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Journal of Chromatography B, 674 (1995) 293–297

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

Determination of schizandrin in human plasma by gas chromatography–mass spectrometry

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First received 14 March 1995; revised manuscript received 4 July 1995; accepted 4 July 1995

Abstract

Schizandrin (SZ) is one of the lignan components from Schisandra fruits. A highly sensitive and precise method for the determination of SZ in human plasma was developed involving selected-ion monitoring with gas chromatography–mass spectrometry using a fused-silica capillary column. A 0.1-ml plasma sample was used for solid-phase extraction. A good linear relationship was obtained in the concentration range studied (2.0–500 ng/ml) and the method was sufficiently accurate and precise to support clinical pharmacokinetic studies. After oral administration of SZ at a dose of 15 mg to healthy male subjects, the average value of the maximum plasma concentration of SZ was 96.1 ± 14.1 ng/ml. The plasma concentration of this substance could be monitored for 8 h after administration.

1. Introduction

The fruits of *Schisandra chinensis* BAILL. (Shizandraceae) are used as antitussives and as tonics under the names "Hoku-(or Kita-)-gomishi" in Japan and "Wu-wei-zi" in China, and are prescribed in some Kampo medicines such as "Sho-seiryu-to". SZ is one of the lignan components isolated from the unhydrolysed fraction of seed oil of this plant [1–3], and has been reported to ameliorate scopolamine-induced amnesia in the spatial cognitive and passive avoidance task in male Wistar rats [4]. This effect of SZ is related to the activation of the cholinergic system [5,6]. Hence SZ is an important compound to be considered when studying the pharmacological effects of Kampo medicines.

To investigate the pharmacokinetics of SZ, a highly sensitive and precise method for the determination of SZ in human plasma was developed, involving selected ion monitoring (SIM) with gas chromatography–mass spectrometry (GC–MS) using a fused-silica capillary column.

2. Experimental

2.1. Materials

SZ was obtained by the isolation procedure reported by Ikeya et al. [3] and 14-deuteromethoxyschizandrin (SZ-d_3) was synthesized in our laboratory for use as an internal

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0378-4347/95/$09.50
SSDI 0378-4347(95)00298-7

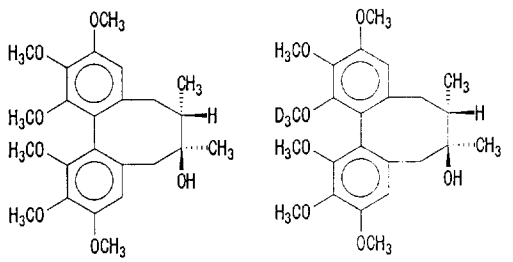

Fig. 1. Structures of schizandrin (**1**) and the internal standard (**2**).

standard (Fig. 1). Briefly, 418 mg of gomisin H [7] (99%) was dissolved in acetone-d_6 (3 ml), and excess iodomethane-d_3 and potassium carbonate were added to the solution. After stirring for 3 h at 50°C, the solvent was evaporated to dryness under reduced pressure. The residue was purified by TLC using hexane–acetone (3:2). The zone with UV absorption (254 nm) was extracted with chloroform–methanol (4:1) and the extract was concentrated. The product was recrystallized from hexane–diethyl ether to give SZ-d_3 as colourless prisms (311 mg).

Acetone-d_6, iodomethane-d_3 and TLC plates (Kieseigel 60 F_{254}) were purchased from Merck (Darmstadt, Germany). Other reagents were all of analytical-reagent grade and purchased from Wako (Osaka, Japan). Cartridge columns (Bond Elut C_{18}, 1 ml), used for sample preparation, were obtained from Analytichem International (Harbor City, CA, USA).

2.2. Apparatus

Analyses by GC–MS were carried out on a JEOL (Tokyo, Japan) AX-505H double-focusing mass spectrometer coupled to a Hewlett-Packard (Avondale, PA, USA) Model 5890 gas chromatograph. GC separation was achieved on an SPB-1 fused-silica capillary column (15 m × 0.25 mm I.D., 0.25 μm film thickness) from Supelco (Bellefonte, PA, USA). Injections were performed using a Hewlett-Packard Model 7673 autosampler in the splitless mode, with the split valve opened 1 min after injection. Helium was used as the carrier gas at an inlet pressure of 35 kPa and the temperature of the column was programmed to rise from 60 to 290°C at 20°C/min. Both the injector and the GC–MS interface were maintained at 290°C. The mass-selective detector was operated in the electron impact (EI) ionization mode with an ionization potential of 70 eV.

2.3. Extraction procedure

The cartridge column was preconditioned with 1 ml of methanol and 1 ml of distilled water. A 0.1 ml plasma sample, 0.1 ml of an aqueous internal standard solution (200 ng/ml) and 0.8 ml of water–methanol (1:1, v/v) solution were introduced on to the cartridge column. After the washing step with 1 ml of water and 1 ml of hexane, the analyte fraction was eluted with 0.5 ml of acetone. After the eluate had been evaporated to dryness under reduced pressure, the residue was dissolved in 0.2 ml of acetone, and 2 μl of this solution were used for GC–MS analysis.

2.4. Calibration graph

Samples for the calibration graph were prepared from drug-free human plasma spiked with SZ at levels ranging from 2.0 to 500.0 ng/ml. The extraction procedure for the sample was as described above. A calibration graph for SZ was constructed by plotting the ratios of m/z 432 peak areas derived from SZ to m/z 435 peak areas derived from SZ-d_3. Analysis was performed in the selected-ion monitoring (SIM) EI mode.

3. Results

3.1. Mass spectra

Mass spectra of SZ and SZ-d_3 obtained in the EI mode are shown in Fig. 2. SZ produced a mass spectrum dominated by m/z 432 (M^+) and SZ-d_3 produced m/z 435 (M^+), which were selected for quantification.

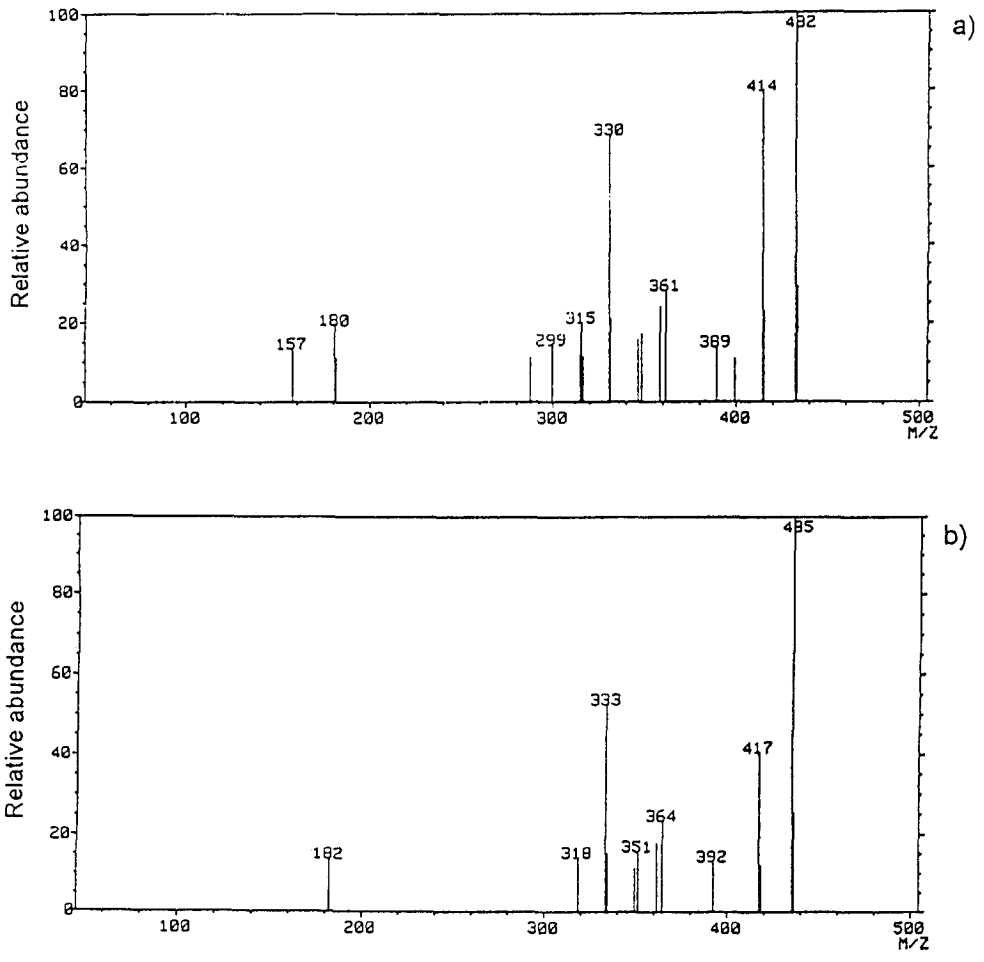

Fig. 2. Mass spectra of (a) SZ and (b) the internal standard.

3.2. Linearity

The peak-area ratio of SZ (m/z 432) to the internal standard (m/z 435) (y) was found to be linear in the range of measurements (x, ng/ml), and the following equation was obtained: $\log y = 1.066 \log x + 1.482$ ($r = 1.0000$, $n = 5$).

3.3. Precision

The intra-day precision, expressed as coefficient of variation (C.V.), is shown in Table 1. The precisions for concentrations of 2.0, 20.0, 100.0 and 500.0 ng/ml SZ were 1.1, 3.3, 0.7 and 1.7%, respectively.

The inter-day precision was studied over five days, and the results are given in Table 2. The precision ranged from 1.6 to 7.1%, and the accuracy, defined as (amount found/amount

Table 1
Intra-assay precision results for plasma samples spiked with SZ ($n = 5$)

Amount added (ng/ml)	C.V. (%)
2.0	1.1
20.0	3.3
100.0	0.7
500.0	1.6

Table 2
Inter-assay precision and accuracy results for plasma samples spiked with SZ ($n = 5$)

Amount added (ng/ml)	C.V. (%)	Accuracy[a] (%)
2.0	4.2	100.0 ± 2.7
20.0	7.1	104.5 ± 3.6
100.0	4.6	102.8 ± 2.9
500.0	1.6	98.3 ± 0.8
Mean	–	101.4 ± 1.3

[a] (Found/added) · 100. Results are means ± S.E.

added) · 100 (%), reached from 98.3% to 108.9% throughout the four concentrations examined. The lower limit of quantification was 2.0 ng/ml for SZ. The absolute recovery of SZ added to human plasma (1000 ng/ml) through all extraction steps was 99.0% ($n = 5$).

3.4. Stability

To test the stability of SZ, plasma samples spiked with various concentrations of SZ were stored at −20°C for 30 days, and results were compared with those for samples analysed immediately. The data in Table 3 show that stability of SZ was good after storage for 1 month at −20°C.

3.5. Application

The procedure was used to obtain pharmacokinetic data for SZ in plasma following oral administration to six healthy male subjects. Plasma samples were obtained just before and 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after administration

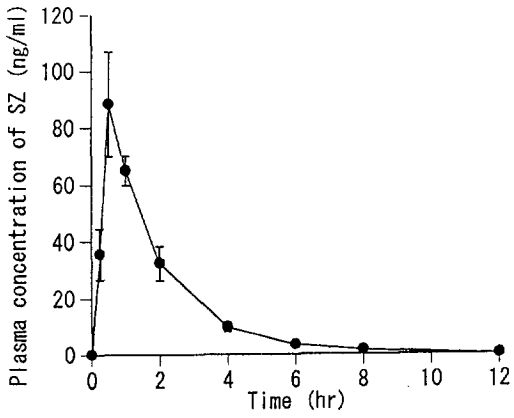

Fig. 3. Plasma concentration of SZ after oral administration at a dose of 15 mg to healthy volunteers. Each value represents the mean ±S.E. for six subjects.

at a dose of 15 mg. The average value of the maximum plasma concentration of SZ was 96.1 ± 14.1 ng/ml (0.5–2.0 h). The plasma concentration of this substance could be monitored for 8 h after administration (Fig. 3).

4. Conclusions

A simple and reliable GC–MS method was developed for the determination of SZ in human plasma. Only 100 μl of plasma and a simple extraction step were required. This method has been used successfully in our laboratory for the analysis of large numbers of human plasma samples for pharmacokinetic studies. This method could also be applied to other biological matrices.

Table 3
Stability of SZ

Sample treatment	SZ concentration (ng/ml)			
	2	20	100	500
Samples analysed immediately (A)	2.06 ± 0.05	19.0 ± 0.6	99.9 ± 0.8	510.7 ± 8.0
Samples stored at −20°C for 30 days (B)	2.12 ± 0.15	20.7 ± 0.4	104.4 ± 0.8	501.9 ± 11.2
Stability (%)[a]	102.9	108.9	104.5	98.3

[a] Stability = (B/A) · 100 (%).

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