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Note

Gas chromatographic method for the determination of vincamine in blood

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Vincamine is an efficient drug for treatment of the cerebral microcirculation system[1]. Because of difficulties in the quantitation of this compound in blood, its pharmacokinetics have been studied by use of ^{14}C-labelled vincamine[2] or by a spectrophotometric and thin-layer chromatographic method from urine. There have been few references in the literature to the analysis of vincamine. A recent gas chromatographic–mass spectrometric (GC–MS) method employed multiple ion detection for a silylated derivative of vincar[illegible]e[3]. However, the above methods are not suitable for routine analysis in man. We therefore report a convenient, sufficiently sensitive and reliable method for the determination of vincamine in blood by gas chromatography, without a derivatization step and without the use of radioactive isotopes. The total assay time is less than 30 min.

EXPERIMENTAL

Instrumentation

A 300 FF gas chromatograph (Girdel, Paris, France) was used with a Girdel 15489 nitrogen flame ionization detector (NFID) and a Servotrace PU recorder (Sefram, Paris, France) having a sensitivity of 1 mV. The stainless-steel column (2 m × 2.17 mm) was packed with 3% OV-1 (Pierce, Rockford, Ill., U.S.A.) on Gaschrom Q (100–120 mesh) (Applied Science Labs., State College, Pa., U.S.A.) and conditioned at 275° for 12 h with 35 ml/min of nitrogen (U quality). The chromatographic conditions were as follows: volume of sample injected, 1–2 μl; injector temperature, 260°; detector temperature, 280°; column temperature, 260° (isothermal); carrier gas (nitrogen) flow-rate, 35 ml/min; hydrogen flow-rate, 30 ml/min; air flow-rate, 350 ml/min; sensitivity and attenuation, 1 × 16; chart speed, 2.5 mm/min.

Reagents

The reagents used were reagent grade (E. Merck, Darmstadt, F.G.R.). Standard solutions (1 mg/ml) were prepared by directly dissolving diazepam or vincamine base in chloroform and were stored at 4° in a cold chamber.

Procedure

To 0.5 ml of Biotrol 00A human serum (Biotrol, Paris, France) in a glass tube

(8 ml) were added 1.5 μg of diazepam as internal standard, 1–5 μg of vincamine base (from the standard solutions) and 100 μl of 1 *N* sodium hydroxide solution. The mixture was shaken and extracted twice with 3 ml of diethyl ether with vigorous stirring. The organic fractions were mixed, dried over anhydrous sodium sulphate and then evaporated under a stream of air on a water bath at 50°. The residue was dissolved in 20 μl of methanol by shaking ultrasonically for 30 sec. 1–2 μl of this mixture were injected into the gas chromatograph.

RESULTS

The retention times (relative to methanol) during standardization of the peak-height ratio were 1.6 min for the internal standard (A) and 3 min for vincamine (B)

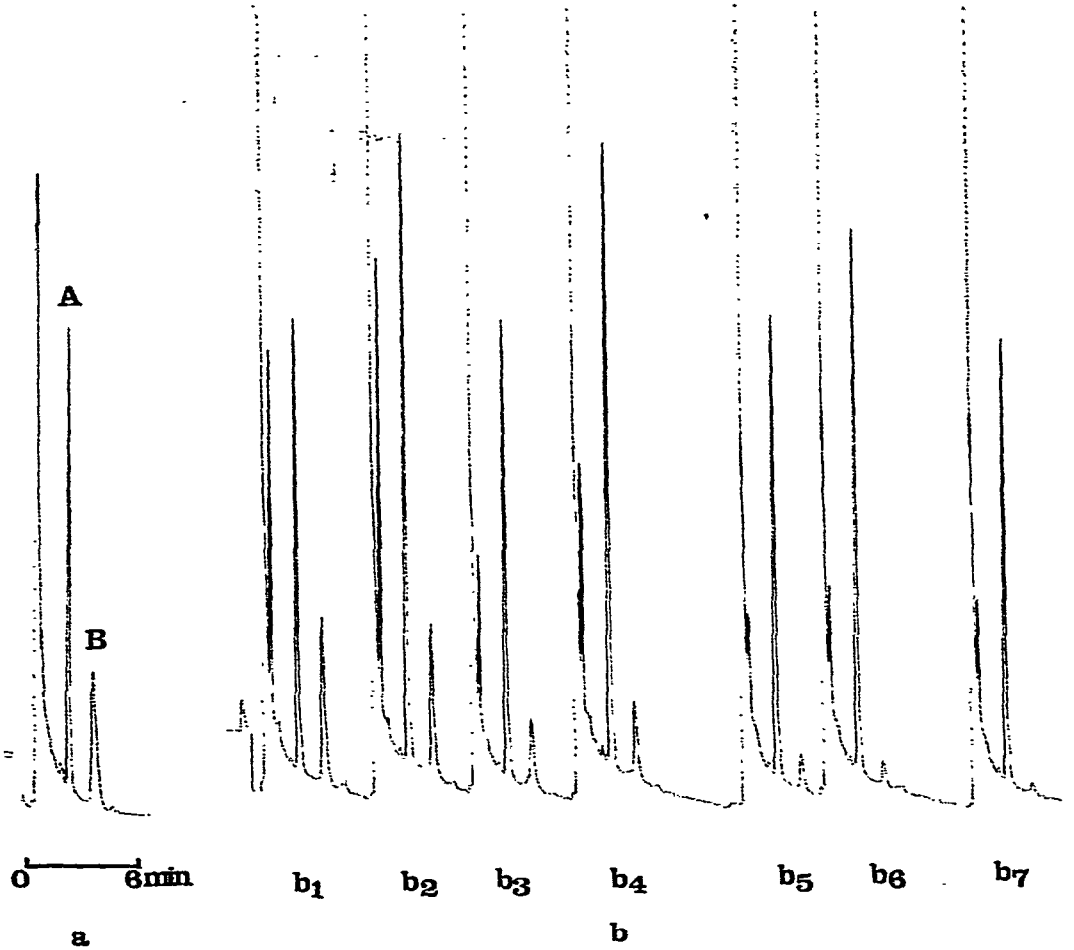

Fig. 1. Gas chromatogram obtained (a) during standardization of the peak-height ratio for vincamine (B) and diazepam (A), and (b) from samples of dog blood after a single IV bolus dose of 4 mg/kg of vincamine. Blood samples were withdrawn 10 (b_1), 20 (b_2), 40 (b_3) and 60 (b_4) min and 2 (b_5), 3 (b_6) and 6 (b_7) h after introduction of drug.

(Fig. 1a). The use of a nitrogen selective detector strongly decreases the tailing peak of the solvent (methanol) owing to its specific detection[4–6]. Fig. 1b, represents the chromatograms from preliminary assays of vincamine in dog blood at 10, 20, 40 and 60 min and 2, 3 and 6 h after intravenous administration of 4 mg/kg of vincamine. The method may be applicable to pharmacokinetic studies in man. The chromatograms of blanks were similar to that of b7, fig. 1.

The standard curve (Fig. 2) of peak-height ratio *versus* amount of vincamine added was linear in the range encountered clinically (0–5 μg). The recovery of vincamine from normal human serum samples spiked with 2 μg was 98.5 $\pm$ 3.5%. The precisions between runs and within runs were 8 and 3.5% respectively.

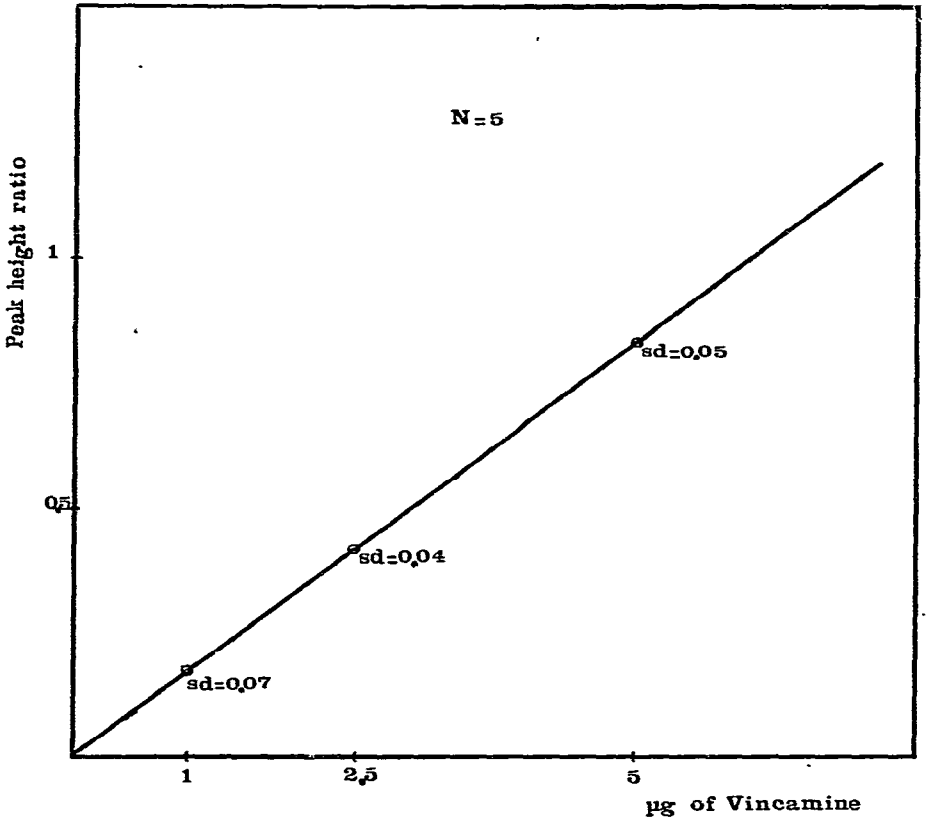

Fig. 2. Standard curve of the peak height ratio (vincamine to internal standard) *versus* the amount of vincamine added. *N* is the number of assays for each point (1, 2.5 and 5 μg of vincamine) on the standard curve.

Under the described conditions, the only interferences were with quinine and quinidine in their on-column methylated forms and with chloroquine.

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REFERENCES

1 L. Szporny, *Acta Pharmacol.*, 28 (1975) 87.
2 K. Ventouras, P. Schulz, E. Doelker, J. Boucherat and P. Buri, *Pharm. Acta Helv.*, 51 (1976) 334.
3 A. Zune and U. Rapp, Application Note, Varian Mat., Bremen, No. 23 (1976).
4 J. H. Goudie and D. Burnett, *Clin. Chim. Acta*, 43 (1973) 423.
5 M. A. Moulin and H. Kinsun, *Clin. Chim. Acta*, 75 (1977) 491.
6 H. Kinsun, M. A. Moulin and E. C. Savini, *J. Pharm. Sci.*, in press (1977).