ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 599–603

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

LC determination of Z-338, novel gastroprokinetic agent in dog plasma by SCX solid phase extraction

Shigeru Furuta *, Haruka Miyahara, Tohru Sugimoto, Hiroshi Sano

Central Research Laboratories, Zeria Pharmaceutical Co., Ltd., 2512-1 Oshikiri, Kohnan-machi, Ohsato-gun, Saitama-ken 360-0111, Japan

Received 11 October 2000; received in revised form 2 November 2000; accepted 12 November 2000

Abstract

A simple high-performance liquid chromatographic assay with using UV detection (266 nm) was developed to determine a novel gastroprokinetic agent, Z-338 in dog plasma. The extraction procedure using solid-phase extraction with a Isolute SCX column produces extremely clean eluates and a high recovery. Intra- and inter-day variabilities were lower than 5%. The limit of quantitation of the method was 2.5 ng/ml. This assay was applied to the monitoring of Z-338 concentrations in dogs after oral administration. The method also appeared rapid, simple and suitable for therapeutic Z-338 monitoring. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Z-338; SCX

1. Introduction

Z-338, (*N*-(*N*′,*N*′-diisopropylaminoethyl)-[2-(2-hydroxy-4,5-dimethoxybenzoylamino) -1,3-thiazole-4-yl] carboxyamide monohydrochloride trihydrate is a novel gastroprokinetic agent, which is synthesized by Zeria Pharmaceutical Co., Ltd (Fig. 1). In pre-clinical assessments, Z-338 improved delayed gastric emptying models due to its enhancement of postprandial gastric antral motor activity. Z-338 may be useful for the treatment of functional dyspepsia and gastroesophageal reflux disease (GEAR) [1–3].

In this study, we have developed an assay suitable for pharmacokinetic studies of Z-338 in dogs. This paper describes the validation of a high-performance liquid chromatographic (HPLC) method and time-profile of plasma concentration of Z-338 in dogs.

2. Experimental

2.1. Chemicals and standards

Z-338 and internal standard (I.S., *N*-(*N*′,*N*′-diisopropyl-aminoethyl)-[2-(2-methoxy-4,5-dimethoxybenzoyl-amino)-1,3-thiazole-4-yl] carboxyamide monohydrochloride tri-hydrate) for HPLC analysis, were synthesised in our laborato-

* Corresponding author. Tel.: +81-048-536-3456; fax: +81-048-539-1072.

E-mail address: ken-taisya@zeria.co.jp (S. Furuta).

0731-7085/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0731-7085(00)00593-8

ries. Solvents and reagents were of HPLC or analytical grade. Stock solutions of Z-338 and I.S. were prepared by methanol. Working solutions were obtained by diluting stock solutions with 50% methanol for Z-338 and water for I.S. Each solution was stored at 5°C until analysis. Plasma controls were prepared by spiking 990 μl of blank plasma with 10 μl of working solutions to their final concentration from 2.5 to 5000 ng/ml of Z-338 free base.

2.2. HPLC system

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-6A pump, an SIL-6A autoinjector (with a 150 μl injection loop), a SCL-6A system controller, an SPD-6A UV detector set at 266 nm and a CR-7A integrator. Analytes were separated at 45°C by CTO-6A (Shimadzu, Kyoto, Japan) on an analytical column (Capcell Pak C18, UG-120, 250 mm × 4.6 mm I.D., 5 μm, Shiseido, Tokyo, Japan) with a guard column (35 mm × 4.6 mm I.D., Shiseido, Tokyo, Japan). The mobile phase was a 0.6% potassium dihydrogen phosphate buffer (pH 3) -methanol (51:49, v/v) containing 10 mM sodium 1-octanesulfonic acid and pumped at a flow-rate of 1 ml/min.

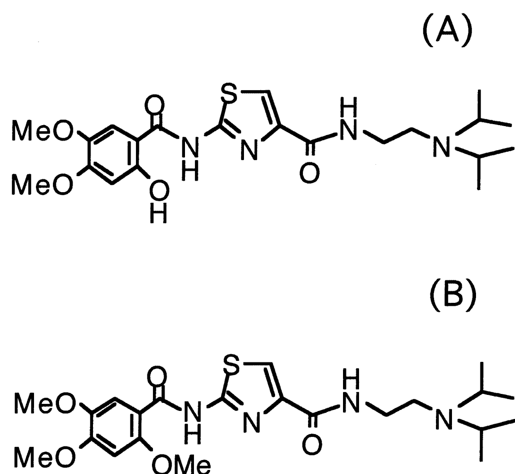

Fig. 1. Chemical structures of Z-338: (A) and; I.S. (B).

2.3. Sample preparation

Z-338 was extracted from plasma using 100 mg Isolute benzenesulfonic acid (SCX) solid-phase extraction cartridges (International Solvent Technology, Mid Glamorgan, UK) by vacuum at an approximate rate of 1 ml/min. A plasma sample (1 ml) was applied after application of 50 μl of I.S. solution (10 μg/ml) to a cartridge previously conditioned successively with 3 ml of 50% methanol, 5 ml of 0.1 N hydrochloric acid–acetonitrile (1:9, v/v), 3 ml of methanol and 3 ml of water. The cartridge was then washed with 3 ml of water and Z-338 was eluted with 5 ml of 0.1 N hydrochloric acid–acetonitrile (1:9, v/v). The eluate was evaporated to dryness under a stream of nitrogen at 70°C. The residue was dissolved with 250 μl of mobile phase and centrifuged at 1000 *g* for 10 min. The supernatant was transferred into the autosampler vial and 70 μl was injected into the HPLC system.

2.4. Pharmacokinetic study

Six month-old male LRE-strain beagle dogs (Covance Research Products Inc., MI, USA) weighing approximately 10 kg were used. Dogs were fasted for 16 h and fed at 6 h after administration.

Z-338 was administered orally with the cannula to five dogs at a dose of 30 mg/kg. Blood was collected using a heparinized cylinder through a forelimb cephalic vein at 15, 30 min, 1, 2, 4, 6, 8, 11, 24 and 48 h after administration. Blood samples were centrifuged at 1000 × g. for 10 min to separate plasma, which were stored at − 80°C until analysis. The plasma samples were thawed at room temperature.

2.5. Assay validation

Standard curves were constructed with plasma controls spiked with 2.5, 5, 10, 50, 100, 500, 1000 and 5000 ng/ml of Z-338. Plasma concentrations were calculated from linear regression analysis of the peak area ratios (analyte/I.S.) vs. concentration curves using a weight factor as 1/(concentration).

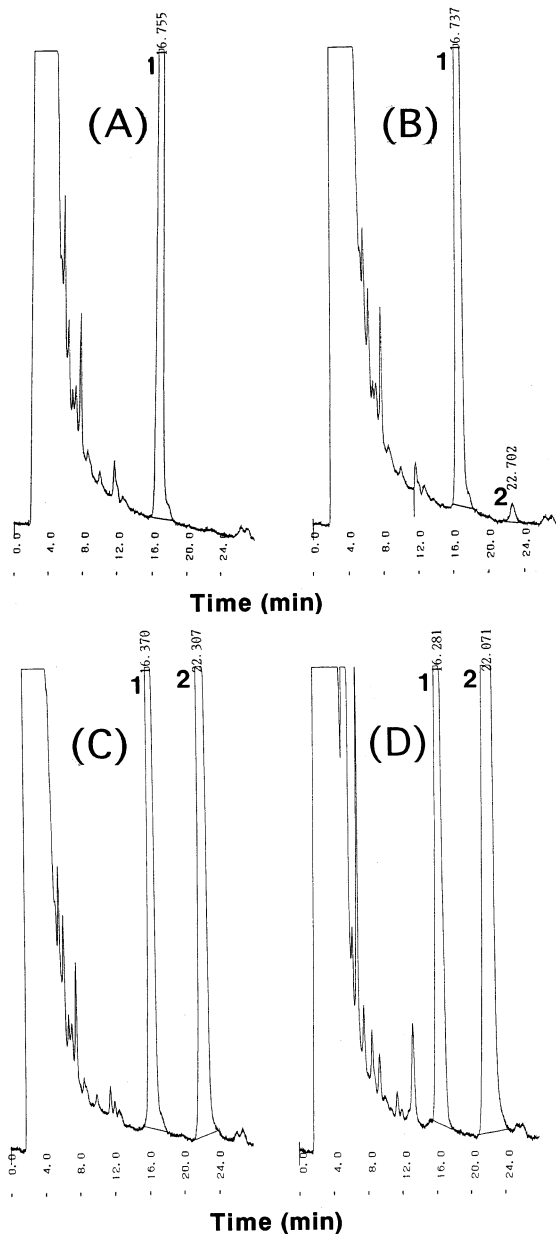

Fig. 2. Typical HPLC chromatograms on measurement of dog plasma samples: (A) blank plasma; (B) plasma sample containing 2.5 ng/ml of Z-338 free base; (C) plasma sample containing 500 ng/ml of Z-338 free base; (D) plasma sample at 30 min after administration of Z-338 at a dose of 30 mg/kg. Peaks: 1 = I.S.; 2 = Z-338.

The within- and inter-day precision and accuracy were determined on fifplicate measurements of three Z-338 concentrations (10, 500 and 5000 ng/ml). All samples were spiked with Z-338 on day 1, and extracted and analyzed on three days ($n = 5$). The recovery yield was determined by comparing the peak area's ratios of extracted samples to those of nonprocessed standard solutions ($n = 3$ for each concentration). The stability of the stock solutions of Z-338 and I.S. and plasma samples was also evaluated over 1 month.

2.6. Data analysis

Pharmacokinetic parameters such as the maximal concentration (C_{max}), time required until the maximal concentration was obtained (T_{max}) and the area under the plasma concentration–time curve (AUC) and half-life on terminal phase ($t_{1/2}$) were calculated from plasma drug concentrations. AUC was calculated by the trapezoidal method and the area of the tail was calculated from the last point divided by elimination slope.

3. Results

3.1. Chromatography

Chromatograms representing a blank plasma sample ($n = 3$) and plasma spiked with 2.5 and 500 ng/ml of Z-338 are presented in Fig. 2. Retention times of Z-338 and I.S. were ca. 22 and 16 min, respectively. There was no interfering peak for the blank plasma. The limit of quantitation was 2.5 ng/ml, and the accuracy was 11.3%. The limit of detection was 1 ng/ml for signal/noise = 3.0.

3.2. Linearity

The standard curves were linear from 2.5 and 5000 ng/ml with correlation coefficients greater than 0.9999. The accuracys calculated from the standard curve were $-5.8 \sim 8.1\%$, showed the good agreement (Table 1).

3.3. Recovery

The recovery of Z-338 from plasma varied as 92.2 ± 0.5% at 10 ng/ml, 90.0 ± 0.6% at 500 ng/ml and 91.0 ± 0.7% at 5000 ng/ml. The recovery of I.S. from plasma was 87.6 ± 1.1% at 500 ng/ml(mean ± SE, $n = 3$).

3.4. Precision and accuracy

The intra- and inter-day precision of fifplicate injections at 10, 500 and 5000 ng/ml are presented in Table 2. For the intra-day study, coefficients of variation (CV) were 1.2, 3.9 and 4.0%, and mean accuracy were 0.6, 3.0 and −1.3% at 10, 500 and 5000 ng/ml, respectively.

For the inter-day study for 3 days, CV were 0.9 ∼ 1.2, 1.3 ∼ 3.9 and 0.7 ∼ 4.0% at 10, 500 and 5000 ng/ml, respectively. The mean accuracy for 3 days were −1.2 ∼ 0.6, −4.0 ∼ 3.0 and −2.7 ∼ 1.4% at 5, 500 and 5000 ng/ml, respectively.

3.5. Stability of the analyte

The between-day coefficients of variation as calculated from the results of the stability test of stock solutions and plasma samples were within 15% under conservation condition. Thus Z-338 and I.S. in methanol was stable for 1 month when solutions were stocked at 5°C, and plasma samples were stable for 1 month at −80°C. In addition, Z-338 and I.S. were found to be stable in vials on the autoinjector carousel for at least 48 h.

Table 1
Linearity of the caliburation curves of Z-338 concentration in dog plasma($n = 3$)[a]

Concentration added (ng/ml)	Peak area ratio (CV%)	Concentration found (ng/ml) (CV%)	Accuracy (%)
2.5	0.0060 (1.1)	2.7 (2.6)	8.1
10	0.0254 (1.9)	10.1 (0.7)	0.8
100	0.2463 (3.9)	94.2 (2.0)	−5.8
500	1.2599 (3.5)	480.3 (1.2)	−3.9
5000	13.1983 (3.9)	5026.6 (0.2)	0.5
Correlation coefficients (CV%)	Slop (CV%)	Intercept (CV%)	
0.9999 (0.0)	381.2 (3.9)	0.4 (37.0)	

[a] Concentration of analyte = slop × (peak area ratio) + intercept.

Table 2
Variation of the measurement of Z-338 concentration in dog plasma[a]

	Concentration added (ng/ml)	Concentration added (ng/ml)	C.V. (%)	Accuracy
1st day	10	10.1 ± 0.1	1.2	0.6
	500	514.8 ± 19.9	3.9	3.0
	5000	4936.1 ± 196.0	4.0	−1.3
2nd day	10	10.0 ± 0.1	1.2	−0.3
	500	479.9 ± 6.4	1.3	−4.0
	5000	4865.1 ± 89.7	1.8	−2.7
3rd day	10	9.9 ± 0.1	0.9	−1.2
	500	499.7 ± 9.4	1.9	−0.1
	5000	5067.6 ± 33.6	0.7	1.4

[a] Data are the mean ± S.D. for five samples.

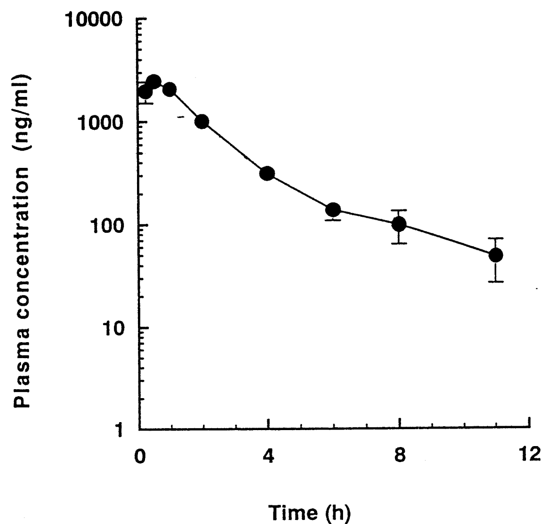

Fig. 3. Plasma concentrations after oral administration of Z-338 to dogs. (dose; 30 mg/kg p.o.) Data are the mean ± S.E.($n = 5$). Plasma concentrations showed the Z-338 free base.

3.6. Pharmacokinetic study

Plasma concentrations of Z-338 after oral administration of Z-338 at a dose of 30 mg/kg are shown in Fig. 3. The plasma Z-338 level reached a C_{max} of 2643.3 ng/ml at 0.4 h after administration. At 24 h after administration, mean plasma concentrations was under the limit of quantitation. The $t_{1/2}$ was 2.9 h, and AUC was 5997.3 ng h/ml.

4. Discussion and conclusion

This report describes the methodology and validation of the HPLC assay using SCX solid-phase extraction for the determination of Z-338 in dog plasma. In preliminary study, Z-338 was also strongly held by the C_{18} and C_8 cartridges, however, the contaminants in plasma (for example, proteins and lipids) could not be completely eliminated by cleaning up. The non-polar cartridges retained more endogenous materials [4], resulting in more extraneous peaks in the chromatograms. Thus we considered another cleanup method using a silica-based cation-exchange cartridge. The SCX cartridges used exhibit a dual mode of action, a cation-exchange and an apolar mechanism[4–6]. Since Z-338 which has thiazole ring and diisopropylaminoethyl moiety is a basic and lipophilic compound, both modes are capable of retaining Z-338 completely in plasma. Furthermore, we found that I.S. was not extracted from SCX cartridge (ca. 50%) by acetonitrile, thus acetonitrile containing hydrocloride was used for elute solvents. This extraction procedure produces extremely clean eluates and allows for approximately 90% extraction efficiency by 0.1 N hydrochloric acid–acetonitrile (1:9, v/v). Moreover, under our conservation condition, no degradation of Z-338 and I.S. could be evidenced. Thus this method is reproducible, precise, and has been successfully applied to pharmacokinetic studies in dogs.

In the pharmacokinetic study, five animals were used, and no interfering components were observed in the chromatograms of plasma samples before and after administation of Z-338. The absolute accuracy of the quality-control (QC) did not exceed 15%, and the results were acceptable. Z-338 was rapid absorbed from the gastrointestinal tract and was eliminated from plasma within 24 h after oral administration of Z-338 to dogs at a dose of 30 mg/kg. This method also appeared rapid, simple and suitable for therapeutic Z-338 monitoring.

References

[1] T. Kurimoto, K. Yoshida, R. Eta, M. Tochigi, R. Sato, T. Yoneta, H. Tamaki, Z. Itoh, M. Ogishima, K. Taniyama, Arch. Pharmacol. 358 (1998) R356.

[2] S. Ueki, Y. Matsunaga, T. Matsumura, Y. Hori, T. Yoneta, T. Kurimoto, H. Tamaki, Z. Itoh, Arch. Pharmacol. 358 (1998) R351.

[3] M. Ogishima, M. Kaibara, S. Ueki, T. Kurimoto, K. Taniyama, J. Pharmacol. Exp. Ther. 294 (2000) 33–37.

[4] M.G. Knize, C.P. Salmon, E.C. Hopmans, J.S. Felton, J. Chromatogr. A 763 (1997) 179–185.

[5] M. Horie, K. Saito, N. Nose, H. Oka, H. Nakagawa, J. Chromatogr. B. 655 (1994) 47–52.

[6] M. Horie, K. Saito, N. Nose, H. Oka, H. Nakagawa, J. Chromatogr. B., 655 (1994) 47–52; L. Millerioux, M. Brault, V. Gualano, A. Mignot, J. Chromatogr. A, 729 (1996) 309–314.