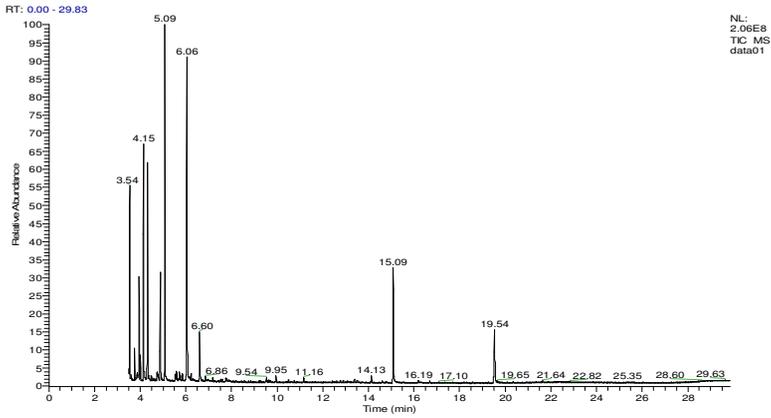
Table 3
Real concentrations of PAHs in seawater samples from Sozopol, pretreated by different SPE Sorbent: Bond Elut Plexa VARIAN and *C18 Agilent in identical condition of area - pH = 8 and S = 16.4

PAHs	Concentration [$\mu\text{g}/\text{cm}^3$]		Concentration [$\mu\text{g}/\text{cm}^3$]		Concentration [$\mu\text{g}/\text{cm}^3$]		Concentration [$\mu\text{g}/\text{cm}^3$]	
	1	1.1*	2	2.2*	3	3.3*	4	4*
Naphthalene	10.62	10.07	5.8	8.73	12.37	7.25	7.78	4.87
Acenaphthylene	0.181	21.84	0.24	19.4	0.22	16.9	0.164	10.19
Acenaphthene	0.216	23.92	0.21	18.882	0.22	16.6	0.144	10.3
Fluorene	0.186	24.9	0.29	20.89	0.161	17.69	0.158	10.556
Phenanthrene	0.230	25.0	0.71	21.156	0.276	17.5	0.177	9.1
Anthracene	0.310	25.12	0.70	20.993	0.235	17.86	0.043	9.09
Fluoranthene	0.369	25.01	0.55	19.3	0.256	16.127	0.230	7.6
Pyrene	0.210	24.5	0.63	18.68	0.295	15.6	0.162	7.5
Benzo[a]anthracene	nf	17.7	0.22	15.3	nf	12.25	nf	5.1
Chrysene	0.3	19.1	1.44	17.4	0.010	13.29	nf	5.76
Benzo[b]fluoranthene	0.331	17.24	0.05	17.77	nf	12.7	nf	5.22
Benzo[k]fluoranthene	1.486	16.88	1.90	16.0	0.944	11.58	0.089	4.65
Benzo[a]fluoranthene	0.36	16.36	0.16	15.65	nf	11.45	nf	4.7
Dibenzo[a,h]anthracene	nf	17.18	0.68	16.94	nf	12.26	nf	5.25
Indeno[1,2,3-cd]pyrene	nf	15.07	0.55	14.56	nf	10.15	nf	4.7
Benzo[ghi]perylene	0.579	15.9	0.65	15.18	nf	9.28	nf	4.54





SPE Sorbent: Bond Elut Plexa VARIAN



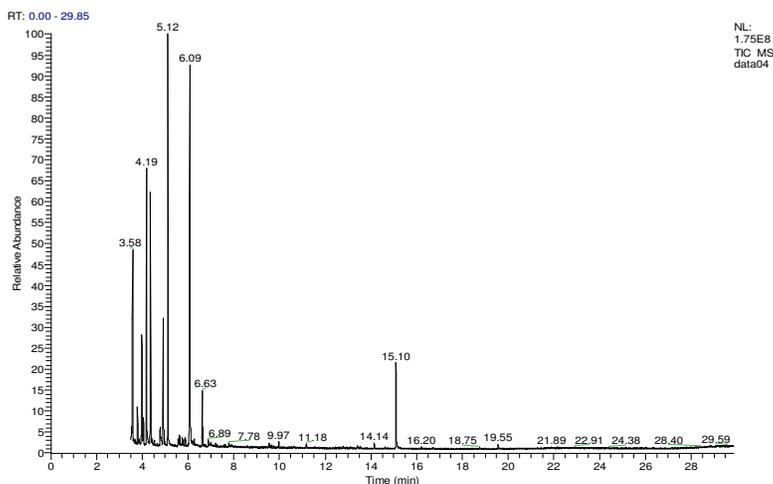


Fig. 2. Chromatogram of seawater samples analysis from Sozopol (pH = 8 and S = 16.4)

Conclusions

Depending of our application or sample type (seawater), we will appreciate the difference the Plexa and C18 range of products makes. Matrix interferences can result in significantly decreased analytical sensitivity due to ion suppression. C18 sorbents gives you higher recoveries in cleaner extracts, which translates into better sensitivity. C18 delivers high recoveries regardless of whether absolute or relative calculations are used. This indicates that ion suppression is minimized and maximum sensitivity is achieved. Relative recovery calculations are routinely used, but mask the effects of ion suppression, which are normalized.

For analysis of seawater samples, C18 is better sorbents because:

- Extremely retentive for non-polar compounds,
- Effective for desalting aqueous mixtures,
- The most hydrophobic, bonded silica sorbent.

References

- [1] Dabestani R, Ivanov IN. *Photochem Photobiol.* 1999;70:10-34. DOI: 10.1111/j.1751-1097.1999.tb01945.x.
- [2] Braga RS, Barone PMVB, Galvao DS. *J Mol Struct.* 1999;464:257-266. DOI: 10.1016/S0166-1280(98)00557-0.
- [3] Jones KC, Stratford JA, Waterhouse KS, Vogt NB. *Environ Sci Technol.* 1989;23:540-550. DOI: 10.1021/es00063a005.
- [4] Futoma DJ, Smith S, Smith TE, Tanaka J. *Polycyclic Aromatic Hydrocarbons in Water Systems.* Boca Raton: CRC Press; 1983. P. 25.
- [5] Pensado L, Blanco E, Casais MC, Mejuto MC, Martinez E, Carro AM, Cela R. *J Chromatogr A.* 2004;1056:121-30. DOI: 10.1016/j.chroma.2004.04.066.
- [6] Method 550.0, Washington: US Environmental Protection Agency; 1990.
- [7] Van de Merbel NC, Hageman JJ, Brinkman UATH. *J Chromatogr.* 1993;634:1-29. DOI: 10.1016/0021-9673(93)80308-U.

- [8] Carabias-Martinez R, Rodríguez-Gonzalo E, Paniagua-Marcos PH, Hernández-Méndez J. *J Chromatogr A*. 2000;869:427-439. DOI: 10.1016/S0021-9673(99)01218-2.
- [9] Hennion MC. *J Chromatogr A*. 1999; 856: 3-54. DOI: 10.1016/j.bbr.2011.03.031.
- [10] Carabias-Martinez R, Rodríguez-Gonzalo E, Dominguez-Álvarez J, Hernández-Méndez J. *J Chromatogr A*. 2000;869:451-461. DOI: 10.1016/S0021-9673(99)01273-X.
- [11] Buchholtz KD, Pawliszyn J. *Anal Chem*. 1994;66:160-167. DOI: 10.1021/ac00073a027.
- [12] Boyd-Boland AA, Magdic S, Pawliszyn J. *Analyst*. 1996;121:929-937. DOI: 10.1039/AN9962100929.
- [13] Díaz Ramos MC, Suárez A, Rúbies A, Companyó R. Laboratori de l'Agència de Salut, Publica de Barcelona, Spain; Determination of 24 PAHs in Drinking Water; Application Note. 2011.
- [14] Qingling L, Xiaolin X, Lee Chun-Sen Fr, Xiaoru W. *Science in China Series B: Chem*. 2006;49:481-491. DOI: 10.1007/s11426-006-2026-5.

OZNACZANIE WWA W WODACH MORZA CZARNEGO METODĄ GC/MS Z ZATĘŻANIEM POPRZEZ EKSTRAKCJĘ DO FAZY STAŁEJ

Abstrakt: Oznaczanie WWA w przybrzeżnych wodach morskich należy przeprowadzać z wielką starannością, aby uniknąć dużych strat w trakcie pobierania próbek i ich przechowywania. Wynika to z właściwości hydrofobowych WWA i ich skłonności do adsorpcji na powierzchniach, z którymi mają kontakt. Najlepszą techniką rozdzielania WWA jest chromatografia gazowa sprzężona ze spektrometrią masową. Z powodu małych stężeń WWA w próbkach wody konieczne jest ich zatężenie przed analizą chromatograficzną. Stosuje się kilka technik zatężania WWA, jednak najlepsze wyniki daje ekstrakcja do fazy stałej (SPE). Głównym celem pracy było dostosowanie i wdrożenie stosunkowo prostej i szybkiej metody chromatografii gazowej do oznaczania poszczególnych WWA w próbkach wód przybrzeżnych i wód płynących. W pracy przeanalizowano wydajność i precyzję ekstrakcji WWA przez zatężanie w fazie stałej z wykorzystaniem dwóch różnych sorbentów (Bond Elut: C18 i dysków Plexa SPE), najczęściej stosowanych w analizie próbek wodnych.

Słowa kluczowe: wielopierścieniowe węglowodory aromatyczne (WWA), ekstrakcja do fazy stałej (SPE), różne sorbenty (Bond Elut: C18 i dyski Plexa SPE), analiza wód przybrzeżnych