



Short communication

Development of simple and rapid LC–MS/MS method for determination of celecoxib in human plasma and its application to bioequivalence study

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ABSTRACT

A suitable liquid chromatography tandem mass spectrometry (LC–MS/MS) method to determine celecoxib in human plasma is needed for bioequivalence and pharmacokinetic studies of celecoxib preparations. The present study describes a simple, rapid, reproducible, and reliable LC–MS/MS method to determine celecoxib concentrations in human plasma. After one-step liquid–liquid extraction (LLE) using methyl *tert*-butyl ether (MTBE), celecoxib and atorvastatin (internal standard, IS) were eluted on a Luna HILIC column with an isocratic mobile phase, consisting of 10 mM ammonium formate buffer (adjusted to pH 3.0 with formic acid):methanol (5:95, v/v) at a flow rate of 0.2 mL/min. The achieved lower limit of quantitation (LLOQ) was 10 ng/mL ($S/N > 10$) and the standard calibration curve for celecoxib was linear (correlation coefficients were >0.9995) over the studied concentration range (10–2000 ng/mL). The inter- and intra-assay coefficients of variation ranged from 1.15% to 4.93% and 1.08% to 7.81%, respectively. The chromatographic run time for each plasma sample was <2 min. The developed method was successfully applied to a bioequivalence study of celecoxib in healthy Korean male volunteers.

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1. Introduction

Celecoxib is a non-steroidal anti-inflammatory drug and a selective cyclooxygenase (COX)-2 inhibitor used to treat osteoarthritis, rheumatoid arthritis, acute pain, painful menstruation, and menstrual symptoms with an improved side-effect profile. Celecoxib also reduces the number of colon and rectum polyps in patients with familial adenomatous polyposis [1,2]. Celecoxib is well absorbed and extensively metabolized in humans with $<3\%$ of the dose excreted unchanged [3]. Celecoxib is methyl-hydroxylated and further oxidized to the corresponding carboxylic acid, and only a small amount is excreted in the urine as a glucuronide [3]. CYP2C9 is the major enzyme mediating methyl hydroxylation [4,5] and the manufacturer's drug information recommends caution when administering celecoxib to poor CYP2C9 substrate metabolizers, because they might develop abnormally high plasma levels. Although the pharmacokinetic properties of celecoxib in healthy volunteers have been described previously [6–13], no report has been issued for Koreans.

Several analytical methods have been used to determine celecoxib concentrations in human plasma with using various analytical techniques, such as high performance liquid chromatography–ultraviolet detection (HPLC–UV) [6–10,14,15], liquid chromatography–mass spectrometry (LC–MS) [11], and LC tandem mass spectrometry (MS/MS) [12,13]. However, these methods suffer from a number of disadvantages, a large volume of plasma (≥ 0.5 mL) [6–8,10–12,14], lengthy run times (≥ 4 min) [6–15] and the lack of an internal standard (IS) [15]. Thus, our objective was to develop a reliable, faster, and high throughput method with simple LLE extraction preparation and a 2 min run time, for the routine determination of celecoxib in human plasma.

The analytical method described in this study was fully validated and successfully used to assess the bioequivalence of two marketed pharmaceutical formulations of 200 mg celecoxib capsules in Koreans.

2. Experimental

2.1. Chemicals and reagents

Celecoxib (100.6% purity) was supplied from Il Hwa Pharm. (Kyunggi-Do, Korea) and atorvastatin calcium was supplied from Sigma–Aldrich (St. Louis, MO, USA), respectively. Methyl *tert*-butyl ether (MTBE) and methanol were obtained from J.T. Baker

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(Phillipsburg, NJ, USA); ammonium formate from Sigma–Aldrich (St. Louis, MO, USA). Distilled water was obtained from an Aqua MAX™-Ultra water purification system (Young Lin Instrument, Kyuggi-Do, Korea). All other chemicals and solvents were of the highest analytical grades available. The test medication, Celest Capsule [200 mg celecoxib, Il Hwa Pharm. (Kyunggi-Do, Korea)] and the reference medication, Celebrex Capsule [200 mg celecoxib, Pfizer Pharm. (Seoul, Korea)] were supplied in the form of capsules.

2.2. Instrumentation and analytical conditions

The HPLC system was Agilent 1100 series (Agilent, USA). Chromatographic separation was achieved using a Luna HILIC (50 mm × 2.0 mm i.d., 3 μm, Phenomenex, USA) analytical column protected by HILIC guard column (4 mm × 2.0 mm i.d., 3 μm, Phenomenex, USA) at 45 °C. The isocratic mobile phase consisted of methanol–10 mM ammonium formate buffer adjusted to pH 3 with formic acid (95:5, v/v) at a flow rate of 0.2 mL/min. The solution filtered using 0.22 μm membrane and ultrasonically degassed prior to use.

Mass spectrometric detection was performed on a PE Sciex API 2000 triple quadrupole mass spectrometer equipped with an ESI source (AB Sciex, Toronto, Canada). Data acquisition was performed with Analyst™ 1.5 software (AB Sciex, Toronto, Canada). The mass spectrometer was operated in the negative ion mode. Optimized instrument settings specific celecoxib and IS were as follows: curtain gas was 10 psi, ion source gas 1 was 60 psi, ion source gas 2 was 60 psi, ionspray voltage was –4500 V, turbo heater temperature was 500 °C. The precursor ions of celecoxib and IS were formed using declustering potentials of –47 and –35 V, respectively, and their precursor ions were fragmented at collision energies of –27 and –58 eV by collision-activated dissociation with nitrogen at a pressure setting of 5 (arbitrary units). Both quadrupoles were maintained at unit resolution.

2.3. Preparation of standard and quality control (QC) samples

Stock solutions of celecoxib (1 mg/mL) and atorvastatin (IS) (1 mg/mL calculated as free base) were prepared by dissolving the accurately weighed reference compounds in 100% methanol and DMSO, respectively. The stock solution of celecoxib was further diluted with 100% methanol to give a series of standard solution with concentrations of 100, 500, 1000, 2000, 5000, 10,000 and 20,000 ng/mL. A solution of containing 2000 ng/mL of IS was prepared with 100% methanol. Calibration curves were prepared by spiking pooled blank plasma with working solutions to final celecoxib concentrations of 10, 50, 100, 200, 500, 1000 and 2000 ng/mL. Quality control (QC) samples were prepared in the same way at concentrations of 30, 200 and 1600 ng/mL for celecoxib. All the solutions were stored at –20 °C.

2.4. Sample preparation

200 μL volume of human plasma was added to 20 μL of IS (20 μg/mL). This sample solution was extracted with 1.5 mL of MTBE by vortexing. After centrifuged at 20,800 × g at 4 °C, the upper organic layer was transferred to another tube (Microtube, Axygen® Scientific, California, USA) and evaporated to dryness at 50 °C under a gentle stream of nitrogen. The residue was reconstituted in 80 μL of mobile phase by vortexing for 3 min and centrifuged at 20,800 × g at 4 °C for 10 min. Aliquot of this solution (5 μL) was injected into the LC–MS/MS system.

2.5. Method validation

The method was validated for specificity, LLOQ (10 ng/mL), recovery, accuracy, precision, stability and linearity for validation of bioanalytical methods [16].

2.5.1. Specificity and LLOQ

The specificity of the method was measured by analysis of six blank plasma samples of different origin for interference at the retention times of the analyte and IS. Specificity was assessed by comparing chromatograms of a blank human plasma sample, a blank human plasma spiked with celecoxib (10 ng/mL) and IS (20 μg/mL), and human plasma samples of a subject 3 h after an oral administration of 200 mg celecoxib.

2.5.2. Linearity

The linearity of the calibration curves, ranging of 10–2000 ng/mL, was validated with five different calibration curves. The calibration curves ($y = ax + b$) were constructed using the weighted regression method ($1/x^2$) of peak area ratios of celecoxib to IS (y) versus actual concentrations (x).

2.5.3. Precision and accuracy

In order to assess the intra- and inter-day precision and accuracy were performed on the same day ($n = 5$, at each concentration) and on five consecutive days at four concentrations (10, 100, 500 and 2000 ng/mL). Precision is expressed as coefficient of variance (C.V.), at each concentration. The accuracy of the assay was defined as a percentage of the measured concentration over the theoretical concentration. The acceptance criterion for each back-calculated standard concentration was 15% deviation from the nominal value, except for LLOQ, which was set at 20%. The LLOQ was determined as the concentrations with a signal to noise (S/N) ratio of 10.

2.5.4. Extraction recovery and matrix effects

The relative recovery, absolute matrix effect and process efficiency was determined at three QC concentrations (30, 200 and 1600 ng/mL, $n = 3$) of celecoxib and IS (20 μg/mL, $n = 9$). The relative recovery was measured by comparing the peak areas obtained from plasma samples spiked before extraction with those from plasma samples spiked after extraction. The absolute matrix effect was measured by comparing the peak response of plasma samples spiked before extraction with those of the pure standards containing equivalent amounts of the celecoxib and IS prepared in mobile phase. The process efficiency was measured by comparing the peak response of plasma samples spiked after extraction with those of the pure standards containing equivalent amounts of the celecoxib and IS prepared in mobile phase.

2.5.5. Stability

The stabilities of celecoxib in human plasma were evaluated by analyzing replicates ($n = 3$, at each concentration) of plasma samples at three QC concentrations (30, 200 and 1600 ng/mL): freeze–thaw stability after 3 freeze–thaw cycles at –70 °C; long-term stability at –70 °C for 2 weeks; short-term stability at room temperature, 4 °C and –70 °C for 24 h; auto-sampler stability at 4 °C for 26 h. In addition, the stability of the celecoxib working solutions was evaluated.

2.6. Application to bioequivalence studies

The validated method was used to determine the plasma concentrations of celecoxib in a bioequivalence study. The subjects were hospitalized (Kyung Hee University Medical Center, Seoul, Korea) and the study protocol was approved by the Korean Food and Drug Administration (KFDA) and the Institutional Review Board of

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