Journal of Chromatography, 77 (1973) 234–239

CHROM. 6529

Note

A rapid method of thin-layer chromatography to aid the identification of quaternary nitrogen drugs

The quaternary nitrogen drugs present some problems when identifying them in a mixture of drugs or in tissues. The compounds are not extracted by organic solvents and remain in the aqueous extract after acidic and basic drugs have been removed[1]. This extract can be analyzed by ion-exchange chromatography, paper chromatography or column chromatography on Sephadex gels[2], but these methods are relatively time-consuming. Thin-layer chromatography (TLC) in the usual solvent systems results in the quaternary compounds remaining near the point of application, which is also a disadvantage of some methods of paper chromatography (see the data in the book edited by CLARKE[1]). This note describes a TLC method by which microgram amounts of quaternary nitrogen drugs can be separated and tentatively identified in less than 30 min. A similar procedure for the identification of some muscle relaxants has been described by others[3], with data on various solvent systems and chromogenic reagents.

Thin layers of Silica Gel H (Merck, G.F.R.) were spread on microscope slides (75 × 25 mm) by pouring a slurry of the gel in chloroform onto the surface, then pushing the slide under a bar held at an appropriate height above the glass to give layers 0.30 to 0.35 mm thick. This method produced more uniform layers than were obtained by dipping the slides into the slurry. They were allowed to dry in air at room temperature before use. The samples consisted of 1 μl of 0.1 *N* HCl containing 5 μg of the drug salts as shown in Table I. The chromatograms were developed in 250-cm³ wide-mouthed jars using 1 *M* HCl (5 min development time) or 1 *M* HCl–ethanol (1:1) for 15–20 min until the solvent front had travelled between 50 and 55 mm. The jars had been allowed to saturate with solvent vapour, but the chromatograms were developed immediately without equilibration. The slides were then dried with a hair-dryer and sprayed with Dragendorff's reagent (ref. 1, p. 799).

From Table I it can be seen that the R_F values in the ethanolic solvent were uniformly greater than in the plain acid solvent. Two broad groupings of the drugs could be made on the basis of their chromatographic behaviour. Compounds which had hydroxyl groups (the pyridinium oximes, the choline compounds and edrophonium) moved faster in each solvent than the second grouping of drugs which had no hydroxyl groups (hexamethonium, pentolinium) or had bulky hydrocarbon substituents (clidinium). Paraquat was unique in that it showed little change in R_F value between the two solvent systems. Chromatography on standard 200-mm plates (0.25-mm layers spread by conventional techniques) gave R_F values very similar to those obtained on the microscope slides, although the development times were extended to 40 min for 1 *N* HCl and 150 min for the ethanolic solvent. The rate of migration of the compounds on the microscope slides was somewhat variable

TABLE I

R_F VALUES (× 100) FOR VARIOUS QUATERNARY AND NON-QUATERNARY DRUGS IN TWO SOLVENT SYSTEMS

2-PAM iodide = 2-hydroxyiminomethyl-1-methylpyridinium iodide; TMB-4 dibromide = 1,1'-trimethylene bis(4-hydroxyiminomethylpyridinium bromide); toxogonin dichloride = bis(4-hydroxyiminomethylpyridinium-1-methyl) ether dichloride.

Drug	*1 M HCl*	*1 M HCl–ethanol (1:1)*
A. Quaternary compounds		
Clidinium bromide	00	01
Gallamine triethiodide	00	08
Pentolinium bitartrate	04	19
Hexamethonium dibromide	07	24
Paraquat dichloride	22	25
Decamethonium diiodide	06	34
Prostigmine bromide	14	47
d-Tubocurarine chloride	12	54
Bretylium tosylate	10	56
2-PAM iodide	37	60
Acetylcholine chloride	41	60
Carbamoylcholine chloride	51	61
Choline perchlorate	55	61
Toxogonin dichloride	40	63
TMB-4 dibromide	42	66
Edrophonium chloride	36	68
B. Non-quaternary compounds		
Chlordiazepoxide	26	45
Adrenaline	near 100	near 100
Strychnine creatinine sulphate	near 100	near 100
Ephedrine	56	near 100
Atropine sulphate	27	75
Neostigmine sulphate	23	69
Procaine hydrochloride	37	75

from experiment to experiment (for instance, $R_F \times 100$ values for 2-PAM ranged from 37 to 43 in 1 *M* HCl); therefore the data of Table I give the relative rates only and marker compounds would be required to identify a sample. Resolution of mixtures of similar compounds on the small slides was not good, but was much better on the 200-mm plates. For instance, a mixture of pentolinium, hexamethonium and decamethonium produced a single long spot on the microscope slide in 1 *M* HCl–ethanol (1:1), but good resolution of the three components was achieved on the longer plates. The sensitivity of the Dragendorff reagent varied with the compound being tested. Paraquat gave the best reaction, followed by decamethonium and related compounds, the pyridinium oximes, and then the choline group, which gave a weak reaction. Detection of 300 ng of paraquat was certain, whereas 1 μg of 2-PAM was difficult or impossible to detect. The use of an iodoplatinate spray did not improve the sensitivity. The properties of some non-quaternary basic drugs in this chromatographic system are given in Table I for comparison with the quaternary compounds. Some of the drugs were satisfactorily separated, although there was a tendency to travel near the solvent front. Exposure to iodine vapour was found to be a more sensitive way of detecting these compounds than the use of the Dragendorff spray.

Chromatography was also tried on commercially-prepared plastic sheets coated with Silica Gel F_{254} (E. Merck, G. F. R.). This was not successful since the layer was not readily wetted by the aqueous solvent systems used, and consequently long development times and erratic chromatography resulted.

In summary, the main advantage of the method described is that it provides a comparatively rapid means of tentatively identifying quaternary nitrogen drugs. For confirmation of identity more complete isolation of the material can be made, using the knowledge gained by TLC. A similar procedure for detection of narcotics by TLC on small pieces of silica gel sheets has been described[4], and such "mini" systems would seem to have general utility.

Australian Defence Scientific Service, H. D. CRONE
Defence Standards Laboratories, E. M. SMITH
Melbourne, Vic. 3032 (Australia)

1 J. V. JACKSON, in E. G. C. CLARKE (Editor), *Isolation and Identification of Drugs*, Pharmaceutical Press, London, 1969, p. 28.
2 H. D. CRONE, *J. Chromatogr.*, 60 (1971) 185.
3 C. WOLLMANN, S. NAGEL AND E. SCHEUBE, *Pharmazie*, 21 (1966) 605.
4 L. K. HO, H. H. LOH AND F. L. WAY, *J. Chromatogr.*, 65 (1972) 577.

First received October 17th, 1972; revised manuscript received December 5th, 1972