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Nitroarenes in Airborne Particulate Organic Extracts Collected from Upper Silesia – Identification and Quantitative Analysis

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The silicic acid column chromatography was employed to isolate the nitroarene fraction from the dichloromethane extracts of airborne particulate matter collected in twelve places in Upper Silesia region. The selective isolation of nitroarenes from PAH and carbazoles was acquired by means of converting nitrocompounds into pentafluoropropylamides.
The use GC–MS system and capillary GC in the analysis of amides made it possible to identify nitrofluorene, nitropyrene, nitrofluoranthene in the most of analysed samples. It also helped to make the quantitative determination of these compounds.

Zastosowano chromatografię kolumnową na kwasie krzemowym do wyodrębnienia frakcji nitroarenów z ekstraktów dichlorometanowych pyłowych zanieczyszczeń powietrza zebranych w dwunastu miejscach w rejonie Górnego Śląska. Przeprowadzenie związków nitrowych w pentafluoropropyloamidy umożliwiło selektywne oddzielenie tych związków od WWA i karbazoli.
Zastosowanie analizy GC–MS oraz kapilarnej GC pozwoliło zidentyfikować nitrofluoren, nitropiren i nitrofluoranten w większości analizowanych próbek. Przeprowadzono również oznaczenia ilościowe tych składników.

The existence of the polycyclic aromatic hydrocarbon nitrogen derivatives in the air creates a great threat to the people's health. It refers, in particular, to the nitroarenes and even their lower molecular mass representatives. These compounds, in distinction to the majority of PAH (polycyclic aromatic hydrocarbons), as direct-acting mutagens not demanding the presence of metabolic activating systems, are distinguished by a specially strong biological activity, *cf.* Rosenkranz *et al.* [1], Tokiwa *et al.* [2], Fu *et al.* [3]. The extensive research upon the toxic influence of nitroarenes on living organisms proved that these compounds also demonstrate a strong genotoxic and cancerogenic activity (Rosenkranz *et al.* [1,4]).

Incomplete fuels' and waste materials' combustion and, above all, the changes of aromatic hydrocarbons under the influence of other reactive pollutants are the processes responsible for the emission of nitroarenes to atmosphere. The research conducted in the simulated atmosphere condition showed that nitroarenes are the product of homogeneous gas reactions of two-, three-, and four-ring PAHs with OH radicals and/or N_2O_5; the presence of NO_x and O_3 is essential to their formation (Arey *et al.* [5]). Smaller quantities of nitrocompounds (especially the derivatives of fluorene, fluoranthene and pyrene) are produced as the result of heterogeneous nitration reactions of the hydrocarbons adsorbed on the particles of dust and soot with NO_2 and in the presence of HNO_3 traces (Arey *et al.* [6]).

The selective isolation of nitroarenes from the complex environmental samples is complicated; the identification and quantitative analyses of these compounds require the use of high quality analytical apparatus and expensive standards.

The isolation of PAH nitro derivatives from the particulate extracts is most often conducted with the aid of high performance liquid chromatography, *cf.* Arey *et al.* [7], Nielsen [8], or the column adsorption chromatography (Jäger [9], Campbell *et al.* [10]).

The qualitative analysis and quantitative determination are made using the GC–MS system (Schuetzle *et al.* [11]) and the capillary column gas chromatography with nitrogen selective detectors (Tejada *et al.* [12]). The investigations of this group of air pollutants have not been made in Poland until now.

EXPERIMENTAL

The airborne particulate samples from non-heating season (June, July, August, 1991) were collected on glass fiber filters using the Staplex sampler. These filters made it possible to retain the airborne particles of 0.01–10 μm in diameter. The average rate of air stream was 80 $m^3 h^{-1}$. The sampling was performed by „Sanepid" (Regional Administration Unit for Control of Epidemic and Hygiene Promotion in Katowice) in the twelve monitoring places of the Upper Silesia region.

Soxhlet extraction of the airborne samples was carried out with methylene chloride for twelve hours as recommended by Bodzek *et al.* [13].

The list of filters from the places of sample collecting, the mass of particulates, the amount of air going through the filter is presented in Table 1. It also shows the contents of organic extract and the fraction enriched with nitroarenes.

Table 1. The list of investigated glass fiber filters from summer season, contents of organic extract and fraction enriched in nitroarenes

Place of sample collection	Air volume*, m^3	Airborne particulate matter, mg	Organic extract		Contents of fraction enriched in nitroarenes in organic extract, % (Fig. 1)
			mg	Content in airborne particulate matter, %	
Katowice	4879	576.3	74.9	13.0	9.3
Bytom	3759	807.8	211.4	26.2	3.4
Chrzanów	3330	278.0	114.1	41.0	3.5
Błędów	4498	192.8	97.5	50.6	2.6
Łaziska	6230	913.6	230.8	25.3	1.7
Mysłowice	6632	622.8	216.5	34.8	4.4
Olkusz	5321	570.0	203.7	35.7	2.3
Rudnik	3355	248.2	192.2	52.1	4.1
Tarnowskie Góry	4544	248.8	127.7	51.3	4.9
Toszek	2307	182.9	63.0	34.4	2.9
Zabrze	4806	471.0	183.5	38.9	4.8
Zebrzydowice	5284	515.8	194.9	37.8	1.8

*The differences of filtered air volume are the results of samplers damage during the collection of airborne particulate samples.

Chemicals

Silicic acid (Mallinckrodt, 100 mesh) was used as the stationary phase in chromatographic separations. Analytical grade organic solvents (dichloromethane, *n*-hexane, benzene) were used for extraction and as the eluents. Copper(II) chloride, $NaBH_4$ (POCh, anal. grade), triethylamine and pentafluoropropionic anhydride (PFPA) (Aldrich, 99 %) were used to obtain the fluoroamide derivatives.

2-Nitronaphthalene, 2-nitrofluorene, 9-nitroanthracene, 1-nitropyrene, 3-nitrofluoranthene, 6-nitrochrysene (Aldrich Chem. Co., Sigma Chem. Co. and Fluka AG) were used as the standards.

Analytical system

Separation of nitroarene fraction

The analytical procedure of separating nitroarene fraction from the dichloromethane extracts has been presented in Fig. 1.

Chromatographic separations were carried out in the glass columns (30 cm×1.8 cm) packed with silicic acid containing 5 % of water. More details about the stationary phase preparation and chromatographic separation have been presented in earlier work by Warzecha *et al.* [14].

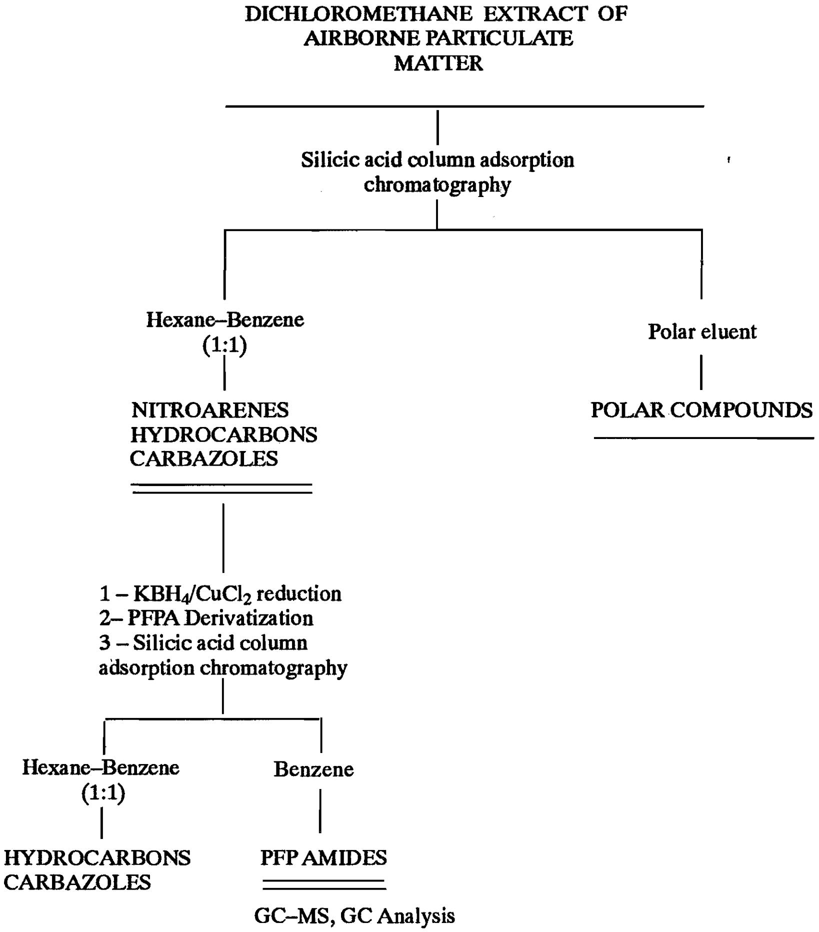

Figure 1. Procedure for the isolation of nitroarenes from particulate extracts: PFPA – pentafluoropropionic anhydride, PFP Amides – pentafluoropropylamide derivatives

Chromatographic separations were carried out in the glass columns (30 cm×1.8 cm) packed with silicic acid containing 5 % of water. More details about the stationary phase preparation and chromatographic separation have been presented in earlier work by Warzecha *et al.* [14].

Preparation of amides

The reduction of nitroarenes to aromatic amines, as well as acylation of amines to fluoroamide derivatives was conducted according to procedure used by Campbell *et al.* [10]. The fraction of fluoroamide derivatives was separated on silicic acid using the following eluents: a mixture of *n*-hexane–benzene (1:1) for the elution of the fraction containing polycyclic aromatic hydrocarbons and carbazoles; benzene for the elution of amide fraction. The way of obtaining of the fluoroamide derivatives is shown in Fig. 2.

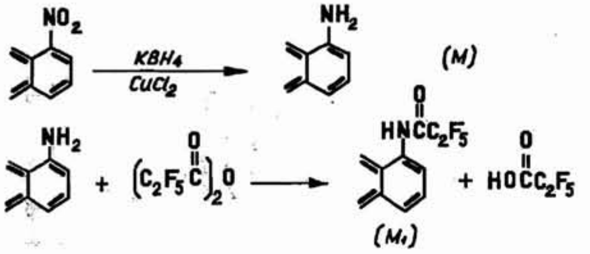

Figure 2. Derivatization of nitroarenes to pentafluoropropylamides

Gas chromatography–mass spectrometry (GC–MS) and gas chromatography (GC)

A Shimadzu QP 2000 GC–MS system for identification of individual compounds has been used. A gas chromatograph was equipped with a fused silica capillary column (25 m× 0.20 mm i.d.) coated with CBP-5 stationary phase (0.25 µm).

The following chromatographic conditions were set: linearly programmed oven temperature from 60°C (2 min), to 280°C (4°C min^{-1}), 280°C (5 min); injector temperature 270°C; interface temperature 280°C. The carrier gas was helium, the column flow: 1.2 ml min^{-1} and split 1/50. A Shimadzu quadrupole mass spectrometer was operated in the electron impact mode (70 eV electron energy, 300°C).

The identification analysis has been made on the ground of comparison of the retention times and mass spectra of standards with the same parameters of compound from investigated samples.

Gas chromatographic work was carried out on a Shimadzu Model A-14 gas chromatograph with FID detector and a splitless injection system. The fused silica capillary column (30 m× 0.25 m, 0.25 µm film thickness) coated with the stationary phase SE 54 (Supelco, Chemin du Lavasson, Switzerland) was used for the quantitative analysis. Samples of 1 µl (benzene solutions) were injected into the column. The GC conditions were the following: injection temperature 280°C, FID detector temperature 300°C; carrier gas helium, flow rate 2 ml min^{-1}; temperature program: 60°C (2 min), 60–150°C (20°C min^{-1}), 150–300°C (4°C min^{-1}), 300°C (10 min).

Calibration graphs were obtained by injection of synthetized fluoroamide derivatives of nitroarene model compounds together with the internal standard (β,β′-binaphthyl). The quantities of amides have been calculated by the internal standard method (*cf.* Katz [15]). Then the quantities of nitroarenes have been calculated on the basis of reaction course presented in Fig. 2. The reduction and acylation yield of nitroarenes was assumed to be 80 % (Warzecha *et al.* [14]).

RESULTS AND DISCUSSION

Separation of nitroarenes and identification analysis

Both the present research work and previous studies by Warzecha *et al.* [14] aimed at the elaboration of the method efficient enough to separate the nitroarenes from other compounds included in dichloromethane extracts. These studies proved that the silicic acid column chromatography allows to isolate nitroarenes at losses not exceeding 5 % by weight. However, considering the similar polarity, the nitroarene fraction isolated this way includes also moderately polar PAH and carbazoles (Fig. 1).

The reduction of nitroarenes to amines, then their acylation with pentafluoropropionic anhydride and the isolation of the fraction of acquired amides one more time on silicic acid column leads to the separation of nitroarenes from PAH and carbazoles. The degree of reaction of standard nitroarenes to amide derivatives was on the average 80 % (Table 2).

Table 2. GC–MS analysis of fluoroamide derivatives of nitroarene standards and yield of reduction/acylation reactions

Standard	Molecular mass	Yield of reduction/acylation reactions %	Molecular ion $(N{-}H{+}C_3F_5O)^+(M_1)^+$	Fragment ions $(M_1{-}C_3F_5)^+$ $(M_1{-}147)^+$	Fragment ions $(M_1{-}C_3F_5O{-}HCN)^+$ $(M_1{-}147{-}27)^+$	Retention time, min
2-Nitronaphthalene	173	80	289	142	115	25.50
2-Nitrofluorene	211	78	327	180	153	36.40
9-Nitroanthracene	223	82	339	192	165	39.15
3-Nitrofluoranthene	247	81	363	216	189	45.26
1-Nitropyrene	247	79	363	216	189	46.51
6-Nitrochryzene	273	80	389	242	215	53.98

M – molecular mass of amine obtained from nitroarene reduction.
M_1 – mass of amide molecular ion obtained from amine acylation using PFPA.
C_3F_5O – pentafluoropropyl (PFP).

Table 3. GC–MS analysis of amides fraction separated from summer extracts

Pentafluoropropylamide derivatives of	Retention time*, min	*m/z* (relative abundance)* $(M + 146^+)(M_1)^+$	*m/z* (relative abundance)* $(M_1{-}147)^+$	*m/z* (relative abundance)* $(M_1{-}147{-}27)^+$	Name of region where nitroarene was identified
2-Nitrofluorene	36.43	327 (100)	180 (53.7)	153 (39.2)	Błędów Chrzanów Tarnowskie Góry Toszek Zebrzydowice
1-Nitropyrene and/or 3-nitrofluoranthene	46.55	363 (100)	216 (97.9)	189 (52.5)	Błędów, Zebrzydowice Zabrze Chrzanów Mysłowice Olkusz Toszek

M – molecular mass of amine.
M_1 – mass of amide molecular ion.
*Some data for the sample collected in Błędów were presented.

Employing the GC–MS system to the identification of nitro compounds by means of the analysis of their amide derivatives allowed to identify nitroarenes explicitly thanks to the presence of very characteristic peaks corresponding to molecular and fragment ions in the mass spectra of the amides (Table 3). Total ion chromatogram and pentafluoroamide mass spectra of adequate nitro compounds isolated from the particulate extract from Błędów are shown in Figs. 3 and 4.

The presence of biologically active 2-nitrofluorene and 1-nitropyrene and/or 3-nitrofluoranthene in the samples collected in Błędów, Zabrze, Chrzanów, Mysłowice, Olkusz, Tarnowskie Góry, Toszek and Zebrzydowice was discovered. These lower molecular mass derivatives of PAH prevail among the nitroarenes detected by other authors in airborne particulate extracts from urban regions (*cf.* Nielsen [8], Arey *et al.* [16], Ramdahl *et al.* [17], Pitts [18]).

It could not be excluded that the other low molecular nitroarenes (nitronaphthalene, nitroanthracene) are present in the air of the Silesia region. However their concentrations in investigated samples were probably too low to detect them in the system GC–MS which has been used.

Quantitative analysis

The identified nitroarenes' content in the analysed extracts is shown in Table 4. The chromatographic conditions employed did not lead to the full separation of 1-nitropyrene from 3-nitrofluoranthene; thus, the total content of both the nitro compounds was determined.

Table 4. Concentration of nitroarenes identified in particulate samples from summer season

Sampling place / Amount 10^{-2} ng in m^3 of air	Błędów	Zabrze	Chrzanów	Mysłowice	Olkusz	Tarnowskie Góry
2-Nitrofluorene	8.5	–	28.8	–	–	3.2
1-Nitropyrene and/or 3-nitrofluoranthene	12.9	34.9	15.6	10.5	10.5	–

Typical gas chromatogram of nitroarene fluoroamide derivatives separated from organic extract is shown in Fig. 5.

Data presented in literature show that the content of nitroarenes in the atmosphere of urban regions' does not exceed, as a rule, 1 ng m^{-3} of air (*cf.* Nielsen [8], Arey *et al.* [16]). The quantities of nitroarenes determined in the airborne particulate matter extracts from some places in the Upper Silesia region also do not exceed this value (Table 4). One may suspect in the atmosphere of the industrially developed region, *e.g.* such as the Upper Silesia, not only high content of PAH, but also higher content of toxic nitroarenes as well (Bodzek *et al.* [19]).

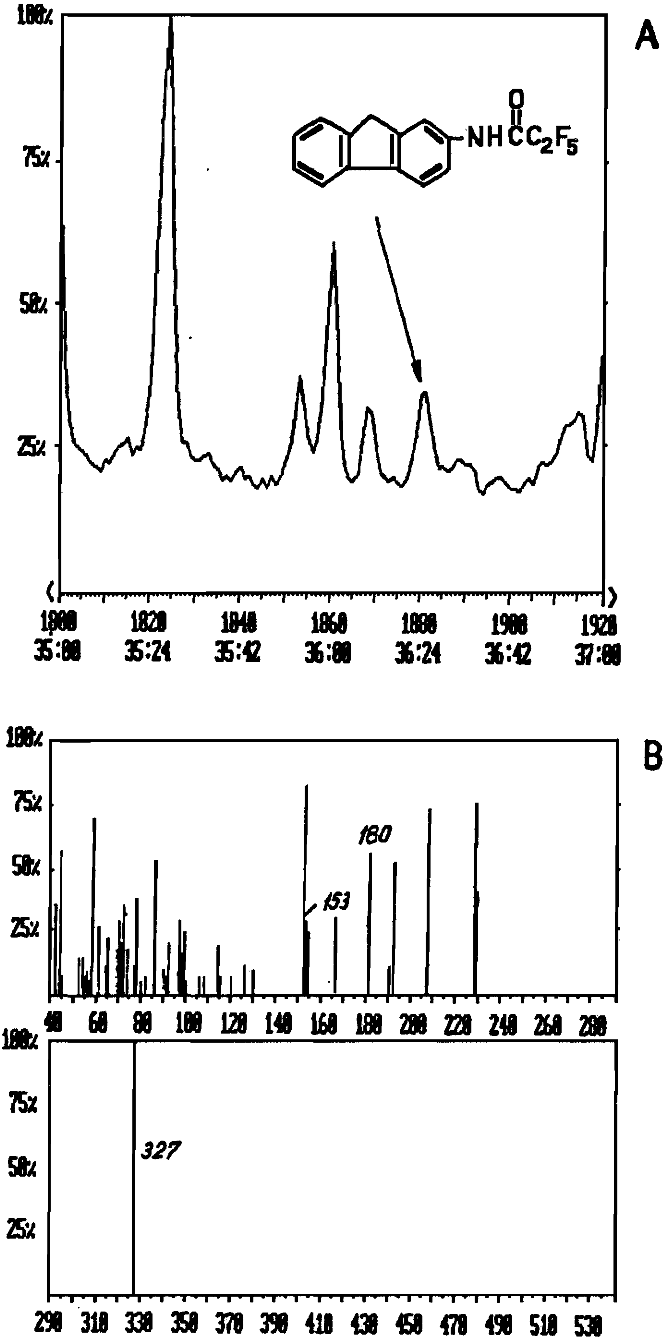

Figure 3. Total ion chromatogram (A) and mass spectrum (B) of 2-nitrofluorene fluoroamide derivative isolated from sample collected in Błędów

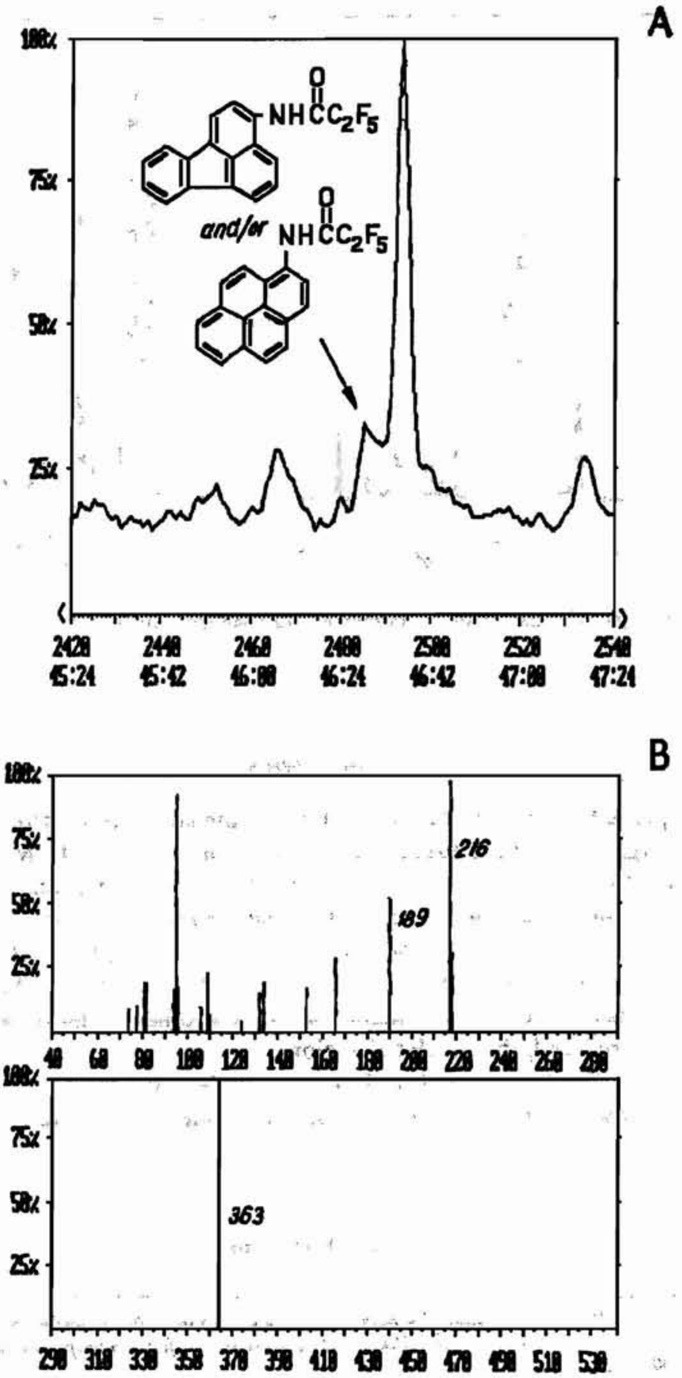

Figure 4. Total ion chromatogram (A) and mass spectrum (B) of 1-nitropyrene and/or 3-nitrofluoranthene fluoroamide derivative isolated from sample collected in Błędów

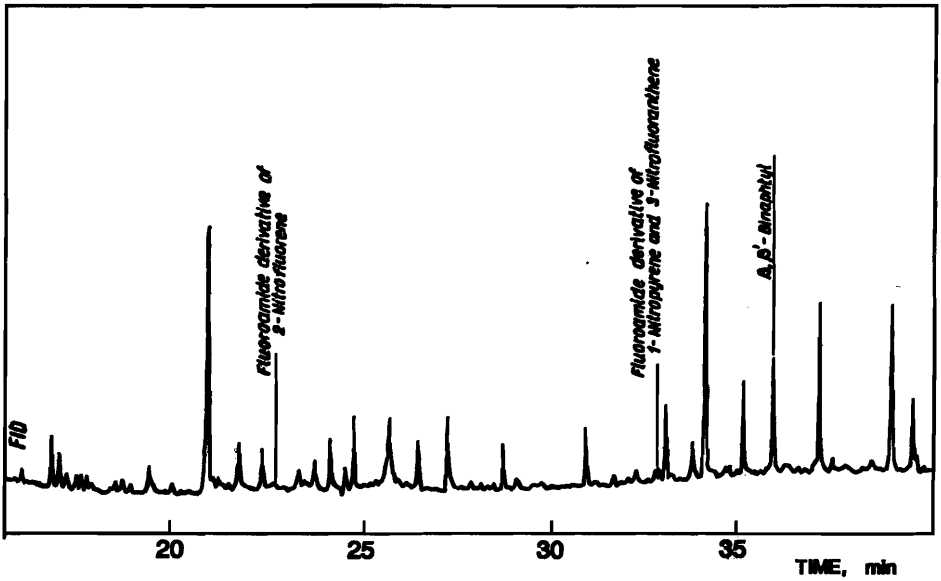

Figure 5. Typical gas chromatogram of nitroarene fluoroamide derivatives fraction separated from organic extract

Conclusions

The employed procedure comprising the column adsorption chromatography, the methods of organic chemistry (reduction, acylation) and the GC–MS, GC analysis made possible the selective isolation, identification and estimation of nitrofluorene, nitropyrene and nitrofluoranthene content in the Upper Silesia air.

The presence of these compounds that show strong mutagenic activity may catch one's attention. These compounds unlike the basic hydrocarbons (fluorene, pyrene, fluoranthene) may be decisive of human beings and other living organisms health's in highly industrial and, thus polluted regions.

The method which was employed in these studies may be used for the analysis of the samples taken from other elements of environment *i.e.* not necessarily airborne particulates.

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REFERENCES

1. Rosenkranz H. S. and Mermelstein R., *Mutat. Res.*, **114**, 217 (1983).
2. Tokiwa H. and Ohnishi Y., *CRC. Crit. Rev. Toxicol.*, **17**, 23 (1986).
3. Fu P. P., Chou M. W. and Beland F. A., *Effects of nitro substitution on the in vitro metabolic activation of polycyclic aromatic hydrocarbons, IN: Polycyclic aromatic hydrocarbons carcinogenesis: structure–activity relation ships*, Vol. II, Yang S. K., and Silverman B. D., Eds., CRC Press, Boca Raton, 1988, pp. 37–65.
4. Rosenkranz H. S. and Klopman G., *Mutat. Res.*, **199**, 95 (1988).
5. Arey J., Atkinson R., Aschmann S. M. and Schuetzle D., *Polyc. Arom. Comp.*, **1**, 33 (1990).
6. Arey J., Zielińska B., Atkinson R. and Winer A. M., *Environ. Sci. Technol.*, **22**, 457 (1988).
7. Arey J., Atkinson R., Zielińska B. and Mc Elroy P. A., *Environ. Sci. Technol.*, **23**, 321 (1989).
8. Nielsen T., *Anal. Chem.*, **55**, 286 (1983).
9. Jäger J., *J. Chromatogr.*, **152**, 575 (1978).
10. Campbell R. M. and Lee M. L., *Anal. Chem.*, **56**, 1026 (1984).
11. Schuetzle D. and Hampton C. V., *Gas chromatography/mass spectrometry in air pollution studies, IN: Mass spectrometry in environmental sciences*, Karasek F. W., Hutzinger O. and Safe S., Eds., Plenum Publ. Corp., 1984, pp. 159–193.
12. Tejada S. B., Zweidinger R. B. and Sigsby J. E., *Anal. Chem.*, **58**, 1827 (1986).
13. Bodzek D., Luks-Betlej K., Warzecha L. and Tyrpień K., *Chem. Anal.*, **35**, 693 (1990).
14. Warzecha L., Janoszka B. and Bodzek D., *Int. J. of Environ. Anal. Chem.* (1992) (in press).
15. Katz E., *(Ed.) Quantitative analysis using chromatographic techniques*, Perkin Elmer, USA 1988.
16. Arey J., Zielińska B., Atkinson R. and Winer A. M., *Atmos. Environ.*, **21**, 1437 (1987).
17. Ramdahl T., Zielińska B., Arey J., Atkinson R., Winer A. M. and Pitts J. N. Jr., *Nature*, **321**, 425 (1986).
18. Pitts J. N., Jr., *Atmos. Environ.*, **21**, 2531 (1987).
19. Bodzek D., Luks-Betlej K. and Warzecha L., *Atmos. Environ.* (1992) (in press).

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