Electron-Capture GLC Determination of Pseudoephedrine in Serum

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Abstract □ A GLC assay was developed for pseudoephedrine in serum using 3-methylamino-1-phenyl-1-propanol as an internal standard. After extraction from serum with benzene under alkaline conditions, pseudoephedrine was derivatized with pentafluorobenzyl bromide and quantitated by electron-capture GLC. The method has a detection limit of ~0.02 μg/ml of serum using 1-ml samples.

Keyphrases □ Pseudoephedrine—analysis, electron-capture GLC, human serum, bioavailability □ Vasoconstrictors (adrenergic)—pseudoephedrine, electron-capture GLC analysis, human serum, bioavailability □ GLC, electron capture—analysis, pseudoephedrine, human serum, bioavailability

Various analytical methods for pseudoephedrine (I) determination in biological fluids have been reported, including GLC methods with flame-ionization (1–3), electron-capture (4, 5), and nitrogen-specific (6) detection. The flame-ionization and nitrogen-specific detector procedures have low sensitivity, while the electron-capture procedure requires a time-consuming derivatization. Kuntzman *et al.* (7) prepared the acetyl derivative of pseudoephedrine with tritiated acetic anhydride and quantitated it by scintillation counting. However, this method lacks specificity.

The report describes a rapid and specific GLC method for pseudoephedrine determination in serum utilizing electron-capture detection of the pentafluorobenzyl derivative. The method is sufficiently sensitive for clinical pharmacokinetic evaluations after a single pseudoephedrine dose. Expected serum concentrations range from ~0.8 to 0.1 μg/ml during the 24-hr interval following oral ingestion of 180 mg of pseudoephedrine hydrochloride (7).

EXPERIMENTAL

Reagents and Materials—Pseudoephedrine hydrochloride[1], the internal standard[2], and pentafluorobenzyl bromide[3] were used as supplied. The other chemicals were analytical reagent grade.

Apparatus—The samples were extracted at 100 cpm on a two-speed reciprocating shaker[4] and centrifuged at 10° in a refrigerated centrifuge[5]. Sample solutions in test tubes were mixed by vortexing[6]. The pentafluorobenzyl derivatives were prepared at high temperature on a heating block[7].

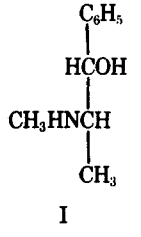

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Table I—Assay Reproducibility of Serum Pseudoephedrine Concentration

Theoretical, μg/ml	Observed[a] Mean ± *SD*, μg/ml	*RSD*, %
0.05	0.055 ± 0.008	14.4
0.10	0.108 ± 0.009	8.1
0.20	0.218 ± 0.014	6.5
0.40	0.388 ± 0.015	3.8
0.60	0.552 ± 0.024	4.4
1.00	1.029 ± 0.060	5.8

[a] Mean of three determinations at each concentration.

GLC—A reporting gas chromatograph[8] equipped with a computing integrator[9], a ^{63}Ni-constant-current electron-capture detector[10], and an automatic sampler[11] were used. The 1.21-m (4-ft) × 4-mm i.d. glass column was packed with 5% OV-225[12] on Gas Chrom Q[12] (80–100 mesh). The column was conditioned at 250° for 16 hr with argon–methane (95:5)[13] at 20 ml/min before being connected to the detector. The carrier gas cylinder was fitted with an oxygen trap filter[14].

The samples were chromatographed using a carrier gas flow rate of 44 ml/min, with the injector, column oven, and detector maintained at 200, 190, and 300°, respectively.

Standards—A 100-μg/ml stock solution of pseudoephedrine was prepared by dissolving 24.4 mg of pseudoephedrine hydrochloride in 200 ml of distilled water. The 1-μg/ml working serum pseudoephedrine standard was prepared by diluting 1.0 ml of the stock solution to 100 ml with drug-free serum. Other serum standards were prepared by further diluting the 1-μg/ml serum standard with drug-free serum.

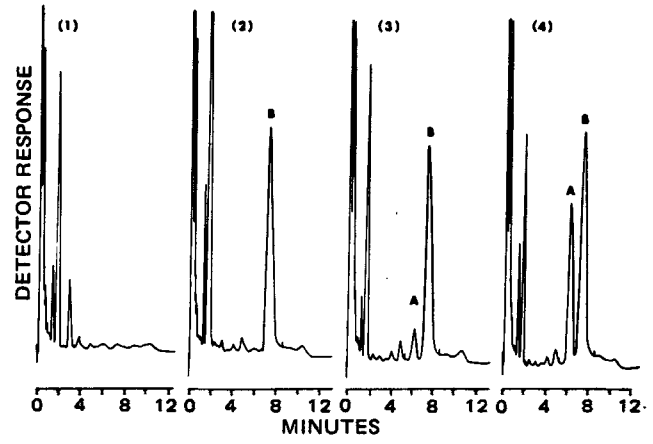

Figure 1—*Chromatograms of extracted serum samples. Key: 1, serum blank; 2, serum blank containing internal standard; 3, serum pseudoephedrine standard at 0.10 μg/ml; 4, serum pseudoephedrine standard at 0.60 μg/ml; A, pseudoephedrine; and B, internal standard.*

[1] NF reference standard.
[2] 3-Methylamino-1-phenyl-1-propanol, Abbott internal reference standard, A-10261, Abbott Laboratories, North Chicago, Ill.
[3] Pierce Chemical Co., Rockford, IL 61105.
[4] Eberbach Corp., Ann Arbor, Mich.
[5] Model RC-3, Sorvall, Newtown, Conn.
[6] Vortex Genie model K-550-GT, Scientific Industries, Springfield, Mass.
[7] Dri-block DB-3, Techne, Princeton, N.J.
[8] Hewlett-Packard model 5830A.
[9] Hewlett-Packard model 18850A.
[10] Hewlett-Packard model 18803A.
[11] Hewlett-Packard model 7671A.
[12] Applied Science Laboratories, State College, Pa.
[13] Matheson Gas Products, Elk Grove Village, Ill.
[14] Altech Associates, Arlington Heights, Ill.

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Table II—Assay Sensitivity

Serum Pseudoephedrine, μg/ml	Peak Area Ratio, Pseudoephedrine to Internal Standard
0.05	0.060
	0.072
	0.075
0.10	0.127
	0.125
	0.111
0.20	0.243
	0.219
	0.220
Linear Regression Parameters	
Number of points	9
Slope	1.057
y-Intercept	0.0158
Correlation coefficient	0.992
Calculated sensitivity[a], μg/ml	0.020

[a] Sensitivity was defined as the concentration calculated by linear regression analysis to give a peak area ratio greater than the y-intercept 95% of the time.

An internal standard stock solution, 100 μg/ml, was prepared by dissolving 20 mg of internal standard in 200 ml of methanol. The 1-μg/ml working internal standard was prepared by diluting 1 ml of the internal standard stock solution to 100 ml with distilled water.

Assay—To 1.0 ml of serum in 20-ml screw-capped test tubes fitted with polytef-lined caps were added 0.5 ml of the working internal standard, 1 ml of 0.1 *M* Na_3PO_4, and 6 ml of benzene. The samples were extracted by shaking at 100 cpm for 10 min. After centrifugation at 3000 rpm for 5 min, 5-ml aliquots of the organic phase were transferred to 15-ml screw-capped conical test tubes containing 1 ml of 0.1 *N* HCl. The pseudoephedrine and the internal standard were back-extracted into the acidic solution by shaking for 5 min.

Following centrifugation, the organic phase was removed by aspiration. The aqueous solution was alkalinized with 1 ml of 1 *N* NaOH and extracted with 2.5 ml of benzene for 5 min. Then 2 ml of the benzene extract was transferred to a screw-capped conical test tube and evaporated to dryness at 45° with filtered air[15]. To the residue were added 0.1 ml of 0.1 *M* K_2HPO_4, 0.1 ml of ethanol, and 0.1 ml of 2 mg of pentafluorobenzyl bromide/ml of ethanol. The mixture was capped after vortexing and reacted at 100° for 1 hr.

After the mixture had cooled to room temperature, 1 ml of 0.1 *M* K_2HPO_4 and 2.5 ml of benzene were added, and the solution was extracted for 5 min. Then 2 ml of the benzene extract was evaporated to dryness at 40° with filtered air to remove excess pentafluorobenzyl bromide. The residue was dissolved in 1 ml of benzene by vortexing, and a 2-μl aliquot was chromatographed.

Calculations—The peak area ratios of pseudoephedrine to the internal standard were plotted *versus* the pseudoephedrine concentration expressed as micrograms per milliliter of serum. Values for unknown pseudoephedrine concentrations in serum were calculated by the least-squares regression method from the calibration curve.

RESULTS AND DISCUSSION

Under the described assay conditions, derivation of pseudoephedrine and the internal standard with pentafluorobenzyl bromide yielded unique derivatives with GLC retention times of 6.3 and 7.5 min, respectively (Fig. 1). Pentafluorobenzyl derivatives of ephedrine and norephedrine have retention times of 5.1 and 21.8 min, respectively. The pseudoephedrine and internal standard derivatives were well resolved from coextracted endogenous compounds in serum.

[15] Air purifier, Koby Inc., Marlboro, Mass.

Table III—Assay Accuracy of Serum Pseudoephedrine Concentration

Unknown	Theoretical, μg/ml	Observed, μg/ml	Recovery, %
1	0.050	0.056	112.0
2	0	0	—
3	0.600	0.556	92.7
4	0.300	0.309	103.0
5	1.000	0.964	96.4
6	0.150	0.157	104.7
7	0.400	0.375	93.8
8	0.075	0.076	101.3
9	0.800	0.755	94.4
10	0.500	0.472	94.4
11	0.100	0.110	110.0
12	0.200	0.213	106.5
Average			100.8

Assay reproducibility was estimated by analyzing replicate standards (n = 3) ranging from 0.05 to 1.00 μg of pseudoephedrine/ml. Relative standard deviations (Table I) ranged from 14.4 to 3.8%, with the largest deviations associated with concentrations of <0.1 μg/ml. Assay sensitivity, defined as the pseudoephedrine concentration calculated by linear regression analysis that gave a peak area ratio response greater than the y-intercept 95% of the time, was ~0.02 μg/ml (Table II). The accuracy of the assay was estimated by analyzing serum samples containing known pseudoephedrine quantities under blind conditions. The average recovery was 100.8 ± 6.9% (n = 11) (Table III).

The extraction efficiency, compared to extraction of the standard in water, averaged 104.0 ± 2.2% (mean ± *SD*) for triplicate serum pseudoephedrine samples at 1 μg/ml. This value was calculated by dividing the peak area ratios of the extracted serum samples by the peak area ratios of the water samples.

This study indicated that the described GLC method can be employed to quantitate serum pseudoephedrine levels in a single-dose bioavailability study.

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