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Microbore high-performance liquid chromatographic determination of cisapride in rat serum samples using column switching

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Abstract

For the determination of cisapride from serum samples, an automated microbore high-performance liquid chromatographic method with column switching has been developed. After serum samples (100 μl) were directly injected onto a Capcell Pak MF Ph-1 pre-column (10×4 mm I.D.), the deproteinization and concentration were carried out by acetonitrile–phosphate buffer (20 m*M*, pH 7.0) (2:8, v/v) at valve position A. At 2.6 min, the valve was switched to position B and the concentrated analytes were transferred from MF Ph-1 pre-column to a C_{18} intermediate column (35×2 mm I.D.) using washing solvent. By valve switching to position A at 4.3 min, the analytes were separated on a Capcell Pak C_{18} UG 120 column (250×1.5 mm I.D.) with acetonitrile–phosphate buffer (20 m*M*, pH 7.0) (5:5, v/v) at a flow-rate of 0.1 ml/min. Total analysis time per sample was 18 min. The linearity of response was good (r=0.999) over the concentration range of 5–200 ng/ml. The within-day and day-to-day precision (CV) and inaccuracy were less than 3.7% and 3.8%, respectively. The mean recovery was 96.5±2.4% with the detection limit of 2 ng/ml.

Keywords: Cisapride

1. Introduction

Cisapride, ±*cis*-4-amino-5-chloro-*N*-[1-{3(4-fluorophenoxy)propyl} - 3 - methoxy - 4 - piperidinyl] - 2 - methoxybenzamide monohydrate, is widely used as a prokinetic drug in the treatment of the disorders of gastrointestinal motility [1–3].

Reversed-phase high-performance liquid chromatography (HPLC) methods with UV [4,6] or fluorescence detection [5] have been reported for the quantification of cisapride in plasma and tissue samples. They are not suitable for routine determination of cisapride in serum samples. Those methods require large sample volumes (~1 ml) [4,6] and sample clean-up, using multiple liquid–liquid extraction [4,5] or solid-phase extraction [6] and evaporation which is tedious, time consuming and laborious.

The need for a rapid, sensitive and reliable method for the analysis of cisapride in serum samples has been emphasized by the increased use in the neonate and elderly patients. Column switching is thought to be reasonable for the sample preparation of cisapride from serum samples because the on-line technique improves reliability and sample throughput and shortens total analysis time as well as minimizing

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sample loss or change [7–11]. This study describes the development of an automated microbore HPLC method using column switching for cisapride in serum samples without pre-purification steps. The present method offers practical advantages over the conventional liquid–liquid [4,5] or solid-phase extraction [6] with respect to the speed, sensitivity, sample throughput and sample volume.

2. Experimental

2.1. Materials and reagents

Cisapride was a gift from Saehan Pharmaceutical (Seoul, South Korea). Methanol and acetonitrile (HPLC grade) were obtained from Burdick and Jackson (Muskegon, MI, USA) and all other reagents were the highest purity available.

Stock solution of cisapride was prepared in methanol to yield a final concentration of 1 mg/ml and stored at −20°C. Working standard solutions were prepared on the day of analysis by diluting the stock solution with water or serum. The serum samples were passed through a 0.2-μm membrane filter (Millipore, MA, USA).

2.2. Chromatographic system

The semi-microbore LC system consisted of the Nanospace SI-1 series (Shiseido, Tokyo, Japan), i.e., two 2001 pumps, a 2002 UV–Vis detector, a 2003 autosampler, a 2004 column oven, a 2012 high-pressure switching valve and a 2009 degassing unit. The systems were operated by Syscon and data handling was performed by S-MicroChrom 4.1. The instrument arrangement for the automated column switching system is shown in Fig. 1.

A pre-column used for on-line sample preparation was a Capcell Pak MF Ph-1 column (10×4 mm I.D., Shiseido). A Capcell Pak C_{18} UG 120 (35×2 mm I.D., Shiseido) and Capcell Pak C_{18} UG 120 (250×1.5 mm I.D., Shiseido) were used as an intermediate column and main separation column, respectively.

Washing solvent for on-line trace enrichment was acetonitrile–phosphate buffer (20 m*M*, pH 7.0) (2:8, v/v) and acetonitrile–phosphate buffer (20 m*M*, pH 7.0) (5:5, v/v) was used in main separation. The column temperature was 35°C and the wavelength of detection was 268 nm.

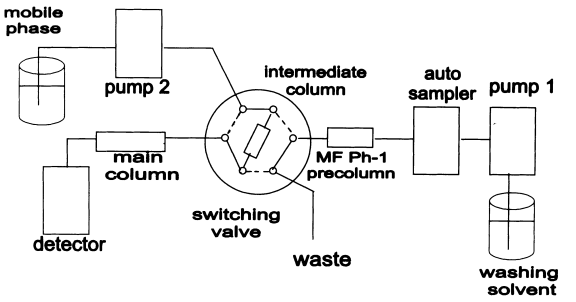

Fig. 1. Schematic diagram of a column switching system. ——, Position A; - - -, position B.

2.3. Analytical procedure

The serum sample (100 μl) was injected onto a MF Ph-1 pre-column and serum proteins were washed out from the pre-column using a washing solvent at a flow-rate of 1 ml/min (valve position A). The intermediate column and analytical column were equilibrated using the mobile phase. From 2.6 min to 4.3 min after sample injection (valve position B), cisapride zone was transferred from the pre-column onto the head of an intermediate C_{18} column by the washing solvent at a flow-rate of 0.5 ml/min. From 4.3 min to 18 min (valve position A), the analytes enriched on an intermediate C_{18} column were separated on a microbore C_{18} column with 0.1 ml/min of acetonitrile–phosphate buffer (20 m*M*, pH 7.0) (5:5, v/v). In the meanwhile, the MF Ph-1 pre-column was equilibrated with a washing solvent.

2.4. Method validation

The limit of detection (LOD) for cisapride was determined as the concentration of drug giving a signal-to-noise ratio greater than 3:1. Cisapride was spiked in serum over the concentration range of 5–200 ng/ml and quantitated to evaluate the recovery. The precision [defined as the coefficient of variation (CV) of replicate analysis] and the accuracy (defined as the bias between added and found concentration) of the method were also evaluated [13].

3. Results and discussion

3.1. Chromatography and column switching procedure

In the present study, microbore HPLC was used for the determination of cisapride from serum samples because there are many advantages such as increased sensitivity, high resolution, economic and environmental aspects. As shown in Fig. 2, there were no interfering peaks at the retention time (15.1 min) of cisapride. On-line trace enrichment using column switching technique is appropriate for the sample clean-up of cisapride from serum samples due to low concentration and sample throughput.

In the column switching technique [7–11], the choice of the pre-column packing, washing solvent and valve switching time is crucial in order to obtain complete recovery and remove interference components.

Capcell Pak MF Ph-1 was chosen as the pre-column because it possesses long polyoxyethylene chains and phenyl groups on the surface of 80 Å silica in order to limit the access of large molecules such as proteins and retain drug molecules longer [12]. In order to determine the composition of washing solvent and the appropriate valve switching time, the retention of cisapride on MF Ph-1 pre-column was examined using different mixtures of acetonitrile and phosphate buffer (20 m*M*, pH 7.0) (Fig. 3). Since cisapride is lipophillic (log $P=3.96$) and weak base ($pK_a=7.83$), the mixture of acetonitrile and pH 7.0 buffer solution was appropriate as a washing solvent. To remove serum proteins and concentrate cisapride in due time, 20% acetonitrile in phosphate buffer (20 m*M*, pH 7.0) was chosen as the washing solvent.

The intermediate column (35×2 mm I.D.) was used to protect a main column and save the analysis time. Without an intermediate column, it takes 8.5 min to transfer the concentrated cisapride fraction from MF Ph-1 pre-column to an analytical column at a flow-rate of 0.1 ml/min.

MF Ph-1 pre-column was exchanged after injection of 50 serum samples (equivalent to 5.0 ml serum). The intermediate and main column showed no decrease in efficiency after more than 300 injections of serum samples.

3.2. Method validation

The calibration curve of peak areas versus the concentrations of cisapride in serum was linear in the range of 5–200 ng/ml. The mean (±SD) regression

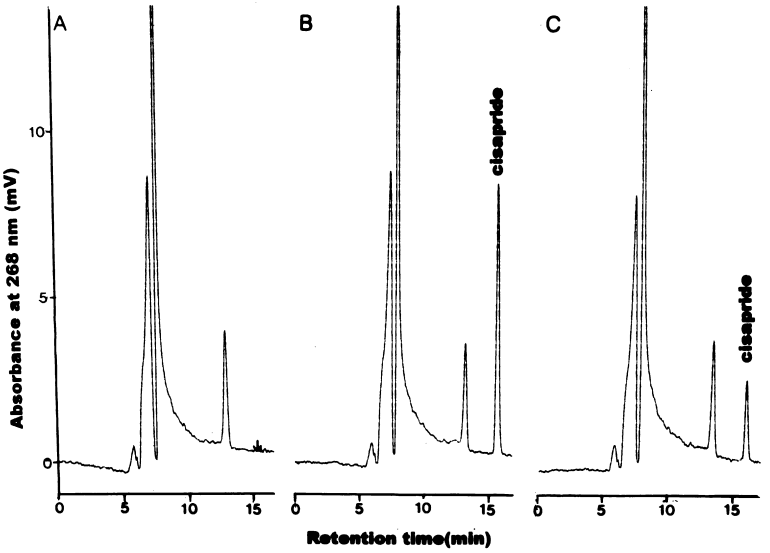

Fig. 2. Chromatograms of (A) blank serum, (B) blank serum spiked with cisapride (50 ng/ml), and (C) serum sample from a rat at 30 min after an oral administration of 1.5 mg/kg cisapride.

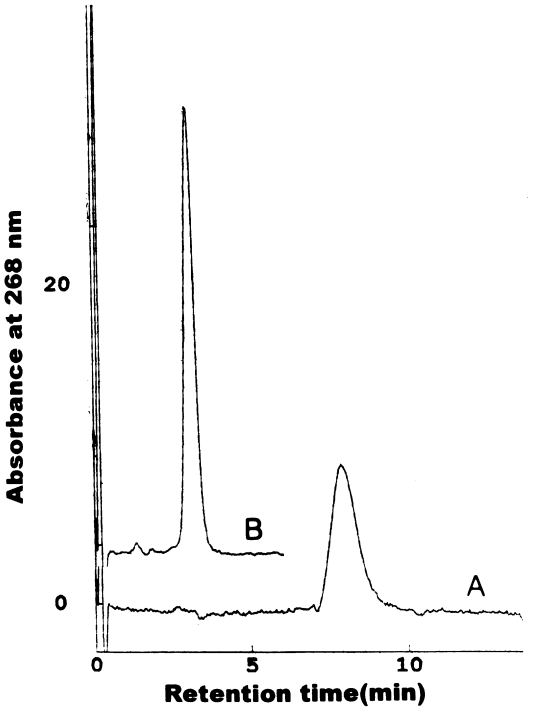

Fig. 3. Retention of cisapride on MF Ph-1 column (10×4 mm I.D.). Conditions: mobile phases; acetonitrile–phosphate buffer (20 m*M*, pH 7.0), (A) 15:85, v/v and (B) 20:80, v/v, flow-rate; 1 ml/min, injection volume; 100 μl.

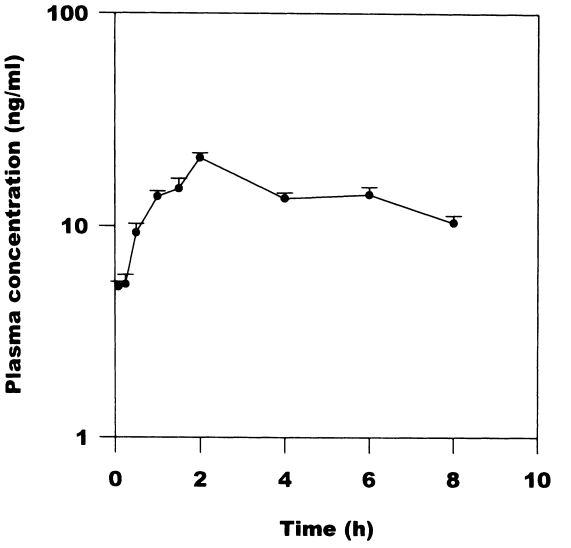

Fig. 4. Mean serum concentration versus time plot of cisapride in rats after an oral administration of 1.5 mg/kg cisapride. Each point with vertical bar represents the mean and standard error of five rats.

equation from twelve replicate calibration curves on different days was $y=2054\ (\pm 77)x-411\ (\pm\ 56)$ (where, y=peak area, x=cisapride concentration) with the correlation coefficient of 0.999 (±0.0003). The LOD of cisapride was 2 ng/ml using 100 μl of serum. The mean recovery of cisapride from serum sample was 96.5±2.4%. The precision and accuracy of the assay are shown in Table 1. The found amount deviated from −3.8% to 3.4% of the added amount in the spiked serum samples. The assay was precise because the CV was less than 3.7%.

3.3. Application of the method to biological samples

The present method has been successfully applied to the analysis of more than 150 serum samples from rats after an oral or intravenous administration of cisapride. The chromatogram of a rat serum sample is shown in Fig. 2c. Fig. 4 shows a mean serum concentration versus time plot of cisapride following an oral administration of cisapride (1.5 mg/kg) to rats.

Table 1
Reproducibility of cisapride in serum samples

Added amount (ng/ml)	Found amount (ng/ml)		CV (%)	
	Within-day[a]	Day-to-day[b]	Within-day[a]	Day-to-day[b]
5	5.0	5.2	3.7	3.7
10	10.1	9.9	3.4	3.5
50	48.1	49.6	2.2	1.2
100	100.6	100.1	0.7	0.5

[a] $n=4$.
[b] $n=6$.

4. Conclusion

To determine cisapride directly from serum sample, an automated microbore HPLC method using column switching has been developed and showed excellent sensitivity, reproducibility, specificity and speed. This method was successfully applied to evaluate the pharmacokinetics of cisapride in rats without time-consuming sample clean-up.

Acknowledgements

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