Selective Assay Procedure for Chlorpropamide in the Presence of Its Decomposition Products

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Abstract □ A colorimetric method for the quantitative determination of chlorpropamide in the presence of its decomposition products is described. The procedure is based on the interaction of *n*-propylamine (liberated *in situ* from chlorpropamide) and ninhydrin to form a blue color which is measured spectrophotometrically at 584 mμ. No color is produced by *p*-chlorobenzenesulfonamide or di-*n*-propylurea, two possible decomposition products of chlorpropamide. *n*-Propylamine, which may be present in decomposed samples, is removed by aqueous acid extraction prior to color development thus making the analytical procedure specific for unchanged chlorpropamide. The merits of the colorimetric procedure over conventional ultraviolet-spectrophotometric procedures are shown by analysis of chlorpropamide tablets which had been subjected to excess heat and moisture. A thin-layer chromatographic procedure for the separation and detection of chlorpropamide and its possible decomposition products is described.

Keyphrases □ Chlorpropamide tablets—analysis □ Degradation products, chlorpropamide—analysis effects □ TLC—separation, identification □ Colorimetric analysis—spectrophotometer □ Ninhydrin—color reagent

Recently a colorimetric method for the selective determination of tolbutamide by reaction with ninhydrin has been published (1). The method has now been extended to chlorpropamide {1-[(*p*-chlorophenyl)sulfonyl-3-propylurea}, another commonly used oral hypoglycemic agent. The method is based on the generation of *n*-propylamine *in situ* by heating an ethanolic solution of chlorpropamide, and the subsequent interaction of the amine with ninhydrin. Because of its specificity for chlorpropamide, the procedure offers advantages over some of the commonly used assay procedures.

Spectrophotometric procedures as described in the USP XVII and elsewhere (2–4) are nonspecific for the assay of chlorpropamide if a UV-absorbing degradation product, such as *p*-chlorobenzenesulfonamide, is present in the sample. Similarly, total nitrogen determination as described in BP 1963 (5) is unsatisfactory if nitrogen-containing contaminants are present. Titrimetric procedures (6, 7) are equally imprecise for degraded samples, as both the active drug and *p*-chlorobenzenesulfonamide would utilize alkali. Colorimetric procedures for the determination of sulfonylurea drugs in biological fluids and/or in pharmaceutical dosage forms have utilized 1-fluoro-2,4-dinitrobenzene (8), picric acid (9), and ascorbic acid (10) but these have not been applied for the determination of intact drug in the presence of its decomposition products in pharmaceutical dosage forms.

The purpose of this communication is to describe the ninhydrin method as applied to unchanged and decomposed chlorpropamide formulations.

EXPERIMENTAL

Reagents—Absolute ethanol, BP or USP specifications; 0.3% ninhydrin (reagent grade) in absolute ethanol.

Standard Reference Solution—Chlorpropamide USP reference standard (125 mg.), previously dried in vacuum at 60° for 2 hr., in absolute ethanol (250 ml.).

Determination of Chlorpropamide—*A. Assay Procedure for Chlorpropamide in the Presence of* n-*Propylamine*—Twenty tablets were weighed and finely powdered. A weighed aliquot equivalent to about 125 mg. of chlorpropamide was quantitatively transferred into a 250-ml. separator containing 20 ml. 1 *N* hydrochloric acid. The mixture was extracted successively with six 25-ml. portions of chloroform. The chloroform solutions were combined in a 250-ml. volumetric flask, made to volume with chloroform, mixed, and filtered through Whatman paper No. 30 or the equivalent. The first 15 ml. of filtrate was discarded. A 25-ml. aliquot of the filtrate was evaporated to dryness with a rotary film evaporator, and the residue was dissolved in about 25 ml. absolute ethanol. A small boiling chip was added to promote continuous and uniform boiling. The solution was refluxed gently in a stirred paraffin oil bath maintained at 96–100°. After 90 min., the solution was cooled, transferred quantitatively into a 50-ml. volumetric flask, and made to volume with absolute ethanol.

A 4.0-ml. aliquot of the ethanolic solution was pipeted into a 50-ml. round-bottom flask, then 3 ml. ninhydrin solution and 0.3 ml. water were added. The reaction solution was further diluted with about 35 ml. absolute ethanol and refluxed gently for 5 hr. as described above. It was cooled, transferred quantitatively to a 50-ml. volumetric flask, and made to volume with absolute ethanol. The absorbance of the sample solution and that of chlorpropamide standard carried simultaneously through the assay procedure were measured at 584 mμ against the reagent blank.

The calculations are as follows:

$$\text{mg. chlorpropamide per tablet} = \frac{A_u}{A_s} \times \frac{W_s \times W_o}{W_u}$$

where A_u = absorbance of sample solution; A_s = absorbance of standard reference solution; W_s = weight of standard chlorpropamide (mg.); W_u = weight of sample (mg.); W_o = tablet average weight (mg.).

B. Procedure for Chlorpropamide in the Absence of n-*Propylamine*—For samples not containing *n*-propylamine (as determined by TLC), the following modified procedure was used.

A weighed aliquot of the powdered tablets containing about 125 mg. of chlorpropamide was quantitatively transferred to a 250-ml. volumetric flask with the aid of absolute ethanol. The mixture was made to volume with absolute ethanol, shaken for 10 min., and filtered. A 25-ml. aliquot of the filtrate was pipeted into a 100-ml. round-bottom flask, a small boiling chip was added to promote continuous and uniform boiling. Then the assay was carried out as above starting at "The solution was refluxed gently in a stirred paraffin oil bath . . . ."

Determination of p-Chlorobenzenesulfonamide—An accurately measured 40-ml. aliquot of the chloroform filtrate obtained as described under *Determination of Chlorpropamide* (Procedure A) was evaporated to dryness with a rotary film evaporator. The residue was transferred quantitatively to a 50-ml. volumetric flask with the aid of absolute ethanol, and the resulting solution made up to volume with the same solvent and mixed. A 5-ml. aliquot of this solution was pipeted into a 200-ml. volumetric flask, and treated as directed in USP XVII, p. 130 under the "Assay for Chlorpropamide," beginning with "add 0.01 *N* hydrochloric acid to volume

Table I—Analysis of Chlorpropamide

	Ninhydrin Method				USP Method			
	% of Label Claim, av.	Anal. Range	*SD*	No. of Detns.	% of Label Claim, av.	Anal. Range	*SD*	No. of Detns.
Modified Procedure[a]								
Simulated tablets[b]	100.7	99.5–101.7	0.98	4	102.6	102.1–102.9	0.36	4
Simulated decomposed tablets[c]	100.9	99.8–102.2	1.0	4	116.0	115.0–116.7	0.71	4
Chlorpropamide tablets (commercial formulation)	103.7	102.8–104.3	0.59	6[d]	105.5	105.2–106.1	0.46	7
	102.6	101.4–104.0	0.87	18[d]	104.8	101.0–107.7	2.12	8
	102.7	101.5–103.4	0.75	6[d]	103.9	101.0–107.7	2.46	7
	103.2	101.6–103.9	0.86	6[d]	—	—	—	—
Acid Aqueous Shake-out Procedure								
Simulated tablets[b]	99.8	98.7–100.8	1.0	4	—	—	—	—
Simulated decomposed tablets[c]	101.2	100.6–101.7	0.54	4	—	—	—	—
Chlorpropamide–phenformin mixture[a]								
A[e]	96.5	95.2–97.7	1.0	6	—	—	—	—
B[f]	96.3	95.2–97.7	0.98	5	—	—	—	—

[a] Acid extraction stage omitted (Procedure B). [b] Chlorpropamide—250 mg. plus excipients such as cornstarch, calcium carbonate, magnesium stearate, and mannitol. [c] Chlorpropamide—225 mg., *p*-chlorobenzenesulfonamide—25 mg. (10%) plus excipients as in *b*. [d] Performed on separate aliquots of 20 tablets. [e] Mixture containing 125 mg. chlorpropamide and 25 mg. phenformin HCl (17.3%) in absolute ethanol. [f] Mixture containing 125 mg. chlorpropamide and 50 mg. phenformin HCl (28.5%) in absolute ethanol.

. . ." The absorbance of the sample was measured at 232 mμ using a 1 in 40 solution of ethanol in 0.01 *N* hydrochloric acid as the blank. At the same time, the absorbances of the standard reference chlorpropamide and *p*-chlorobenzenesulfonamide solutions having concentrations of 10 and 7 mcg./ml., respectively (final dilutions were made as above using a 1 in 40 solution of ethanol in 0.01 *N* hydrochloric acid) were measured at 232 mμ.

Calculations—The absorbance contribution of chlorpropamide (A_1) at 232 mμ was determined from the true concentration (C_1) of chlorpropamide (as calculated from the amount of chlorpropamide determined by the ninhydrin procedure) and the absorbance of a reference chlorpropamide solution at 232 mμ:

$$A_1 = A_s \times \frac{C_1}{C_s}$$

where A_s = absorbance at 232 mμ of the final standard reference chlorpropamide solution; C_s = concentration (approx. 10 mcg./ml.) of the final standard reference chlorpropamide solution; C_1 = true concentration of chlorpropamide in final diluted sample as calculated by the formula C_1 = mg. chlorpropamide/tablet obtained by ninhydrin procedure × weight of sample taken (mg.) × 8 × 10^{-5} divided by average tablet weight.

The absorbance (A_2) due to *p*-chlorobenzenesulfonamide in the final diluted sample at 232 mμ was determined by substracting (A_1) from the total absorbance (A_T) of the final diluted sample solution:

$$A_2 = A_T - A_1$$

The concentration (C_2) of *p*-chlorobenzenesulfonamide in the final diluted sample solution was determined by the equation:

$$C_2 = C_3 \times \frac{A_2}{A_3}$$

where A_2 = absorbance due to *p*-chlorobenzenesulfonamide in the final diluted sample as determined above; A_3 = absorbance of reference *p*-chlorobenzenesulfonamide solution having final concentration of C_3 mcg./ml. (approx. 7 mcg./ml.).

The amount of *p*-chlorobenzenesulfonamide (II) per dosage unit was calculated by the equation:

$$\text{mg. of II} = C_2 \times 12{,}500 \times \frac{\text{average tablet weight}}{\text{weight of sample taken}}$$

Thin-Layer Chromatography (TLC)—The chromatoplates were prepared using standard equipment (11). Silica Gel GF and a layer thickness of 250 μ were used throughout. The chromatoplates were air-dried and used without activation. Quantities of the ethanolic solutions of the sample equivalent to 50 mcg. of chlorpropamide and decomposition product standards equivalent to 5–10 mcg. were spotted. The plate was inserted in a suitable jar equilibrated with a solvent mixture of benzene–methanol–concentrated ammonia (70:30:2). When the solvent front reached the 15-cm. mark (about 30 min.), the plate was removed and air-dried. Examination under short-wave UV light enabled the detection of the aromatic compounds. Spraying with 0.5% ethanolic ninhydrin solution (after allowing the ammonia to evaporate) followed by heating at 80–100° for 5–10 min. caused the formation of a purple spot for *n*-propylamine at R_f 0.07 (limiting sensitivity 1 mcg.). Chlorine treatment followed by benzidine reagent spray (12) gave immediately blue spots for chlorpropamide (R_f 0.3) and *p*-chlorobenzenesulfonamide at R_f 0.6 (limiting sensitivities of 1 and 0.5 mcg., respectively). Spraying with potassium iodoplatinate reagent (13) enabled the detection of di-*n*-propylurea as a light brown spot at R_f 0.5.

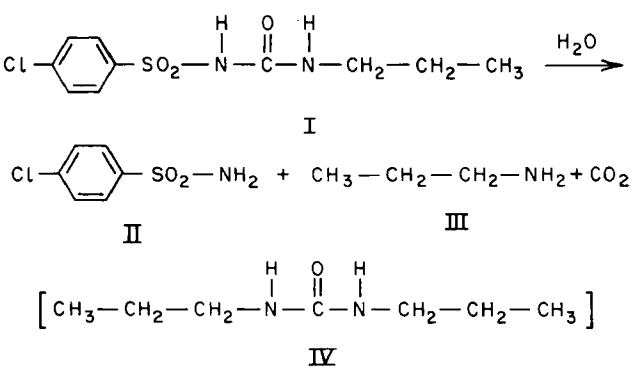
Table II—Amount of Chlorpropamide in Decomposed Commercial Formulation[a]

Decomposition Period, Days	Assay Values for Chlorpropamide[b] USP Method, %	Ninhydrin Method,[c] %
0	103.9	102.7
5	102.7	96.4
10	97.4	88.6
15	97.0	88.0
20	96.5	85.5

[a] Stored at 70° and 75% relative humidity. [b] Calculated relative to the label claim. [c] Procedure using acid extraction step was employed.

RESULTS AND DISCUSSION

The decomposition of chlorpropamide (I) can be envisioned as shown in Scheme I by analogy with the proposed mechanisms for



Scheme I

other sulfonylureas (14, 15). *p*-Chlorobenzenesulfonamide (II) will be encountered in all the hydrolyzed samples but the relative amounts of Components III and IV would be dependent on the pH of the medium.

Both chlorpropamide and the sulfonamide II exhibit maximum absorption at 232 mμ. Their molar absorptivity values are also similar—16,500 and 15,235, respectively—so that a direct spectrophotometric analysis of a chlorpropamide sample which had undergone 10% decomposition would give an apparent assay value of 99%. The assay procedure involving ninhydrin is capable of differentiating between these two closely related compounds.

The blue color formed during the assay of chlorpropamide arises from the interaction of *n*-propylamine (generated *in situ*) and ninhydrin, and is stable for at least 1 hr. The reaction was found to be analogous to that observed for the assay of tolbutamide (1) in which the colorimetric assay is dependent upon the liberation of *n*-butylamine and subsequent interaction with ninhydrin. In addition to the sulfonamide II, *n*-propylurea and di-*n*-propylurea did not react with ninhydrin. Since the assay procedure is based upon the interaction of *n*-propylamine and ninhydrin, any propylamine formed by previous degradation of chlorpropamide had to be removed prior to the color development step by an aqueous acid extraction procedure. For the analysis of chlorpropamide formulations which are free from propylamine (as determined by TLC), the acid-extraction stage can be omitted (modified Procedure B) and the sample dissolved directly in absolute ethanol.

Experiments undertaken to determine optimum conditions for reproducible and maximum color formation indicated that (*a*) a molar ratio of 14:1 of ninhydrin to chlorpropamide was satisfactory for analysis although a molar ratio of 20:1 would still give reproducible color intensities; (*b*) 0.46 to 1.1% water be present in the final reaction solution (the amount utilized in the method is the equivalent of 0.69%); (*c*) a prereflux stage of 90 min. for the sample prior to the addition of ninhydrin be utilized; and (*d*) the sample and reagent be gently refluxed for at least 5 hr. Under the conditions, Beer's law was obeyed for a concentration up to at least 34 mcg./ml. of chlorpropamide.

Application of the modified procedure (acid extraction step omitted) to simulated chlorpropamide tablets and to simulated decomposed samples free of amine but containing *p*-chlorobenzenesulfonamide gave the results shown in Table I. Analytical range and standard deviation values demonstrate the accuracy and precision of the method. Four aliquots of 20 tablets of a commercial formulation of chlorpropamide free of amine were analyzed in this way (Table I). The results appear satisfactory in comparison with those obtained from the USP analyses and with data obtained with simulated formulations.

When applied to simulated chlorpropamide tablets and to simulated decomposed tablets, the method of analysis employing the acid extraction step (Procedure A) gave results (Table I) virtually identical with those obtained by modified procedure having the aqueous acid shake-out step omitted. This procedure involving aqueous acid shake-out was also applied to chlorpropamide tablets which had been subjected to hydrolytic conditions. Weighed aliquots of powdered tablets were stored in individual open vials in a chamber maintained at a temperature of 70° and a relative humidity of about 75% (by means of a saturated sodium chloride solution). The analyses were performed on these partially decomposed samples at various time intervals. The results are presented in Table II. As anticipated, the USP XVII spectrophotometric procedure gave results higher than the true values. TLC showed that the decomposition products were *p*-chlorobenzenesulfonamide and *n*-propylamine. Di-*n*-propylurea was not detected.

Data in Table III show the recoveries of sulfonamide II from simulated decomposed chlorpropamide tablets. In the case of tolbutamide (1) where the absorptivity values of the active drug and its decomposition product, *p*-toluenesulfonamide, were virtually identical, it was possible to deduce the amount of *p*-toluenesulfonamide by subtracting the assay result of the ninhydrin method from that obtained by the USP procedure. However, the absorptivity values of chlorpropamide and the sulfonamide II differ considerably —59.6 and 79.5, respectively—so that this factor must be taken into account in calculating the amount of Compound II.

Phenformin [(1-phenethylbiguanide; $C_6H_5 \cdot CH_2 \cdot CH_2 \cdot NHC(=NH)—NHC(=NH)NH_2$] is sometimes prescribed concomitantly with chlorpropamide (16). In anticipation of the possible time when this combination is formulated, mixtures of these compounds were examined to determine if the guanide compound would interfere with the analysis of chlorpropamide when the acid extraction stage is omitted. The results (Table I) indicate that it did not. The recoveries, though reasonably precise, were slightly low. In theory at least, this could be attributed to an interaction between the phenformin hydrochloride and the *n*-propylamine liberated *in situ*. However, phenformin hydrochloride should not interfere with the quantitative recoveries of chlorpropamide if the sample is analyzed by the procedure using the acid extraction step, as the former will be retained in the aqueous acidic phase.

Table III—Amount of *p*-Chlorobenzenesulfonamide in Simulated Decomposed Tablets[a]

	Assay Values for Chlorpropamide				Assay Values for *p*-Chlorobenzenesulfonamide[c]	
	USP Method		Ninhydrin Method[b]			
	mg.	%[d]	mg.	%[d]	mg.	%[d]
Simulated decomposed tablets	262.6	116.7	229.0	101.7	25.34	10.1
	258.8	115.0	226.8	100.8	24.0	9.6
	261.7	116.3	228.3	101.5	25.0	10.0
	261.3	116.1	226.4	100.6	26.3	10.5

[a] Formulation same as in Table I, *i.e.*, chlorpropamide—225 mg., *p*-chlorobenzenesulfonamide—25 mg. (10%) plus excipients. [b] Procedure using acid extraction step was employed. [c] Calculated as described under *Determination of p-Chlorobenzenesulfonamide*. [d] Calculated relative to the label claim.

The proposed method of analysis has been found to be selective for chlorpropamide in the presence of its decomposition products. It should, therefore, be of value for carrying out the accelerated stability studies of the sulfonylurea drugs. It should also find application for the determination of the intact drug in the biological fluids since the two urinary metabolites, *p*-chlorobenzenesulfonamide and *p*-chlorobenzenesulfonylurea (17), do not undergo this reaction.

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