Simultaneous Quantitation of 13 Active Components in SimiaoYong’an Decoction Using High-Performance Liquid Chromatography with Diode Array Detection

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SimiaoYong’an decoction, a traditional Chinese medicine formula consisting of four herbs, has been widely used for the treatment of gangrene disease. However, its clinical application is restricted due to the lack of an effective quality control method that covers the main active compounds in the formula. In this study, a high-performance liquid chromatography with diode-array detection (HPLC–DAD) method was established for the simultaneous determination of 13 active compounds including harpagide, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, ferulic acid, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C, angoroside C, harpagoside, cinnamic acid, glycyrhrizic acid, and ligustilide. Separation of these compounds was achieved using a Kromasil 100-5-C18 column with a gradient elution program consisting of acetonitrile and 0.4% phosphoric acid solution. The specificity, linearity, precision, repeatability, and accuracy tests were implemented to validate the method. The validated method was successfully applied for determination of 13 components from several finished batches of SimiaoYong’an decoction. The results demonstrated that the established method was accurate, reliable, and could be used as a suitable quality control method for the quantification of SimiaoYong’an decoction.

Keywords: Active components, quantitative analysis, SimiaoYong’an decoction, traditional Chinese medicine

1 Introduction

SimiaoYong’an decoction (SYD) is a famous Chinese herbal formula firstly recorded in Hua Tuo Shen Yi Mi Zhan in Han Dynasty and then listed in Yan Fang Xin Bian by Bao Xiang’ao in Qing Dynasty [1]. SYD is composed of Lonicerae japonicae flos (dried flower bud or opening flower of Lonicera japonica Thunb.), Scrophulariae radix (dried root of Scrophularia ningpoensis Hemsl.), Angelicae sinensis radix (dried root of Angelica sinensis [Oliv.] Diels.), and Glycyrrhiza uralensis (dried root of Angelica sinensis [Oliv.] Diels.), Glycyrrhizae radix et rhizoma (dried root and rhizome of Glycyrrhiza uralensis Fisch.). It has been used for approximately a thousand years to treat gangrene, clear heat, remove toxicity, and alleviate pain [2, 3]. Modern pharmacological studies have shown that SYD has broad clinical applications for treating thromboangiitis obliterans [4], atherosclerosis [5], diabetic foot [6], and gouty arthritis [7–9]. According to the compatibility theory of Chinese medicine of SYD, Lonicerae japonicae flos, Scrophulariae radix, and Glycyrrhizae radix et rhizoma, while the constituents from Angelicae sinensis radix were not taken into account [1]. According to the compatibility theory of Chinese medicine of SYD, Lonicerae japonicae flos, the monarch herb which plays the most critical role in the formula, was confirmed to contain organic acids, flavonoids, iridoid glycosides, and saponins; particularly organic acids such as chlorogenic acid, neochlorogenic acid, cryptochlorogenic acid, isochlorogenic acid A, isochlorogenic acid B, and isochlorogenic acid C make a great contribution to its anti-inflammatory, anti-bacterial, and antioxidant effects [11–14]. Similarly, Scrophulariae radix as the minister herb, which enhance the efficacy of the monarch herb, is mainly composed of iridoids (such as harpagide and harpagoside), phenylethanoid glycosides (such as angoroside C), and organic acids (such as cinnamic acid) [15–17]. On the other hand, the assistant herb, Angelicae sinensis radix, supported the monarch and minister herbs to reach target positions and exert their effects. It mainly consists of organic acids (such as ferulic acid) and volatile oils (such as ligustilide), which exert many pharmacological activities such as anti-thrombosis, anti-inflammatory, and antimicrobial [18–20]. Glycyrrhizae radix et rhizoma, used as supplementary herb to coordinate with other herbs, is mainly composed of flavonoids and saponins, and glycyrhrizic acid is the typical constituents with extensive pharmacological effects as well as high content in its water extract [21, 22]. Based on the above, thirteen compounds, which have certain pharmacological effects and relatively high contents for quantitative analysis, were selected for the quality

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control of SYD: neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, isochlorogenic acid B, isochlorogenic acid A and isochlorogenic acid C from Lonicerae japonicae flos, harpagide, angoroside C, harpagoside and cinnamic acid from Scrophulariae radix, ferulic acid and ligustilide from Angelicae sinensis radix, and glycyrrhizic acid from Glycyrrhizae radix et rhizoma. The chemical structures of these compounds are shown in Figure 1. In this study, a method of high-performance liquid chromatography with diode-array detection (HPLC–DAD) was established for simultaneous determination of multiple active compounds for the first time, which will contribute to the establishment of a comprehensive quality control method of SYD.

2 Experimental

2.1 Chemicals, Reagents, and Materials. The four crude herbs of several different batches, Lonicerae japonicae flos, Scrophulariae radix, Angelicae sinensis radix, and Glycyrrhizae radix et rhizoma, were purchased from Nanjing Haiyuan Chinese Medicine Tablet Co., Ltd. (Nanjing, China) and Sichuan Mianyang Pharmaceutical Co., Ltd., Taiji Group (Mianyang, China). Their botanical origins were identified by the corresponding author, and voucher specimens were deposited at the School of Pharmacy, Shanghai Jiao Tong University. The reference standards of neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, ferulic acid, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C, angoroside C, harpagoside, cinnamic acid, glycyrrhizic acid, and ligustilide (purity, >98%) were purchased from Sichuan Weikeqi Biological Co., Ltd. (Chengdu, China). Reference standard of harpagide (purity, 96.8%) was purchased from the National Institutes for Food and Drug Control (Beijing, China). Acetonitrile, methanol, and phosphoric acid of HPLC grade were obtained from ANPEL Laboratory Technologies Inc. (Shanghai, China). Ultrapure water was prepared by a Milli-Q system (Millipore, MA, USA).

Figure 1. Chemical structures of the thirteen marker constituents in SYD. The numbers (1)–(13) are as follows: (1) harpagide, (2) neochlorogenic acid, (3) chlorogenic acid, (4) cryptochlorogenic acid, (5) ferulic acid, (6) isochlorogenic acid B, (7) isochlorogenic acid A, (8) isochlorogenic acid C, (9) angoroside C, (10) harpagoside, (11) cinnamic acid, (12) glycyrrhizic acid, and (13) ligustilide
2.2 Chromatographic Conditions and Instrumentation. Analyses were performed on an Agilent 1200 HPLC system, equipped with a quaternary solvent delivery system, an on-line degasser, an autosampler, a column temperature controller, and a DAD detector. A Kromasil 100-5-C18 (250 mm × 4.6 mm, 5 μm) column was employed during the experiment with a flow rate of 1.0 mL/min. The column temperature was maintained at 38 °C, and the injection volume was 10 μL. The mobile phase was composed of water (with 0.4% phosphoric acid) (A) and acetonitrile (B) with the following gradient elution: 0–3 min, 95% A; 3–18 min, 95%–89% A; 18–45 min, 89%–80% A; 45–55 min, 80%–67% A; 55–60 min, 67%–50% A; 60–70 min, 50%–0% A, and reequilibration for 5 min. The wavelength was set at 210 nm for harpagide; 254 nm for glycyrrhizic acid; 278 nm for chlorogenic acid, harpagoside, and cinnamic acid; and 327 nm for neochlorogenic acid, cryptochlorogenic acid, ferulic acid, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C, angoroside C, and ligustilide.

2.3 Preparation of Sample Solutions. According to the original composition of SYD, the four herbs including Lonicerae japonicae flos (11.25 g), Scrophulariae radix (11.25 g), Angelicae sinensis radix (7.5 g), and Glycyrrhizae radix et rhizoma (3.75 g) were weighed accurately, mixed, and then immersed in 10 fold volume of distilled water for 30 min. Subsequently, the mixture was decoced for 40 min in a casserole. The extracted solution was centrifuged at 4000 rpm for 15 min, and the supernatant was concentrated under reduced pressure to a final volume of 100 mL.

For HPLC analysis, 1 mL methanol was added to equal volume of SYD solution in a centrifuge tube. After being vortexed for 3 min, the mixture was centrifuged at 12,000 rpm for 10 min. The supernatant was then transferred into a 2 mL dark brown volumetric flask and bring to volume by 50% methanol solution. The prepared solution was filtered through a 0.22 μm membrane filter before injection into the HPLC–DAD system for analysis.

2.4 Preparation of Standard Solutions. Stock standard solution of the 13 reference compounds was prepared by dissolving them with 50% methanol in a 10 mL dark brown volumetric flask and then diluted to a series of proper concentrations of mixed standard solutions. The concentrations of the 13 reference compounds were the following: harpagide: 438.50, 350.80, 175.40, 87.70, 43.85, 21.93, and 10.96 μg/mL; neochlorogenic acid: 153.62, 122.90, 61.45, 30.72, 15.36, 7.68, and 3.84 μg/mL; chlorogenic acid: 1416.23, 1132.99, 566.49, 283.25, 141.62, 70.81, and 35.41 μg/mL; cryptochlorogenic acid: 254.64, 203.71, 101.85, 50.93, 25.46, 12.73, and 6.37 μg/mL; ferulic acid: 110.90, 88.72, 44.36, 22.18, 11.09, 5.55, and 2.77 μg/mL; isochlorogenic acid B: 219.71, 175.77, 87.89, 43.94, 21.97, 10.99, and 5.49 μg/mL; isochlorogenic acid A: 461.26, 369.01, 184.50, 92.25, 46.13, 23.06, and 11.53 μg/mL; isochlorogenic acid C: 324.02, 259.22, 129.61, 64.80, 32.40, 16.20, and 8.10 μg/mL; angoroside C: 154.98, 123.99, 61.99, 31.00, 15.50, 7.75, and 3.87 μg/mL; harpagoside: 120.74, 96.59, 48.30, 24.15, 12.07, 6.04, and 3.02 μg/mL; cinnamic acid: 167.58, 134.06, 67.03, 33.52, 16.76, 8.38, and 4.19 μg/mL; glycyrrhizic acid: 396.22, 316.98, 158.49, 79.24, 39.62, 19.81, and 9.91 μg/mL; and ligustilide: 134.94, 107.95, 53.98, 26.99, 13.49, 6.75, and 3.37 μg/mL. All the solutions were stored at 4 °C and filtered through a 0.22 μm membrane filter before injection.

2.5 Preparation of Negative Control Samples of SYD. The three negative control samples of SYD were prepared by removal of one herb from the prescriptions, respectively. The rest of herbs were accurately weighed according to the prescription of SYD and prepared by the same procedure described in Section 2.3.

2.6 Method Validation. The analytical method was validated for specificity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, repeatability, stability, and accuracy in accordance with Chinese Pharmacopoeia (ChP) 2015 [23]. The specificity of the method was evaluated by comparing the chromatograms of standard solution, sample solution, and negative control samples. The standard curves for the linearity assay were based on plotting the peak areas versus the corresponding concentrations of each analyte. Linearity test solutions for the assay method were prepared from stock solution, as detailed in Section 2.4. To determine the LODs and LOQs, the lowest diluted solutions for calibration use were further diluted to a series of concentrations. They were then analyzed until the signal-to-noise (S/N) ratio for each analyte was about 3 for LOD and 10 for LOQ. The intra-day and inter-day precision were assessed by analyzing six replicates of
3 Results and Discussion

3.1 Optimization of Chromatographic Conditions.

The types of chromatographic column, mobile phase compositions, gradient elution program, column temperature, and detection wavelength were all tested to optimize the separation conditions for HPLC analysis. Different columns including Kromasil 100-5-C$_{18}$ (250 mm × 4.6 mm, 5 μm), Agilent XDB-C$_{18}$ (250 mm × 4.6 mm, 5 μm), and Shimadzu shim-pack C$_{18}$ (250 mm × 4.6 mm, 5 μm) were tested, and the results showed that Kromasil 100-5-C$_{18}$ column was the most suitable one, since it gave better peak shapes and a good separation of selected analytes. The mobile phases with different acids (formic acid and phosphoric acid) and acidity of this method.

Furthermore, different gradient elution programs were also optimized. We tried to shorten the analysis time and simplify the gradient elution systems, but peaks for isochlorogenic acid C and angoroside C, as well as harpagoside and cinnamic acid, have not been completely separated except for the current gradient elution program mentioned above. The ultraviolet (UV) wavelength of the DAD for the quantification of each component was selected at 210 nm (harpagide), 254 nm (glycyrrhizic acid), 278 nm (chlorogenic acid, harpagoside, and cinnamic acid), and 327 nm (neochlorogenic acid, cryptochlorogenic acid, ferulic acid, isochlorogenic acid C, isochlorogenic acid A, isochlorogenic acid B, chlorogenic acid, and cinnamic acid, glycyrrhizic acid, and ligustilide) based on retention time and UV spectra compared with those of the reference standards, and each of the 13 compounds had the higher UV absorption and could be determined without interference from other components at the selected UV wavelength.

3.2 Method Validation.

The analytical method was validated for specificity, linearity, LOD, LOQ, precision, repeatability, stability, and accuracy.

3.2.1 Specificity.

Representative chromatograms of the mixed standard solution, SYD sample, and negative control samples are shown in Figure 2. The chromatographic peaks of the 13 compounds were identified by comparing the HPLC retention time and UV spectra with those of reference compounds. The retention times of the harpagide, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, ferulic acid, isochlorogenic acid B, isochlorogenic acid A, isochlorogenic acid C, angoroside C, harpagoside, cinnamic acid, glycyrrhizic acid, and ligustilide were observed at 10.50, 14.28, 20.52, 22.38, 37.06, 45.70, 47.07, 51.08, 52.20, 58.17, 59.17, 62.63, and 68.96 min, respectively. In addition, no interfering peak in the negative control samples was observed under the established chromatographic conditions, which further confirmed the specificity of this method.

3.2.2 Linearity, LOD, and LOQ.

The linear regression equation, correlation coefficient ($R^2$), linear range, LODs, and LOQs are presented in Table 1. Calibration curves of the 13 analytes showed good linearity, with $R^2$ values of $\geq$0.9998 in the seven tested concentration ranges. The LODs and LOQs of the 13 analytes were 0.28–2.74 and 0.92–9.91 μg/mL, respectively.

3.2.3 Precision, Repeatability, and Stability.

The results of the intra-day and inter-day precision, repeatability, and stability are summarized in Table 2. RSD values were used to evaluate the variations. The results showed that the values of intra- and inter-day precision were in the range of 0.22%–0.43% and stability were in the range of 0.76%–3.87%.

### Table 1. Regression equation, correlation coefficient ($R^2$), linear range, LODs, and LOQs of the 13 compounds in SYD

| Compounds          | Regression equation | $R^2$ | Linear range (μg/mL) | LOD (μg/mL) | LOQ (μg/mL) |
|--------------------|---------------------|-------|----------------------|-------------|-------------|
| Harpagide          | $y = 3.695x + 7.0046$ | 0.9998 | 10.96–438.50         | 2.74        | 6.85        |
| Neochlorogenic acid| $y = 37.477x + 17.061$ | 0.9999 | 3.84–153.62          | 0.96        |             |
| Chlorogenic acid   | $y = 13.935x + 84.033$ | 0.9999 | 35.41–141.36         | 0.89        | 2.53        |
| Cryptochlorogenic acid | $y = 34.690x + 26.642$ | 0.9999 | 6.37–254.64          | 0.35        | 1.06        |
| Ferulic acid       | $y = 67.728x − 2.1599$ | 0.9999 | 2.77–110.90          | 0.28        | 0.92        |
| Isochlorogenic acid B | $y = 35.64x + 4.1952$ | 0.9999 | 5.49–219.71          | 0.39        | 0.92        |
| Isochlorogenic acid A | $y = 39.729x + 21.961$ | 0.9999 | 11.53–461.26         | 0.47        | 1.92        |
| Isochlorogenic acid C | $y = 39.754x + 13.611$ | 0.9999 | 8.10–324.02          | 0.68        | 2.03        |
| Angoroside C       | $y = 15.82x − 16.328$ | 1     | 3.87–154.98          | 0.97        | 3.87        |
| Harpagoside        | $y = 30.962x + 13.778$ | 0.9998 | 3.02–120.74          | 0.75        | 3.02        |
| Cinnamic acid      | $y = 100.57x + 82.294$ | 0.9998 | 4.19–167.58          | 0.42        | 1.40        |
| Glycyrrhizic acid  | $y = 6.1048x + 9.0114$ | 0.9999 | 2.91–396.22          | 2.45        | 8.91        |
| Ligustilide        | $y = 14.324x + 7.6034$ | 0.9999 | 3.37–134.94          | 0.56        | 1.69        |
The established method laid a good foundation for the quality control of SYD, while an effective and systematic quality control method of SYD solutions were then determined by simultaneously applying the validated method above. The results are summarized in Table 4. Among the 13 components, chlorogenic acid was the most abundant compound (7.20–10.90 mg/g) followed by glycyrrhizic acid (3.23–7.62 mg/g), and ligustilide had the lowest amount (0.06–0.17 mg/g). Not only that, the average contents of 13 marker compounds were different in triplicates of 6 batches of SYDs. The contents of cinnamic acid showed the biggest differences (0.17 ± 0.08 mg/g), followed by harpagoside (0.42 ± 0.19 mg/g). We consider that it was probably due to the different origin of these crude herbs. It was reported that the contents of main constituents in Lonicerae japonicae flos, Scrophulariae radix, Angelicae sinensis radix, and Glycyrrhizae radix et rhizoma from different production areas differed greatly [24–27]. In order to ensure the efficiency of SYD, the crude drugs composed of SYD should be chosen from suitable production areas. In addition, the extraction temperature or time in the decocting process might also influence the content of each component in SYD. Further studies will be carried out to investigate the quality of each herb in SYD formula from different cultivation areas, and the decocting conditions of SYD formula, as well as the correlation between chromatographic fingerprints of chemical constituents and quality, so as to establish a comprehensive quality control system of SYD.

4 Conclusion

In the present study, an HPLC–DAD method was established and successfully applied for the simultaneous quantification of 13 major components in SYD for the first time to our knowledge. Thirteen marker compounds which have certain pharmacological activities were derived from the four individual herbs in SYD and could fully reflect the quality of the prescription. Validation of the method showed good linearity, repeatability, intra- and inter-day precision, and accuracy. The established method laid a good foundation for the quality control of SYD, while an effective and systematic quality control method for SYD still has a long way to go and requires further investigations.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Table 3. Recovery of 13 compounds in SYD (n = 3)

| Compounds          | Original (μg/mL) | Spiked (μg/mL) | Detected (μg/mL) | Recovery (%) | RSD (%) |
|--------------------|-----------------|---------------|-----------------|--------------|---------|
| Harpagoside        | 73.08           | 35.39         | 107.35          | 102.61       | 0.81    |
| Neochlorogenic acid| 15.66           | 8.97          | 24.89           | 102.90       | 1.02    |
| Chlorogenic acid   | 288.25          | 144.79        | 439.83          | 104.69       | 0.65    |
| Cryptochlorogenic acid | 31.86     | 14.37         | 46.75           | 103.65       | 0.87    |
| Isochlorogenic acid A | 57.65      | 31.60         | 89.09           | 99.49        | 1.55    |
| Isochlorogenic acid B | 220           | 13.54         | 35.47           | 104.39       | 0.81    |
| Ferulic acid       | 4.63            | 2.25          | 6.92            | 101.36       | 0.40    |
| Isocorylchogenic acid B | 23.65     | 15.14         | 39.11           | 102.14       | 2.46    |
| Cinnamic acid      | 7.35            | 4.53          | 8.92            | 99.43        | 0.29    |
| Isochlorogenic acid A | 31.86      | 17.94         | 33.42           | 98.98        | 0.52    |
| Isochlorogenic acid B | 45.31       | 22.49         | 48.29           | 102.14       | 1.31    |
| Angoside C         | 60.67           | 31.54         | 54.73           | 98.57        | 0.57    |
| Harpagoside        | 7.35            | 4.53          | 8.92            | 99.43        | 0.29    |
| Cinnamic acid      | 2.44            | 2.47          | 4.95            | 99.43        | 0.76    |
| Glycyrrhizic acid  | 60.67           | 31.52         | 53.06           | 102.76       | 0.87    |
| Ligustilide        | 2.76            | 2.48          | 5.13            | 97.47        | 0.05    |

Table 4. Contents of 13 components in six batches of SYD

| Compounds          | Contents (mg/g) |
|--------------------|-----------------|
|                   | Batch 1 | Batch 2 | Batch 3 | Batch 4 | Batch 5 | Batch 6 | Mean ± SD |
| Harpagoside        | 2.50    | 1.26    | 1.57    | 1.43    | 1.62    | 2.43    | 1.80 ± 0.53 |
| Neochlorogenic acid| 0.43    | 0.58    | 0.53    | 0.49    | 0.46    | 0.55    | 0.51 ± 0.06 |
| Chlorogenic acid   | 8.46    | 7.40    | 8.27    | 10.90   | 7.20    | 1.10    | 8.72 ± 1.48 |
| Cryptochlorogenic acid | 0.93  | 1.07    | 0.62    | 1.15    | 0.91    | 1.13    | 0.97 ± 0.20 |
| Ferulic acid       | 0.36    | 0.21    | 0.25    | 0.44    | 0.24    | 0.24    | 0.29 ± 0.09 |
| Isocorylchogenic acid B | 0.67  | 0.83    | 0.71    | 0.75    | 0.69    | 0.82    | 0.75 ± 0.07 |
| Isocorylchogenic acid A | 1.57  | 1.38    | 1.29    | 1.82    | 1.33    | 1.99    | 1.56 ± 0.26 |
| Isocorylchogenic acid C | 1.26  | 1.45    | 1.33    | 1.53    | 1.25    | 1.56    | 1.40 ± 0.14 |
| Angoside C         | 0.66    | 0.40    | 0.33    | 0.67    | 0.48    | 0.76    | 0.55 ± 0.17 |
| Harpagoside        | 0.76    | 0.33    | 0.28    | 0.43    | 0.48    | 0.26    | 0.42 ± 0.19 |
| Cinnamic acid      | 0.24    | 0.14    | 0.10    | 0.29    | 0.17    | 0.09    | 0.17 ± 0.08 |
| Glycyrrhizic acid  | 7.62    | 3.46    | 3.23    | 4.31    | 3.31    | 6.41    | 4.92 ± 1.73 |
| Ligustilide        | 0.17    | 0.06    | 0.07    | 0.09    | 0.10    | 0.14    | 0.10 ± 0.04 |
References

1. Liu, Y.; Chi, S.; Wang, W.; Su, L.; Liu, B. Molecules 2017, 22, 1–16.
2. Liu, Y. F.; Tu, S. H.; Chen, Z.; Wang, Y.; Hu, Y. H.; Dong, H. Evid. Based Complement. Alternat. Med. 2014, 2014, 1–7.
3. Wang, Y. N.; Liu, H.; Zhang, J. S.; Ma, W. G.; Lu, Y.; Meng, F. X. Integ. Med. Res. 2015, 4, 57–58.
4. Li, N.; Qu, X.; Lin, S.; Li, J.; Li, H.; Lin, H.; Lv, G.; Tian, W.; Li, G.; Lin, Z. J. Jilin Univ. 2013, 39, 264–267.
5. Xu, Y.; Zhang, J.; Li, M.; Li, L.; Peng, L.; Zhang, G.; Yang, C.; Zhou, Y. Chin. J. Basic Med. Tradit. Chin. Med. 2012, 18, 161–163.
6. Zhao, Y.; Liu, H.; Zhang, Y.; Ma, W. G.; Lu, Y.; Meng, F. X.; Wang, Y. N. Integr. Med. Res. 2015, 4, 57–58.
7. Li, N.; Qu, X.; Lin, S.; Li, J.; Wu, B.; Liu, Q.; Tian, W.; Li, G.; Lin, Z. J. Jilin Univ. 2013, 39, 264–267.
8. Guo, S.; Cui, X.; Jiang, M.; Bai, L.; Tian, X.; Guo, T.; Liu, Q.; Zhang, L.; Ho, C.; Bai, N. J. Food Drug Anal. 2017, 25, 417–424.
9. Liu, Q.; Qin, K.; Shen, B.; Cai, H.; Cai, B. Acta Chromatogr. 2015, 27, 697–709.
10. Chen, Y. H.; Qi, J.; Hu, J.; Wu, Y.; Ye, M. J. Nat. Med. 2014, 12, 47–54.
11. Jing, J.; Chen, C. O.; Xu, L.; Jin, D.; Cao, X.; Mok, D. K.; Parekh, H. S.; Chen, S. J. Pharm. Biomed. Anal. 2011, 56, 830–835.
12. Ji, J.; Huang, X. Y.; Du, X. J.; Sun, W. J.; Zhang, Y. M. Nat. Prod. Res. 2009, 23, 775–780.
13. Wei, W. L.; Zeng, R.; Gu, C. M.; Qu, Y.; Huang, L. F. J. Ethnopharmacol. 2016, 190, 116–141.
14. Li, J.; Hua, Y.; Ji, P.; Yao, W.; Zhao, H.; Zhong, L.; Wei, Y. Pharm. Biol. 2016, 34, 1881–1890.
15. Deng, S.; Chen, S. N.; Yao, P.; Nikolic, D.; van Breemen, R. B.; Bolton, J. L.; Fong, H. H.; Farnsworth, N. R.; Pauli, G. F. J. Nat. Prod. 2006, 69, 536–541.
16. Zhang, Q.; Ye, M. J. Chromatogr. A 2009, 1216, 1954–1969.
17. Long, J.; Liang, B.; Li, S.; Chen, Z. J. Sep. Sci. 2017, 40, 4847–4856.
18. China Pharmacopoeia Committee. Pharmacopoeia of the People’s Republic of China (IV), China, Medical Science Press, Beijing 2015, pp. 374–377.
19. Qian, Z. M.; Li, H. J.; Li, P.; Ren, M. T.; Tang, D. Chem. Pharm. Bull. 2007, 55, 1073–1076.
20. Zhang, Y.; Cao, G.; Ji, J.; Cong, X.; Wang, S.; Cai, B. J. Sep. Sci. 2011, 34, 1429–1436.
21. Peng, Z.; Bi, Z. M.; Li, P.; Qi, L. W.; Yi, L.; Tang, D. Chromatographia 2008, 67, 973–978.
22. Wei, S. S.; Yang, M.; Chen, X.; Wang, Q. R.; Cui, Y. J. Chin. J. Nat. Med. 2015, 13, 232–240.