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Assessment of a developed HPLC-MS/MS approach for determining plasma eupatorin in rats and its application in pharmacokinetics analysis

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Eupatorin, a bioactive compound extracted from Java tea (*Orthosiphon stamineus*), possesses potent anti-cancer, anti-inflammatory and vasodilation activities. To date, no pharmacokinetics studies on eupatorin have yet been performed. Here, we established and validated a sensitive and selective LC-MS/MS (liquid chromatography-tandem mass spectrometry) approach for determining plasma eupatorin in rats. Chromatographic fractionation was conducted on a Wonda Cract ODS-2 C18 Column (4.6 mm × 150 mm, 5 μm) with a mobile phase containing aqueous 0.1% formic acid and acetonitrile using a flow rate of 0.8 ml min^{-1}. In multiple reaction monitoring mode, precursor-to-product ion transitions for quantification of eupatorin and the internal standard were set at 343.1 → 328.1 and 252.0 → 155.9, respectively. The intra- and inter-day precision and accuracy were found to be below 6.72% and within ±8.26% in rat plasma, respectively. Meanwhile, all values of the matrix effect, recovery and stability were within the accepted ranges. Furthermore, we carried out the pharmacokinetic analysis using the developed method. The pharmacokinetic study revealed that while the C_{max} (maximum plasma concentration) of eupatorin and time for reaching the C_{max} (T_{max}) were 974.886 ± 293.898 μg L^{-1} and 0.25 h, respectively, the half-life was 0.353 ± 0.026 h. This study will be of great significance to the research on the pharmacology, clinical pharmacy and drug action mechanism of eupatorin.

1. Introduction

Breast cancer is among the most common female malignancies in the world, as well as a leading cause of mortality in women.[1,2] In recent years, flavonoids have been shown to possess a wide variety of anti-cancer effects.[3] Therefore, the intake of certain flavonoid-containing food could contribute to prevention of the cancer development at an early stage in the patients. As a potential anti-tumor agent against breast cancer, eupatorin (5,3′-di-hydroxy-6,7,4′-tri-methoxy-flavone) exhibited inhibitory effects on the invasion and migration of MDA-MB-231 and MCF-7 cells *via* suppressed Akt pathway as well as cell cycle blockade.[4,5] Eupatorin has been identified as a natural methoxy flavonoid that widely exists in Java tea (*Orthosiphon stamineus*, OS), the traditional Chinese medicinal herb commonly used as a diuretic as well as for renal system diseases in Southeast Asia

and Europe.[6–8] Multiple studies have shown that, in addition to anti-breast cancer effect, eupatorin has the effect on inhibiting proliferation and inducing apoptosis in other types of cancer cells.[9–11] Moreover, the pharmacological study found that eupatorin possesses strong anti-inflammatory[12,13] and vasodilation activities.[14] Thus, it is of great significance to study this compound in depth due to its potential application prospect and clinical value.

Pharmacokinetics is the subject of studying the drug changes *in vivo*, which has important implications for the studies of pharmacology, clinical medicine and elucidation of the mechanism of drug action. Meanwhile, the pharmacokinetic study plays an important role in evaluating the concentration–effect relationship, designing new drug delivery systems and optimizing drug delivery scheme.[15] Although the pharmacokinetic analysis of OS has been reported,[16] there is no study on the pharmacokinetics of eupatorin. Here, we aimed to undertake a pharmacokinetics analysis of eupatorin in rats and to elucidate its changes and rules under the influence of organism.

High Performance Liquid Chromatography (HPLC)[17–19] and ultra-performance liquid chromatography–triple quadrupole mass spectrometry (UPLC-QQQ/MS)[20] have been successfully utilized to determine eupatorin. LC-MS/MS approach is known to be widely used in drug analysis due to its high selectivity and

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sensitivity.[21] In the present work, we employed the LC-MS/MS approach for determining plasma eupatorin in rats, while conducting a pharmacokinetics study using this method. The experimental results will be helpful to better understand the pharmacokinetic law of eupatorin in rats, thereby laying a basis for further pharmacological and clinical research.

2. Materials and methods

2.1. Reagents and compounds

The following compounds and reagents were purchased as follows: eupatorin with a purity of more than 98.94% (855-96-9) from Chengdu Desite Co., Ltd (Chengdu, China); HPLC-grade acetonitrile, methanol and formic acid from Fisher Scientific (Waltham, MA, USA); Purified water from Wahaha Group Co., Ltd (Hangzhou, China); CMC–Na (sodium carboxymethyl cellulose) from Guangfu Technology Development Co., Ltd. (Tianjin, China. Sulfamethoxazole was obtained from the National Institutes for Food and Drug Control, Beijing, China).

2.2. Experimental apparatus and parameters

An Agilent Technologies Series 1200 system with an Applied Biosystems/MDS SCIEX 3200 QTRAPTM system were used to conduct the experiments. Chromatographic fractionation was performed on a Wonda Cract ODS-2 C18 column (4.6 mm × 150 mm, 5 μm) using a mobile phase comprising aqueous 0.1% formic acid (solvent A) and acetonitrile (solvent B). The flow rate and injection volume were set at 0.8 ml min^{-1} and 10 μl, respectively. The following gradient elution was carried out: 0–3 min, eluent B 10–61%; 3–10 min, eluent B 61–85%; and 10–13 min, eluent B 85–95%.

A QTRAP-3200 system including Turbo V sources and turbo-ion spray interface in multiple reaction monitoring mode was utilized to conduct the mass spectrometry. The parameters for the spectrometric measurements were set as follows: curtain gas 35 psi, nebulizer gas 60 psi, heater gas 65 psi, temperature for the turbo gas 650 °C, and ion spray voltage −4500 V. The precursor/product ion pairs of eupatorin and sulfamethoxazole were 343.1→328.1 and 252.0→155.9, respectively. The following software systems were used: Analyst 1.6.2 software (AB SCEIX, USA) for data acquisition; Peakview 2.0 software (AB SCEIX, USA) and MultiQuant 3.0 software (AB SCEIX, USA) for data processing.

2.3. Preparation of standard solutions and quality control sample solutions

0.16 mg ml^{-1} of eupatorin was prepared with methanol as the stock solution. Then, a serial dilution using methanol was made to prepare standard working solutions at 3.125, 6.25, 12.5, 25, 50, 100, 200, 400, and 800 ng ml^{-1} as well as quality control (QC) sample solutions, including low 6.25 ng ml^{-1}, medium 50 ng ml^{-1}, and high quality control 600 ng ml^{-1}. Preparation of the internal standard (IS, sulfamethoxazole) working solution (400 ng ml^{-1}) with methanol was performed. All solutions were kept in the refrigerator.

2.4. Sample preparation

Extraction of eupatorin or IS from the blank plasma *via* protein precipitation was carried out using methanol. 100 μl of IS solution and 300 μl of methanol were spiked to 100 μl of the blank plasma. After being vortexed for 3 min, the mixture was subjected to a 21,380g centrifugation for 10 min at 4 °C. The collected supernatant was evaporated by nitrogen gas and re-dissolved into 100 μl of methano l. The sample was purified by twice centrifugation (21,380g) for 10 min, and a 10 μl of the supernatant was loaded on the LC-MS/MS for analysis.[22]

2.5. Method validation for pharmacokinetics

The suitability of the developed method used for this investigation was evaluated under the guidelines for bioanalytical method validation issued by US Food and Drug Administration.[23]

2.5.1. Specificity and selectivity. The blank plasma, blank plasma mixed with eupatorin and IS, as well as plasma samples following oral medication of eupatorin were processed and analyzed to determine specificity and selectivity of the developed method.[24]

2.5.2. Linearity and sensitivity. 100 μl of IS working solution, 100 μl of above standard solutions and 200 μl of methanol were spiked to 100 μl of the blank plasma. The mixture was vortexed for 3 min, followed by a 21,380g centrifugation for 10 min. After being dried by nitrogen gas, the collected supernatant was re-dissolved into 100 μl of methanol. The sample was purified by twice centrifugation (21,380g) for 10 min, and a 10 μl of the supernatant was loaded on the LC-MS/MS for analysis. The data was processed using MultiQuant 3.0 software to obtain the standard curve of the target compound. In the standard curve, LLOQ (lower limit of quantification) indicates the lowest analyte concentration that can be accurately quantified.[25]

2.5.3. Precision and accuracy. Six replicates of QC samples at three different concentrations (low, medium and high) were assayed to determine intra- and inter-day precision and accuracy that were indicated by the relative standard deviation (RSD) and relative error (RE), respectively.[26] And RSD was expected to be below 15%, while the RE should be within ±15%.[27]

2.5.4. Recovery and matrix effect. ① 100 μl of each QC sample solution at low, medium, and high concentrations, 200 μl of methanol and 100 μl of IS working solution were spiked to 100 μl of the blank plasma. QC samples were prepared according to the above protocol. Six replicates of each concentration were prepared in parallel.

② 200 μl of methanol and 100 μl of IS working solution were spiked to 100 μl of the blank plasma. The mixture was processed according to the method above, then 100 μl of each QC sample solution at three different concentrations was added. For each concentration, six replicates were prepared in parallel.

③ 200 μl of methanol and 100 μl of IS working solution were spiked to 100 μl of water. The mixture was processed according to the method above, followed by the addition of 100 μl of each QC sample solution at the three concentration levels. Each concentration was prepared for six replicates in parallel.

The calculation formula of recovery rate was presented as: recovery rate = ①/② × 100%.[28]

The calculation formula of matrix effect was presented as: matrix effect = ②/③ × 100%.[29]

2.5.5. Stability. The analyte stability in rat plasma at the three QC concentration levels was evaluated under indicated conditions below ($n = 6$). For testing the short-term and long-term stabilities, the samples were kept at 25 °C for 24 h and −20 °C for one month before analysis, respectively. To assay the freeze–thaw stability, samples were subjected to three freeze–thaw cycles prior to test. For evaluating the stock solutions stability, samples were kept at 4 °C for one month prior to study. RE was used to define the stability, and the analyte was considered stable if the RE was within ± 15%.[30]

2.6. Pharmacokinetics analysis

Eighteen male SD rats (200–250 g, 12–14 weeks old) were obtained from the Laboratory Animal Research Center of the Hebei Medical University in China. All experimental procedures involving animals were performed under the guidelines for Care and Use of Laboratory Animals issued by the Hebei Committee, and protocols were approved by Ethics Committee of the Hebei

Medical University. Animals were reared at the temperature ranging 22–25 °C and relative humidity of 55–60% under a 12 h light–dark cycle, and were freely fed for at least one week before the experiments. For this study, rats were subjected to a 12 h of fasting with free access to water before the experiment. The rats were administered *via* gavage with eupatorin in a 0.5% CMC–Na solution (50 mg kg^{-1}).

For plasma preparation, a single blood sample (0.3 ml for each time point) was collected from the canthus of rat eyes at 0, 0.033, 0.083, 0.117, 0.167, 0.25, 0.5, 1, 2, 3, 4, 5 and 7 h after administration, respectively, followed by an immediate centrifugation at 1920 × g for 10 min. After centrifugation, these samples were mixed and stored at −80 °C. The Drug and Statistics 3.0 software program[31–33] (DAS 3.0, http://www.drugchina.net) was used to calculate pharmacokinetic parameters of eupatorin in the non-compartmental model.

3. Results and discussion

3.1. Optimization of chromatographic and mass spectrometry conditions

It has been demonstrated that chromatographic conditions, especially the mobile phase composition, are critical for
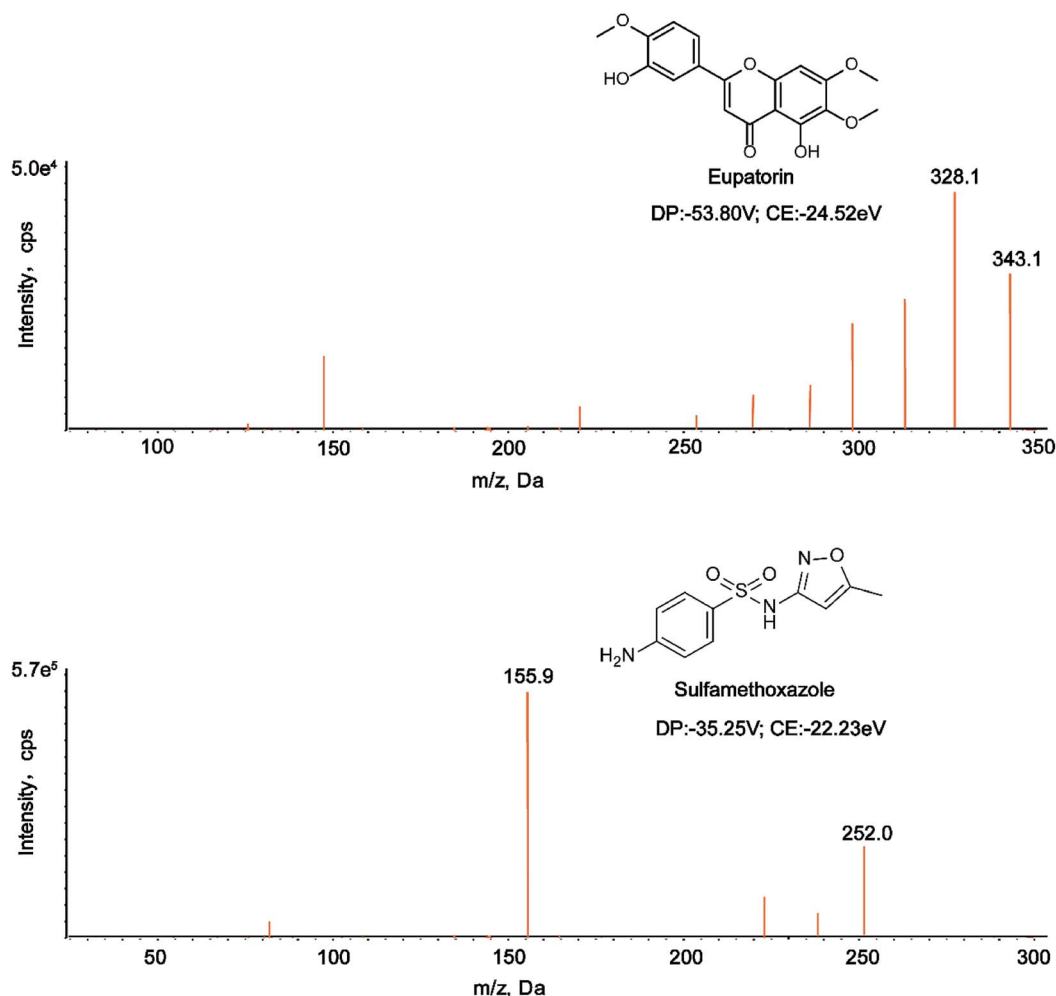

Fig. 1 The chemical structures, MS/MS spectra, DP and CE of eupatorin and sulfamethoxazole in negative mode.
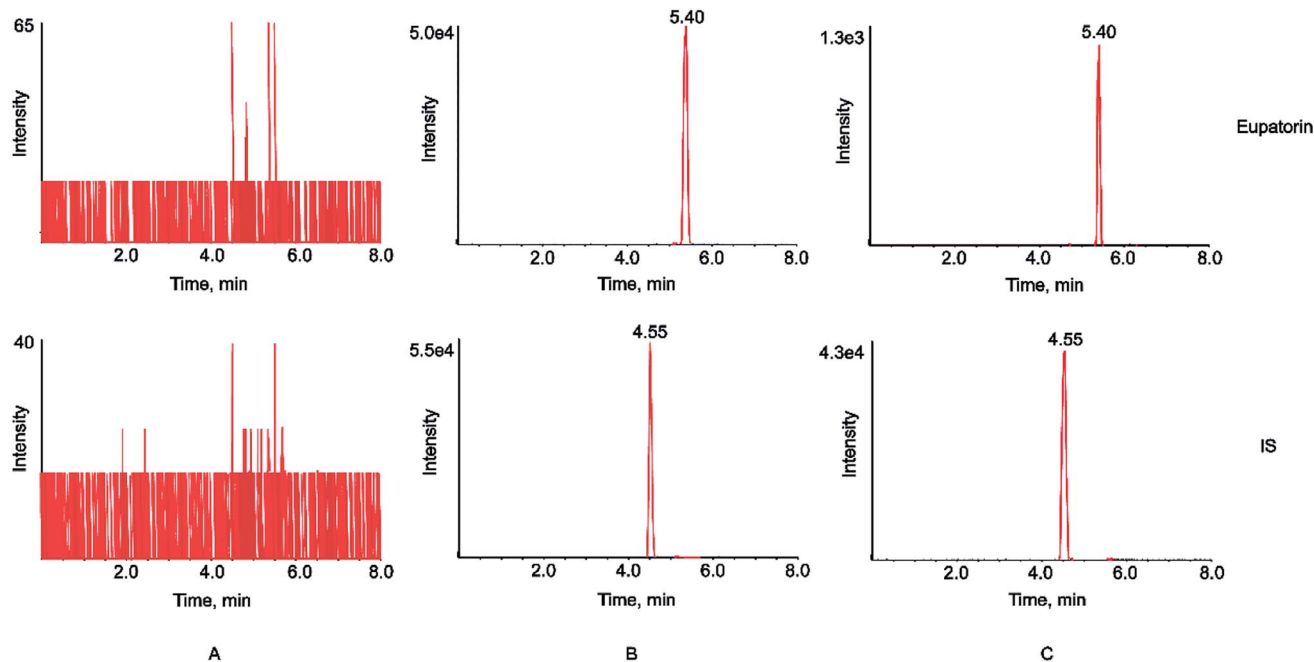
Fig. 2 The MRM chromatograms of rat blank plasma (A), plasma mixed with eupatorin and sulfamethoxazole (B), and plasma samples following oral medication of eupatorin (C).

Table 1 Intra- and inter-day precision and accuracy of plasma eupatorin in rats

| Conc. (ng ml^{-1}) | Intra-day ($n = 6$) | | | Inter-day ($n = 6$) | | |
	Measured conc. (ng ml^{-1})	Precision, RSD (%)	Accuracy, RE (%)	Measured conc. (ng ml^{-1})	Precision, RSD (%)	Accuracy, RE (%)
6.25	6.16 ± 0.41	6.72	−1.43	6.48 ± 0.37	5.68	3.72
50	53.38 ± 2.55	4.77	6.76	54.13 ± 3.23	5.97	8.26
600	574.02 ± 15.80	2.75	−4.33	613.91 ± 22.01	3.59	2.32

obtaining satisfactory chromatographic performance such as peak symmetry, sensitivity, and short run time, as well as proper ionization.[34,35] In this study, we made a comparison between acetonitrile–water and methanol–water mobile phase systems, and found that acetonitrile–water system was superior to methanol–water counterpart. Strikingly, the addition of 0.1% formic acid into water of the mobile phase could not only improve chromatographic peak shape, but also increase the analyte sensitivity. The mass spectrometry conditions were also optimized in the study. The response values of the analyte and IS in negative ion mode were higher than those in positive ion

mode. In addition, parameters such as declustering potential (DP) and collision energy (CE) were optimized to achieve the best responses of the analyte and IS. The selected precursor-product ion pairs of eupatorin and IS were 343.1/328.1 and 252.0/155.9, respectively. The chemical structures, MS/MS spectra, DP and CE of eupatorin and IS were presented in Fig. 1.

3.2. Method validation

3.2.1. Specificity and selectivity. The typical chromatograms of the blank plasma, blank plasma mixed with eupatorin

Table 2 The mean recoveries and matrix effects of plasma eupatorin in rats ($n = 6$)

| Analyte | Spiked concentration (ng mL^{-1}) | Extraction recoveries | | Matrix effects | |
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
Eupatorin	6.25	83.56	7.04	81.66	1.60
	50	81.17	6.16	88.97	3.01
	600	90.01	2.24	85.80	6.57

Table 3 The stability of plasma eupatorin in rats ($n = 6$)

Storage condition	Eupatorin		
	Nominal conc. (ng ml^{-1})	Found conc. (ng ml^{-1})	RE (%)
Short-term	6.25	6.14 ± 0.385	−1.70
	50	52.70 ± 2.149	5.39
	600	609.05 ± 29.597	1.51
Long-term	6.25	6.27 ± 0.400	0.27
	50	52.10 ± 1.948	4.20
	600	597.79 ± 30.462	−0.37
Freeze–thaw cycles	6.25	6.66 ± 0.134	6.54
	50	51.53 ± 0.970	3.07
	600	603.05 ± 30.867	0.51
Stock solution	6.25	6.37 ± 0.421	1.98
	50	54.30 ± 2.661	8.60
	600	621.57 ± 19.355	3.59

and IS, as well as plasma samples following oral medication of eupatorin were depicted in Fig. 2. The retention times of eupatorin and internal standard were 5.40 min and 4.55 min, respectively, showing no marked endogenous interference on the blank sample during the retention time of eupatorin and internal standard.

3.2.2. Linearity and sensitivity. Upon data acquisition of a number of standard samples, MultiQuant 3.0 software was used to generate the linear regression curve and calculate the correlation coefficient of the analyte. The regression eupatorin was $y = 0.004x - 0.0089$ ($r = 0.9907$), while the linear range and lower limit of quantification were 3.125–1600 ng ml^{-1} and 3.125 ng ml^{-1}, respectively. These results showed a good linear correlation between the concentration and response value of eupatorin.

3.2.3. Precision and accuracy. Results of intra- and inter-day precision and accuracy of eupatorin were summarized in Table 1. As shown in the table, the intra- and inter-day RSDs were ≤6.72% and 5.97%, respectively, while the corresponding REs were within ±6.76% and ±8.26%, respectively. This study validated a precise, reliable and reproducible method for determining plasma eupatorin in rats.

3.2.4. Recovery and matrix effect. As summarized in Table 2, the mean recoveries of eupatorin at three different concentration levels were determined to be 81.17–90.01%, while the corresponding matrix effects ranged from 81.66% to 88.97%. All RSD values were less than 7.04%.

3.2.5. Stability. Table 3 summarized the stability data of eupatorin at low, medium, and high concentrations under four distinct conditions. The data showed that eupatorin was stable during the 24 h storage at 25 °C (RE: −1.70–5.39), 30 day storage at −20 °C (RE: −0.37–4.20), or after three freeze–thaw cycles (RE: 0.51–6.54). In addition, good stability was observed in the stock solution of eupatorin kept at 4 °C for one month (RE: 1.98–8.60).

3.3. Pharmacokinetic study

Here, the developed LC-MS/MS approach was successfully used in the pharmacokinetic analysis of plasma eupatorin in rats.
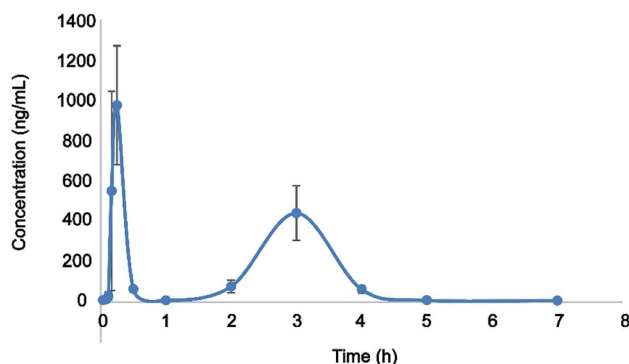

Fig. 3 The concentration–time curve of plasma eupatorin in rats after single oral medication (50 mg kg^{-1}). The data were represented as mean ± SD, $n = 6$.

Table 4 The pharmacokinetic parameters of plasma eupatorin in rats following oral medication ($n = 6$)

Pharmacokinetic parameters	Eupatorin
C_{max} (µg L^{-1})	974.886 ± 293.898
T_{max} (h)	0.25
$T_{1/2}$ (h)	0.353 ± 0.026
AUC$_{0-t}$ (µg l^{-1} h^{-1})	831.224 ± 246.677
AUC$_{0-\infty}$ (µg l^{-1} h^{-1})	831.313 ± 246.661
CLz/F (L kg^{-1} h^{-1})	25.718 ± 7.266
Vz/F (L kg^{-1})	13.265 ± 4.457

The concentration–time curve of eupatorin in rat plasma was depicted in Fig. 3. Table 4 listed various pharmacokinetic parameters such as the C_{max} (maximum plasma concentration), T_{max} (time for reaching the maximum concentration), $T_{1/2}$ (the half-life), the area after administration under the concentration–time curve (AUC$_{0-t}$, AUC$_{0-\infty}$), CLz/F (the clearance), and Vz/F (volume of distribution). All these parameters were subjected to the analysis using the non-compartment model and indicated by mean ± SD.

The pharmacokinetic study revealed that the C_{max} and T_{max} were 974.886 ± 293.898 µg L^{-1} and 0.25 h, respectively, indicating that eupatorin was rapidly absorbed by the gastrointestinal system. Meanwhile, the $T_{1/2}$ was 0.353 ± 0.026 h, showing that the analyte has a short residence time in the body and can be administered multiple times to improve clinical efficacy. In addition, we found that the mean plasma concentration displayed double peaks after oral medication of eupatorin; the first and second peaks occurred at around 0.25 h and 3 h, respectively. Consistent with the previous studies,[29,36,37] the above phenomenon may be related to the heterogeneity of gastrointestinal absorption. It has been suggested that multiple absorption sites are located on distinct parts of the gastrointestinal system. Given the difference in the permeability of the lumen endocardium at different sites, the absorption time and rate vary among the different parts after oral administration. The absorbed drug superimposed in the blood, and the double peak phenomenon of the drug–time curve appeared.[38,39] In

addition, it could be caused by the irregular gastric emptying.[40–42] It has been shown that the irregular gastric emptying could be a main reason for the double peaks in plasma concentration profiles. However, the underlying mechanism of this observation in the pharmacokinetics analysis remains to be further investigated. Overall, this study can lay a theoretical foundation for the preclinical and clinical application of eupatorin.

4. Conclusions

This study established and assessed a rapid and sensitive LC-MS/MS analytical approach for determining plasma eupatorin in rats, while successfully conducting a pharmacokinetic analysis using the developed method. We showed that following oral medication, eupatorin was quickly absorbed, reaching the maximum blood concentration and displaying the secondary absorption. Moreover, eupatorin had a short residence time in the body, as indicated by its short half-life, and can be administered multiple times to improve the clinical efficacy. Thus, these findings could lay a foundation for the clinical application of eupatorin and provide technical support for consuming eupatorin-containing food and drugs.

Conflicts of interest

The authors have no conflicts of interest to disclose.

References

1 W. G. Ihraiz, M. Ahram and S. K. Bardaweel, *Acta Pharm.*, 2020, **70**, 179–190.

2 A. Tellez, C. Rodriguez-Padilla, D. M. Juarez-Garcia, L. Jaime-Bernal, T. Sanchez-Jauregui, D. Almaraz-Castruita and H. Vielma-Ramirez, *Am. J. Clin. Hypn.*, 2020, **62**, 298–310.

3 D. M. Kopustinskiene, V. Jakstas, A. Savickas and J. Bernatoniene, *Nutrients*, 2020, **12**, 457.

4 N. A. Razak, N. Abu, W. Y. Ho, N. R. Zamberi, S. W. Tan, N. B. Alitheen, K. Long and S. K. Yeap, *Sci. Rep.*, 2019, **9**, 1514.

5 V. Androutsopoulos, R. R. Arroo, J. F. Hall, S. Surichan and G. A. Potter, *Breast Cancer Res.*, 2008, **10**, R39.

6 R. Pariyani, I. S. Ismail, A. Azam, A. Khatib, F. Abas, K. Shaari and H. Hamza, *J. Pharm. Biomed. Anal.*, 2017, **135**, 20–30.

7 M. A. Hossain and S. M. Mizanur Rahman, *Arabian J. Chem.*, 2015, **8**, 218–221.

8 N. D. Yuliana, A. Khatib, A. M. Link-Struensee, A. P. Ijzerman, F. Rungkat-Zakaria, Y. H. Choi and R. Verpoorte, *Planta Med.*, 2009, **75**, 132–136.

9 K. Lee, D. Hyun Lee, Y. J. Jung, S. Y. Shin and Y. H. Lee, *Appl. Biol. Chem.*, 2016, **59**, 193–199.

10 S. Estevez, M. T. Marrero, J. Quintana and F. Estevez, *PLoS One*, 2014, **9**, e112536.

11 I. Doleckova, L. Rarova, J. Gruz, M. Vondrusova, M. Strnad and V. Krystof, *Fitoterapia*, 2012, **83**, 1000–1007.

12 M. Laavola, R. Nieminen, M. F. Yam, A. Sadikun, M. Z. Asmawi, R. Basir, J. Welling, H. Vapaatalo, R. Korhonen and E. Moilanen, *Planta Med.*, 2012, **78**, 779–786.

13 M. F. Yam, V. Lim, I. M. Salman, O. Z. Ameer, L. F. Ang, N. Rosidah, M. F. Abdulkarim, G. Z. Abdullah, R. Basir, A. Sadikun and M. Z. Asmawi, *Molecules*, 2010, **15**, 4452–4466.

14 M. F. Yam, C. S. Tan, M. Ahmad and R. Shibao, *Eur. J. Pharmacol.*, 2016, **789**, 27–36.

15 I. H. Baek, *Xenobiotica*, 2019, **49**, 734–739.

16 Z. Guo, B. Li, J. Gu, P. Zhu, F. Su, R. Bai, X. Liang and Y. Xie, *Molecules*, 2019, **24**, 3057.

17 N. H. Saidan, A. F. Aisha, M. S. Hamil, A. M. Majid and Z. Ismail, *Pharmacogn. Res.*, 2015, **7**, 23–31.

18 M. F. Yam, E. A. Mohamed, L. F. Ang, L. Pei, Y. Darwis, R. Mahmud, M. Z. Asmawi, R. Basir and M. Ahmad, *J. Acupunct. Meridian Stud.*, 2012, **5**, 176–182.

19 G. Akowuah, I. Zhari, I. Norhayati, A. Sadikun and S. Khamsah, *Food Chem.*, 2004, **87**, 559–566.

20 Z. Guo, X. Liang and Y. Xie, *J. Pharm. Biomed. Anal.*, 2019, **164**, 135–147.

21 C. Liang, J. Yin, Y. Ma, X. Zhang and L. Zhang, *J. Pharm. Biomed. Anal.*, 2020, **177**, 112835.

22 L. Li, R. Feng, X. Feng, Y. Chen, X. Liu, W. Sun and L. Zhang, *RSC Adv.*, 2020, **10**, 10552–10558.

23 U.S. Department of Health and Human Services, *Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Guidance for Industry*, Bioanalytical Method Validation, 2018.

24 F. Zhang, L. Sun, J. Zhai, T. Xia, W. Jiang, M. Li, S. Gao, X. Tao, W. Chen and Y. Chai, *BioMed Res. Int.*, 2019, **2019**, 1854323.

25 W. Zhao, L. Pang, D. Xu and N. Zhang, *Molecules*, 2015, **20**, 9084–9098.

26 L. Zhao, W. Li, Y. Li, H. Xu, L. Lv, X. Wang, Y. Chai and G. Zhang, *J. Chromatogr. Sci.*, 2015, **53**, 1185–1192.

27 B. Yang, L. Xie, S. Peng, K. Sun, J. Jin, Y. Zhen, K. Qin and B. Cai, *J. Sep. Sci.*, 2019, **42**, 2341–2350.

28 E. Ezzeldin, M. Iqbal, R. Al-Salahi and T. El-Nahhas, *J. Pharm. Biomed. Anal.*, 2019, **166**, 244–251.

29 X. Zhang, J. Guan, H. Zhu and T. Niu, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2014, **971**, 126–132.

30 C. Zhao, S. Han, S. Yang and W. Xin, *Biomed. Chromatogr.*, 2019, **33**, e4667.

31 B.-H. Bao, A. Kang, Y. Zhao, Q. Shen, J.-S. Li, L.-Q. Di and J.-X. Li, *J. Chromatogr. B*, 2017, **1052**, 60–65.

32 M. Shi, M. Xu and L. Yin, *J. Pharm. Pharmacol.*, 2020, **72**, 1061–1071.

33 L. Wang, X. Ren, Y. He, G. Cui, Z. Wei, J. Jia, J. Cao, Y. Liu, B. Cong, Y. Niu and K. Yun, *Eur. J. Drug Metab. Pharmacokinet.*, 2020, **45**, 477–485.

34 M. Ding, W. Ma, X. Wang, S. Chen, S. Zou, J. Wei, Y. Yang, J. Li, X. Yang, H. Wang, Y. Li, Q. Wang, H. Mao, X. M. Gao and Y. X. Chang, *J. Ethnopharmacol.*, 2019, **242**, 112055.

35 Z. Wang, Q. Wu, Y. Meng, Y. Sun, Q. Wang, C. Yang, Q. Wang, B. Yang and H. Kuang, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2015, **985**, 164–171.

36 R. Shi, S. Qiao, D. Yu, X. Shi, M. Liu, X. Jiang, Q. Wang and L. Zhang, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2011, **879**, 1625–1632.

37 Z. Zhu, L. Zhao, X. Liu, J. Chen, H. Zhang, G. Zhang and Y. Chai, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2010, **878**, 2184–2190.

38 S. K. Thapa, M. Upadhyay, T. H. Kim, S. Shin, S. J. Park and B. S. Shin, *Molecules*, 2019, **24**, 2037.

39 M. Zhang, X. Ren, S. Yue, Q. Zhao, C. Shao and C. Wang, *Molecules*, 2019, **24**, 3117.

40 Y. Metsugi, Y. Miyaji, K.-i. Ogawara, K. Higaki and T. Kimura, *Pharm. Res.*, 2007, **25**, 886–895.

41 H. Ding, Y. Liu, Z. Zeng, H. Si, K. Liu, Y. Liu, F. Yang, Y. Li and D. Zeng, *Res. Vet. Sci.*, 2012, **93**, 374–377.

42 B. Ji, Y. Zhao, P. Yu, B. Yang, C. Zhou and Z. Yu, *Talanta*, 2018, **190**, 450–459.