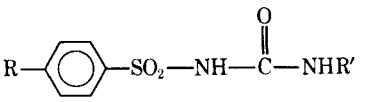
Gas Chromatographic Method for Determination of Tolbutamide and Chlorpropamide

KHALID SABIH and KHAWLA SABIH

Abstract □ Gas-liquid chromatographic methods for the measurement of tolbutamide and chlorpropamide in blood and urine and in pharmaceutical preparations have been developed. In these procedures the drugs were extracted from acidified plasma or urine with chloroform and converted to the corresponding methyl derivative by treatment with dimethylsulfate in the presence of base. The methyl derivatives of these drugs were analyzed on DC-200 coated on diatomaceous earth (Gas Chrom Q). The method has considerable specificity and sensitivity and can measure as little as 0.1 mcg. of these drugs.

Keyphrases □ Tolbutamide in biological fluids—determination □ Chlorpropamide in biological fluids—determination □ Column chromatography—separation, purification □ TLC—identity □ IR spectrophotometry—identity □ GLC—analysis

Tolbutamide (I) and chlorpropamide (II) are sulfonylurea derivatives and are widely used as oral hypoglycemic drugs in the treatment of diabetes mellitus. Few methods are available for the determination of the concentration of these and related drugs in blood or other biological fluids or materials.



I, R = CH_3, R′ = *n*-Bu
II, R = Cl, R′ = *n*-Pr

Mesnard and Crockett (1) described a method for the determination of sulfonylurea derivatives in urine. This method involved hydrolysis of these drugs in the presence of a base to obtain the amine, which in turn was treated with a solution of picric acid to produce a colored material. This color was compared with that obtained with a standard. A colorimetric method was described by Spingler (2), which also involved the hydrolysis of tolbutamide to butyl amine followed by formation of a yellow color by reacting butyl amine with dinitrofluorobenzene. Leal (3) precipitated tolbutamide as its silver salt and identified it by its melting point. Kalinowski and Korzyliski (4) and Voicu (5) described a mercurocoulometric method for tolbutamide determination. A photometric method has been described (6) for the determination of tolbutamide in pharmaceuticals. This method involved treatment of a solution of the drug in dimethylformamide (DMF) with a solution of ascorbic acid in DMF, and absorbance was measured at 530 mμ. Thin-layer chromatographic methods were also described recently (7, 8) using silica gel G and GF. A UV method was also described (9) for the determination of several sulfonylurea derivatives. Some of these methods are not very specific and some are not very sensitive.

The difference in absorption and metabolism rates of these drugs and their success as oral antidiabetics has stimulated the search for a gas chromatographic method to determine these drugs in biological material and in pharmaceutical preparations. This report describes a gas chromatographic method for the determination of tolbutamide and chlorpropamide in blood and urine and in pharmaceutical preparations. This method involves the conversion of these drugs to their methyl derivatives by treatment of their respective sodium or potassium salt with dimethylsulfate.

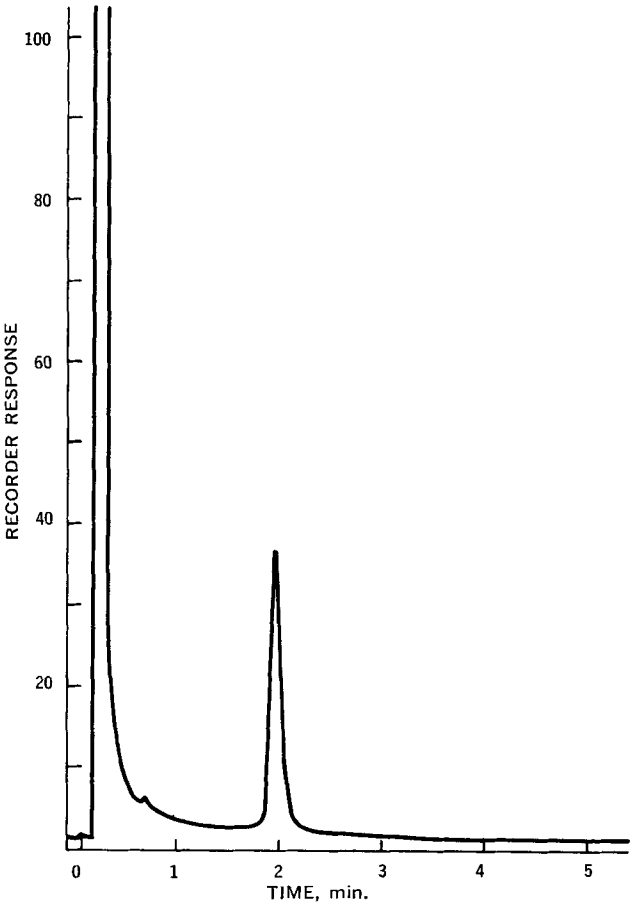


Figure 1—*Typical chromatogram of methylated tolbutamide. The curve represents a 5-μl. injection of 40 mcg. of the drug in 100 μl. chloroform.*

EXPERIMENTAL

Reagents—Tolbutamide (The Upjohn Co., Kalamazoo, Mich.); chlorpropamide (Chas. Pfizer and Co., Inc., New York, N. Y.); and dimethyl sulfate (Matheson, Coleman & Bell, Norwood, Ohio) were used. Chloroform redistilled analytical reagent, methanol analytical reagent, and heptane redistilled were used. Methanolic solution of potassium carbonate was made by mixing 1 ml. of 5–10% aqueous solution of K_2CO_3 with 9 ml. of MeOH. Acetate buffer 0.2 *M*, pH 5.6, was made by mixing 4.8 ml. of AcOH solution (0.2 *M;* 11.55 ml. in 1000 ml. H_2O) and 45.2 ml. of NaOAc solution (0.2 *M;* 16.4 g. in 1000 ml. H_2O), and the mixture was diluted with water to 1000 ml.

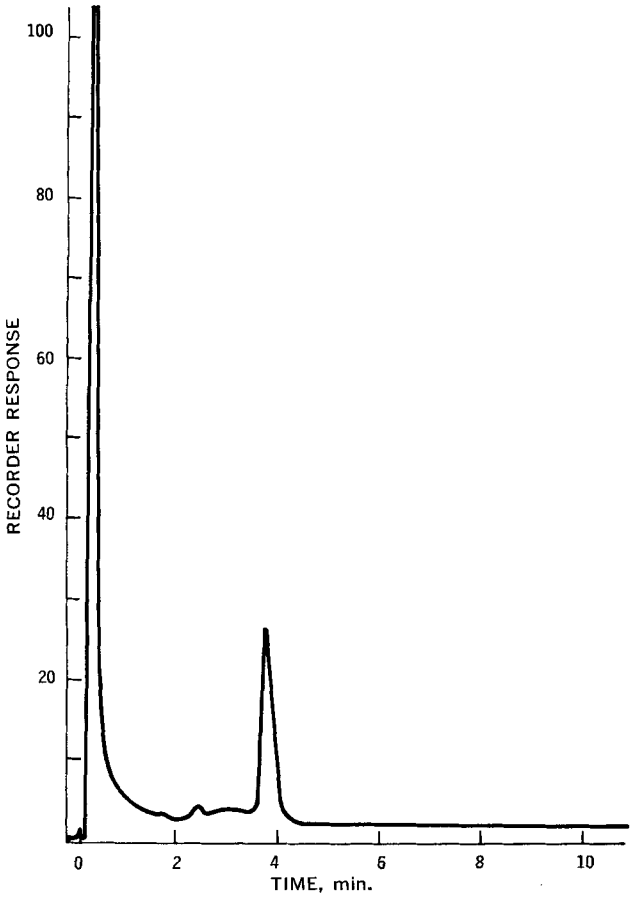


Figure 2—*Typical chromatogram of methylated chlorpropamide. The curve represents a 5-μl. injection of 28 mcg. of the drug in 100 μl. chloroform.*

Apparatus—An F and M model 5755 B gas chromatograph equipped with a flame-ionization detector was used for chromatography. The column was 1.37 m. (4.5 ft.), 0.33 cm. (0.125 in.) stainless steel tubing packed with diatomaceous earth (Gas-Chrom Q), 80–100 mesh, coated with 5% DC-200. The operating temperatures used for tolbutamide were: column, 205–210°; detector, 320°; and injection port, 330°. Helium was used as the carrier gas with a flow rate of 50 ml./min. For analysis of chlorpropamide, column temperature was 180° and the helium flow rate was 40 ml./min.

Procedure—*Standards*—A stock solution of 5 mg. of each sulfonylurea drug in 10 ml. chloroform was prepared. Aliquots containing 0, 10, 20, 40, 60, 80, and 100 mcg. were placed in separate centrifuge tubes; the solvent was removed under a stream of air, and each residue was dissolved in 1 ml. of methanolic potassium carbonate followed by 0.1 ml. of dimethyl sulfate. The resulting mixtures were heated on a water bath at 70° for 5 min. Following removal of the methanol at that temperature by a steady stream of air, 1 ml. 0.2 *M* acetate buffer, pH 5.6, was added and followed by 5 ml. of heptane. Extraction was aided by the use of a vortex mixer. After centrifugation, 4 ml. of the heptane extract from each tube was transferred to a separate 5-ml. flask from which the heptane was evaporated with a stream of air. The resulting residues were dissolved in 100 μl. of chloroform. One- to five-microliter samples were injected onto the column under the conditions cited above. Figures 1 and 2 show typical chromatograms of tolbutamide and chlorpropamide, respectively.

Pharmaceutical Preparations—One tablet of tolbutamide or chlorpropamide was pulverized in a mortar to a fine powder and triturated with 50 ml. ethanol. The mixture was then filtered and the filtrate transferred to a 100-ml. volumetric flask and made to volume with ethanol. Aliquots were placed in separate centrifuge tubes and the ethanol was removed under a stream of air. The residues were then converted to the methyl derivatives and chromatographed as described.

Biological System—A stock solution containing 5 mg. tolbutamide or 5 mg. chlorpropamide in 10 ml. 5% aqueous K_2CO_3 was prepared. The proper volume of either solution was added to plasma or urine in amounts of 0, 2.5, 5, 10, 20, 30, and 40 mcg./ml. Two milliliters of the resulting samples were acidified with 1 ml. of saturated solution of NaH_2PO_4 and then extracted with 10 ml. of chloroform by shaking on a vortex mixer for 2 min. The phases were separated by centrifugation and the aqueous layer was removed by aspiration. Five milliliters of the chloroform extracts was transfered to centrifuge tubes and the solvent was removed under a stream of air. Each residue was treated with Me_2SO_4 and chromatographed as described. One-milliliter plasma samples containing either of the sulfonylurea drugs were acidified with 1 ml. of saturated solution of NaH_2PO_4 and extracted with five volumes of chloroform by shaking carefully for 2 min. Layers were separated by centrifugation (2000 r.p.m. for 5–10 min.); emulsions were broken by stirring with a wooden stick and recentrifuged for another 5–10 min. The aqueous layer was aspirated and four-fifths of the chloroform layer was placed in a second centrifuge tube. Solvent was removed under a stream of air, and the sulfonylurea residues were converted to their respective methyl derivatives and chromatographed as described.

Identification of the Methyl Derivative of Tolbutamide—A solution of 1.0 g. tolbutamide in 30 ml. of 10% water–methanol was placed in a three-necked 150-ml. flask fitted with a reflux condenser and a magnetic stirrer. To this solution was added 2.0 g. of K_2CO_3 followed by 20 ml. of dimethyl sulfate. The reaction was allowed to proceed at 70° by heating on a water bath with stirring for 15 min. The reaction mixture was then cooled and extracted three times with 100-ml. portions of heptane. Removal of heptane under reduced pressure gave 0.9 g. of a semisolid material. The original reaction mixture was then treated with 20 ml. of 0.2 *M* acetate buffer, pH 5.6, and extracted twice with 100-ml. portions of heptane. Removal of heptane under reduced pressure yielded 0.2 g. of a semisolid material. TLC of both extracts using silica gel and chloroform as the moving phase showed only one uniform spot with an R_f value of 0.55. In comparison, tolbutamide did not move under these conditions. The product was purified by column chromatography using silica gel (Merck) and eluted with chloroform. Removal of chloroform under pressure gave 1.0 g. of a colorless oil, which solidified on standing overnight in a cool place, m.p. 33°.

Anal.—Calcd. for $C_{13}H_{20}N_2O_3S$: C, 54.93; H, 7.04; N, 9.85; Found: C, 55.49; H, 7.36; N, 9.96.

Identification of the Methyl Derivative of Chlorpropamide—A solution of 1.0 g. chlorpropamide in 30 ml. of 10% water–methanol was converted to its methyl derivative using the same procedure listed for tolbutamide. Purification of the methyl derivative

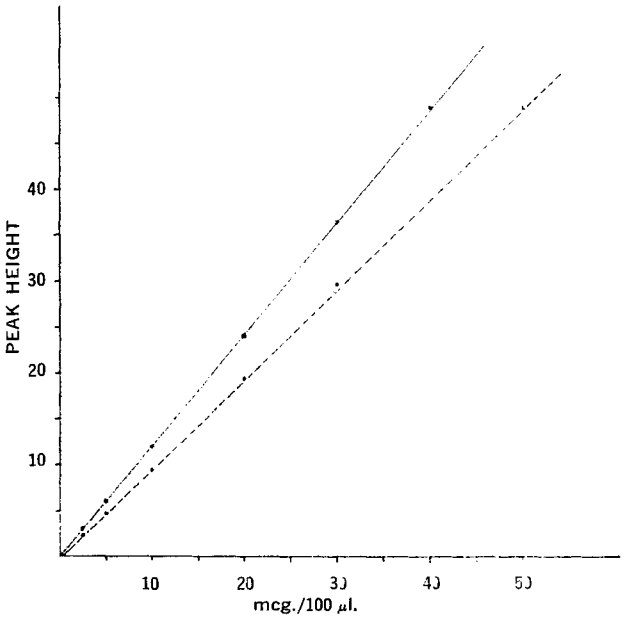


Figure 3—*Relationship between peak height and amount injected. Five microliters injected in each case. Key: ——, tolbutamide; and - - -, chlorpropamide.*

Table I—Recovery of Tolbutamide from Plasma

Amount Added, mcg.	Amount Recovered, mcg.	Recovery, %
5	4.8	96
10	9.8	98
20	19.4	97
30	30.0	100
40	40.8	102
60	57.6	96

was performed by column chromatography using silica gel (Merck) and eluted with chloroform. Removal of the chloroform under reduced pressure gave a colorless oily liquid which did not solidify on standing.

Anal.—Calcd. for $C_{11}H_{15}ClN_2O_3S$: C, 45.44; H, 5.13; N, 9.67; Found: C, 45.89; H, 5.39; N, 9.32.

RESULTS

Attempts to chromatograph tolbutamide and chlorpropamide on four different stationary phases of varying selectivity were not successful. In both cases, two peaks were obtained. The first peak eluted very close to the solvent front and the second peak showed a marked tailing under different temperature and flow-rate settings.

The methyl derivatives of tolbutamide and chlorpropamide were obtained in quantitative yield and were identified by microanalysis and IR spectroscopy. These derivatives were found to be *N*-methyl tolbutamide and *N*-methyl chlorpropamide.

By utilizing conditions cited in the method, the retention time of methylated tolbutamide was 2.0 min. and that of methylated chlorpropamide was 3.8 min. Chromatograms of control samples did not contain any peak with the retention time of the methyl derivative of tolbutamide or chlorpropamide. The retention times of pure methyl derivatives and that prepared from extracted blood or urine were identical. Chromatograms of extracts of control plasma did not contain any peaks with the retention time of either methyl derivative. Addition of increasing amounts of methylated derivatives to extracts of plasma containing the drugs produced an increase in the respective peak height having the retention time of the standard. The relationship between peak height and quantity of the drugs is linear between 1–50 mcg. in both cases as shown in Fig. 3.

To test the reproducibility of the gas chromatographic method, three samples of plasma containing known amounts of the sulfonyl ureas were made. Each sample was divided into four equal parts; the drugs were extracted from each part and converted to the methyl derivatives as described in the method. Gas chromatography of these samples gave the same peak height in each case with a standard error of ±0.52.

In a separate experiment to test the adequacy of the extraction procedure, varying amounts of the drugs were added to plasma samples and the chromatograms of these samples were compared with that of a standard. The recovery ranged between 96–102% of added tolbutamide and 94–100% of added chlorpropamide as shown in Tables I and II, respectively. The extraction method is also applicable to other biological fluids such as urine.

Table II—Recovery of Chlorpropamide from Plasma

Amount Added, mcg.	Amount Recovered, mcg.	Recovery, %
5	4.7	94
10	9.7	97
20	20.0	100
30	28.8	96
40	39.6	94

The methylation procedure of these sulfonylurea drugs was also tested for its completion. Known amounts of these drugs were added to plasma or water and extracted and methylated. The amount methylated was then determined from a standard curve obtained with a pure methylated drug. This showed about 95–100% methylation.

Other commonly used drugs, *e.g.*, phenobarbital, pentobarbital, glutethimide, and diphenylhydantoin, showed no interfering peaks in the same region as methylated tolbutamide or chlorpropamide. These drugs can also be methylated and analyzed using the procedure described in the *Experimental* section.

The amounts of tolbutamide and chlorpropamide were determined using the external standard technique.

DISCUSSION

The presence of two peaks in the chromatograms of unchanged tolbutamide can be attributed to the decomposition of the drug on the column. Methylation of tolbutamide stabilized the molecule and prevented this decomposition. Furthermore, the methylation process was advantageous because it shortened the retention time and reduced the possibility of loss of tolbutamide on the column during chromatography. In the case of chlorpropamide, the molecule decomposed on the column to give a chromatogram of two peaks similar to that of tolbutamide. Methylation of chlorpropamide produced only one symmetrical peak with fairly short retention time.

The IR spectrum of tolbutamide shows, among other bands, a strong absorption band at 1710–1690 $cm.^{-1}$, which is due to the carbonyl (C=O) stretching vibrations. The IR spectrum of methylated tolbutamide shows some differences due to the introduction of the methyl group. However, the spectrum again shows a strong absorption band at 1710–1690 $cm.^{-1}$, which again may be attributed to the presence of a carbonyl group in the molecule. This indicated that the methyl group was introduced on the nitrogen atom rather than the oxygen atom to give the *N*-methyl derivative.

The GLC method described is accurate, sensitive, and specific for the measurements of tolbutamide and chlorpropamide concentration in blood and other biological fluids and can be used in clinical laboratories. The method is also applicable to determine the amount of these drugs in pharmaceutical preparations such as tablets. The method requires approximately 2 hr. for analysis of several samples and the gas chromatographic step takes less than 5 min.

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