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Note

Quantitative determination of vinpocetine in human plasma by capillary gas chromatography—mass spectrometry

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Vinpocetine, [3α,16α]-eburnamenine-14-carboxylic acid ethyl ester (Fig. 1), is marketed in Hungary, Japan and several other countries for the treatment of cerebral diseases originating from vascular or cerebral metabolic disturbances [1–7]. Pharmacokinetic investigations revealed very low plasma concentrations [8,9] and we suspected that the elimination half-life of about 4 h had been underestimated, because the detection limit of the assay of 1 ng/ml (gas chromatography with nitrogen flame ionisation detection [10]) may be not sensitive enough, especially for studies with oral dosing [11].

Another method using direct mass fragmentographic analysis [12] proved to be non-specific [13]. Therefore, a method has been developed using fused-silica capillary gas chromatography with direct coupling to a mass spectrometer, which resulted in a detection limit of 0.1 ng/ml.

EXPERIMENTAL

Chemicals, reagents and glassware

Vinpocetine and the internal standard, apovincaminic acid methyl ester, were supplied by Gedeon Richter (Budapest, Hungary). *n*-Hexane and methanol (both LiChrosolv from Merck, Darmstadt, F.R.G.) were used without further purification.

The glassware was treated with washing-up liquid, 0.1 mol/l hydrochlorid acid, 0.1 mol/l sodium carbonate solution, doubly distilled water, *n*-hexane and three times with methanol.

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Instrumentation

The analyses were performed on a modified Hewlett-Packard 5995A gas chromatographic—mass spectrometric (GC—MS) system consisting of a quadrupole spectrometer, an HP 5710 gas chromatograph and an HP 9825T data system.

The mass spectrometer was operated in the selected-ion monitoring (SIM) mode with an electron ionization voltage of 70 eV. The source pressure was 10^{-5} Torr. The temperatures of the GC—MS transfer line, analyser and ion source were 270, 210 and 210°C, respectively. The dwell time of the selected ions was 100 ms.

A split/splitless injector (270°C) from Gerstel Labormechanik (Mülheim/Ruhr, F.R.G.) was used in the splitless mode. An ULTRA 1 (OV-1) fused-silica chemically bonded capillary column (12.5 m×0.2 mm I.D.) from Hewlett-Packard (Palo Alto, CA, U.S.A.) was fixed through the transfer line about 0.2 cm from the ion source block. This modification of the interface was constructed by Gerstel Labormechanik.

A volume of 2 μl was injected at a column flow-rate (helium) of 0.8 ml/min and an oven temperature of 150°C. The oven temperature was further programmed at 16°C/min to 270°C, where it was held for 4.5 min.

Assay

In a centrifuge tube 1 ml of plasma and 10 ng of internal standard in 40 μl methanol were shaken twice with 3 ml of *n*-hexane for 5 min at 110 rpm (Labshaker; Braun, Melsungen, F.R.G.) and then centrifuged for 6 min at 2800 *g*. The organic phase was transferred into a 5-ml conical vial and evaporated to dryness under nitrogen. The residue was dissolved in 20 μl of methanol and 2 μl were injected into the GC—MS system. The recoveries of both vinpocetine and the internal standard were 95%.

Fig. 1 shows the mass fragmentograms of (A) vinpocetine and (B) the internal standard. In both instances the base peak M − 29 was chosen for SIM (*m/e* 307.1 and 321.1). The peak-area ratios of vinpocetine to the internal standard were used for further calculations.

Quality-control samples of 0.1 ng/ml were included to check the daily performance. Plasma samples spiked with 50 ng/ml vinpocetine were stored at −20°C for up to eight months without any sign of degradation. As vinpocetine is slightly light-sensitive, the samples and extracts were protected from direct light.

RESULTS AND DISCUSSION

Fig. 2 shows the SIM chromatograms for plasma samples containing 0.1 and 2 ng/ml vinpocetine and 10 ng/ml internal standard. No interfering peaks were observed in blank plasma.

Four calibration runs were carried out in duplicate on four successive days. Linear regression analysis yielded the equation $y = 0.00151\ (\pm 0.0006) + 0.067\ (\pm 0.002)\ x$. The correlation coefficient was 0.9999. As the intercept is almost zero, one concentration is sufficient for calibration. Therefore, plasma samples spiked with 10 ng/ml vinpocetine were used for daily calibration. From the results

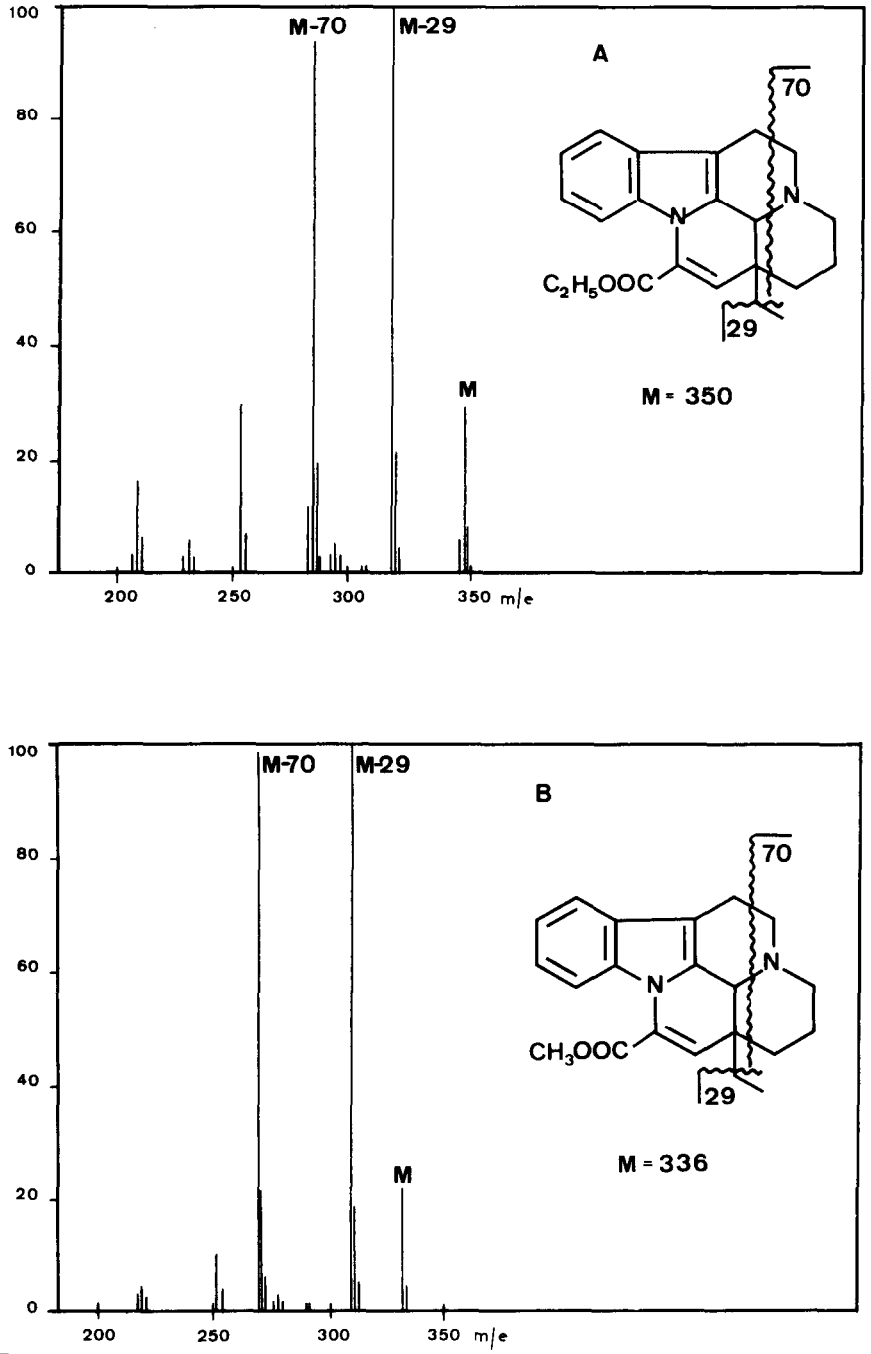

Fig. 1. Mass spectra of (A) vinpocetine and (B) internal standard.

in Tables I and II we defined 0.1 ng/ml as a practical limit of detection.

We modified the extraction procedure and the detection method used in the original method for assaying vinpocetine in biological samples [10]. Polgár and

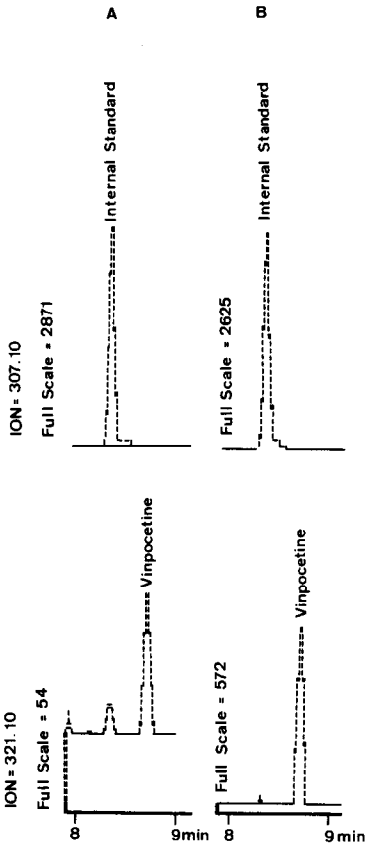

Fig. 2. SIM chromatograms of a plasma sample containing (A) 0.1 ng/ml and (B) 2 ng/ml vinpocetine; 10 ng/ml internal standard was added to each sample before extraction.

Vereczkey [10] alkalinized their samples and extracted them with diethyl ether, but when we tried to reproduce their method we found that the extracts were not clean enough for our purposes. Eliminating the alkalinization step and extracting

TABLE I

BETWEEN-DAY VARIATION ($n=4$)

Theoretical vinpocetine concentration (ng/ml)	Vinpocetine found (ng/ml)	Coefficient of variation (%)
0.1	0.12	10.12
0.2	0.22	9.15
0.5	0.54	4.27
1.0	1.06	5.36
5.0	5.11	2.09
30.0	28.26	0.31
50.0	46.76	1.97

TABLE II

WITHIN-DAY VARIATION ($n=8$)

Theoretical vinpocetine concentration (ng/ml)	Vinpocetine found (ng/ml)	Coefficient of variation (%)
0.1	0.11	9.41
0.5	0.50	6.21
1.0	1.00	2.30
2.0	1.97	1.90
5.0	5.02	2.08
10.0	9.99	1.78
20.0	20.00	3.66

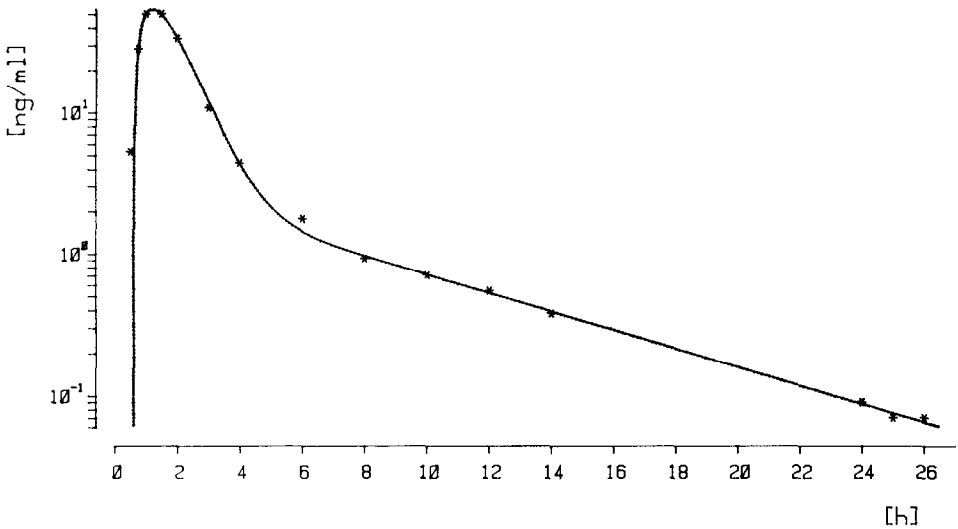

Fig. 3. Time course of plasma concentrations in a human subject receiving 80 mg of vinpocetine orally.

the samples directly with *n*-hexane yielded much cleaner extracts without affecting the recovery. We were then able to confirm the limit of sensitivity to be 1 ng/ml when a nitrogen flame ionisation detector was used. The GC—MS method described here now provides an even better sensitivity of 0.1 ng/ml.

During a human tolerance study with single increasing doses of 40–360 mg of vinpocetine, we found an average elimination half-life of 9 h. Fig. 3 shows an example of the time course of vinpocetine plasma concentrations after oral dosing of 80 mg to a healthy volunteer. It can be seen that the terminal elimination phase begins at about 6 h and that all of the subsequent concentrations are below 1 ng/ml and would not have been detected using the GC method with nitrogen flame ionisation detection.

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