Monofluorinated polycyclic aromatic hydrocarbons as internal standards to monitor trace enrichment and desorption of their parent compounds during solid-phase extraction

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Abstract

Solid-phase extraction (SPE) is an established sample-preparation technique for clean-up procedures of polycyclic aromatic hydrocarbons (PAHs). The suitability of monofluorinated polycyclic aromatic hydrocarbons (F-PAHs) as a novel set of internal standards, was tested for two widely used SPE sorbents, octyl-bonded silica and styrene–divinylbenzene copolymer. The elution profiles of eight F-PAHs and their parent PAHs, taken from the priority pollutant list of the US-Environmental Protection Agency, were compared for demineralized water and waste water. In both instances, the match of the elution profiles was exceptionally close. The eight F-PAHs and the corresponding PAHs were determined by reversed-phase liquid chromatography using acetonitrile–water gradient elution with UV detection at 254 nm. The UV spectra of the F-PAHs show a small bathochromic shift compared with the parent PAHs. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

The determination of polycyclic aromatic hydrocarbons (PAHs) — ubiquitous environmental contaminants of proven carcinogenicity [1,2] — in matrices such as soil, food, air and water typically involves extraction, clean-up and pre-concentration in order to isolate the analytes from the matrix, to remove interferences, and to improve the sensitivity of the method. Taking aqueous samples as an example, many laboratories involved in the environmental control and monitoring of water systems use solid-phase extraction (SPE) as a routine sample preparation tool [3,4]. However, even with this well-known procedure, losses during solvent evaporation, early breakthrough, slow desorption, matrix interactions or other such problems may adversely affect the recoveries of the PAHs. By using one or more properly selected internal standards it is possible to determine the extent of such losses and, if necessary, to correct for them. Since such problems may arise for low- as well as high-molecular-mass PAHs, it is established practice to use several internal standards

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in such analyses — with deuterated PAHs generally being recommended. However, as was argued in a recent paper [5], deuterated PAHs are high priced, are not freely available to all workers in the field and cannot be used for all types of study.

As an alternative, we propose to use monofluorinated PAHs (F-PAHs). F-PAHs are very similar to PAHs in terms of chemical and physical properties. Furthermore, to our best knowledge they do not occur naturally. On-going studies reveal the potential of F-PAHs as internal standards and/or markers in gas (GC) and liquid (LC) chromatography [6], in Shpol′skii spectroscopy [7] and during in vivo metabolic studies [8]. First investigations in this area were carried out by the group of Andersson and Ballschmiter [9], who attempted to use polyfluorinated PAHs (FF-PAHs) as internal standards in gas chromatography using flame ionization detection (GC–FID). At the same time, we studied the behavior of poly- and perfluorinated PAHs in liquid chromatography (LC) [10]. In this article we will discuss the potential of using F-PAHs as internal standards (ISs) for SPE procedures. To this end, two SPE sorbents were used, an octyl-bonded silica and a styrene–divinylbenzene copolymer. In order to make the experiments representative for a broad group of PAHs, we selected four pentafused compounds (acenaphthylene, fluorene, fluoranthene, benzo[k]fluoranthene), a linear-condensed (naphthalene) and a pericondensed (pyrene) compound and two angular-condensed polyarenes (phenanthrene, chrysene) from the list of US-Environmental Protection Agency (EPA) priority pollutants. The SPE procedures were carried out with spiked distilled water and waste water.

2. Experimental

2.1. Materials

The following F-PAHs were used: 1-fluoronaphthalene, 5-fluoroacenaphthylene 1-fluorophenanthrene, 1-fluoropyrene, 2-fluorofluorene, 3-fluorochysene, 3-fluorofluoranthene and 9-fluorobenzo[k]fluoranthene. Based on GC–FID, the purity of all compounds considered was at least 98%. The syntheses involved reactions such as oxidative photocyclization [11], the Schiemann reaction [12],

cycloaddition [13] and flash vacuum thermolysis [14]. The F-PAHs are now commercially available through Chiron (Trondheim, Norway). The parent PAHs were obtained from Aldrich (Zwijndrecht, The Netherlands).

Bakerbond 60 Å SPE octyl disposable extraction cartridges (3 ml, 200 mg, 40 μm average particle diameter) and Bakerbond 2,7 Å SPE SDB-1 disposable extraction cartridges (3 ml, 200 mg styrene–divinylbenzene copolymer, 43–125 μm particle diameter) were purchased from J.T. Baker (Deventer, The Netherlands). HPLC-grade acetonitrile and HPLC-grade methanol were obtained from J.T. Baker. LC-grade water was obtained by distillation followed by quartz distillation.

2.2. Waste water pretreatment

Five litre batches of effluent sampled at the municipal sewage treatment plant of Amstelveen, The Netherlands, were sterilized by adding 1 ml of a 1 M silver azide solution to 1 l of waste water, sedimented overnight and decanted. The treated effluent sample was then filtered over a glass fiber filter (type GF/D; Whatman, Maidstone, Kent, UK) and next over a 0.45 μm membrane filter (type HA; Millipore, Etten-Leur, The Netherlands). The samples were stored at 4°C in dark brown glass bottles.

2.3. SPE procedure

Distilled water and pretreated waste water samples were spiked with 1:1 (w/w) mixtures of PAHs and F-PAHs to obtain the following concentrations which are similar to the concentration levels in National Institute of Standards and Technology (NIST) SRM 1647: 4 ng/μl of naphthalene and 1-fluoronaphthalene, 3 ng/μl of acenaphthylene and 5-fluoroacenaphthylene, 1 ng/μl of fluorene and 2-fluorofluorene, 0.7 ng/μl of phenanthrene and 1-fluorophenanthrene, 1.6 ng/μl of fluoranthene and 3-fluorofluoranthene, 1.7 ng/μl of pyrene and 1-fluoropyrene, 0.7 ng/μl of chrysene and 3-fluorochysene, 0.9 ng/μl of benzo[k]fluoranthene and 9-fluorobenzo[k]fluoranthene. Mixtures with F-PAH/PAH mass ratios of 1:10 and 10:1 were also prepared from these starting solutions.

The octyl-bonded silica and the SDB-1 cartridges were conditioned with 15 ml of acetonitrile, followed by twice 3 ml distilled water–acetonitrile (85:15, v/v). The sample solutions were loaded onto the octyl columns (100 ml) and on the SDB-1 columns (1000 ml) under reduced pressure, using a Baker SPE-12G glass column processor.

After loading of the samples, the columns were washed with 3 ml of distilled water–acetonitrile (85:15, v/v). After drying by air the analytes were eluted from the columns with acetonitrile using 100-µl steps for the octyl, and 1-ml steps for the SDB-1 cartridges, and centrifugation at 3000 rpm. The eluates were collected in glass vials.

2.4. LC–UV analysis

The LC system consisted of a HP1100 (Hewlett-Packard, Amstelveen, The Netherlands), G1316A dual-pistol high-pressure gradient LC pump and a HP 1100 diode array detection (DAD)-UV, G1315A detector. All LC solvents were degassed with a HP 1100, G1322A degasser. Data acquisition was performed on an HPChemStation 4.0 running on a 485er PC. A HP 1100, G1313A autosampler auto-mated the sample handling. A 250 mm×3.0 mm I.D., 5 µm, 120 Å, HPLC Baker bond PAH-16 Plus column including a guard column (J.T. Baker) was used.

The linear gradient programme was as follows: 50% acetonitrile (0 min) to 60% (5 min) and, next, to 100% (15 min; hold to 25 min) and, finally, a return to 50% (30 min), all at a flow of 0.5 ml/min. The UV spectra were recorded during the LC separation on the HP 1100 DAD-UV with water–acetonitrile as the solvent.

3. Results and discussion

3.1. LC–UV of F-PAHS and PAHS

Gradient elution with acetonitrile–water using a C_{18}-bonded silica column is the preferred method of LC separation of PAHs. Fig. 1 shows the separation obtained for the set of eight F-PAHs and the corresponding parent PAHs. All compounds were baseline-separated, with the resolution of the F-PAHs and parent PAHs being greater than 1.3 in all cases except 1-fluoronaphthalene and acenaphthylene for
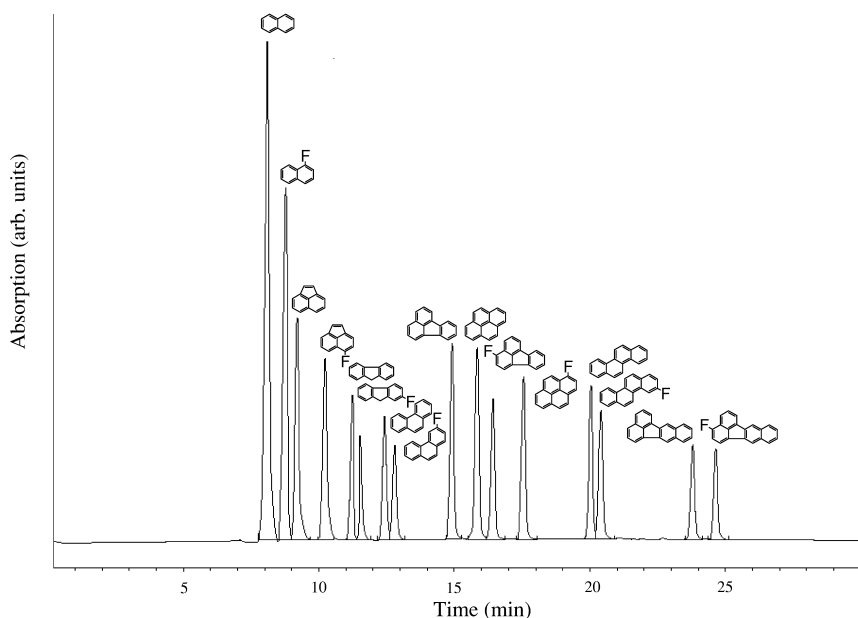

Fig. 1. Gradient LC–UV$_{254}$ chromatogram of eight EPA PAHs and their monofluorinated analogs. Analyte concentrations, ca. 100 µg/l. For other conditions, see text.

which the resolution was 1.2. The slightly higher retention of the fluorinated analogs probably reflects their more liphophilic character caused by the reduction of the London forces, which outweighs the influence of the dipole creation.

As regards UV detection, the introduction of fluorine was found to cause no changes in the structure of the spectra, the only effect being a shift of the absorption maxima to higher wavelengths (0.5–5 nm) depending on the parent PAH and the pattern of substitution. On the basis of these data one can conclude that analyte detectability will be similar for the parent and monofluorinated compounds, which gives added value to the use of F-PAHs as internal standards.

The small bathochromic effect in the UV spectra can be explained on the basis of hyperconjugation. In the F-PAHs, the six free orbital electrons of the fluorine atom are not involved in the covalent σ-carbon–fluorine bond, and are available for secondary π-bonding. Because of the similar size of carbon and fluorine *p*-orbitals, their overlap is excellent. In all F-PAHs, the hyperconjugation causes an extension of the conjugated system which increases the resonance stabilization energy. Because of the different electron densities at different positions in the aromatic system, the position of the fluorine substituent determines how strong the shift will be.

3.2. Comparison of F-PAH and PAH behavior in SPE

A comparison of the behavior of the two analyte classes of interest in an SPE procedure should, of course, emphasize the study of the desorption profiles. Therefore, the sorption step was studied only briefly. Experiments with naphthalene, 1-fluoro-naphthalene, acenaphthylene and 5-fluoro-acenaphthylene as test compounds showed that, for the octyl cartridges, the breakthrough volumes were at least 100 ml and, for the copolymer cartridges, at least 1000 ml. These were the largest volumes tested in each case because 0.1–1.0 l samples cover the range typically found in real-life applications. For the rest, the two sorbents were selected because they are widely used in many laboratories and, also, reflect somewhat different analyte–stationary phase interactions, viz. weak non-polar interactions with

the alkyl chains in the case of the bonded silica, and strong π–π plus weak non-polar interactions in the case of the styrene–divinylbenzene copolymer.

As regards desorption, a series of 100-μl elution steps with pure acetonitrile were used for the 100-ml experiments, and of 1-ml elutions for the 1000 ml studies. In addition, care was taken to target the study on the desorption profiles by omitting any solvent-evaporation steps which will easily cause losses of the more volatile aromatic hydrocarbons. All experiments were carried out in triplicate on three different cartridges, and three F-PAH/PAH mass ratios were used, 1:10, 1:1 and 10:1.

As an example, Fig. 2 shows the desorption profiles of four F-PAHs (right-hand side frames) and their corresponding parent PAHs (left-hand side frames) for octyl cartridges and when using a 1:1 mass ratio. Not too surprisingly, the second 100-μl desorption step is the most efficient one in all instances. Obviously, under the present conditions and for the analytes selected, a volume of 500–700 μl of acetonitrile is required to effect quantitative desorption. However, neither this observation nor any subsequent attempt to improve the desorption profiles is the main issue here. Rather, one should consider that, if the three plots recorded for each of the eight test compounds are a good estimate of the run-to-run differences obtained with disposable cartridges (and when using manual operation), the correspondence of data for each F-PAH/PAH pair on each individual cartridge, is excellent. It is, actually, somewhat surprising that this is true during all stages of the desorption process.

To demonstrate that it is not the nature of the sorbent that causes the close elution similarity, the experimental results for the same analytes as above, but now for SDB-1 as the sorbent, are shown in Fig. 3. There are obvious differences with the situation encountered for the octyl cartridges, such as the early elution maximum for naphthalene and 1-fluoro-naphthalene, and the much wider range of desorption volumes required, viz. from about 3.5 to 8 ml, but the general trend of close similarity of the profiles for each F-PAH/PAH is clearly present again.

Two further remarks should be made here. The cartridge-to-cartridge repeatability is, in the present case, distinctly better for the copolymer as compared with the octyl cartridges. However, with three car-
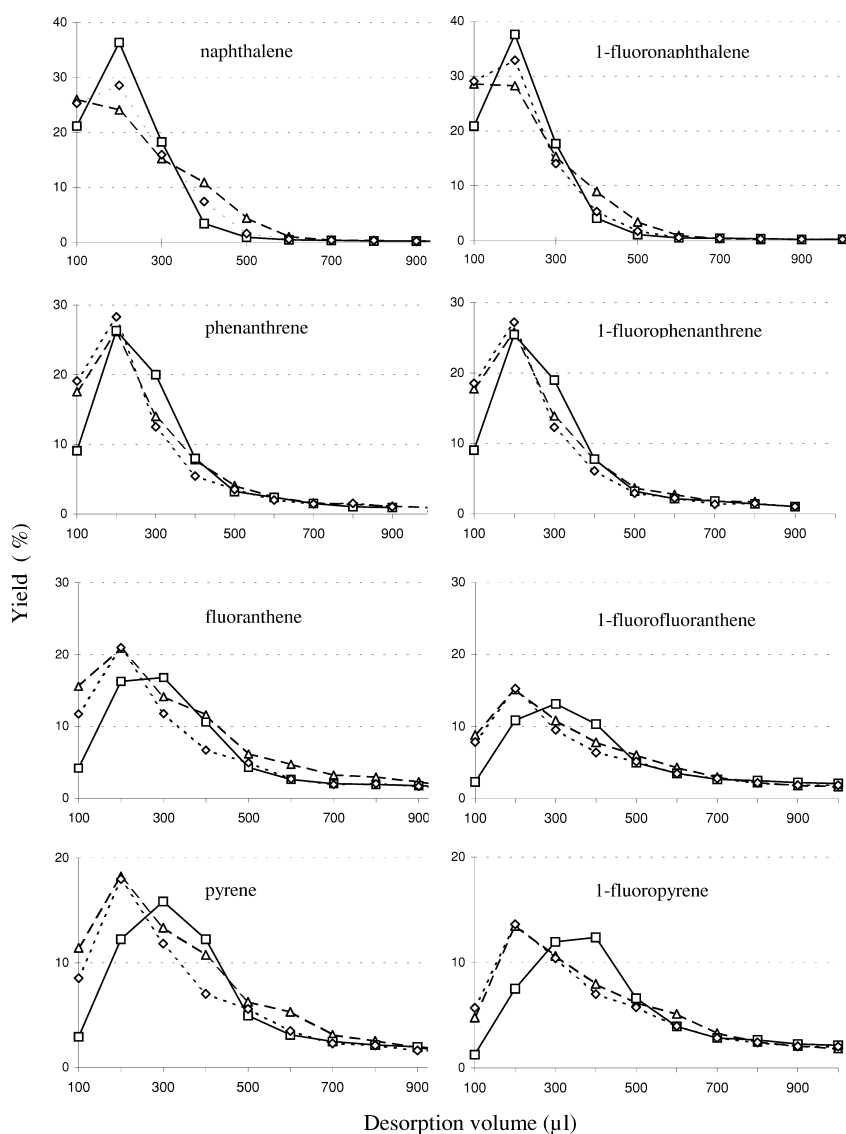
Fig. 2. Desorption profiles of naphthalene, fluoranthene, pyrene and phenanthrene and their monofluorinated analogs, from 40 μm, 60 Å disposable Bakerbond SPE Octyl extraction columns (3 ml, 200 mg). Conditions: 100 ml water sample, spiked with 100 μg/l of each of the test analytes; desorption with 100-μl aliquots of acetonitrile; analysis with LC–UV$_{254}$, injection volume, 10 μl.

tridges per analyte, no conclusions should be drawn. Secondly, there are occasionally small differences between the profile of a parent and a monofluorinated PAH. A good example is fluoranthene/1-fluorofluoranthene with their similar (and repeatable) peak profiles, but a required desorption volume of 8 and 7 ml, respectively. In other words, even with results as satisfactory as those shown here, due care

(safety margin!) has to be taken when designing SPE protocols. Results for the four PAH/F-PAH pairs not illustrated in Figs. 2 and 3 fully confirmed what was concluded above. In addition, for six of the PAH/F-PAH pairs SPE studies on both sorbents were also performed as mass ratios of 10:1 and 1:10. In all instances, the results — expressed as peak area ratios 1:1 were the same to within ±2% as those found at
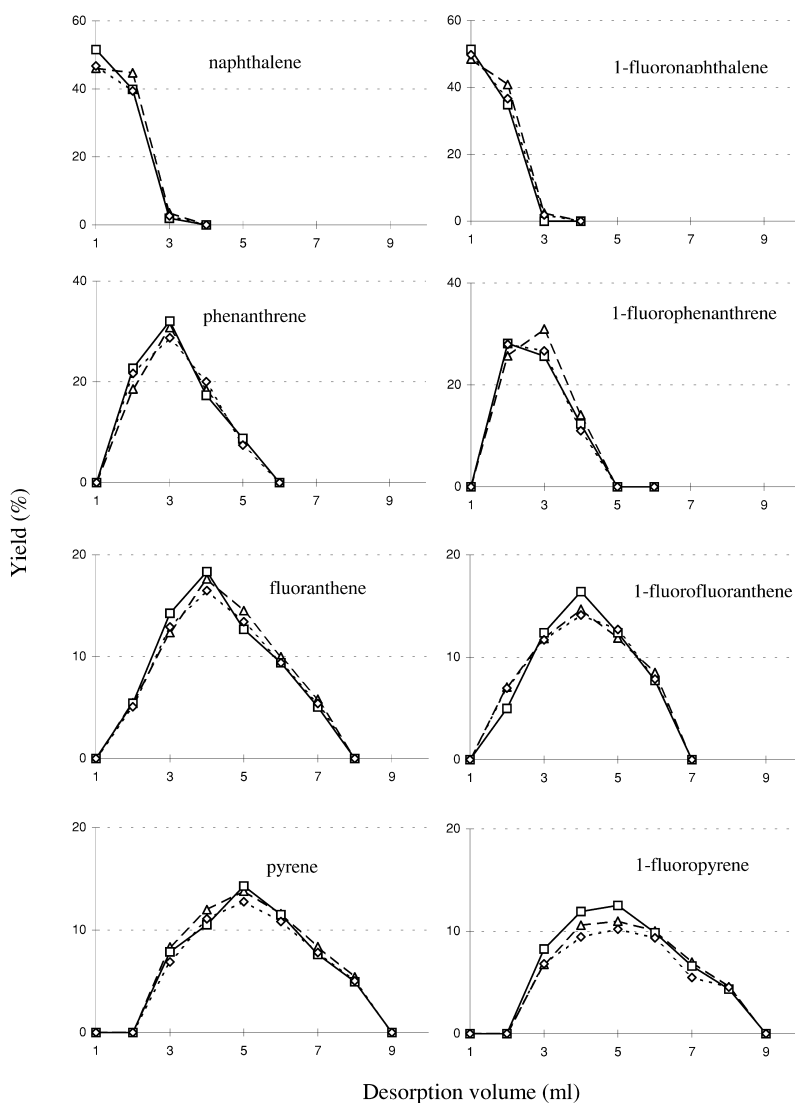
Fig. 3. Desorption profiles of napthalene, phenanthrene, fluoranthene and pyrene and their monofluorinated analogs, from 43 to 125 μm, 2.7 Å disposable Bakerbond SPE SDB-1 extraction columns (3 ml, 200 mg). Conditions: 1000 ml water sample, spiked with 100 μg/l of each of the test analytes: desorption with 1-ml aliquots of acetonitrile; analysis with LC–UV$_{254}$; injection volume, 10 μl.

the 1:1 mass ratio. This is a rewarding outcome because the results were calculated after the second desorption step — i.e., after desorption with 200 μl (octyl) or 2.0 ml (SDB-1). As the profiles of the Figs. 2 and 3 show, quantitative elution has, then, not yet been achieved which makes the comparison rather challenging.

Finally, in order to clearly demonstrate the usefulness of adding an appropriate internal standard, the

relative standard deviations (RSDs) were calculated from the experimental data for all parent PAHs without and with F-PAH-based correction. Fig. 4 shows results for both octyl-bonded silica and SDB-1 copolymer. The dramatic improvement achieved when using an internal standard needs little comment. It is evident that the RSD values clearly reflect systematic errors (from losses due to volatility to inefficient desorption) in the absence of F-PAH
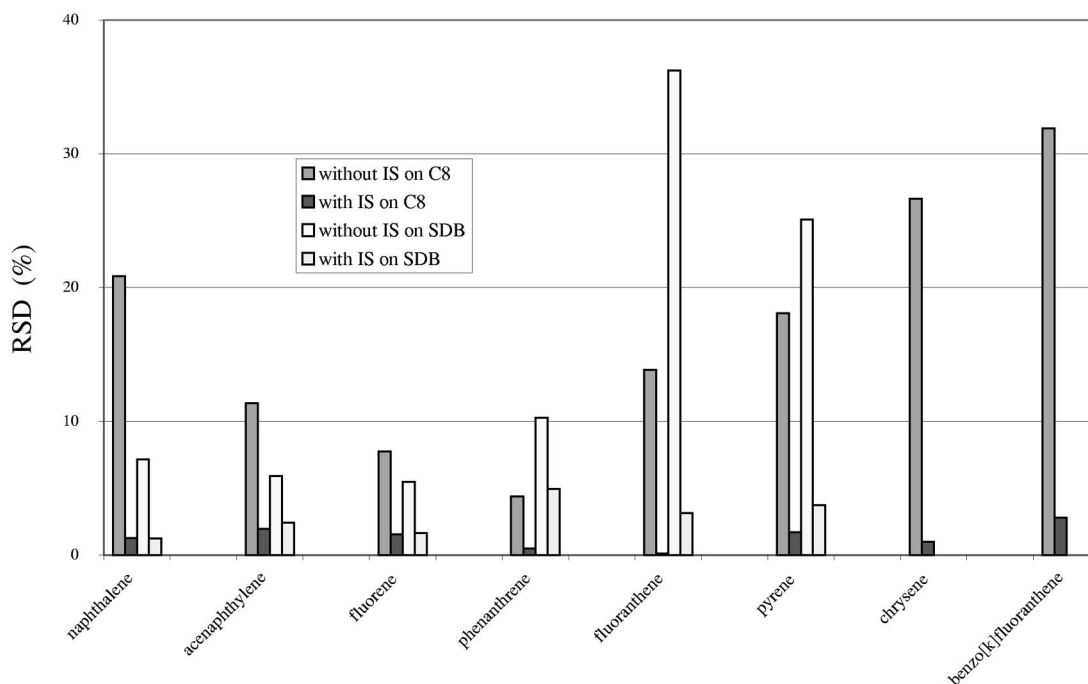
Fig. 4. Relative standard deviations ($n=3$) of PAH desorptions from C8 and SDB-1 SPE materials with and without using the corresponding F-PAH as internal standard.

correction, while the residual RSDs calculated after correction show an essentially random distribution. Caused by inefficient desorption higher condensed PAHs show high RSD values, which increase with the capacity of sorbents material and cartridge. In summary, the above results leave little to be desired and one can only conclude that the use of F-PAHs as internal standards in SPE-based PAH analysis can be recommended with some confidence.

3.3. Application: waste water

In view of the general complexity of waste water, the effluent of a municipal sewage treatment plant was selected for a real-life test of the F-PAH/PAH approach. We used the six-step SPE procedure of Junk and Richard [15] which is summarized in the Experimental section. This procedure involves a two-step elution: firstly, the more polar sample constituents are removed by washing with water–acetonitrile (85:15; v/v) and, subsequently, the non-polar compounds (including the PAHs and F-PAHs) are desorbed with pure acetonitrile. Fig. 5 shows

typical results of one such analysis in which 100 ml of waste water was spiked with 100 μg/l of each of the analytes of interest. Two things are obvious: (i) the recoveries are lower for waste water, which is probably due to the presence of much non-polar material in the samples. However, the various profiles have essentially the same shape as those of Fig. 2, (ii) more importantly, although the various F-PAH/PAH pairs require rather different volumes of acetonitrile for their near-complete elution, the behavior of each individual F-PAH and the corresponding parent PAH is remarkably alike.

The application of our method deals with the possibility of interfering peaks. This can be avoided by increasing the selectivity, e.g., by the use of fluorescence detection instead of UV.

4. Conclusions

The desorption profiles recorded for eight mono-fluorinated PAHs and the corresponding parent compounds during SPE of 100–1000 ml water samples
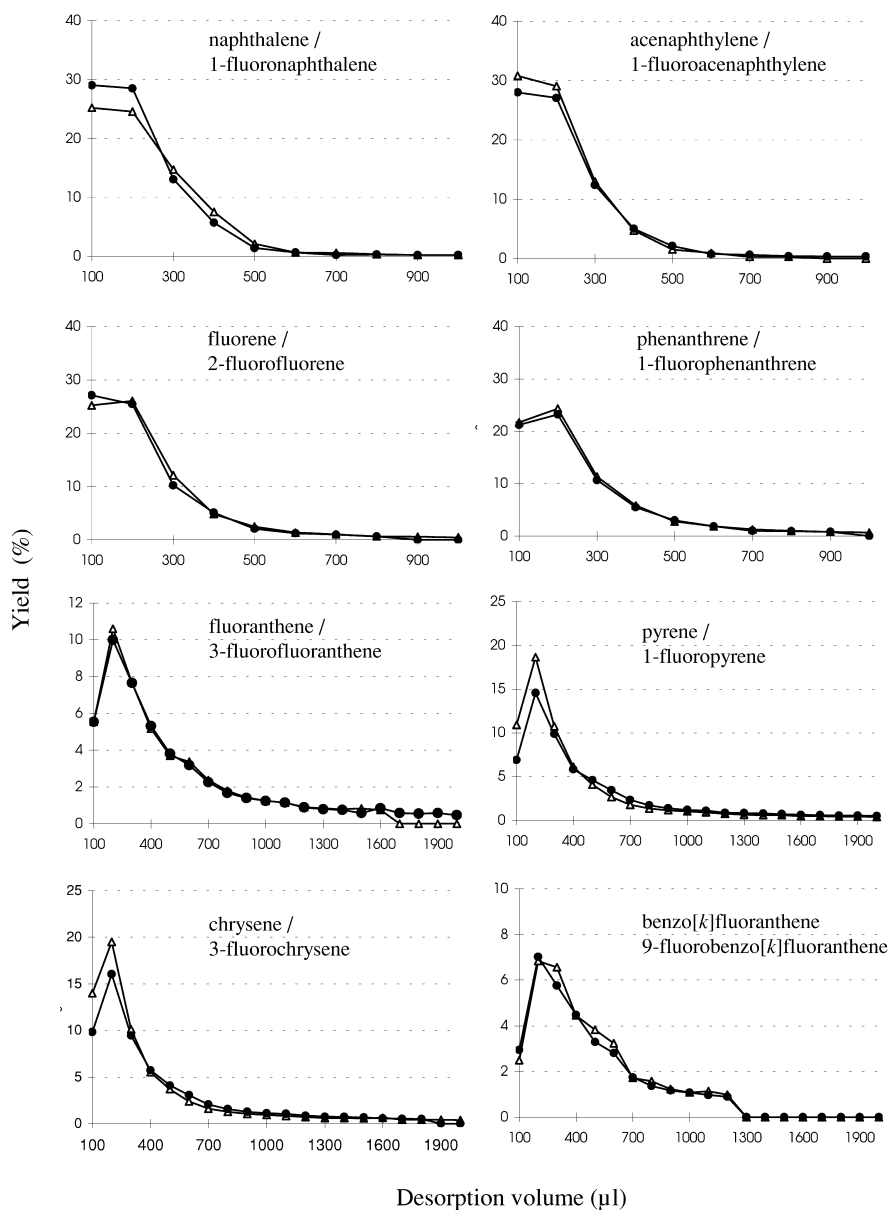
Fig. 5. Desorption profiles of eight PAHs (black dots) and their corresponding F-PAHs (open triangles), isolated from 100 ml waste water, spiked with 100 µg/l of each of the test analytes. Desorption yields were measured in 100-µl steps, using acetonitrile and an octyl SPE column; analysis with LC–UV$_{254}$, injection volume, 10 µl.

on alkyl-bonded silica and a styrene–divinylbenzene copolymer are remarkably similar. This was found for aqueous standard solutions as well as spiked waste water.

The present results, including those regarding LC separation and UV absorbance spectra, fully confirm earlier preliminary findings on the markedly analogous behavior of PAHs and F-PAHs. This similarity in physicochemical properties can be explained on the basis of the near-equality of the antagonistic effects in monofluorinated PAHs of the introduction of a permanent dipole and the decrease of the

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London forces. These findings, and the fact that they do not naturally occur in the environment, make F-PAHs most interesting surrogate standards for PAHs in sample preparation procedures. Losses caused in SPE by e.g., breakthrough or incomplete desorption can be monitored and, if necessary, corrected by using the F-PAHs as internal standards. On-going work on liquid–liquid extraction and pressurized liquid extraction procedures shows equally promising results.

Acknowledgements

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