Table III—Statistical Analysis: Absolute Error and Percent Deviation from Average

Dihydrophenylglycine	Phenylglycine	Cyclohexylglycine	Tetrahydrophenylglycine
99.1	101.9	98.4	105.9
102.4	95.8	100.4	92.7
101.4	101.9	97.6	94.0
98.3	96.9	105.3	106.6
$\bar{x}$ = 100.3	99.1	100.4	99.8
σ = ±1.92	±3.24	±3.46	±7.47
RSD, % = 1.91	3.27	3.45	7.48

RESULTS AND DISCUSSION

The NMR spectrum of a four-component mixture with dihydrophenylglycine (II) as the major component and phenylglycine (I), tetrahydrophenylglycine (III), and cyclohexylglycine (IV) as minor components is shown in Fig. 1. Tetramethylammonium bromide was chosen as the internal standard because it is readily available and gives a single NMR signal at 3.36 ppm; this signal does not interfere with the NMR spectra of the four components.

The NMR signal characteristic of each component is indicated in Fig. 1 and listed in Table I. Once these characteristic NMR signals have been established and assigned, the method is straightforward. The remaining operation is to integrate the respective signals carefully relative to the signal of a known weight of the internal standard. These data are sufficient to calculate the amount of each component present in the mixture by Eqs. 1 and 2.

The results for a series of four-component mixtures prepared for analysis by the NMR method are shown in Table II. Since two components are present in amounts less than 10%, the accuracy and precision of this complex mixture are reasonably adequate (Table III). The tetrahydrophenylglycine content is found by difference due to the overlap of this signal with part of the NMR signal from cyclohexylglycine.

The developed method has the advantage over existing methods in that no prior separation of the components is required. An additional advantage is that qualitative identification of the individual components is obtained from their NMR spectra.

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ACKNOWLEDGMENTS AND ADDRESSES

Received February 6, 1975, from *Research and Development, Smith Kline & French Laboratories, Philadelphia, PA 19101*

Accepted for publication July 8, 1975.

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Electron-Capture GLC Determination of Phenylpropanolamine as a Pentafluorophenyloxazolidine Derivative

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Abstract □ A simplified procedure is described for an electron-capture GLC determination of phenylpropanolamine in blood plasma. The method is based on derivatization of phenylpropanolamine with pentafluorobenzaldehyde at room temperature without prior extraction of the drug from plasma. The derivative, pentafluorophenyloxazolidine, is readily extracted from plasma into a small volume of hexane. Samples usually can be injected directly into the gas chromatograph without concentration of the hexane solution. Data are presented to illustrate the suitability of the method for dosage form bioavailability evaluation from plasma phenylpropanolamine levels achieved after acute oral administration of a typical dosage form.

Keyphrases □ Phenylpropanolamine—GLC analysis, plasma □ GLC—analysis, phenylpropanolamine, plasma □ Adrenergic agents—phenylpropanolamine, plasma

Previously reported methods for GLC determination of phenylalkanolamines and related compounds in plasma are based upon extraction of the amine from plasma followed by conversion to an electron-capturing perfluoroacyl- or pentafluorobenzaldehyde derivative (1–10). While this approach provides adequate sensitivity, the extraction procedure necessary to isolate the relatively water-soluble phenylalkanolamine from plasma sometimes gives rise to troublesome interfering peaks in the chromatogram.

A marked improvement in assay simplicity, together with excellent sensitivity and reliability, can be achieved by derivatization of phenylpropanolamine directly in plasma. The principle of the method involves reaction of the phenylpropanolamine in plasma with pentafluorobenzaldehyde to yield an electron-capturing derivative, which is then readily extracted into a small volume of hexane. The derivative forms within 1 hr at room temperature and is extremely stable under the assay conditions.

EXPERIMENTAL

Chromatographic Conditions—The gas chromatograph[1] was equipped with both an alkali flame-ionization detector and a 250-mCi tritium electron-capture detector. Glass columns, 2 m in length, 0.625 cm o.d., 0.20 cm i.d., were packed with 100–120-mesh

[1] Varian Aerograph model 2700, Varian Instrument Division, Palo Alto, Calif.

Gas Chrom Q coated with 1.25% OV-17[2]. Columns were conditioned for 36 hr at 275° and were treated *in situ* with a silylating agent[3]. Nitrogen carrier gas flow was 30 ml/min. For alkali flame-ionization detection, hydrogen flow was 27 ml/min and air flow was 300 ml/min. The column temperature was 190°, the injector temperature was 245°, and the detector temperature was 210°.

Reagents—The following were used: phenylpropanolamine hydrochloride NF; pentafluorobenzaldehyde[4], 98%; 2,4-dinitrofluorobenzene[4]; and hexane[5], pesticide grade. Other materials were reagent grade.

The marker, 2,4-dinitrophenyl-*N,N*-diethylamine, was prepared from 2,4-dinitrofluorobenzene and diethylamine and was twice recrystallized from ethanol.

The pentafluorobenzaldehyde, although adequately pure when fresh, gave numerous interfering peaks in the chromatogram within a few days after the original container was opened. Therefore, the aldehyde was shaken with an excess of saturated aqueous sodium bisulfite solution, and the precipitated aldehyde bisulfite compound was collected and recrystallized from aqueous ethanol. The aldehyde bisulfite compound is stable and can be stored for use in this form.

Estimation of Phenylpropanolamine in Plasma—Into 13-mm glass culture tubes (acid washed and siliconized[6]) were placed 3 ml of plasma, approximately 20 mg of aldehyde bisulfite reagent, two drops of 10% aqueous potassium hydroxide, and 2 ml of a hexane solution containing 640 ng of the marker, 2,4-dinitrophenyl-*N,N*-diethylamine. The tubes were capped[7] and rocked for 1 hr at room temperature while protected from direct light. The tubes were then centrifuged, and 2-μl samples of the hexane solution were injected into the gas chromatograph. The concentration of phenylpropanolamine in plasma was determined by comparison of peak height ratios and a standard curve.

Calibration Curve—Known amounts of phenylpropanolamine hydrochloride were added to 2.5 ml of human plasma, volume was made up to 3 ml with distilled water, and these solutions were carried through the same procedure as already described.

Similar standard curves were obtained by adding phenylpropanolamine hydrochloride to distilled water rather than to plasma.

RESULTS

Figure 1 shows a typical chromatogram for electron-capture

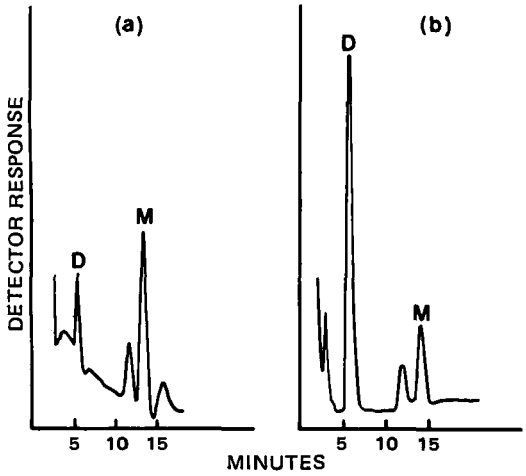


Figure 1—(a) *Chromatogram obtained after spiking 3 ml of pooled human plasma with phenylpropanolamine hydrochloride to provide a concentration of 13.3 ng/ml; attenuation 32.* (b) *Chromatogram obtained from assay of a 2-ml plasma sample from a human subject 4 hr after oral ingestion of 50 mg of phenylpropanolamine hydrochloride; attenuation 64. The symbols D and M refer to drug and marker peaks, respectively.*

[2] Applied Science Laboratories, State College, Pa.
[3] Silyl-8, Pierce Chemical Co., Rockford, Ill.
[4] Aldrich Chemical Co., Milwaukee, Wis.
[5] Matheson, Coleman and Bell, East Rutherford, N.J.
[6] Siliclad, Clay Adams, Division of Becton, Dickinson and Co., Parsippany, N.J.
[7] Stoppers lined with Teflon (du Pont).

Table I—Standard Curve Obtained for Derivatization, Extraction, and GLC Quantitation of Phenylpropanolamine Hydrochloride Added to Plasma and Water

Medium	Drug[a]/Marker[b]	Weight Ratio	Weight Ratio: Peak Height Ratio	Weight Ratio: Peak Height Ratio
Plasma	1.1 ng/32 ng	0.034	0.15	0.23
Plasma	2.2 ng/32 ng	0.069	0.33	0.21
Plasma	3.3 ng/32 ng	0.103	0.50	0.21
Plasma	5.5 ng/32 ng	0.172	0.86	0.20
Plasma	11.0 ng/32 ng	0.344	1.58	0.22
Plasma	200 ng/640 ng	0.312	1.55	0.20
Plasma	300 ng/640 ng	0.469	2.44	0.19
Plasma	400 ng/640 ng	0.625	3.09	0.20
Plasma	500 ng/640 ng	0.781	3.70	0.21
Water	300 ng/640 ng	0.469	2.28	0.21
Water	500 ng/640 ng	0.781	3.94	0.20
Water	0/640 ng	0	0.03	—
Plasma	0/640 ng	0	0.00	—

[a] Phenylpropanolamine hydrochloride in a 3-ml solution. [b] 2,4-Dinitrophenyl-*N,N*-diethylamine.

quantitation of phenylpropanolamine in spiked human plasma and in plasma obtained from a human subject after oral ingestion of a phenylpropanolamine dosage form.

Table I illustrates the linearity of a calibration curve obtained for phenylpropanolamine hydrochloride added to plasma in amounts ranging from 1.1 to 500 ng/3 ml of plasma. Data for four replicate determinations at 13 and 130 ng/ml indicate a relative standard deviation of less than 5% at both concentrations.

Figure 2 presents typical plasma levels of phenylpropanolamine in two human subjects who ingested 50 mg of phenylpropanolamine hydrochloride in tablet form after an overnight fast.

DISCUSSION

The principle of this analytical method rests on the reaction of pentafluorobenzaldehyde with phenylpropanolamine to form a derivative, which is proposed to be a pentafluorophenyloxazolidine (Scheme I). Reaction of β-aminoalcohols with aldehydes to produce oxazolidines was reported previously (11–14). In some cases, however, the product may exist as a mobile tautomeric system with the corresponding Schiff base.

Bergmann (15), in his comprehensive review, discussed the structure, reactions, and synthesis of oxazolidines. Neelakantan (16, 17) and Neelakantan and Molin-Case (18) did extensive studies on the stereospecific reaction of aromatic aldehydes with ephedrine and ψ-ephedrine. Indeed, this reaction is an induced asymmetric synthesis. The reaction of aldehyde bisulfites with ephedrine also pro-

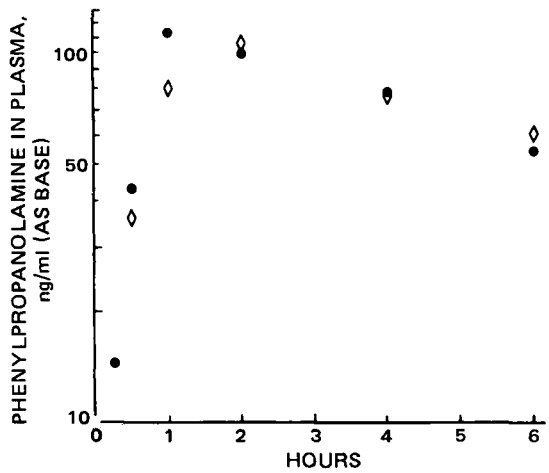
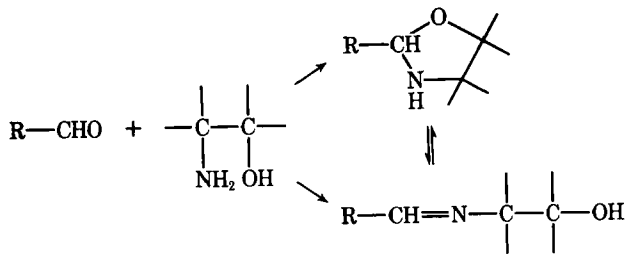


Figure 2—*Plasma phenylpropanolamine levels in two human subjects following ingestion of 50 mg of phenylpropanolamine hydrochloride in tablet form.*



Scheme I

ceeds by a stereospecific mechanism. These α-aminoalkanesulfonates readily give the corresponding oxazolidines in the presence of base (17). The absolute configurations and structures were confirmed by X-ray diffraction for the benzaldehyde–ephedrine product (18).

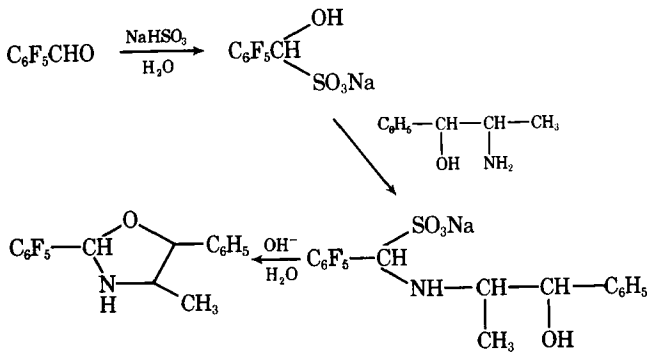
In the present case, pentafluorobenzaldehyde or its bisulfite addition compound reacts with phenylpropanolamine in the presence of base. The product obtained is sparingly soluble in water and readily soluble in aprotic solvents such as hexane and benzene. The product is easily hydrolyzed by acids and very stable in aqueous base. The reaction involves facile splitting of water in an aqueous medium and goes to completion at room temperature in 1 hr. Both thermodynamic and steric factors probably contribute to the ease of formation of this oxazolidine.

Moffat and Horning (6) reported the formation of a Schiff base from pentafluorobenzaldehyde and β-hydroxy-β-phenylethylamine by heating in acetonitrile for 1 hr at 60° and, after addition of *N,O*-bis(trimethylsilyl)acetamide, further heating for 1 hr at 60°. Even in this case, however, formation of an oxazolidine, with subsequent ring opening with *N,O*-bis(trimethylsilyl)acetamide, cannot be ruled out. Indeed, the reactivity of oxazolidines cannot unambiguously be differentiated from that of Schiff bases in many chemical reactions. Physical properties such as boiling point, dipole moment, magnetic susceptibility, and UV spectra also cannot establish with certainty the structure of these products.

In mass fragmentography, benzyl cleavage would occur predominantly in both cases. Molecular refraction and IR spectra might be of assistance, but ^{13}C-NMR might be most useful. The ^{13}C-NMR spectra would be free of complications from the methylene protons. From the pronounced difference in the geometry of the two possible structures, it might be possible to assign the molecular structure of the reaction product. This study is in progress.

On the basis of currently available information, Scheme II is suggested for the derivatization.

Indirect evidence for the oxazolidine structure is provided from the reaction of both ephedrine and phenylpropanolamine with



Scheme II

benzaldehyde. In both cases, the products are formed readily at room temperature. Both products are readily extracted with hexane, are stable to base, and are easily hydrolyzed by acid. Their respective retention time and sensitivity to an alkali flame-ionization detector are very similar.

Although the structure of the pentafluorobenzaldehyde–phenylpropanolamine derivative remains to be confirmed, the derivative provides a simple, reliable, and sensitive method for quantitation of phenylpropanolamine. No back-extractions or preliminary cleanups are involved. Since the reaction goes to completion at room temperature in 1 hr, there is no possibility of decomposition due to heating. The product is readily extracted into hexane, and usually no concentration of the hexane solution is required. If desired, however, the hexane extract can be concentrated by evaporation of solvent without loss of the derivative or marker.

The derivative has been shown to be stable in hexane for days. This derivative avoids problems such as partial hydrolysis of silyl ether groups in the presence of moisture, nitrogen to oxygen migrations, and similar complications which may arise with electron-capture derivatives of the perfluoroacyl type.

As shown in Fig. 1, spiked plasma samples yield several additional, unidentified, chromatographic peaks. These peaks also appear in drug-free plasma, but they do not interfere with phenylpropanolamine quantitation. Figure 2, illustrating typical plasma phenylpropanolamine levels following a single oral administration of 50 mg of phenylpropanolamine hydrochloride in tablet form, indicates that the assay procedure is quite adequate for assessment of bioavailability.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 20, 1974, from the *College of Pharmacy, University of Kentucky, Lexington, KY 40506*

Accepted for publication July 14, 1975.

Supported by a research grant from Warner-Lambert Research Institute, Morris Plains, N.J.

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