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Short communication

Improved gas chromatographic–mass spectrometric method for the quantitative determination of vinpocetine in human plasma

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Abstract

An improved and validated method for the determination of vinpocetine in human plasma using a combination of a solid-phase extraction as a cleaning step followed by gas chromatography–mass spectrometry (GC–MS) has been presented. Quantitation has been carried out with apovincaminic acid methyl ester as internal standard. A limit of detection of 0.01 ng/ml, limit of quantitation of 0.10 ng/ml, as well as a satisfactory accuracy, improved precision and prolonged capillary column life have been achieved. © 1997 Elsevier Science B.V.

Keywords: Vinpocetine

1. Introduction

The trace determination of drugs in plasma presents a challenging analytical problem. In particular, the quantitation of vinpocetine in plasma has been proved difficult [1–10].

Vinpocetine, [3α,16α]-eburnamenine-14-carboxilic acid ethyl ester (Fig. 1a) is used in the therapy of different cerebrovascular disorders [11,12]. The drug improves the cerebral utilization of oxygen and protects the brain cells against ischaemic anoxia [13]. It dilates the cerebral blood vessels and increases the cerebral blood flow [14,15]. The pharmacokinetic investigations of vinpocetine have been conducted by direct mass fragmentography [1,2], gas chromatography (GC) with nitrogen–phosphorus detection [3–7] and GC–mass spectrometry (MS) methods [8–10]. Czira at al. [2], Polgár and Vereczkey [3] and Miskolczi and coworkers [6,7] used alkalization of the plasma samples before liquid extraction with diethyl ether. Hammes and Weyhenmeyer [9] and Lohmann and Dingler [10] described a GC–MS method for the quantitative determination of vinpocetine in human plasma eliminating the alkalization step. They extracted vinpocetine directly with *n*-hexane. We discovered that these extracts are not pure enough for GC–MS analysis. For example, the *n*-hexane extracts contain large amounts of cholesterol that contaminates the capillary column and changes its efficiency. The lowest reported limit of quantitation is 0.25 ng/ml plasma [8–10] and no recent review has described its improvement.

To establish a better procedure for sample cleanup and obtain a more reliable determination of vinpocetine in human plasma we used a solid-phase extraction (SPE) technique with an RP-8 cartridge.

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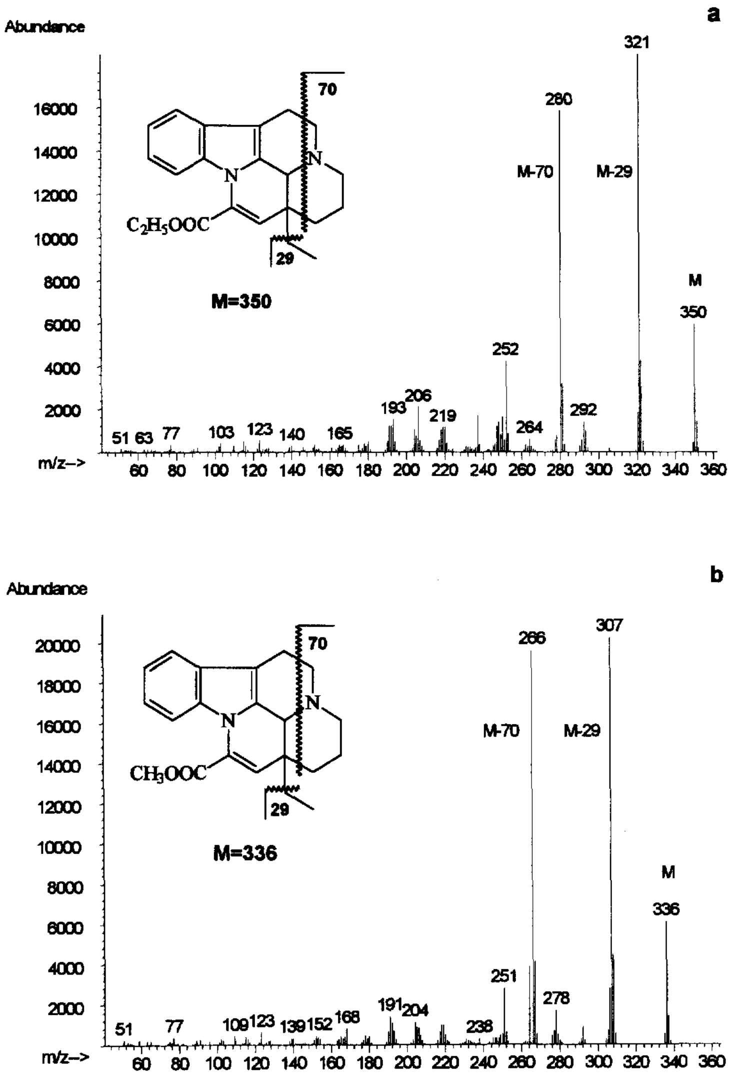

Fig. 1. Mass spectra and structures of vinpocetine (a) and apovincamine (b).

2. Experimental

2.1. Equipment

The GC–MS equipment consists of Hewlett-Packard 5890 SERIES II Plus GC and 5972 MSD (Hewlett-Packard, Palo Alto, CA, USA) and an autosampler HP 7673 with controller. The column was a HP Ultra 1 fused-silica (25 m×0.2 mm I.D.) with cross-linked methyl silicone gum stationary phase with a film thickness 0.33 μm, fixed through the transfer line about 2 mm from the ion source block. The transfer line temperature was 280°C; ionization voltage was 70 eV; vacuum $1.47 \cdot 10^{-3}$ Pa. A split–splitless injector was used at 270°C with split opening time 1.0 min after the injection. The carrier gas was helium at a flow-rate of 1.0 ml/min. An HP G 1034C MS ChemStation Software has been used. The column temperature program began at 150°C, increased at 30°C/min to 250°C, then increased at 7°C/min to the final temperature of 280°C, which was held for 2.4 min. A stabilization step at 300°C for 3.1 min was included. The injection frequency in the automatic process was 16 min 25 s.

Apovincaminic acid methyl ester was used as internal standard (I.S.). The selected-ion monitoring (SIM) mode for identification and quantitation has been applied on the following conditions: group 1 for I.S.: m/z 307.10 and 307.20, (Fig. 1b); group 2 for vinpocetine: m/z 321.10 and 321.20, (Fig. 1a), both at dwell time 70 ms, EMV delta 800 V. User tune at masses: 219; 264; 414 was used for daily calibration of the MSD.

2.2. Chemicals and reagents

Vinpocetine was prepared in NIHFI (Sofia, Bulgaria), apovincaminic acid methyl ester was obtained from Covex (Madrid, Spain), human drug free plasma was from the National Center for Blood Transfusion. The solvents used were: 2-propanol for chromatography, chloroform for chromatography, methanol and *n*-hexane (both analytical-reagent grade) obtained from Merck (Darmstadt, Germany) and toluene, scintillation grade from Koch-Light (Haverhill, Suffolk, UK). The SPE was carried out with "Backer-10 SPE" cartridges, 500 mg, 6 ml reversed-phase (RP) octylsilane (C_8) Bonded Silicagel from J.T. Baker (Phillipsburg, NJ, USA). All solutions were prepared with 2-propanol, as Lohmann and Dingler [10] report that the dissolution of vinpocetine in methanol results in the transesterification to apovincaminic acid methyl ester. Both stock solutions of vinpocetine and I.S. were made at a concentration of 50 μg/ml in 2-propanol, stored at 4°C and protected from direct light. The I.S. working solution was prepared by a 500-fold dilution of the stock solution to give a final concentration of 100 ng/ml.

2.3. Analytical procedure

2.3.1. Preparation of the calibration samples

Solutions for the preparation of calibration samples were made at the following concentrations of vinpocetine: 10, 25, 30, 50, 100, 200, 400, 500 and 600 ng/ml in 2-propanol. 1.0 ml of each solution was diluted with blank plasma to 100 ml. The final concentrations of the calibration samples were: 0.10, 0.25, 0.50, 1.00, 2.00, 4.00 and 6.00 ng/ml plasma. All plasma samples were stored at −20°C in plastic tubes.

2.3.2. Sample preparation

A 2 ml volume of plasma and 50 μl working solution of I.S. (2.5 ng/ml plasma) were mixed at 100 rpm in a test-tube for 30 s and diluted to 6 ml with bidistilled water. The SPE-RP cartridge was conditioned with 4 ml methanol and 6 ml bidistilled water. The sample was aspirated through a SPE-RP cartridge. The cartridge was washed with two volumes bidistilled water and 1 ml mixture of bidistilled water–2-propanol (2:1, v/v). After vacuum drying of the cartridge vinpocetine and I.S. were eluted with 2×2 ml 2-propanol and the total eluate was evaporated to dryness at 45°C by a vacuum evaporator. The dry residue was dissolved in 300 μl chloroform, transferred into a microvial and evaporated to dryness at room temperature. The residue was dissolved in 50 μl toluene and 2 μl of it were injected into the GC–MS system.

2.3.3. Washing of SPE-RP cartridges and glassware

After the elution the extraction columns were regenerated by rinsing with chloroform, methanol and bidistilled water. The glassware was washed with a detergent solution, soaked in a bichromic mixture, rinsed with bidistilled water, soaked in 0.02 *M* NaOH and finally rinsed with bidistilled water.

3. Results

The method was validated in terms of selectivity, sensitivity, linearity, accuracy, precision, analytical recovery and stability.

3.1. Selectivity

The SIM chromatograms for blank plasma and calibration plasma samples contained 0.10 and 2.00 ng/ml vinpocetine and 2.5 ng/ml I.S. are presented in Fig. 2. No interfering peaks have been observed in blank plasma.

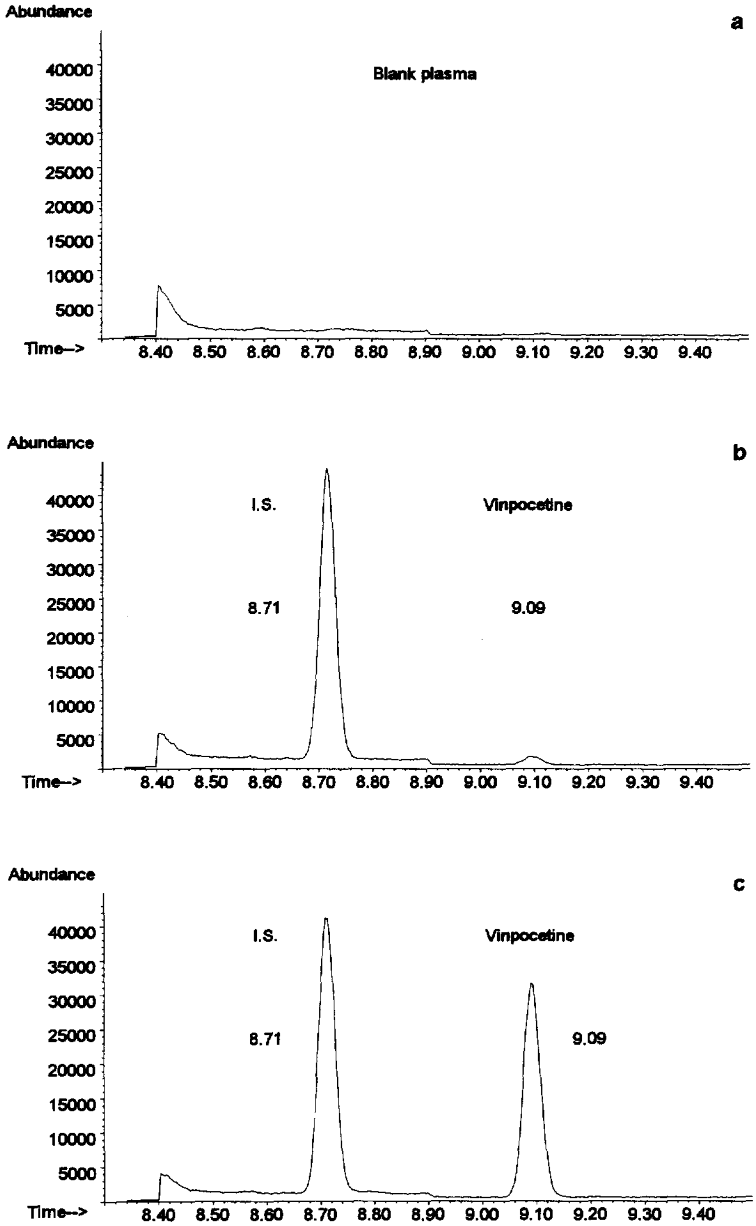

Fig. 2. SIM chromatograms for blank plasma (a) and plasma samples containing 0.10 (b) and 2.00 (c) ng/ml vinpocetine and 2.5 ng/ml I.S.

3.2. Sensitivity

The detection limit, defined as 3-times the baseline noise for the plasma sample examined was 0.01 ng/ml human plasma. The limit of quantitation, defined as the lowest concentration assessed with acceptable accuracy and precision (below 15%), was 0.10 ng/ml plasma.

Table 1
Intra-day variation ($n=6$)

Concentration of vinpocetine added (ng/ml)	Concentration of vinpocetine found (ng/ml)	Coefficient of variation (%)	Accuracy (%)
0.10	0.105	5.13	5.00
0.25	0.244	7.55	−2.40
0.50	0.519	4.58	3.80
1.00	0.998	3.10	−1.20
2.00	1.950	3.36	−2.50
4.00	4.037	1.67	0.93
6.00	5.986	0.72	−0.23

3.3. Precision and accuracy

Seven concentration levels, each replicated six times have been measured for the evaluation of intra-day precision and accuracy (Table 1).

The replicates at each level for the calibration range from 0.10 to 6.00 ng/ml plasma have been measured daily. Sixteen calibration curves were constructed on sixteen consecutive days. Table 2 presents inter-day precision and accuracy ($n=32$).

3.4. Linearity and curve fitting

The calibration curve was studied over the concentration range from 0.10 to 6.00 ng/ml plasma and shown to be linear. In general, the r^2 values obtained were at a range of 0.999. For a better fitting a quadratic regression has been chosen: $y=ax^2+bx+c$, ($a{\sim}10^{-3}\div10^{-4}$), where y is the response ratio and x=(peak area of vinpocetine)/(peak area of I.S.). The equation obtained from seven calibration levels ($n=6$) was:Response ratio $= 6.97\cdot10^{-3}x^2 + 1.03x - 7.62\cdot10^{-3}$

3.5. Analytical recovery

The analytical recovery was determined at all concentration levels (each with six repetitions) as the ratio of the peak area of vinpocetine obtained from the calibration plasma sample to the peak area of vinpocetine obtained from the calibration solution at the same concentration. The recovery for the internal standard was calculated in the same way. The values of the absolute mean analytical recovery were 71% and 60% for vinpocetine and I.S., respectively.

Table 2
Inter-day variation ($n=32$)

Concentration of vinpocetine added (ng/ml)	Concentration of vinpocetine found (ng/ml)	Coefficient of variation (%)	Accuracy (%)
0.10	0.094	6.90	−6.00
0.25	0.237	7.44	−5.12
0.50	0.551	3.19	10.20
1.00	1.003	3.11	0.30
2.00	1.920	5.80	−3.89
4.00	4.054	4.85	1.35
6.00	5.980	1.59	−0.33

3.6. Stability

Under storage conditions for more than three months neither the plasma calibration samples nor the stock solutions showed any degradation.

4. Conclusions

A suitable GC–MS method was set up to guarantee a reliable determination of vinpocetine in human plasma. The extracts obtained by our extraction procedure have been considerably cleaner compared to the ones described in the literature [1–10]. The most important advantages are: (1) limit of detection 0.01 ng/ml plasma; (2) limit of quantitation 0.10 ng/ml plasma; (3) improvement in the method precision and accuracy; (4) sparing of the capillary column and MS detector; (5) clean extracts of large volumes of plasma samples (2 ml and more) providing a reliable determination of low vinpocetine concentrations.

Time consuming purification procedures have been avoided because of the high degree of purification of the plasma samples using SPE-RP extraction. The results from the validation proved that the method described is suitable for pharmacokinetic studies.

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