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Semi-micro high-performance liquid chromatographic analysis of tiropramide in human plasma using column-switching

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Abstract

A rapid and sensitive column-switching semi-micro high-performance liquid chromatography method was developed for the direct analysis of tiropramide in human plasma. The plasma sample (100 μl) was directly injected onto Capcell Pak MF Ph-1 precolumn where deproteinization and analyte fractionation occurred. Tiropramide was then eluted into an enrichment column (Capcell Pak UG C_{18}) using acetonitrile–potassium phosphate (pH 7.0, 50 mM) (12:88, v/v) and was analyzed on a semi-micro C_{18} analytical column using acetonitrile–potassium phosphate (pH 7.0, 10 mM) (50:50, v/v). The method showed excellent sensitivity (limit of quantification 5 ng/ml), and good precision (C.V. $\leqslant$ 5.9%), and speed (total analysis time 20 min). The calibration curve was linear in the concentration range 5–200 ng/ml (coefficient of determination equal to 0.998). This method was successfully applied to the pharmacokinetic study of tiropramide in volunteers.

Keywords: Tiropramide; Column-switching HPLC

1. Introduction

Tiropramide, α-(benzoylamino)-4-[2-(diethylamino)ethoxy]-*N*,*N*-dipropylbenzene-propanamide (Fig. 1), has been used as antispasmodic drug in the treatment of irritable colon and biliary dyskinesia [1,2]. Tiropramide was extensively metabolized to *N*-desethyltiropramide, *N*-desethyl-*N*-despropyltiropramide, *N*-despropyltiropramide and hydroxytiropramide via *N*-desethylation, *N*-despropylation, and hydroxylation at *N*-propyl after oral administration of tiropramide to rats [3] and human [4,5]. There are few methods described for the determination of tiropramide in biological fluids, include gas–liquid chromatography (GLC) [4] or GLC/mass spectrometry [5]. These methods required time-consuming sample clean-up procedures such as liquid–liquid extraction or solid-phase extraction prior to instrumental analysis. Therefore, the aim of the present study was to develop a rapid, sensitive and accurate semi-micro high-performance liquid chromatography (HPLC)

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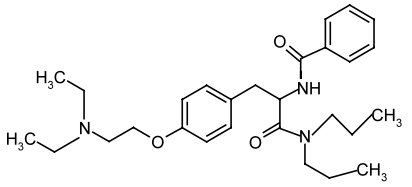

Fig. 1. Chemical structure of tiropramide.

method using column-switching for the determination of tiropramide in human plasma without pre-purification steps. The applicability of the assay method was demonstrated in the pharmacokinetic study of tiropramide in healthy volunteers.

2. Experimental

2.1. Materials and reagents

Tiropramide hydrochloride and Tiropa® tablets (100 mg) were obtained from Daewong Pharm. Co. (Seoul, Korea). HPLC grade methanol and acetonitrile were purchased from Burdick & Jackson, Inc. (Muskegon, MI). Stock solution of tiropramide (1 mg/ml) was prepared by dissolving tiropramide hydrochloride in water and aliquots were spiked to drug-free human blank plasma to obtain the calibration plasma standards at six concentrations of 5, 10, 20, 50, 100 and 200 ng/ml. Plasma samples were filtered with low protein binding membrane syringe filter (0.22 μm, PVDF, Millipore, Bedford, MA).

2.2. Chromatographic conditions

The schematic diagram of the column-switching system for semi-micro LC is shown in Fig. 2. The system consisted of the Nanospace SI-2 series (Shiseido, Tokyo, Japan), i.e. two 3001 pumps, a 3002 UV–VIS detector, a 3023 auto-injector, a 3004 column oven, a 3012 high pressure switching valve and a 3009 degassing unit. The system was operated by Syscon (Shiseido) and the signals were processed by S-MicroChrom (Shiseido). The columns used in this on-line extraction system were a precolumn (Capcell Pak MF Ph-1, 20 × 4 mm I.D., 5 μm, Shiseido, polymer-coated mixed function precolumn), an enrichment column (Capcell Pak C_{18} UG 120, 35 × 2 mm I.D., 5 μm) and an analytical column (Capcell Pak C_{18} UG 120, 250 × 1.5 mm I.D., 5 μm).

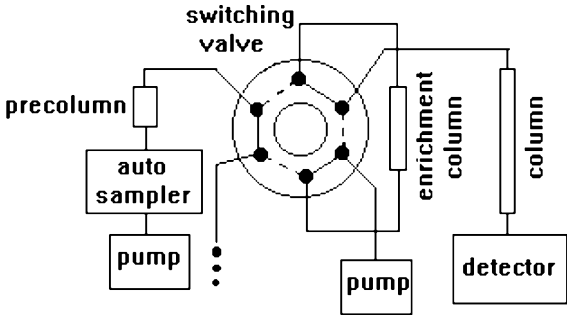

Fig. 2. Schematic diagram of a column-switching semi-micro HPLC. – – – – –, position A; --------, position B.

The mobile phase for the primary separation of tiropramide in the precolumn and concentration in the enrichment column was acetonitrile–potassium phosphate (pH 7.0, 50 mM) (12:88, v/v) with a flow rate of 0.5 ml/min. The mobile phase for the analytical column was acetonitrile–potassium phosphate (pH 7.0, 10 mM) (50:50, v/v) at a flow rate of 0.1 ml/min. The precolumn and the analytical column were maintained at 30 °C and the enrichment column was run at room temperature. The detection wavelength was 230 nm.

2.3. Analytical procedure

The operation of this column-switching semi-micro HPLC consists of three main steps: sample loading and primary separation, enrichment of the analyte fraction and chromatographic separation [6–9].

When the column-switching valve was at the A position, an aliquot of filtered plasma sample (100 μl) was loaded to precolumn and primary separation of tiropramide from plasma proteins were performed using acetonitrile–potassium phosphate (pH 7.0, 50 mM) (12:88, v/v). Subsequently, the valve was switched to the B position and tiropramide fraction was eluted from the precolumn and concentrated in the enrichment column by acetonitrile–potassium phosphate (pH 7.0, 50 mM) (12:88, v/v). Position B was maintained from

4.5 to 7.5 min after injection of plasma sample. Then, the valve position was returned to A and tiropramide concentrated in the enrichment column were separated on an analytical column using acetonitrile–potassium phosphate (pH 7.0, 10 mM) (50:50, v/v) at a flow rate of 0.1 ml/min (7.5–18 min).

2.4. Method validation

Limit of quantitation (LOQ) for tiropramide was determined as the concentration of drug giving a signal to noise ratio greater than 5:1. The recovery of tiropramide from plasma was determined by the analysis of fixed amounts of tiropramide in plasma, followed by replicate injection of the same amount of a standard in 5 μl mobile phase directly onto the analytical column providing the 100% value. Six tiropramide-spiked plasma standard samples over the concentration range of 5–200 ng/ml were quantified to evaluate the linearity, precision (the coefficient of variation (C.V.) of replicate analysis) and accuracy (the bias between theoretical and actual concentration). Within-day and day-to-day precision and accuracy were evaluated from six experiments in a day and six consecutive days, respectively.

2.5. Pharmacokinetics of tiropramide in human

Fourteen healthy male volunteers (age: 25.3 ± 2.8 years), fasted for 12 h, received a single oral dose of tiropramide tablet (100 mg, Tiropa® tablets) with 20 ml of water. Blood samples (1 ml) were withdrawn from the forearm vein at 0.5, 1, 1.5, 2, 3, 4, 6 and 10 h post dosing, transferred to Vacutainer tubes and centrifuged. Following centrifugation ($3000 \times g$, 15 min, 4 °C), plasma samples were transferred to eppendorf tubes and stored at −70 °C prior to analysis. Drug concentrations were determined as the mean of duplicate samples. The peak concentration (C_{max}) and the time to peak concentration (T_{max}) were determined by visual inspection from each volunteer's plasma concentration–time plots for tiropramide. Area under the plasma concentration–time curve (AUC) was calculated by the linear trapezoidal method from 0 to 10 h. Plasma elimination half-life ($t_{1/2}$) was determined from the descending slope of the concentration–time profile after logarithmic transformation of the concentration data.

3. Results and discussion

No HPLC method for the determination of tiropramide in biological samples has previously been reported. In this study, semi-micro reverse-phase HPLC method using column-switching for the direct analysis of tiropramide in plasma samples was chosen because of some advantages such as high sensitivity (0.5 ng tiropramide), small sample volume (100 μl), no sample pre-purification step, reliability and lower organic solvent consumption.

In column-switching [6–9], the choice of the precolumn packing, washing solvent and column-switching time is crucial in order to obtain complete recovery and clean chromatograms. A polymer-coated mixed function Capcell Pak MF Ph-1, which possesses long polyoxyethylene chains and phenyl groups on the surface of 80 Å silica to limit the access of large molecules while retaining analytes longer [6], was used as the precolumn. The separation profile of tiropramide in plasma on the precolumn was evaluated using acetonitrile–potassium phosphate (pH 7.0, 50 mM) (12:88, v/v) to obtain the good recovery and to determine the appropriate time for column-switching. Tiropramide was retained in the precolumn during the exclusion of the plasma proteins which was seen at the position of void volume and then, the peak of tiropramide appeared from 4.5 to 7.5 min. Therefore, the eluate of precolumn from 4.5 to 7.5 min after injection of plasma sample was transferred to the enrichment column by switching the valve to B position. The enrichment column was used in order to save the analysis time and protect the precolumn from high pressure. Without an enrichment column, it might take 15 min to transfer tiropramide fraction from the precolumn to analytical column at a flow rate of 0.1 ml/min.

Typical chromatograms of blank plasma, plasma spiked with tiropramide and plasma sam-

ple obtained after a single oral administration of tiropramide tablet to a volunteer are shown in Fig. 3. There was no interference peak at the retention time of tiropramide (13.5 min).

MF Ph-1 precolumn was exchanged after injection of 35 plasma samples (equivalent to 3.5 ml plasma). The enrichment and analytical columns showed no decrease in efficiency after the analysis of more than 300 plasma samples.

The correlation of peak area with the concentrations of tiropramide in plasma was linear over the range 5–200 ng/ml. Within-day linear regression for tiropramide ($n = 6$) was $y = 764\ (\pm 50)x + 805\ (\pm 547)$ [y: peak area, x: concentration of tiropramide (ng/ml)] with coefficient of determination (r^2) of 0.999 (± 0.001) and day-to-day regression equation ($n = 6$) was $y = 773\ (\pm 51)x + 137\ (\pm 128)$ with r^2 of 0.998 (± 0.001). Relative standard deviation for the slope and determination coefficient were smaller than 6.6 and 0.1%, respectively. LOQ for tiropramide was 5 ng/ml with 100 μl plasma and sufficient for the therapeutic monitoring of tiropramide. Mean absolute recovery of tiropramide from plasma samples was $92.5 \pm 3.5\%$ ($n = 5$). The within-day and day-to-day precision and accuracy of the assay were shown in Table 1. Actual amount was deviated from −2 to 1.8% of the theoretical amount in the spiked plasma samples and the assay was precise because C.V. was less than 5.9%.

The suitability of this method was proved in the pharmacokinetic study of tiropramide in man. Fig. 4 shows plasma concentration–time plot of tiropramide after a single oral dose of commercial tiropramide tablet (100 mg) to 14 healthy male volunteers. The pharmacokinetic parameters, such as AUC, C_{max}, T_{max}, and $t_{1/2}$ of tiropramide were 377 ± 220 ng h/ml, 111 ± 62 ng/ml, 1.6 ± 0.6 h, and 6.3 ± 2.0 h, respectively. These values are comparable to the corresponding parameters obtained by single oral dose of 100 mg tiropramide in the report of Arigoni et al. [4].

In conclusion, an automated semi-micro HPLC method using column-switching without time-consuming sample clean-up steps has been described for the direct analysis of tiropramide from human plasma samples. This method showed the excellent sensitivity (5 ng/ml using 100 μl plasma), reproducibility, specificity, and speed (20 min/sample). The applicability of the method was

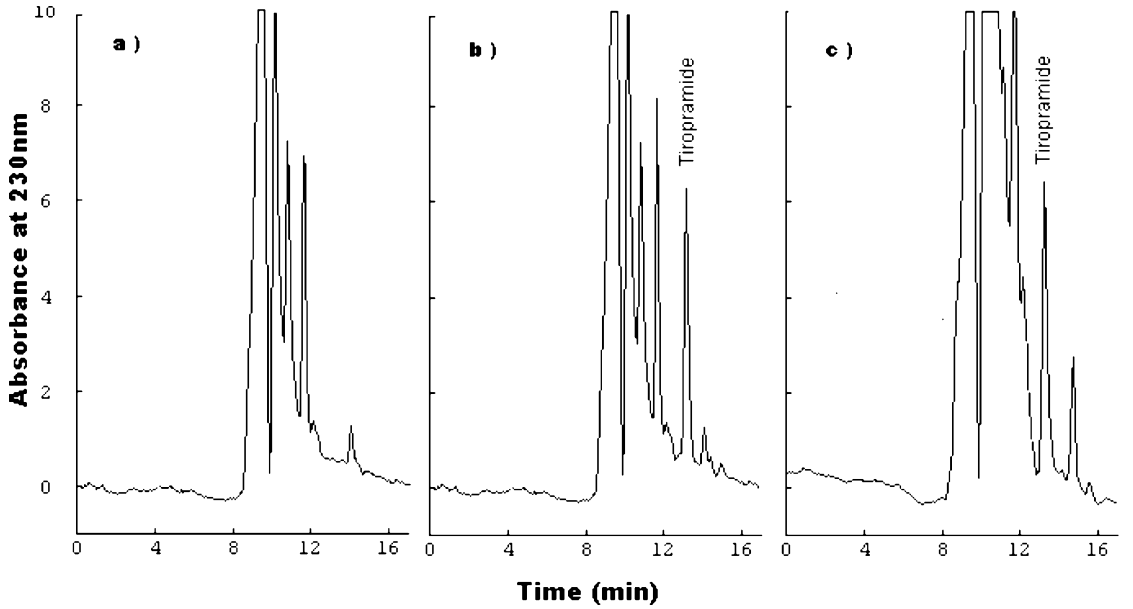

Fig. 3. Representative chromatograms of (a) blank plasma, (b) blank plasma spiked with tiropramide (50 ng/ml), and (c) plasma sample at 4 h after an oral dosing of 100 mg tiropramide to a healthy male volunteer.

Table 1
Precision and accuracy of tiropramide in human plasma samples ($n = 6$)

Theoretical concentration (ng/ml)	Concentration found (ng/ml)		C.V. (%)	
	Within-day	Day-to-day	Within-day	Day-to-day
5	5.1	4.9	5.1	5.9
10	10.1	10.3	4.8	5.0
20	19.8	20.3	3.4	3.5
50	50.9	51.1	2.7	3.4
100	99.8	100.0	2.9	3.3
200	198.0	199.3	1.9	2.1

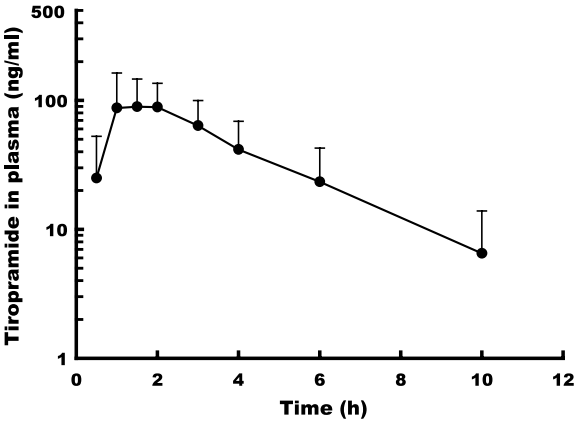

Fig. 4. Mean plasma concentration–time plot of tiropramide after a single oral dose of tiropramide (100 mg) to 14 male volunteers. Each point represents the mean ± S.D.

demonstrated in the study of pharmacokinetic disposition of tiropramide in man.

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