Determination and Pharmacokinetics and Bioavailability of *O*-Demethyl Nuciferine in Mice by UPLC–MS/MS

Haiya Wu^{1†}, Mengrou Lu^{2†}, Jiamin He³, Miaoling Huang³, Aote Zheng³, Meiling Zhang³, Congcong Wen^{4*} and Jufen Ye^{5*}

¹The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325000, China

²Cellular Biomedicine Group (Shanghai), Inc., 333 Guiping Road, Xuhui, Shanghai 200233, China

³Analytical and Testing Center, School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou 325035, China

⁴Laboratory Animal Centre, Wenzhou Medical University, 325035 Wenzhou, China

⁵Department of Ultrasound, The Second People's Hospital of Lishui, Lishui 323000, China

Received: 05 March 2018; accepted: 02 May 2018

In this study, a precise, rapid, and accurate ultra-performance liquid chromatography–tandem mass spectrometer (UPLC–MS/MS) method for the quantitation of *O*-demethyl nuciferine in mouse blood was developed, and pharmacokinetics of *O*-demethyl nuciferine was studied for the first time after sublingual injection and gavage. The study was performed with an UPLC ethylene bridged hybrid (UPLC BEH) (2.1 mm × 50 mm, 1.7 μm) column at 30 °C, using diazepam as the internal standard (IS). The mobile phase consisted of acetonitrile–10 mmol/L ammonium acetate (containing 0.1% formic acid), with a flow rate of 0.4 mL/min for 4 min run time. Multiple reaction monitoring (MRM) modes of *m/z* 282.1→219.0 for *O*-demethyl nuciferine and *m/z* 296.2→265.1 for IS were utilized to conduct quantitative analysis. Protein in mouse blood was directly precipitated with acetonitrile for sample preparation. The linear range was 1–500 ng/mL with *r* > 0.995, and the lower limits of quantification (LLOQ) was 1 ng/mL. The intra- and inter-day precision of *O*-demethyl nuciferine in mouse blood were RSD < 14% and RSD < 15%, respectively. The accuracy ranged from 89.0% to 110.7%, with a recovery higher than 88.9%, while the matrix effect was between 103.1% and 108.7%. We further applied this UPLC–MS/MS method to the pharmacokinetic study on *O*-demethyl nuciferine after sublingual injection and gavage and determined the bioavailability to be 6.4%.

Keywords: *O*-demethyl nuciferine, mice, UPLC–MS/MS, bioavailability, pharmacokinetics

Introduction

Lotus leaves from *Nymphaeaceae* has been recognized as a precious Chinese medicine for both pharmaceutical and food usage and is widely farmed in China for its high economic value [1, 2]. The major active ingredients of lotus leaf are alkaloids such as nuciferine, *O*-demethyl nuciferine, and *N*-demethyl nuciferine, whose pharmacological activities contribute significantly to hypolipidemic, weight-losing, antibacterial, antiviral, seductive, and anticonvulsive effects, despite the rather low content in lotus leaves [3–7].

Pharmacokinetics studies of the absorption, distribution, metabolism, and excretion of drugs has been extended to the exploration on the mechanism, complex systemic interactions, and dynamics of drugs in vivo [8, 9]. Such studies may provide the theoretical fundamentals for drug development by revealing the absorption and distribution profile in vivo; therefore, pharmacokinetic studies on *O*-demethyl nuciferine is critical for advanced clinical applications. Few nuciferine pharmacokinetics and tissue distribution studies have been reported on mice and rats [10, 11]. Despite such mere reports on nuciferine, no literature has been reported on pharmacokinetic study of *O*-demethyl nuciferine in mice or rats to the best of our knowledge. Therefore, we need to develop a bioanalytical method for determination of *O*-demethyl nuciferine in biological matrix for pharmacokinetics.

Compared with liquid chromatography–tandem mass spectrometer (LC–MS/MS), ultra-performance LC–MS/MS (UPLC–MS/MS)

has higher detection sensitivity with more powerful separation and analytical capability, which are essential for studying Chinese medicine with complex contents and the in vivo metabolism in such compound systems [12–14]. Herein, by taking advantage of the powerful UPLC–MS/MS, for the first time, we developed a precise, rapid, and accurate method for the determination of *O*-demethyl nuciferine in mouse blood using UPLC–MS/MS, and we further studied the pharmacokinetics and bioavailability after sublingual injection and gavage using the newly developed UPLC–MS/MS.

Materials and Methods

Chemicals and Reagents. *O*-demethyl nuciferine (purity > 98%, Figure 1a) and diazepam (internal standard, IS) (purity > 98%, Figure 1b) were purchased from the Chengdu Mansite Pharmaceutical Co., Ltd. (Chengdu, China). Chromatography-grade methanol and acetonitrile were purchased from Merck Company (Darmstadt, Germany). Chromatography-grade ammonium acetate was purchased from Tedie Ltd. (Ohio, USA). Ultrapure water was prepared by Millipore Milli-Q purification system (Bedford, MA, USA). Institute of Cancer Research (ICR) mice (male, 20–22 g) were purchased from Laboratory Animal Center of Wenzhou Medical University. Blank mouse blood was collected from adult healthy male ICR mice.

Instrumentation and Conditions. XEVO TQS-micro triple quadrupole mass spectrometer and ACQUITY I-Class UPLC were from Waters Corp. (Milford, MA, USA). MassLynx 4.1 software (Waters Corp.) was used for data acquisition and instrument control.

* Authors for correspondence: bluce494949@163.com (Congcong Wen), 2846896387@qq.com (Jufen Ye).

† These authors contributed equally to this work.

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium for non-commercial purposes, provided the original author and source are credited, a link to the CC License is provided, and changes - if any - are indicated.

Figure 1. Mass spectrum of *O*-demethyl nuciferine (A) and diazepam (IS) (B)

O-demethyl nuciferine and IS were separated using an UPLC ethylene bridged hybrid (UPLC BEH) C18 column (2.1 mm × 50 mm, 1.7 μm, Waters, USA) maintained at 30 °C. The mobile phase consisted of acetonitrile and ammonium acetate solution (containing 0.1% formic acid) with gradient elution at a flow rate of 0.4 mL/min. The gradient elution conditions were as follows: 0–0.2 min, acetonitrile 10%; 0.2–1.5 min, acetonitrile 10%–75%; 1.5–2.0 min, acetonitrile 75%; 2.0–2.5 min, acetonitrile 75%–10%; and 2.5–4.0 min, acetonitrile 10%. The total run time was 4.0 min.

Nitrogen was used as the desolvation gas (900 L/h) and cone gas (50 L/h). Ion monitoring conditions consisted of a capillary voltage of 2.0 kV, a source temperature of 150 °C, and a desolvation temperature of 450 °C. Multiple reaction monitoring (MRM) modes of m/z 282.1→219.1 for *O*-demethyl nuciferine and m/z 285.1→193.2 for IS were utilized to conduct quantitative analysis (Figure 1).

Calibration Standards and Quality Control Samples.

The stock solutions of *O*-demethyl nuciferine (1.0 mg/mL) and IS (1.0 mg/mL) were prepared in methanol–water (50:50). The working solutions of *O*-demethyl nuciferine were prepared from the stock solution by dilution with methanol. The working solution of IS (20 ng/mL) were prepared from the stock solution by dilution with acetonitrile. All solutions were stored at 4 °C and were brought to room temperature before use.

O-demethyl nuciferine calibration standards were prepared by spiking appropriate working solutions into blank mouse blood (1, 5, 10, 20, 50, 100, 200, and 500 ng/mL). Quality-control (QC) samples were prepared in the same manner as the calibration standards, in three different concentrations of mouse blood (2, 90, and 450 ng/mL).

Sample Preparation. An aliquot of 100 μL of the IS working solution (20 ng/mL) was added to 20 μL of the collected blood sample in a 1.5 mL centrifuge tube, followed

by 1.0 min of vortex-mixing. After centrifugation at 14,900 g for 10 min at 4 °C, 80 μL of the supernatant was collected into the liner tube of autosampler vials, and 2 μL of the supernatant was injected into the UPLC–MS/MS system for analysis.

Method Validation. In order to thoroughly validate the proposed UPLC–MS/MS method for *O*-demethyl nuciferine analysis [15–17], tests, such as selectivity, matrix effect, linearity, accuracy, precision, recovery, and stability tests, were conducted according to the guidelines from the Food and Drug Administration (FDA), United States.

The selectivity of the method was evaluated by analyzing six lots of blank mouse blood, blank blood spiked with *O*-demethyl nuciferine and IS, and mouse blood sample.

To assess the linearity, calibration curves were obtained by analyzing the spiked calibration samples (1–500 ng/mL in mouse blood). Peak area ratios of *O*-demethyl nuciferine to IS were plotted against *O*-demethyl nuciferine concentrations.

Accuracy and precision were evaluated by the determination of LLOQ and QC samples (1, 2, 90, and 450 ng/mL) in six replicates over three consecutive days. Intra-day and inter-day precision is expressed as relative standard deviation (RSD). Intra-day and inter-day accuracy was determined as the extent to which the mean value corresponded to the true value.

To evaluate the matrix effect, blank mouse blood was extracted and then spiked with the *O*-demethyl nuciferine at 1, 2, 90, and 450 ng/mL. The matrix effect was defined as comparing the corresponding peak areas to those of standard solutions prepared with acetonitrile and 10 mmol/L ammonium acetate solution (containing 0.1% formic acid) (1:1, v/v) at equivalent concentrations.

The recovery of *O*-demethyl nuciferine was evaluated by comparing the peak area of the extracted QC samples with that of the reference QC solutions.

Figure 2. Representative UPLC-MS/MS chromatograms of *O*-demethyl nuciferine and diazepam (IS). (A) Blank mouse blood, (B) blank blood spiked with *O*-demethyl nuciferine (2 ng/mL) and IS (20 ng/mL), and (C) a mouse blood sample after intragastrical administration

Carry-over was assessed following injection of a blank mouse blood sample immediately after 3 repeats of the upper limit of quantification (ULOQ) and the response was checked [18].

Stability of *O*-demethyl nuciferine in mouse blood was evaluated by analyzing the peak area of the mouse blood QC sample in three *O*-demethyl nuciferine concentrations (2, 90, and

450 ng/mL) exposed to three different storage conditions. These results were compared with those of freshly-prepared standard samples. Short-term stability was determined after incubating the spiked samples at room temperature for 2 h and the ready-to-inject samples in the autosampler. Freeze-thaw stability was evaluated after complete freeze-thaw cycle (-20°C to room temperature). Long-term stability was assessed after storing the samples at -20°C for 30 days.

Reproducibility of the analytical method was further evaluated by re-analysis of incurred samples [19]. A total of 9 samples (8.3% of the total number of samples) were re-analyzed and included samples from samples collected from six mice after oral administration [20]. The re-analysis data for *O*-demethyl nuciferine were compared with the data from the original assay.

Pharmacokinetic Study. Twelve mice were randomly divided into two groups and numbered 1–12. 4.0 mg of *O*-demethyl nuciferine were dissolved in 0.01% HCl solution to prepare 1.0 mg/mL drug solution freshly before use. For mice #1–6, 3 mg/kg of *O*-demethyl nuciferine was sublingually injected, while mice #7–12 were gavaged with 15 mg/kg. Blood samples (20 μL) were collected from the tail vein into 1.5 mL polythene tubes at 0.0833, 0.5, 1, 1.5, 2, 3, 4, 8, and 12 h after *O*-demethyl nuciferine administration.

The samples were processed following “Sample preparation”, then analyzed with UPLC–MS/MS. Area under the curve (AUC) of *O*-demethyl nuciferine concentration versus time, mean retention time (MRT), blood clearance (CL), apparent volume of distribution (V), maximum concentration in blood (C_{max}), and half-life ($t_{1/2}$) were analyzed by DAS (Drug and Statistics) software (version 2.0, China Pharmaceutical University) in the non-compartment mode for fitting the pharmacokinetic parameters. Absolute bioavailability was defined as the percentage AUC ratio of oral administration to intravenous injection.

Results and Discussion

Method Optimization. Selection of electrospray ionization (ESI) in positive and negative ion modes is frequently assessed [21–24]. Since *O*-demethyl nuciferine is an alkaloid with a more basic nature, we demonstrated that ESI with positive ion detection resulted in higher sensitivity in comparison with the negative ion detection mode.

Chromatography column and mobile phase are critical in separating endogenous interferents from analyte and IS [25–27]. To optimize the conditions, we investigated a variety of columns, such as BEH C18 (2.1 mm $\times$ 50 mm, 1.7 μm), BEH C18 (2.1 mm $\times$ 100 mm, 1.7 μm), and HSS T3 (2.1 mm $\times$ 100 mm, 1.8 μm), and we found that BEH C18 (2.1 mm $\times$ 50 mm, 1.7 μm) gave the optimal peaking time and optimal effect to separate interferents from analyte and IS. We also explored different gradient elution solutions ranging from acetonitrile–0.1% formic acid and acetonitrile–10 mmol/L ammonium acetate solution (containing 0.1% formic acid) to methanol–0.1% formic acid and methanol–10 mmol/L ammonium acetate solution (containing 0.1% formic acid). The result showed that acetonitrile–10 mmol/L ammonium acetate (containing 0.1% formic acid) was the optimal combination of the mobile phase that provided peak and retention time in separating interferents from analyte and IS. Therefore, the following experiments were performed with BEH C18 (2.1 mm $\times$ 50 mm, 1.7 μm) column and acetonitrile–10 mmol/L ammonium acetate (containing 0.1% formic acid) as the mobile phase solution.

The content of the blood samples is considerably more complicated than plasma. Elimination of proteins and potential interferents is another critical step before UPLC–MS/MS analysis [20, 28]. We investigated liquid–liquid extraction with ethyl

acetate, chloroform, and diethyl ether with and without alkalification by sodium carbonate first; direct precipitation with methanol, acetonitrile, and methanol–acetonitrile (1:1, v/v) has also been evaluated. Results showed that acetonitrile precipitation produced the best recovery and acceptable matrix effect. Therefore, 100 μL acetonitrile was used to precipitate the endogenous impurities from 20 μL of blood. As UPLC–MS/MS analysis requires a rapid, simple, and convenient sample preparation method with acceptable recovery and matrix effect, acetonitrile precipitation method was chosen for this study.

Selection of internal standard is also crucial during method establishment [29–31]. We compared several compounds such as berberine, magnoflorine, diazepam, and jatrorrhizine. We selected diazepam based on its similarity with *O*-demethyl nuciferine in chromatography retention time and mass ionization.

Detecting *O*-demethyl nuciferine in blood using UPLC–MS/MS provides higher sensitivity and consumes less time compared with conventional HPLC [32, 33], given that 4 min of total run time may spare a generous amount of time and reagents. In addition, the relatively low LLOQ (1 ng/mL) of *O*-demethyl nuciferine facilitated the determination of the lower drug concentration in blood at sampling time points.

Method Validation. Figure 2 showed typical UPLC–MS/MS chromatograms of a blank blood sample, a blank blood sample spiked with either *O*-demethyl nuciferine or IS, and a tail vein blood sample. The retention time of *O*-demethyl nuciferine and IS was 1.66 and 1.83 min, respectively. There were no interfering endogenous substances observed during the retention time of *O*-demethyl nuciferine, indicating the feasibility of the method.

The peak area ratios versus concentrations were fitted with linear regression over the concentration range of 1–500 ng/mL for *O*-demethyl nuciferine in mouse blood. The equation utilized to express the calibration curve was $y = 0.0131049c + 0.0246397$ ($r = 0.9952$), where y represents the ratios of *O*-demethyl nuciferine peak area to that of IS, and c represents the drug concentration in blood. The LLOQ for the determination of *O*-demethyl nuciferine in blood was 1 ng/mL with a signal-to-noise ratio of 11. The precision and accuracy at LLOQ were 13.6% and 108.2%, respectively. The LLOD of *O*-demethyl nuciferine was 0.2 ng/mL with a signal-to-noise ratio of 3.

Table 1 showed that the intra-day precision was $\text{RSD} < 14\%$, while inter-day precision was $\text{RSD} < 15\%$. The accuracy ranged from 89.0% to 110.7%, with a recovery higher than 88.9% and a matrix effect between 103.1% and 108.7%. This result suggested that the precision, accuracy, recovery, and matrix effect of UPLC–MS/MS method met the requirements of pharmacokinetic study on *O*-demethyl nuciferine.

None of the *O*-demethyl nuciferine showed any significant peak ($\geq 20\%$ of the LLOQ and 5% of the IS) in blank samples injected after the ULOQ samples. Adding 1.5 extra minutes to the end of the gradient elution effectively washed the system between samples, thereby eliminating carry-over.

Results showed that *O*-demethyl nuciferine was stable under short-term room temperature (2 h), freeze–thaw cycling, and long-term (-20°C , 30 days) storage conditions, as the bias in concentrations was within $\pm 12\%$ of the nominal values, and RSD was less than 13%. This remarkable stability justified the method to be suitable for pharmacokinetic study.

A total of 11 samples were re-analyzed. The differences between the re-assay concentrations and the original concentrations were all less than 10% of their mean values and met the acceptance criteria for incurred sample reanalysis.

Pharmacokinetic Study. Using the validated UPLC–MS/MS method, we performed pharmacokinetic study on mice with sublingual injection or gavage with *O*-demethyl nuciferine. Considering the low blood volume in mice, only 20 μL of blood

Table 1. Accuracy, precision, matrix effect, and recovery of *O*-demethyl nuciferine in mouse blood

Concentration (ng/mL)	Accuracy (%)		Precision (RSD%)		Matrix effect (%)	Recovery (%)
	Intra-day	Inter-day	Intra-day	Inter-day		
1	108.2	110.7	13.6	14.6	106.3	90.8
2	103.0	96.6	5.1	6.4	107.0	88.9
90	95.8	89.0	4.3	4.9	108.7	92.6
450	97.8	102.7	5.6	4.6	103.1	92.4

were collected at each time point. Serum collection by centrifugation on such small volume was rather impractical; therefore, we directly precipitated the blood with acetonitrile, followed with UPLC–MS/MS analysis on *O*-demethyl nuciferine concentration in the whole blood. The curve of *O*-demethyl nuciferine concentration vs. time post-administration was plotted in Figure 3. Figure 3A suggested that the absorption after gavage was low, and expressing the y-axis into log 10 (Figure 3B) visualized the absorption process in a clearer manner. The primary pharmacokinetic parameters for fitting with a non-compartmental model analysis were summarized in Table 2,

Figure 3. Mean blood concentration–time profile after intragastrical (15 mg/kg) and intravenous (3 mg/kg) administration of *O*-demethyl nuciferine. y-axis, Linear (A), Log10 (B)**Table 2.** Primary pharmacokinetic parameters after *O*-demethyl nuciferine administration in mice

Parameters	Unit	IG (15 mg/kg)	IV (3 mg/kg)
AUC _(0–t)	ng/mL*h	63.3 ± 12.4	198.4 ± 37.6
AUC _(0–∞)	ng/mL*h	64.7 ± 12.0	198.9 ± 37.7
MRT _(0–t)	h	1.93 ± 0.29	0.78 ± 0.17
MRT _(0–∞)	h	2.28 ± 0.44	0.82 ± 0.21
<i>t</i> _{1/2z}	h	2.73 ± 0.55	1.76 ± 0.41
CL _{z/F}	L/h/kg	237.7 ± 39.8	15.6 ± 3.1
<i>V</i> _{z/F}	L/kg	960.2 ± 339.1	39.5 ± 11.2
<i>C</i> _{max}	ng/mL	47.9 ± 7.4	359.5 ± 107.1

where the AUC of sublingual administration was greater than that of gavage with a *O*-demethyl nuciferine bioavailability of 6.4%, while the CL was significantly lower than that of gavage administration. This indicated that a portion of the drug was metabolized before entering the blood during oral intake, which may result in a strong first-pass effect.

There has not been reports on the pharmacokinetic study on *O*-demethyl nuciferine in mice or rats, and the reports on nuciferine, a chemical with similar structure with *O*-demethyl nuciferine, were also scarce [10, 11]. Gu et al. studied pharmacokinetics of nuciferine in rats with a HPLC–MS/MS method, where 2.0, 5.0, and 1.0 mg/kg nuciferine oral administration and 0.2 mg/kg IV resulted in *t*_{1/2} of 1.5 ± 0.8 h, 0.8 ± 0.4 h, 1.0 ± 0.6 h, and 0.6 ± 0.2 h and CL of 55.5 ± 22.0 L/h/kg, 58.6 ± 39.9 L/h/kg, 52.5 ± 15.6 L/h/kg, and 1.8 ± 0.5 L/h/kg, respectively, while the bioavailability was 3.8%, 4.2%, and 3.9%. Xu et al. carried out the pharmacokinetic study on nuciferine in rats with the UPLC–MS/MS method. Gavage with 15 mg/kg and IV administration of 2 mg/kg of nuciferine resulted in *t*_{1/2} of 3.8 ± 1.1 h and 1.2 ± 1.0 h and CL of 22.5 ± 10.4 L/h/kg and 13.8 ± 3.7 L/h/kg, with a bioavailability of 17.9%. In our study, gavage of 15 mg/kg and IV injection of 3 mg/kg of *O*-demethyl nuciferine exhibited *t*_{1/2} of 2.73 ± 0.55 h and 1.76 ± 0.41 h and CL of 237.7 ± 39.8 L/h/kg and 15.6 ± 3.1 L/h/kg, respectively. While the *t*_{1/2} of *O*-demethyl nuciferine was similar to that of nuciferine in rats, the CL of gavage was significantly different. This may be attributed to the subtle variation on the structures of the compounds, in addition to the difference between mice and rats.

Conclusion

In this study, we developed a precise, rapid and accurate UPLC–MS/MS method for the quantitation of *O*-demethyl nuciferine with a linear range of 1–500 ng/mL and LLOQ of 1 ng/mL using 20 µL of mouse blood directly precipitated with acetonitrile. We further applied this UPLC–MS/MS method to the pharmacokinetic study on *O*-demethyl nuciferine for the first time after sublingual injection and gavage and determined the bioavailability to be 6.4%. By comparing the primary pharmacokinetic parameters of gavage and intravenous injection, we revealed that gavage administration of *O*-demethyl nuciferine may result in strong first-pass effect.

Acknowledgements. This study was supported by grants from Wenzhou Municipal Science and Technology Bureau (Y20150178 and Y20170699).

References

- Yan, K.; Zhu, H.; Xu, J.; Pan, H.; Li, N.; Wang, L.; Yang, H.; Liu, M.; Gong, F. *Front. Pharmacol.* **2017**, *8*, 409.
- Tang, X.; Tang, P.; Liu, L. *Molecules* **2017**, *22*, 1036.
- Zeng, Z.; Xu, Y.; Zhang, B. *Biol. Trace Elem. Res.* **2017**, *176*, 321–327.
- Cho, B. O.; Che, D. N.; Yin, H. H.; Shin, J. Y.; Jang, S. I. *Biomed. Pharmacother.* **2017**, *89*, 819–826.
- Ye, L. H.; Kong, L. T.; Yan, M. Z.; Cao, F. R.; Wang, L. S.; Liao, Y. H.; Pan, R. L.; Chang, Q. J. *Ethnopharmacol.* **2016**, *194*, 913–917.
- Yan, M. Z.; Chang, Q.; Zhong, Y.; Xiao, B. X.; Feng, L.; Cao, F. R.; Pan, R. L.; Zhang, Z. S.; Liao, Y. H.; Liu, X. M. *J. Agric. Food Chem.* **2015**, *63*, 9277–9285.
- Lee, K.; Kim, J.; Lee, N.; Park, S.; Cho, H.; Chun, Y. *J. Exerc. Nutrition Biochem.* **2015**, *19*, 25–30.

8. Li, S. P.; Cheng, X. M.; Wang, C. H. *J. Ethnopharmacol.* **2017**, *203*, 127–162.
9. Cheng, H. S.; Ton, S. H.; Kadir, K. A. *Phytochem. Rev.* **2017**, *16*, 159–193.
10. Gu, S.; Zhu, G.; Wang, Y.; Li, Q.; Wu, X.; Zhang, J.; Liu, G.; Li, X. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2014**, *961*, 20–28.
11. Xu, Y.; Bao, S.; Tian, W.; Wen, C.; Hu, L.; Lin, C. *Int. J. Clin. Exp. Med.* **2015**, *8*, 17612–17622.
12. Pang, H. Q.; Tang, Y. P.; Cao, Y. J.; Tan, Y. J.; Jin, Y.; Shi, X. Q.; Huang, S. L.; Sun, D. Z.; Sun, J.; Tang, Z. S.; Duan, J. A. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2017**, *1061–1062*, 372–381.
13. Wang, L.; Sang, M.; Liu, E.; Banahene, P. O.; Zhang, Y.; Wang, T.; Han, L.; Gao, X. *J. Pharm. Biomed. Anal.* **2017**, *140*, 45–61.
14. Wang, S.; Li, D.; Pi, J.; Li, W.; Zhang, B.; Qi, D.; Li, N.; Guo, P.; Liu, Z. *J. Pharm. Pharmacol.* **2017**, *69*, 1540–1551.
15. Xu, W.; Zhang, Y.; Zhou, C.; Tai, Y.; Zhang, X.; Liu, J.; Sha, M.; Huang, M.; Zhu, Y.; Peng, J.; Lu, J. J. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2017**, *1061–1062*, 314–321.
16. Wang, Z.; Zhao, Q.; Li, L.; Hu, P.; Dong, K.; Chen, S.; Jiang, J. *J. Pharm. Biomed. Anal.* **2017**, *132*, 1–6.
17. Wang, W.; Zheng, X.; Wang, H.; Wang, L.; Jiang, J.; Hu, P. *J. Pharm. Biomed. Anal.* **2017**, *139*, 205–214.
18. Williams, J. S.; Donahue, S. H.; Gao, H.; Brummel, C. L. *Bioanalysis* **2012**, *4*, 1025–1037.
19. Akrami, A.; Hsieh, F.; Almon, V.; Bruenner, B. A.; James, C.; Wong, P. *J. Pharm. Biomed. Anal.* **2011**, *55*, 1104–1110.
20. 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*, 131–137.
21. Zhang, Q.; Wen, C.; Xiang, Z.; Ma, J.; Wang, X. *J. Pharm. Biomed. Anal.* **2014**, *90*, 134–138.
22. Hu, L. F.; Lin, G. Y.; Wang, X. Q.; Xu, R. A.; Zhang, X. H.; Zhang, C. H. *Lat. Am. J. Pharm.* **2011**, *30*, 10–16.
23. Hu, L. F.; Wang, X. Q.; Jiang, H. Y.; Xu, X. G.; Xu, R. A.; Lin, G. Y. *Lat. Am. J. Pharm.* **2010**, *29*, 839–844.
24. Ma, J. S.; Zhang, Y. Q.; Zhang, M. L.; Fan, X. F.; Wang, Z. B.; Shentu, Y. P.; Wang, Z. Y.; Wang, X. Q. *Lat. Am. J. Pharm.* **2011**, *30*, 613–618.
25. Ye, W.; Chen, R.; Sun, W.; Huang, C.; Lin, X.; Dong, Y.; Wen, C.; Wang, X. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2017**, *1060*, 144–149.
26. Gullick, D. R.; Mott, K. B.; Bartlett, M. G. *Biomed. Chromatogr.* **2016**, *30*, 772–789.
27. Yang, X. K.; Ma, Y. J.; Li, N.; Cai, H. J.; Bartlett, M. G. *Anal. Chem.* **2017**, *89*, 813–821.
28. 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: Anal. Technol. Biomed. Life Sci.* **2015**, *990*, 118–124.
29. van der Nagel, B. C. H.; Versmissen, J.; Bahmany, S.; van Gelder, T.; Koch, B. C. P. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2017**, *1060*, 367–373.
30. Simiele, M.; Ariaudo, A.; De Nicolo, A.; Favata, F.; Ferrante, M.; Carcieri, C.; Bonora, S.; Di Perri, G.; De Avolio, A. *J. Pharm. Biomed. Anal.* **2017**, *138*, 223–230.
31. Rousset, M.; Titier, K.; Bouchet, S.; Dutriaux, C.; Pham-Ledard, A.; Prey, S.; Canal-Raffin, M.; Molimard, M. *Clin. Chim. Acta* **2017**, *470*, 8–13.
32. Fang, B.; Bao, S.; Wang, S.; Chen, M.; Chen, B.; Su, K.; Wen, C.; Zhou, Y.; Wang, X.; Jin, Y. *Biomed. Chromatogr.* **2017**, *31*, e3826.
33. Wang, J.; Zhao, T.; Kong, J.; Peng, H.; Lv, P.; Li, J.; Cao, X.; Zhang, S. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.* **2017**, *1061–1062*, 270–274.