CHROM. 6099

A thin-layer chromatography screening technique for thiazide diuretics in urine*

Thiazide diuretics are of such minor consequence toxicologically that their recognition when investigating cases of acute drug overdosage is not of prime importance. Nevertheless, they are widely prescribed and their presence in urine may have to be distinguished from that of other drugs such as barbiturates, benzodiazepines and even sulphonamides, which are commonly sought by thin-layer chromatography (TLC). Furthermore, when urines from patients are being studied analytically to monitor the drugs which they have actually taken, as distinct perhaps from those that have been prescribed, it is essential to be able to identify any of the thiazide diuretics, since these so frequently occur.

Paper chromatography (PC) and TLC of the thiazide diuretics have been described utilising location by ultraviolet light (UV) (254 nm), 1,2-naphthaquinone-4-sulphonate and sodium pentacyanoaminoferrate[1-3]. Trials of these methods showed them to be unsuitable for the detection of the drugs in urine following therapeutic doses of 10–80 mg per day, since they were either too slow or subject to interference from endogenous components of urine. Colorimetric assays involving diazotisation after hydrolysis have been described for chlorothiazide[4], hydrochlorothiazide[5], bendrofluazide[6], and frusemide[7]. The present work involves the application of the BRATTON–MARSHALL reaction[8] to the detection of diuretics after separation by solvent extraction and TLC. The structures of the thiazide diuretics studied are shown in Table I.

Experimental

Thin-layer chromatography. Plates were prepared from a slurry of Silica Gel G (30 g) in water (65 ml). Layers of thickness 250 μ were prepared and dried at 110° for 30 min. Reference solutions of the drugs in ethyl acetate (1 μg/μl) were prepared.

Location regents. The following reagents were used: (1) sodium nitrite (1 % in 1 % H_2SO_4); (2) ammonium sulphamate (5 % aq.); (3) N-(1-naphthyl)-ethylenediamine dihydrochloride (1 % in 80 % acetone).

Extraction procedure. Urine (10 ml) was acidified with hydrochloric acid (1 ml, 1 *N*) and extracted with ethyl acetate (10 ml) for 10 min on a mechanical shaker. After centrifugation, the aqueous layer was aspirated off and the organic layer washed with lead acetate (10 ml, 5 % aq.). The ethyl acetate was then transferred to another tube and evaporated to dryness.

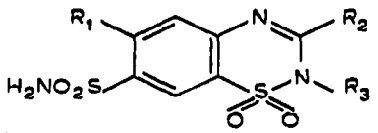
Hydrolysis. Dried urine extracts and pure drugs were hydrolysed with sodium hydroxide (1 ml, 10 *N*) at 15 p.s.i. for 15 min. The samples were then acidified and extracted with ethyl acetate (10 ml). The organic layer was evaporated to dryness in a 10-ml conical tube.

Detection. The dried unhydrolysed extracts were reconstituted with acetone (100 μl) and 30-μl aliquots were applied to the thin-layer plate, together with 10 μl of the reference solutions of the pure drugs. Similar amounts of the hydrolysed extracts and standards were applied to a second plate. The plates were developed in an un-

* Reprints from: New Cross Hospital, Poisons Unit, Avonly Road, New Cross, London S.E. 14, Great Britain.

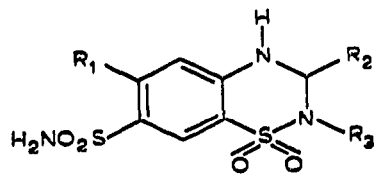
TABLE I

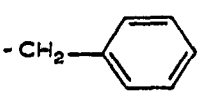
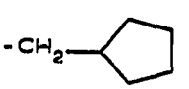
Chlorothiazide derivatives



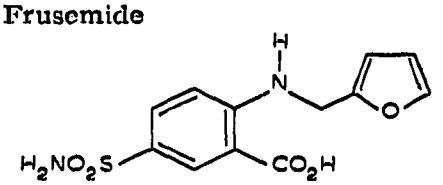
Drug	R_1	R_2	R_3
Chlorothiazide	–Cl	–H	–H

Hydrochlorothiazide derivatives



Drug	R_1	R_2	R_3
Hydrochlorothiazide	–Cl	–H	–H
Hydroflumethiazide	$-CF_3$	–H	–H
Bendrofluazide	$-CF_3$		–H
Cyclopenthiazide	–Cl		–H

Frusemide



saturated tank containing a mixture of ethyl acetate–benzene (8:2). After development, the unhydrolysed drugs were sprayed with concentrated hydrochloric acid and placed in an oven at 100° for 10 min. The hydrolysis products were then located by spraying both plates with the BRATTON–MARSHALL reagents. Results were improved by drying the plates with a hair drier in between each spray.

Discussion

For simple screening purposes, the thiazide diuretics are readily distinguished

by chromatographing unhydrolysed urine extracts, followed by "on-plate" hydrolysis and application of the BRATTON–MARSHALL spray sequence. R_F values and the colours produced are quite distinct for the six diuretic drugs under study (Table II). It is clear that if sulphonamide drugs are present in the urine sample, these will also be detected by this procedure. These can be distinguished by: (a) spraying the unhydrolysed plate with *p*-dimethylaminobenzaldehyde spray (1 % in ethanol + conc. HCl (10 ml)), which gives lemon yellow colours with sulphonamides[9] but not with thiazides; (b) concomitant chromatography of unhydrolysed and hydrolysed urine extracts: a free sulphonamide drug will give spots of the same R_F value in both extracts, on the other hand, a thiazide diuretic will be chromatographed firstly as the unchanged drug and secondly as the hydrolysis products, (Table III), thus giving two distinct R_F values; and (c) the colours produced by diazotising the hydrolysed diuretics and free sulphonamide drugs are different. The use of a lead acetate wash to remove urinary pigments from solvent extracts was suggested by FRAHM *et al.*[10] and was found to clarify the subsequent chromatograms. The benzodiazepine group of drugs are also capable of diazotisation following hydrolysis to the corresponding 2-aminobenzophenones. Analysis of urine extracts from patients receiving therapeutic doses of nitrazepam and diazepam in conjunction with thiazide drugs did not reveal any extraneous chromatogram spots. The method has been shown to be very satisfactory for the detection of thiazide diuretics in urine from patients receiving therapeutic doses of these drugs.

TABLE II

R_F VALUES OF THIAZIDE DIURETICS IN ETHYL ACETATE–BENZENE (8:2) (ref. 3)

Drug	*R_F value*	*Colour after diazotisation*
Chlorothiazide	0.26	Salmon pink
Hydrochlorothiazide	0.52	Salmon pink
Cyclopenthiazide	0.94	Salmon pink
Hydrofluazide	0.78	Orange-pink
Bendrofluazide	0.98	Orange-pink
Frusemide	0.15	Cerise

TABLE III

HYDROLYSIS PRODUCTS OF THIAZIDE DIURETICS

Compound	*Parent drug*	*R_F ethyl acetate–benzene (8:2)*	*Colour after diazotisation*
2-Amino-4-chlorobenzoic acid-5-sulphonamide	Frusemide	0.29 (streak)	Cerise
4-Amino-6-chlorobenzene-1,3-disulphonamide	Chlorothiazide Hydrochlorothiazide Cyclopenthiazide	0.80	Salmon pink
4-Amino-6-trifluoromethylbenzene-1,3-disulphonamide	Bendrofluazide Hydroflumethazide	0.93	Orange-pink

The author wishes to thank Dr. R. GOULDING and Dr. B. WIDDOP for their advice and encouragement during the course of this work. I also wish to thank S. J. HARSTON of Hoechst Pharmaceuticals for the supply of Frusemide.

Poisons Unit, Guy's Hospital, B. G. OSBORNE
London S.E. 1 (Great Britain)

1 V. B. PILSBURY AND J. V. JACKSON, *J. Pharm. Pharmacol.*, 18 (1966) 713.
2 R. MAES, M. GIJBELS AND L. LARUELLE, *J. Chromatogr.*, 53 (1970) 408.
3 R. ADAM AND C. L. LAPIÈRE, *J. Pharm. Belg.*, 19 (1964) 79.
4 J. E. BAER, H. LORRAINE LEIDY, A. V. BROOKS AND K. H. BEYER, *J. Pharmacol. Exp. Ther.*, 125 (1959) 295.
5 H. SHEPPARD, T. F. MOWLES AND A. J. PLUMMER, *J. Amer. Pharm. Ass. (Sci. Ed.)*, 49 (1960) 722.
6 J. J. PIALA, J. W. POUTSIAKA, C. I. SMITH, J. C. BURKE AND B. N. CRAVER, *J. Pharmacol. Exp. Ther.*, 134 (1961) 273.
7 P. HADJU AND P. HAÜSSLER, I, *Arzneim.-Forsch.*, 14 (1964) 709.
8 A. C. BRATTON AND E. K. MARSHALL, *J. Biol. Chem.*, 128 (1939) 537.
9 E. G. C. CLARKE AND D. J. HUMPHRIES, *J. Pharm. Pharmacol.*, 22 (1970) 845.
10 V. M. FRAHM, A. GOTTESLEBEN, K. SOEHRING, *Pharm. Acta Helv.*, 38 (1963) 785.

Received March 17th, 1972