GLC Determination of Phendimetrazine in Human Plasma, Serum, or Urine

G. R. RUDOLPH, J. R. MIKSIC ˣ, and M. J. LEVITT

Received February 5, 1982, from the *Biodecision Laboratories, Pittsburgh, PA 15213.* Accepted for publication May 19, 1982.

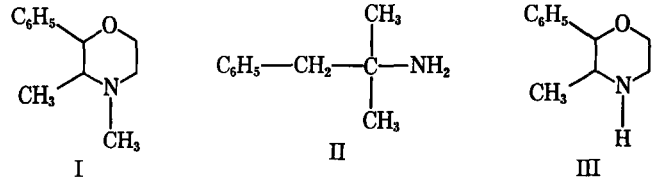
Abstract □ A sensitive, specific, and quantitative GLC method is described for the determination of phendimetrazine in plasma, serum, or urine. An internal standard was used, which was extracted along with the drug. This mixture then was acetylated to improve the chromatographic separation. A concentration as low as 2 ng/ml of phendimetrazine could be measured from 2 ml of sample using a nitrogen-phosphorous detector. Linearity extended from 2 to 500 ng/ml, and the coefficient of variation was 7%. The method was shown to be applicable to a single-dose bioavailability study.

Keyphrases □ GLC—determination of phendimetrazine in human plasma, serum, or urine □ Phendimetrazine—GLC determination in human plasma, serum, or urine □ Bioavailability—GLC determination of phendimetrazine in human plasma, serum, or urine

Phendimetrazine (I) is a sympathomimetic amine with anorectic activity used in nonprescription products for appetite suppression (1). As a condition of marketing phendimetrazine tartrate drug products in the United States, the Food and Drug Administration (FDA) has required bioavailability testing since 1973. To measure serum or plasma concentrations following a single dose of 105 mg in a bioavailability study, an analytical method with a sensitivity of ~5 ng/ml was required.

Phendimetrazine has been measured by TLC (2) and GLC (3, 4). These methods lack sufficient sensitivity to be useful for pharmacokinetic studies after a single 105-mg dose of drug. One GLC assay procedure does have adequate sensitivity (5), but only if 5 ml of serum is analyzed. Moreover, it was confirmed that the internal standard employed in this procedure was unstable, as previously reported (6).

The assay procedure described in this paper uses GLC with nitrogen-phosphorous detection to measure as little as 2 ng/ml of I in a 2-ml sample. Phentermine (II) is added to the samples to serve as an internal standard. Acetic anhydride is used to form an *N*-acetyl derivative of II which is suitable for GLC. Phenmetrazine (III), a metabolite of phendimetrazine (7), is also acetylated providing a derivative which does not interfere with the analysis of I.



I II III

EXPERIMENTAL

Reagents and Chemicals—All chemicals were used as supplied. Phendimetrazine tartrate[1], phentermine hydrochloride[2], and phenmetrazine[3] were pharmaceutical grade. Sodium chloride, potassium hydroxide, anhydrous ethyl ether, and acetic anhydride were ACS certified reagent grade[4]. HPLC grade toluene[4] was used. Phentermine hydrochloride was dissolved in deionized water (450 ng/ml) for use as the internal standard. Standards and controls were prepared by diluting aqueous solutions of phendimetrazine tartrate with pooled plasma, serum, or urine previously checked for interfering peaks. Concentrations of I at 0, 2, 5, 10, 25, 50, 80, and 150 ng/ml in serum or plasma and 0, 10, 25, 50, 80, 150, 200, 300, and 400 ng/ml in urine were analyzed. Standards stored at −15° were stable for at least 3 months.

Instrumentation—Analyses were performed on a gas chromatograph[5] equipped with a nitrogen-phosphorous detector, an auto injector[6], a recording integrator, and a 1.5-m × 2.0-mm i.d. glass column containing 3% SP-2100 on 80–100 mesh Supelcoport[7]. The column temperature was 140° during each analysis and was raised to 200° for 5 min following elution of the internal standard. The injection port and detector temperatures were 210 and 290°, respectively. Prepurified nitrogen[8] was used as a carrier gas at 30 ml/min. Samples were mechanically vortexed and shaken. Two-milliliter glass autoinjector vials with polytef-lined, red rubber caps and aluminum seals were used[9].

Analytical Procedure—Internal standard solution (700 μl, equivalent to 315 ng of phentermine hydrochloride) and sodium chloride (1 ± 0.2 g) were added to 2.0 ml of plasma, serum, or urine in a 16 × 125-mm screw-cap tube. The sample was made basic by the addition of 2 *N* KOH (300 μl). After brief mixing, I and II were extracted into ethyl ether (3 ml)

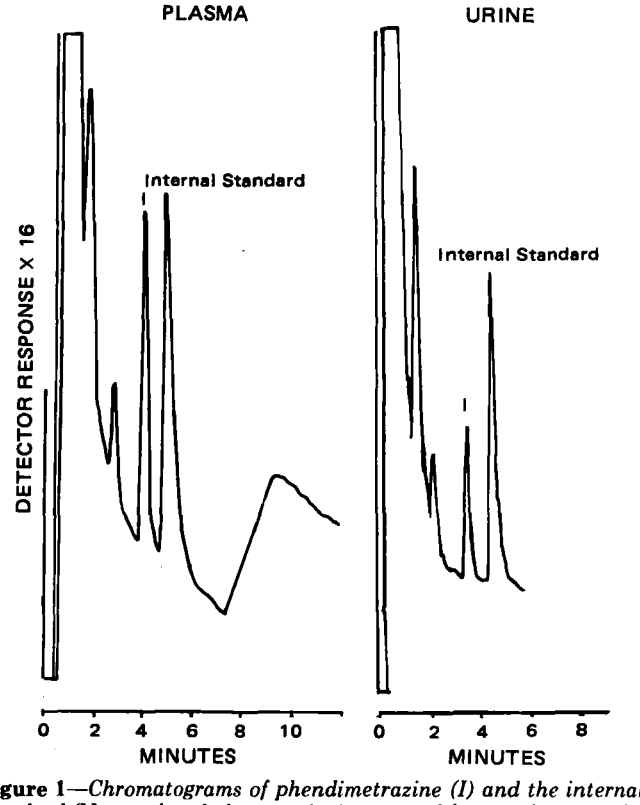


Figure 1—*Chromatograms of phendimetrazine (I) and the internal standard (N-acetylated phentermine) extracted from a plasma and a urine sample. The phendimetrazine concentrations were 39 ng/ml in plasma and 1621 ng/ml in urine (diluted 40-fold).*

[1] KV Pharmaceuticals, St. Louis, Mo.
[2] Beecham Laboratories, Bristol, Tenn.
[3] Preludin tablets, Boehringer Ingelheim, Elmsford, N.Y.
[4] J.T. Baker Chemical Co., Phillipsburg, N.J.
[5] Model 5840 A, Hewlett-Packard, Avondale, Pa.
[6] Model 7672 A, Hewlett-Packard, Avondale, Pa.
[7] Supelco, Bellefonte, Pa.
[8] Air Reduction Co., Pittsburgh, Pa.
[9] Models 5080-8712 and -8713, Hewlett-Packard, Avondale, Pa.

0022-3549/83/0500-0519$01.00/0

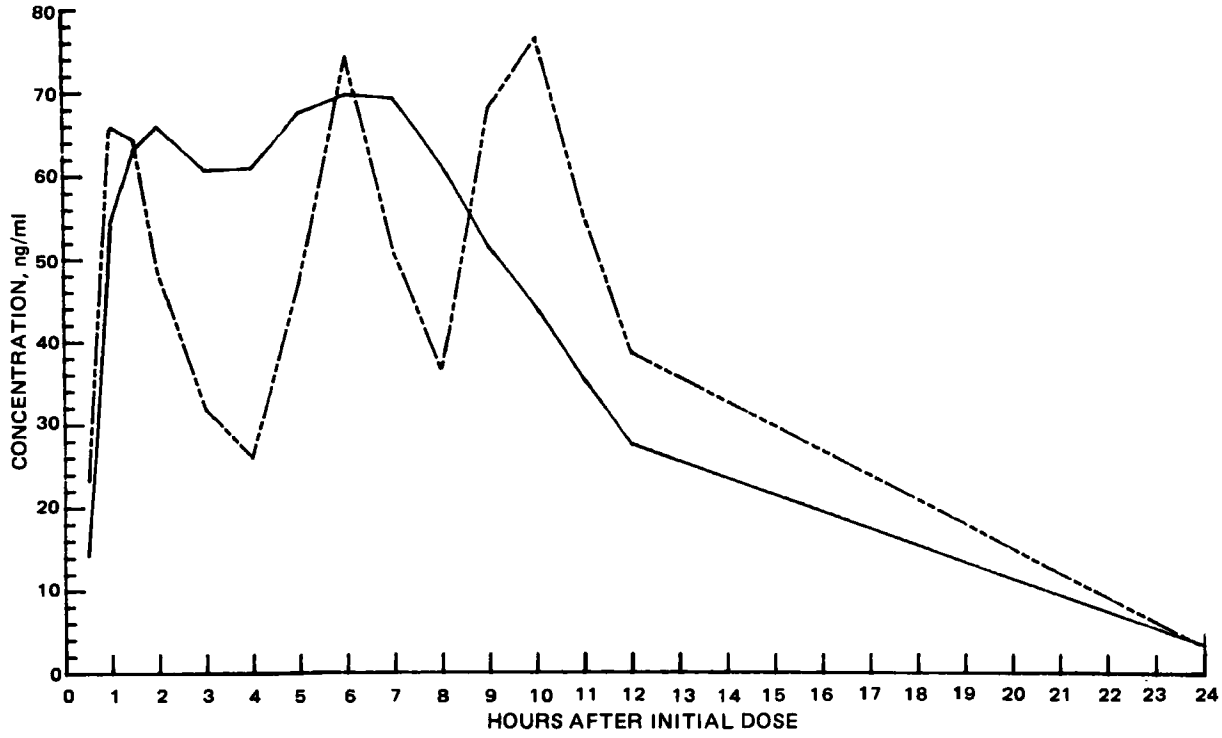


Figure 2—*Average plasma levels of phendimetrazine in 20 males who received a 105-mg controlled-release product at 0 hr or a 35-mg immediate-release product at 0, 4, and 8 hr. Key: (- - -) immediate release; (——) controlled release.*

by shaking for 10 min, and then the ether layer was transferred to another 16 × 125-mm tube. The drug and internal standard were then extracted from the ether into 0.1 *N* HCl (1.0 ml) by vortexing for 2 min. After centrifuging at 2800×*g* for 5 min, the ether layer was aspirated and discarded. Toluene (300 μl) and 10 *N* KOH (100 μl) were added to the remaining aqueous layer. After vortexing for 2 min and centrifuging at 2800×*g* for 5 min, the toluene layer was transferred to an injector vial. Acetic anhydride (50 μl) was added, the vial was capped, mixed briefly, and allowed to stand for at least 5 min. An aliquot (4 μl) then was injected into the gas chromatograph.

Human Bioavailability Study—A two-way crossover study was performed in 20 males (age, 18–37 years; weight, 60–89 kg). All of the subjects had hematology, blood chemistry, and urinalysis values within the normal range. The subjects received no medication for 7 days prior to the study and only the prescribed medications during the study. All the subjects were administered oral doses of enteric-coated ammonium chloride starting two days before dosing in order to maintain urine pH <7. They were fasted overnight at least 10 hr predose and for 2 hr postdose. A light breakfast was eaten 2 hr after initial dosing.

In each phase of the study, half of the subjects were administered a single 105-mg controlled-release phendimetrazine tartrate capsule[10] at 0 hr. The remaining subjects were administered one 35-mg immediate-release phendimetrazine tartrate tablet[11] at 0, 4, and 8 hr. Each dose was administered with 177 ml of water.

Blood samples (10 ml) were drawn from an antecubital vein into heparinized evacuated blood collection tubes[12] at intervals from 0 to 48 hr after initial dosing. The blood samples were centrifuged at 2000×*g* for 10 min within 10 min of collection. The plasma samples were transferred to polystyrene culture tubes[13] and stored at −15° until analyzed. Urine samples were collected at 12-hr intervals for 48 hr after initial dosing and were stored at −15° until analyzed.

Calculations—Peak height ratios were calculated by dividing the height of the phendimetrazine peak by the height of the acetylated internal standard peak. A best-fit line was determined by linear regression for standards ranging from 0 to 150 ng/ml (to 400 ng/ml for urine) that were processed along with the samples. The peak height ratio for each sample was used to calculate its concentration from the best-fit line. Comparable results were obtained when peak area ratios were used instead of peak height ratios. Control samples consisting of phendimetrazine added to plasma at 20 and 75 ng/ml and to urine at 20 and 158 ng/ml were also analyzed with each set of samples, and day-to-day coefficients of variation were determined from these controls.

[10] Rexar Pharmaceutical Corp., Valley Stream, N.Y.
[11] Plegine, Ayerst Laboratories, New York, N.Y.
[12] Model 6480, Becton-Dickinson, Rutherford, N.J.
[13] Model 2054, Falcon, Oxnard, Calif.

RESULTS AND DISCUSSION

Chromatography—The retention times of phendimetrazine and *N*-acetyl phentermine were 4.1 and 4.8 min, respectively (Fig. 1). Because some serum and plasma samples had a late-eluting peak at ~10 min, the column temperature was programmed to increase to 200° for 5 min after the internal standard peak was completely eluted.

Acetic anhydride was added to improve the chromatographic separation and eliminate a metabolic interference. In the absence of acetic anhydride, phentermine was poorly resolved from the solvent front as a tailing peak (retention time of 1.4 min), and phenmetrazine (a metabolite of phendimetrazine) was incompletely resolved from the phendimetrazine peak. Injection of acetic anhydride remaining from the acetylation reaction had no perceptible effect on column performance and, therefore, was left in the reaction mixture.

Extraction and Derivatization—The total recovery of phendimetrazine from plasma or serum was 73% ($n = 5$, $CV = 4\%$) of that obtained with a single extraction from aqueous solutions. Gel formation was encountered during the initial extraction with ethyl ether but was eliminated by the addition of salt and gentle shaking.

The reaction of acetic anhydride with phentermine was complete within 5 min at room temperature. Acetic anhydride obtained from one other alternate source produced a large peak at the retention time of phendimetrazine. Redistillation of this anhydride did not prevent the appearance of the interfering peak. Alternate bottles from the manufacturer listed in this paper had no interfering peaks.

Internal Standard—Biogenic amines such as pseudoephedrine, phenylpropanolamine, phentermine (underivatized), and diethylpropion were unsuitable as internal standards, because they were unstable, produced tailed chromatographic peaks, or had undesirable retention times. The internal standard used in this procedure, phentermine, was extracted with phendimetrazine and the mixture treated with acetic anhydride. Phentermine formed a stable *N*-acetyl compound which afforded a suitable retention time and peak shape.

Assay Sensitivity, Linearity, and Precision—Phendimetrazine standards in plasma and serum produced linear responses over the range of 2 to at least 200 ng/ml. Urine standards gave linear responses to at least 500 ng/ml. The standard curves typically had regression coefficients >0.99. The lower limit of quantitation of the analysis was 2 ng/ml from

an initial 2-ml sample. The day-to-day coefficients of variation (n = 24) of the procedure over a 3-week period, during which ~1000 plasma samples were analyzed, were 7% at 20 ng/ml and 6% at 75 ng/ml. The day-to-day coefficients of variation (n = 5) for the urine analyses were 6% at 20 ng/ml and 4% at 158 ng/ml. Within-day reproducibility for the plasma assay with n = 5 at concentrations of 2 and 50 ng/ml was CV = 8.7 and 1.3%, respectively.

Bioavailability Study Results—The assay procedure was used to measure the plasma and urine concentrations of phendimetrazine in 20 subjects in a two-way crossover bioavailability study (Fig. 2). The peak concentration of 70 ng/ml observed after one 35-mg dose of phendimetrazine tartrate and the elimination half-life of 2 hr observed for the immediate-release product were similar to those previously reported (5, 7). The average total recovery in urine over 48 hr after a 105-mg dose amounted to 5.72 ± 3.01 mg for the controlled-release formulation and 4.72 ± 2.89 mg for the immediate-release formulation. The assay procedure had adequate sensitivity to measure phendimetrazine in plasma at 24 hr, even for the subjects who received the immediate-release formulation. No predose plasma or urine samples had interferences at the phendimetrazine or internal standard retention times.

REFERENCES

(1) *Fed. Reg.*, **44**, 47618 (1979).

(2) R. T. Macnab, *Can. J. Med. Technol.*, **25**, 43 (1965).

(3) A. H. Beckett, *J. Pharm. Pharmacol.*, **19**, 273 (1967).

(4) D. V. Canfield, P. Lorimer, and R. L. Epstein, *J. Forensic Sci.*, **22**, 429 (1977).

(5) H. D. L. Hundt, E. C. Clark, and F. O. Muller, *J. Pharm. Sci.*, **64**, 1041 (1975).

(6) S. M. Walters, *ibid.*, **69**, 1206 (1980).

(7) A. H. Beckett and A. Raisi, *J. Pharm. Pharmacol.*, **28**, Suppl: 40P (1976).

ACKNOWLEDGMENTS

The authors thank Charles Bon and Mimi Passarello for the bioavailability analyses and statistics.

Determination of Benzalkonium Chloride in the Presence of Interfering Alkaloids and Polymeric Substrates by Reverse-Phase High-Performance Liquid Chromatography

DENNIS F. MARSH ˣ **and LLOYD T. TAKAHASHI**

Received October 9, 1981, from *Allergan Pharmaceuticals, Inc., Irvine, CA 92713.* Accepted for publication May 24, 1982.

Abstract □ A specific assay for the analysis of benzalkonium chloride in the presence of interfering substances was conducted. The approach involved complexing benzalkonium chloride in an ophthalmic system with methyl orange, extraction of the complex into 1,2-dichloroethane, and subsequent analysis by reverse-phase high-performance liquid chromatography. Since the method separates each homologue of benz*alkonium chloride*, homologues not resident in the ophthalmic system were added as internal standards to improve both recovery and precision in the method.

Keyphrases □ High-performance liquid chromatography—determination of benzalkonium chloride, ophthalmic systems, complex with methyl orange □ Benzalkonium chloride—high-performance liquid chromatography, ophthalmic systems, complex with methyl orange

Various nonspecific dye extraction methods (1–3) have been developed for the determination of benzalkonium chloride. Recently, a specific analysis for benzalkonium chloride in aqueous solution by high-performance liquid chromatography (HPLC) was developed (4). This method involves direct injection of the aqueous formulation onto the chromatographic column. However, this method of analysis proved unsatisfactory for ophthalmic systems containing polymeric material. Polyvinyl alcohol, for example, precipitates under assay conditions, thus plugging the HPLC column. Suspended particulate matter in the formulation, such as steroid suspensions, also precludes the use of the method for the same reason.

Another difficulty encountered with the direct injection procedure is the interference of active alkaloids with the benzalkonium chloride during chromatography. Benzalkonium chloride is generally present in ophthalmic systems at the antimicrobial level of 0.004%, while active ingredients are present in considerably greater concentration. Also, active ingredients have considerably higher extinction coefficients in the UV than does the benzalkonium chloride preservative. These factors make benzalkonium chloride difficult to detect by HPLC if its retention time and that of the active ingredients are at all similar.

The purpose of this paper is to describe an extraction procedure to determine benzalkonium chloride in problem systems which preclude direct injection of the samples.

EXPERIMENTAL

Apparatus—The HPLC consisted of a pump[1], an automatic sampler[2], a reverse-phase microcyano column[3], a 254-nm detector[4], and a recorder[5]. Peak integrations were performed with a laboratory data system[6].

Reagents and Solvents—The mobile phase was 58% acetonitrile[7] (UV grade) and 42% 0.161 *M* sodium propionate at pH 5.35. Sodium carbonate[8] (7.5 g) was mixed with distilled water in a 2000-ml volumetric flask. Propionic acid[9] (12 ml) was added, and the solution was brought to volume (2000 ml) with distilled, deionized water. This solution was mixed with 2800 ml of acetonitrile.

Preparation of the C_{10} and C_{18} Homologues of Benzalkonium Chloride (as the Methyl Orange Complex)—In a 1000-ml round-bottom flask 22.1 g (0.1 mole) of 1 bromodecane[10], 13.5 g (0.1 mole) of *N,N*-dimethylbenzylamine[9], 500 ml of acetonitrile, and 30 ml of dimethylformamide[9] were added. This solution was allowed to reflux (86°)

[1] Model 6000A, Waters Associates, Milford, Mass.
[2] Wisp 710B, Waters Associates, Milford, Mass.
[3] μBondapak CN (10-μm particle size, 30-cm long × 4-mm i.d. column), Waters Associates, Milford, Mass.
[4] Model 440, Waters Associates, Milford, Mass.
[5] Omniscribe, Houston Instruments, Austin, Tex.
[6] Model 3352B, Hewlett-Packard, Fullerton, Calif.
[7] Burdick & Jackson Laboratories, Muskegon, Mich.
[8] Mallinckrodt, Inc., St. Louis, Mo.
[9] J. T. Baker Chemical Co., Phillipsburg, N.J.

0022-3549/83/0500-0521$01.00/0