Problems of PAH quantification by GC–MS method using isotope-labelled standards

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Article history:
Received 5 June 2008
Received in revised form 10 December 2008
Accepted 16 December 2008
Available online 25 December 2008

Keywords:
Sediments
Polycyclic aromatic hydrocarbons
Internal standard
Extraction
Detection
GC–MS system

1. Introduction

The gas chromatography–mass spectrometry (GC–MS) system is routinely used today in environmental analysis of trace organic compounds.

In the case of every device, work of which is based on the relative principle, suitable standard substances are needed.

With the use of a MS-detector, stable isotope-labelled materials' analogous of the native analyte, are a convenient internal standard. They can be used for tracing and compensating analytes' losses during the particular stages of analytical procedure, such as cleaning or diluting, and variations in instrument settings and sensitivity. In determination of diverse physical forms of the same individual chemical species is a typical example of an activity from the scope of physical speciation analysis [8,9]. In this case, required is the determination of analytes present in the following forms:

- dissolved in the aqueous phase;
- associated with suspended particulate matter;
- associated (as result of bioaccumulation) with organs and tissues of living organisms;
- associated with sediments.

Most environmental particulates consist of solid minerals covered with organic matter where the native analyte is sorbed. For this reason numerous mechanisms of sorption of organic substances to particulate matter are possible [10,11]. When the internal standard is added with solvent to the particle surface—being a solid sample, it cannot be assumed that the internal standard is identically associated with corresponding analyte. The reason is that both the analytes and internal standards are transferred to the sample matrix in different way (the internal standard is added with the organic solvent, what is connected with the solvation by solvent molecule, not by water, as it take place in case of native analytes) and the degree of sorption might then differ. Usually the internal standard is (at least partially) more loosely connected with the matrix than the analytes are. Thus, the extraction efficiency of native analyte

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doi:10.1016/j.talanta.2008.12.037
and internal standard might differ [12]. It is especially significant in case of extraction techniques characterized by low recovery of analytes.

There are several methods of adding internal standard to the examined solid samples known. In guidelines and recommendations available, information about the proper working conditions with biological samples with a relatively high content of lipid matter are included. However, the internal standard recovery from complex samples differs depending on the matrix type and technique of standard addition [13].

Most often, the internal standard is added to the sample in a small (0.1–1 ml) amount of organic solvent. There are also used more complicated systems based on application of rotating equipment for uniform coating and reduction of large solvent volumes [14]. Nevertheless, no method enable adding the internal standard in the way truly imitating association of native analytes with sample matrix, especially in the case of environmental analyses, where samples with very complex matrix are analysed.

In this article, problems connected with the quantitative analysis of polycyclic aromatic hydrocarbons in sediment samples using GC–MS system were raised.

The aim of conducted work was to assess the influence of the following factors on the results obtained: calibration of the GC–MS system, internal standard addition technique and the amount of internal standard added.

2. Experimental

2.1. Reagents and standards

High-purity HPLC-grade dichloromethane, acetone and methanol were purchased from Sigma–Aldrich (Germany).

A standard mixture of PAHs in methanol was purchased from Restek Corporation, USA, and consisted of: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benz[b]fluoranthene, benz[k]fluoranthene, benz[a]pyrene, indeno[123-cd]pyrene, dibenz[a]anthracene, benz[g[h]i]perylene. The mixture contained 2000 µg/ml of each PAH.

Certified naphthalene-d8 and benz[a]anthracene-d12 (2000 µg/ml in dichloromethane) standards were from Supelco (USA); Copper powder and silica gel were from J.T Baker. Reference material – river-sediment METRANAL2 was from Promochem (Poland).

2.2. Gas chromatographic analysis

All experiments were performed using a TraceGC gas chromatograph (ThermoQuest, Finningan) equipped with a mass spectrometric detector TraceMS (ThermoQuest, Finningan) and cold on-column injector, using a RtX-5MS column (30 m; 0.25 mm; 0.25 μm). The carrier gas (helium) was maintained at a constant pressure of 70 kPa. The GC temperature was programmed as follows: from 40 to 120 °C at a rate of 40 °C min⁻¹; till 280 °C at a rate 5 °C min⁻¹ where it was held for 12 min. The MS was operated in electron ionization (EI) mode with the ion source temperature of 220 °C. The mass spectrometer was operated in selected ion monitoring mode; the following ions were monitored: \((m/z)\) 127, 128, 136, 151, 152, 153, 154, 165, 166, 176, 178, 202, 203, 226, 228, 240, 250, 252, 276, 277, 278, 279.

2.3. Procedure of calculation of the amount of analytes introduced to the chromatographic column

In order to perform the calculations of the quantity of analytes in the sample dosed to the chromatographic column, samples were introduced subsequently:

- first—the standard-solution, wherein the content of analytes and internal standard is well-known;
- second—the investigated sample, containing internal standards in predefined quantity.

During the studies two standard solutions were used. Standard solution I contained analytes from PAH group at concentrations of 133 ng/ml as well as deuterated PAHs at concentration of 167 ng/ml; standard solution II contained analytes at concentrations of 53.5 ng/ml and 66.5 ng/ml, respectively.

The quantity of analytes in the investigated sample was calculated on the basis of formula presented below:

\[
p_{X}\text{pr}/m_{X}\text{pr} = p_{X}\text{st}/m_{X}\text{st}
\]

where \(p_{X}\text{pr}\) is the peak area of a determined substance \(X\) on a chromatogram obtained after injecting extract from a sediment sample into the chromatographic system, \(m_{X}\text{pr}\) is the mass of a determined substance \(X\) on a chromatogram obtained by dosing extract from a sediment sample into the chromatographic system, \(p_{D}\text{pr}\) is the peak area of a deuterated standard \(D\) on a chromatographic system by dosing extract from a sediment sample into the chromatographic system, \(m_{D}\text{pr}\) is the mass of a determined deuterated standard \(D\) on a chromatogram obtained by dosing extract from a sediment sample into the chromatographic system; \(p_{X}\text{st}\) is the peak area of a determined substance \(X\) on a chromatogram obtained by dosing standard solution into the chromatographic system, \(m_{X}\text{st}\) is the mass of a determined substance \(X\) on a chromatogram obtained by dosing standard solution into the chromatographic system, \(p_{D}\text{st}\) is the peak area of a determined deuterated standard \(D\) on a chromatogram obtained by dosing standard solution into the chromatographic system and \(m_{D}\text{st}\) is the mass of a determined deuterated standard \(D\) on a chromatogram obtained by dosing standard solution into the chromatographic system.

2.4. Calibration of the GC–MS system

The standard curves for analytes were prepared by setting the linear dependence between the chromatographic peak area for the given substance and the amount of the substance in the sample introduced into the control-measurement device. The calibration of the GC–MS system was conducted for 16 analytes from PAH group, and for two isotope-labelled analytes (used as an internal standard). Ten solutions of the each standard analyte in dichloromethane were prepared in the following quality ranges: 1–120 pg and 160–3200 pg of the analyte in the sample dosed.

The standard solution samples were dosed into the chromatographic column in the volume of 2 μl. On the basis of the obtained measuring points (every point was an average from the three independent determinations) the calibration curves were prepared.

2.5. Investigation of the influence of standard addition techniques on the result of quantitative analysis of the analytes from PAH group

Investigation of the influence of standard solution addition on the result of quantitative analysis was performed with the use of certified reference material (Metranal 2).

Three different procedures of the standard addition to the investigated samples were applied:

Type I:

- samples filled with sediment (each containing approximately 1 g of certified sediment) were wetted with acetone, 10 or
100 ng of internal standards in methanol solution was added, samples were left until dry at room temperature, and next 3 ml of dichloromethane was added.

Type II:
- to samples filled with sediment (each containing approximately 1 g of certificated sediment) 10 or 100 ng of internal standards in methanol solution was added, and next 3 ml of dichloromethane was added.

Type III:
- samples filled with sediment (each containing approximately 1 g of certificated sediment) were wetted with solvent (dichloromethane, 3 ml), next 10 or 100 ng of internal standards in methanol solution was added.

The influence of the amount of added standard on the determination results was also investigated. For this purpose 6 series of 5 sediment samples were prepared. The internal standard was added according to the three different procedures (described above). To three series of samples 10 ng of internal standards was added (variant A), and to the next three series 100 ng of internal standards was added (variant B).

Prepared samples were left in automatic shaker for 24 h. Then extracts were purified on the hand-made small glass columns filled with silica gel and a layer of activated copper [15]. The excess of the solvent from final extracts was removed in the stream of the inert gas (nitrogen). Final volume of extract was 1 ml. The extract samples were dosed to the chromatographic column in the volume of 2 μl.

3. Results and discussion

3.1. Calibration of the GC–MS system

While using deuterated standards to determine PAHs, it is necessary to observe the following:

1. the relative response factor for each of the compounds vs. the deuterated internal standard should be determined individually. In practice, it means injecting standard solution every several or so times of extract from sediment samples injection into the GC–MS system;

2. deuterated standards should be added to sediment in quantities such that their concentrations in the extract are close to the concentration in standard solution;

3. concentrations of deuterated standards in the extract should be close to the concentrations of determined PAHs.

Quantitative calculations in a method using deuterated standards are based on the assumption that Eq. (1) is true. In other words, relative response factors for substances determined in a sample and standard solution are equal.

Calibration curves for deuterated standards and determined PAHs can be described with linear regression equations (Table 1), and they are different for lower (1–120 pg in injection) and higher (160–3200 pg in injection) concentration ranges.

Fig. 1a and b presents calibration curves of deuterated standards (naphthalene-d8 and benz[a]anthracene-d12) and respective PAHs (naphthalene and benz[a]anthracene) for two concentration ranges, the lower and the higher.

Direction coefficients ‘a’ of calibration curves for deuterated compounds are several times smaller than direction coefficients of respective PAHs (Table 1). Coefficients of direction for naphthalene-d8 are 2.27 (for lower concentrations) and 1.78 (for higher concentrations) times smaller than for naphthalene, while coefficients of direction for benz[a]anthracene-d12 are 4.25 times lower (for lower concentrations) and 7.1 times lower (for higher concentrations) than for benz[a]anthracene. A lower sensitivity of the mass detector with regards to deuterated compounds probably results from the higher ionization energy of the C–D bond than the C–H bond.

So an assumption about the equality of relative response factors in the extract from a sample and standard solution (Eq. (1)) is true only when response coefficients for a deuterated standard and a determined substance are found in the same range of linearity.

3.2. Investigation of the influence of standard addition techniques on the result of quantitative analysis of the analytes from PAH group

For variant A of standard addition (addition of 10 ng of internal standards to the sample) results were calculated on the basis of the
formula (1) using standard solutions I and II, whereas for variant B of standard addition (addition of 100 ng of internal standards to the sample) results were calculated using standard solutions I.

Results obtained are presented in the form of analytes recovery, under assumption that the reference value corresponds to 100%. Determination results of analytes content from the PAH group in sediment samples obtained using different standard addition techniques are not statistically different among each other (Figs. 2–4). It indicates that in every case the recovery of internal standard is similar, independently on addition technique. However it could be observed that results closer to the certified value were obtained in

Fig. 1. Calibration curves obtained during the chromatographic analysis of series of standard solutions samples for naphthalene (N) and deuterated naphthalene (N-d8) and for benz[a]anthracene (B(a)A) and deuterated benz[a]anthracene (B(a)A-d12) in lower (a) and higher (b) concentration ranges.

Fig. 2. Comparison of the determination results of analytes from the PAH group in sediment samples, obtained using three different standard addition techniques; variant A (addition of 10 ng of internal standard); results were calculated using standard solution I.
variant B (addition of 100 ng of internal standards to the sample), while results obtained in variant A (addition of 10 ng of internal standards to the sample) are lower. Additionally, in case of addition of internal standard in the quantity of 100 ng (variant B), the closest to the certified value results were obtained in case of the I type of the standard addition technique (sediment + acetone + standard).

For variant A (addition of 10 ng of internal standard) no difference were observed during results recalculating to standard solution I and II, what confirms the proper assumption of the formula (1). However it could be observed that results closer to the certified value were obtained during results recalculating to standard solution I, independently on standard addition technique.

Fig. 5 shows results of PAH determination in sediment (reference material: Metranal 2) after dosing 10 and 100 ng of deuterated standards into the sediment sample.

The results of PAH determinations after injecting 100 ng of deuterated standards in methanol solution (all areas, for standard, deuterated and determined substances were in the higher measurement range) are close to the reference values.

PAH determination results after injecting 10 ng of deuterated standards in methanol solution (areas of deuterated standards were in the lower measurement range) were considerably lower than the reference value (marked in white in the Fig. 5). Correction of determination results for PAHs using a correction coefficient (Table 1) gives results conforming with reference values.

On the basis of the results obtained it can be stated that the technique of internal standard addition does not influence the obtained determination results, whereas the amount of added standard is essential. Additionally, the closest results to the certified value were obtained in case of the addition of 100 ng of standard (variant B) to a sediment wetted with acetone (I type). Therefore, this technique is the best one from all investigated (truly imitates association of analytes with sample matrix, which occurs in aqueous environment). It gives an additional advantage—in case of adding internal stan-

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**Fig. 3.** Comparison of the determination results of analytes from the PAH group in sediment samples, obtained using three different standard addition technique; variant A (addition of 10 ng of internal); results were calculated using standard solution II.

**Fig. 4.** Comparison of the determination results of analytes from the PAH group in sediment samples, obtained using three different standard addition technique; variant B (addition of 100 ng of internal standard); results were calculated using standard solution I.
Fig. 5. Comparison reference values with PAH determinations after injecting 10 and 100 ng of deuterated standards in methanol solution, and with corrected results.

dards to sediment sample wetted with acetone it is not necessary to previously dry or freeze-dry a sample.

4. Conclusions

Development of analytical procedures applied in determination of PAHs in sediments was connected with successive trials of solving the problems appearing. The possibility of usage isotope labeled compounds, as recovery standards, in multistage procedure of PAHs isolation and determination, substantially contributed to the improvement of determinations’ accuracy and precision. Essential points of procedure based on application of deutered standards are:

- The lower coefficient of the mass spectrometer’s response for deutered PAHs analogous than for nondeutered one. Probably it results from the difference in ionization potential of these compounds. Appropriate application of the coefficient of the detector’s response for deutered and nondeutered analytes in the formula used for quantitative determinations (see formula (1)) allows to obtain correct determination results PAHs, present in the sediment samples.

- Technique of the internal standard addition to the sediment sample. The standard should be added in such a manner that since the sample addition till the final determinations both the analyte and the internal standard behave in the same way, that is, e.g. can be extracted with the same efficiency. In order to meet this condition, it is necessary to add the internal standard in such a way to enable it connecting with the matrix in the closest way to the form of association between the analyte and the sediment. Thank to that, the accuracy of the results obtained is independent on the recovery degree, of course in case, when the value of the obtained analytical signal is higher than the limit of detection of a given method. Results obtained indicate that wetting of the sediment sample with acetone, spiking of acetone with deutered standards, intensive stirring of the sample (in order to equalize the concentrations of standards in the sample volume) and undisturbed evaporation of the acetone (for approximately 12 h) allow to achieve a similar binding form between standards added and sediment matter.

Acknowledgement

This work has been partially financed in the framework of grant attributed by State Committee of Scientific Research.

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