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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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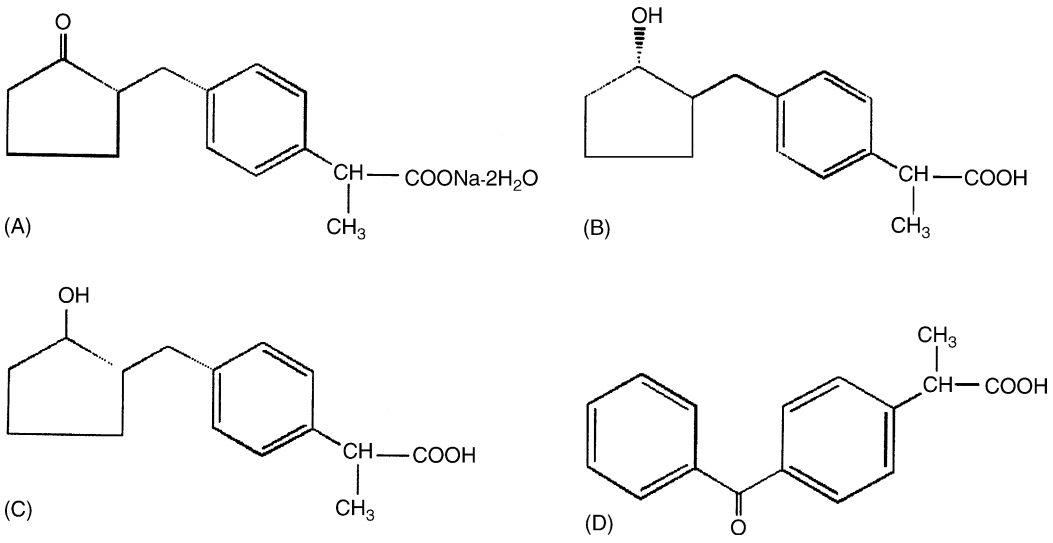

Fig. 1. Chemical structures of (A) loxoprofen sodium dihydrate, its (B) *trans*- and (C) *cis*-alcohol metabolites, and (D) ketoprofen (I.S.).

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