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## THE USE OF THIN-LAYER CHROMATOGRAPHY AND MASS SPECTROMETRY FOR THE RAPID ESTIMATION OF TRACE QUANTITIES OF AIR POLLUTANTS

J. R. MAJER, R. PERRY AND M. J. READE

*Department of Chemical Engineering, University of Birmingham, Birmingham 15 (Great Britain)*

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### SUMMARY

The combination of thin-layer chromatography with high-resolution mass spectrometry results in a highly sensitive rapid method of analysis for polycyclic aromatic hydrocarbons. Use of a micro centrifuge technique facilitates transfer of the eluted samples from the chromatogram to the mass spectrometer and enables limits of detection in the picogram region to be obtained. In conjunction with such other techniques as paper and thin-layer chromatography, the integrated ion-current method offers great potential as an analytical technique.

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### INTRODUCTION

The necessity to actively control air pollution has led to an increased interest in sensitive and specific methods of analysis for air pollutants. Of particular concern has been the identification and estimation of polycyclic aromatic hydrocarbons in that many of these substances are carcinogenic<sup>1</sup> in character and may well be associated with the incidence of lung cancer in polluted environments.

The existence of a large variety of other substances in association with the polycyclic compounds present in the atmosphere necessitates a preliminary analytical separation. For this such techniques as sublimation, distillation, solvent extraction, column, paper and thin-layer chromatography have all been used with varying degrees of success<sup>2</sup>. Following separation, estimation of the individual compounds is normally carried out by UV absorption or fluorescence measurements. In a recent review SAWICKI and co-workers claimed limits of detection by these methods as about 1  $\mu\text{g}$  for the UV absorption method<sup>3</sup> and between 1 and 10 ng for the fluorescence method<sup>4</sup>.

The only other technique that has been extensively used in the analysis of polycyclic aromatic hydrocarbons is gas chromatography. Although it cannot compete in sensitivity with the fluorescence methods, it has the advantage that a simultaneous analysis of many of the more volatile polycyclic compounds can be carried out on a routine basis.

More recently, however, the advantages of using high-resolution mass spectrometry in the identification and estimation of polycyclic compounds was demonstrated<sup>5</sup>. The ability of these substances to sublime rapidly into the ion source of the mass spectrometer facilitates the use of an integrated ion-current technique and enables detection limits in the subpicogram region to be attained. The sensitivity of this mass spectrometric technique is exploited by its use in conjunction with TLC. Transfer of the separated sample from the chromatogram is accomplished by use of a micro centrifuge method. By this procedure some 50% of the sample from the chromatogram is directly introduced into the mass spectrometer ion source for detection. The technique has the advantage that substances of differing mass that are eluted together can be resolved. Furthermore, the limits of detection for polycyclic compounds are several orders of magnitude lower than that of the fluorescence method.

## EXPERIMENTAL

### *Materials*

All polycyclic aromatic hydrocarbons were obtained from commercial sources.

### *Thin-layer chromatography*

Prepared TLC plates were obtained from "Camlab" Cambridge, England (250  $\mu$  cellulose powder MN300 on glass). Dimethylformamide-water (70:30) was used as solvent. Development was performed by the ascending method in the usual way.

### *Centrifuge tubes*

These were made from capillary tube (O.D. 6 mm, I.D. 1 mm). They had an overall length of 6 cm and were tapered into a funnel of diameter 1 cm at the open end.

### *Mass spectrometry*

Measurements were carried out with a G.E.C.-A.E.I. MS9 mass spectrometer fitted with a direct insertion probe.

### *Analytical procedure*

The mass spectrum of the individual polycyclic compound is first recorded by evaporating a microgram quantity into the source of the mass spectrometer. On examination of the resulting mass spectrum a characteristic ion is selected for the quantitative examination. With the polycyclic compounds this is frequently the molecular ion as it carries a high percentage of the total ion current.

Calibration curves are prepared by chromatographing standard quantities of each polycyclic compound in the weight range required. For quantities greater than 10 ng the position of the resulting spot can readily be determined by examination of the chromatogram under UV light. For smaller quantities, the sample must be run alongside a larger standard sample and the spot position determined with the aid of a template. The spot is then marked out, removed with a spatula and transferred to the capillary centrifuge tube with the aid of a platinum scoop and camel hair brush. The polycyclic compound is extracted from the cellulose by stirring with between 20 and 50  $\mu$ l of solvent and centrifuging the resulting suspension.

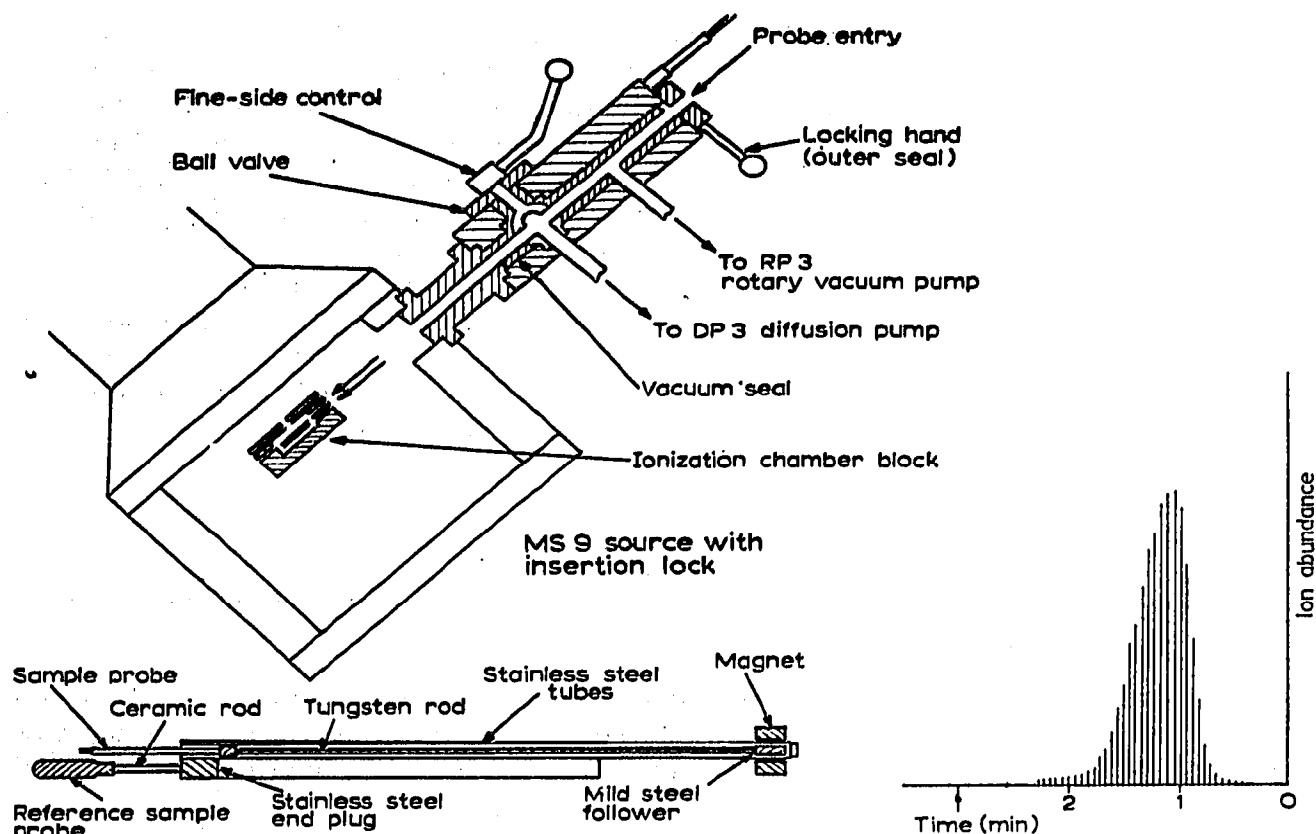


Fig. 1. MS9 source and direct insertion probe. The probe used in this work was not fitted with a reference sample probe.

Fig. 2 (bottom right). A typical integrated ion-current curve of a polycyclic hydrocarbon.

A measured volume of the sample solution is then transferred into the sample probe (Fig. 1). This is introduced into the vacuum system of the mass spectrometer through the insertion lock and held in the cool part of the system until the solvent has been evaporated off. A reference compound, heptacosafuoro-tri-*n*-butylamine, is introduced into the source by an alternative inlet, in order to calibrate the mass scale of the instrument. The sample probe is then lowered into the heated part of the source to allow evaporation of the sample whilst the rise and fall of the ion current is being recorded. Fig. 2 indicates a typical ion-current curve for one of the polycyclic compounds. The area under this curve is directly proportional to the amount of sample evaporated into the ion source.

## RESULTS AND DISCUSSION

Use of the integrated ion-current (IIC) method for the estimation of polycyclic compounds is facilitated by their characteristic properties in that they generally have low vapour pressures at room temperature and yet readily sublime without decomposition at temperatures between 200 and 500°. Furthermore, fragmentation results in the molecular ion carrying a high percentage of the total ion current making its use ideal for analytical purposes.

TABLE I

LIMIT OF DETECTION OF POLYCYCLIC COMPOUNDS BY THE IIC METHOD

<i>Polycyclic compound</i>	<i>m/e of molecular ion</i>	<i>Reference peak</i>	<i>Decade</i>	<i>Limit of detection (g)</i>
Fluorene	166	164	1.012715	$1 \times 10^{-12}$
Anthracene	178	169	1.053724	$1 \times 10^{-11}$
Fluoranthene	202	181	1.116111	$1 \times 10^{-12}$
Pyrene	202	181	1.116501	$1 \times 10^{-11}$
Benzo[ <i>a</i> ]anthracene	228	219	1.041574	$5 \times 10^{-13}$
Benzo[ <i>a</i> ]pyrene	252	231	1.091370	$5 \times 10^{-14}$
Benzo[ <i>g,h,i</i> ]perylene	276	264	1.045721	$1 \times 10^{-14}$
Coronene	300	264	1.136401	$1 \times 10^{-14}$

Initially calibration curves were constructed using standard solutions of each polycyclic compound. These were linear in all ranges between  $10^{-6}$  and  $10^{-12}$  g. By decreasing the concentration of these solutions, the limits of detection were found for several polycyclic aromatic hydrocarbons at an instrument gain of 1000 (Table I). These results do not represent the ultimate sensitivity of the method in that a further amplification of the ion current is possible which would lower the limit of detection by at least a further two orders of magnitude.

TABLE II

ANALYSIS OF A MIXTURE OF THREE POLYCYCLIC COMPOUNDS

<i>Polycyclic compound</i>	<i>Quantity introduced into mixture (ng)</i>	<i>Quantity detected (ng)</i>
Anthracene	20	21.5
Benzo[ <i>a</i> ]anthracene	20	22.7
Benzo[ <i>a</i> ]pyrene	20	21.0

The analysis of a mixture is summarized in Table II. Here successive aliquots of a solution are evaporated into the mass spectrometer source while the ion current is being recorded at the appropriate *m/e* value corresponding to the molecular ion.

Linear calibration curves are also obtained for each polycyclic compound following TLC. Fig. 3 illustrates typical examples for concentrations in the nanogram range using an instrument gain of 300.

Provided that the position of the eluted sample spot is known, it is possible to transfer samples in the picogram range from the chromatogram to the mass spectrometer. Extraction of the polycyclic compound from the cellulose adsorbent can be carried out in a micro centrifuge tube with as little as 20  $\mu$ l of solvent, enabling a large part of the eluted sample to be used in the mass spectral assay. It should be pointed out, however, that the sensitivity of the technique can be even further increased by decreasing the thickness of the adsorbent layer. In this way a smaller quantity of solvent would be required for the extraction. Table III lists the  $R_F$  values of polycyclic compounds separated as a mixture by TLC. Here satisfactory

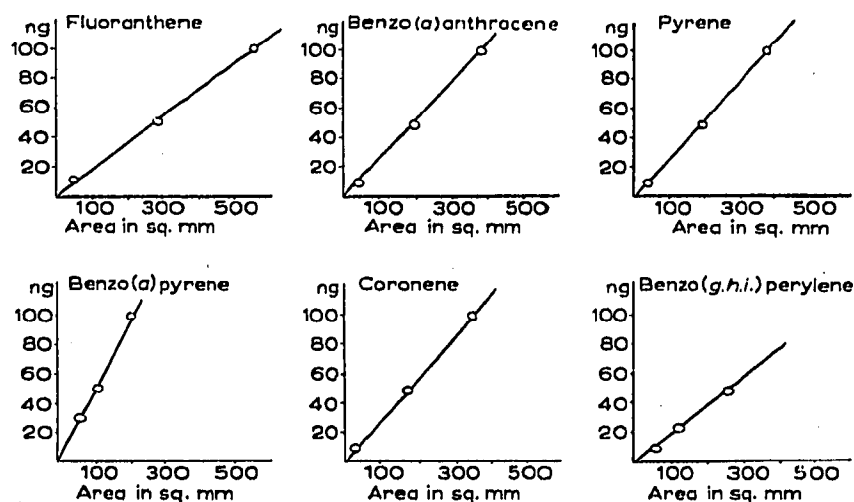


Fig. 3. Integrated ion-current calibration curves of polycyclic compounds after elution from the TLC plate.

TABLE III

SEPARATION OF POLYCYCLIC COMPOUNDS BY TLC

<i>Polycyclic compound</i>	<i>R<sub>F</sub> value</i>
Fluoranthene	0.73
Benzo[ <i>a</i> ]anthracene	0.68
Benzo[ <i>a</i> ]pyrene	0.62
Benzo[ <i>g,h,i</i> ]perylene	0.56
Coronene	0.45

resolution was obtained enabling ready identification of each substance by the IIC technique. A typical analysis is given in Table IV.

In the field of air pollution this mass spectrometric technique, in conjunction with either TLC or GC, offers great potential. For instance, the rapid estimation of benzo[*a*]pyrene and benzo[*k*]fluoranthene in the atmosphere<sup>6</sup> could be carried out by this procedure. With limits of detection in the picogram region, much smaller volumes of air could be sampled and changes in concentration of these carcinogens could readily be monitored.

TABLE IV

ANALYSIS OF A MIXTURE OF POLYCYCLIC COMPOUNDS FOLLOWING SEPARATION BY TLC

<i>Polycyclic compound</i>	<i>Quantity introduced into mixture (ng)</i>	<i>Quantity detected (ng)</i>
Fluoranthene	10	9.8
Benzo[ <i>a</i> ]anthracene	10	10.0
Benzo[ <i>a</i> ]pyrene	10	11.6
Benzo[ <i>g,h,i</i> ]perylene	10	12.1
Coronene	10	9.4

Further, it is well known that methyl derivatives of tetracyclic aromatic hydrocarbons are far more carcinogenic than their parent compounds. For example, chrysene is comparatively inactive as a carcinogen whereas its 4-, 5- and 6-derivatives have activities rated as +, +++ and + respectively<sup>1</sup>. The chromatographic separation of such compounds from the parent hydrocarbon is often impossible and the analysis of the mixture by UV absorption is unreliable. Such a problem could be readily resolved by the IIC method in that the substances concerned have differing mass numbers.

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#### DISCUSSION

AMOS: In the petroleum industry we are very concerned with possible air pollution by polycyclic aromatics as a result of pyrolysis of petroleum products. Of particular interest is the measurement of the benzo[*a*]pyrene content, one of the most potent carcinogens known. We have found it very difficult to separate benzo[*a*]pyrene from the less potent benzo[*e*]pyrene by TLC and consequently have been unable to accurately measure benzo[*a*]pyrene concentrations by mass spectrometry. Have you found it possible to make this measurement at low concentrations without recourse to such techniques as UV fluorescence spectroscopy?

PERRY: According to SAWICKI it is possible to resolve benzo[*a*]pyrene and benzo[*e*]pyrene by TLC. The integrated ion-current technique could therefore be used to monitor concentrations of these substances in the  $10^{-12}$  range. In an earlier paper<sup>5</sup> we have shown that these substances in low concentration can also be partially resolved by mass spectrometry.