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Short Communication

Determination of vincamine in human plasma by high-performance liquid chromatography with ultraviolet detection

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ABSTRACT

A simple and rapid high-performance liquid chromatographic method was developed for the determination of vincamine in human plasma. Plasma samples were buffered at pH 9 and after extraction with *tert.*-butyl methyl ether back-extracted into 0.017 *M* orthophosphoric acid. Propranolol was used as the internal standard. An aliquot was injected on to a high-performance liquid chromatographic system using a C_{18} reversed-phase column and an acetonitrile–phosphate buffer containing triethylamine (30:70) as mobile phase. Detection was performed with an ultraviolet detector at 273 nm. The method had good accuracy and precision and the detection limit (0.3 ng/ml with a signal-to-noise ratio of 3:1) allowed the assessment of vincamine concentrations in plasma in pharmacokinetic studies on healthy human volunteers.

INTRODUCTION

Vincamine, 14,15-dihydro-14β-hydroxy-(3α,16γ)-eburnamenine-14-carbonic acid methyl ester, one of the major indole alkaloids from *Vinca minor* [1], is commonly used as a vasodilator in the treatment of cerebrovascular diseases [2].

Analytical methods previously described for the determination of vincamine include radioisotope techniques [3], gas chromatography–mass spectrometry [4], gas chromatography with nitrogen–phosphorus flame-ionisation detection [5,6] and high-performance liquid chromatography (HPLC) [7,8]. However, these methods are time-consuming or lack selectivity or an adequate detection limit.

This paper describes a reliable and simple HPLC method for the determination of vincamine in plasma, which was successfully used in pharmacokinetic studies in humans.

EXPERIMENTAL

Chemicals and reagents

Vincamine and propranolol were obtained from Sigma (St. Louis, MO, USA). Potassium dihydrogenphosphate, phosphoric acid, triethylamine, sodium hydroxide (all p.a. grade), acetonitrile (HPLC-grade) and *tert.*-butyl methyl ether (nanograde) were purchased from E. Merck (Darmstadt, Germany).

HPLC-grade water was produced with an Elgastat-UHQ apparatus. The borate buffer for plasma extraction was obtained by adding 0.5 *M* NaOH to a 0.5 *M* H_3BO_3 solution to a pH of 9.

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Internal standard

Propanolol was used as an internal standard (I.S.). A stock solution was prepared in methanol (1 mg/ml) and a working solution (5 μg/ml) for addition to the plasma samples was obtained by dilution in methanol.

Standard solutions and calibration standards

A stock solution of vincamine was prepared in methanol at a concentration of 1 mg/ml. This stock solution was then diluted further to yield appropriate working solutions for the preparation of the calibration standards.

The calibration standards were prepared by adding 20 μl of the I.S. working solution and 20 μl of a suitable standard solution to drug-free plasma, to obtain vincamine concentrations in the range 1–200 ng/ml.

Apparatus and chromatographic conditions

The HPLC system consisted of a solvent delivery system pump, an ISS-100 autosampler and an LC 95 UV visible detector set at 273 nm, coupled to an Omega work-station integrator (all from Perkin-Elmer, Norwalk, CT, USA).

The analytical column used was a Supelcosil LC 18 DB column, 150 mm × 4.6 mm I.D., 5 μm particle size (Supelco, Bellefonte, PA, USA), protected by a 20 mm × 2.1 mm I.D. guard column (Upchurch Scientific, Oak Harbor, WA, USA) filled with Perisorb RP-18, 30–40 μm particle size, from E. Merck.

The mobile phase consisted of acetonitrile–0.02 *M* potassium dihydrogenphosphate containing 0.1% triethylamine with the pH adjusted to 3 with 1 *M* phosphoric acid (30:70, v/v) and filtered through a 0.45-μm nylon membrane (Zetapore-Nylon 66 from Supelco). The chromatography was performed isocratically at a flow-rate of 1 ml/min at room temperature, yielding a back-pressure of 75 bar.

Extraction procedure

Frozen samples were allowed to thaw at room temperature. To 1 ml of plasma, 20 μl of I.S. solution, 0.5 ml of 0.5 *M* borate buffer (pH 9) and 5 ml of methyl *tert.*-butyl ether were added in 12-ml capacity tubes fitted with PTFE-lined screw-caps.

After vortex-mixing (1 min) and centrifugating (3 min at 1500 *g*), the tubes were placed in a freezer (10 min at −80°C). The ether phase was transferred to another series of conical tubes containing 150 μl of 0.017 *M* H_3PO_4 and back-extracted by agitating for 1 min on a vortex mixer.

After centrifugating for 3 min at 1500 *g*, the upper organic layer was drawn off with a Pasteur pipette connected to a flask under vacuum. Then the aqueous residue was flushed with a stream of nitrogen at 40°C to remove the residual ether and tranferred into the autosampler vial. A 100-μl aliquot was injected on to the column.

Calibration and calculations

Six calibration samples covering the expected concentration range were extracted and processed daily with the unknown samples.

The calibration graph was constructed by linear least-squares regression of vincamine and the I.S. peak-height ratios against vincamine concentrations. The vincamine concentration in unknown samples was calculated by interpolating the peak-height ratios, obtained from the relevant chromatograms, with the calibration graph.

Accuracy and precision

To assess the accuracy and precision of the method, quality control samples at concentrations of 37.5 and 3.75 ng/ml were prepared by spiking 1-ml aliquots of blank plasma with vincamine standard solutions. These standard samples were prepared before analysis, stored at −20°C, and analysed with the unknown samples.

To determine the intra-assay precision of the analytical procedure, seven quality control samples for each concentration were analysed on the same day. The between-day reproducibility of the assays was determined from the mean concentrations of the quality control samples (n = 2–7) found on each testing day (seven days over a ten-day period).

RESULTS AND DISCUSSION

Chromatographic performance

Under these chromatographic conditions, a rapid and adequate separation of vincamine and the I.S. was achieved; the retention times were about 4.9 and 6.9 min, respectively.

The use of a base-deactivated stationary phase and the addition of triethylamine to the mobile phase improves the peak symmetry and the efficiency of the system by reducing the interaction of the basic compounds with the adsorption sites on the stationary phase.

Linearity

A linear correlation between vincamine and I.S. peak-height ratio and vincamine concentration was found for the concentration range 0–200 ng/ml and the calibration graph could be described by the equation: $y = 46.0x - 0.072$.

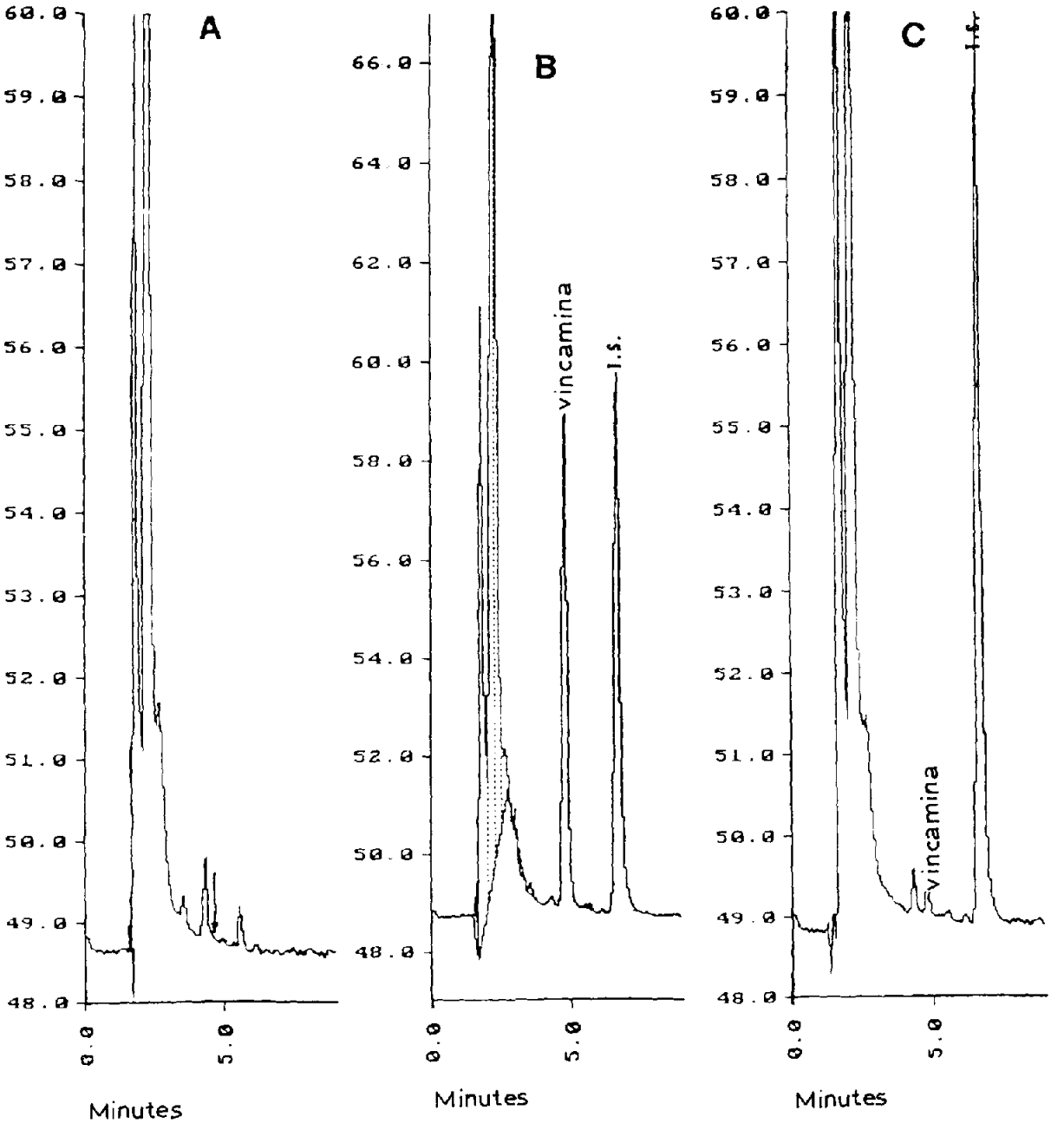

Fig. 1. Representative chromatograms from: (A) pre-dose plasma sample (the arrow indicates vincamine retention time); (B) plasma sample 2 h after administration of a 30-mg oral dose of vincamine to a healthy subject (estimated to contain 43.3 ng/ml vincamine); and (C) plasma sample 24 h after administration of a 30-mg oral dose of vincamine to a healthy subject (estimated to contain 0.7 ng/ml vincamine).

TABLE I

INTRA-ASSAY AND INTER-ASSAY (BETWEEN-DAY) PRECISION AND ACCURACY OF THE DETERMINATION OF VINCAMINE IN HUMAN PLASMA

Concentration (ng/ml)		Accuracy (%)	C.V. (%)
Added	Found		
Intra-assay (n=7)			
37.5	36.5	97.3	3.8
3.75	3.72	99.2	4.4
Inter-assay (n=10)			
37.5	37.3	99.5	2.9
3.75	3.85	102.7	3.3

The correlation coefficient (r^2) was generally greater than 0.998.

Accuracy and precision

Intra-assay (within-day) and inter-assay (between-day) coefficients of variation (C.V.), determined from the standard samples for quality control, were < 5%; the accuracy was 97–103% (Table I).

At the lowest calibration point (1 ng/ml), the total C.V. (n = 10) was 8.9% and the mean accuracy 97.6%.

Lower limit of detection and selectivity

The detection limit of vincamine was 0.3 ng/ml, with a signal-to-noise ratio of 3. The detection sensitivity could be further improved by setting the UV detector to 232 nm, but this value was considered sufficient for these purposes and it was preferred not to impair the selectivity.

No interfering peaks were found in more than 26 analysed blank plasma samples at the retention times corresponding to the compounds (Fig. 1A).

Extraction efficiency

The absolute recoveries of vincamine and the I.S. following the whole sample preparation procedure were determined by comparing the peak heights obtained from the calibration samples to those of the corresponding unextracted standard solutions. The recoveries of vincamine and I.S. from plasma were always >88%.

Stability

The compounds were stable in the autosampler vials at room temperature for at least 16 h.

Applications

The method has been successfully applied to the determination of vincamine concentrations in plasma samples from a bioequivalence study in

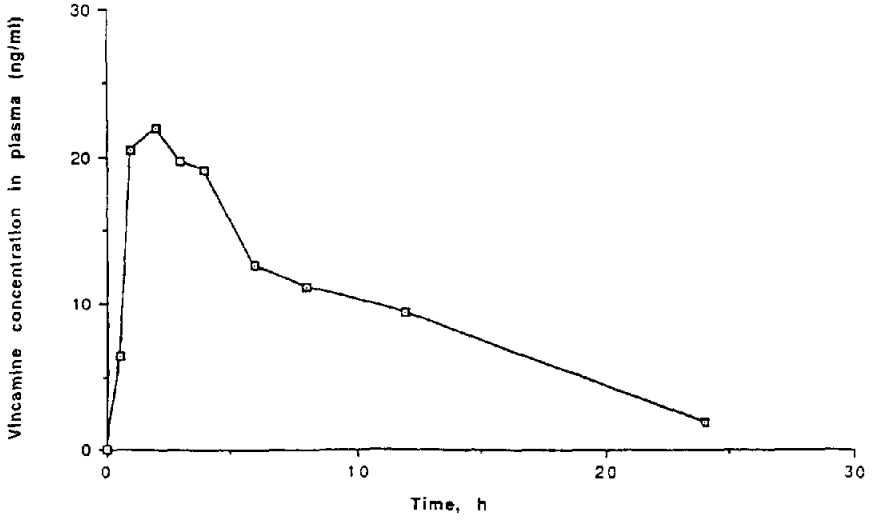

Fig. 2. Time course of the plasma concentration of vincamine after the administration of 30 mg of vincamine in a controlled release dosage form to a healthy subject.

volunteers following the single oral administration of 30 mg of vincamine in a controlled release dosage form.

Fig. 1B and C shows representative chomatograms from the plasma assay and Fig. 2 the time course of vincamine plasma concentrations in a healthy subject.

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