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Note

High-performance liquid chromatographic determination of vincamine

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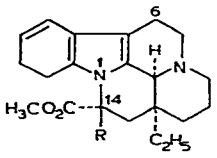
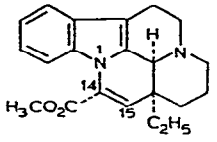
Vincamine (I) [eburnamenine-14-carboxylic acid, 14,15-dihydro-14-hydroxy-, methyl ester (3α, 14β, 16α)] is an efficient vasodilator for the treatment of the cerebral microcirculation system[1,2]. Pharmacokinetic studies of this product require a rapid, sensitive and specific assay. Methods for determining the vincamine content of biological fluids include radioisotope techniques[3] and gas chromatography–mass spectrometry[4,5]. However, these techniques are not suitable for routine analysis. More convenient and reliable methods for the determination of vincamine in blood by gas chromatography without a derivatization step[6] and by *in situ* spectrophotometry[7] have also been described. However, they are time consuming and lack sensitivity.

We report here a method for the measurement of vincamine using reversed-phase high-performance liquid chromatography (HPLC). Chromatography is performed at alkaline pH on bonded octadecylsilane with papaverine as an internal standard. The proposed method requires minimal sample preparation times and can determine as little as 5–10 ng of vincamine in 4 min. Moreover, the same system has been extended to the assay of vincamine either as a bulk drug or as a component of pharmaceutical preparations, even in the presence of the following impurities: 14-epivincamine (II) [eburnamenine-14-carboxylic acid, 14,15-dihydro-14-hydroxy-, methyl ester (3α, 14α, 16α)] and apovincamine (III) [eburnamenine-14-carboxylic acid, methyl ester (3α, 16α)].



I R = β –OH

II R = α –OH



III

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EXPERIMENTAL

Materials

HPLC-grade methanol (LiChrosolv; Merck, Darmstadt, G.F.R.) was used. Water was distilled from an all-glass still and then passed through a 0.45-μm membrane filter (Millipore Type HA). Vincamine, 14-epivincamine and apovincamine are purchased from Ricerchimica (Milan, Italy) and papaverine and ammonium carbonate from Sigma (St. Louis, MO, U.S.A.).

Solutions

Standard solutions were prepared by dissolving vincamine and papaverine (0.02%, w/v), 14-epivincamine and apovincamine (0.004%, w/v) in methanol. Both liquid and solid dosage forms were weighed accurately and diluted with methanol to the desired volume (C_I = 0.02%, w/v).

To urine (diluted 1:10 with water) and plasma samples (0.5 ml) in a glass tube (10 ml) were added 4 μg of papaverine, 4–20 μg of vincamine and 200 μl of 1 *M* sodium hydroxide solution. The mixture was adjusted to 2 ml with distilled water to give diluted urine and plasma samples for assay by HPLC. Alternatively, diluted urine and plasma samples were extracted three times with 5 ml of diethyl ether by vortex mixing, followed by centrifugation. The ethereal phases were transferred by Pasteur pipette into a small tube, dried over anhydrous sodium sulphate and evaporated to dryness at 30°C under a stream of dry nitrogen. The residue was dissolved in 200 μl of methanol and vortexed for 20 sec, then 10 μl of this solution were injected into the liquid chromatograph.

Chromatographic conditions

The chromatographic system consisted of a Model 6000 A solvent delivery system, a Model U6K universal injector, a Model 440 ultraviolet detector and a Model 730 data module (Waters Assoc., Milford, MA, U.S.A.). Ultraviolet absorption was measured at 254 nm. Samples were chromatographed at room temperature over a 300 × 3.9 mm I.D. stainless-steel column packed with μBondapak C_{18} (10 μm) (Waters Assoc.). To extend the column life a pre-column packed with Bondapak C_{18}/Corasil was inserted between the pump and the injector. The mobile phase was methanol–0.01 *M* ammonium carbonate (75:25, v/v) at a flow-rate of 2 ml/min. The pH of the mobile phase was about 7.8–7.9; retention times were constant to ±2% over a 30-day period.

RESULTS AND DISCUSSION

Papaverine, 14-epivincamine, vincamine and apovincamine had capacity factors of 1.3, 2, 3.7 and 7.2, respectively (Fig. 1).

Calibration graphs of peak-height ratio (vincamine to papaverine) *versus* vincamine concentration in the range 0.2 to 0.8 μg/ml were constructed from give replicate injections (Fig. 2).

The determination of vincamine in methanol extracts of bulk drugs or pharmaceutical preparations and in diluted urine and plasma and in ether extracts thereof was studied (Fig. 3).

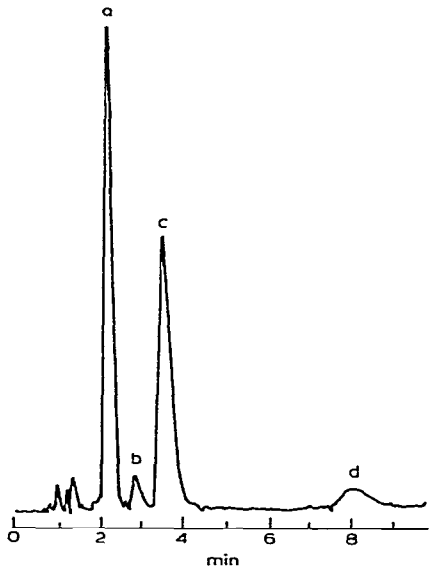

Fig. 1. High-performance liquid chromatogram of (a) papaverine, (b) 14-epivincamine, (c) vincamine and (d) apovincamine.

The results suggest that for urine samples ether extraction is not necessary, and direct analysis of diluted urine (1:10) is preferred. The mean recovery in the specified concentration ranges was 99.8 ± 1.5% (n = 12) and the precision over a 30-day period was ±3.5%, using different urine samples. For plasma samples, ether extraction is recommended in order to prevent contamination of the column by plasma proteins. Ether extraction, although time consuming, results in a more accurate pro-

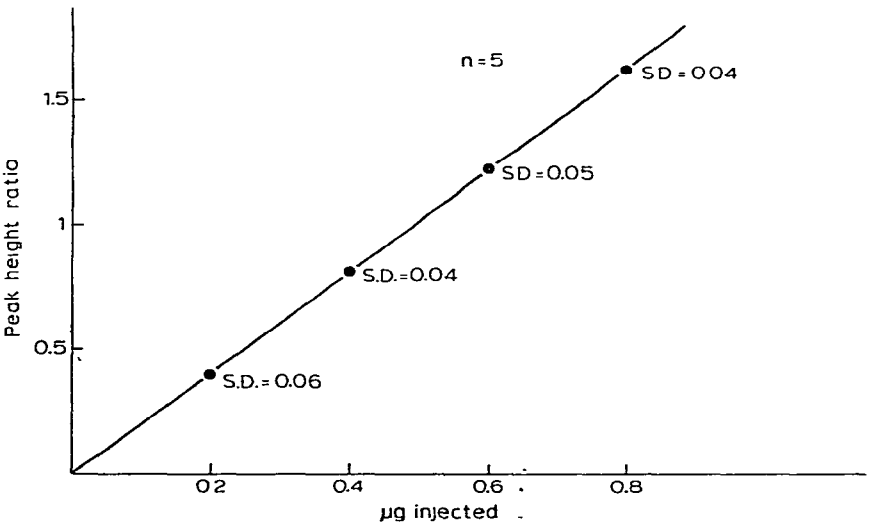

Fig. 2. Calibration graph of peak-height ratio (vincamine to papaverine) *versus* amount of vincamine injected.

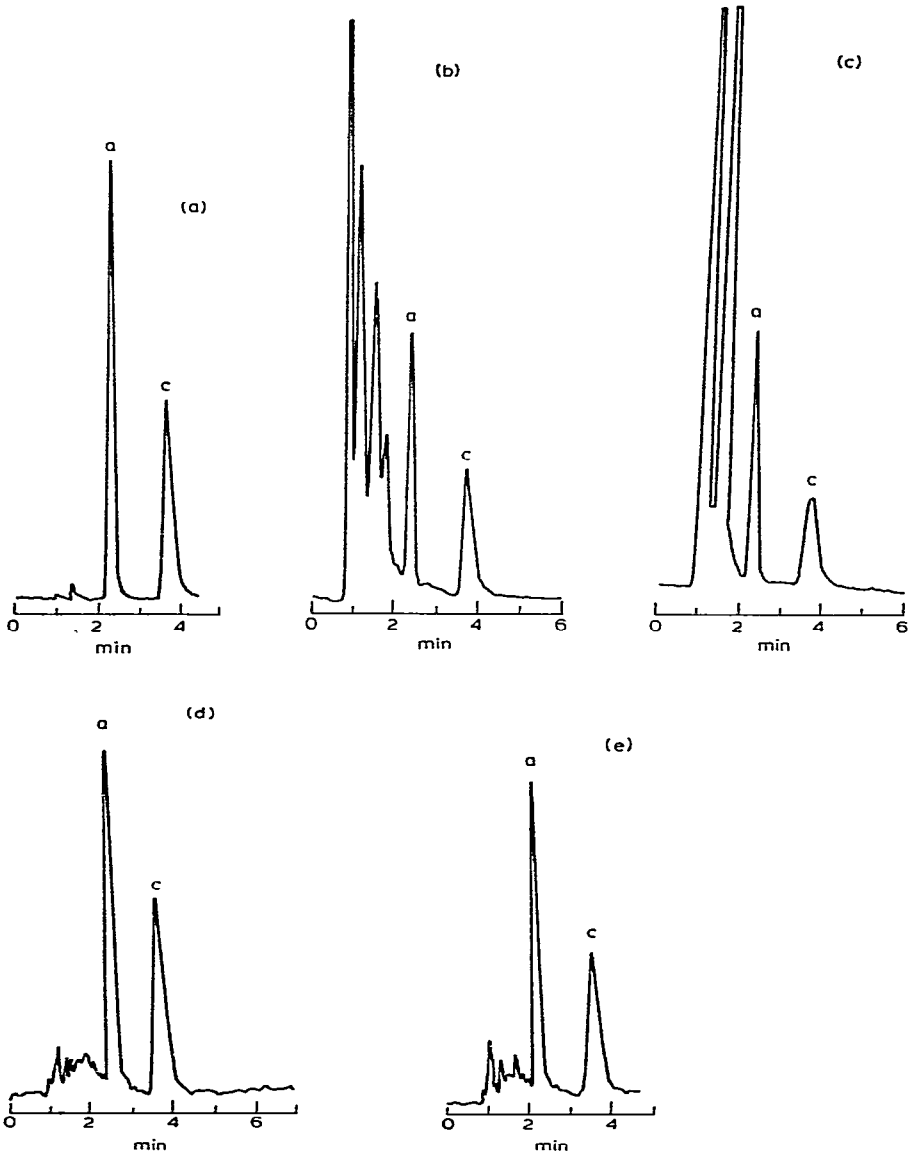

Fig. 3. High-performance liquid chromatogram of internal standard and vincamine in drugs and urine and plasma; samples. Eluent: methanol–0.01 *M* ammonium carbonate (75:25, v/v), flow-rate 2 ml/min. Column: 300 × 3.9 mm, μBondapak C_{18}. UV detection at 254 nm. (a) Drugs; (b) diluted urine; (c) diluted plasma; (d) ether extract of diluted urine; (e) ether extract of diluted plasma.

cedure, with a mean extraction recovery of 100.5 ± 1.3% (n = 12) and a precision of ±2.9%.

The HPLC assay described is simple, rapid and provides a sensitivity of 5–10 ng of vincamine in plasma and urine. No interfering peaks were noted in plasma or urine samples from different sources. Moreover, the urine assay allows the quanti-

tation of total vincamine in urine, as the conjugated drug is easily hydrolysed at alkaline pH.

In conclusion, the proposed method is accurate, sensitive and offers a useful routine alternative to gas chromatography or *in situ* spectrophotometry.

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* *Editor's Note:* See also the paper by C. Dubruc, H. Caqueret and G. Bianchetti, *J. Chromatogr.*, 204 (1981) 335–339.