网络出版时间：2013-01-21 14:56
网络出版地址：http://www.cnki.net/kcms/detail/11.2863.R.20130121.1456.011.html

Fingerprint analysis of Ophiopogonis Radix by HPLC-UV-ELSD coupled with chemometrics methods

Ning Li, Yanyun Che, Liang Zhang, Jiayu Zhang, Yuan Zhou, Yong Jiang, Pengfei Tu*

State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing 100191, China

Abstract: A new, simple and reliable method using HPLC-UV-ELSD was developed to generate the fingerprint of Ophiopogonis Radix. Homoisoflavonoids and steroidal saponins were determined simultaneously in a single run. A total of 27 Ophiopogonis Radix samples were analyzed, and 18 reference substances were used for the identification of the common peaks. The fingerprint was further analyzed by chemometrics methods including similarity analysis (SA), hierarchical clustering analysis (HCA) and principal component analysis (PCA). The results indicated that the combination of chromatographic fingerprint and chemometrics analysis could be used for the geographical differentiation and quality evaluation of Ophiopogonis Radix.
Keywords: Ophiopogonis Radix; *Ophiopogon japonicus*; HPLC-UV-ELSD; Fingerprint; Chemometrics; Quality evaluation

CLC number: R284 **Document code:** A **Article ID:** 1003–1057(2013)1–55–09

1. Introduction

As a famous traditional Chinese medicine (TCM), Ophiopogonis Radix, the tuberous roots of *Ophiopogon japonicus* (L. f.) Ker-Gawl, known as Maidong in Chinese, have been used to cure acute and chronic inflammation and cardiovascular diseases for thousands of years[1]. Nowadays it is also clinically used in the alternative treatments for cancer[2]. Previous phytochemical investigations have revealed that the major bioactive constituents of *O. japonicus* are homoisoflavonoids, steroidal saponins and polysaccharides[3,4].

In China, *O. japonicus* is mainly cultivated in the provinces of Sichuan and Zhejiang. Ophiopogonis Radix from Sichuan province, konwn as “Chuan-Maidong (CMD)”, is usually harvested one year after planting, while Ophiopogonis Radix from Zhejiang province, konwn as “Zhe-Maidong (ZMD)”, requires two to three years from planting to harvest[5]. ZMD is generally considered to be of superior quality than CMD[6,7]. However, the two different geographical sources of Ophiopogonis Radix are often difficult to be differentiated, especially when the materials have been powdered or extracted.

The quality evaluation of Ophiopogonis Radix is a challenging task. Several studies[6–11] have been conducted on the fingerprint analysis and the discrimination of CMD and ZMD by high performance liquid chromatography (HPLC) fingerprint. Unfortunately, because of the chemical complexity of Ophiopogonis Radix, most of these works[7–11] examined the homoisoflavonoids and the saponins separately. Only a few studies[6] focused on the examination of the two categories of compounds simultaneously using the combination of HPLC, ultraviolet (UV) and evaporative light scattering detector (ELSD). Furthermore, owing to the lack of reference substances, most of the characteristic peaks in the HPLC fingerprint could not be identified.

Chemometrics has developed impressively in recent years. It provides powerful tools in extracting useful information from the huge amounts of data obtained in the fingerprint, and it is proved to be valuable in the classification, discrimination and quality evaluation of medicinal plants[12,13].

During our investigation on the chemical constituents of CMD[1,14], a series of homoisoflavonoids and steroidal saponins have been isolated. In the present study, a new, simple and reliable fingerprint method was developed by HPLC-UV-ELSD combining the chemometrics methods such as similarity analysis (SA), hierarchical clustering analysis (HCA) and principal component analysis (PCA) for the geographical differentiation and quality evaluation of Ophiopogonis Radix.

2. Experimental

2.1. Chemicals and materials

2.1.1. Chemicals

HPLC-grade acetonitrile was purchased from Tianjin Biaoshiqi Science and Technology Development Co., Ltd. (Tianjin, China). HPLC-grade methanol was purchased from Tianjin Xihua Special Type Reagent Factory. The water used in the experiment was double distilled. Other chemicals and solvents were of analytical grade.

Received date: 2012-08-25.
Foundation items: National Key Technology R&D Program “New Drug Innovation” of China (Grant No. 2012ZX09304-005, 2012ZX09301002-002-002).
*Corresponding author. Tel.: 86-10-82802750;
E-mail: pengfeitu@vip.163.com

doi:10.5246/jcps.2013.01.007

2.1.2. Plant materials

The plant materials of Ophiopogonis Radix (14 samples of CMD and 13 samples of ZMD) involved in this work were listed in Table 1. All the samples were authenticated by the corresponding author (Prof. Pengfei Tu). The voucher specimens were deposited at Herbarium of Peking University Modern Research Center for Traditional Chinese Medicine, Beijing, China.

2.1.3. Reference substances

Reference substances including ophiopojaponin C (S-1), ophiogenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (S-2), pennogenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucopyranoside (S-3), 14-hydroxy diosgenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→4)]-*β*-D-glucopyranoside (S-4), pennogenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (S-5), 14-hydroxy diosgenin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside (S-6), ophiopogonin D (S-7), sprengerinin C (S-8), 7,2′-dihydroxy-5,8,4′-trimethoxy-6-methyl homoisoflavanone (H-1), 5,7,4′-trihydroxy-3′-methoxy-6,8-dimethyl homoisoflavanone (H-2), ophiopogonanone E (H-3), 5,7,2′-trihydroxy-4′-methoxy-6,8-dimethyl homoisoflavanone (H-4), 5,7,2′-trihydroxy-3′,4′-methylenedioxy-6,8-dimethyl homoisoflavone (H-5), ophiopogonanone A (H-6), 5,7-dihydroxy-4′-methoxy-6-methyl homoisoflavanone (H-7), methylophiopogonone A (H-8), methylophiopogonanone A (H-9) and methylophiopogonanone B (H-10) (Fig. 1) were isolated from CMD. Their structures were elucidated by MS, ^{1}H- and ^{13}C NMR spectroscopy and confirmed by comparing the data with literature values. The purity of these reference substances was greater than 98% by the peak area normalization method using HPLC-UV or HPLC-ELSD.

2.2. Chromatography system and conditions

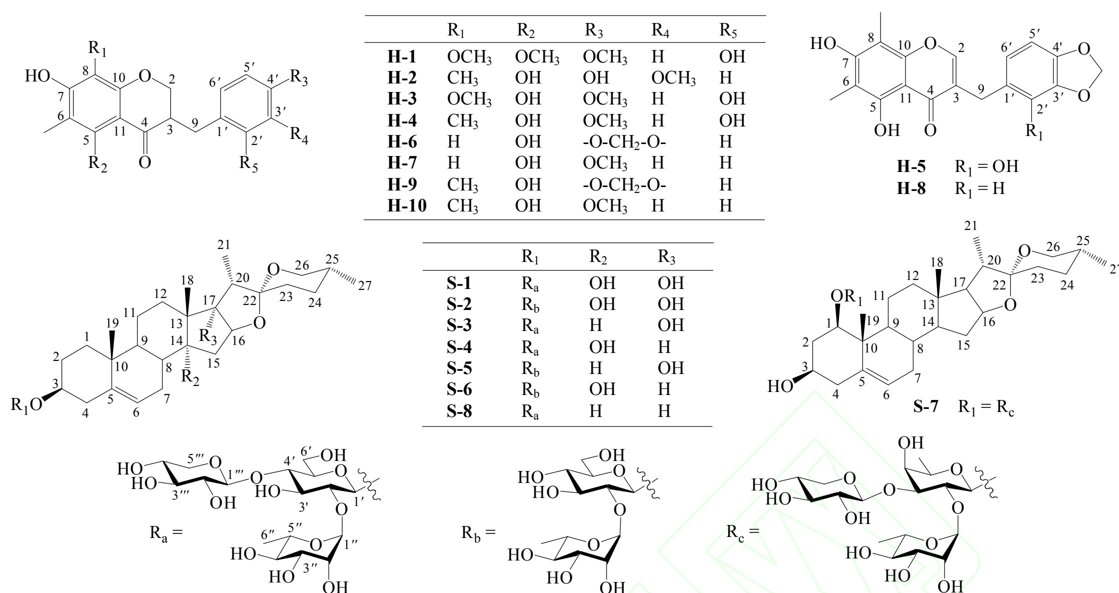
HPLC analysis was performed on an Agilent 1100 HPLC system (Agilent Technologies, USA) comprising a vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment and a photodiode array detector. The separation was carried out on an Agilent SB-C_{18} (250 mm×4.6 mm, 5 μm) column. An elution system composed of acetonitrile–methanol (3:1, v/v) as solvent A and 0.1% formic acid in water as solvent B was applied for the fingerprint analysis. The linear gradient program was as follows: 0–10 min, 30% A; 10–25 min, 30%–48% A; 25–45 min, 48% A; 45–50 min, 48%–55% A; 50–65 min, 55%–65% A; 65–80 min, 65%–90% A; 80–85 min, 90% A. The mobile phase flow rate was 1.0 mL/min. The column temperature was maintained at 30 °C. The chromatogram was monitored at a wavelength of 296 nm. The injection volume was 20 μL for CMD and 10 μL for ZMD. A Sedere Sedex 75 ELSD detector (Sedere, France) was connected to the HPLC instrument with a tandem mode. Compressed air was used as the nebulizing gas. The nebulizing gas flow rate was 2.5 L/min. The drift tube temperature was set at 40°C.

For HPLC-MS^n analysis, an Agilent 6320 ion trap mass spectrometer was connected on the HPLC instrument via

Table 1. A summary of Ophiopogonis Radix involved in this work

Sample No.	Sample type	Geographical origin	Provider	Year of collection
1[a]	CMD	Santai, Sichuan	Sichuan Daidaiweiben Agriculture Science and Technology Co., Ltd.	2011
2[a]	CMD	Santai, Sichuan	Sichuan Daidaiweiben Agriculture Science and Technology Co., Ltd.	2011
3[a]	CMD	Santai, Sichuan	Sichuan Daidaiweiben Agriculture Science and Technology Co., Ltd.	2011
4[a]	CMD	Santai, Sichuan	Sichuan Daidaiweiben Agriculture Science and Technology Co., Ltd.	2011
5[a]	CMD	Santai, Sichuan	Sichuan Daidaiweiben Agriculture Science and Technology Co., Ltd.	2011
6[a]	CMD	Santai, Sichuan	Sichuan Daidaiweiben Agriculture Science and Technology Co., Ltd.	2011
7[a]	CMD	Santai, Sichuan	Ya'an Sanjiu Pharmaceutical Co., Ltd.	2009
8	CMD	Sichuan	Shineway Pharmaceutical Co., Ltd.	2010
9	CMD	Sichuan	Chengdu Hehuachi Chinese Herbal Medicine Market	2010
10	CMD	Sichuan	Beijing Tongrentang Co., Ltd.	2011
11	CMD	Santai, Sichuan	Local herb market in Santai	2011
12	CMD	Santai, Sichuan	Local herb market in Santai	2011
13	CMD	Sichuan	Anguo Herb Market	2011
14	CMD	Sichuan	Anguo Herb Market	2011
15	ZMD	Xinjie, Xiaoshan, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2012
16	ZMD	Simen, Yuyao, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2012
17	ZMD	Tingpang, Sanmen, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2012
18	ZMD	Kandun, Cixi, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2012
19	ZMD	Chongshou, Cixi, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2012
20	ZMD	Longshan, Cixi, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2012
21	ZMD	Shengshan, Cixi, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2012
22	ZMD	Chongshou, Cixi, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2011
23[a]	ZMD	Xiaolin, Cixi, Zhejiang	Chiatai Qingchunbao Pharmacentical Co., Ltd.	2011
24	ZMD	Andong, Cixi, Zhejiang	Local herb market in Andong	2011
25	ZMD	Kandun, Cixi, Zhejiang	Local herb market in Kandun	2011
26	ZMD	Fuhai, Cixi, Zhejiang	Local herb market in Fuhai	2011
27	ZMD	Shengshan, Cixi, Zhejiang	Local herb market in Shengshan	2011

[a] Ophiopogonis Radix cultivated according to Good Agricultural Practice (GAP).



	R_1	R_2	R_3	R_4	R_5
H-1	OCH_3	OCH_3	OCH_3	H	OH
H-2	CH_3	OH	OH	OCH_3	H
H-3	OCH_3	OH	OCH_3	H	OH
H-4	CH_3	OH	OCH_3	H	OH
H-6	H	OH	$-O-CH_2-O-$		H
H-7	H	OH	OCH_3	H	H
H-9	CH_3	OH	$-O-CH_2-O-$		H
H-10	CH_3	OH	OCH_3	H	H

	R_1	R_2	R_3
S-1	R_a	OH	OH
S-2	R_b	OH	OH
S-3	R_a	H	OH
S-4	R_a	OH	H
S-5	R_b	H	OH
S-6	R_b	OH	H
S-8	R_a	H	H

Figure 1. Structures of the reference substances.

an electrospray ionization (ESI) interface. The HPLC effluent was introduced into the ESI source in a post-column splitting ratio of 1:4. The ESI-MS^n was performed in negative ionization mode with source settings as follows: nebulizer gas pressure of 25.00 psi; dry gas flow rate of 10.00 L/min; electrospray voltage of the ion source of 3500 V; capillary temperature of 350°C; compound stability of 100%; trap drive level of 100%; AutoMS(n) operation mode; collision energy of 1 V; SmartFrag Start Ampl of 30%, SmartFrag End Ampl of 200%. Nitrogen (>99.99%) and He (>99.99%) were used as sheath and damping gas, respectively. The Agilent 6300 series trap control workstation (Version 6.1) was used for the data processing.

2.3. Sample preparation

All the samples were dried at 60°C for 12 h before use. The dried material was pulverized to 40 meshes. Each sample (2.00 g) was weighted accurately and placed into a 100 mL flask containing 50 mL of methanol. The mixture was sonicated for 30 min at room temperature. The extract was filtered and concentrated to dryness under reduced pressure to give a residue. The residue was dissolved in water (5 mL) and subjected to a solid phase extraction C_{18} column (Agela, Cleanert C_{18}, 500 mg/3 mL) eluting with water (2 mL), 40% methanol (2 mL) and 100% methanol (2 mL), sequentially. The 100% methanol eluant was evaporated to dryness under reduced pressure and dissolved in methanol to 1 mL. The solution was filtered through 0.45 μm membrane before HPLC analysis.

2.4. Data analysis

Data analysis was performed by ChemPattern 2.0, a professional software developed by Chemmind Technologies Co., Ltd, Beijing, P. R. China.

3. Results and discussion

3.1. Optimization of sample preparation

As mentioned in the literature[6], the presence of large amount of saccharides in Ophiopogonis Radix samples interfered with the detection of the nonsaccharide components. Methanol was selected as the extraction solvent in view of its favorable solubility for homoisoflavonoids and steroidal saponins, and its relatively low solubility for saccharides. In addition, a solid phase extraction procedure was applied for the further concentration of homoisoflavonoids and steroidal saponins.

3.2. Optimization of HPLC conditions

Among the major bioactive components of Ophiopogonis Radix, homoisoflavonoids can be detected by UV detector at a wavelength of 296 nm with high sensitivity and specificity. However, steroidal saponins are difficult to detect by UV detector, because of the lack of UV chromophore. ELSD is a universal and non-specific mass detector based on the detection of solute molecules by light scattering after nebulization and evaporation of the mobile phase[15]. It can serve as a complementary tool for the detection of steroidal saponins. Therefore, in the present study, a combination of UV and ELSD was used for the simultaneous determination of homoisoflavonoids and steroidal saponins.

Different mobile phases, such as methanol–water, acetonitrile–water and acetonitrile–methanol–water were compared. Acetonitrile–methanol–water provided the best resolutions. Formic acid was added to improve the peak shapes. Five columns including Waters Symmetry C_{18}, Kromasil ODS, Agilent XDB-C_{18}, Agilent Extend-C_{18} and Agilent SB-C_{18} were tested, and the Agilent SB-C_{18} column was finally selected for the analysis.

3.3. Validation of methodology

The precision of the method was determined by six replicate injections of the same sample solution in a day. The similarities of the six chromatograms were more than 0.98. The relative standard deviations (RSD) of the relative retention time (RRT) and the relative peak area (RPA) of selected peaks were less than 2.0% and 5.0%, respectively. The repeatability tests were performed with six independently prepared sample solutions. The similarities of the six chromatograms were more than 0.98. The RSD of RRT and RPA of selected peaks were found below 2.0% and 5.0%, respectively. The stability of the method was determined with one sample solution at different time points (0, 2, 4, 8, 12 and 24 h). The similarities of the six chromatograms were more than 0.98. The RSD of RRT and RPA of selected peaks were less than 2.0% and 5.0%, respectively.

3.4. HPLC-UV-ELSD fingerprint analysis of Ophiopogonis Radix

3.4.1. Fingerprint of Ophiopogonis Radix and the identification of the common peaks

The established method was applied to the analysis of 27 Ophiopogonis Radix samples (Fig. 2). The common patterns were shown in Figure 3. In the UV fingerprint of CMD, 22 peaks (labeled as 1a to 22a) were selected as the common peaks. Peaks 1a, 5a, 7a, 9a, 11a, 12a, 13a, 14a and 15a were identified as H-1, H-2, H-3, H-4, H-6, H-7, H-8, H-9 and H-10, respectively, by comparing their retention times and MS^n data (Table 2) with reference substances. In the ELSD fingerprint of CMD, 33 peaks (labeled as 1b to 33b) were selected as the common peaks. Peaks 2b, 3b, 4b, 5b, 6b, 7b, 8b, 9b, 11b, 17b, 18b, 19b and 20b were identified as H-1, S-1, S-2, H-3, S-3, S-4, S-5, S-6, H-6, H-9, H-10, S-7 and S-8, respectively, by comparing their retention times and MS^n data (Table 2) with standard compounds. In the UV fingerprint of ZMD, 26 peaks (labeled as 1c to 26c) were selected as the common peaks. Peaks 5c, 6c, 7c, 8c, 10c, 11c, 14c and 15c were identified as H-2, H-3, H-4, H-5, H-6, H-7, H-9 and H-10, respectively, by comparing their retention times and MS^n data (Table 2) with reference substances. In the ELSD fingerprint of ZMD, 33 peaks (labeled as 1d to 33d) were selected as the common peaks. Peaks 16d, 17d, 20d and 21d were identified as H-6, H-7, H-9 and H-10, respectively, by comparing their retention times and MS^n data (Table 2) with standard compounds.

3.4.2. Similarity analysis

The similarities were calculated based on the entire chromatographic profiles by correlation coefficient method. The similarity of each UV chromatogram comparing to the UV common pattern of CMD is shown in Figure 4A. The similarities of CMD were above 0.95, while those of ZMD were found below 0.75. The similarity of each UV chromatogram comparing to the UV common pattern of ZMD is shown in Figure 4B. The similarities of ZMD were more than 0.99, while those of CMD were less than 0.75. As for the ELSD fingerprint, comparing to the common pattern of CMD, the similarity of each fingerprint of CMD was above 0.70, while that of ZMD were less than 0.40 (Fig. 4C). Comparing with the common pattern of ZMD, the similarities of all the fingerprint of ZMD were more than 0.95, while those of CMD were below 0.45 (Fig. 4D). Based on the similarity analysis, the two different geographical sources of Ophiopogonis Radix could be clearly classified.

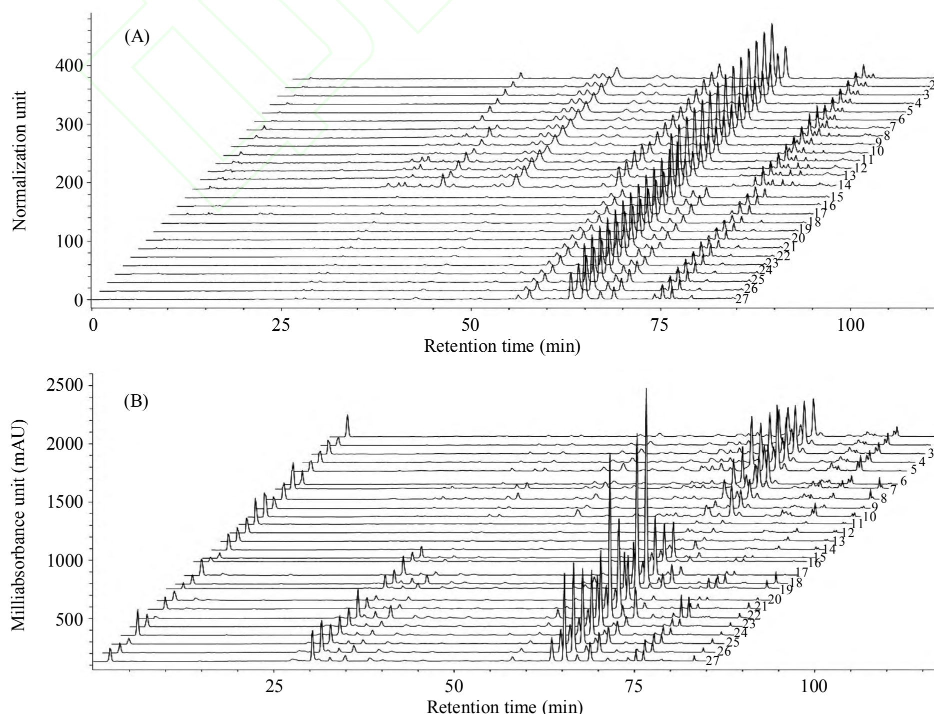


Figure 2. Fingerprint (A. UV 296 nm; B. ELSD) of 27 samples of Ophiopogonis Radix.

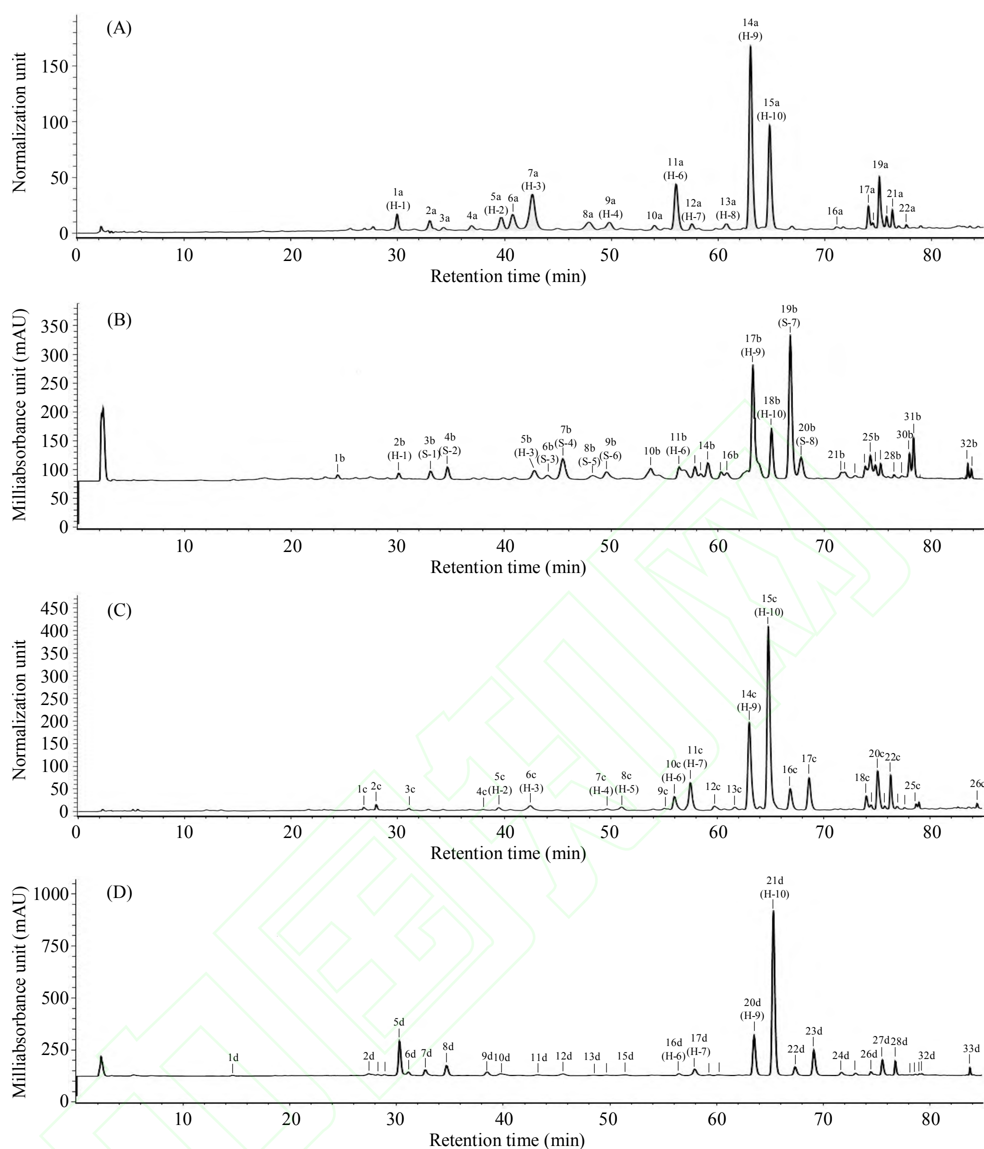


Figure 3. Fingerprint common patterns (A. UV 296 nm common pattern of CMD; B. ELSD common pattern of CMD; C. UV 296 nm common pattern of ZMD; D. ELSD common pattern of ZMD) of Ophiopogonis Radix.

Table 2. Identification of the common peaks in the fingerprint of Ophiopogonis Radix by HPLC-MS^n

Reference substances	Retention time t_R (min)	$[M–H]^-$ (m/z)	MS^2 (m/z)	MS^3 (m/z)	Peak No.
H-1	29.9	373	183	168	1a, 2b
S-1	32.9	885	753	607	3b
S-2	34.5	753	607	445	4b
H-2	39.7	343	207	192, 179, 163	5a, 5c
H-3	42.6	359	344, 169*, 154	154	7a, 5b, 6c
S-3	43.8	869	737	591	6b
S-4	45.3	869	737	591	7b
S-5	47.8	737	591	–	8b
S-6	49.2	737	591	–	9b
H-4	49.7	343	153	111	9a, 7c
H-5	50.9	355	327, 218, 205*	161, 149, 137	8c
H-6	56.0	327	205, 192*, 164	164	11a, 11b, 10c, 16d
H-7	57.6	313	192	164	12a, 11c, 17d
H-8	60.9	339	311*, 218	218, 205	13a
H-9	63.1	341	206*, 178	178	14a, 17b, 14c, 20d
H-10	64.9	327	206*, 178	178	15a, 18b, 15c, 21d
S-7	66.7	853	721	575	19b
S-8	67.8	853	721	575	20b

* Precursor ion.

3.4.3. Hierarchical clustering analysis

The raw data derived from the fingerprint was pre-processed by normalization before HCA. Euclidean distance and the nearest neighbor method were applied in this study. The result of HCA based on the total 27 UV fingerprint was shown in Figure 5A. The samples were divided into two well separated clusters. All the samples of CMD were grouped into one cluster, and the samples of ZMD were classified into another cluster. A detailed analysis revealed that the samples of CMD could be further divided into three sub-clusters. The GAP samples (samples 1–6) were grouped into one sub-cluster. One sample from another GAP base (sample 7) gathered with three commercial samples (samples 8–10) to form another sub-cluster. The other four commercial samples (samples 11–14) were classified into the third sub-cluster. In addition, the samples of ZMD collected in 2012 (samples 15–21) and 2011 (samples 22–27) fell into two separate sub-clusters. The result of HCA based on the total 27 ELSD fingerprint was shown in Figure 5B. The samples of CMD and ZMD could be clearly classified into two clusters. Six GAP samples of CMD (samples 1–6) gathered into one sub-cluster, which further blended with sample 7, a sample from another GAP base, and sample 8. In all, HCA was helpful for the discrimination of CMD and ZMD. By using this methodology, minor differences in the samples could be revealed, and the relationship among the samples could be represented more clearly.

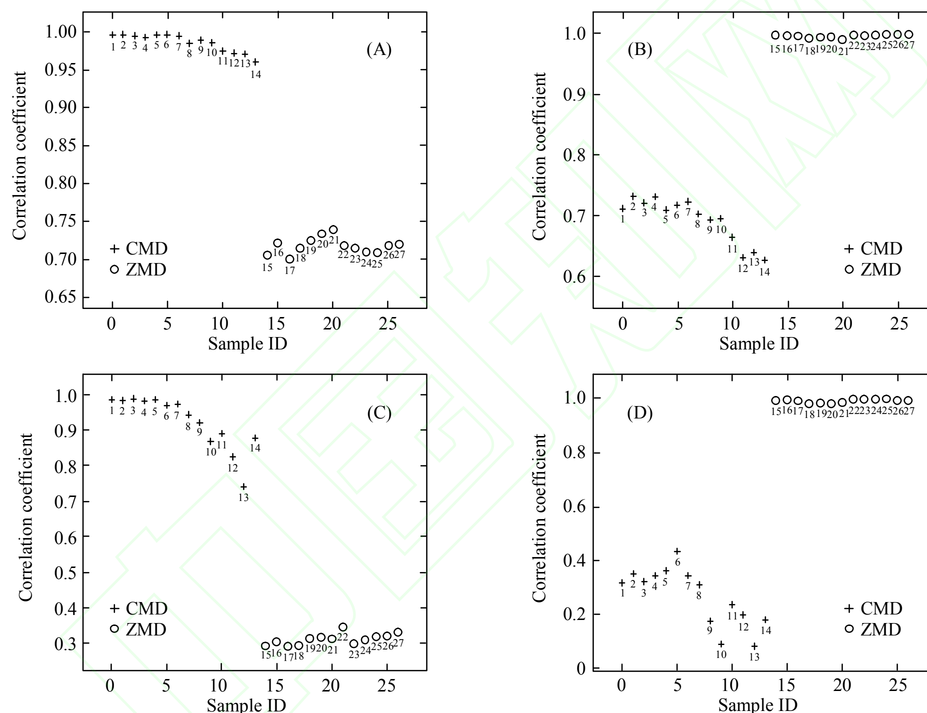


Figure 4. SA of the fingerprint of Ophiopogonis Radix. (A) The similarities of 27 UV fingerprint comparing to the UV common pattern of CMD; (B) The similarities of 27 UV fingerprint comparing to the UV common pattern of ZMD; (C) The similarities of 27 ELSD fingerprint comparing to the ELSD common pattern of CMD; (D) The similarities of 27 ELSD fingerprint comparing to the ELSD common pattern of ZMD.

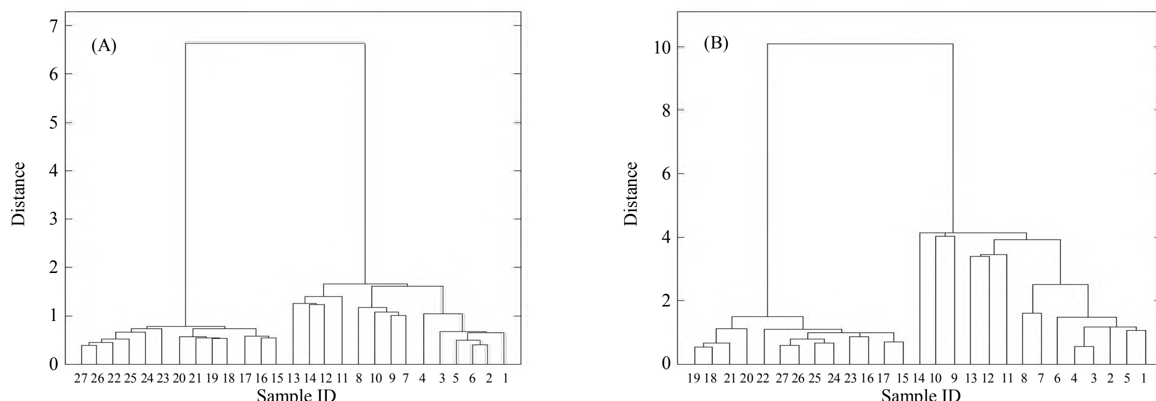


Figure 5. HCA dendrograms of the fingerprint of 27 samples of Ophiopogonis Radix. (A) Based on the UV fingerprint; (B) Based on the ELSD fingerprint.

3.4.4. Principal component analysis

PCA is an unsupervised multivariate data analysis approach[16–18]. It can be used for reducing the dimensions of multivariate data sets to obtain a new set of variables called principal components (PCs). By this approach, the significant data variance can be described by just a few PCs. PC1 accounts for most of the data variance, PC2 accounts for most of the remaining variance, and so on. Each object has a score value on each PC, and each variable is likewise associated with a loading on each PC. The scores plots give information about the similarities and the clustering tendency of the objects, while the loading plots reveal the contribution of the original variables.

In order to find the characteristic peaks, which were most informative for the classification of the samples, PCA was conducted. The raw data derived from the fingerprint was preprocessed by normalization before PCA. PCA based on the total 27 UV fingerprint was shown in Figure 6. The first two PCs (PC1 and PC2) accounted for more than 95% of the total variance. In the PC1–PC2 scores plots, the samples of CMD and ZMD could be clearly classified according to their scores on PC1 (Fig. 6A). In the loading plots, it was found that PC1 had good correlation with peaks 2a, 6a, 7a/6c, 11a/10c, 12a/11c, 14a/14c, 15a/15c, 16c and 17c (Fig. 6B). The results indicated that these peaks could serve as chemical markers for the discrimination of CMD and ZMD. PCA based on the total 27 ELSD fingerprint was shown in Figure 6. The first two PCs (PC1 and PC2) represented more than 90% of the total variance. In the PC1–PC2 scores plots, the samples of CMD and ZMD could be separated according to their scores on PC1 (Fig. 6C). In the loading plots, it was noticeable that peaks 2b/5d, 7b/12d, 10b, 17b/20d, 18b/21d, 19b/22d, 20b, 23d, 25b and 31b/31d contributed the most to PC1 (Fig. 6D). These peaks were important for the classification of CMD and ZMD.

PCA based on the UV fingerprint of 14 samples of CMD was shown in Figure 7. The first two PCs (PC1 and PC2) accounted for more than 88% of the total variance. In the PC1-PC2 scores plots, the samples were clustered into three different groups (Fig. 7A). The result was accordant with HCA of the UV fingerprint in general. In the loading plots, it was found that PC1 was most correlated with peaks 1a, 2a, 3a, 5a, 6a, 8a, 9a, 11a, 15a and 19a (Fig. 7B). Peaks 1a, 7a, 11a, 14a, 18a, 19a and 21a showed the greatest influence on PC2 (Fig. 7C). PCA based on the ELSD fingerprint of 14 samples of CMD was shown in Figure 7. The first two PCs (PC1 and PC2) represented more than 75% of the total variance. In the PC1–PC2 scores plots, sample 8 gathered with seven GAP samples (samples 1–7) to form a group, while the distribution of other samples seemed to be random (Fig. 7D). In the loading plots, PC1 showed good correlation with peaks 2b, 7b, 10b, 11b, 17b, 18b, 20b, 21b, 24b, 25b and 31b (Fig. 7E). PC2 was mostly influenced by peaks 16b, 17b, 19b, 23b, 24b, 25b and 31b (Fig. 7F).

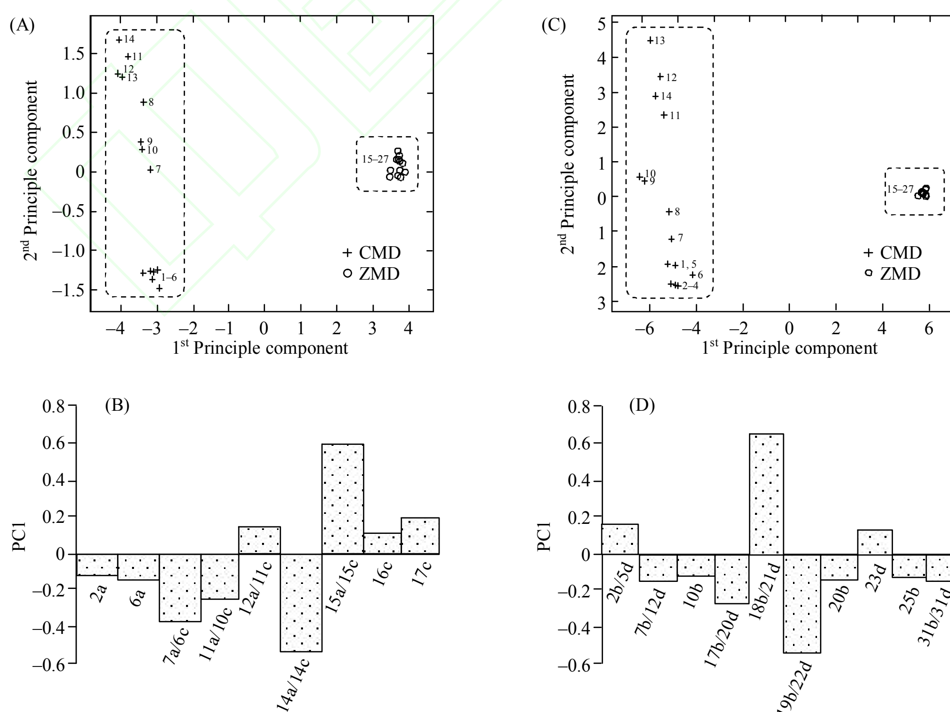


Figure 6. PC1-PC2 scores plots (A. Based on the UV fingerprint; C. Based on the ELSD fingerprint) and PC1 loading plots (B. Based on the UV fingerprint; D. Based on the ELSD fingerprint) obtained by PCA of 27 samples of Ophiopogonis Radix.

PCA based on the UV fingerprint of 13 samples of ZMD was shown in Fig. 8. The first two PCs (PC1 and PC2) accounted for more than 86% of the total variance. In the PC1-PC2 scores plots, the samples collected in 2012 (samples 15–21) and 2011 (samples 22–27) could be classified according to their scores on PC1. The loading plots indicated that that PC1 was correlated with peaks 2c, 5c, 7c, 8c, 9c, 10c, 11c, 15c, 16c, 17c, 20c and 22c.

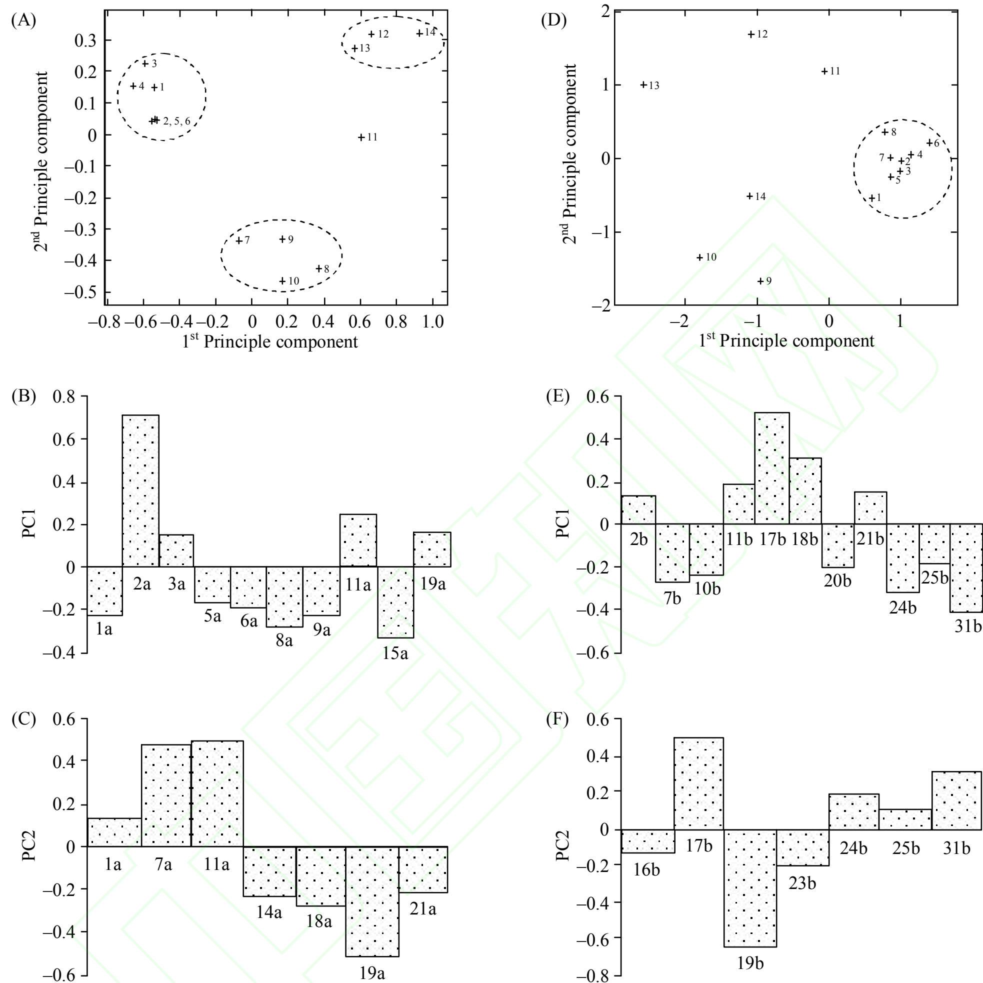


Figure 7. PC1-PC2 scores plots (A. Based on the UV fingerprint; D. Based on the ELSD fingerprint), PC1 loading plots (B. Based on the UV fingerprint; E. Based on the ELSD fingerprint) and PC2 loading plots (C. Based on the UV fingerprint; F. Based on the ELSD fingerprint) obtained by PCA of 14 samples of CMD.

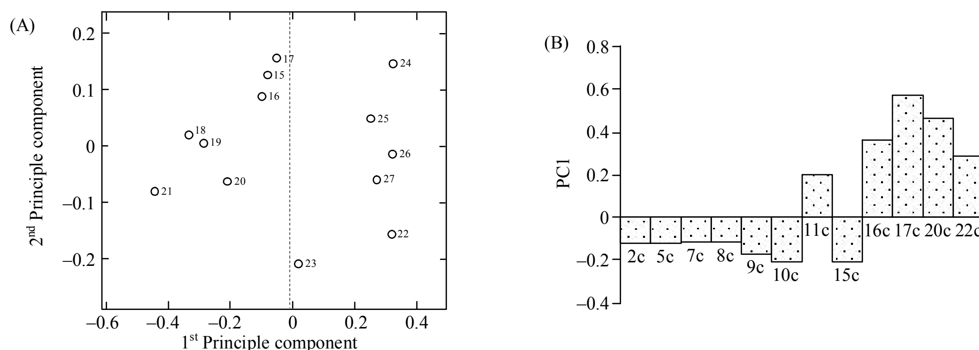


Figure 8. PC1-PC2 scores plots (A) and PC1 loading plots (B) obtained by PCA of UV fingerprint of 13 samples of ZMD.

4. Conclusions

A new, simple and reliable fingerprint method was developed by HPLC-UV-ELSD for the quality evaluation of Ophiopogonis Radix. Multiple bioactive components including homoisoflavonoids and steroidal saponins were determinated simultaneously in a single run. The fingerprint was analyzed by chemometrics methods including SA, HCA and PCA. SA provided a simple way to differentiate CMD from ZMD rapidly. HCA revealed further details of the relationship among the samples, and minor differences between the samples could be observed. The characteristic peaks, which were most informative for the classification of the samples, were further highlighted by PCA. This study provided an example for the quality evaluation of TCM by using a combination of fingerprint and chemometrics analysis.

Acknowledgements

We are grateful to Mr. Runtao Tian (Chemmind Technologies Co., Ltd, Beijing, China) for providing the software of ChemPattern 2.0 and for his technical support. We are also pleased to thank Mr. Jianbing Zhang, Mr. Zhenfeng Li and Ms. Mulan Wang (Chiatai Qing-chunbao Pharmacentical Co., Ltd, Hangzhou, China) for providing the samples of ZMD. This work was financially supported by National Key Technology R&D Program “New Drug Innovation” of China (Grant No. 2012ZX09304-005, 2012ZX09301002-002-002).

References

[1] Li, N.; Zhang, J.Y.; Zeng, K.W.; Zhang, L.; Che, Y.Y.; Tu, P.F. *Fitoterapia.* **2012**, *83*, 1042–1045.

[2] Du, Q.; Hu, B.; Shen, K.P. *J. Chin. Med. Mater.* **2010**, *33*, 1512–1516.

[3] Jiang, H.B.; Huang, J.; Guo, M.J.; Zou, P.; Tian, X.Q. *Acta Pharm. Sin.* **2007**, *42*, 118–126.

[4] Yang, M.P.; Wu, H.; Yin, L.; Zhang, X.; Duan, J.A. *Chin. Arch. Tradit. Chin. Med.* **2008**, *26*, 2169–2171.

[5] Yu, B.Y.; Xu, G.J. *Chin. Tradit. Herb. Drugs.* **1995**, *25*, 205–210.

[6] Liu, L.; Lu, Y.; Shao, Q.; Cheng, Y.Y.; Qu, H.B. *J. Sep. Sci.* **2007**, *30*, 2628–2637.

[7] Lin, Y.N.; Zhu, D.N.; Qi, J.; Qin, M.J.; Yu, B.Y. *J. Pharm. Biomed. Anal.* **2010**, *52*, 757–762.

[8] Lin, Y.N.; Shida, Y.; Yuan, B.; Cheng, Z.H.; Yu, B.Y.; Zhu, D.N. *J. Chin. Pharm. Univ.* **2005**, *36*, 538–542.

[9] Dong, Z.Q.; Zhu, J.; Jin, G.Z. *Spec. Wild Economic Animal Plant Res.* **2007**, *29*, 20–22.

[10] Chen, Y.G.; Wu, G.Q.; Dai, J.D.; Li, H. *Chin. J. Inf. Tradit. Chin. Med.* **2008**, *15*, 56–58.

[11] Liu, J.; Chen, X.F.; Yang, W.Y.; Zhang, S.P.; Du, G.; Liu, W.G. *Chin. Tradit. Herb. Drugs.* **2010**, *41*, 1875–1881.

[12] Du, J.; Wan, D.G.; Huang, L.F.; Chen, S.L.; Qin, M.J. *J. Med. Plants Res.* **2011**, *5*, 4001–4008.

[13] Tistaert, C.; Dejaegher, B.; Vander Heyden, Y. *Anal. Chim. Acta.* **2011**, *690*, 148–161.

[14] Jiang, Y.; Duan, C.L.; Chai, X.Y.; Hua, H.M.; Tu, P.F. *China J. Chin. Mater. Med.* **2007**, *32*, 1111–1114.

[15] Li, F.J.; Zhang, H.Y.; Li, Y.; Yu, Y.J.; Chen, Y.L.; Xie, M.F.; Duan, G.L. *Chromatographia.* **2012**, *75*, 187–191.

[16] Guo, S.; Duan, J.A.; Shen, J.; Xia, N.; Tang, Y.P.; Meng, F.W.; Shang, E.X.; Qian, D.W. *J. Liq. Chromatogr. Relat. Technol.* **2011**, *34*, 2048–2062.

[17] Ni, Y.N.; Song, R.M.; Kokot, S. *Anal. Methods.* **2012**, *4*, 171–176.

[18] Wang, L.B.; Wang, X.B.; Kong, L.Y. *Biochem. Syst. Ecol.* **2012**, *40*, 138–145.

HPLC-UV-ELSD结合化学计量学方法研究麦冬的指纹图谱

李宁, 车彦云, 张梁, 张加余, 周渊, 姜勇, 屠鹏飞*

北京大学医学部 药学院 天然药物及仿生药物国家重点实验室, 北京 100191

摘要: 本文利用HPLC-UV-ELSD技术建立了一种新的麦冬化学指纹图谱分析方法。该方法简单、可靠, 可同时对高异黄酮及甾体皂苷两类成分进行检测。在此基础上, 对27批麦冬药材的指纹图谱进行了分析。采用18个对照品对部分共有峰进行了指认。采用相似度分析、聚类分析、主成分分析等化学计量学方法对指纹图谱进行了进一步分析。结果显示, 色谱指纹图谱结合化学计量学方法可用于不同产地麦冬的鉴定及药材的质量评价。

关键词: 麦冬; HPLC-UV-ELSD; 指纹图谱; 化学计量学; 质量评价