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Stability-Indicating High-Performance Liquid Chromatographic Determination of Chlorpropamide, Tolbutamide, and Their Respective Sulfonamide Degradates

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Abstract □ A quantitative high-performance liquid chromatographic method for the determination of chlorpropamide, tolbutamide, and their respective hydrolysis products, *p*-chlorobenzenesulfonamide and *p*-toluenesulfonamide, in solid dosage forms was developed. The method is stability indicating and can be used to determine the sulfonamide hydrolysis product and the intact drug in the presence of minor degradates. Method reproducibility, demonstrated by repeated injections of a calibration standard, was 1.21%. The lower limit of quantitation of the hydrolysis products, *p*-chlorobenzenesulfonamide and *p*-toluenesulfonamide, was 0.2 μg/5-μl injection. The accuracy of the method for intact drugs was determined by comparison of the HPLC results to those obtained by the appropriate USP or BP assays. The mean of the results obtained by the two methods differed by 0.7% for chlorpropamide and 0.3% for tolbutamide. Pure drug samples were spiked with amounts of the hydrolysis products ranging from 20 to 120% of the intact drug content. The mean percent recovery for *p*-chlorobenzenesulfonamide was 98.6%; for *p*-toluenesulfonamide, it was 100.6%. A qualitative TLC procedure for the detection of chlorpropamide, *p*-chlorobenzenesulfonamide, dipropylurea, propylurea, *n*-propylamine, tolbutamide, *p*-toluenesulfonamide, dibutylurea, butylurea, and *n*-butylamine is also described.

Keyphrases □ High-performance liquid chromatography—analysis of chlorpropamide, tolbutamide, *p*-chlorobenzenesulfonamide, *p*-toluenesulfonamide, stability in solid pharmaceutical dosage forms □ Chlorpropamide—high-performance liquid chromatographic analysis of stability in solid pharmaceutical dosage forms, sulfonamide degradates □ Tolbutamide—high-performance liquid chromatographic analysis of stability in solid pharmaceutical dosage forms, sulfonamide degradates □ Sulfonamides—degradation products of chlorpropamide and tolbutamide, high-performance liquid chromatographic analysis □ Antidiabetic agents—chlorpropamide, tolbutamide, high-performance liquid chromatographic analysis of stability and sulfonamide degradation products

The antidiabetic agents chlorpropamide and tolbutamide decompose under various experimental conditions. The major degradation route is hydrolysis (1, 2), mainly to *p*-chlorobenzenesulfonamide or *p*-toluenesulfonamide, but thermal dissociation in various solvents has been reported also (3, 4).

The USP XIX (5) spectrophotometric assay for tablets containing these drugs lacks specificity since the sulfonamide degradates retain the UV chromophore of the intact drug. The BP 1973 (6) titrimetric procedures are selective because the sulfonamide degradates are weak acids that are not neutralized by the aqueous solution of sodium hydroxide. However, the method cannot be used to assay highly discolored samples due to interference in the visual detection of the end-point.

Colorimetric procedures have been reported (2, 7, 8), but these methods require prior separation of the degradates before the intact drug can be quantitated. A high-performance liquid chromatographic (HPLC) method for the quantitation of sulfonylureas in pharmaceuticals has been reported (9), but separation and quantitation of the hydrolysis products were not demonstrated. An HPLC procedure for the determination of chlorpropamide in tablet formulations was described (10) but was not suitable for resolution of the sulfonamide from the main drug.

This paper describes a TLC technique for the detection of the major degradates of chlorpropamide and tolbutamide and an HPLC assay of intact chlorpropamide, tolbutamide, and their sulfonamide impurities, *p*-chlorobenzenesulfonamide and *p*-toluenesulfonamide, in solid dosage formulations.

EXPERIMENTAL

Materials—Chlorpropamide[1] and tolbutamide[2] were recrystallized from acetone and found to meet USP XIX (5) specifications. *p*-Chlorobenzenesulfonamide[3], *p*-toluenesulfonamide[4], *n*-propylurea[3], *n*-butylurea[4], *n*-dipropylurea[5], *n*-dibutylurea[6], *n*-propylamine[7], *n*-butylamine[3], micronized prednisone[8], acetic acid[9], hydrochloric acid[9], chlo-

[1] F. W. Horner Ltd., Montreal, Quebec, Canada.
[2] Paul Maney Laboratories, Toronto, Ontario, Canada.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Eastman Organic Chemicals, Rochester, N.Y.
[5] Authentic samples, B.P. Commission.
[6] K & K Laboratories, Plainsview, N.Y.
[7] J. T. Baker Chemical Co., Phillipsburg, N.J.
[8] Roussel (Canada) Ltd., Montreal, Quebec, Canada.
[9] Analytical reagent grade.

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Table I—TLC Data [a]

Compound	R_f Values Absolute (× 100)	Relative [b]	Absolute (× 100)	Relative [b]
	Sil G-25 UV 254		Silica Gel G/MH	
Chlorpropamide	42	1.0	50	1.0
p-Chlorobenzene-sulfonamide	30	0.71	36	0.72
Dipropylurea	20	0.48	25	0.52
Propylurea	12	0.29	14	0.28
n-Propylamine	0	0	0	0
	Silica Gel 60 F-254		Silica Gel G/HL	
Tolbutamide	44	1.0	35	1.0
p-Toluenesulfon-amide	29	0.66	24	0.69
Dibutylurea	35	0.80	30	0.86
Butylurea	16	0.36	16	0.46
n-Butylamine	0	0	0	0

[a] Development and visualization details given in the text. [b] Relative to chlorpropamide or tolbutamide.

roform[9], absolute ethanol[9], ethyl acetate[10], n-hexane[10], tetrahydrofuran[10], toluene[9], sodium hypochlorite solution[11], and phenothiazine[12] were used as received.

TLC Procedures—For chlorpropamide, 0.25-mm Sil G-25 UV 254[13] or silica gel G/MH[14] plates were used. For tolbutamide, 0.25-mm silica gel 60 F-254[15] or silica gel G/HL[14] plates were used.

The solvent system was toluene–ethyl acetate–chloroform–acetic acid (80:15:5:10). The detection agents were: A, 0.2% ninhydrin aerosol[7]; B, 0.6% (v/v) aqueous sodium hypochlorite; C, 1% (w/v) phenothiazine in chloroform (prepared fresh each week); and D, 20% (v/v) acetic acid in ethanol.

Sample Preparation—An accurately weighed amount of powdered tablet was tumbled[16] for 30 min in a screw-capped test tube with sufficient acetone to yield a 25-mg/ml final solution. The sample was centrifuged, 10 μl of the supernate was applied to the appropriate TLC plate, and the plate was developed 15 cm in a filter paper-lined chromatographic chamber, which had been equilibrated with the solvent system for 1 hr prior to use.

Visualization—The plate was air dried, and the origin was sprayed with Detection Agent A to disclose n-propylamine in chlorpropamide or n-butylamine in tolbutamide. Detection Agent B was then applied. The plate was dried with a warm air stream until free of chlorine odor and then sprayed consecutively with Detection Agents C and D. The main component and the potential degradates appeared as green spots on a white background. The R_f values are given in Table I.

HPLC Procedure—*Apparatus*—A liquid chromatograph[17] equipped with a fixed wavelength UV detector (254 nm)[17], a septumless injector port[17], and a computing integrator[18] were used. The detector was operated at an attenuation of 0.02 absorbance unit full scale (aufs), and the integrator output to the recorder was attenuated ×16 throughout. Integrator malfunction would necessitate peak height quantitation of the degradates. If peak height is to be utilized for the degradate determination, the signal from the chromatograph electrometer to the recorder would be 0.02 aufs. At this signal level, 2% impurity levels were seen and measured easily.

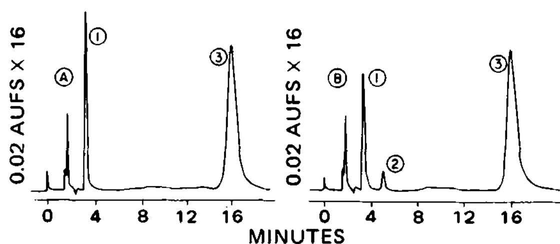

Figure 1—*Typical HPLC chromatograms from the chlorpropamide stability study of an intact formulation (A) and the same formulation exposed to 60° and 70% RH for 6 weeks (B). Key: 1, chlorpropamide; 2, p-chlorobenzenesulfonamide; and 3, prednisone.*

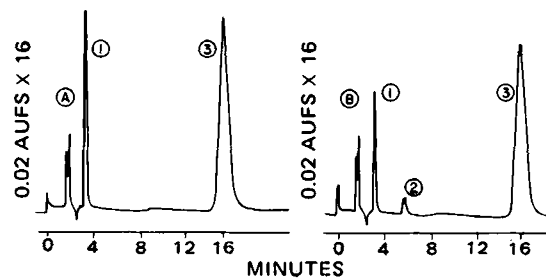

Figure 2—*Typical HPLC chromatograms from the tolbutamide stability study of an intact formulation (A) and the same formulation exposed to 60° and 70% RH for 6 weeks (B). Key: 1, tolbutamide; 2, p-toluenesulfonamide; and 3, prednisone.*

Column—A 250 × 3.2-mm i.d. column was packed with 10-μm diameter silica gel[19], using a balanced density slurry technique (11). All chromatographic operations were performed at ambient temperature and at a flow rate of 60 ml/hr (35 bar).

Mobile Phase—A mixture of 4% absolute ethanol, 8% tetrahydrofuran, and 0.06% acetic acid in n-hexane was prepared as required, degassed (reflux, 5 min), and stored in the solvent reservoir of the instrument.

Internal Standard Solution—A solution of micronized prednisone in ethyl acetate (0.70 mg/ml) was used.

Linearity—Solutions containing 0.3–3.0 mg/ml of chlorpropamide and tolbutamide (15–150% of label claim) were assayed; 2 mg/ml of drug substance was chosen as the 100% level. Solutions containing 0.04–1.2 mg/ml of p-chlorobenzenesulfonamide and p-toluenesulfonamide (2–60% of label claim) were prepared and analyzed.

Calibration Standards—A calibration standard was prepared in a 50-ml glass tube to contain accurately weighed amounts (about 50 mg) of chlorpropamide or tolbutamide and about 10 mg of the respective impurity.

System Check Sample—Powdered, well-mixed composites were prepared from a minimum of 20 tablets of chlorpropamide or tolbutamide previously shown by TLC to contain a sulfonamide impurity. The tablet composites were analyzed repeatedly for intact drug and impurity content. An aliquot of this powdered tablet substance, equivalent to ~50 mg of intact drug, was accurately weighed into a 50-ml glass tube.

Chromatography—To the calibration standard tube and the system check sample tube were added 4.0 ml of 10% (v/v) aqueous hydrochloric acid and 25.0 ml of the internal standard solution. Both tubes were agitated for 30 min on a shaker[20] and centrifuged[21] for 5 min. Duplicate 5-μl aliquots of the organic phases of both tubes were chromatographed.

If the check sample assay fell within the limits of experimental error (Table II), the system was established as functioning satisfactorily and tablet formulations were sampled. The system check sample was injected periodically to detect any changes within the chromatographic system.

Tablet Analysis—Powdered tablet substance aliquots, equivalent to ~50 mg of drug, were accurately weighed into separate 50-ml glass tubes and treated as previously described.

Calculations—Peak area ratio was determined by electronic integration of the sample and the internal standard.

RESULTS AND DISCUSSION

The ideal stability assay would measure the undegraded drug and all possible degradates without prior separation. A chromatographic technique such as HPLC generally comes closest to meeting this criterion. Preliminary TLC stability studies showed that the main degradation products of chlorpropamide and tolbutamide are the respective sulfonamides and alkylamines. The alkylamines, propyl and dipropylurea, and butyl and dibutylurea did not interfere with the HPLC assay because these compounds give no absorbance at 254 nm. Prior separation of these

[10] Glass distilled, low UV cutoff, Burdick & Jackson Laboratories, Muskegon, Mich.
[11] Javex 1-10 dilution, Bristol-Myers Canada Ltd., Toronto, Canada.
[12] Fisher Scientific Co., Fair Lawn, N.J.
[13] Macherey-Nagel precoat, Brinkmann Instruments, Rexdale, Ontario, Canada.
[14] Analtech precoat, Analtech Inc., Newark, Del.
[15] Merck precoat, E. Merck, Darmstadt, Germany.
[16] Multi-purpose rotator, Scientific Industries, Springfield, Mass.
[17] Model 4100, Varian Aerograph, Palo Alto, Calif.
[18] Autolab System 1, Spectra-Physics, Santa Clara, Calif.
[19] LiChrosorb Si-60, Brinkmann Instruments, Rexdale, Ontario, Canada.
[20] Eberbach Corp., Ann Arbor, Mich.
[21] Model K, International Equipment Co., Needham Heights, Mass.

Table II—HPLC Linearity Data

Compound	Range, mg/ml	Correlation Coefficient[a], r^2	Slope[b]	y-Intercept[c]
Chlorpropamide	0.3–3.0	0.9997	0.0825	−0.00164
p-Chlorobenzenesulfonamide	0.04–1.2	0.9986	0.0522	−0.00059
Tolbutamide	0.3–3.0	0.9996	0.0576	−0.00130
p-Toluenesulfonamide	0.04–1.2	0.9998	0.0576	−0.00347

[a] Five points were subjected to regression analysis with each compound. [b] The slope was obtained by dividing the count ratio of the compound to the internal standard by the weight ratio of the compound to the internal standard. [c] The y-coordinate for a value of $x = 0$.

compounds from the intact drug and the sulfonamide impurity was not necessary.

An important prerequisite for an HPLC stability study is separation reproducibility over time. The forward-phase technique on microparticulate silica gel fulfills this need. The chromatograms in Figs. 1 and 2 are typical of the separation achieved.

Chlorpropamide and tolbutamide exhibited linear responses between 0.3 and 3.0 mg/ml (1.5 and 15 μg/injection). The respective sulfonamide impurities exhibited linear responses between 0.04 and 1.2 mg/ml (0.2 and 6.0 μg/injection) (Table II).

The precision of the quantitation of the main drugs and the respective sulfonamide impurities was determined by analyzing a degraded formulation on different days (Table III). The reproducibility of the chromatographic system over a single day was shown by injecting 12, 5-μl aliquots of the ethyl acetate layer of a calibration standard (2.90 mg/ml of

Table III—HPLC Assay of Degraded[a] Chlorpropamide (A) and Tolbutamide (B) Composites on Different Days

Day	Composite A: Chlorpropamide by HPLC, %	Composite A: p-Chlorobenzenesulfonamide by HPLC, %	Composite A: Total by HPLC, %	Composite A: Assay by USP XIX, %	Composite B: Tolbutamide by HPLC, %	Composite B: p-Toluenesulfonamide by HPLC, %	Composite B: Tolbutamide by BP 1973, %
1	79.0	18.3			87.6	10.9	
2	79.8	18.2			86.2	11.2	
3	78.4	18.2			85.9	10.9	
4	79.1	19.1					
Mean assay (% label claim)	79.1 (±0.6[b])	18.5 (±0.4[b])	97.6	96.0	86.6 (±0.9[b])	11.0 (±0.2[b])	83.9

[a] Stored at 60° and 70% RH for 3 months. [b] Standard deviation.

Table IV—HPLC Analysis of Impurity-Free Chlorpropamide and Tolbutamide Tablet Grinds Spiked with Known Amounts of the Respective Sulfonamide

Aliquot	Chlorpropamide: Intact Drug, % label claim	p-Chlorobenzenesulfonamide Spike: Calc., %	Found by HPLC, %	Recovery, %	Tolbutamide: Intact Drug, % label claim	p-Toluenesulfonamide Spike: Calc., %	Found by HPCL, %	Recovery, %
1	99.3	11.3	10.4	92.0	98.8	11.7	11.4	97.4
2	101.2	21.2	20.5	96.7	99.9	28.5	28.5	100.0
3	101.1	46.1	47.9	103.8	99.4	32.4	32.7	100.9
4	99.6	57.2	58.3	101.8	100.1	66.2	68.9	104.1
Mean recovery, %	100.3			98.6	99.6			100.6
CV, %	±1.0			±5.4	±0.6			±2.8
Mean assay, %	101.0[a,b]				99.3[a,c]			

[a] Assay value obtained on tablet triturate before spiking with impurity. [b] USP XIX. [c] BP 1973.

Table V—Assay of Chlorpropamide Formulations by USP XIX and HPLC Procedures

Formulation	Storage Condition	USP XIX, Chlorpropamide, % label claim	HPLC: Chlorpropamide, % label claim	HPLC: p-Chlorobenzenesulfonamide[a]	HPLC: Total, % label claim
1	Ambient	98.1	97.8	Nil	97.8
1	60°, 70% RH for 3 months	96.0[b], 80.1[c]	79.1	18.7	97.8
2	Ambient	103.4	106.1	Nil	106.1
2	60°, 70% RH for 3 months	101.1[b], 70.8[c]	66.7	39.6	106.3
3	Ambient	101.0	98.8	Nil	98.8
3	60°, 70% RH for 3 months	99.5[b], 71.6[c]	70.7	32.1	102.8

[a] Expressed as equivalent weight of intact drug. [b] Uncorrected for p-chlorobenzenesulfonamide. [c] Corrected assay value determined by calculating the contribution of p-chlorobenzenesulfonamide to USP XIX assay from the known absorptivity of this compound and its actual level in the formulation as determined by HPLC.

Table VI—Assay of Tolbutamide Formulations by BP 1973 and HPLC Procedures

Formulation	Storage Condition	BP 1973, Tolbutamide, % label claim	HPLC: Tolbutamide, % label claim	HPLC: p-Toluenesulfonamide[a]	HPLC: Total, % label claim
1	Ambient	97.7	99.1	Nil	99.1
1	60°, 70% RH for 3 months	83.9	86.9	11.0	97.9
2	Ambient	99.3	101.4	Nil	101.4
2	60°, 70% RH for 3 months	95.7	97.2	3.9	101.1
3	Ambient	98.6	99.7	Nil	99.7
3	60°, 70% RH for 3 months	72.9	73.4	25.7	99.1

[a] Expressed as equivalent weight of intact drug.

chlorpropamide and 0.697 mg/ml of the internal standard). The relative standard deviation of the peak area ratios was 1.21%. The lower limit of quantitation[22] of *p*-chlorobenzenesulfonamide or *p*-toluenesulfonamide was 0.2 μg/5-μl injection. This impurity level is ~2% of the label claim of chlorpropamide or tolbutamide.

Several injections at the low impurity level were made to analyze the error involved in quantitating the sulfonamide impurity. For *p*-toluenesulfonamide at the 1.95% level, the relative standard deviation of the count ratio of the substance to the internal standard was 3.77%, an estimate of the error expected at the lower limit of quantitation.

The accuracy of the intact drug quantitation was established by analysis of individual tablet composites of chlorpropamide and tolbutamide, shown to be free of impurities by TLC. The samples were analyzed by the official method and also by HPLC. Additional weighings of these samples were spiked with known amounts of the respective sulfonamide impurity and reanalyzed by the HPLC method (Table IV).

To establish that quantitative partitioning of the intact drug and the related sulfonamide impurity was obtained, accurately weighed tablet composite samples, equivalent to 50 mg of the intact drug, were partitioned between 10, 20, and 30 ml of ethyl acetate and 4 ml of 10% (v/v) aqueous hydrochloric acid. Duplicate 5-μl aliquots of the organic layer were chromatographed. The assay values obtained after partition with 20 and 30 ml of ethyl acetate were not significantly different. The partitioning experiment was repeated with tablets that had been partially degraded by exposure to 60° and 70% relative humidity (RH). The total amount of intact drug and impurity obtained after partition with 20 and 30 ml of ethyl acetate was not significantly different. These data indicated that 25 ml of ethyl acetate was sufficient to extract completely the intact drug and impurities from degraded formulations.

The time required for complete extraction was determined by shaking replicate samples for 15, 30, and 45 min with 4.0 ml of 10% aqueous hydrochloric acid solution and 25.0 ml of the internal standard solution. There was no difference in assay value after 30 min of shaking. The aqueous layers of a calibration standard and of a decomposed formulation extracted with ethyl acetate for 30 min were chromatographed. No peaks were detected in these layers, indicating that complete extraction had taken place.

The system check sample in combination with the calibration standard indicated the status of the total system. Reproducible check sample values showed that the calibration standard factors were correct and that the sampling procedure had been carried out properly. For chlorpropamide and *p*-chlorobenzenesulfonamide, the relative standard deviations of these calibration factors were 1.42 and 3.11%, respectively, over ~8 weeks. For tolbutamide and *p*-toluenesulfonamide, the relative standard deviations of the calibration factors were 1.39 and 2.37%, respectively. These values indicate that the method is reproducible over an extended time.

[22] Defined as the level of impurity that, on repeated analysis, gives a relative standard deviation of ~4%.

Tablet formulations of chlorpropamide and tolbutamide stored for 3 months at ambient temperature (range of 21–23°) and ambient relative humidity and replicate samples stored at 60° and 70% RH (12) were analyzed by HPLC for the intact drug and the sulfonamide degradates. The samples were also analyzed by the appropriate compendial method.

Chlorpropamide samples were analyzed by the USP XIX (5) spectrophotometric technique. From the known absorptivity of *p*-chlorobenzenesulfonamide and its HPLC-determined level in the formulation, the contribution to the measured total absorbance was estimated, and the absorbance due to the intact drug was calculated. The percent recoveries of the undegraded drug determined by the two methods are given in Table V.

Similarly, the BP 1973 (6) titrimetric procedure was utilized for the tolbutamide formulations (Table VI). Because the degradate, *p*-toluenesulfonamide, is not titratable, this method allowed for a direct determination of the intact drug. The method was not applicable to some decomposed samples because discoloration in the tablets interfered with the end-point.

Comparison of the data in Tables V and VI indicates that the HPLC method is equivalent or superior to existing techniques with respect to accuracy. The HPLC method could be used to determine levels of the sulfonamide impurities in amounts less than 2% by increasing the amount of sample injected onto the column; however, the levels of chlorpropamide and tolbutamide might exceed the linear range of the system. The TLC method can be used to follow the stability profile of formulations in a stability study and to estimate semiquantitatively the concentration of impurities.

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