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Column-switching high-performance liquid chromatographic method for the determination of zaltoprofen in rat plasma

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Abstract

A direct injection column-switching high-performance liquid chromatography (HPLC) method was developed and validated for quantification of zaltoprofen in rat plasma. Following dilution with mobile phase A, i.e. acetonitrile-10 mM potassium phosphate buffer (pH 6.8) (12:88, v/v) samples were directly injected to the pre-column without sample pre-purification step. After endogenous plasma components were eluted to waste, the system was switched and the analyte was eluted to the trap column. Zaltoprofen was then back-flushed to the analytical column for separation with mobile phase B, i.e. acetonitrile–10 mM potassium phosphate buffer (pH 6.8) (35:65, v/v) and quantification with an ultraviolet detector at 230 nm. The calibration curve was linear in the concentration range of 40–5000 ng mL^{-1}. This method has been fully validated and shown to be specific, accurate and precise. The method is simple, rapid and the sample preparation is minimal and appears to be useful for the pharmacokinetic study of zaltoprofen.

Keywords: Zaltoprofen; Column switching; High-performance liquid chromatography (HPLC); Rat plasma; Direct injection

1. Introduction

Sample extraction procedure before any chromatographic analysis in bioanalysis remains the major challenge for the determination of drugs in biological matrix. Numerous laborious and time consuming off-line sample clean-up procedures based on liquid–liquid extraction (LLE) or solid-phase extraction (SPE) have been reported to get rid of some interferences. And besides, there are literatures introducing not only turbulent flow chromatography (TFC) which is a relatively new technique used for rapid extraction and analysis of drugs from bioanalytical fluids but also restricted-access media (RAM) using technicque that is a relatively new one, uses interaction between RAM and small analytes and exclusion of big molecules [1–3]. Nowadays, due to the increasing demand for automation and high throughput analysis, on-line sample preparation procedures are increasingly used [4]. And especially, column-switching systems via a switching valve have great advantages in permitting the direct injection of protein rich samples, such as plasma [5–7]. In case of non-steroidal anti-inflammatory drugs (NSAIDs) used for the treatment of rheumatoids (RA), some drug substances such as naproxen, ibuprofen and ketoprofen have been reported about the bioanalytical method using column-switching technique [4,8–11]. Some of NSAIDs cause adverse reactions such as gastrointestinal impairment disqualifying them from long-term administration [10,12]. In order to eliminate adverse reactions on the gastrointestinal tract, 2-(10,11-dihydro-10-oxodibenzo[b,f] thiepin-2-yl) propionic acid (zaltoprofen) (Soleton®; Nippon Chemiphar Co., Japan) was recently developed (Fig. 1) [10,13,14]. Its anti-inflammatory analgesic efficacy and safety have been confirmed by various human or animal experiments [10,11,13–15]. Nevertheless, there is no known study published in the literature that provides information on the bioanalytical method of zaltoprofen in plasma.

To minimize the possible introduction of analytical artifacts through excessive sample manipulation, an analytical method

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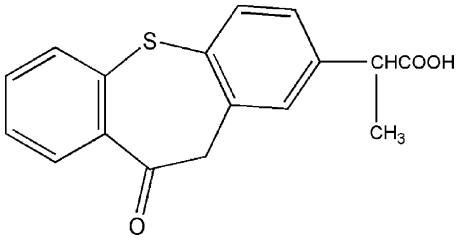

Fig. 1. The chemical structure of 2-(10,11-dihydro-10-oxodibenzo[b,f] thiepin-2-yl) propionic acid (zaltoprofen).

was developed and validated in our laboratory using high-performance liquid chromatography with column switching [5,7,16]. The column switching technique avoids off-line procedures for sample clean-up [5,6,17]. This method allowed plasma samples to be directly injected onto a fully automated HPLC system, thereby resulting in an accurate assay without internal standards. The method was validated in the concentration range of 40–5000 ng mL^{-1} in plasma.

2. Experimental

2.1. Chemicals

Zaltoprofen was kindly donated by CJ Pharm. Co. (Seoul, Korea). HPLC grade acetonitrile was purchased from J.T. Baker (Deventer, The Netherlands). Analytical grade dipotassium hydrogenphosphate and potassium dihydrogenphosphate were obtained from Wako (Osaka, Japan). Water was purified with a Ultimate Reverse Osmosis system (Barnstead, Dubuque, USA). And the others used as reagents were of analytical or HPLC grade.

2.2. Equipments

The semi-microcolumn SI-2 nanospace HPLC system (Shiseido, Tokyo, Japan) consisted of two 3001 pumps, a 3023 autosampler with a sample cooler, a 3004 column oven, a UV–vis 3002 detector, a two-flow channel 3009 degasser and a high pressure six-port switching 3012 dual valve. The system included a pre-column, a trap column and an analytical column. The chromatograph, switching valves and autosampler were controlled by a S-MicroChrom 21 software (Shiseido, Tokyo, Japan) as a fully automated system. Data were acquired and processed on the software.

2.3. Chromatographic conditions

Chromatographic separation was achieved on a 150 mm × 1.5 mm, 5 μm Capcell pak C_{18} column (Shiseido, Tokyo, Japan) with a 50 mm × 4.6 mm, 5 μm Capcell pak mixed-funtional (MF) ph-1 pre-column (Shiseido, Tokyo, Japan) and a 35 mm × 2.0 mm, 5 μm Capcell pak C_{18} trap column. The column temperatures of the pre-column and the analytical column were maintained at 40 °C, and the trap column was operated at ambient temperature. The mobile phase A (Pump A) was acetonitrile-10 mM potassium phosphate buffer (pH 6.8) (12:88, v/v) and the mobile phase B (Pump B) was acetonitrile-10 mM potassium phosphate buffer (pH 6.8) (35:65, v/v). The flow rate of mobile phase A was 500 μl min^{-1} and mobile phase B was 100 μl min^{-1}. The autosampler was set with an injection volume of 40 μl, a rack temperature of 4 °C and a run time of 15 min. The absorbance wavelength on the UV detector was set at 230 nm.

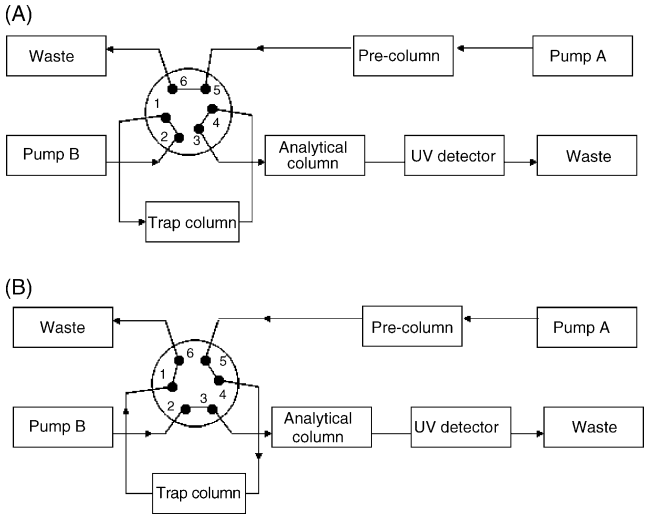

Fig. 2. Schematic diagram of the column switching HPLC system using a six-port switching valve. Step 1: valve A, step 2: valve B, step 3: valve A.

2.4. Column-switching procedure

Fig. 2 shows a schematic diagram of the automated system. The system was operated according to the following procedure where valve positions and switchover times are in parentheses.

Step 1 (valve A; 0–3 min): a plasma sample is injected onto the pre-column and the pre-column was washed by mobile phase A at a flow rate of 500 μl min^{-1} in order to remove plasma proteins and other endogenous interferences. Data acquisition starts in detector. Step 2 (valve B; 3–5 min): the valve was switched from positions A–B and the enriched compounds were eluted from pre-column to trap column in the back-flush mode. Step 3 (valve A; 5–13 min): afterwards, the flow path of the mobile phase was returned to the initial condition and enriched compounds were eluted from trap column to analytical column for separating the analyte in the analytical column from co-eluted endogenous compounds with the mobile phase B.

2.5. Stock solution and sample preparation

A amount of about 1.0 mg of zaltoprofen was accurately weighed into volumetric flask and diluted to 10 mL volume with methanol to obtain stock solution (100 μg ml^{-1}). Stock solution was made for the preparation of calibration standards. The concentrations of zaltoprofen to be used for the preparation of calibration standards were: 80, 300, 1500, 4000, 7000 and 10,000 ng mL^{-1}. They were obtained from serial dilution of stock solution with mobile phase A. The diluted standard

solutions were spiked with the plasma samples (50:50). Final concentrations for calibaration standards were 40, 150, 750, 2000, 3500 and 5000 ng mL^{-1}. After then, this mixtures were centrifuged at 1000 × *g* at 4 °C for 15 min. The supernatants were analyzed without sample clean up procedure in off-line.

2.6. Validation study

The method was validated prior to the analyses of rat plasma samples according to the guidance of bioanalytical method validation [18]. The limit of quantification, specificity, linearity, accuracy, precision and stability of zaltoprofen in plasma sample were assessed and evaluated.

2.6.1. Accuracy and precision

The intra-day accuracy (% Bias) and precision (coefficient of variation; CV) were estimated by analyzing five replicates containing zaltoprofen at four different concentrations i.e. 40, 150, 2000 and 5000 ng mL^{-1} in a day. The inter-day accuracy (%) and precision (CV) were determined by analyzing five replicates at the four different concentrations within a week. The acceptance criteria for intra-day and inter-day accuracy and precision is below 15% bias or CV except at the lower limit of quantitation (LLOQ), for which bias and precision should be below 20%.

2.6.2. Linearity

The linearity of the method was evaluated by analysis of standard curve samples, which were prepared by adding blank plasma into the working standard solutions. The final concentrations of zaltoprofen in the final prepared samples were 40, 50, 100, 150, 500, 750, 1000, 2000, 3500 and 5000 ng mL^{-1}. Calibration curve was generated by plotting peak areas against drug concentrations. The coefficient of determination (r^2) was determined.

2.6.3. Stability

The stability tests of zaltoprofen in plasma were carried out by evaluating the freeze and thaw stability, short, long-term temperature stability, stock solution stability and post-preparative stability. The tests of stability were assessed using two concentrations of samples, i.e. 150 and 2000 ng mL^{-1} as low, high concentration and at least three aliquots. In the freeze and thaw stability test, the samples were stored at −74 °C between three freeze/thaw cycles for 24 h and the samples were thawed by allowing them to stand (unassisted) at room temperature for approximately an hour. The short-term stability was assessed after storage at room temperature for 4–24 h and long-term stability was assessed after storage of samples more than longer period of last sample analysis. Stock solution of zaltoprofen was kept at room temperature for 6 h and then analyzed. The post-preparative stability was evaluated about the stability of sample in the autosampler.

The samples were processed using the same procedure as described in the sample preparation section. Samples were considered stable if assay values were within the acceptable limits of accuracy (i.e. ±15%).

2.7. Animal study

Male Sprague–Dawley (SD) rats (body weight: 250–300 g) were kept under conventional conditions of animal house and experiments started after a week of acclimatization period. Rats were fasted overnight before the dosing day and they had free access to food and water after 4 h of post dosing. Animals were anesthetized with an inhalation of ether. Zaltoprofen was administered orally at a dose of 1.0 mg kg^{-1} body weight as a suspension (in 1.0% sodium carboxymethylcellulose). Blood samples were collected approximately 0.2–0.25 mL at 0, 0.08, 0.25, 0.5, 1, 1.5, 2, 4, 8, 12, 24, 32 and 48 h post-dosing. Heparinized blood samples were harvested by centrifugation (Micro12, Han-Il Science Industrial Co., Inchun, Korea) at 10,770 × *g* for 15 min and stored at −74 °C immediately until analysis. After plasma samples of rats were diluted with mobile phase A (50:50), the mixtures were centrifuged at 1000 × *g* at 4 °C for 15 min. The supernatants were analyzed without sample clean up procedure.

3. Results and discussion

3.1. Method development

The major issue in the development of a direct injection column-switching method for zaltoprofen in plasma was, considering the viscosity of blood sample and large size of endogenous components, the separation of zaltoprofen from endogenous components without sample pre-purification, namely direct injection, sensitivity, rapidity of analysis and the retention of the compound into the trap column. Several different factors were taken into account to optimize this parameter: columns with high efficiency stationary phases, mobile phases at differents pHs and with different percentages of organic solvent [5,8,16,19]. High organic modifier solvent contents may cause buffer precipitation which can be the cause of clogging in pre-column and tubing. To avoid protein precipitation and get appropriate retention time, the concentration of the organic modifier and the ionic strength of the mobile phase applied in this chromatographic system was optimized. In addition, the contents of organic solvent in mobile phase B are much higher than mobile phase A, because enriched compounds trapped in the trap column can be eluted to analytical column.

When developing a column-switching method at first, the switching time must be determined. The elution profile of the sample matrix on the pre-column applied was determined by direct connection to the UV detector set at 230 nm. The column switching system finally developed provided on adequate clean up of plasma as shown by the absence of interfering peaks in blank plasma samples (Fig. 3). The selectivity of the assay was checked by measurement of drug-free plasma. No endogenous interferences were observed. The reduced and focused transferring time also limited the transfer of unwanted late eluting interfering compounds from the trap column to the analytical column.

The lower limit of quantification is 40 ng mL^{-1} (S/N = 5:1). And analyte peak was identifiable, discrete and reproducible

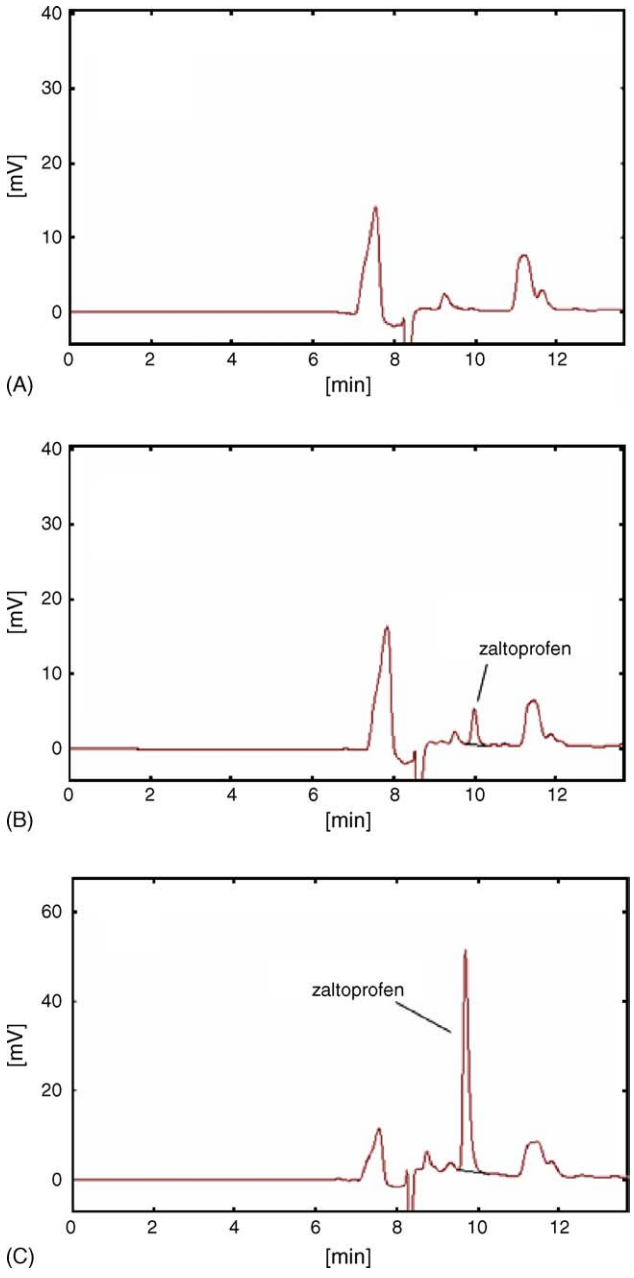

Fig. 3. Representative column-swtiching HPLC chromatograms of rat plasma samples. (A) Blank plasma; (B) plasma spiked with zaltoprofen (40 ng mL^{-1}); (C) plasma sample obtained 1 h after oral administraion of 1 mg kg^{-1} zaltoprofen to a rat.

with a precision of 20% and on accuracy of 80–120%. Fig. 3 shows typical chromatograms obtained from samples of blank rat plasma (A), plasma spiked with zaltoprofen (B) and rat plasma sample obtained at 1 h (C) after administration of 1 mg kg^{-1} of zaltoprofen to a rat. Visual examination of the HPLC-UV chromatograms of blank samples obtained during the validation, including the rat plasma from six different sources, indicated high specificity. The analytes were chromatographically resolved and no significant interferences from endogenous material were observed.

The calibration curve was generated by plotting peak areas against the concentrations of zaltoprofen at 40–5000 ng mL^{-1}. The coefficient of determination (r^2) was greater than 0.999.

3.2. Accuracy and precision

The accuracy and precision of HPLC data collected begin with a well-behaved chromatographic system. The system suitability specifications and tests were evaluated for providing assistance in achieving this purpose [18].

Table 1 shows the results of inter- and intra-day accuracy and precision of zaltoprofen. All the accuracy and precision of inter-day and intra-day were within the specified ranges and therefore acceptable [18,20].

3.3. Stability

Drug stability in a biological fluid is a function of the storage conditions, the chemical properties of the drug, the matrix and the container system. The analyte was stable during sample collection and handling, after short-, long-term storage, after going through freeze and thaw cycles and during the analytical process. Conditions used in stability experiments reflected situations likely to be encountered during actual sample handling and analysis. The stability of analyte in stock solution was also proven [18].

The analyte was found to be stable and the range of accuracy was from 94 to 115%. Zaltoprofen was shown to be stable in rat plasma at room temperature for at least 4 h and the post-preparative samples were stable in autosampler for at least 24 h (Table 2).

3.4. Application of the method

This method has been successfully applied to the analysis of rat plasma samples proving the advantage of the method devel-

Table 1
Accuracy and precision for the determination of zaltoprofen in rat plasma[a]

Concentration of zaltoprofen (ng mL^{-1})	Intra-day			Inter-day		
	Found (ng mL^{-1})	Accuracy (%)	Precision CV (%)	Found (ng mL^{-1})	Accuracy (%)	Precision CV (%)
40.0	43.0	107.5	0.8	36.3	90.7	5.9
150.0	154.6	103.1	0.3	146.3	97.6	6.7
2000.0	1949.1	97.5	1.0	1991.1	99.6	3.5
5000.0	5087.9	101.8	0.4	5009.3	100.2	2.5

[a] Number of replicates = 5.

Table 2
Stability of zaltoprofen in rat plasma[a]

Test of stability	Accuracy of zaltoprofen (%)	
	Low QCs[b]	High QCs[c]
Freeze and thaw	97.6	98.6
Short-term, temperature	98.6	97.1
Long-term	107.3	95.1
Stock solution	98.8	98.8
Post-preparative	99.1	98.6

[a] Number of aliquots = 3.
[b] Low QCs of low quality control samples are 150.0 ng mL^{-1}.
[c] High QCs of low quality control samples are 2000.0 ng mL^{-1}.

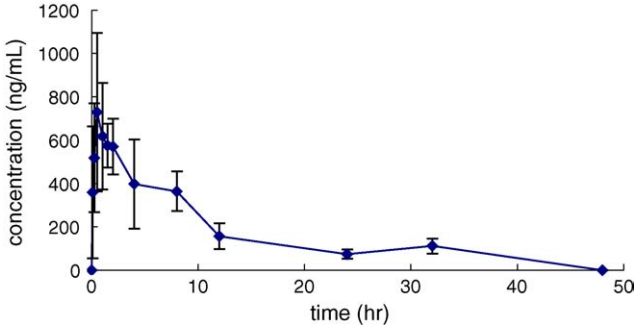

Fig. 4. Plasma concentration-time curve of zaltoprofen after oral administration of 1 mg kg^{-1} of zaltoprofen to male SD rats. Mean values (±S.D.) are given (n = 6).

oped in this study. Fig. 4 shows a plasma concentration curve obtained during animal studies in rats. The plasma concentration of zaltoprofen was found from 47.7 to 1286.8 ng mL^{-1}.

4. Conclusion

A direct injection column-switching HPLC method was developed for the quantification of zaltoprofen in rat plasma. The method is very simple and sample preparation is minimal. The method has been fully validated and shown to be sensitive, accurate, precise.

This method will be able to be useful for human pharmacokinetic studies of zaltoprofen.

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