Differentiation of Rhizoma Et Radix Polygoni Cuspidati from Closely Related Herbs by HPLC Fingerprinting

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An HPLC-DAD fingerprinting profile of Rhizoma Et Radix Polygoni Cuspidati was established basing on the consistent chromatographic features of 24 authentic herb samples. The major types of chemical constituents, stilbenes and anthraquinones, were analyzed and included in the fingerprint. Eight common peaks of Polygonum Cuspidatum were identified by using HPLC-MS. The developed fingerprint was applied to differentiate Rhizoma Et Radix Polygoni Cuspidati from Radix Polygoni Multiflori and Radix Et Rhizoma Rhei. Although the three herbs belong to the family of Polygonaceae, the results indicated that these could be differentiated by using the established method.

Key words fingerprint; Polygonaceae; Rhizoma Et Radix Polygoni Cuspidati; HPLC-DAD-MS; quality control

Rhizoma Et Radix Polygoni Cuspidati (Huzhang, HZ), the stem and root of *Polygonum Cuspidatum* SIEB. *et* ZUCC., belongs to the family of Polygonaceae. It is one of the most generally used traditional Chinese medicinal materials in China and is listed in the Chinese Pharmacopoeia (2005 edition).[1)] Clinically it is used in prescriptions and composite formulae to activate blood circulation, remove blood stasis, expel wind and dampness, dredge the meridians and clear away heat evil and toxic material, *etc*.[1,2)]

The stem and root of crude drug contain stilbenes, anthraquinones and flavones as the major chemical constituents. Examples included resveratrol, polydatin (piceid), emodin, physcion, chrysophanol, rhein, anthraglycoside A, anthraglycoside B, fallacinol, questinol, *etc*.[2—5)]

Pharmacological and clinical studies indicated that several chemical components in HZ are bioactive with reported activities. Resveratrol, which is also available in grapes but in a much lower content,[6,7)] has demonstrated biological activities in inhibiting growth of several bacteria and fungi,[8,9)] lowering blood cholesterol level[2,9)] and exhibiting anti-inflammatory property.[9)] Emodin can act as a strong inhibitor of protein tyrosine kinase to inhibit the growth of cancer cell.[9,10)] Polydatin, the most abundant chemical component in HZ, can inhibit platelet aggregation,[2,4,5,8,9)] lower blood cholesterol,[2,4)] enhance blood flow in capillaries[2,4,5)] and act as a tranquilizing agent.[2,9)]

Both Radix Polygoni Multiflori (Heshouwu, HSW) and Radix Et Rhizoma Rhei (Dahuang, DH), are members of family Polygonaceae. Although they can be differentiated from each other simply based on their morphological appearances, however, precise authentication on powder or extracted forms always encountered difficulties. Representative fingerprinting profiles of HZ, HSW and DH were therefore generated with aims to differentiate among each other by identities and relative amounts of chemical constituents. Authentication using chemical profile for HZ was reported previously but these were mainly focused on qualitative and/or quantitative analysis of one to two constituents which are considered inadequate.[6,11—18)] Therefore, a comprehensive qualitative HPLC analysis of HZ using 13 characteristic peaks was conducted. Multiple batches of authenticated samples in different cultivation origins were collected for generating representative chromatogram. Altogether 24 HZ, 9 HSW and 7 DH samples were analyzed and eight common compounds were identified in the HZ fingerprint (Table 1, Fig. 1). A reproducible method of generating HPLC fingerprint of HZ was developed which can be used for distinguishing HZ samples from other related herbs.

In order to determine the optimal detection wavelength for the characteristic chromatograms of HZ, HPLC-diode array detection (DAD) associated with three dimensional plots of retention time-absorbance-wavelength (3D-plots) was used. The entire chemical profile of HZ in the chromatographic window was analyzed by HPLC-MS. The developed fingerprinting profile with major chemical constituents was applied to evaluate the quality of HZ. Besides, the Computer Aided Similarity Evaluation System was used to determine the similarity of chromatographic patterns among HZ, HSW and DH by calculating the correlation coefficients and generating a simulative mean chromatogram as a reference standard chromatogram for each respective herb in the current study.[19,20)] The relative retention time (RRT) and relative peak area (RPA) of each characteristic peak with respect to the reference peak in chromatograms were calculated. The generated data can be used in the differentiation of the three herbs as well as applied in the quality assessment of herbs and their related products.

Experimental

Instrumentation An Agilent 1100 series HPLC-DAD system comprising a vacuum degasser, binary pump, autosampler, thermostated column compartment and DAD (Agilent, Palo Alto, CA, U.S.A.) was used for acquiring chromatograms, UV spectra and 3-D plots. For mass spectrometric measurement in identifying characteristic peaks, an Applied Biosystems/PE-SCIEX API 365 LC-MS-MS system with atmospheric pressure chemical ionization source (Applied Biosystems, Foster City, CA, U.S.A.) was used. Branson 5210E-MTH ultrasonic bath (Branco Ultrasonics Corporation, CT, U.S.A.) was used for sample extraction.

For chromatographic analysis, an Alltima C_{18} column (250 mm×4.6 mm i.d., 5 μm) with a suitable guard column (C_{18}, 7.5 mm×4.6 mm i.d., 5 μm) was used. The mobile phase consisted of 0.4% formic acid in deionized water (A) and acetonitrile (B) using gradient program of 15—20% (B) in 0—20 min, 20—40% (B) in 20—40 min, 40—100% (B) in 40—60 min and

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100% (B) in 60—65 min. The flow rate was 1.0 ml/min, the injection volume was 10 μl and column temperature was maintained at 30 °C. DAD detector was set at 290 nm for acquiring chromatograms.

Table 1. A Summary of the Tested Samples

No.	Sample code	Source	Origin	Year of collection
1	HZ-1	Hong Kong	Guangxi, China	2005
2	HZ-2	Hong Kong	Guangxi, China	2005
3	HZ-3	Hong Kong	Guangxi, China	2005
4	HZ-4	Hong Kong	Guangxi, China	2005
5	HZ-5	Hong Kong	Guangxi, China	2005
6	HZ-6	Hong Kong	Guangxi, China	2005
7	HZ-7	Hong Kong	Guangxi, China	2005
8	HZ-8	Sichuan	Abeizhou, Sichuan, China	2006
9	HZ-9	Sichuan	Huaxi, Sichuan, China	2006
10	HZ-10	Sichuan	Changdu, Sichuan, China	2006
11	HZ-11	Sichuan	Aoping, Sichuan, China	2006
12	HZ-12	Sichuan	Changdu, Sichuan, China	2006
13	HZ-13	Sichuan	Changdu, Sichuan, China	2006
14	HZ-14	Sichuan	Changdu, Sichuan, China	2006
15	HZ-15	Sichuan	Changdu, Sichuan, China	2006
16	HZ-16	Sichuan	Changdu, Sichuan, China	2006
17	HZ-17	Sichuan	Emei, Sichuan, China	2006
18	HZ-18	Guangzhou	Guangxi, China	2006
19	HZ-19	Guangzhou	Guangxi, China	2006
20	HZ-20	Guangzhou	Guangxi, China	2006
21	HZ-21	Guangzhou	Guangxi, China	2006
22	HZ-22	Guangzhou	Guangxi, China	2006
23	HZ-23	Guangzhou	Guangxi, China	2006
24	HZ-24	Guangzhou	Guangxi, China	2006
25	HSW-1	Hong Kong	Shanghai, China	2005
26	HSW-2	Hong Kong	Anhui, China	2005
27	HSW-3	Hong Kong	Henan, China	2005
28	HSW-4	Guangdong	Lushi, Henan, China	2005
29	HSW-5	Guangdong	Fanchang, Anhui, China	2005
30	HSW-6	Guangdong	Zunyi, Guizhou, China	2005
31	HSW-7	Guangdong	Zunyi, Guizhou, China	2005
32	HSW-8	Guangdong	Deqing, Guangdong, China	2005
33	HSW-9	Guangdong	Deqing, Guangdong, China	2005
34	DH-1	Hong Kong	Sichuan, China	2005
35	DH-2	Hong Kong	Sichuan, China	2005
36	DH-3	Hong Kong	Sichuan, China	2005
37	DH-4	Hong Kong	Sichuan, China	2006
38	DH-5	Hong Kong	Sichuan, China	2006
39	DH-6	Hong Kong	Sichuan, China	2006
40	DH-7	Hong Kong	Sichuan, China	2006

Chromatographic analysis for the LC-MS was carried out on a C_{18} column (Alltech, Alltima, 250 mm×4.6 mm i.d., 5 μm) at ambient temperature with a sample injection volume of 10 μl. The mobile phase, gradient conditions and flow rate were identical to those used in HPLC-DAD for HPLC fingerprinting. Perkin-Elmer SCIEX API 365 triple-quadrupole mass spectrometer (Applied Biosystems), equipped with an ion-spray (pneumatically assisted electrospray) interface was employed.

Solvents and Chemicals Analytical grade methanol (Labscan, Bangkok, Thailand) was used for the preparation of sample and standard solutions. HPLC grade acetonitrile (Labscan, Bangkok, Thailand), deionized water generated from a Milli-Q water system (Millipore, Bedford, MA, U.S.A.) and formic acid (Unichem, Warsaw, Poland) were used for preparation of mobile phase.

Reference Compounds Resveratrol, polydatin, emodin, physcion, rhein, chrysophanol and 2,3,5,4′-tetrahydroxystilbene-2-*O*-β-D-glucoside were purchased from the Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China).

Plant Materials Samples of HZ, HSW and DH were procured from a number of retailers in Hong Kong and collected in several cultivation bases in mainland China. The sources of the plant materials are listed in Table 1. These herbal samples were authenticated by Prof. Zhao Zhong-Zhen in the School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China. Voucher specimens were deposited in the Herbarium Centre, Hong Kong Baptist University. The samples were cut into small pieces, ground into powder and passed through a 20-mesh (0.9 mm) sieve. The ground powder was kept in cool and dry condition before use.

Sample Preparation An accurately weighed sample powder of 0.5 g was introduced into a 60 ml amber vial and 25 ml methanol was added. The mixture was sealed and sonicated for 60 min. It was centrifuged at 3500 rpm for 10 min. The extract was filtered through a 0.45 μm membrane filter. An aliquot of 10 μl solution was injected for HPLC analysis.

Data Analysis of Chromatogram The simulative mean chromatograms of HZ, HSW and DH were generated and the correlation coefficients of chromatograms of the samples were calculated by using the Computer Aided Similarity Evaluation System. The similarities of the chromatograms of HZ, HSW and DH were analyzed. The RRT and RPA of each characteristic peak with respect to the reference peak in chromatograms of HZ were also calculated.

Results and Discussion

Optimization of Chromatographic Conditions The choice of detection wavelength is a crucial step for developing a reliable fingerprint. For previous related chromatograms of HZ, those wavelengths commonly used for UV detection of polydatin and resveratrol were 291, 300, 303, 305 or 306 nm.[1,6,7,11—16] For emodin, the wavelengths usually used for quantitative analysis were 252, 254, 288 or 437 nm.[1,12,17,18,21] Besides, both resveratrol and emodin also demonstrated a strong absorption at 299 nm.[12] Therefore,

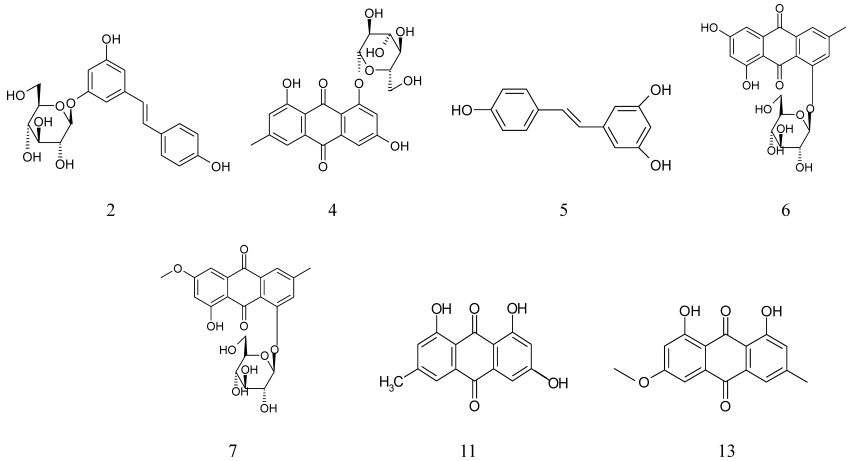

Fig. 1. Chemical Structures of the Seven Identified Chemical Compounds in HPLC Fingerprints of HZ: (2) Polydatin; (4) Emodin-1-*O*-β-D-glucopyranoside; (5) Resveratrol; (6) Anthraglycoside B; (7) Anthraglycoside A; (11) Emodin; (13) Physcion

Table 2. The Relative Retention Time (RRT) and Relative Peak Area (RPA) of Characteristic Peaks in Polygonum Cuspidatum Samples in Different Cultivated Areas

Peak/ Compound No.	Compound	Guangxi (HZ 1—7)		Sichuan (HZ 8—17)		Guangxi (HZ 18—24)	
		RRT	RPA	RRT	RPA	RRT	RPA
1	—	0.37±0.001	0.77±0.480	0.37±0.001	0.75±0.661	0.37±0.001	0.45±0.381
2	Polydatin	0.57±0.001	3.80±2.091	0.57±0.001	6.37±5.612	0.57±0.001	3.83±2.202
3	—	0.83±0.001	0.66±0.272	0.83±0.001	0.81±0.530	0.83±0.001	0.45±0.321
4	Emodin-1-*O*-*β*-D-glucopyranoside	0.91±0.001	0.62±0.350	0.91±0.001	0.55±0.491	0.91±0.001	0.48±0.270
5	Resveratrol	1.00	1.00	1.00	1.00	1.00	1.00
6	Anthraglycoside B	1.10±0.001	3.90±2.201	1.10±0.001	4.57±3.821	1.10±0.001	2.48±1.130
7	Anthraglycoside A	1.17±0.001	0.37±0.290	1.17±0.002	0.61±0.463	1.17±0.001	0.29±0.240
8	—	1.21±0.001	0.46±0.240	1.22±0.001	0.51±0.391	1.22±0.001	0.28±0.101
9	—	1.31±0.001	0.24±0.210	1.31±0.001	0.35±0.260	1.31±0.001	0.20±0.092
10	—	1.43±0.001	0.10±0.040	1.43±0.001	0.06±0.030	1.43±0.001	0.06±0.050
11	Emodin	1.60±0.001	2.92±1.120	1.60±0.001	2.32±1.062	1.60±0.001	2.39±0.960
12	—	1.61±0.001	0.43±0.173	1.61±0.001	0.16±0.071	1.61±0.002	0.17±0.091
13	Physcion	1.78±0.001	0.45±0.212	1.78±0.001	0.40±0.200	1.78±0.001	0.39±0.170

RRT and RPA are the ratio of retention time and peak area of each characteristic peak with reference to peak 5 (reference peak). The values of RRT and RPA are expressed as mean±S.D.

based on the previous experience and our trial, 290 nm was chosen as the optimal detection wavelength in the fingerprint study.

For the choice of extraction solvent, methanol, ethanol, 50% ethanol were used in previous studies.[6,7,11—18] The chemical compounds of interests were found soluble in these solvent systems. However, methanol was chosen as the extraction solvent in this study instead of ethanol and 50% ethanol. From a structural viewpoint, most of the chemical components such as compounds **2**, **5**, **11** and **13** in Rhizoma Et Radix Polygoni Cuspidati contain hydroxyl functionalities.[2—5] Using a relatively more polar solvent can enhance the extraction efficiency. Therefore, methanol gave the optimized extraction efficiency[22] in compare with ethanol. Besides, 60 min extraction at ambient temperature was retrieved to be the optimized conditions for sonication.

Method reproducibility and repeatability were evaluated by five injections of a sample solution (HZ-17) and five replicated analysis of samples (HZ-1), respectively. For reproducibility, the relative standard deviation (RSD) of retention time and peak area of compounds **2**, **5**, **11**, **13** were found within the ranges of 0.0003—0.021% and 0.29—2.73%, respectively. For method repeatability, the RSD of the RPA of compounds **2**, **11** and **13** in sample (HZ-1) were found to be 0.55, 1.65 and 1.31% (n=5), respectively.

HPLC Fingerprint of Rhizoma Et Radix Polygoni Cuspidati Altogether 24 authentic samples collected from Guangxi and Sichuan were analyzed. 13 common peaks and 8 compounds (including Rhein) were identified in the fingerprint. In general, the chromatographic patterns were found consistent to each other despite the peaks abundance were varied. Among the characteristic peaks, the peak abundance of compound **5** (resveratrol) were found generally consistent in all 24 chromatograms. Besides, peak 5 also eluted at a reasonable time within the chromatographic windows and possessed known pharmacological activities. Therefore, it was chosen as the reference peak.

Fingerprint of Rhizoma Et Radix Polygoni Cuspidati from Various Cultivation Origins The medicinal effects of traditional Chinese medicine are always influenced by the contents of active chemical components and therefore varied with the cultivated origins.[23] From the fingerprint profiles, the variation can be visualized in terms of peak abundance. By analyzing the RRT and RPA of each characteristic peak with respect to the reference peak, the quantitative relationship of Rhizoma Et Radix Polygoni Cuspidati from different cultivated areas was established (Table 2). The results retrieved that samples from Sichuan always contain highest contents of compounds **2**, **3**, **6**, **7**, **8** and **9**. Among these, compounds **2** and **7** were found almost 2-fold to those acquired in Guangxi.[11] However, the contents of compounds **5**, **11** and **13** were almost the same over the two origins. Therefore, Rhizoma Et Radix Polygoni Cuspidati from Sichuan can be easily distinguished from Guangxi by simply comparing the RPA of compounds **2** and **7**. It is worth noting that in previous studies, Rhein and chrysophanol appeared as characteristic chemical constituents in HZ.[24,25] However, our results retrieved that they were not present in all HZ samples. Therefore, both rhein and chrysophanol were not included as characteristic peaks in the fingerprinting profile of HZ.

Similarity Match of Chromatograms of Rhizoma Et Radix Polygoni Cuspidati For HZ, the correlation coefficient of each chromatogram to their simulative mean chromatogram was found as 0.956±0.025 (mean±S.D., n=24). The RRT and RPA of each characteristic peak with respect to the reference peak were analyzed (Table 3). The result showed that the relative contents of chemical components in samples from Guangxi and Sichuan were similar. The chromatographic patterns in the two origins were consistent (Figs. 3a—d).

The identities of seven compounds were elucidated in the chromatogram. Although the remaining six peaks cannot be structurally identified at the moment, they are classified as stilbene- and anthraquinone-typed compounds based on the absorption wavelengths. The simulative mean chromatogram of the 24 samples of HZ can therefore be used as a representative fingerprint of HZ to differentiate itself from HSW and DH (Fig. 3a).

Differentiation of Rhizoma Et Radix Polygoni Cuspidati from Radix Polygoni Multiflori HZ, HSW and DH

Table 3. The Relative Retention Time (RRT) and Relative Peak Area (RPA) of 13 Characteristic Peaks in Polygonum Cuspidatum

Peak/ Compound No.	Compound	HZ (n=24)	
		RRT	RPA
1	—	0.37±0.001	0.65±0.542
2	Polydatin	0.57±0.001	4.76±4.011
3	—	0.83±0.001	0.65±0.421
4	Emodin-1-*O*-*β*-D-glucopyranoside	0.91±0.001	0.53±0.392
5	Resveratrol	1.00	1.00
6	Anthraglycoside B	1.10±0.001	3.65±2.830
7	Anthraglycoside A	1.17±0.001	0.43±0.370
8	—	1.22±0.001	0.42±0.290
9	—	1.31±0.001	0.27±0.211
10	—	1.43±0.001	0.08±0.041
11	Emodin	1.60±0.001	2.49±1.020
12	—	1.61±0.001	0.24±0.161
13	Physcion	1.78±0.001	0.41±0.192

RRT and RPA are the ratio of retention time and peak area of each characteristic peak with respect to peak 5 (reference peak). The values of RRT and RPA are expressed as mean±S.D.

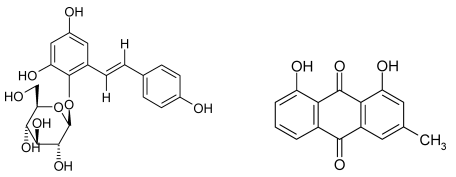

Fig. 2. Chemical Structures of the Identified Chemical Compounds in HPLC Fingerprints of HSW and DH (a) 2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucoside; (b) Chrysophanol

belong to the family Polygonaceae and contain similar types of chemical constituents, for instance, anthraquinone. The present developed HPLC fingerprint can be used to differentiate HZ from HSW and DH in terms of chromatographic similarities, compounds identities and their quantities. Nine batches of HSW collected from various origins were analyzed (Table 1). In general, the chromatograms of the nine HSW were similar to each other (Fig. 4). The correlation coefficient of each chromatogram to their simulative mean chromatogram was 0.962±0.043 (mean±S.D., n=9). However, the chromatographic profile of HSW was drastically different in compare with HZ in terms of characteristic peaks. The correlation coefficient of the nine HSW chromatograms to HZ simulative mean chromatogram (Fig. 6a) was only 0.087±0.055 (mean±S.D., n=9) and the correlation coefficient of the simulative mean chromatogram of HSW (Fig. 6b) to that of HZ was 0.078. Although compounds **6** and **11** were found in both species, they remain the highest peaks in HZ but in a much lower contents in HSW. Besides, the major compound 2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucoside (peak A) can be used as a distinctive feature to differentiate HSW from HZ (Figs. 2a, 3a, 4). On the other hand, compounds **2** and **5** were only available in HZ. Therefore, HZ and HSW can be differentiated from each other by comparing the relative quantity of compounds **6** and **11**, the presence of 2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucoside, or compounds **2** and **5** in their respective chromatograms.

Differentiation of Rhizoma Et Radix Polygoni Cuspidati from Radix Et Rhizoma Rhei Seven batches of DH were collected and analyzed to generate a mean chromatogram for differentiation from HZ (Fig. 6c). The correlation coefficient of each chromatogram to their simulative mean chromatogram was 0.947±0.021 (mean±S.D., n=7). However, the chromatographic profile of DH was found considerably different when compared with HZ in terms of characteristic chemical components. The correlation coefficient of the seven DH chromatograms to simulative mean chromatogram of HZ was only 0.303±0.047 (mean±S.D., n=7) and the correlation coefficient of the simulative mean chromatogram of HZ to that of DH was 0.318. Compounds **6**, **11** and **13** were found co-existed in both HZ and DH, with compounds **6** and **11** as the most abundant peaks in HZ (Figs. 6a, c). In addition, unidentified peak B and chrysophanol (peak C) were characteristic peaks in DH (Figs. 2b, 3a, 5). Furthermore, compounds **2** and **5** were the characteristic peaks in HZ chromatogram which were not present in DH. Therefore, HZ and DH can be distinguished from each other by either assessing the contents of compounds **6** and **11** or the presence of compounds **2**, **5** and chrysophanol in their fingerprinting profiles.

Identification of Chemical Components in HZ Fingerprints Five compounds were unequivocally identified together with 3 other compounds (peak 4, 6, 7) tentatively assigned basing on their on-line APCI-MS and UV data. Their retention time, APCI-MS and UV data are listed in Table 4.

Peaks 2, 5, 11 and 13 were unequivocally identified as polydatin, resveratrol, emodin and physcion together with rhein in the spiked studies. The protonated molecular ions $[M-H]^-$ were observed in the APCI-MS spectra for all compounds. Apart from the protonated molecular ion, other characteristic fragments were also present which can provide further evidences on their chemical identities. For polydatin, the characteristic fragment ions corresponding to $[M+HCOOH-H]^-$ and $[aglycone-H]^-$ by loss of the glucosidic unit (162 u) at m/z 435 and 227 were found. For emodin, the characteristic fragment ions corresponding to $[2M-H]^-$ and $[M-CO-H]^-$ at m/z 539 and 241 were located. For physcion, the characteristic fragment ions corresponding to $[M-COCH_3-H]^-$ and $[M-COCH_3-CO-H]$ at m/z 240 and 212 were identified. For rhein, the characteristic fragment ions corresponding to $[M+HCOOH-H]^-$, $[M-CO-H]^-$, $[M-CO_2-H]^-$ and $[M-2CO-H]^-$ at m/z 329, 255, 239 and 227 were noted. The UV results were found in agreement with those reported in literature.[26—29]

Owing to the unavailability of authentic compounds, the identities of peaks 4, 6 and 7 were tentatively elucidated using their UV and APCI-MS data with those in literatures. Protonated molecular ions $[M-H]^-$ were found in the APCI-MS spectra for all three compounds. For peak 7, the strongest fragment ion at m/z 283 was also assigned as $[aglycone-H]^-$. For peaks 4 and 6, the strongest fragment ion at m/z 269 was due to $[aglycone-H]^-$ with another fragment ion at m/z 241 corresponding to $[aglycone-CO-H]$. Concerning the relative retention ability and previous published works,[27] peaks 4, 6 and 7 were identified as emodin-1-*O*-*β*-D-glucopyranoside, anthraglycoside B and anthraglycoside A, respectively. Another piece of indirect evidence was about the contents of emodin and physcion, which increased no-

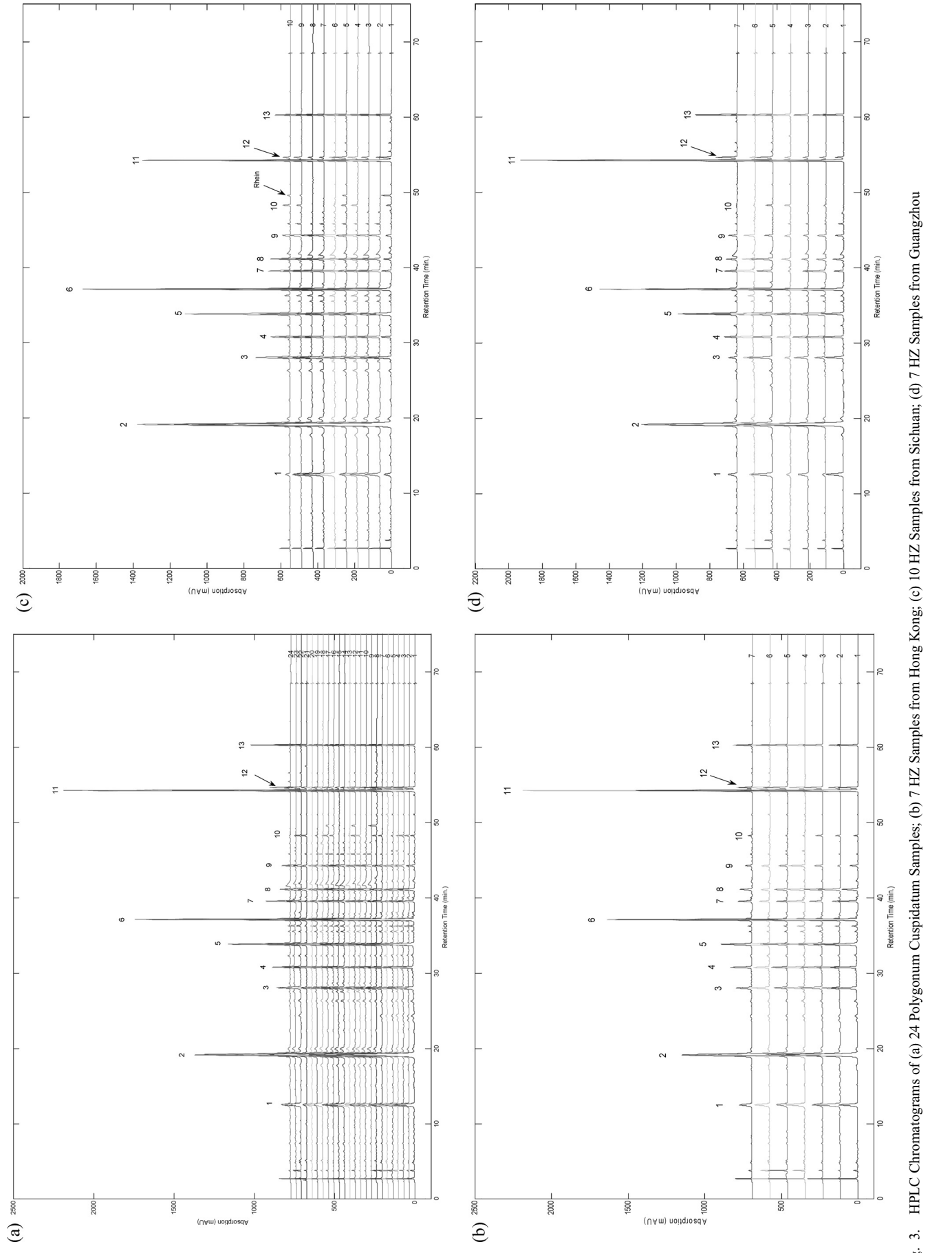

Fig. 3. HPLC Chromatograms of (a) 24 Polygonum Cuspidatum Samples; (b) 7 HZ Samples from Hong Kong; (c) 10 HZ Samples from Sichuan; (d) 7 HZ Samples from Guangzhou

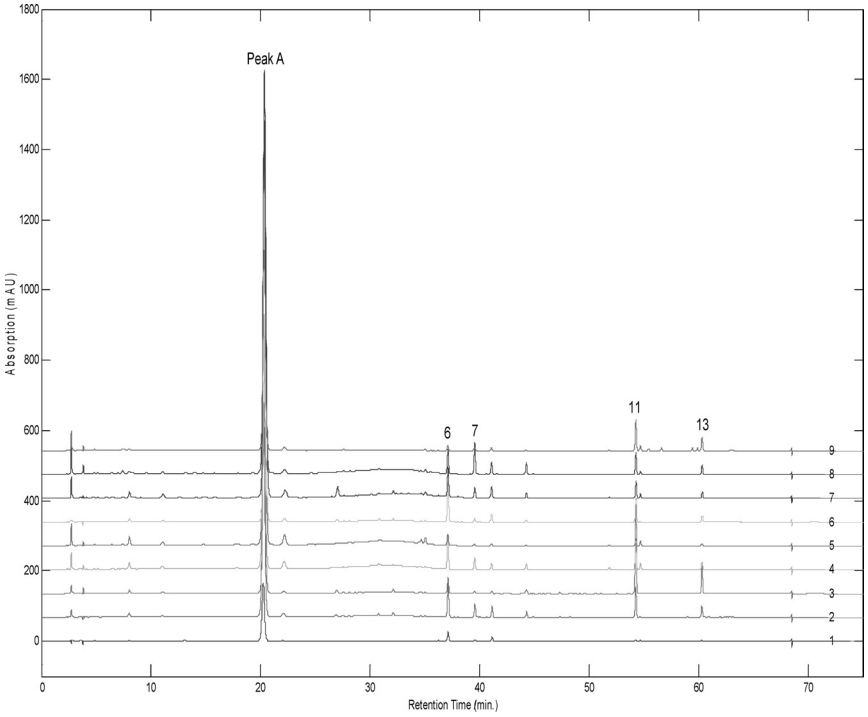

Fig. 4. HPLC Chromatogram of 9 Polygoni Multiflori Samples: Peak (6) Anthraglycoside B; (7) Anthraglycoside A; (11) Emodin; (13) Physcion; Peak A: 2,3,5,4′-tetrahydroxystilbene-2-*O*-*β*-D-glucoside

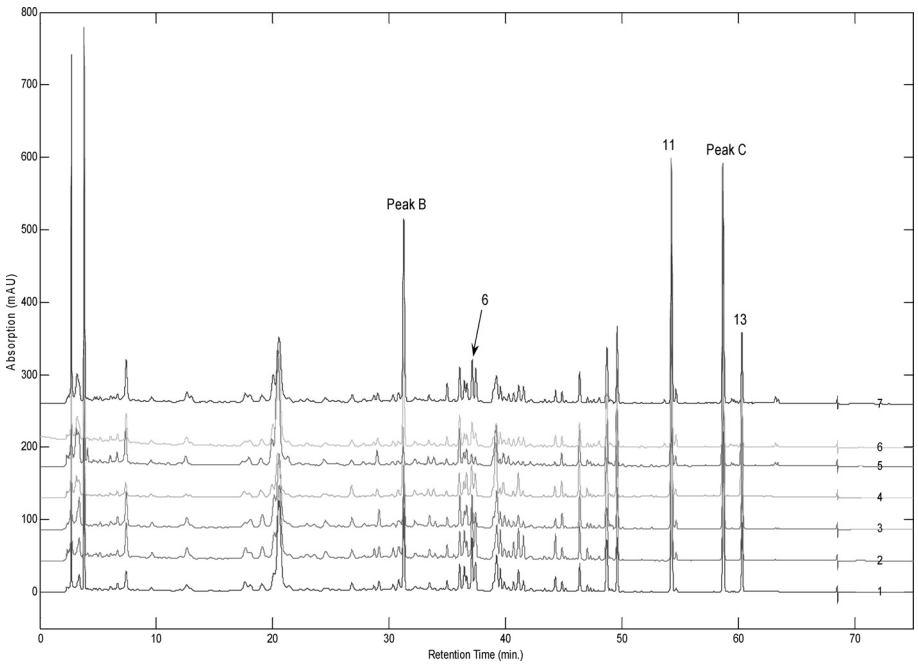

Fig. 5. HPLC Chromatogram of 7 Rheum Tanguticum Samples: Peak (6) Anthraglycoside B; (11) Emodin; (13) Physcion; Peak B: Unidentified Peak; Peak C: Chrysophanol

tably after hydrolysis (The results will be published in another paper soon). It is worth noting that the UV data were also in agreement with those in literature.[26—28)]

Conclusion

Rhizoma Et Radix Polygoni Cuspidati has been used as a traditional Chinese medicine for many years. Previously, the quality assessment was mainly relied on one or two marker compounds. In our study, altogether 24 authentic samples were collected and developed as a comprehensive fingerprint representing the entire chemical profile. The chromatograms of 24 HZ samples were found to be consistent with respect to the correlation coefficients of the chromatograms despite the contents of chemical components were varied among different cultivated origins. The simulative mean chromatogram of the 24 tested samples was therefore established and applied

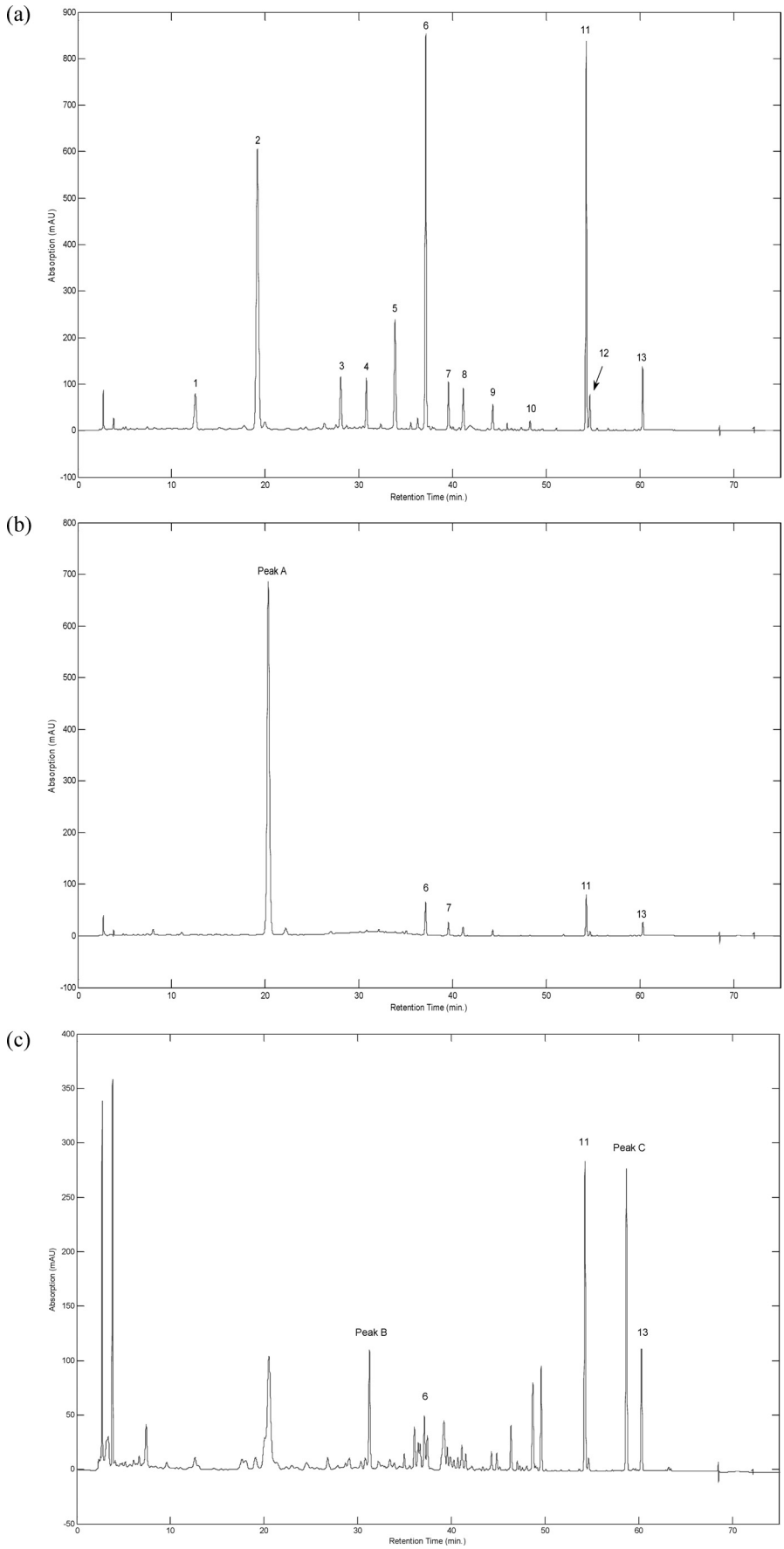

Fig. 6. Simulative Mean Chromatograms of (a) Polygonum Cuspidatum; (b) Polygoni Multiflori (c) Rheum Tanguticum with Identified Peaks: (2) Polydatin; (4) Emodin-1-*O*-*β*-D-glucopyranoside; (5) Resveratrol; (6) Anthraglycoside B; (7) Anthraglycoside A; (11) Emodin; (13) Physcion; Peak A: 2,3,5,4′-Tetrahydroxystilbene-2-*O*-*β*-D-glucoside; Peak B: Unidentified Peak; Peak C: Chrysophanol

Table 4. Chromatographic and Spectrometric Data of Eight Identified Compounds of Polygonum Cuspidatum

Peak/ Compound No.	t_R (min)	$[M-H]^-$ (m/z)	Other negative ions (m/z)	λ_{max} (nm)	Identification
2	20.33	389	435, 227	318, 303, 230	Polydatin
4	30.85	431	269, 241	286, 250, 225	Emodin-1-*O*-β-D-glucopyranoside
5	34.93	227	—	303, 230	Resveratrol
6	36.98	431	539, 269, 241	282, 272, 224	Anthraglycoside B
7	40.79	445	491, 283	282, 272, 244	Anthraglycoside A
—	49.81	283	329, 255, 239, 227	286, 258, 226, 202	Rhein[a)]
11	54.82	269	539, 241	288, 268, 253, 221	Emodin
13	61.22	283	240, 212	287, 266, 222	Physcion

a) Rhein was only found in five batches of Sichuan samples.

as a representative HPLC fingerprint of HZ.

The representative HPLC fingerprint was used as a way of total quality assessment and apply to differentiate Rhizoma Et Radix Polygoni Cuspidati from Radix Polygoni Multiflori and Radix Et Rhizoma Rhei. Although the three species belong to the same family Polygonaceae, they can be distinguished by either from the variation in chromatographic profiles or the contents of certain chemical constituents.

Five compounds (peaks 2, 5, 11, 13 and rhein) were unequivocally identified and three compounds (peaks 4, 6, 7) were tentatively assigned based on their on-line APCI-MS and UV data. In addition, by using the Computer Aided Similarity Evaluation System, the similarities of chromatograms for HZ, HSW and DH were analyzed and their respective mean chromatograms of samples were generated.

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