GLC Determination of Chlorphenesin Carbamate in Serum

D. G. KAISER[x] and S. R. SHAW

Abstract □ A method for the determination of chlorphenesin carbamate in human serum was developed to measure the concentrations of intact drug in circulation after therapeutic doses (≥400 mg). The method, based on a chloroform extraction of the buffered (pH 7.6) specimen and subsequent GLC analysis of the trimethylsilyl derivatives of the extract residue, is sensitive to 0.5 μg chlorphenesin carbamate/ml serum. Statistical analyses indicate an average recovery (±*SD*) of 98.3 ± 7.8%. Mass spectrometric analysis, in conjunction with GLC, confirmed the specificity of the method for intact drug. The procedure was successfully applied to drug absorption studies in humans.

Keyphrases □ Chlorphenesin carbamate—GLC analysis in human serum □ GLC—analysis, chlorphenesin carbamate in human serum

Chlorphenesin carbamate[1] [3-(*p*-chlorophenoxy)-1,2-propanediol 1-carbamate, I] is a well-tolerated, orally active agent effective in the treatment of skeletal muscle trauma and inflammation (1–5). The pharmacology (6), physical and analytical characterization (7–12), metabolism in humans and animals (13–16), and pharmacokinetics in humans (17) have been reported for this drug.

The only available data for serum levels of I in humans were obtained by Forist and Judy (17), following oral administration of large single doses (2–3 g); they utilized modifications of the procedure developed by Morgan *et al.* (18) for the determination of mephenesin carbamate and methocarbamol. The method involved: (*a*) alkaline hydrolysis of I in diluted serum to yield chlorphenesin (II), (*b*) chloroform extraction of II, (*c*) periodate oxidation of II (8, 9), and (*d*) spectrophotometric determination of the resulting formaldehyde by reaction with chromotropic acid.

Determination of serum I levels at therapeutic doses (≥400 mg) required a more sensitive and specific method. To meet this need, a GLC procedure was developed and is the subject of this report. Recently, GLC methods were described for the determination of II in plasma (20) and glyceryl guaiacolate in blood (21).

EXPERIMENTAL

Reagents and Materials—Compound I was synthesized[2]. Hydrocarbon-stabilized chloroform[3], *n*-docosane[4], and silylating reagent[5] (1-ml ampuls) were used as supplied. Stock solutions of *n*-docosane (35 μg/ml) and I (25, 50, 75, and 100 μg/ml) in hydrocarbon-stabilized chloroform were stored in glass containers. McIlvaine's buffer (0.5 *M*, pH 7.6) was prepared by dissolving 26.61 g dibasic sodium phosphate and 1.24 g citric acid in sufficient water to make 1 liter of solution. Silicone gum rubber (OV-17) on 60–80-mesh Gas Chrom Q (3% w/w) was used as supplied[6].

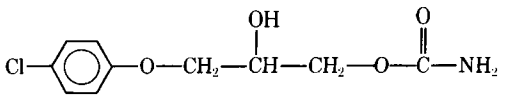
Instrumentation—A two-speed reciprocating shaker[7] was used for shaking the samples in the horizontal position. A mixer[8] was used to aid in preparing the silyl derivatives. Chromatographic measurements were made with a gas chromatograph[9] equipped with a hydrogen flame-ionization detector and a −0.2–1.0-mv recorder[10]. All cylinders of gases used for chromatography (helium, hydrogen, and oxygen) were fitted with filters containing molecular sieve 4Å.

Chromatographic Conditions—All chromatography was conducted on U-shaped glass columns [1.8 m (6 ft) × 3 mm i.d.] of 3% (w/w) OV-17 on 60–80-mesh Gas Chrom Q. All newly prepared columns were preconditioned at 250° for 1 hr without carrier gas flow and for 16 hr with a carrier gas flow of 10 ml/min. During analysis, the column, injection port, and detector block were maintained isothermally at 220, 220, and 250°, respectively. Helium, hydrogen, and oxygen flow rates were 60, 40, and 400 ml/min, respectively. Under these conditions, *n*-docosane and the disubstituted trimethylsilyl derivative of I have retention times of 3.0 and 6.2 min, respectively (Fig. 1). The monosubstituted trimethylsilyl derivative of I has a retention time of 7.2 min.

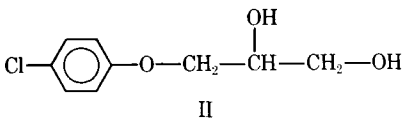
Assay Procedure—*Preparation of Standards*—Pipet aliquots of the I chloroform stock solution equivalent to 2.5, 5.0, 7.5, and 10.0 μg into glass-stoppered centrifuge tubes. Add 0.1-ml aliquots of the *n*-docosane in chloroform stock solution. Evaporate to dryness with a gentle stream of nitrogen gas. Add 1 ml control serum to each centrifuge tube and mix well with the mixer. Prepare an appropriate blank. Add 0.5 ml McIlvaine's buffer to all standards and extract in the same manner as described later for the serum specimens.

Preparation of Samples—Place 0.1-ml aliquots of the *n*-docosane stock solution in a series of glass-stoppered centrifuge tubes and evaporate to dryness with a gentle stream of nitrogen gas. Add 1 ml serum, 0.5 ml McIlvaine's buffer, and 4 ml hydrocarbon-stabilized chloroform. Shake in the horizontal position for 20 min, and centrifuge for 10 min at 2000 rpm. Transfer a 2-ml aliquot of the chloroform layer to a fresh glass-stoppered centrifuge tube and evaporate to dryness with a gentle stream of nitrogen gas. Wash down the walls of the centrifuge tube with 0.5 ml chloroform and evaporate to dryness with nitrogen gas. Add 0.1 ml silylating reagent to each chloroform extract residue. Rotate each tube to permit the reagent to contact the lower 1.2 cm (0.5 in.) of the centrifuge tube wall, and allow the reagent to react for at least 2 hr. Then inject a 2-μl aliquot of the reaction mixture for analysis into the chromatograph.

Calculations—The peak heights for *n*-docosane and the disubstituted trimethylsilyl derivative of I are measured. Peak height ratios are obtained by dividing the peak height of the I derivative by the peak height of *n*-docosane. Calibration curves for known



I



II

[1] Maolate, The Upjohn Co.
[2] Research Division, The Upjohn Co.
[3] Matheson, Coleman and Bell, Milwaukee, Wis.
[4] Analabs, Inc., Hamden, Conn.
[5] Sil-Prep, Applied Science Labs., State College, Pa.
[6] Applied Science Labs., State College, Pa.
[7] Eberbach and Sons, Ann Arbor, Mich.
[8] Vortex model K-500, Scientific Industries, Queen's Village, N.Y.
[9] F & M model 400, Hewlett-Packard Co., Avondale, Pa.
[10] Electronik 15, Honeywell, Inc., Philadelphia, Pa.

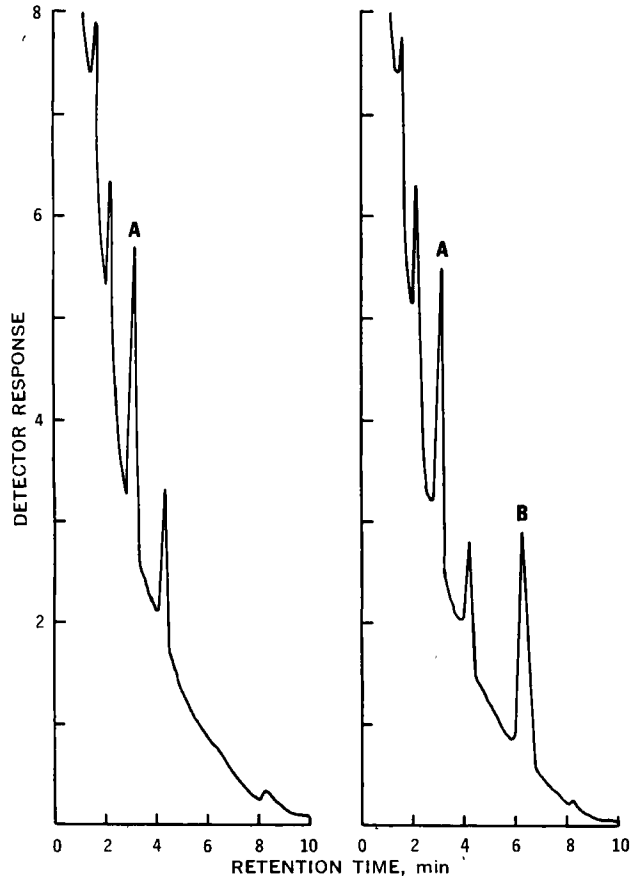


Figure 1—*GLC of human serum extracts. Left: normal serum specimen. Right: serum specimen from subject at 1 hr after dose 30 of a multiple-dose regimen (400 mg every 6 hr). Key: A, n-docosane internal standard; and B, disubstituted trimethylsilyl derivative of I.*

concentrations of I in serum are prepared by plotting peak height ratios *versus* I concentration expressed as micrograms per milliliter serum. Values for unknown concentrations of I in serum specimens, obtained in the same manner, are then read directly from the graph or calculated from the slope of the standard curve.

Drug Administration to Humans—Four normal adult male volunteers, ages 28–37 years and ranging in body weight from 66.4 to 87.3 kg, received 400 mg I in hard-filled gelatin capsules every 6 hr for a total of 30 doses. At zero time (just prior to the 30th dose) and at 0.5, 1, 2, 3, 4, 6, and 8 hr postadministration of the 30th dose, blood specimens (20 ml) were withdrawn. The serum was harvested and stored at −18°.

RESULTS AND DISCUSSION

Silylation of Chlorphenesin Carbamate—The thermal stabilities of carbamates show a marked dependence upon the degree and type of *N*-substitution (22). Structural transformations observed during GLC of intact carbamates show a similar dependence (23). Preparation of suitable derivatives, as well as careful selection of column supports and chromatographic temperatures, circumvents or minimizes thermal degradation of carbamates during GLC analysis (24). Trimethylsilyl derivatives retard the thermal breakdown of pesticidal carbamates and ureas, resulting in better symmetry of the GLC peaks (25). As shown in Fig. 2, four drug-related materials, with retention times of 1.25, 1.5, 6.2, and 7.2 min, were observed in the chromatogram of a I silylation mixture. GLC in conjunction with IR and mass spectrometry indicated that the materials were the: (*a*) disubstituted trimethylsilyl derivative of II (R_T = 1.25 min), (*b*) monosubstituted trimethylsilyl derivative of II (R_T = 1.5 min), (*c*) disubstituted trimethylsilyl derivative of I (R_T = 6.2 min), and (*d*) monosubstituted trimethylsilyl derivative of I (R_T = 7.2 min).

Table I—Recovery of Chlorphenesin Carbamate from Human Serum

Added, μg/ml	Found, μg/ml	Recovery, %
1.0	0.95	95.0
2.5	2.43	97.2
5.0	4.48	89.6
7.5	7.69	102.5
10.0	9.39	93.9
15.0	16.76	111.7
	Mean ± *SD* = 98.3 ± 7.8	

A series of samples containing known amounts of I and *n*-docosane was prepared to determine the optimal reaction time for formation of the disubstituted trimethylsilyl derivative. To minimize thermal degradation, the injection port flash heater was maintained at the same temperature as the column. The results indicated that formation of the disubstituted derivative was completed within 2 hr and that little, if any, monosubstituted derivative was present (Fig. 3). Stability studies showed that the I disubstituted derivative was stable for at least 72 hr in the silylating reagent. In the presence of known amounts of water, the I disubstituted derivative was rapidly hydrolyzed to the monosubstituted derivative. GLC also showed increased amounts of the II monosubstituted derivative, consistent with the greater thermal instability of *N*-unsubstituted carbamates. Small amounts of the II disubstituted derivative, found in the chromatograms, resulted from thermal cleavage of I mono- and disubstituted derivatives and subsequent silylation in the flash heater of the chromatograph. Ancillary studies showed that increasing the temperature of the injection port flash heater markedly increased the amount

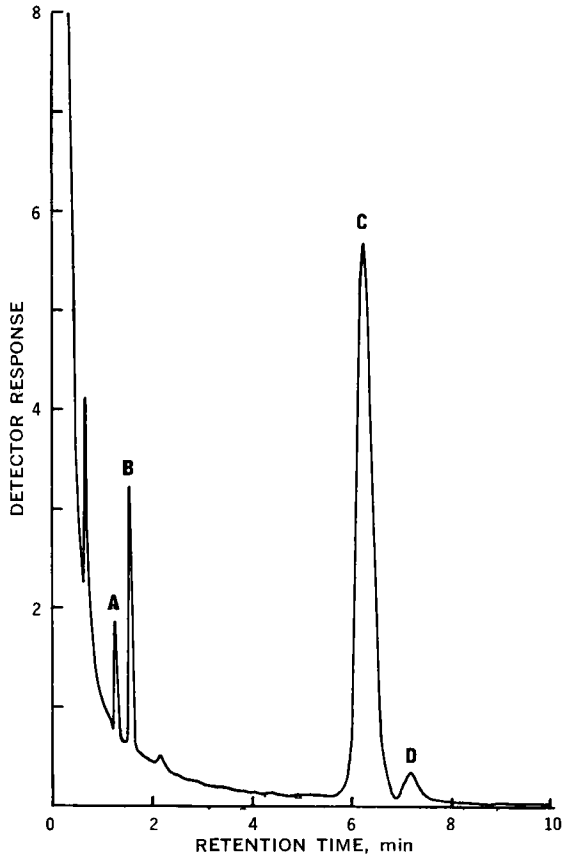


Figure 2—*GLC of chlorphenesin carbamate silylation mixture. Reaction time = 30 min. Key; A, disubstituted trimethylsilyl derivative of II; B, monosubstituted trimethylsilyl derivative of II; C, disubstituted trimethylsilyl derivative of I; and D, monosubstituted trimethylsilyl derivative of I.*

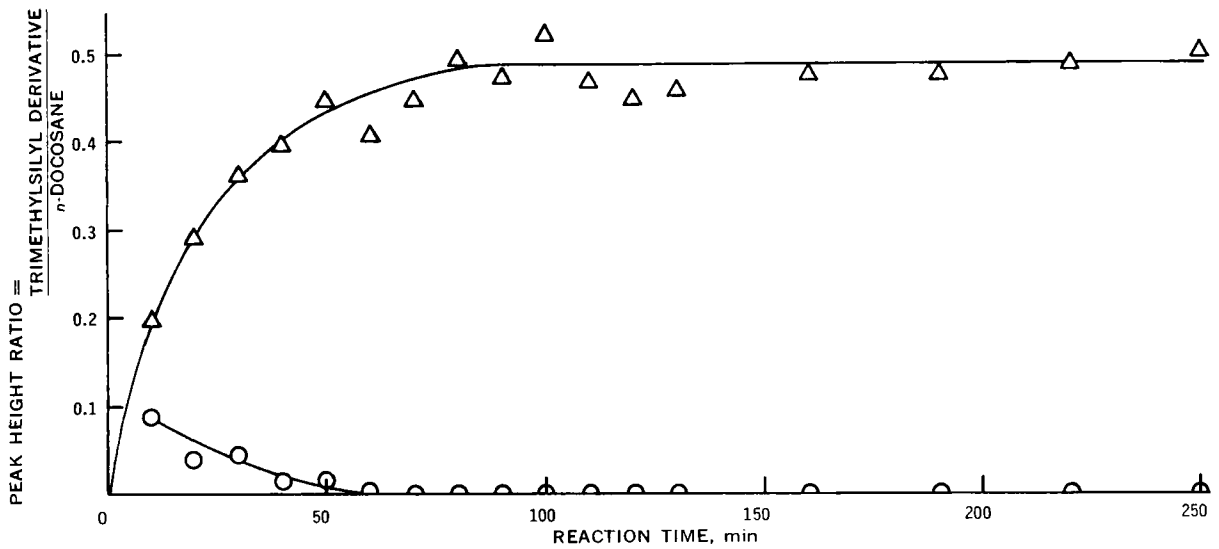


Figure 3—*Effect of reaction time on formation of I trimethylsilyl derivatives. Key: △, disubstituted derivative; and ○, monosubstituted derivative.*

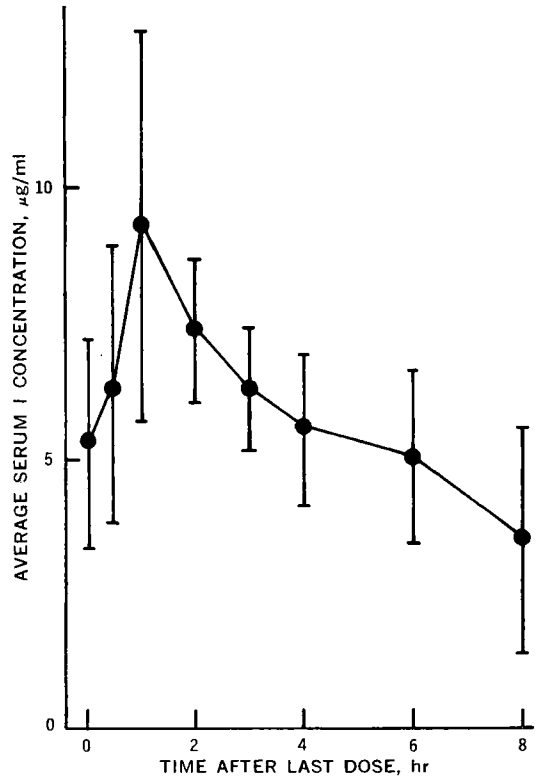


Figure 4—*Average* (±SD) *serum I concentrations* versus *time in humans* (n = 4) *after dose 30 of a multiple-dose regimen (400 mg every 6 hr).*

of the II monosubstituted derivative found in the chromatogram of the I disubstituted derivative.

Selection of Internal Standard—Pilot studies, using 3-(*p*-chlorophenoxy)-1,2-propanediol cyclic carbonate as an internal standard, showed that it had a retention time of about 6.7 min as compared to 6.2 min for the I disubstituted trimethylsilyl derivative. As shown in Fig. 2, the monosubstituted derivative of I had a retention time of 7.2 min. To ensure that no hydrolysis of the I disubstituted derivative had occurred, it was highly desirable to maintain this region of the chromatogram free of other materials. *n*-Docosane was subsequently selected to replace 3-(*p*-chlorophenoxy)-1,2-propanediol cyclic carbonate as the internal standard. Although *n*-docosane eluted from the chromatograph in a region where there was no interference, it only served as a measure of sample injection reproducibility.

Assay Sensitivity and Specificity—At a sensitivity of 3.2×10^{-10} amp/mv, 0.98 μg of I as its disubstituted trimethylsilyl derivative produced a full-scale response. However, under the assay conditions described, the lower limit of assay detection sensitivity for I in extracts of human serum is 0.5 μg/ml of the original sample aliquot. This value is based on a sample signal equivalent to 2% of full-scale response. Under the assay conditions described, a linear relationship between detector response and concentration is obtained for I over the 0–20-μg/ml range. Quantification from a standard curve is adequate. Analysis of serum specimens from drug-treated human subjects, using GLC in conjunction with mass spectrometry, showed that the material responding to the assay is the disubstituted trimethylsilyl derivative of I. Known metabolites of I—*viz.*, *p*-chlorophenoxylactic acid, *p*-chlorophenoxyacetic acid, and *p*-chlorophenol (13), do not interfere in the assay.

Recovery Experiments—Known amounts of I and *n*-docosane in chloroform were evaporated to dryness in centrifuge tubes and water or serum was added. The samples were thoroughly mixed, 0.5 ml McIlvaine's buffer was added, and the solution was extracted with chloroform. All extract residues were silylated and analyzed chromatographically. The results (Table I) indicate that recovery of I from serum is essentially quantitative (98.3 ± 7.8%) as compared to simple aqueous samples.

Serum Levels of Chlorphenesin Carbamate in Humans—Results from the measurement of serum I concentrations in normal human subjects, after multiple-dose oral drug administration, demonstrate the utility of the analytical methodology (Fig. 4). A peak mean (±*SD*) level of I (9.29 ± 3.62 μg/ml) was observed at 1 hr postadministration, indicating rapid drug absorption from hard-filled gelatin capsules. The serum drug disappearance half-life, as estimated graphically from the average serum drug concentrations, was 4.6 hr, showing good agreement with the calculated half-lives (2.32–4.30 hr) following single-dose oral administration of 2–3 g drug (17). The combined results from these investigations show that the GLC method can be used for: (*a*) determining serum I concentrations after therapeutic doses (≥400 mg) of drug, (*b*) evaluating the pharmacokinetics, and (*c*) selecting an optimum dosage regimen for I administration to humans.

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Effects of Colestipol Hydrochloride on Drug Absorption in the Rat I: Aspirin, L-Thyroxine, Phenobarbital, Cortisone, and Sulfadiazine

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Abstract □ The effects of colestipol hydrochloride, a hypocholesterolemic bile acid-binding anion-exchange polymer, on the GI absorption of five drugs commonly used in humans were studied in the rat. Colestipol hydrochloride was given orally in single doses of 71.5 or 214.5 mg/kg, equivalent to 5 (usual single dose) or 15 g, respectively, in a 70-kg human; controls received equal amounts of microcrystalline cellulose. Single oral doses of labeled drugs were given concurrently with colestipol hydrochloride and control in the human therapeutic dose range on a milligram per kilogram basis. Subsequent changes in serum drug levels were measured at several time intervals and evaluated mathematically by a one-compartment open model. The high dose of colestipol hydrochloride reduced the rate of absorption of aspirin-carboxyl-^{14}C from 8.36 to 4.68 hr^{-1} and increased the absorption half-life from 0.102 to 0.165 hr. Peak serum radioactivity was reduced by 27%, and the area under the time–concentration curve was reduced by 15%. The low dose of colestipol hydrochloride reduced peak radioactivity and the area under the curve of L-thyroxine-^{14}C (uniformly labeled) by 22 and 25%, respectively. The high dose of colestipol hydrochloride also reduced these parameters; however, the reduced total absorption increased the apparent rate of absorption from 0.169 to 0.270 hr^{-1} and reduced the absorption half-life from 4.24 to 2.68 hr. Colestipol hydrochloride did not affect the absorption of phenobarbital-2-^{14}C, cortisone acetate-4-^{14}C, or sulfadiazine-^{35}S. These results indicate that colestipol hydrochloride can inhibit absorption of some concurrently administered drugs from the GI tract of the rat.

Keyphrases □ Colestipol hydrochloride—effects on aspirin, L-thyroxine, phenobarbital, cortisone, and sulfadiazine absorption, rat □ Absorption, drug—effects of colestipol hydrochloride on various drugs, rat □ Antilipemic agents—effects of colestipol hydrochloride on absorption of aspirin, L-thyroxine, phenobarbital, cortisone, and sulfadiazine, rat □ Anion-exchange polymers, colestipol hydrochloride—effects on absorption of various drugs, rat

Colestipol hydrochloride[1] is a high molecular weight anion-exchange polyethylenepolyamine polymer with 1-chloro-2,3-epoxypropane which may have approximately one of five amine nitrogens protonated as the chloride salt. Investigators have shown that orally administered polymer binds bile salt anions in the small intestine and reduces serum cholesterol levels in experimental animals (1–3) and in humans (4–8). Since colestipol may also bind other substances, including drugs that might be used as concurrent therapy in hypercholesterolemic subjects, the effects of the polymer on the absorption of a series of drugs in the rat were studied. Results with radiolabeled aspirin, L-thyroxine, phenobarbital, cortisone, and sul-

[1] Colestid, The Upjohn Co., Kalamazoo, Mich. The official generic name (USAN) for the material reported here is colestipol hydrochloride; colestipol is used as an abbreviation in the text.