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## VACUUM SUBLIMATION OF POLYNUCLEAR AROMATIC HYDROCARBONS SEPARATED BY THIN-LAYER CHROMATOGRAPHY FOR DETECTION WITH SHPOL'SKII LOW-TEMPERATURE FLUORESCENCE

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### SUMMARY

A method for the vacuum sublimation of polynuclear aromatic hydrocarbons (PAHs) from thin-layer chromatographic plates is described. Low-temperature fluorescence spectroscopy, utilizing the Shpol'skii effect, is used for the detection and identification of benzo[*a*]pyrene, pyrene and benzo[*ghi*]perylene in samples from different sources. The recoveries of these PAHs were optimized by varying the time and temperature of sublimation.

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### INTRODUCTION

Fractional sublimation at normal or reduced pressure has been used with different techniques and in various applications for the separation and purification of organic compounds that have similar properties<sup>1-5</sup>. It has also been utilized as a method for the recovery of volatile compounds from air-borne particulate matter<sup>6-13</sup>. However, sublimation is also a rapid and convenient method for the transfer of small amounts of sample from a thin-layer chromatographic plate to some other analytical system. In this investigation it has been shown to be a good substitute for the conventional solvent extraction technique<sup>14-17</sup>.

This paper describes a method for recovering polynuclear aromatic hydrocarbons (PAHs) from a cellulose thin-layer plate. It also demonstrates a useful method of measuring fluorescence at low temperature, utilizing the Shpol'skii effect. This method was developed especially for the preparation of samples for Shpol'skii low-temperature fluorescence spectroscopy, although it can be applied to other fields.

### EXPERIMENTAL

#### *Apparatus*

Sublimation was carried out at reduced pressure in an all-glass device as shown in Fig. 1. Fluorescence at ambient temperature was measured using 3.00-ml Spectrosil cuvettes in a Perkin-Elmer MPF 2A instrument; spectra obtained at 77 °K were measured in a home-built fluorescence spectrophotometer<sup>18</sup>.

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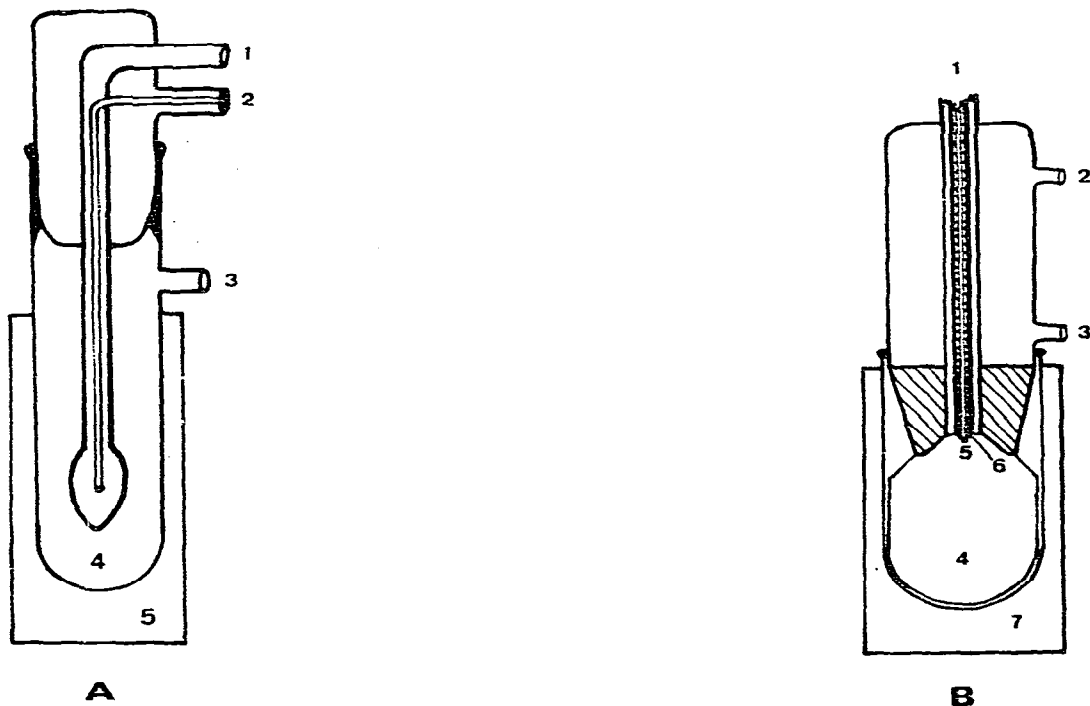


Fig. 1. All-glass vacuum sublimation apparatus. (A) 1 = water outlet; 2 = water inlet; 3 = connection to vacuum pump; 4 = vacuum chamber; 5 = oven. (B) Not used for recovery studies, only for small amounts of sample: 1 = connection to vacuum pump; 2 = water outlet; 3 = water inlet; 4 = vacuum chamber; 5 = removable glass capillary; 6 = PTFE tube; 7 = oven.

#### Standard solutions

Pyrene, coronene (Fluka, Buchs, Switzerland), benzo[*a*]pyrene (Sigma, St. Louis, Mo., U.S.A.) and benzo[*ghi*]perylene (Koch-Light, Colnbrook, Great Britain) were used without further purification. The concentrations of the standard solutions varied between 20 and 80 ng  $\mu\text{l}^{-1}$ . The solvents used were cyclohexane (pro analysi grade; Merck, Darmstadt, G.F.R.) and ethanol (spectrograde; Kemetyl, Stockholm, Sweden).

#### Procedure

Thin-layer plates coated with 30% acetylated cellulose (Macherey, Nagel & Co., Düren, G.F.R., Polygram Cel 300, 30% AC) were cleaned by development in ethanol-methylene chloride-water (10:5:1) to the top of the plates. The different solutions of the standards were applied on these plates with a micropipette; 5  $\mu\text{l}$  of solution contained 100–400 ng of sample, depending on the compound. The same micropipette was used to inject standard reference compounds directly into the measuring cuvette, so as to avoid any discrepancy in volume between different pipettes.

The thin-layer plates were developed with the mobile phase described and protected from direct UV light. After development the spots, observed under a UV lamp, were marked and cut out with a pair of scissors. This procedure eliminates the

losses that occur if the layer is scraped off. The spot with the absorbed sample was placed in the vacuum chamber (Fig. 1A), which was connected to a Humboldt-Hereaus vacuum pump. The temperature in the oven was monitored and stabilized ( $\pm 1^\circ\text{C}$ ) by a temperature regulator. The temperature on the cold finger was about  $8\text{--}10^\circ\text{C}$ . The pressure in the vacuum chamber was *ca.* 0.1 torr, which was reached in 60 sec and maintained during the whole procedure. After sublimation was completed, the pressure was equalized and the cold finger washed with the desired solvent. The recovery was calculated from the room-temperature fluorescence using peak-area measurements and a calibration graph.

## RESULTS AND DISCUSSION

Figs. 2-5 show the recoveries of four different polynuclear aromatic hydrocarbons as a function of temperature and time. It is clear that these functions vary owing to the different boiling points of the components.

Earlier workers reported 95% recoveries of coronene by sublimation at  $270^\circ\text{C}$  and 1 torr<sup>9</sup> and of pyrene at  $100^\circ\text{C}$  and 1 mmHg<sup>7</sup>. The time needed for sublimation of pyrene was 5 min. Both of these experiments were carried out with pure standards, which were either placed directly on a glass-fibre filter or applied as a solution, the solvent being allowed to evaporate before the sublimation.

Fig. 2 shows our results for the sublimation of pyrene from thin-layer plates. The difference in recovery between  $100^\circ\text{C}$  and  $165^\circ\text{C}$  is small and is negligible after 15 min. A recovery of over 90% is achieved within 5 min at both temperatures. Figs. 3 and 4 show the recoveries of benzo[*a*]pyrene and benzo[*ghi*]perylene. The recoveries of both components exceeded 90% at  $165^\circ\text{C}$  and  $200^\circ\text{C}$ , respectively.

Fig. 5 shows the recovery of coronene. Because of its higher boiling point ( $525^\circ\text{C}$  at 760 mmHg) it is necessary to use a higher sublimation temperature in order to achieve a reasonable recovery within 1 h. The upper temperature limit when using this plastic thin-layer plate is  $220^\circ\text{C}$ . Higher temperatures will cause degradation of the plastic film on which the layer is coated, and thus depositing on the cold finger a yellow slick that is insoluble in cyclohexane and ethanol. This limitation makes the sublimation of coronene difficult, but 90% recovery is reached in about 1 h at  $220^\circ\text{C}$ .

Each point in the figures is the mean of five measurements. The variation in the recoveries was erratic for time periods of less than 5 min but became stable after this period of sublimation. There is also a slight tendency for erratic recoveries on prolonged sublimation. Table I gives the standard deviation of the percentage recovery of benzo[*a*]pyrene with sublimation time. The four compounds investigated represent a boiling range from  $393^\circ\text{C}$  (pyrene) to  $525^\circ\text{C}$  (coronene). Hence it should be possible to apply this sublimation technique to other polynuclear aromatic hydrocarbons within this or lower boiling ranges.

We have used this vacuum sublimation method to prepare samples from thin-layer chromatography for low-temperature fluorescence spectroscopy. Many polynuclear aromatic hydrocarbons show a so-called quasilinear spectrum when dissolved in an *n*-alkane and frozen to  $90^\circ\text{K}$  or lower<sup>18,19</sup>. This method possesses certain advantages over fluorescence at ambient temperature. The many well resolved emission maxima in these spectra are specific for each hydrocarbon. Therefore, substances that cannot be separated can be identified, even when superposition occurs.

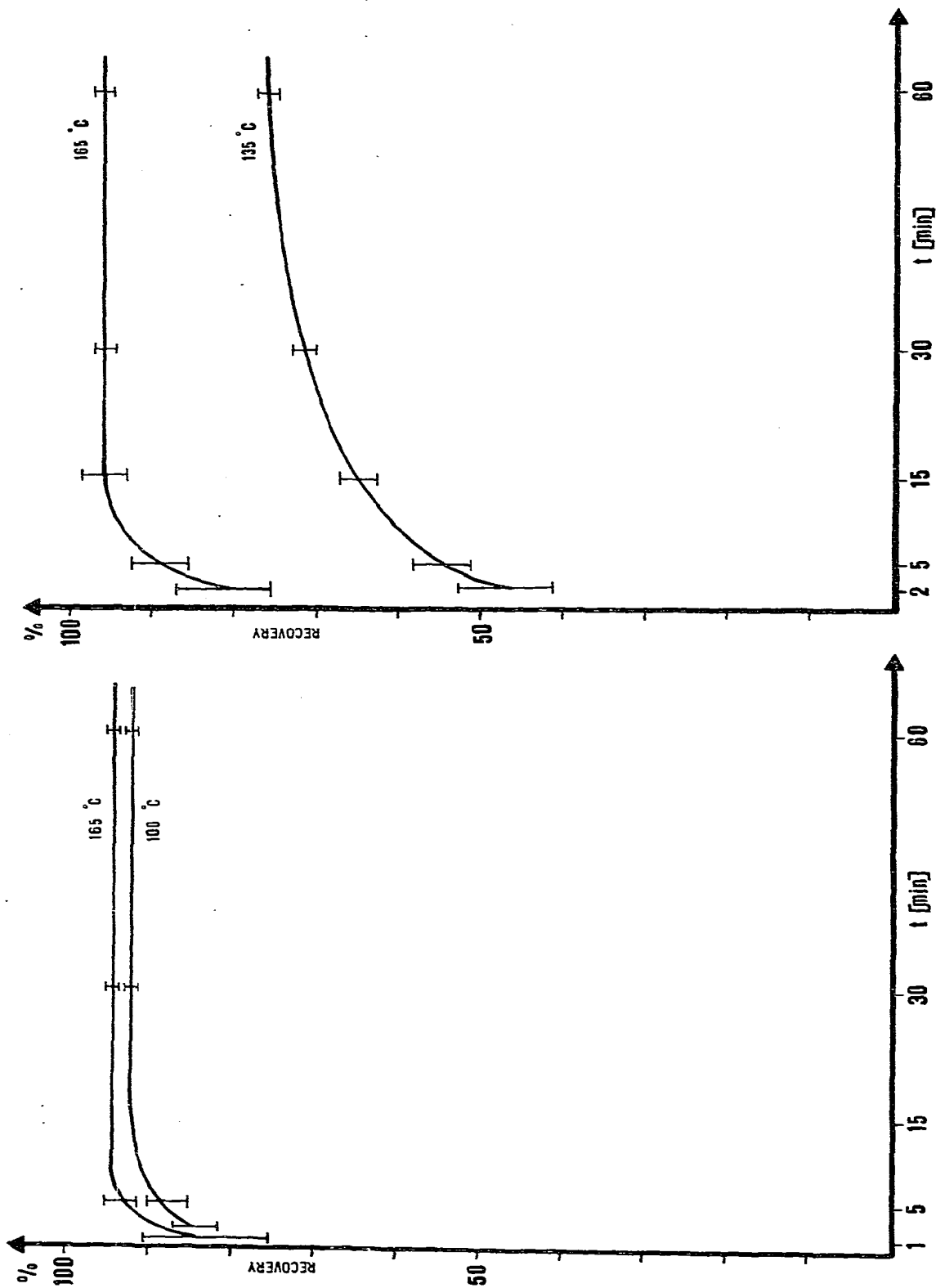


Fig. 2. Vacuum sublimation of pyrene from a thin-layer plate. Bars show minimum and maximum recoveries from five measurements.

Fig. 3. Vacuum sublimation of benzo[*a*]pyrene from a thin-layer plate. Bars as in Fig. 2.

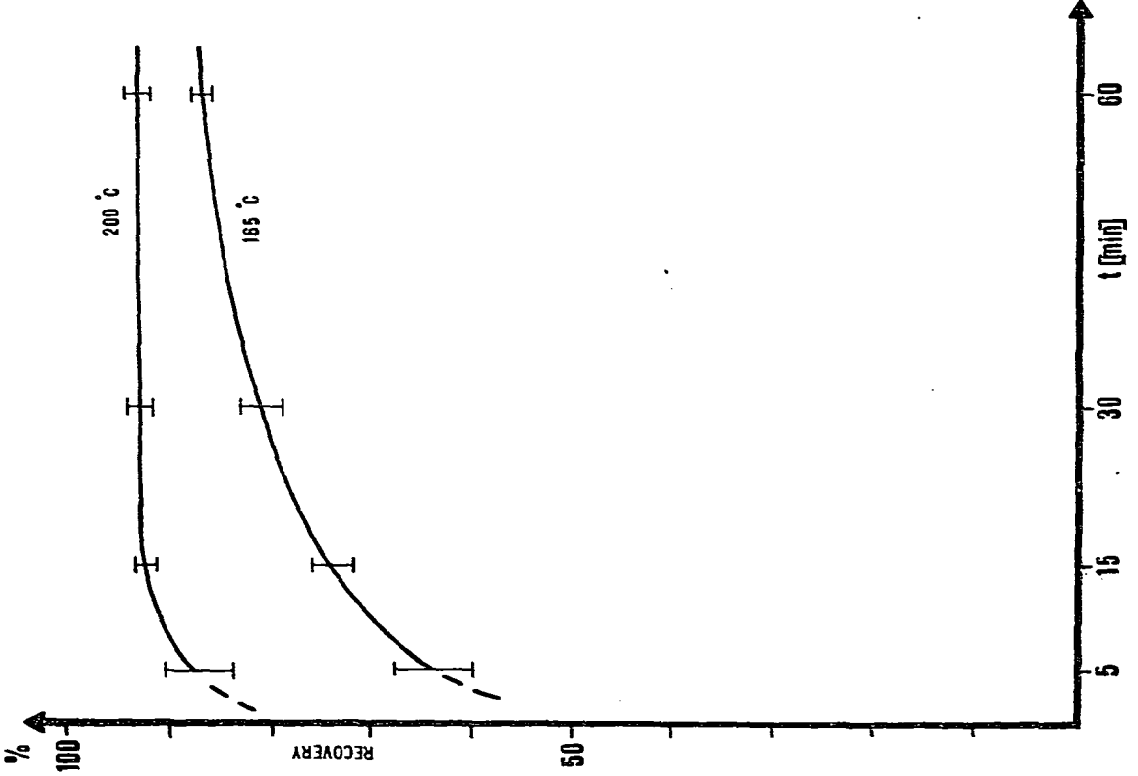
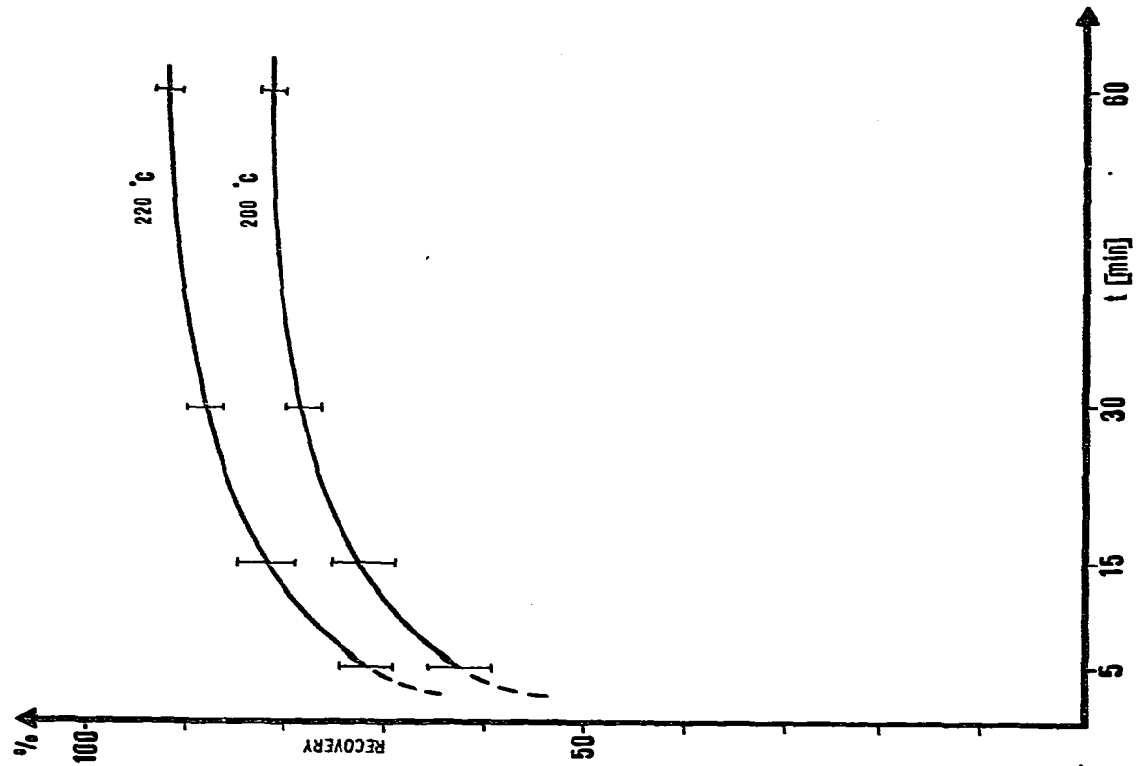


Fig. 4. Vacuum sublimation of benzofluoranthene from a thin-layer plate. Bars as in Fig. 2.

Fig. 5. Vacuum sublimation of coronene from a thin-layer plate. Bars as in Fig. 2.

TABLE I  
PRECISION OF RECOVERY OF BENZO(*a*)PYRENE

Time (min)	Recovery $\pm$ standard deviation (%)	
	(135 °C)	(165 °C)
2	47 $\pm$ 6.6	81 $\pm$ 6.8
5	54 $\pm$ 4.5	89 $\pm$ 4.7
15	65 $\pm$ 3.0	96 $\pm$ 4.1
30	72 $\pm$ 2.9	96 $\pm$ 2.8
60	77 $\pm$ 3.3	96 $\pm$ 3.4

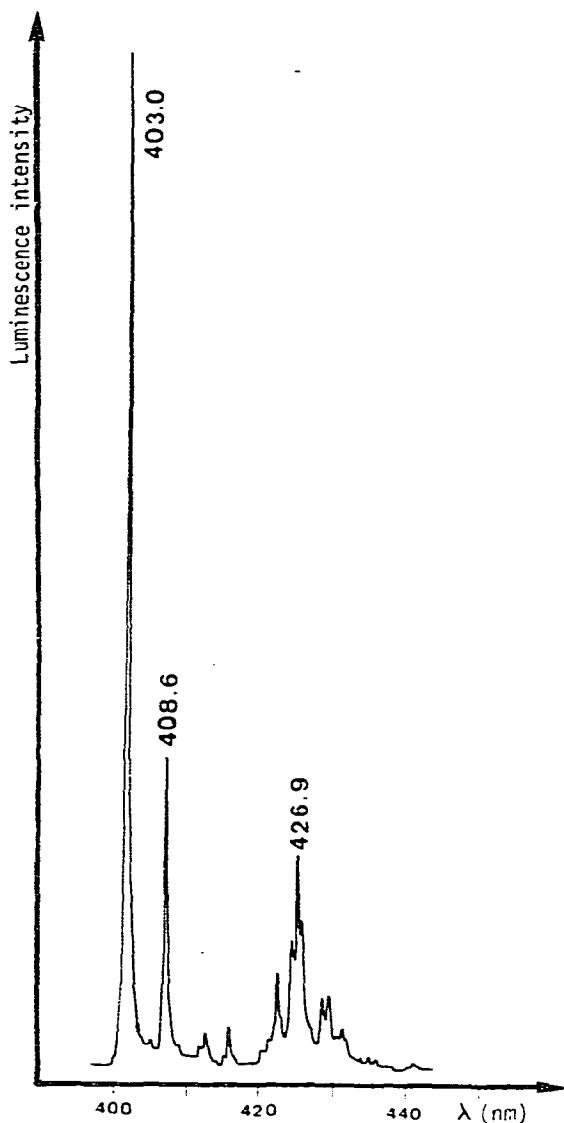


Fig. 6. Low-temperature fluorescence spectrum of a sublimated thin-layer chromatographic fraction of automobile exhaust. Excitation wavelength, 365 nm; scanning rate, 12.5 nm·min<sup>-1</sup>; slit width, 100 μm; temperature, 77 °K. Compound identified: benzo(*a*)pyrene. Solvent: *n*-octane.

In order to evaluate the method we used extracts from automobile exhausts. Samples were taken by using a proportional sampling technique which uses a collecting device similar to that used by Grimmer *et al.*<sup>20</sup>. This system gave a particulate phase collected on a glass-fibre filter and a condensed water phase, both of which were combined prior to the thin-layer chromatography. The filter was extracted by vacuum sublimation at 300 °C and 0.1 torr for 1 h<sup>12</sup> and the collected sublimate was dissolved in cyclohexane. The aqueous phase was extracted with cyclohexane according to Grimmer *et al.*<sup>20</sup>. The cyclohexane phase was gently evaporated at 30 °C under a flow of nitrogen. The residue was dissolved in cyclohexane and added to the filter extract. The combined extract was separated by thin-layer chromatography using the described system, and spots with the same  $R_F$  values as those of the reference sub-

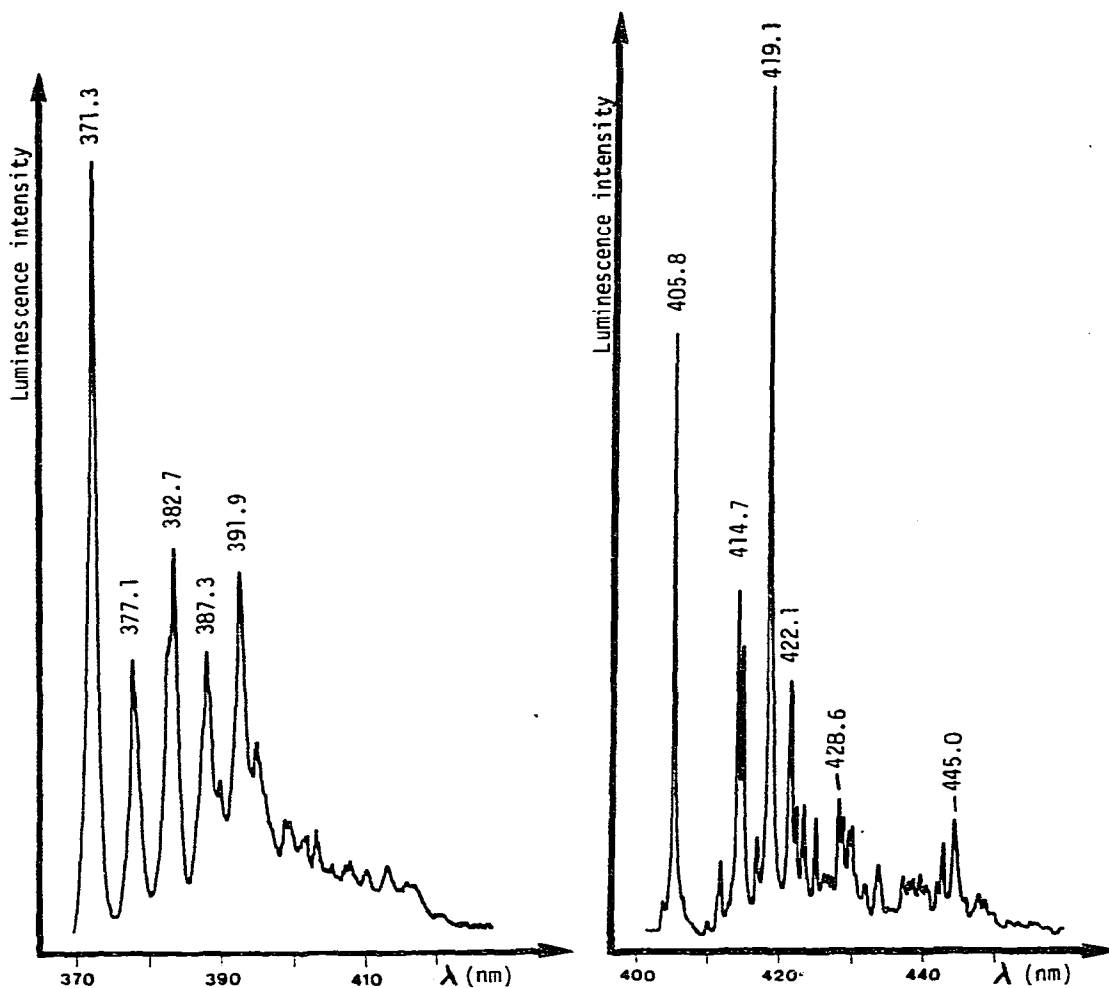


Fig. 7. Low-temperature fluorescence spectrum of a sublimated thin-layer chromatographic fraction of automobile exhaust. Conditions as in Fig. 6. Compound identified: pyrene. Solvent: *n*-pentane.

Fig. 8. Low-temperature fluorescence spectrum of a sublimated thin-layer chromatographic fraction of automobile exhaust. Conditions as in Fig. 6. Compound identified: benzo[ghi]perylene. Solvent: *n*-hexane.

stances were cut out and treated as described earlier. After sublimation, the isolated substances were dissolved in *n*-pentane, *n*-hexane or *n*-octane, depending on the compound that was suspected to be present. As the quality of the low-temperature fluorescence spectra is dependent on the frozen solvent matrix<sup>18</sup> a variety of solvents is necessary.

With this mobile phase and acetylated cellulose, benzo[*a*]pyrene occurs as a well isolated spot<sup>21</sup>. Fig. 6 shows its quasi-linear spectrum in *n*-octane at 77 °K. The appearance of the intense peak at 403.0 nm is typical and all of the other emission maxima are in good accordance with those of the standard component.

Figs. 7 and 8 show spectra from pyrene and benzo[*ghi*]perylene also isolated from automobile exhausts. The maxima in these spectra are in good agreement with those obtained from standard substances<sup>18</sup>, and structures could be assigned reliably to the components in this mixture, even though they cannot be separated by this chromatographic system.

After analysing samples from other expected sources of PAHs, such as an alumina refinery and from coke production, we believe that this fluorescence technique in combination with thin-layer chromatography and vacuum sublimation could be a useful analytical method in screening tests. The procedure is rapid and results in the unambiguous identification of such a critical substance as benzo[*a*]pyrene.

#### ACKNOWLEDGEMENTS

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