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Research Article

Validated UPLC-MS/MS Method for Determination of Futibatinib and Its Pharmacokinetics in Beagle Dogs

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Futibatinib, a highly selective, irreversible potent fibroblast growth factor receptor (FGFR) inhibitor, has been proved to be effective in clinical trials of intrahepatic cholangiocarcinoma (ICCA) patients. An ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method to determine the concentration of futibatinib in beagle dog plasma was developed and validated for the study of pharmacokinetics. After the plasma protein was removed by acetonitrile precipitation, futibatinib was detected and derazantinib was used as the internal standard (IS). Futibatinib and IS were separated in an UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm) with acetonitrile and 0.1% formic acid as the mobile phase, and the flow rate was 0.3 mL/min. Under the positive ion condition of an electrospray spray ion (ESI+) source, multireaction detection was used, and the ion pairs for futibatinib and IS were m/z 418.99 ⟶ 295.97 and 468.96 ⟶ 382.00, respectively. Futibatinib had a good linear relationship in the linear range of 0.5~100 ng/mL; the lower limit of quantification (LLOQ) was 0.5 ng/mL. The RSDs of the intraday and interday precision were all less than 10.70%, and the RE value of accuracy was between −3.87% and 3.28%. The extraction recovery of futibatinib was more than 80%, and the matrix effect was around 100%, and futibatinib was found to be stable under four experimental conditions. The new optimized and validated UPLC-MS/MS method was an effective tool to determine the concentration of futibatinib in plasma and has been successfully applied to the pharmacokinetics of futibatinib in beagle dogs. This method would also be used to study drug-drug interaction (DDI).

1. Introduction

Intrahepatic cholangiocarcinoma (ICCA) is a rare malignancy and is one of the types of cholangiocarcinoma (CCA) [1]. According to the studies, the morbidity and mortality rates of ICCA have shown a particularly significant increase in recent years [2]. There are many factors that contribute to ICCA, such as cholestasis, persistent biliary inflammation, intrahepatic bile duct stones, hepatitis virus infection, and cirrhosis [3]. Clinical symptoms in ICCAs are not evident in the early stages, and when the disease progresses to the middle and advanced stages, obvious signs or symptoms such as right upper abdominal distension, jaundice, and nausea appear [1, 3]. Currently, effective treatments for ICCA are hepatic resection and chemotherapy, but the vast majority of patients have progressed to an unresectable advanced stage [4, 5].

The receptor tyrosine kinase (RTK) family is known to have four fibroblast growth factor receptors (FGFR1, FGFR2, FGFR3, and FGFR4), and the FGFR signaling pathway is involved in the regulation of numerous biological processes, including cell survival, proliferation, differentiation, and angiogenesis [5]. Thus, abnormal FGFR signaling can lead to the occurrence and transformation of many different cancer types [6, 7]. In recent years, gene sequencing studies have found that ICCA has the most potent molecular targets for FGFR. Therefore, FGFR inhibitors have become a new therapeutic target for ICCA patients. As a result, a number of FGFR inhibitors are currently being developed, some of which have demonstrated clinical benefit and safety in trials [8, 9]. However,

as the use of FGFR inhibitors in ICCA increases, the main challenge is facing drug resistance due to acquired mutations [10].

Futibatinib (Figure 1(a)), also known as TAS-120, is a highly selective irreversible effective FGFR inhibitor, which has been proved to be effective in clinical trials of ICCA patients. It has a low risk of acquired drug resistance mutations and can inhibit various FGFR (FGFR 1–4) aberrations [10]. In preclinical experiments, futibatinib exhibited antiproliferative activity against tumor cell lines with various FGFR genomic aberrations from different tissue sources (stomach, bladder, lungs, endometrium, etc.) [11–13]. Especially in ICCA patients, clinical trial response rates in antitumor activity were higher than in any other tumor type [11].

The results of trials suggested that the maximum tolerated dose (MTD) of futibatinib was given orally once a day at a dose of 20 mg [10–13]. In the first human study of futibatinib phase I, the C_{max} values of MTD were 256.70 ng/ml on days 1 and 170.58 ng/ml on days 21, the T_{max} was 1.9 h and 3.5 h after administration, and $t_{1/2}$ was 2.94 h and 3.44 h, respectively, on days 1 and 21 [11].

Currently, futibatinib is undergoing Phase II/III trials [10, 11]. However, as a new drug, there are few reports on the detection and pharmacokinetics of futibatinib, and one reports the detection of futibatinib in human liver microsomes to estimate its metabolism in vivo by UPLC/MS methods, and futibatinib was a medium clearance drug with a predicted $CL_{H,int}$ of 2075 mL/min [14], which suggested that futibatinib was metabolized by CYP450. Meanwhile, futibatinib has an inactivation effect on CYP3A, and fut can be used as an exemplary targeted covalent inhibitor [15].

Therefore, it is necessary to establish a method for the determination of futibatinib in plasma samples and to be used in pharmacokinetic studies. Consequently, the purpose of this experiment was to establish a UPLC-MS/MS method to determine the concentration of futibatinib in beagle dog plasma, and derazantinib was used as the internal standard (IS, Figure 1(b)). At the same time, the novel developed and validated UPLC-MS/MS method was successfully used to study the pharmacokinetics of futibatinib in beagle dogs.

2. Materials and Methods

2.1. Instruments. Waters ACQUITY UPLC instrument included a binary solvent manager with an online degassing function, a sample manager, and a column with active preheating at high temperatures (Waters, USA). Waters XEVO TQD triple quadrupoles mass spectrometer equipped with electrospray ionization (ESI) source (Waters, USA) was used. Other instruments included FA1004B electronic balance, ultra pure water machine (UPR-II-5/10TU), and high-speed refrigerated centrifuge (H1650R).

2.2. Chemical Agent. Futibatinib standard and derazantinib standard (purity ≥98%) were purchased from Beijing Sunflower Science and Technology Development Co., Ltd. HPLC pure methanol, acetonitrile, and formic acid were purchased from Tianjin Kemio Chemical Reagent Co., Ltd. Ultra-pure water was prepared by ultra-pure water machine.

2.3. Solutions Preparation. The 10 mg futibatinib standard was precisely weighed in a 10 mL volumetric flask, dissolved in methanol, and volumized to a scale to obtain 1 mg/ml standard stock solution. 1 mg/ml IS stock solution was prepared by the same method. The stock solution of 1 mg/ml futibatinib was diluted 10 times with methanol to obtain the standard application solution of 100 μg/ml, 10 μg/ml, 1 μg/ml, and 100 ng/ml for calibration curve and quality control (QC) samples.

Different volumes of standard application solution were added to different volumes of blank beagle dog plasma to obtain the calibration standards with concentrations of 0.5, 1, 2.5, 5, 10, 25, 50, and 100 ng/mL, respectively. QC samples were separately prepared using the same way at three different concentration levels, including the low quality control (LQC, 1.0 ng/mL), middle quality control (MQC, 10 ng/mL), and high quality control (HQC, 75 ng/mL). The ISTD working solution at a concentration of 100 ng/mL was obtained by dilution of its stock solution with methanol.

2.4. Preparation of Plasma Samples. Plasma samples were pretreated with acetonitrile to precipitate plasma proteins. Generally speaking, 100 μL beagle dog plasma sample was put into a 1.5 mL Eppendorf tube, then 50 μL IS working solution was added and mixed. 200 μL of acetonitrile was added and mixed for 2 min and then centrifuged for 10 min at 4°C and 6.743 × g. Finally the supernatant was transferred to the sample bottle, and 2 μL of the supernatant was injected directly into the LC-MS/MS system for analysis.

2.5. UPLC-MS/MS Conditions. The chromatographic column was a Waters Acquity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm). Acetonitrile and 0.1% formic acid aqueous solution were used as the mobile phase. The gradient elution was as follows: 0–0.5 min, acetonitrile kept at 10%; 0.5–1.0 min, acetonitrile increased from 10% to 90%; 1.0–1.4 min, acetonitrile kept at 90%; 1.4–1.5 min, acetonitrile decreased to 10%; 1.5–2.5 min, acetonitrile remained at 10% until the end of the analysis. The flow rate was 0.3 mL/min. The temperature of the column was maintained at 40°C, and the temperature of the autosampler tray was set at 4°C.

By adopting the electrospray ionization (ESI) interface, in the positive ion and multiple reaction monitoring (MRM) mode, the mass spectrometer had realized data measurement. The parent ions and daughter ions used for quantification were as follows: m/z 418.99⟶295.97 for futibatinib and m/z 468.96⟶382.00 for IS, respectively. The cone voltage of futibatinib and ISTD were 30 V and 20 V, and the collision energy of futibatinib and ISTD were 25 V and 25 V, respectively. The dwell time was 162 ms. The control of the experimental instrument and the collection of data were completed by MassLynx4.1 software (Waters Corp).

Figure 1: The chemical structural formulas: futibatinib (a) and IS (b).

2.6. Method Validation. In this experiment, the basic parameters that must be examined for method validation include specificity, linearity, LLOQ, precision, accuracy, recovery, matrix effect (ME), and stability. The validation of this method was carried out in accordance with the guidelines for the determination of biological samples [16, 17].

The analytical method should be able to distinguish between the target analyte, IS, and endogenous components in the matrix. At least six beagle dog blank plasma should be used to demonstrate selectivity, which is generally acceptable when the response of the interfering component is less than 20% of the response of LLOQ of analyte and less than 5% of the IS response.

The response of the instrument to the analyte within the specified concentration range was evaluated to obtain a standard curve. The concentrations of the calibration standard of futibatinib were as follows: 0.5, 1, 2.5, 5, 10, 25, 50, and 100 ng/ml. The least square method was used to perform linear regression with the peak area ratio (futibatinib/IS) and the corresponding concentration. The lowest concentration of standard curve was the LLOQ. The back calculated concentration of the calibration standard sample should generally be within ±15% of the marked value, and the back calculated concentration of LLOQ should be within ±20%.

Six LQC, MQC, HQC, and LLOQ samples were prepared for detection and analysis. On the same day, the intraday precision was calculated, and the interday precision was obtained by tracking for three consecutive days. The precision was expressed by the relative standard deviation (RSD %), and the accuracy was expressed in terms of relative error (RE %).

The extraction recovery was obtained by calculating the ratio of the response value of the analyte recovered from the biological sample matrix to the response value of the standard. The ME was evaluated by calculating the ratio of the peak area in the presence of the matrix to the corresponding peak area without the matrix.

Six LQC, MQC, and HQC samples were prepared and tested for stability under the following 4 conditions: room temperature for 12 h, autosampler (4°C) 12 h, −20°C~25°C freeze-thaw cycle 3 times, and −20°C long-term stability (4 weeks).

2.7. Animal Experiments. Six healthy adult beagles (weighing 7 to 9 kg) were purchased from Hubei Yizhicheng Biotechnology Co., Ltd., Shiyan, Hubei, and the animal production license No. was SCXK2021 (Hubei)−0020. They were housed in a room at 25~27°C, with a 12/12 h light/dark cycle, 40%~60% humidity, and were fed twice every day, with free access to water. All beagle dogs were fasted to eat for 12 h but allowed to drink water before experiment. The animals were authorized by the Animal Laboratory of Henan University of Science and Technology and also were cared on the basis of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Six beagle dogs were randomly numbered, each of which was given 0.67 mg/kg futibatinib orally in a single dose. The blood samples (1.5 mL) were taken from the cephalic vein of the forelimb or the small saphenous vein of the hind limbs at 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 9, 12, and 24 h after oral administration. All blood samples were anticoagulated with heparin and were centrifuged at $606 \times g$ for 10 min. The plasma was separated and stored at −20°C until test.

2.8. Pharmacokinetic Study. The established UPLC-MS/MS method was used to detect beagle dog plasma to obtain the concentration of futibatinib. The samples were analyzed according to the analysis batch, and each analysis batch was accompanied by standard curve and QC samples. The drug concentration data was processed by DAS (Drug and Statistics, version 2.0), calculating the main pharmacokinetic parameters T_{max}, C_{max}, $t_{1/2}$, MRT, CL, Vd, and AUC, and then was expressed as an arithmetic mean ± standard deviation, Mean ± SD.

3. Results and Discussion

3.1. Method Validation and Improvement. UPLC-MS/MS has the advantages of high sensitivity, strong specificity, short analysis time, and good reproducibility. Therefore, it is often used in the detection of biological samples and the study of pharmacokinetics [18, 19].

Different mobile phase systems such as methanol-water, methanol −0.1% formic acid aqueous solution, acetonitrile-water, and acetonitrile −0.1% formic acid aqueous solution were investigated in this experiment in order to better determine the compounds to be tested. The results showed that

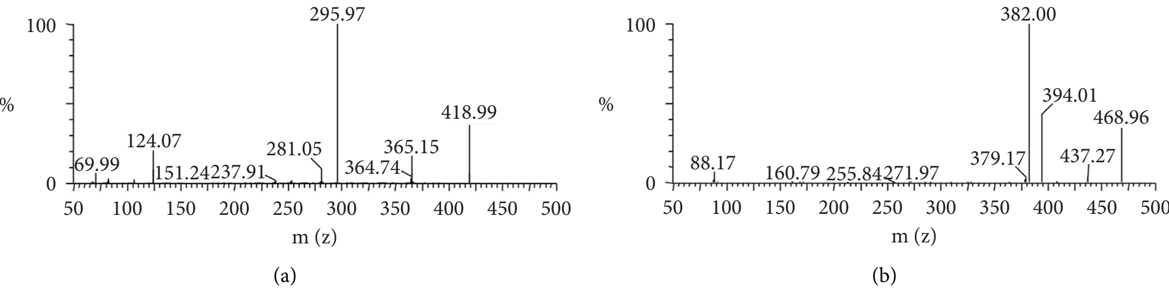

Figure 2: The mass spectra of of futibatinib (a) and IS (b).

the components to be tested had a strong response under the gradient elution system of acetonitrile −0.1% formic acid aqueous solution, and the endogenous components in plasma did not interfere with the determination of futibatinib and IS. The chromatographic peaks were good, so acetonitrile −0.1% formic acid aqueous solution was finally selected as the mobile phase.

In order to select the appropriate ion pair for each substance to be measured, the mass spectrometry parameters were optimized in this experiment. The responses of the substance to be measured in positive and negative ion modes were investigated. The results showed that the substance to be measured in positive ion mode had a higher response, so the experiment was carried out in positive ion mode. At the same time, the conditions such as spray voltage, air curtain gas, atomization gas, auxiliary heating gas, and auxiliary gas temperature were optimized. Under the final detection conditions, the response values of futibatinib and IS were strong, and the peak area was the largest. The mass spectra of futibatinib and IS are shown in Figure 2.

In this experiment, derazantinib was selected as the IS, which is a potent pan-FGFR kinase inhibitor too [3, 20]. Under the chromatographic and mass conditions in this experiment, the retention time of futibatinib and IS was the same (1.50 min). Although the retention time was the same, futibatinib and IS did not interfere with each other, because in mass spectrometry detection, the mass charge ratio of substances was used for qualitative and quantitative detection (parent ions and daughter ions were scanned and detected in their respective channels), and this was also the advantage of mass spectrometry.

According to the green analytical chemistry, the amounts and toxicity of reagents, generated waste, energy requirements, the number of procedural steps, miniaturization, and automation are just a few of the multitude of criteria considered when assessing an analytical methodology's greenness [21]. The method of precipitating plasma protein with acetonitrile was adopted, which reduced the sample processing steps. At the same time, a lower flow rate, less injection volume (2 μL), and shorter detection time were adopted to reduce the generation of waste.

3.2. Specificity. Under this experimental condition, futibatinib and IS were detected in their respective channels, with good peak shape and no interference from endogenous substances. The retention time of futibatinib was about 1.50 min, and the retention time of IS was about 1.50 min. See Figure 3.

3.3. Linearity and LLOQ. In this study, the plasma drug concentration of futibatinib had a good linear relationship in the range of 0.5~100 ng/mL. The standard curve regression equation was as follows: $y = 2.25 \times 10^{-2}x - 1.67 \times 10^{-2}$, $r = 0.999\ 3$, where y represents the ratio of the peak area of futibatinib to ISTD, and x represents the plasma concentration of futibatinib. The LLOQ of futibatinib was 0.5 ng/mL.

3.4. Precision and Accuracy. The results of the precision and accuracy are listed in Table 1, which showed that the precision (% RSD) did not exceed 10.70% and the accuracy (% RE) was in the range of −3.87% to 3.28% at LQC, MQC, and HQC.

3.5. Recovery and ME. The results of recovery and ME are shown in Table 2, and the results showed that the extraction recovery of the LQC, MQC, and HQC samples exceeded 80%. The ME of the LQC, MQC, and HQC samples ranged from 98.08% to 103.21%, which demonstrated that the ME did not affect the determination of futibatinib in beagle dog plasma.

3.6. Stability. The stability of futibatinib under 4 conditions was investigated, and the RE of all samples ranged from −4.17% to 0.94%, which indicated that futibatinib was stable under the experimental conditions and no significant degradation was observed. The stability results are listed in Table 3.

3.7. Pharmacokinetic Application. The established UPLC-MS/MS method was used to detect the concentration of futibatinib in the beagle dog plasma and was successfully applied to a pharmacokinetic study of futibatinib in beagle dogs. After futibatinib was given orally to six healthy beagle dogs at a single dose of 0.67 mg/kg, the plasma mean drug concentration-time curve is shown in Figure 4. The pharmacokinetic parameters of futibatinib were analyzed using DAS2.0 statistical analysis and are listed in Table 4. The pharmacokinetic results showed that futibatinib was

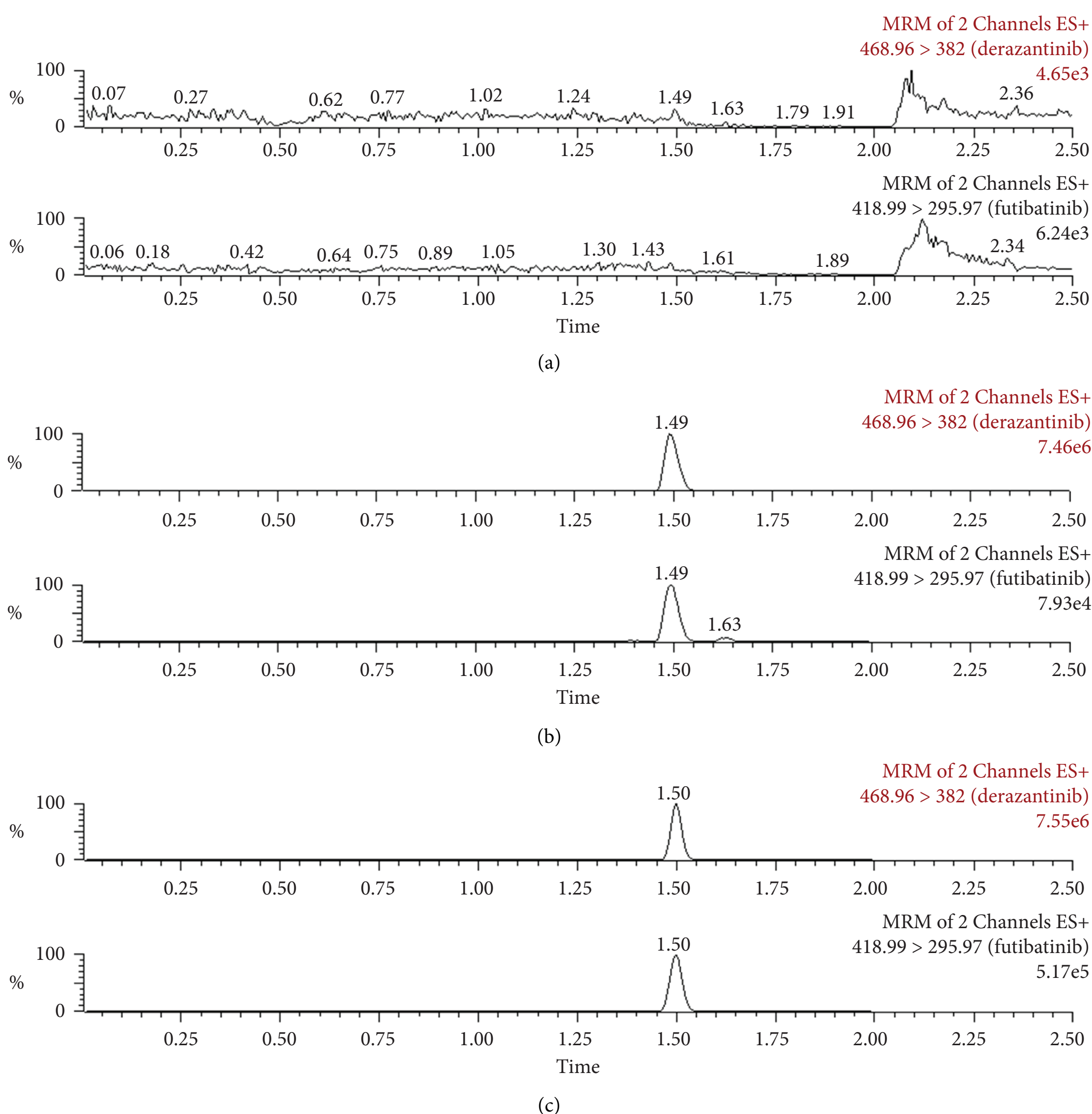

Figure 3: Representative chromatograms of futibatinib. (a) A blank plasma sample; (b) a blank plasma sample containing futibatinib (LLOQ) and IS; (c) a plasma sample collected 20 min after oral administration of 0.67 mg/kg futibatinib.

Table 1: Precision and accuracy of futibatinib in the beagle plasma ($n = 6$).

Added (ng/mL)	Intraday			Interday		
	Found (ng/mL)	RSD (%)	RE (%)	Found (ng/mL)	RSD (%)	RE (%)
0.5	0.51 ± 0.02	4.26	1.33	0.51 ± 0.01	2.34	1.11
1.0	1.02 ± 0.06	6.00	1.50	0.99 ± 0.02	1.62	−0.67
10	9.61 ± 1.03	10.70	−3.87	10.33 ± 0.33	3.22	3.28
75	72.71 ± 5.98	8.22	−3.06	74.68 ± 1.82	2.44	−0.43

Table 2: Recovery and ME of futibatinib in the beagle dog plasma ($n = 6$).

Skipped (ng/mL)	Recovery (%)		Matrix effect (%)	
	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
1.0	81.22 ± 4.16	5.12	98.08 ± 5.35	5.46
10	83.95 ± 4.10	4.89	103.21 ± 6.43	6.23
75	85.34 ± 3.61	4.24	101.94 ± 4.26	4.18

Table 3: The stability of futibatinib in the beagle plasma ($n = 6$).

Added (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 (%)
1.0	7.85	−4.17	8.89	−2.50	8.41	0.50	9.68	−2.00
10	10.41	−3.67	8.86	−1.62	5.87	4.82	10.47	−0.68
75	2.58	0.20	6.45	0.59	6.38	−1.67	3.11	0.94

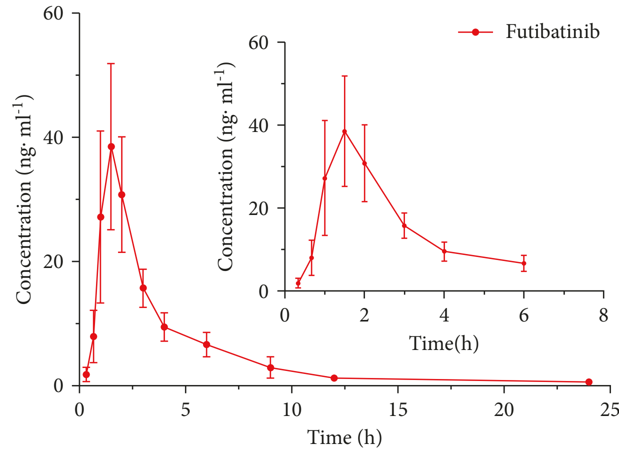

Figure 4: Plasma concentration-time after oral administration of 0.67 mg/kg futibatinib in 6 beagle dogs.

Table 4: Pharmacokinetic parameters of futibatinib after 0.67 mg/kg oral administration to 6 beagle dogs.

Parameters	Futibatinib
$t_{1/2}$ (h)	3.75 ± 1.08
T_{max} (h)	1.50 ± 0.45
C_{max} (ng/mL)	44.47 ± 10.02
$MRT_{0\longrightarrow t}$ (h)	4.53 ± 0.62
$MRT_{0\longrightarrow\infty}$ (h)	5.21 ± 0.63
Vz/F (L/kg)	28.61 ± 7.68
CL$z/F$ (L/h)	5.35 ± 0.55
$AUC_{0\longrightarrow t}$ (ng·h/mL)	124.51 ± 13.38
$AUC_{0\longrightarrow\infty}$ (ng·h/mL)	126.43 ± 14.26

absorbed rapidly after oral administration, reaching a peak concentration of 44.47 ng/ml at about 1.50 h.

4. Conclusions

The UPLC-MS/MS method established in this research had high separation efficiency, high sensitivity, fast analysis speed (only 2.5 min), and a wide application range. After validation, the UPLC-MS/MS method had successfully been used for the first time for the determination and pharmacokinetics of futibatinib in beagle dogs and it will be used in the study of DDI, including the effects of western medicine, Chinese herbal medicine, or food on the pharmacokinetics of futibatinib in future studies.

Data Availability

The experimental data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] S. Rizvi, S. A. Khan, C. L. Hallemeier, R. K. Kelley, and G. J. Gores, "Cholangiocarcinoma-evolving concepts and therapeutic strategies," *Nature Reviews Clinical Oncology*, vol. 15, no. 2, pp. 95–111, 2018.

[2] A. Bergquist and E. von Seth, "Epidemiology of cholangiocarcinoma," *Best Practice & Research Clinical Gastroenterology*, vol. 29, no. 2, pp. 221–232, 2015.

[3] S. Sarcognato, D. Sacchi, M. Fassan et al., "Cholangiocarcinoma," *Pathologica*, vol. 113, no. 3, pp. 158–169, 2021.

[4] B. Doherty, V. E. Nambudiri, and W. C. Palmer, "Update on the diagnosis and treatment of cholangiocarcinoma," *Current Gastroenterology Reports*, vol. 19, no. 1, 2017.

[5] P. C. Lee, A. Hendifar, A. Osipov, M. Cho, D. Li, and J. Gong, "Targeting the fibroblast growth factor receptor (FGFR) in advanced cholangiocarcinoma: clinical trial progress and future considerations," *Cancers*, vol. 13, no. 7, p. 1706, 2021.

[6] G. King and M. Javle, "FGFR inhibitors: clinical activity and development in the treatment of cholangiocarcinoma," *Current Oncology Reports*, vol. 23, no. 9, 2021.

[7] G. Aitcheson, A. Mahipal, and B. V. John, "Targeting FGFR in intrahepatic cholangiocarcinoma [iCCA]: leading the way for precision medicine in biliary tract cancer [BTC]?" *Expert Opinion on Investigational Drugs*, vol. 30, no. 4, pp. 463–477, 2021.

[8] L. Chen, Y. Zhang, L. Yin et al., "Fibroblast growth factor receptor fusions in cancer: opportunities and challenges," *Journal of Experimental & Clinical Cancer Research*, vol. 40, no. 1, p. 345, 2021.

[9] A. Kommalapati, S. H. Tella, M. Borad, M. Javle, and A. Mahipal, "FGFR inhibitors in oncology: insight on the management of toxicities in clinical practice," *Cancers*, vol. 13, no. 12, p. 2968, 2021.

[10] A. Rizzo, A. D. Ricci, and G. Brandi, "Futibatinib, an investigational agent for the treatment of intrahepatic cholangiocarcinoma: evidence to date and future perspectives," *Expert Opinion on Investigational Drugs*, vol. 30, no. 4, pp. 317–324, 2021.
[11] F. Meric-Bernstam, R. Bahleda, C. Hierro et al., "Futibatinib, an irreversible FGFR1-4 inhibitor, in patients with advanced solid tumors harboring *FGF/FGFR* aberrations: a phase I dose-expansion study," *Cancer Discovery*, vol. 12, no. 2, pp. 402–415, 2022.
[12] R. Bahleda, F. Meric-Bernstam, L. Goyal et al., "Phase I, first-in-human study of futibatinib, a highly selective, irreversible FGFR1-4 inhibitor in patients with advanced solid tumors," *Annals of Oncology*, vol. 31, no. 10, pp. 1405–1412, 2020.
[13] H. Sootome, H. Fujita, K. Ito et al., "Futibatinib is a novel irreversible FGFR 1-4 inhibitor that shows selective antitumor activity against FGFR-deregulated tumors," *Cancer Research*, vol. 80, no. 22, pp. 4986–4997, 2020.
[14] L. W. T. Tang and E. C. Y. Chan, "Quantification of the irreversible fibroblast growth factor receptor inhibitor futibatinib by UPLC-MS/MS: application to the metabolic stability assay in human liver microsomes for the estimation of its *in vitro* hepatic intrinsic clearance," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 214, no. ID, Article ID 114731, 2022.
[15] L. W. T. Tang, J. Fu, S. K. Koh, G. Wu, L. Zhou, and E. C. Y. Chan, "Metabolic activation of the acrylamide michael acceptor warhead in futibatinib to an epoxide intermediate engenders covalent inactivation of CYP3A," *Drug Metabolism & Disposition*, vol. 50, no. 7, pp. 931–941, 2022.
[16] Us Food and Drug Administration, *Guidance for Industry: Bioanalytical Method Validation*, US Department of Health and Human Services, US FDA, Center for Drug Evaluation and Research, Rockville, MD, USA, 2018.
[17] Chinese Pharmacopoeia Commission, *Guidelines for Validation of Quantitative Analysis Methods for Biological Samples, Pharmacopoeia of the People's Republic of China*, China Medical Science and Technology Press, Beijing, China, 2020.
[18] H. Guo, M. Chen, M. Li, M. Hu, B. Chen, and C. Zhou, "Pharmacokinetic comparisons of mangiferin and mangiferin monosodium salt in rat plasma by UPLC-MS/MS," *Journal of Chemistry*, vol. 201912 pages, Article ID 9272710, 2019.
[19] S. L. Li, Y. L. Zhu, Y. Zhang, S. H. Liu, X. D. Wang, and X. J. Qiu, "Development and validation of UPLC-MS/MS method for determination of enasidenib in rat plasma and its pharmacokinetic application," *International Journal of Analytical Chemistry*, vol. 20207 pages, Article ID 5084127, 2020.
[20] V. Mazzaferro, B. F. El-Rayes, M. Droz Dit Busset et al., "Derazantinib (ARQ 087) in advanced or inoperable FGFR2 gene fusion-positive intrahepatic cholangiocarcinoma," *British Journal of Cancer*, vol. 120, no. 2, pp. 165–171, 2019.
[21] F. Pena-Pereira, W. Wojnowski, and M. Tobiszewski, "AGREE-analytical GREEnness metric approach and software," *Analytical Chemistry*, vol. 92, no. 14, pp. 10076–10082, 2020.