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Note

Capillary gas chromatographic determination of vincamine in plasma

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Vincamine, the main alkaloid of *Vinca minor*, is used as a vasodilator in the treatment of cerebrovascular affections. In order to study the pharmacokinetics of a new sustained-release formulation, an analytical method for the determination of vincamine in plasma was developed. Analytical methods previously described for the determination of vincamine include gas chromatography (GC) [1, 2], gas chromatography—mass spectrometry [3], radioisotope techniques [4] and high-performance liquid chromatography (HPLC) [5—7].

The HPLC method described by Pietta et al. [5] seemed very attractive but, although they claimed it to be suitable for pharmacokinetic studies, the data given by the authors suggest that it is not sensitive enough to measure accurately plasma concentrations as low as 10 ng/ml. The HPLC method of Dubruc et al. [7] could not be investigated owing to the absence of an appropriate detector. The GC method on packed columns described by Kinsun and Moulin [1] could not be applied as the detection limit calculated by these authors was 0.25 μg/ml of plasma, a value much too high for our purposes. The GC method of Gazdag et al. [2] on packed columns was developed to achieve separation of *Vinca* alkaloids and not for quantitation in blood. Therefore, and in order to achieve high sensitivity and specificity, we have developed a capillary GC procedure using a nitrogen—phosphorus flame-ionization detector.

EXPERIMENTAL

Vincamine hydrochloride and ethaverine hydrochloride were of pharmacopoeial purity and supplied by Bios (Brussels, Belgium). All other reagents were analytical-reagent grade and purchased from E. Merck (Darmstadt, F.R.G.).

Apparatus

A Carlo Erba HRGC gas chromatograph was used, equipped with an on-column injector, a nitrogen—phosphorus detector, a Vitatron recorder and a Varian Vista CDS 401 chromatographic data system.

Chromatography

Chromatography was performed on a glass capillary WCOT OV-1 column (Alltech Europe, Eke, Belgium) (12 m × 0.5 mm I.D., film thickness 0.4 μm). Helium was used as the carrier gas (0.7 ml/min) and as the make-up gas (30 ml/min). The oven was programmed as follows: from 130°C to 210°C at 30°C/min, hold for 5 min, then to 250°C at 5°C/min. A cold on-column injection of 1 μl was performed. The detector temperature was set at 300°C. Flowrates were as follows: hydrogen, 30 ml/min; air, 300 ml/min. The detector attenuation was 2—32 and the range 10^{-11} A/mV.

Extraction procedure

To 4 ml of plasma, 4 ml of a 1 M carbonate—bicarbonate buffer (pH 10) and 500 μl of internal standard solution were added in silanized centrifuge tubes. The mixture was homogenized by vortexing and 4 ml of n-hexane were added. Partitioning was performed by gentle mechanical shaking for 15 min. After centrifugation (1900 g), the organic phase was transferred to a silanized tube and a second extraction with 4 ml of hexane was performed on the aqueous phase. A back-extraction of the combined organic fractions with two 5-ml portions of 0.1 M hydrochloric acid was then carried out. The combined acid fractions were adjusted to pH 10 with a 5% solution of sodium carbonate and re-extracted twice with 4-ml portions of hexane. The combined hexane fractions were evaporated to dryness under a stream of nitrogen at 40°C. Each extract was reconstituted just prior to chromatography with 20 μl of toluene; 1 μl was injected.

Assay standards

Plasma standards were prepared by spiking drug-free plasma with vincamine hydrochloride solutions in double-distilled water to give final concentrations of 20—200 ng/ml of plasma. The plasma standards were taken through the entire procedure.

Internal standard solution

A solution containing 400 μg of ethaverine hydrochloride per 100 ml of double-distilled water was prepared daily.

Quantitation

The peak-area ratio of vincamine to the internal standard was used for quantitation. Calibration graphs were obtained by plotting the peak area ratios for the plasma standards against concentration. The calibration graph was linear in the concentration range studied (0—200 ng/ml of plasma).

RESULTS AND DISCUSSION

Extraction procedure

As the number of samples to be analysed was quite large, attention was paid to the development of a rapid extraction method. A number of simple solvent extraction procedures were tested using diethyl ether, n-hexane or n-heptane—isoamyl alcohol (98:2) as the extractant. However, they all yielded extracts with various interfering peaks. A solid extraction using commercially available reversed-phase octadecylsilane-bonded cartridges yielded unacceptable results in our hands. We finally decided to perform an extraction procedure with a back- and re-extraction step. This procedure yielded very clean extracts. No deterioration of the chromatographic quality was observed after injection of more than 500 plasma extracts. The percentage overall recovery of the extraction procedure yielded a mean of 55.6% (S.D. = 2.9%; n = 7).

Precision and determination limit

The within-day precision of the entire procedure was evaluated at the 100 ng/ml level by analysing replicate spiked plasma samples. A coefficient of variation of 5.1% was found. Recovery was complete. The limit of determination, defined as the concentration that yields a signal-to-noise ratio of at least 10 above the blank, was 3 ng/ml. This was considered to be sufficient for our purpose.
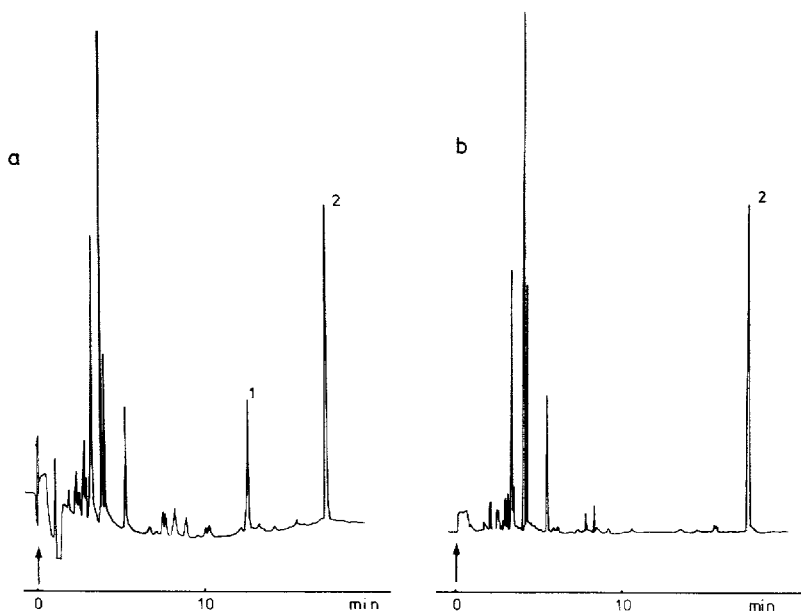

Fig. 1. (a) Chromatogram of plasma from a volunteer withdrawn 2 h after ingestion of a 60-mg dose of vincamine hydrochloride. Peaks: 1 = vincamine, 2 = internal standard. Concentration found: 50 ng/ml. (b) Chromatogram of plasma from the same volunteer before vincamine administration, spiked with internal standard (peak 2).
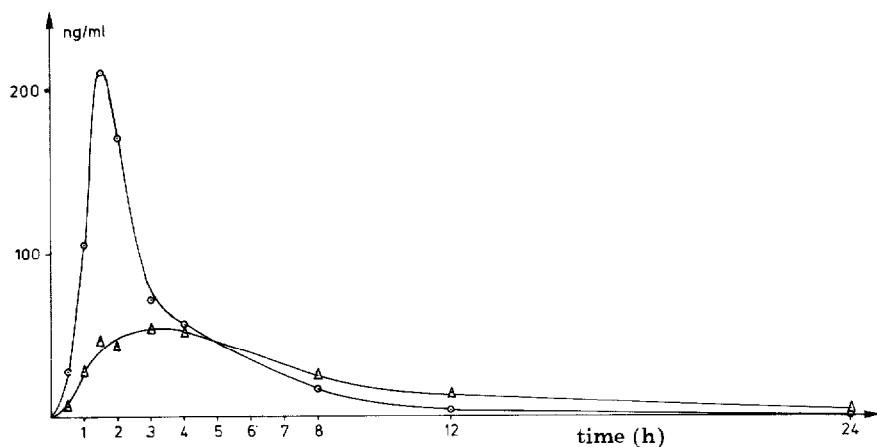
Fig. 2. Mean plasma concentration of vincamine hydrochloride as a function of time of six volunteers each receiving an oral 60-mg dose of vincamine as a simple formulation (○) and as a sustained-release formulation (△).
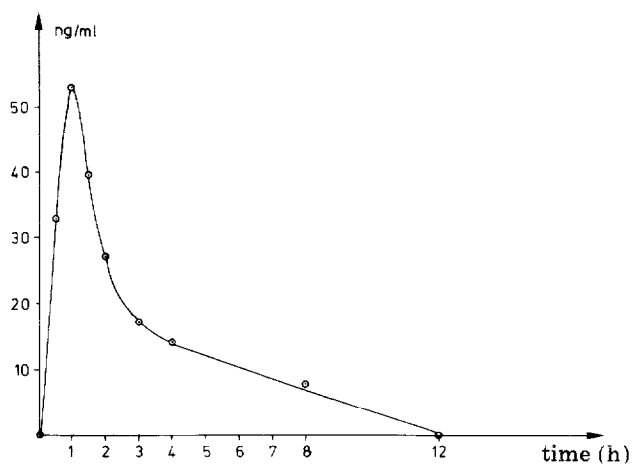

Fig. 3. Mean plasma concentration of vincamine hydrochloride as a function of time of six volunteers each receiving an oral 20-mg dose of vincamine.

TABLE I

PHARMACOKINETIC PARAMETERS OF VINCAMINE FORMULATIONS

Values are mean ± S.D.

Parameter	20-mg Tablets	60-mg Tablets	60-mg Sustained-release tablets
Area under the curve (ng/ml h)	172 ± 45	595 ± 293	500 ± 197
Time to reach peak (h)	1.17 ± 0.52	1.50 ± 0.32	2.08 ± 0.97
Drug concentration at peak (ng/ml)	64 ± 35	242 ± 172	70 ± 29
Half-life (h)	3.45 ± 0.71	2.65 ± 1.25	7.03 ± 3.61
Elimination constant (h^{-1})	0.21 ± 0.04	0.30 ± 0.10	0.14 ± 0.10

Pharmacokinetic study

The first study was performed applying a randomized cross-over design on six healthy volunteers with a one-week wash-out period. A sustained-release preparation of vincamine (60 mg) was compared with a normal preparation (60 mg). A chromatogram of a plasma extract from a volunteer taken 2 h after administration of 60 mg of vincamine is shown in Fig. 1a. The chromatogram of a blank plasma extract from the same volunteer taken before intake of the drug is shown in Fig. 1b. The results of the pharmacokinetic study are reported in Fig. 2. In a second study the time course of vincamine in the plasma of six other volunteers, each receiving orally a single dose of 20 mg of vincamine, was determined. The results are given in Fig. 3.

A number of pharmacokinetic parameters were calculated using a computer program on Apple 2 [8]. The results are summarized in Table I.

CONCLUSION

The proposed method permits a reliable and precise determination of vincamine in plasma and is successfully applicable to pharmacokinetic studies.

ACKNOWLEDGEMENTS

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