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Identification and determination of ephedrine and its congeners in urine by gas chromatography

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A sensitive and specific assay for ephedrine-type compounds in urine is described. The procedure involves the removal of neutral and acidic compounds, then extraction of the bases with ether. This extract, after concentration, is injected into a flame ionisation gas chromatograph equipped with a 2% polyethylene glycol 6000, alkaline treated, column at 165°. Linear detector response between 5 and 100 μg base/ml urine was observed (peak height with respect to an internal marker) and replicate analyses indicated good reproducibility. No interfering compounds have been encountered nor any "on column" decomposition. The amines were further identified by chromatography of their acetone derivatives.

METHODS have been described for the identification and determination of ephedrine and norephedrine in biological materials (Richter, 1938; Axelrod, 1953; Heimlich, MacDonnell, Flanagan & O'Brien, 1961), but they lack sensitivity and specificity and are tedious for routine analysis. Gas chromatography of ephedrine, norephedrine and pseudoephedrine has been reported, but primarily from a qualitative aspect (Parker, Fontan & Kirk, 1962, 1963; Brochmann-Hansson & Svendsen, 1962). The adaptation of this technique to a specific quantitative determination of ephedrine and related compounds in the presence of each other in biological fluids, is described herein.

Experimental

Reagents. Analar diethyl ether, freshly distilled. Hydrochloric acid, 5 N. Sodium hydroxide solution, 5 N. Internal marker solution: (2,6-dimethylphenoxy)ethylamine hydrochloride; a solution equivalent to 10 μg base/ml in 0·1 N hydrochloric acid.

Apparatus. A Perkin Elmer F 11 chromatograph equipped with a flame ionisation detector and a 0 to 5 mv Leeds and Northrup Speedomax G recorder, Model S were employed. The chromatographic column was stainless steel tubing $\frac{1}{8}$ inch o.d., 1 metre in length, and packed with 80–100 mesh Chromosorb G, acid-washed and treated with dimethyldichlorosilane. This was coated with 5% w/w potassium hydroxide and 2% w/w polyethyleneglycol 6000. It was conditioned for 24 hr under the operating conditions: oven temperature, 165°; injection block temperature about 250°; hydrogen pressure, 14 lb/sq. in; air pressure, 25 lbs/sq. in; nitrogen flow rate, 40 ml/min; stream split ratio, 1:5. The column was silanized *in situ* with 2 × 5 μl hexamethyldisilazane before use.

PROCEDURE

To internal marker solution (1·0 ml), in a glass stoppered centrifuge tube, was added 1 ml to 5 ml urine and 0·1 ml 5 N hydrochloric acid.

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The solution was diluted to 6·1 ml and extracted with 3 × 2·5 ml diethyl ether, centrifuging to break any emulsion. The ether extract was rejected and urine made alkaline with 5 N sodium hydroxide (0·5 ml). A further 3 × 2·5 ml diethyl ether extracts were combined in a 15 ml Quickfit test tube, finely tapered at the base, and concentrated to about 50 μl at 42°; 3·0 to 5·0 μl of this solution was injected onto the column using a 10 μl Hamilton syringe. The concentration of the sympathomimetic amine was obtained by calculating the ratio of peak heights of the amine to that of the internal marker and relating this to a previously constructed calibration curve of the amine in urine.

The acetone derivatives of the amines (Brochmann-Hanssen & Svendsen, 1962), were formed by adding acetone (1 ml) to the ether concentrate in the tapered test tube; the solution was allowed to stand for 2 hr, concentrated to about 50 μl at 60° and 3·0 to 5·0 μl injected onto the column.

The amines in urine at various pH values were stored at 4° and the amine content determined periodically. The amines in the ether extract and concentrate were also determined before and after storage at 4° for 24 hr.

Results and discussion

Well resolved symmetrical peaks were obtained for the amines (Fig. 1), however, it was not possible to separate ephedrine and pseudoephedrine under the described operating conditions. These two amines were differentiated and identified, as were the other amines, by their retention times and by the characteristic peak-shift on formation of the respective acetone derivatives (Table 1). Methylephedrine being a tertiary amine

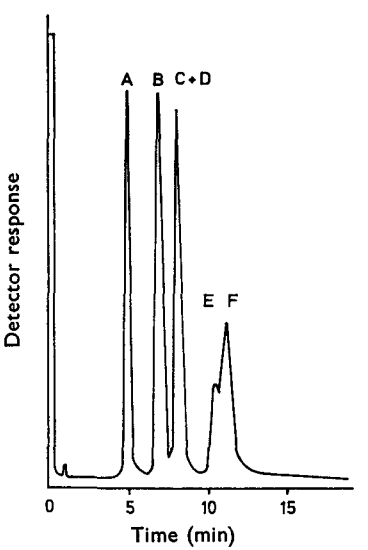

FIG. 1. Separation of a mixture containing A, internal marker; B, methylephedrine; C, ephedrine; D, pseudoephedrine; E, norpseudoephedrine; and F, norephedrine extracted from urine.

did not form an acetone derivative. Conversion of the other amines to their acetone derivatives did not go to completion under the described conditions.

TABLE 1. RETENTION TIMES OF SOME EPHEDRINE CONGENERS

Compound	Retention time in min	
	Base	Acetone deriv.
Internal marker, (2,6-dimethylphenoxy)ethylamine	4·9	8·0
Methylephedrine	6·8	Not formed
Pseudoephedrine	8·2	3·6
Ephedrine	8·2	4·0
Norpseudoephedrine	10·5	4·2
Norephedrine	11·3	4·0

A consistent linear detector response was obtained for all the individual amines over the range 5–100 μg base/ml urine. Sixteen analyses of a urine sample containing 20 μg amines/ml gave a standard deviation of 0·64.

No interfering substances were found in the many samples of urine analysed and no deterioration of the amines occurred when they were stored (4 days at 4°) in urine. Both the ether extract and concentrate were stable at 4° for 24 hr. Alkaline treatment of the column support was necessary for symmetrical peaks and "on column" decomposition of the amines was not observed; this can probably be attributed to the silanized steel tubing used (cf. Vessman & Schill, 1962; Vessman, 1964). The decomposition of ephedrine-type amines reported by Parker & others (1962) is probably an artifact caused by the use of acetone solutions of the amines.

The advantages of this method over those previously reported are its specificity as shown by the absence of interference from normal urinary constituents, the short analysis time (16 samples may be analysed in about 6 hours), and the ease of determination of a mixture of the amines. Furthermore, although 5 μg/ml was the lowest concentration determined in the present study, by the use of larger volumes of urine and smaller amounts of internal marker much smaller concentrations may be estimated.

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