J. Pharm. Pharmac., 1966, **18**, 713–720 Received April 29, 1966

Identification of the thiazide diuretic drugs

VALERIE B. PILSBURY AND J. V. JACKSON

A method for detecting and identifying ten thiazide diuretic drugs in tablets, gastric washings and urine by ultraviolet spectrophotometry and paper chromatography is described. The thiazides can be detected by absorption maxima in the region 264 to 294mμ at concentrations of 5 μg/ml or lower. Identification is by ascending high temperature reverse phase paper chromatography using tributyrin treated paper and developing for 20 min at 90° with a phosphate buffer (pH 7·4). Further differentiation is obtained with the solvent system amyl alcohol—0·880 ammonia (9:1) on Whatman No. 1 paper. The thiazides are located as absorbing or fluorescent spots in ultraviolet light (2534 Å) and these are confirmed by the stable red colour given by an alkaline sodium 1,2-naphthaquinone-4-sulphonate spray reagent. Many of these diuretics are co-extracted with barbiturates and may interfere with barbiturate determinations.

NON-mercurial oral diuretic drugs are unlikely suicidal agents because of their relatively low toxicity, but their widespread use ensures that they will be encountered during routine screening for poisons or in the identification of unknown pharmaceutical preparations. Most of these compounds have a thiazide structure (i.e. a substituted 1,3-benzene-disulphonamide). Chlorothiazide (6-chlorobenzo-1,2,4-thiadiazine-7-sulphonamide-1,1-dioxide) may be considered as the parent compound.

Many of the published methods for detecting and estimating these drugs rely on hydrolysis, diazotisation and coupling to form a coloured azo-dye which can be estimated colorimetrically. This is the basis of the procedure used by Sheppard, Mowles & Plummer (1960) to determine chlorothiazide and hydrochlorothiazide in urine, and by Bermejo (1961) to estimate hydroflumethiazide and bendroflumethiazide. But although the procedure is very sensitive it is not specific.

Spectrophotometric determination of chlorothiazide and hydrochlorothiazide has been reported by Marciszewski (1960), and also by Charnicki, Bacher, Freeman & DeCesare (1959) who used it for the estimation of chlorothiazide in tablets. Sunshine & Gerber (1963) have investigated the ultraviolet and infrared spectra of hydrochlorothiazide and Kaye (1961) the ultraviolet spectrum of chlorothiazide. While the British Pharmacopoeia and the British Pharmaceutical Codex 1963 use ultraviolet absorption spectra in the assay of chlorothiazide, hydrochlorothiazide, hydroflumethiazide, benzthiazide and bendrofluazide, crystal tests were used to detect chlorothiazide, hydrochlorothiazide, hydroflumethiazide and acetazolamide by Groenewegen (1960) and to detect chlorothiazide and hydrochlorothiazide by Zoetten (1960).

The spectrophotometric and chromatographic behaviour of acetazolamide and chlorothiazide has been examined by Kraemar & Vacek (1960) and by Kala (1961), who also studied chlorazanil and hydrochlorothiazide.

No systematic scheme for the identification of this group of drugs appears to have been published and many of the reported methods assume prior knowledge of the compounds sought. We now report on

From the Metropolitan Police Forensic Science Laboratory, Toxicology Department, 2 Richbell Place, Holborn, London, W.C.1.

the spectrophotometric and chromatographic examination of these thiazides, and also of frusemide which has been included because it has some structural similarities and is an important diuretic.

ULTRAVIOLET SPECTROPHOTOMETRY

Standard solutions of the diuretics containing 10 μg/ml were prepared with distilled water made alkaline to pH 10 (Universal Indicator paper) with 2N ammonia solution. These solutions were stable for several days whereas standard solutions prepared in dilute sodium hydroxide deteriorate in a few hours. The ultraviolet absorption spectra of the solutions

TABLE 1. MELTING POINTS, ULTRAVIOLET ABSORTPION AND CHROMATOGRAPHIC DATA OF THE THIAZIDE DIURETICS

Approved name	M.p. °C.	pH 10		pH 2		Paper chromatography			
		λ max	$E(1\%, 1\text{ cm})$	λ max	$E(1\%, 1\text{ cm})$	Rf values system 1	Rf values system 2	Ultra-violet light	**NQS
Polythiazide[1] ..	209	264 315	460 80	270 310	550 135	0·88	0·05	Absorbs	++
Methyclothiazide[2] ..	216	264 318	420 60	270 315	525 120	0·73	0·20	Absorbs	++
Hydroflumethiazide[3]	226 –229 decomp.	274 330	450 80	273 325	580 100	0·62	0·06	Fluores.	+
Cyclopenthiazide[4] ..	380	273 320	375 40	272 315	470 45	0·80	0·06	Absorbs	+
Hydrochlorothiazide[5]	273	274 325	620 110	272 315	760 90	0·08	0·62	Absorbs	++
Bendrofluazide[6] ..	262	275 335	480 100	273 330	620 120	0·22	0·64	Fluores.	++
Chlorothiazide[7] ..	342	294 315S	630	280 272, 294S 305S	580	0·05	0·75	Absorbs	++
Benzthiazide[8] ..	241	298 315	290 270	282 300S	190	0·36	0·56	Absorbs	+
Teclothiazide[9] ..	230 decomp.	270 315	350 80	265 310	455 80	0·72	0·21	Absorbs	++
Frusemide[10]	216	272 333	810 200	275 340	860 250	0·75	0·17	Fluores. green	++

(S = Shoulder.) (++ = Strong positive. + = Positive.)

* With the exception of chlorothiazide and hydrochlorothiazide, the compounds in the Table have not been examined by both chromatographic and spectrophotometric methods so far as we are aware.
** See reagents, p. 717.

[1] 6-Chloro-3,4-dihydro-2-methyl-3-(2,2,2-trifluoroethylthiomethyl)benzo-1,2,4-thiadiazine-7-sulphonamide-1,1-dioxide[Nephril, Renese].
[2] 6-Chloro-3-chloromethyl-3,4-dihydro-2-methylbenzo- 1,2,4-thiadiazine-7-sulphonamide 1,1-dioxide [Enduron].
[3] 3,4-Dihydro-6-trifluoromethylbenzo-1,2,4-thiadiazine-7-sulphonamide 1,1-dioxide [Di-Ademil, Hydrenox, Naclex, Rontyl].
[4] 6-Chloro-3-cyclopentylmethyl-3-4-dihydrobenzo-1,2,4-thiadiazine-7-sulphonamide 1,1-dioxide [Navidrex].
[5] 6-Chloro-3,4-dihydrobenzo-1,2,4-thiadiazine-7-sulphonamide 1,1-dioxide [Dichlotride, Direma, Esidrex, Esidrex, Hydril, Hydro-Diuril, Hydrodiuril, Hydrosaluric, Hydrothide, Oretic].
[6] 3-Benzyl-3,4-dihydro-6-trifluoromethylbenzo-1,2,4-thiadiazine-7-sulphonamide 1,1-dioxide [Aprinox, Centyl, Neo-Naclex].
[7] 6-Chlorobenzo-1,2,4-thiadiazine-7-sulphonamide 1,1-dioxide [Alurene, Chlorotride, Diuril, Minzil, NeoDema, Salisan, Saluric, Saluretil, Yadalan].
[8] 3-Benzylthiomethyl-6-chlorobenzo-1,2,4-thiadiazine-7-sulphonamide 1, 1-dioxide [Fovane, Dytide].
[9] 6-Chloro-3,4-dihydro-3-trichloromethylbenzo-1,2,4-thiadiazine-7-sulphonamide 1,1-dioxide [Deplet].
[10] 4-Chloro-*N*-furfuryl-5-sulphamoylanthranilic acid [Lasix, Furosemide].

were determined between 230 and 350 mμ with a Hilger & Watts H.999 automatic recording spectrophotometer. The sample and reference solutions were then acidified with sulphuric acid and the absorption spectra again determined.

The results indicated that small changes in the pH value do not cause significant variations in the spectra but diagnostic features can be obtained if both acid and alkaline spectra are recorded. Thus hydroflumethiazide, cyclopenthiazide, hydrochlorothiazide, bendrofluazide and frusemide exhibit little or no variation in the position of the maxima at 272 to 275 mμ but polythiazide, methyclothiazide, chlorothiazide, benzthiazide and teclothiazide show changes greater than 5 mμ. When the alkaline solutions of polythiazide and methyclothiazide are acidified and replotted the strong absorption maxima at 264 mμ shifts to a higher wavelength (270 mμ) whereas the absorption maxima of chlorothiazide, benzthiazide and teclothiazide move to lower wavelengths. Eight of the ten diuretics examined show a significant increase in the absorption maxima at 260 to 275 mμ on changing from alkaline to acid pH. Distinctive characteristics are obtained for eight of the ten diuretics studied, the unresolved pair being polythiazide and methyclothiazide.

The main purpose of this examination of the thiazide ultraviolet spectra was to detect and identify these drugs; the extinction values shown in Table 1 are therefore intended only as an indication of the sensitivity of the method and for approximate quantitative determination of the drugs. They should not be used for accurate spectrophotometric assays. Rogers (1964) has stressed that particular care is needed to avoid spuriously low results, and recommends the adoption of a procedure in which the extinction of the sample is compared with that of a reference substance under the same conditions. Table 2 gives the values published for five

TABLE 2. COMPARISON OF PUBLISHED ULTRAVIOLET SPECTROPHOTOMETRIC DATA

Compound	Solvent	Wave-length absorp-maximum mμ	$E(1\%, 1\text{ cm})$	Wave-length absorp-maximum mμ	$E(1\%, 1\text{ cm})$	Reference
Chlorothiazide ..	0·1N NaOH	292	430	—	—	British Pharmacopoeia 1963
	,,	291	425	—	—	Marciszewski (1961)
	,,	292	—	—	—	Charnicki & others (1959)
	0·5N NaOH	294	444	—	—	Kaye (1961)
	H_2SO_4	278	301	—	—	Kaye (1961)
	Ethanol	280	—	—	—	Kraemar & Vacek (1960)
Hydrochlorothiazide	Ethanol	271	660	317·5	120	British Pharmacopoeia 1963
	Methanol and trace HCl	271	654	317	130	Merck Index (1960)
	Methanol	271	—	—	—	Rehm & Smith (1960)
	0·1N NaOH	273	525	—	—	Marciszewski (1961)
	0·1N NaOH	272	520	322	90	Sunshine & Gerber (1963)
	H_2SO_4	271	650	314	110	Sunshine & Gerber (1963)
Bendrofluazide ..	0·01N NaOH	273	413	329	80	British Pharmacopoeia 1963
Hydroflumethiazide	0·01N NaOH	274	460	333	95	British Pharmacopoeia 1963
Benzthiazide.. ..	0·01N ethanolic HCl	283	284	—	—	British Pharmaceutical Codex 1963

of these drugs and shows the variations in both wavelength and extinction values. These variations are probably due to instrumental differences, type of solvent, and possibly to the instability of some of these compounds. A note on the stability of the benzothiazines has been published by Yamana & Koike (1961), and Arizan & Sterescu (1960), Rehm & Smith (1960) and Marciszewski (1960) have reported the presence of 4-amino-6-chlorobenzene-1,3-disulphonamide as an impurity in some samples of chlorothiazide and hydrochlorothiazide.

PAPER CHROMATOGRAPHY

The two solvent systems chosen, namely the high temperature reverse-phase system (Street, 1962) and the amyl alcohol-ammonia system (Jackson, 1958) give good distribution of this group of drugs and are already widely used. Brief details of these systems are given below. The Rf values obtained are shown in Table 1. The use of both systems permits unequivocal identification of any particular thiazide diuretic (see Table 3).

TABLE 3. R_c VALUES OF THE THIAZIDE DIURETICS, WHERE R_c = DISTANCE MOVED BY THIAZIDE/DISTANCE MOVED BY CHLOROTHIAZIDE

Name	Rc values for system 1	Rc values for system 2
Chlorothiazide	1	1·0
Bendrofluazide	4	0·85
Hydrochlorothiazide	6	0·83
Benzthiazide	7	0·75
Hydroflumethiazide	12	0·08
Teclothiazide	14	0·28
Frusemide	15	0·23
Methyclothiazide	15	0·27
Cyclopenthiazide	17	0·08
Polythiazide	18	0·07

Location. Most of the common location reagents did not react with these compounds, but experiments with sodium 1,2-naphthaquinone-4-sulphonate indicated that it might provide a selective group reagent. Feigl (1954) mentions the use of this compound to detect substances containing two removable hydrogen atoms attached to carbon or nitrogen. Coloured paraquinoid condensation products are formed.

The drugs were located with ultraviolet light (2534 Å), and an alkaline solution of sodium naphthaquinone-4-sulphonate (NQS) was sprayed on the chromatograms giving stable orange-red colours with the thiazide diuretics. This reagent did not react with other solvent-soluble acidic and neutral drugs (e.g., salicylates, barbiturates, carbamates, hydantoins, phenacetin, glutethimide), and provides a useful confirmatory test for the thiazide drugs, distinguishing them from other drugs which give absorbing or fluorescent spots on chromatograms. Mercurous nitrate solution may also be used to locate synthetic diuretics (Kala, 1961), but this reagent reacts with many other drugs including barbiturates.

Experimental

REAGENTS

Tributyrin solution. Prepare a solution containing 10% v/v glycerol tributyrate (tributyrin) in acetone. *Buffer solution pH* 7·4. Dissolve 1·5 g potassium dihydrogen phosphate and 7·9 g disodium hydrogen phosphate in 1 litre distilled water. *NQS reagent.* Prepare a saturated solution of sodium 1,2-naphthaquinone-4-sulphonate (NQS) in 50% v/v ethanol and water. *Solvent system* 1. Mix together amyl alcohol (180 ml) and concentrated ammonia solution (20 ml) (sp.gr. 0·880). Shake well. *Thiazide standards.* Prepare a solution containing 10 mg/ml of the relevant thiazide diuretic in acetone.

GENERAL PROCEDURE

System 1. Samples of the thiazide standard and of the unknown were applied in 1 μl and 5 μl quantities on the baseline of a Whatman No. 1 paper to give spot sizes less than 5 mm. The paper was then developed by the ascending method with solvent system 1 at room temperature for $6\frac{1}{2}$ hr. No equilibration is necessary with this solvent.

System 2. A sheet of Whatman No. 3 paper cut to fit a small chromatography tank was dipped in the tributyrin solution, blotted and dried at room temperature. Samples of the thiazide standards and the unknown were applied to the prepared paper as before and the chromatogram was developed by the ascending method in the phosphate buffer solution at 90° $\pm$ 5° for 20 min.

Location. The dried chromatograms were examined by ultraviolet light (2534 Å) and the fluorescent or absorbing spots marked with pencil. The chromatograms were then lightly sprayed with 0·1N sodium hydroxide solution and with the NQS reagent. The thiazides appeared as stable orange spots within 5 to 15 min.

EXTRACTION

The methods of extraction were those used in routine screening of pharmaceutical and biological samples submitted for qualitative analysis for poisons and drugs. They are not necessarily the most efficient, but they have been developed to meet the particular problem of searching for and identifying an unknown substance.

Tablets. The tablet is crushed in a mortar and triturated with distilled water (3 $\times$ 20 ml). The combined water extracts are filtered through a Whatman No. 1 paper and the filtrate is examined at pH 10 and pH 2 with a recording ultraviolet spectrophotometer. If a diuretic drug is indicated the solution is acidified, saturated with ammonium sulphate, and extracted three times with an equal volume of ether. After drying with anhydrous sodium sulphate, the solvent is filtered and evaporated and the residue taken up in a small amount of ethanol for paper chromatography.

Urine or gastric washings. The sample is acidified with dilute hydrochloric acid and saturated with solid ammonium sulphate, filtered, and the

filtrate extracted with 2 volumes of ether by shaking in a separating funnel for ½ min. The ether layer is separated and the aqueous layer returned to the separating funnel. The ether extraction is repeated and the ether extracts pooled. The aqueous phase is retained for extraction of basic drugs if required. The ether extracts are washed with 2 × 10 ml of freshly prepared saturated sodium bicarbonate solution. The bicarbonate washings are separated, and labelled "A". The ether is now extracted with 2 × 20 ml of 0·1N sodium hydroxide solution and these extracts are labelled "B".

The "A" and "B" extracts contain the strong and weak acidic drugs respectively. Most of the thiazide diuretics are extracted into the "B" group but chlorothiazide, hydrochlorothiazide and frusemide are in group "A".

The bicarbonate extracts "A" are acidified, re-saturated with ammonium sulphate, filtered and ether extracted. The ether extract is dried with anhydrous sodium sulphate, treated with a small amount of charcoal, filtered and evaporated. The residue is taken up in a known volume of distilled water and filtered. The filtrate is made alkaline with ammonia, and examined in an ultraviolet spectrophotometer. If the solution exhibits an absorption maximum between 264 and 300 mμ it is acidified and replotted.

The sodium hydroxide extract "B" may sometimes be examined directly in the ultraviolet spectrophotometer but if this extract is cloudy or coloured it should be treated as extract "A". The thiazides may be recovered from these solutions after ultraviolet examination by acidification, saturation and ether extraction. The residues obtained after evaporation of the ether are dissolved in the minimum volume of acetone for chromatographic examination.

Discussion

The qualitative analysis of pharmaceuticals is frequently required in forensic science. Many of these analyses are of the "general unknown" type, that is to say the analyst has no prior knowledge of the type of drug; it is important therefore that any scheme of analysis should be generally applicable and without too many specialised procedures. Once such a scheme has been established it is essential to know how new drugs will behave with the reagents and solvent systems employed, and whether they will interfere with the familiar chromatographic and spectral patterns of the established drugs and poisons. The use of the diuretic drugs in heart diseases and in the treatment of barbiturate poisoning by "forced diuresis" indicated that an examination of their analytical behaviour should be useful to the forensic scientist and clinical biochemist.

This investigation of the ultraviolet spectrophotometric properties of the diuretic thiazides shows that the $E(1\%, 1\text{ cm})$ values of these drugs are so good that, despite their low water solubility, they can be easily detected by simple aqueous extraction of tablets and ultraviolet spectrophotometric screening. Thus, maxima in the 270 to 280 mμ and 310 to 315 mμ regions

at pH 2 provide the analyst with a good clue to the presence of these drugs and confirmation is obtained by replotting at pH 10 followed by paper chromatographic examination.

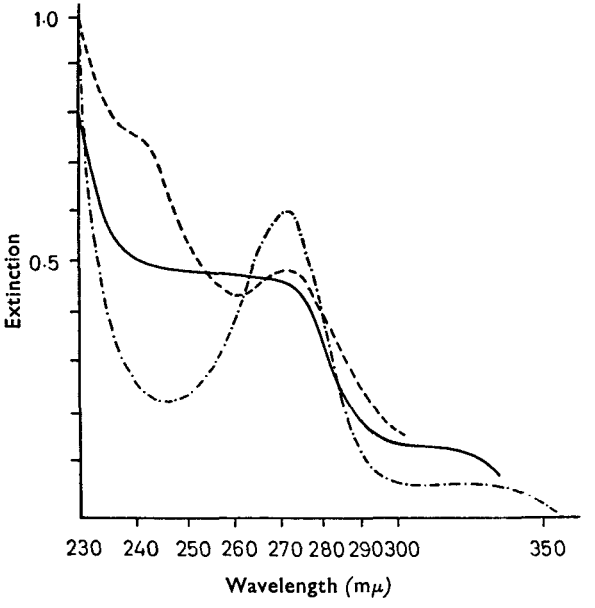

FIG. 1. Ultraviolet spectra of the weak acid group (Residue 'B') obtained from a urine containing equal quantities of pentobarbitone and polythiazide. The typical 240 mμ maximum of pentobarbitone at pH 10 is partially obscured and the 255 mμ maximum at pH 13 is obliterated by the presence of polythiazide. — · — · — pH 2; — — — pH 10; —— pH 13.

Many of these drugs can be extracted along with the barbiturates. This can cause distortion of the typical barbiturate spectra, e.g. the characteristic 240 mμ peak at pH 10 may be partially obscured and the 255 mμ peak at pH 14 of the 5,5-di-substituted barbiturates may be almost obliterated by the presence of these diuretics. The spectra obtained from the weak acid group (residue B) when equal quantities of polythiazide and pentobarbitone were added to a urine sample, are shown in Fig. 1. Quantitative barbiturate estimations based on the difference between the extinction at 260 mμ at pH 14 and pH 10 are seriously affected. Methods based on the 240 mμ differential absorption at pH 10 and pH 2 are less disturbed.

Additional maxima obtained between 270 and 315 mμ should however warn the analyst that he is dealing with a mixture; paper chromatographic examination would then confirm this fact. The presence of unusual absorbing or fluorescing spots on the chromatogram which do not react with the common acidic group location reagents (e.g. ferric chloride, cobalt nitrate) should be investigated by spraying with the NQS reagent.

The thiazide diuretics therefore do not offer an insurmountable obstacle to the detection and determination of the barbiturates if a full spectral plot and paper chromatographic examination are made.

References

Arizan, S. & Sterescu, M. (1960). *Revue Chim., Buc.* **11**, 297–298.
Bermejo, J. (1961). *Galenica Acta*, **14**, 255–264.
Charnicki, W. F., Bacher, F. A., Freeman, S. A. & DeCesare, D. H. (1959). *J. Am. Pharm. Ass., Sci. Edn*, **48**, 656–659.
Feigl, F. (1954). In *Spot Tests and Organic Applications*, Vol. 11, p. 221, London: Elsevier Publishing Co.
Groenewegen, H. (1960). *Pharm. Weekbl. Ned.*, **95**, 345–350.
Jackson, J. V. (1958). In *Chromatographic Techniques*, editor, Smith, I., p. 217. London; Heinemann.
Kala, H. (1961). *Pharmazie*, **16**, 297–305.
Kaye, S. (1961). In *Handbook of Emergency Toxicology*, 2nd edn, p. 144. Springfield; Charles C. Thomas.
Kraemar, J. & Vacek, J. (1960). *Čolká Farm*, **9**, 497–502.
Marciszewski, H. (1960). *Chemia analit.*, **5**, 617–624.
Marciszewski, H. (1961). *Dissnes pharm., Warsz.*, **13**, 233–241.
Rehm, C. R. & Smith, J. B. (1960). *J. Am. pharm. Ass., Sci. Edn.*, **49**, 386–389.
Rogers, A. R. (1964). *J. Pharm. Pharmac.*, **16**, 433–434.
Sheppard, H., Mowles, T. F. & Plummer, A. J. (1960). *J. Am. pharm. Ass., Sci. Edn*, **49**, 722.
Street, H. V. (1962). *J. forens. Sci.*, **7**, 222.
Sunshine, I. & Gerber, S. R. (1963). In *Spectrophotometric Analysis of Drugs*, pp. 46, 154. Springfield; Charles C. Thomas.
Yamana, T. & Koike, H. (1961). *J. pharm. Soc. Japan*, **81**, 783–784.
Zoetten, E. (1960). *Pharm. Weekbl. Ned.*, **95**, 174–176.