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METHODOLOGY

Simultaneous Determination of Parecoxib and Its Metabolite Valdecoxib Concentrations in Beagle Plasma by UPLC-MS/MS and Application for Pharmacokinetics Study

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Abstract: A method for the simultaneous determination of parecoxib and its metabolite valdecoxib in beagle plasma by UPLC-MS/MS was developed and validated. After the plasma was extracted by acetonitrile precipitation, the analytes were separated on an Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm) using acetonitrile-formic acid as the mobile phase in gradient mode. The analytes were monitored by multiple reaction monitoring (MRM) in electrospray negative ion mode. The mass transfer pairs were m/z 368.97→119.01 for parecoxib, m/z 312.89→118.02 for valdecoxib, and m/z 379.98→316.02 for celecoxib (internal standard, IS). The correlation coefficients of parecoxib and valdecoxib ranged from 5 to 4000 ng/mL were greater than 0.9998. The recovery of parecoxib and valdecoxib was greater than 82.54%. The inter- and intra-day precision RSD values were 1.36~3.65% and 2.28~5.91%, respectively. The accuracy of RE values were −1.38%~1.96%. Finally, the matrix effect (ME) and stability were also within acceptable criteria. This method had been successfully applied to the pharmacokinetics of parecoxib and valdecoxib in beagle plasma after injection of parecoxib (1.33 mg/kg, intramuscular injection).

Keywords: parecoxib, valdecoxib, UPLC-MS/MS, pharmacokinetics

Introduction

Nonsteroidal anti–inflammatory drugs (NSAIDs) were widely used clinically for the relief of osteoarthritis, various fevers, and various pain symptoms. NSAIDs act by suppressing cyclooxygenase cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) enzymes.[1,2] COX-1 was important for maintaining homeostasis, such as platelet aggregation, and regulating gastric acid secretion.[3,4] COX-2 was the leading cause of pain and inflammation.[4] NSAIDs have severe gastrointestinal symptoms, nephrotoxicity, hepatotoxicity, platelet dysfunction and other adverse reactions.[5–7] COX-2 inhibitors has a lower risk of detrimental gastrointestinal (GI) effects, and the analgesic effect of COX-2 selective NSAIDs was the same as that of nonselective NSAIDs.[3,4] In addition, COX-2 inhibitors did not affect platelet aggregation and therefore has a lower risk of perioperative bleeding.[7]

Parecoxib (Figure 1A) was a prodrug of valdecoxib (Figure 1B), a COX-2 selective inhibitor that could be given intravenously and intramuscularly.[8] In the clinical, parecoxib was mainly used for the short-term treatment of postoperative pain, and it could also be used for the treatment of perioperative analgesia to

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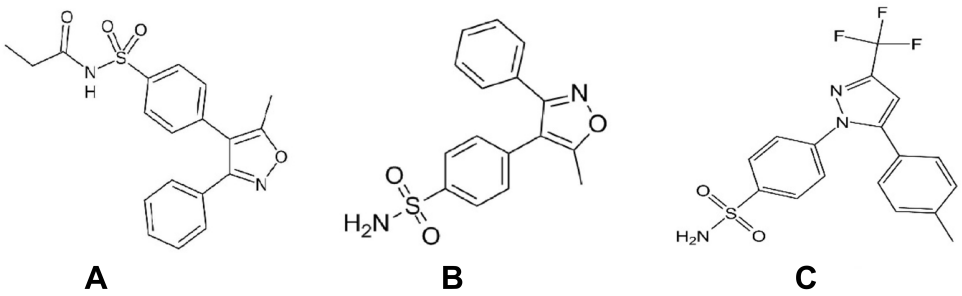

Figure 1 The chemical structure of parecoxib, valdecoxib and celecoxib (IS) (**A**) parecoxib, (**B**) valdecoxib, (**C**) celecoxib (IS).

prevent or reduce severe postoperative acute pain.[9] For a long time, opioids has been one of the most important drugs for the treatment of postoperative moderate and severe pain.[10] But as the understanding deepens, more and more doctors and patients have realized that opioids were not safe. Related studies showed that there were several adverse reactions (respiratory depression,[11] addiction,[12] nausea and confusion[13]) and the risk of hyperalgesia after opioid administration, which were positively correlated with dose.[14] In addition to analgesic effect, opioids could also activate the injury promoting mechanism in the body, leading to increased sensitivity of the body to pain, thus inducing opioid induced hyperalgesia (OIH) and chronic post-surgical pain (CPSP).[15,16] Compared with opioids, COX-2 inhibitors could effectively reduced the synthesis of peripheral and central prostaglandins, inhibited hypersensitivity to pain and improved the pain threshold.[17] The analgesic efficacy of parecoxib had been confirmed in several studies including anesthesiology,[8] orthopedics,[18] gynecology,[19] and general surgery.[20] Parecoxib was a second-generation specific inhibitor of COX-2, that could be rapidly converted to its metabolite valdecoxib by liver enzyme hydrolysis. Compared with other selective COX-2 inhibitors, valdecoxib was more likely to cause severe, potentially lethal skin reactions, including severe erythema multiforme and toxic epidermal necrolysis. So, the valdecoxib tablet Bextra was withdrawn from the market.[21] The combination of fast and long-lasting analgesic properties and good safety of parecoxib provided a better choice for postoperative analgesia.[8]

Parecoxib was rapidly and almost completely converted to valdecoxib and propionic acid in cats with $t_{1/2}$ about 24 mins.[22] The elimination of valdecoxib was extensively carried out in a variety of ways in the liver, including cytochrome CYP3A4 and CYP2C9 enzyme metabolism and sulfonamide glucose hydroformylation (about 20%).[23] The rapid onset and long-lasting analgesic effects of parecoxib were related to valdecoxib. Therefore, there was a need for a rapid and sensitive method for evaluating the pharmacokinetics of parecoxib and valdecoxib. Nowadays, HPLC with an UV, HPLC with DAD and HPLC-MS/MS has been widely used to analyze valdecoxib and parecoxib.[21,24] However, these research methods were relatively complex, with low sensitivity and long analysis time. LC-MS/MS had been widely used for the determination of drug concentrations in various biological matrices, which had many advantages such as short time, high sensitivity and strong specificity. So, LC-MS/MS would be an ideal method for the determination of parecoxib and valdecoxib. Although it has been reported to detect valdecoxib and parecoxib by LC-MS/MS.[25,26] In contrast, the experimental animal in our experiment was the beagle, which was closer to human drug metabolism. The plasma sample processing method we used was also simpler and the mobile phase conditions were easier to achieve. The analysis time of our methods was only 3 min, and the analysis time reported was 7.5 mins.[26] We have more data on plasma drug concentrations and the experimental data were more accurate after injection of parecoxib (1.33 mg/kg, intramuscular injection).

Therefore, we developed a new rapid and sensitive UPLC-MS/MS method for simultaneous determination of parecoxib and valdecoxib, and described the pharmacokinetic characteristics of parecoxib and valdecoxib in beagles after injection of parecoxib.

Materials and Methods

Chemicals and Reagents

Parecoxib (purity >98%), valdecoxib (purity >98%) and celecoxib (purity >98%, IS, Figure 1C) were obtained from Sigma (USA). Methanol and acetonitrile of LC-grade were purchased from Tianjin Kermel Chemical Reagent Co., Ltd. Trifluoroacetic acid was procured from Sigma-Aldrich. Other chemicals were analytical grade.

Instrumentation and Conditions

The analysis was carried out on a Waters UPLC system (USA). Two analytes and IS were separated on an Acquity BEH C18 column (2.1 × 50 mm, 1.7 μm) by gradient elution with the mobile phase of 0.1% formic acid (A) and acetonitrile (B) at the temperature of 45°C and the flow rate of 0.4 mL/min. The gradient program was as follows: 0.00–0.50 min, 10% B; 0.50–1.00 min, 10→90% B; 1.00–2.00 min, 90% B; 2.00–2.10 min, 90→10% B; 2.10–3.00 min, 10% B. The column temperature was set at 45°C and the auto-sampler was conditioned at 4°C.

Mass spectrometry was measured by XEVO TQ-S triple quadrupole mass spectrometer with an electrospray ionization (ESI) interface in negative ionization mode. Multiple reaction monitoring (MRM) conditions are shown in Table 1 and Figure 2. The Masslynx V4.1 software were used to get the data.

Preparation of Calibration Standards

Ten milligrams of parecoxib, valdecoxib and celecoxib were accurately weighed, respectively, in a 10 mL volumetric flask, dissolved in methanol and volume to the scale. Through gradient dilution of the original solution, various working solutions of calibration curve and quality control (QC) in methanol were obtained. Plasma standard solutions with parecoxib and valdecoxib concentrations of 5, 10, 50, 100, 500, 1000, 2000 and 4000 ng/mL were prepared, respectively. QC samples in plasma were similarly prepared, and the concentrations were set 10, 800, and 3000 ng/mL for parecoxib and valdecoxib, respectively.

Sample Preparation

Fifty microlitre of beagle plasma was accurately drawn to 1.5 mL of EP tube, 10 μL of diazepam internal standard working solution (50 ng/mL) was added, then vortex for 15 s. 200 μL of acetonitrile was added to the mixture and vortexed for 1.0 min. The mixture was centrifuged at 10,000 r/min for 15 min, and 2 μL of the supernatant was taken into the UPLC-MS/MS system for detection.

Method Validation

The UPLC-MS/MS method was validated in accordance with the guidelines of the United States Food and Drug Administration (FDA).[27]

Specificity

The specificity was assessed by comparing the chromatograms of individual blank beagle plasma samples, blank plasma spiked with parecoxib, valdecoxib and IS, and a plasma sample after injection of parecoxib (1.33 mg/kg, intramuscular injection).

Linearity and Carryover Effect

A series of concentrations of parecoxib and valdecoxib QC samples were prepared in triplicate on three consecutive days to evaluate linearity of the method. The peak areas of parecoxib or valdecoxib were A_s and the peak area of the IS was A_i, the ratio of A_s/A_i was the ordinate (y), the ratio of the concentration of parecoxib or valdecoxib to the concentration of IS was plotted on the abscissa (x), with a weighted factor ($1/\chi^2$). The lower limit of quantitation (LLOQ) was defined as the lowest concentration of the calibration curve of parecoxib and valdecoxib. The carryover test was performed by injecting a blank plasma sample spiked with IS (50 ng/mL) or parecoxib and valdecoxib (4000 ng/mL) followed by injecting a blank sample. In this blank sample, each analyte should be less than 20% of the LLOQ.

Accuracy and Precision

The precision and accuracy were analyzed using QC samples at 10, 800 and 3000 ng/mL concentration levels for parecoxib and valdecoxib with six replicates at each concentration on three consecutive validation days. The precision was expressed in terms of relative standard deviation (RSD%≤15%), which were determined by comparing the measured concentration to its true value. The accuracy was expressed in terms of relative error (RE %≤±15%), which were determined by comparing the measured value minus true value concentration to its true value.

Table 1 MS Parameters of Two Analytes and IS

Analytes	ESI Source	RT (min)	Parent (m/z)	Daughter (m/z)	Cone (V)	Collision (V)
Parecoxib	–	1.41	368.97	119.01	10	38
Valdecoxib	–	1.36	312.89	118.02	30	25
Celecoxib	–	1.48	379.98	316.02	20	20

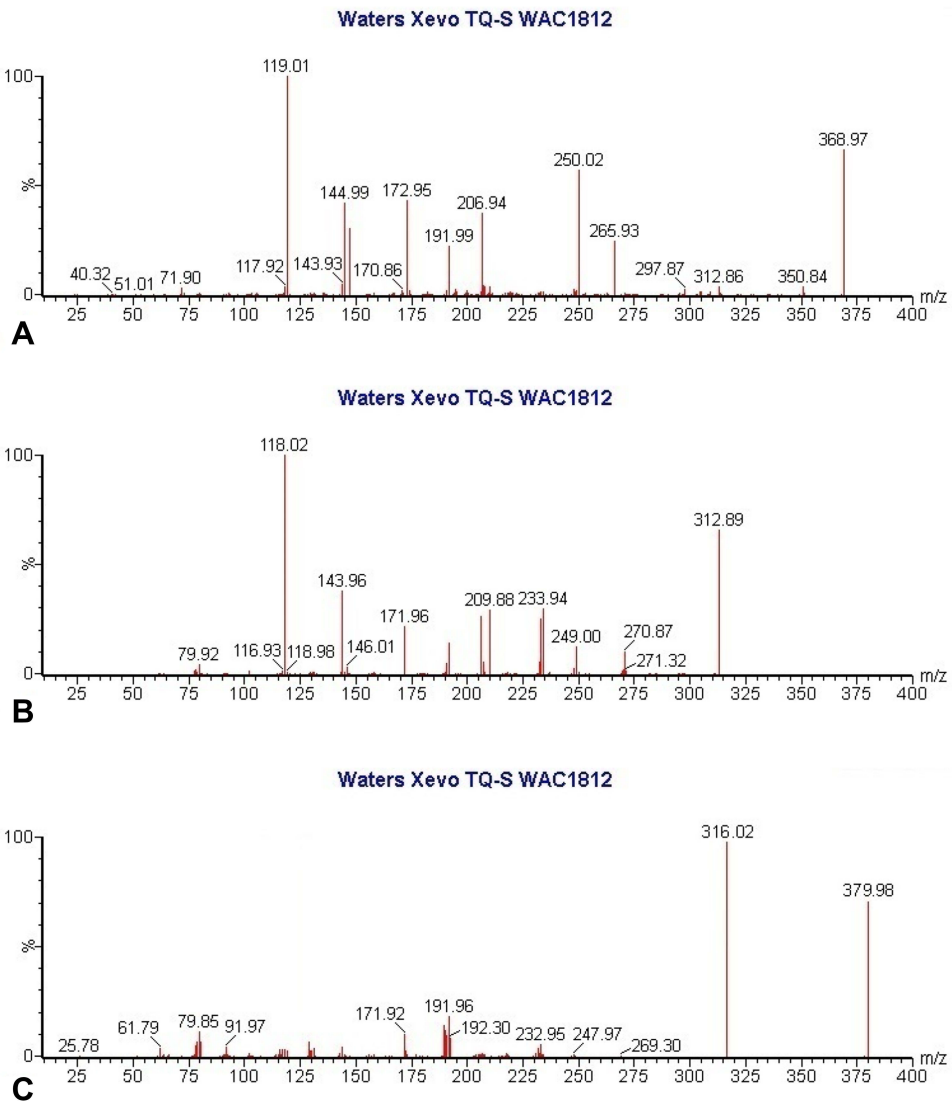

Figure 2 The ion transitions from parent ion to daughter ion of (**A**) Parecoxib, (**B**) valdecoxib, (**C**) Celecoxib.

Recovery and Matrix Effect

The extraction recovery of parecoxib and valdecoxib was evaluated by repeating six times at 10, 800 and 3000 ng/mL, respectively. The extraction recovery of parecoxib and valdecoxib were compared by comparing the peak area of the conventional pretreated QC sample with the peak area after extraction of the corresponding concentration of blank plasma (after extraction). The ME of parecoxib and valdecoxib was measured with six different beagle plasma at 10, 800 and 3000 ng/mL. The ME was evaluated by comparing the peak area ratio of the analyte in the sample after extraction and the corresponding water substitution sample. The ME values between 85% and 115% were acceptable.

Stability

The QC samples replicated six times under each condition were analyzed to evaluate their stability at 10, 800 and 3000 ng/mL for parecoxib and valdecoxib, respectively. The short-term temperature stability of using untreated QC samples was assessed at room temperature for 12 h. The auto-sampler tray stability of using untreated QC samples was assessed for 12 h in processed samples. The freeze-thaw stability was assessed after three freeze-thaw cycles using the QC samples (−20°C to 25°C). The long-term stability was assessed after storage of the untreated QC samples at −20°C for at least 4 weeks. The RE was less than ± 10% and the RSD was less than 15%, and the sample was considered stable.

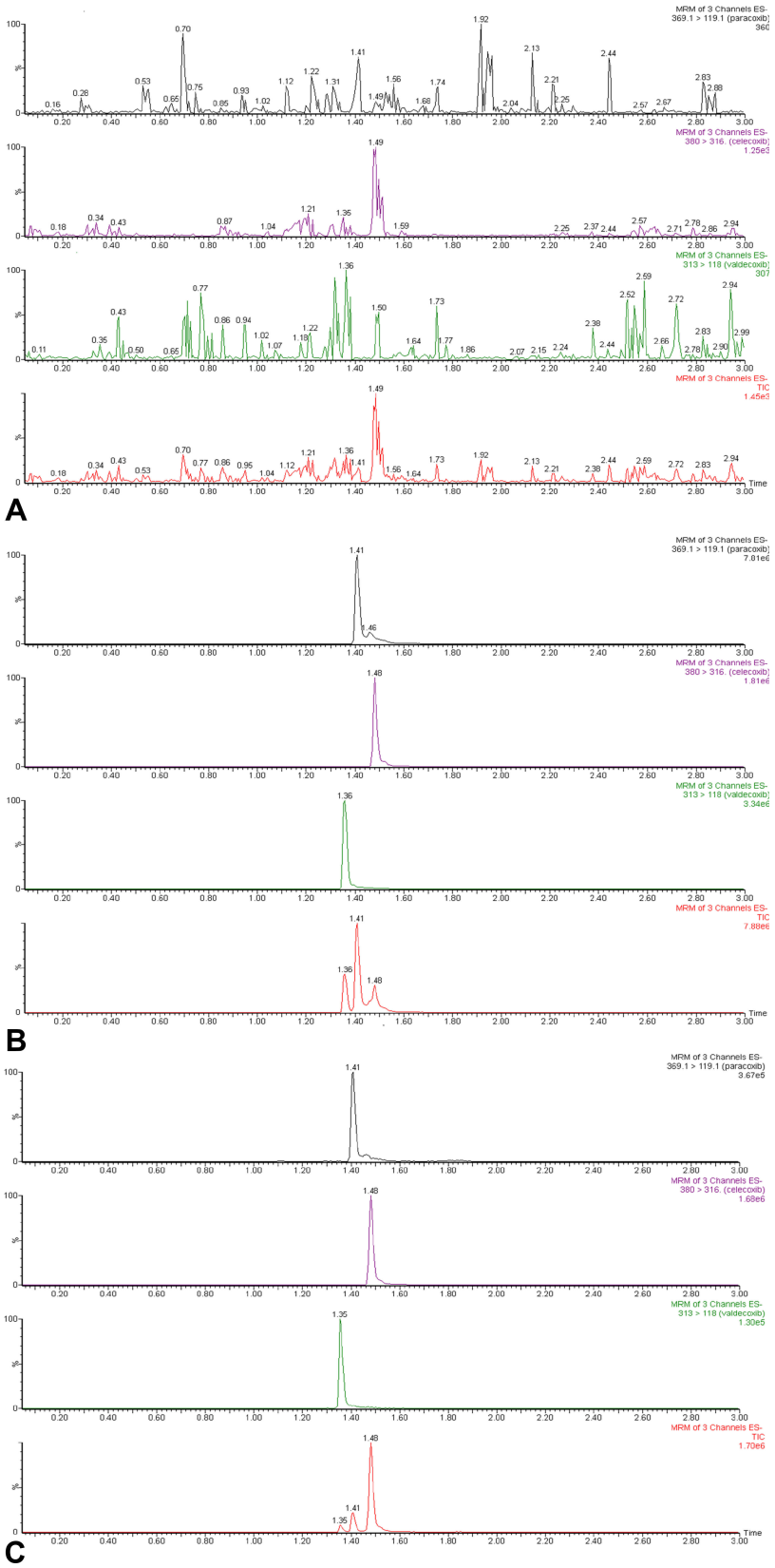

Figure 3 (**A**) a blank plasma sample, (**B**) a blank plasma sample spiked with parecoxib, valdecoxib and IS, (**C**) a beagle plasma sample 1.5 h after injection of parecoxib.

Stock Solution Stability

The stock solutions stability of the parecoxib, valdecoxib and IS (10 μg/mL) at room temperature stability and freeze stability were investigated by six replicates tests. The room temperature stability was evaluated comparing the portion of the stock solution stored at room temperature for 24 h against the remainder of the stock solution stored in a −20°C freezer. The freezing stability was evaluated by comparing the newly configured stock solution with the stock solution stored in a −20°C freezer for 3 months. The solution was considered to be stable if the test value was within acceptable accuracy (RE%≤±10%) and precision (RSD%≤15%).

Pharmacokinetic Study

Six healthy beagles weighing from 5.20 to 7.15 kg were selected. These beagles were provided by the Experimental Animal Center of Henan University of Science and Technology (Henan, China). The experiment obtained the necessary approval from the Animal Ethics Committee of Henan University of Science and Technology. The experiment was approved according to the Laboratory animals-guidelines for ethical review of welfare (GB/T 35892–2018). The institutional approval number for the preclinical study of this experiment was 2,018,080,004. After ten o'clock in the evening before the experiment, the beagles were free to drink water but could not eat. Blood samples (0.5 mL) were collected from the forelimb cephalic vein or the small saphenous vein of the hind limb and put into heparinized tubes at 0.17, 0.33, 0.67, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 9.00, 12.00 and 24.00 h after injection of parecoxib (1.33 mg/kg, intramuscular injection). After centrifugation of the blood samples, the supernatant was taken and frozen at −20°C until analysis.

Data Analysis

The pharmacokinetic parameters of the two analytes were calculated by the noncompartmental analysis using DAS software (version 2.0). The concentration data of parecoxib and valdecoxib in each beagle dog were analyzed and the pharmacokinetic parameters were evaluated.

Results and Discussion

Method Development

We had established an UPLC-MS/MS method for the determination of parecoxib and valdecoxib concentrations in beagle plasma. The method has high sensitivity and short analysis time (3 min).

The endogenous substances in the beagle plasma samples did not interfere with the determination of the content, and the specificity of the method was higher. The RSD values of inter- and intra-day precision of parecoxib and valdecoxib were need to be less than 15%, the precision results were good, the recovery rate was high, and the stability of plasma samples were stable.

In this experiment, we finally found that Acquity UPLC BEH C18 column was more suitable for experimental requirements. With acetonitrile and 0.1% formic acid as the mobile phase, parecoxib, valdecoxib and IS were well separated. The 0.1% formic acid could further improve the chromatographic and mass response curves. We also evaluated flow rates, gradient elution procedures, column temperatures, injection volumes, and more. In addition, we found that parecoxib and valdecoxib showed high specificity in the negative mode. At the same time, MS parameters were optimized with infusion and flow injection analysis. For more details, please refer to "Instrumentation and Conditions".

Protein precipitation was a common method of plasma sample treatment at present, which had the advantage of

Table 2 Regression Equation, Linear Ranges, Correlation Coefficients and LLOQ of Two Analytes

Analytes	Regression Equation	Linear Ranges (ng/mL)	R^2	LLOQ (ng/mL)
Parecoxib	y = 0.0151 x - 0.1342	5–4000	0.9999	5
Valdecoxib	y = 0.1744 x - 0.0206	5–4000	0.9998	5

Table 3 Precision and Accuracy of Parecoxib and Valdecoxib in Beagle Plasma (n=6, Mean ± SD)

Compounds	Spiked (ng/mL)	Intra-Day		Inter-Day	
		RSD (%)	RE (%)	RSD (%)	RE (%)
Parecoxib	10	3.65	−1.38	5.91	−0.33
	800	2.81	−0.57	3.85	0.45
	3000	1.67	−0.76	2.28	−0.54
Valdecoxib	10	3.22	−0.51	4.71	−0.49
	800	2.39	1.96	3.91	−0.43
	3000	1.36	−0.68	2.41	−0.25

Table 4 The Recoveries and ME of Parecoxib, Valdecoxib and IS in Beagle Plasma (n=6, Mean ± SD)

Compounds	Spiked (ng/mL)	Recoveries (%)	ME (%)
Parecoxib	10	83.46 ± 2.70	99.64 ± 2.95
	800	83.06 ± 3.98	100.17 ± 4.76
	3000	87.58 ± 0.62	99.75 ± 4.15
Valdecoxib	10	88.87 ± 2.23	102.29 ± 2.33
	800	89.69 ± 0.94	99.98 ± 1.99
	3000	82.54 ± 2.37	100.50 ± 4.29
IS	50	81.53 ± 3.34	97.13 ± 5.54

relatively easy and faster removal of proteins and potential interference with sample preparation, different types of precipitants had been tested to extract analyte.[28–30] After multiple screenings, we chose acetonitrile the method of protein precipitation method with acetonitrile. Acetonitrile could provide higher protein precipitation efficiency and better reproducibility for analytes.

Several internal standard candidates were selected for the experiment. Considering into account retention time, impurity interference and other factors, we employ celecoxib as an internal standard for negative ion mode.

Method Validation

Specificity

The specificity of the sample was examined by comparing the chromatograms of the blank plasma samples of beagle, plasma samples with parecoxib, valdecoxib and IS, and the beagle plasma samples after injection of parecoxib (1.33 mg/kg, intramuscular injection). Under the above experimental condition, parecoxib, valdecoxib and IS were well separated from endogenous substances. Representative chromatograms are shown in Figure 3. No significant interferences were found in the chromatograms of six randomly selected beagle plasma samples at the respective retention position of analyte. The mean retention times of parecoxib, valdecoxib and celecoxib (IS) were 1.41, 1.36 and 1.48 min, respectively.

Linearity and Carryover Effect

The standard curve and LLOQ of parecoxib and valdecoxib in this study are shown in Table 2. It could be seen that the standard curve of parecoxib and valdecoxib had a good linear relationship. The results of the carryover test showed that the analyzer did not detect residual analyte or IS injected into the sample at the next injection. In the UPLC-MS/MS analysis, carryover did not affect the determination of parecoxib and valdecoxib.

Precision and Accuracy

The intra- and inter-day precision and accuracy of parecoxib and valdecoxib were investigated and shown in Table 3. The precision (% RSD) and accuracy (% RE) for parecoxib and valdecoxib under investigation did not exceed 10%. The results indicated that the method was reliable, accurate and reproducible.

Recovery and ME

The recovery and ME values were investigated and shown in Table 4. The recovery values were all between 83.06% and 89.69% and the ME was all between 97.13% and 102.29%. These results indicated that this method was reliable.

Stability

The stability of parecoxib and valdecoxib in beagle plasma were evaluated under different conditions. The stability test results are shown in Table 5. It could be seen from the experimental results that parecoxib and valdecoxib were stable under the experimental conditions.

Table 5 The Stability of Parecoxib and Valdecoxib in Beagle Plasma (n=6, Mean ± SD)

Compounds	Spiked (ng/mL)	Room Temperature, 12 h		Autosampler 4 °C, 12 h		Three Freeze-Thaw		−20°C, 4 weeks	
		RSD(%)	RE(%)	RSD(%)	RE(%)	RSD(%)	RE(%)	RSD(%)	RE(%)
Parecoxib	10	2.76	−0.41	3.09	0.37	2.91	−0.75	1.78	−1.96
	800	2.49	2.94	2.52	3.95	1.98	2.38	1.46	0.33
	3000	2.11	0.23	2.21	−1.20	1.57	−0.42	1.79	0.60
Valdecoxib	10	2.47	−0.59	3.12	−1.04	2.09	0.27	4.64	−1.88
	800	3.84	−0.74	4.44	−2.71	4.58	1.70	3.06	−1.93
	3000	1.09	−1.09	2.50	−0.73	1.21	1.90	2.28	−0.29

Table 6 The Stock Solution Stability of Parecoxib, Valdecoxib and IS in Beagle Plasma (n=6)

Compounds	Spiked (μg/mL)	Room Temperature, 12 h		−20°C, 3 weeks	
		RSD (%)	RE (%)	RSD (%)	RE (%)
Parecoxib	10	3.72	−1.83	2.59	2.67
Valdecoxib	10	3.10	1.33	3.51	−2.17
IS	10	3.56	−3.33	4.29	−2.83

Table 7 Pharmacokinetic Parameters of Parecoxib and Valdecoxib After Intramuscular Injection of 1.33 mg/kg Parecoxib (n=6, Mean ± SD)

Parameters	Parecoxib	Valdecoxib
$t_{1/2}$ (h)	3.39±2.32	2.27±1.22
T_{max} (h)	0.20±0.07	1.36±0.34
$MRT_{(0-t)}$ (h)	1.50±0.18	2.46±0.45
$MRT_{(0-\infty)}$ (h)	1.66±0.25	2.52±0.47
C_{max} (ng/mL)	1967.59±418.18	1944.84±247.68
$AUC_{(0-t)}$ (ng·h/mL)	2502.79±370.09	4960.31±630.49
$AUC_{(0-\infty)}$ (ng·h/mL)	2508.19±368.28	4967.02±629.81

Abbreviations: $t_{1/2}$, Half-life; T_{max}, Time of peak concentration; $MRT_{(0-t)}$, Mean residence time of 0-t time; $MRT_{(0-\infty)}$, Mean residence time of 0-infinity time; C_{max}, Peak concentration; $AUC_{(0-t)}$, Area under curve of 0-t time; $AUC_{(0-\infty)}$, Area under curve of 0-infinity time.

Stock Solution Stability

Under the experimental conditions, the stock solution stability is shown in Table 6. It can be seen from the experimental results that the parecoxib, valdecoxib and IS stock solutions were stabilized.

Pharmacokinetic Study

The pharmacokinetic parameters of parecoxib and valdecoxib included T_{max}, C_{max}, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, $t_{1/2}$, MRT were determined. The calculation of non-compartmental is listed in Table 7. The curve of plasma concentrations-time of parecoxib and valdecoxib was shown in Figure 4. After intramuscular injection dosage, the concentration of parecoxib in the beagle rapidly decreased and was metabolized to valdecoxib. The $t_{1/2}$ of valdecoxib was about 2.27 h, and the T_{max} was about 1.36 h. Parecoxib and valdecoxib were metabolized faster in beagles after muscle administration. The UPLC-MS/MS method for detecting parecoxib and valdecoxib concentrations in this study could be used for the pharmacokinetic study of parecoxib in beagle.

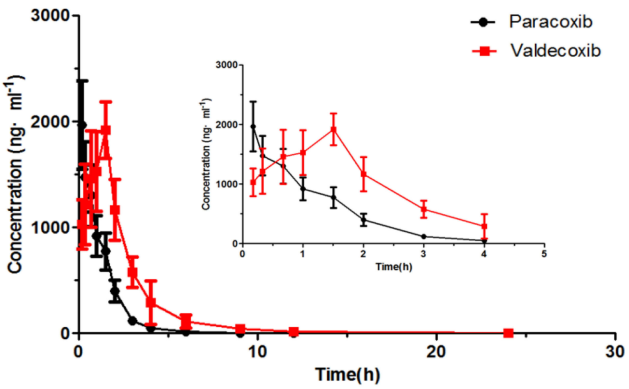

Figure 4 The mean plasma concentration-time curve of parecoxib and valdecoxib (zoomed 1 h to 4 h pharmacokinetic profile).

Conclusion

This study established a sensitive, rapid and specific UPLC-MS/MS method for simultaneous determination of parecoxib and its active metabolite valdecoxib in beagle plasma. This method required a simple acetonitrile precipitation process with a short analysis time (3.0 min). This method was successfully applied to the pharmacokinetic study of beagle, which provided a reference for the study of drug interactions.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Dwivedi AK, Gurjar V, Kumar S, Singh N. Molecular basis for non-specificity of nonsteroidal anti-inflammatory drugs (NSAIDs). *Drug Discov Today*. 2015;20(7):863–873. doi:10.1016/j.drudis.2015.03.004
2. Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. *J Lipid Res*. 2009;50(Suppl):S29–34. doi:10.1194/jlr.R800042-JLR200
3. Rostom A, Muir K, Dube C, et al. Gastrointestinal safety of cyclooxygenase-2 inhibitors: a cochrane collaboration systematic review. *Clin Gastroenterol Hepatol*. 2007;5(7):818–828, 828 e811–815; quiz 768. doi:10.1016/j.cgh.2007.03.011
4. Hawkey CJ. COX-1 and COX-2 inhibitors. *Best Pract Res Clin Gastroenterol*. 2001;15(5):801–820. doi:10.1053/bega.2001.0236
5. Schafer AI. Effects of nonsteroidal antiinflammatory drugs on platelet function and systemic hemostasis. *J Clin Pharmacol*. 1995;35(3):209–219. doi:10.1002/j.1552-4604.1995.tb04050.x
6. Lanas A, Garcia-Rodriguez LA, Arroyo MT, et al. Risk of upper gastrointestinal ulcer bleeding associated with selective cyclo-oxygenase-2 inhibitors, traditional non-aspirin non-steroidal anti-inflammatory drugs, aspirin and combinations. *Gut*. 2006;55(12):1731–1738. doi:10.1136/gut.2005.080754

7. Nadesalingam K, Kirby D. Cardiovascular safety of non-steroidal anti-inflammatory drugs. *Br J Gen Pract*. 2013;63(617):632. doi:10.3399/bjgp13X675377
8. Schug SA, Parsons B, Li C, Xia F. The safety profile of parecoxib for the treatment of postoperative pain: a pooled analysis of 28 randomized, double-blind, placebo-controlled clinical trials and a review of over 10 years of postauthorization data. *J Pain Res*. 2017;10:2451–2459. doi:10.2147/JPR.S136052
9. Kranke P, Morin AM, Roewer N, Eberhart LH. Patients' global evaluation of analgesia and safety of injected parecoxib for postoperative pain: a quantitative systematic review. *Anesth Analg*. 2004;99(3):797–806. (). doi:10.1213/01.ANE.0000133139.68208.92
10. Joshi GP, Rawal N, Kehlet H, et al. Evidence-based management of postoperative pain in adults undergoing open inguinal hernia surgery. *Br J Surg*. 2012;99(2):168–185. doi:10.1002/bjs.v99.2
11. Nagappa M, Weingarten TN, Montandon G, Sprung J, Chung F. Opioids, respiratory depression, and sleep-disordered breathing. *Best Pract Res Clin Anaesthesiol*. 2017;31(4):469–485. doi:10.1016/j.bpa.2017.05.004
12. Vadivelu N, Lumermann L, Zhu R, Kodumudi G, Elhassan AO, Kaye AD. Pain control in the presence of drug addiction. *Curr Pain Headache Rep*. 2016;20(5):35. doi:10.1007/s11916-016-0561-0
13. Lee YZ, Lee RQ, Thinn KK, Poon KH, Liu EH. How patients fare after anaesthesia for elective surgery: a survey of postoperative nausea and vomiting, pain and confusion. *Singapore Med J*. 2015;56(1):40–46. doi:10.11622/smedj.2015008
14. Plein LM, Rittner HL. Opioids and the immune system - friend or foe. *Br J Pharmacol*. 2018;175(14):2717–2725. doi:10.1111/bph.v175.14
15. Guastella A, Latchman J, Tofthagen CS. Opioid-induced hyperalgesia: clinical implications for advanced practice nurses in oncology. *Clin J Oncol Nurs*. 2017;21(3):294–296. doi:10.1188/17.CJON.294-296
16. Huang A, Azam A, Segal S, et al. Chronic postsurgical pain and persistent opioid use following surgery: the need for a transitional pain service. *Pain Manag*. 2016;6(5):435–443. doi:10.2217/pmt-2016-0004
17. Malan TP, Marsh G, Hakki SI, Grossman E, Traylor L, Hubbard RC. Parecoxib sodium, a parenteral cyclooxygenase 2 selective inhibitor, improves morphine analgesia and is opioid-sparing following total hip arthroplasty. *Anesthesiology*. 2003;98(4):950–956. doi:10.1097/00000542-200304000-00023
18. Nakata K, Hanai T, Take Y, et al. Disease-modifying effects of COX-2 selective inhibitors and non-selective NSAIDs in osteoarthritis: a systematic review. *Osteoarthr Cartilage*. 2018;26(10):1263–1273. doi:10.1016/j.joca.2018.05.021
19. Zhang H, Liu X, Jiang H, Liu Z, Zhang XY, Xie HZ. Parecoxib increases muscle pain threshold and relieves shoulder pain after gynecologic laparoscopy: a randomized controlled trial. *J Pain Res*. 2016;9:653–660. doi:10.2147/JPR.S115889
20. Bajaj P, Ballary CC, Dongre NA, Baliga VP, Desai AA. Role of parecoxib in pre-emptive analgesia: comparison of the efficacy and safety of pre- and postoperative parecoxib in patients undergoing general surgery. *J Indian Med Assoc*. 2004;102(5):272,274, 276–278.
21. Giorgi M, Saccomanni G, Del Carlo S, Manera C, Lavy E. Pharmacokinetics of intravenous and intramuscular parecoxib in healthy Beagles. *Vet J*. 2012;193(1):246–250. doi:10.1016/j.tvjl.2011.11.005
22. Kim TW, Vercelli C, Briganti A, Re G, Giorgi M. The pharmacokinetics and in vitro/ex vivo cyclooxygenase selectivity of parecoxib and its active metabolite valdecoxib in cats. *Vet J*. 2014;202(1):37–42. doi:10.1016/j.tvjl.2014.07.025
23. Fenton C, Keating GM, Wagstaff AJ. Valdecoxib: a review of its use in the management of osteoarthritis, rheumatoid arthritis, dysmenorrhoea and acute pain. *Drugs*. 2004;64(11):1231–1261. doi:10.2165/00003495-200464110-00006
24. Yu QY, Yu QL, Wu L, et al. Validated stability-indicating RP-HPLC method for the determination of parecoxib sodium and degradation impurities in bulk drug. *Curr Pharm Anal*. 2017;13(3):271–278. doi:10.2174/1573412912666160418165539
25. Liu M, Yu Q, Li P, et al. Simultaneous determination of parecoxib sodium and its active metabolite valdecoxib in rat plasma by UPLC-MS/MS and its application to a pharmacokinetic study after intravenous and intramuscular administration. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2016;1022:220–229. doi:10.1016/j.jchromb.2016.04.009
26. Jin XL, Zhou F, Liu Y, et al. Simultaneous determination of parecoxib and its main metabolites valdecoxib and hydroxylated valdecoxib in mouse plasma with a sensitive LC-MS/MS method to elucidate the decreased drug metabolism of tumor bearing mice. *J Pharmaceut Biomed*. 2018;158:1–7. doi:10.1016/j.jpba.2018.05.034
27. US Food and Drug Administration. *Guidance for Indus-try: Bioanalytical Method Validation*. Rockville, MD, USA: US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research; 2018. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical method-validation-guidance-industry. Accessed August 10, 2018..
28. Zhu YL, Li SL, Chen KL, Ma KP, Wu DQ, Qiu XJ. Effect of Chinese herb Danzhi Xiaoyao pills on pharmacokinetics of venlafaxine in Beagles. *Drug Des Dev Ther*. 2019;13:3343–3355. doi:10.2147/DDDT.S221927
29. Mao ZS, Wang X, Li BX, et al. A simplified LC-MS/MS method for rapid determination of cycloserine in small-volume human plasma using protein precipitation coupled with dilution techniques to overcome matrix effects and its application to a pharmacokinetic study. *Anal Bioanal Chem*. 2017;409(11):3025–3032. doi:10.1007/s00216-017-0249-2
30. Ye WJ, Lin XJ, Zhang YT, et al. Quantification and pharmacokinetics of alpinetin in rat plasma by UHPLC-MS/MS using protein precipitation coupled with dilution approach to eliminate matrix effects. *J Pharmaceut Biomed*. 2018;152:242–247. doi:10.1016/j.jpba.2017.12.046