Received: 4 December 2016 | Revised: 23 April 2017 | Accepted: 26 April 2017

DOI: 10.1002/chir.22715

REGULAR ARTICLE

WILEY

Enantioselective analysis of ibuprofen enantiomers in mice plasma and tissues by high-performance liquid chromatography with fluorescence detection: Application to a pharmacokinetic study

Katarzyna Przejczowska-Pomierny | Monika Włodyka | Agnieszka Cios | Elżbieta Wyska

Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University Medical College, Cracow, Poland

Correspondence
K. Przejczowska-Pomierny, Department of Pharmacokinetics and Physical Pharmacy Jagiellonian University Medical College, Medyczna 9, 30-688 Cracow, Poland.
Email: katarzyna.przejczowska@doctoral.uj.edu.pl

Abstract

A direct fluorometric high-performance liquid chromatography (HPLC) method was developed and validated for the analysis of ibuprofen enantiomers in mouse plasma (100 μl) and tissues (brain, liver, kidneys) using liquid–liquid extraction and 4-tertbutylphenoxyacetic acid as an internal standard. Separation of enantiomers was accomplished in a Chiracel OJ-H chiral column based on cellulose tris(4-methylbenzoate) coated on 5 μm silica-gel, 250 x 4.6 mm at 22 °C with a mobile phase composed of n-hexane, 2-propanol, and trifluoroacetic acid that were delivered in gradient elution at a flow rate of 1 ml min^{-1}. A fluorometric detector was set at: $\lambda_{excit.}$ = 220 nm and $\lambda_{emis.}$ = 290 nm. Method validation included the evaluation of the selectivity, linearity, lower limit of quantification (LLOQ), within-run and between-run precision and accuracy. The LLOQ for the two enantiomers was 0.125 μg ml^{-1} in plasma, 0.09 μg g^{-1} in brain, and 0.25 μg g^{-1} in for liver and kidney homogenates. The calibration curves showed good linearity in the ranges of each enantiomers: from 0.125 to 35 μg ml^{-1} for plasma, 0.09–1.44 μg g^{-1} for brain, and 0.25–20 μg g^{-1} for liver and kidney homogenates. The method was successfully applied to a pharmacokinetic study of ibuprofen enantiomers in mice treated i.v. with 10 mg kg^{-1} of racemate.

KEYWORDS

(R,S)-2-(4-isobutylphenyl)propionic acid, Chiracel OJ-H, chiral separation, compartment modelling, normal phase

1 | INTRODUCTION

Ibuprofen, (R,S)-2-(4-isobutylphenyl)propionic acid, is a nonsteroidal antiinflammatory drug (NSAID) that is widely used for the treatment of acute and chronic pain, fever, osteoarthritis, rheumatoid arthritis, and related diseases.[1] It contains a chiral carbon atom located on its propionic side chain and, therefore, it exists in two stereoisomeric forms: (+)-S and (−)-R. Although ibuprofen is commonly marketed as a racemic drug formulation, the pharmacodynamic, pharmacokinetic, and toxicological properties of its enantiomers are different.[2-5] Ibuprofen exerts antiinflammatory activity mainly by inhibiting cyclooxygenase (COX)-1 and COX-2 and there is evidence that (+)-S-ibuprofen is much more potent than its antipode.[6] Recent studies have also demonstrated other mechanisms of action of this drug that are independent of COX, such as: an inhibition of leukocyte function and production, an influence on nitric oxide (NO) production, an inhibition of production of transcription factors, MAP kinase, nuclear

receptors, heat shock proteins, cytokines and antibodies, a reduction of apoptosis, and an increase of endogenous cannabinoids in the central nervous system[7,8] having positive effects on memory deficits in the Alzheimer's animal model[9] and reducing the risk of various human cancers.[10] In the human body, 53–65% of (−)-R-ibuprofen is inverted to the active form (+)-S-ibuprofen.[2,5,6] This inversion is enzymatic and proceeds via formation of thioester of (−)-R-ibuprofenyl adenylate with acyl coenzyme A (CoA).[6,8,11,12] It has been reported that this biochemical reaction is tissue- and species-specific. It is both a presystemic[13] and systemic process, which takes place mainly in the liver and kidneys. In general, in mammals inversion of (−)-R-ibuprofen to (+)-S-ibuprofen is unidirectional, although Chen et al. observed bidirectional chiral inversion of ibuprofen in guinea pigs and, minimally, in rats and rabbits.[14] Due to differences in pharmacokinetics and pharmacodynamics between both ibuprofen enantiomers, it is necessary to measure their plasma concentrations separately following administration of racemic ibuprofen.

There are several assays to determine ibuprofen enantiomers in biological fluids described in the literature. High-performance liquid chromatography (HPLC) coupled with UV detection is the most frequently analytical technique used.[11,13,15-24] In addition, HPLC methods with fluorescence[1,25] or mass spectrometry[26-29] detection was applied. Methods using HPLC with UV detection are characterized by a relatively low cost, wide availability, and high precision and accuracy, but they are the least sensitive. Higher sensitivity may be achieved using fluorescence or mass spectrometry detectors. Unfortunately, the HPLC/ tandem mass spectrometry (MS/MS) systems are not available in many laboratories, whereas most of validated HPLC-fluorescence methods for ibuprofen enantiomer quantification are indirect,[1,25] i.e., they require a derivatization process. This means that sample preparation is extensive and long-lasting. Furthermore, formation of diastereomeric derivatives may lead to the obtaining of a false concentration of enantiomers due either to chiral impurities in the reagent or to the racemization during the process of derivatization.[30] To avoid these problems, direct enantiomeric analysis using enantioselective chiral stationary phases may be advisable. In the literature, several direct HPLC methods to quantify ibuprofen enantiomers in biological fluids can be found but they were coupled with UV detection.[11,13,17-19,23,24]

The aim of this study was to develop and validate a direct enantioselective HPLC method with fluorescence detection for quantitative determination of ibuprofen enantiomers in mouse plasma and tissues. Furthermore, the method was applied to a pharmacokinetic study following intravenous (i.v.) administration of a racemic drug in mice.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Ibuprofen sodium salt, 4-tertbutylphenoxyacetic acid, used as an internal standard (IS), and trifluoracetic acid (TFA) were purchased from Sigma-Aldrich (Germany). n-Hexane, 2-propanol, ethyl acetate, methanol, and hydrochloric acid were of HPLC grade and were obtained from Merck (Germany). Deionized water used during the experiment was prepared in-house using a Hydrolab water purification system (Poland) with a 0.2 μm microfiltration capsule. Blank blood samples and tissues (brain, liver, and kidneys) used for the validation of the analytical method were collected from healthy CD-1 mice. Plasma was obtained by centrifugation (10 min at 3000 rpm) of blood containing heparin (Polfa, Poland) as anticoagulant and all samples were stored at −80 °C (Skadi Telstar, Spain) until the time of analysis.

2.2 | Instrumentation and chromatographic conditions

The analysis of ibuprofen enantiomers was performed with a Hitachi HPLC system (Japan) consisting of a pump (model L-2130), an autosampler (model L-2200), a column oven (model L-2350), a fluorometric detector (model L-2485), and a computer, Optiplex 745 (DELL) with EZChrom Elite Client/Server v. 3.2 software for data collection and analysis. Separation of both enantiomers and the IS was achieved at ambient temperature (22 ± 1 °C) using a Chiracel OJ-H chiral column based on cellulose tris(4-methylbenzoate) coated on 5 μm silica-gel, 250 x 4.6 mm (Daicel Chemical Industries, Japan) protected with a guard column 10 x 4 mm (Daicel Chemical Industries) with the same packing material.

The mobile phase consisted of n-hexane with TFA mixed in a 1000:0.664 (v/v) ratio (A) and pure 2-propanol (B) that were delivered in gradient elution at a flow rate of 1 ml min^{-1}. Gradient elution for plasma samples was as follows: 98% A for 9.5 min, 98–90% A from 9.5 to 13 min, 90–98% A from 13 to 16 min, and 98% A from 16 to 22 min. In turn, gradient elution for liver, kidneys, and brain samples was as follows: 99% A for 9.5 min, 98.5–92% A from 9.5 to 12 min, hold 90% A from 13 to 14 min, 90–99% A from 14 to 19 min, and 99% A from 19 to 23 min. The total time of analysis was 23 min. The injection volume was 20 μl for plasma and 30 μl for tissue samples.

The spectrofluorimetric detector was operated at an excitation wavelength of 220 nm and an emission wavelength of 290 nm. These settings were developed before the validation process. As the excitation wavelength, the maximum of absorbance of ibuprofen in mobile phase was selected, whereas the emission wavelength was developed experimentally.

2.3 | Preparation of standard solutions

A stock solution of racemic ibuprofen sodium salt (500 μg ml^{-1} of each enantiomer) and IS (1 mg ml^{-1}) were prepared in methanol and were kept at 4 °C. The stock solution of ibuprofen was subsequently diluted in methanol to prepare working standard solutions in the ranges of each enantiomer: 0.125–30 μg ml^{-1} for plasma, 0.25–20 μg g^{-1} for liver and kidneys, and 0.09–1.44 μg g^{-1} for brain. IS solutions (IS_1 = 125 μg ml^{-1}, IS_2 = 62 μg/ml, and IS_3 = 31 μg ml^{-1}) were prepared by diluting the stock solution with methanol.

2.4 | Sample preparation

Ibuprofen enantiomers were isolated from plasma and tissue homogenates by liquid–liquid extraction. Frozen plasma samples were thawed at room temperature and vortex-mixed briefly (Reax top, Heidolph, Germany). Then 100 μl of the plasma samples was transferred to a glass tube and IS_1 working solution (125 μg ml^{-1}, 20 μl) was added. The samples were acidified with 100 μl of 1 M HCl, mixed briefly on the vortex mixer, and extracted with 3 ml of ethyl acetate/n-hexane (30:70, v/v) mixture for 10 min on a shaker (VXR Vibrax, IKA, Germany). After centrifugation (Universal 32, Hettich, Germany) at 3000 rpm for 15 min, the organic layers were transferred into conical glass tubes and evaporated to dryness at 37 °C in the water bath under a gentle stream of nitrogen. Frozen mouse tissues (brain, liver, and kidneys) were thawed at room temperature, weighed, and then homogenized (4 mg g^{-1}) in distilled water with a tissue homogenizer TH220 (Omni International, USA). The tissue homogenates (200 μl for liver and kidneys and 500 μl for brain) were transferred to the glass tubes. Then 20 μl of IS working solution (IS_1 for kidneys, IS_2 for liver, and IS_3 for brain) was added and samples were acidified with 100 μl of 1 M HCl. After vortex mixing for 15 s, 1 ml of NaCl solution (20 g/100 ml) was added to clear samples from proteins. Next, the samples were vortex mixed again and extracted with 4 ml of ethyl acetate/n-hexane (30:70, v/v) mixture for 10 min on a shaker. After centrifugation at 3000 rpm for 15 min, the organic layers were transferred into conical glass tubes and evaporated to dryness at 37 °C under a gentle stream of nitrogen. The residues were reconstituted in 100 μl of mobile phase components in 99(A):1(B) ratio, vortexed for 1 min, and 20 (plasma) or 30 μl (tissue homogenates) were injected into the HPLC system.

2.5 | Method validation

The method validation was performed in accordance to US Food and Drug Administration (FDA) Guidance for Industry (www.fda.gov/downloads/drugs/.../guidances/ucm368107.pdf). Throughout the study, the suitability of the chromatographic system was monitored by calculating the capacity factor (k'), the resolution (R), the selectivity (α), and column efficiency (N) for ibuprofen enantiomers and IS in the eluting systems used for plasma and tissue samples.

The selectivity of the method was evaluated by analyzing extracts of plasma and tissue homogenates (brain, liver, and kidneys) from six different CD-1 mice to investigate the potential interference in the peak regions of ibuprofen enantiomers and IS in chromatograms.

The calibration curves were prepared in the drug-free plasma or tissue homogenates by spiking them with 20 μl of each of the standard working solutions to obtain the final concentrations of ibuprofen enantiomers: 0.125, 0.25, 0.5, 2.5, 5, 10, 20, and 30 μg ml^{-1} for plasma samples; 0.09, 0.18, 0.36, 0.72, 1.08, and 1.44 μg g^{-1} for brain samples; and 0.25, 0.5, 1, 5, 10, and 20 μg g^{-1} for liver and kidney samples. Three replicates were prepared for each concentration, then the samples were submitted to the extraction and analytical procedures described above. The peak area ratios of ibuprofen enantiomers to IS were plotted against spiked ibuprofen concentrations for the evaluation of linearity and the coefficients of linear correlation. The regression lines were used to calculate concentrations of ibuprofen enantiomers in the unknown plasma, brain, liver, and kidney samples.

The sensitivity of the method was characterized by the lower limit of quantification (LLOQ) values, which were established using five drug-free samples of plasma or appropriate tissue homogenates spiked with a known amount of ibuprofen racemate that after the extraction procedure were analyzed with a coefficient of variation (CV) of ≤20% and a relative error (RE) of ≤20%.

Precision and accuracy were determined within a run and between runs for plasma samples and within a run for tissues samples. Ibuprofen enantiomer quality control (QC) samples were prepared at three concentrations: 0.5 μg ml^{-1} for plasma samples, 0.36 μg g^{-1} for brain samples, and 0.5 μg g^{-1} for liver and kidney samples as low quality control (LQC); 5 μg g^{-1} for plasma samples, 0.72 μg g^{-1} for brain samples, and 5 μg ml^{-1} for liver and kidney as medium quality control (MQC); and 20 μg ml^{-1} for plasma samples, 1.44 μg g^{-1} for brain samples, and 10 μg g^{-1} for liver and kidney samples as high quality control (HQC). In the interassay study each quality control sample was analyzed in five replicates. In the intraassay study quality control samples in duplicate were analyzed for 5 consecutive days. The precision was expressed as the coefficient of variation and the accuracy as the relative error.

The extraction recovery of analytes was determined by comparing the peak area of analytes in the extracted QC samples with mean peak area of analytes obtained by a direct injection of standard solutions of analytes and IS at corresponding concentrations.

Prestudy stability studies of ibuprofen enantiomers were carried out in many laboratories in the past.[20,29-31] In all these experiments both (−)-R- and (+)-S-ibuprofen was stable in short and long stability studies and also during freeze/thaw stability evaluations. Therefore, in these studies

postpreparative stability was only determined. To this end, the QC samples after first injection into the chromatographic system were maintained in the autosampler at 10 °C for 12 h and after that time they were injected again.

2.6 | Pharmacokinetic study in mice

The validated method was applied to a pharmacokinetic study in mice treated with a single dose of 10 mg kg^{-1} racemic ibuprofen i.v. The experimental study was approved by the First Local Ethical Committee on Animal Testing of the Jagiellonian University (Krakow, Poland).

Male CD-1 mice weighing 25–30 g were used in this study. Animals were housed under controlled environmental conditions (temperature 22 ± 1 °C) with a 12-h dark/light cycle. They had free access to water but 12 h before ibuprofen dosing they were fasted. Racemic ibuprofen was diluted in 0.9% saline and used within 1 day. Mice received the drug in a single i.v. dose of 10 mg kg^{-1} mouse weight (4 ml kg^{-1}). Animals were exsanguinated at 5, 15, 30, 45, 90, 120, 240, and 360 min postdose (n = 3–4 per timepoint). Blood was collected into heparinized tubes and plasma was separated by centrifugation at 3000 rpm for 10 min and stored at −80 °C until analysis. Additionally, at every timepoint brain, liver, and kidneys were harvested.

Pharmacokinetic analyses were performed using Phoenix WinNonlin program v. 6.3 (Pharsight, Certara, Mountain View, CA). Concentration versus time profiles of both ibuprofen enantiomers in plasma were analyzed simultaneously using a one- or two-compartment model with unidirectional conversion of (−)-R to (+)-S-ibuprofen (Figure 1). The unidirectional conversion has been confirmed in a study with the use of perfused mouse liver performed in our laboratory (data not shown). To reduce the number of model parameters, in the first step the total elimination rate constant (k_{10Rt}) for (−)-R-enantiomer was estimated using a two-compartment pharmacokinetic model. Then the obtained value of 0.094 min^{-1} was used in pharmacokinetic modeling to calculate the first-order elimination rate constant k_{10R} according to the equation: $k_{10R} = 0.094 – k_{RS}$ (where k_{RS} is the first-order rate constant representing the unidirectional conversion of (−)-R- to (+)-S-ibuprofen). The final model was selected on the basis of visual inspection of the fitting, examination of residuals, parameter precision, and Akaike and Bayesian information criteria. Concentration versus time profiles of ibuprofen enantiomers in plasma were also analyzed by means of a noncompartmental approach using the same pharmacokinetic program. This method was also used in relation to the concentration–time relationships obtained in the studied tissues (brain, liver, and kidneys). The peak concentration (C_{max}) and the time to reach the peak concentration (t_{max}) in tissues were obtained directly from the concentration versus time data. The terminal elimination rate constant (λ_z) was assessed by linear regression and terminal half-life ($t_{0.5\lambda z}$) was calculated as $\ln 2/\lambda_z$. Area under the concentration–time curve (AUC) from time zero to the last sampling time at which concentrations were at or above the LLOQ (AUC_{0-t}), was calculated by the linear trapezoidal rule. Clearance was calculated as D/AUC and mean residence time (MRT) as AUMC/AUC, where AMUC is the area under the first moment curve.

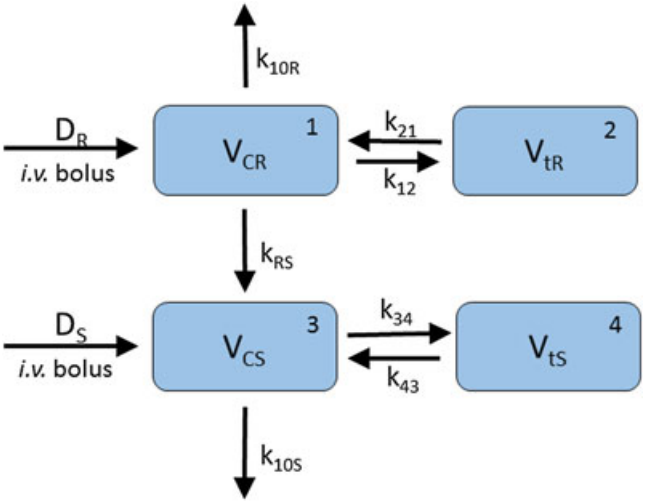

FIGURE 1 Proposed pharmacokinetic model for ibuprofen enantiomers in mice plasma after i.v. administration of the racemic drug at a dose of 10 mg kg^{-1}. V_{CR} and V_{CS} are volumes of the central compartments for (−)-R- and (+)-S-ibuprofen, respectively; k_{10R} and k_{10S} are the first-order elimination rate constants of both enantiomers; k_{12}, k_{21} and k_{34}, k_{43} are the first-order distribution rate constants of (−)-R- and (+)-S-enantiomer, respectively, and k_{RS} is the first-order rate constant representing the unidirectional conversion of (−)-R- to (+)-S-ibuprofen

3 | RESULTS AND DISCUSSION

In this study we developed and validated a sensitive enantioselective HPLC method with fluorimetric detection. Until now, the method where the Chiracel OJ-H chiral column was used to separate both enantiomers of ibuprofen directly, and 4-tertbutylphenoxyacetic acid was employed as an internal standard, has not been described in the literature. Moreover, the pharmacokinetics and tissue distribution of ibuprofen enantiomers in mice was not extensively studied in the past, whereas this species is frequently used to study the pharmacodynamics of ibuprofen.[9,10]

3.1 | Chromatographic conditions

To avoid the long-lasting derivatization process, we chose to separate ibuprofen enantiomers using a chiral stationary phase column Chiracel OJ-H containing cellulose tris(4-methylbenzoate) coated on 5 μm silica-gel. This column is recommended to separate ibuprofen enantiomers on a producer's website (https://search.daicelchiral.com/name)

with the HPLC method coupled with UV detection and using isocratic normal phase eluting (n-hexane:2-propanol:TFA, 98:2:0.1). Cellulose-based chiral stationary phases (Chiralcel OJ) have been used for the chiral separation of ibuprofen enantiomers in one study, but they were analyzed after derivatization into their amide.[32] In turn, Ducret et al.[33] used this column for the resolution of ibuprofen esters. The Chiralcel OJ stationary phase was also utilized for the thermodynamic study of enantioseparation of arylpropionic acids.[34] Tang evaluated the influence of the mobile phase composition on the enantioseparation of ibuprofen, ketoprofen, albuterol, acebutolol, propafenone, betaxolol, methylphenidate, and homatropine using a cellulose-based chiral stationary phase.[35] In turn, Valdermara et al. validated a direct enantioselective method of ibuprofen using the Chiracel OJ column and UV detection, although that study was not performed using biological fluids.[23]

In the present study, we used for the first time the Chiracel OJ-H column and a fluorometric detector to quantify ibuprofen enantiomers in mouse plasma and tissues. Because of the liquid–liquid extraction procedure applied to isolate both analytes, we needed to add an internal standard to the analyzed samples. The internal standards, which were used by other authors and HPLC/UV methods were useless in the HPLC/fluorimetric assay. Among the different compounds that were tested in the present study, for example: salicylic acid, acetylsalicylic acid, naproxen, ketoprofen, flurbiprofen, diclofenac, nimesulid, 4-aminosalicylic acid, p-coumaric acid, p-chlorophenoxyacetic acid, and p-tolylacetic acid, only one of them that is 4-tertbutylphenoxyacetic acid was extracted from plasma and tissue homogenates in acidic conditions repetitively with a recovery of 68.89 ± 6.75% (CV = 9.80%) for plasma and of 71.39 ± 0.46% (CV = 0.65%), 76.53 ± 6.54% (CV = 8.54%), and 78.72 ± 4.17% (CV = 5.31) for brain, liver, and kidney homogenates, respectively. In addition, it gave a satisfactory detector response at an excitation wavelength of 220 nm and an emission wavelength of 290 nm. Because the IS had a long retention time under isocratic chromatographic conditions, the gradient elution was applied leading to the total runtime of 23 min per sample.

3.2 | Suitability of the method

Chromatographic parameters, such as resolution, selectivity, capacity factor, and column efficiency for ibuprofen enantiomers and IS in two eluting systems are listed in Table 1. The calculated resolution values between each peak-pair were no less than 1.5 and selectivity was not less than 1.1. k' values were higher than 1 and lower than 10, which indicates enough space between unretained compounds and desired peaks and not too slow elution.

3.3 | Selectivity

Figure 2 shows the typical chromatograms of the blank mouse plasma (A), brain (D), liver (G), and kidney (J) homogenates. From this figure, no interfering peaks from endogenous substances were observed at the retention times of both enantiomers and the IS.

3.4 | Linearity

The linearity of the calibration curves for ibuprofen enantiomers in mouse plasma was estimated using each enantiomer concentration in the range from 0.125 to 35 μg ml^{-1}. As presented in Table 2, the calibration curves were linear in the tested concentration range as indicated by high coefficients of determination (R^2 = 0.999) for both enantiomers.

The linearity of the calibration curves for ibuprofen enantiomers in mouse tissues was estimated using each enantiomer concentration in the ranges: 0.09–1.44 μg g^{-1} for brain, and 0.25–20 μg g^{-1} for liver and kidney homogenates. Similarly, all of the calibration curves were linear and the coefficients of determination ranged from 0.99755–0.9989 (Table 3).

3.5 | Sensitivity

The LLOQ in plasma was 0.125 μg ml^{-1} (2.0 ng on-column) for the two enantiomers with the coefficient of variation (CV) less than 20% (CV = 18.70% for (−)-R and CV = 18.96% for (+)-S-ibuprofen). In the case of tissue homogenates we

TABLE 1 System performance parameters of ibuprofen enantiomers and IS for two gradient eluting compositions: one used for plasma and the second for tissue samples (n = 5)

	Gradient elution for plasma samples				Gradient elution for tissue samples			
Compound	**k'**	**R**	**α**	**N**	**k'**	**R**	**α**	**N**
(−)-R-ibuprofen	1.66 (0.48)*	1.53 (0.05)	1.18 (0.09)	3086 (0.77)	2.88 (0.45)	1.64 (0.56)	1.11 (0.08)	5796 (0.82)
(+)-S-ibuprofen	1.95 (0.39)			3805 (0.71)	3.19 (0.46)			5118 (0.83)
IS	5.17 (0.13)	14.41 (0.08)	2.65 (0.28)	9350 (0.34)	5.51 (0.35)	10.57 (0.49)	1.72 (0.57)	12298 (0.37)

*Values are means (RSD%). RSD% = (Standard Deviation/Mean) × 100.

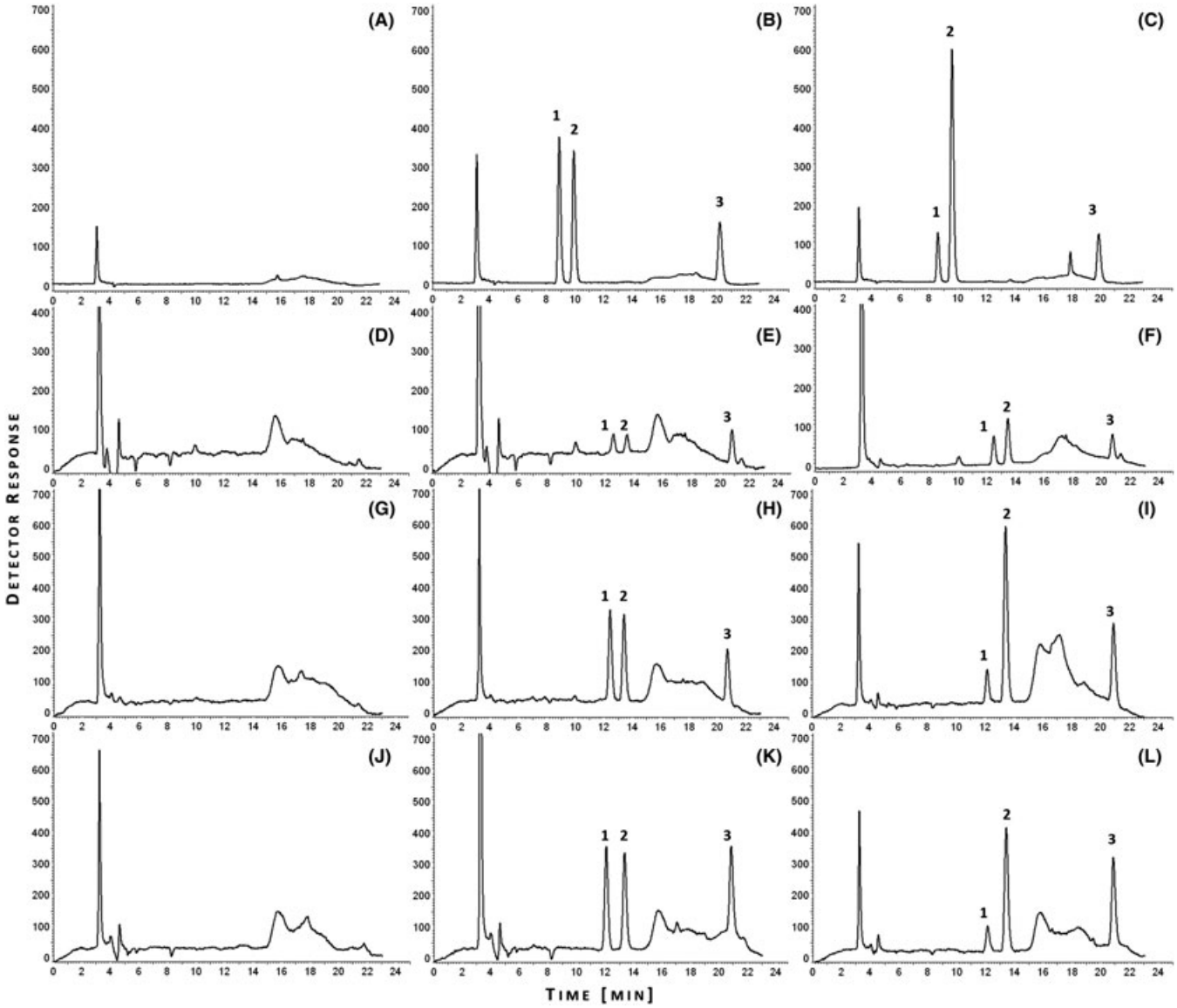

FIGURE 2 HPLC chromatograms of blank mouse plasma (a) brain (D), liver (G), and kidney (J) homogenates; plasma containing ibuprofen enantiomers at concentrations of 10 μg ml^{-1} (B), brain (E), liver (H), and kidney (K) homogenates containing ibuprofen enantiomers at concentrations of 0.36 μg g^{-1}, 5 μg g^{-1}, and 5 μg g^{-1}, respectively; plasma (C), brain (F), liver (I), and kidneys (L) samples collected 15 min after i.v. administration of racemic ibuprofen at a dose of 10 mg kg^{-1} to CD-1 mice. Peaks: 1- (−)-R-ibuprofen, 2- (+)-S-ibuprofen, 3- IS

achieved an LLOQ of 0.09 μg g^{-1} for brain, and 0.25 μg g^{-1} for liver and kidneys, which gave about 2.5 ng for brain and about 3 ng for liver and kidneys of each enantiomer on-column.

3.6 | Accuracy and precision

The accuracy and precision of the method was determined in both within-day and between-day assays for plasma and within-day for brain, liver, and kidney homogenates. Tables 2 and 3 demonstrate the results obtained with three concentrations of both enantiomers. As can be seen, neither CVs nor relative errors exceeded a value of 15%, which indicates that the validated method has a good precision and accuracy in the tested concentration ranges.

3.7 | Recovery

The liquid–liquid extraction from the acidified environment was selected for isolation of both enantiomers from the plasma and tissue samples. Several procedures of extraction were examined. In the case of extraction of ibuprofen enantiomers from plasma samples, 1 M HCl was selected from a group of others acidifiers: 1 M H_2SO_4, 0.1 M HCl, and 0.1 M CH_3COONa (pH = 4). A few extraction mixtures

TABLE 2 Validation parameters of the method for the enantioselective analysis of ibuprofen in mouse plasma

	(−)-R-ibuprofen	(+)-S-ibuprofen
Recovery ± SD% (CV%)		
0.5 μg ml^{-1}	81.17 ± 7.37 (9.08)	74.15 ± 8.66 (11.64)
5.0 μg ml^{-1}	68.71 ± 8.32 (12.10)	67.85 ± 9.55 (14.07)
20.0 μg ml^{-1}	65.10 ± 2.26 (3.46)	65.00 ± 2.50 (3.84)
Linearity (0.125–35 μg ml^{-1})	y = 0.1476x + 0.0057	y = 0.1476x – 0.0104
R^2	0.99912	0.99901
LLOQ (μg ml^{-1})	0.125	0.125
Precision (CV%, n = 5)	18.70	18.96
Accuracy (RE%)	10.22	12.61
Intraday precision (CV%, n = 5)		
LQC (0.5 μg ml^{-1})	3.21	4.24
MQC (5.0 μg ml^{-1})	13.45	4.13
HQC (20.0 μg ml^{-1})	5.32	5.11
Interday precision (CV%, n = 5)		
LQC (0.5 μg ml^{-1})	9.30	6.53
MQC (5.0 μg ml^{-1})	4.04	3.76
HQC (20.0 μg ml^{-1})	3.72	5.94
Intraday accuracy (RE%, n = 5)		
LQC (0.5 μg ml^{-1})	6.44	13.31
MQC (5.0 μg ml^{-1})	3.89	6.58
HQC (20.0 μg ml^{-1})	6.13	2.96
Interday accuracy (RE%, n = 5)		
LQC (0.5 μg ml^{-1})	10.25	12.43
MQC (5.0 μg ml^{-1})	6.10	5.67
HQC (20.0 μg ml^{-1})	1.28	0.34

were tested and different recoveries were achieved, whereby the highest one was observed for extraction with dichloromethane, which was about 85% for both enantiomers and n-heptane/2-propanol (95:5, v/v) or n-hexane/2-propanol (95:5, v/v) mixtures, ranging about 80% for both enantiomers. Despite relatively high recoveries, these extraction mixtures were not selected because of interfering peaks observed at the retention times of the analytes or IS in chromatograms or a lengthy evaporation process when using the n-heptane/2-propanol mixture. The lowest recovery was observed during extraction with methyl-tert-butyl-ether, which in addition gave dirty samples. The best selectivity was achieved when using ethyl acetate/n-hexane (30:70, v/v) as an extraction mixture with a good recovery of about 70%, and finally this mixture was selected for the extraction process. To isolate both analytes from tissue homogenates, several methods of sample purification were tested: proteins precipitation with cooled methanol or acetonitrile and 10% trifluoroacetic acid solution, but the best method for sample purification was to vortex the samples with 1 ml of NaCl solution in water (20 g/100 ml). The best selectivity was achieved in the case of the double extraction process, where in the first step homogenate was extracted from acidic samples by the ethyl acetate/n-hexane (30:70, v/v) mixture and then the organic phase was transferred to a new tube and 0.1 ml of 2 M NaOH was added. Then, after vortex mixing and centrifugation, the water phase was acidified and extracted with the same extraction mixture. Despite the good selectivity, a small recovery was achieved (about 45%). Because of the small volume of distribution of ibuprofen, low concentrations of this drug are expected in tissues. Therefore, the procedure with a single extraction with the ethyl acetate/n-hexane (30:70, v/v) mixture after NaCl protein precipitation was selected for tissue homogenates. The selectivity was improved by starting the eluting process with solvent A and B at a ratio: 99:1%. Tables 2 and 3 show the obtained recoveries from plasma, brain, liver, and kidney homogenates, respectively. The coefficients of variation were

TABLE 3 Validation parameters of the method for the enantioselective analysis of ibuprofen in mouse brain, liver, and kidneys

	(−)-R-ibuprofen	(+)-S-ibuprofen
BRAIN		
Recovery ± SD % (CV%)		
0.36 μg g^{-1}	71.88 ± 5.63 (7.84)	74.93 ± 5.74 (7.66)
0.72 μg g^{-1}	72.06 ± 2.25 (3.12)	73.39 ± 3.04 (4.14)
1.44 μg g^{-1}	74.28 ± 3.37 (4.53)	74.44 ± 2.20 (2.95)
Linearity (0.09–1.44 μg g^{-1})	y = 1.5872x + 0.0012	y = 1.5835x – 0.0395
R^2	0.99773	0.99755
LLOQ (μg g^{-1})	0.09	0.09
Precision (CV%, n = 5)	15.18	6.56
Accuracy (RE%)	11.42	11.79
Inter-day precision (CV%, n = 5)		
LOQ (0.36 μg g^{-1})	10.65	6.11
MOQ (0.72 μg g^{-1})	1.63	5.71
HQC (1.44 μg g^{-1})	4.85	4.75
Inter-day accuracy (RE%, n = 5)		
LOQ (0.36 μg g^{-1})	9.76	4.69
MOQ (0.72 μg g^{-1})	2.57	0.27
HQC (1.44 μg g^{-1})	0.95	2.51
LIVER		
Recovery ± SD % (CV%)		
0.5 μg g^{-1}	79.87 ± 2.85 (3.56)	78.32 ± 6.31 (8.05)
5.0 μg g^{-1}	76.99 ± 5.82 (7.56)	75.31 ± 6.60 (8.76)
10.0 μg g^{-1}	75.19 ± 3.96 (5.26)	72.38 ± 2.13 (2.95)
Linearity (0.25–20 μg g^{-1})	y = 0.2015x + 0.0098	y = 0.1965x – 0.0134
R^2	0.9989	0.99856
LLOQ (μg g^{-1})	0.25	0.25
Precision (CV%, n = 5)	9.34	5.38
Accuracy (RE%)	11.85	4.61
Inter-day precision (CV%, n = 5)		
LOQ (0.5 μg g^{-1})	0.90	2.36
MOQ (5.0 μg g^{-1})	0.63	1.87
HQC (10 μg g^{-1})	1.74	1.99
Inter-day accuracy (RE%, n = 5)		
LOQ (0.5 μg g^{-1})	4.02	11.98
MOQ (5.0 μg g^{-1})	7.20	2.00
HQC (10 μg g^{-1})	0.99	1.15

(*Continues*)

TABLE 3 (Continued)

	(−)-R-ibuprofen	(+)-S-ibuprofen
KIDNEY		
Recovery ± SD % (CV%)		
0.5 μg g^{-1}	79.88 ± 1.39 (1.73)	78.38 ± 0.69 (0.88)
5.0 μg g^{-1}	74.43 ± 5.03 (6.75)	75.39 ± 4.96 (6.58)
10.0 μg g^{-1}	75.43 ± 2.05 (2.72)	74.32 ± 5.03 (7.25)
Linearity (0.25–20 μg g^{-1})	y = 0.0932x + 0.0298	y = 0.0923x + 0.0164
R^2	0.9985	0.99875
LLOQ (μg g^{-1})	0.25	0.25
Precision (CV%, n = 5)	12.11	14.14
Accuracy (RE%)	3.99	2.00
Inter-day precision (CV%, n = 5)		
LOQ (0.5 μg g^{-1})	1.43	4.31
MOQ (5.0 μg g^{-1})	6.12	7.83
HQC (10 μg g^{-1})	8.96	9.23
Inter-day accuracy (RE%, n = 5)		
LOQ (0.5 μg g^{-1})	6.09	12.39
MOQ (5.0 μg g^{-1})	3.92	5.81
HQC (10 μg g^{-1})	3.52	4.10

lower than 15%, thus confirming the repeatability of the extraction procedures.

3.8 | Postprocessing stability

The results of postprocessing stability studies revealed that there was no change in the area of the peaks of both ibuprofen enantiomers and IS after keeping samples in the autosampler at 10 °C for 12 h, provided that samples were tightly closed to prevent evaporation of the solvent.

3.9 | Pharmacokinetics of ibuprofen enantiomers in mice

The developed method was applied for the determination of ibuprofen enantiomers in the pharmacokinetic study performed in CD-1 mice, which were given 10 mg kg^{-1} of racemic ibuprofen i.v. Based on the standard goodness-of-fit criteria, a two-compartment model presented in Figure 1 best describes the pharmacokinetics of the studied enantiomers.

Figure 3 presents the mean observed and pharmacokinetic model-predicted plasma concentration–time profiles of ibuprofen enantiomers and Table 4 shows the estimated pharmacokinetic parameters and their respective CVs. From this figure, the model very well captured the observed concentrations of both enantiomers that

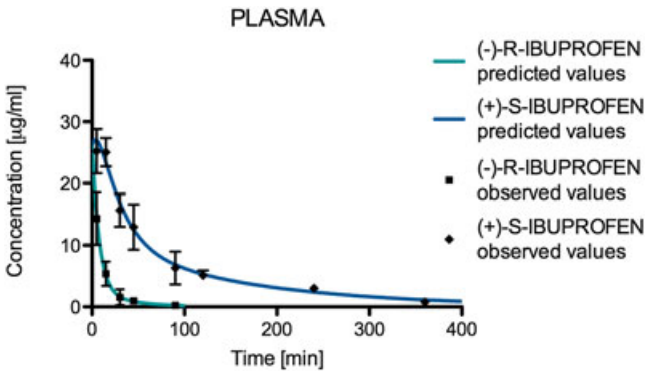

FIGURE 3 The mean observed (symbols ± SD) and pharmacokinetic model predicted (lines) plasma concentration–time profiles of ibuprofen enantiomers after intravenous administration of racemic drug at a dose of 10 mg kg^{-1} to mice

may be further confirmed by the relatively low CV values presented in Table 4.

The volumes of distribution of both enantiomers were comparable (188.01 and 190.94 ml kg^{-1} for (−)-R- and (+)-S-ibuprofen, respectively). Similarly, no differences between volumes of distributions of ibuprofen enantiomers were observed in rats[16] and dogs[36] after i.v. administration of one enantiomer or the racemic drug. In turn, Cheng et al. noticed some differences in healthy humans after i.v. administration of each enantiomer (Vss was 7.4 and 8.8 L for (−)-R- and (+)-S-ibuprofen, respectively).[5] The inversion of

TABLE 4 Pharmacokinetic parameters of ibuprofen enantiomers estimated following i.v. administration of a single dose of 10 mg kg^{-1} of racemic ibuprofen to mice

Parameters	Final estimate	CV [%]
V_{CR} [ml kg^{-1}]	188.01	4.86
V_{CS} [ml kg^{-1}]	190.94	5.92
k_{10R} [1 min^{-1}]	0.038 (0.094–0.056)	---
k_{10S} [1 min^{-1}]	0.018	20.37
k_{RS} [1 min^{-1}]	0.056	58.82
K_{12} [1 min^{-1}]	0.027	39.64
K_{21} [1 min^{-1}]	0.037	52.43
K_{34} [1 min^{-1}]	0.018	47.43
K_{43} [1 min^{-1}]	0.016	34.14

V_{CR} volume of thecentral compartment for (−)-R-ibuprofen, V_{CS} volume of the central compartment for (+)-S-ibuprofen; k_{10R} and k_{10S} first order elimination rate constants; k_{RS} first-order inversion rate constant; k_{12}, k_{21}, k_{34}, and k_{43} distribution rate constants.

(−)-R- to (+)-S-enantiomer (k_{RS} = 0.056 min^{-1}) was 3½ times faster than the elimination of (+)-S- (k_{10S} = 0.018 min^{-1}) and (−)-R-enantiomer (k_{10R} = 0.038 min^{-1}) from the central compartment. The rate of this biochemical process in rats was almost the same (k_{RS} = 0.06 min^{-1}) but the elimination rate constants were higher (k_{10R} = 0.05 min^{-1} and k_{10S} = 0.1 min^{-1}) than those observed in the present study in mice.[16] A relatively large difference was observed in the elimination half-lives of both enantiomers in mice that were 7.37 ($t_{0.5R}$ = 0.693/(k_{10R} + k_{RS})) and 38.5 min ($t_{0.5S}$ = 0.693/k_{10S}) for (−)-R- and (+)-S-ibuprofen, respectively. Slightly higher differences were observed by Wang et al. in rats, who found that $t_{0.5}$ values of both enantiomers differed about 6 times after i.v. administration, but only 3 times after oral drug intake.[11] Smaller differences were observed in dogs, where after i.v. administration of individual enantiomer or racemic ibuprofen the ratio of $t_{0.5\ (+)\text{-S-ibuprofen}}$/$t_{0.5\ (-)\text{-R-ibuprofen}}$ was only 2.[36] In human, there were small differences in $t_{0,5}$ values of each enantiomer after oral intake[2,13,37] or no differences after i.v.[5] or oral administration of ibuprofen.[38] The distribution rates constants were also different for both enantiomers. As a result, V_{dss} calculated as: (1 + k_{12}/k_{21})V_c were 325.21 and 405.75 ml kg^{-1} for (−)-R- and (+)-S-ibuprofen, respectively.

Figure 4 shows the concentration versus time profiles of both enantiomers in studied tissues, and pharmacokinetic parameters estimated based on the noncompartmental analysis are listed in Table 5. The collected data indicate that the highest concentrations of both enantiomers were observed in plasma and the lowest in brain tissue

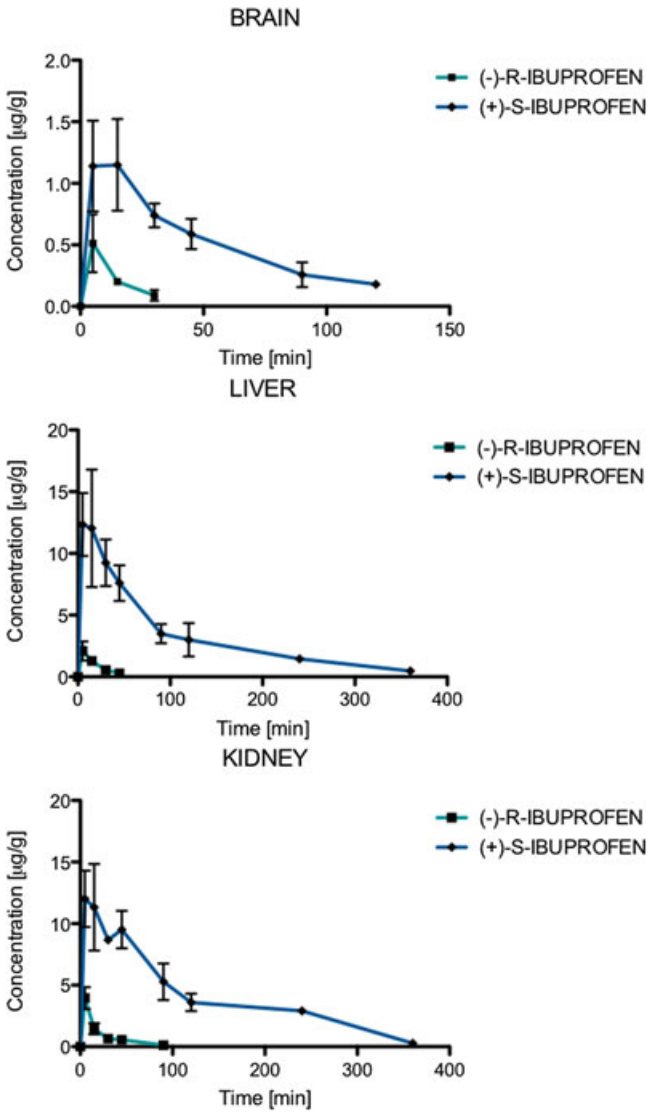

FIGURE 4 Mean brain, liver, and kidney concentration–time profiles of ibuprofen enantiomers following a single i.v. administration of 10 mg kg^{-1} racemic drug to mice. Symbols represent the means ± SD of 3–4 mice

(Figure 4, Table 5). Moreover, $AUC_{0\text{-}t}$ of (+)-S-ibuprofen in plasma and tissues was considerably higher than that of its optical antipode. (+)-S- to (−)-R-ibuprofen AUC ratios were 8.98, 9.10, 27.56, and 19.14 in plasma, brain, liver, and kidneys, respectively. The values of $t_{0.5\lambda z}$ assessed in plasma, brain, liver, and kidneys were 4-, 4-, 6-, 2.5-times higher, respectively, for (+)-S-enantiomer in comparison to (−)-R-enantiomer. Tissue-to-plasma AUC ratios of (−)-R-ibuprofen were 0.03, 0.18, and 0.32 for brain, liver, and kidneys respectively, whereas for (+)-S-enantiomer these values were 0.03, 0.54, and 0.69. The main reasons for all these differences are the unidirectional inversion of (−)-R- to (+)-S-enantiomer in the body and a slower elimination of (+)-S-antipode from plasma and all studied tissues. Taken together, these

TABLE 5 Plasma, brain, liver, and kidney pharmacokinetic parameters of ibuprofen enantiomers in mice after a single i.v. dose of 10 mg kg^{-1} of racemic drug using noncompartmental analysis

Pharmacokinetic parameters	(−)-R-ibuprofen	(+)-S-ibuprofen
PLASMA		
t_{max} [min]	5.0	5.0
C_0 [μg ml^{-1}]	14.32	25.23
$AUC_{0\text{-}t}$ [μg min ml^{-1}]	239.88	2155.91
CL [ml min^{-1} kg^{-1}]	20.16	2.20
$t_{0.5\lambda z}$ [min]	22.65	89.39
MRT [min]	24.39	113.83
BRAIN		
t_{max} [min]	5	15
C_{max} [μg g^{-1}]	0.51	1.15
$AUC_{0\text{-}t}$ [μg min g^{-1}]	7.03	64.04
$t_{0.5\lambda z}$ [min]	10.09	42.95
MRT [min]	15.94	60.48
LIVER		
t_{max} [min]	5	5
C_{max} [μg g^{-1}]	2.11	12.34
$AUC_{0\text{-}t}$ [μg min g^{-1}]	42.38	1169.19
$t_{0.5\lambda z}$ [min]	14.08	90.35
MRT [min]	21.70	113.82
KIDNEYS		
t_{max} [min]	5	5
C_{max} [μg g^{-1}]	3.95	12.01
$AUC_{0\text{-}t}$ [μg min g^{-1}]	77.29	1479.43
$t_{0.5\lambda z}$ [min]	24.06	64.31
MRT [min]	27.96	115.68

results indicate that after i.v. administration of racemic ibuprofen to mice a stereoselective disposition of this drug occurs, which is in agreement with the data from pharmacokinetic studies in other species and humans.[5,11,16,19,36,39]

4 | CONCLUSION

In the present study, a direct enantioselective HPLC method coupled with fluorescence detection for quantitative determination of ibuprofen enantiomers in mouse plasma and tissues was developed and validated. The proposed method is simple, sensitive, and selective, meaning that ibuprofen enantiomers could be measured in biological samples with acceptable accuracy and precision. Finally, this method was successfully applied to the pharmacokinetic study of ibuprofen enantiomers after i.v. administration of the racemic drug to CD-1 mice, where a small volume of plasma (100 μl) and tissue homogenates (0.2–0.5 ml) was used for the analysis.

REFERENCES

1. Canaparo R, Muntoni E, Zara GP, et al. Determination of ibuprofen in human plasma by high-performance liquid chromatography: Validation and application in pharmacokinetic study. *Biomed Chromatogr*. 2000;14:219-226.
2. Lee E, Williams K, Day R, Graham G, Champion D. Stereoselective disposition of ibuprofen enantiomers in man. *Br J Clin Pharmacol*. 1985;19:669-674.
3. Evans AM. Enantioselective pharmacodynamics and pharmacokinetics of chiral non-steroidal antiinflammatory drugs. *Eur J Clin Pharmacol*. 1992;54:237-256.
4. Evans AM. Comparative pharmacology of S(+)-ibuprofen and (RS)-ibuprofen. *Clin Rheumatol*. 2001;20(Suppl 1):S9-S14.
5. Cheng H, Rogers JD, Demetriades JL, Holland SD, Seibold JR, Depuy E. Pharmacokinetics and Bioinversion of ibuprofen enantiomers in humans. *Pharm Res*. 1994;11:824-830.
6. Davies NM. Clinical pharmacokinetics of ibuprofen. The first 30 years. *Clin Pharmacokinet*. 1998;34:101-154.
7. De La Cruz JP, Reyes JJ, Ruiz-Moreno MI, Lopez-Villodres JA, Jebrouni N, Gonzalez-Correa JA. Differences in the in vitro antiplatelet effect of dexibuprofen, ibuprofen, and flurbiprofen in human blood. *Anesth Analg*. 2010;111:1341-1346.
8. Rainsford KD. Ibuprofen: Pharmacology, efficacy and safety. *Inflammopharmacology*. 2009;17:275-342.
9. McKee AC, Carreras I, Hossain L, et al. Ibuprofen reduces Aβ, hyperphosphorylated tau and memory deficits in Alzheimer mice. *Brain Res*. 2008;1207:225-236.
10. Xie G, Sun Y, Nie T, et al. Phospho-ibuprofen (MDC-917) is a novel agent against colon cancer: Efficacy, metabolism, and pharmacokinetics in mouse models. *J Pharmacol Exp Ther*. 2011;337:876-886.
11. Wang Y. Pharmacokinetics of ibuprofen enantiomers in rats after intravenous and oral administration of ibuprofen arginate. *Acta Pharm Sin*. 2012;47:88-93.
12. Hao H, Wang G, Sun J. Enantioselective pharmacokinetics of ibuprofen and involved mechanisms. *Drug Metab Rev*. 2005; 37:215-234.
13. Ding G, Liu Y, Sun J, et al. Effect of absorption rate on pharmacokinetics of ibuprofen in relation to chiral inversion in humans. *J Pharm Pharmacol*. 2007;59:1509-1513.
14. Chen C-S, Shieh W-R, Lu P-H, Harriman S, Chen C-Y. Metabolic stereoisomeric inversion of ibuprofen in mammals. *Biochim Biophys Acta - Protein Struct Mol Enzymol*. 1991;1078:411-417.
15. Bannwarth B, Lapicque F, Pehourcq F, et al. Stereoselective disposition of ibuprofen enantiomers in human cerebrospinal fluid. *Br J Clin Pharmacol*. 1995;40:266-269.
16. Itoh T, Maruyama J, Tsuda Y, Yamada H. Stereoselective pharmacokinetics of ibuprofen in rats: Effect of enantiomer-enantiomer interaction in plasma protein binding. *Chirality*. 1997;9:354-361.

17. Fornasini G, Monti N, Brogin G, et al. Preliminary pharmacokinetic study of ibuprofen enantiomers after administration of a new oral formulation (ibuprofen arginine) to healthy male volunteers. *Chirality*. 1997;9:297-302.

18. Główka FK. 1998HPLC methodology for determination of ibuprofen enantiomers to be used in pharmacokinetics. *Chem Anal*. 1998;43:79-84.

19. Vermeulen B, Remon JP. Validation of a high-performance liquid chromatographic method for the determination of ibuprofen enantiomers in plasma of broiler chickens. *J Chromatogr B*. 2000;749:243-251.

20. Teng XW, Wang SW, Davies NM. Stereospecific high-performance liquid chromatographic analysis of ibuprofen in rat serum. *J Chromatogr B*. 2003;796:225-231.

21. Zhao X, Chen D, Li K, Wang D. Sensitive liquid chromatographic assay for the simultaneous determination of ibuprofen and its prodrug, ibuprofen eugenol ester, in rat plasma. *Yakugaku Zasshi*. 2005;125:733-737.

22. De Oliveira ARM, Cesarino EJ, Bonato PS. Solid-phase microextraction and chiral HPLC analysis of ibuprofen in urine. *J Chromatogr B*. 2005;818:285-291.

23. Valderrama P, Romero AL, Imamura PM, Poppi RJ. Figures of merit in the quantification of ibuprofen enantiomers by chiral HPLC. *J Chromatogr Sci*. 2009;47:777-780.

24. Ye J, Yu W, Chen G, Shen Z, Zeng S. Enantiomeric separation of 2-arylpropionic acid nonsteroidal antiinflammatory drugs by HPLC with hydroxypropyl-β-cyclodextrin as chiral mobile phase additive. *Biomed Chromatogr*. 2010;24:799-807.

25. Tan SC, Jackson SHD, Swift CG, Hutt AJ. Enantiospecific analysis of ibuprofen by high performance liquid chromatography: Determination of free ant total drug enantiomer concentrations in serum and urine. *Chromatographia*. 1997;46:23-32.

26. Bonato PS, Del Lama MPFM, De Carvalho R. Enantioselective determination of ibuprofen in plasma by high-performance liquid chromatography-electrospray mass spectrometry. *J Chromatogr B*. 2003;796:413-420.

27. Szeitz A, Edginton AN, Peng HT, Cheung B, Riggs KW. A validated enantioselective assay for the determination of ibuprofen in human plasma using ultra performance liquid chromatography with tandem mass spectrometry (UPLC-MS / MS). *Am J Anal Chem*. 2010;2:47-58.

28. Ogawa S, Tadokoro H, Sato M, Higashi T. Enantioselective determination of ibuprofen in saliva by liquid chromatography/tandem mass spectrometry with chiral electrospray ionization-enhancing and stable isotope-coded derivatization. *J Pharm Biomed Anal*. 2014;98:387-392.

29. Cavalcanti JL, Lanchote VL, Pereira MPM, Moraes NV, Lepera JS. Analysis of ibuprofen enantiomers in rat plasma by liquid chromatography with tandem mass spectrometry. *J Sep Sci*. 2014; 37:944-949.

30. Ahn HY, Shiu GK, Trafton WF, Doyle TD. Resolution of the enantiomers of ibuprofen; comparison study of diastereomeric method and chiral stationary phase method. *J Chromatogr B*. 1994;653:163-169.

31. Główka FK, Karaźniewicz M. High performance capillary electrophoresis method for determination of ibuprofen enantiomers in human serum and urine. *Anal Chim Acta*. 2005;540:95-102.

32. Van Overbeke A, Baeyens W, Van Den Bossche W, Dewaele C. Enantiomeric separation of amide derivatives of some 2-arylpropionic acids by HPLC on a cellulose-based chiral stationary phase. *J Pharm Biomed Anal*. 1994;12:911-916.

33. Ducret A, Trani M, Pepin P, Lortie R. Chiral high performance liquid chromatography resolution of ibuprofen esters. *J Pharm Biomed Anal*. 1998;16:1225-1231.

34. Kang GW, Ho Ko J, Jo CW. Thermodynamic study of enantioseparation of arylpropionic acids with a chiralcel OJ-H stationary phase. *J Liq Chromatogr Relat Technol*. 2005;28:513-526.

35. Tang Y. Significance of mobile phase composition in enantioseparation of chiral drugs by HPLC on a cellulose-based chiral stationary phase. *Chirality*. 1996;8:136-142.

36. Hae-Young A, Amidon GL, Smith DE. Stereoselective systemic disposition of ibuprofen enantiomers in the dog. *Pharm Res*. 1991;8:1186-1190.

37. Zheng C, Hao H, Wang G, et al. Chiral separation of ibuprofen and chiral pharmacokinetics in healthy chinese volunteers. *Eur J Drug Metab Pharmacokinet*. 2008;33:45-51.

38. Geisslinger G, Schuster O, Stock KP, Loew D, Bach GL, Brune K. Pharmacokinetics of S(+)- and R(−)-ibuprofen in volunteers and first clinical experience of S(+)-ibuprofen in rheumatoid arthritis. *Eur J Clin Pharmacol*. 1990;38:493-497.

39. Knihinicki RD, Day RO, Graham GG, Williams KM. Stereoselective disposition of ibuprofen and flurbiprofen in rats. *Chirality*. 1990;2:134-140.

How to cite this article: Przejczowska-Pomierny K, Włodyka M, Cios A, Wyska E. Enantioselective analysis of ibuprofen enantiomers in mice plasma and tissues by high-performance liquid chromatography with fluorescence detection: Application to a pharmacokinetic study. *Chirality*. 2017;1-12. https://doi.org/10.1002/chir.22715