Journal of Chromatography, 204 (1981) 335–339
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,155

DETERMINATION OF VINCAMINE IN HUMAN PLASMA USING AUTOMATED HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A specific and sensitive high-performance liquid chromatographic method for the quantitative determination of vincamine at therapeutic concentrations in plasma is described. The column was packed with Spherisorb ODS 5 μm, and the mobile phase was acetonitrile–potassium phosphate (0.02 *M*, pH 2.3) (50:50) with a flow-rate of 10 ml/min. Detection was at 230 nm. Using automated large-volume injection of a non-eluting solvent (0.02 *M* potassium phosphate) the method was capable of the analysis of a large number of samples daily. The coefficient of variation of the procedure was 4.8% at a plasma vincamine concentration of 10 ng/ml.

INTRODUCTION

Vincamine is one of the major indole alkaloids derived from *Vinca minor*[1]. Despite its widespread use in the treatment of cerebrovascular disorders[2] there have been no simple and reliable analytical methods described for the measurement of vincamine at therapeutic concentrations in biological fluids. Those methods which have been described either lack specificity[3] and sensitivity[4] or require very elaborate and expensive instrumentation[5]. The present described method is based on reversed-phase high-performance liquid chromatography (HPLC) with UV detection and automatic injection. Because of its simplicity and speed of operation the technique offers many advantages over existing methods.

EXPERIMENTAL

Standards and reagents

Vincamine, (3α,14β,16α)-14,15-dihydro-14-hydroxyeburnamenine-14-carboxylic acid methyl ester, and its internal standard desoxyvincaminamide, (3α,14β,16α)-14,15-dihydroeburnamenine-14-carboxamide, were synthesized at the laboratories of Synthélabo-Lers at Bagneux.

The following reagents were used: glass-distilled diethyl ether, acetonitrile, potassium and sodium phosphate, phosphoric acid (Merck, Darmstadt, G.F.R.).

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Stock solutions

Standard solutions of vincamine (1 μg/ml) and desoxyvincaminamide (10 μg/ml) in acetonitrile were prepared and kept at 4°C. Under these conditions the solutions were stable for at least one month.

Calibration curve and quantification

Vincamine and desoxyvincaminamide standard curves were prepared by adding 10, 50, 100 and 200 ng of vincamine and 200 ng of desoxyvincaminamide to 1 ml of drug-free plasma. The plasma standards were processed exactly the same way as the samples according to the method described below.

The ratio of the peak areas for vincamine/desoxyvincaminamide were used to calculate a calibration curve, the slope of which was used in the quantification of vincamine in plasma.

Extraction procedure

For each analysis 1 ml of plasma sample was used, and 200 ng of a solution of desoxyvincaminamide internal standard (10 ng/μl in acetonitrile) were added to each sample in a glass centrifuge tube. 1 ml of phosphate buffer (pH 8) and 8 ml of glass-distilled diethyl ether were added and the tubes stoppered and shaken vigorously for 10 sec at room temperature on a Vortex mixer. The tubes were then centrifuged for 5 min at 1000 *g*. The ether phase was transferred to another series of clean glass tubes, and evaporated under a gentle stream of nitrogen at 37°C. The dry residue was dissolved into 880 μl of 0.02 *M* potassium phosphate (pH 2.3, adjusted with phosphoric acid) and transferred into vials for automatic injection.

Apparatus

An LDC Constametric II G HPLC pump equipped with an LDC Spectro-Monitor III UV spectrophotometer was used with a stainless-steel column (15 cm × 4.6 mm I.D.) packed with ODS Spherisorb 5 μm (Phase Separations, Queensferry, Great Britain).

The automatic injector, Micromeritics model 725, holding 64 samples, was coupled to a Perkin-Elmer Σ10 integrator–calculator.

Operating conditions

The eluent was acetonitrile–potassium phosphate (0.02 *M*, pH 2.3, adjusted with phosphoric acid) (50:50) at a flow-rate of 1 ml/min. The detector was set at $\lambda = 230$ nm.

RESULTS AND DISCUSSION

The chromatogram obtained from blank plasma to which known amounts of vincamine and desoxyvincaminamide were added is shown in Fig. 1 together with those obtained from drug-free plasma and from a patient receiving chronic treatment with vincamine. The vincamine and desoxyvincaminamide peaks were well separated, and no interfering peaks from endogenous substances or concomitant therapy were observed.

To determine the best pH for the extraction of vincamine, the peak heights

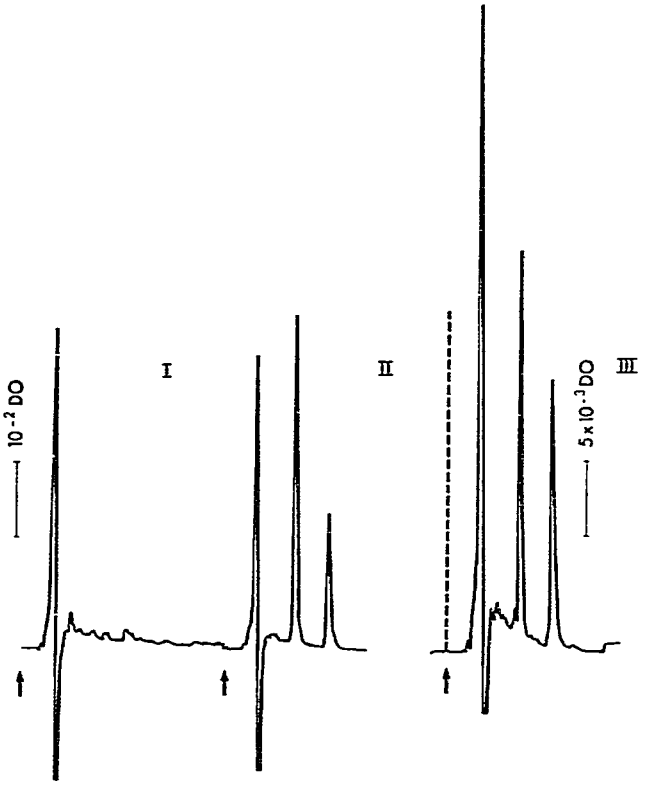

Fig. 1. Typical chromatogram obtained after extraction of 1 ml of plasma. I, Blank. II, Spiked with vincamine and its internal standard (200 ng). III, From a patient treated with vincamine.

obtained after extraction of drug from plasma at different pH values were measured and the optimum pH was found to be 8.0, giving a recovery of 99% for vincamine and 92% for desoxyvincaminamide. The chromatographic characteristics of vincamine and desoxyvincaminamide are listed in Table I.

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF VINCAMINE AND DESOXYVINCAMINAMIDE

	Vincamine	*Desoxyvincaminamide*
pK_a	8.39	8.03
pH of extraction	8	8
Solvent	Ether	Ether
Recovery after extraction (%)	99	92
Limit of sensitivity (ng/ml)	10	
Retention time (min)	6.49	4.37
Height equivalent to one theoretical plate (μm)	36.2	35.0

A standard calibration curve obtained after the extraction of vincamine from plasma is shown in Fig. 2, and the coefficient of variation of the method at different concentrations is reported in Table II.

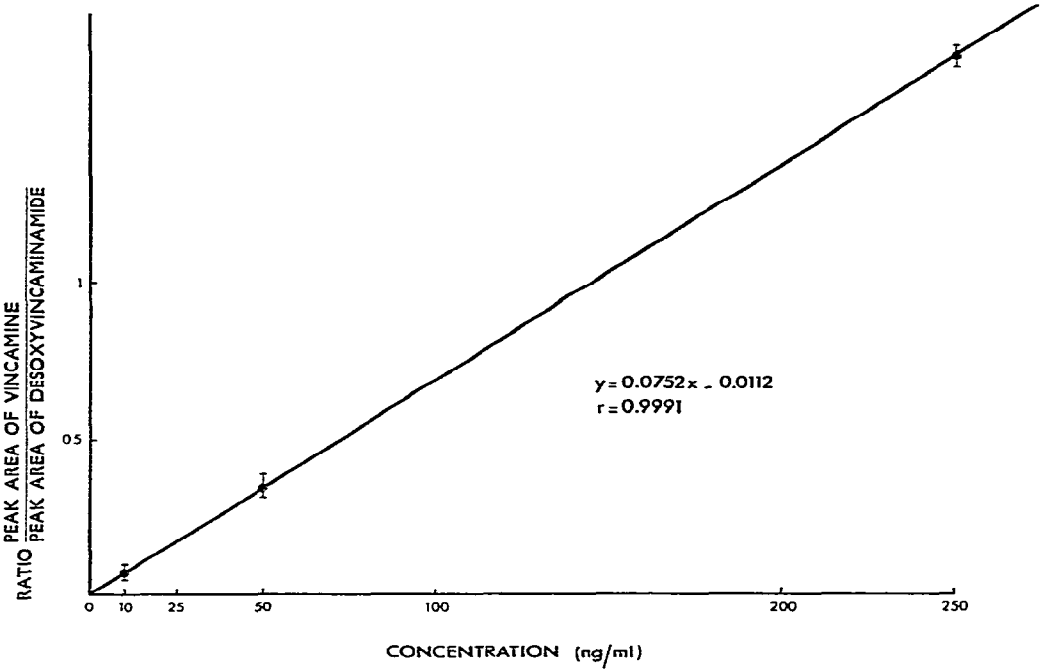

Fig. 2. Standard calibration curve obtained after extraction of vincamine from plasma.

The injection loop had a volume of 500 μl, but the drug extract was redissolved in 880 μl of solvent in order to fill the vials and rinse the loop between each injection. This was possible without any problem of band-broadening because of the choice of a non-eluting solvent (the aqueous phase of the eluent, potassium phosphate 0.02 *M*). Under these conditions the dry extract containing vincamine was perfectly redissolved and concentrated on the top of the HPLC column, without any distortion in its equilibrium. Moreover, the sensitivity of the method was as good as that obtained when injecting the total volume of a sample redissolved in only 30 μl of acetonitrile in order to prevent band-broadening. In this latter situation, it was obviously impossible to use automatic injection.

The method was found suitable for pharmacokinetic studies and the monitoring of plasma levels in patients receiving chronic treatment with vincamine. This was clearly demonstrated in a bioavailability study comparing chronic treatment with two different formulations of vincamine in ten elderly patients.

TABLE II

COEFFICIENT OF VARIATION OF THE METHOD AT DIFFERENT CONCENTRATIONS

Number of determinations: 10.

Vincamine concentration added to plasma (ng/ml)	*Mean value of concentration found*	*S.D.*	*Coefficient of variation*
10	11.2	0.52	4.8
50	47.2	1.93	4.1
250	248.9	11.20	4.5

The results indicate that the method described is not only specific and sensitive, but also very simple and rapid. The coupling of an automatic injector holding 64 samples with an integrator–calculator allows a large number of analysis to be carried out in one day.

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