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Journal of Chromatography A, 823 (1998) 179–187

JOURNAL OF CHROMATOGRAPHY A

Solving coelution problems on a smectic-liquid crystalline polysiloxane capillary column for the determination of priority polycyclic aromatic hydrocarbons in environmental samples

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Abstract

A comparison of the separation efficiency of a liquid-crystalline polysiloxane capillary column (smectic phase) and a DB-5 column (5% phenyl-substituted methyl polysiloxane) is made for the analysis of the 16 US Environmental Protection Agency polycyclic aromatic hydrocarbons (PAHs) and three isomeric, nonpriority PAHs (triphenylene, benzo[*j*]fluoranthene and dibenzo[*a*,*c*]anthracene) which have been shown to occur in environmental samples. The three isomeric compounds which coelute on the DB-5 column are well separated on the smectic phase. The analysis of a soil sample shows that some priority PAHs tend to be overestimated on DB-5 type columns. The smectic phase is therefore an attractive alternative for confirmation and correction of final quantitative results.

Keywords: Soil; Liquid crystalline stationary phases; Environmental analysis; Stationary phases, GC; Polycyclic aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are an important group of organic contaminants which are widespread in the environment as a result of human activities. They are produced by incomplete combustion of fossil and synthetic fuels as well as naturally by forest fires and possibly microbiological synthesis. Some PAHs exhibit carcinogenic and/or mutagenic properties and are listed by the US Environmental Protection Agency (EPA) and the European Community as priority pollutants. Due to their physicochemical properties, the compounds, especially the higher molecular mass PAHs, are hardly degradable and tend to accumulate in the biosphere [1]. Although several hundreds of PAHs exist, most of the studies determine just a limited amount of them, namely the 16 better known EPA priority PAHs. Their analysis involves extraction with different organic solvents, clean-up procedures by means of solid-phase extraction (SPE) and final determination by high-resolution gas chromatography (HRGC) with flame ionization detection (FID) or mass selective (MS) detection and high-performance liquid chromatography (HPLC) with time programmed fluorescence detection (FD). Applying HRGC PAH are normally measured on nonpolar stationary phases like 5% phenyl methylpolysiloxane or 100% methylpolysiloxane capillary columns. Separation problems are however observed on these nonpolar stationary phases for closely eluting PAH isomers like the different benzofluoranthenes, dibenzoanthracenes and others. Therefore, column lengths of 50 m or more are used to increase

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0021-9673/98/$ – see front matter
PII: S0021-9673(98)00302-1

resolution, increasing the total chromatography time significantly. The use of liquid crystals in gas chromatography was introduced by Kelker in 1963 [2]. The liquid-crystalline stationary phases add some unique selectivity to chromatographic separations of rigid planar molecules like PAHs. The smectic phase exhibits a layered structure similar to the C_{18} structure in reversed-phase liquid chromatography (RPLC). Liquid-crystal layers have successfully been applied to PAHs [3–15] as well as to other organic pollutants like polychlorinated biphenyls, dibenzodioxins and dibenzofurans [16–19].

The aim of this study was to evaluate the separation efficiency of the smectic column for the 16 EPA priority PAHs including some closely eluting isomers and to make a comparison with a 5% phenyl-methylpolysiloxane stationary phase used in routine analysis of these molecules. Finally, a noncertified soil sample, containing native PAHs, was analyzed and quantitative results were compared on both types of columns.

2. Experimental

2.1. PAH standards

A PAH mixture containing the 16 EPA priority PAHs (naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benzo[*a*]anthracene (BaA), chrysene (CHR), benzo[*b*]fluoranthene (BbF), benzo[*k*]-fluoranthene (BkF), benzo[*a*]pyrene (BaP), indeno-[1,2,3-*cd*]pyrene (IND), dibenzo[*a,h*]anthracene (Da,hA), benzo[*g,h,i*]perylene (BGP)) at a concentration of 10 ng/μl each compound in toluene and deuterated internal standards $^2H_{10}$-acenaphthene (ACE-D10), $^2H_{12}$-chrysene (CHR-D12), $^2H_8$-naphthalene (NAP-D8), $^2H_{12}$-perylene (PER-D12) and $^2H_{10}$-phenanthrene (PHE-D10) at a concentration of 2000 μg/ml each compound, dissolved in the same solvent, were purchased from Dr. Ehrensdorfer, Augsburg, Germany. Calibration solutions (0.003–5 μg/ml) were prepared by dilution of the stock solution with toluene. The final concentration of the deuterated internal standards in the calibration solutions were 2 μg/ml. Nonpriority PAH (triphenylene (TRI), benzo[*j*]fluoranthene (BjF), dibenzo[*a,c*]anthracene (Da,cA)) were obtained as crystalline material in a purity >95%.

2.2. Organic solvents and reagents

Methanol, hexane, dichloromethane (DCM) and toluene for organic trace analysis (SupraSolv) from Merck (Darmstadt, Germany). DCM was handled with special care, observing safety precautions, using efficient fume hoods and wearing protective gloves. Anhydrous sodium sulphate (Na_2SO_4) was from Merck of analytical-reagent grade. Before use, sodium sulphate was activated at 600°C for 12 h. Potassium hydroxide pellets (KOH) GR grade were from Merck. Solid phase: silica gel 40 (particle size 0.063–0.2 mm) from Merck was first activated at 300°C for 12 h. Deactivation with water (5% w/w) was performed 12 h before use. Aluminium oxide 90 (particle size 0.063–0.2 mm) from Merck was activated at 110°C for 12 h and deactivated in the same way as silica gel.

2.3. Sample pretreatment and characterization

The soil sample was dried at 40°C and milled to the fraction <0.5 mm. The particle size distribution was as follows: sand 17.6%, silt 75.2% and clay 25.7%. The organic C-content was 36%.

2.4. Extraction of the soil sample and clean-up

Extraction and clean-up were performed as previously published [20]. Briefly the soil sample (2.5 g, $n=4$) was digested in 2 *M* KOH–methanol for 2 h at 70°C. Deuterated internal standards were added at the initial of the extraction. After liquid–liquid partitioning with hexane the concentrated nonpolar phase was cleaned on a double layer column consisting of silica gel and aluminium oxide (1:1 w/w). Elution of the PAH fraction was achieved with hexane–DCM (4:1, v/v). After reconcentration the PAH eluate was dissolved in toluene and analyzed by HRGC–MS.

2.5. Analysis of PAHs by HRGC–MS

A Hewlett-Packard 5890 Series II GC and HP

7673 autosampler was fitted with either a 12 m×0.2 mm I.D., 0.15 μm film thickness SB-smectic liquid crystalline capillary column (Dionex) or a 50 m× 0.20 mm I.D., 0.33 μm film thickness DB-5 MS fused-silica column (J&W). A deactivated retention gap (2 m×0.53 mm) was used to protect the analytical column. The column end was directly interfaced to a HP 5971 A mass selective detector. Samples (1 μl) were injected into an on column injector system and chromatographed at 100°C, 60°C/min to 150°C, 3°C/min to 270°C for 10 min (SB-smectic column); 100°C for 1 min to 300°C, 2.5°C/min for 20 min (DB-5 MS column). The carrier gas was helium at a linear gas velocity of 62 cm s^{-1} (smectic column) and 34 cm s^{-1} (DB-5 MS column) respectively. The analysis was carried out using the injector system in the oven tracking mode to maximize chromatographic resolution. The oven tracking mode maintains the injector temperature 3°C higher than the oven temperature program to optimize repeatability. The mass spectrometer was operated under electron impact (EI) ionization with a 70 eV ionization voltage. Quantification using the internal standard method was based on SIM as follows: NAP/NAP-D8 *m/z* 128.1, 136.2, ACY/ACE/ACE-D10 *m/z* 152.1, 154.1, 164.3 FLU *m/z* 166.1, PHE/ANT/PHE-D10, *m/z* 178.1, 188.1, FLT/PYR *m/z* 202.2, BaA/TRI/CHR/CHR-D12 *m/z* 228.2, 240.2, BbF/BkF/BjF/BaP/PER-D12 *m/z* 252.2, 264.4 IND/BGP/Da,cA/Da,hA *m/z* 276.2, 278.2. Electron multiplier voltage (EMV) was 2700 V absolute, dwell time for ion groups was 150 ms. The MS temperature parameters were as follows: injector 103°C, transfer line 280°C. The MS detector was set for highest sensitivity of the analytes using a manual tune (based on autotune) that optimized ions *m/z* 131, 219 and 264. GC–MS instrumental control, data acquisition and processing were provided by a Vectra 486/33 VL computer equipped with a Hewlett-Packard G1034C CHEMSTATION software.

3. Results and discussion

For the determination of PAHs in different environmental matrices, including certified reference materials (CRM), the two main analytical techniques are GC–MS and HPLC–FD. GC–MS on 5% phenyl-substituted methylpolysiloxane stationary phases is the typical procedure for the quantitative determination of the 16 priority PAHs. The separation on such a column is shown in Fig. 1. Typically there are four closely eluting pairs of PAHs: PHE/ANT, BaA/CHR, BbF/BkF and IND/Da,hA which cause separation problems in real sample analysis as column efficiency often decreases with the number of injections. This is especially true for the last three pairs of PAHs. Moreover a second problem is associated with the lack of separation of other important isomers on the 5% phenyl-substituted methylpolysiloxane stationary phase, namely CHR and TRI, BbF — and BjF and Da,hA — and Da,cA (Fig. 2) which are not all resolved even on 50-m columns (Fig. 1, windows 3,4,5) but do occur in environmental matrices [21]. A correct quantification however, is of great importance, especially for PAHs having a carcinogenic or mutagenic potential (BaA, BbF and Da,hA). Improved resolution of these PAH would be beneficial. The use of a smectic phase for the separation of the 16 EPA priority PAHs was successfully introduced some time ago [22]. The separation of the PAHs on the smectic phase including the major coeluting isomers TRI, BjF and Da,cA is presented in Fig. 3 together with the extracted ion chromatograms showing the ion traces *m/z* 228 (BaA/TRI/CHR), *m/z* 252 (BbF/BkF/BjF/BaP) and *m/z* 278 (Da,hA/Da,cA) (Fig. 4). The isomeric PAHs are well resolved with resolution factors ≥1.2 which corresponds to a baseline separation. This separation was achieved on a 12-m column as retention of the PAHs on the smectic phase turned out to be much stronger compared to the DB-5 column. There are, however some disadvantages of the smectic phase which limits its use for routine analysis: the separation potential is rather restricted for PAHs which differ only in the number of double bonds (ACY/ACE, peaks 2,3 in Fig. 3). Moreover, we have observed problems regarding the reproducibility of retention times and an increasing bleed due to the loss of the stationary phase with extended use.

Retention time differences can be compensated by using internal standards (deuterated PAH) as time reference peaks whereas the bleeding rate can be minimized by keeping the electron multiplier voltage

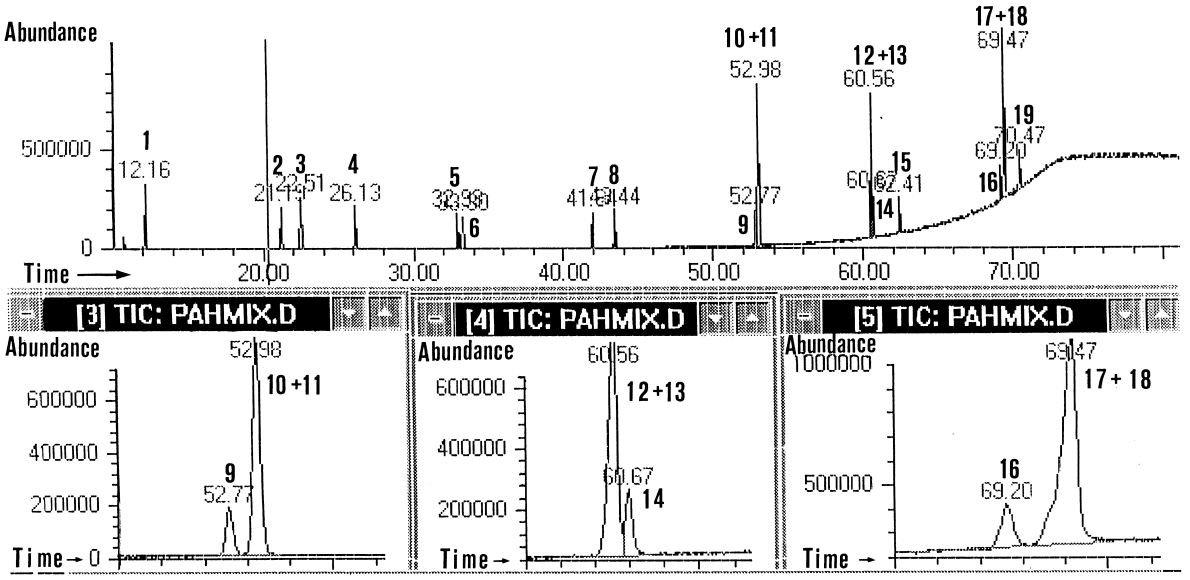

Fig. 1. Separation of the 16 EPA priority PAHs on a DB-5 column showing coelution of the isomeric, nonpriority PAH; 1=NAP, 2=ACY, 3=ACE, 4=FLU, 5=PHE, 6=ANT, 7=FLT, 8=PYR, 9=BaA, 10=TRI, 11=CHR, 12=BbF, 13=BjF, 14=BkF, 15=BaP, 16=IND, 17=Da,cA, 18=Da,hA, 19=BGP (for peak identification see Section 2.1).

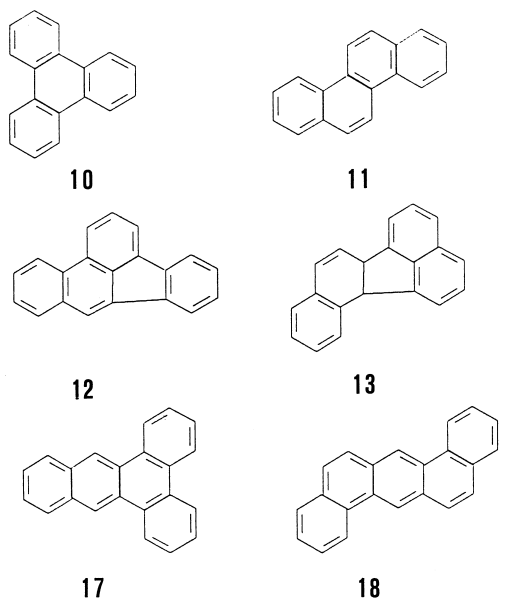

Fig. 2. Chemical structures of closely eluting isomeric PAHs: TRI (10), CHR (11), BbF (12), BjF (13), Da,cA (17) and Da,hA (18).

of the MS system as low as possible. Smectic phases can therefore provide precious information on selected isomers which frequently coelute on other nonpolar columns.

Finally the suitability of the smectic phase for the separation of PAH mixtures was tested by extracting a soil sample. The total ion chromatogram of the PAH eluate is shown in Fig. 5 together with the ion traces m/z 228, 252, 276, 278 (Fig. 6). Clearly the three isomers TRI, BjF and Da,cA can be identified in the extract and are well separated. The quantification results of the 16 priority PAHs obtained on the DB-5 and on the smectic phase column are summarized in Table 1. As expected the three priority PAH CHR, BbF and Da,hA are overestimated on the DB-5 column. Preliminary investigations with other soil samples have confirmed the presence of these coeluting isomers and they seem to play an important role in the determination of the 16 EPA priority PAHs. Therefore analysts should consider confirming the GC results on another stationary phase capable of separating isomeric PAH. The smectic column would be a good choice.

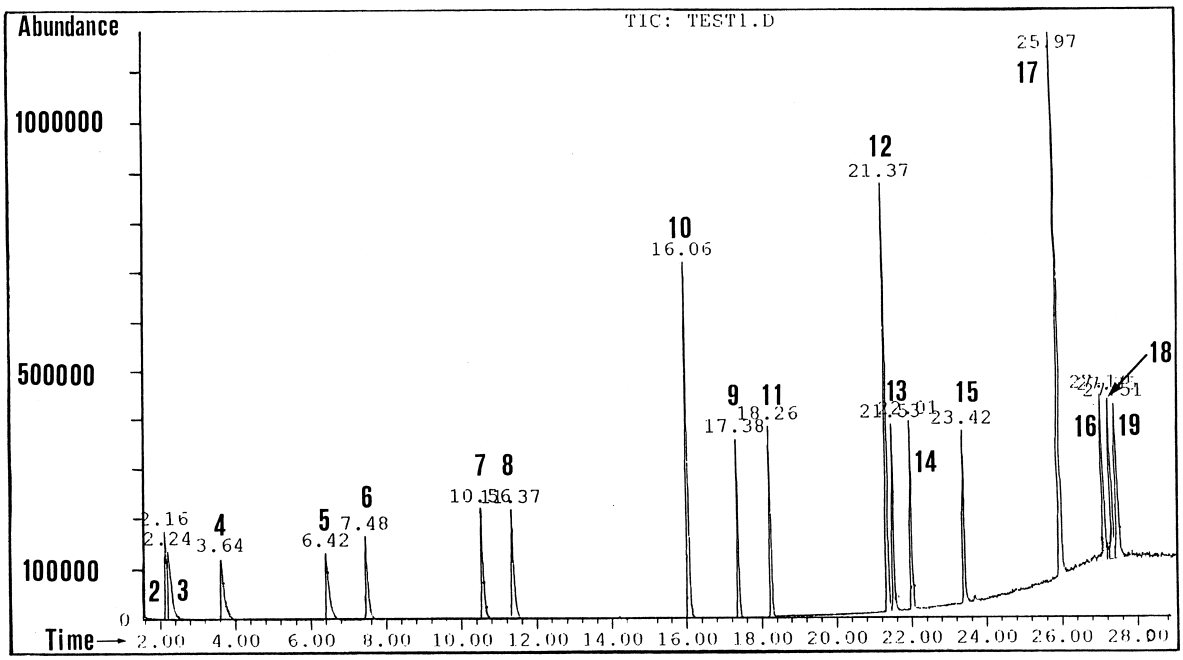

Fig. 3. Separation of the 16 EPA priority PAHs on a smectic phase together with the nonpriority PAHs (NAP not shown).

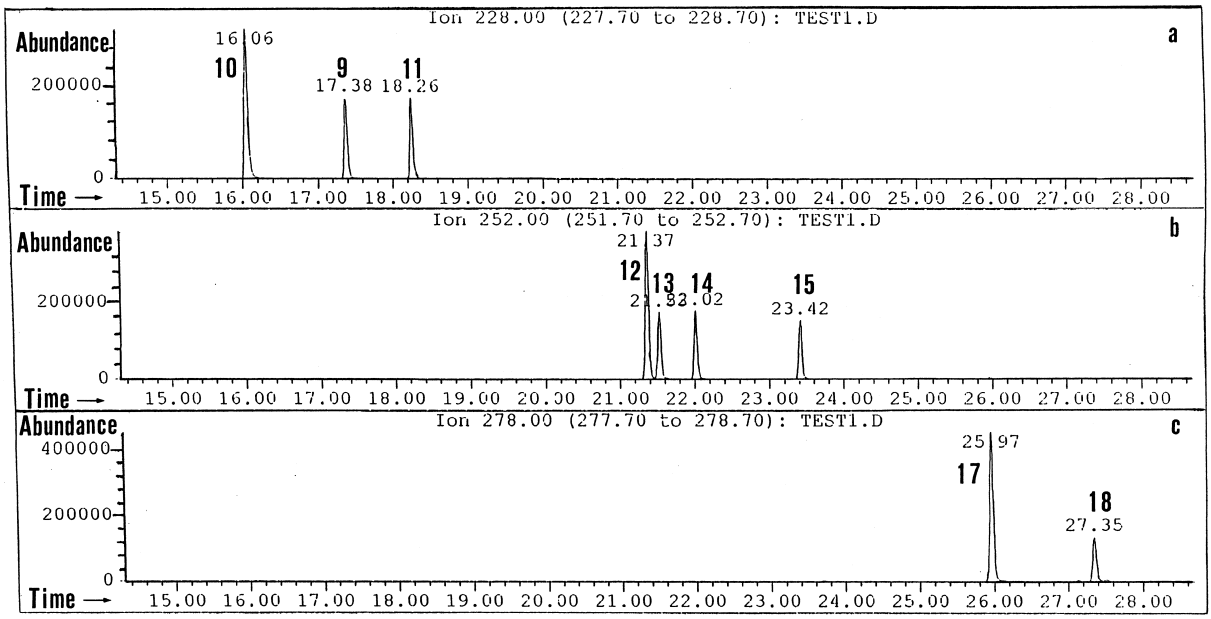

Fig. 4. Ion traces of *m/z* 228 (a), 252 (b) and 278 (c) showing the separation efficiency of the smectic phase for isomeric PAHs (peaks 10/11, 12/13, 17/18).

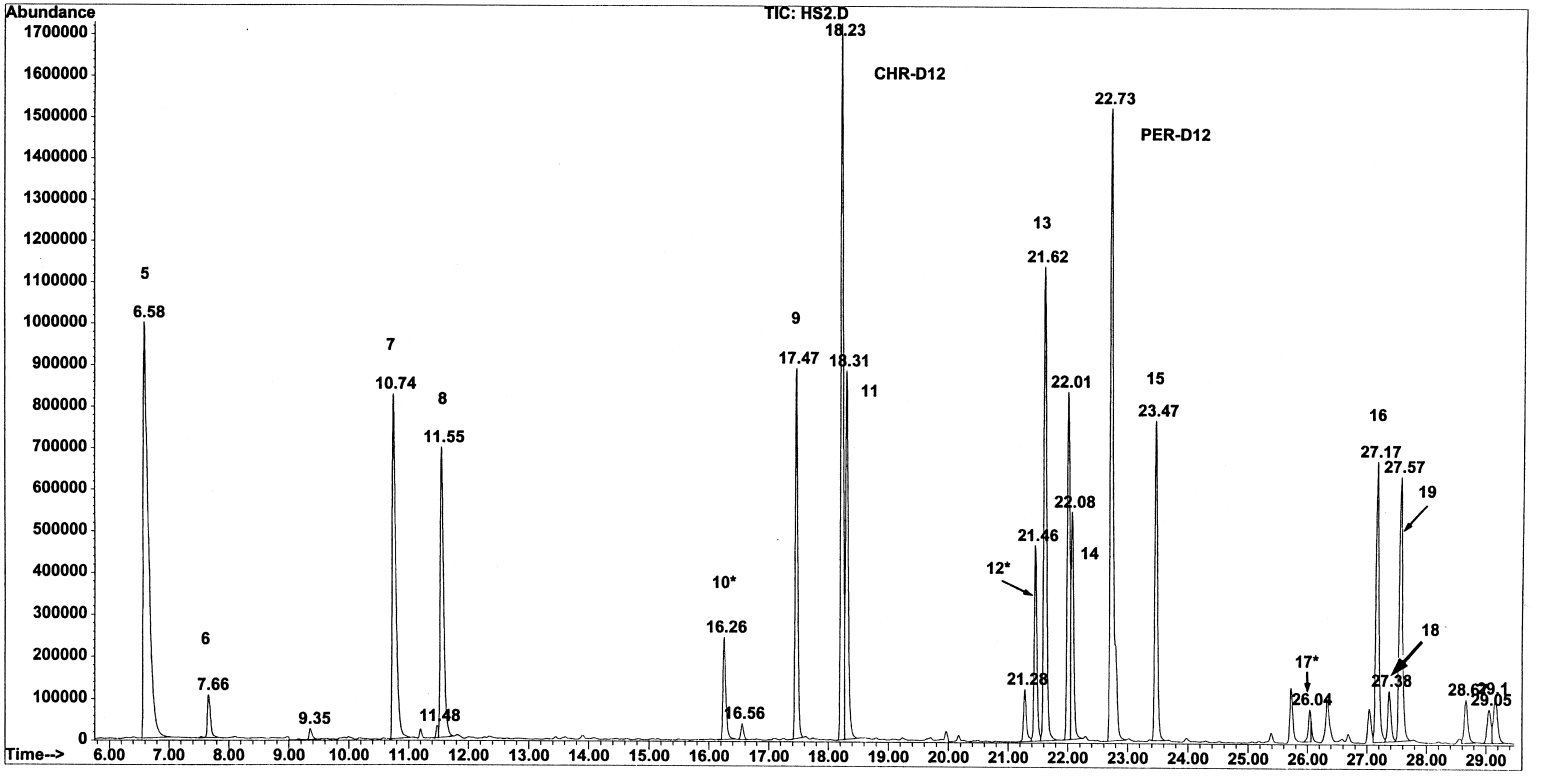

Fig. 5. Total ion chromatogram (TIC) of the soil sample extract containing PAHs; isomeric PAHs are marked with an asterisk (*) (peaks 10, 12 and 17).

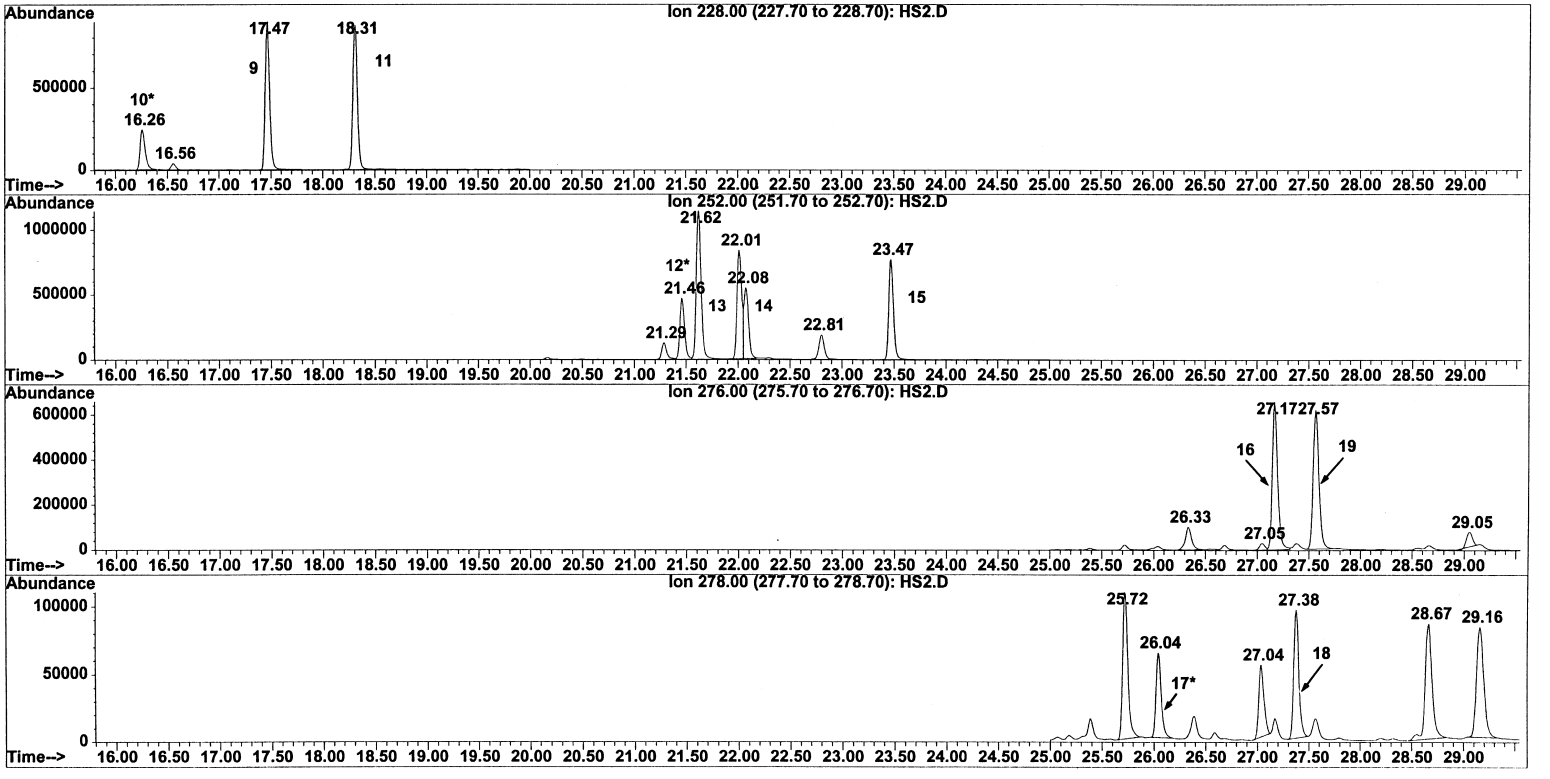

Fig. 6. Ion traces of m/z 228, 252, 276 and 278 of the soil sample proving the presence of the isomeric, nonpriority PAH.

Table 1
Comparison of quantitative results of the 16 EPA priority PAHs as well as three nonpriority PAHs (c) obtained on a DB-5 and smectic column from a soil sample ($n=4$)

PAHs	μg/kg dry mass	
	DB-5 column	Smectic column
Naphthalene	N.q.	N.q.
Acenaphthylene	10 (3)[a]	72 (4)[b]
Acenaphthene	21 (3)	14 (6)
Fluorene	31 (15)	52 (9)
Phenanthrene	470 (4)	464 (8)
Anthracene	103 (10)	87 (12)
Fluoranthene	722 (3)	698 (7)
Pyrene	559 (2)	517 (8)
Benzo[*a*]anthracene	493 (4)	449 (8)
Triphenylene[c]		156 (6)
Chrysene	655 (4)[d]	454 (10)
Benzo[*b*]fluoranthene	553 (6)[e]	323 (9)
Benzo[*j*]fluoranthene[c]		198 (8)
Benzo[*k*]fluoranthene	433 (9)	495 (14)
Benzo[*a*]pyrene	499 (10)	456 (16)
Indeno[1,2,3-*cd*]pyrene	374 (14)	420 (17)
Dibenzo[*a,c*]anthracene[c]		46 (13)
Dibenzo[*a,h*]anthracene	97 (13)[f]	61 (14)
Benzo[*g,h,i*]perylene	456 (4)	370 (11)
Sum	5476	5332

[a] Relative standard deviation (%).
[b] Interference.
[c] Nonpriority PAHs.
[d] Sum of CHR+TRI.
[e] Sum B(b)+B(j)F.
[f] Sum of Da,cA+Da,hA.
N.q.: not quantified.

4. Conclusions

Quantification of priority PAHs are normally performed on 5% methyl-substituted methylpolysiloxane capillary columns (DB-5). However, these columns do not separate isomeric PAHs like TRI, BjF and Da,cA which have been shown to occur in environmental matrices. An attractive alternative is the use of a liquid-crystalline polysiloxane column (smectic phase) which is capable of resolving these isomeric PAH. Analyzing a soil sample on a smectic phase column, the presence of these isomeric PAHs could clearly be proved. Therefore, the analysis of the priority PAHs on DB-5 type columns tend to overestimate the compounds CHR, BbF and Da,hA. Confirmation and/or correction of the results on a column having a high resolution potential like the smectic phase would certainly improve the confidence in the final results.

Acknowledgements

The authors are grateful to the Sulzer Innotec laboratory, Winterthur for providing the soil sample.

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