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ORIGINAL RESEARCH PAPER

Determination of sarecycline by UPLC-MS/MS and its application to pharmacokinetic study in rats

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ABSTRACT

Sarecycline is a narrow-spectrum antibiotic for the treatment of acne, which is a chronic inflammatory disease of the hair follicle sebaceous glands. In the study, UPLC-MS/MS was used to establish a rapid and accurate analytical method. The sarecycline was determined with pozitotinib as internal standard (IS) in rat plasma. An ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) could perform chromatographic separation with the mobile phase (methanol: water of 0.1% formic acid) with gradient elution. The ions of target fragment were m/z 488.19→410.14 for sarecycline and m/z 492.06→354.55 for pozitotinib, which could quantify the electrospray ionization of positive multiple reaction monitoring (MRM) mode. The linear calibration curve of the concentration range was 1–1,000 ng/mL for sarecycline with a lower limit of quantification (LLOQ) of 1 ng/mL. The mean recovery was between 82.46 and 95.85% for sarecycline and pozitotinib in rat plasma. RSD for precision of inter-day and intra-day were between 3.24 and 13.36%, and the accuracy ranged from 105.26 to 109.75%. The developed and validated method was perfectly used in the pharmacokinetic study and bioavailability of sarecycline after intravenous and oral administration in rats.

KEYWORDS

sarecycline, UPLC-MS/MS, pharmacokinetic study, bioavailability

INTRODUCTION

Acne (also known as acne vulgaris) is a common chronic skin disease, usually found in the face, chest, and back, etc., which is characterized by acne, papules, pustules, etc. It mainly caused by skin damage. Acne often occurs in adolescents, and naturally reduces or heals in adulthood [1]. The occurrence of acne is mainly due to elevated testosterone levels, blockage of hair follicle sebaceous glands, excessive bacterial growth and inflammation, and excessive secretion of sebum (*Propionibacterium acnes*: a normal gram-anaerobic bacterium that is parasitic on acne) [2, 3]. Among them, *P. acnes* shows a vital role in the occurrence of acne. According to incomplete statistics, by 2015, acne affected 633 million people worldwide and became the eighth most common disease in the world [4, 5]. Therefore, the treatment of acne has become an urgent problem to be solved.

For moderate or severe inflammatory lesions (such as papules, pustules, etc.), it plays two important roles on the antibacterial treatment-one is to inhibit the growth and metabolism of *P. acnes*, reducing the release of inflammatory mediators; and the other is to alleviate local inflammatory reaction, avoiding scar formation. The most commonly used antibiotics for antibacterial drugs are tetracyclines (such as minocycline, doxycycline, etc.), macrolides (such

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as erythromycin, etc.). However, these drugs have certain limitations, such as vertigo symptoms of minocycline, gastrointestinal reactions of erythromycin and doxycycline [6]. At the same time, due to the widespread use of systemic or local broad-spectrum antibiotics in treating acne recently, the drug resistance phenomenon of the bacteria is significantly increased, and there is cross-resistance, which reduces the efficacy of the drug [7]. Recently, it has been reported that a new type of oral narrow tetracycline antibiotic sarecycline (Fig. 1), which has been proven to be effective in Phase 3 trials to treat acne, and has been marketed by Paratek and Allergan. The US FDA approved sarecycline for the treatment of non-nodular was moderate to severe acne patients over 9 years of age [8]. Compared with the existing tetracyclines, sarecycline has excellent antibacterial properties, but its activity range is narrower [9]. Paratek Pharmaceuticals also indicated that sarecycline was also effective in the treatment of rosacea [10]. Its usage and dosage are calculated based on the weight of the person.

To date, few literatures have involved analytical methods for the detection of sarecycline in rat plasma, especially the sarecycline on rat about bioavailability. A UPLC-MS/MS (chromatography-tandem mass spectrometry of ultra-performance liquid) was used to determine the concentration of sarecycline in plasma of the rat, which was verified by accuracy, specificity, stability, recovery and precision etc. UPLC-MS/MS is significantly superior to other analytical techniques in terms of method sensitivity, speed and resolution, it has been demonstrated in bioanalytical and pharmaceutical research applications for a wide range for a long time [11, 12]. In this study, pharmacokinetics and bioavailability studies were successfully carried out by methodological validation of sarecycline.

EXPERIMENTAL

Chemicals and reagents

Analytical reagent grade formic acid was provided by Sigma-Aldrich (St. Louis, MO, USA). Sarecycline and poziotinib were both purchased from the Beijing Sunflower and Technology Development Co. Ltd. (Beijing, China). Chromatography grade acetonitrile and methanol were prepared by Fisher Scientific Co. (Fair Lawn, New Jersey, USA).

Instruments and LC-MS/MS conditions

ACQUITY I-Class UPLC and a XEVO TQD mass spectrometer were purchased from Waters Corp., Milford, MA, USA. Binary Solvent Manager (BSM) and Sample Manager with Flow-Through Needle (SM-FTN). The ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm) separates sarecycline and poziotinib maintained at 40 °C. Water (0.1% formic acid) and methanol make up the mobile phase with gradient elution. The flow rate was set at 0.4 mL/min. The initial methanol increased from 10 to 30% (0–0.6 min), rapidly raised from 30 to 80% (0.6–1 min), and then increased again from 80 to 95% (1–2 min) slowly, maintained at 95% (2–2.5 min), decreased to 10% (2.5–2.6 min) lastly. All the compounds were analyzed within 3 min and the SM-FTN was washed after each injection with methanol-water solution.

Mass spectrometry was obtained by mass spectrometry detection with a triple quadrupole mass analyzer with better specificity, selectivity, and sensitivity, and an electrospray ionization mode that causes ions that pass through to repelions from the droplets. The optimal MS parameters were defined as follows: The capillary voltage was 1 kV; the cone voltage was 35 V; collision energy was set at 23 V and 16 V for sarecycline and poziotinib, respectively. The multiple reaction monitoring mode of m/z 488.19→410.14 for sarecycline and m/z 492.06→354.55 for poziotinib were used for quantitative analysis. Qualitative analysis for sarecycline was m/z 488.19→410.14. All sample data was acquired and the instrument was controlled by Masslynx 4.1 software (Waters Corp.).

Preparation of standard and quality control (QC) samples

We prepared a standard solution of sarecycline and poziotinib from the corresponding stock solution with methanol-water at a concentration of 0.5 mg/mL. Calibration plots were prepared by adding suitable working solutions to blank rat plasma, and the range is 1–1,000 ng/mL for sarecycline. Similarly, three plasma concentrations (2, 80 and 800 ng/mL) of sarecycline quality control (QC) samples were prepared separately, and their storage environment was –20 °C.

Sample preparation

Plasma samples containing sarecycline were thawed at room temperature. 20 μL of an equal amount of IS solution (0.5 μg/mL), 100 μL of plasma sample and 200 μL of acetonitrile

Fig. 1. Mass spectra and chemical structures of sarecycline (A) and poziotinib (B, IS)

were added in each 1.5 mL centrifuge tube, and then vortexed for 1 min with a vortex instrument. The mixture was centrifuged for 10 min at a speed of 13,000 rpm, followed by injecting 2 μ L supernatant into a UPLC-MS/MS system.

Assay validation

The performance of specificity, precision, recovery and stability etc. were tested strictly, according to the guidelines set by the EMA (European Medicines Agency) and US FDA (Food and Drug Administration) [13] and literatures [14–22], to comprehensively and deeply verify bioanalytical methods. The validation run of one set of standards and five QC samples, were performed within three consecutive days. The rat blank plasma was mixed with the prepared sarecycline three different concentrations (2, 80 and 800 ng/mL) of working solution to prepare three high-quality, medium-quality and low-quality control samples of sarecycline.

Specificity

The specificity was evaluated through analyzing blank plasma, blank plasma spiked with sarecycline and IS, and rat plasma sample acquired after dosing sarecycline. The integrity of the UPLC-MS/MS chromatograms of the blank and verified samples was compared, while some endogenous material interference was also avoided.

Precision and accuracy

The precision was determined by preparing at least five samples per concentration (2, 80 and 800 ng/mL) in the determination of intra-assay RSD. In order to obtain inter-assay RSD, continuous preparation and determination on different days (one analysis batch per day), at least three consecutive analysis batches of not less than 45 samples of the analysis results. The accuracy was obtained by repeatedly measuring the known concentration of the sample to be tested, which is usually expressed by the relative error (RE) of the measured concentration of the QC sample and the labeled concentration.

Linearity and lower limit of quantification (LLOQ)

A standard curve was constructed by selecting standard samples on three different days. The peak area ratio of sarecycline and IS was compared with the analyte concentration, and the equation and correlation coefficient were calculated by weighted ($1/x$) least squares method and plotted as a sarecycline standard curve. The lower limit of quantitation (LLOQ) was the lowest drug concentration in a sample that meets the precision and accuracy requirements and represents the sensitivity of the method. The sample concentration was greater than 10 of the signal/noise ratio, the precision-relative standard deviation was less than 20% and the accuracy was between 80 and 120% of the indicated concentration.

Recovery and matrix effect

The extraction recovery was compared to the unextracted standards by analysis of the extracted samples at 3

concentrations (2, 80 and 800 ng/mL). The IS was measured in the same manner. To evaluate the matrix effect, five different lots of blank rat plasma were extracted and then spiked with the analyte at 2, 80 and 800 ng/mL. At the time of matrix effect evaluation, three concentrations (2, 80 and 800 ng/mL) of sarecycline QC samples and 5 different batches of blank rat plasma were vortex mixed. The value of the matrix effect was then used to calculate the value, so as the IS processed in the same way.

Stability

Samples of three concentrations (2, 80 and 800 ng/mL) of sarecycline were stored under different conditions and times, and the average value was determined after repeated measurements for more than 3 times to evaluate the stability of sarecycline. There are several conditions, including refrigerated at 4 °C for 6 h, room temperature for 24 h, storage at –20 °C for 14 days and three freeze/thaw cycles (storage at –20 °C and thawing at room temperature) was determined in our study ($n = 5$). IS (50 ng/mL) was also processed in a similar way to estimate the stability [23].

Application to pharmacokinetic studies

Twelve Male SD (Sprague-Dawley) rats (220 $\pm$ 20 g) were obtained from the Experimental Animal Center of Wenzhou Medical University. The diet was forbidden 12 h before the drug was administered except water. A single intravenous and oral dose of sarecycline (dissolved in CMC-Na solution) was administered at 5 and 10 mg/kg. After oral administration of sarecycline 0.0833, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h, blood samples (0.3 mL) were taken from the tail vein of the rats into a 1.5 mL centrifuge tube (Heparin has been added to the tube to prevent blood clotting). The sample was immediately centrifuged (3,500 rpm, time 10 min) and the supernatant was taken. The storage environment was –20 °C in the refrigerator until analysis.

C_{max} , AUC, CL, V_d , $t_{1/2}$ were calculated using DAS software (Drug and Statistics, Version 3.2.8, The People's Hospital of Lishui, China) and by non-compartment model. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Wenzhou Medical University (NO. wyd2017-0010) and were following the Guide for the Care and Use of Laboratory Animals. Absolute bioavailability (Fabs) was the non-intravenous dose-corrected AUC divided by AUC intravenous, calculated as follows.

$$F_{abs} = 100 \times \frac{AUC_{po} \times Dose_{iv}}{AUC_{iv} \times Dose_{po}}$$

RESULTS AND DISCUSSION

Optimization of the methods

To optimize the in vivo sample processing method, water of 0.1% formic acid and methanol were used as the mobile

Fig. 2. Representative UPLC-MS/MS chromatograms of sarecycline and poziotinib (IS), (A) blank plasma; (B) blank plasma spiked with sarecycline (2 ng/mL) and IS; (C) a rat plasma sample 3 h after oral administration of single dosage 10 mg/kg sarecycline

phase, and a gradient elution method was applied to obtain a good peak shape. In positive ion mode, sarecycline and poziotinib acquire protonated precursor molecular ions $[M+H]^+$. Our results showed that for sarecycline, the main ions detected was m/z 488.19→410.14, and for poziotinib (IS), m/z was 492.06→354.55, with the peak time of 1.78 and 1.90 min. The method of protein precipitation with methanol provided better matrix effects and higher recovery than liquid-liquid extraction. This method exhibits high detection sensitivity with a total run time of only 3 min for each sample.

Develop liquid chromatography conditions to segregate interfering compounds from sarecycline and poziotinib. We systematically adjusted the mobile phase modifier, gradient elution type and flow rate to obtain the best chromatographic conditions. Therefore, a combination of water of 0.1% formic acid-methanol, 0.4 mL/min flow rate and ACQUITY UPLC HSS T3 column (2.1×100 mm, $1.8 \mu\text{m}$) was used in our study. Compared to the UPLC BEH C18 column (2.1×50 mm, $1.7 \mu\text{m}$), our chromatographic conditions provide less peak diffusion and higher sensitivity. Methanol was chosen to quantify Sarecycline because the strong peak response produced by acetonitrile may affect the results of the quantitative analysis.

In order to obtain the accuracy of sarecycline, biological samples need to be carefully prepared. In our study, methanol was used instead of acetonitrile as a method of protein precipitation solvent as a result of its accredited recovery and better precipitation, and in addition, it was fast and simple.

Specificity

Fig. 2 showed typical chromatograms of blank plasma, blank plasma spiked with sarecycline and poziotinib, and plasma sample acquired at 3 h after oral dosing sarecycline (10 mg/kg). There were no interfering endogenous substances observed between the analytes.

Linearity, Precision, accuracy and the LLOQ

The calibration curve of sarecycline was demonstrated to be linear in the concentration range of 1–1,000 ng/mL. The equation was: $y = 0.00197147 \times x - 0.0015151$, $r = 0.9993$. The LLOQ was 1 ng/mL, which determined the lowest concentration of sarecycline in plasma. Calibration curves of sarecycline in plasma, assessed by performing back-calculated concentrations, showed <15% deviation from nominal values at all concentrations, and the deviation was <20% for the LLOQ. The precision of intra-day and inter-day was <13.11 and 13.36%. The range of accuracy was from 105.26 to 109.75% at each QC level.

Recovery and matrix effect

Mean recoveries of sarecycline were better than 82.46%. The results demonstrated that the values were within the

Table 1. The intra-day and inter-day precision, accuracy, recovery and matrix effect of sarecycline in rat plasma ($n = 5$)

Concentration (ng/mL)	RSD (%)		Accuracy (%)		Recovery (%)	Matrix effect (%)
	Intra-day	Inter-day	Intra-day	Inter-day		
2.00	13.11	13.36	109.75	107.84	82.46	88.39
80.00	3.59	4.57	106.90	106.19	95.85	91.58
800.00	3.24	4.57	107.59	105.26	90.97	92.89

Table 2. Stability of sarecycline under various conditions ($n = 5$)

Condition	Concentration (ng/mL)	Measured (ng/mL)	RSD (%)	Accuracy (%)
Room temperature, 24 h	2	2.07 ± 0.10	4.61	103.53
	80	85.40 ± 3.94	4.61	106.75
	800	833.86 ± 19.05	2.28	104.23
Freeze/thaw	2	2.16 ± 0.16	7.23	108.11
	80	86.76 ± 2.84	3.27	108.45
	800	856.26 ± 31.51	3.68	107.03
−20 °C 14d	2	2.10 ± 0.18	8.4	104.91
	80	84.96 ± 2.97	3.49	106.2
	800	852.83 ± 29.96	3.51	106.6
4 °C, 6h	2	2.12 ± 0.21	10.12	105.75
	80	88.35 ± 2.06	2.33	110.44
	800	842.18 ± 37.11	4.41	105.27

Fig 3. Mean plasma concentration time profile after intravenous (5 mg/kg) and oral administration (10 mg/kg) of sarecycline in six rats

acceptable range. The matrix effect of sarecycline measurements at three QC levels were between 88.39% and 92.89% ($n = 5$). All results suggested that plasma matrix did not significantly affect the results (shown in Table 1).

Stability

The results of stability showed that the analytes were stable under the storage conditions, and the deviation of the average value of the QC samples repeatedly measured more than 3 times is within $\pm 15\%$ of the measured value. All the stability data were shown in Table 2.

Table 3. Primary pharmacokinetic parameters after oral and intravenous administration of sarecycline in rats ($n = 6$)

Parameters	oral administration ($n = 6$)	intravenous administration ($n = 6$)
$AUC_{(0-t)}$ ($\mu\text{g/h/L}$)	3,275.75 ± 596.06	2,264.03 ± 449.69
$AUC_{(0-\infty)}$ ($\mu\text{g/h/L}$)	3,281.55 ± 594.53	2,266.78 ± 450.63
$MRT_{(0-t)}$ (h)	4.28 ± 0.36	3.51 ± 0.64
$MRT_{(0-\infty)}$ (h)	4.3 ± 0.34	3.53 ± 0.65
$t_{1/2z}$ (h)	1.74 ± 0.45	2.04 ± 0.65
T_{max} (h)	3 ± 0.63	0.08 ± 0.00
Vz/F (L/kg)	7.85 ± 2.34	6.41 ± 1.3
CLz/F (L/h/kg)	3.15 ± 0.67	2.3 ± 0.55
C_{max} ($\mu\text{g/L}$)	615.1 ± 103.69	673.13 ± 157.79

Pharmacokinetic application

The method for the determination of rat plasma, pharmacokinetic study and bioavailability of sarecycline was successfully applied in rats, with the drug-time curve shown in Fig. 3. The pharmacokinetic parameters of oral and intravenous administration were calculated in a non-compartmental model in Table 3. The data showed that C_{max} was 615.1 ± 103.69 ng/mL after oral sarecycline. After 3 h, the blood drug concentration began to decrease; after intravenous administration, the C_{max} was 673.13 ± 157.79 ng/mL, and the plasma concentration began to decrease after 0.08 h. The drop in drug concentration might lie in the distribution from plasma to other organs and tissues. According to

reports, the absolute bioavailability of sarecycline was as high as 72.38%.

CONCLUSION

A rapid and accurate analytical method was developed and validated for the quantification of sarecycline in rat plasma, pharmacokinetic study and bioavailability of sarecycline by UPLC-MS/MS. The method has better specificity, sensitivity (LLOQ, 1 ng/mL), and required micro-volume aliquots of 2 µL, which greatly shortens the analysis time (3 min) and its qualitative and quantitative functions are more perfect. The results from the preclinical pharmacokinetics study illustrate that the absolute bioavailability of sarecycline was 72.38% in rats. The validated method was perfectly used in the pharmacokinetic study and bioavailability of sarecycline after intravenous and oral administration on rats.

Conflict of interest: The authors declare no conflict of interest.

Ethical statement: All applicable institutional guidelines for the care and use of animals were followed. This study was approved by Laboratory Animal Ethics Committee Committee of Wenzhou Medical University & Laboratory Animal Centre of Wenzhou Medical University (wydw 2019-0651).

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REFERENCES

- Williams, H. C.; Dellavalle, R. P.; Garner, S. *Lancet* **2012**, 379, 361.
- Fox, L.; Csongradi, C.; Aucamp, M.; du Plessis J.; Gerber, M. *Molecules* **2016**, 21, 1063.
- Jović, A.; Marinović, B.; Kostović, K.; Čeović, R.; Basta-Juzbašić, A.; Bukvić Mokos, Z. *Acta. Dermatovenerol. Croat.* **2017**, 25, 1133.
- Hay, R. J.; Johns, N. E.; Williams, H. C.; Bolliger, I. W.; Dellavalle, R. P.; Margolis, D. J.; Marks, R.; Naldi, L.; Weinstock, M. A.; Wulf, S. K.; Michaud, C.; Murray, J. L.; Naghavi, M. *J. Invest. Dermatol.* **2014**, 134, 1527.
- Disease, G. B. D.; Injury, I.; Prevalence, C. *Lancet* **2016**, 388, 1545.
- Zhanel, G.; Critchley, I.; Lin, L.Y.; Alvandi, N. *Antimicrobial Agents. Chemother.* **2018**, 63, e01297.
- Oprica, C.; Emtestam, L.; Hagströmer, L.; Nord, C.E. *Acta. Derm. Venereol.* **2007**, 87, 246.
- Deeks, E. D. *Drugs* **2019**, 79, 325.
- Leyden, J. J.; Sniukiene, V.; Berk, D. R.; Kaoukhov, A. *J. Drugs Dermatol.* **2018**, 17, 333.
- Karpiuk, I.; Tyski, S. *Przegl. Epidemiol.* **2015**, 69, 723.
- Churchwell, M. I.; Twaddle, N. C.; Meeker, L. R.; Doerge, D. R. *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci.* **2005**, 825, 134.
- Vijaya Bhaskar Reddy, A.; Venugopal, N.; Madhavi, G.; Gangadhara Reddy, K.; Madhavi, V. *J. Pharm. Biomed. Anal.* **2013**, 84, 84.
- Kaza, M.; Karaźniewicz-Łada, M.; Kosicka, K.; Siemiątkowska, A.; Rudzki, P. *J. Pharm. Biomed. Anal.* **2019**, 165, 381.
- Matuszewski, B. K.; Constanzer, M. L.; Chavez-Eng, C. M. *Anal. Chem.* **2003**, 75, 3019.
- Wang, X. Q.; Wang, S. H.; Ma J. S.; Ye, T.; Lu, M. R.; Fan, M.; Deng, M. J.; Hu, L. F.; Gao, Z. M. *J. Pharm. Biomed. Anal.* **2015**, 115, 368.
- Rocci, M. L., Jr.; Devanarayan, V.; Haughey, D. B.; Jardieu, P. *AAPS J.* **2007**, 9, E336.
- Fast, D. M.; Kelley, M.; Viswanathan, C. T.; O'Shaughnessy, J.; King, S. P.; Chaudhary, A.; Weiner, R.; DeStefano, A. J.; Tang, D. *AAPS J.* **2009**, 11, 238.
- Dong, Y. Y.; Mao, B. P.; Guan, H. G.; Bai, Y. F.; Chi, B. H.; Shan, Y. Y.; Lian, Q. Q.; Ge, R. S. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* **2015**, 1002, 234.
- Fang, B. M.; Bao S. H.; Wang, S. H.; Chen, M. L.; Chen, B. B.; Su, K.; Wen, C. C.; Zhou, Y. F.; Wang, X. Q.; Jin, Y. P. *Biomed. Chromatogr.* **2017**, 31, e3826.
- Ma, J. S.; Wang, S. H.; Huang, X. L.; Geng, P. W.; Wen, C. C.; Zhou, Y. F.; Yu, L. S.; Wang, X. Q. *J. Pharm. Biomed. Anal.* **2015**, 111, 131.
- Wang, S. H.; Wu, H. Y.; Huang, X. L.; Geng, P. W.; Wen, C. C.; Ma, J. S.; Zhou, Y. F.; Wang, X. Q. *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci.* **2015**, 990, 118.
- Wen C. C.; Cai, J. Z.; Lin, C. L.; Ma, J. S.; Wang, X. Q. *Xenobiotica* **2014**, 44, 743.
- Du, J. B.; Ma, Z. Y.; Zhang, Y. F.; Wang, T.; Chen, X. Y.; Zhong, D. F. *Bioanalysis* **2014**, 6, 2343.