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Note

Determination of 11-bromovincamine in human plasma by high-performance liquid chromatography

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11-Bromovincamine (Fig. 1) is a cerebrovascular agent chemically and pharmacologically related to vincamine, the major alkaloid of *Vinca minor* L. Apocynaceae. The metabolism of this drug has been previously studied [1]. In order to provide information necessary for the clinical development of the drug, a specific and reliable analytical method for the measurement of 11-bromovincamine in biological fluids is required. The related compound vincamine has been measured using spectrophotometry [2], fluorimetry [3], gas—liquid chromatography [4], gas chromatography utilising mass spectrometric detection [5] and more recently by high-performance liquid chromatography (HPLC) [6, 7].

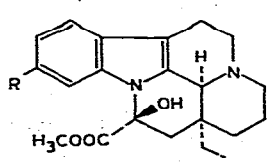

Fig. 1. Chemical structures of 11-bromovincamine (R = Br) and vincamine (R = H).

This paper describes an HPLC method for the measurement of 11-bromovincamine in human plasma. The method employs reversed-phase chromatography and utilises vincamine as an internal standard, achieving a sensitivity of 10 ng/ml from a 1-ml plasma sample. The method can also be used for the analysis of vincamine, in which case 11-bromovincamine could serve as an internal standard.

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EXPERIMENTAL

Materials

All reagents were of analytical grade and all inorganic reagents were prepared in freshly glass-distilled water. Acetonitrile was HPLC grade (Fisons Scientific Apparatus, Loughborough, Great Britain) and diethyl ether was freshly redistilled prior to use.

Standard solutions of 11-bromovincamine and vincamine were prepared at concentrations equivalent to 1 mg free base per ml and 10 μg free base per ml in acetonitrile and stored at 4°C, under which conditions they were stable for at least 1 month. Samples of 11-bromovincamine as the hydrogen fumarate salt and vincamine were supplied by Sandoz (Basle, Switzerland).

Extraction procedure

Plasma samples (1 ml) were transferred into conical centrifuge tubes (10 ml), spiked with internal standard (40 μl, containing 400 ng vincamine) and made alkaline by the addition of 0.5 ml of a 0.88 ammonia—water (1 : 10, v/v) mixture. The samples were extracted with diethyl ether (5 ml) for 10 min using a rotary mixer. The extracts were centrifuged at 2000 *g* for 10 min and the separated ether layer carefully transferred to another conical centrifuge tube. The ether was evaporated to dryness under nitrogen at 37°C and the walls of the tube rinsed with more ether to ensure that all the residue was at the bottom of the tube. The ether was again evaporated and the residue dissolved in mobile phase (50 μl). After centrifugation at 2000 *g* for 10 min, the clear solution was transferred to autosampling vials and injected into the chromatograph.

Calibration procedure

Samples of control (drug-free) plasma (1 ml) were spiked with 11-bromovincamine at concentrations equivalent to 20, 50, 150, 250, 350 and 500 ng free base per ml and with internal standard at a fixed concentration equivalent to 400 ng/ml. The samples were submitted to the extraction procedure described previously.

Instrumentation

The liquid chromatograph consisted of a Waters Model 6000A pump (Waters Assoc., Cheshire, Great Britain) fitted to an LC—UV variable-wavelength UV detector (Pye Unicam, Cambridge, Great Britain) operated at 232 nm. Injection was performed automatically using a WISP™ autosampler (Waters Assoc.). Chromatograms were recorded using a Trilab Model III computing integrator (Trivector Systems, Sandy, Great Britain) which automatically computed the peak area ratio measurements.

Chromatography

Chromatography was performed in a reversed-phase mode. The column was constructed of stainless steel (30 cm × 0.4 cm I.D.) and packed with μBondapak C_{18} (mean particle diameter 10 μm) (Waters Assoc.). A precolumn constructed of stainless steel (7 cm × 0.2 cm I.D.) and dry-packed with pellicular Co:Pell® ODS (particle diameter 25—37 μm) (Whatman, Maidstone, Great

Britain) was installed in series in front of the analytical column to protect it from contamination and was changed if the back pressure in the system increased beyond reasonable limits (e.g. ca. 280 bar). The mobile phase was composed of 35% (v/v) acetonitrile in aqueous sodium dihydrogen orthophosphate (0.1%, w/v) adjusted to pH 3.5 with phosphoric acid. A flow-rate of 2 ml/min was maintained.

Fig. 2 illustrates the separation of 11-bromovincamine from vincamine (internal standard) with retention times of 6.8 min and 3.6 min, respectively.

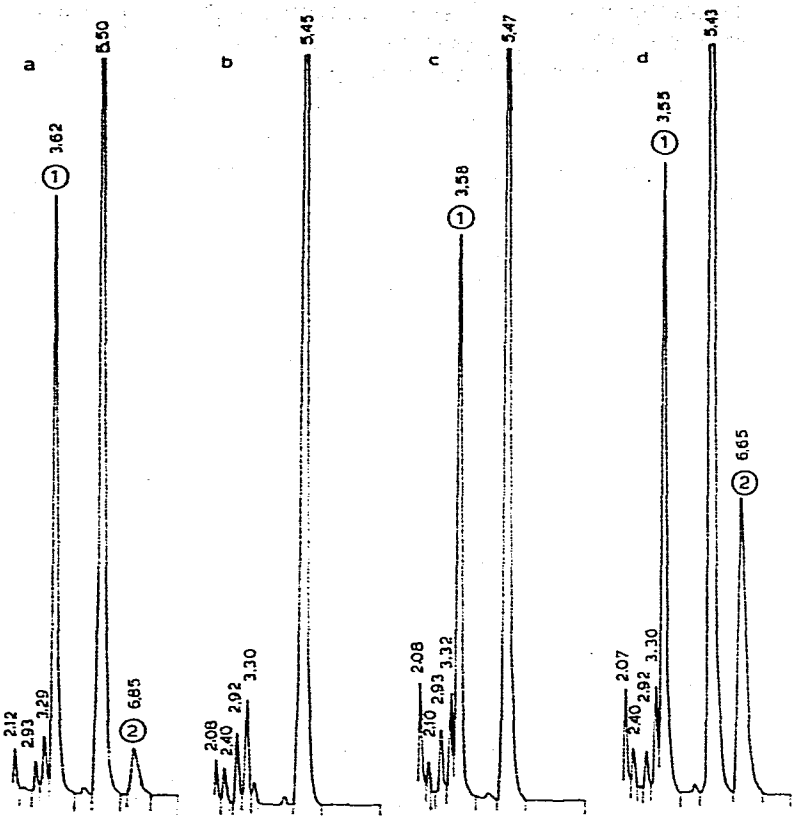

Fig. 2. Chromatograms of (a) extracted plasma standard containing (1) vincamine, internal standard and (2) 11-bromovincamine (50 ng/ml); (b) predose (blank) plasma; (c) predose (blank) plasma containing internal standard; and (d) postdose plasma containing 11-bromovincamine (268 ng/ml). Column: 30 cm × 4 mm I.D. containing μBondapak C_{18}; solvent system: 35% (v/v) acetonitrile—aqueous sodium dihydrogen orthophosphate (0.1%, w/v), pH 3.5; flow-rate, 2 ml/min; detector, UV at 232 nm; 1 V output to recording integrator.

Studies in humans

The method of analysis was applied to plasma samples from two healthy male volunteer subjects obtained during a study of the metabolism of 11-bromovincamine after each had received a single oral dose of 40 mg of 11-bromo[^{3}H]vincamine hydrogen fumarate salt in aqueous solution. The conditions of the volunteer studies were similar to those described by Mayo et al. [1].

RESULTS AND DISCUSSION

Precision of measurement

Extraction and measurement at each concentration over the calibration range was repeated on five occasions. The precision of the method for the measurement of 11-bromovincamine in plasma, as indicated by the coefficient of variation of peak area ratio measurements of drug to internal standard (Table I), were ± 12% at 20 ng/ml, ± 2% at 250 ng/ml and ± 1% at 500 ng/ml.

The coefficient of variation of peak area ratio measurements of a non-extracted mixture of 11-bromovincamine and internal standard analysed routinely was ± 1.4% throughout the analysis of all plasma samples assayed.

TABLE I

RECOVERY AND PRECISION MEASUREMENTS OF 11-BROMOVINCAMINE FROM PLASMA

Concentration of 11-bromovincamine added to plasma (ng/ml)	Recovery* (%)	Coefficient of variation (%)
20	94	12
50	96	5
150	92	4
250	94	2
350	96	1
500	97	1
Mean	95 ± 2 S.D.	

*Mean of 5 determinations at each concentration.

Accuracy

The calibration line for the measurement of 11-bromovincamine in plasma was constructed from five replicate measurements at each of six concentrations over the range, and the plot of peak area ratio against concentration was linear ($y = 0.0031x - 0.0090$, $r = 0.9997$), where y is the peak area ratio and x is the concentration of 11-bromovincamine (ng/ml). The accuracy of the method as defined by the 95% confidence limits of the least squares regression line, i.e., taking the calibration line as an estimate of the concentration of 11-bromovincamine in plasma, was ± 53%, ± 4% and ± 2% at 20 ng/ml, 250 ng/ml and 500 ng/ml, respectively.

Recovery and limit of detection

The recovery of internal standard (vincamine) from plasma at a concentration of 400 ng/ml was 98 ± 6% S.D. ($n = 5$). The mean recovery of 11-bromovincamine from plasma over the concentration range 20—500 ng/ml was determined by comparison of peak area ratio measurements of non-extracted standards to those of standards corrected for 100% recovery of internal standard and was 95 ± 2% S.D. (Table I).

No interfering peaks were present in predose (blank) plasma samples with the same retention time as 11-bromovincamine (Fig. 2b). The limit of detec-

TABLE II

CONCENTRATIONS OF 11-BROMOVINCAMINE (ng/ml) IN THE PLASMA OF TWO HUMAN SUBJECTS AFTER A SINGLE ORAL DOSE OF 40 mg OF DRUG

Time (h)	Subject No.		Mean
	1	2	
0.25	268	503	386
0.50	385	551	468
0.75	460	771	616
1.0	495	601	548
1.5	395	452	424
2.0	311	298	305
3.0	139	235	187
4.0	90	235	163
5.0	71	210	141
6.0	62	186	124
8.0	40	127	84
10.0	26	88	57
12.0	24	62	43
16.0	17	41	29
24.0	<10	15	<10

tion under the experimental conditions used with a 1-ml plasma sample was 10 ng/ml, set by the sensitivity of the computing integrator.

Concentrations of 11-bromovincamine in plasma

After single oral doses of 40 mg of 11-bromovincamine administered as an aqueous solution to two human subjects, the mean peak drug concentration was 616 ng/ml and occurred at 0.75 h after dosing (Table II). Mean concentrations of 11-bromovincamine declined apparently biphasically with half-lives of 1.3 and 4.6 h, respectively.

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