

**Analysis of Dissolved BTEX and PAHs in Seawater Following an Oil Spill:
Development of Sensitive, Operational Methods for Rapid Diagnosis**

February 25, 2011

Anne-Laure BALCON, Rami KANAN, Sophie VAN GANSE and Julien GUYOMARCH *

Cedre
715 rue Alain Colas – CS 41836
29218 BREST cedex 2
France
Julien.Guyomarch@cedre.fr

* corresponding author

ABSTRACT

The latest oil spills which have occurred in recent years have shown an increasing demand for detailed assessments of the chemical contamination induced in the water column. Even at low concentrations, hydrocarbons are known to generate impacts, and sanitary questions can be raised. Moreover, non-contaminated and contaminated areas must be distinguished; hence the necessity for developing tools sensitive enough to obtain reliable information on baseline levels.

In this context, *Cedre* has developed different analytical methods which can be partially performed on-site, and which can provide results within a few days, even in the case of pollution far from the laboratory. On the one hand, the SBSE (Stir Bar Sorptive Extraction) technique, performed on-site and coupled with subsequent GC/MS analysis in the laboratory, can be used to quantify PAHs at low levels, around nanogram per liter (ng/L). This paper discusses the possibility of adapting this technique to develop a more operational tool. The laboratory protocol can be adapted to avoid the shipment of chemicals, but the resulting increased variability and loss of sensitivity must be assessed.

On the other hand, BTEX (Benzene, Toluene, Ethylbenzene and Xylenes) can be analysed using a new device which uses the same equipment as SBSE. However, conditions required to keep samples cold or frozen during shipment cannot always be attained, and alternative possibilities have been investigated. The SBSE technique can also provide answers but analytical reliability must be checked prior to application in the field.

INTRODUCTION

The identification and quantification of semi-volatile contaminants dissolved in water is generally performed in the laboratory after several stages following sampling, mainly extraction, concentration and purification processes. These analyses are in most cases time-consuming, and the many stages involved increase the risk of contamination, particularly when dealing with trace levels. Moreover, in the case of oil spills, the rapid

shipment of samples can be difficult, and the conditions of their preservation questionable. The comparison of various extraction techniques used to analyze water samples has been discussed, and showed the reliability of Stir Bar Sorptive Extraction (SBSE), particularly in comparison with traditional liquid-liquid extractions (Hyötyläinen and Riekkola, 2007).

Consequently, SBSE performed on-site combined with subsequent analysis in the laboratory represents an interesting solution for most non-classical situations (low amount of sample, difficult access to the location of interest, difficulties in sending samples or chemicals, ...). This technique, which was successfully applied in previous monitoring studies conducted in order to assess baseline levels, was also developed in the context of oil spills. It must be noted that this technique was also tested with a field GC/MS; however some limitations appeared as regards the injection system (Roy *et al.*, 2005, *Talanta*). Finally, it appeared that some volatiles organic compounds (VOCs) such as BTEX could also be quantified by SBSE (Demeestere *et al.*, 2007), and a new device, based on the Dynamic Headspace (DHS) technique and using the same instrumentation as SBSE, was also assessed for these molecules.

MATERIAL AND METHODS

Principle of the SBSE Analyses

SBSE (Stir Bar Sorptive Extraction) developed by Gerstel (Mülheim an der Ruhr, Germany) consists in the concentration of apolar dissolved compounds by the apolar phase (Polydimethylsiloxane) deposited on a stir bar (Kawaguchi *et al.*, 2006). Extraction is ensured by stirring at 900 rpm for 2 hours. Quantification is achieved by introducing internal standards at the beginning of the sample preparation procedure. These standards (5 perdeuterated PAHs) are introduced into a methanolic solution in order to achieve complete solubilization, and this final 10% methanol content also prevents compounds from being sorbed on the glass walls. After the extraction, the bars are recovered, rinsed with distilled water, dried over paper and placed on the automatic sampler in order to thermally desorb the compounds which are then introduced into the GC/MS coupling.

Principle of the DHS Technique

The Dynamic Headspace (DHS) technique consists in purging dissolved volatile organic compounds (VOCs) with helium. The VOCs are then trapped and concentrated on a sorbent. These molecules are then thermally desorbed for subsequent GC/MS analysis. Extraction yields are corrected using a deuterated internal standard (Toluene d_8) introduced as a methanolic solution to achieve a final methanol concentration close to 10%.

Sample preparation

Calibration solutions

The solutions were prepared from certified reference material purchased from LGC Standards (Molsheim, France) as regards semi-volatile compounds: CUS 9305, which contains 21 PAHs at the concentration of 1 µg/mL in methanol, and CUS 9207, which contains the corresponding internal standards: Naphthalene d₈, Biphenyl d₁₀, Phenanthrene d₁₀, Chrysene d₁₂ and Benzo[*a*]pyrene d₁₂ at the concentration of 1 µg/mL in acetone. For volatile organics compounds, the Gasoline Range Organics solution was used, which contains BTEX (Benzene, Toluene, Ethylbenzene and Xylene) and also trimethylbenzenes and naphthalene, at the concentration of 2000 µg/mL of methanol. Toluene d₈, purchased from Sigmaaldrich (St. Louis, MO, USA), was used as internal standard. All the calibrations curves were obtained by extracting and analyzing water samples spiked with target molecules and corresponding internal standards.

Water-accommodated fractions (WAFs)

Water-accommodated fractions were prepared at 20°C according to guidelines established by CROSSERF (Singer et al., 2000). These experiments were conducted in order to transfer molecules from the oil to the water phase only by solubilization.

The principle of this experiment was to introduce oil at the water surface of a closed flask. To avoid significant transfer to the air phase, the headspace had to be lower than 25% of the whole volume of the flask. The oil/water ratio was set at 1/10,000, and agitation was ensured by using a magnetic stirrer. This agitation was set at a minimum level to prevent oil from being dispersed in the water column. The experimental device was set in an air-conditioned room at 20°C for 24 hours to reach the equilibrium. The oil used was an Arabian crude oil topped at 150°C in order to simulate a slight weathering at sea. Finally, a water sample was collected from a tap located at the bottom of the flask.

Samples analyses

Prior to the extraction by SBSE, 100 mL water samples were added with 10 mL of methanol containing the 5 perdeuterated internal standards at the concentration of 1 ng/mL (final concentration of 100 ng/L relatively to water). For the quantification of BTEX, toluene d₈ was also added to obtain a final concentration in water of 100 ng/L. The stir bar was then introduced in the sample and the stirring performed, for 1 hour for BTEX and 2 hours for PAHs.

Prior to the DHS analyses, 10 mL water samples were added with 1 mL of methanol containing toluene d₈ used as an internal standard at the concentration of 100 ng/mL (final concentration of 1 µg/L relatively to water).

Simplified sample preparation for field applications

In order to avoid the shipment of chemicals, which may be time-consuming or even problematic, a simplified procedure was assessed for PAH quantification. Internal standards used for SBSE analyses were directly added onto the stir bar, in the same proportion as the regular protocol (10 μ L of CUS 9207). Extractions were then performed without addition of methanol. In order to assess the stability of the spiking, a delay (from 0 to 31 days) was observed between the addition of internal standards and the analysis of stir bars. Finally, the influence of this simplified protocol was estimated by quantifying PAHs contained in a WAF with spiked bars (addition of internal standards from 0 to 14 days before extraction) and according to the standard protocol. All these experiments were performed in triplicates.

Instrumentation and analytical conditions

PAHs by SBSE

The analyses were performed using a Thermal Desorption Unit (TDU) combined with a Cooled Injection System (CIS) from Gerstel (Mülheim an der Ruhr, Germany) mounted on a 7890 Agilent GC system coupled to an Agilent 5975 mass spectrometer (Agilent Technologies, Little Falls, DE, USA) as illustrated picture 1. The analytical system was equipped with an automated sampler MPS2 (Gerstel). Desorption was achieved at 300 °C for 10 minutes under an helium flow of 50 mL/min in the splitless mode and with a transfer line maintained at 300 °C. The desorbed compounds were cryofocused in a cooled injection system (CIS-4, Gerstel) at 10 °C and then transferred to the HP-5MS column (30 m x 0.25 mm i.d. x 0.25 μ m film thickness, constant helium flow of 1 mL/min) by a rapid increase of the CIS temperature (from 10°C to 300°C at 12°C/s). For the analysis of PAHs, the oven program of temperature was: from 50 °C (1 min) to 150°C at 10°C/min, and then to 320°C (5 min) at 5°C/min. The mass spectrometer was operated in Selected Ion Monitoring (SIM) with a minimum of 1.5 scan/s. The quantification was performed by using the molecular ion of each PAH. The target molecules were quantified relatively to the perdeuterated PAHs (internal standards) using a calibration curve (from 0.1 ng/L to 100 ng/L).

BTEX analyses by DHS and SBSE

Volatiles Organic Compounds were concentrated on a Tenax phase by using the Gerstel Dynamic Headspace (DHS) module mounted on the automated sampler. The analyses were performed by using the TDU/CIS-GC/MS system described previously. The 10 mL water sample was purged for 6 minutes by an helium flow of 25 mL/min after an incubation period of 5 minutes at 30°C (stirring at 500 rpm with a 1 second stop every 10 seconds). The Tenax was then dried for 3 minutes by an helium flow of 50 mL/min, and then introduced in the TDU for subsequent thermal desorption. As regards the SBSE

technique, the stir bar was directly introduced in a desorption tube placed on the sampler. Conditions of analyses were then similar for both DHS and SBSE.

Desorption was achieved at 300 °C for 10 minutes under an helium flow of 50 mL/min in the splitless mode and the transfer line at 300 °C. The desorbed compounds were cryofocused in programmable temperature vaporisation (PTV) injector (CIS-4, Gerstel) at 0 °C. Analytes were then transferred to the HP-5MS column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, constant helium flow of 1 mL/min) by a rapid increase of the CIS temperature (from 0°C to 300°C at 12°C/s). The oven program of temperature was: from 20 °C (1 min) to 80°C (3 min) at 20°C/min, and then to 250°C (3 min) at 60°C/min. The mass spectrometer was operated in Selected Ion Monitoring (SIM) at a minimum of 1.5 scan/s and the quantification was performed by using a representative fragment of each compound. The target molecules were quantified respectively to toluene d_8 by using a calibration curve (0.1 ng/L to 100 ng/L for SBSE and from 10 ng/L to 100 µg/L for DHS).

RESULTS OF ANALYSES ON STANDARD SOLUTIONS

SBSE analyses of PAHs

The performances of the SBSE coupled to a GC/MS analysis were determined from 7 levels of concentrations with 3 repetitions per level. Figure 1 illustrates a reconstructed chromatogram obtained for individual concentrations of 100 ng/L.

Limits of detection and quantification are presented in Table 1. It must be noted that alkylated derivatives could not be analyzed directly: for real samples, it was assumed that the sensitivity of the method was similar for alkylated compounds and corresponding parents PAHs. These limits, in the range of sub-ng/L, are particularly low and relate to the concentrations frequently met in coastal seawater (Singer *et al.*, 2000). Moreover, linearity was checked for concentrations from 0.1 to 100 ng/L, thus allowing a wide range of applications, from baseline levels to significant levels of contamination. Finally, extraction yields, calculated according to the 5 deuterated PAHs responses compared to direct injection, were assessed around 85%.

DHS analyses of BTEX

The analysis of BTEX using the DHS technique was first optimized in order to specify the optimum conditions at the various stages of the analysis. Many parameters were considered: DHS extraction, thermal desorption of compounds using TDU/CIS coupling, separation and shape of peaks by gas chromatography The analytical conditions described previously were established using various experimental plans as numerous parameters were involved at each stage of the protocol (temperatures, incubation time, purge flows, ...).

Figure 2 presents the reconstructed chromatogram obtained for BTEX and trimethylbenzenes for individual concentrations of 10 µg/L. The limits of quantification were not defined but it can be reasonably assumed, by comparing abundance peaks and results obtained by SBSE, that these limits may be in the range of 10-50 ng/L. Extraction

yields, calculated according to the Toluene d_8 response compared to direct injection, were assessed around 20%.

SBSE analyses of BTEX

First, the extraction time was established by comparing the abundances obtained for various durations, from 15 minutes to 3 hours. As the equipment configuration used to thermally desorb and analyze compounds was the same as for the DHS technique, the same conditions were adopted for the quantification of BTEX extracted by the stir bar. Figure 3 presents the reconstructed chromatogram obtained for BTEX and trimethylbenzenes for individual concentrations of 100 ng/L. Table 2 presents the limits of detection and quantification, also in agreement with performances required for monitoring studies.

ANALYSES OF WATER-ACCOMMODATED FRACTIONS

Analyses of PAHs by the standard protocol

Figure 4 presents some of the PAHs and alkylated PAHs which can be analyzed by SBSE in a water-accommodated fraction prepared with an oil/water ratio of 1/10 000 and diluted 100 times. This chromatogram shows that peaks can be easily detected and separated, and no interferences with compounds generated by the PDMS desorption were observed.

The linearity of the method was also assessed for concentrated solutions in order to determine the potential limitations of the method in the case of high contamination levels as generally observed in the case of oil spills. The concentrations of compounds measured on the WAF diluted 100 times (1%) were also determined for lower dilutions (10, 20 and 50%), and even on the WAF itself (100%). Theoretical concentrations were calculated considering the value obtained for the 1% sample, which represented the highest dilution, less liable to be affected by the saturation of the PDMS phase. Figure 5 presents the correlation between the predicted and calculated concentrations for dibenzothiophene. This figure shows that the linearity is acceptable (slope close to 0.9), even for a wide range of concentrations and high levels of contamination (from 2 to 200 $\mu\text{g/L}$).

Development of simple procedure for operational use

The spiking proved to be stable over time, as illustrated in figure 6. Abundances were not significantly different within a delay of up to 1 month, which is sufficient considering applications in the field.

These pre-spiked bars were used to quantify the 21 parent PAHs contained in a water soluble fraction prepared with an oil/water ratio of 1/10,000 and diluted 1000 times. Results of analyses presented figure 7 do not show significant differences over time for pre-spiked bars but it appears that the modified protocol slightly underestimates

concentrations. This could be due to the complete extraction of internal standards by this protocol whereas an equilibrium is established in standard conditions between the water and PDMS phase. As the PAH quantification was performed using the same calibration curve for both cases, the simplified approach could be greatly improved using spiked bars to establish the calibration curves.

CONCLUSION

The SBSE extraction, in combination with a GC/MS analysis, proved to be a powerful tool for the quantification of PAHs and BTEX, either at trace levels (in the sub-ng level) or for significant contamination of the water column as generally observed during oil spills. Taking into account the high toxicity of these molecules, even at low levels (Goanvec et al., 2008), SBSE represents an interesting solution for monitoring studies which should be carried out following an oil spill. Moreover, limits of quantification were in agreement with the sensitivity required to assess contaminations relatively to Environmental Quality Standards (EQS) for priority substances and certain other pollutants, in inland surface waters and coastal waters, established within the European Water Framework Directive (European Parliament and Council, 2008). Over and above its analytical performances, this technique proved to be quite flexible as the extraction could be performed directly in the field by non-specialists provided care is taken to avoid contamination. Moreover, the same equipment could also be used to directly analyze BTEX in water samples.

To broaden the possibilities of applying the SBSE technique in the field, some simplified procedures were also developed in order to avoid any shipment of chemicals which can sometimes be problematic. This approach showed promising results, close to the standard protocol, and could be rapidly improved by adapting the calibration procedure.

Finally, all these developments must be confirmed, particularly as concerns the conservation of BTEX on the stir bar after extraction, as molecules are liable to be affected by significant losses due to their volatility (in this study, analyses were performed immediately after the extraction).

ACKNOWLEDGEMENTS

Most of the analytical developments were performed within the MIGRHYCAR project financially supported by the French National Agency for Research

REFERENCES

DEMEESTERE K., DEWULF J., DE WITTE B., VAN LANGENHOVE H. Sample preparation for the analysis of volatile organic compounds in air and water matrices. *Journal of Chromatography A*. 2007, vol. 1153, n° 1-2, pp. 130–144

EUROPEAN PARLIAMENT, COUNCIL. *DIRECTIVE 2008/105/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council*. Official Journal of the European Union, 24.12.2008, L 348, pp.84-97

GOANVEC C., THERON M., LACOUÉ-LABARTHE T., POIRIER E., GUYOMARCH J., LE FLOCH S., LAROCHE J., NONNOTTE L., NONNOTTE G. Flow Cytometry for the Evaluation of Chromosomal Damage in Turbot *Psetta maximus* (L.) Exposed to the Dissolved Fraction of Heavy Fuel Oil in Sea Water: A Comparison with Classical Biomarkers. *Journal of Fish Biology*. 2008, vol. 73, n°2, pp. 395-413

HYÖTYLÄINEN T.; RIEKKOLA M.-L. Potential of effective extraction techniques and new analytical systems for profiling the marine environment. *Trends in analytical chemistry (Regular ed.) A*. 2007, vol. 26, n° 8, pp. 788-808

KAWAGUCHI M., ITO R., SAITO K., NAKAZAWA H. Novel stir bar sorptive extraction methods for environmental and biomedical analysis. *Journal of Pharmaceutical and Biomedical Analysis*. 2006, vol. 40, n°3, pp. 500–508

ROY G., VUILLEMIN R., GUYOMARCH J. On-site Determination of Polynuclear Aromatic Hydrocarbons in Seawater by Stir Bar Sorptive Extraction and Thermal Desorption GC-MS. *Talanta*. 2005, vol. 66, n°3, pp. 540-546

SINGER M.M.; AURAND D.; BRAGIN G.E.; CLARK J.R.; COELHO G.M.; SOWBY M.L.; TJEERDEMA R.S. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. *Marine pollution Bulletin*. 2000, vol. 40 n°11, pp. 1001-1040

Table 1. Quantification ions and limits of detection (LOD) and quantification (LOQ) of parent PAHs

Compound	Quantification ion	LOD (ng/L)	LOQ (ng/L)
Naphthalene	128	0.11	0.38
Benzothiophene	134	0.07	0.24
Biphenyl	154	0.04	0.12
Acenaphthylene	152	0.04	0.14
Acenaphthene	154	0.03	0.10
Fluorene	166,	0.03	0.11
Phenanthrene	178	0.02	0.08
Anthracene	178	0.03	0.09
Dibenzothiophene	184	0.02	0.05
Fluoranthene	202	0.04	0.12
Pyrene	202	0.04	0.13
Chrysene	228	0.07	0.23
Benz[<i>a</i>]anthracene	228	0.05	0.16
Benzo[<i>b</i>]fluoranthene	252	0.02	0.06
Benzo[<i>k</i>]fluoranthene	252	0.02	0.06
Benzo[<i>e</i>]pyrene	252	0.03	0.08
Benzo[<i>a</i>]pyrene	252	0.07	0.11
Perylene	252	0.02	0.08
Indeno[1,2,3- <i>cd</i>]pyrene	276	0.07	0.24
Dibenz[<i>a,h</i>]anthracene	278	0.09	0.31
Benzo[<i>ghi</i>]perylene	276	0.10	0.34

Table 2. Quantification ions and limits of detection (LOD) and quantification (LOQ) of main compounds

Compound	Quantification ion	LOD (ng/L)	LOQ (ng/L)
Benzene	78	0.10	0.32
Toluene	91	0.11	0.38
Etylbenzene	91	0.10	0.32
m,p-Xylene	91	0.09	0.30
o-Xylène	91	0.19	0.63
1,3,5-Trimethylbenzene	120	0.09	0.29
1,2,4-Trimethylbenzene	120	0.11	0.35



Picture 1. GC/MS equipment used for SBSE and DHS analyses

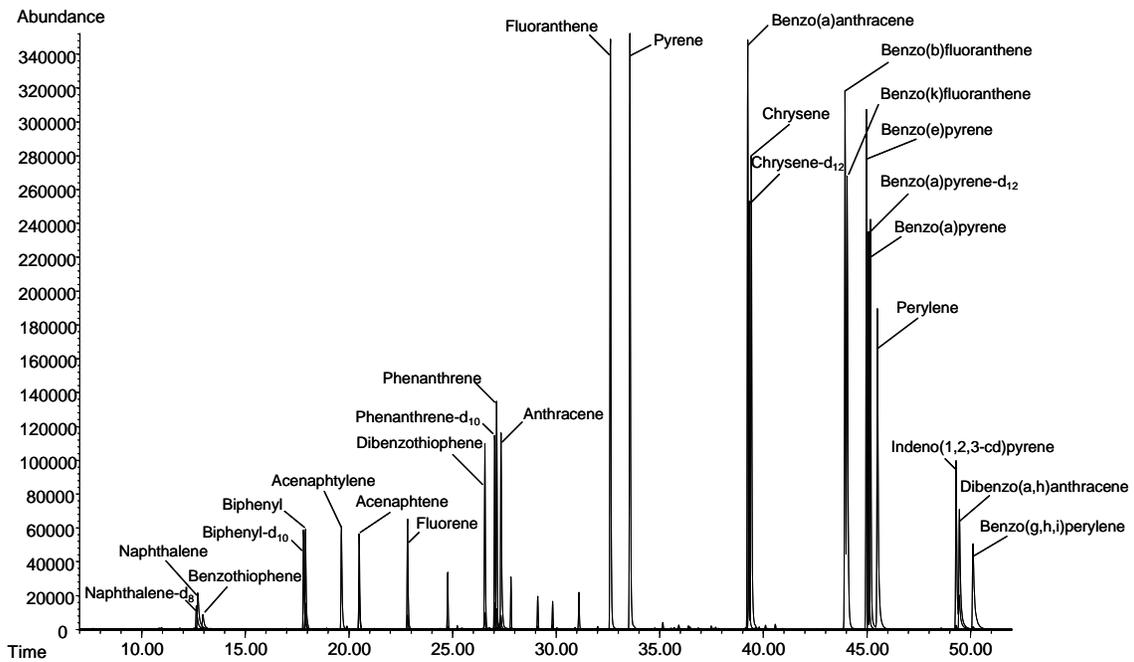


Figure 1. Reconstructed chromatogram of the 21 PAHs and corresponding internal standards at the concentration of 100 ng/L

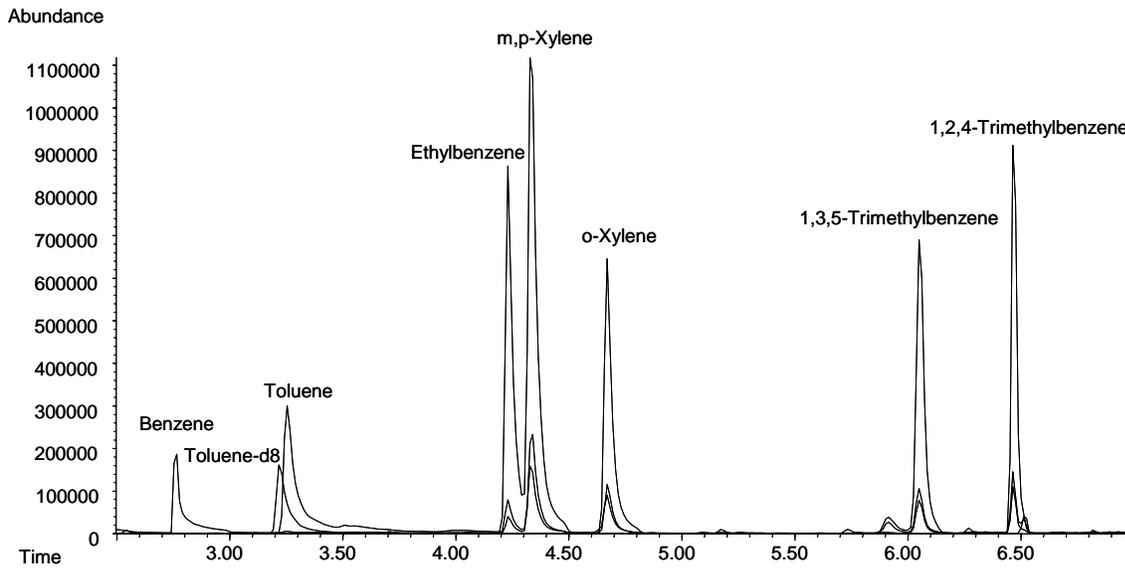


Figure 2. Reconstructed chromatogram of BTEX and trimethylbenzenes and corresponding internal standard (Toluene d₈) at the concentration of 10 µg/L

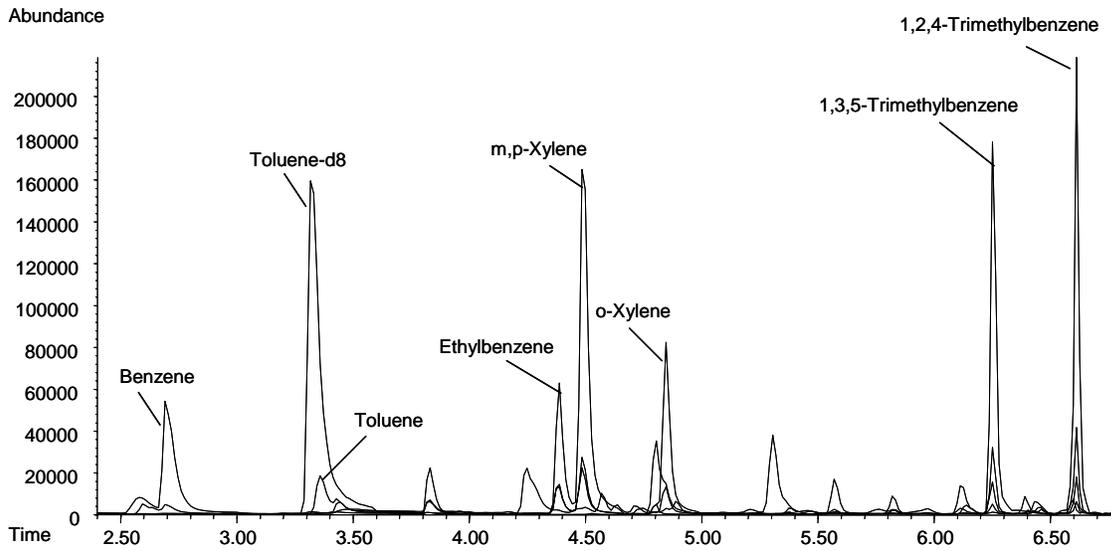


Figure 3. Reconstructed chromatogram of BTEX and trimethylbenzene and corresponding internal standard (Toluene d₈) at the concentration of 100 ng/L

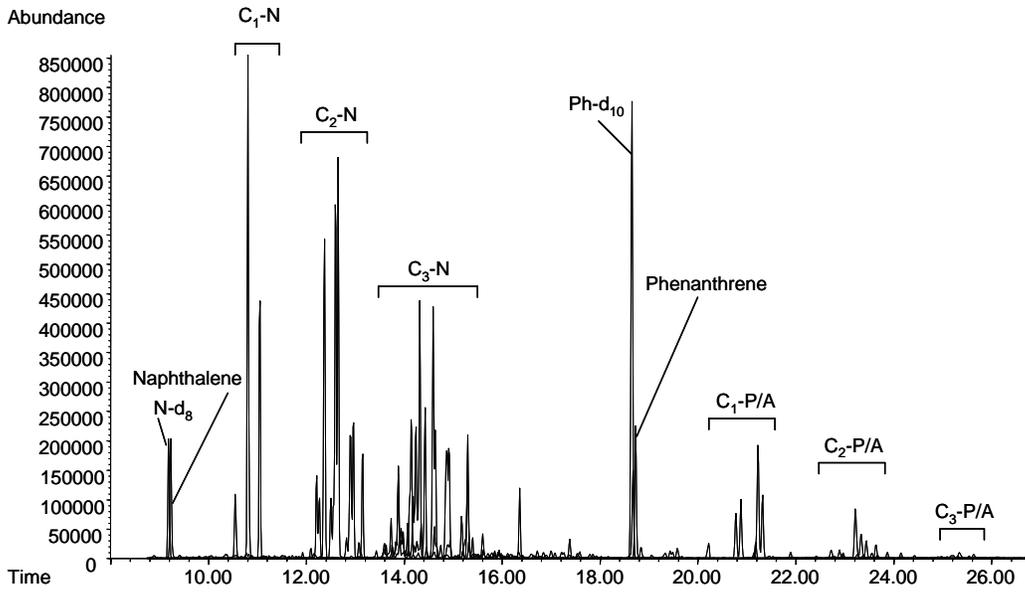


Figure 4. Reconstructed chromatogram of Naphthalene, Phenanthrene, Anthracene and corresponding alkylated derivatives, and internal standards (Naphthalene d₈ and Phenanthrene d₁₀ at the concentration of 100 ng/L)

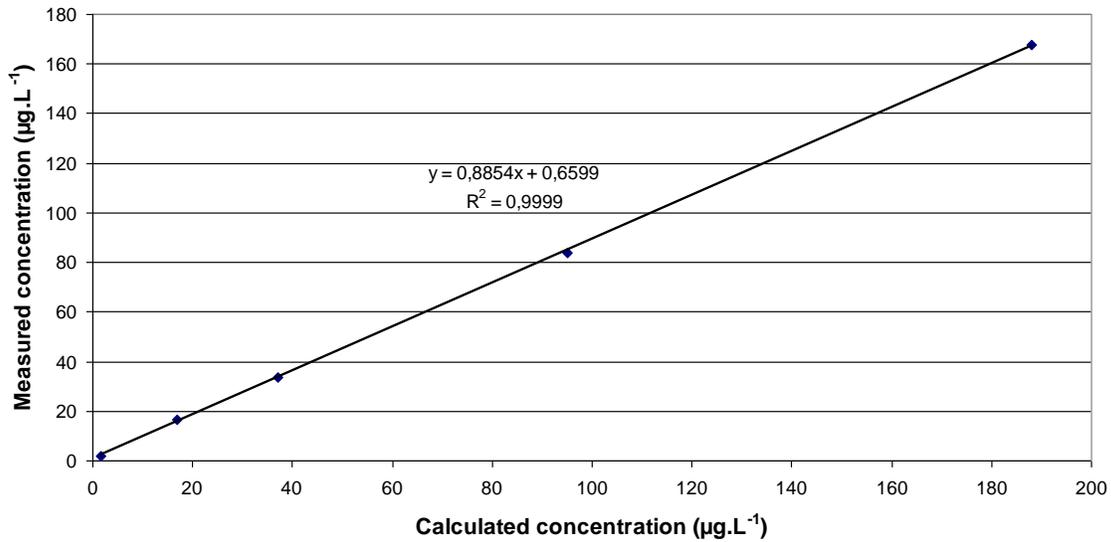


Figure 5. Comparison of calculated and measured concentrations of dibenzothiophene considering dilutions by 100, 10, 5 and 2, and pure water accommodated fraction (100-times dilution as reference for calculated values)

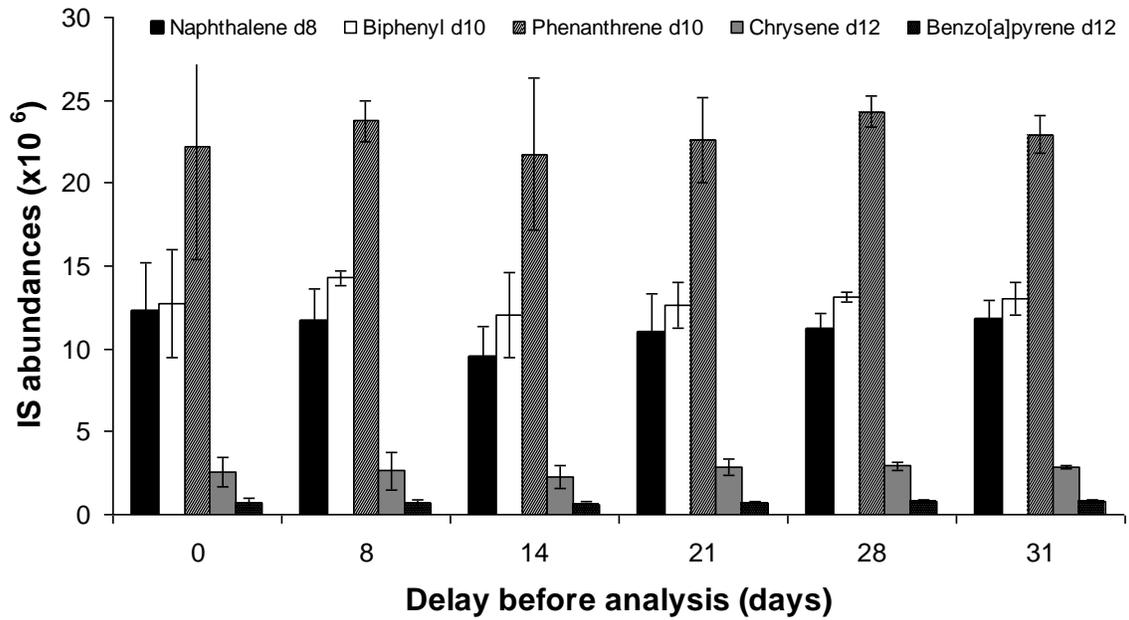


Figure 6. Comparison of the abundances of internal standards with various delays, from 0 to 31 days, between spiking and extraction

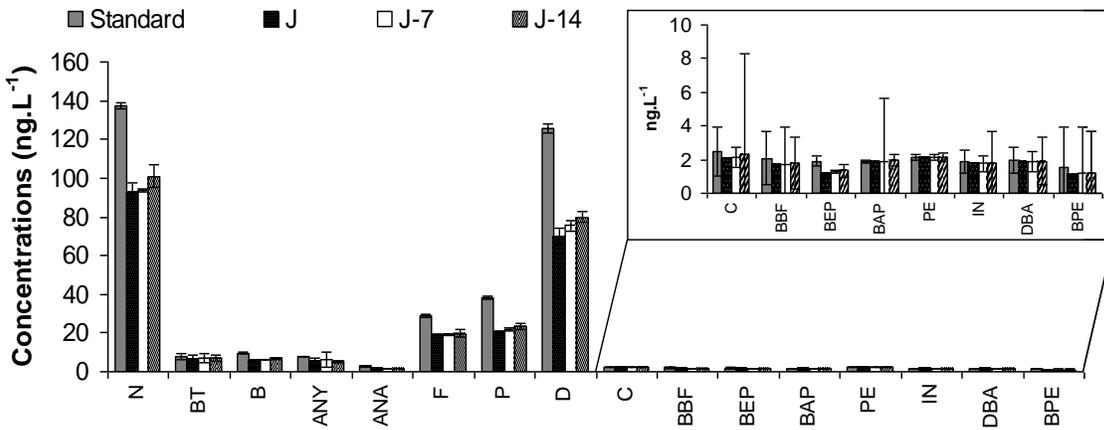


Figure 7. Comparison of PAH quantifications for the standard protocol and using pre-spiked stir bars, from 0 to 14 days before extraction