

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/51464916>

Development of a novel method combining HPLC fingerprint and multi-ingredients quantitative analysis for quality evaluation of traditional Chinese medicine preparation

Article in *Talanta* · August 2011

DOI: 10.1016/j.talanta.2011.04.059 · Source: PubMed

CITATIONS

47

READS

115

7 authors, including:



Dao-quan Tang

Xuzhou Medical College

41 PUBLICATIONS 285 CITATIONS

SEE PROFILE

Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Development of a novel method combining HPLC fingerprint and multi-ingredients quantitative analysis for quality evaluation of traditional chinese medicine preparation

Dong-Zhi Yang^{a,b}, Yi-Qiang An^b, Xiang-Lan Jiang^a, Dao-Quan Tang^{a,b,*}, Yuan-Yuan Gao^a, Hong-Tao Zhao^c, Xiao-Wen Wu^c

^a Department of Pharmaceutical Analysis, Xuzhou Medical College, Xuzhou Jiangsu 221004, China

^b Key Laboratory of New Drug and Clinical Application, Xuzhou Medical College, Xuzhou Jiangsu 221004, China

^c Student of School of Pharmacy, Xuzhou Medical College, Xuzhou Jiangsu 221004, China

ARTICLE INFO

Article history:

Received 25 January 2011

Received in revised form 12 April 2011

Accepted 21 April 2011

Available online 19 May 2011

Keywords:

Fingerprint analysis

Quality control

Multi-ingredient quantitative analysis

Shuang-huang-lian (SHL) oral liquid

High-performance liquid chromatography (HPLC)

ABSTRACT

A novel method combining high performance liquid chromatography (HPLC) fingerprint and simultaneous quantitative analysis of multiple active components was developed and validated for quality evaluation of one type of traditional Chinese medicine preparations: Shuang-huang-lian (SHL) oral liquid formulation. For fingerprint analysis, 45 peaks were selected as the common peaks to evaluate the similarities among several different SHL oral liquid preparations collected from manufacturers. Additionally, simultaneous quantification of eleven markers, including chlorogenic acid, caffeic acid, rutin, forsythiaside, scutellarin, baicalin, forsythin, luteoloside, apigenin, baicalein and wogonin, was performed. Statistical analysis of the obtained data demonstrated that our method has achieved desired linearity, precision and accuracy. Finally, concentrations of these eleven markers in SHL oral liquid prepared by different manufacturers in China were determined. These results demonstrated that the combination of HPLC chromatographic fingerprint and simultaneous quantification of multi-ingredients offers an efficient and reliable approach for quality evaluation of SHL oral liquid preparations.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Shuang-huang-lian (SHL) is a traditional Chinese formula, the preparation of which includes three medicinal herbs: *Flos Lonicerae*, *Radix Scutellariae* and *Fructus Forsythiae*. SHL preparations, in various formulations, are commonly used to treat acute upper respiratory tract infection, acute bronchitis and pneumonia [1–3]. Particularly, SHL oral liquid has been widely applied as effective clinical therapeutics [4]. Previous studies on the three medicinal herbs have revealed the presence of phenylpropanoids and flavonoids. These components have been hypothesized to be the main bioactive ingredients of SHL preparations [5]. Due to various factors, such as different cultivation areas and climatic conditions, the level of these components may vary significantly in different SHL oral liquid preparations. Thus, a global and systematic quality standard for quality assessment is imperative. In fact, studies on SHL preparations have been reported previously [6], and a method

has been documented in the Ch. P to evaluate the quality of the SHL oral liquid [7]. However, the quality control of traditional Chinese medicines (TCM) usually only focuses on a single component or a limited number of components [8]. Unlike synthetic drugs, it is well known that medicinal herbs and their preparations generally exert their therapeutic effects through the synergic effects of the multiple active ingredients and the multi-targets they are targeting [9]. We believe that a more effective and reliable quality control method for SHL oral liquid should include the assay of a set of major active components rather than a single species, or only a handful of the active species. Our study was initiated to achieve this goal. In addition, we propose that an ideal chemical quality control method of herbal medicine should consist of two aspects: one is the qualitative and quantitative analysis of several major components and the other is the analysis of chemical fingerprint [10,11]. Chemical fingerprint has been introduced and adopted by the World Health Organization (WHO) [12], State Food and Drug Administration (SFDA) of China (2000) [13] and other authorities as a strategy for quality assessment of herbal medicines.

Compared with conventional analytical approaches, fingerprint technique emphasizes on the integral characterization of a complex system with a quantitative degree of reliability. Using this method, a particular herbal preparation with complex constituents can be

* Corresponding author at: Department of Pharmaceutical Analysis, Xuzhou Medical College, Xuzhou Jiangsu 221004, China. Tel.: +86 516 83262136; fax: +86 516 83262136.

E-mail address: tdq993@hotmail.com (D.-Q. Tang).

identified and distinguished from other closely related species [14–16]. As a novel approach to identify and control the quality of herbal samples, chromatographic fingerprint technique has been regarded as one of the most rational and powerful approaches for the quality evaluation of herbal preparations [17,18]. Recently, chromatographic fingerprint, especially high-performance liquid chromatography (HPLC) fingerprint [19], has been widely accepted and is attracting ever-increasing attention owing to both the high separation efficiency and high detection sensitivity of the technique. Chromatographic fingerprint can give an overall view of all components in TCM and demonstrates both the 'sameness' and 'differences' among various samples successfully. However, one drawback is that it can only show results of similarity calculated based on the relative value using pre-selected marker compound as a reference standard, and minor differences between very similar chromatograms might not be distinguishable [20]. Thus, the chemical pattern recognition methods such as multi-ingredients quantitative analysis should be taken into consideration for reasonable definition of the class of herbal medicine.

Up to now, several methods have been developed for SHL preparations. Cao et al.'s work [21] focused on the correlation between fingerprint of the preparations and that of the raw herbs. Yan et al.'s [22] work studied qualitative and quantitative analysis of SHL fenzhenji by FTIR. However, both studies give quantitative information of only two constituents (baicalin and chlorogenic acid), which could not represent the ideal comprehensive evaluation of the quality of herbal medicine. Obviously, it is desirable to identify more components in TCM to serve as markers and set up a method by combining chromatographic fingerprint and quantification of multi-ingredients to evaluate the quality of SHL oral liquid formulation effectively.

As mentioned above, the major bioactive components of SHL oral liquid preparations are phenylpropanoids and flavonoids. Hence, eleven compounds: Chlorogenic acid, caffeic acid, rutin, forsythin, forsythiaside and flavonoids including scutellarin, baicalin, luteoloside, apigenin, baicalein and wogonin, were selected for analysis and evaluation of SHL oral liquid. To our knowledge, no method is available for the simultaneous quantification of these eleven components by HPLC. We have strategically established chromatographic fingerprinting profile and simultaneous determination of eleven compounds for the assessment of the quality of SHL oral liquid by HPLC coupled with diode array detection (DAD). In addition, the contents of eleven components in four samples from different manufacturers was determined to establish the effectiveness of the method.

2. Material and methods

2.1. Chemicals and reagents

Chlorogenic acid, caffeic acid, rutin, forsythiaside, scutellarin, baicalin, forsythin, luteoloside, apigenin, baicalein, and wogonin ($\geq 98.0\%$) (see Fig. 1) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (NICPP, Beijing, China). HPLC grade-methanol was obtained from Fisher Scientific (Fisher Scientific, USA). Purified water was used from a Milli-Q system (Millipore, Bedford, MA, USA). Commercial product SHL-A (lot no. 09110451, 09111021, 09121417, 09122431, 09123117, 10012371, 10012412, 10020473, 10020724, 10032454), SHL-B (lot no. 100510), SHL-C (lot no. 09122124) and SHL-D (lot no. 20100201) were purchased from Sanjing Pharmaceutical Co., Ltd. (Harbin, China), Xinsheng Pharmaceutical Co., Ltd. (Nanyang, China), Tailong Pharmaceutical Co., Ltd. (Zhengzhou, China), and Changzheng-xinkai Co., Ltd. (Suzhou, China), respectively. Other reagents were all of analytical grade. SHL-A (lot no. 09110451) was selected as the sample for chromatographic conditions and sub-

sequent method validation. All aqueous solutions were made up in deionized water. The reference standards stock solutions of the eleven compounds were prepared in methanol and stored in brown vials at 4 °C. All solutions were diluted to the desired concentration with methanol–water (50:50, v/v) prior to use.

2.2. Chromatographic system

Instrumentation for analysis was a Shimadzu 20A separation module equipped with a DGU-20As degasser, a SIL-20A autosampler, a CTO-20AC column oven and a SPD-M20A UV–vis diode array detector (Shimadzu, Japan). System control and data analysis were carried out using Lcsolution software (Shimadzu, Japan). Different columns such as Agilent Zorbax SB-C₁₈ (Akzo Nobel, Sweden), Kromasil C₁₈ (Agilent, U.S.), Sepax GP-C₁₈ (Sepax, U.S.) and Grace BDS C₁₈ (Grace, U.S.) were used for chromatography system. The mobile phase was composed of acetonitrile and 0.1% formic acid in gradient elution mode. The flow rate of the mobile phase was kept at 1.0 ml/min and the volume ratio of acetonitrile was changed as follows: 0–7 min, 7%; 7–10 min, 7–10%; 10–15 min, 10–14%; 15–20 min, 14–15%; 20–35 min, 15–16%; 35–40 min, 16–20%; 40–58 min, 20%; 58–75 min, 20–28%; 75–90 min, 28–37%; 90–105 min, 37%. The effluent from the column was detected by a diode array detector and the detection wavelength was set at 278 nm. The temperature of the column was kept at 35 °C and the sample injection volume was 10 μ l.

2.3. Sample pretreatment

For the LC procedure an aliquot of 2.00 ml SHL oral liquid was used. This aliquot was diluted in methanol–water (50:50, v/v) in a volumetric flask of 10 ml and then pretreated in an ultrasonic water bath for 30 min. The supernatant was filtered through a 0.45 μ m nylon filter membrane and 10 μ l of which was injected into HPLC system.

2.4. Data analysis

Date analysis for chromatographic fingerprint was performed by use of the professional software 'Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine' (Version 2004A), which is recommended by SFDA. Using this software, the correlative coefficient for samples were calculated and the similarities of different chromatograms were compared with the mean chromatogram among the samples tested.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

Columns types, mobile phase compositions, gradient elution procedure, flow rate of the mobile phase, and column temperature were optimized respectively to achieve good separation of as many peaks as possible within a short analysis time. Different types of column including Agilent Zorbax SB-C₁₈ (5 μ m, 250 mm \times 4.6 mm), Kromasil C₁₈ (5 μ m, 250 mm \times 4.6 mm), Sepax GP-C₁₈ (5 μ m, 250 mm \times 4.6 mm) and Grace BDS C₁₈ (5 μ m, 250 mm \times 4.6 mm) were evaluated. All four types of column exhibited similar chromatography behavior (see Fig. 2) and the backpressure were all in the range of 10.5–12.4 MPa. Agilent Zorbax SB-C₁₈ was selected because it exhibited relatively shorter retention time than other columns. On the basis of several trials using different mobile phase, including methanol–water, acetonitrile–water, methanol–0.1% phosphoric acid, acetonitrile–0.1% phosphoric acid, acetonitrile–0.1% formic acid and acetonitrile–0.2% formic acid, acetonitrile–0.1% formic was selected as the most appropriate

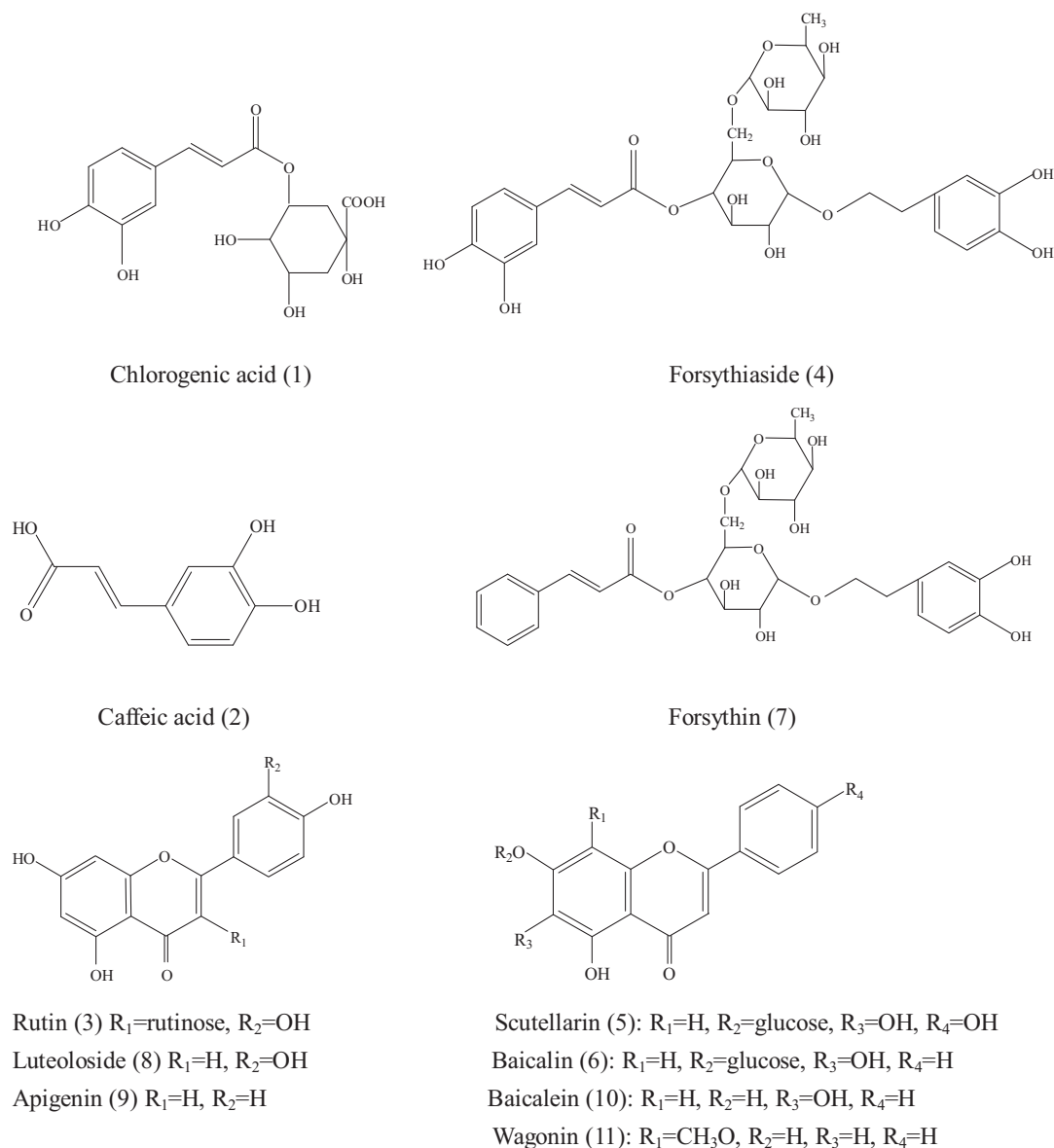


Fig. 1. Molecular structures of the eleven compounds.

eluent. Under the optimized linear gradient mode, a sufficiently large number of peaks on the chromatogram were achieved within 105 min. The effects of temperature and flow rate were investigated, and 35 °C and 1 ml/min were found to be optimal parameters.

The UV spectra of the compounds were obtained at 220, 254, 278, 320, and 360 nm by diode array detector under the chromatography conditions as described in chromatography system. Results indicated that more peaks were detected at 220 and 254 nm than other detection wavelengths, however, more interference was also observed at these two wavelengths. Although signal response for flavonoids are higher at 320 and 360 nm, some ester glycosides, such as forsythiaside, could only be detected at about 278 nm. In order to detect more common peaks while achieving precise detection of eleven components, the most appropriate wavelength was set as 278 nm.

3.2. Chromatographic fingerprint analysis

The process of standardization included the selection of 'common peaks' in chromatograms and the normalization of retention times of all common peaks. Furthermore, the total area of the

common peaks must be more than 90% of the whole area in one chromatogram. Using the proposed method, HPLC-DAD chromatograms of different SHL oral liquid samples from the same manufacturer were acquired. The average chromatogram from the 10 batches was regarded as the standardized characteristic fingerprint. Among these components, baicalin represents as a high-level and stable content, therefore it was chosen as the reference peak. As shown in Fig. 3, there were 45 common peaks shown in all samples. All common peaks' relative retention time (RRA) and relative peak area (RPA) were obtained with reference to this substance. Their relative standard deviation (RSD) values of the RRA were less than 1.1%, which demonstrated good stability and reproducibility of the fingerprint analysis by HPLC. Their similarity indexes calculated based on similarity system theory of ten samples were higher than 0.997, which meant that the common peaks were in good correlation. Meanwhile, chromatographic fingerprints of four samples of SHL oral liquid from different manufacturers were also performed. They shared the similar chromatographic patterns with the similarity indexes higher than 0.959. However, the RSD values of RPA from above 10 batches samples collected from the same manufactures were very high (12.6–72.7%). Variation of the RSD might be due

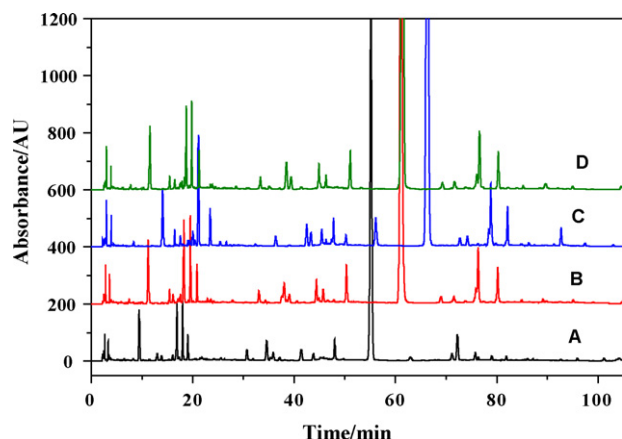


Fig. 2. The chromatogram of SHL oral liquid separated on different column. (A): Agilent Zorbax SB-C₁₈; (B): Kromasil C₁₈; (C): Grace BDS C₁₈; (D): Sepax GP-C₁₈.

to a number of factors, such as different origin, production process, storage conditions, alternative environment and another collection time, etc. To obtain a more comprehensive evaluation for SHL oral liquid, the quantification of multi-ingredients had to be combined with chromatographic fingerprint.

As we have already known, flavonoids and phenylpropanoids were the main and effective components in SHL oral liquid. So, phenylpropanoids, including chlorogenic acid, caffeic acid, forsythiaside and forsythin, and flavonoids such as rutin, scutellarin, baicalin, luteoloside, apigenin, baicalein and wogonin were analyzed. By comparing with standards, we showed that the eleven compounds could be well separated under the previously specified conditions.

3.3. Quantitative analysis

The HPLC method was validated by defining the linearity, limits of quantification and detection, identification and quantification of the analytes, repeatability, precision, stability and recovery.

3.3.1. Linearity, limits of quantification and detection

Series of standard solutions comprising of eleven compounds were freshly prepared in methanol–water (50:50, v/v) and were used to determine linear range of the analytes. The results of cal-

ibration were summarized in Table 1 and good correlations were found between the peak area (y) and concentration of tested compounds (x) ($r > 0.9995$) within test ranges. The limit of detection (LOD) and the lower limit of quantification (LLOQ) values of individual compounds listed in Table 1 clearly indicated that the analytical method was acceptable with excellent sensitivity.

3.3.2. Repeatability, precision and stability

Repeatability of this method was obtained by analyzing six different samples using the same preparation procedure. RSD values of component content and retention time of eleven compounds were all less than 2.0%, which satisfied the criteria of quantitative analysis.

Intra-day and inter-day variability was used to evaluate precision. Six sample solutions respectively prepared as described above and mixed standard solutions of eleven compounds at low, medium and high concentrations on 1 day ($n=6$) and on five consecutive days, were analyzed, respectively. Three groups of concentrations were chosen based on the linear range. The low concentrations were 2 times LLOQ, medium and high concentrations were the half and 90% of the upper limit of quantification (ULOQ), respectively. The results indicated that the mean intra-day RSD values of the retention time and peak area were less than 0.2% and 0.9%, and the calculated mean inter-day RSD values of the retention time and peak area were less than 0.3% and 1.1%, respectively.

For the stability test, retention time and peak area of twelve compounds in sample solution were analyzed in 0, 2, 4, 8, 16, 32 and 48 h. RSD values of the retention time and peak area of eleven compounds were less than 0.3% and 2.0%, respectively. These results suggested that it was feasible to analyze samples within 2 days.

3.3.3. Accuracy

The accuracy of the method was validated by measuring recovery through standard addition method. A known amount (low, medium and high) of the eleven standard references were spiked into samples, and then extracted according to the section of 'sample pretreatment'. The extracted solution was analyzed by the proposed HPLC method. Quantity of each component was subsequently obtained by use of the corresponding calibration plots. Each set of samples was analyzed three times. The RSD values were in the range of 0.9–4.8% and recoveries of analytes varied from 96.0% to 101.1%. Above results exhibited the reliability and accuracy for the measurement of these constituents.

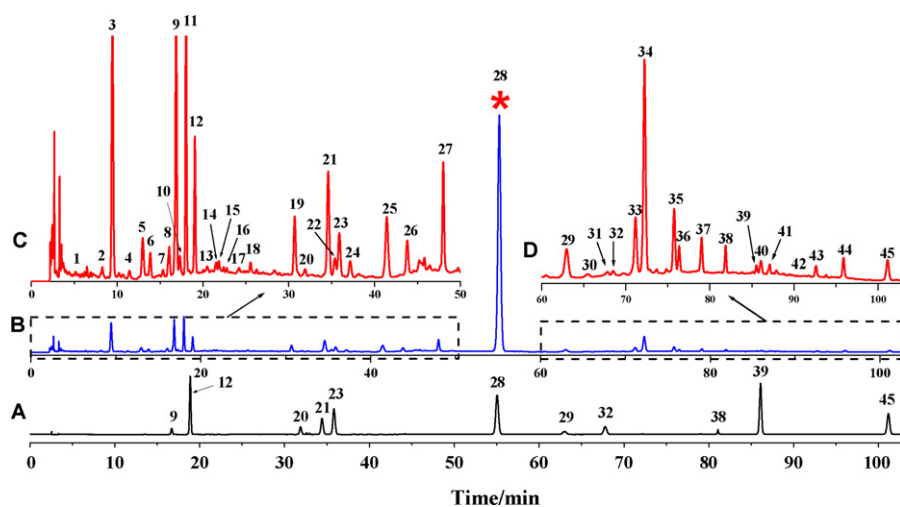


Fig. 3. The chromatogram of SHL oral liquid and eleven compounds. ((A). Chromatogram of eleven compounds; (B). Chromatogram of SHL oral liquid. (C) The amplified chromatogram from 0 min to 50 min. (D) The amplified chromatogram from 60 min to 105 min; 9. Chlorogenic acid, 12. Caffeic acid, 20. Rutin, 21. Forsythiaside, 23. Scutellarin, 28. Baicalin, 29. Forsythsin, 32. Luteoloside, 38. Apigenin, 39. Baicalein, 45. Wogonin).

Table 1
Calibration plots, LOD, and LLOQ for the eleven compounds.

Compound	Linearity range ($\mu\text{g/ml}$)	Calibration equation $y = a + bx^a$	LLOQ ^b ($\mu\text{g/ml}$)	LOD ^b ($\mu\text{g/ml}$)	Correlation factor (r)
Chlorogenic acid	0.04–5.98	$y = 1.0 \times 10^6 x - 42034$	0.02	0.006	0.9997
Caffeic acid	0.05–2.40	$y = 3.0 \times 10^6 x - 13945$	0.03	0.009	1
Rutin	0.12–1.80	$y = 8.2 \times 10^5 x - 4240.1$	0.06	0.02	0.9995
Forsythiaside	0.24–3.62	$y = 1.0 \times 10^6 x - 4049.8$	0.1	0.03	1
Scutellarin	0.04–1.64	$y = 4.0 \times 10^6 x - 11753$	0.03	0.006	0.9996
Baicalin	0.21–31.44	$y = 3.0 \times 10^6 x - 93674$	0.1	0.04	0.9999
Forsythin	0.12–1.76	$y = 6.5 \times 10^5 x - 3918$	0.10	0.02	0.9998
Luteoloside	0.03–0.91	$y = 3.0 \times 10^6 x - 13095$	0.02	0.008	0.9999
Apigenin	0.003–0.10	$y = 3.0 \times 10^6 x - 137.67$	0.003	0.0006	0.9996
Baicalein	0.01–1.86	$y = 6.0 \times 10^6 x - 23659$	0.01	0.003	0.9999
Wogonin	0.06–1.00	$y = 6.0 \times 10^6 x + 14739$	1.5	0.5	0.9998

^a y and x are, respectively, the peak areas and concentrations ($\mu\text{g/ml}$) of the analytes.

^b The LLOQ was defined as the concentration for which the signal-to-noise ratio was 10 and the LOD was defined as the concentration for which the signal-to-noise ratio was 3.

Table 2
Concentrations of the eleven compounds in SHL oral liquid samples.

Compound	Concentration (mg/ml, mean \pm S, $n = 3$)			
	A ^a	B ^b	C ^c	D ^d
Chlorogenic acid	10.31 \pm 0.10	19.00 \pm 0.11	21.65 \pm 0.15	20.85 \pm 0.05
Caffeic acid	1.50 \pm 0.05	3.45 \pm 0.15	2.85 \pm 0.10	2.70 \pm 0.05
Rutin	1.10 \pm 0.12	1.65 \pm 0.11	2.15 \pm 0.05	1.85 \pm 0.05
Forsythiaside	5.70 \pm 0.11	9.81 \pm 0.35	11.15 \pm 0.50	10.86 \pm 0.13
Scutellarin	0.55 \pm 0.05	1.05 \pm 0.15	1.10 \pm 0.05	1.00 \pm 0.11
Baicalin	66.50 \pm 0.65	114.00 \pm 0.84	154.45 \pm 1.72	151.45 \pm 0.63
Forsythin	3.15 \pm 0.15	6.05 \pm 0.10	6.15 \pm 0.11	5.85 \pm 0.21
Luteoloside	0.18 \pm 0.03	0.21 \pm 0.04	0.32 \pm 0.08	0.28 \pm 0.03
Apigenin	0.19 \pm 0.04	0.27 \pm 0.02	0.47 \pm 0.06	0.37 \pm 0.05
Baicalein	0.14 \pm 0.03	0.09 \pm 0.04	0.09 \pm 0.04	0.10 \pm 0.03
Wogonin	0.19 \pm 0.04	0.49 \pm 0.07	0.42 \pm 0.04	0.39 \pm 0.03

SHL oral liquid.

^a A: Sanjing.

^b B: Xinsheng.

^c C: Tailong.

^d D: Changzheng-xinkai.

3.4. Simultaneous quantitative analysis of eleven constituents of SHL oral liquid

The quality control of SHL preparations is carried out by determining the content of baicalin, chlorogenic acid, and forsythine in the Ch. P 2010 [7]. As mentioned above, *Fols Lonicerae*, *Radix Scutellariae* and *Fructus Forsythiae* are the herbs to produce this SHL preparation. Beside baicalin, chlorogenic acid and forsythine, there are many other active components. Hence, quantitative analysis of multiple

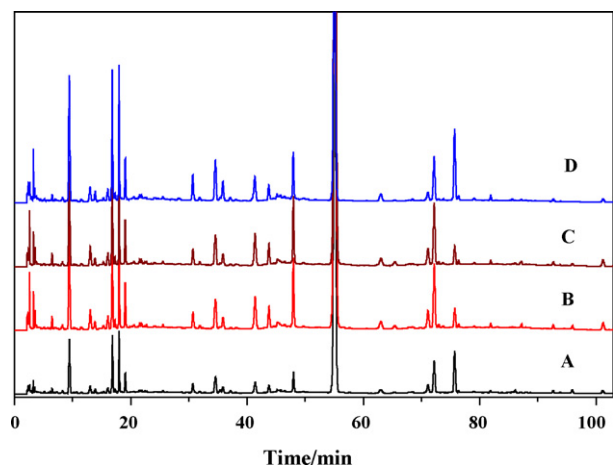


Fig. 4. The chromatogram of four SHL oral liquid samples from different manufacturers. ((A): SHL-A; (B): SHL-B; (C): SHL-C; (D): SHL-D).

active ingredients combined with chromatographic fingerprint is desired.

From the results obtained above, we are confident that our proposed method is sufficient, validated and applicable. The developed method was applied to the simultaneous determination of chlorogenic acid, caffeic acid, rutin, forsythoside, scutellarin, baicalin, forsythine, luteoloside, apigenin, baicalein, and wogonin in four SHL oral liquid samples obtained from different manufacturers located in various provinces in China (Fig. 4). Each sample was determined in triplicate, and the peaks in chromatograms were identified by comparing the retention times and on-line UV spectra with those of the standards. As shown in Table 2, for the content of the eleven compounds in four samples, the concentration of each analyte varied greatly among the different samples, which was probably due to growing condition, climate, and drug processing of crude herbs. So, detection of a single or only several components could not control the quality of SHL oral liquid effectively. It is obvious that combination of chromatographic fingerprint and simultaneous determination of multiple gradients is essential.

4. Conclusion

To the best of our knowledge, this is the first report on chromatographic fingerprint analysis and simultaneous determination of eleven active compounds in SHL oral liquid formulations by HPLC-DAD. This novel evaluation approach can overcome the deficiencies of previously described methods revealing the complexity and synergistic effects of samples' constituents from the same or different manufacturers. It provides much more qualitative infor-

mation than any other singular evaluation. Data analysis on the four SHL oral liquid samples suggested that the concentration of the eleven compounds varied significantly from different locations of China. The proposed method had been elucidated to be a simple, sensitive, accurate and reliable quality control procedure for SHL oral liquid samples.

Acknowledgments

The authors acknowledge support for this work by the Xuzhou Natural Science Foundation of China (no. XX10A052), A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions and Jiangsu Province Student Innovation Project (621).

References

- [1] J. Han, M. Ye, H. Guo, M. Yang, B.R. Wang, D.A. Guo, *J. Pharm. Biomed. Anal.* 44 (2007) 430–438.
- [2] J.X. Ye, W. Wei, L.H. Quan, C.Y. Liu, Q. Chang, Y.H. Liao, *J. Pharm. Biomed. Anal.* 52 (2010) 625–630.
- [3] T.K. Huang, *A Handbook of the Composition and Pharmacology of Common Chinese Drugs*, 1st ed., China Medico-Pharmaceutical Sciences and Technology Publishing House, Beijing, 1994.
- [4] L.J. Ni, L.G. Zhang, J. Hou, W.Z. Shi, M.L. Guo, *J. Ethnopharmacol.* 124 (2009) 79–86.
- [5] M. Yang, J.B. Sun, Z.Q. Lu, G.T. Chen, S.H. Guan, X. Liu, B.H. Jiang, M. Ye, D.A. Guo, *J. Chromatogr. A* 1216 (2009) 2045–2062.
- [6] C.Y. Dai, X.Y. Gao, B. Tang, Y. Fu, H.A. Liu, *Spectrosc. Spect. Anal.* 30 (2010) 358–362.
- [7] National Pharmacopoeia Committee, *Chinese Pharmacopoeia*, China Medico-Pharmaceutical Sciences and Technology Publishing House, Beijing, 2010, pp. 611–612.
- [8] L. Liu, Z. Suo, J. Zheng, *Chinese J. Chromatogr.* 24 (2006) 247–250.
- [9] P.S. Xie, S.B. Chen, Y.Z. Liang, X.H. Wang, R.T. Tian, R. Upton, *J. Chromatogr. A* 1112 (2006) 171–180.
- [10] H.Z. Lian, Y.N. Wei, *Talanta* 71 (2007) 264–269.
- [11] D.Q. Tang, D.Z. Yang, A.B. Tang, Y.Y. Gao, X.L. Jiang, J. Mou, X.X. Yin, *Anal. Bioanal. Chem.* 396 (2010) 3087–3095.
- [12] World Health Organization, *WHO Guidelines for the Assessment of Herbal Medicine*, Munich, 1991.
- [13] State Food and Drug Administration of China, *Technical Requirements for the Development of Finger-prints of TCM Injections*. SFDA, Beijing, 2000.
- [14] Y.H. Lu, W. Song, X.H. Liang, D.Z. Wei, X.L. Zhou, *Chromatographia* 70 (2009) 125–131.
- [15] J.H. Zeng, G.B. Xu, X. Chen, *Med. Chem. Res.* 18 (2009) 158–165.
- [16] C.M. Xiong, J.L. Ruan, Y. Tang, Y.L. Cai, W. Fang, Y. Zhu, D.N. Zhou, *Chromatographia* 70 (2009) 117–124.
- [17] Y. Li, T. Wu, J. Zhu, L. Wan, Q. Yu, X. Li, Z. Cheng, C. Guo, *J. Pharmaceut. Biomed.* 52 (2010) 597–602.
- [18] S.A. Wang, H.Q. Ma, Y.J. Sun, C.D. Qiao, S.J. Shao, S.X. Jiang, *Talanta* 72 (2007) 434–436.
- [19] H. Zhu, Y. Wan, H. Liang, Q. Chen, P. Zhao, J. Tao, *Talanta* 81 (2010) 129–135.
- [20] C.J. Xu, Y.Z. Liang, F.T. Chau, Y.V. Heyden, *J. Chromatogr. A* 1134 (2006) 253–259.
- [21] Y.H. Cao, L.C. Wang, X.J. Yu, J.N. Ye, *J. Pharmaceut. Biomed.* 41 (2006) 845–856.
- [22] S. Yan, M.L. Xu, Y. Tu, H.F. Li, S.Q. Sun, *Spectrosc. Spect. Anal.* 29 (2009) 1558–1561.