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Monofluorinated polycyclic aromatic hydrocarbons: characteristics and intended use in environmental analysis

G. Luthe* and U. A. Th. Brinkman

Free University, Department of Analytical Chemistry and Applied Spectroscopy, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

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First studies of a series of eight monofluorinated polycyclic aromatic hydrocarbons indicate that they are a promising set of internal standards and markers in the trace-level environmental analysis of their parent polycyclic aromatic hydrocarbons. The selected examples deal with solid-phase extraction, LC separation, Shpolśkii spectroscopy and metabolic study.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) and especially the sixteen EPA-PAHs, are compounds that have to be determined very frequently in environmental studies. Deuterated PAHs[1] are generally recommended as internal standards but because of their high price they are not freely available to all analysts and/or not practically useful for all types of study, primarily of the possibility of scrambling of deuterium and hydrogen atoms. Methylated PAHs are sometimes recommended as alternatives. However, because of their less close analogy to the parent PAHs and, even more, because of their occurrence in the natural environment,[2] they are less than ideal replacements. In our opinion, monofluorinated PAHs (F-PAHs) should be considered as a more promising alternative. Firstly, as a result of the antagonistic effects of the creation of a dipole moment and the reduction of the London forces caused by fluorine substitution, F-PAHs are closely similar to the parent PAHs in terms of chemical and physical properties. Secondly, according to present-day experience, F-PAHs do not occur in the natural environment[3] and are not being produced in appreciable amounts for any industrial, medical or other related purpose.

Until recently the synthesis of most F-PAHs was considered to be complicated, which did not encourage the systematic study of this class of compounds. However, by using procedures such as oxidative photocyclization,[4] the Schiemann reaction[5] cycloaddition[6] and flash vacuum thermolysis,[7] we have recently been able to synthesize relatively large amounts of eight (model) F-PAHs which cover the range from naphthalene through acenaphthylene, fluorene, phenanthrene, chrysene, pyrene and fluoranthene to benzo[*k*]fluoranthene. Conse-

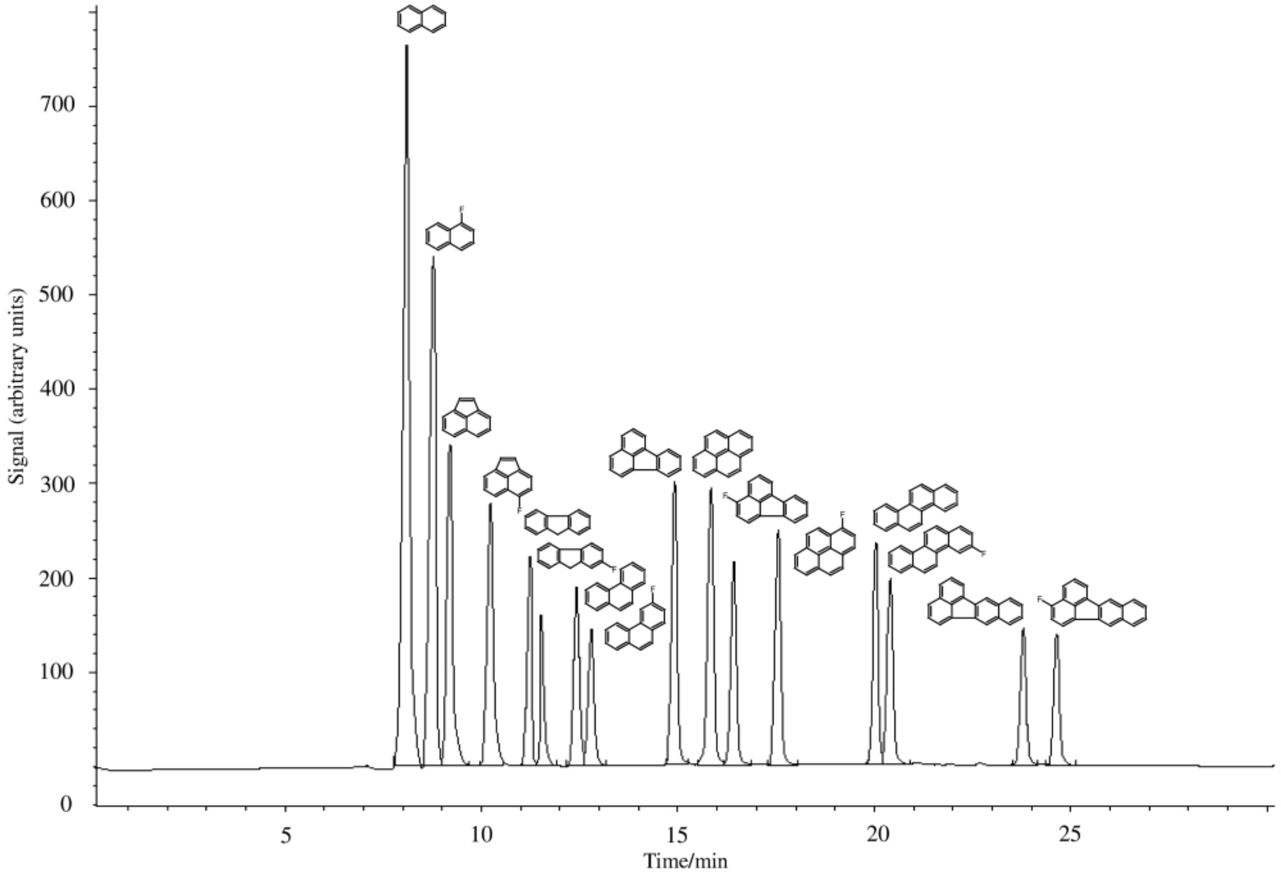

Fig. 1 LC-UV chromatogram of eight EPA PAHs and their monofluorinated analogues. Conditions: 50% acetonitrile (0.0 min), 60% (5.0 min), 100% (15.0–25.0 min), 50% (30.0 min); detected at 254 nm; analyte concentrations, *ca.* 100 μg l^{-1}; injection volume, 10 μl.

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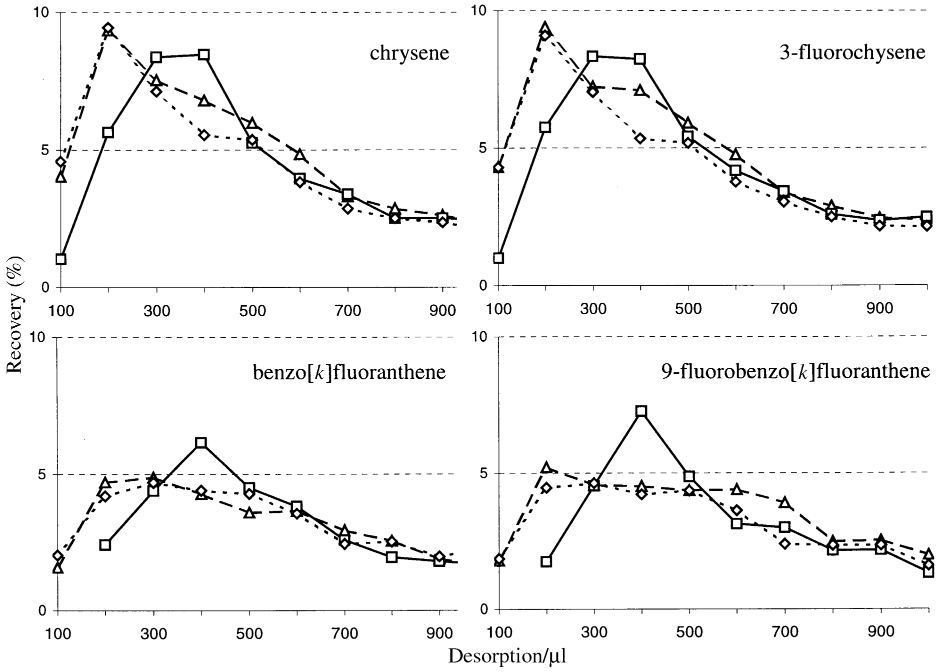

Fig. 2 Elution profiles of chrysene, benzo[*k*]fluoranthene and their monofluorinated analogues, from 40 µm, 60 Å disposable Bakerbond SPE Octyl extraction columns (3 ml, 200 mg). Conditions: 100 ml water sample, spiked with 100 µg l^{-1} of each of the test analytes; desorption with 100 µl aliquots of acetonitrile; analysis with LC-UV; detection at 254 nm; injection volume, 10 µl.

quently, it is now possible to study the characteristics and behaviour of a representative set of F-PAHs and, therefore, to evaluate their potential usefulness as markers and internal standards in trace-level environmental analysis.

The present communication discusses some relevant characteristics and intended applications to illustrate the practicability of our approach.

Experimental

Materials

1-Fluoronaphthalene, 1-fluoroacenaphthylene, 2-fluorofluorene, 1-fluorophenanthrene, 3-fluorochrysene, 1-fluoropyrene, 3-fluorofluoranthene and 9-fluorobenzo[*k*]fluoranthene were synthesised using procedures based on the reactions mentioned in the Introduction. Their purity, which was checked by means of GC-FID was found to be >98%. The content of the parent PAH and higher fluorinated PAHs invariably was less than 0.5%. Dilute solutions in appropriate solvents were prepared immediately before use. I-PAHs will be made available by CHIRON, Trondheim, Norway.

The corresponding parent PAHs were purchased from Aldrich (Zwijndrecht, the Netherlands) and were treated in the same way as the F-PAHs.

All other chemicals and solvents were of the highest quality which is commercially available, and were obtained from J. T. Baker (Deventer, the Netherlands).

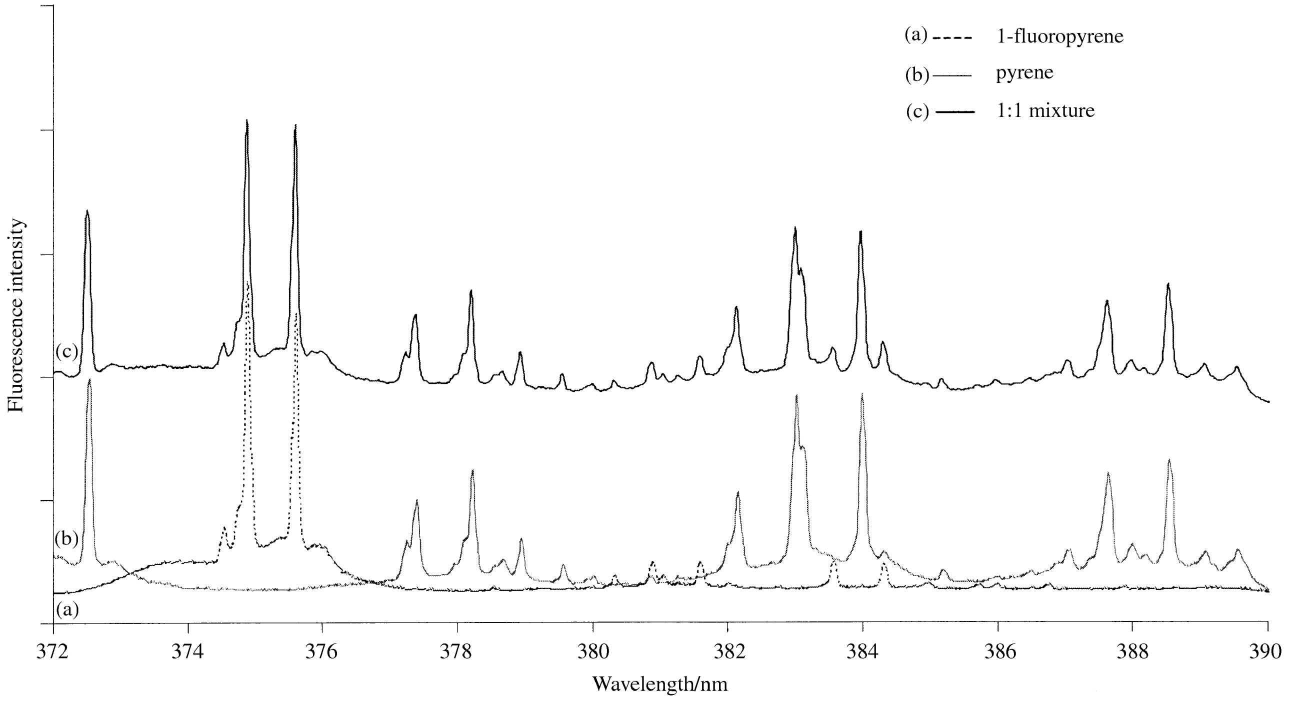

Fig. 3 Shpolŝkii spectra of pyrene and 1-fluoropyrene. Conditions: hexane as matrix, temperature 11 K, analyte concentrations 10^{-6} M.

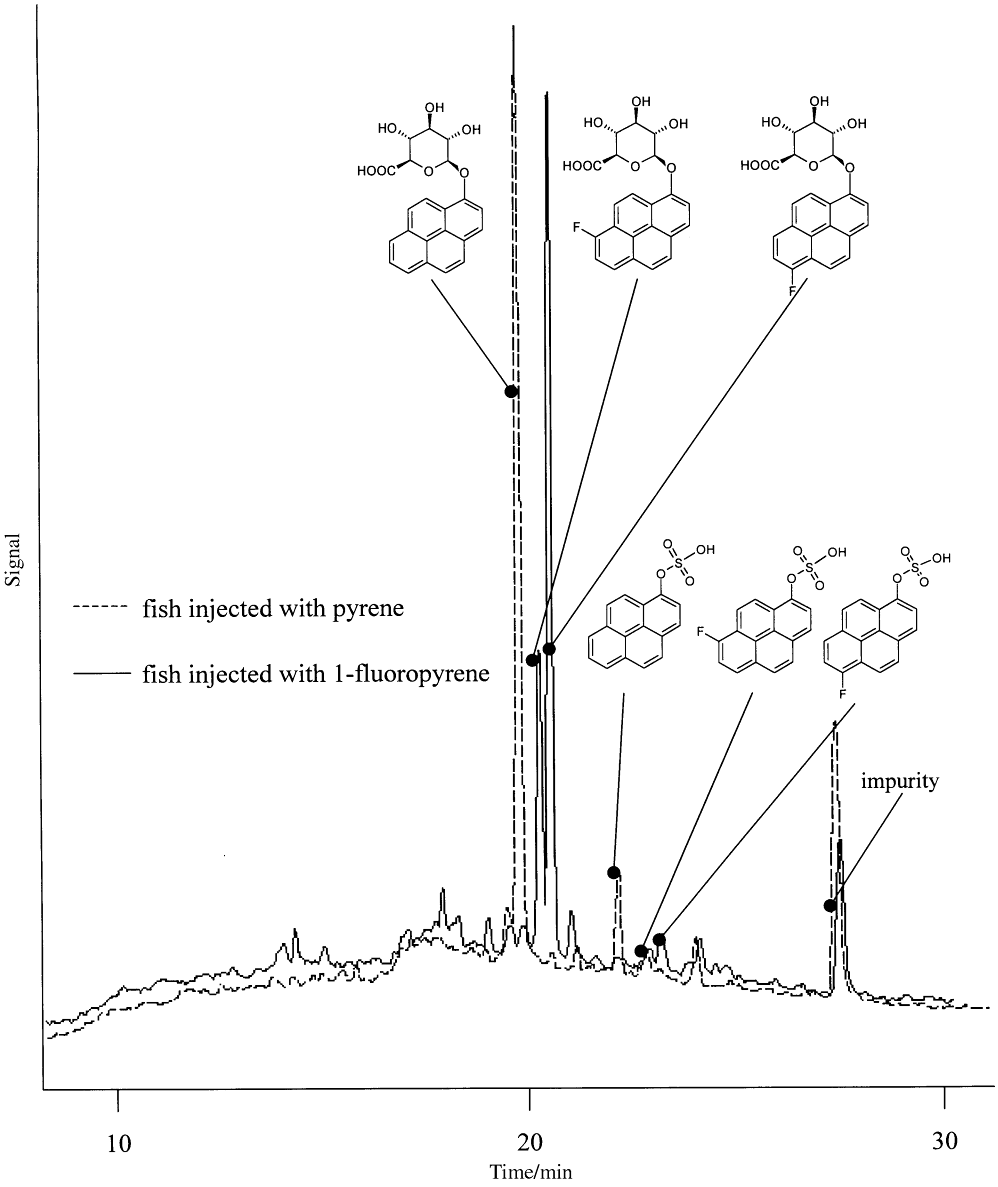

Fig. 4 LC-UV analysis of water-soluble metabolites of 1-fluoropyrene and pyrene present in flatfish, *Platichthys flesus*. LC conditions: C_{18} Vydac column, 5% acetonitrile (0 min), 90% (40–50 min), 5% (60 min) in water, detection at 339 nm. For exposure conditions, see text.

Methods

All instrumentation and procedures used were similar to or the same as those repeatedly reported in the literature. Specifically, for column liquid chromatography (LC), solid-phase extraction (SPE) and Shpolśkii spectroscopy, one should consult references, 1, 8 and 9 respectively. Details relevant for the present study are included in the appropriate sections below.

Results and discussion

For a first appraisal of the practicability of the goal we have in mind, experiments were performed which should reflect a variety of uses of the F-PAHs, *viz.* as calibration standards, internal recovery standards and markers.

LC separation

Gradient elution with acetonitrile–water using a C_{18}-bonded silica column is the preferred method of LC separation of the parent PAHs. Fig. 1 shows the separation of the eight F-PAHs and the corresponding EPA-PAHs. Detection was by UV absorbance measurement at 254 nm. All compounds were baseline-separated, with the resolution of the F-PAHs and their parent PAHs being greater than 1.3 in all cases except 1-fluoronaphthalene and acenaphthylene for which the resolution was 1.2. The slightly higher retention of the fluorinated analogues probably reflects their more liphophilic character caused by the reduction of the London forces. Based on the close similarity of the UV-VIS absorbance spectra of the F-PAHs and PAHs, analyte detectability can be said to be essentially the same. Fluorine causes no change in the structure of the UV spectra, just a small bathochromic effect because of hyperconjugation, of 0.5 nm.

Solid-phase extraction

Sample handling of environmental microcontaminants receives increasing attention, one main reason being that most losses of analytes occur during that part of the analytical procedure. If F-PAHs would be used as recovery standards, one prerequisite is that their behaviour on SPE cartridges should closely resemble that of the parent compounds. 100 ml of a spiked aqueous test solution containing naphthalene, chrysene, benzo[*k*]fluoranthene and their monofluorinated analogues were loaded on a disposable C_8-bonded silica SPE column (3 ml, 200 mg, 40 μm average particle diameter, 60 Å). Elution was undertaken with 100 μl aliquots of acetonitrile, which were collected and subjected to analysis by means of LC-UV using the procedure outlined above. In each recorded instance, the procedure was carried out in triplicate on three different cartridges, the individual results being presented by the individual plots shown in each frame in Fig. 2. If the three plots for each individual analyte are considered to be a fair estimate of the run-to-run differences to be expected with disposable cartridges and manual operation, then the correspondence of the data for each F-PAH/PAH pair, shown in Fig. 2, can be said to be excellent, irrespective of the completeness or non-completeness of the desorption. The same similarity was observed for the naphthalene pair (data not included in Fig. 2).

Shpolśkii spectroscopy

Shpolśkii spectroscopy is a cryogenic technique which requires temperatures below 30 K and provides high-resolution fluorescence and phosphorescence spectra both with lamp and laser excitation. The large number of experimental parameters can easily affect the spectral intensity; however, the shape of the spectra remains the same. In other words, Shpolśkii spectroscopy is primarily a qualitative detection technique. However, this does not make the technique less useful for *e.g.*, distribution and fate studies provided the spectra of the monofluorinated and non-fluorinated compounds can easily be distinguished. One successful example for pyrene and 1-fluoropyrene is shown in Fig. 3. The 0–0 transition region of the fluorinated analogue is seen to be significantly shifted, and its recognition will not meet with any problems.

On-going work shows that similarly good results can be obtained for all F-PAHs currently available, with the 0–0 transitions showing shifts ranging from 0.5 to 5.8 nm in the fluorescence mode.[10] Interpretation of the observed spectral differences is a logical next step.

Metabolic study

Today, *in vitro* and *in vivo* metabolic studies of PAHs in a variety of biota are a topic of much interest. Also here, F-PAHs may well start to play a role, even though this role can be rather different from that exemplified in the previous sections. After all, although F-PAHs generally are closely similar to their parents, the fluorine substituent can also cause the blocking of active or non-active positions on the PAH molecule and, consequently, change the charge distribution and, also, the metabolic routes.

As an example, a typical result from a study on water-soluble metabolites of 1-fluoropyrene and pyrene in flatfish, *Platichthys flesus*, is shown in Fig. 4. Briefly, flatfish caught in a non-polluted area, were injected intraperitoneally with 1-fluoropyrene or pyrene in DMSO. After a 7 day exposure, bile was collected and analysed by LC-UV, according to the procedure given above, but with detection at 339 nm. The traces of Fig. 4 show results for fish injected with pyrene and its fluorinated analogue; the fluorinated analogues and parent compounds can easily be separated by LC. 1-Fluoropyrene and pyrene (retention times: 44.6 and 43.4 min, respectively) are seen to have been completely converted to phase II metabolites, sulfates and glucuronides of pyrene and 1-fluoropyrene. If an electrophilic aromatic substitution mechanism is assumed, the ratios of the isomers are found to be in agreement with the electron density of 1-fluoropyrene. Position 6 has a higher electron density than position 8[11] and, indeed, the 1,6-isomers are the main, and the 1,8-isomers the minor products.

Conclusion

The present preliminary study on the behaviour of a set of eight monofluorinated PAHs and their parent compounds shows that the fluorinated analogues are promising candidates for a variety of analytical purposes, *e.g.*, as markers, tracers and internal standards. The general characteristics of the fluorine-substituted aromatic compounds are closely similar to those of the parent PAHs, but detailed inspection reveals that differentiation is possible whenever this is required. Further study of this interesting aspect is currently in progress. Such studies will, however, include work at environmental trace levels down to 10 ng l^{-1}.

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