Journal of Chromatography, 575 (1992) 289–294
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6228

Short Communication

Identification and quantification of ergotamine in human plasma by gas chromatography–mass spectrometry

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(First received August 19th, 1991; revised manuscript received November 25th, 1991)

ABSTRACT

A highly sensitive and simple gas chromatographic–mass spectrometric method is described for the identification and quantification of ergotamine in plasma or serum. Ergotamine is extracted with chloroform from the alkalinized sample and detected by electron ionization mass spectrometry. This analytical method was selected for an intense high-mass ion ideal for the specific quantification. It shows good linearity in the range from 50 pg/ml to 50 ng/ml for ergotamine in plasma. The practicability of this method is demonstrated by determining the plasma concentration of ergotamine in a sample from a patient.

INTRODUCTION

Ergotamine is one of the ergot alkaloids that have been widely used in the preventive and curative treatment of migraine and migraine variant headaches [1]. The therapeutic doses of ergotamine in general range from 0.5 to 1.5 mg by parenteral administration [2] and from 2 to 5.0 mg by oral route [3]. Ergotamine is rapidly metabolized in the liver and excreted mainly in the bile, and its bioavailability is very low. Hence, the plasma concentration of the parent drug after a single oral dose is of the order of pg/ml or ng/ml [5]. Therefore, an analytical method for the determination of ergotamine needs both high sensitivity and good separation from any coextracted endogenous compounds.

Several analytical methods have been developed to determine ergotamine in plasma. The first method described was radioimmunoassay (RIA) [4], but because it uses lysergic acid antisera for determination of ergopeptides it is not specific or sensitive enough for pharmacokinetic investigations. Edlund [5] reported the development of a high-performance liquid chromatographic (HPLC) method for ergotamine. This method exhibited good sensitivity (100 pg/ml from 3.0 ml of plasma) for the pharmacokinetic studies. But the HPLC technique cannot unambiguously identify an unknown compound in a patient's sample. For toxicological purposes, gas chromatography–mass spectrometry (GC–MS) is preferable. De Zeeuw *et al.* [6] described a GC–MS method for the detection of ergotamine in blood after acid hydrolysis. It enables identification of ergotamine and some of its degradation products.

The purpose of our study was to develop a

0378-4347/92/$05.00

highly sensitive, fast and selective GC–MS method capable of both the identification and the reproducible quantification of ergotamine in plasma or serum.

EXPERIMENTAL

Chemicals and reagents

Ergotamine tartrate was obtained from Sandoz Pharma (Basel, Switzerland). Flunitrazepam used as an internal standard was obtained from Hoffman La Roche (Basel, Switzerland). Ammonia (25%, v/v), chloroform and ethanol were purchased from Merck (Darmstadt, Germany). All solvents were of spectroscopically pure grade and used without further purification.

Gas chromatography–mass spectrometry

GC–MS data were recorded with a Saturn mass spectrometer (Varian Assoc., Sunnyvale, CA, USA) interfaced with a Varian 3400 gas chromatograph fitted with a 1077 split–splitless injector and 1094 septum-equipped temperature-programmable injector (SPI) operated in the SPI mode. A Varian fused-silica capillary column (30 m × 0.25 mm I.D.), with cross-linked 5% phenyl/95% methyl silicone (film thickness 0.25 μm) was coupled to the mass spectrometer ion trap. The head pressure of carrier gas (helium) was 1.034 bar. The operating conditions were: transfer line temperature, 280°C; injector temperature, programmed from 120 to 240°C at 80°C/min, maintained at 240°C for 2.5 min; column oven temperature, programmed from 60 to 280°C at 35°C/min with a final hold for 10 min; emission current, 10 μA; Fil/Mul delay, 200 s; scanning mass range, m/z 25–330. Data handling and chromatogram plotting were carried out by means of a Compaq 386/20e personal computer and an Epson FX-850 printer.

Preparation of the standard solution

Ergotamine solutions were freshly prepared every week because of its instability. Standard stock solutions were prepared in concentrations of 0.1 and 2.5 μg/ml in ethanol. Blank plasma samples were spiked with appropriate amounts of ethanolic ergotamine solution for standard curves.

Extraction procedure of samples

Plasma samples collected from a patient were stored at −20°C until used. The internal standard (1 μl of 10 μg/ml flunitrazepam in ethanol) was mixed with 3.0 ml of plasma, and 2.0 ml of 25% ammonia and 8.0 ml of chloroform were added to this solution. The sample was mixed on a shaker for 10 min and then centrifuged at 900 *g* for 8 min. The supernatant was discarded and the chloroform extract was evaporated to dryness with a PVK 650 rotating vacuum evaporator (Dogrel, St. Margrethen, Switzerland), then the residue was reconstituted with 100 μl of ethanol. An aliquot of 1–10 μl was injected into the gas chromatograph.

RESULTS AND DISCUSSION

Extraction

Ergotamine is a hydrophobic compound of high relative molecular mass (582). In order to avoid coextraction of endogenous compounds, it is desirable to use a solvent with a polarity as low as possible for its extraction from the biological matrix. Haering *et al.* [3] used hexane–1-butanol (6:1) for the first extraction step. After back-extraction into sulphuric acid the organic phase was discarded. Dichloromethane was used for reextraction after alkalinization of the samples with 1.0 *M* ammonium chloride buffer. Edlund [5] used cyclohexane–1-butanol (9:1) to perform several extraction steps for HPLC determination. These extraction procedures are time-consuming because of the multiple steps required and the low volatility of the organic solvents.

The use of chloroform as an extraction solvent is preferable in our experience because ergotamine tartrate becomes free ergotamine base after alkalinization, and this form is freely soluble in chloroform. Our simple extraction step results in clean chromatograms. Fig. 1 shows that no endogenous substances interfere with the peaks of ergotamine and the internal standard. The recovery experiments for the extraction of ergota-

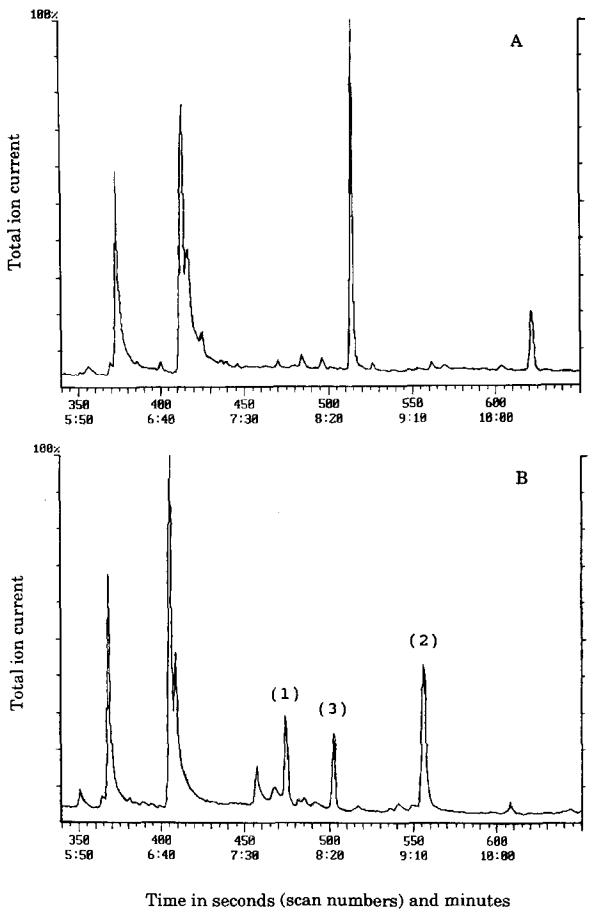

Fig. 1. Typical chromatograms of (A) a blank plasma extract and (B) a spiked plasma sample extract. Peaks: 1 = ergotamine (20 ng); 2 = internal standard (20 ng); 3 = phthalate from septum.

mine from plasma were done as follows. The standard sample was prepared by adding ergotamine to a blank plasma, then performing the extraction as usual. The internal standard in ethanol was added at the end of evaporation. As a reference, a blank plasma sample was extracted without any addition. After evaporation of the chloroform extract, ergotamine and the internal standard in ethanol were added to the residue, and the sample was dried again. The residues of both the standard sample and the reference sample were finally diluted with the same volume (100 μl) of ethanol and then injected. The recovery rate was calculated by comparing the peak-area ratio of ergotamine to the internal standard in the standard sample with that in the reference sample. The recoveries were 94.8 ± 1.5% (n = 6) at 0.10 μg/ml and 92.8 ± 6.2% (n = 3) at 0.10 ng/ml.

Chromatograms and mass spectra of plasma extract

Ergotamine is prone to thermal decomposition during gas chromatography [7]. We also found at least two peaks for a pure standard using a high injector temperature (260°C) and a high column temperature (240°C). The chromatographic conditions, especially optimal temperature programming of the injector and the column, were crucial in order to obtain a sharp and reproducible peak for ergotamine with a constant electron-impact ionization (EI) mass spectrum. This peak probably corresponds to the cyclic peptide part of ergotamine only and the lysergic acid moiety is not recovered at all. The total-ion current chromatogram of ergotamine and the internal standard extracted with chloroform from a spiked plasma is displayed in Fig. 1B. The retention times of ergotamine and the internal standard were 7.56 and 9.20 min, respectively. As shown in Fig. 1, ergotamine and the internal standard are well separated from endogenous compounds.

The probable ionization scheme for ergotamine is given in Fig. 2, and the mass spectra of ergotamine and the internal standard are depicted in Fig. 3. No molecular ion M (m/z 582) for ergotamine was found with the EI mode. Major fragment ions of m/z 244, 153, 125, 91, 70 and 43 were found, as reported previously [2]. In addition, the major fragment ion of m/z 314 was observed in directly injected standards as well as in extracts from spiked plasma. It probably corresponds to the cyclic peptide of ergotamine (Fig. 2). The identity of this ion peak with ergotamine in plasma extracts was confirmed by the following features: (1) identical retention time; (2) identical EI spectrum with the standard; (3) no interference from blank plasma extract.

Calibration curve

The ions m/z 314 and 315 were selected for

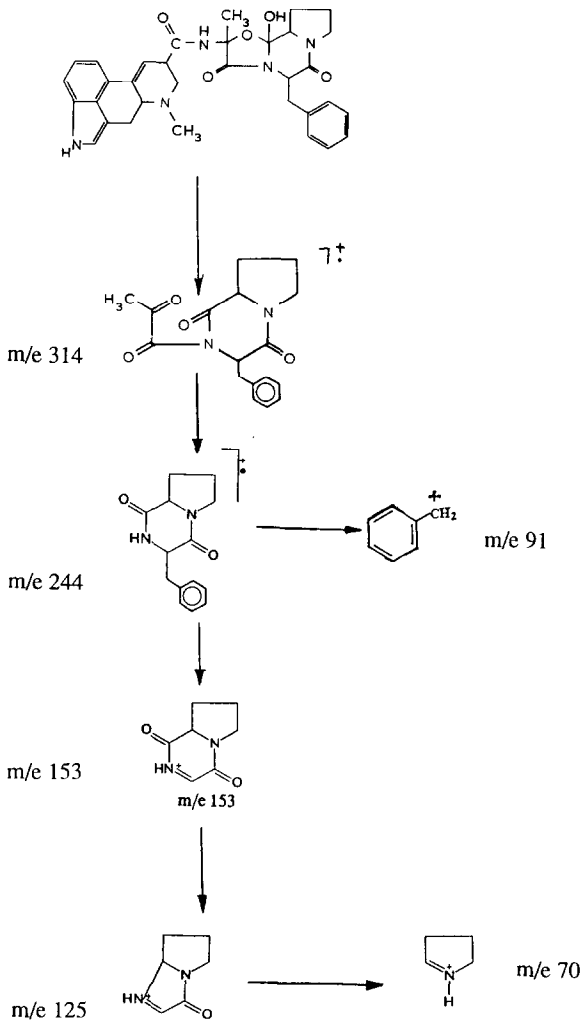

Fig. 2. Probable ionization scheme for ergotamine in the EI mode.

quantification because they were constant major fragment ions and had strong intensities in the mass spectrum of ergotamine. Although flunitrazepam is not chemically related to ergotamine, it was chosen as the internal standard because it has the same major fragments, an appropriate retention time and the same recovery rate during extraction from plasma as ergotamine. Compared with literature data [2] and our own recording on a sector field instrument, the EI spectrum of flunitrazepam by the ion-trap instrument shows a shift of one mass unit. This may be due to protonation in the ionization chamber of ion-trap detector.

Fig. 4 depicts the selected-ion recordings of extracts from a spiked plasma (A) and from a patient's serum (B). The calibration curve is linear over a range from 50 pg/ml to 2.0 ng/ml ergotamine. The equation is $y = 0.0845 + 1.110x$ ($r = 0.9985$, $n = 7$). The linearity extends to 50 ng/ml if an appropriate amount of internal standard (100 ng/ml) is added (data not shown).

Reproducibility and accuracy of the analytical method

The precision and accuracy of this analytical method were studied by measuring spiked plasma samples containing between 0.10 and 2.00 ng/ml and between 5 and 50 ng/ml. Two different amounts of the internal standard were used: 10 ng/ml for the range 0.1–2.0 ng/ml and 100 ng/ml for the range 5–50 ng/ml (Tables I and II). The day-to-day coefficient of variation (C.V.) was 4–10% and the within-run C.V. was 2.3–5.2%. The accuracy ranged from 98 to 104% of the added amount.

These results show that the method is highly reproducible and accurate despite the fact that only a decomposition product of ergotamine is assayed. The described method was applied to a sample drawn from a patient taking "over-the-counter" drugs containing ergotamine in unknown dose for migraine: 213 pg/ml in serum was determined.

CONCLUSION

The GC–MS method described in this paper has several advantages over previously published techniques. Identification of ergotamine from the EI spectrum and its quantification by using two intense high-mass ions are performed in the same run. Sample preparation was simplified to a one-step procedure using a single solvent. The method is sufficiently sensitive for the determination of ergotamine in samples from patients.

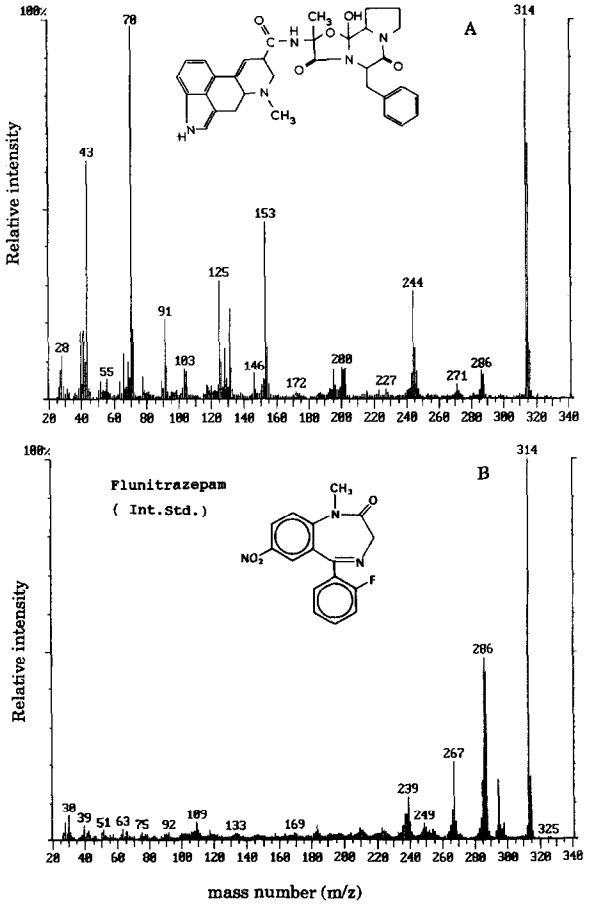

Fig. 3. Typical mass spectra of (A) ergotamine and (B) the internal standard, from a plasma extract, obtained with an ion-trap instrument.

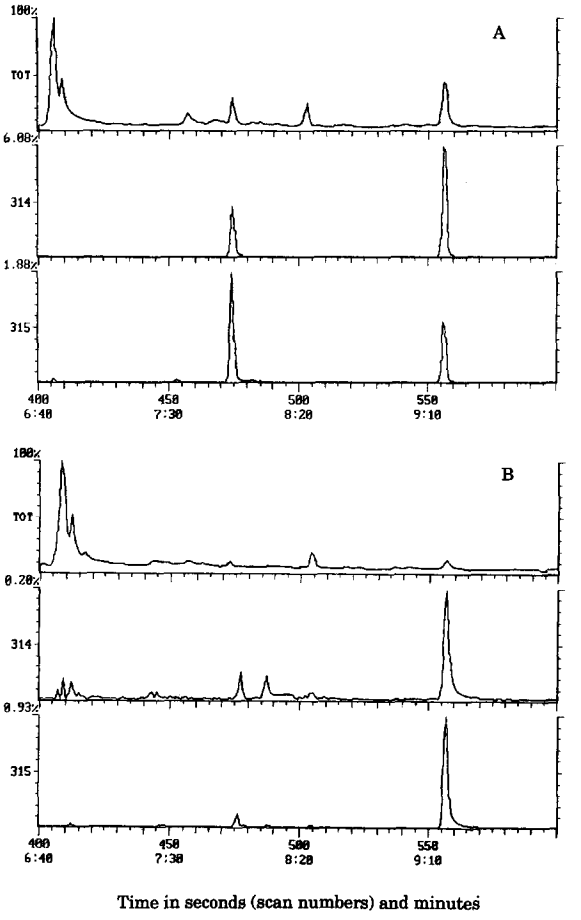

Fig. 4. Total-ion chromatograms and high-resolution selected-ion recordings of ergotamine and the internal standard in extracts of (A) a spiked plasma and (B) a patient's serum.

TABLE I

ACCURACY AND DAY-TO-DAY PRECISION FOR THE DETERMINATION OF ERGOTAMINE IN HUMAN PLASMA

Concentration added (ng/ml)	Concentration found (mean ± S.D., $n=4$) (ng/ml)	Accuracy (%)	C.V. (%)
0.100	0.098 ± 0.01	98.0	10
0.250	0.252 ± 0.01	101	3.9
0.500	0.519 ± 0.05	104	10
1.50	1.49 ± 0.09	99.0	6.1
2.00	1.99 ± 0.09	99.5	4.5

TABLE II

ACCURACY AND WITHIN-RUN PRECISION FOR THE DETERMINATION OF ERGOTAMINE IN HUMAN PLASMA

Concentration added (ng/ml)	Concentration found (mean ± S.D., $n=4$) (ng/ml)	Accuracy (%)	C.V. (%)
5.00	5.16 ± 0.21	103	4.0
10.0	9.81 ± 0.51	98.1	5.2
25.0	25.3 ± 0.59	101	2.3
50.0	50.4 ± 1.4	101	2.7

ACKNOWLEDGEMENT

The authors thank Sandoz Pharma AG (Basel, Switzerland) for supplying ergotamine tartrate standard.

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