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Short communication

Fast screening method for the profile analysis of polycyclic aromatic hydrocarbon metabolites in urine using derivatisation–solid-phase microextraction[1]

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Abstract

A method for the qualitative analysis of various metabolites of naphthalene, phenanthrene and pyrene is presented. The method uses SPME with a 85-μm polyacrylate fibre for extraction, headspace silylation with BSTFA without any catalyst for on-fibre derivatisation and GC–MS in the SIR mode for separation and detection. The suitability of the method for profile analysis of PAH metabolites is shown by analysing a smokers urine after enzymatic cleavage (and additionally after spiking with the target analytes) and spiked water. The method exhibits satisfactory separation of all investigated metabolites and no interferences due to matrix peaks. © 1998 Elsevier Science B.V.

Keywords: Polynuclear aromatic hydrocarbons; Hydroxypyrene; Hydroxyphenanthrene; Hydroxynaphthalene

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a well known group of environmental carcinogens which are often underestimated with regard to their potential to cause cancer diseases [1]. Their most important source is the incomplete combustion of organic materials [2–4]. Ingestion, inhalation and resorption are the main pathways into the human body [5].

A useful and direct approach to assess human exposure and PAH uptake is to measure PAH metabolites in urine [6]. The suitability of 1-hydroxypyrene, the major metabolite of pyrene, to estimate the individual exposure of PAHs and to act as a representative measure of the quantity of PAHs, taken up by the described pathways, has been demonstrated in numerous papers, pioneered by Jongeneelen et al. in 1987 [7]. The measurement of these metabolites thus enables the assessment of the internal dose of toxic substances and provides an additional important information source for biomonitoring of occupational and environmental human exposure in combination with the determination of emission concentrations and model calculations [8].

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[1]Dedicated to Prof. Christian R. Noe, Institute for Pharmaceutical Chemistry (Frankfurt/Main), on the occation of his 50th birthday.

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Simultaneous detection of various metabolites enables profile analysis, which enlarges the information scope and appears to be a suitable tool to study possible causes of profile changes via effect monitoring. According to Grimmer et al. [1], the individual urinary metabolite profile owing to the PAHs inhaled is invariant, whereas significant inter-individual differences occur. To confirm this assumption a thorough monitoring of exposed persons is necessary.

Solid-phase extraction (SPE) in combination with high-performance liquid chromatography (HPLC) has been most commonly used for profile analysis [7,9,10]. Though bearing in mind the possibility of automated analysis [11,12], the separation performance is not satisfactory, because some of the target metabolites exhibit coelution. There again, gas chromatography (GC) generally delivers better separation performance, either, but the sample preparation steps are time-consuming and require considerable amounts of solvent and separation materials [1,13,14].

The objective of this investigation is to pave the way for the development of a fast, simple and straightforward method for the profile analysis of various PAH metabolites in urine. The method uses solid-phase micro extraction (SPME) as a solvent-free, rapid and inexpensive extraction technique for organic compounds from aqueous matrices [15,16]. The extraction is followed by on fibre silylation to obtain analytes more appropriate for gas chromatographic–mass spectrometric analysis.

2. Experimental

2.1. Materials and equipment

1-, 2-, 3-, 4-, and 9-Hydroxyphenanthrene and 1-hydroxypyrene were obtained from Dr. Ehrenstorfer (Augsburg, Germany). 1- and 2-Hydroxynaphthalene were received from Sigma (St. Louis, MO, USA), 2- and 4-hydroxypyrene were gifts from the Institute for Analytical Chemistry, University of Vienna, Austria. 85-μm polyacrylate SPME fibres, fibre holders and N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) were obtained from Supelco (Bellefonte, PA, USA). β-Glucuronidase (approx. 100 000 units/ml) and sulfatase (up to 5000 units/ml) from *Helix pomatia* were obtained from Sigma (St. Louis, MO, USA).

Table 1 shows the composition of the standard solution used for spiking purposes.

The gas chromatography–mass spectrometry (GC–MS) system consisted of a GC 8000 gas chromatograph and a MD 800 mass spectrometer (Fisons Instruments, Rodano, Italy). Separation was performed on a DB5MS fused-silica capillary column, 30 m×0.25 mm I.D., 0.25 μm film thickness (J&W Scientific, Folsom, CA, USA). Carrier gas was helium with an inlet pressure set to 1 bar. A glass liner with an I.D. of 3 mm was used. The temperature program for the GC run was: 100°C held for 1 min, 15°C/min to 160°C, 8°C/min to 305°C, held for 7 min. The split/splitless injector was set to 270°C with a splitless period of 3 min during desorption of the fibre.

Table 1
Ion masses of the target hydroxy-PAHs, their concentration in the standard solution (solvent: acetonitrile) and limits of detection

Compound	Ion masses (m/z)	Concentration (μg/ml)	Limits of detection (μg/l)
1-Hydroxynaphthalene	216, 201	0.08	0.03
2-Hydroxynaphthalene	216, 201	0.092	0.03
1-Hydroxyphenanthrene	266, 251	0.05	0.04
2-Hydroxyphenanthrene	266, 251	0.05	0.06
3-Hydroxyphenanthrene	266, 251	0.05	0.04
4-Hydroxyphenanthrene	266, 251	0.05	0.03
9-Hydroxyphenanthrene	266, 251	0.105	0.14
1-Hydroxypyrene	290, 275	0.05	0.05
2-Hydroxypyrene	290, 275	0.076	0.03
4-Hydroxypyrene	290, 275	0.096	0.23

The mass spectrometer was operated in the electron impact (EI) mode at an ionisation energy of 70 eV. The ion source temperature was 250°C, and the GC–MS interface set to 280°C. The analyses were performed in the selected ion monitoring (SIM) mode detecting two masses simultaneously for each compound as summarised in Table 1.

2.2. Sampling and derivatisation

After adding 1 μl enzyme solution per ml urine, urine samples were hydrolysed enzymatically at 37°C for about 16 h [7] and stored at −20°C until analysis.

SPME was performed by immersing the fibre into 5 ml sample liquid for 45 min at 35°C under magnetic stirring. The sample was either urine, urine spiked with 50 μl stock solution, blank water or water spiked with 50 μl standard solution. Following extraction, the fibre was placed in the headspace of 10 μl BSTFA in a 2 ml autosampler vial and left for 45 min at 60°C. Then the fibre was transferred to the hot injection port of the GC and desorbed for 3 min.

3. Results and discussion

The SPME–derivatisation procedure was developed using PAH metabolites, which are known to be present as background burden in the urine of nonexposed persons. These are hydroxylated naphthalenes, phenanthrenes and pyrenes. To cover the additional PAH impact by smoking [17], a smokers urine (about 20 cigarettes per day) was used for demonstrating the applicability of the method to native urine. Most of the hydroxy-PAHs mentioned in this investigation can be analysed by GC without derivatisation, so that the completeness of the silylation reaction can be monitored easily. Derivatisation, however, yielded a better separation of the isomers,

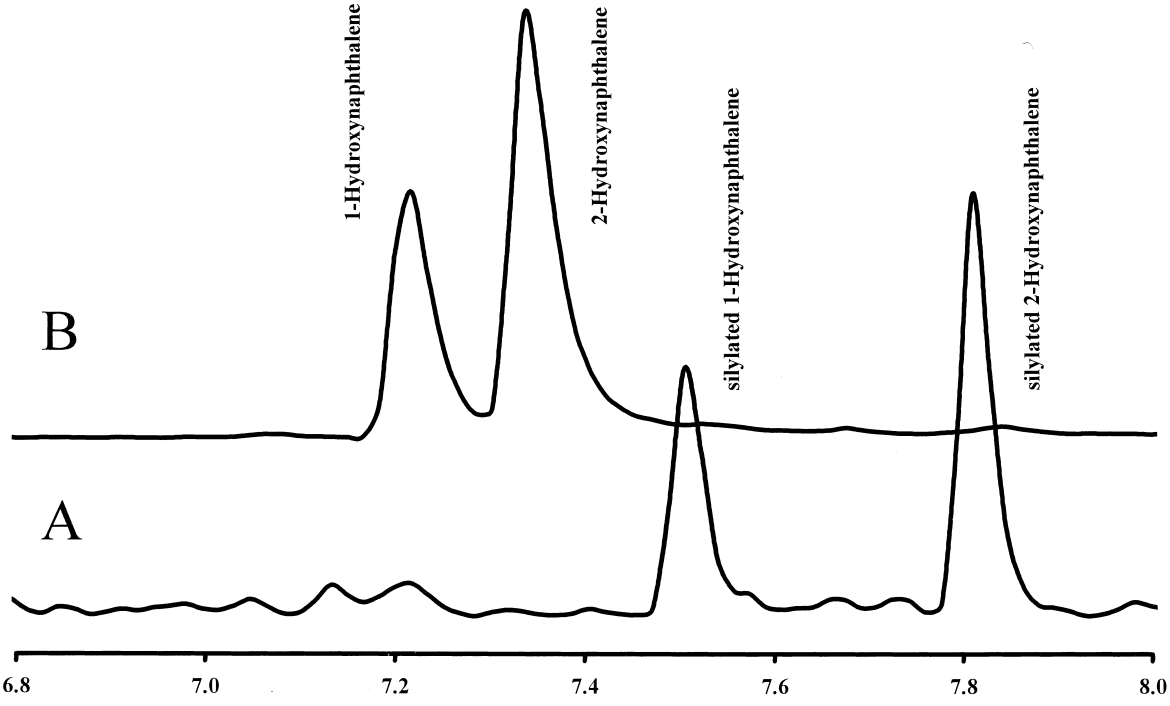

Fig. 1. Analysis of spiked water using SPME: (A) sum of the mass traces 144 (hydroxynaphthalenes) and 216 (silylated hydroxynaphthalenes) after derivatisation with BSTFA. (B) Mass trace 144 (hydroxynaphthalene) without silylation. Conditions as described in Section 2.

since the silylated metabolites could be separated from each other whereas most of the parent compounds exhibited coelution and strong tailing peaks. Moreover, the detection sensitivity increased markedly for the derivatives. Fig. 1 shows as an example the analysis of a water sample spiked with hydroxynaphthalenes. Trace B was obtained by SPME without silylation, whereas for trace A the headspace silylation step between SPME and injection was performed. The absence of the parent compounds in trace A indicate complete derivatisation.

The next figures illustrate the analysis of four different samples for hydroxylated naphthalenes (Fig. 2), phenanthrenes (Fig. 3) and pyrenes (Fig. 4), respectively: in each case, trace A shows the background of blank water, B a smokers urine, C the same urine spiked with standards, both urine samples after enzymatic hydrolysis. Trace D was obtained from water spiked with the same amount of standards. The figures present only the data of one of the two ions measured for each compound group, because the second mass traces have only been used for identification proposes via ion ratio measurement and are quite similar to the traces presented. The chromatographic separation of the individual silylated metabolites is sufficient for quantification, though complete resolution is not achieved. Table 1 contains preliminary detection limits, calculated via the background noise level $+3\sigma$ of the background noise [18] of the mass traces of the standard (trace D in each figure) shown in Figs. 2–4. These values are quite close to that published by other authors [7,12,19].

Compared to the standard chromatograms, no significant additional matrix peaks appear in the urine samples. This improvement is based both on

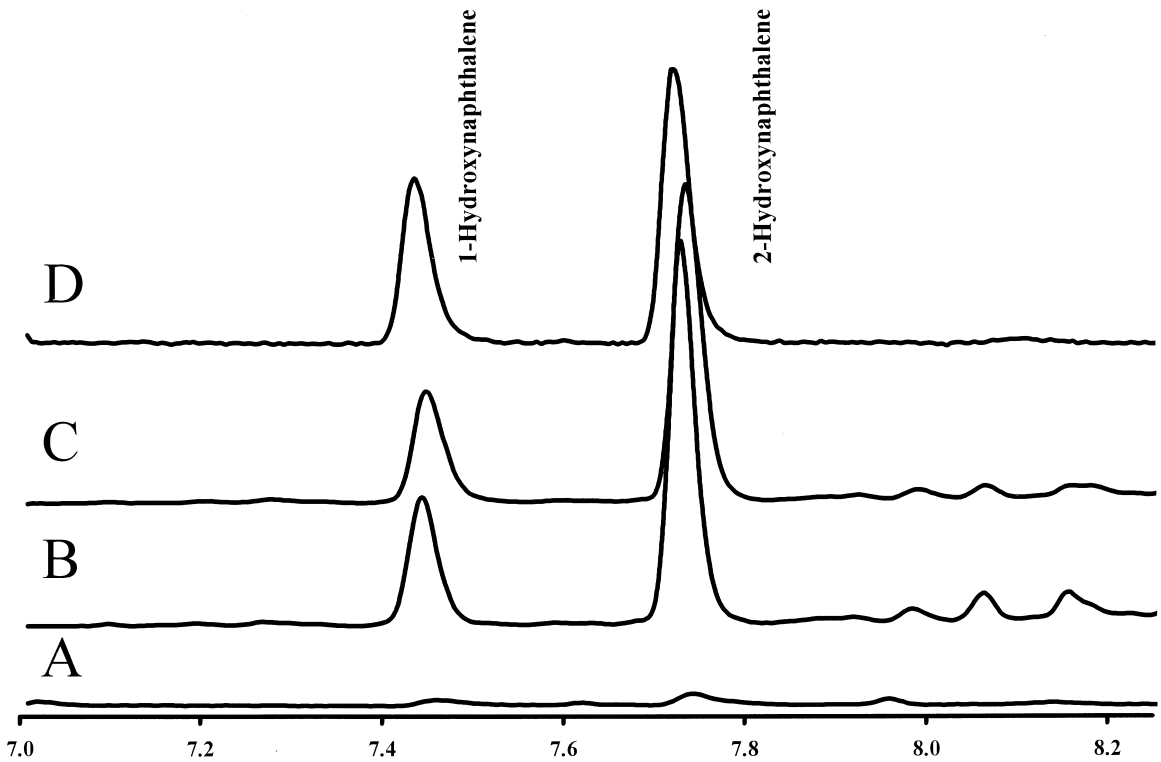

Fig. 2. Selected ion chromatogram, m/z 216 (trimethylsilyl-derivatives of hydroxynaphthalenes) after SPME sampling and derivatisation with BSTFA. (A) Blank water, (B) 5 ml enzymatically hydrolysed urine, (C) 5 ml enzymatically hydrolysed urine, spiked with 50 μl stock solution and (D) 5 ml water spiked with 50 μl stock solution. Conditions as described in Section 2.

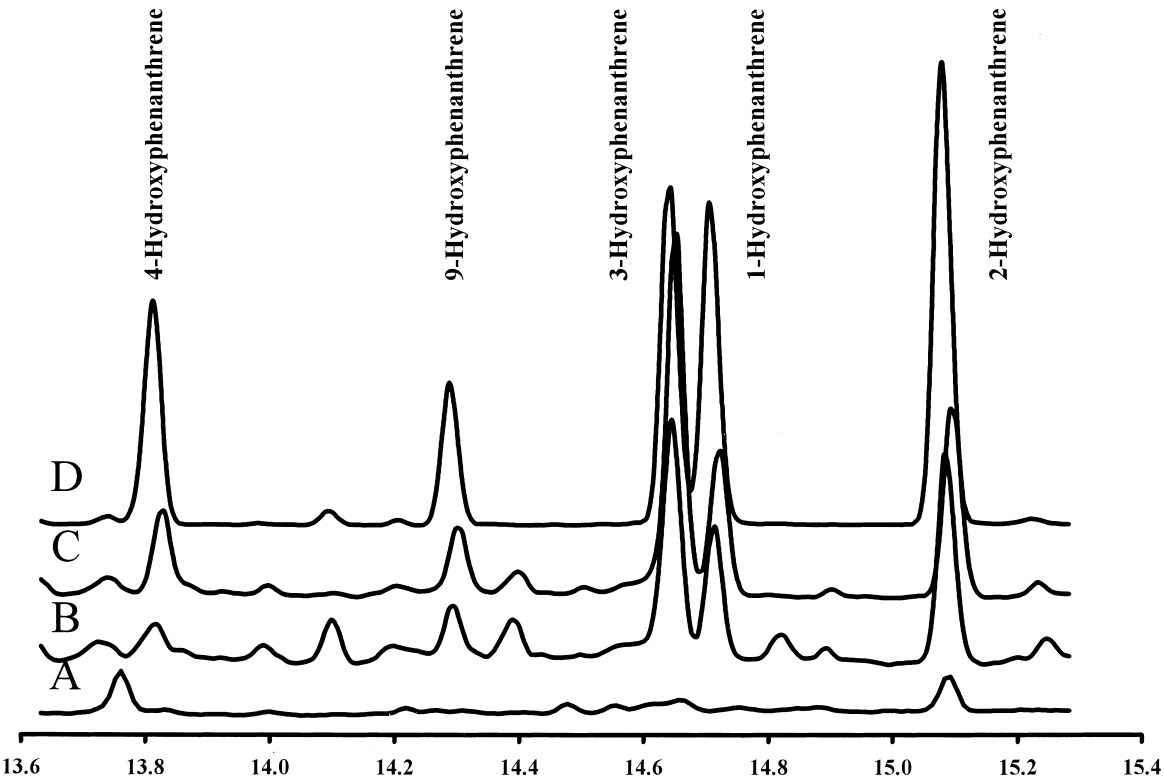

Fig. 3. Selected ion chromatogram, m/z 261 (trimethylsilyl-derivatives of hydroxyphenanthrenes) after SPME sampling and derivatisation with BSTFA. (A) Blank water, (B) 5 ml enzymatically hydrolysed urine, (C) 5 ml enzymatically hydrolysed urine, spiked with 50 μl stock solution and (D) 5 ml water spiked with 50 μl stock solution. Conditions as described in Section 2.

the extraction selectivity of the polyacrylate fibre and the mass-selective detection.

To check for carryover effects, pure water was analysed after a concentrated standard (about 5 μg/l of each compound), using the same fibre for both analyses. Under the applied conditions (270°C injector temperature and 3 min of desorption time in the injector) no carryover was detected. The analyte responses from spiked urine were generally lower than from water containing the same hydroxy-PAH concentrations. One possible explanation could be the matrix influence. Urine includes plenty of other substances with similar chemical properties, which are able to compete in the SPME coating with the PAH metabolites. Such phenomena have been already reported for phenols in tap water [20].

The presented method is currently under optimisation. The effects of sampling time, derivatisation time, pH, temperature etc., have to be examined in more detail. Performance data like detection limit or standard deviation will be determined with the final method, even though the already achieved sensitivity allows for the detection of the target compounds in background concentrations.

The method is easy to perform, it is straightforward, consumes no solvents and exhibits mild conditions for extraction and derivatisation. Only a small amount of sample is necessary and handling operations with urine are restricted to one pipetting step.

On the other hand, the method is not easy to automate, because until now there has been no autosampler available with the desired features. The obtained specificity and selectivity adhere to a great

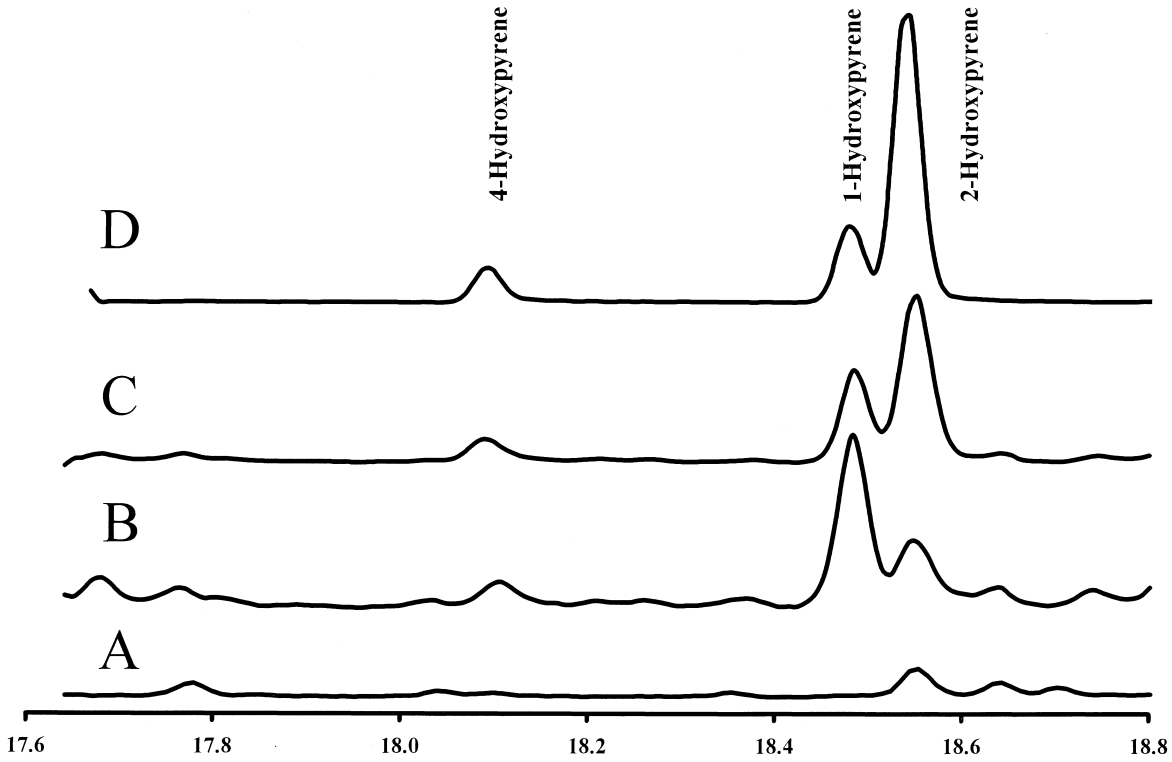

Fig. 4. Selected ion chromatogram, m/z 290 (trimethylsilyl-derivatives of hydroxypyrenes) after SPME sampling and derivatisation with BSTFA. (A) Blank water, (B) 5 ml enzymatically hydrolysed urine, (C) 5 ml enzymatically hydrolysed urine, spiked with 50 μl stock solution and (D) 5 ml water spiked with 50 μl stock solution. Conditions as described in Section 2.

extend to the use of the MS-SIR detection method: the relatively high costs of the necessary instrumentation (which moreover requires a highly skilled operator) obviously has to be considered as a certain disadvantage.

4. Conclusion

A method for the profile analysis of various metabolites of naphthalene, phenanthrene and pyrene is presented, which restricts the analytic procedure to the following steps: extraction by SPME with a 85-μm polyacrylate fibre followed by headspace silylation with BSTFA and GC–MS in the SIR mode for measurement. The method was tested for its applicability to metabolite profile analysis using a smokers urine (native and spiked, both after enzymatic cleavage) and spiked water as samples. Though not yet optimised, the method exhibits satisfactory separation of all metabolites under investigation without interference by extraneous matrix peaks.

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