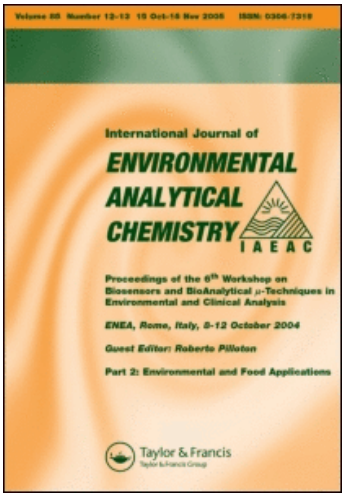

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Identification Of Oxygen Derivatives Of Polycyclic Aromatic Hydrocarbons In Airborne Participate Matter Of Upper Silesia (Poland)

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IDENTIFICATION OF OXYGEN DERIVATIVES OF POLYCYCLIC AROMATIC HYDROCARBONS IN AIRBORNE PARTICULATE MATTER OF UPPER SILESIA (POLAND)

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Organic material extracted from airborne particulate matter collected in various places of Upper Silesia has been separated by column liquid adsorption chromatography. Six fractions of different polarity have been eluted. These fractions have undergone careful spectral analysis, thin layer chromatography and gas chromatography-mass spectrometry. Several PAH oxygen derivatives which have not been found in air from our Region so far were identified. Their biological activity is higher than activity of basic PAH structures. These compounds can be responsible to a high degree for interference in living organism functions in this highly polluted region.

KEY WORDS: Oxy-derivatives of PAH's, separation, GC-MS analysis.

INTRODUCTION

Because of the progressive environment degradation intensive investigations have been carried out on the determination of toxic organic compounds emitted to the atmosphere. The studies conducted on the composition of environmental samples, including separation and identification of polycyclic aromatic hydrocarbons (PAH), have often disregarded the problem of their oxygen and nitrogen derivatives. The source of these compounds are, to a great degree, the hydrocarbon changes in the atmosphere under the influence of light and as a result of interactions with other reactive species, mainly NO_2, O_2, O_3, SO_3.[1-4] Changes of this type lead to the formation of i.a., nitrogen derivatives of PAH's, ketones, aldehydes, acids, esters, anhydrides, phenoles and quinones[5,6]—and thus, of compounds with similar structures to those of hydrocarbon metabolites.[3,4] It results from literature data that these compounds often exhibit a higher biological activity than the parent PAH.[7] The determination and identification of PAH and their heterocyclic derivatives, or derivatives with

functional groups, is difficult because these compounds occur side by side, forming complex multicomponent mixtures.[5]

The aim of the paper was to suggest a simple procedure for the separation of oxygen compounds from airborne particulate matter, as well as for the identification of the dominant components by chromatographic and spectral methods.

MATERIALS AND METHODS

Airborne particulate matter was collected in glass fiber filters by the Katowice Province Sanitary-Epidemiological Station during winter (sample A) and summer (sample B) seasons.

Separation of the heterocyclic compounds fraction

In order to optimize the separation stage of organic material from airborne particulate matter methylene chloride as a suitable extraction solvent was selected. The selection criterion was the result of channel thin-layer chromatography,[8] making possible the estimation of the percentage fractions of PAH and heterocompounds in the mixtures. The extraction of the filter papers was carried out in a Soxhlet apparatus for 24h. After this time the solvent was evaporated in a rotary evaporator.

Fractionation of the oxy-PAH derivatives concentrates

Concentrates of oxygen derivatives were separated from the total extracted organic material by means of adsorption column chromatography on silica gel with 10% water contents.[9] The separation diagram is shown in Figure 1. Elution was carried out successively with: hexane, 25% CH_2Cl_2 in *n*-hexane, methylene chloride and methanol. The testing of the chromatographic column (0,85 × 20 cm) was carried out using a standard mixture of PAH and heterocompounds. The recoveries of pyrene and 2-nitrofluorene were about 87% and 69%, respectively. For each of the eluates (5 ml), UV-VIS spectra were executed in a Specord type spectrometer. On the basis of the diagram of elution volume of the standards,[10] and the spectra in ultraviolet range of the eluates they were combined into fractions in accordance with the enumeration given in Figure 1, evaporated to dryness and then, subjected to GC-MS analysis.

An alternative way of fractionating the extracts i.e., separating PAH from heterocompounds and separating fractions of oxygen compounds of different polarity, was the technique of thin-layer band chromatography.[11] The extract of airborne particulate matter (5 mg in 0.5 ml of methylene chloride) was superimposed in bands on plates with silica gel, while the developing phase was a mixture of methylene chloride with methanol (10:0.5). Separation was carried out in a "sandwich" chamber.[12] The bands of gel differing in their fluorescence colour in the UV light 254 nm lamp and R_f values, were scrubbed off the plate, extracted with methylene chloride, filtered, and then the solvent was evaporated to the volume of about 200 µl. The recoveries of pyrene and 2-nitrofluorene were 63% and 60%

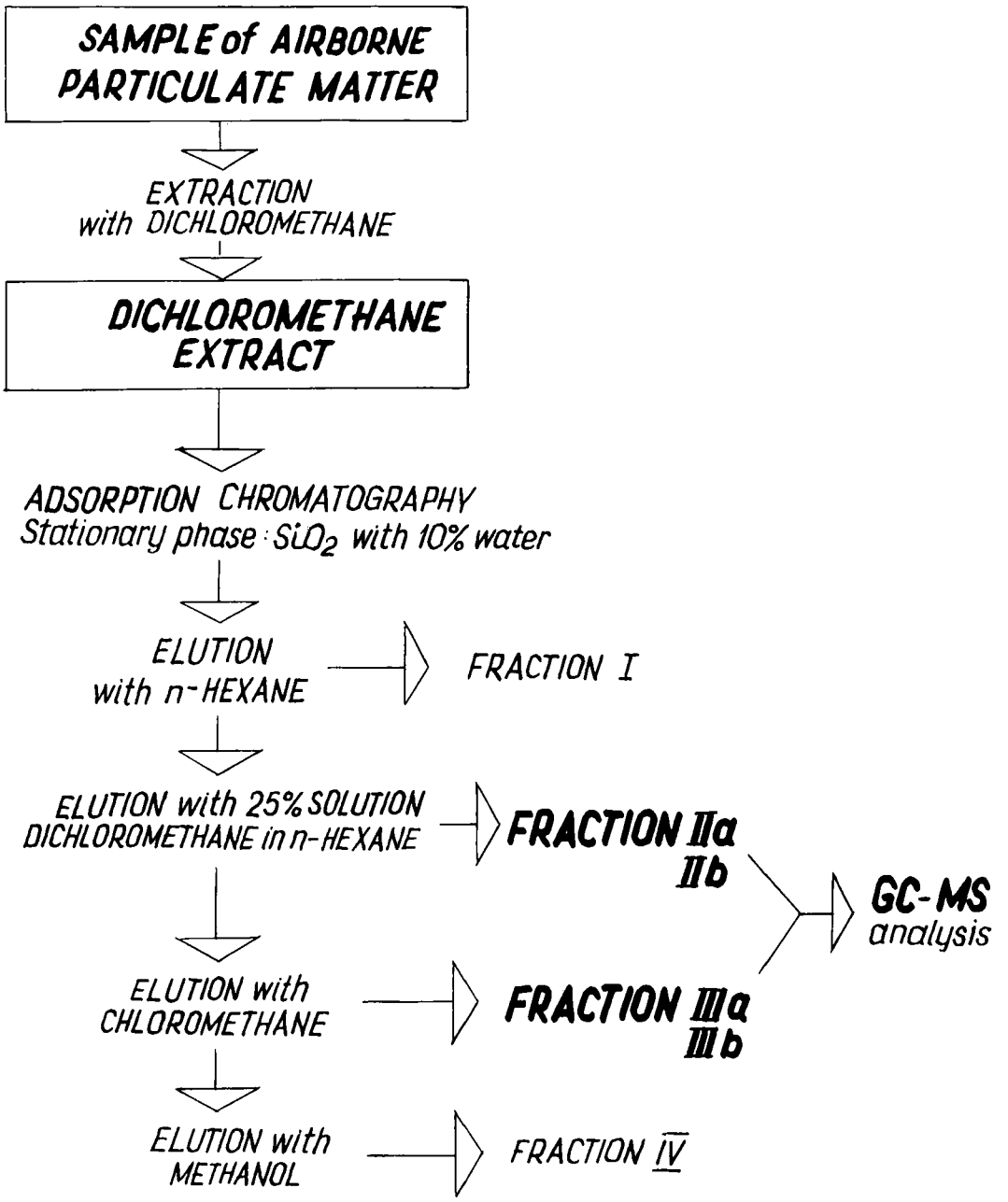

Figure 1 Analytical scheme of PAH's derivatives from air particulate matter extracts.

respectively. The separated fractions were analyzed by GC-MS under identical conditions as the fractions separated by column adsorption chromatography.

Identification of functional groups by thin-layer chromatography

The optimum conditions of TLC separation of the standard substances containing various functional groups were determined, selecting the types of mobile and stationary phases.[10] The tests were made with various developers selecting such substances that give coloured, specific reactions with particular functional groups.[13] Also, limits of detection of particular functional groups were determined.[11]

Fractions from the previous chromatographic separations were superimposed on the plates with silica gel in the form of 0,5 μg/μl methylene chloride solutions. The quantity of the tested solution superimposed once on to the plate was about 10 μg. Chromatograms were developed with a mixture of methylene chloride and methanol (10:0.5) over the distance of 15 cm. After drying, chromatograms were observed at the UV light 254 nm lamp, and sprayed with the developing solutions.

Analysis by gas chromatography—mass spectrometry

The qualitative composition was carried out by GC-MS using a Shimadzu GC-14A gas chromatograph coupled to a Shimadzu QP-2000 mass detector. The conditions of analysis were: helium flow 1,2 cm^3/min, splitter of carrier gas stream 1:50. Temperatures: injector 270° C, interface 200° C, column furnace 300° C. A 25 m/0,20 mm i.d. fused silica capillary column coated with CBP-5 phase (film thickness 0,25 μm) was temperature programmed as follows:

—for non-polar fractions:

60° C (3 min), 25° C/min to 200° C, 4° C/min to 300° C, 300° C (12 min)

—for polar fractions:

60° C (3 mins), 5° C/min to 300° C, 300° C (10 min)

—for moderately polar fraction IIa:

70° C (3 min), 7° C/min to 300° C, 300° C (10 min)

The interpretation of the spectra was carried out on the basis of:

—a comparison of retention times and mass spectra of the standard substances and the components of the samples analyzed.

—a comparison of the mass spectra registered for the components corresponding to the particular peaks in the chromatograms with the mass spectra found in catalogues.[14]

—an analysis of the characteristic fragment ions present in the spectra and their correlation with the literature data on fragmentation.[15–17]

Table 1 Weight percentages of chromatographic fractions.

Extract	*Percentages of fractions*						*Separation losses*
	Fr I	*Fr IIa*	*Fr IIb*	*Fr IIIa*	*Fr IIIb*	*Fr IV*	*%*
Winter season	5	3	4	22	50	10	7
Summer season	6	5	2	5	48	30	8

RESULTS AND DISCUSSION

It has been found that methylene chloride is a suitable solvent for extracting heterocyclic hydrocarbon compounds from airborne particulate matter. This is consistent with the literature data preferring this solvent for extraction, for the purpose of obtaining a material of increased biological activity.[18,19]

The use of adsorption chromatography on silica gel with 10% water content led to a separation of the organic material into six fractions (in accordance with the diagram from Figure 1). Percentage (by weight) of the corresponding fractions are given in Table 1 for both summer and winter samples.

Detailed analysis by TLC (results given in Table 2) and GC-MS (results in Table 3), led to the following data in relation to qualitative composition:

Fraction I—eluted with *n*-hexane, includes hydrocarbons and non-polar oxygen compounds (such as e.g. xanthone). None of the determined functional groups were found in these fractions. GC-MS analysis showed the presence of 16 PAH in a sample from the summer season and 13 PAH in a sample from the winter season. These were hydrocarbons whose presence in the samples of this kind had been found in earlier work.[20] Fraction I did not exceed 6% by weight of the total mass of the extracts.

Fraction IIa—eluted with a mixture of methylene chloride-*n*-hexane (3:1), contained carbazole and its derivatives and nitrocompounds, such as nitroanthracene and nitrofluorene. The above mentioned compounds occurred in the samples from both seasons. The determi-

Table 2 Presence of functional groups in separated fractions from air particulate matter from Upper Silesia Region in the winter (A) and summer (B) sample.

Sample	*A (winter season)*					*B (summer season)*				
Functional group / *Fraction*	*I*	*IIa*	*IIb*	*IIIa*	*IIIb*	*I*	*IIa*	*IIb*	*IIIa*	*IIIb*
-OH	-	-	-	+	+	-	-	-	+	+
-COOH	-	-	-	+	/+/	-	-	-	+	+
C=O	-	+	+	+	-	-	+	+	+	-
$-NO_2-$	-	-	-	-	-	-	/+/	-	/+/	-
-N=	-	-	-	/+/	/+/	-	-	-	+	+

- lack of colouration spots
/+/ weak colouration spots
+ strong colouration spots

Table 3 Qualitative composition of fractions separated by column adsorption chromatography from A and B samples determined by GC-MS method.

Sample A			*Sample B*		
Retention time	*$(M)^+$*	*Compound*	*Retention time*	*$(M)^+$*	*Compound*
		Fraction I (n-hexane)			
		16 PAH			13 PAH
		Fraction IIa (25% CH_2Cl_2 in *n*-hexane)			
20.96	167	carbazole	18.00	144	dioxane derivative
26.71	223	nitroanthracene	20.95	167	carbazole
27.55	237	methylnitroanthracene	24.11	204	carbonyl compound
29.78	227	pirenecarbonitrile	25.05	211	2-nitrofluorene
26.66	223	nitroanthracene			
27.51	237	methylnitroanthracene			
		Fraction IIb (25% CH_2Cl_2 in *n*-hexane)			
27.16	180	9-fluorenone	27.10	180	9-fluorenone
34.40	212	methylnaphthalene carboxylic acid anhydride	43.46	258	carbonyl compound
		Fraction IIIa (CH_2Cl_2)			
13.30	128	benzenedicarbonitrile	21.88	144	1-/2-naphthol
21.63	220	derivative of phenol	28.60	179	acridine
21.68	144	1-/2-naphthol	28.60	180	phenalenone
28.68	138	hydroxybenzoic acid	31.80	196	xanthone
30.81	180	phenalenone	32.18	208	anthraquinone
32.28	208	anthraquinone acid anhydride	33.56	198	naphthalene dicarboxylic
32.36	167	indenepiridine methylphenanthraquinone	34.91	222	methylanthraquinone/
34.41	148	carbonyl compound methylanthraquinone	35.58	222	methylphenanthraquinone/
41.90	230	benzanthrone/ benzophenanthrone			
		Fraction IIIb (CH_2Cl_2)			
14.01	129	quinoline	26.30	179	azaarene
21.63	220	derivative of phenol	28.15	178	carbonyl compound
28.11	178	carbonyl compound	32.30	167	indenepiridine
28.60	179	acridine	34.41	148	carbonyl compound
32.28	208	anthraquinone			

nations of the functional groups by means of TLC also showed the presence of a carboxyl group, however, it was not possible to identify univocally any compound with this substituent. The percentages of this fraction were negligible (up to 5%).

Fraction IIb—eluted as above, with a mixture methylene chloride-*n*-hexane (3:1) should contain, in accordance with TLC results, carbonyl compounds. By GC-MS analysis, 9-fluorenone was found. This compound has also been identified in samples of airborne particulate matter from various places in Europe.[5,6,21] In our investigations, this compound appeared both in winter and summer samples. In Figure 2a its mass spectra is presented. Mass participation of fraction IIb to the whole extract was also not very high (up to 4%).

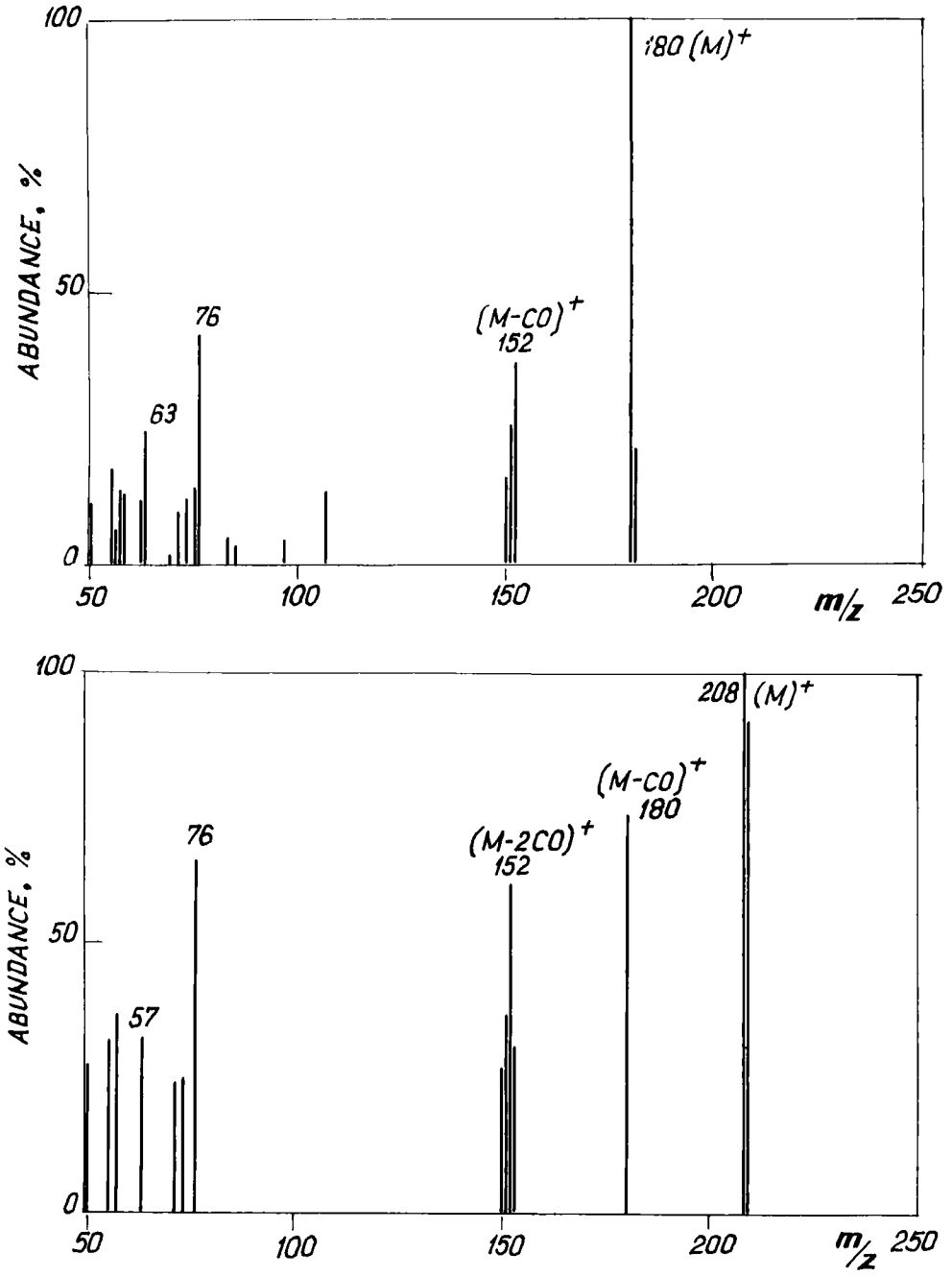

Figure 2 Mass spectra of identified compounds in the investigated samples. a) 9-fluorenone; b) 9,10-anthraquinone.

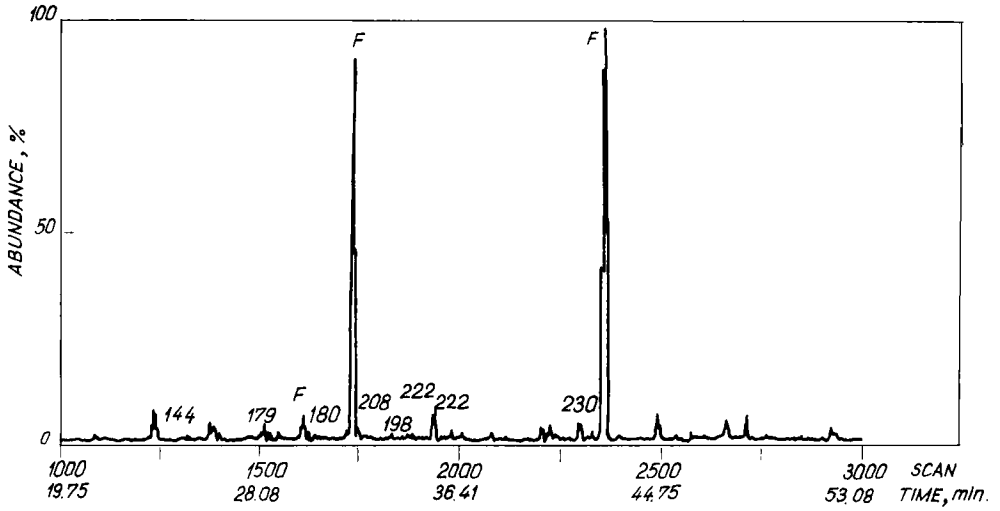

Figure 3 Chromatogram of fraction IIIa from summer sample with indication of peaks of identified compounds. 144-naphthol; 179-acridine; 180-phenalenone; 196-xanthone; 198-naphthalenedicarboxylic acid anhydride; 208-anthraquinone; 222-methylanthraquinone/methylphenanthraquinone; F-esters of 1,2-benzenedicarboxylic acid.

Fractions IIIa—eluted with methylene chloride, contained the highest variety of oxygen compounds. TLC analysis showed the presence of oxygen functional groups: hydroxyl, carbonyl, and also a nitro group, as well as nitrogen in an aromatic ring. Among chemical species in the samples from both seasons 1 and 2-naphthols, 1-phenalenone, anthraquinone were found. In a sample from the summer season also acridine, xanthone, benzanthrone and/or benzophenanthrone, methyl derivatives of anthraquinone and phenanthrenquinone were found.

In Figure 3 is shown the chromatogram of fraction IIIa from the summer season with marked peaks of the compounds identified. Percentages by weight of fraction IIIa were reasonably dependent. The sample from the winter season contained over 22% of this fraction, whereas the one from the summer season represented only 5% by weight.

Fraction IIIb—also eluted with methylene chloride, was a complex mixture of more polar oxygen and nitrogen compounds. Among the compounds found in the samples from both seasons were carbonyl derivatives of unknown structure (M 178 and 148) and indenepiridine, and in a sample from the winter season also quinoline, acridine and anthraquinone. In Figure 2b the mass spectra of anthraquinone is shown. Mass participation of these fractions in the extracts tested were similar in both seasons (about 50% each).

Fraction IV—eluted with methanol was not subjected to detailed identification analysis because of a very strong differentiation of their chemical character.

The separation by band TLC technique, used alternatively for fractionation of the organic material separated from the airborne particulate matter led to the separation of 13 fractions differing in the range of R_f values and fluorescence colour observed at the UV lamp.

Table 4 Compounds identified in one sample separated by TLC.

band number	*Range of R_f values*	*Proposed compound*	*$(M)^+$*
1	0,26–0,28	owing to a lack of standards, fractions	
2	0,35–0,37	1–3 have not been analysed	
3	0,37–0,39		
4	0,39–0,42	benzenedicarbonitrile	128
		quinoline	129
		1-/2-naphthol	144
		naphthofuran imidazo (2,1-a) isoquinoline	168
		acridine	179
5	0,42–0,43	benzenedicarbonitrile 128	
		aza-arene	185
		"	213
6	0,43–0,47	aza-arene	179
7	0,47–0,57	carbonyl compound	256
		aza-arene	199
8	0,57–0,68	benzenedicarbonitrile	128
		9,10- anthraquinone	208
		fluorenecarbonitrile	191
		anthrone	194
9	0,68–0,73	benzenedicarbonitrile	128
		carbazole	167
		9- fluorenone	180
		fluorenecarbonitrile	191
		nitroanthracene	223
10	0,73–0,79	benzenedicarbonitrile	128
		fluorenecarbonitrile	191
		nitrofluorene	211
		nitroanthracene	223
		nitropyrene	247
		carbonyl compound	274
		" "	278
11	0,79–0,83	benzenedicarbonitrile	128
		carbonyl compound	178
12	0,83–0,92	nitronaphthalene	173
		PAH	
13	0,92–0,95	PAH	

Qualitative composition of the fractions determined by GC-MS is given in Table 4. Among the compounds identified, the presence of 15 PAH has been found in the bands with the highest R_f values (bands 12 and 13). In the bands of lower R_f values nitro-PAH derivatives have been identified, carbazole, aromatic ketones, quinones, esters, and nitriles. In the bands closest to the start, naphthols and compounds with nitrogen in an aromatic ring were found. In Figure 4 is presented, a representative chromatogram of a fraction separated from band 4. Bands 1,2,3 with R_f values below 0,39 were not analyzed because of the lack of standards and the lack of characteristic ions in the registered mass spectra.

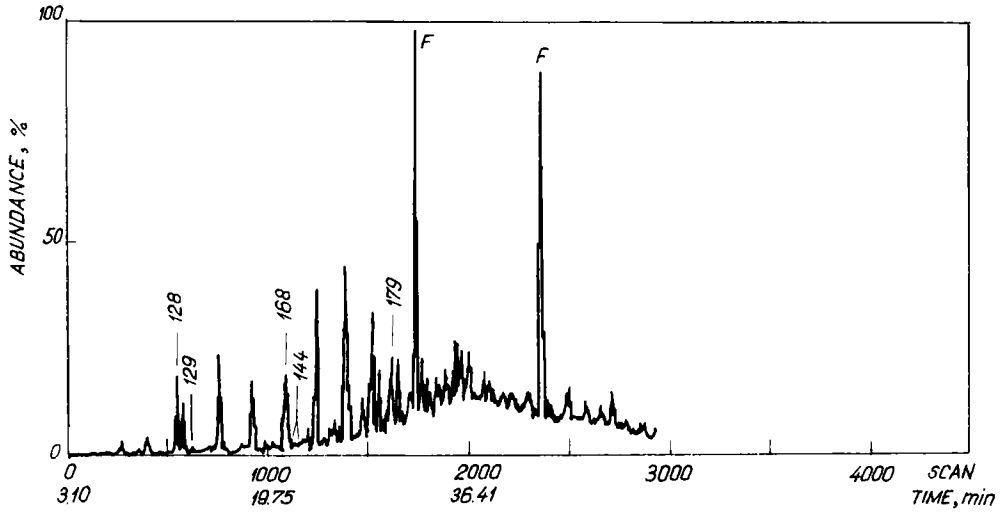

Figure 4 Chromatogram of the selected fraction from TLC band 4 from the Bledów sample, with indication of peaks of identified compounds. 128-benzenedicarbonitrile; 129-quinoline; 144-naphthol; 168-naphthofuran/imidazo (2,1-a)isoquinoline; 179-acridine; F-esters of 1,2-benzenedicarboxylic acid.

CONCLUSION

Methylene chloride may be a suitable solvent for the extraction of airborne particulate matter with the purpose of separating the organic material enriched in heterocompounds. The application of column adsorption chromatography, using silica gel with 10% water contents, made possible a separation of PAH from the oxygen derivatives, and the separation of the latter into fractions of similar chemical character. A convenient and alternative way of preparing PAH concentrates and their oxygen derivatives for analysis by GC-MS is the separation of extracts by the thin-layer chromatography band technique. In both methods recoveries of standard compounds (pyrene and 2-nitrofluorene) were good enough.

Application of GC-MS analysis for the qualitative determination of moderately polar fractions made possible an identification of several individual oxygen derivatives, nitro and azaarenes. These compounds, producing disturbances in the functioning of living organisms, have not been identified so far in airborne particulate matter in our country.

The mass balance of column chromatography fractionation shows that nearly 80% of the organic material extracted with methylene chloride correspond to fractions containing moderately polar oxygen compounds, of which it was possible to identify univocally i.a.: 9-fluorenone, anthraquinone, benzanthrone, 1-phenalenone, methyl derivatives of anthraquinone and phenanthrone, xanthone.

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