The determination of ephedrine plasma levels by a gas chromatographic method

Gas chromatography has been the method of choice in the specific quantitative determination of ephedrine and its metabolite norephedrine in urine (Beckett & Wilkinson, 1965a, b; Welling, Lee & others, 1971). We have evolved a rapid technique that increases the sensitivity such that concentrations of ephedrine occurring in plasma after oral administration of therapeutic doses of ephedrine-containing compounds can be measured.

A Perkin-Elmer F.11 gas chromatograph fitted with a flame ionization detector and 2 m glass column, $\frac{1}{4}$ inch o.d., packed with 8% Carbowax 20 m, 2% KOH on Chromosorb W 80—100 mesh was used. The oven temperature was 180°, injection block temperature 250°, hydrogen pressure 21 lb inch^{-2}, air pressure 25 lb inch^{-2}, and nitrogen flow rate 60 ml min^{-1}. The reagents were Analar diethyl ether—double distilled; ammonium hydroxide sp. gr. 0·88; Analar potassium chloride; internal marker solution: 300 ng phendimetrazine acid tartrate (Ayerst Laboratories Ltd.) per ml of distilled water. Standard solutions of ephedrine hydrochloride containing the equivalent of 300 and 75 ng base ml^{-1} in distilled water.

Lithium-heparinized blood was centrifuged at 4000 rev min^{-1} immediately after collection from 2 subjects. The supernatant was divided into 3 ml portions and known amounts of the standard solution of ephedrine were added to each.

Internal marker solution (1 ml), potassium chloride (0·2 g) and 0·88 ammonium hydroxide (2 ml) were added and the mixture was extracted with 3 × 4 ml diethyl ether. The pooled extract was evaporated to 20 μl in a tapered glass tube (Beckett & Wilkinson, 1965a). Duplicate 4 μl injections were made immediately onto the column for each concentration and the mean peak height ratio (ephedrine : internal marker) was plotted against ephedrine plasma concentration (ng base ml^{-1}). A typical chromatogram is shown in Fig. 1.

Freshly taken blood was used for calibration purposes since variable extractability and interfering chromatographic peaks were observed using bottled or dried blood. For the latter reason, ammonia replaced the more commonly used sodium hydroxide (Beckett & Wilkinson, 1965a; Welling & others, 1971).

The flame ionization detector response was linear over the range 12·5—400 ng base ml^{-1}.

Application to plasma levels. Duplicate 3 ml plasma samples were subjected to the

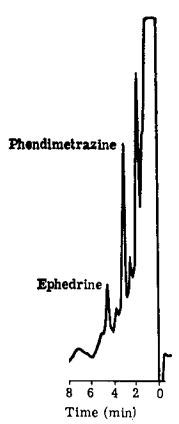

FIG. 1. Chromatogram of plasma containing ephedrine (50 ng base ml^{-1}).

analytical procedure described above, omitting the ephedrine standard. Ephedrine plasma concentrations were calculated by reference to the calibration curve.

Accuracy and reproducibility were determined by assaying twelve plasma samples each containing the equivalent of 25 ng ephedrine base per ml. The coefficient of variation calculated from this assay was 9·7% (mean 23·75; s.d. $\pm$ 2·30).

No breakdown of drug was observed after 50, 100 and 150 ng base per ml was added to plasma (3 ml) and stored at 4° for 4 days.

The plasma concentration of ephedrine expressed as base was measured in a patient receiving chronic therapy with a compound tablet containing ephedrine* three times daily. The baseline concentration 15 h after the night dose was 20 ng ml^{-1}. Following administration of one tablet, the concentration rose to 95 ng ml^{-1} at 4 h, falling to 65 ng ml^{-1} at 6 h. The other compounds present in the tablet did not interfere with the assay, and the sensitivity of the method (the minimum measurable level is 12·5 ng ml^{-1}) is therefore sufficient to accurately determine the range of plasma levels obtained after the administration of therapeutic doses of ephedrine-containing preparations.

Operations designed to increase sensitivity relative to background—for example derivatization (Cummins & Fourier, 1969) or extraction under acidic conditions (Campbell, 1969; Cummins & Fourier, 1969)—were omitted for the sake of speed. Use of pure oxygen instead of compressed air to increase detector sensitivity (Campbell, 1969) was of no value in the present method: although overall sensitivity was increased, ephedrine response relative to background was not, as the extraction technique of Campbell was not used.

To the authors' knowledge, no plasma ephedrine assay has hitherto been reported. Cummins & Fourier (1969) published a serum pseudoephedrine assay which involved a 12 h standing period; they did not state the minimum measurable level but inspection of their calibration curve suggests a figure of about 20 ng ml^{-1} and the authors suggested that the method could be adapted for ephedrine. In the present method, duplicate samples can be extracted and chromatographed inside 1 h.

We wish to thank Mr. J. C. Mountain (Ayerst Laboratories Ltd., Camberley, Surrey) for the supply of phendimetrazine acid tartrate.

* Franol Plus tablet: 15 mg ephedrine sulphate, 8 mg phenobarbitone, 120 mg theophylline, 10 mg thenyldiamine hydrochloride.

Asthma Research Council Clinical Pharmacology Unit,
Department of Medicine, Cardiothoracic Institute,
Brompton Hospital, London SW3 6HP, U.K.

M. E. PICKUP
J. W. PATERSON

March 26, 1974

REFERENCES

BECKETT, A. H. & WILKINSON, G. R. (1965a). *J. Pharm. Pharmac.*, **17**, Suppl. 104S–106S.
BECKETT, A. H. & WILKINSON, G. R. (1965b). *Ibid.*, **17**, *Suppl.* 107S–108S.
CAMPBELL, D. B. (1969). *Ibid.*, **21**, 129–131.
CUMMINS, L. M. & FOURIER, M. J. (1969). *Anal. Lett.*, **2**, 403–409.
WELLING, P. G., LEE, K. P., PATEL, J. A., WALKER, J. E. & WAGNER, J. G. (1971). *J. pharm. Sci.*, **60**, 1629–1634.