facilitate identification by optical crystallographic methods. Dilituric acid rarely crystallized out of the reaction solution in the concentration utilized; however, dilituric acid crystals appeared as small square- to rectangular-shaped tablets with a low α index (n = 1.388), running parallel to the crystal length and showing a low extinction angle (9°) on the top face.

Dextroamphetamine crystallized out as thin rectangular platelets that showed bright first- to second-order polarization colors under crossed nicols as compared to its *dl*-isomer, which was observed as poorly formed acicular prisms showing a high birefringence. *d*-Methamphetamine appeared as large, well-formed, lamellar platelets twinned on 001, showing bright polarization colors and a front face extinction angle of 33°. Its *dl*-isomer occurred as bundles of elongated rods with a notably small $2V$ = 8°. Both isomers are monoclinic crystals as compared to the two amphetamine isomers which show orthorhombic symmetry.

Derivatives of *l*-ephedrine, racephedrine, *dl*-chlorpheniramine, methapyrilene, and diphenhydramine crystallized out as six-sided platelets. The two ephedrine isomers crystallized out as large hexagonal platelets. *l*-Ephedrine showed orthorhombic symmetry, while racephedrine crystallized out as a large, yellow, triclinic platelet with a 38° extinction angle on the front face. *dl*-Chlorpheniramine had a crystal size smaller than the ephedrines and also showed monoclinic symmetry with an extinction angle of 15° on the top face. Methapyrilene and diphenhydramine appeared as monoclinic crystals with extinction angles of 33 and 42°, respectively, on the top face. However, diphenhydramine occurred as stellate rosettes, and the crystals showed a large $2V$ = 75°; methapyrilene appeared as a diamond-shaped crystal with a moderate $2V$ = 48°.

Propoxyphene diliturate occurred as long, acicular, monoclinic crystals that crystallized out in stellate rosettes. The side face of this crystal showed a 15° extinction angle. The mephentermine derivative was a long, acicular, monoclinic crystal with an extinction angle of 27° appearing on the top face; the phentermine diliturate, which was of the same crystal habit, showed orthorhombic symmetry.

Pseudoephedrine, phenylephrine, and papaverine diliturates crystallized out as prisms. Derivatives of both pseudoephedrine and phenylephrine were elongated in form but were distinguished easily from each other since pseudoephedrine had an orthorhombic symmetry and a low $2V$ = 36° while phenylephrine appeared as a triclinic crystal with a $2V$ = 70° and a front face extinction angle of 42°. The papaverine diliturate was observed as an almost equant triclinic prism with multifaceted sides.

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High-Pressure Liquid Chromatographic Analysis of Phenylpropanolamine in Human Plasma following Derivatization with *O*-Phthalaldehyde

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Abstract □ A high-pressure liquid chromatographic analysis of phenylpropanolamine in human plasma following extraction, back-extraction, and *O*-phthalaldehyde derivatization is presented. Using fluorescence detection, the method was sufficiently sensitive to quantitate as little as 5 ng of drug/ml of plasma; the coefficient of variation below 100 ng/ml ranged between 5.7 and 2.8%. Plasma concentration data following a single 25-mg dose of phenylpropanolamine hydrochloride in 12 healthy volunteers demonstrate the application of the analytical method.

Keyphrases □ High-pressure liquid chromatography—phenylpropanolamine analysis in human plasma, *O*-phthalaldehyde derivatization □ Phenylpropanolamine—high-pressure liquid chromatographic analysis in human plasma, *O*-phthalaldehyde derivatization □ Derivatization—*O*-phthalaldehyde, high-pressure liquid chromatographic analysis in human plasma

There is great interest in measuring plasma phenylpropanolamine concentrations following single therapeutic doses (25 mg of phenylpropanolamine hydrochloride) of this sympathomimetic agent. GLC following formation of a heptafluorobutyryl derivative (1) was used previously (2, 3) to study phenylpropanolamine pharmacokinetics and was adequate when doses of 60 mg or more were administered or when plasma concentrations were >40 ng/ml and 2 ml of plasma was available for each determination. TLC following acetylation with tritiated acetic anhydride (4) suffers from a high coefficient of variation and nonlinearity.

After preliminary studies of GLC and liquid chromatography with absorbance detection were found to have inadequate specificity and sensitivity, the present method was developed. Previous investigators (5–7) showed that *O*-phthalaldehyde, in the presence of 2-mercaptoethanol, reacts with primary amines to form highly fluorescent products, 1-alkylthio-2-alkyl-substituted isoindoles (8). Replacing 2-mercaptoethanol with ethanethiol was reported to provide a more stable product (9), but the latter is difficult to work with due to its unpleasant odor.

A procedure for extraction of phenylpropanolamine and an internal standard from plasma, derivatization with *O*-phthalaldehyde, separation by high-pressure liquid chromatography (HPLC), and detection by fluorescence

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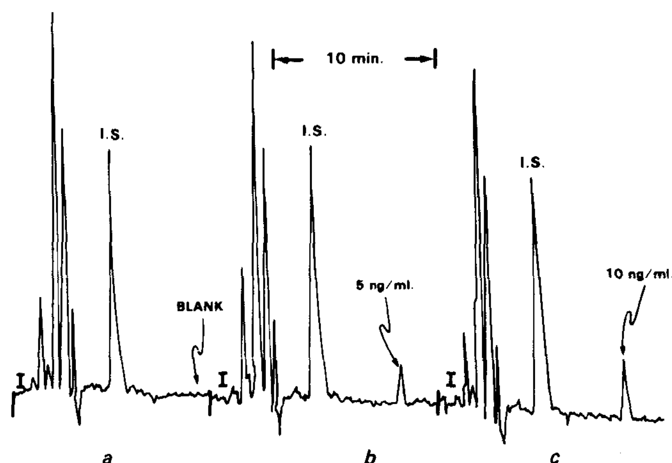

Figure 1—*HPLC tracings for plasma. Key:* a, *blank plasma;* b, *5 ng of phenylpropanolamine added to 1 ml of plasma; and* c, *10 ng of phenylpropanolamine added to 1 ml of plasma.*

is presented in this report. The procedure can differentiate plasma with as little as 1 ng/ml from a blank and can quantitate plasma concentrations above 5 ng/ml with accuracy and precision suitable for pharmacokinetic studies.

EXPERIMENTAL

Materials—UV grade butyl chloride[1], 1-butanol[1], methanol[1], and acetonitrile[1] were employed without further preparation. Reagent grade ammonium acetate[2], boric acid[3], and ACS grade acetic acid[3] also were used as received. A derivatization solution was prepared by dissolving 50 mg of crystalline *O*-phthalaldehyde[4] in 2 ml of methanol, adding 0.2 ml of 2-mercaptoethanol[5], and diluting to 100 ml with 0.4 *M* borate buffer (pH 10.5). Phenylpropanolamine hydrochloride[6] and viloxazine hydrochloride[7] [2-(2-ethoxyphenoxymethyl)tetrahydro-1,4-oxazine hydrochloride, ICI-58,834] were used as received.

Apparatus—The HPLC system consisted of a pump[8], an injector[9], a 5-μm ODS column[10], and a fluorescence detector[11]. Excitation was at 230 nm (sensitivity, photomultiplier tube voltage setting, at 380 and time constant of 2.0 sec), and no filters were employed between the flowcell and photomultiplier tube. The window of the photomultiplier served as a 280-nm cutoff filter. The detection range was set at 0.2 with the recorder[12] input set to provide tracings corresponding to 0.2 and 0.02 and a chart speed of 0.5 cm/min.

Plasma Extraction—One milliliter of plasma, 0.1 ml of 0.1 *N* NaOH, and 8.0 ml of extraction solution (10% v/v 1-butanol in butyl chloride with 0.60 μg of viloxazine/ml as the internal standard) were placed in a 15-ml centrifuge tube. This mixture was vortexed for 60 sec and centrifuged for 5 min, and the organic layer (upper) was transferred to a second 15-ml centrifuge tube containing 300 μl of 1% acetic acid.

Back-extraction was effected by vortexing for 60 sec, and the layers were separated by centrifugation. The organic layer (upper) was discarded by aspiration, leaving the aqueous layer for derivatization. This acetic acid back-extract was stable for several days.

Derivatization—A 50-μl syringe was used to transfer 40 μl of a 1% acetic acid back-extract into a 4.0-ml glass tube to which 20 μl of the *O*-phthalaldehyde reagent then was added, and the solution was vortexed for 10 sec. The reaction was allowed to proceed exactly 60 sec, and then 200 μl of 1% acetic acid was added. The solution was vortexed, and 100 μl was injected immediately into the HPLC system.

Chromatography—A mobile phase was prepared by dissolving 10.4 g of ammonium acetate in 2400 ml of water and adding 8.0 ml of acetic acid and sufficient acetonitrile to obtain a 40% solution by volume (pH 5.6). With a flow rate of 1.8 ml/min in the HPLC system, a pressure of 2000 psig resulted. The retention times of viloxazine and the phenylpropanolamine derivative were 5.8 and 8.2 min, respectively.

Clinical Study—As part of a bioavailability study, 12 normal healthy subjects received single 25-mg doses of phenylpropanolamine hydrochloride in solution following an overnight fast. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hr in heparin-containing vacuum containers[13]. The plasma was separated immediately by centrifugation and frozen within 20 min. All samples were maintained frozen at −10° until assayed.

[1] Burdick & Jackson, Muskegon, Mich.
[2] J. T. Baker, Phillipsburg, N.J.
[3] Mallinckrodt, St. Louis, Mo.
[4] Fluoropa, Durrum Chemical Corp., Palo Alto, Calif.
[5] Eastman Kodak Co., Rochester, N.Y.
[6] Kindly supplied by A. H. Robins Co., Richmond, Va.
[7] Kindly supplied by ICI Americas Inc., Wilmington, Del.
[8] Model 6000A, Waters Associates, Milford, Mass.; model 110A, Altex Scientific, Berkeley, Calif.
[9] Model U6K, Waters Associates, Milford, Mass.; L.D.C. Rheodyne 7125, Rheodyne Inc., Cotati, Calif.
[10] Laboratory Data Control, Riviera Beach, Fla.
[11] Schoeffel S.F.970, Kratos, Westwood, N.J.
[12] Omniscribe, Houston Instruments, Austin, Tex.

Table I—Precision and Accuracy of Phenylpropanolamine Analysis

Actual Concentration[a], ng/ml	Number of Samples	Mean Concentration, ng/ml	*SD*	*CV*, %
17.3	12	17.2	0.98	5.7
20.0	12	20.1	1.13	5.7
40.0	10	38.8	1.72	4.4
50.0	20	49.8	2.13	4.3
80.6	12	80.3	2.22	2.8
100	20	101	3.20	3.2
150	10	148	6.33	4.3
200	24	201	7.68	3.8

RESULTS AND DISCUSSION

Figure 1 presents chromatograms for plasma containing 0, 5, and 10 ng of phenylpropanolamine/ml. Analysis of predose plasma samples from 22 human subjects and several batches of pooled human plasma presented no chromatographic peaks that interfered with the internal standard or phenylpropanolamine derivative. A previous report (10) suggested that no significant metabolism of phenylpropanolamine occurs, so *in vivo* interference by metabolites probably was not a problem. The ratio of the peak height of the phenylpropanolamine derivative to the viloxazine peak height varied linearly with the plasma phenylpropanolamine concentration range studied (*i.e.*, <200 ng/ml). A typical standard curve (20 points between 5 and 200 ng/ml), with the peak height ratio as the abscissa and plasma phenylpropanolamine as the ordinate, had a slope of 27.94 ng/ml and an intercept of −0.32 ng/ml with a correlation coefficient of 0.9976.

Table I demonstrates the precision and accuracy of the method following a single therapeutic dose. These data were collected over several weeks and thus demonstrate reproducibility. The lowest tabulated standard was 17.3 ng/ml; however, the method can be employed for concentrations of 5 ng/ml with slightly less precision (*i.e.*, coefficient of variation of 8%).

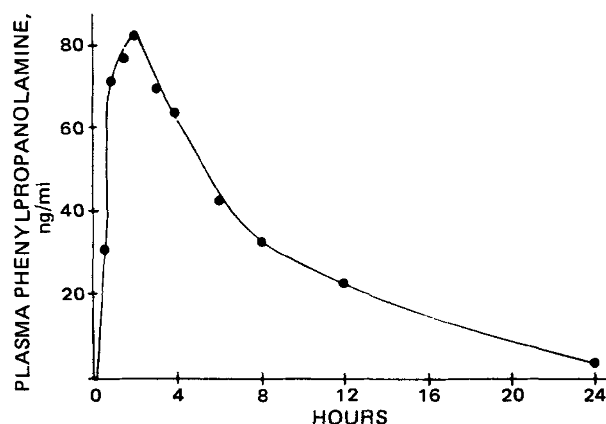

Figure 2—*Mean plasma phenylpropanolamine for 12 subjects following a single 25-mg dose of phenylpropanolamine hydrochloride in solution.*

[13] Vacutainers.

Figure 2 presents the mean plasma concentration–time data for the 12 subjects who received 25 mg of phenylpropanolamine hydrochloride. The concentrations were within the quantitative range of the described method. The samples were assayed in duplicate with 2 weeks between duplicate determinations of each sample. The precision of these assays is indicated by the mean coefficient of variation of 3.0% for duplicates. A repeated analysis of 24 samples after 6 months at −10° revealed no significant differences, thus indicating sample stability.

The general approach presented in this report produced very clean chromatograms and thus should be considered for other primary amines.

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COMMUNICATIONS

Drug–Disintegrant Interactions: Binding of Oxymorphone Derivatives

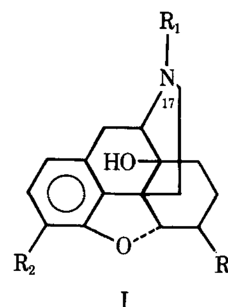
Keyphrases □ Drug–disintegrant interactions—binding of oxymorphone derivatives to disintegrating agents, dissolution testing, Freundlich adsorption isotherm □ Disintegrating agents—carboxymethylcellulose sodium, sodium starch glycolate, povidone, and modified cornstarch, binding to oxymorphone derivatives compared □ Oxymorphone derivatives—binding to disintegrating agents compared, Freundlich adsorption isotherm □ Freundlich adsorption isotherm—binding of oxymorphone derivatives to disintegrating agents compared, dissolution testing □ Analgesics, narcotic—oxymorphone, binding to disintegrating agents compared, dissolution testing

To the Editor:

Since the USP first established a disintegration standard in 1948, the search for good disintegrating agents for tablet formulations has intensified. Starch had been used as the primary tablet disintegrant and, in most cases, was relatively effective. However, recognition of the importance of bioavailability and compendial dissolution test requirements spurred the search for new disintegrants. An ideal disintegrant would improve both disintegration and dissolution and be effective in small amounts.

The search has produced several new disintegrating agents, most notably internally cross-linked carboxymethylcellulose sodium[1], sodium starch glycolate[2], cross-linked polyvinyl pyrrolidone[3] (povidone), and modified cornstarch[4].

During the development of a tablet formulation with a rapid disintegration/dissolution rate profile for a synthetic narcotic agonist–antagonist analgesic with the general structure of oxymorphone (I), extensive binding was noted between I and cross-linked carboxymethylcellulose sodium, and lower recovery resulted during dissolution testing. However, the binding to sodium starch glycolate was substantially less extensive, and no binding was detected for carboxymethylcellulose sodium, cornstarch, and modified cornstarch.



The binding of I to cross-linked carboxymethylcellulose sodium and sodium starch glycolate followed the Freundlich adsorption isotherm as shown in Fig. 1. The binding was sensitive to variation in solution pH (Fig. 2). Maximum binding was achieved for both cross-linked carboxy-

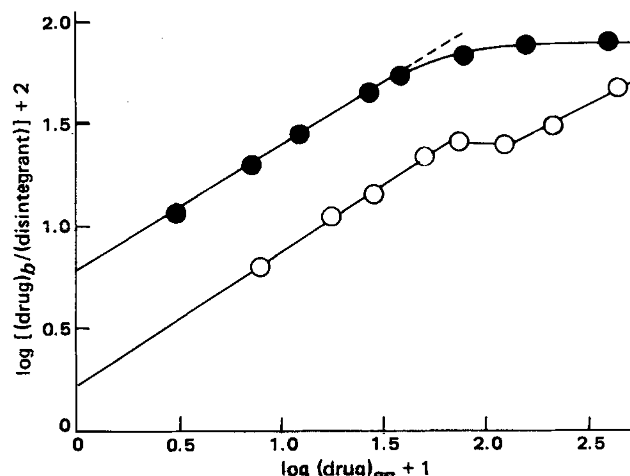


Figure 1—*Freundlich adsorption isotherm for the interaction of oxymorphone derivative (I) with cross-linked carboxymethylcellulose sodium (●) and sodium starch glycolate (○) in distilled water at room temperature.*

[1] Ac-Di-Sol, FMC Corp., Philadelphia, PA 19103.
[2] Explotab, E. Mendell Co., Carmel, NY 10512.
[3] PVP-XL, GAF Co., New York, NY 10020.
[4] StaRx 1500, Staley & Co., Decatur, IL 62525.

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