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The identification, assay and purity determination of chlorpropamide, glibenclamide and tolbutamide and their tablet preparations by thin-layer chromatography

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Abstract: A thin-layer chromatographic procedure is described for the identification and quantitative determination of chlorpropamide, glibenclamide and tolbutamide in both powder and tablet form. The coefficient of variation of the method is 0.6–0.7%, and results show good agreement with those obtained by the B.P. methods of assay. The TLC procedure has the advantage of greater specificity, and can also be used to identify and limit degradation products that may be present.

Keywords: *Assay; chlorpropamide; glibenclamide; tolbutamide; thin-layer chromatography.*

Introduction

The *British Pharmacopoeia* [1] contains monographs for three antidiabetic sulphonylurea drugs, chlorpropamide, glibenclamide and tolbutamide, and their tablet preparations. The specifications given for these closely related compounds lack uniformity, however, and this can cause some confusion, particularly as regards positive identification.

Gas–liquid chromatographic procedures have been used to separate tolbutamide from chlorpropamide [2–4] and from glibenclamide [5]. High-performance liquid chromatographic methods of assay have also been developed which can separate tolbutamide from chlorpropamide [6–9] and from glibenclamide [10], or can be used for all three compounds [11].

Surborg *et al.* [12] have shown that the three drugs can be separated by thin-layer chromatography (TLC). The TLC system used in the present work, however, is a modification of that used in the *British Pharmacopoeia* (B.P.) for Glibenclamide. A single tablet extraction technique is used, and the procedure provides a combined test of identification, assay and purity that is applicable for all three drugs. The assay is more

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specific than the B.P. spectrophotometric assays for tablets of chlorpropamide or glibenclamide, since these measure sulphonamide degradation products, in addition to the sulphonylurea.

Materials and Methods

Materials

Pre-coated TLC silica gel F_{254} plates (Merck, Darmstadt, F.R.G.), thickness 0.25 mm, were used. Mobile phase: cyclohexane–chloroform–glacial acetic acid–ethanol (96%), (10:7:2:1, v/v/v/v). Standard solutions used were: 5 mg/ml each of chlorpropramide, glibenclamide or tolbutamide in dichloromethane–acetone (2:1, v/v). Methanolic hydrochloric acid B.P. (0.01 M) and methanolic sodium hydroxide B.P. (0.01 M) were used. Chlorpropamide (Pfizer Ltd.), glibenclamide and tolbutamide (Hoechst UK Ltd.) were kindly supplied by the manufacturers. Ultraviolet absorbance measurements were made using a Pye Unicam SP500 (Series 2) spectrophotometer and a matched pair of 1 cm silica cells.

Preparation of test solutions

Powder. Weigh accurately about 125 mg of chlorpropamide, glibenclamide or tolbutamide, dissolve and dilute to 25.0 ml in dichloromethane–acetone (2:1, v/v).

Tablets. Weigh and powder 20 tablets. *Glibenclamide tablets.* Extract an accurately-weighed quantity of the powdered tablets, equivalent to 25 mg of glibenclamide, by shaking with four quantities, each of 5 ml, of the dichloromethane–acetone. Centrifuge and separate the supernatant liquid after each extraction. Evaporate the combined extracts to dryness *in vacuo* at a temperature not exceeding 40°, and dissolve the residue in dichloromethane–acetone to produce 5.0 ml of solution. *Chlorpropamide and tolbutamide tablets.* Shake an accurately weighed quantity of the powdered tablets, equivalent to 125 mg of chlorpropamide or tolbutamide, with 20 ml of the dichloromethane–acetone for 5 min, and add sufficient of the solvent mixture to produce 25.0 ml. Centrifuge and separate the supernatant liquid.

Chromatographic assay

Following the procedure given under "Thin-layer chromatography" in the B.P. [1], apply 100 μl aliquots of the test and standard solutions, and of the solvent blank (dichloromethane–acetone). Develop the plate to a height of 15 cm, allow it to dry in a stream of air, and examine it under an ultraviolet lamp having a maximum output at about 254 nm. Mark an area 1.5 × 1.5 cm around each of the main spots from the test and standard solutions and around a corresponding area of silica gel from the blank. Quantitatively remove the silica gel from within each marked area, and transfer separately to a glass-stoppered centrifuge tube. Add 5.0 ml of methanolic sodium hydroxide to each tube, and shake for 10 min. Centrifuge for 5 min at 2000 rev/min, and separate the clear solution from each tube by means of a teat pipette. Dilute 1.0 ml of the solution to 10.0 ml with methanolic hydrochloric acid. Measure the absorbances of the test and standard dilutions against the blank dilution as reference at 232 nm for chlorpropamide, 230 nm for glibenclamide, and 228 nm for tolbutamide. Calculate the content per tablet of chlorpropamide, glibenclamide or tolbutamide.

Results and Discussion

The solvent system used for TLC in the B.P. test for Glibenclamide, chloroform–cyclohexane–ethanol (96%)–glacial acetic acid (9:9:1:1, v/v/v/v), gave R_f values for chlorpropamide, glibenclamide and tolbutamide of 0.36, 0.33 and 0.39 respectively. These values were too close for satisfactory quantitative assay. Table 1 shows that better separation was achieved by using the modified solvent system proposed. The R_f values shown in the table were not significantly changed when the amounts of the parent sulphonylurea drugs added to the plate were increased from 10 to 500 μg, as in the recommended TLC assay procedure. Glibenclamide, chlorpropamide and tolbutamide can be readily distinguished from each other, and from their degradation products. 1,3-Dipropylurea, which has been reported [13] as a decomposition product of chlorpropamide, is not readily detected using the ultraviolet lamp, but is adequately separated from chlorpropamide, and will not interfere with its determination. Its light absorption at 232 nm is in any case relatively weak.

Table 1
R_f values for chlorpropamide, glibenclamide, tolbutamide, their degradation products, and other hypoglycaemic sulphonylureas using the proposed solvent system. (Spots were obtained using 10 μl of a 0.10% w/v solution of each compound.)

Compound	R_f value		Degradation product	R_f value	
	Absolute	Relative*		Absolute	Relative*
Carbutamide	0.19	0.51			
Glipizide	0.23	0.62			
Metahexamide	0.33	0.89			
Glibenclamide	0.37	1.0	4-(2-{5-Chloro-2-methoxybenzamido}-ethyl) benzenesulphonamide	0.12	0.32
			Methyl *N*-4-(2-{5-chloro-2-methoxybenzamido}ethyl) benzenesulphonylsulphonylcarbamate	0.23	0.62
Glibornuride	0.41	1.11			
Acetohexamide	0.43	1.16			
Chlorpropamide	0.44	1.19	4-Chlorobenzenesulphonamide	0.26	0.70
			1,3-Dipropylurea	0.38†	1.03
Tolazamide	0.49	1.32			
Tolbutamide	0.49	1.32	4-Methylbenzenesulphonamide	0.27	0.73

* Relative to glibenclamide.
† Visualized by means of iodine vapour.

The chromatographic solvent proposed in this work can be used in place of that specified for thin-layer chromatography in the B.P. under "Related substances" to test the purity of chlorpropamide and glibenclamide in powder and tablet form. The tests can thereby be carried out on the same chromatoplate as the assay, with the same developing solvent.

The chromatographic procedure does not resolve chlorpropamide from acetohexamide or tolbutamide from tolazamide. Procedures for identifying these compounds based on their infrared spectra are, however, given in the *United States Pharmacopeia* [14].

Calibration graphs of absorbance versus concentration of the sulphonylurea solution applied to the chromatoplate complied with Beer's Law over the range 2.0–6.0 mg/ml for

chlorpropamide, glibenclamide and tolbutamide. The relative standard deviation of the assay is 0.6–0.7%, calculated from results (shown in Table 2) which were obtained by repeating the chromatographic procedure using ten aliquots of each standard solution.

Table 2
Precision of thin-layer chromatographic assay procedure. Aliquots (100 μl) of 5 mg/ml solutions of the different compounds were applied to the chromatoplate

Solution	Wavelength (nm)	Absorbance		Standard deviation (n = 10)
		Mean	Range	
Chlorpropamide*	232	0.573	0.568–0.580	0.004
Glibenclamide*	230	0.551	0.545–0.558	0.004
Tolbutamide*	228	0.497	0.493–0.501	0.003
TLC blank†	232	0.004	0.002–0.006	0.001
	230	0.005	0.004–0.007	0.001
	228	0.006	0.004–0.008	0.001

* Absorbances measured against TLC blank.
† Absorbances measured against solvent blank.

Table 3
Comparison of the proposed thin-layer chromatographic procedure and the *British Pharmacopoeial* methods [1] for the determination of chlorpropamide, glibenclamide and tolbutamide in some commercial tablet formulations

Sample	Type of tablet	Average mass per tablet (g)	Drug content as percentage of stated amount			
			TLC method		B.P. method	
			Mean		Mean	
1	Chlorpropamide 100 mg	0.125	100.2 100.5	100.4	100.2 100.6	100.4
2	Chlorpropamide 100 mg	0.137	104.6 104.4	104.5	105.3 104.9	105.1
3	Glibenclamide 5 mg	0.163	99.2 99.6	99.4	101.6 102.2	101.9
4	Glibenclamide 5 mg	0.161	98.6 99.0	98.8	100.8 101.2	101.0
5	Tolbutamide 500 mg	0.642	96.9 98.7	97.8	97.3 97.3	97.3
6	Tolbutamide 500 mg	0.653	96.6 97.9	97.2	98.2 97.8	98.0

Absorbance measurements, obtained after direct dilution of the standard solutions used, showed the mean recoveries from the TLC plates to be 98.5% for chlorpropamide, 97.7% for glibenclamide and 98.0% for tolbutamide. The use of methanolic hydrochloric acid for elution of the chromatoplate instead of methanolic sodium hydroxide also resulted in quantitative recoveries, but gave higher blank values, ranging from 0.025 to 0.031 AU. The relative standard deviation was consequently increased to 1.0–1.4%. Final absorbance measurements were made after dilution in methanolic hydrochloric acid to improve the sensitivity of the assay, since readings are higher in acid solution.

Various commercial tablet formulations were assayed by the TLC procedure, and the results compared with those obtained by the B.P. methods. The chlorpropamide and glibenclamide tablets assayed complied with corresponding pharmacopoeial tests for

Table 4
The precision of the proposed thin-layer chromatographic assay procedure applied to the determination of chlorpropamide, glibenclamide and tolbutamide in commercial tablet formulations

Chlorpropamide tablets 100 mg (average mass 0.125 g)		Glibenclamide tablets 5 mg (average mass 0.163 g)		Tolbutamide tablets 500 mg (average mass 0.642 g)	
Sample weight (g)	Drug content*	Sample weight (g)	Drug content*	Sample weight (g)	Drug content*
0.1645	99.7	0.9836	99.0	0.1786	96.9
	100.2		99.4		95.5
0.1571	101.5	0.8025	98.8	0.1612	96.3
	99.1		99.8		97.1
0.1714	99.3	0.7836	99.0	0.1534	95.5
	100.1		100.2		96.8
0.1590	100.0	0.8158	99.2	0.1888	95.3
	99.2		98.3		96.5
0.1438	99.3	0.7938	99.8	0.1636	95.9
	98.0		100.8		97.1
Mean	99.6		99.4		96.3
Relative standard deviation (%)	0.9		0.7		0.7

* As a percentage of the stated amount.

"Related substances". Both samples of glibenclamide tablets, however, showed spots corresponding to 4-(2-{5-chloro-2-methoxybenzamido}-ethyl) benzenesulphonamide, while still conforming to the B.P. requirement, which allows up to 2.4% of this impurity to be present. They also showed a faint spot at an R_f value of 0.47, which does not correspond to that found for either of the impurities normally controlled by the test. Nevertheless, Table 3 shows that good agreement can be obtained by the two methods of assay. The marginally lower results obtained for glibenclamide are probably due to the greater specificity of the TLC method.

The overall precision of the tablet assay procedure was determined using a sample from each type of tablet. Five quantities from each powdered sample were weighed and extracted, and the chromatographic procedure was carried out in duplicate for each extract. The relative standard deviations of the assays ranged from 0.7 to 0.9% (Table 4).

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