Journal of Chromatography, 103 (1975) 208–210

CHROM. 7816

Note

Investigation of direct thin-layer chromatography–mass spectrometry as a drug analysis technique

G. J. DOWN and S. A. GWYN
Chemistry Division, Department of Scientific and Industrial Research, Lower Hutt (New Zealand)
(Received July 9th, 1974)

Thin-layer chromatography (TLC) and mass spectrometry can be combined in two main ways. In the first[1,2], a compound is eluted off the adsorbent and the resulting solution evaporated on a mass spectrometer inlet device. In the second, the compound is inserted into the mass spectrometer while still on the adsorbent. The latter direct method is the subject of this investigation.

The literature reports work which used the direct combination of the techniques in the analysis of simple aromatics[3], alicyclic carboxylic acids, sugar derivatives, and acylated amino acids and peptides[4]. The present study was undertaken to determine the conditions and limits for extension of this technique to more complex molecules, namely several drugs frequently encountered in analyses carried out by this laboratory.

MATERIALS AND METHODS

TLC was carried out on glass plates coated with Merck (Darmstadt, G.F.R.) GF_{254} silica gel (0.5 mm). BDH (Poole, Great Britain) AnalaR solvents were used for elution. The drugs were applied to the plates from standard solutions (10 mg/ml). After development, the spots were located under UV light (254 nm). The mass spectra were run on an AEI MS-30 mass spectrometer fitted with a heated solid insertion probe.

The silica gel containing the compound was removed from the plate and placed in glass capillary tubing (10 mm) with one end sealed (the standard MS-30 sample tip). The open end was plugged with glass wool. Diffuse thin-layer spots could not always be completely accommodated in the sample tip.

The mass spectrometer ion source was maintained at 150° and 70 eV ionisation energy. Samples were volatilised with the heater incorporated in the probe tip and spectra were run at 50° intervals up to 300–350° (the upper limit of the probe temperature control). These spectra were compared with reference spectra obtained from samples evaporated into sample tips without preliminary TLC (normal probe samples).

RESULTS AND DISCUSSION

Table I shows the important parameters for the series of drugs studied.

TABLE I

SUMMARY OF RESULTS

Solvent systems: (A) ammonia solution (s.g. 0.91)–methanol (1.5:100); (B) acetic acid–ethanol–water (30:50:20)[5]; (C) chloroform.

Compound	*Molecular weight*	*Highest m/e in mass spectrum of TLC sample*	*Eluting solvent*	*Normal practical limit* (μg)*	*Detection limit** (μg)*
Mepyramine	285	256	A	50	30
Phenazone	188	188	A	20	10
Caffeine	194	194	A	15	10
Methaqualone	250	250	A	20	10–15
Amylobarbitone	226	211	A	20	5–10
Diazepam	284	284	A/C	25	10
Chlorpheniramine	274 (230)***	230	A	40	20
Amitriptyline	277	232	A	15	10
Promethazine	284	213	B	20	5
Trimipramine	294	294	B	20	5–10
Propoxyphene	339 (266)***	208	B	5	5
Chlorpromazine	318	318	B	40	5–10
Codeine	299	—	B	—	—
Methadone	309	—	B	—	—

* Lower limit using only mass spectrometric data.

** Lower limit if additional information is available and mass spectrometry is used to check the identity of a specimen.

*** Rearrangement evident in normal probe spectrum. Highest *m/e* in brackets.

Mass spectral background

The number and intensity of background peaks was markedly dependent on the solvents used for elution. Although AnalaR solvents were used in all cases, some systems gave more numerous and intense background peaks than others. In particular, spectra run after elution with light petroleum (b.p. 40–60°) contained many intense peaks characteristic of high-molecular-weight hydrocarbons. Elution with methanol gave peaks characteristic of phthalate plasticisers.

Chloroform gave some background peaks up to about *m/e* 100 below 100–150° but these almost completely disappeared at higher temperatures. The ammonia–methanol mixtures gave some of the lowest background interferences encountered. Some large background peaks were produced by Clarke's T4 solvent system[5] up to about 120° (mostly *m/e* 60 and below, probably from the acetic acid). The intensity of these dropped markedly at higher temperatures.

Background spectra were run of silica gel which had no compound on it but which had been eluted with the solvent. This silica gel was removed from the plate at several points between the solvent front and the point of application of the spot. The number and intensity of the background peaks did not change significantly with position on the plate.

Temperature

At probe temperatures of 250–300° all of the drugs except codeine and methadone produced high-quality spectra. Below this range peak intensities were low except

for diazepam and propoxyphene, which gave reliable spectra at 200°. Contrary to expectations, very little evidence was shown of increased rearrangement or decomposition with increased temperature, even at 350°.

In addition to spectra run at the 50° intervals mentioned above, single ion monitoring was used to study the variation of sample volatilisation with temperature. Results for several compounds showed that about 90% of the material volatilised off the adsorbent at 300° or above. However, good quality spectra of some compounds were obtained at lower temperatures.

Reproducibility

Spectra obtained by the normal solid probe method usually contained only a few major ions which, for some compounds, did not include a significant parent ion. In most cases, volatilising the compound off silica gel caused one or two of these main peaks to disappear, sometimes with the appearance of one or two new major peaks. The main peaks in the spectra were found to be quite reproducible, the relative peak intensities varying by up to 20% usually and occasionally up to 30%. This consistency was found through virtually the whole temperature range over which spectra were obtainable (the variations being greatest at low probe temperatures) and, to a large extent, over the range of quantities used with the variations again greatest at lower levels.

General

The quality of the spectrum, for a given amount of drug, was related to the size of the TLC spot in a non-linear manner. The relationship between spot size and qnantity of material was also found to be non-linear. The combined influence of these two factors tended to give a threshold below which spectra were poor but above which they were reliable. It was found, in agreement with the work of Heyns and Grützmacher[4], that the threshold lay near the point at which the compound to silica gel ratio was 1 or 2%.

The combined TLC–mass spectrometry technique was found to be applicable to most of the drugs used, the notable exceptions being codeine and methadone. Both of these compounds failed to give useful spectra even using 60 μg and 350°. To be useful as a routine analysis technique for drugs, a collection of spectra off silica gel for compounds which are most commonly encountered is required. The lower workable limits may be improved by solvent redistillation and running background spectra for subtraction from the sample spectrum. However, as mentioned above, the background tends to be driven off before the compound starts to volatilise to any great extent so these extra measures would probably not be worth the inconvenience. No inherent limitations were found that would prevent extension of the combined technique to a wider range of drugs and other complex molecules.

REFERENCES

1 M. J. Rix, B. R. Webster and I. C. Wright, *Chem. Ind. (London)*, (1969) 452.
2 M. A. Schwartz, P. Bommer and F. M. Vane, *Arch. Biochem. Biophys.*, 121 (1967) 508.
3 F. T. Deverse, E. Gipstein and L. G. Lesoine, *Instrum. News*, 18, No. 2 (1967) 16.
4 K. Heyns and H. F. Grützmacher, *Angew. Chem.*, 74 (1962) 387.
5 E. G. C. Clarke, *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1969, p. 48.