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Application of planar chromatography to the analysis of polycyclic aromatic hydrocarbons and their derivatives in environmental samples

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Abstract

The main aspects of using planar chromatography to isolate and identify polycyclic aromatic hydrocarbons (PAHs) and their derivatives in environmental samples are described. Several carbonyl, nitro polycyclic aromatic compounds, azaarenes, and polar PAHs, which are very dangerous for human health, were identified. These results have been confirmed by gas chromatography coupled with mass spectrometry. Progress in the application of these techniques using different stationary and mobile phases, as well as in the modernization of chromatographic chambers, are presented. © 1997 Elsevier Science B.V.

Keywords: Particulate matter; Sewage sludges; Polynuclear aromatic hydrocarbons

1. Introduction

Planar chromatography is a type of liquid chromatography consisting of the following techniques: thin-layer chromatography (TLC) or high-performance thin-layer chromatography (HPTLC), forced flow planar chromatography (FFPC) [overpressured layer chromatography (OPLC) or rotation planar chromatography (RPC)] and paper chromatography (PC). The principles of these techniques, particularly of TLC, have been known for many years, but, after a short period of stagnation, TLC has become an important analytical separation technique [1,2]. Modern TLC is widely used due to automation of the instrumentation involved, its reproducibility and the ability to quantitate results accurately [1,3].

The theories, methodology, instrumentation and applications of planar chromatography have been reviewed [3–8]. Classical TLC remains a popular and inexpensive method for the separation of complex mixtures [9], requiring little instrumentation, however, confirmation of the identity of compounds is generally performed using high-performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS) [10].

The advantages of TLC are that, independently of the various analytical stages, minimum clean-up is required before samples can be analysed; parallel separation and direct comparison of standards and sample components can be performed; the stationary phases are disposable; static detection; the procedure is highly selective and flexible [5] and it provides an opportunity to determine substances of low volatility.

The disadvantages of TLC are the lack of com-

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plete automation in comparison with other analytical methods; compounds sensibility to air and/or light as well as the difficulties in determining highly volatile compounds. The on-line coupling of TLC with flame ionization detection (FID) is very convenient for these compounds.

For structural elucidation in combination with chromatographic techniques, infrared spectroscopy (IR) is not as successful as mass spectroscopy [1,5,10] (off-/on-line mode). Additional information for structural elucidation can be obtained by coupling TLC with MS–MS detection [11]. Highly selective and sensitive analysis of single compounds in complex matrices is also possible using LC–MS–MS [12].

Planar chromatography has been used to analyse many compounds, especially organic agents, which contaminate air, water, food, sludge and other elements of the natural environment [3,13–17].

Polycyclic aromatic hydrocarbons (PAHs) and their derivatives, with different functional groups and/or heteroatoms in the molecules, are emitted first of all during combustion processes (of coal, gas, wood, oil, biomass), depending on the combustion conditions and they usually occur (particularly those that are the most dangerous for human health) suspended in particulate matter in the air [18–22]. Some PAH derivatives can be formed by photochemical reactions in the atmosphere, however, they may also be the products of PAH degradation e.g. in sewage sludge [23].

The products of PAH conversions depend on the nature of the substrate to which they are adsorbed. However, the structure of PAHs is one of the dominant factors determining their reactivity [24–26]. Parameters such as the surface area of particulate matter or sewage sludge, their size, their pH values, and their metal and carbon content may affect the rates of PAH degradation [27,28].

Many derivatives are often formed during the sampling and storage of samples and during the analytical procedures [27,29,30].

Usually, the determination of these compounds requires a multistage analytical scheme, using various chromatographic methods [15,31–37].

Planar chromatography has been used to analyse PAHs [15,31,38] and their derivatives [30,39,40] as a preliminary separation stage, with or without sample clean-up, and to identify functional groups and individual compounds, and as a subfractionation method, particularly prior to the use of other analytical methods.

2. Experimental

2.1. Model PAHs and retention of their derivatives

Firstly, investigations on the separation of model PAHs and their derivatives were carried out using classic saturated chambers. Since spots of compounds broadened near the solvent front, channel chromatography was used to find out which solvent would be the best for the separation of organic material isolated from particulate matter [31]. This technique concentrates separated compounds and enables an approximate evaluation to be made of the main compounds contained in the samples being investigated [31,41].

For this purpose, the standard PAH mixture was separated on a silica gel layer using dichloromethane (DCM)–*n*-hexane (1:1, v/v) as the mobile phase.

Nitrogen and oxygen PAH derivatives were also investigated.

Nitrogen derivatives, such as PAHs containing nitro groups, were separated on plates that were coated with a silica gel layer, using DCM and a mixture of DCM with *n*-hexane and methanol (MeOH) as the mobile phases. The chromatograms, developed using a mixture of *n*-hexane–DCM (1:1, v/v), were observed before and after being sprayed with reducing agent [42,43] under UV light.

Some oxygen derivatives were separated using silica gel layers developed with DCM–hexane or DCM–MeOH mixtures in a 'DS' sandwich chamber [33].

Standard azaarenes were investigated using silica gel and cellulose as the stationary phases, with DCM–methanol and dimethylformamide (DMF)–water as the mobile phases, respectively [44].

Primary amines rarely occur in air, but their presence in sewage sludge has not been ruled out. TLC analysis of the model aminoarenes was carried out using reversed-phase RP-18, with acetonitrile–water (9:1, v/v) as the mobile phase [45].

The retention behaviour of the more polar PAHs,

such as 1-hydroxypyrene, which may occur in environmental samples [46], has been investigated on C_{18} reversed stationary phase [47]. A mixture of acetonitrile–water (3:2, v/v) was used to develop the chromatograms.

2.2. Visualization

The detection of PAHs was carried out by UV illumination at λ=254 and 366 nm. Individual derivatives of PAHs were also identified by UV illumination before and after characteristic reactions with development reagents [47] and/or treatment with reagent vapour [44] for increasing or quenching fluorescence intensity [42]. Some, e.g. 1-aminoanthraquinone, but not many, derivatives of PAHs, are coloured in visible light. The characteristics of the polar derivatives of PAHs, before and after being sprayed with Fast blue salt B, are given in Table 1.

Detection of functional groups was performed by spraying the plates with specific reagents [2,48], which were observed using both visible and UV light (λ=245 and 366 nm). The limits of detection of particular functional groups in the standards used were determined.

2.3. Identification of the functional groups of PAHs in environmental samples

Using the given analytical conditions (presented in Section 2.1), PAHs and their derivatives were separated and the functional groups were identified in organic material from airborne particulate matter and sewage sludge. Raw extracts (or fractions of them), isolated by liquid–solid chromatography (LSC) and solid-phase extraction (SPE), were investigated. Details of the methodology used and the results of determinations are described in Refs. [32,33,45,53].

2.4. Semipreparative separations

On the basis of the separation results for model PAHs and their derivatives, the investigated extracts of the organic material of particulate matter and sewage sludge were linearly applied on the plates. The bands of sorbents containing compounds with similar R_F values were scraped or cut out of the plate, extracted, filtered and evaporated to the required volume, so that they could be identified using GC–MS [42,47].

The technique of thin-layer band chromatography was used initially for PAHs isolation from airborne

Table 1
Colours of the spots of hydroxy-PAHs separated on RP-18 phase with acetonitrile–water (3:2, v/v) as the mobile phase under UV illumination before and after treatment with visualizing agent.

Compound	Colour of the spots				Detection limits (ng)
	Under UV illumination		After being sprayed with Fast Blue B salt		
	at λ=365 nm	at λ=254 nm	in visible light	at λ=365 nm	
2,7-Dihydroxynaphthalene	Pale pink	Slate	Pink	Dark violet	10
1,5-Dihydroxynaphthalene	Pale pink	Slate-blue	Pink	Violet	10
1,3-Dihydroxynaphthalene	Pale pink	Pale slate	Brown–pink	Dark violet	7
2,4-Dihydroxyquinoline	Violet	Violet	Salmon	Orange	8
2,4-Dinitro-1-naphthol	Dark yellow	Yellowish	Yellow	Brown	10
2-Hydroxymethylanthracene	Slate yellow	Dark violet			50
2,3-Dihydroxynaphthalene	Pale violet	Greenish	Bluish	Pale yellow	30
2-Naphthol	Pale pink	Slate blue	Pink	Dark violet	10
1-Naphthol	Pale pink	Pale slate	Pink	Dark violet	10
9-Hydroxyphenanthrene	Pale violet	Dirty violet	Pink	Dark violet	6
1-Hydroxypyrene	Intense violet	Dark yellow	Yellowish	Salmon	3
4-Hydroxyacridine	Yellow	Greenish	Grey	Greenish	20
1,8-Dihydroxyanthraquinone	Yellow	Yellow	Yellow	Brown	10
8-Hydroxyquinoline	Greenish	Dark violet	Slate pink	Yellowish	50

particulate matter [49]. For this purpose, a horizontal chamber with the distributor of the eluent was used [50,51]. The cyclohexane extract was separated on silica gel TLC plates using a DCM–*n*-hexane (1:1, v/v) mixture. The fractions containing polycyclic compounds were obtained by scraping the bands extracted with cyclohexane from the plate, and then analyzing them using GC–MS, under the conditions described earlier [49].

PAHs from sewage sludge samples were isolated in a similar way but using a horizontal DS-chamber (without a distributor of the eluent) [52].

For this purpose, the DMF extracts from sewage sludge were separated on a silica gel layer in a 'DS' sandwich chamber and developed using DCM–*n*-hexane (1:3, v/v). The bands of silica gel with R_F values from 0.45 to 0.80 were extracted by DCM, then filtered and evaporated to dryness. The isolated PAH concentrates were qualitatively analyzed using the GC–MS method.

A similar procedure was used for the separation of PAH derivatives from extracts of airborne particulate matter. To determine the functional groups in the extracts investigated, after TLC separation, eleven bands with different R_F values and fluorescence under UV light were obtained, marked, removed from the plates and extracted with DCM. After evaporation of the solvent, each fraction was qualitatively analyzed using GC–MS [32,42].

Moderately polar fractions of airborne particulate matter isolated using the procedure described earlier [32] were separated for isolation of polar PAH derivatives on the basis of data from TLC and OPLC. The fractions being investigated were separated on aluminium plates coated with RP-18 stationary phase and were developed using an acetonitrile–water (3:2, v/v) mixture in a DS sandwich chamber. The sorbent bands with R_F values from 0.05–0.35 were cut out of the plate, extracted with methanol and analyzed using the GC–MS method [47].

Semipreparative TLC was also used to isolate azaarenes and aminoarenes from moderately polar fractions of DMF extracts of sewage sludge. Azaarenes were separated on a silica gel layer and developed with DCM–methanol (10:0.5, v/v), while aminoarenes were separated on an aluminium oxide layer with DCM–hexane–diethyl ether (1:1:1, v/v) as the mobile phase. Azaarene and aminoarene concentrates were isolated from the cut-out bands by extraction with DCM–MeOH (1:1, v/v) and DCM–benzene (2:1, v/v), respectively, and then were analyzed by GC–MS [45,53].

3. Results and discussion

The use of the above-mentioned conditions for TLC and OPLC analyses has made the separation of PAHs and their derivatives, which were performed in horizontal chambers, possible.

The R_F values of model PAHs containing different numbers of aromatic rings and different degrees of condensation, which were separated on silica gel plates using various volumes of DCM–*n*-hexane as the mobile phases, are presented in Table 2. In both cases, it is possible to isolate PAH concentrates from the plate (R_F value above 0.45) for further analysis.

The horizontal chamber with distributor of the eluent (Fig. 1a) enabled better separation results to be obtained than could be achieved using a classical saturated chamber, however, the types of solvents that could be used were limited (in the case of more aggressive solvents, such as DCM, this chamber may be damaged). In addition, introduction of solvent through the distributor was sometimes interrupted. The use of this method for the isolation of PAH concentrates from environmental samples enabled the identification (by GC–MS) of parent PAHs containing two to five aromatic rings and of their alkyl derivatives, i.e. thirteen compounds in particulate matter (Fig. 2A) and sixteen compounds in sewage sludge from the most industrialized region of Poland (Fig. 2B).

The isolation of PAHs from sewage sludge samples was performed using the horizontal DS-chamber

Table 2
R_F values of model PAHs on silica gel developed in 'DS' sandwich chamber with DCM–*n*-hexane mixtures

Compound	R_F	
	DCM–*n*-hexane (1:1, v/v)	DCM–*n*-hexane (1:3, v/v)
Anthracene	0.73	0.68
Pyrene	0.71	0.62
Benzo[*a*]pyrene	0.70	0.51
Coronene	0.69	0.50

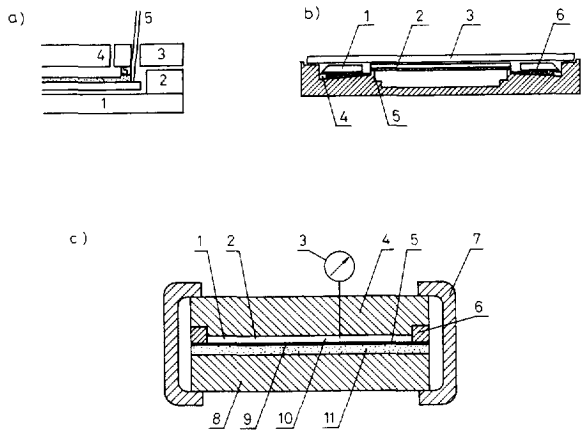

Fig. 1. Cross section through: (a) Sandwich chamber with distributor of the eluent: 1=base of the chamber; 2=limiting plate; 3, 4=cover plates; 5=capillary connection between container and eluent distributor. (b) DS-chamber during development of chromatograms: 1=container cover plates; 2=chromatographic plates; 3=main glass lid; 4=eluent container; 5=threshold; 6=eluent. (c) The closed overpressured chamber: 1=gas inlet; 2=safety valve; 3=manometer; 4=methyl polymethacrylate plate; 5=eluent inlet system; 6=rubber seal; 7=metal connection between base and cover; 8=metal base; 9=elastic membrane; 10=space with a compressed gas; 11=chromatographic plates [57,58].

(Fig. 1b). The development process was carried out by bringing the eluent into direct contact with the sorbent surface, without causing disturbances, as there is a simple way of delivering eluent from the shallow eluent reservoir [54] that was inside the chamber. A convenient developmental process, the chemical resistance of the body chamber material and the ability to separate a large number of samples were also advantages of this chamber.

Both, the sandwich chamber with the eluent distributor and the horizontal DS-chamber required minimum solvent consumption, protecting the environment from waste solvent vapours [55]. The means of eluent delivery to the sorbent layer in each of the horizontal chambers are presented in Fig. 1.

Detection of the functional groups, such as $-NO_2$, $-OH$, $-COOH$, $=N-$, $=CO$ and $-NH_2$, in the TLC separation of moderate and more polar fractions isolated from raw extracts by means of SPE or column liquid chromatography have indicated the complexity of environmental samples. The determination of the functional groups of PAH derivatives is also possible in raw extracts.

In the case of oxygen and nitrogen PAH derivatives, separated on a silica gel layer, the best results were obtained with DCM–methanol (10:0.5, v/v) as the mobile phase. These results are shown in Table 3. This solvent system enables PAHs to be separated from their derivatives and heterocycles in organic material from environmental samples.

Nitro compounds were also chromatographed on silica gel plates eluted with *n*-hexane–DCM (1:1, v/v). The R_F values obtained are presented in Table 4.

A comparison of the R_F values of azaarenes are presented in Table 5. It was concluded that better separation of azaarenes was achieved on a cellulose layer with DMF–water (20:80, v/v) than on a silica gel as the stationary phase with DCM–methanol (10:0.5, v/v) as the mobile phase.

The R_F values of the polar PAHs that occur most often in environmental samples are presented in Table 6, their characteristics after separation on RP-18 phase and their detection limits are given in Table 1.

Firstly, the TLC results show that the investigated fractions from extracts of particulate matter consist of oxygen derivatives of PAHs such as aromatic ketones, aldehydes, phenols, and nitrogen compounds i.e. nitro-PAHs and azaarenes. In extracts from sewage treatment plants, amino groups were also found [45].

Secondly, modernization of chromatographic chambers lead to the shortening of the separation time. The comparison of the development time was realized for example of hydroxy-PAH separation by the use of RP C_{18} stationary phase and acetonitrile–water (3:2, v/v) as the mobile phase. The separation of these compounds (carried out over a distance of 6 cm) were almost twice as long on glass plates (22 min) than on aluminium plates (12 min) that were coated with the same stationary phase and chromatographed under the same conditions in a DS-horizontal sandwich chamber. On the other hand, the development time for chromatograms on aluminium plates coated with RP C_{18} phase was shorter in an overpressured chamber (7.5 min) than in a sandwich chamber (12 min).

Additionally, the use of an overpressured chamber excluded the effect of the vapour phase on the chromatographic process (Fig. 1c) [56], however,

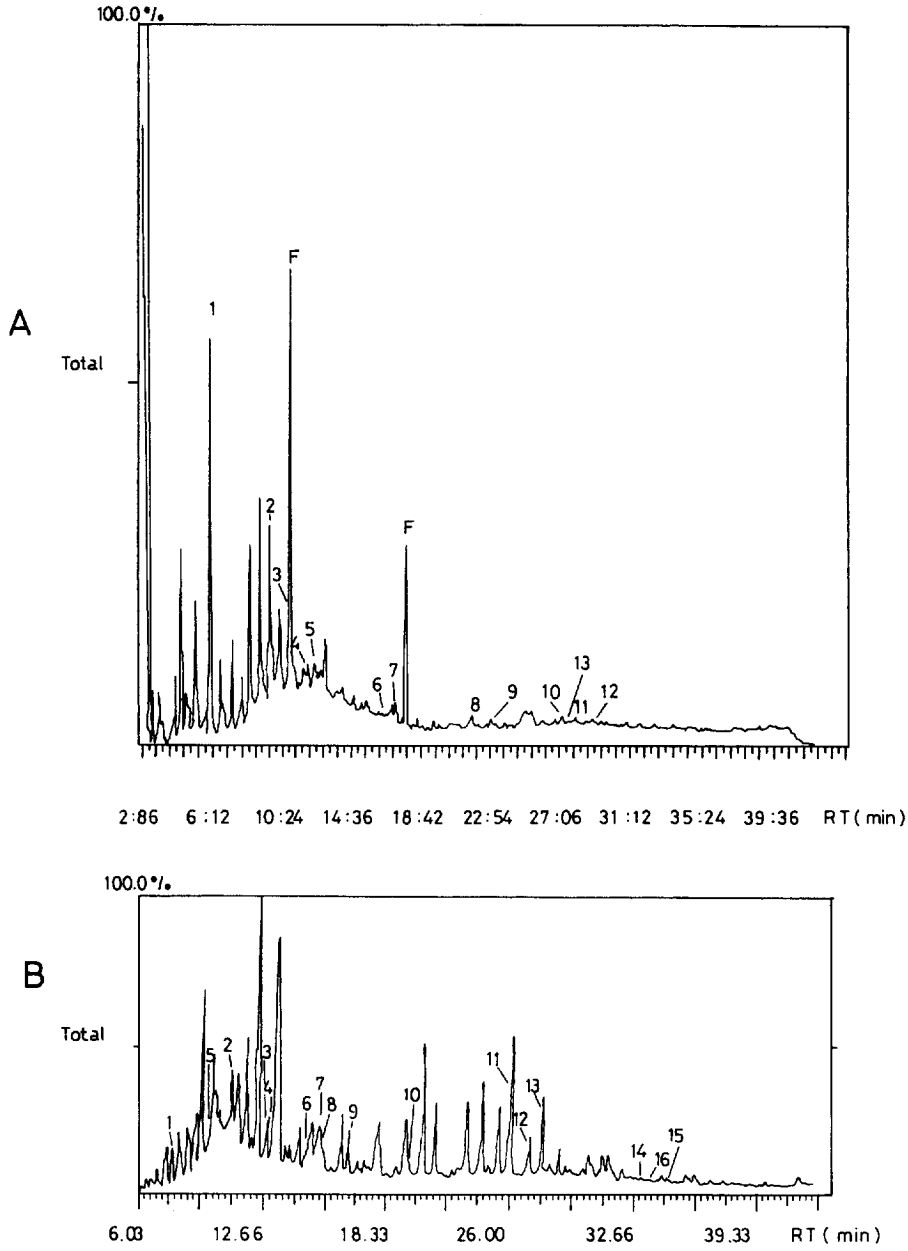

Fig. 2. Total ion chromatograms of PAH fractions isolated by TLC from: (A) Airborne particulate matter: 1=naphthalene; 2=anthracene; 3=fluorene; 4=methylanthracene; 5=fluoranthene; 6=pyrene; 7=2,2-diphenylanthracene; 8=benz[*a*]anthracene; 9=chrysene; 10=benzo[*b*]fluorathene; 11=benzo[*a*]pyrene; 12=indeno[1,2,3-*cd*]pyrene; 13=benzo[*ghi*]perylene. (B) Sewage sludge (waste water treatment plant in Siemianowice): 1=naphthalene; 2=alkylbiphenyl; 3=anthracene; 4=phenanthrene; 5=fluorene; 6=fluoranthene; 7=pyrene; 8=dihydrofluoranthene/dihydropyrene; benzo[*a*]fluorene; 10=benzo[*c*]fluorene; 11=benz[*a*]anthracene; 12=chrysene; 13=dimethylpyrene; 14=benzo[*k*]fluoranthene; 15=benzo[*e*]pyrene; 16=benzo[*a*]pyrene; RT=retention time.

Table 3
R_F of oxygen and nitrogen derivatives of PAHs with different functional groups, separated on silica gel

Compound	R_F	
	DCM–*n*-hexane (1:1, v/v)	DCM–MeOH (10:0.5, v/v)
9-Nitroanthracene	0.46	0.80
2-Nitrofluorene	0.37	0.71
Carbazole	0.33	0.68
Fluoren-9-one	0.24	0.63
1-Aminoanthraquinone	0.11	0.59
Acridine	0.07	0.45

Table 4
R_F values of nitroarenes on a silica gel layer with DCM–*n*-hexane (1:1, v/v) as the mobile phase

Compound	R_F
9-Nitronaphthalene	0.60
1-Nitronaphthalene	0.50
2-Nitrofluorene	0.48
1-Nitropyrene	0.45
1,3-Dinitropyrene	0.34
2,7-Dinitrofluorene	0.26

there is a little problem with a special preparation of the plates.

Moreover, separations using the distributor of the eluent or DS-sandwich chamber and overpressured chamber consumed similar and very low solvents volumes, but the development time was shortest when the mobile phase flow was forced, as in the overpressured chamber [47].

The results of GC–MS analysis have not always confirmed to the results obtained using planar chromatography analysis of investigated environmental samples, but most PAHs and their derivatives have been identified using both methods. An example of the results obtained using GC–MS to identify polar PAHs after TLC semipreparative isolation and the probable structures of the identified polar compounds is shown in Fig. 3. The names of the identified polar compounds, which are present in air particulate matter, as indicated in Ref. [46], and their TLC characteristics are shown in Table 1. The example shown in Fig. 4 is the mass spectrum of the very dangerous, carcinogenic, 1-nitropyrene [42], which was identified in the moderately polar fraction from particulate matter. Additionally, GC–MS lead to the identification of some PAH acidic anhydrides, esters and nitriles [32].

4. Conclusions

Planar chromatography is an inexpensive method, the main advantage of which is its minimum solvent consumption in comparison to other liquid separation techniques (LSC, HPLC). The equipment required for this technique has been improved recently, e.g. through the development of different kinds of chromatographic chambers. Each of the chambers used was produced in Poland and they are still being improved. Forced flow development mode, such as OPLC, has assured constant mobile phase velocity. For this reason, it may be essential in the optimization process of the determination of PAHs, their derivatives and metabolites, e.g. hydroxy-PAHs by HPLC method.

Another advantage of planar chromatography is that standards and samples can be separated simultaneously and, therefore, can be compared directly.

Table 5
R_F values of azaarenes separated on silica gel and on a cellulose layer with DCM–MeOH (10:0.5, v/v) and DMF–water (20:80, v/v) as the mobile phases, respectively

Compound	R_F	
	Silica gel, DCM–MeOH (10:0.5, v/v)	Cellulose DMF–water (20:80, v/v)
Benzo[*h*]quinoline	0.80	0.74
Azafluorene	0.57	0.62
Phenantridine	0.52	0.56
Benzo[*f*]quinoline	0.53	0.55
Acridine	0.56	0.42

Table 6
R_F values of polar PAH derivatives separated on RP-18 stationary phase with acetonitrile–water as the mobile phase

Compound	R_F			
	Acetonitrile–water (1:1, v/v)	Acetonitrile–water (3:2, v/v)	Acetonitrile–water (2:1, v/v)	Acetonitrile–water (4:1, v/v)
1,8-Dihydroxyquinoline	0.06	0.15	0.19	0.37
4-Hydroxyquinoline	0.08	0.17	0.23	0.38
8-Hydroxyquinoline	0.09	nd	0.29	nd
1-Hydroxypyrene	0.09	0.19	0.26	0.46
2-Hydroxymethylanthraquinone	0.22	0.25	nd	0.51
3-Hydroxyphenanthrene	0.25	0.27	0.33	0.63
1-Naphthol	0.40	0.45	0.52	0.72
2-Naphthol	nd	0.47	0.56	0.76
2,3-Dihydroxynaphthalene	0.42	0.55	0.69	nd
1,3-Dihydroxynaphthalene	0.43	0.68	0.76	nd
2,4-Dihydroxyquinoline	0.59	0.69	nd	0.71
2,4-Dinitro-1-naphthol	0.59	0.70	0.76	0.79
2,7-Dihydroxynaphthalene	0.58	0.73	0.83	0.91

nd=not detected.

This could be useful in the analysis of complex environmental samples. The determination of chemical classes of compounds on TLC plates after separation of organic material isolated from air particulate matter and sewage sludge samples shows that these samples are multicomponent mixtures of organic compounds, among which, derivatives of PAHs with different functional groups have been identified. In addition, easy post-chromatographic visualization techniques lead to the identification of many individual PAHs and their derivatives.

Planar chromatography is an analytical method with many complementary possibilities, in combination with other analytical techniques (e.g. GC–MS).

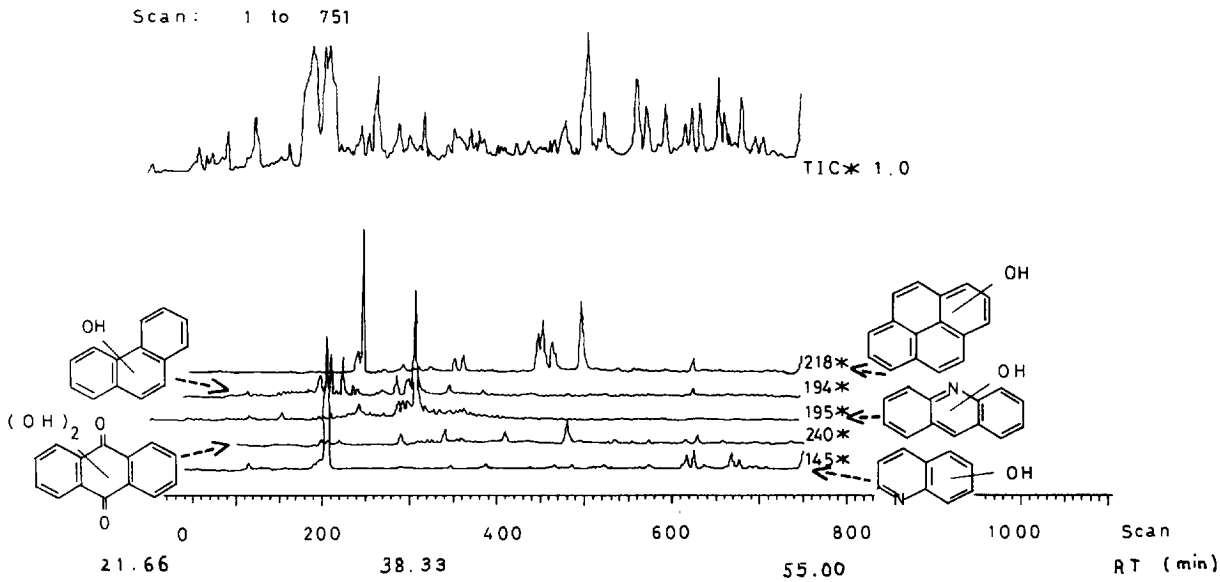

Fig. 3. GC–MS chromatogram of polar PAHs isolated by band TLC.

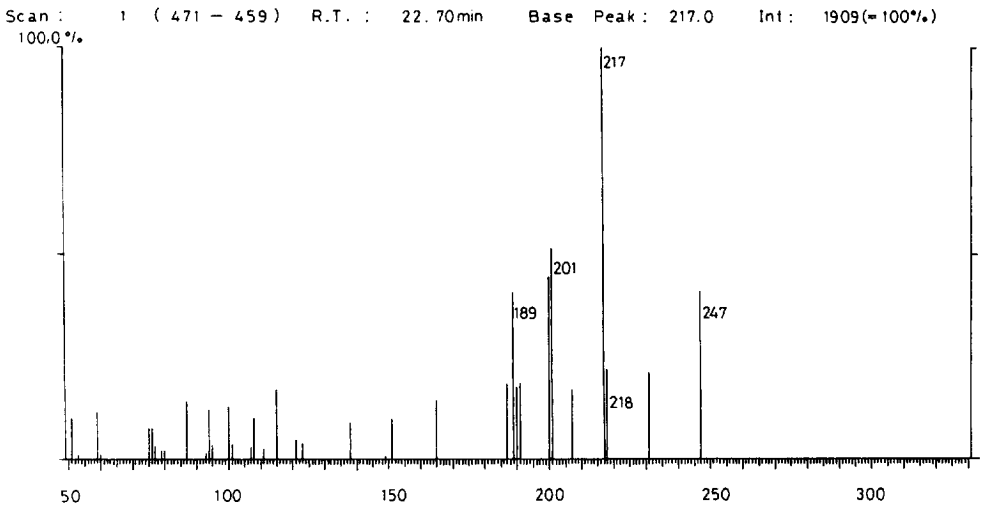

Fig. 4. Mass spectrum of 1-nitropyrene that was identified in airborne particulate matter from Upper Silesia.

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