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Note

Sensitive assay for pseudoephedrine and its metabolite, norpseudoephedrine in plasma and urine using gas—liquid chromatography with electron-capture detection

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Different techniques have been used to determine pseudoephedrine (PS) and its major metabolite, norpseudoephedrine (NPS) in biological fluids. These techniques include gas—liquid chromatography (GLC) with electron-capture [1—4] and nitrogen-specific [5] detection, radioimmunoassay [6, 7] and high-performance liquid chromatography (HPLC) [8]. However, these methods either have low sensitivity [1, 2] or require large plasma sample sizes [3—6]. Some of the above methods are applicable only to plasma determination [1, 4, 6, 7] and one, only to urine determination [8].

This paper describes a sensitive and specific electron-capture GLC assay of PS and NPS in plasma and urine. The assay is a modification of that described by Lin et al. [2]. However, the toxic extraction solvent, benzene is replaced by toluene and the derivatisation reagent, heptafluorobutyric anhydride by trifluoroacetic anhydride (TFAA). Also a different internal standard was used. More importantly the sensitivity has been increased so that concentrations as low as 50 ng/ml for both PS and NPS in plasma and urine can be detected readily from a 100-μl sample.

EXPERIMENTAL

Reagents and chemicals

Toluene Distal grade (Fisons, Loughborough, Great Britain), 0.01 *M* hydrochloric acid, 4 *M* sodium hydroxide, TFAA (Pierce and Warriner, Chester, Great Britain), pseudoephedrine (Sigma, Poole, Great Britain), norpseudo-

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ephedrine hydrochloride and the internal standard (IS), N-benzylethanolamine (both from Aldrich, Gillingham, Great Britain) were used.

All aqueous solutions were prepared using glass-distilled water unless otherwise stated.

Instrumentation

A gas chromatograph (Model F30, Perkin-Elmer, Beaconsfield, Great Britain), modified to allow samples to be injected from an automatic sampler (Model 7670A, Hewlett-Packard, Winnersh, Great Britain) was used with a pulse-modulated ^{63}Ni electron-capture detector. A coiled glass column (1.8 m × 4 mm I.D.) packed with GP 2% SP-2510-DA on 100—120 mesh Supelcoport (Supelco, Rayleigh, Great Britain) was used isothermally at 121°C. The column was conditioned at 280°C for 24 h (carrier gas, 50 ml/min, was used for the last 22 h only) and silylated if necessary before use. The injection port temperature was 170°C and the detector 263°C. The carrier gas was methane—argon (5:95, v/v) flowing at 90 ml/min. The pulse repetition frequency was 2 kHz, nominal.

The chromatograms, the chromatographic peak areas, the retention times and the area ratios of the PS or NPS peak to the IS peak were calculated and recorded by a data system (Spectra-Physics, St. Albans, Great Britain) which consisted of a central processor (SP 4000), a data interface (SP 4020) and a printer/plotter (SP 4050).

The calibration curve was obtained by the method of internal standardisation. The peak area ratios of PS to IS or NPS to IS in the standard sets were plotted against the standard concentrations. The best straight line was calculated using the least squares linear regression method (Texas Instruments SR-51-II calculator).

Sample preparation

Standards of PS and NPS were prepared by adding the appropriate volume of an aqueous stock solution (1 mg/ml) to blank (i.e. drug-free) plasma or urine to give final concentrations of each compound of 50, 100, 200, 500 and 1000 ng/ml.

To each plasma or urine standard (100 μl) in a 10-ml Sovirel tube (V.A. Howe, London, Great Britain) were added 100 μl of N-benzylethanolamine aqueous solution (IS, 2.5 μg/ml). The mixture was made alkaline with 100 μl of 4 *M* sodium hydroxide solution and toluene (1 ml) was added. The tube was screw-capped, mixed for 30 min along its long axis at 25 oscillations/min and then centrifuged at 1000 *g* for 10 min. As much toluene as possible was taken into a 10-ml BC24/C14T conical centrifuge tube (Quickfit and Quartz, Corning, Stone, Great Britain) using a pasture pipette (Kullman Glass, Whitby, Great Britain). After adding 50 μl TFAA, the tube was vortex-mixed, stoppered with a glass stopper and then left standing at room temperature for 4 h. The toluene layer was washed with 3 ml of ice-cold 0.01 *M* hydrochloric acid and then transferred to a microvial (Hewlett-Packard). The vial was capped and 2 μl of the toluene layer were injected into the gas chromatograph for analysis. Care was taken to ensure that no aqueous layer was transferred with the toluene at any step. The plasma or urine samples were analysed in the same way as the standards. Where necessary the urine samples were diluted with water and the corresponding standards made up in water.

Validation

An estimate of the assay precision for PS and NPS was obtained by carrying out replicate analyses of the standards over the range of 50—1000 ng/ml.

RESULTS

Symmetrical, adequately resolved peaks were obtained for PS, NPS and IS after derivatisation with TFAA (Figs. 1 and 2). The retention times for the TFAA derivatives of PS (PS-TFAA), IS (IS-TFAA) and NPS (NPS-TFAA) were 574, 770, and 1316 sec respectively. Although other substances extracted by the method appeared in the chromatograms, the data system used had no problems in identification and quantitation of the peaks of interest. The results for the method precision and the calibration curves for PS and NPS are summarized in Tables I and II. Both calibration curves were linear over the concentration range 50—1000 ng/ml.

A plasma profile from a healthy fasting male volunteer after taking two 60-mg pseudoephedrine hydrochloride tablets is shown in Fig. 3. Absorption of PS was rapid and NPS was not detected in the plasma samples from this study. The plasma half-life of PS was calculated as 4.3 h which was within the range found

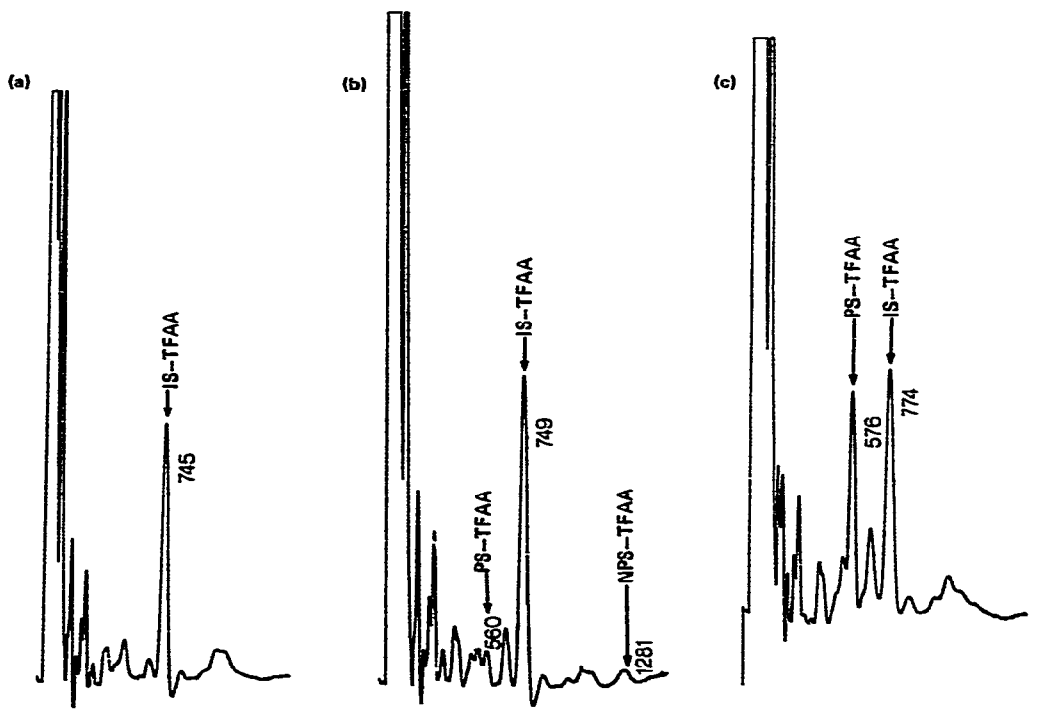

Fig. 1. Typical chromatograms of human plasma after extraction and derivatisation with TFAA. (a) Blank plasma containing the internal standard, N-benzylethanolamine (IS, 2.5 μg/ml). (b) Plasma standard containing pseudoephedrine (PS, 50 ng/ml), norpseudoephedrine (NPS, 50 ng/ml) and IS (2.5 μg/ml). (c) Plasma sample containing PS (461 ng/ml) and IS (2.5 μg/ml). Time in sec.

TABLE I

THE ASSAY PRECISION WHEN PSEUDOEPHEDRINE REPLICATE STANDARDS IN PLASMA OR URINE WERE ANALYSED

	Pseudoephedrine concentration (ng/ml)											
	In plasma						In urine					
	0	50	100	200	500	1000	0	50	100	200	500	1000
No. of replicates	6	6	6	6	6	6	6	5	5	6	5	4
Mean value of peak area ratio	0	0.047	0.092	0.25	0.605	1.233	0	0.043	0.121	0.241	0.643	1.295
Standard deviation	0	0.0087	0.0117	0.0213	0.0439	0.1139	0	0.0060	0.0173	0.0424	0.0473	0.1133
Percentage of standard deviation (%)	0	18.7	12.7	8.5	7.3	9.2	0	13.9	14.3	17.6	7.3	8.8
Correlation coefficient				0.9997						0.9999		
Slope value				0.00124						0.00131		
Intercept				−0.0123						−0.0124		

TABLE II

THE ASSAY PRECISION WHEN NORPSEUDOEPHEDRINE REPLICATE STANDARDS IN PLASMA OR URINE WERE ANALYSED

	Norpseudoephedrine concentration (ng/ml)											
	In plasma						In urine					
	0	50	100	200	500	1000	0	50	100	200	500	1000
No of replicates	6	6	6	6	6	6	6	6	5	6	5	4
Mean value of peak area ratio	0	0.065	0.119	0.258	0.597	1.203	0	0.058	0.126	0.260	0.572	1.128
Standard deviation	0	0.0117	0.0146	0.0159	0.0335	0.1103	0	0.0098	0.0071	0.037	0.0598	0.1048
Percentage of standard deviation (%)	0	18.0	12.3	6.2	5.6	9.2	0	16.9	5.6	14.5	10.4	9.3
Correlation coefficient				0.9999						0.9995		
Slope value				0.00120						0.00112		
Intercept				0.0043						0.0118		

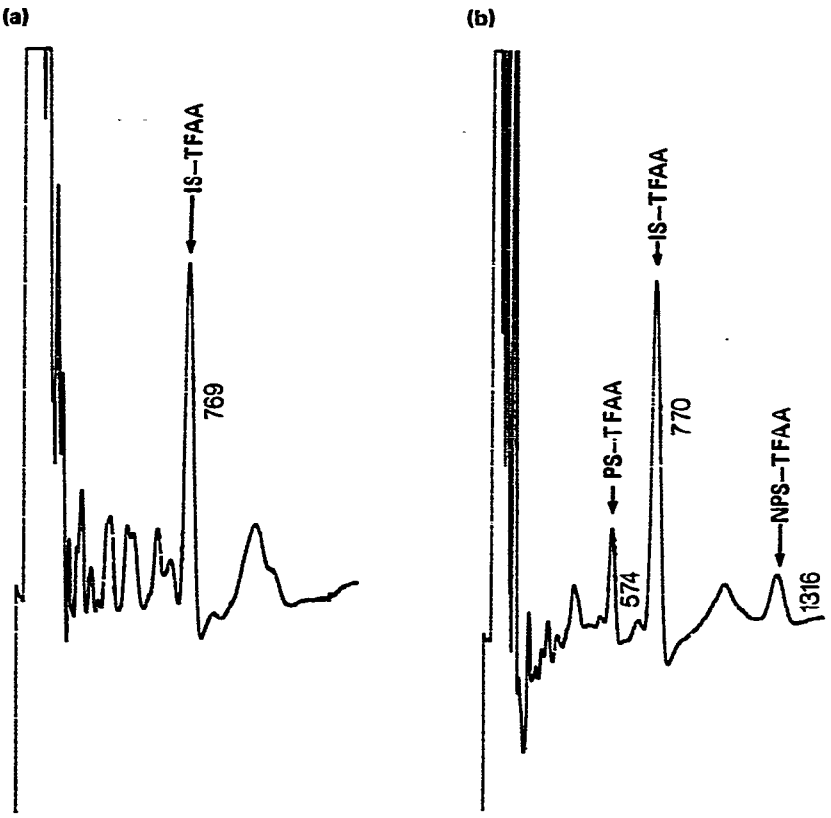

Fig. 2. Typical chromatograms of human urine after extraction and derivatisation with TFAA. (a) Blank urine containing the internal standard, N-benzylethanolamine (IS, 2.5 μg/ml). (b) Urine standard containing pseudoephedrine (PS, 200 ng/ml), norpseudoephedrine (NPS, 200 ng/ml) and IS (2.5 μg/ml).

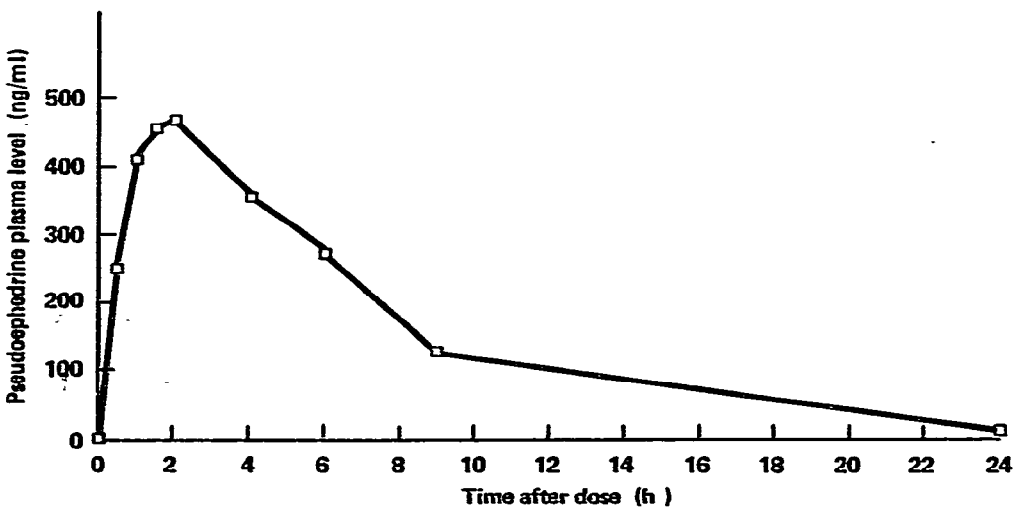

Fig. 3. Plasma profile of pseudoephedrine from a healthy male volunteer after taking two 60-mg pseudoephedrine hydrochloride tablets.

by Kuntzman et al. [6]. The plasma level at 24 h was approaching the sensitivity limit of the assay (about 20 ng/ml).

Cumulative urinary excretion of PS and NPS is given in Table III. In the 0—24-h urine, 43.4% of the dose was excreted as PS and 1.2% as NPS. The rate of urinary excretion of PS is consistent with the calculated plasma half-life in this subject.

TABLE III

EXCRETION OF PSEUDOEPHEDRINE AND NORPSEUDOEPHEDRINE IN URINE FOLLOWING A 120-mg ORAL DOSE OF PSEUDOEPHEDRINE HYDROCHLORIDE

Urine excretion time after dose (h)	Urine pH	Urine volume (ml)	Total amount excreted (mg)	
			Pseudoephedrine	Norpseudoephedrine
0	6.5	—	0	0
0—1.5	6.0	89	8.12	0.07
1.5—6	6.0	342	19.28	0.31
6—9	6.8	234	4.40	0.15
9—24	6.5	618	10.90	0.52

CONCLUSION

The described method is useful for bioavailability and pharmacokinetic studies as the extraction is simple and rapid, the sensitivity is good and only a small sample size is required. Other substances present in plasma and urine are extracted but when chromatographed under the described conditions, they were adequately resolved from the peaks of interest on the chromatograms, so that reliable quantitation for both PS and NPS was obtained. This assay provides good sensitivity and precision for monitoring levels of PS in plasma and PS and NPS in urine after a single therapeutic dose of PS.

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