# High-Pressure Liquid Chromatographic Determination of Chlorpropamide in Tablet Formulations

D. MOLINS, C. K. WONG, D. M. COHEN, and K. P. MUNNELLY ˣ

**Abstract** □ A method was developed for the quantitative determination of chlorpropamide in tablet formulations by high-pressure liquid chromatography after homogenization of the sample with methanol.

**Keyphrases** □ Chlorpropamide tablets—high-pressure liquid chromatographic analysis after homogenization with methanol □ High-pressure liquid chromatography—analysis, chlorpropamide tablets

Chlorpropamide is an oral hypoglycemic agent of the sulfonylurea class. It is indicated in uncomplicated diabetes mellitus of the stable, mild, or moderately severe, nonketotic, maturity-onset type that cannot be completely controlled by diet alone. Various methods for the determination of chlorpropamide have been reported, including GLC (1), TLC (2, 3), titrimetry (4, 5), and spectrophotometry (5–9). However, these methods do not have the rapidity, simplicity, and sensitivity found in high-pressure liquid chromatographic (HPLC) methodology.

Recently, a quantitative HPLC method for sulfonylureas involving reverse-phase chromatography was published (10). A simple, direct, and extremely rapid HPLC procedure for the quantitation of chlorpropamide in tablet formulations after homogenization of the sample with methanol is the subject of this report.

## EXPERIMENTAL

**Equipment**—A liquid chromatograph[1], operated at ambient temperature and equipped with a UV detector for monitoring the column effluent at 254 nm, was used. The column was 1-m × 2.1-mm (i.d.) stainless steel tubing, dry packed with silica gel[2] (140–200 mesh) obtained by fractionation through U.S. standard sieves[3]. A variable-speed homogenizer[4] equipped with a 250-ml cup was also used.

**Reagents**—USP chlorpropamide reference standard was dried at 60° for 2 hr before use. Bis(dodecyl) phthalate[5], practical grade, was the internal standard. Analytical reagent grade methanol[6] was the mobile phase.

**Preparation of Standard Solution**—Dissolve approximately 300 mg of bis(dodecyl) phthalate in 300 ml of methanol. Accurately weigh approximately 200 mg of chlorpropamide standard, transfer quantitatively to a 100-ml volumetric flask, and dissolve in and dilute to volume with methanol. Pipet 5 ml of the bis(dodecyl) phthalate solution and 5 ml of the chlorpropamide standard solution into a 50-ml volumetric flask and dilute to volume with methanol.

**Preparation of Sample Solution**—Determine the average tablet weight of 20 tablets. Pulverize the tablets, weigh an amount equivalent to 250 mg of chlorpropamide, and transfer the powder to a 250-ml homogenizer cup. Pipet 100 ml of methanol into the cup and homogenize for 3 min at maximum speed in an ice bath to dissolve the chlorpropamide completely. Centrifuge the suspension, pipet 5 ml of the supernate and 5 ml of the bis(dodecyl) phthalate solution into a 50-ml volumetric flask, and dilute to volume with methanol.

**Chromatography**—Condition the column for 24 hr with the mobile phase at a flow rate of 0.5 ml/min. This procedure is necessary for newly packed columns; conditioning is not required for previously used columns. Inject 3 μl of the standard solution and adjust either the pressure or flow rate so that the chlorpropamide

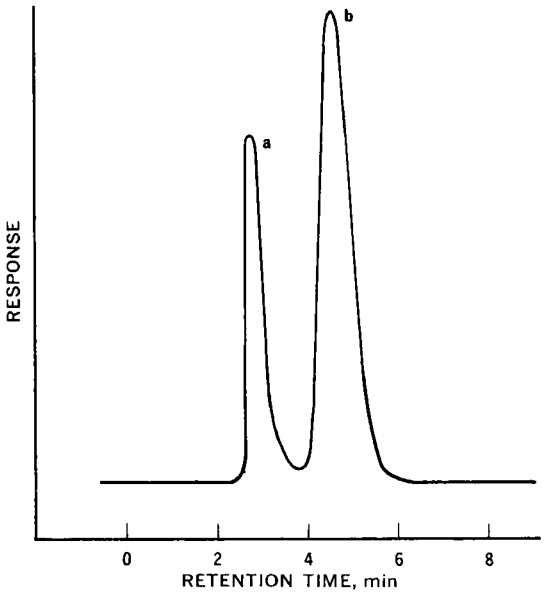


**Figure 1**—*Representative chromatogram. Key: a, bis(dodecyl) phthalate; and b, chlorpropamide.*

**Table I**—Percent Recovery[a] of Chlorpropamide from Spiked Placebos

| Day | Weight Number | Injection 1 | Injection 2 |
|---|---|---|---|
| 1 | 1 | 101.3 | 102.1 |
| | 2 | 101.1 | 98.9 |
| | 3 | 98.4 | 99.6 |
| | 4 | 98.7 | 101.3 |
| 2 | 1 | 100.8 | 98.7 |
| | 2 | 100.0 | 100.8 |
| | 3 | 100.4 | 98.4 |
| | 4 | 99.2 | 99.2 |
| 3 | 1 | 98.7 | 100.4 |
| | 2 | 101.4 | 102.7 |
| | 3 | 100.4 | 98.4 |
| | 4 | 100.4 | 99.2 |
| 4 | 1 | 99.6 | 101.3 |
| | 2 | 96.6 | 100.4 |
| | 3 | 100.4 | 98.8 |
| | 4 | 99.2 | 100.0 |

[a] Overall average recovery = 99.9%.

[1] DuPont 820.
[2] Silica gel 60 (0.063–0.200 mm), Brinkmann Instruments, Westbury, N.Y.
[3] Newark Wire Cloth Co., Newark, N.J.
[4] Sorvall Omni-Mixer.
[5] Eastman Kodak.
[6] Mallinckrodt.

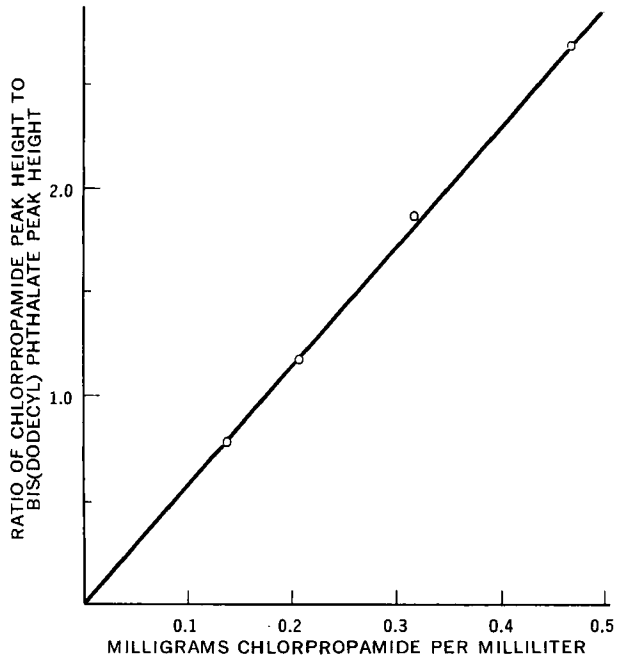


**Figure 2**—*Relationship of varying amounts of chlorpropamide to a constant bis(dodecyl) phthalate concentration.*

exhibits a retention time of approximately 5 min. Repeat if necessary.

Inject 3 μl of the standard solution followed by two 3-μl injections of the sample solution and one 3-μl injection of the standard solution, allowing sufficient time between injections for development of the chromatograms. The peak heights obtained are used for the calculations.

**Calculations**—Calculate the quantity of the chlorpropamide in milligrams per tablet according to the following formula:

$$\text{mg/tablet} = \frac{R_{spl} \times \text{standard weight (mg)} \times \text{average weight (mg)}}{R_{std} \times \text{sample weight (mg)}} \quad \text{(Eq. 1)}$$

where $R_{spl}$ is the average ratio of the chlorpropamide peak height to the bis(dodecyl) phthalate peak height in the sample solution injection, and $R_{std}$ is the average ratio of the chlorpropamide peak height to the bis(dodecyl) phthalate peak height in the standard solution before and after the sample injections.

## RESULTS AND DISCUSSION

A typical chromatogram of chlorpropamide and bis(dodecyl) phthalate is shown in Fig. 1. Figure 2 shows the linear relationship of varying amounts of chlorpropamide to a constant amount of bis(dodecyl) phthalate.

The accuracy and precision of the method were tested by the following experiment. Four weights of a placebo blend in which known quantities of chlorpropamide had been added were assayed per day for 4 consecutive days. The average percent recovery (Table I) was 99.9 with 95% confidence limits of 99.4–100.4. The estimates of precision (Table II) were obtained using the analysis of variance statistical technique. Ninety-five percent of individual results will not vary from each other (*i.e.*, from the mean) by more than ±2.7%. The standard error for the average of two injections per sample was ±0.9%.

The proposed HPLC method was compared to a UV spectroscopic assay (6) and to a modification of a TLC method (2); the latter utilizes silica gel GF plates and chloroform–acetone–butanol–water (90:95:5:2.5) as the developing solvent. The results obtained (Table III) by the proposed method were in good agreement with the UV and TLC assays. All samples were within the requirements of USP XVIII for chlorpropamide tablets by the HPLC method.

**Table II**—Estimates of Precision for Determination of Chlorpropamide in Tablet Formulations

| Day | Number of Weights per Day | Number of Injections per Weight | Estimates of Precision[a], % |
|---|---|---|---|
| 1 | 1 | 1 | ±2.7 |
| 1 | 1 | 2 | ±1.9 |
| 1 | 2 | 2 | ±1.4 |
| 1 | 3 | 2 | ±1.1 |
| 1 | 4 | 2 | ±1.0 |
| Injection to injection within a weight on a day | | | ±2.7 |

[a] Ninety-five percent of the individual results or averages of 2, 3, 6, or 12 results will not vary from each other by more than the percentages quoted. These estimates include variability due to days, weights, and injections. The estimate of precision for injection to injection within a weight on a day excludes variability due to days and weights.

**Table III**—Comparison of HPLC, UV, and TLC Methods for Determination of Chlorpropamide in Tablet Formulations[a]

| Sample | HPLC | UV | TLC |
|---|---|---|---|
| 1 | 245 | 264 | 256 |
| 2 | 255 | 259 | 251 |
| 3 | 256 | 259 | 249 |
| 4 | 249 | 255 | 246 |
| 5 | 250 | 249 | 263 |
| 6 | 249 | 257 | 257 |
| 7 | 252 | 259 | 249 |
| 8 | 258 | 256 | 244 |
| Average | 252 | 257 | 252 |

[a] All results are reported as milligrams per tablet based on a composite sample of five tablets.

Concerning the behavior of the silica gel column, the retention times increased with use time and the column lost its efficiency after 2–3 weeks of continuous use. However, excellent reproducibility was obtained from column to column. A possible explanation of this behavior is that separation is accomplished by both adsorption and partition chromatography and is, therefore, dependent on the moisture content or activity of the silica gel.

## REFERENCES

(1) K. Sabih and K. Sabih, *J. Pharm. Sci.*, **60**, 1669(1971).

(2) M. G. Hutzul and C. F. Wright, *Can. J. Pharm. Sci.*, **3**, 4(1968).

(3) K. C. Guven, S. Geegil, and O. Pekin, *Eczacilik Bul.*, **8**, 158(1966).

(4) L. O. Guerello and J. Dobrecky, *Rev. Asoc. Bioquim. Argent.*, **33**, 178(1968).

(5) J. Dobrecky and R. J. Calleja, *Rev. Fac. Quim. Farm.*, **8**, 44(1969).

(6) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970.

(7) K. K. Kaistha, *J. Pharm. Sci.*, **58**, 235(1969).

(8) A. Alessandro, R. Emer, and G. Abbondanza, *Gazz. Med. Mil.*, **116**, 827(1966).

(9) P. Mesnard and R. Crockett, *Congr. Sci. Pharm. Conf. Commun. 21st*, **1961**, 702.

(10) W. F. Beyer, *Anal. Chem.*, **44**, 1312(1972).

## ACKNOWLEDGMENTS AND ADDRESSES

Received May 23, 1974, from *Quality Control, Pfizer Inc., Brooklyn, NY 11206*

Accepted for publication July 25, 1974.

[x] To whom inquiries should be directed.