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## DETERMINATION OF AZA-HETEROCYCLIC COMPOUNDS IN ATMOSPHERIC DUST BY A COMBINATION OF THIN-LAYER AND GAS CHROMATOGRAPHY

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### SUMMARY

A simplified and relatively rapid method, based on a gas chromatographic determination preceded by a thin-layer chromatography, for the separation of heterocyclic nitrogen compounds in atmospheric particulate matter has been developed. The benzene extract of the dust is applied to a silanized silica gel plate and developed with benzene-hexane-pyridine(4:5:1). In this way, aza-heterocyclic compounds are separated from other classes of components, such as polynuclear hydrocarbons and paraffins. The portion containing aza-heterocyclic compounds is extracted again and examined by gas chromatography on a high-efficiency glass capillary column coated with Versamid 900.

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### INTRODUCTION

A problem of great importance in air pollution studies is that of the detection and determination of polynuclear aza-aromatic compounds in airborne particulate matter. Although the biological action of the majority of such compounds has not yet been investigated, several members of this class of compounds have been demonstrated to have toxic and carcinogenic properties<sup>1</sup>.

The amount of aza-heterocyclic compounds present in urban air is relatively small (sub-parts per billion range) compared with the total aromatic hydrocarbon content and, consequently, a selective extraction combined with highly sensitive analytical techniques is required to monitor the former compounds.

Many different systems have been suggested for separating and determining aza-heterocyclic compounds in the organic dust fraction. The techniques most commonly used are based on a chromatographic separation and determination utilizing ultraviolet and fluorescence spectrophotometry. Sawicki and co-workers<sup>2,3</sup> reported column and thin-layer chromatographic separations of heterocyclic and polynuclear hydrocarbons; fluorescence spectra were used for characterizing the compounds directly on the plates. Analogous separations have been achieved by Lederer and Roch<sup>4</sup> by using paper chromatography with various aqueous solvents. Gas chromatography

also has been applied to the investigation of many heterocyclic nitrogen compounds after solvent extraction from atmospheric dust and the fractionation of different classes of compounds<sup>5</sup>.

Detailed examinations of each of these methods showed that many difficulties have to be overcome and that none of the methods is satisfactory for use in routine analysis. The sample has to be extracted for many hours and large volumes of solvents have to be used. Consequently, in the subsequent steps it is necessary to concentrate the solution, with the risk of losing or destroying some of the compounds.

Column chromatography is a lengthy procedure; many fractions have to be collected and many measurements with the spectrophotometer or fluorimeter are required in order to obtain a complete analysis.

Paper chromatographic methods are useful in that the techniques involved are simple, but the disadvantages are the length of time needed for separations and the difficulty of separating some of the aza-heterocyclic compounds.

Thin-layer chromatography is recommended for the separation of complex mixtures of polycyclic compounds prior to their spectral characterization; only small samples are used and the analysis is rapid. However, the separation is not complete; as there are too many compounds in dust to be separated on a single chromatogram and sometimes two-dimensional development in two different solvent systems is required. In the last method, although the gas chromatographic procedure is more sensitive and accurate, numerous steps in the preparation of the sample are necessary before its injection into the gas chromatograph.

The separation of aza-heterocyclic compounds from aliphatic and polynuclear hydrocarbons by partition between two solvents (cyclohexane and nitromethane) and evaporation of the solution to a very small volume is also a time-consuming technique. In this procedure, owing to the complex nature of the molecules of the compounds present in the dust, each of which may possess several organic functional groups, a sharp separation cannot be achieved and some aza-heterocyclic compounds may be found in the polycyclic hydrocarbon fraction<sup>5</sup>.

Because of these difficulties, we have studied a procedure based on gas chromatography for the separation of the heterocyclic nitrogen compounds, in which we have tried to eliminate all of the preliminary steps by using thin-layer chromatography for the preparation of the samples to be injected into the gas chromatograph. Gas chromatography, carried out with a glass capillary column coated with a new stationary phase operating with temperature programming, gives good separations of many of the most important compounds. Retention times are used for the identification of peaks and by using an internal standard it is possible to establish the relative amounts present.

## EXPERIMENTAL AND RESULTS

### *Compounds*

The following compounds of the three main classes present in particulate matter were examined.

*Heterocyclic nitrogen compounds.* Quinoline, 3-methylisoquinoline, 2,6-dimethylquinoline, benzo(*h*)quinoline, phenanthridine, benzo(*f*)quinoline, 3-methylbenzo(*f*)quinoline, 3-methylbenzo(*b*)quinoline, 2-methylindole, carbazole, benzo(*a*)-

acridine, benzo(c)acridine, 2-methylacridine, 10-methylbenzo(c)acridine, benzo(c)-cinnoline, 1-azafluoranthene, 7-azafluoranthene, 1-azapyrene, 7-azapyrene, 9-(3'-pyridyl)anthracene, 9-(4'-pyridyl)anthracene, 10-(3'-pyridyl)-1,2-benzanthracene and 10-(4'-pyridyl)-1,2-benzanthracene.

**Polycyclic hydrocarbons.** Fluoranthene, pyrene, 3,4-benzofluoranthene, 3-methylpyrene, 1,2-benzopyrene, 3,4-benzopyrene, phenanthrene, 1,2-benzoanthracene, methylcholanthrene, anthracene, 1,2-benzofluorene, chrysene and 1,2,5,6-dibenzanthracene.

**Paraffins.** C<sub>11</sub>, C<sub>12</sub>, C<sub>13</sub>, C<sub>14</sub>, C<sub>15</sub>, C<sub>18</sub>, C<sub>20</sub>, C<sub>22</sub>, C<sub>24</sub> and C<sub>28</sub>.

#### Thin-layer chromatography

Thin-layer chromatography was used as a preliminary step to separate the above three classes of compounds. Alumina (Type E), silica gel and silanized silica gel (Merck) were examined as adsorbents and solvents with different polarities, hexane, benzene and pyridine, were used as developers.

Smooth glass plates (20 × 20 cm) were coated with a 500- $\mu$ m thickness of adsorbent by means of an applicator. The development was performed by the ascending method in the usual manner. The fluorescence colours of the polynuclear compounds were marked under a UV lamp and the alkanes were detected by spraying the plates with a concentrated H<sub>2</sub>SO<sub>4</sub>-KMnO<sub>4</sub> mixture (universal reagent).

The best separation of the three classes of compounds was obtained by using silanized silica gel as the adsorbent and benzene-hexane-pyridine (4:5:1) as the developing solvent. Silica gel and alumina have a very strong attraction for the aza-nitrogen atom and consequently, while the polycyclic and paraffinic hydrocarbons are eluted as only one spot<sup>6</sup>, the aza-heterocyclic compounds are separated according to the extent of steric hindrance around the aza-nitrogen atom. When the aza-nitrogen atom is sterically protected, the *R<sub>F</sub>* value of the aza-compound is approximately the same as

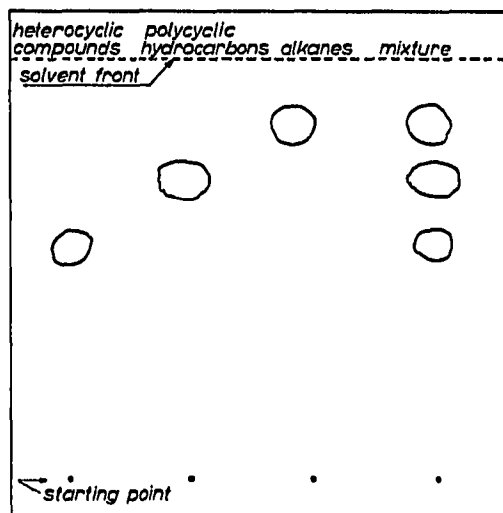


Fig. 1. Separation of heterocyclic nitrogen compounds from paraffinic and polycyclic hydrocarbons by thin-layer chromatography. Silanized silica gel plate, 500  $\mu$ m thick; solvent, benzene-hexane-pyridine (4:5:1).

that of the corresponding aromatic hydrocarbon; when the aza-nitrogen atom is not sterically protected, the  $R_F$  value of the aza-compound is lower than that of the corresponding aromatic hydrocarbon. In order to minimize this selective adsorption by the active sites of the adsorbent, we used silanized silica gel, which is sensitive mainly to the nature of the  $\pi$ -electronic system of the adsorbate molecule.

With a benzene-hexane mixture, the aza-heterocyclic compounds were not resolved from the polynuclear hydrocarbons and were found to cover a wide range of  $R_F$  values; some were strongly retained near the starting point.

By using the basic mixture benzene-pyridine, all of the aza-heterocyclic compounds were eluted together with a high  $R_F$  value (0.92), but the corresponding spot was not sufficiently separated from that of the polycyclic hydrocarbons ( $R_F$  0.85); at the same time, the paraffins were located on the solvent front.

The addition of an apolar component to this polar mixture decreased the  $R_F$  values of paraffins, polycyclic hydrocarbons and aza-heterocyclic compounds ( $R_F$  0.90, 0.76 and 0.55, respectively) and enabled a better separation to be obtained. Fig. 1 shows a chromatogram obtained on a silanized silica gel plate developed with benzene-hexane-pyridine (4:5:1).

#### *Gas-liquid chromatography*

Gas chromatographic separations of aza-heterocyclic compounds were carried out with a Carlo Erba Fractovap Model GI chromatograph equipped with a flame ionization detector and a linear temperature programmer. The column consisted of a 40-m glass capillary of 0.29 mm I.D., coated with Versamid 900. A 3% solution of this phase in chloroform was made to flow very slowly (10 cm/min) through the capillary and the solvent was then volatilized in a stream of nitrogen. This column has an efficiency of about 60,000 theoretical plates for benzophenone (capacity ratio,  $K' = 2.5$ ). The flow-rate through the column was 0.5 ml/min at room temperature with a carrier gas inlet pressure of 0.35 kg/cm<sup>2</sup>.

Versamid 900 offers some advantage in comparison with other liquid phases previously used<sup>5</sup>. Because of the higher solubility of the aza-compounds in a basic phase and the neutralization of the adsorption effect from the glass walls, symmetrical peaks are obtained; in fact, the functional groups of the phase form hydrogen bonds with the silanol groups of the glass and a spontaneous deactivation of the support takes place.

For a complete evaluation of the aza-heterocyclic compounds, as is usually required in air pollution investigations, it is of interest to determine compounds with a wide range of boiling-points; for this purpose, programmed-temperature chromatography is very convenient. Good separations have been achieved for many compounds and the analysis of a standard aza-heterocyclic mixture, ranging in boiling-point from quinoline to pyridylbenzanthracenes, has been obtained, as shown in Fig. 2.

Column bleeding was found to be negligible and almost no baseline drift was observed.

#### *Examination of atmospheric dust for aza-heterocyclic compounds*

Some dust samples were examined for their content of aza-heterocyclic compounds.

Particulate matter (1-2 g) was collected from air by means of a high-volume air

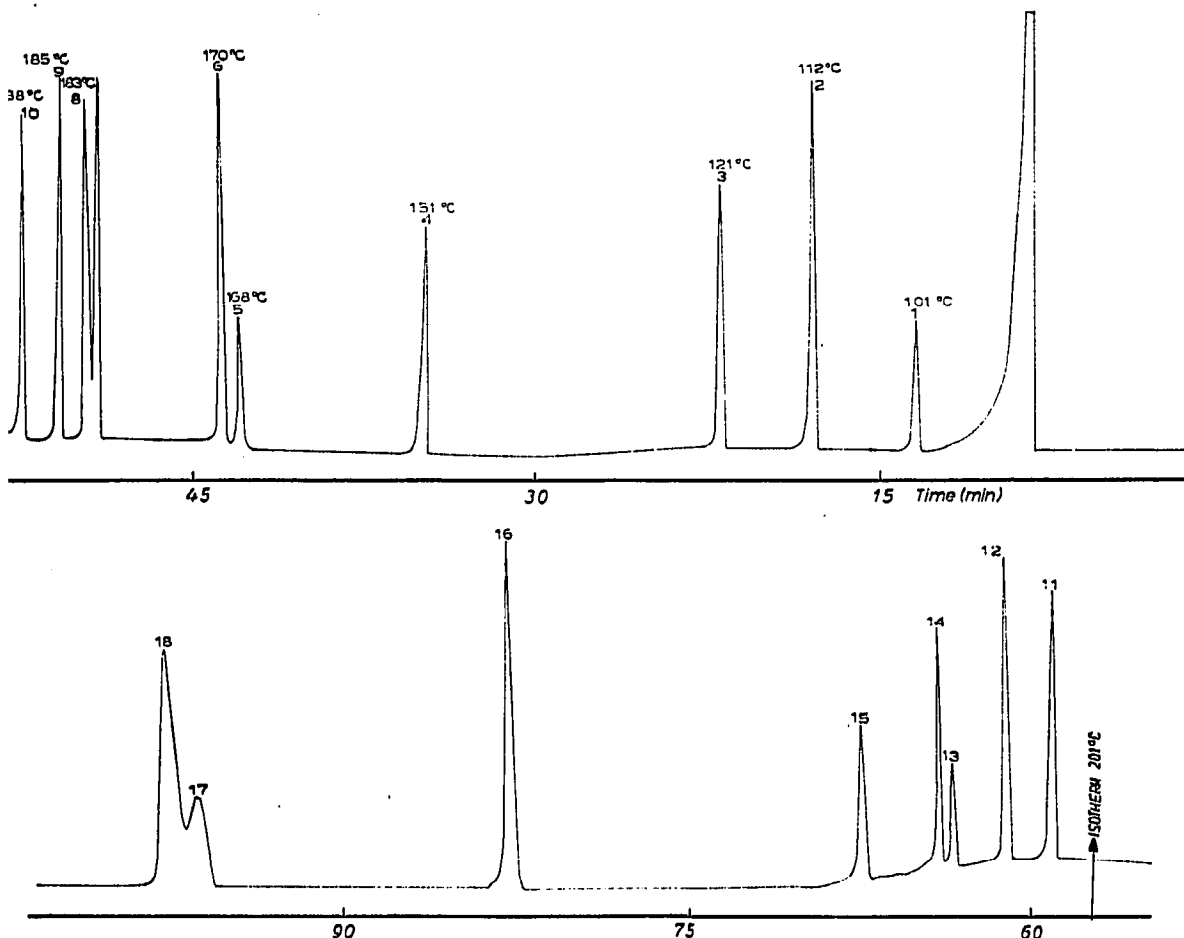


Fig. 2. Programmed-temperature gas chromatogram of a standard aza-heterocyclic mixture (88–201° at 2.5°/min). Glass capillary column coated with Versamid 900. Peaks (see also Fig. 3): 1 = quinoline; 2 = 3-methylisoquinoline; 3 = 2,6-dimethylquinoline; 4 = 2-methylindole; 5 = carbazole; 6 = benzo(*h*)quinoline; 7 = phenanthridine; 8 = 10-methylbenzo(*c*)acridine and benzo(*f*)quinoline; 9 = 3-methylbenzo(*b*)quinoline and 3-methylbenzo(*f*)quinoline; 10 = benzo(*a*)acridine and 2-methylacridine; 11 = benzo(*c*)cinnoline; 12 = 1-azafluoranthene; 13 = 7-azafluoranthene; 14 = 1-azapyrene; 15 = 4-azapyrene; 16 = benzo(*c*)acridine; 17 = 9-(3'-pyridyl)anthracene and 9-(4'-pyridyl)anthracene; 18 = 10-(3'-pyridyl)-1,2-benzanthracene and 10-(4'-pyridyl)-1,2-benzanthracene.

pump (Staplex) on a glass-fibre filter. The filter plus the dust was extracted in a Soxhlet extractor with 200 ml of benzene for about 8 h. The extract was concentrated to a small volume (*ca.* 1 ml) and transferred on to a silanized silica gel thin-layer plate along a 10-cm line. A standard mixture of aza-heterocyclic compounds was placed on one side of the plate, which was developed with the solvent mixture benzene-hexane-pyridine (4:5:1). After drying the plate, it was observed under a UV lamp and the fluorescent part containing the aza-compounds was scraped off the plate into a small sintered test-tube. The compounds were then extracted two or three times with

small portions of benzene; the benzene fractions were collected in a test-tube with a conical end and the solvent was evaporated to a volume of a few microlitres. For quantitative analysis, an internal standard (benzo(*h*)quinoline) was added. The benzene solution was analyzed by programmed-temperature gas chromatography with the column described and some of the compounds were identified by comparing their retention times with those of standard compounds.

The chromatogram of an atmospheric dust extract containing aza-heterocyclic compounds is shown in Fig. 3. The quantitative determination was limited to the compounds that have been identified, and the results are given in Table I.

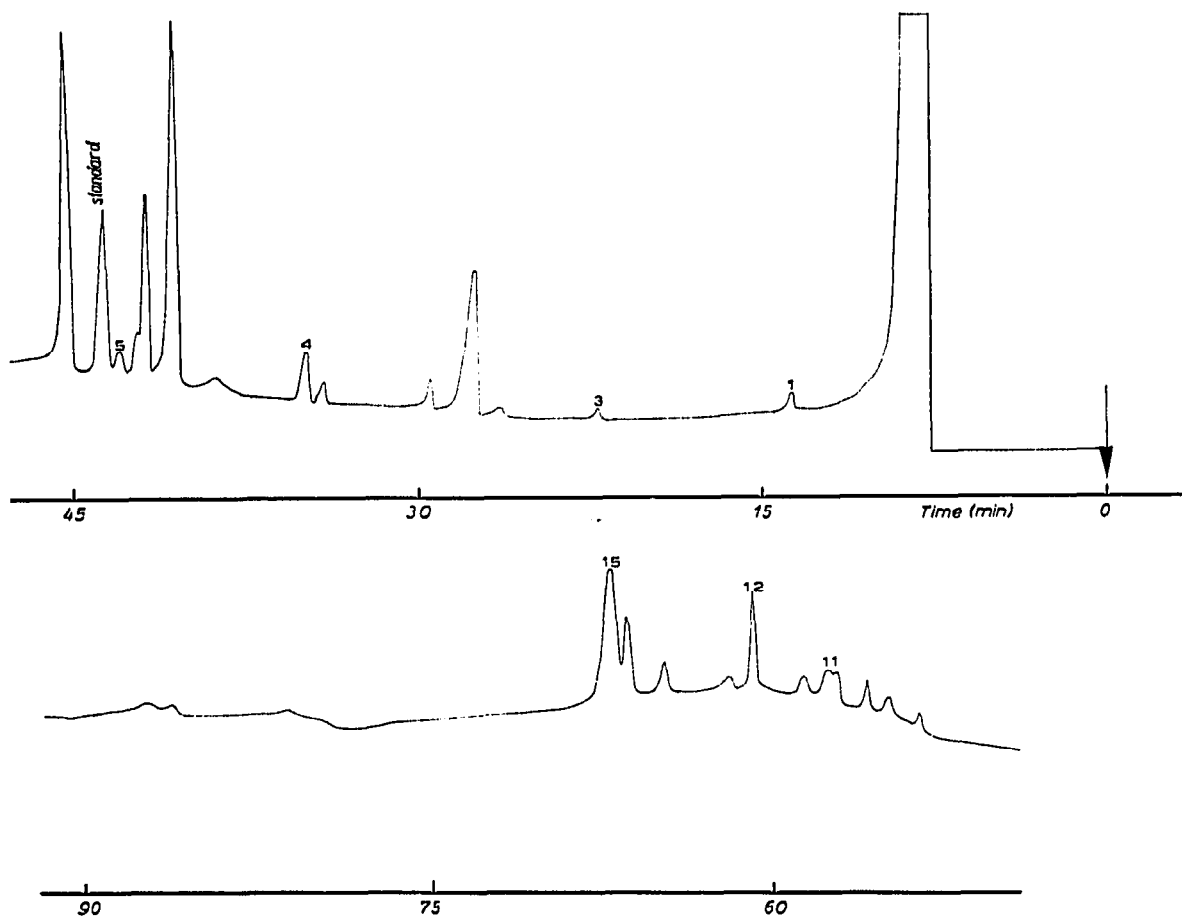


Fig. 3. Gas chromatogram of a sample of an atmospheric dust extract. Conditions and peaks as in Fig. 2.

In order to establish the efficiency of the analytical procedure, known amounts of aza-heterocyclic compounds were added to the particulate matter and a recovery in the extract of *ca.* 90% was obtained.

TABLE I

CONCENTRATIONS OF AZA-HETEROCYCLIC COMPOUNDS IN ATMOSPHERIC DUST  
Samples taken in Rome during November and December, 1972.

<i>Compound</i>	<i>Concentration</i> ( $\mu\text{g per } 1000 \text{ m}^3 \text{ of air}$ )
Quinoline	0.6
2,6-Dimethylquinoline	0.3
2-Methylindole	2.0
Carbazole	1.1
Benzo(c)cinnoline	1.0
1-Azafluoranthene	3.0
4-Azapyrene	13.1

## CONCLUSIONS

The procedure described, which combines thin-layer and gas chromatography, offers a number of advantages in comparison with other analytical methods suggested for the examination of aza-heterocyclic compounds adsorbed in airborne particles.

Thin-layer chromatography is a rapid and simple procedure for isolating and purifying the sample and its use, prior to the injection of the compounds into the gas chromatographic column, greatly improves the quality of the chromatograms, as unwanted compounds are eliminated. Gas chromatography permits one to work with small amounts of samples and has a high resolution and short analysis time for evaluating the individual constituents.

The simplicity of the separation conditions enables this procedure to be used with confidence for routine analysis.

## ACKNOWLEDGEMENT

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