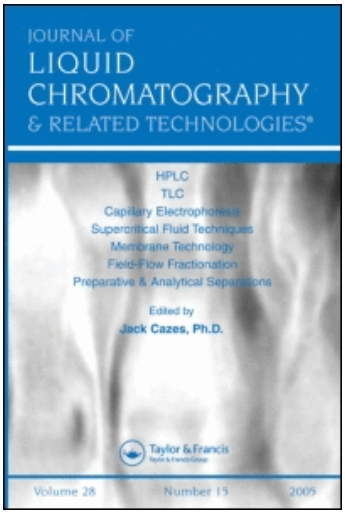

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Ion-Pair Liquid Chromatographic Analysis of Phenylpropanolamine in Plasma and Urine by Post-Column Derivatization with O-Phthalaldehyde

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ION-PAIR LIQUID CHROMATOGRAPHIC ANALYSIS OF PHENYLPROPANOLAMINE IN PLASMA AND URINE BY POST-COLUMN DERIVATIZATION WITH O-PHTHALALDEHYDE

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ABSTRACT

A high pressure liquid chromatographic analysis of phenylpropanolamine in plasma and urine by post-column derivatization with o-phthalaldehyde is described. Plasma samples are extracted with methylene chloride under alkaline conditions. Urine is diluted with mobile phase without extraction. Using fluorescence detection, the method is sufficiently sensitive (2 ng/ml in 0.5 ml of plasma and 0.5 mcg/ml in 0.2 ml of urine) so that phenylpropanolamine concentrations in plasma or urine may be measured for up to 24 hours following a 75 mg oral dose. Coefficients of variation for inter-day and intra-day precision are less than 10%.

INTRODUCTION

Phenylpropanolamine (Fig. 1), a sympathomimetic amine, is widely used as a decongestant and an appetite suppressant. For

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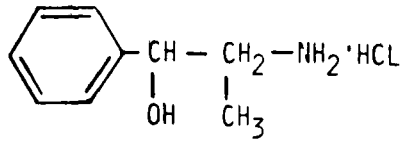
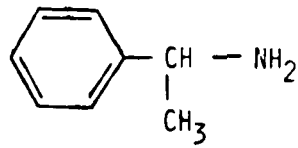


PHENYLPROPANOLAMINE HCL



α- METHYLBENZYLAMINE (INTERNAL STD)

FIGURE 1.

pharmacokinetic and bioavailability studies, phenylpropanolamine concentrations in biological fluids has been determined by gas liquid chromatography (GLC) following extraction and derivatization using an electron capture detector[1] or a nitrogen selective detector[2,3]. High-performance liquid chromatography following extraction[4] and pre-column derivatization[5-7] have also been reported. A post-column derivatization method has also been described recently for urine sample measurements[8].

A sensitive, rapid method for separation of phenylpropanolamine by HPLC followed by post-column in-line derivatization with o-phthalaldehyde and fluorometric detection is presented here for both plasma and urine. Only 0.5 ml of plasma or 0.2 ml of urine is needed for this assay.

EXPERIMENTAL

Apparatus

The HPLC consists of two pumps (Model 110, Beckman Inc., San Jose, CA), an automatic sample processor (WISP 710B, Waters Associates, Milford, MA), 5 μm Ultrasphere ODS columns (Beckman Inc., San Jose, CA), 4.6 mm X 15 cm for the plasma assay and 4.6 mm X 25 cm for the urine assay. A "T" mixing connector and a 10 ft. long coiled teflon tubing (I.D. 0.027") are used as the post column in-line reactor. A fluorescence detector Shimadzu Rf-530 (InstruSpec, Inc., Concord, CA) and an integrator (Model 3390A, Hewlett-Packard, Santa Clara, CA) were also used.

Reagents and Materials

Acetonitrile (J.T. Baker Chem. Co., Phillipsburg, NJ) and methylene chloride (Burdick and Jackson Lab. Inc., Muskegon, MI) were HPLC grade. Phenylpropanolamine HCl (Ciba-Geigy Pharm Div., Summit, NJ), α-methylbenzylamine (Pfaltz & Bauer, Inc., Stamford, CT), o-phthalaldehyde (Sigma Chem. Co., St. Louis, MO), tetramethylammonium chloride (Fluka Chem. Co., Hauppauge, NY), and sodium 1-heptane sulfonate (Regis Chem. Co., Morton Grove, IL) were obtained commercially. Water was purified through a Nanopure apparatus (Barnstead Co., Boston, MA). All other reagents were analytical or reagent grade.

Chromatographic Conditions

The mobile phase for the plasma assay was prepared by mixing 2400 ml of acetonitrile, 1600 ml of water, 0.8 gm of sodium 1-heptane sulfonate and 12 ml of glacial acetic acid. For the urine assay, the mobile phase was prepared by mixing 1400 ml of acetonitrile, 2600 ml of water, 32 ml of 1% tetramethylammonium chloride aqueous solution and 12 ml glacial acetic acid. The solvents were degassed and filtered before used.

The post column o-phthalaldehyde solution was prepared by dissolving 100 gm of boric acid in 3.9 liters of water, adjusting the pH to 10.4 with 50% KOH, adding 8 ml of 2-mercaptoethanol and 40 ml of 8% o-phthalaldehyde methanolic solution. The solution is stable for weeks. Both the mobile phase and the post-column solution were pumped at a flow rate of 1.0 ml/min in both plasma and urine assays. The fluorescence detector was set at 340 nm for excitation and 418 nm for emission. The retention times for phenylpropanolamine and methylbenzylamine (the internal standard, Fig.1) were 8.5 min and 10.3 min, respectively in plasma (Fig. 2) and 11.3 min and 13.3 min, repectively in urine (Fig. 3).

Sample Preparation

Plasma samples (0.5 ml) were extracted with 5 ml of methylene chloride solution containing internal standard and 100 μl of 0.5 M

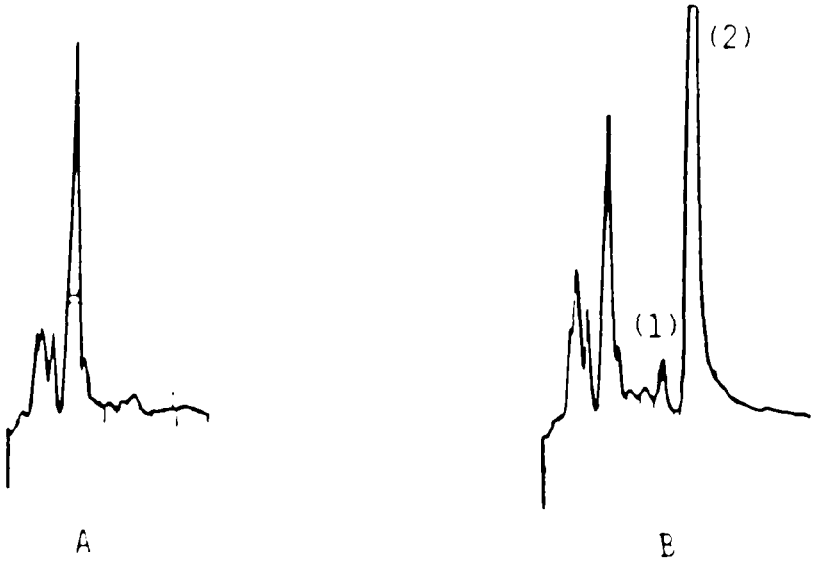


FIGURE 2. HPLC CHROMATOGRAMS OF (A) BLANK PLASMA EXTRACT AND (B) AN EXTRACT OF PLASMA CONTAINING PHENYLPROPANOLAMINE (4 NG/ML) (1) AND α-METHYLBENZYLAMINE (2).

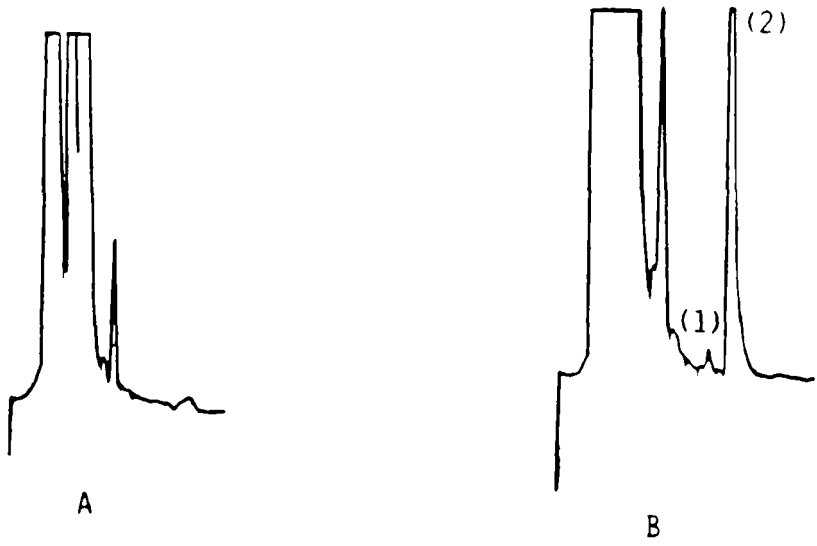


FIGURE 3. HPLC CHROMATOGRAMS OF (A) BLANK URINE AND (B) URINE CONTAINING PHENYLPROPANOLAMINE (1 MCG/ML) (1) AND α-METHYLBENZYLAMINE (2).

K_2HPO_4 (pH 11) for 15 minutes in a rotator. After centrifuging at 3000 G for 10 minutes, the plasma layer was discarded and the organic layer transferred to a test tube containing 50 µl of 0.1% HCl in acetonitrile. The solution was then evaporated to dryness under nitrogen, 200 µl of mobile phase was added to the residue and the solution was vortexed for 1 minute. A 40 to 100 µl aliquot of this solution was injected onto the column. Urine samples (0.2 ml) were diluted with 2 ml of the mobile phase containing the internal standard and mixed by vortexing for 1 minute; a 2 to 10 µl aliquot of the resulting solution was delivered onto the column.

RESULTS

Calibration curves using ten different concentrations ranging from 2 to 200 ng/ml in the plasma assay and eleven different concentrations from 0.5 to 150 mcg/ml in the urine assay were

obtained by calculating the ratio of the peak height of phenylpropanolamine to that of the internal standard versus their respective concentrations. Over these concentration ranges, the standard curves were linear for both plasma and urine assays with r^2 values greater than 0.9990 (see Table 1). However, these

TABLE 1

LINEARITY OF REPRESENTATIVE CALIBRATION CURVES

PLASMA					URINE				
		CALCULATED (ng/ml)					CALCULATED (mcg/ml)		
SPIKED CONC ng/ml	PEAK HEIGHT	FROM ALL DATA	FROM 0–40	FROM 40–200	SPIKED CONC mcg/ml	PEAK HEIGHT	FROM ALL DATA	FROM 0–15	FROM 15–150
2	0.039	3.08	2.28		0.5	0.033	0.33	0.58	
4	0.067	4.68	3.92		1	0.055	0.75	0.97	
6	0.101	6.61	5.92		2	0.111	1.83	1.94	
10	0.166	10.3	9.73		4	0.224	3.99	3.92	
16	0.271	16.3	15.9		8	0.462	8.54	8.08	
30	0.521	30.5	30.6		15	0.857	16.1	15.0	16.1
40	0.676	39.3	39.7	40.5	30	1.565	29.7		29.6
80	1.323	76.1		76.9	50	2.654	50.5		50.9
120	2.049	117.		118.	80	4.087	77.9		77.9
200	3.556	203.		202.	100	5.28	101.		101.
					150	7.864	150.		150.
Intercept		−0.0146	0.0002	−0.0430			0.0159	−0.0005	0.0180
Slope		0.0176	0.0170	0.0178			0.0522	0.0572	0.0522
r^2		0.9991	0.9996	0.9988			0.9997	0.9999	0.9996

standard curves showed significant deviations at the lower concentrations. If two calibration curves are calculated separately over ranges of 2 to 40 ng/ml and 40 to 200 ng/ml for the plasma assay and 0.5 to 15 mcg/ml and 15 to 150 mcg/ml for the urine assay, the accuracy at the lower concentrations is greatly improved, as shown in Table 1. Therefore, two standard curves were used for calculating the concentrations of clinical samples.

Precision and Accuracy

Precision of the method over the entire working range was determined with the analysis of spiked samples. In Tables 2 and 3, the interday and intraday precision for four different plasma concentrations are presented. Tables 4 and 5 detail the interday and intraday precision of the method at four different urine concentrations. Average CVs for the method were less than 10% at all concentrations.

TABLE 2
INTER-DAY PRECISION PHENYLPROPANOLAMINE HCL IN PLASMA

1	2	3	4	5	6	MEAN	SD	%CV
HIGH CONCENTRATION (SPIKED: 100 ng/ml)								
105	95.6	92.3	95.8	98.2	97.5	97.4	4.20	4.31%
MEDIUM CONCENTRATION (SPIKED: 50.0 ng/ml)								
53.3	42.8	49.9	49.8	47.5	56.2	49.9	4.64	9.30%
LOW CONCENTRATION (SPIKED: 20.0 ng/ml)								
21.8	19.3	20.7	19.3	20.1	20.1	20.3	0.93	4.60%
X-LOW CONCENTRATION (SPIKED: 10.0 ng/ml)								
10.6	9.92	10.4	10.0	10.0	9.09	10.1	0.52	5.19%

TABLE 3
INTRA-DAY PRECISION FOR PHENYLPROPANOLAMINE HCL IN PLASMA

1	2	3	4	5	6	MEAN	SD	%CV
HIGH CONCENTRATION (SPIKED: 100.0 ng/ml)								
95.1	90.6	98.6	96.7	96.2	97.5	95.8	2.79	2.91%
MEDIUM CONCENTRATION (SPIKED: 50 ng/ml)								
45.5	50.6	50.8	49.3	50.2	53.1	49.9	2.51	5.03%
LOW CONCENTRATION (SPIKED: 20 ng/ml)								
19.9	20.1	20.9	19.1	20.7	19.8	20.1	0.64	3.19%
X-LOW CONCENTRATION (SPIKED: 10.0 ng/ml)								
9.49	9.39	10.6	10.8	9.86	9.12	9.88	0.68	6.88%

TABLE 4
INTER-DAY PRECISION PHENYLPROPANOLAMINE HCL IN URINE

1	2	3	4	5	6	MEAN	SD	%CV
HIGH CONCENTRATION (SPIKED: 100 mcg/ml)								
110	98.7	99.3	99.3	96.4	93.6	99.5	5.54	5.57%
MEDIUM CONCENTRATION (SPIKED: 50.0 mcg/ml)								
54.9	47.0	47.8	49.6	46.3	46.3	48.6	3.29	6.76%
LOW CONCENTRATION (SPIKED: 20.0 mcg/ml)								
11.1	10.3	10.2	9.43	9.42	8.68	9.86	0.85	8.62%
X-LOW CONCENTRATION (SPIKED: 10.0 mcg/ml)								
1.08	0.92	1.11	1.02	0.93	1.10	1.03	0.08	7.78%

TABLE 5
INTRA-DAY PRECISION FOR PHENYLPROPANOLAMINE HCL IN URINE

1	2	3	4	5	6	MEAN	SD	%CV
HIGH CONCENTRATION (SPIKED: 100.0 mcg/ml)								
109	103	105	109	101	110	106	3.92	3.69%
MEDIUM CONCENTRATION (SPIKED: 50 mcg/ml)								
52.4	53.2	52.3	54.7	54.5	54.7	53.6	1.17	2.18%
LOW CONCENTRATION (SPIKED: 10 mcg/ml)								
9.32	9.46	9.18	10.8	8.98	10.9	9.77	0.84	8.60%
X-LOW CONCENTRATION (SPIKED: 1.0 mcg/ml)								
0.98	1.05	1.02	1.05	1.07	1.04	1.04	0.03	2.88%

Extraction Efficiency

Spiked plasma samples were assayed in triplicate at four different concentrations. All samples were treated as described previously except that for extraction, the internal standard was not added. After the organic solution was evaporated to dryness, the internal standard was added and evaporated again and reconstituted with mobile phase. Peak height ratios were compared with those obtained from samples containing equivalent concentrations of phenylpropanolamine and internal standard without extraction. Recoveries obtained ranged between 79 and 87% (Table 6).

DISCUSSION

Previous HPLC methods used to determine phenylpropanolamine in biological fluids are relatively time consuming with respect

TABLE 6
RECOVERY OF EXTRACTION (N=3)

Conc. Spiked	Peak Height Ratio After Extraction	Peak Height Ratio W/Out Extraction	Recovery
10 ng/ml	0.215	0.274	79.3
20 ng/ml	0.393	0.477	82.4
50 ng/ml	0.978	1.127	86.8
100 ng/ml	1.924	2.229	86.3

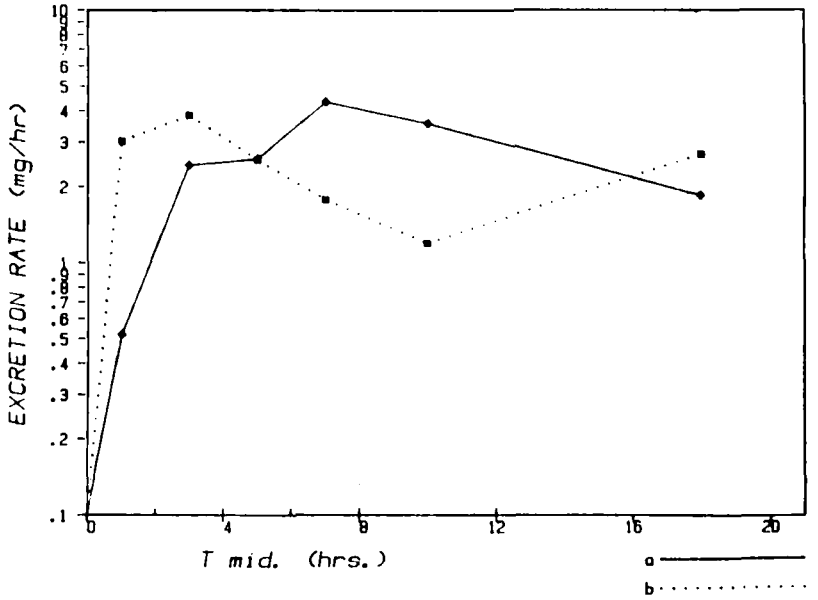


FIGURE 4. PLASMA PHENYLPROPANOLAMINE CONCENTRATION - TIME PROFILE FOR ONE HUMAN VOLUNTEER (a) GIVEN A SUSTAINED-RELEASE TABLET (75 MG) ONLY AT 0 HR (SOLID LINE); (b) GIVEN A SOLUTION (37.5 MG) AT 0 AND AT 12 HR (DASHED LINE).

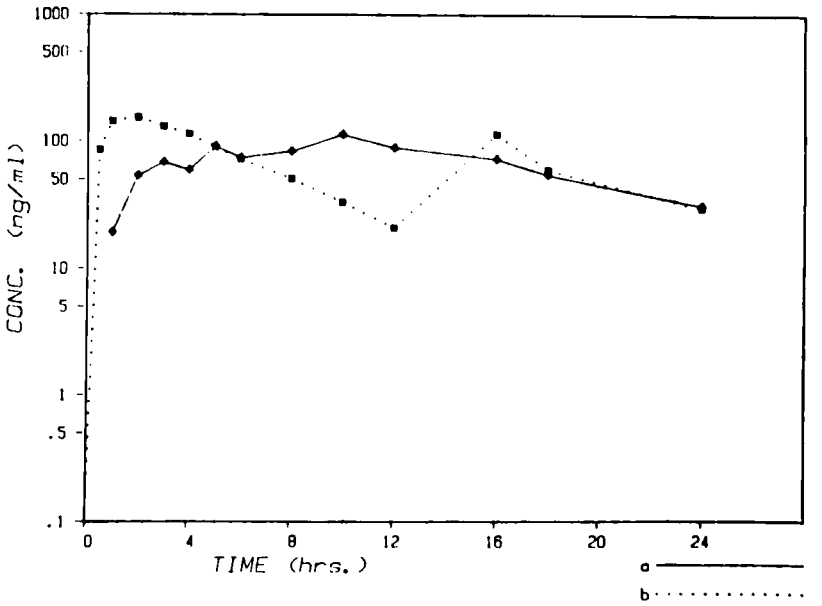


FIGURE 5. URINE PHENYLPROPANOLAMINE EXCRETION RATE - TIME PROFILE FOR ONE HUMAN VOLUNTEER (a) GIVEN A SUSTAINED-RELEASE TABLET (75 MG) ONLY AT 0 HR (SOLID LINE); (b) GIVEN A SOLUTION (37.5 MG) AT 0 AND AT 12 HR (DASHED LINE).

to sample preparation or relatively insensitive to detection of low concentrations in plasma. The method described here is rapid, specific and relatively simple for both plasma and urine. This method is sufficiently sensitive, accurate and precise so that reliable measurements of phenylpropanolamine concentrations may be obtained for plasma and urine samples up to 24 hours following a 75 mg oral dose. This is exemplified in Figures 4 and 5 where plasma concentrations and urinary excretion rates of phenylpropanolamine are depicted for one normal volunteer who received a 75 mg sustained release tablet and on separate occasions, 2 doses of phenylpropanolamine as a 37.5 mg solution at 0 and 12 hours. The HPLC method described here has been utilized in bioavailability studies involving over 1000 biological samples.

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