Note

Determination of vincamine by high-performance liquid chromatography with dual-wavelength ultraviolet detection

A. AMATO, G. CAVAZZUTTI, L. GAGLIARDI, M. PROFILI and V. ZAGARESE

Istituto Superiore di Sanità, Via Regina Elena, Rome (Italy)

F. CHIMENTI

Istituto di Chimica Farmaceutica e Tossicologica, University of Rome, Rome (Italy)

and

D. TONELLI* and E. GATTAVECCHIA

Istituto di Scienze Chimiche, University of Bologna, Via San Donato 15, 40127 Bologna (Italy)

(Received June 23rd, 1983)

Vincamine [(3α,14β,16α)-14,15-dihydro-14-hydroxyeburnamenine-14-carboxylic acid methyl ester], one of the major indole alkaloids from *Vinca minor*[1] is commonly used in the treatment of cerebrovascular diseases[2,3]. Various analytical methods are available for determining vincamine and include gas liquid chromatography (GLC)[4,5], GLC–mass spectrometry[6,7], fluorescence spectroscopy[8], radioisotope techniques[9] and thin-layer chromatography (TLC) followed by direct spectrophotometric determination[10]. Sometimes, these methods, although requiring expensive instrumentation, present some analytical difficulties in terms of sensitivity and selectivity[11]. Recently, reversed-phase high-performance liquid chromatography (HPLC) has been successfully employed for the determination of vincamine in pharmaceutical prepartions and biological fluids using either ion suppression[12,13] or ion-pair techniques[11]. In order to accomplish a survey of marketed pharmaceutical products in our laboratory, an HPLC method which allows rapid, sensitive and selective assays and purity determinations for vincamine has been developed. The method, here reported, is based on ion-pair reversed-phase HPLC with dual-wavelength detection. Chromatography is performed at pH 3.0 on bonded octadecylsilane with pindolol as an internal standard, setting the detector either at $\lambda = 232$ or 273 nm.

EXPERIMENTAL

Reagents

Vincamine was obtained from Sigma (St. Louis, MO, U.S.A.). Pindolol, 4-[2-hydroxy-3-(isopropylamino)propoxy]indole, was supplied by Sandoz (Basel, Switzerland). Perchloric acid (70% aqueous solution) and sodium perchlorate monohydrate were purchased from Farmitalia–Carlo Erba (Milan, Italy). All chemicals utilized were analytical-reagent quality and were used without further purification. HPLC-grade methanol and acetonitrile (Farmitalia–Carlo Erba) were used. Water was deionized and doubly distilled in glass. All solvents and solutions were filtered

through a Millipore filter, pore size 0.45 μm (Millipore, Bedford, MA, U.S.A.), and vacuum degassed by sonication before use.

Apparatus

A Varian (Zug, Switzerland) Model 5000 liquid chromatograph equipped with a variable-wavelength UV detector (Varichrom UV 50), a Valco AH 60 injection valve and a Waters Assoc. (Milford, MA, U.S.A.) Model 730 integrator-recorder were used. The analytical column was a Varian Micro Pack MCH-10 (300 × 4.6 mm I.D.).

HPLC conditions

The operating conditions were as follows: mobile phase, acetonitrile-0.005 M sodium perchlorate (pH 3.0, adjusted with perchloric acid) (45:55) at a flow-rate of 2 ml/min; measured pressure, 1200 p.s.i.; column temperature, 25°C; injection volume, 10 μl; detector wavelengths, 232 and 273 nm; detector sensitivity, 0.16 a.u.f.s.

Calibration curves

Working solutions were prepared by dissolving 2.5, 5.0, 7.5, 10 and 12.5 mg of vincamine in 50 ml of methanol containing 7.5 mg of pindolol. These solutions were processed using the HPLC conditions described above. The ratios of the peak areas for vincamine–pindolol obtained at both 232 and 273 nm were used to calculate the calibration curves, the slopes of which were used in the determination of vincamine in some medicinal specialties. The response factors relative to the internal standard and calculated from the weight ratio, were 2.50 at 232 nm and 0.86 at 273 nm.

Assay of pharmaceutical formulations

The following dosage forms of three proprietary preparations (A, B, C) marketed in Italy, containing vincamine as active principle, have been examined: 20-mg tablets, vials containing 15 mg/ml and oral solutions containing 0.75 g per 100 ml.

Amounts of liquid dosage forms equivalent to 7.5 mg of vincamine were evaporated to dryness at room temperature (20°C) under a stream of dry nitrogen and the residue was dissolved in 50 ml of methanol containing 7.5 mg of pindolol.

Ten tablets were ground, the equivalent of 7.5 mg vincamine was weighed and extracted three times with 15 ml of methanol containing the internal standard, then diluted to a volume of 50 ml with the same methanolic solution. All solutions were filtered through a 0.45-μm filter (Millipore) and kept refrigerated in the dark at 4°C. Aliquots (each 10 μl) of these solutions were injected into the liquid chromatograph.

RESULTS AND DISCUSSION

Fig. 1A and B shows typical chromatograms of a standard solution of vincamine and pindolol obtained setting the detector at 232 and 273 nm. Vincamine and pindolol had capacity factors of 3.53 and 1.36, respectively. The analysis time was 8 min. HPLC detection was performed at the two wavelengths corresponding to the two maxima of the UV spectrum of vincamine. Pindolol was chosen as internal standard since this compound shows an UV absorption spectrum similar to that of
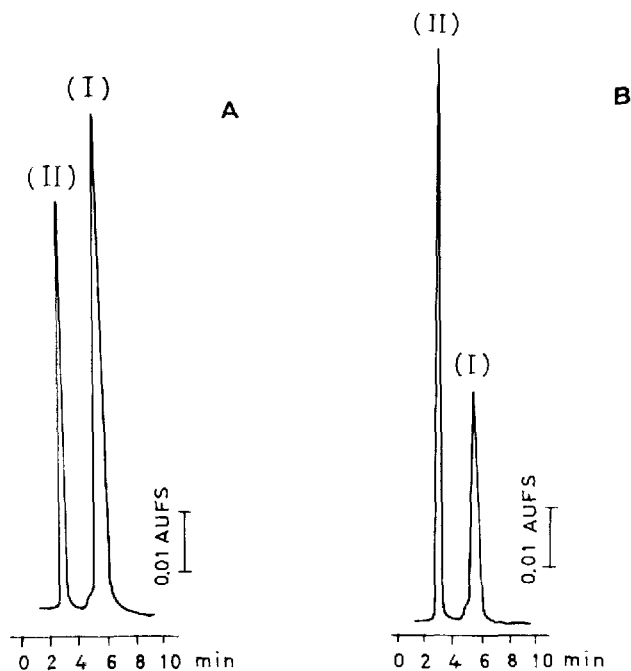
Fig. 1. Typical chromatograms of vincamine (I) and internal standard (II) obtained at (A) 232 nm and (B) 273 nm. Retention times: II, 162 sec; I, 315 sec.

vincamine when dissolved in the mobile phase used for HPLC, as can be seen from Fig. 2A and B. Calibration graphs of peak-area ratio (vincamine to pindolol) *versus* amount of vincamine injected were constructed from five consecutive injections (Fig. 3). The reproducibility of the method was very satisfactory, the resulting coefficient of variation being less than 1.5%. Linearity was observed up to 15 µg injected. The
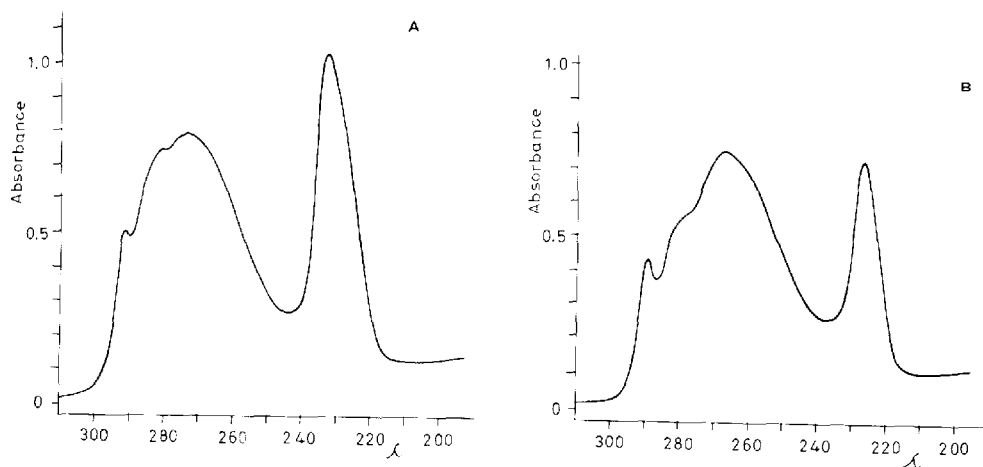

Fig. 2. UV absorption spectra of (A) vincamine and (B) pindolol in acetonitrile 0.005 *M* sodium perchlorate (pH 3.0) (45:55).
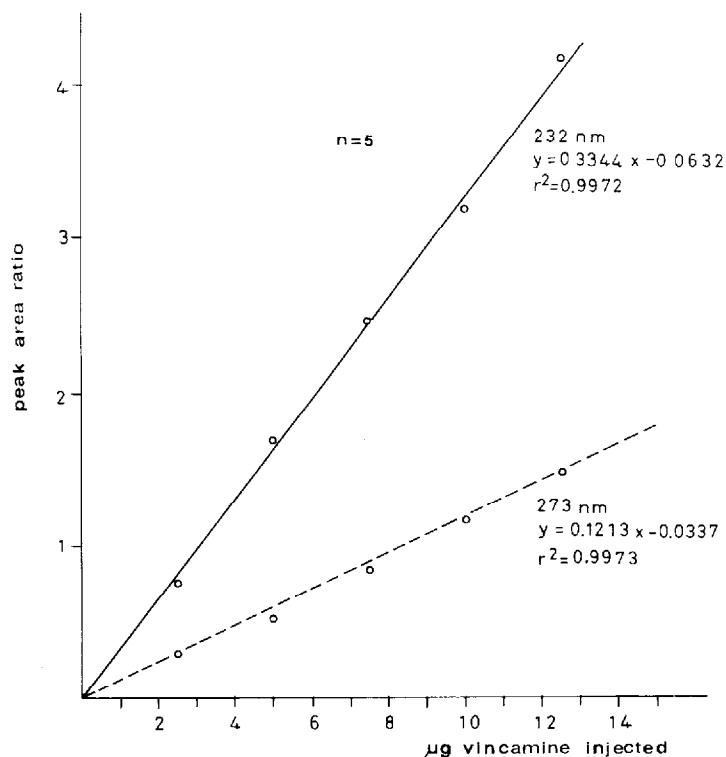
Fig. 3. Calibration graphs of peak-area ratio (vincamine to pindolol) *versus* amounts of vincamine injected, obtained at $\lambda = 232$ and 273 nm.

TABLE I

HPLC ASSAY OF VINCAMINE IN VARIOUS DOSAGE FORMS OF THREE MEDICINAL SPECIALTIES

Each value is the mean from five determinations.

Medicinal specialty	Recovery (%) at 232 nm (mean ± S.D.)	Recovery (%) at 273 nm (mean ± S.D.)	$\dfrac{Area_{232}}{Area_{273}}$
A			
Vials	97.4 ± 1.3	96.9 ± 0.9	2.796
Tablets	98.9 ± 1.1	98.2 ± 0.9	2.785
Oral solution	99.3 ± 1.2	100.2 ± 1.3	2.791
B			
Vials	98.1 ± 1.4	98.2 ± 1.6	2.714
Tablets	99.7 ± 1.1	98.9 ± 1.2	2.700
Oral solution	101.3 ± 1.2	99.7 ± 1.1	2.693
C			
Vials	98.3 ± 1.3	98.2 ± 1.2	2.745
Tablets	100.4 ± 1.6	101.3 ± 1.4	2.780
Oral solution	99.6 ± 1.2	98.3 ± 1.1	2.784

detection limits, calculated as the response of twice the noice level, were approx. 5 and 10 ng at 232 and 273 nm, respectively. The applicability of the HPLC method for assaying vincamine and for checking its purity in pharmaceutical preparations is demonstrated by the data in Table I.

The possibility of performing the detection at two wavelengths makes it most unlikely that interfering peaks will alter the results when complex samples are to be assayed. The identification of vincamine made on the basis of retention time is corroborated by the measurement of the peak-area ratio at 232 and 273 nm. This measurement is of primary importance in the control of purity of samples and resulted in a value of 2.756 for a vincamine reference standard.

In conclusion, the proposed method is rapid, accurate, sensitive and offers a useful way of checking the purity of a vincamine sample. Moreover, on the basis of the results showed above, the HPLC assay can obviously be extended to the determination of pindolol in pharmaceutical preparations where this drug is present as a vasodilator.

REFERENCES

1 W. I. Taylor, in W. I. Taylor and N. R. Farnsworth (Editors), *The Vinca Alkaloids*, Marcel Dekker, New York, 1973, p. 187.
2 M. Have, in W. I. Taylor and N. R. Farnsworth (Editors), *The Vinca Alkaloids*, Marcel Dekker, New York, 1973, p. 305.
3 L. Szporny, *Acta Pharmacol.*, 28 (1975) 87.
4 H. Kinsun and M. A. Moulin, *J. Chromatogr.*, 144 (1977) 123.
5 M. Gazdag, K. Mihalyfi and G. Szepesi, *Z. Anal. Chem.*, 309 (1981) 105.
6 P. Devaux, E. Godbille and R. Viennet, *Recent Dev. Mass Spectrom. Biochem. Med.*, 2 (1979) 191.
7 H. O. Hoppen, R. Heuer and G. Siedel, *Biomed. Mass Spectrom.*, 5 (1978) 133.
8 H. Iven and C. P. Siegers, *Arzneim.-Forsch.*, 27 (1977) 1248.
9 K. Ventouras, P. Schulz, E. Doelker, J. Boucherai and P. Brin, *Pharm. Acta Helv.*, 51 (1976) 334.
10 Z. V. Robakidze, V. Yu Vachnadze and K. S. Mudzhiri, *Izv. Akad. Nauk Gruz. SSR, Ser. Khim.*, 3 (1977) 223.
11 C. Dubruc, H. Caqueret and G. Bianchetti, *J. Chromatogr.*, 204 (1981) 335.
12 G. Szepesi and M. Gazdag, *J. Chromatogr.*, 204 (1981) 341.
13 P. Pietta, A. Rava and E. Catenacci, *J. Chromatogr.*, 210 (1981) 149.