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A rapid method for simultaneous determination of 14 phenolic compounds in Radix Puerariae using microwave-assisted extraction and ultra high performance liquid chromatography coupled with diode array detection and time-of-flight mass spectrometry

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ABSTRACT

A microwave-assisted extraction (MAE) and ultra high performance liquid chromatography coupled with diode array detection and time-of-flight mass spectrometry (UHPLC-DAD-TOF-MS) method was developed for simultaneous determination of 14 phenolic compounds in the root of *Pueraria lobata* (Wild.) Ohwi and *Pueraria thomsonii* Benth. Operational conditions of MAE were optimized by central composite design (CCD). The optimized result was 65% ethanol as extraction solvent, 17 mL of extraction volume, 100 °C of extraction temperature and 2 min of hold time. A Zorbax SB C_{18} (50 mm × 4.6 mm I.D., 1.8 μm) and gradient elution were used during the analysis. The chromatographic peaks of 14 investigated compounds in samples were successfully identified by comparing their retention time, UV spectra and TOF mass data with the reference substances. All calibration curves showed good linearity ($r > 0.9997$) within the test ranges. The intra-day and inter-day variations were less than 1.77% and 2.88%, respectively. The developed method was successfully applied to determine the investigated compounds in 10 samples of Radix Puerariae Lobatae and Radix Puerariae Thomsonii, respectively. The result indicated that MAE and UHPLC-DAD-TOF-MS system might provide a rapid method for the quality control of Radix Puerariae.

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1. Introduction

Radix Puerariae (RP) has been widely used as herbal medicine and dietary supplement in eastern Asia [1,2]. It includes dried Radix Puerariae Lobatae (RPL) and Radix Puerariae Thomsonii (RPT) [3]. The phenolic compounds in RP have been demonstrated to have multiple pharmacological activities, such as effect on reproductive organ development [4], prevention of bone loss [5], anti-cancer action [6,7], neuroprotective effect [8,9], estrogenic activity [10,11] and anti-oxidative activity [12,13]. Therefore, it is necessary to develop a method for the rapid identification and quantification of these phenolic compounds. Up to now, a number of extraction methods, including low temperature soaking [14,15], ultrasonic extraction (UE) [13,16–18], reflux extraction (RE) [3,19,20] and pressurized liquid extraction (PLE) [16,21] have been developed for extraction of phenolic compounds from RP. But these methods usually need long extraction time and large amount of solvent consumption. Meanwhile, high performance thin-layer chromatography (HPTLC) [18], high performance capillary electrophoresis (HPCE) [19,20] and high performance liquid chromatography (HPLC) [1,21–24] were used to analyze phenolic compounds in RP. However, these technologies suffered from long analysis time [1,18,21–24], low resolution [18], low sensitivity [18] and/or few analytes [1,19,21,22].

As a fast and effective extraction method, microwave-assisted extraction (MAE) was first reported by Ganzler et al. [25]. And then it was widely used in sample preparation like extracting isoflavones from soybean [26] and drying isoflavones extract from RPL [27]. Modern physical chemistry studies indicated that the large dielectric constant solvent, such as water and ethanol, absorbs microwave energy and produces intense molecular vibration, which leads to simultaneous heating up of whole solvent and samples [28]. Thus, MAE using water and ethanol as a mixture solvent could obtain high extraction efficiency. Comparing with other techniques such as PLE, RE and UE, MAE reduces extraction time, solvent consumption and increases extraction efficiency. According to the previous report, MAE was applied to the extraction of RP [29]. However, some chemical properties of puerarin, such as solubility, were different from isoflavones aglycones, puerarin was chosen as the only evaluating indicator to optimize the extraction condition, which could

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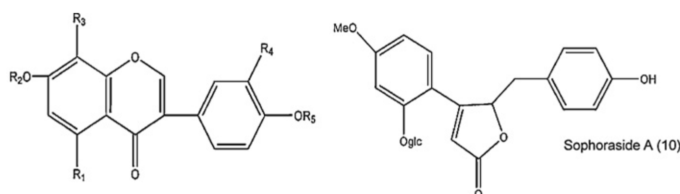

	Compounds	R1	R2	R3	R4	R5	Formula	Accurate Mass
1	puerarin-4'-*O*-glucoside	-H	-H	-glc	-H	-glc	$C_{27}H_{30}O_{14}$	578.1636
2	puerarin-3'-methyoxy-4'-*O*-glucoside	-H	-H	-glc	-OMe	-glc	$C_{28}H_{32}O_{15}$	608.1741
3	puerarin-4',7-*O*-glucoside	-H	-glc	-H	-H	-glc	$C_{27}H_{30}O_{14}$	578.1636
4	puerarin	-H	-H	-glc	-H	-H	$C_{21}H_{20}O_{9}$	416.1107
5	6"-*O*-xylosylpuerarin	-H	-H	-glc-xyl	-H	-H	$C_{26}H_{28}O_{13}$	548.1530
6	mirificin	-H	-H	-glc-api	-H	-H	$C_{26}H_{28}O_{13}$	548.1530
7	daidzin	-H	-glc	-H	-H	-H	$C_{21}H_{20}O_{9}$	416.1107
8	3'-methoxypuerarin	-H	-H	-glc	-OMe	-H	$C_{22}H_{22}O_{10}$	446.1213
9	genistin	-OH	-glc	-H	-H	-H	$C_{21}H_{20}O_{10}$	432.1056
10	sophoraside A						$C_{24}H_{26}O_{10}$	474.1526
11	ononin	-H	-glc	-H	-H	-Me	$C_{22}H_{22}O_{9}$	430.1264
12	daidzein	-H	-H	-H	-H	-H	$C_{15}H_{10}O_{4}$	254.0579
13	genistein	-OH	-H	-H	-H	-H	$C_{15}H_{10}O_{5}$	270.0528
14	formononetin	-H	-H	-H	-H	-Me	$C_{16}H_{12}O_{4}$	268.0736

Fig. 1. Chemical structure of 14 investigated compounds. glc, β-D-glucose; xyl, β-D-xylose; api, β-D-apiose; Me, methyl.

not provide the comprehensive optimum extraction condition for isoflavones glycosides and aglycones in RP.

The analyses of Chinese medicines (CMs) generally cost long time due to the complicated matrix. Fortunately, ultra high performance liquid chromatography (UHPLC) has been proved to be a rapid chromatographic analytic tool, which performed multi-component analysis with satisfactory separation, good resolution and sensitivity [30]. Nowadays, mass spectrometry (MS) has been widely used for identification of chemical components in CMs. Especially, time-of-flight mass spectrometry (TOF-MS) has various advantages including high resolution, accurate mass measurement and high sensitivity [31]. Thus, UHPLC coupled with TOF-MS may provide a rapid qualitative and quantitative analysis method for CMs.

In this paper, it was the first time to report a MAE coupled with UHPLC-DAD-TOF-MS system for rapid determination of the major components in Radix Puerariae. Furthermore, the contents of 14 phenolic compounds, namely puerarin-4′-*O*-glucoside, puerarin-3′-methyoxy-4′-*O*-glucoside, daidzein-4′,7-*O*-glucoside, puerarin, mirificin, daidzin, 6″-*O*-xylosylpuerarin, 3′-methoxypuerarin, genistin, sophoraside A, ononin, daidzein, genistein and formononetin, in RPL and RPT were also compared.

2. Experimental

2.1. Chemicals, reagents and materials

Methanol and formic acid (HPLC grade) for UHPLC analysis were purchased from Merck (Darmstadt, Germany). Absolute ethanol (AR grade) used for extraction purpose was obtained from Riedel-de Haën (Seeize, Germany). Deionized water was purified by a Millipore Milli-Q purification system (Millipore, Bedford, MA, USA).

Puerarin, daidzin, daidzein and genistein were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The 10 phenolic compounds, puerarin-4′-*O*-glucoside, puerarin-3′-methyoxy-4′-*O*-glucoside, daidzein-4′, 7-*O*-glucoside, mirificin, 6″-*O*-xylosylpuerarin, 3′-methoxypuerarin, genistin, sophoraside A, ononin and formononetin (Fig. 1), were isolated from the root of *Pueraria lobata* (wild.) Ohwi by Li et al. in our lab. The plant material was collected from Jinzhai county, Anhui province. The dried material was extracted with 95% aqueous ethanol under reflux three times. The extract was filtered and concentrated in vacuum to yield a brownish residue, which was suspended in water and then successively extracted with petroleum ether, ethyl acetate and *n*-butanol to yield four fractions. The process of separation and purification mainly used silica gel column, macroporous resin column chromatography and preparative chromatography, respectively [32]. The purity of all compounds is more than 95% (Determined by HPLC). The structures are confirmed by their UV, MS, ^{1}H NMR and ^{13}C NMR data compared with the data from literatures [32–36].

Ten samples of RPL and 10 samples of RPT were collected from 19 different places. The RP was dried with in a universal oven with forced convection (FD115, Tuttlingen, Germany) at 40 °C for 4 days. The dried sample was ground using Sample Mill (model YF102, Ruian Yongli Pharmacy Machinery Company, China), and the powder was sieved. Particles with the size between 10 and 120 mesh (0.125–2 mm, I.D.) was collected for the study. The botanical origins of the material were identified by Professor Yuecheng Li. The voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao, China.

2.2. Sample preparation

2.2.1. Microwave-assisted extraction

MAE was carried out using a CEM MARS (Matthews, NC, USA) system equipped with 100 mL Teflon vessel. In brief, 0.3 g powder (particle size, 0.18–0.35 mm) was accurately weighed into a Teflon vessel and extracted under the optimized condition, including solvent, 65% ethanol; extraction volume, 17 mL; temperature, 100 °C; ramp time, 2 min; hold time, 2 min; microwave power, 600 W and the number of extraction cycles, 1. Then the extract was transferred into 50 mL volumetric flask which was made up to its volume with water and filtered through a 0.22 μm nylon membrane filter (Tianjin Jinteng Experiment Equipment Co., Ltd, China) prior to injection into the UHPLC system.

2.2.2. Ultrasonic extraction

UE was performed as described by Lee et al. with minor modification using a ultrasonic cleaner (model HS20500D, China) [16]. In

brief, 0.3 g powder was accurately weighed into a 50 mL flask and extracted with 30 mL 65% ethanol for 30 min at room temperature. Then, the extract was made up to its volume with water and filtered through a 0.22 μm nylon membrane filter prior to injection into the UHPLC system.

2.2.3. Reflux extraction

In brief, 0.3 g RPL powder was accurately weighed into a conical flask and extracted with 65% ethanol 50 mL for 30 min. The extract was filtered through a 0.22 μm nylon membrane filter before UHPLC analysis.

2.2.4. Pressurized liquid extraction

Sample preparation was performed using pressurized liquid extraction on a Dionex ASE 200 system as described by Wan et al. (Dionex Corp. Sunnyvale, CA, USA) under optimized conditions [21]. In brief, 0.3 g powder of RPL was mixed with diatomaceous earth in a proportion (1:2) and placed into an 11 mL stainless steel extraction cell, respectively. The extraction cells were placed into the carousel and the samples were extracted under the extraction conditions: solvent, methanol; temperature, 140 °C; static extraction time, 10 min; pressure 1500 psi; flush volume, 60%; static cycle, 1. The extract was transferred into a 50 mL volumetric flask which was brought up to its volume with water and filtered through a 0.22 μm nylon membrane filter before injected into the UHPLC system for analysis.

2.3. UHPLC-DAD-TOF-MS system

2.3.1. UHPLC chromatography

The qualitative and quantitative analyses were performed on an Agilent 1200 Series UHPLC system (Agilent Technologies, Waldbronn, Germany), equipped with a microvacuum degasser, a high pressure binary pump, an autosampler, a column compartment coupled with a carrier for heat exchanger (1.6 μL), a diode array detector and an Agilent 6210 TOF-MS, connected to a Masshunter software (A02.02). A Zorbax SB C_{18} (Agilent, 50 mm × 4.6 mm I.D., 1.8 μm) was used. The mobile phase constituted of A (0.1% formic acid) and B (methanol) with gradient elution: 0–3 min, 20–30% B; 3–4 min, 30–32% B; 4–8 min, 32–57% B. Flow rate was 2.0 mL/min and it had been split before mobile phase into MS system. The split ratio was adjusted at 3:1 between DAD and TOF-MS using a micro-splitter valve (Upchurch Scientific Oak Harbor, WA, USA). Backpressure was about 400 bar. The injection volume was 4 μL. The column temperature was set at 46 °C. UV spectra were collected from 190 nm to 400 nm which were used for qualitative analysis. Peaks were detected at 250 nm which were used for validation of method and assay.

2.3.2. Mass spectrometry

The UHPLC system coupled with an Agilent 6210 TOF-MS (Agilent Corp., Santa Clara, CA, USA), which was equipped with an electrospray ionization (ESI) source. The parameters of ion source were as follows: acquisition mode, negative mode; capillary voltage, 4000 V; drying gas (N_2) temperature, 330 °C; drying gas flow rate, 12 L/min; nebulizer gas (N_2) pressure, 20 psi. MS conditions: mass range, 100–1000 *m*/*z*; fragmentor voltage, 300 V; skimmer voltage, 60 V. The instrument performed automatic autotuning using a reference mass correction system, which introduced a constant flow (100 μL/min) of calibrating solution containing the ESI-TOF tuning mix reference solution and API-TOF reference mass solution (Agilent Technologies, USA).

2.4. Calibration curves

Stock solution containing 14 reference compounds were prepared and diluted to appropriate concentrations for construction of calibration curves. Each concentration of the mixed standard solution was injected in duplicates, and then the calibration curves were constructed by plotting the peak area versus the concentration of each analyte.

2.5. LOD and LOQ

The stock solution containing 14 reference compounds were diluted to a series of appropriate concentrations, and an aliquot of the diluted solutions were injected into UHPLC for analysis. 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.

2.6. Precision, accuracy and repeatability

Intra- and inter-day variations were chosen to determine the precision of the developed method. For intra-day variability test, the mixed standards solutions were analyzed for six replicates within 1 day, while for inter-day variability test, the solutions were examined in duplicates for consecutive 3 days. Variations were expressed by relative standard deviation (RSD).

The recovery was used to evaluate the accuracy of the method. A known amount of standards were added into a certain amount (0.15 g) of RPL2 sample. The mixture was extracted and analyzed using the method mentioned above. Three replicates were performed for the test. To confirm the repeatability, six replicates of the same samples (RPL2) were extracted and analyzed as mentioned above. The RSD value was calculated as a measurement of method repeatability.

3. Results and discussion

3.1. Optimization of MAE

Optimizing MAE conditions should consider the interaction of different extraction factors and the linear relationship between response and variables. In order to reveal the complicated interaction and relationship, a statistical analysis method, central composite design was selected to optimize MAE parameters. The overall desirability (OD) [37], the geometric mean of the contents of 14 target compounds were used as marker to evaluate the extraction efficiency.

Before CCD optimizing MAE parameters, a preliminary experiment has been performed. In the preliminary experiment, extraction factors including particle size, temperature, volume, ethanol concentration, power, ramp time and hold time were studied. Particles with the size between 10 and 120 mesh (0.125–2 mm, I.D.) were collected for the study. The result (Fig. 2A) showed that the range of optimal particle size was 0.18–0.35 mm, which was consistent with *Chinese Pharmacopoeia* (2005 edition) [3]. In order to avoid excessive temperature and overpressure problem, the CEM MARS system could be set at a fixed temperature value by automatic adjusting microwave power. When microwave power was set at 600 W, the extraction energy was enough. Ramp time was investigated from 0.5 to 5 min, the result showed 30 mL extraction solvent could be heated to 140 °C within 2 min. Fig. 2B showed that the peak areas of 14 investigated compounds have no significant difference when extraction time was longer than 2 min. Thus, the other three factors, temperature, volume, ethanol concentration were selected as CCD factors and the power was set at 600 W. Ramp time and hold time was set at 2 min, respectively. The ranges

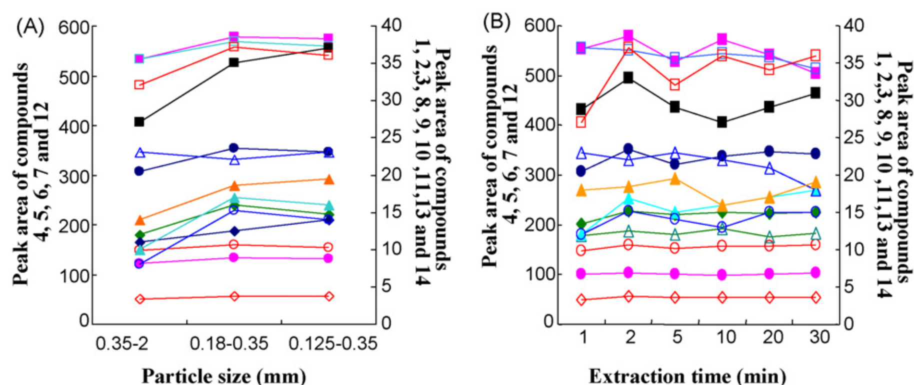

Fig. 2. Effects of particle size (A) extraction time (B) on extraction efficiency of the investigated compounds. (◆) puerarin-4′-*O*-glucoside (1), (△) puerarin-3′-methyoxy-4′-*O*-glucoside (2), (●) puerarin-4′,7-*O*-glucoside (3), (■) puerarin (4), (○) 6″-*O*-xylosylpuerarin (5), (●) mirificin (6), (◇) daidzin (6), (▲) 3′-methoxypuerarin (7), (△) genistin (8), (▲) sophoraside A (10), (○) ononin (11), (□) daidzein (12), (■) genistein (13) and (□) formononetin (14).

and the levels of the variables (temperature, volume, ethanol concentration) investigated in this study were given in Table 1. The experiments were performed in random order to avoid systematic error.

By applying multiple regression analysis to the experimental data, the results of the CCD were fitted to a second-order polynomial equation. Thus, a mathematical regression model for OD fitted in the coded factors was given as follows

$$Y = 9.9482 + 0.1777X_1 + 0.3919X_2 + 0.5911X_3 + 0.00188X_1X_2 + 0.00139X_1X_3 - 0.00079X_2X_3 - 0.001369X_1^2 - 0.015986X_2^2 - 0.005571X_3^2$$

where Y was the response, that was the OD of 14 phenolic compounds contents and X_1, X_2 and X_3 were the coded values of the test variables temperature, volume, and ethanol concentration, respectively. The significance of each coefficient was determined by Student's *t*-test and *P*-values, and the results showed that ethanol concentration played the main effect on the extraction efficiency. The prediction optimization values were calculated using the second-order polynomial equation. The result was that X_1 (temperature) was 100 °C, X_2 (volume) was 17 mL and X_3 (ethanol concentration) was 51%.

To consider the interaction of different extraction parameters, the three-dimensional profiles of multiple non-linear regression models were depicted in Fig. 3A–C, respectively. From Fig. 3, we could found that the effect of ethanol concentration on the extraction efficiency was most obvious. Fig. 3A showed the interaction between temperature and extraction volume, the maximum extraction efficiency was obtained at temperature of 100 °C and extraction volume of 17 mL. Fig. 3B drew surface responses among temperature and ethanol concentration, the optimal temperature of 100 °C and ethanol concentration of 51%. Fig. 3C showed that the optimal extraction efficiency was obtained at ethanol of 51% and extraction volume of 17 mL.

The prediction optimization values, including temperature (80 °C, 90 °C, 100 °C, 110 °C and 120 °C), ethanol concentration (20%, 35%, 50%, 65% and 80%), and extraction volume (10 mL, 15 mL, 17 mL, 20 mL and 25 mL), were validated using univariate method. When one of the parameters, including temperature, ethanol concentration and extraction volume, was optimized, the others were set at the predication optimization value (temperature, 100 °C; ethanol, 51%; extraction volume, 17 mL). The results showed the optimization values of temperature and extraction volume were the same as the results of CCD (Fig. 4A and B), but ethanol concentration of univariate method was 65%. Because 65% ethanol concentration was a real experiment result rather than predicted value (Fig. 4C). At last, temperature was set at 100 °C, extraction volume was 17 mL and ethanol concentration was 65%. The extraction time of MAE was optimized by performing consecutive three times extractions on the same sample under the optimized MAE conditions. After one time extraction, the target compounds were almost undetectable. It was suggested that the MAE with one cycle was enough.

Table 1
The central composite design matrix of three test variables in coded and natural units along with the observed responses.

No.	X_1	X_2	X_3	Temperature (°C)	Volume (mL)	Ethanol (%)	OD[a]
1	−1	−1	−1	76	9	22	0.720
2	1	−1	−1	124	9	22	0.660
3	−1	1	−1	76	25	22	0.646
4	1	1	−1	124	25	22	0.675
5	−1	−1	1	76	9	80	0.815
6	1	−1	1	124	9	80	0.797
7	−1	1	1	76	25	80	0.795
8	1	1	1	124	25	80	0.859
9	−1.668	0	0	60	17	51	0.864
10	1.688	0	0	140	17	51	0.954
11	0	−1.668	0	100	4	51	0.937
12	0	1.668	0	100	30	51	0.860
13	0	0	−1.668	100	17	2	0.532
14	0	0	1.668	100	17	100	0.692
15–20	0	0	0	100	17	51	0.951

[a] Overall desirability.

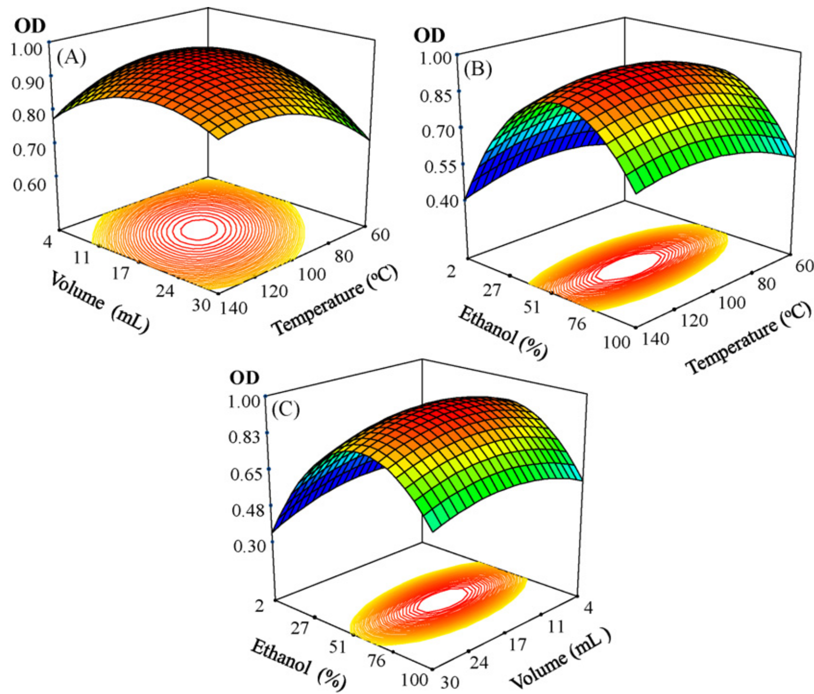

Fig. 3. Response surface for overall desirability (OD) response function of 14 isoflavones. (A) Volume (mL, X_2) vs. temperature (°C, X_1). Ethanol (%, X_3) is held at its optimum. (B) Ethanol (%, X_3) vs. temperature (°C, X_1). Volume (mL, X_2) is held at its optimum. (C) Ethanol (%, X_3) vs. volume (mL, X_2), Temperature (°C, X_1) is held at its optimum.

3.2. Comparison of MAE, PLE, RE and UE

The extraction efficiency of MAE for RPL was compared with those extraction methods of PLE, RE and UE. Fig. 5 showed that the OD of 14 compounds extracted by MAE was higher than those of PLE, RE and UE. The chromatograms of some phenolic compounds with low content (<2 mg/g) obtained from different extraction methods were similar, such as puerarin-3′-methyoxy-4′-*O*-glucoside, genistein and formononetin. However, for some major phenolic compounds (>6 mg/g), such as puerarin, mirificin, daidzin and daidzein, the peak areas of them extracted by MAE were similar to that by PLE but obviously more than that by RE and UE. The difference may be derived from high temperature and high pressure extraction which facilitate dissolution of major com-

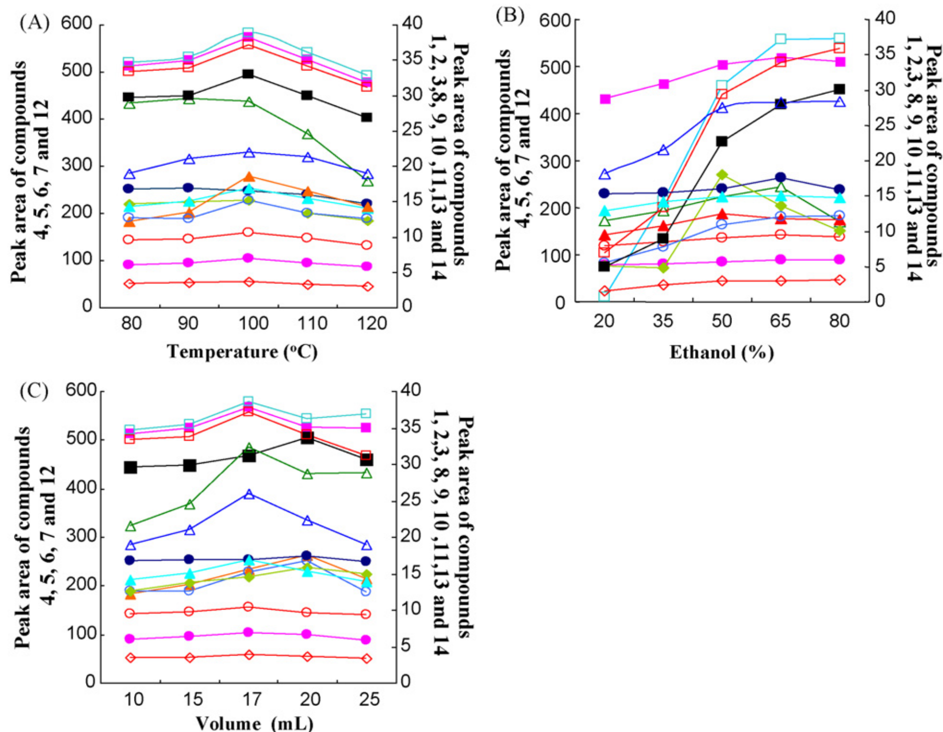

Fig. 4. Validation of predication optimization values including temperature (A), ethanol concentration (B), extraction volume (C). Condition: To determine one of the parameters including temperature, ethanol concentration and extraction volume, the others were set at the predication optimization value (temperature, 100 °C; ethanol, 51%; extraction volume, 17 mL). The legends are the same as Fig. 2.

Table 2
Linear regression data, limit of detection (LOD), limit of quantification (LOQ) of the investigated compounds.

Analytes	Linear regression data			LOD (μg/mL)	LOQ (μg/mL)
	Regression equation	r	Test range (μg/mL)		
Puerarin-4′-*O*-glucoside	$y = 1.4562x - 0.9131$	0.9998	2.23–262.22	0.04	0.14
Puerarin-3′-methyoxy-4′-*O*-glucoside	$y = 0.9941x + 0.8641$	0.9996	2.47–215.70	0.10	0.35
daidzein-4′,7-*O*-glucoside	$y = 1.3067x + 0.8060$	0.9999	2.63–281.15	0.07	0.27
Puerarin	$y = 2.0577x + 2.5902$	0.9997	8.31–554.72	0.01	0.02
6″-*O*-xylosylpuerarin	$y = 1.2971x + 1.5779$	0.9999	4.10–284.25	0.04	0.11
Mirificin	$y = 1.3497x + 1.4293$	0.9999	2.72–163.46	0.02	0.08
Daidzin	$y = 1.3262x + 1.1097$	0.9999	2.97–213.44	0.06	0.20
3′-Methoxypuerarin	$y = 0.6704x + 0.0152$	1.0000	1.70–146.06	0.13	0.41
Genistin	$y = 0.4270x + 0.1650$	0.9999	1.18–110.29	0.06	0.22
Sophoraside A	$y = 0.2682x - 0.0481$	1.0000	2.18–204.57	0.36	0.65
Ononin	$y = 0.4520x - 0.0511$	1.0000	2.41–209.53	0.29	0.41
Daidzein	$y = 1.6005x + 2.3021$	0.9996	1.64–211.81	0.08	0.27
Genistein	$y = 2.2812x - 0.2585$	1.0000	1.88–175.68	0.09	0.39
Formononetin	$y = 2.4141x + 1.4023$	0.9999	1.68–204.50	0.09	0.29

pounds. Moreover, MAE had the advantages of fast extraction speed and small amount of solvent consumption. In this paper, CEM MARS system was used as an extract instrument. It can perform 12 samples in one cycle. So compared to other extraction methods, the main advantage of MAE is the considerable reduction in extraction time and solvent consumption.

3.3. Validation of method

The linearity, regression, and linear ranges of 14 analytes were performed using the developed UHPLC method (Table 2). The correlation coefficient values ($r > 0.9997$) indicated good correlations between the investigated compounds concentrations and their peak area within the test ranges. The LOD and LOQ were less than 0.36 μg/mL and 0.65 μg/mL (Table 2), and the overall intra- and inter-day variations (RSD) of the 14 analytes were less than 1.77% and 2.88%, respectively. The developed method had good accuracy and repeatability. The recoveries were between 94.9% and 102.9% (Table 3), and the repeatability present as RSD ($n = 6$) was between 1.1% and 3.9%.

3.4. Qualitative analysis of the compounds in RPL and RPT

3.4.1. TOF mass spectrometry analysis of 14 reference compounds

The UHPLC-DAD chromatograms of 14 references were shown in Fig. 6. Aiming at identifying the main chromatographic peaks in

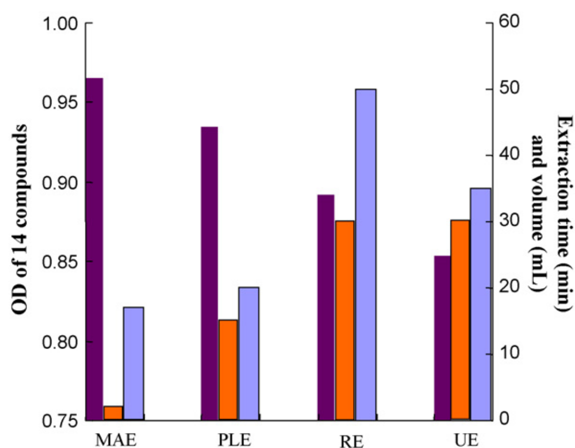

Fig. 5. Comparison of four extraction method including microwave-assisted extraction (MAE), pressure liquid extraction (PLE), reflux extraction (RE) and ultrasonic extraction (UE) on overall desirability (OD, ■) extraction time (min, ■) and solvent volume (mL, ■).

RPL and RPT, retention time (RT), ultraviolet spectra (UV), and TOF-MS data were acquired and shown in Table 4. In the negative mode, all the isoflavones revealed quasi-molecular ion $[M-H]^-$ in the MS spectrum. $[M-28]^-$, $[M-29]^-$, $[M-30]^-$ ions were observed in the

Table 3
Recoveries for the assay of 14 compounds in Radix Puerariae Lobatae.

Analytes	Originals (μg)	Spiked (μg)	Found (μg)[a]	Recovery (%)[b]	RSD (%)
Puerarin-4′-*O*-glucoside	48.9	23.5	72.7	101.3	2.9
		46.9	95.7	99.8	3.1
		70.4	119.7	100.6	2.2
Puerarin-3′-methyoxy-4′-*O*-glucoside	34.1	16.0	50.5	102.5	1.2
		32.1	65.9	99.1	2.1
		48.1	80.4	96.3	1.3
Daidzein-4′,7-*O*-glucoside	171.9	86.0	255.9	97.7	3.1
		171.9	343.3	99.7	2.3
		257.9	423.3	97.5	2.5
Puerarin	4353.0	2061.5	6474.3	102.9	1.2
		4123.0	8595.6	102.9	2.6
		6184.5	10704.5	102.7	1.3
6″-*O*-xylosylpuerarin	1985.9	952.9	2916.9	97.7	3.1
		1905.8	3918.4	101.4	1.7
		2858.7	4741.7	96.4	2.7
Mirificin	1299.7	643.9	1914.6	95.5	2.7
		1287.8	2601.7	101.1	1.7
		1931.7	3192.8	98.0	1.4
Daidzin	763.2	371.6	1139.3	101.2	1.9
		743.2	1518.3	101.6	1.0
		1114.8	1824.5	95.2	1.1
3′-Methoxypuerarin	332.0	165.2	503.1	103.6	2.9
		330.4	661.1	99.6	2.6
		495.6	828.1	100.1	1.1
Genistin	496.5	238.2	740.4	102.4	0.7
		476.4	970.5	99.5	1.5
		714.6	1212.5	100.2	2.0
Sophoraside A	312.7	166.4	471.9	95.7	3.2
		332.8	640.2	98.4	1.6
		499.2	802.9	98.2	1.3
Ononin	417.9	229.0	639.6	96.8	3.1
		458.0	852.5	94.9	1.5
		687.0	1082.9	96.8	1.6
Daidzein	1468.7	764.3	2244.5	101.5	1.0
		1528.6	2994.2	99.8	1.0
		2292.9	3738.7	99.0	0.7
Genistein	75.6	47.8	123.2	99.6	1.7
		95.6	173.6	102.5	0.2
		143.4	220.0	100.7	1.6
Formononetin	99.3	54.6	155.0	102.1	0.9
		109.2	207.8	99.4	1.0
		163.8	264.7	101.0	0.6

[a] The data was present as average of three determinations.
[b] Recovery (%) = 100% × (amount found − original amount)/amount spiked.

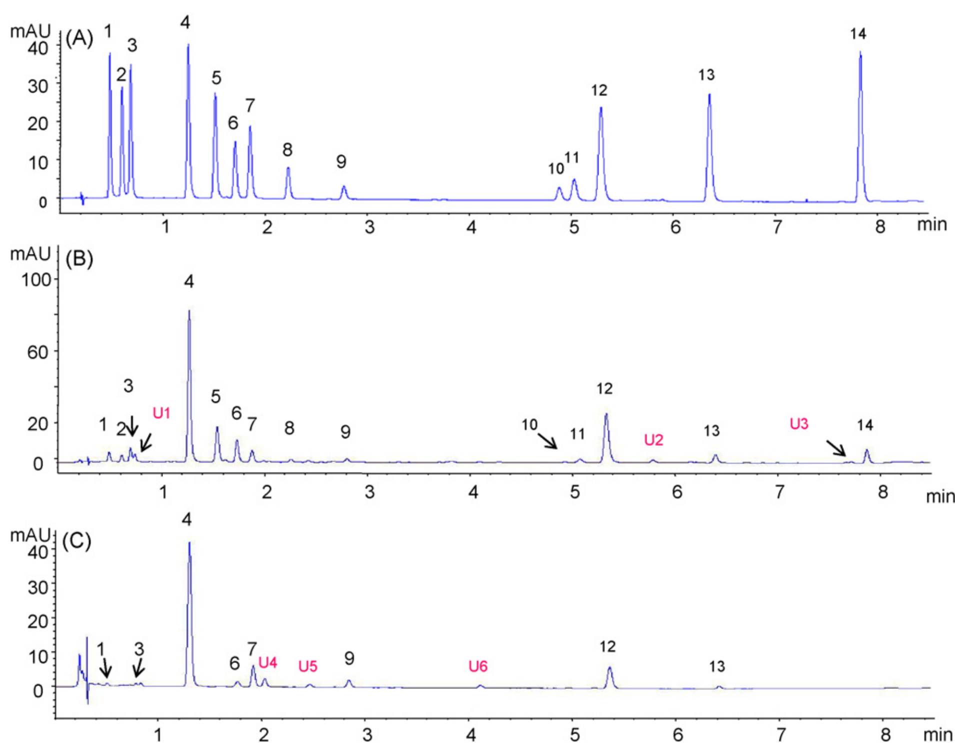

Fig. 6. Typical UHPLC chromatograms of (A) mixed standards, (B) RPL and (C) RPT. puerarin-4′-*O*-glucoside (1), puerarin-3′-methyoxy-4′-*O*-glucoside (2), daidzein-4′,7-*O*-glucoside (3), puerarin (4), 6″-*O*-xylosylpuerarin (5), mirificin (6), daidzin (7), 3′-methoxypuerarin (8), genistin (9), sophoraside A (10), ononin (11), daidzein (12), genistein (13), formononetin (14) and unknown peaks (U1–U6).

fragments of these isoflavones, which were attributed to the neutral loss of CO, CHO, CH_2O caused by the cleavage of C-ring. Additionally, the characteristic ion $[M\text{-}C_4H_8O_4]^-$ of isoflavones C-glucoside was observed in the MS spectrum. However, because of the structure similarity of xylose and apiose, the common ion $[M\text{-}C_4H_7O_3]^-$ could be found in the MS spectrum of 6″-O-xylosylpuerarin and mirificin. Thus, it was almost impossible to discriminate them by MS data, and the difference only existed in the retention time of HPLC. Therefore, the identification of investigated compounds was carried out by comparison of their retention time, UV spectra and MS spectrum. The 14 chromatographic peaks were unambiguously identified as puerarin-4′-*O*-glucoside (1), 3′-methyoxy-4′-*O*-glucopyranoside (2), 4′,7-*O*-glucopyranoside (3), puerarin (4), 6″-*O*-xylosylpuerarin (5), mirificin (6), daidzin (7), 3′-methoxypuerarin (8), genistin (9), sophoraside A (10), ononin (11), daidzein (12), genistein (13) and formononetin (14).

3.4.2. Qualitative analysis of six unknown chromatographic peaks in RPL and RPT

Being a high resolution mass spectrum, TOF-MS could perform accurate mass measurement, which gives elemental composition of parent and fragment ions. Furthermore, the in-source collision induced dissociation (CID) technique was applied in our experiment to acquire sufficient structure information from TOF-MS. Six compounds U1–U6 were tentatively identified from RP species by comparison of UV spectra and MS spectrum, including three isoflavone C-glycosides (U1, U4 and U5) and three isoflavone aglycones (U2, U3 and U6) (Table 4).

Peaks U1, U4 and U5 at 0.81 min, 2.11 min and 2.55 min revealed a series of diagnostic ions $[M\text{-}C_4H_8O_4{-}H]^-$, $[M\text{-}C_4H_8O_4\text{-}H_2O{-}H]^-$, $[M\text{-}C_4H_8O_4\text{-}CO{-}H]^-$. $[M\text{-xyl-}C_4H_7O_3{-}H]^-$ and $[M\text{-xyl-}C_4H_7O_3\text{-}CO{-}H]^-$ in their TOF-MS data. Comparing the molecular and fragment ions with those produced by puerarin, peaks U1 and U4 belonged to hyroxypuerarin and methoxypuerarin, respectively. According to their literature [38,39], these two compounds were tentatively identified as 3′-hydroxypuerarin (U1) and 3′-methoxydaidzin (U4). Peak U5 exhibited $[M{-}H]^-$ ion at m/z 563.1408. In its mass spectrum, a prominent characteristic ion $[M\text{-api-}C_4H_7O_3{-}H]^-$ indicated that it was an isomer of api-glc C-glycosidic isoflavone. Peak U5 was tentatively identified as 6″-*O*-Apiosyl based on its TOF-MS information and literature [40].

Peak U2 generated $[M\text{-}CH_3{-}H]^-$ ion at m/z 268.0377 which was due to methyl losing at ring C. Comparing the molecular and fragment ions with those produced by formononetin, Peak U2 was identified as biochanin A [41]. Peak U3 generated $[M{-}H]^-$ ion at m/z 281.0461 indicating a molecular formula of $C_{16}H_9O_5$. The fragment ions at m/z 253.0510, 223.0398, 195.0451 were attributed to the loss of two CO and CH_2O. According to its fragmentation pattern and previous literature [42], peak U3 was tentatively identified as pseudobaptigenin [43]. The parent ion of peak U6 was the same as daidzein. The fragmentation pathway of peak U6 was similar to daidzein. In addition, two UV absorption maximum were observed at 250 nm and 310 nm, which was similar with isoflavone. So peak U6 were tentatively identified as isodaidzein [44].

3.5. Quantitative analysis of the investigated compounds in RPL and RPT

The developed MAE and UHPLC method were applied to the simultaneous quantification of 14 investigated compounds. The typical UHPLC-DAD chromatograms of RPL and RPT were shown in Fig. 6, and their contents were listed in Table 5. It was recognized that isoflavones puerarin-4′-*O*-glucoside (1), 4′,7-*O*-glucopyranoside (3), puerarin (4), mirificin (6), daidzin (7), genistin (9), daidzein (12) and genistein(13) are the coexistent constituents. However, the contents of these compounds were different distinctly in RPL and RPT species. Peak 4, 5, 6, 7, 9, 12 and 14 were the major common components in RPL, and the contents of them were much higher than those in RPT. Moreover, 6″-*O*-xylosylpuerarin (5) and formononetin (14) could not be detected in

Table 4
The retention time, UV and MS characteristics of the main detected peaks in RPL and RPT.

Peak No.	Identification	Retention time (min)	Proposal ions	Measured mass (*m*/*z*)	Elemental composition	Error (ppm)	UV λ_{max} (nm)
1	Puerarin-4′-*O*-glucoside	0.56	$[M-H]^-$	577.1571	$C_{27}H_{29}O_{14}$	1.4212	250, 310
			$[M-C_4H_8O_4-H]^-$	457.1143	$C_{23}H_{21}O_{10}$	0.6107	
			$[M-C_4H_8O_4-glc-H]^-$	295.0618	$C_{17}H_{11}O_5$	2.0428	
2	Puerarin-3′-methyoxy-4′-*O*-glucoside	0.68	$[M-1]^-$	607.1682	$C_{28}H_{31}O_{15}$	2.2326	250, 285
			$[M-C_4H_8O_4-H]^-$	487.1268	$C_{24}H_{23}O_{11}$	2.5459	
			$[M-C_4H_8O_4-glc-H]^-$	325.0713	$C_{18}H_{13}O_6$	−1.4210	
			$[M-C_4H_8O_4-glc-CH_3-H]^-$	310.0476	$C_{17}H_{10}O_6$	−2.2152	
3	Daidzein-4′,7-*O*-glucoside	0.77	$[M-H]^-$	577.1555	$C_{27}H_{29}O_{14}$	−1.3509	250, 300
			$[M-glc-H]^-$	415.1040	$C_{21}H_{19}O_9$	1.3102	
			$[M-glc-glc-H]^-$	253.0503	$C_{15}H_9O_4$	−1.3139	
4	Puerarin	1.37	$[M-H]^-$	415.1045	$C_{21}H_{19}O_9$	2.5147	250, 310
			$[M-C_4H_8O_4-H]^-$	295.0622	$C_{17}H_{11}O_5$	3.3985	
			$[M-C_4H_8O_4-H_2O-H]^-$	277.0507	$C_{17}H_9O_4$	0.2436	
			$[M-C_4H_8O_4-CO-H]^-$	267.0667	$C_{16}H_{11}O_4$	1.5629	
5	6″-*O*-xylosylpuerarin	1.65	$[M-H]^-$	547.1467	$C_{26}H_{27}O_{13}$	1.8002	250, 310
			$[M-xyl-C_4H_7O_3-H]^-$	295.0618	$C_{17}H_{11}O_5$	2.0428	
			$[M-xyl-C_4H_7O_3-CO-H]^-$	267.0668	$C_{16}H_{11}O_4$	1.9374	
6	Mirificin	1.85	$[M-H]^-$	547.1466	$C_{26}H_{27}O_{13}$	1.6175	250, 310
			$[M-api-C_4H_7O_3-H]^-$	295.0619	$C_{17}H_{11}O_5$	2.3818	
			$[M-api-C_4H_7O_3-CO-H]^-$	267.0664	$C_{16}H_{11}O_4$	0.4396	
7	Daidzin	1.99	$[M-H]^-$	415.1036	$C_{21}H_{19}O_9$	0.3466	250, 310
			$[M-glc-H]^-$	253.0501	$C_{15}H_9O_4$	−2.1043	
8	3′-Methoxypuerarin	2.38	$[M-H]^-$	445.1153	$C_{22}H_{21}O_{10}$	2.8738	250, 310
			$[M-glc-H]^-$	283.0605	$C_{16}H_{11}O_5$	−2.4631	
			$[M-glc-CH_3-H]^-$	268.0384	$C_{15}H_8O_5$	2.5291	
			$[M-glc-CH_3-CO-H]^-$	240.0434	$C_{14}H_8O_4$	2.4684	
9	Genistin	2.94	$[M-H]^-$	431.1061	$C_{21}H_{19}O_{10}$	−0.2216	250, 310
			$[M-glc-H]^-$	269.0449	$C_{15}H_9O_5$	−2.4053	
10	Sophoraside A	5.05	$[M-H]^-$	473.1443	$C_{24}H_{25}O_{10}$	−2.1578	290, 315
			$[M-glc-H]^-$	311.0933	$C_{18}H_{15}O_5$	2.5800	
			$[M-glc-CO_2-H]^-$	267.1030	$C_{17}H_{15}O_3$	1.2425	
			$[M-glc-CO_2-CH_3-H]^-$	252.0800	$C_{16}H_{12}O_3$	3.2014	
11	Ononin	5.18	$[M-H]^-$	429.1181	$C_{22}H_{21}O_9$	−2.3447	250, 310
			$[M-glc-H]^-$	267.0656	$C_{16}H_{11}O_4$	−2.5558	
			$[M-CH_3-H]^-$	252.0421	$C_{15}H_8O_4$	−2.8069	
12	Daidzein	5.47	$[M-H]^-$	253.0509	$C_{15}H_9O_4$	1.0571	250, 310
			$[M-CH_2O-H]^-$	223.0405	$C_{14}H_7O_3$	1.9378	
			$[M-CH_2O-CO-H]^-$	195.0456	$C_{13}H_7O_2$	2.2910	
13	Genistein	6.50	$[M-H]^-$	269.0461	$C_{15}H_9O_5$	2.0548	260, 330
14	Formononetin	7.94	$[M-H]^-$	267.0670	$C_{16}H_{11}O_4$	2.6862	250, 310
			$[M-CH_3-H]^-$	252.0432	$C_{15}H_8O_4$	1.5574	
			$[M-CH_3-CHO-H]^-$	223.0404	$C_{14}H_7O_3$	1.4895	
			$[M-CH_3-CHO-CO-H]^-$	195.0457	$C_{13}H_7O_2$	2.8037	
			$[M-CH_3-CHO-2CO-H]^-$	167.0506	$C_{12}H_7O$	2.1640	
U1	3′-Hydroxypuerarin	0.81	$[M-1]^-$	431.0985	$C_{21}H_{19}O_{10}$	0.2998	250, 310
			$[M-C_4H_8O_4-H]^-$	311.0560	$C_{17}H_{11}O_6$	−0.3596	
			$[M-C_4H_8O_4-H_2O-H]^-$	293.0460	$C_{17}H_9O_5$	1.5453	
U2	Biochanin A	5.90	$[M-H]^-$	283.0614	$C_{16}H_{11}O_5$	0.7163	250, 310
			$[M-CH_3-H]^-$	268.0377	$C_{15}H_8O_5$	−0.0824	
			$[M-CH_3-CHO-H]^-$	239.0346	$C_{14}H_7O_4$	−1.5998	
			$[M-CH_3-CHO-CO-H]^-$	211.0402	$C_{13}H_7O_3$	0.6265	
			$[M-CH_3-CHO-2CO-H]^-$	183.0452	$C_{12}H_7O_2$	0.2560	
U3	Pseudobaptigenin	7.80	$[M-H]^-$	281.0461	$C_{16}H_9O_5$	1.9671	250, 310
			$[M-CO-H]^-$	253.0510	$C_{15}H_9O_4$	1.4522	
			$[M-CO-CH_2O-H]^-$	223.0398	$C_{14}H_7O_3$	−1.2005	
			$[M-2CO-CH_2O-H]-$	195.0451	$C_{13}H_7O_2$	−0.2724	
U4	3′-Methoxypuerarin	2.11	$[M-H]^-$	445.1127	$C_{22}H_{21}O_{10}$	−2.9673	250, 310
			$[M-C_4H_8O_4-H]^-$	311.0563	$C_{17}H_{11}O_6$	0.6048	
			$[M-C_4H_8O_4-CO-H]^-$	283.0611	$C_{16}H_{11}O_5$	−0.3434	
U5	6″-*O*-Apiosyl	2.55	$[M-H]^-$	563.1408	$C_{26}H_{27}O_{14}$	0.3025	250, 310
			$[M-xyl-C_4H_7O_3-H]^-$	311.0564	$C_{17}H_{11}O_6$	0.9263	
			$[M-xyl-C_4H_7O_3-CO-H]^-$	283.0615	$C_{16}H_{11}O_5$	1.0696	
U6	Isodaidzein	4.23	$[M-H]^-$	253.0507	$C_{15}H_9O_4$	0.2667	250, 310
			$[M-CH_2O-H]^-$	223.0402	$C_{14}H_7O_3$	0.5928	
			$[M-CH_2O-CO-H]^-$	195.0457	$C_{13}H_7O_2$	2.8037	

all RPT samples. In order to evaluate the variation of RPL and RPT, hierarchical cluster analysis was performed base on the contents of 14 phenolic compounds of 20 tested samples. Between-groups linkages method, one of effective methods for the analysis of variance between clusters, was applied and Squared Euclidean distance was selected as measurement. Fig. 7 showed the dendrogram, which was divided into two main clusters. Though the chemical constituents of the two species were quite similar, the contents of the investigated isoflavones were significantly different. Cluster I was mainly RPT samples (except one *P. lobata* (wild.) Ohwi sample from Dujiangyan, Sichuan Province), which contained much lower contents of isoflavones than those in cluster II. The

Table 5
The contents (mg/g) of 14 investigated compounds in Radix Puerariae Lobatae and Radix Puerariae Thomsonii.

Sample		Contents (mg/g) (RSD%, $n=2$)														
No.	Sources	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total[a]
RPT1	Hengfeng, Jiangxi	0.04[b](1.03)	–[c]	0.18(3.24)	3.70(0.22)	–	1.29(0.31)	0.28(3.02)	–	1.36(0.43)	0.11(4.26)	–	0.74(0.12)	0.06(3.73)	–	7.76
RPT2	Yifeng, Jiangxi	0.09(1.83)	–	0.25(0.73)	6.98(0.57)	–	0.65(1.91)	2.03(1.02)	–	2.84(0.34)	–	–	1.37(0.14)	0.11 (4.69)	–	14.32
RPT3	Shanxi Market	0.09(2.74)	0.07(1.60)	0.12(0.83)	5.64(0.04)	–	0.54(0.31)	1.34(0.19)	–	2.07(0.17)	–	–	1.98(0.05)	0.18(1.55)	–	12.03
RPT4	Wuzhou, Guangxi	0.08(2.39)	0.05(3.67)	0.15(0.89)	5.40(0.04)	–	0.37(0.56)	1.13(0.20)	–	2.04(0.39)	–	–	2.07(0.16)	0.26(1.69)	–	11.55
RPT5	Macao Market	0.09(4.09)	–	0.10(3.38)	5.54(0.38)	–	1.28(0.07)	0.85(0.22)	0.10(2.37)	1.12(0.30)	0.20(3.26)	0.10(2.34)	0.22(0.09)	0.03(3.85)	–	9.63
RPT6	Taiwan Market	0.08(0.95)	0.07(1.27)	0.11(2.35)	6.59(0.07)	–	0.36(0.84)	1.38(0.11)	–	1.69(0.12)	0.19(4.26)	0.14(3.46)	1.48(0.02)	0.12(1.56)	–	12.21
RPT7	Renshou, Sichuan	0.10(2.84)	0.06(3.75)	0.03(4.35)	3.71(0.13)	–	0.36(0.56)	0.88(0.06)	–	1.51(0.19)	0.16(4.46)	0.06(4.12)	0.82(0.03)	0.08(3.48)	–	7.77
RPT8	Dujiangyan, Sichuan	0.07(3.26)		0.22(2.17)	3.64(0.24)	–	1.34(0.97)	1.54(1.27)	0.34(1.49)	2.08(1.65)	0.32(2.56)	0.15(3.56)	1.65(0.34)	0.14(2.32)	–	11.49
RPT9	Meishan, Sichuan	0.09(3.25)	0.05(3.24)	0.17(1.45)	4.32(0.58)	–	0.74(2.32)	1.86(2.08)	–	1.40(1.24)	0.28(3.02)	0.11(2.33)	2.33(0.58)	0.17(1.65)	–	11.52
RPT10	Jiangsu Market	0.08(2.22)	0.11(2.75)	0.17(2.34)	5.67(0.69)	–	0.65(2.86)	1.97(1.88)	0.27(2.11)	2.09(0.97)	0.21(2.78)	0.21(2.47)	1.62(0.36)	0.28(1.69)	–	13.16
RPL1	Ningguo, Anhui	1.16(1.98)	1.02(0.63)	2.07(0.17)	55.99(0.16)	23.71(0.14)	16.44(0.11)	13.10(0.12)	2.72(2.31)	6.27(0.47)	1.13(0.34)	2.87(0.32)	3.19(0.15)	0.19(0.73)	0.08(2.76)	149.94
RPL2	Macheng, Hubei	0.33(3.72)	0.23(4.35)	1.16(2.16)	29.17(0.38)	13.40(0.34)	8.77(0.45)	5.15(0.59)	2.24(1.57)	3.35(0.48)	2.11(1.59)	2.82(1.14)	9.91(0.08)	0.51(0.51)	0.67(0.09)	79.82
RPL3	Yichang, Hubei	1.44(0.37)	0.94(0.47)	4.09(0.23)	55.38(0.08)	17.91(0.03)	14.08(0.08)	12.37(0.16)	1.79(0.37)	6.05(0.07)	1.27(1.83)	5.39(1.09)	2.86(1.73)	0.20(0.57)	0.24(0.86)	124.02
RPL4	Zaoyang, Hubei	1.32(0.11)	1.11(2.00)	3.39(0.71)	50.67(0.16)	16.32(0.17)	11.44(0.15)	10.61(0.20)	1.69(0.34)	5.23(0.25)	1.07(0.93)	3.24(0.36)	2.41(0.35)	0.22(0.30)	0.08(3.40)	108.79
RPL5	Yuexi, Anhui	1.26(2.86)	0.88(2.62)	7.08(4.39)	37.42(0.28)	27.38(0.30)	11.80(0.31)	13.84(0.36)	2.55(0.35)	8.50(0.38)	0.44(3.42)	1.79(0.30)	2.17(0.10)	0.16(1.32)	0.01(4.21)	115.26
RPL6	Liuan, Anhui	0.98(2.44)	1.91(1.36)	4.11(1.38)	47.93(0.07)	12.78(0.07)	7.70(0.03)	12.12(0.05)	1.58(0.85)	7.43(0.22)	0.77(1.50)	6.20(0.14)	2.98(0.61)	0.24(1.54)	0.10(0.42)	106.84
RPL7	Jishou, Hubei	3.66(0.38)	2.07(0.24)	3.65(0.67)	58.43(0.18)	26.66(0.20)	19.61(0.13)	12.21(0.17)	1.67(0.98)	7.91(0.72)	1.62(1.65)	2.75(1.42)	6.87(1.42)	0.39(1.03)	0.23(1.41)	147.74
RPL8	Zaoyang, Hubei	0.54(1.24)	0.53(0.98)	1.21(2.39)	47.70(0.22)	22.23(0.19)	17.10(0.16)	5.23(0.50)	0.73(0.57)	2.81(0.25)	0.95(1.51)	0.37(2.64)	3.56(0.55)	0.10(0.37)	0.41(2.53)	103.11
RPL9	Hanyan, Jiangsu	2.41(0.41)	1.70(1.41)	5.15(0.40)	61.31(0.20)	23.44(0.18)	18.81(0.16)	10.86(0.14)	1.39(0.15)	7.22(0.10)	0.83(0.89)	3.98(0.11)	3.32(0.21)	0.26(1.12)	0.15(0.90)	140.88
RPL10	Dujiangyan, Sichuan	0.34(1.65)	–	0.93(1.34)	7.56(0.76)	3.26(1.72)	1.96(1.54)	1.05(0.78)	0.14(2.76)	1.39(0.47)	–	0.92(1.39)	8.96(0.77)	0.37(2.16)	0.34(2.08)	27.22

[a] The sum of 14 compounds including puerarin-4′-*O*-glucoside (1), puerarin-3′-methyoxy-4′-*O*-glucoside (2), daidzein-4′,7-*O*-glucoside (3), puerarin (4), 6″-*O*-xylosylpuerarin (5), mirificin (6), daidzin (7), 3′-methoxypuerarin (8), genistin (9), sophoraside A (10), ononin (11), daidzein (12), genistein (13), formononetin (14).
[b] The data was present as average of duplicates.
[c] Under the limit of detect.

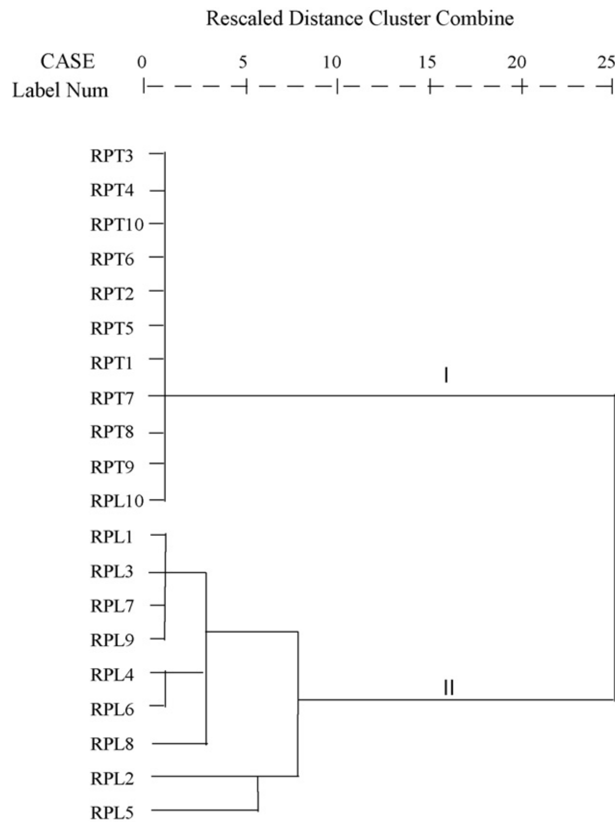

Fig. 7. Dendrogram of hierarchical cluster analysis for the 20 tested samples of Radix Puerariae. The hierarchical clustering was performed by SPSS software. Between-group linkage method was applied, and Squared Euclidean distance was selected as measurement. Dendrogram resulting from the 14 investigated compounds contents of the tested samples. The 20 samples are shown in Table 5.

total amount of isoflavones of RPT and RPL were ranged from 7.76 mg/g to 14.32 mg/g and 79.82 mg/g to 149.94 mg/g, respectively (Fig. 7, Table 5). Among these investigated compounds, the contents of puerarin, mirificin and daidzin were relatively higher than others. Especially, the contents of puerarin were ranged from 3.64 mg/g to 6.98 mg/g in RPT and 7.56 mg/g to 61.31 mg/g in RPL, which was corresponding to previous report for the determination of puerarin in RP [20]. Additionally, hierarchical cluster analysis was also performed by using the contents of puerarin, mirificin and daidzin. The result was consistent with that obtained from 14 compounds (Fig. 7). Therefore, puerarin, mirificin and daidzin may be the reasonable markers to distinguish RPT and RPL.

4. Conclusions

A rapid qualitative and quantitative method for simultaneous determination of 14 major constituents in two Radix Puerariae species by UHPLC-DAD–TOF-MS was developed. In this study, MAE showed outstanding extraction efficiency compared with other conventional extraction methods. Meanwhile, six unknown chromatographic peaks were tentatively identified by accurate mass measurement of TOF-MS. The results indicated that MAE combined with UHPLC analysis was an excellent method for the quality evaluation of RPL and RPT comprehensively.

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