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Direct and simultaneous analysis of loxoprofen and its diastereometric alcohol metabolites in human serum by on-line column switching liquid chromatography and its application to a pharmacokinetic study

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Abstract

A simple, rapid, and accurate column-switching liquid chromatography method was developed and validated for direct and simultaneous analysis of loxoprofen and its metabolites (*trans*- and *cis*-alcohol metabolites) in human serum. After direct serum injection into the system, deproteinization and trace enrichment occurred on a Shim-pack MAYI-ODS pretreatment column (10 mm × 4.6 mm i.d.) by an eluent consisting of 20 mM phosphate buffer (pH 6.9)/acetonitrile (95/5, v/v) and 0.1% formic acid. The drug trapped by the pretreatment column was introduced to the Shim-pack VP-ODS analytical column (150 mm × 4.6 mm i.d.) using acetonitrile/water (45/55, v/v) containing 0.1% formic acid when the 6-port valve status was switched. Ketoprofen was used as the internal standard. The analysis was monitored on a UV detector at 225 nm. The chromatograms showed good resolution, sensitivity, and no interference by human serum. Coefficients of variations (CV%) and recoveries for loxoprofen and its metabolites were below 15 and over 95%, respectively, in the concentration range of 0.1–20 μg/ml. With UV detection, the limit of quantitation was 0.1 μg/ml, and good linearity ($r = 0.999$) was observed for all the compounds with 50 μl serum samples. The mean absolute recoveries of loxoprofen, *trans*- and *cis*-alcohol for human serum were 89.6 ± 3.9, 93.5 ± 3.2, and 93.7 ± 4.3%, respectively. Stability studies showed that loxoprofen and its metabolites in human serum were stable during storage and the assay procedure. This analytical method showed excellent sensitivity with small sample volume (50 μl), good precision, accuracy, and speed (total analytical time 18 min), without any loss in chromatographic efficiency. This method was successfully applied to the pharmacokinetic study of loxoprofen in human volunteers following a single oral administration of loxoprofen sodium (60 mg, anhydrate) tablet.

Keywords: Loxoprofen; Alcohol metabolites; On-line column switching

1. Introduction

Loxoprofen sodium, sodium (±)2-[4-(2-oxocyclopentylmethyl)phenyl]propionate dihydrate (Fig. 1), a 2-phenylpropionate non-steroidal anti-inflammatory drug (NSAID), has marked analgesic and antipyretic activities and relatively weak gastrointestinal ulcerogenicity [1]. The mechanism of action of loxoprofen is inhibition of prostaglandin biosynthesis by its action on cyclooxygenase. However, loxoprofen itself is not the major in vivo inhibitor. After oral administration, loxoprofen sodium is absorbed as the free acid rather than the sodium salt from the gastrointestinal tract, which causes only weak irritation of the gastric mucosa, and is then converted to an active metabolite by reduction of the ketone carbonyl to the *trans*-OH form. The active isomer has the 2*S*, 1′*R*, 2′*S* configuration (Fig. 1), which potently inhibits prostaglandin biosynthesis [2–4].

Several HPLC methods have been reported for analyzing loxoprofen in human plasma [5–7] and urine [6–9]. Prior to injection, sample pretreatment was required to remove protein and/or coupling with a chiral reagent. These pretreatment methods included precipitation by organic solvents, resulting in decreased efficiency, and there are no reports showing the simultaneous measurement of loxoprofen and its diastereometric alcohol metabolites by column-switching HPLC.

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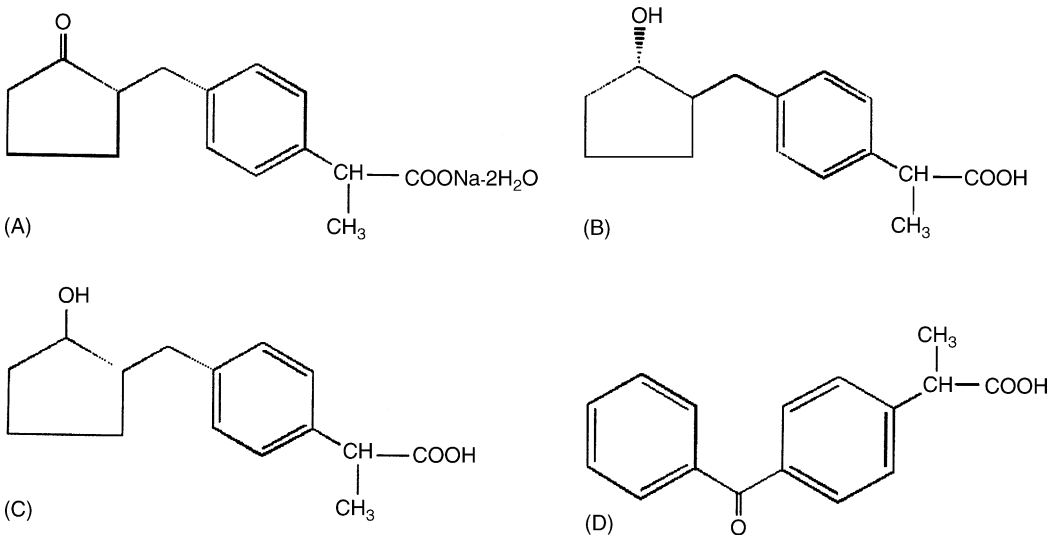

Fig. 1. Chemical structures of (A) loxoprofen sodium dihydrate, its (B) *trans*- and (C) *cis*-alcohol metabolites, and (D) ketoprofen (I.S.).

When the number of biological samples is particularly large, such as in bioavailability and pharmacokinetic studies, manual procedures [5–9] become tedious and time-consuming. However, the automated column-switching technique and on-line extraction system presented here is fast and simple to use, with sample enrichment and clean-up performed by on-line liquid-solid extraction via a short pretreatment column [10]. In addition, serum drug levels are easily determined by UV detector, without losing accuracy or sensitivity. The key factors that affect on-line column-switching include the stability of the bonded phase and the mobile phase composition used in elution and de-salting. A simple, rapid, sensitive, and reliable column-switching HPLC method for the determination of loxoprofen and its metabolites in human serum was required to examine their pharmacokinetics.

Our objective was to develop and validate a straightforward column-switching and UV detection system for the direct and simultaneous analysis of loxoprofen and its metabolites, where serum samples required only simple deproteinization before HPLC application. We then used this system to study the pharmacokinetics of loxoprofen in young adult male Korean volunteers following a single oral administration of loxoprofen sodium (60 mg, anhydrate) tablet.

2. Experimental procedures

2.1. Chemicals and reagents

Loxoprofen sodium (99.9%, Fig. 1A), (±)-2-[4-(*trans*)-(1′*R*,2′*S*)-2′-hydroxycyclo pentylmethyl]-phenyl]propionic acid (*trans*-alcohol, >95.0% purity, Fig. 1B), and (±)-2-[4-(*cis*)-(1′*R*,2′*R*)-2′-hydroxycyclo pentylmethyl]-phenyl]propionic acid (*cis*-alcohol, >95.0% purity, Fig. 1C) were kindly supplied by KyungDong Pharmaceutical Co. (Seoul, Republic of Korea). Ketoprofen (internal standard, Fig. 1D) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol and acetonitrile (HPLC grade) were purchased from Fischer Scientific (Fair Lawn, NJ, USA), and other chemicals were of HPLC grade or highest quality available. HPLC grade water was obtained from a Milli-Q water purification system (Millipore Co., Milford, MA, USA) and used throughout the study. The mobile phase components, such as potassium dihydrogenate phosphate, potassium monohydrogenate phosphate and ammonium acetate, were filtered through a 0.45-μm pore size membrane filter prior to mixing.

2.2. Instruments

The HPLC system consisted of a Shimadzu LC-VP system (Kyoto, Japan) equipped with pumps (model LC-10AD*vp*) and an autosampler (model SIL-HTC), a degasser (model DGU-14A), a column oven (model CTO-10AC*vp*), a UV/Vis detector (model SPD-10A*vp*), and Shimadzu CLASS-VP software. The instrument arrangement for the automated column-switching system and system flow diagram is shown in Fig. 2. The pretreatment column used for on-line sample preparation was the Shim-pack MAYI-ODS (50-μm particle size, 10 mm × 4.6 mm i.d., Shimadzu, Kyoto, Japan), using 20 mM phosphate buffer (pH 6.9)/acetonitrile (95/5, v/v). A Shim-pack VP-ODS column (5-μm particle size, 150 mm × 4.6 mm i.d., Shimadzu) was used as the main analytical column. The analytical mobile phase used acetonitrile/water (45/55, v/v) containing 0.1% formic acid. Detection was carried out at 225 nm with the UV detector. The column temperature was maintained at 30 °C.

2.3. Calibration standards and quality control samples

Stock solutions of loxoprofen, its *trans*- and *cis*-alcohols, and ketoprofen, were prepared in methanol at concentration of 1 mg/ml and kept at 4 °C. Serum calibration standards for loxoprofen and its metabolites were prepared at concentrations of 0.1, 0.5, 1, 5, 10 and 20 μg/ml in drug-free, pooled serum obtained from eight different volunteers. In the same manner, quality control (QC) samples at low (0.5 μg/ml of serum), medium (5 μg/ml

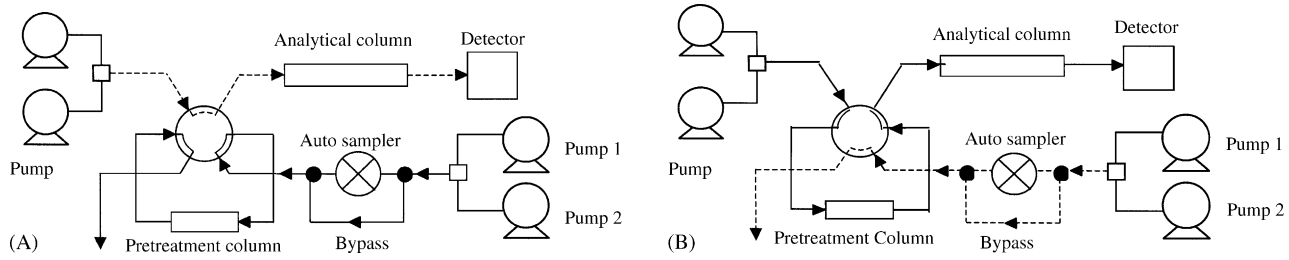

Fig. 2. Flow diagram of the column-switching system; (A) position A (pretreatment); (B) position B (analysis).

of serum), and high (10 μg/ml of serum) concentration, were prepared to evaluate accuracy and precision.

2.4. Analytical procedures

2.4.1. Step 1

To prepare the sample for assay, an aliquot of 100 μl of ketoprofen solution (100 μg/ml in methanol) was added to a serum sample (1 ml) by vortex-mixing for 30 s. Serum samples (1.1 ml) were filtered with a polyvinylidene-fluoride (PVDF) syringe filter (13 mm, 0.45-μm pore size, Millipore, Bedford, MA, USA) and transferred to autosampler vials. An aliquot (50 μl) of filtered sample was injected onto the pretreatment column by the autosampler. At the time of sample injection, the column-switching valve was placed in position A (Fig. 2A). Protein and other interfering compounds were eluted with 20 mM phosphate buffer (pH 6.9)/acetonitrile (95/5, v/v) and 0.1% formic acid at a flow rate of 0.3 and 2 ml/min, respectively. During this step, macromolecules such as proteins, which cannot enter the pore interior blocked by the water soluble polymer on the outer surface of pretreatment column, are easily eluted and not retained by the stationary phase. Other organic, low molecular weight compounds such as drugs, however, permeate into the pore interior and are retained by the stationary phase of the inner surface. The analytical column was filled with the analytical mobile phase, which was acetonitrile/water (45/55, v/v) containing 0.1% formic acid, at a flow rate of 1 ml/min.

2.4.2. Step 2

The column-switching valve was shifted to position B (Fig. 2B) at 3.0 min to move samples containing the target compounds from the pretreatment column to the analytical column. The analytical mobile phase was acetonitrile/water (45/55, v/v) containing 0.1% formic acid. The flow rate was 1.0 ml/min. During sample analysis, the pretreatment column line was washed with acetonitrile/50 mM ammonium acetate (60/40, v/v) at a flow rate of 1.0 ml/min by switching the valve in pump 2 (Fig. 2).

2.4.3. Step 3

The switching valve was shifted to position A (Fig. 2) again at 11 min after sample injection for disconnecting the pretreatment column from the analytical column. For 4 min, the analytical column was washed with a linear gradient of acetonitrile/water (45/55, v/v) containing 0.1% formic acid from 100 to 20%, and acetonitrile/water (80/20, v/v) from 0 to 80%. The total flow rate was 1 ml/min. From 15 to 18 min after sample injection, the mobile phase flowing into the analytical column was changed again to the analytical mobile phase at a flow rate of 1.0 ml/min by switching the valve in pump. Finally, the analytical column was equilibrated with the analytical mobile phase for 3 min. On the other hand, the pretreatment column was washed with acetonitrile/50 mM ammonium acetate (60/40, v/v) at a flow rate of 1.0 ml/min from 11 to 15 min after sample injection. From 15 to 18 min, the pretreatment column was equilibrated again with 20 mM phosphate buffer (pH 6.9)/acetonitrile (95/5, v/v) and 0.1% formic acid at a flow rate of 0.3 and 2 ml/min, respectively, by switching the valve in pump 2. The total running time was 18 min.

2.5. Method validation

2.5.1. Specificity

The interference by endogenous compounds was assessed by analyzing standards of loxoprofen, its *trans*- and *cis*-alcohol, each drug-free serum samples, serum spiked with loxoprofen and its metabolites, and serum samples obtained from subjects given loxoprofen sodium tablets. All peaks with the retention times of loxoprofen or its metabolites were confirmed using a photodiode array detector (SPD-10A*vp*, Shimazdu, Kyoto, Japan).

2.5.2. Sensitivity

The lower limit of quantitation (LLOQ) was defined as the lowest concentration yielding a precision with less than 20% (CV) and accuracy between 80 and 120% of the theoretical value. The LLOQ was 0.1 μg/ml for loxoprofen and its metabolites in nine replicate samples.

2.5.3. Linearity

The linearity of the calibration curve for loxoprofen and its metabolites was assessed in the range of 0.1–20 μg/ml in serum samples. Straight-line regression equations were weighted (weighting factor: 1/concentration) and are presented with their correlation coefficients. The *P*-value was calculated by SPSS program.

2.5.4. Precision and accuracy

In order to assess the intra- and inter-day precision and accuracy of the assay, QC samples were prepared as described above. The intra-day precision of the assay was assessed by calculating the coefficients of variation (CV) for the analysis of QC samples in five replicates, and inter-day precision was determined

through the analysis of QC samples on five consecutive days. Accuracy was determined by comparing the calculated concentrations to known concentrations with calibration curves.

2.5.5. Recovery

The absolute recoveries of loxoprofen and its metabolites from human serum were determined by comparing the peak area obtained using the pretreatment column to that obtained without using it.

The relative recoveries from human serum were assessed by comparison of the peak area from extracted QC samples to that from extracted samples made in water instead of serum. The mean recoveries were determined at low, medium, and high concentrations in five replicates.

2.5.6. Stability

To test the short- and long-term stability of these acidic drugs, two QC samples, one low (1 μg/ml of serum) and one high (10 μg/ml of serum), were stored under different conditions: at room temperature for 0, 4, or 24 h during the extraction; or at −80 °C for 1 month. Stock solution stability was tested at room temperature for 6 h in daylight. The effects of three freeze-thaw cycles on the stability of serum samples were determined with five aliquots each of low and high QC samples. The compounds were considered stable if assay variation was less than 10%.

2.6. Pharmacokinetic studies of loxoprofen

Twenty-four normal, healthy, male Korean volunteers (19–26 years, 54.9–79.6 kg) participated in the pharmacokinetic study of oral loxoprofen after giving written informed consent. All subjects fasted at least 10 h before drug administration and continued to fast up to 4 h thereafter. They abstained from consumption of alcohol or xanthine-containing foods and beverages during the study according to the FDA and KFDA (Korea Food and Drug Administration) regulation. Each volunteer received a single oral dose of a loxoprofen sodium (60 mg, anhydrate) tablet with 240 ml of spring water.

Blood samples were withdrawn from the forearm vein before oral administration and at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240, and 360 min after the oral administration, transferred to Vacutainer® (5 ml, Becton Dickinson and Company, Franklin Lakes, NJ, USA) tubes, and centrifuged. Following centrifugation (3000 *g*, 20 min), serum samples were transferred to polyethylene tubes and immediately stored at −80 °C until analysis.

Pharmacokinetic parameters were calculated by non-compartmental analysis of serum concentration-time curve data using WinNonlin software (Pharsight Corporation, Mountain View, CA, USA) [11]. The peak concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined by individual serum concentration-time profiles for loxoprofen and its metabolites. The area under the serum concentration-time curve ($AUC_{0\text{-}t}$) was calculated by the linear trapezoidal rule from 0 to 360 min. The area under the serum concentration-time curve from zero to time infinity ($AUC_{0\text{-}\infty}$) was calculated as $AUC_{0\text{-}t} + C_t/\lambda_Z$, where C_t is the last measurable concentration. The terminal half-life ($t_{1/2}$) was calculated as $0.693/\lambda_Z$, where λ_Z is terminal rate constant.

3. Results and discussion

3.1. HPLC and column-switching procedure

On-line extraction was accomplished by combining a two-pump HPLC system with a column-switching valve, a pretreatment column, and an analytical column. The autosampler and the two pumps were used to load 50 μl of serum containing loxoprofen, its metabolites and I.S. onto the pretreatment column Shim-pack MAYI-ODS. The reservoir of pump 1 contained 0.1% formic acid in water (v/v) as diluted solution, and pump 2 contained 20 mM phosphate buffer (pH 6.9)/acetonitrile (95/5, v/v) as pretreatment mobile phase or acetonitrile/50 mM ammonium acetate (60/40, v/v) as washing solution. Pump 2 delivered the pretreatment mobile phase for 3 min, followed by washing solution. At 3.0 min after injection, the column-switching valve was shifted to position B, and an analytical mobile phase (acetonitrile/water (45/55, v/v) containing 0.1% formic acid) was directed in reverse through the pretreatment column. The focused analytes were then chromatographed on the analytical column and detected on the UV absorbance detector (225 nm). The column-switching valve was shifted to position A again at 11 min post-injection to condition the pretreatment column and await the next injection.

Although column-switching preparation allows for direct analysis and preconcentration of an analyte, an additional isolation procedure may be necessary to prevent other problem, such as damage to the liquid chromatography system due to protein [10]. Therefore, a simple deproteinization (0.1% formic acid in water, v/v) was applied to prolong the life of the pre-

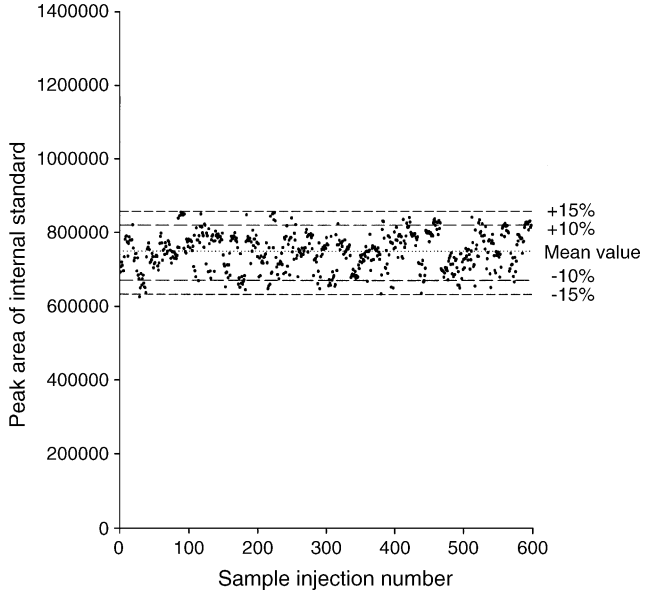

Fig. 3. Scatter plot of sample injection number vs. peak area of internal standard (10 μg/ml).

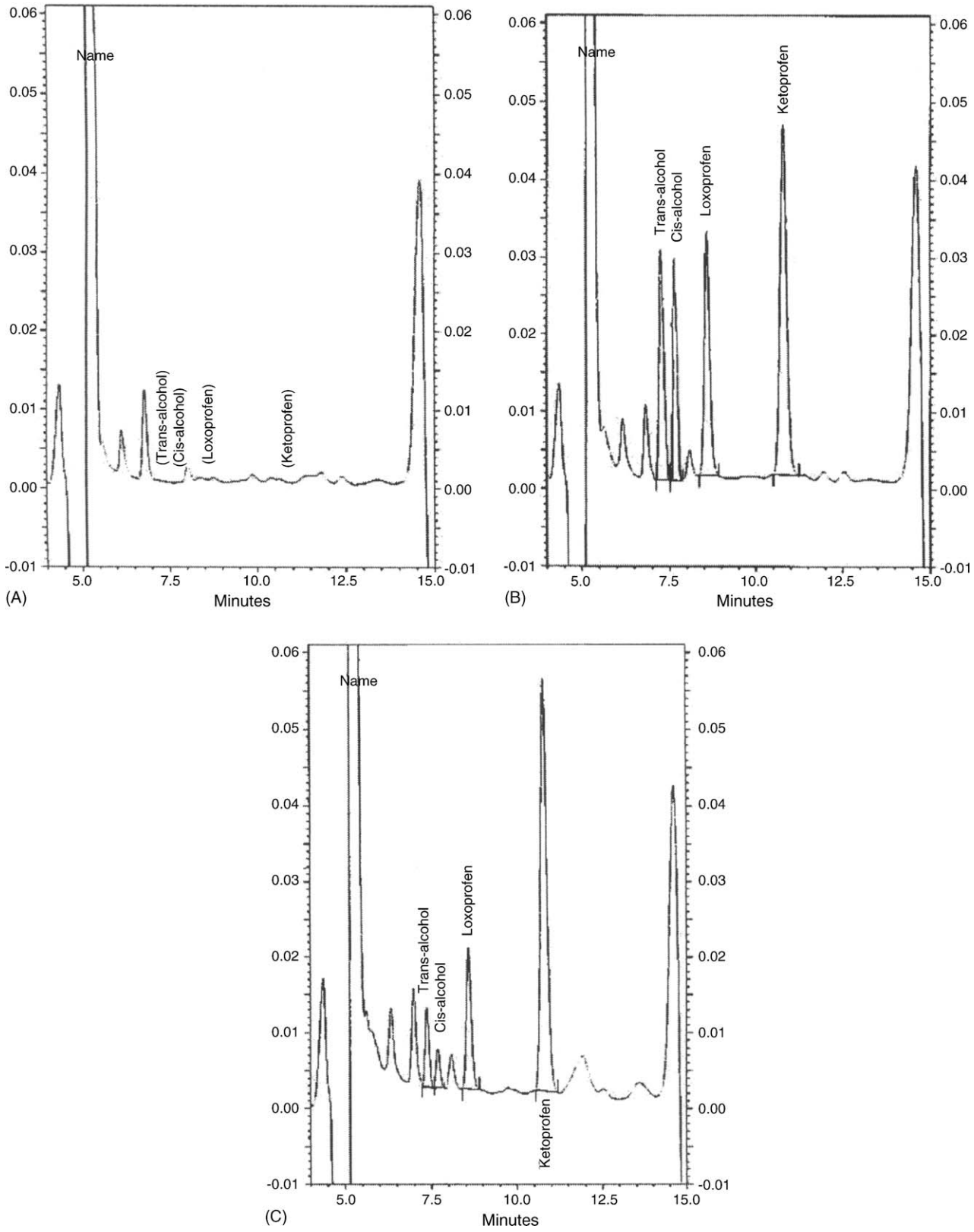

Fig. 4. Chromatograms of (A) blank human serum; (B) blank human serum spiked with loxoprofen (5 μg/ml), *trans*- (5 μg/ml) and *cis*-alcohol (5 μg/ml) containing internal standard (I.S., ketoprofen 10 μg/ml); and (C) human serum sample (2.15, 1.17 and 3.19 μg/ml of *trans*- and *cis*-alcohol and loxoprofen) at 1 h after a single oral dose of loxoprofen sodium (60 mg, anhydrate) tablet to a volunteer.

Table 1
Precision and accuracy of HPLC analysis of loxoprofen and its metabolites in human serum samples

Intra-day ($n=5$)				Inter-day ($n=5$)			
Target conc. (μg/ml)	Concentration found (Mean ± S.D.)	CV (%)	Accuracy (%)	Target conc. (μg/ml)	Concentration found (Mean ± S.D.)	CV (%)	Accuracy (%)
Loxoprofen							
0.5	0.51 ± 0.04	7.84	102.63	0.5	0.48 ± 0.03	6.25	96.12
5	5.17 ± 0.11	2.12	103.44	5	4.83 ± 0.34	7.08	96.58
10	10.54 ± 0.35	3.32	105.41	10	9.75 ± 0.62	6.32	97.54
Trans-alcohol							
0.5	0.49 ± 0.02	4.08	98.21	0.5	0.50 ± 0.02	4.11	99.75
5	5.09 ± 0.32	6.29	101.83	5	4.98 ± 0.27	5.42	99.60
10	10.04 ± 0.51	5.09	100.43	10	9.88 ± 0.71	6.32	97.54
Cis-alcohol							
0.5	0.51 ± 0.02	3.92	101.98	0.5	0.49 ± 0.03	6.12	99.78
5	4.99 ± 0.23	4.62	99.41	5	5.00 ± 0.38	7.62	100.22
10	10.11 ± 0.42	4.06	101.14	10	10.01 ± 0.65	7.11	101.02

treatment column. No striking change was observed even after more than 600 serum samples (50 μl each) had been analyzed by this pretreatment column (Fig. 3). Subsequent development used an acidic mobile phase (acetonitrile/water (45:55, v/v) containing 0.1% formic acid) to improve assay sensitivity. The acidic mobile phase accelerated the elution of the analytes, sharpened the peaks, and enhanced their sensitivity.

A heart-cut technique using column-switching HPLC is a very simple and efficient method. The Shim-pack VP-ODS column with a mobile phase of acetonitrile/water (45/55, v/v) containing 0.1% formic acid resulted in short chromatographic run times (18 min) with satisfactory separation of loxoprofen, its metabolites and I.S. The retention times for *trans*- and *cis*-alcohol, loxoprofen, and I.S. were approximately 7.2, 7.6, 8.5, and 10.8 min, respectively (Fig. 4).

3.2. Specificity

Fig. 4 shows typical chromatograms for blank serum, blank serum spiked with loxoprofen, its metabolites, and I.S., and a serum sample taken from a human subject 1 h after the oral administration of loxoprofen sodium (60 mg, anhydrate) tablet. Endogenous compounds, such as proteins, were eluted by the pretreatment column and the focused analytes were separated rapidly by the analytical column, with complete baseline resolution between peaks of interest. We confirmed the identical peak spectrum with the diode array detector. No chromatographic interference derived from endogenous substances or system peaks were observed.

3.3. Sensitivity

The lower limit of quantitation (LLOQ) was defined as those quantities that were 10-fold of the background noise, with precision errors of less than 20% (CV), and accuracy between ±20% (bias). The LLOQ for serum samples was 0.1 μg/ml for all three compounds. The mean accuracy of loxoprofen and the *trans*- and *cis*-alcohols in serum samples were 107.89, 96.24, and 92.38%, with CV of 3.11, 6.24 and 9.52% at the LLOQ, respectively.

3.4. Linearity

The calibration curves for loxoprofen and its metabolites were linear over the concentration range of 0.1–20 μg/ml in human serum. The least squares regression was weighted (weighting factor: 1/concentration) and presented with correlation coefficients (r). The mean (±S.D.) regression equations from nine replicate calibration curves on different days for human serum: $y=(0.1090\pm0.00373)x-(0.0018\pm0.00054)$ for loxoprofen, $y=(0.0877\pm0.00315)x-(0.0011\pm0.00035)$ for *trans*-alcohol, and $y=(0.0858\pm0.00336)x-(0.0025\pm0.00053)$ for *cis*-alcohol (where, y = peak-area ratio, x = concentration), showed significant linearities ($r=0.9999$, 0.9994 and 0.9983, $P<0.01$, respectively).

Table 2
Absolute and relative recoveries of loxoprofen and its metabolites from human serum

Concentration (μg/ml)	Absolute recovery (%, mean ± S.D., $n=5$)	Relative recovery (%, mean ± S.D., $n=5$)
Loxoprofen		
0.5	89.3 ± 2.8	95.2 ± 7.0
5	85.9 ± 1.9	93.8 ± 5.5
10	92.4 ± 4.6	95.4 ± 5.1
Trans-alcohol		
0.5	94.3 ± 3.1	95.5 ± 5.2
5	92.0 ± 2.1	97.8 ± 4.9
10	94.4 ± 4.7	99.9 ± 7.4
Cis-alcohol		
0.5	92.2 ± 2.2	94.0 ± 6.0
5	95.6 ± 4.1	93.3 ± 4.9
10	93.3 ± 6.5	94.7 ± 5.7

Table 3
Pharmacokinetic parameters of loxoprofen and its metabolites after a single oral administration of loxoprofen sodium (60 mg, anhydrate) tablet to human volunteers ($n = 24$, mean ± S.D.)

Pharmacokinetic parameters	Loxoprofen	*Trans*-alcohol	*Cis*-alcohol
C_{max} (μg/ml)	4.78 ± 0.99	2.36 ± 0.46	0.73 ± 0.17
T_{max} (min)	27.7 ± 4.39	51.92 ± 9.90	64.62 ± 28.32
$t_{1/2}$ (min)	64.46 ± 9.68	94.91 ± 23.47	187.68 ± 67.16
$AUC_{0-\infty}$ (μg min/ml)	446.37 ± 100.08	380.89 ± 117.31	219.61 ± 86.49

3.5. Precision and accuracy

Table 1 shows a summary of intra- and inter-day precision and accuracy. The intra-day accuracies for loxoprofen, *trans*- and *cis*-alcohol were 102.63–105.41, 98.21–101.83 and 99.41–101.98% with precision (CV) less than 7.84, 6.29, and 4.62%, respectively. The inter-day accuracies for loxoprofen, *trans*- and *cis*-alcohol ranged from 96.12 to 97.54, 97.54 to 99.75, and 99.78 to 101.02% with precision (CV) less than 7.08, 6.32 and 7.62%, respectively. These results indicate that our method has a satisfactory accuracy, precision, and reproducibility.

3.6. Recovery

The extraction recoveries of loxoprofen were determined at low (0.5 μg/ml), medium (5 μg/ml) and high (10 μg/ml) concentrations in five replicates (Table 2). The mean absolute recoveries of loxoprofen, *trans*- and *cis*-alcohol for human serum were 89.6 ± 3.9, 93.5 ± 3.2, and 93.7 ± 4.3%, respectively. The mean relative recoveries of loxoprofen, *trans*- and *cis*-alcohol for human serum were 94.7 ± 5.2, 97.3 ± 5.7 and 94.3 ± 4.8%, respectively. We could obtain good recovery from the deproteinization of serum samples on the Shim-pack MAYI-ODS pretreatment column with a pretreatment mobile phase for 3 min after sample injection.

3.7. Stability

Two QC samples (low and high) of loxoprofen, *trans*- and *cis*-alcohol were stable in serum at room temperature for 24 h in daylight, with variation of less than 7.26%. They were also stable for 1 month at −80 °C in serum (CV was less than 4.34%), and after three freeze-thaw cycles changes in peak area were within acceptable limits (≤6.84%). Finally, the storage of stock solutions at room temperature for 6 h in daylight produced no significant decreases in peak areas.

3.8. Pharmacokinetics of loxoprofen in human

We used this method to determine loxoprofen pharmacokinetics after a single oral administration of loxoprofen sodium (60 mg, anhydrate) tablet to twenty-four healthy male Korean volunteers. Fig. 5 shows the mean (±S.D.) serum concentration-time curves of loxoprofen and its metabolites in twenty-four subjects. The pharmacokinetic parameters such as C_{max}, T_{max}, AUC and $t_{1/2}$ of loxoprofen and its metabolites are shown in Table 3. These parameters were similar to results of other investigators [6–7].

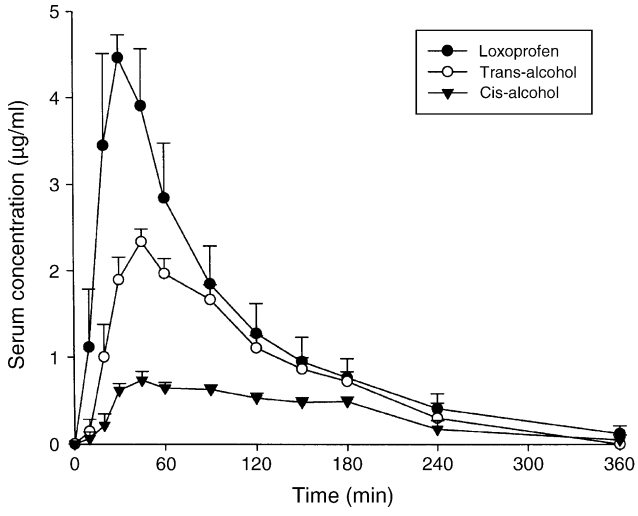

Fig. 5. Mean (±S.D., $n = 24$) serum concentration-time profiles of loxoprofen and its metabolites following a single oral administration of loxoprofen sodium (60 mg, anhydrate) tablet in twenty-four male volunteers. Vertical bar represents the standard deviation of the mean.

4. Conclusion

The performance of column-switching HPLC using MAYI-ODS was investigated for loxoprofen and its alcohol metabolites. MAYI-ODS resulted in a highly efficient on-line extraction of the target drugs with minimal nonspecific matrix effects. The column-switching technique is automated by software control and requires only 18 min of total run time. The method involved direct serum injection in a liquid chromatograph with UV detection and column switching, which resulted in on-line sample purification, preconcentration, and separation using programmed chromatographic conditions. The method shows good overall recovery, accuracy, precision, and a low detection limit of loxoprofen and its metabolites with UV detection. The present method was successfully applied to a pharmacokinetic study of loxoprofen and its metabolites to human volunteers. We have extensively used this technique to investigate the population pharmacokinetics of loxoprofen, and these results will be presented elsewhere.

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