Development and validation of an UPLC-ESI-MS/MS method for determination of dehydroevodiamine, limonin, evodiamine, and rutaecarpine in Evodiae Fructus

Yang Zhao¹², Yunling Zhao¹², Xin Zhou¹², Xiaojian Gong¹²

¹The Research Center for Quality Control of Nature Medicine, Guizhou Normal University, Guiyang 550001, Guizhou, ²Key Laboratory for Information System of Mountainous Area and Protection of Ecological Environment of Guizhou Province, Guizhou Normal University, Guiyang 550001, Guizhou, China

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ABSTRACT

Objective: Evodiae Fructus (EF), one of the most widely used traditional Chinese medicines, mainly consists of alkaloids, is widely used for the treatments of headache and gastrointestinal disorders. In this study, a sensitive and reliable UPLC-ESI-MS/MS method was developed for qualitative determination of dehydroevodiamine, limonin, evodiamine, and rutaecarpine.

Materials and Methods: Chromatographic separations were accomplished on a Phenomenex Kinetex XB-C18 column (2.1 x 150 mm, 1.7 μm) by using a gradient elution profile with a mobile phase consisting of 0.5% formic acid in water (A) and acetonitrile (B). Detection was performed using multiple reactions monitoring mode under ESI in the positive ion mode.

Results: The results showed good linearity over the investigated concentration ranges (R² > 0.9900) for the analytes. The limit of quantitations (LOQs) were 6.88 ng/mL for dehydroevodiamine, 18.6 ng/mL for limonin, 6.24 ng/mL for evodiamine, and 2.56 ng/mL for rutaecarpine, respectively. Intraday and interday precisions (relative standard deviations, %) were < 5% and accuracies ranged from 92% to 106%.

Conclusion: The validated method was successfully applied to assay the contents of the four compounds in EF samples from different regions, with which just 10 min was needed to analyze each sample.

Key words: Evodiae Fructus, Quantitative determination, UPLC-ESI-MS/MS

INTRODUCTION

Evodiae Fructus (EF), the dried and nearly ripe fruits of Evodia rutaecarpa (Juss.) Benth., Evodia rutaecarpa (Juss.) Benth. var. officinalis (Dode) Huang, or Evodia rutaecarpa (Juss.) Benth. var. bodinieri (Dode) Huang,¹ is widely used as one of the traditional Chinese medicines (TCMs) with anti-inflammatory,²,³ antinociceptive,⁴,⁵ anthelmintic,⁶,⁷ antidiarrheal,⁸ anti-anoxic,⁹,¹⁰ and antibacterial effects.¹¹

Alkaloids are traditionally regarded as the major bioactive compounds in EF not only because a number of types of alkaloids were isolated from the herb,¹²-¹⁴ but also pharmacological and clinical studies indicated that alkaloids from EF had antipolysarcous,¹⁵ cardiotonic,¹⁶,¹⁷ central stimulative,¹⁸ vasodilatory,¹⁹,²⁰ and anticancer activities.²¹-²³ Evodiamine and rutaecarpine were specified as the biomarkers for quality assessment on EF in Chinese Pharmacopoeia (CP) (edition 2005),²⁴ and another compound, limonin, was used combined with evodiamine and rutaecarpine in CP (edition 2010)²⁵ for better assessment on this herb. In previous researches, TLC, HPLC with UV detector or MS as well as capillary electrophoresis (CE) were applied to quantitate or to identify these alkaloids in EF.²⁵-³² To date, these developed methods have played very important roles in assessment on EF and EF-derived products. However, some of them either need quite long analysis time, or use complicated elution programs or mobile phases. As one of the herbs having been studied for a long time in our laboratory, quantitation of the chemical compounds and chromatographic fingerprinting methods on it were developed, which were then applied to
analyze EF samples from different regions of China. In light of the genuineness of EF in Guizhou province, the established quantitative methods have been applied to analyze the marker compounds in different EF samples harvested from different resources all over the country. However, because of the high polarity, dehydroevodiamine, another chemical compound with high yield in EF, needs be united with buffer salt solution to enhance its retention behavior in chromatographic column.

This study describes the development and validation of a new, simple, and reliable UPLC-ESI-MS/MS method for simultaneous assay of the four marker compounds with high yields including dehydroevodiamine, limonin, evodiamine, and rutaecarpine in EF. The established method will be widely accepted and approved with the popularity of MS and the requirements of fast and efficient assays in routine work.

MATERIALS AND METHODS

Chemicals and materials
Reference standards including dehydroevodiamine, limonin, evodiamine, and rutaecarpine were isolated from EF in the Laboratory of the Research Center for Quality Control of Natural Medicine, Guizhou Normal University, Guiyang, China. The chemical structures of these standards were confirmed based on their UV, MS, 1H NMR, and 13C NMR data and by comparing their spectral data with the ones reported in literatures. On the basis of UV, MS, NMR, and HPLC, each reference standard was considered to have a purity of 98% or more.

MS-grade acetonitrile and formic acid were purchased from TEDIA Co. (Fairfield, OH, USA) and ROE Scientific Inc. (DE, USA), respectively. All other reagents were of analytical grade. Robust purified water was used as mobile phase in this study.

Nineteen batches of EF samples were authenticated as E. rutaecarpa (Juss.) Benth, and all the voucher specimens were deposited in the Research Center for Quality Control of Natural Medicine, Guizhou Normal University. Then, they were stored in sealed bottles before use in dry environment to avoid moisture and chemical changes.

Preparation of reference standard solutions
Stock standard solutions were prepared by dissolving accurately weighed individual reference compound in methanol. Working standard solutions containing each of the four compounds were prepared by diluting the stock solutions with methanol to a series of proper concentrations. All the stock and working solutions were stored at 4°C until analysis.

Preparation of EF samples
All the samples were prepared according to the method described in CP (edition 2010). In brief, approximately 0.5 g of each pulverized EF sample (50 mesh) was accurately weighed into a 50-mL conical flask with 25 mL of 80% ethanol (v/v) added, which was then extracted using ultrasonication for 40 min (100 W, 40 kHz) after being soaked for 1 h. The supernatant was filtered through a 0.45-μm membrane for UPLC-MS/MS analysis.

UPLC-MS/MS analysis
Chromatographic separations were carried out using an Accela 1250 UHPLC system equipped with an Accela 1250 photo diode array (PDA) detector, an Accela HTC PAL autosampler, and an Accela 1250 binary pump. Separations were achieved on a Phenomenex Kinetex XB-C18 column (2.1 × 150 mm, 1.7 μm). The mobile phase consisted of 0.5% formic aqueous solution (A) and acetonitrile (B), and the gradient elution program was as follows: 0-3 min, 20-70% B; 3-10 min, 70-80% B. The column temperature was maintained at 25°C. The flow rate was 200 μL/min, and the injection volume was 5 μL.

Mass spectrometric analyses were performed on a TSQ quantum ultra triple-quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with an ESI interface in positive mode. The MS instrument parameters were as follows: sheath gas flow rate, 35 (arbitrary units); auxiliary gas flow rate, 15 (arbitrary units); spray voltage, 3000 V; vaporizer temperature, 400°C; capillary temperature, 350°C; capillary voltage, 30 V; and tube lens offset, 170. Helium was used as the collision gas for collision-induced dissociation. Quantitation was performed using multiple reactions monitoring (MRM) mode. Three product ions were optimized for each parent ion of dehydroevodiamine, limonin, evodiamine, and rutaecarpine. The collision energy, tube lens offset, and collision pressure for each parent ion-product ion transition are displayed in Table 1. Data acquisition and processing were performed using the Xcalibur 2.1 data system and LCquan 2.6 quantitation software.

RESULTS AND DISCUSSIONS

Method development
Optimization of LC conditions
The optimization of chromatographic separation was mainly guided by the requirement for assuring assay specificity and reducing the analytical run time. The mobile phase composition was modified by adding formic acid in water to enhance the ionization efficiencies of the compounds. Compared with other mobile phases, the solvent consisting of acetonitrile and 0.5% formic acid aqueous solution provided the lowest pressure and the highest ionization efficiency, which was ultimately
selected as our mobile phase system. Dehydroevodiamine, evodiamine, and rutaecarpine are alkaloids, of which the structures and fragmentation mechanisms are similar. In order to eliminate the undesirable cross-talk effects that might exist and affect the accuracy of MS quantification, a complete chromatographic resolution was achieved. Meanwhile, flow rate and column temperature were adjusted to obtain an acceptable resolution with no cross-talk observed. Under the optimized chromatographic conditions, the four analytes were separated within 10 min. A representative MRM chromatogram of the extract of EF obtained from sample no. 1 is shown in Figure 1.

**Optimization of MS parameters**

Ionization of the four analytes was attempted both in negative mode and in positive mode with the ESI source. The results revealed that dehydroevodiamine, evodiamine, and rutaecarpine gave strong responses in positive ESI mode, whereas limonin showed weak sensitivities in both the two modes. Therefore, positive mode was selected for detection of the four compounds. Furthermore, as dehydroevodiamine, evodiamine, and rutaecarpine are nitrogen heterocyclic ring compounds, 0.5% formic acid aqueous solution was used in this study to enhance the ionization efficiencies. To quantify the four analytes more specifically and sensitively, three product ions were selected for each parent ion [Figure 2].

**Method validation**

**Linearity, LOD, and LOQ**

The calibration curve for each analyte was established with different appropriate concentrations in triplicate. Low-concentration and high-concentration calibration curves were constructed in this study, because the concentrations of the analytes in different samples varied greatly. The calibration curves of the four compounds were constructed by plotting the peak areas versus the concentrations of compounds injected. As shown in Table 2, the correlation coefficient ($R^2$) was $\geq 0.9900$ for each calibration curve. The stock standard solution of each analyte was further diluted to a series of concentrations with methanol for limit of detection (LOD) ($\sigma/n$ ratio at 3) and LOQ ($\sigma/n$ ratio at 10), respectively. As shown in Table 2, LODs and LOQs were 0.98-6.25 and 2.56-18.6 ng/mL, respectively.

**Precision, repeatability, and stability**

Precision was evaluated by analyzing the mixed standards solution under the optimal separation conditions six times in one day for intraday variation and twice a day on three consecutive days for interday variation. The relative standard deviations (RSDs) of intra- and interday assays were 1.11-3.65% and 1.81-4.21%, respectively. Repeatability was tested by analyzing six different working solutions prepared from sample no. 1. Stability of the analytes in the final extract at room temperature was investigated by triplicate injection of the sample solution at 0, 2, 4, 6, 8, 10, and 12 h. All the results are expressed as RSDs, which are shown in Table 3, showing the overall repeatability and stability variations are <4%.

**Accuracy**

The recovery test was used to evaluate the accuracy of the method by analyzing the tested samples spiked with one

### Table 1: The collision energy, tube lens offset, and collision pressure for each parent ion-product ions transitions

| Analytes       | Transition | Tube lens offset (V) | Collision pressure (m Torr) | Collision energy (eV) |
|----------------|------------|----------------------|----------------------------|-----------------------|
| Dehydroevodiamine | 301.84→256.91 | 103 | 2.4 | 57 |
|                | 301.84→285.91 |       | 38  |
|                | 301.84→287.02 |       | 25  |
| Limonin        | 470.98→104.87 | 101 | 2.4 | 41 |
|                | 470.98→160.83 |       | 24  |
|                | 470.98→425.06 |       | 17  |
| Evodiamine     | 303.84→76.95  | 92  | 2.4 | 62 |
|                | 303.84→133.87 |       | 27  |
|                | 303.84→160.85 |       | 19  |
| Rutaecarpine   | 288.07→114.85 | 115 | 2.4 | 47 |
|                | 288.07→270.93 |       | 28  |
|                | 288.07→272.96 |       | 31  |

Capillary temperature, 350°C; vaporizer temperature, 450°C; sheath gas pressure, 35 arb; Aux gas pressure, 15 arb; spray voltage, 3000 V

### Table 2: Regression data, LODs, and LOQs of the four analytes

| Analytes       | Regressive equation | $R^2$ | Liner range (ng/mL) | LOD (ng/mL) | LOQ (ng/mL) |
|----------------|---------------------|-------|---------------------|-------------|-------------|
| Dehydroevodiamine | $Y=5.35 \times 10^3 + 5.15 \times 10^3$ | 0.9999 | 11.9-1.19$\times 10^3$ | 2.08 | 6.88 |
|                | $Y=1.86 \times 10^3 + 1.68 \times 10^3$ | 0.9982 | 1.19$\times 10^3$-2.38$\times 10^4$ |       |             |
| Limonin        | $Y=6.48 \times 10^3 - 8.51 \times 10^2$ | 0.9989 | 30.5-610             | 6.25 | 18.6 |
|                | $Y=4.42 \times 10^3 + 7.37 \times 10^3$ | 0.9988 | 610-1.22$\times 10^4$ |       |             |
| Evodiamine     | $Y=1.88 \times 10^3 - 7.70 \times 10^2$ | 0.9997 | 8.92-3.57$\times 10^2$ | 3.17 | 6.24 |
|                | $Y=1.86 \times 10^3 + 2.41 \times 10^4$ | 0.9999 | 357-1.78$\times 10^3$ |       |             |
| Rutaecarpine   | $Y=5.26 \times 10^3 + 1.62 \times 10^3$ | 0.9990 | 3.88-194             | 0.98 | 2.56 |
|                | $Y=3.27 \times 10^3 + 1.01 \times 10^3$ | 0.9998 | 194-970              |       |             |

LODs: Limit of detection; LOQs: Limit of quantitation
level (100%) of the four reference standards. Six replicate analyses were carried out and the percent recoveries of the analytes were calculated. The results shown in Table 4 indicate that the developed analytical method provides acceptable accuracy with the recoveries from 92.73% to 105.71% for the analytes concerned.

Figure 1: Representative MRM chromatograms of (a) 80% ethanol blank and (b) mixed standards, and (c) sample solution. (a) Dehydroevodiamine; (b) limonin; (c) evodiamine; and (d) rutaecarpine.
All the results obtained indicate that the established method in this study is satisfactory for quantitative determination of dehydroevodiamine, limonin, evodiamine, and rutaecarpine simultaneously in EF samples.

### Sample analysis

The validated analytical method was subsequently applied for the analyses of the four compounds in 19 batches of EF samples from different regions of China. The detailed information of the herbs and the content results are summarized in Table 5. To begin with, the results showed that the concentrations of the analytes varied markedly in different samples. The contents of the four analytes were between 0.0020% and 0.2943%, 0.0154% and 1.9340%, 0.0004% and 0.1137%, and 0.0176% and 0.6025%, respectively. Then, the samples from Pan’an, Jinhua, Zhejiang, was found to have the highest content of dehydroevodimine, the one from Taizhou, Zhejiang, was found to have the highest content of limonin, and the one from Xingyi, Guizhou, had the highest contents of evodiamine and rutaecarpine. It is worth noting that dehydroevodimine is one of the chemical compounds found with high content in EF by phytochemistry study in our lab. Together with the discovery in our serum pharmacochemistry study that it is one of the compounds which can be absorbed into the blood (data not shown), we suggest that it should be designated as anther biomarker to assess the qualities of EF samples.

Principal components analysis (PCA), a mathematical procedure that uses orthogonal transformation to convert

| Compounds          | Precision | Repeatability | Stability |
|--------------------|-----------|---------------|-----------|
|                    | Intraday (n=6) | Interday (n=6) |           |
|                    | Mean (peak area) | RSD (%) | Mean (peak area) | RSD (%) | Mean | RSD |
| Dehydroevodiamine  | 4.01×10^6 | 3.65 | 3.95×10^6 | 1.81 | 0.1802 | 1.71 | 0.1795 | 2.84 |
| Limonin            | 7.07×10^4 | 1.65 | 7.27×10^4 | 3.35 | 0.5340 | 1.26 | 0.5568 | 3.37 |
| Evodiamine         | 5.63×10^5 | 2.46 | 5.38×10^5 | 4.21 | 0.0789 | 3.57 | 0.0783 | 3.68 |
| Rutaecarpine       | 1.61×10^5 | 1.11 | 1.58×10^5 | 3.86 | 0.2144 | 0.90 | 0.2359 | 0.47 |

RSD: Relative standard deviation

| Table 4: The recovery results of the method (n=6) |
|-----------------------------------------------|
| Analytes | Sample weight (g) | Contained (mg) | Added (mg) | Found (mg) | Recovery (%) | Mean recovery (%) ±SD |
|---------|-------------------|----------------|------------|------------|---------------|------------------------|
| Dehydroevodiamine | 0.2509 | 0.4521 | 0.4476 | 0.8841 | 96.51 | 99.21±5.08 |
|          | 0.2514 | 0.4530 | 0.4476 | 0.8628 | 92.07 | 93.78 |
|          | 0.2501 | 0.4507 | 0.4476 | 0.8518 | 92.07 | 95.46 |
|          | 0.2491 | 0.4501 | 0.4476 | 0.8728 | 93.78 | 92.64 |
|          | 0.2493 | 0.4492 | 0.4476 | 0.8756 | 94.65 | 92.73±2.04 |
| Limonin  | 0.2500 | 1.3350 | 1.3562 | 2.6587 | 97.60 | 93.57±2.26 |
|          | 0.2506 | 1.3382 | 1.3562 | 2.5876 | 92.12 | 92.73±2.04 |
|          | 0.2504 | 1.3371 | 1.3562 | 2.5968 | 92.88 | 92.73±2.04 |
|          | 0.2491 | 1.3302 | 1.3562 | 2.6145 | 94.70 | 94.70 |
|          | 0.2493 | 1.3313 | 1.3562 | 2.5876 | 92.64 | 92.64 |
|          | 0.2519 | 1.3451 | 1.3562 | 2.5854 | 91.45 | 91.45 |
| Evodiamine | 0.2509 | 0.1980 | 0.1889 | 0.4039 | 109.02 | 105.71±2.93 |
|          | 0.2514 | 0.1984 | 0.1889 | 0.4032 | 108.44 | 108.44 |
|          | 0.2501 | 0.1973 | 0.1889 | 0.3995 | 107.03 | 107.03 |
|          | 0.2498 | 0.1971 | 0.1889 | 0.3896 | 101.91 | 101.91 |
|          | 0.2501 | 0.1973 | 0.1889 | 0.3956 | 104.96 | 104.96 |
|          | 0.2493 | 0.1967 | 0.1889 | 0.3911 | 102.91 | 102.91 |
| Rutaecarpine | 0.2500 | 0.5360 | 0.5217 | 1.0241 | 93.56 | 92.73±2.04 |
|          | 0.2506 | 0.5373 | 0.5217 | 1.0389 | 96.15 | 96.15 |
|          | 0.2504 | 0.5369 | 0.5217 | 1.0144 | 91.54 | 91.54 |
|          | 0.2491 | 0.5341 | 0.5217 | 1.0149 | 92.17 | 92.17 |
|          | 0.2493 | 0.5345 | 0.5217 | 1.0188 | 92.83 | 92.83 |
|          | 0.2519 | 0.5401 | 0.5217 | 1.0104 | 90.15 | 90.15 |

SD: Standard deviation
Figure 2: Spectra of product ions of (a) dehydroevodiamine, (b) limonin, (c) evodiamine, and (d) rutaecarpine
Zhao, et al.: Determination of dehydroevodiamine, limonin, evodiamine, and rutaecarpine in Evodiae Fructus

High throughput data into uncorrelated variables called principal components (PCs) to simplify the analyses, was widely used in the chemical pattern recognition and discrimination on TCMs. In this study, the contents of the four analytes were used as variables for PCA with SOLO (Eigenvector Research, Inc., Wenatchee, WA). Normalize (2-norm, length = 1) and mean center were used for data reprocessing before PCA was performed. A two-component (the first two components) model was obtained, cumulatively accounting for 94.98% of total variance [Figure 3], based on which the PCA scores plot [Figure 4] was generated. From the scores plot, we can see intuitively that EF-2, EF-3, EF-4, EF-5, EF-11, EF-12, EF-13, EF-15, EF-18, and EF-19 are clustered tightly in group “A”, EF-6, EF-14, and EF-17 are clustered in group “B”, and EF-1 and EF-10 are distributed in group “C”. The distribution of samples means that the EF samples with similar contents of the four analytes are clustered into the same group. In other words, the qualities of the samples in the same group are more similar compared with others. Additionally, EF-7, EF-8, EF-9, and EF-16 are distributed far away from the three groups. It is worth noting that EF-8, EF-9, and especially EF-16 are located near the elliptic line (the confident limit at 95%), indicating that these three EF samples are the most different from other EF samples.

To find out how the variables (contents of the four analytes in this study) contribute to the positions of different EF samples in the scores plot, PC1 and PC2 loadings plots were generated. The PC1 loadings plot [Figure 5a] indicates

### Table 5: Collected information and contents of the four compounds in Evodiae Fructus (n=3)

| Sample no. | Sources | Contents (%) |
|------------|---------|--------------|
|            |         | Dehydroevodiamine | Limonin | Evodiamine | Rutaecarpine |
| EF-1       | Guangling, Tongren, GZ | 0.1802 | 0.5340 | 0.0789 | 0.2144 |
| EF-2       | Shiqian, Tongren, GZ | 0.0020 | 0.4860 | 0.0093 | 0.1805 |
| EF-3       | Jiangkou, Tongren, GZ | 0.0178 | 1.1332 | 0.0093 | 0.1805 |
| EF-4       | Jiangkou, Tongren, GZ | 0.1121 | 0.8070 | 0.0004 | 0.0176 |
| EF-5       | Yuping, Tongren, GZ | 0.0320 | 1.1378 | 0.0036 | 0.0250 |
| EF-6       | Dejiang, Tongren, GZ | 0.0463 | 0.0154 | 0.0246 | 0.1687 |
| EF-7       | Dejiang, Tongren, GZ | 0.0666 | 0.0557 | 0.0157 | 0.1328 |
| EF-8       | Dejiang, Tongren, GZ | 0.0228 | 0.0305 | 0.0320 | 0.0200 |
| EF-9       | Dejiang, Tongren, GZ | 0.0495 | 0.0158 | 0.0877 | 0.2483 |
| EF-10      | Fenggang, Zunyi, GZ | 0.2709 | 0.7663 | 0.0801 | 0.2352 |
| EF-11      | Liuzhi, Liupanshui, GZ | 0.1223 | 0.9351 | 0.0145 | 0.1431 |
| EF-12      | Daozhen, Zunyi, GZ | 0.1390 | 0.7612 | 0.0554 | 0.2054 |
| EF-13      | Guiyang Tongjitang pharmacy | 0.1645 | 1.1710 | 0.0077 | 0.0771 |
| EF-14      | Xingyi, GZ | 0.2058 | 0.1811 | 0.1137 | 0.6025 |
| EF-15      | Liuzhi, Liupanshui, GZ | 0.0903 | 1.1644 | 0.0316 | 0.1380 |
| EF-16      | Kaili, Guiyang, GZ | 0.1796 | 0.1786 | 0.0622 | 0.1955 |
| EF-17      | Shiquan, Ankang, SX | 0.1386 | 0.0667 | 0.0756 | 0.3807 |
| EF-18      | Pan’an, Jinghua, ZJc | 0.2943 | 1.5908 | 0.0167 | 0.0725 |
| EF-19      | Taizhou, ZJc | 0.2927 | 1.9340 | 0.0212 | 0.0818 |

**Notes:**
- Guizhou province, Shanxi province, Zhejiang province

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**Figure 3:** The cumulative variance of the four generated principal components. The first two components account for 94.98% of the total variance.

**Figure 4:** PCA scores plot of the 19 EF samples using the contents of the four analytes as input data.
that contents of dehydroevodiamine, evodiamine, and rutaecarpine contribute to positive positions of EF samples in PC1 in the scores plot. What it means is that samples with higher contents of the three analytes are placed to the right in the PCA scores plot. Meanwhile, higher content of limonin makes samples get lower PC1 scores, placing them on the left in the scores plot. The combined action of the contents ultimately decides the position of each sample in the scores plot.

EF-9 from Dejiang, Tongren, Guizhou, gets the second highest content of evodiamine at 0.0877%, the third highest content of rutaecarpine at 0.2483%, and the second lowest content of limonin at 0.0158%, making it be placed to the most right in the scores plot. In a similar way, EF-7, EF-8, and the samples from group B (EF-6, EF-14, and EF-17) have relatively higher contents of dehydroevodiamine, evodiamine, and rutaecarpine and relatively lower contents of limonin, resulting in their higher PC1 scores (>1.5). In contrast, EF-4 from Jiangkou, Tongren, Guizhou, has the lowest content of evodiamine at 0.0004% and the lowest content of rutaecarpine at 0.0176%. EF-2 from Shijian, Tongren, Guizhou, has the lowest content of dehydroevodiamine at just 0.0020%. EF-3 from Jiangkou, Tongren, Guizhou, contains the second lowest content of dehydroevodiamine. EF-5 from Yuping, Tongren, Guizhou, contains the second lowest content of evodiamine at 0.0036% and rutaecarpine at 0.0250% in all the samples. These content characteristics make them locate in the left of other samples. Other samples from group “A” also have lower contents of dehydroevodiamine, evodiamine, and rutaecarpine as well as higher content of limonin. Therefore, all the samples from group “A” get PC1 scores <0. It is interesting to mention that although EF-18 from Pan’an, Jinhua, Zhejiang, and EF-19 from Taizhou, Zhejiang, have the highest and the second highest contents of dehydroevodiamine at 0.2943% and 0.2927%, respectively, they also have the second highest and the highest contents of limonin at 1.5908% and 1.9340%, respectively, at the same time. The combined action of the contents ultimately makes the two samples lie in the left.

According to PC2 loadings plot, the contents of dehydroevodiamine and limonin mainly contribute to positive positions of EF samples in PC2, conversely, the contents of evodiamine and rutaecarpine contribute to negative positions of EF samples in PC2. However, due to nearer distances to zero line, contents of evodiamine and rutaecarpine play weaker roles in samples’ positions in PC2 compared with those of dehydroevodiamine and limonin. EF-8 has the third lowest content of dehydroevodiamine. EF-9 has the second highest content of evodiamine and the third highest content of rutaecarpine. They are both located at the bottom of scores plot and are both from Dejiang of Guizhou. It is worth noting that the contents of the four analytes of EF-16, regarded as the outlier in all the EF samples, are not the most or the least. The concentrations of the four markers are in the middle. That’s why it is positioned near the elliptic line. The sample is from the Guiyang city.

Through the PCA scores plot, the similarities of the analyzed EF samples can be obtained clearly and intuitively. Then, PCA loading plots tell us how the variables (contents of the four analytes in this study) contribute to the positions of the samples in the scores plot. The method can help us to assess on the qualities of EF samples from different sources quickly and visually.

**CONCLUSIONS**

EF is regarded as one of the geoauthentic herbs of Guizhou province and is one of the important herbs in many compound preparations. Evodiamine and rutaecarpine were required to be assayed in CP (edition 2010) for quality control of EF, whereas limonin was designated as another characteristic chemical marker in CP (edition 2010). As one of the chemical compounds with high yield from EF samples through the phytochemistry study in our lab, dehydroevodiamine is recommended as another chemical marker for quality assessment on this herb. Therefore, in this study, a sensitive, accurate, and precise UPLC-ESI-MS/MS method for assay of dehydroevodiamine, limonin, evodiamine, and rutaecarpine was established, with which 19 batches of EF samples from different regions were analyzed. Each analysis can be finished in 10 min. With
the population of MS instrument, the method can be accepted and applied widely for assessment on EF and EF-derived products.

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