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Effect of solvent polarity on the determination of oxo- and nitro-polycyclic aromatic hydrocarbons using capillary gas chromatography with splitless injection

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ABSTRACT

The importance of solvent polarity in the injection conditions for the determination of oxo- and nitro-polyclic aromatic hydrocarbons in capillary gas chromatography was investigated. When standard solutions of these compounds were analysed by high-resolution gas chromatography using splitless injection, peak splitting with two or more maxima was obtained when methanol and acetonitrile were used, in the preceding steps no peak splitting was observed with dichloromethane or acetone. This splitting can be eliminated using a retention gap or by increasing the initial column temperature. Response factors for all these compounds at different initial temperatures and different solvents were studied. The best results were obtained using acetonitrile, an initial temperature of 60°C and a retention gap. Reproducibility gave a relative standard deviation from 1.8 to 6.7 when measured by peak area; detection limits (signal-to-noise ratio of 2:1) using a flame ionization detector ranged from 129 pg for 2-methyl-1-nitronaphthalene to 1.5 ng for 9,10-phenanthroquinone.

INTRODUCTION

Increasing attention is being devoted to the determination of polycyclic aromatic hydrocarbons (PAHs) containing heteroatoms or polar functional groups in the molecule (PAH derivatives) in environmental samples, mainly of atmospheric aerosols and diesel exhausts [1–3], as it has been shown that these species act as direct mutagens in the Ames test [4]. General concern about the possible health effects of PAH derivatives, particularly those containing nitro, hydroxy and carbonyl groups, has led to a need for the specific determinations of these compounds.

Owing to the extremely complex composition, dilution and variability of the soluble organic fraction (SOF) extracted from particles, the extract must be subjected to an efficient clean-up and enrichment procedure to provide samples suitable for gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS) or high-performance liquid chromatography (HPLC). The published fractionation methods, some of which are designed for specific samples, are usually carried out using low- and high-resolution liquid chromatography and solvents of various polarities. The polar fractions which contain the PAH derivatives are eluted with solvents such as dichloromethane, acetone, methanol or acetonitrile [5–7]; direct injection of these fractions in high-resolution gas chromatography (HRGC) without changing the solvent may be useful. Moreover, on-line coupling of HPLC and GC using polar solvents might be advisable as about 80% of HPLC analyses are performed by a reversed-phase mechanism; the use of polar solvents and water in coupling has been studied [8–10].

The solvent is one of the most important factors in improving sensitivity in HRGC. The solvent effect was first described by Grob and Grob [11,12] for the determination of hydrocarbons by splitless injection; it is now considered to be an important mechanism for some of the most frequently used injection modes, such as splitless and on-column injection. Cold-trapping and the solvent effect are two

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factors that lead to a recondensation of the sample components at the top of the chromatographic column [12–14]. The solvent effect requires conditions which recondense a large portion of the solvent in the first part of the column. Grob and Grob [15] have emphasized that this is controlled by four independent factors: initial column temperature, volatility of the solvent, amount of solvent and injection time. By selecting these variables correctly, the effect can be optimized for any combination of sample and column.

Although the solvent effect in splitless and on-column injection in capillary gas chromatography has been discussed, showing that band broadening or splitting appear to be the result of increasing solvent polarity [16,17], few quantitative results on the response factors caused by solvents of different polarities have been published. Lee *et al.* [18] and Brindle and Li [19] have studied the effect of the solvent in the analysis of PAHs by HRGC. These workers indicate that different solvents can drastically change the response of the analyte under the same chromatographic conditions, and that the best results are obtained using non-polar solvents such as isooctane or xylenes.

For more polar compounds few data have been published, but, generally, these compounds and polar solvents lead to band broadening and splitting [20,21]. In this work we studied the effect of the solvent in the determination of oxo- and nitro-PAHs by capillary GC. The relationship between the optimum initial temperature of the column and the boiling point of the solvent, the use of retention gaps and other factors that might effect the performance in the GC system are reported.

EXPERIMENTAL

Working conditions

A DANI (Milan, Italy) Model 3800 HRGC gas chromatograph equipped with a flame ionization detector was used for all determinations. DB-5 (J&W Scientific, Rancho Cordova, CA, USA) 30 m × 0.25 mm I.D. (film thickness 0.25 μm) fused-silica capillary columns with a retention gap 2.5 m × 0.25 mm I.D., deactivated) were used with splitless injection. The operating temperatures were: injection port, 250°C; detector, 300°C; and temperature programme as shown in Table I. Flow-rates were as follows: hydrogen, 30 ml min^{-1}; air, 280 ml min^{-1}; detector makeup gas (nitrogen), 30 ml min^{-1}. The linear velocity of the carrier gas (helium) was 30 cm s^{-1} at 60°C. The splitless valve was on for 45 s; 1 μl of each sample was injected onto the column by hot needle injection. A Merck–Hitachi (Tokyo, Japan) Model D-2000 integrator was used to measure peak areas and heigths for the subsequent calculation of response factors.

Materials

The compounds studied are listed in Table II and were provided by Carlo Erba (Milan, Italy), EGA Chemie (Steinheim, Germany), Fluka (Buchs, Switzerland), Jessen-Chimica (Geel, Belgium) or Merck (Darmstadt, Germany). Acetone and dichloromethane (Panreac, Barcelona, Spain) were distilled and acetonitrile and methanol were of HPLC grade (Merck).

A stock solution of the compounds studied was prepared containing 1 mg ml^{-1} of each in acetonitrile. The 20 μg ml^{-1} solutions in the various sol-

TABLE I

TEMPERATURE PROGRAMME

Program No.	Initial temperature (°C)	Initial time (min)	Rate of increase (°C/min)	Final temperature (°C)	Final time (min)
1	40	2	6	260	15
2	60	2	5	260	15
3	80	4	4	260	15
4	100	4	4	260	15

TABLE II

RETENTION TEMPERATURES OF OXO- AND NITRO-PAHs IN ACETONITRILE USING PROGRAMME NO. 2

Peak No.	Compound	Symbol	Retention temperature (°C)
1	1,4-Naphthoquinone	1,4-NQ	145
2	5-Nitroindan	5-NI	160
3	2-Methyl-1-nitronaphthalene	2-M-1-NN	173
4	2-Nitronaphthalene	2-NN	174
5	9-Fluorenone	9-Fl	185
6	Acenaphthenequinone	ACQ	199
7	9-Nitroanthracene	9-NA	226
8	9,10-Phenanthroquinone	9,10-PQ	229
9	Benzanthrone	BZ	257
10	1-Nitropyrene	1-NP	268

vents were prepared by dilution of appropriate volumes of this stock solution. Pyrene was used as an internal standard.

RESULTS AND DISCUSSION

Effect of solvent and temperature

The solvents studied were dichloromethane, acetone, methanol and acetonitrile, which all have different boiling points and polarities and are generally used in the clean-up of the medium and high polar fractions of aerosol samples in which the PAH derivatives are found.

Peak splitting was observed when methanol and acetonitrile were used as sample solvents. Figs. 1 and 2 show the sample gas chromatograms for these two solvents obtained with the DB-5 column at different initial column temperatures. For the two sol-

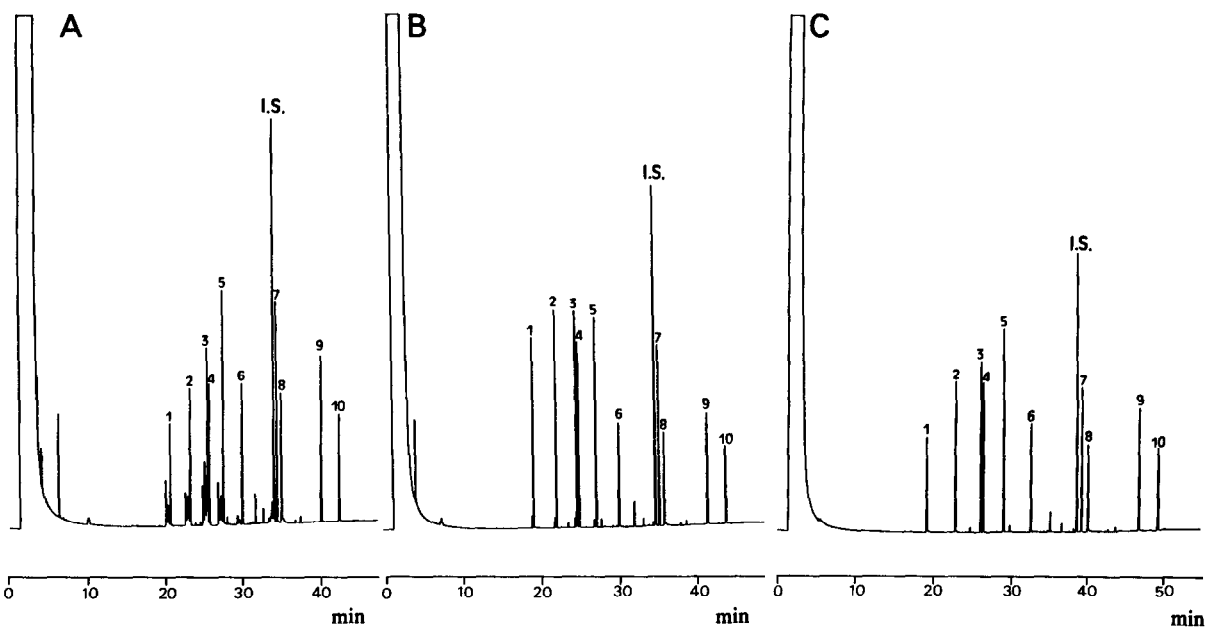

Fig. 1. Chromatograms of the oxo-PAH and nitro-PAH, 1 μl of 20 μg ml^{-1} in methanol. Column DB-5. Initial temperature: (A) 40°C, (B) 60°C and (C) 80°C. For peak identification, see Table II.

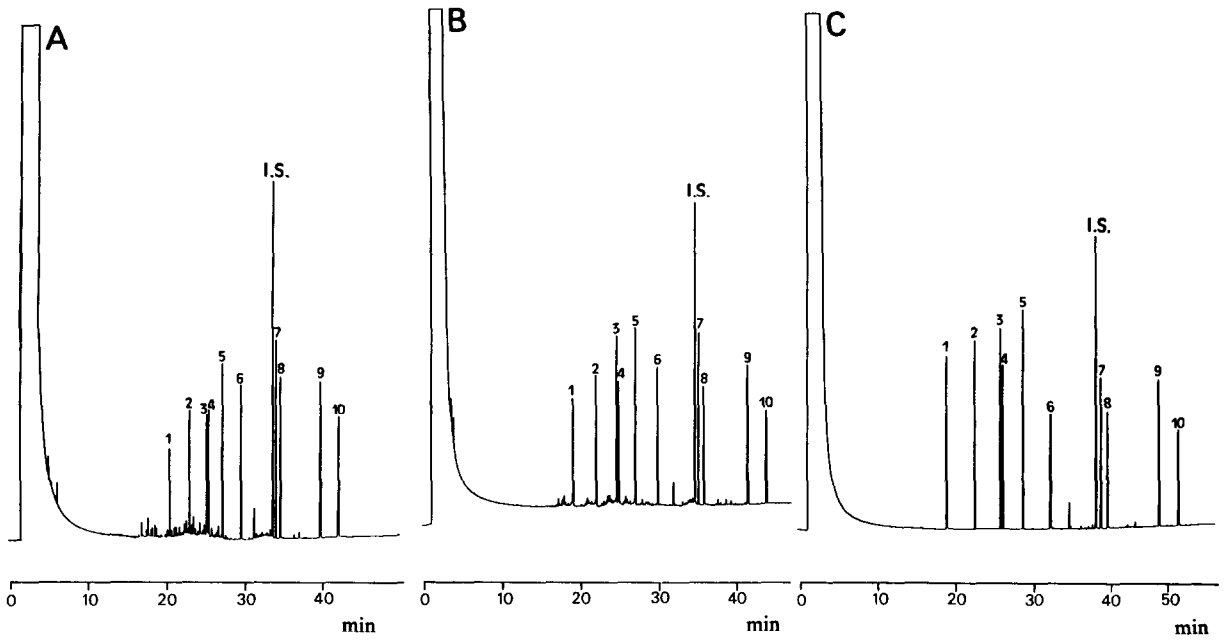

Fig. 2. Chromatograms of the oxo-PAH and nitro-PAH, 1 μl of 20 μg ml^{-1} in acetonitrile. Column DB-5. Initial temperature: (A) 40°C, (B) 60°C and (C) 80°C. For peak identification, see Table II.

vents, splitting was observed at two temperatures (60 and 40°C). An explanation for peak splitting observed in splitless and on-column injection has been provided in detail by Grob [16,22]. Grob points out than when two maxima are observed in a split peak, these correspond to an uneven distribution of the liquid sample injected into the flooded section of the column. Increasing the temperature tends to keep the solutes within the rear portion of the flooded zone, and at a high enough temperature, this focusing effect essentially eliminates peak splitting. These phenomena are illustrated in Figs. 1 and 2. When the initial column temperature is raised, the length of the flooding zone is reduced, providing additional focusing, and the splitting disappears. In this instance several maxima are observed in a split peak for both solvents, probably due to the difference between the polarity of the solvent and the stationary phase. The polar solvent did not wet the non-polar stationary phase and the sample was sprayed into the column in the form of micro-droplets. This leads to a pattern of multiple peak distortion, which can be eliminated if the column inlet has a much lower retention power than the coated column. In practice this means that an uncoated inlet section, a "retention gap" [17,23] should be used. Fig. 3 shows the chromatograms obtained for the two solvents at 40°C using a "retention gap", in which no splitting is observed.

Column temperature and temperature programming are two factors affecting the resolution and sensitivity. In our study the temperature programmes were optimized for each initial temperature to obtain a high enough resolution, and were the same for the four solvents studied. To determine the effect of the initial temperature on the peak height and area, the samples were injected at different initial temperatures from 40 to 100°C and the chromatograms were obtained. Peak heights and peak areas were determined and the relative peak height and peak area of each compound were calculated based on the values obtained at 40°C. These relative peak heights for each compound, temperature and solvent are shown in Table III, in which low values are observed for most of the compounds and solvents at high temperatures. The changes in areas are smaller owing to a simultaneous increase in peak width, so a low initial temperature near to or below the boiling point of the solvent seems to give the best results, as has been pointed out by

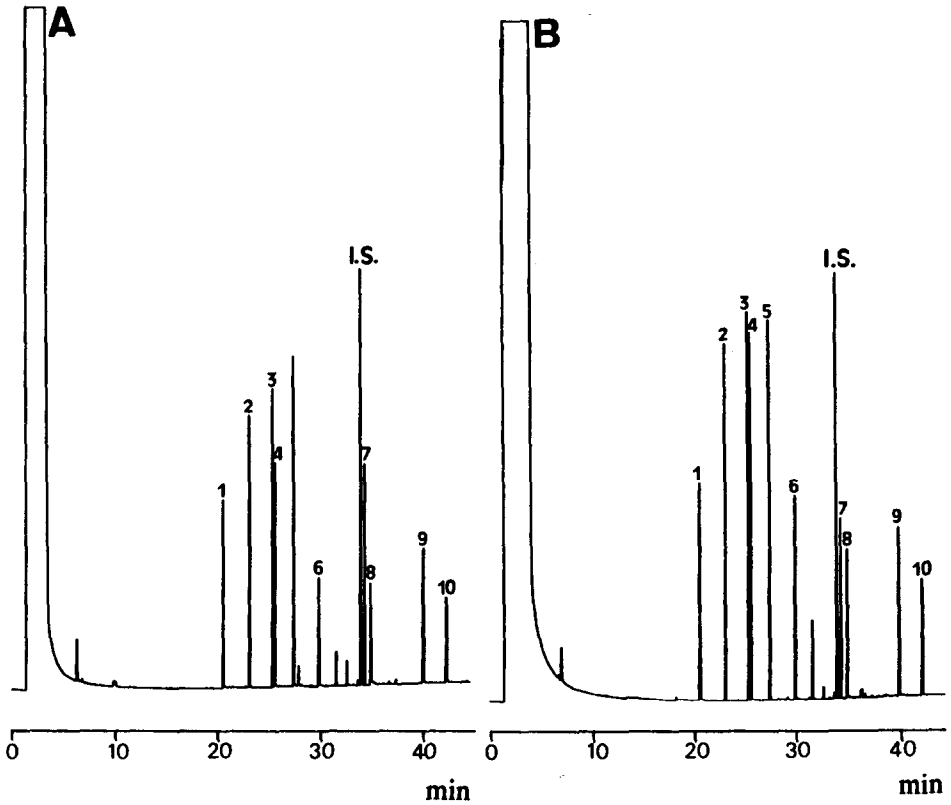

Fig. 3. Chromatograms of the oxo-PAH and nitro-PAH, 1 μl of 20 μg ml^{-1} in (A) methanol and (B) acetonitrile. Column DB-5. Initial column temperature 40°C; retention gap 2.5 m. For peak identification, see Table II.

Grob [16] for *n*-alkane compounds. Indeed, initial temperature also affected the resolution and peak shape, as shown in Fig. 4, in which the peak height-to-peak width ratios for the first two eluted compounds (1,4-naphthoquinone and 5-nitroindan) at different initial temperatures are shown. Decreases in this ratio at high initial column temperatures were observed for the two compounds in all the solvents studied and were related to a decrease in the solvent effect. Decreases at low initial column temperatures were only observed for the first eluted peak (1,4-naphthoquinone) in acetonitrile, and this may be due to the slow evaporation of the solvent. There seems to be a critical inital temperature, above or below which the solvent loses its efficiency; this effect was not observed for the other solvents studied because the working temperatures were not far enough below their boiling points.

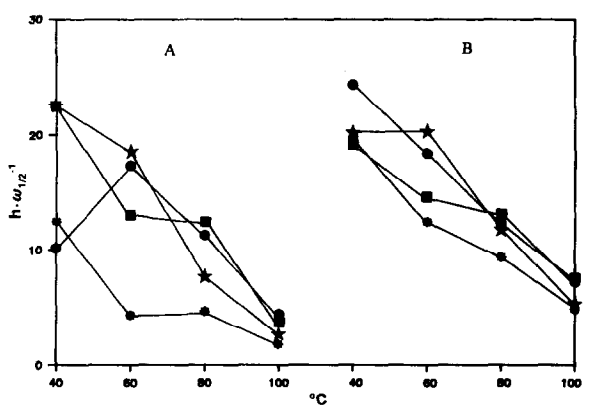

Fig. 4. Effect of column temperature on the peak shape (height–width) ratio of solvent effect. (A) 1,4-naphthoquinone and (B) 5-nitroindan. Key: ■ = dichloromethane; ★ = acetone; * = methanol; ● = acetonitrile.

To optimize the chromatographic conditions, response factors for each oxo- and nitro-PAH in the four solvents studied relative to dichloromethane at 40°C were calculated. These factors are listed in Table IV. From the results obtained for the height response factors, we can conclude that the best condi-

TABLE III

RELATIVE PEAK HEIGHTS IN DIFFERENT SOLVENTS AT INITIAL TEMPERATURES OF 40, 60, 80 AND 100°C

Compound[a]	Relative peak height (%)															
	Dichloromethane (40.0°C)[b]				Acetone (56.0°C)				Methanol (65.0°C)				Acetonitrile (81.6°C)			
	100°C	80°C	60°C	40°C	100°C	80°C	60°C	40°C	100°C	80°C	60°C	40°C	100°C	80°C	60°C	40°C
1,4-NQ	41	76	73	100	36	62	93	100	41	64	58	100	59	95	130	100
5-NI	62	84	84	100	54	82	103	100	52	70	82	100	49	64	88	100
2-M-1-NN	66	79	76	100	65	87	94	100	57	72	90	100	64	63	88	100
2-NN	72	63	70	100	67	83	98	100	71	84	104	100	53	55	83	100
9-Fl	83	75	91	100	68	78	82	100	77	78	89	100	69	69	96	100
ACQ	86	67	86	100	79	61	73	100	138	127	89	100	86	68	88	100
9-NA	86	70	91	100	78	79	76	100	82	80	86	100	101	101	108	100
9,10-PQ	101	72	74	100	78	73	88	100	119	108	77	100	85	95	101	100
BZ	96	134	87	100	101	112	96	100	124	112	112	100	89	104	112	100
1-NP	90	139	68	100	93	99	83	100	124	119	123	100	84	100	103	100

[a] For explanation of solvent abbreviations, see Table II.
[b] Boiling point.

TABLE IV

RELATIVE RESPONSE FACTORS IN DIFFERENT SOLVENTS AT INITIAL TEMPERATURES OF 40, 60, 80 AND 100°C

Compound[a]	Relative response factor															
	Dichloromethane (40.0°C)[b]				Acetone (56.0°C)				Methanol (65.0°C)				Acetonitrile (81.6°C)			
	100°C	80°C	60°C	40°C	100°C	80°C	60°C	40°C	100°C	80°C	60°C	40°C	100°C	80°C	60°C	40°C
1,4-NQ	41	76	73	100	33	57	86	92	25	39	35	61	41	66	91	70
5-NI	62	84	84	100	51	78	97	95	47	63	74	90	58	75	102	117
2-M-1-NN	66	79	76	100	55	74	80	85	47	60	76	84	68	67	93	106
2-NN	72	63	70	100	55	68	80	82	51	60	75	72	60	62	94	113
9-Fl	83	75	91	100	78	89	94	115	76	76	87	98	77	78	107	112
ACQ	86	67	86	100	84	65	78	106	77	70	49	56	89	70	91	103
9-NA	86	70	91	100	85	87	83	109	88	86	93	108	87	87	93	86
9,10-PQ	101	72	74	100	83	78	94	106	94	85	61	79	97	108	115	114
BZ	96	134	87	100	109	121	104	108	138	125	125	112	122	143	154	138
1-NP	90	139	68	100	98	105	87	105	129	123	128	104	116	138	143	138

[a] For explanation of solvent abbreviations, see Table II.
[b] Boiling point.

tions were acetonitrile as solvent at an initial column temperature of 60°C, as there was a marked loss of efficiency in the first peak eluted at 40°C in acetonitrile.

There are other factors that must be optimized. For instance, the injection port temperature affects the amount of sample retained in the capillary section of the column or in the retention gap in the bottom of the injector, and peak splitting can be observed owing to this warm connection. To study this effect, the injection port temperature was increased from 200 to 300°C. No difference was observed in the peak areas or peak heights. An increase in the inlet vent flow, which can be achieved by increasing the split ratio to 1:100, gives solvent peaks with a better shape due to a faster removal of the last traces of vapour from the vaporizing chamber.

As is well known, the peak splitting depends on the solvent, on the solutes and on the stationary phase. In Fig. 5 it can be seen that when a more polar stationary phase (DB-17) was used without a retention gap, peak splitting was obtained using acetonitrile as solvent, but with a different pattern; now two maxima appear rather than multiple maxima as happens when DB-5 is used (Fig. 2A). This lower peak splitting may be due to the different acetonitrile wettability of both stationary phases, giving a less homogeneous flooding zone for the DB-5 stationary phase.

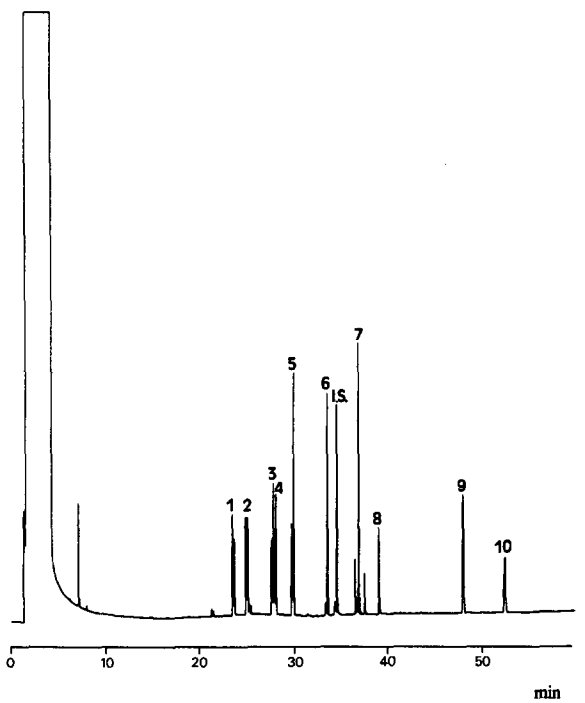

Fig. 5. Chromatogram of the oxo-PAH and nitro-PAH, 1 μl of 20 μg ml^{-1} in acetonitrile. Column DB-17. Initial column temperature 40°C. For peak identification, see Table II.

Quality parameters

Calibrations for oxo- and nitro-PAHs in acetonitrile were carried out under the optimum conditions, with concentrations in the range 2–20 μg ml^{-1}. Peak area was used as the response. The correlation coefficients of calibration graphs in the concentration range 2–20 μg ml^{-1} were better than 0.9990 for all oxo- and nitro-PAHs.

Five replicate determinations of 8 ng (8 μg ml^{-1} solution) of each oxo- and nitro-PAH in acetonitrile were carried out under the optimum conditions to determine the precision in the analysis of PAH derivatives. Relative standard deviations (R.S.D.) in the range 1.8–6.7% based on peak area were obtained.

The detection limits for the determination of oxo- and nitro-PAHs by GC with a flame ionization detector ranged from 129 pg for 2-methyl-1-nitronaphthalene to 1.5 ng for 9,10-phenanthroquinone when determinations were made in acetonitrile (Table V). These values are higher than those obtained

TABLE V

DETECTION LIMITS AND PRECISION OF OXO- AND NITRO-PAHs IN ACETONITRILE

Chromatographic conditions: programme 2.

Compound[a]	Detection limit[b] (pg)	Precision R.S.D. %
1,4-NQ	130	2.5
5-NI	135	2.3
2-M-1-NN	129	2.3
2-NN	132	2.0
9-Fl	133	1.8
ACQ	189	4.1
9-NA	408	1.9
9,10-PQ	1500	2.0
BZ	399	4.6
1-NP	404	6.7

[a] For explanation of solvent abbreviations, see Table II.
[b] As 2 S.D.

for the PAHs with non-polar solvents such as isooctane or toluene [19], showing that the polar solvents are less efficient in transferring the compounds to the column.

CONCLUSIONS

When methanol and acetonitrile were used as solvents of oxo- and nitro-PAHs in HRGC with non-polar phases, peak splitting was observed, showing multiple maxima for acetonitrile, which are related to the poor wetting ability of this solvent. We conclude that the optimum initial column temperature depends on the boiling point of the solvent; the highest response factors were obtained using acetonitrile at an initial temperature of 60°C. These results could be applied to the determination of oxo- and nitro-PAHs in the fractions obtained from the clean-up of environmental samples.

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