Determination of eugenitin in mouse blood by ultra-performance liquid chromatography-tandem mass spectrometry and its application to a pharmacokinetic study

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ABSTRACT

Eugenitin is a non-volatile chromone derivative which is always found in dried flower buds of Syzygium aromaticum L. (Merr.) & L.M. Perry. Until now, there were no reports about the pharmacokinetics of eugenitin in biological fluids. A UPLC-MS/MS method developed to determine eugenitin in mouse blood. The blood samples were prepared by protein precipitation with acetonitrile. Chrysin (internal standard, IS) and eugenitin were gradient eluted by mobile phase of acetonitrile and water (0.1% formic acid) in a BEH C18 column. The multiple reaction monitoring (MRM) of m/z 221.1→206.0 for eugenitin and m/z 255.1→152.9 for IS with an electrospray ionization (ESI) source was used for quantitative detection. The calibration curve ranged from 0.5 to 500 ng/mL (r > 0.995). The accuracy ranged from 98 to 113%, the precision was less than 12%, and the matrix effect was between 86 and 94%, the recovery was better than 81%. The developed method was successfully used for pharmacokinetics of eugenitin in mice after intravenous (5 mg/kg) and oral (20 mg/kg) administration, and the absolute availability of eugenitin was 12%.

KEYWORDS

UPLC-MS/MS, eugenitin, mouse, bioavailability, determination

INTRODUCTION

Eugenitin is a non-volatile chromone derivative which is always found in dried flower buds of Syzygium aromaticum L. (Merr.) & L.M. Perry [1]. It is also a metabolite of the endophyte Mycoleptodiscus indicus (V.P. Sahni) B. Sutton. It has been reported that eugenitin was isolated from the culture of endophytic fungus BCC 54265 and the endophytic mitosporic Dothideomycete sp LRUB20 [2, 3]. Many studies have shown that eugenitin can activate the recombinant GH 11 endoxylanase and Aspergillus niger glucoamylase [1].

Pharmacokinetic, was a science that uses mathematical analysis to deal with the dynamic processes of drugs in the body [4–8]. Until now, there were no reports about the pharmacokinetics of eugenitin in biological fluids. Therefore, it was necessary to develop a UPLC-MS/MS method for the pharmacokinetics. In this study, UPLC-MS/MS was used to detect eugenitin in mouse blood and its pharmacokinetics was studied. It provides a theoretical basis for the further development and utilization of eugenitin.
EXPERIMENTAL

Chemicals and animals
Eugenitin and chrysin (IS) (both purity >98%) were obtained from Chengdu Munster biotechnology Co. Ltd (Chengdu, China). Milli-Q water system was purchased from Millipore Sigma (Burlington, MA, USA). HPLC grade formic acid, acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Institute of Cancer Research (ICR) mice (male, 20–22 g) were from Wenzhou Medical University (Wenzhou, China).

Instrument and condition
XEVO TQS-micro triple quadrupole mass spectrometry with an ACQUITY H-Class UPLC (Waters Corp, Milford, MA, USA) was used for analysis.

UPLC BEH C18 (1.7 μm, 2.1 mm × 50 mm) column was for separation, it was set at 30 ºC. The mobile phase consisted of acetonitrile and water (0.1% formic acid). The gradient conditions with as follows: 0–0.2 min, 10% acetonitrile; 0.2–1.4 min, 10–85% acetonitrile; 1.4–2.0 min, 85% acetonitrile; 2.0–2.1 min, 85-10%, acetonitrile; 2.1–3.5 min, 10% acetonitrile. The flow rate was set at 0.4 mL/min.

The mass condition was set: dry gas (nitrogen) of 800 L/h, capillary voltage of 2.5 kV, the temperature for drying gas of 400 ºC. It was operated in an ESI positive mode and MRM, m/z 221.1→206.0 for eugenitin (cone voltage 8 v, collision voltage 14 v) and m/z 255.1→152.9 for IS (cone voltage 70 v, collision voltage 22 v), Fig. 1.

Calibration standards
The stored solutions of eugenitin (1.0 mg/mL) and chrysin (1.0 mg/mL) were prepared in methanol and water (1:1, v/v). Working standard solutions of eugenitin were diluted by methanol (5, 20, 50, 200, 500, 1,000, 2,000, and 5,000 ng/mL). The IS working standard solution (100 ng/mL) was diluted with acetonitrile.

Calibration standards of eugenitin were spiked working standard solutions to blank mouse blood into 0.5, 2, 5, 20, 50, 100, 200, and 500 ng/mL. Three quality control (QC) samples were prepared in same manner (1, 45 and 450 ng/mL).

Sample preparation
Acetonitrile (100 μL, containing IS 100 ng/mL) was added into 20 μL blood, mixed for 1.0 min, and centrifuged at 13,000 rpm for 15 min. The supernate (2 μL) was injected into UPLC-MS/MS for analysis.

Method validation
The method validation performed according to the US Food and Drug Administration (FDA) bioanalytical guidelines [9].

Pharmacokinetics
All experimental procedures and protocols were approved by the Animal Care Committee of Wenzhou Medical University (Wydw 2019-0982). Six mice was given eugenitin (20 mg/kg) by oral administration and another six mice was given eugenitin (5 mg/kg) by intravenous administration. The blood samples (20 μL) were withdrawn from caudal

Fig. 1. Chemical structure and mass spectrum of eugenitin and chrysin (IS)
vein after administration at 0.167, 0.5, 1, 1.5, 2, 3, 4, 6 h, and stored at −20 °C until analysis.

The data determined by UPLC-MS/MS was fitted by the DSA 2.0 (Shanghai, China).

RESULTS

Method validation

Figure 2 exhibited the UPLC-MS/MS of eugenitin and IS in mouse blood, and no interference was found.

The equation of the calibration curve (0.5–500 ng/mL) of eugenitin was: $y = 0.0023 \times 0.0015 \ (r = 0.9992, \ n = 6)$, $y$ represented the ratio of the peak area of eugenitin to that of IS, and $x$ was the concentration of eugenitin. The LOQ was 0.5 ng/mL. The precision and accuracy of the LOQ were 12 and 113%, respectively.

The accuracy ranged from 98 to 113%, the precision was less than 12%, and the matrix effect was between 86 and 94%, the recovery was better than 81%, in Table 1.

The stability of eugenitin in variations condition (room temperature for 2 h, 3 freezing and thawing cycles, −20 °C for 30 days) was acceptable, the accuracy was within 86 and 110%, and precision was less than 13%.

Pharmacokinetics

The main pharmacokinetic parameters of eugenitin were fitted by the one-compartment model, Table 2. The blood concentration of eugenitin was showed in Fig. 3. No literature has been reported on the pharmacokinetics of eugenitin in rats or mice. The bioavailability was 12%.

DISCUSSION

The mass spectrometry conditions were optimized. We chose the positive mode for the response of the eugenitin

![Fig. 2. Eugenitin and IS chromatograms obtained by UPLC/MS/MS in mouse blood. (A) a blood samples after oral administration, (B) the blank blood samples spiked with eugenitin (5 ng/mL) and IS, (C) a blank blood sample](image-url)

| Concentration (ng/mL) | Precision (%) | Accuracy (RSD%) | Matrix Effect (%) | Recovery (%) |
|-----------------------|---------------|-----------------|------------------|-------------|
|                       | Intra-day     | Inter-day       | Intra-day        | Inter-day   |              |              |
| 0.5                   | 12            | 12              | 113              | 110         | 94           | 85           |
| 1                     | 11            | 11              | 106              | 103         | 90           | 81           |
| 45                    | 5             | 5               | 98               | 99          | 91           | 85           |
| 450                   | 7             | 6               | 102              | 104         | 86           | 83           |
Table 2. Main Pharmacokinetic study of eugenitin after oral and intravenous administration

| Parameters | Unit     | po (20 mg/kg) | iv (5 mg/kg) |
|------------|----------|---------------|--------------|
| AUC(0–t)   | ng/mL*h  | 47 ± 12       | 101 ± 22     |
| AUC(0–∞)   | ng/mL*h  | 74 ± 19       | 138 ± 31     |
| t1/2       | h        | 69.3          | 0.8 ± 0.1    |
| V1         | L/kg     | 45 ± 16       |              |
| CL         | L/h/kg   |               | 38 ± 9       |
| V1/F       | L/kg     | 28,697 ± 8,580|              |
| CL/F       | L/h/kg   | 287 ± 86      |              |
| Cmax       | ng/mL    | 26 ± 10       | 180 ± 58     |
| Bioavailability |        |              | 12%          |

was stronger than that in the negative ion mode. Then fragment peaks with relatively high fragments were selected as quantitative ion pairs, m/z 221.1→206.0 for eugenitin (cone voltage 8 v, collision voltage 14 v) and m/z 255.1→152.9 for IS (cone voltage 70 v, collision voltage 24 v), were shown in Fig. 1.

The different mobile phase was tested, such as acetonitrile and 0.1% formic acid in water, acetonitrile and 10 mmol/L ammonium acetate, acetonitrile and water, methanol and 0.1% formic acid in water, and methanol and 10 mmol/L ammonium acetate, methanol and water. The acetonitrile and 0.1% formic acid in water was used as the mobile phase because it achieved the better peak, and suitable retention time.

Choosing suitable sample treatment method was very important [10–14]. The extraction efficiency of acetonitrile (around 83%) were better than methanol (around 72%) and ethyl acetate (around 50%). And the matrix effects of acetonitrile were acceptable (around 90%), and acetonitrile was used in this work.

It was also an important task to select the IS during the method establishment [15–18]. Several compounds including chrysin, astragalin, rubiadin, midazolam was compared. It was shown that chrysin had a better peak shape, and the peak time was similar to that of eugenitin, and it was used in this work.

UPLC-MS/MS was applied to the quantitative analysis of eugenitin in mouse blood, which was much faster than HPLC. The AUC(0–t) of were 101 ± 22 ng/mL·h and 47 ± 12 ng/mL·h for intravenous and oral administration.

CONCLUSIONS

A simple UPLC-MS/MS method was developed for determination of eugenitin in mouse with the LOQ of 0.5 ng/mL. The developed method was successfully applied to the pharmacokinetics in mice, and the bioavailability was 12%, it showed that the oral absorption was not good.

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REFERENCE

1. Andrioli, W. J.; Damasio, A. R.; Silva, T. M.; da Silva, V. B.; Maller, A.; Nanayakkara, N. P.; Silva, C. H.; Polizeli, M. L.; Bastos, J. K. Biotechnol. Lett. 2012, 34, 1487–92.
2. Isaka, M.; Palasarn, S.; Sommai, S.; Laksanacharoen, P.; Srichomthong, K. Nat. Prod. Res. 2018, 32, 1506–11.
3. Chomcheon, P.; Wiyakrutta, S.; Sriubolmas, N.; Ngamrojanavanich, N.; Mahidol, C.; Ruchirawat, S.; Kittakoop, P. Phytochemistry 2009, 70, 121–7.
4. Zhang, X.; Xiang, Q.; Zhao, X.; Ma, L.; Cui, Y. J. Clin. Pharm. Ther. 2019, 44, 163–73.
5. Millar, S. A.; Stone, N. L.; Yates, A. S.; O’Sullivan, S. E. Front Pharmacol. 2018, 9, 1365.
6. Weng, Q. H.; Chen, Y. C.; Zhong, Z. Q.; Wang, Q. Q.; Chen, L. G.; Wang, X. Q. J. Pharm. Biomed. Anal. 2020, 16, 960–6.
7. Xie, H. L.; Lu, X. J.; Jin, W. Q.; Zhou, H.; Chen, D. X.; Wang, X. Q.; Zhou, Y. F. J. Pharm. Biomed. Anal. 2020, 16, 438–45.
8. Zhang, Z. N.; Sun, Z.; Ye, Y. Z.; Wang, X. Q. J. Pharm. Biomed. Anal. 2020, 16, 520–8.
9. US Department of Health and Human Services, F.a.D.A. Guidance for Industry: Bioanalytical Method Validation, 2013.
10. Deng, M.; Li, P.; Zhang, Q.; Bao, S.; Lin, G. J. Pharm. Biomed. Anal. 2013, 32, 769–73.
11. Zhu, J.; Sun, R.; Wen, C.; Ma, Y. E.; Lin, G. Latin Am. J. Pharm. 2014, 33, 506–10.
12. Liu, J.; Sun, L.; Chen, Y.; Chen, L.; Weng, Q.; Wu, S. Latin Am. J. Pharm. 2018, 37, 2114–20.
13. Ma, J.; Wang, X. J. Pharm. Biomed. Anal. 2021, 195, 113894.
14. Ma, J.; Wang, S.; Huang, X.; Geng, P.; Wen, C.; Zhou, Y.; Yu, L.; Wang, X. J. Pharm. Biomed. Anal. 2015, 111, 131–7.
15. Li, T. R.; Ye, W. J.; Huang, B. G.; Lu, X. J.; Chen, X. X.; Lin, Y. J.; Wen, C. C.; Wang, X. Q. J. Pharm. Biomed. Anal. 2019, 168, 133–7.
16. Ye, W.; Chen, R.; Sun, W.; Huang, C.; Lin, X.; Dong, Y.; Wen, C.; Wang, X. J. Chromatogr. B Analyt Technol. Biomed. Life Sci. 2017, 1060, 144–9.
17. Shen, X. W.; Ma, J. S.; Wang, X. Q.; Wen, C. C.; Zhang, M. L. Biomed. Res. Int. 2020, 2020, 8247270.
18. Liu, Z. Z.; Liu, H. M.; Wu, Y. Z.; Xu, X. X.; Ma, J. S. Latin Am. J. Pharm. 2020, 39, 1116–21.

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