Journal of Chromatography, 584 (1992) 261–266
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6610

Short Communication

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

Tomio Takamatsu, Kasuhiko Yamazaki and Masanori Kayano

Department of Clinical Chemistry, Tokyo Research Laboratories, Eisai Co., Ltd., 4-6-10, Koishikawa, Bunkyo-ku, Tokyo 112-88 (Japan)

Fumihito Takenaka, Masakatsu Hasui and Tetsuo Ohkawa

Sekisui Clinical Laboratory, Sekisui Chemical Co., Ltd., 2-1, Hyakuyama, Shimamoto-cho, Mishima-gun, Osaka 618 (Japan)

(First received July 28th, 1992; revised manuscript received September 29th, 1992)

ABSTRACT

A gas chromatographic–mass spectrometric method was developed to determine eperisone hydrochloride, 4′-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride, in human plasma over the concentration range 0.2–40 ng/ml. Excellent sensitivity was achieved by selection of a favorable fragment ion, *m/z* 98, of eperisone and reduction of heat decomposition of eperisone by using a splitless injector and a shortened capillary column. The method described here allows the determination of plasma concentrations as low as 0.2 ng/ml, the concentration attained 6 h after a single oral administration of 50 mg. At eperisone hydrochloride concentrations higher than 0.5 ng/ml, the mean inter-day variation of accuracy of the assay was less than 12%.

INTRODUCTION

Eperisone hydrochloride, 4′-ethyl-2-methyl-3-piperidinopropiophenone hydrochloride (Fig. 1), is a skeletal muscle relaxant that inhibits spinal cord nervous activity [1]. Eperisone hydrochloride (Myonal) is clinically widely used in Japan for treatment of spastic paralysis and is administered orally at a dose of 50–100 mg [2].

As a result of extensive first-pass metabolism, very low plasma concentrations of eperisone are observed [3]. To characterize the disposition of eperisone, an extremely sensitive analytical technique, capable of measuring a concentration of 0.2 ng/ml at 8 h after oral administration of eperisone, is required. However, the packed-column gas chromatographic–mass spectrometric (GC–MS) method previously described by Tanaka and Tsutsumi [14] can only determine eperisone hy-

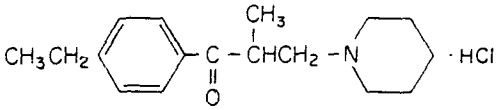

Fig. 1. Structure of eperisone hydrochloride.

Correspondence to: T. Takamatsu, Department of Clinical Chemistry, Tokyo Research Laboratories, Eisai Co., Ltd., 4-6-10, Koishikawa, Bunkyo-ku, Tokyo 112-88, Japan.

0378-4347/92/$05.00

drochloride concentrations of 1.0 ng/ml or higher.

This paper reports a sensitive and specific GC–MS method which can determine eperisone plasma concentrations as low as 0.2 ng/ml, the concentrations attained 6 h following a single dose of 50–100 mg.

EXPERIMENTAL

Standards and reagents

Eperisone hydrochloride was synthesized at Eisai (Tokyo, Japan). Tolperisone, 4'-methyl-2-methyl-3-piperidinopropiophenone hydrochloride, was obtained from Sigma (St. Louis, MO, USA). All reagents used were guaranteed-reagent grade.

The stock solution used was 2 μg/ml eperisone hydrochloride in distilled water. The internal standard solution used was 500 ng/ml tolperisone hydrochloride in distilled water.

Equipment and chromatographic conditions

Eperisone was analyzed by GC–MS using a 5890A gas chromatograph (Hewlett-Packard, Avondale, PA, USA) directly interfaced with a VG TRIO-1 mass-selective detector (VG MassLab, Manchester, UK) which operated in the electron-impact selected-ion monitoring (SIM) mode at an electron energy of 70 eV and an ion acceleration potential of 350 V. A cyanopropyl-bonded fused-silica capillary column (CBP10-S50-050, 10 m × 0.33 mm I.D., 0.50 μm thickness, Shimadzu, Kyoto, Japan) was used.

Splitless injection was performed at 200°C. Helium, at 0.14 bar, was used as the carrier gas. The initial oven temperature was 50°C, which resulted in cold trapping of the sample applied to the column. This oven temperature was kept at 50°C for 15 s, increased at 30°C/min to 170°C, and then maintained at 170°C for 6 min. It was finally increased at 30°C/min to 200°C and maintained at 200°C for 7.5 min. The interface and ion source temperatures were 200°C. Eperisone and the internal standard were eluted with retention times of 9.42 and 7.66 min, respectively. Mass spectra of eperisone and tolperisone were obtained in the electron-impact ionization mode, and samples were analyzed by SIM at *m/z* 98. The base peak at *m/z* 98 (M − 161, $[CH_2-\text{piperidine}]^+$) originated from the cleavage of the C–C bond (Fig. 2).

Extraction of analytes

To 1 ml of plasma, 100 μl of the internal standard solution and 1 ml of saturated sodium bicarbonate solution were added. Then, 4 ml of diethyl ether were added, and the mixture was vortex-mixed for 10 min and centrifuged at 1500

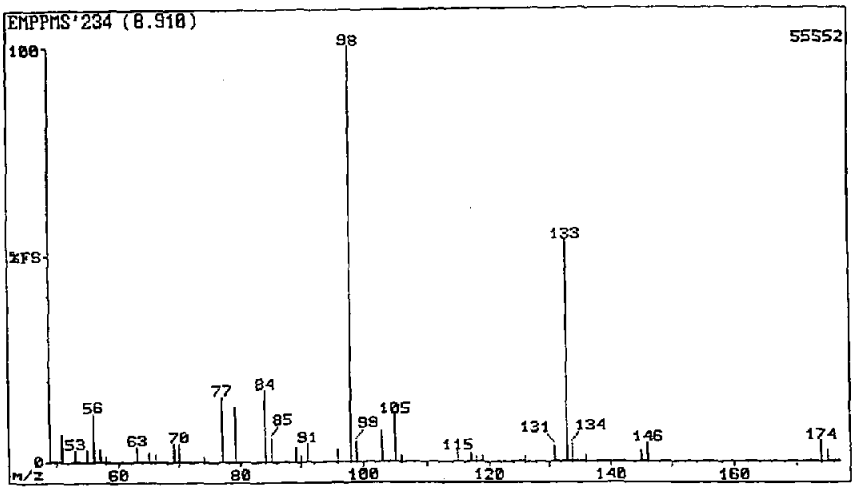

Fig. 2. Mass spectrum of eperisone hydrochloride.

g for 10 min. To the diethyl ether layer, 1 ml of 0.1 M hydrochloric acid was added, and the mixture was vortex-mixed for 10 min. After the mixture was centrifuged at 1500 g for 10 min, the hydrochloric acid layer was isolated. To the hydrochloric acid layer, 1 ml of saturated sodium bicarbonate solution and 4 ml of diethyl ether were added, and the mixture was vortex-mixed for 10 min. After the mixture was centrifuged at 1500 g for 10 min, the diethyl ether layer was isolated and evaporated to dryness at 40°C under a stream of nitrogen gas. This sample was dissolved in 15 μl of methanol, and an aliquot was injected into the GC–MS apparatus.

Calibration curves

Calibration curves were prepared on each day of analysis, using drug-free heparinized plasma obtained from healthy volunteers. A range of standard solutions containing 0.2, 0.5, 0.75, 1, 2.5, 5, 10 and 40 ng/ml eperisone hydrochloride were prepared. Calibration curves were constructed in the ranges 0.2–2.5 and 2.5–40 ng/ml, evaluated by linear regression analysis from the peak-area ratio.

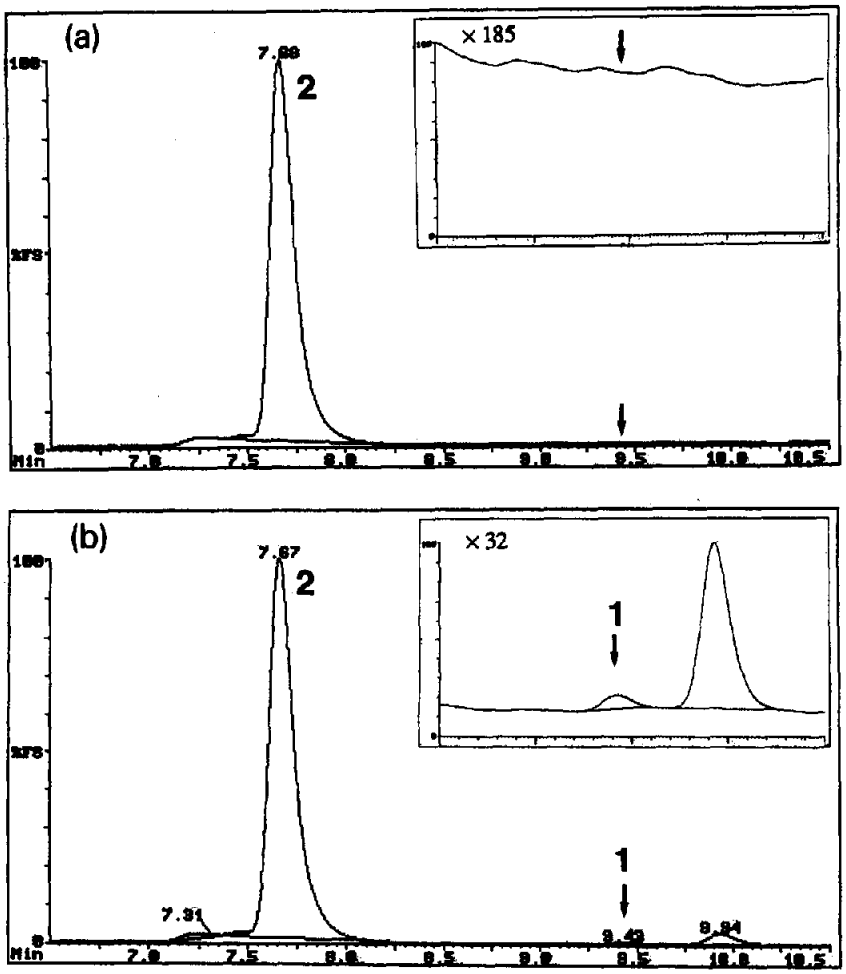

Fig. 3. Chromatograms of extracts from (a) drug-free plasma and (b) plasma from a healthy volunteer 6 h after oral administration of eperisone hydrochloride, containing 0.24 ng/ml eperisone hydrochloride and 50 ng/ml internal standard. Peaks: 1 = eperisone; 2 = internal standard.

Accuracy and reproducibility

In order to ensure that eperisone concentrations determined on different days could be directly compared, quality control samples containing 1.5 and 30 ng/ml eperisone in plasma were prepared by adding a known amount of eperisone hydrochloride solution to 50 ml of drug-free plasma, which was divided into 1.0-ml aliquots and stored at −20°C. The quality control samples were assayed in triplicate for each concentration each day of analysis. The accuracy and precision over the whole concentration range measured, 0.1, 0.2, 1, 15 and 30 ng/ml, were assessed by measurement of the concentration of five samples for each concentration for three days after adding various amounts of eperisone hydrochloride standard solution to drug-free plasma.

RESULTS AND DISCUSSION

Typical chromatograms of plasma extracts from a healthy volunteer before and after oral administration of 50 mg of eperisone hydrochloride are shown in Fig. 3. There were no interfering peaks in the chromatograms of plasma from healthy volunteers. The retention times of eperisone were longer than those of the internal standard, tolperisone. The run time of this chromato-

TABLE I

INTRA-DAY AND INTER-DAY PRECISION AND ACCURACY

The accuracy of the method can be assessed from the difference between the concentration added and the concentration assayed.

Concentration added (ng/ml)	Day	Concentration assayed (mean ± S.D., n = 5) (ng/ml)	C.V. (%)	Accuracy (mean) (%)	Precision (mean) (%)
0.10	1	0.14 ± 0.01	8.7	140	
	2	0.12 ± 0.01	9.3	120	
	3	0.12 ± 0.01	9.2	120	
	Mean ± S.D.	0.13 ± 0.01	9.1	127 ± 11.5	9.1
0.20	1	0.26 ± 0.02	8.1	130	
	2	0.20 ± 0.01	6.1	100	
	3	0.22 ± 0.01	3.8	110	
	Mean ± S.D.	0.23 ± 0.01	6.0	113 ± 15.3	13.5
0.50	1	0.55 ± 0.04	6.8	110	
	2	0.44 ± 0.02	5.3	88	
	3	0.47 ± 0.03	5.5	94	
	Mean ± S.D.	0.49 ± 0.03	5.9	97 ± 11.4	11.7
1.00	1	1.11 ± 0.09	7.6	111	
	2	0.99 ± 0.11	11.0	99	
	3	1.05 ± 0.06	5.8	105	
	Mean ± S.D.	1.05 ± 0.09	8.1	105 ± 6.0	5.7
15.04	1	14.08 ± 0.23	1.6	94	
	2	14.94 ± 0.12	0.8	99	
	3	15.17 ± 0.41	2.7	101	
	Mean ± S.D.	14.73 ± 0.25	1.7	98 ± 3.6	3.7
30.07	1	29.45 ± 0.38	1.3	98	
	2	30.60 ± 1.29	4.2	102	
	3	31.41 ± 0.50	1.6	105	
	Mean ± S.D.	30.49 ± 0.73	2.4	102 ± 3.5	3.5

TABLE II

PLASMA CONCENTRATIONS OF EPERISONE HYDROCHLORIDE

Values were determined during two analytical runs using samples from healthy volunteers after administration of 50 mg of eperisone hydrochloride.

Sample No.	Concentration (ng/ml)		Assay 1/assay 2 (%)
	Assay 1	Assay 2	
1	0.49	0.40	123
2	0.44	0.41	107
3	0.38	0.38	100
4	0.54	0.52	104
5	0.89	0.85	105
6	1.32	1.38	96
7	1.31	1.46	90
8	1.33	1.44	92
9	1.16	1.24	94
10	0.73	0.83	88

graphic system was 10.5 min. The total run time was prolonged to 25 min because of the temperature programme selected.

Calibration curves were constructed for the ranges 0.2–2.5 and 2.5–40 ng/ml. The sample concentration were first calculated using the calibration curve for the low concentration range (*e.g.*, $y = 0.01320x - 0.0007$, $r = 0.9995$) by linear regression analysis, and sample concentrations exceeding this range were calculated using the calibration curve for the high concentration range (*e.g.*, $y = 0.01461x - 0.0066$, $r = 0.9999$).

The stability studies of eperisone in plasma stored at −20°C demonstrated a loss of less than 10% at in eight months. The extraction recovery of eperisone from plasma was 70–80% .

The concentrations of eperisone measured in the quality control plasma samples assessed daily were in good agreement with the expected concentrations. The inter-day variations of accuracy and precision for the determination of eperisone hydrochloride on three days are shown in Table I. At eperisone hydrochloride concentrations higher than 0.5 ng/ml, the mean inter-day variation of accuracy was less than 12%. At the lowest concentration assayed (0.1 ng/ml), the mean inter-day variation of accuracy was less than 30%.

The good accuracy and precision of this method are reflected by the agreement of eperisone concentrations determined during two consecutive analytical runs, using plasma samples obtained from healthy subjects orally administered 50 mg of eperisone hydrochloride (Table II). This technique has been used to determine the concentrations of eperisone in plasma samples from healthy volunteers after oral administration of 50 mg of eperisone hydrochloride. A typical plasma concentration–time curve is shown in Fig. 4, and the half-life of eperisone in plasma after oral administration of the drug using this method is 1–3 h.

The GC–MS method for the determination of eperisone concentrations in plasma reported here has excellent sensitivity, and the lower limit of quantitation is 0.2 ng/ml. This high sensitivity was achieved by the choice of a favourable fragment of eperisone, and by decreasing the heat decomposition of eperisone using of a splitless injector and a short capillary column. The limit of quantitation of plasma eperisone in a previous study [4] utilizing GC–MS was 1 ng/ml, which is not sensitive enough for detection in clinical use. Owing to the excellent sensitivity of this method, we can measure plasma concentrations of eperisone as low as 0.2 ng/ml, which is equal to the plasma concentration attained 6 h after a single oral administration of 50 mg of eperisone, with a high degree of accuracy and precision. The half-

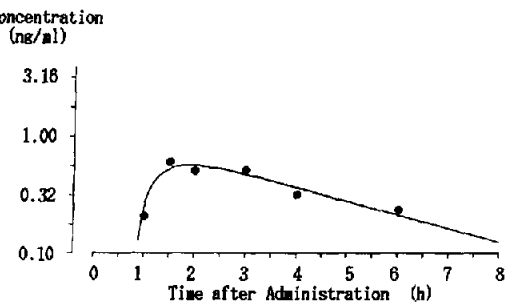

Fig. 4. Typical plasma concentration–time curve of eperisone hydrochloride. The biexponential relationships fitted to the plasma concentration–time data generated using non-linear least-squares regression analysis are also shown.

life of eperisone can therefore be determined accurately, and the pharmacokinetics of eperisone after oral administration can be characterized reliably.

ACKNOWLEDGEMENT

The authors wish to thank Dr. Wendy Gray for her help in the preparation of this paper.

REFERENCES

1 K. Tanaka, T. Kaneko and K. Yamatsu, *Folia Pharmacol. Jpn.*, 77 (1981) 511.
2 Y. Kuroiwa, I. Sobue, Y. Tazaki, T. Nakanishi, E. Ohotomo and K. Itahara, *Clin. Eval.*, 9 (1981) 391.
3 T. Fujita, T. Takamatsu, T. Hisamoto, J. Tsutsumi, K. Kinoshita and T. Kanai, *Pharmacometrics (Japan)*, 21 (1981) 835.
4 S. Tanaka and J. Tsutsumi, *Clin. Rep.*, 16 (1982) 235.