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Abstract

A high-performance liquid chromatography (HPLC) coupled with diode array detector (DAD) and electrospray ionization mass spectrometry (ESI/MS) method was developed for the simultaneous analysis of fourteen characteristic components, including piceid, 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, rhaponticoside, torachrysone-8-O-β-D-glucoside, emodin-1-O-β-D-glucoside, resveratrol, emodin-8-O-β-D-glucoside, chrysophanol-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, aloe-emodin, rhein, emodin, chrysophanol, and physcion, in roots of *Rheum officinale* Baill. (RO), *Polygonum multiflorum* Thunb. (PM) and *Polygonum reynoutria* Makino. (PR). The sample extraction was conducted on a Dionex pressurized liquid extraction (PLE) system, and the sample analysis was performed under the optimized HPLC-ESI/MS conditions. A comprehensive validation on the developed method was conducted, and the method presented a good sensitivity, repeatability and accuracy. The unique properties of the present method were validated by analyzing 15 related herb samples including 5 RO samples, 5 PM samples and 5 PR samples. Fourteen components were identified by online ESI/MS and by comparison with literature data and standard compounds, and eight of them were quantified by diode array detector (DAD) simultaneously. The key characteristic components for the quality control of a single herb were found by comparing the chemical composition of these three herbs. The overall procedure is highly precise and accurate which is considered suitable for qualitative and quantitative analysis of a large number of samples.

Keywords: HPLC-DAD-ESI/MS; Pressurized liquid extraction; Stilbenes; Anthraquinones; Quality control

Introduction

Polygonaceous herbs play an important role in various traditional medical systems, and roots of *Rheum officinale* Baill. (RO), *Polygonum multiflorum* Thunb. (PM) as well as *Polygonum reynoutria* Makino. (PR) are three of them. RO is one of the most commonly used traditional laxative drugs in Europe and Asia, which officially listed in the British pharmacopoeia,[1] European pharmacopoeia,[2] Chinese pharmacopoeia[3] and Japanese pharmacopoeia.[4] The demand for RO is still increasing and it is obtained mainly from China.[5] PM and PR, which are well known herbs in Asia and also listed in the Chinese pharmacopoeia,[3] were included in our investigations owing to their therapeutic purpose which is partly identical to that of RO in folk medicines.[6-10] Although these related medicinal plants from the same family or genus have been used for similar therapeutic purpose in various traditional medicines, the qualitative and quantitative differences of these related plants regarding chemical composition are usually unknown thereby limiting further pharmacological research and quality evaluation.

A number of studies have been performed on the analyses of the constituents in these medicinal plants by using colorimetry,[1, 2] high performance liquid chromatography (HPLC),[3, 4, 11-13] liquid chromatography-mass spectrometry (LC-MS)[12, 14] and capillary electrophoresis (CE),[15] respectively. However, the existing studies mainly focus on the quantitative analysis of a single herb or on qualitative analysis of various herbs.[3, 4, 12-14] To date, quantitative analysis of various herbs simultaneously by a single method has not been successful, and systematic comparison of the chemical composition in these herbs is seldom reported. Additionally, it is widely accepted that multiple constituents are responsible for the therapeutic effects of herbal products, and the current quality assessment based on a few marker compounds cannot accurately reflect the quality of these herbal products.[16, 17] Therefore, to compare the chemical composition of these three polygonaceous herbs and to find out the key characteristic components for the quality evaluation, the necessity for a quantitative comparison based on chemical identification of the main constituents is urgent.

The present study describes the development of a highly precise and accurate method for comparison of the main chemical constituents in RO, PM as well as PR herbs by using pressurized liquid extraction (PLE) and HPLC-ESI/MS techniques. The results revealed that stilbenes and anthraquinones are the main constituents in these polygonaceous herbs, and that the abundance of the main constituents varied between and within the species. The results also showed that chrysophanol, 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside and piceid are the key characteristic components in RO, PM and PR, respectively. Therefore, these characteristic components should be chosen as the analytical markers for the quality evaluation as well as chemical authentication of these related herbs.

Experimental

Chemicals, reagents and materials

Acetonitrile of HPLC grade was from Lab-scan (Bangkok, Thailand), methanol and the other reagents were of analytical grade and were purchased from Lab-scan (Bangkok, Thailand). Water was purified using a Milli-Q water system (Millipore, Bedford, MA, USA). Piceid, 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, rhaponticoside, resveratrol, aloe-emodin, emodin, rhein, chrysophanol and physcion were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Emodin-8-O-β-D-glucoside and physicoin-8-O-β-D-glucoside were isolated from *Rheum officinale* Baill. in our laboratory and their identities were confirmed by the comparison of their respective NMR and mass spectra with the published data.[18, 19] The purity of these chemical standards was determined to be more than 98% by normalization of the peak areas detected by high performance liquid chromatography-diode array detector (HPLC-DAD). Their chemical structures are shown in Fig. 1.

The identity and sample source of the test samples are listed in Table 1. Identity of the herbs was confirmed by means of geographical origin identification, macroscopic identification, microscopic identification, and physicochemical identification by comparing with voucher specimens. Voucher specimens were deposited in the Herbarium, Sichuan University, China (No. 10:3:8 for RO, No. 22:3:4 for PM, and No. 22:3:10 for PR, respectively).

Insert Fig. 1. and Table 1 here

Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE) was performed on a Dionex ASE 200 PLE system (Dionex, Sunnyvale, CA, USA). The raw materials of these herbs were dried at 45 ^{o}C for 6 h and were grounded into powder of 0.45-0.9 mm (20-40 mesh sieves). Powder of materials (0.2 g) was mixed with diatomaceous earth (0.5 g) and placed into 11-mL stainless steel extraction cell, respectively. The herb samples were extracted under the optimized conditions: solvent, methanol; temperature, 150 ^{o}C; static extraction time, 15 min; pressure, 1200 psi.; static cycle, 1 and 45% of the flush volume. The extract was transferred into a 25-mL volumetric flask, which was filled up to the calibration mark with methanol. The test solution was filtered through a syringe filter (0.45-μm, Alltech, Beerfield, IL, USA), then an 10-μL aliquot of the solution was injected for HPLC-DAD and LC-MS analysis. Sample duplicates were prepared as shown above for analysis.

HPLC-DAD and LC-MS analysis

An Agilent 1100 series HPLC-DAD system consisting of a vacuum degasser, binary pump, autosampler, thermostated column compartment and DAD (Hewlett Packard, Palo Alto, CA USA) was used for quantitative analysis and UV spectra acquisition. UV detector was set to 290 nm.

For chromatographic analyses, an Alltima C_{18} column (250 mm×4.6 mm I.D., 5 μm, Alltech Associates) with an Alltima C_{18} guard column (7.5 mm×4.6 mm I.D., 5 μm) was used. The mobile phase consisted of water (A) and acetonitrile (B) using a gradient program of 15-20% (B) in 0-10 min, 20-53% in 10-40 min, and 53-100% in 40-60 min. The solvent flow rate was 1 mL min^{-1} and the column temperature was set to 30 ^{o}C.

For mass spectrometric analyses, an Applied Biosystems/PE-Sciex API 365 high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI/MS) system (Applied Biosystems, Foster City, AS, USA) was used. All of the operation, the acquiring and analysis of data were controlled by Chemstation software (Agilent Technologies, Palo Alto, CA USA). The LC conditions for LC-MS analysis were the same as those used for HPLC-DAD analysis. The conditions for MS analysis in the positive ion mode were as follows: drying gas (air), flow rate, 8 L min^{-1}; gas temperature, 400 ^{o}C; scan range, 120-500 m/z; orifice voltage, 91 V; focusing voltage, 370 V; electrospray voltage, 5000 V.

Validation of the present method and quantification of the main constituents

Calibration curves were established for each standard compound at different concentrations (1-1000 mg L^{-1} in methanol). Reproducibility was evaluated in intra- and inter-day assays. Chemical standards with 50, 100 and 200% of the quantified levels of constituents were prepared and used with the samples for recovery studies. All herb samples collected in various regions were compared quantitatively using the present method.

Results and discussion

Optimization of pressurized liquid extraction (PLE) conditions

Pressurized liquid extraction (PLE) is a fully automated system, requiring far smaller quantities of solvent than traditional methods for the extraction of the test compounds from solid matrices.[20-22] Regarding extraction of herb samples, methanol and ethanol were evaluated as extraction solvents. The results demonstrated that the methanol extraction efficacy was higher than ethanol extraction efficacy in the PLE experiments, which might be related to low viscosity and high permeability of methanol. Therefore, methanol was used as the solvent for sample extraction and for preparation of the standard solutions. The extraction procedure, including extraction temperature (100, 120, 150 ^{o}C), pressure (1200, 1500, 1800 psi), time (10, 15, 20 min) and flush volume (30, 45, 60 %), was

further optimized and validated using an orthogonal test $L_9(3^4)$. The optimization was evaluated by determining the spiked samples. Spiked samples were prepared by adding appropriate standards to dry blank dregs, and then extracting the spiked samples according to the orthogonal test $L_9(3^4)$. The extract was determined to calculate the recovery and to assess the effect of optimization. Finally, the conditions of PLE were optimized as follows: solvent, methanol; temperature, 150 ^oC; static extraction time, 15 min; pressure, 1200 psi.; static cycle, 1 and 45% of the flush volume (details are listed in Table S1 and S2 of the Electronic Supplementary Information).

Optimization of HPLC conditions

By comparing the HPLC chromatograms of the herbs recorded at different wavelengths from 210 to 500 nm and the corresponding UV absorption maximum for each chemical standard, it was found that a wavelength of 290 nm could represent the profile of the main constituents. The UV absorption maximum for each identified compound is listed in Table 2 and the representative HPLC chromatograms are shown in Fig. 2.

Insert Table 2 and Fig. 2. here

Calibration curves, limits of detection and quantification

Methanol stock solutions contain eight analytes were diluted to appropriate concentration for the construction of calibration curves (1-1000 mg L^{-1} in methanol). Six concentrations of the analyte solution were analyzed in triplicate, and then the calibration curves were constructed by plotting the peak areas versus the concentration of each analyte. The limits of detection (LOD) and quantification (LOQ) under the present chromatographic conditions were determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively. The results are shown in Table 3.

Insert Table 3 here

Precision and accuracy

Intra- and inter-day variations were chosen to determine the precision of the present method. For intra-day variability test, the respective herb samples were analyzed for three replicates within one day; while for inter-day variability test, the solutions were analyzed for inconsecutive three days. Variations were expressed by the relative standard deviations (RSD) for intra- and inter-day, which were less than 1.83% and 2.12%, respectively (see Table 4).

Chemical standards with 50, 100 and 200% of the quantified level of constituents were prepared and used with the samples for recovery. The recoveries of eight analytical markers were calculated,

and the results demonstrated that the overall analytical procedure is reproducible and accurate and is therefore suitable for high throughput analysis of a large number of samples (see Table 5).

Insert Table 4 and Table 5 here

LC-MS analysis of fourteen characteristic components in the herbs

The positive ion mode was found to be more sensitive in the present method, and most of the constituents exhibited their quasi-molecular ions $(M+H)^+$ and $(M+Na)^+$ in this mode. Based on the comparison with standard compounds, eleven peaks were unambiguously identified as piceid (**1**), 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside (**2**), rhaponticoside (**3**), resveratrol (**6**), emodin-8-O-β-D-glucoside (**7**), physicoin-8-O-β-D-glucoside (**9**), aloe-emodin (**10**), rhein (**11**), emodin (**12**), chrysophanol (**13**) and physcion (**14**). Another three peaks were tentatively identified as torachrysone-8-O-β-D-glucoside (**4**), emodin-1-O-β-D-glucoside (**5**) and chrysophanol-8-O-β-D-glucoside (**8**) by comparing their m/z values and UV spectra with literature data.[18, 23-26] The results are listed in Table 2 and the structures of the identified compounds are shown in Fig. 1. From the analysis results, it is obvious that the major types of constituents in these polygonaceous herbs are stilbenes and anthraquinones. Moreover, the results also demonstrated that compounds of similar chemical type exist in plants of the same family or genus.

Quantification of eight characteristic components in the herbs

The PR, PM and RO samples collected from various regions were determined with the present method, and the results are listed in Table 1. From Table 1, it is observed that compounds of the same chemical types (stilbenes and anthraquinones) make up the main constituents of PR, PM and RO herbs, but the difference of stilbenes in the three herbs is more significant than that of anthraquinones. Piceid exists in PR, which has been prescribed for the treatment of cardiovascular diseases; 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside is dominant in PM, which is effective for anti-aging and anti-atherosclerotic, reducing brain pathologic atrophy and promoting learning and memory ability. Thus, PR and PM have been additionally employed as hypolipidemic and tonic, especially against hyperlipidemia and amnesia in Chinese and Japanese folk medicines.[6, 8, 27-30] Although the abundance of rhaponticoside is higher in RO, anthraquinone derivates as laxative components in RO are more potent. Therefore, RO has been mainly used as a botanical source of rhubarb to prescribe for the treatment of constipation.[1-5]

From Table 1 and Fig. 2, it is also observed that emodin-8-O-β-D-glucoside, physcion-8-O-β-D-glucoside, emodin and physcion are common anthraquinone compounds in the three herbs. PR, PM and RO have almost the same anthraquinones, which are, however, different in content. The mean abundance of anthraquinones in RO is higher than that in PR or PM, and the

variation in anthraquinones content between PR and PM is not greatly significant. Additionally, aloe-emodin and chrysophanol are only found in RO. This result revealed that PR and PM have close relationship (be plants of *Polygonum* genus in polygonaceae family), but RO is far away from PR or PM in plant taxonomy.

The above results also demonstrated that piceid, 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, and chrysophanol are the key characteristic components in PR, PM and RO, respectively. Therefore, these characteristic components could be chosen as the analytical markers for the quality evaluation and chemical authentication of the three related polygonaceous herbs.

Conclusions

This study developed an HPLC-ESI/MS method for simultaneous analysis of 14 characteristic components including stilbenes and anthraquinones in roots of *Rheum officinale* Baill., *Polygonum multiflorum* Thunb. as well as *Polygonum reynoutria* Makino.. The key characteristic components of a single herb for quality evaluation were found by comparing the chemical composition of these related polygonaceous herbs. The results showed that the developed method has good precision, accuracy and sensitivity, which is helpful to control the quality and safety of these herbs.

Acknowledgements

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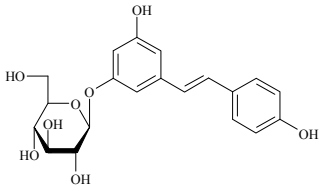
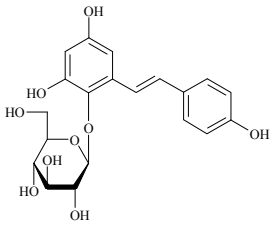
Legends for Figures

Fig. 1 The structures of characteristic compounds in the HPLC chromatograms.

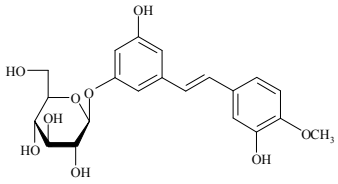
Fig. 2 Typical HPLC chromatograms of (A) *Polygonum reynoutria* Makino., (B) *Polygonum multiflorum* Thunb. and (C) *Rheum officinale* Baill..



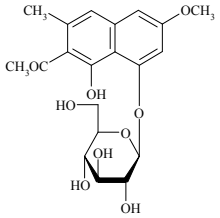
Piceid (**1**)



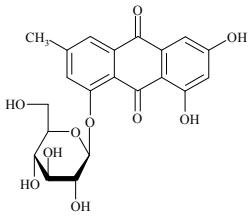
2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside (**2**)



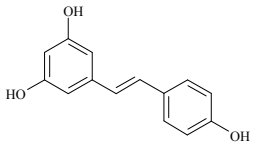
Rhaponticoside (**3**)



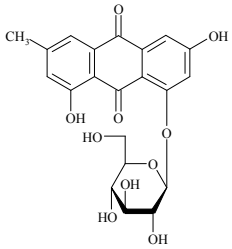
Torachrysone-8-O-β-D-glucoside (**4**)



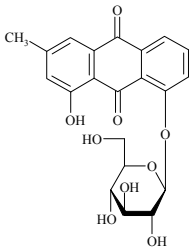
Emodin-1-O-β-D-glucoside (**5**)



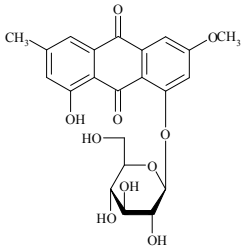
Resveratrol (**6**)



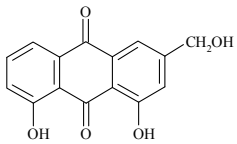
Emodin-8-O-β-D-glucoside (**7**)



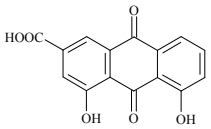
Chrysophanol-8-O-β-D-glucoside (**8**)



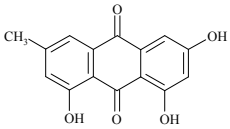
Physcion-8-O-β-D-glucoside (**9**)



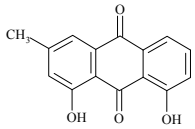
Aloe-emodin (**10**)



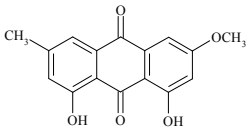
Rhein (**11**)



Emodin (**12**)



Chrysophanol (**13**)



Physcion (**14**)

Fig. 1

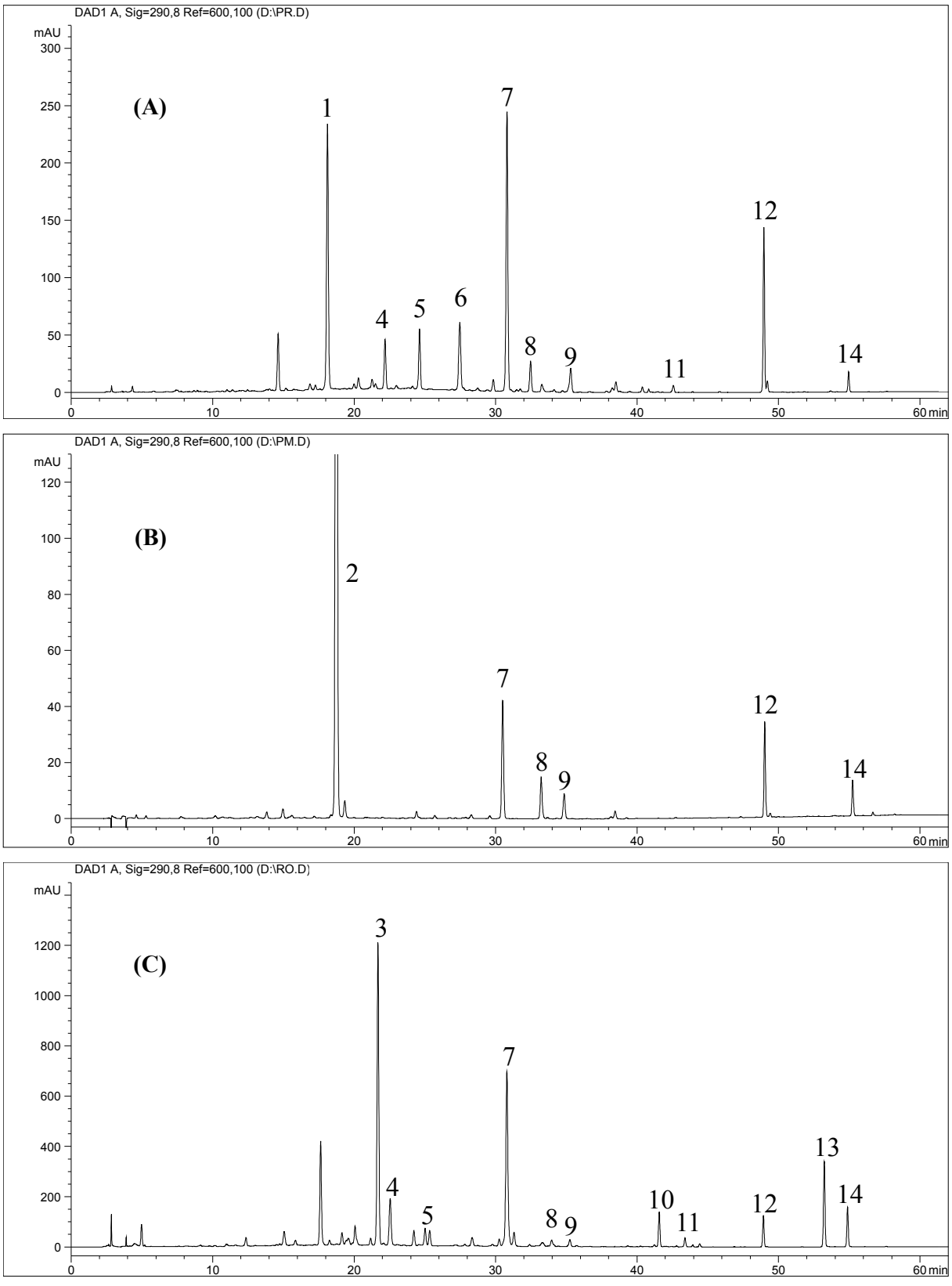

DAD1 A, Sig=290,8 Ref=600,100 (D:\PR.D)
mAU
(A)
1
4
5
6
7
8
9
11
12
14
60 min
DAD1 A, Sig=290,8 Ref=600,100 (D:\PM.D)
mAU
(B)
2
7
8
9
12
14
60 min
DAD1 A, Sig=290,8 Ref=600,100 (D:\RO.D)
mAU
(C)
3
4
5
7
8
9
10
11
12
13
14
60 min

Fig. 2

Legends for Tables

Table 1 Abundance of eight components in the herbal samples

Table 2 The on-line detected chromatographic and spectrometric data of the identified compounds in the HPLC chromatograms

Table 3 Linearity calibration curve factors and limits of detection (LOD) for the investigated compounds

Table 4 Intra- and inter-day variability for the assays of the investigated compounds

Table 5 Recoveries for the assays of the investigated compounds

Table 1

Materials [a]	Source	Abundance of eight compounds (mg g^{-1})							
		(**1**) [b]	(**2**)	(**3**)	(**7**)	(**9**)	(**12**)	(**13**)	(**14**)
PR-1	Zhuxi, Hubei, China	2.29 ± 0.04 [c]	- [d]	-	2.23 ± 0.04	0.89 ± 0.011	1.03 ± 0.012	-	0.31 ± 0.006
PR-2	Yangshuo, Guangxi, China	1.48 ± 0.03	-	-	1.36 ± 0.03	0.54 ± 0.009	1.25 ± 0.009	-	0.52 ± 0.005
PR-3	Dali, Yunnan, China	4.79 ± 0.09	-	-	1.90 ± 0.03	0.61 ± 0.013	0.69 ± 0.008	-	0.26 ± 0.003
PR-4	Minxi, Fujian, China	5.60 ± 0.12	-	-	0.74 ± 0.02	0.47 ± 0.008	1.56 ± 0.020	-	0.37 ± 0.006
PR-5	Xianyang, Shanxi, China	4.27 ± 0.08	-	-	2.82 ± 0.05	0.72 ± 0.011	0.53 ± 0.005	-	0.43 ± 0.009
PM-1	Kaiyang, Guizhou, China	-	30.27 ± 0.17	-	0.37 ± 0.01	0.29 ± 0.007	0.49 ± 0.005	-	0.12 ± 0.002
PM-2	Wanyuan Sichuan, China	-	46.30 ± 0.31	-	1.17 ± 0.03	0.37 ± 0.005	0.09 ± 0.001	-	0.16 ± 0.003
PM-3	Qianjiang, Sichuan, China	-	27.61 ± 0.18	-	0.57 ± 0.01	0.56 ± 0.008	0.07 ± 0.001	-	0.25 ± 0.005
PM-4	Tianlin, Guangxi, China	-	28.65 ± 0.14	-	0.77 ± 0.02	0.20 ± 0.005	0.10 ± 0.001	-	0.18 ± 0.003
PM-5	Yongzhou, Hunan, China	-	38.69 ± 0.25	-	0.53 ± 0.01	0.19 ± 0.002	0.08 ± 0.001	-	0.17 ± 0.002
RO-1	Longxi, Gansu, China	-	-	12.35 ± 0.22	6.17 ± 0.10	1.19 ± 0.015	0.91 ± 0.006	2.41 ± 0.02	1.46 ± 0.019
RO-2	Qingyang, Gansu China	-	-	24.61 ± 0.65	3.65 ± 0.06	1.53 ± 0.020	1.88 ± 0.023	4.38 ± 0.07	2.59 ± 0.032
RO-3	Mianyang, Sichuan China	-	-	25.69 ± 0.43	8.01 ± 0.11	1.33 ± 0.016	0.69 ± 0.008	1.81 ± 0.02	1.29 ± 0.029
RO-4	Nanchuan, Sichuan China	-	-	8.94 ± 0.26	4.64 ± 0.09	0.72 ± 0.013	0.83 ± 0.010	2.47 ± 0.05	2.12 ± 0.028
RO-5	Xining, Qinghai, China	-	-	24.05 ± 0.40	7.59 ± 0.17	1.36 ± 0.026	1.53 ± 0.019	1.89 ± 0.02	2.47 ± 0.032

[a] PR-1 to PR-5: roots of *Polygonum reynoutria*; PM-1 to PM-5: roots of *Polygonum multiflorum*; RO-1 to RO-5: roots of *Rheum officinale*.

[b] (**1**): Piceid; (**2**): 2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside; (**3**): Rhaponticoside; (**7**): Emodin-8-glucoside; (**9**): Physcion-8-glucoside; (**12**): Emodin; (**13**): Chrysophanol; (**14**): Physcion.

[c] Values shown are mean ± SD (n = 3).

[d] Undetected

Table 2

Peak	Retention time (min)	Identification	$[M+H\text{-glu}]^+$ (*m/z*)	$[M+H]^+$ (*m/z*)	$[M+Na]^+$ (*m/z*)	λ_{max} (nm)
1	18.1	Piceid	229	391	413	220, 318
2	18.8	2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside	245	407	429	214, 320
3	21.7	Rhaponticoside	259	421	443	220, 326
4	22.4	Torachrysone-8-O-β-D-glucoside	247	409	431	220, 320
5	24.8	Emodin-1-O-β-D-glucoside	271	433	455	284, 426
6	27.5	Resveratrol	-	229	251	220, 306
7	30.7	Emodin-8-β-D-glucoside	271	433	455	282, 424
8	33.2	Chrysophanol-8-O-β-D-glucoside	255	417	439	282, 426
9	35.1	Physcion-8-β-D-glucoside	285	447	469	272, 422
10	41.6	Aloe-emodin	-	271	293	290, 418
11	43.0	Rhein	-	285	307	284, 422
12	49.0	Emodin	-	271	293	288, 440
13	53.2	Chrysophanol	-	255	277	258, 430
14	55.0	Physcion	-	285	307	288, 436

Table 3

Peak	Compounds	Slope (*A*)	Intercept (*B*)	R^2	LOD (ng)	LOQ (ng)
1	Piceid	31.35	-3.17	0.9999	1.32	5.60
2	2,3,5,4'-Tetrahydroxystilbene -2-O-β-D-glucoside	37.42	-2.85	0.9999	1.48	6.63
3	Rhaponticoside	30.36	-4.74	0.9999	2.29	7.25
7	Emodin-8-β-D-glucoside	18.57	+2.08	0.9999	1.34	5.07
9	Physcion-8-β-D-glucoside	14.83	+1.70	0.9999	1.73	6.12
12	Emodin	39.84	-1.82	0.9998	0.71	3.54
13	Chrysophanol	22.87	+2.38	0.9998	1.70	6.32
14	Physcion	22.57	-1.26	0.9997	0.91	4.28

Table 4

Compounds	Assay on day 1 Determined content (mg g^{-1})	Assay on day 3 Determined content (mg g^{-1})	Assay on day 5 Determined content (mg g^{-1})	Inter-day RSD (%)
Piceid	2.29 ± 1.75 [a]	2.33 ± 1.72	2.27 ± 1.69	1.33
2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside	30.27 ±0.56	30.65 ±0.59	30.04 ±0.61	1.02
Rhaponticoside	12.35 ± 1.78	12.26 ± 1.68	12.14 ± 1.83	0.86
Emodin-8-β-D-glucoside	6.17 ± 1.62	6.05 ± 1.63	6.18 ± 1.67	1.18
Physcion-8-β-D-glucoside	1.19 ± 1.26	1.21 ± 1.16	1.16 ± 1.22	2.12
Emodin	0.91 ± 0.66	0.93 ±1.13	0.94 ± 0.87	1.65
Chrysophanol	2.41 ± 0.83	2.39 ± 1.15	2.34 ± 0.97	1.51
Physcion	1.46 ± 1.30	1.43 ± 1.25	1.48 ± 1.41	1.73

[a] Values shown are mean ± RSD (n = 3).

Table 5

Compounds	Recovery at three spike level [a]			Mean recovery
	50 (%)	100 (%)	200 (%)	(%)
Piceid	97.32 ± 1.72 [b]	98.04 ± 1.36	101.25 ± 1.90	98.87 ± 2.12
2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucoside	99.02 ± 1.43	98.23 ± 1.12	102.98 ± 1.65	100.08 ±2.54
Rhaponticoside	98.17±1.41	101.43 ±1.22	98.75 ±1.06	99.45 ± 1.75
Emodin-8-β-D-glucoside	97.67 ±1.48	99.22 ±0.97	95.34 ± 1.16	97.41 ± 2.00
Physcion-8-β-D-glucoside	103.56 ±1.30	99.94 ±1.53	101.59 ± 1.18	101.70 ± 1.78
Emodin	96.27 ± 0.87	99.31 ± 1.03	98.45 ± 0.94	98.01 ± 1.60
Chrysophanol	95.62 ± 1.21	98.75 ± 1.29	96.84 ± 1.48	97.07 ± 1.63
Physcion	98.72 ±1.32	100.47 ±0.88	101.29 ± 1.06	100.16 ± 1.31

[a] Recovery (%) = 100 × (amount found – original amount)/amount spiked.

[b] Values shown are mean ± RSD (n = 3).

Electronic Supplementary Information

Preparation of spiked samples for optimization of PLE conditions

Spiked samples were prepared by adding appropriate amounts of eight standards solutions to dry blank dregs, and the final contents of the standards in the spiked samples were: piceid, 5 mg/g; 2,3,5,4'-tetrahydroxystilbene-2-O-β-D-glucoside, 15 mg/g; rhaponticoside, 10 mg/g; emodin-8-glucoside, 3 mg/g; physcion-8-glucoside, 1 mg/g; emodin, 0.5 mg/g; chrysophanol, 2 mg/g; physcion, 2 mg/g. After drying, the spiked samples (0.2 g) was mixed with diatomaceous earth (0.5 g) and placed into 11-mL stainless steel extraction cell, respectively. Extraction of the spiked samples according to the orthogonal test $L_9(3^4)$ (Table S1). The extract was transferred into a 25-mL volumetric flask, which was filled up to the calibration mark with methanol. The test solution was determined with the present HPLC method, and the total determined amount of the spiked standards was calculated using the calibration curves in Table 3. The recovery=100*(total amount of the determined standards/ total amount of the spiked standards). From the results in Table S2, the condition of A3B1C2D2 (namely extraction temperature, 150 ^{o}C; pressure, 1200 psi; time, 15 min and flush volume, 45%) was chosen as the optimized PLE conditions.

Table S1 Factors and levels

Levels	A: Temperature (^{o}C)	B: Pressure (psi)	C: Time (min)	D: Flush volume (%)
1	100	1200	10	30
2	120	1500	15	45
3	150	1800	20	60

Table S2 Results of $L_9(3^4)$ orthogonal test

	Four factors				Recovery of the spiked compounds (%)
Test No.	A	B	C	D	
1	1	1	1	1	65
2	1	2	2	2	67
3	1	3	3	3	68
4	2	1	2	3	70
5	2	2	3	1	59
6	2	3	1	2	67
7	3	1	3	2	89
8	3	2	1	3	75
9	3	3	2	1	80
K1	200	224	207	204	
K2	196	201	217	223	
K3	244	215	216	213	
Extreme deviation	48	23	10	19	