Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 173–178

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Determination of oxaceprol in rat plasma by LC–MS/MS and its application in a pharmacokinetic study

Jifeng Gu[a,*], Nianzu Chen[a], Guoqiang Ding[b], Zhen Zhang[a]

[a] Department of Pharmacy, Eye & ENT Hospital of Fudan University, Shanghai 200031, China
[b] Department of Otorhinolaryngology Head and Neck Surgery, Eye & ENT Hospital of Fudan University, Shanghai 200031, China

ARTICLE INFO

Article history:
Received 23 November 2009
Received in revised form 3 August 2010
Accepted 9 August 2010
Available online 19 August 2010

Keywords:
Oxaceprol
LC–MS/MS
Pharmacokinetics
Rat plasma
Oral administration

ABSTRACT

A sensitive method for the quantification of oxaceprol in rat plasma using high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed. Sample pretreatment involved a simple protein precipitation by the addition of 60 μL of acetonitrile–methanol (1:2, v/v) to 20 μL plasma sample volume. Separation was achieved on a Dikma ODS-C18 (5 μm, 150 mm × 4.6 mm) reversed-phase column at 40 °C with acetonitrile/0.1% formic acid–4 mM ammonium acetate in water (35:65,v/v) at a flow rate of 0.6 mL/min. Detection was performed using an electrospray ionization (ESI) operating in negative ion multiple reaction monitoring (MRM) mode by monitoring the ion transitions from *m/z* 172 → 130 (oxaceprol) and *m/z* 153 → 109 (protocatechuic acid, internal standard). The calibration curve of oxaceprol in plasma showed good linearity over the concentration range of 1.25–800 ng/mL. The limit of detection and limit of quantification were 0.400 ng/mL and 1.25 ng/mL, respectively. Intra- and inter-day precisions in all samples were within 15%. There was no matrix effect. The validated method was successfully applied to a preclinical pharmacokinetic study of oxaceprol in rats. After oral administration of 20 mg/kg oxaceprol to rats, the main pharmacokinetic parameters T_{max}, C_{max}, $T_{1/2}$, $V_{z/F}$ and AUC_{0-t} were 1.4 h, 1.2 μg/mL, 2.3 h, 19.7 L/kg and 3.4 mg h/L, respectively.

1. Introduction

Oxaceprol, a synthetic *N*-acetylated derivative of hydroxyproline (Fig. 1(A)), has been used for the treatment of ameliorating pain and stiffness, osteoarthritis and rheumatoid arthritis [1–5], and exhibits a low incidence of gastrointestinal side-effect, particularly in comparison with Non-steroidal anti-inflammatory drugs (NSAIDs) [6–8]. Recently, the efficacy of therapy and tolerability of oxaceprol in the treatment of symptomatic osteoarthritis of knee or hip was further confirmed in a multicentric, randomised, double-blind, placebo-controlled study [9].

To date, a thorough literature search revealed only one study showed limited pharmacokinetic profiles and described a method for the determination of oxaceprol in biological sample [10]. However, the method was costly and time-consuming to synthesize radiolabeled drug for administration to study pharmacokinetics of the unmetabolized drug and that approach was not selective as there was no separation of drug from metabolites.

Oxaceprol is a small molecular with poor ultraviolet absorption. Therefore, high-performance liquid chromatography with ultraviolet detection (HPLC–UV) method is not suitable for the pharmacokinetic study of oxaceprol. Mass spectrometric detection is often considered sensitive and specific, compared with other detection techniques such as ultraviolet and fluorometric detection. So the aim of this study was developing and validating a simple, rapid, and sensitive high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the determination of oxaceprol in plasma. To validate the method with real samples, a trial was undertaken to determine oxaceprol in the plasma of healthy rats, that were administered a single oral dose of 20 mg/kg of body weight.

2. Experimental

2.1. Chemicals and reagents

Oxaceprol (purity >98.2%) was purchased from Weibo Chemical Reagent Co. Ltd. (Guangzhou, China). The internal standard (IS), protocatechuic acid (HPLC purity), was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and methanol (HPLC grade) were purchased from Sigma (USA). HPLC-grade water by a Milli-Q system from Millipore (USA) was used. All other chemicals were of analytical grade. Blank rat plasma (drug free) was prepared in our laboratory.

* Corresponding author. Tel.: +86 21 64377134; fax: +86 21 64377151.
E-mail address: gujifeng1949@126.com (J. Gu).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.08.011

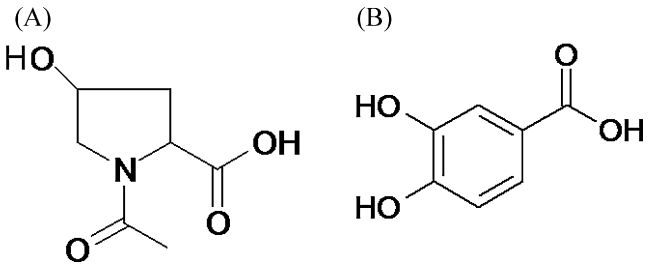

Fig. 1. Chemical structure of: (A) oxaceprol and (B) protocatechuic acid (internal standard, IS).

2.2. Preparation of standard and quality control samples

Stock solutions of oxaceprol (0.4 mg/mL) and IS (0.2 mg/mL) were prepared in methanol and stored at 4 °C. The stock solution of oxaceprol was diluted quantitatively with water to give working standards at concentrations of 12.5, 25.0, 50.0, 100, 400, 1600, 6400 and 8000 ng/mL. The working IS solution was prepared by diluting the IS stock solution with methanol giving a concentration of 2000 ng/mL. Calibration standards of oxaceprol were prepared by dissolving the evaporated working standards with blank rat plasma yielding final concentrations of 1.25, 2.50, 5.00, 10.0, 40.0, 160 and 800 ng/mL. Quality control (QC) samples were prepared at low (2.50 ng/mL), medium (10.0 ng/mL) and high (640 ng/mL) concentrations in the same way as the plasma samples for calibration.

2.3. LC–MS/MS analysis

An Agilent HP1200 series HPLC system (Agilent, USA) coupled to a triple-quadrupole mass spectrometer (API 4000, AB, USA) via an electrospray ionization (ESI) interface was used for analysis. Analyst software (version 1.4.2) was used for sample control, data acquisition and processing.

Oxaceprol and IS were separated on a Dikma ODS-C18 reversed-phase column (5 μm, 150 mm × 4.6 mm) equipped with a guard column maintained at 40 °C. The mobile phase consisted of A: 0.1% formic acid–4 mM ammonium acetate–water and B: acetonitrile (65:35, v/v) and was delivered at a flow rate of 0.6 mL/min. The temperature of the sample cooler in the autosampler was 4 °C. The total run time was 4.0 min. Under these conditions, typical standard retention times were 2.5 min for oxaceprol and 3.5 min for IS.

The mass spectrometer operated in negative electrospray ionization mode (ESI−) was set up for multiple reaction monitoring (MRM) to monitor the transitions 172 → 130 and 153 → 109, for oxaceprol and IS, respectively. Following optimization of the settings, the instrument parameters were set at source temperature 600 °C, ion transfer voltage of −4500 V, curtain gas flow of 30 psi, ion source GS1 flow of 55 psi, ion source GS2 flow of 70 psi, entrance potential (EP) of −10 V. The declustering potential (DP) for oxaceprol and IS were at −48 and −24 V, respectively. The collision energies for oxaceprol and IS were −16 and −20 eV, respectively. The collision cell exit potential (CXP) for oxaceprol and IS were −26 and −16 V, respectively. Resolution was set to unit. Representative mass spectra for oxaceprol (panel A) and IS (panel B) are shown in Fig. 2.

2.4. Sample preparation

An aliquot (20 μL) of plasma was transferred to a test tube, 4 μL of IS and 60 μL of acetonitrile–methanol (1:2, v/v) were added and the sample was vortex-mixed for 60s. To avoiding solvent effect, after centrifuging at 16000 rpm for 10 min at 4 °C, the upper layer of 60 μL was transferred polypropylene tube and further diluted with 60 μL water, then 10 μL of supernatant fluid were injected into the LC–MS/MS system for analysis.

2.5. Assay validation

Specificity was assessed by analysis of six different samples of blank matrix with and without spiking with oxaceprol and IS.

Calibration curves were constructed from working standard solutions of oxaceprol at concentration range 1.25–800 ng/mL by plotting peak area ratio (y) of oxaceprol to the internal standard, versus oxaceprol concentration (x). Linearity was assessed by weighted ($1/x$) linear regression of calibration curves generated in triplicate on three consecutive days using analyte–internal standard peak area ratios.

Quality control samples (2.50, 10.0 and 640 ng/mL) were prepared to evaluate the accuracy, precision, recovery, stability, and matrix effect of the assay. Accuracy (expressed as relative error, RE) and intra- and inter-day precision (expressed as relative standard deviation, R.S.D.) were assessed by assay of five replicate QC samples on three different days. The precision of IS was calculated from the mean and standard deviation of the internal standard peak areas in QC samples.

The lower limit of quantification (LLOQ) was defined as the lowest concentration in the calibration curve that can be determined with accuracy and precision of no more than 20%. The limit of detection (LOD) was defined as a signal to noise ratio of 3:1.

The extraction recovery for the oxaceprol and IS were determined by assaying two batches of samples: plasma extracts spiked with oxaceprol and IS after extraction (batch 1), and plasma spiked with oxaceprol and IS before extraction (batch 2). Oxaceprol of each batch were prepared at levels of 2.50, 10.0 and 640 ng/mL. The extraction recoveries of oxaceprol and IS were calculated as extraction recovery (%) = batch 2/batch 1 × 100%.

Matrix effect was assayed by two ways [11]. First, post column analytes infusion experiment was conducted. A standard solution containing 80 ng/mL of oxaceprol and IS in acetonitrile: water (35:65, v/v) was infused post column via a T connector into the mobile phase at 10 μL/min employing infusion pump. Aliquots of 10 μL of different extracted blank plasma samples were then injected into the column by the autosampler. Any peak at the retention time of oxaceprol and IS indicates ion enhancement while a dip in the baseline would indicate ion suppression [12]. Secondly, the quantitative measure of matrix effect can be termed as Matrix Factor (MF) and evaluated using six different blank plasma lots by measuring a ratio of peak response ratio (analyte/IS) in the presence of matrix to the analyte peak ratio (analyte/IS) response in mobile phase [13]. In the method, biological matrix samples were prepared by spiking post-extracted blank plasma with oxaceprol and IS. The control sample was the same reference solution prepared in mobile phase. Oxaceprol of each batch were prepared at levels of 2.50, 10.0 and 640 ng/mL. Potential sample carry-over was tested by analyzing the upper limit of quantitation (ULOQ, 800ng/mL) calibrator of oxaceprol followed by blank samples.

Stability experiments were performed to evaluate the analyte stability in stocks solutions and in plasma samples under different conditions. Stock solution stability was performed by comparing area response of stability sample of analyte with the area response of sample prepared from fresh stock solutions. The results should be within the acceptable limit of ±10% change for stock solution stability experiment [11]. Room temperature stability, long-term stability, freeze–thaw cycles stability and post-extracted stability were performed at QC levels using five replicates at each level. To meet the acceptance criteria the RE (%) should be within ±15%.

The dilution integrity experiment was performed with an aim to validate the dilution test to be carried out on higher analyte concentrations (above ULOQ), which should be encountered dur-

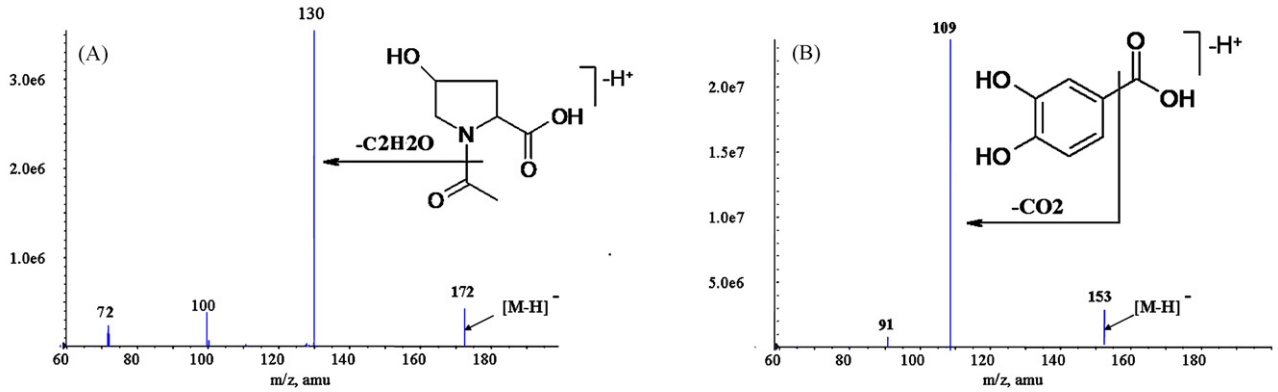

Fig. 2. Full-scan product ion spectra of $[M-H]^-$ ions and fragmentation pathways for oxaceprol (panel A) and IS (panel B).

ing real subject samples analysis. Five replicate oxaceprol samples in plasma prepared at nominal concentrations of 3 times ULOQ (2400 ng/mL) and high QC (640 ng/mL) were diluted 10-fold using blank matrix. The diluted samples were analyzed and the mean concentrations were compared to the nominal value after the dilution factor was applied.

2.6. Pharmacokinetic study

Six Sprague–Dawley rats (250–280 g, three males and three females, purchased from the Animal Center of Shanghai University of Traditional Chinese Medicine, Shanghai, China) were used in a pharmacokinetic study. The rats were fasted for 12 h and had free access to water before experiment. After administration of 20 mg/kg oxaceprol to rats through intragastric gavage (i.g.), blood samples collected by retro-orbital puncture at 0.25, 0.50, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12, and 24 h post-dosing. All were taken into heparinized tubes and centrifuged at 5000 rpm at 4 °C for 5 min to separate the plasma. The plasma obtained was stored at −70 °C until analysis. Plasma concentrations of oxaceprol were measured as described above and expressed as mean ± S.D. (standard deviation, S.D.). Samples that were found to contain concentration above the higher limit of quantification were diluted with blank plasma and then reanalyzed.

Pharmacokinetic parameters were estimated by a non-compartmental method from the plasma concentration–time data with the aid of the program DAS (the Drug and Statistics 2.0 software package). The maximum plasma concentration (C_{max}) and the time to peak concentration (T_{max}) were obtained from the observed data.

The basic objective of an incurred sample reanalysis (ISR) was to reconfirm the initial values and to demonstrate that the assay was reproducible. It was required that greater than 2/3 of reanalyzed samples had a relative difference in concentration of within 20% of the original result [14]. In the study, ISR was performed on 18 plasma samples from six different rats. Two time points were taken up for ISR of which one time point was T_{max} and the second time point was covering the phase of elimination.

3. Results and discussion

3.1. Method development

During the experiment, several internal standards were tested under present experimental conditions like benzoylglycine, rhein and protocatechuic acid. However, benzoylglycine was interfered by a significant endogenous substance in plasma sample and rhein could not give satisfactory retention time under such experimental condition. Protocatechuic acid (Fig. 1(B)) had a moderate retention time and could give molecular ions $[M-H]^-$ as the most intensive precursor ions in negative ion scan mode. Because its chromatographic and mass spectrometric behaviors were similar to those of oxaceprol, protocatechuic acid was selected as the IS.

To maximize the responses of oxaceprol and IS, their standard solutions were directly infused into the mass spectrometer at a flow rate of 10 μL/min. Negative ion mode was chosen because of better sensitivity and MRM was used on account of great advantage in selectivity for the quantification of oxaceprol. In Q1 MS scan mode, oxaceprol and IS gave molecular ions $[M-H]^-$ as the most intensive precursor ions. The major product ions at m/z 130 and 100 in product ion mode (MS2) for oxaceprol were candidates for quantification. However, the product ion at m/z 130 was more abundant than the ion at m/z 100 and relatively stable. Therefore, the transition of m/z 172 → 130 was selected for quantification of oxaceprol. And the precursor → product ion transition of m/z 153 → 109 was selected for IS.

The selection of mobile phase was focused on good peak shape and mass spectral response, as well as a short run time. Acetonitrile resulted in a short run time and a mixture of 0.1% formic acid–4 mM ammonium acetate water solution could achieve excellent peak shape and better ionization efficiency; therefore, these were finally used as mobile phase. A flow rate of 0.6 mL/min could achieve a satisfactory separation and suitable retention time, so the adjusted flow of 0.6 mL/min was chosen.

Different kinds of extraction procedures, including liquid–liquid extraction (LLE) were evaluated during our method development. The high hydrophilicity of oxaceprol makes it difficult to obtain satisfactory recovery by LLE. However, we found that the sample preparation with a protein precipitation procedure had a high recovery (83.8–86.8%). Moreover, the protein precipitation was much simpler, better reproducibility and less time-consuming and therefore utilized in the study.

3.2. Method validation

Blank plasma samples from six sources were screened and no significant endogenous substance was observed at the retention time of the compounds of interest. The representative MRM chromatograms of (a) a blank rat plasma sample, (b) a blank rat plasma sample spiked with oxaceprol and IS, and (c) a plasma sample from a rat after an oral administration of 20 mg/kg oxaceprol are illustrated in Fig. 3.

The LLOQ of oxaceprol was 1.25 ng/mL and the LOD (S/N > 3) was 0.400 ng/mL. The linear regression of the curve for the peak area ratio of oxaceprol to IS (y) versus concentration of oxaceprol (x) was plotted for plasma sample. A typical calibration curve was

Table 1
Precision and accuracy for the determination of oxaceprol in rat plasma by LC–MS/MS method.

Sample concentration (ng/mL)	Average measured concentration (ng/mL)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)	Accuracy RE (%)
2.50	2.30	5.5	6.5	−8.0
10.0	10.4	8.7	6.7	4.0
640.0	623	8.7	5.4	−2.7

Table 2
Stability of oxaceprol in rat plasma at different conditions determined by LC–MS/MS method ($n = 5$).

Sample concentration (ng/mL)	Average measured concentration (ng/mL)	Accuracy RE (%)	Precision R.S.D. (%)
Short-term stability (about 25 °C, 2 h)			
2.50	2.40	-4.0	2.4
10.0	10.7	7.0	1.4
640	697	8.9	2.2
Long-term stability (−70 °C, 2 weeks)			
2.50	2.61	4.0	2
10.0	10.1	1.0	6.2
640	577	−9.8	6.4
Freeze–thaw stability			
2.50	2.66	4.0	3.8
10.0	9.60	−4.0	3.9
640	632	−1.2	1.3
Stability of the post-extracted samples (4 °C, 12 h)			
2.50	2.64	4.0	7.8
10.0	10.5	5.0	6.3
640	651	1.7	1.9

$y = 0.22 + 0.0476x$ ($r = 0.996$). Accuracy and intra-day and inter-day precisions of oxaceprol were less than 10%. The results are presented in Table 1. The precision of IS was less than 15%.

Stock solution of oxaceprol was stable at room temperature for 12 h and at 4 °C for 30 days with mean % change within 2.9%. In plasma, oxaceprol was stable after being placed at room temperature (about 25 °C) for 2 h, stored at −70 °C for two week, and after undergoing three freeze–thaw cycles. The post-extracted oxaceprol was also stable under the autosampler conditions (4 °C) for at least 12 h. Stability data were summarized in Table 2. The stability results in our study showed a reliable stability behavior of oxaceprol under the experimental conditions.

The measured oxaceprol concentrations in plasma following a 10-fold dilution were within ±15% of their nominal values and the precisions (R.S.D. %) were within 8.1% for both 2400 and 640 ng/mL (data not shown). These results indicated that the method was applicable for analyzing matrix-diluted samples whose original concentrations extend beyond the calibration range.

The extraction recoveries for oxaceprol were 85.3 ± 3.3, 83.8 ± 9.8 and 86.8 ± 1.4% at the concentration of 2.50, 10.0 and 640 ng/mL, respectively. The extraction recovery for IS was 89.5 + 3.7%.

Post column infusion experiment showed no ion enhancement or suppression at the retention time of oxaceprol and IS in the

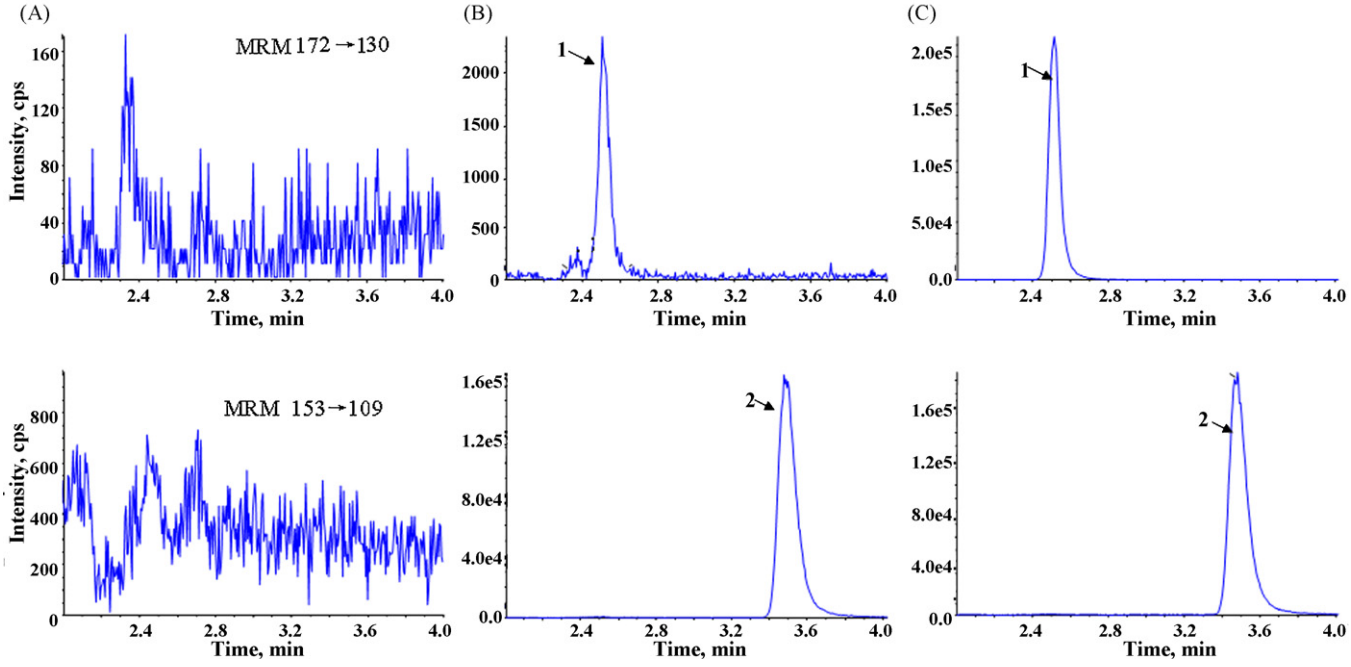

Fig. 3. Typical chromatograms of (A) blank plasma; (B) LLOQ for oxaceprol (1.25 ng/mL) in plasma an IS; (C) a plasma sample from a rat after an oral administration of 20 mg/kg oxaceprol.
Peak 1: oxaceprol; peak 2: IS

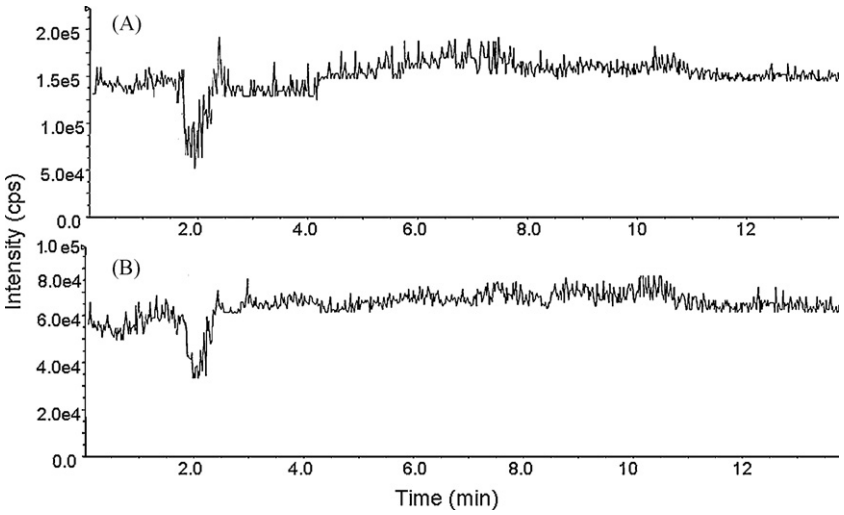

Fig. 4. Post column infusion of oxaceprol (panel A) and IS (panel B) with injection of the extracts of the blank plasma.

Table 3
Matrix factor for analyzing oxaceprol using analyte/IS peak area ratio.

Batch	Analyte/IS peak area ratio		
	2.50 ng/mL	10.0 ng/mL	640 ng/mL
1	1.03	0.95	0.99
2	1.03	1.02	1.00
3	1.00	1.01	1.00
4	1.08	0.95	0.95
5	1.09	1.02	0.97
6	1.05	1.08	1.02
Mean	1.05	1.00	0.99
S.D. (±)	0.03	0.05	0.03
R.S.D. (%)	3.33	4.90	2.58

LC–MS/MS chromatograms (Fig. 4). The matrix effect quantitative values for oxaceprol were summarized in Table 3. These results indicated the assay had no significant matrix ionization suppression or enhancement. The result of carry-over test indicated no carry-over was detected.

The accuracy, precision, recovery, matrix effect and stability tests all met the requirements for the quantitative biological samples [13,15].

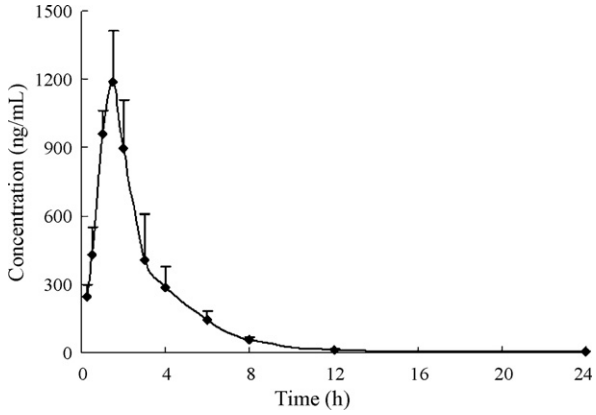

Fig. 5. Mean plasma concentration–time profile of oxaceprol determined by LC–MS/MS method after oral administration of 20 mg/kg oxaceprol in rats. Each point represents the mean ± S.D. (n = 6).

3.3. Pharmacokinetic study

The assay was successfully applied to the determination of oxaceprol in a preclinical pharmacokinetic study. The mean plasma concentration–time profile is presented in Fig. 5. It showed that the concentrations of oxaceprol in rat plasma were quantifiable at least 24 h (C = 3.30 ± 1.70 ng/mL) after oral administration. The main pharmacokinetic parameters of oxaceprol in rats were reported for the first time in Table 4. Oxaceprol reached peak concentra-

Table 4
The pharmacokinetic parameters of oxaceprol in rats following an oral administration of 20 mg/kg oxaceprol (n = 6).

Parameters	Mean	S.D.
$AUC_{(0-24)}$ (mg h/L)	3.396	0.571
$AUC_{(0-\infty)}$ (mg h/L)	3.401	0.572
$MRT_{(0-24)}$ (h)	3.108	0.165
$MRT_{(0-\infty)}$ (h)	3.142	0.195
$T_{1/2}$ (h)	2.261	0.694
T_{max} (h)	1.400	0.224
C_{max} (μg/Ml)	1.232	0.151
$V_{z/F}$ (L/kg)	19.74	6.708

Table 5
Incurred sample reanalysis for rat plasma samples.

Original concentration (ng/mL)	Reassay concentration (ng/mL)	Mean	%Difference
1085	1068	1077	−1.58
860	876	868	1.84
956	912	934	−4.71
940	967	954	2.83
860	800	830	−7.23
1045	1098	1072	4.95
185	201	193	8.29
153	137	145	−11.03
74.9	80.1	77.5	6.71
58.9	50.4	54.7	−15.55
60.9	64.3	62.6	5.43
29.2	27.5	28.4	−6.00
10.1	11.9	11.0	16.36
17.7	15.7	16.7	−11.98
18.1	16.3	17.2	−10.47
3.5	3.12	3.31	−11.48
1.84	1.91	1.88	3.73
2.01	2.18	2.10	8.11

Note: %difference = absolute (reanalyzed value − original value)/average of reanalyzed and original value × 100%.

tion between 1 and 1.5 h in plasma and its $T_{1/2}$ was short, clued to that oxaceprol may be given by sustained and controlled release formulations for maintaining clinical effect. The relative large values of distribution volume (9.6–27.7 L/kg) suggested oxaceprol was easily to distribute into tissues, which is beneficial to the treatment of osteoarthritis. However, the pharmacokinetic parameters, T_{max} and $T_{1/2}$ of oxaceprol in rat we obtained varied from those in dogs in published paper [10]; these may be due to the species differences in experimental animals and the selectivity of analytical method.

Based on the results obtained from the ISR, it was observed that all of the samples were within ±20% of initial concentration value (Table 5), further demonstrating that this method is capable of producing reproducible results over time.

4. Conclusion

A sensitive, specific and accurate method is first described for the quantification of oxaceprol in plasma by LC–MS/MS in negative electrospray ionization mode using MRM and fully validated according to commonly accepted criteria. The method exhibited excellent performance in terms of high selectivity, low LLOQ (1.25 ng/mL), wide linear range (1.25–800 ng/mL), small organic solvent consumption (60 μL) and small plasma volume (20 μL). The method has been successfully used for a pharmacokinetic study in rats after oral administration of oxaceprol, and can be easily extended to the pharmacokinetic study of other species of animal, such as dogs and rhesus macaque.

References

[1] M. Curatolo, N. Bogduk, Clin. J. Pain 17 (2001) 25–32.
[2] A. Veihelmann, A. Hofbauer, H.J. Refior, K. Messmer, Acta Orthop. Scand. 72 (2001) 293–298.
[3] G. Herrmann, D. Steeger, M. Klasser, J. Wirbitzky, M. Fürst, R. Venbrocks, H. Rohde, D. Jungmichel, H.D. Hildebrandt, M.J. Parnham, W. Gimbel, H. Dirschedl, Clin. Rheumatol. 19 (2000) 99–104.
[4] H.W. Bauer, M. Klasser, K.L. von Hanstein, H. Rolinger, G. Schladitz, H.D. Henke, W. Gimbel, K. Steinbach, Clin. Rheumatol. 18 (1999) 4–9.
[5] M.J. Parnham, Biochem. Pharmacol. 58 (1999) 209–215.
[6] E. Juvin, Sem. Hop. 74 (1998) 47–54.
[7] K. Steinbach, H.W. Bauer, Extract. Orthop. 18 (1995) 18–21.
[8] M. Ionac, M.J. Parnham, M. Plauchithiu, K. Brune, Pharmacol. Res. 33 (1996) 367–373.
[9] K. Krüger, M. Klasser, J. Mössinger, U. Becker, Clin. Exp. Rheumatol. 25 (2007) 29–34.
[10] G. Lachmann, B. Siegemund, W. Kusche, Arzneimittelforschung 40 (1990) 200–206.
[11] P. Partani, Y. Modhave, S. Gurule, A. Khuroo, T. Monif, J. Pharm. Biomed. Anal. 50 (2009) 966–976.
[12] B.N. Patel, N. Sharma, M. Sanyal, P.S. Shrivastav, J. Pharm. Biomed. Anal. 49 (2009) 354–366.
[13] C.T. Viswanathan, S. Bansal, B. Brian, J. Anthony, DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, AAPS J. 9 (2007) E30–E42.
[14] J.M. Cunliffe, C.F. Noren, R.N. Hayes, R.P. Clement, J.X. Shen, J. Pharm. Biomed. Anal. 50 (2009) 46–52.
[15] US Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research (CDER), Guidance for Industry, Bioanalytical Method Validation, May 2001.