Determination of Phenylpropanolamine in Serum and Urine by High-Performance Liquid Chromatography

R. DOWSE, J. M. HAIGH, and I. KANFER ˣ

Received June 18, 1982, from the *School of Pharmaceutical Sciences, Rhodes University, Grahamstown 6140, South Africa.* Accepted for publication August 13, 1982.

Abstract □ A high-performance liquid chromatographic analysis of phenylpropanolamine in human serum and urine without prior derivatization is presented. Using direct UV detection the method is sufficiently sensitive to detect 25 ng of drug/ml of serum or urine; the coefficients of variation at 25 ng/ml and 500 ng/ml were 5.16 and 2.12, respectively, in serum. The method involves serum and urine extraction at a basic pH with chloroform, a single back-extraction, and chromatography on a reverse-phase column. Serum and urine data following administration of a single 150-mg sustained-release tablet of phenylpropanolamine hydrochloride in six healthy volunteers demonstrates the suitability of the analytical method.

Keyphrases □ Phenylpropanolamine—determination by high-performance liquid chromatography without prior derivatization, human serum and urine □ High-performance liquid chromatography—phenylpropanolamine, determination in human serum and urine without prior derivatization

Phenylpropanolamine, a sympathomimetic amine, used to relieve congestion of the nasal mucosa and sinuses in the treatment of colds, sinusitus, rhinitis, and hay fever is also used as an appetite suppressant (1). Phenylpropanolamine has been determined in plasma by GLC following derivatization with pentafluorobenzaldehyde (2) and also as the heptafluorobutyryl derivative (3). Urine phenylpropanolamine levels have been analyzed by nitrogen-selective GLC (4, 5). High-performance liquid chromatography (HPLC) has been used for the determination of phenylpropanolamine alone and in pharmaceutical formulations (6–11), but little information has appeared on the HPLC analysis of phenylpropanolamine in biological fluids (12, 13).

Endo *et al.* (12) used a color reaction with sodium β-naphthoquinone-4-sulfonate after extraction from alkaline urine to determine phenylpropanolamine by HPLC, but this method is time consuming and no indication is given as to the levels of phenylpropanolamine determined. The HPLC method described by Mason and Amick (13) involved extraction from plasma, back-extraction, and *o*-phthalicdicarboxaldehyde derivatization after which fluorescence detection was used to determine concentrations of phenylpropanolamine as low as 5 ng/ml.

The purpose of this study was to develop a sensitive, reproducible, and rapid method to determine phenylpropanolamine in serum and urine without prior derivatization.

EXPERIMENTAL

Apparatus—The modular high-performance liquid chromatograph consisted of a constant-flow pump[1], an automated sample injector[2], a variable-wavelength UV detector[3], and a data module[4]. The separation was performed on a 30-cm × 4-mm i.d. column containing microparticulate-bonded (10-μm) octadecylsilane material[5]. The temperature of the column was maintained at 20° [6].

Reagents and Materials—Reagents were of at least analytical reagent grade quality and the acetonitrile[7] was distilled in glass. Phenylpropanolamine hydrochloride[8], ephedrine hydrochloride[9], chloroform[10], and sodium 1-heptanesulfonate[11] were obtained commercially. Water was deionized and then double-distilled in glass.

Chromatographic Conditions—The mobile phase was prepared by mixing acetonitrile (250 ml) with a 0.005 *M* solution of sodium 1-heptanesulfonate in water (750 ml) and adding 1 *M* HCl (2 ml). The solvent mixture (pH 2.5) was deaerated and filtered through a 0.6-μm filter[12]. This mobile phase was used at a flow rate of 1.3 ml/min for the analysis of both serum and urine samples, with a resulting pressure of 230 bar. The wavelength of detection was 220 nm.

Extraction—*Serum*—One milliliter of serum, 1 ml ephedrine hydrochloride solution (0.24 μg/ml), and 50 μl of a saturated solution of sodium carbonate, which adjusted the pH of the mixture to 10, were vortexed for 15 sec in a test tube[13] (16 × 100 mm). Five milliliters of chloroform were added and the tube was stoppered, vortexed for 1 min, and centrifuged at 2000×*g* for 5 min. A plug formed between the aqueous and organic phases, and the aqueous layer above this plug was removed by aspiration and discarded. A pasteur pipet was used to transfer the chloroform extract to a tapered centrifuge tube containing 100 μl of 5% (v/v) acetic acid. The pipet was rinsed with 2 ml of chloroform, which was then added to the original test tube, vortexed for 30 sec, and centrifuged at 2000×*g* for 5 min. The chloroform washings were added to the tapered centrifuge tube, vortexed for 1 min, and centrifuged at 600×*g* for an additional minute. The chloroform layer was reduced by aspiration to ~200 μl and discarded, and the tube was again centrifuged at 2000×*g* for 5 min.

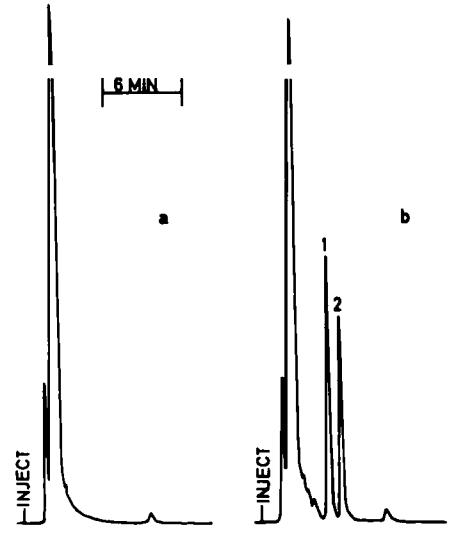

Figure 1—*HPLC chromatograms of (a) blank serum extract and (b) an extract of serum containing phenylpropanolamine (1) and ephedrine (2).*

[1] Model M6000A, Waters Associates, Milford, Mass.
[2] WISP model 710B, Waters Associates, Milford, Mass.
[3] Model LC3, Pye Unicam, Cambridge, England.
[4] Model 730, Waters Associates, Milford, Mass.
[5] μ-Bondapak C_{18}, Waters Associates, Milford, Mass.
[6] Model LC-22 Temperature Controller, Bioanalytical Systems Inc., West Lafayette, Ind.
[7] UV grade, Waters Associates, Milford, Mass.
[8] Chamberlains (Pty) Ltd., Cape Town, South Africa.
[9] E. Merck, Darmstadt, West Germany.
[10] B. D. H. Chemicals, Poole, England.
[11] Aldrich Chemical Co., Milwaukee, Wis.
[12] Type BD, Millipore Corp., Bedford, Mass.
[13] Vacutainer, Becton, Dickinson and Co., Rutherford, N.J.

0022-3549/83/0900-1018$01.00/0

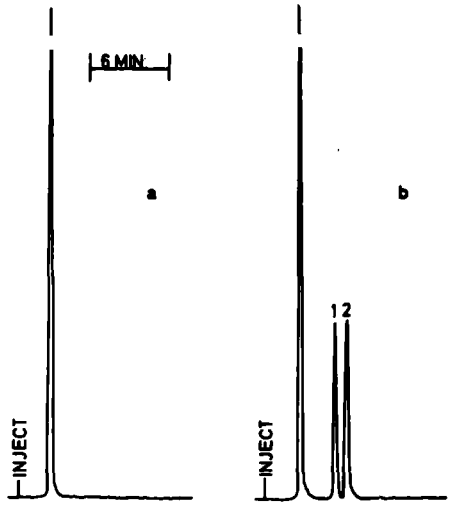

Figure 2—*HPLC chromatograms of (a) blank urine extract and (b) an extract of urine containing phenylpropanolamine (1) and ephedrine (2).*

Aliquots of 15–30 μl of the acetic acid extract were injected directly onto the column. Typical chromatograms are depicted in Fig. 1.

Urine—To 1 ml of urine in a test tube were added an aqueous solution of 1 ml of ephedrine hydrochloride (40 μg/ml) and 50 μl of a saturated solution of sodium carbonate (to adjust the pH to 10). The tube was vortexed for 15 sec, 5 ml of chloroform was added, and the tube was stoppered and vortexed again for 1 min. After centrifugation at 2000×*g* for 5 min, the chloroform extract was transferred with a pasteur pipet to a tapered centrifuge tube containing 1 ml of 5% (v/v) acetic acid. The pipet was rinsed with 2 ml of chloroform, which was then added to the original tube, vortexed for 30 sec, and centrifuged at 2000×*g* for 5 min. The chloroform washings were added to the centrifuge tube, vortexed for 1 minute and centrifuged at 2000×*g* for 5 min. Aliquots of 1–8 μl of the upper acetic acid extract were injected onto the column. Typical chromatograms are depicted in Fig. 2.

Clinical Study—As part of a bioavailability study, six normal healthy volunteers each received one sustained-release tablet containing 150 mg of phenylpropanolamine hydrochloride following an overnight fast. Blood samples were drawn at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 9, 10, 12, and 24 hr after ingestion of the medication; the serum was separated by centrifugation and frozen until assayed. Urine samples were collected at 0, 2, 4, 6, 8, 12, and 24 hr, and representative samples were frozen until analysis. Urine voided at times other than those specified above was collected and treated in the same manner.

RESULTS

Linearity—Calibration curves using five different concentrations of phenylpropanolamine in serum and urine, obtained by plotting the ratio of the peak height of phenylpropanolamine to that of the internal standard (ephedrine) *versus* their respective concentrations, were linear over the concentration ranges studied. The calibration curve in serum (25–500 ng/ml) had a slope of 0.0039 and a *y*-intercept of 0.0509 with a correlation coefficient of 0.9997, while the curve in urine (10–200 μg/ml) had a slope of 0.0265 and a *y*-intercept of 0.0386 with a correlation coefficient of 0.9999.

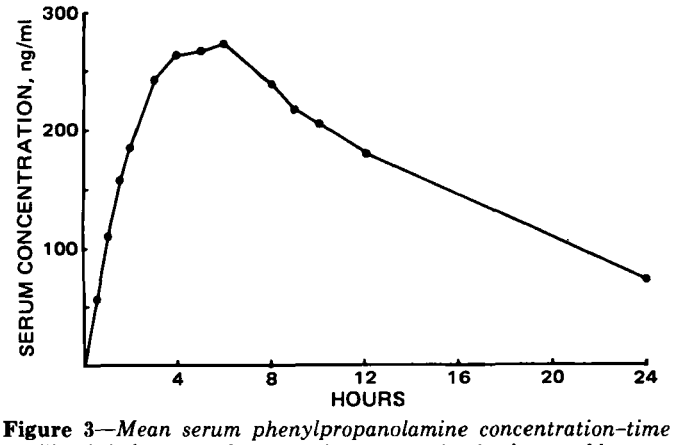

Figure 3—*Mean serum phenylpropanolamine concentration–time profile of six human volunteers given a sustained-release tablet containing 150 mg of drug.*

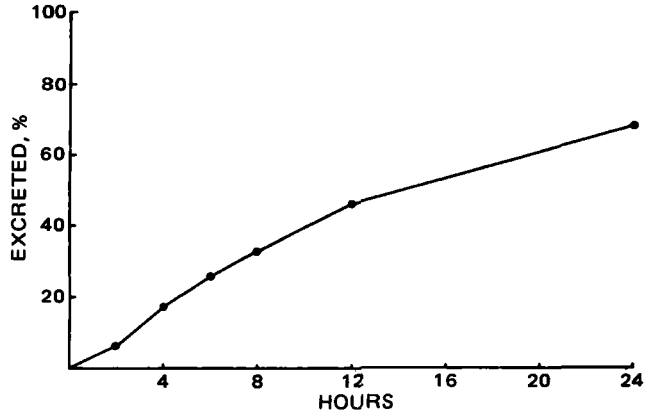

Figure 4—*Mean cumulative urinary phenylpropanolamine concentration–time profile of six human volunteers given a sustained-release tablet containing 150 mg of drug.*

Precision and Accuracy—Within-run precision was assessed by extracting five spiked serum and urine samples each at the upper and lower limits of the concentration ranges studied. The coefficients of variation were found to be 5.16% at 25 ng/ml and 2.12% at 500 ng/ml for serum and 1.66% at 10 μg/ml and 0.33% at 100 μg/ml for urine. Corresponding standard deviations for these samples were 1.26 ng/ml, 10.75 ng/ml, 0.155 μg/ml, and 0.324 μg/ml, respectively. Replicate assays in serum and urine samples determined over a period of 3 months (stored at −10°) revealed no significant differences in phenylpropanolamine concentrations.

Extraction Efficiency—Spiked serum and urine samples were assayed in triplicate at four different concentrations. All samples were extracted as described previously except that the internal standard was added as a final step to each extract. The results were compared with those obtained from the injection of equivalent concentrations of phenylpropanolamine in the internal standard solution. Mean values of 80.39 and 80.20% recovery were obtained for phenylpropanolamine from serum and urine, respectively.

Sensitivity and Detection Limit—Under the conditions of this assay, the detection limit for phenylpropanolamine was 25 ng/ml in serum and 10 μg/ml in urine. These lower limits can be extended, particularly in the case of urine, if smaller volumes of 5% (v/v) acetic acid are used in the back-extraction step.

Serum and Urine Profiles—The mean serum concentration–time profile is presented in Fig. 3. Figure 4 represents the cumulative urinary excretion profile for the six subjects who received one sustained-release tablet containing phenylpropanolamine hydrochloride (150 mg).

DISCUSSION

The method described involved a rapid, relatively simple extraction of phenylpropanolamine from urine and serum. Previous HPLC methods used to determine this drug in biological fluids have involved either derivatization with *o*-phthalicdicarboxaldehyde followed by fluorescence detection (13) or the use of a normal-phase column followed by reaction with sodium β-naphthoquinone-4-sulfonate and subsequent colorimetric determination (12). The latter method was applied to urinalysis only, and concentrations relating specifically to phenylpropanolamine were not reported. Both these methods are relatively time consuming and involve postcolumn manipulations.

Our method eliminates the derivatization step, thus allowing direct determination using a variable-wavelength UV detector. The method is sufficiently sensitive, accurate, and precise for the monitoring of phenylpropanolamine concentrations in small volumes of both serum and urine following oral doses of the drug. In addition, the resulting chromatograms are clean with no interfering peaks due to endogenous constituents. Metabolite interference did not present a problem since no significant metabolism of phenylpropanolamine occurs (14). Retention times of phenylpropanolamine and ephedrine were 4.8 min and 5.7 min,

respectively. In summary, the HPLC method presented here is rapid, precise, and extremely suitable for the determination of phenylpropanolamine in serum and urine.

REFERENCES

(1) "Remington's Pharmaceutical Sciences," 15th ed., Mack Publishing Co., Easton, Pa., 1975, p. 820.

(2) L. Neelakantan and H. B. Kostenbauder, *J. Pharm. Sci.*, **65**, 740 (1976).

(3) L. M. Cummins and M. J. Fourier, *Anal. Lett.*, **2**, 403 (1969).

(4) H. Kinsun, M. A. Moulin, and E. C. Savini, *J. Pharm. Sci.*, **67**, 118 (1978).

(5) E. Appel, *Eur. J. Clin. Pharmacol.*, **8**, 161 (1975).

(6) T. L. Sprieck, *J. Pharm. Sci.*, **63**, 591 (1974).

(7) V. Das Gupta and A. G. Ghanekar, *J. Pharm. Sci.*, **66**, 895 (1977).

(8) T. R. Koziol, J. T. Jacob, and R. G. Achari, *J. Pharm. Sci.*, **68**, 1135 (1979).

(9) A. G. Ghanekar and V. Das Gupta, *J. Pharm. Sci.*, **67**, 873 (1978).

(10) "Over the Counter Cough/Cold Remedies," L. C. du Pont, Technical Report, E 32022.

(11) "Analysis of Pharmaceutical Products," Waters Associates, Publication N68, 1976, p. 11.

(12) M. Endo, H. Imamichi, M. Moriyasu, and Y. Hashimoto, *J. Chromatogr.*, **196**, 334 (1980).

(13) W. D. Mason and E. N. Amick, *J. Pharm. Sci.*, **70**, 707 (1981).

(14) J. E. Sinsheimer, I. G. During, and R. T. Williams, *Biochem. J.*, **136**, 763 (1973).

ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from the South African Council for Scientific and Industrial Research.

High-Performance Liquid Chromatographic Determination of Aspirin and Its Metabolites in Plasma and Urine

SHAMSUL K. BAKAR and SARFARAZ NIAZI ˣ

Received December 3, 1981, from the *Department of Pharmacodynamics, College of Pharmacy, University of Illinois Health Sciences Center, Chicago, IL 60612.* Accepted for publication February 25, 1982.

Abstract □ A simple quantitative method for the rapid determination of aspirin and its metabolites, salicylic acid, salicyluric acid, and gentisic acid, in plasma and urine using *o*-toluic and *o*-anisic acids, respectively, as internal standards was developed. Plasma proteins were precipitated by the addition of acetonitrile and, after centrifugation, the supernatant fluid was injected directly onto a reverse-phase column. The mobile phase consisted of an isocratic mixture of water, methanol, and glacial acetic acid (64:35:1, v/v/v) and the separated components were detected at 238 nm using a UV detector. Concentrations ≥0.5 μg/ml could be quantitated for aspirin or its metabolites in plasma. The peak heights and peak height ratios to the internal standard, *o*-toluic acid, were linear for the concentration range of 0.5–200 μg/ml. The aspirin metabolites in urine were isolated by extracting the acidified urine with ether and then reextracting the material into an aqueous buffer solution at pH 7.0. Twenty microliters of the buffer extract was directly injected onto the column. The separated components were detected and quantitated at 305 nm. Concentrations ≥5 μg/ml of salicyluric acid, salicylic acid, and gentisic acid could be determined accurately. The peak heights and peak height ratios to the internal standard, *o*-anisic acid, were found to be linear for the concentration range of 5–200 μg/ml in urine.

Keyphrases □ Salicylates—aspirin and metabolites, quantitation in urine and plasma, high-performance liquid chromatography □ Metabolites—of aspirin, quantitation in urine and plasma, high-performance liquid chromatography □ High-performance liquid chromatography—quantitation of aspirin and metabolites, urine and plasma

Aspirin is one of the most extensively used drugs. Recently, the importance of monitoring plasma levels of salicylates has been reviewed (1). A number of high-performance liquid chromatographic (HPLC) methods for the analysis of aspirin and its metabolites in plasma and urine have been reported (2–7). Few of the reported methods (3, 6) can determine aspirin in plasma, and they employ time-consuming extraction procedures. Since aspirin is rapidly hydrolyzed in blood and plasma (8), extraction procedures which delay the analysis of the sample will yield inaccurate results, underestimating the quantity of aspirin. Therefore, this work was initiated to develop a method for the rapid analysis of plasma samples for aspirin, salicylic acid, salicyluric acid, and gentisic acid, which does not require an extraction step.

The analysis of urine for the metabolites of aspirin using a simple extraction procedure is also desirable since urine usually contains a large number of acidic components (9, 10). Better results are obtained by eliminating as many undesirable components from the sample as possible before analysis. Injecting the urine onto a column without extraction (7, 11) shortens the useful life of the chromatography column.

EXPERIMENTAL

Materials—Aspirin USP[1], salicylic acid[1], salicyluric acid[1], gentisic acid[1], *o*-toluic acid[1], *o*-anisic acid[1], glacial acetic acid[2], anhydrous ether[3], acetonitrile[4], and methanol[4] were obtained commercially. The mobile phase consisted of water–methanol–glacial acetic acid (64:35:1, v/v/v), filtered and deaerated under reduced pressure.

Instrumentation—A dual-pump high-performance liquid chromatograph[5] equipped with a variable-wavelength UV detector[6], a 20-μl loop injector[7], and a reverse-phase microparticulate column[8] was used. The UV detector was connected to a linear recorder[9].

Stock Solutions—For the standard curves in plasma, aspirin, salicylic acid, salicyluric acid, and gentisic acid were singly dissolved in water–

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] J. T. Baker Chemical Co., Phillipsburg, N.J.
[3] Mallinckrodt, St. Louis, Mo.
[4] Burdick and Jackson Laboratories, Muskegon, Mich.
[5] Perkin-Elmer Series 2 High-Pressure Liquid Chromatograph, Perkin-Elmer, Norwalk, Conn.
[6] Model LC-55 Variable Wavelength Detector, Perkin-Elmer, Norwalk, Conn.
[7] Model 7125 Rheodyne, Berkeley, Calif.
[8] μBondapak C_{18} column, Waters Associates, Milford, Mass.
[9] Linear Instruments, Model 261/MM, Irvine, Calif.

0022-3549/83/0900-1020$01.00/0