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Note

Gas–liquid chromatographic determination of pseudoephedrine and norpseudoephedrine in human plasma and urine

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Methods have been described for the identification and determination of pseudoephedrine (PS) in biological materials. Cummins and Fourier[1] made heptafluorobutyryl (HFB) derivatives of ephedrines and by using gas–liquid chromatography (GLC) with an electron capture detector (ECD) were able to analyze blood levels in man. A different approach was that of Kuntzman *et al.*[2] who made an acetyl derivative of PS with tritiated acetic anhydride and, after thin-layer chromatography, quantified the drug by scintillation counting. Bye *et al.*[3] determined the plasma concentration of PS by GLC using a nitrogen-sensitive detector. We modified the previously published GLC–ECD assay[1] of the HFB derivatives of the ephedrines to allow determination of PS and its observed metabolite, norpseudoephedrine (NPS) in plasma and urine of children with renal tubular acidosis.

EXPERIMENTAL

Reagents

Benzene AR grade, 0.01 *N* hydrochloric acid, 4 *N* sodium hydroxide, pyridine sequential grade (Pierce, Rockford, Ill., U.S.A.) and heptafluorobutyric anhydride (HFBA) (Pierce) were used.

Apparatus

A Varian Aerograph Model 1400 GLC instrument equipped with a scandium tritide ECD and Varian A-25 recorder was used. The chromatographic column was 1/8 in. O.D. × 6 ft. glass, packed with 3% OV-17 on 100–120 mesh Gas-Chrom Q.

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The column was conditioned for 24 h at 275° before using. Nitrogen was used as the carrier gas at a flow-rate of 6 ml/min. The injector and detector temperatures were 210° and 200°, respectively. The column temperature was 150°.

Procedure

Biologic samples, 0.2 ml of serum or 0.1 ml of urine, were placed into 20-ml test tubes to which 0.2 ml of a stock solution of 1-(1)-methylbenzylamine (internal standard) and 0.1 ml of 4 *N* NaOH were added. This mixture was extracted with 3 ml of benzene for 30 min using a rotatory extractor. Following centrifugation at 1000 *g* for 5 min after extraction, 2 ml of the benzene solution was removed and placed in another tube with a PTFE-lined cap. To this tube 0.1 ml of 10% pyridine (diluted with benzene) and 0.02 ml of HFBA were added; the samples were mixed on a Vortex mixer for 20 sec and the tubes were capped. The mixture was allowed to stand at room temperature for 4 h, and then washed three times with 3 ml of cold 0.01 *N* HCl.

Standard curves were derived from assays of duplicate samples of plasma and urine in the concentration ranges 0.14 to 0.84 μg per 0.2 ml for PS, 0.10 to 1.30 μg per 0.2 ml for NPS and 0.05 to 0.5 μg per 0.2 ml for ephedrine (E). The concentration of the ephedrines was obtained by calculating the ratio of peak heights of the ephedrines to that of the internal standard and relating this to a constructed calibration curve of ephedrines in plasma or urine. Plasma and urine samples were frozen for varying periods of time and ephedrines content determined periodically.

RESULTS AND DISCUSSION

Well-resolved symmetrical peaks were obtained for the ephedrines (Fig. 1). PS, E and NPS were differentiated and identified by their retention times. However, the method did not separate NPS from norephedrine (NE). The retention time for the internal standard was 2.8 min, 3.8 min for NE and NPS, 5 min for E, and 7.5 min for PS. This method has the sensitivity required to determine the serum and urine levels of PS in man at the doses used therapeutically (5 mg/kg). As little as 0.7 ng of PS could be easily detected, smaller levels could be measured if necessary by utilizing larger samples.

Because only one extraction was required before derivatization, the method lent itself to multiple sample analysis with minimal effort and equipment. The ECD response was linear between 0 and 1000 ng for the HFB derivatives of PS and NPS. Plots of peak height ratio to concentration of PS, NPS, and E (μg per 0.2 ml plasma) are shown in Fig. 2. The linear equations for each plot (concentration in μg per 0.2 ml) and the regression coefficients are:

Peak height ratio of PS = 0.010 + 1.770 conc. ($r^2 = 0.9967$)

Peak height ratio of NPS = 0.039 + 0.707 conc. ($r^2 = 0.9953$)

Peak height ratio of E = −0.001 + 0.676 conc. ($r^2 = 0.9966$)

No significant difference was noted between standard curves prepared from urine or plasma samples as indicated in Table I for PS. Twelve repeat analyses of a plasma sample containing 0.84 μg per 0.2 ml of PS gave a mean concentration of 0.849 and a standard deviation of 0.018.

The benzene extracts contain less water and other undesirable products, so the derivatization of ephedrines was accomplished without the need for several acid–

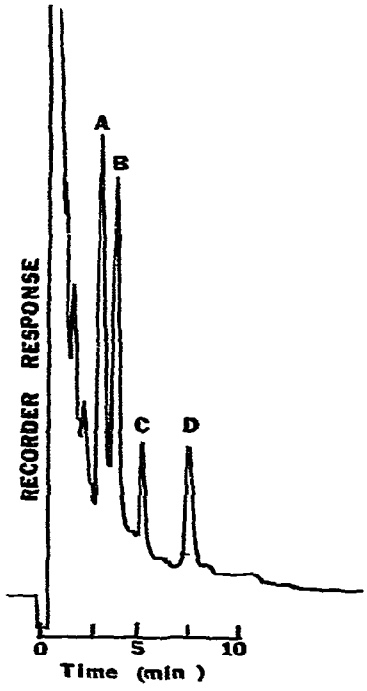

Fig. 1. Chromatogram of the HFB derivatives of L-(−)-*d*-methylbenzylamine (internal standard) (A); DL-norpseudoephedrine (B); ephedrine (C); pseudoephedrine (D).

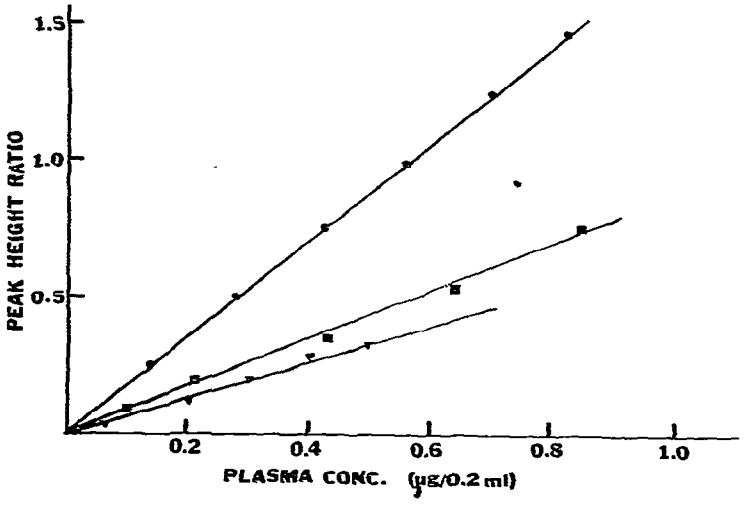

Fig. 2. Calibration curves relating the peak ratios to the concentration of PS (●), NPS (■), and E (▼) present in 0.2-ml plasma samples.

TABLE I

PEAK HEIGHT RATIO *VS.* CONCENTRATION FOR PSEUDOEPHEDRINE IN DUPLICATE PLASMA AND URINE SAMPLES

Concentration (μg per 0.2 ml)	*Peak height ratio*	
	Plasma	*Urine*
0.14	0.26	0.26
	0.26	0.24
0.28	0.48	0.52
	0.50	0.50
0.42	0.75	0.76
	0.74	0.70
0.56	1.00	0.97
	1.07	0.99
0.70	1.27	1.25
	1.25	1.25
0.84	1.47	1.47
	1.48	1.48
Slope	1.770	1.754
Intercept	0.010	0.006
r^2	0.9967	0.9982

base purification steps and drying of the solvent extract. The acid extraction after derivation removes both the pyridine and excess HFBA; thus no interfering substances were found in the many samples analyzed and no deterioration of the ephedrines occurred with storage or freezing (see Table II). The acid washes carried out under cold conditions prevent any hydrolysis that might occur. The HFB derivatives of the ephedrines themselves are stable for weeks in benzene at room temperature.

The method described for analysis of ephedrines with a very sensitive Sc^3H ECD allowed detection of nanogram amounts of ephedrines in plasma and urine. Only small amounts of samples are required. This results in a simplified extraction

TABLE II

STABILITY STUDY OF PSEUDOEPHEDRINE IN FROZEN PLASMA AND URINE SAMPLES

Day	*Measured concentration*	
	Plasma (μg per 0.2 ml)	*Urine (μg per 0.1 ml)*
0	0.22	0.36
2		0.36, 0.39
3	0.22	
4		0.35, 0.35
6	0.23	
7		0.35, 0.36, 0.38
10		0.36, 0.35
20	0.22	0.35, 0.35
33	0.20	
39	0.22	
60	0.24	

procedure and reduces the amount of interfering substances in the samples. A typical plasma concentration–time curve of PS following oral administration of 5 mg/kg dose to a normal volunteer is depicted in Fig. 3. Our laboratory is currently utilizing this GLC assay to characterize the pharmacokinetics and the determinants of renal excretion of PS and NPS. We are studying the possibility of both urine pH and flow dependent excretion kinetics.

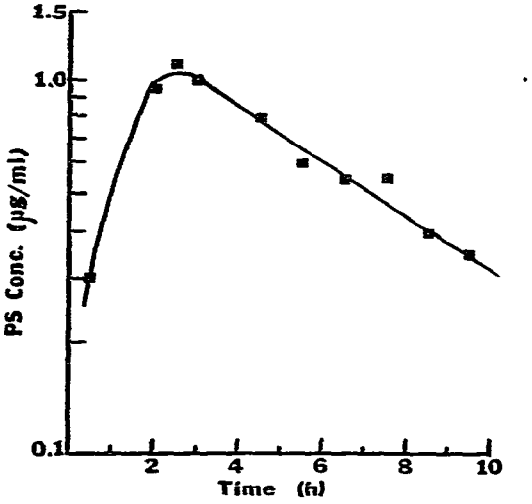

Fig. 3. Typical serum concentration–time curve for pseudoephedrine after oral administration of 5 mg/kg to a normal volunteer.

ACKNOWLEDGEMENT

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