Quality Evaluation of *Astragali Radix* Products by Quantitative Analysis of Multi-components by Single Marker

SUN Jie[1, 2†], JIANG Zhen-zuo[1, 2, 3†], YAN Rui-qing[1, 2], OLALEYE Olajide[1, 2], ZHANG Xiao-long[1, 2], CHAI Xin[1, 2*], WANG Yue-fei[1, 2]

1. Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China
2. Tianjin Key Laboratory of TCM Chemistry and Analysis, Tianjin University of Traditional Chinese Medicine, Tianjin 300457, China
3. Harmonia Biotechnology (Tianjin) Co., Ltd., Tianjin 300457, China

Abstract: **Objective** To develop a quantitative analysis of multi-components by single-marker (QAMS) method for the simultaneous determination of eight components in *Astragali Radix* products, and to examine the feasibility of using the method among the different dosage forms and between two different types of compounds. **Methods** Eight main effective components, campanulin, genistin, ononin, calycosin, genistein, formononetin, methylnissolin, and astragaloside IV were selected as analytes for the quality control of *Astragali Radix* products. Calycosin was selected as the internal reference substance, the content of which was determined by external standard method; the relative correction factors (RCFs) of campanulin, genistin, ononin, genistein, formononetin, methylnissolin, and astragaloside IV were calculated. In total, twelve *Astragali Radix* specimen in decoction pieces, as well as in two different dosage forms, such as granule and oral liquid products, were used for the quality control by both methods of external standard and QAMS. The validity of the QAMS method was evaluated by comparison on the quantitative results of the two methods. **Results** These RCFs were obtained with good reproducibility (RSD < 6.5%) by using ultra high performance liquid chromatography coupled with diode array detector under various chromatographic conditions. Meanwhile, no obvious differences (RSD < 3.98%) were found in the quantitative results of the seven components in twelve samples of *Astragali Radix* products determined by the two methods. **Conclusion** QAMS is a reliable and feasible method in determining the components in products of *Astragali Radix*.

Key words: *Astragali Radix*; quality control; quantitative analysis of multi-components by single-marker; relative correction factor; ultra high performance liquid chromatography coupled with diode array detector

DOI: 10.1016/S1674-6384(13)60040-4

Introduction

Astragali Radix, a kind of frequently-used Chinese materia medica (CMM), is derived from the dried roots of *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge. (Pharmacopoeia Committee of P. R. China, 2010). It has been widely used for centuries in the prevention and treatment of various diseases such as nephritis, diabetes, cancer, etc. (Cheng *et al*, 2004; Xie and Du, 2011; Liu, Zhao, and Chen, 2011). Several modern dosages produced with *Astragali Radix* have been used frequently in clinic, including pill, oral liquid, granule, and injection. Chemical investigations have revealed that the flavonoids and saponins are the two main components in *Astragali Radix* being responsible for a variety of biological activities, such as immuno-modulation, anti-oxidation, and enhancement of cardio-vascular function (Yu *et al*, 2005; Zhang *et al*, 2009; Lu

* Corresponding author: Chai X Address: Tianjin State Key Laboratory of Modern Chinese Medicine, Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China
Tel: +86-22-5959 6163 Fax: +86-22-2749 3265 E-mail: chaixinphd@gmail.com
† These authors contributed equally to this work
Received: February 8, 2013; Revised: May 2, 2013; Accepted: August 6, 2013
Fund: The National Program on Key Basic Research Project (2011CB512008), Tianjin Binhai New Area of Science and Technology Professional Services Projects (2012-XJR13006) and National Key Technology R&D Program (2008BAI51B01)

et al, 2011).

Due to the complexity of chemical constitutions of CMM, the multi-components analysis used for the quality control of CMM is more scientific and reasonable. Several analytical methods, such as HPLC-UV (Wu *et al*, 2005; Liu *et al*, 2006), LC-MS (Gu, Wang, and Fawcett, 2004; Xiao *et al*, 2004; Zhang *et al*, 2005), and HPLC-DAD-ELSD (Yu *et al*, 2007; Song *et al*, 2008) have been used to evaluate the quality of *Astragali Radix*. However, the application of these analytical methods is often restricted by the availability of reference substances and the high costs involved.

A quantitative analysis of multi-components by single marker (QAMS) was first proposed by Wang *et al* (2006) in China for the quality control of *Akebiae Caulis*. In recent years, QAMS method has been extensively used in the quality control of CMM and its compound preparations (Zou *et al*, 2008; Yu *et al*, 2010; He *et al*, 2013). The ruggedness and robustness of relative correction factors (RCFs) in the method of QAMS, investigated by researchers, could demonstrate the wide applicability of QAMS method (Hao *et al*, 2011). In particular, the QAMS standard of *Coptidis Rhizoma* has been adopted by *Pharmacopoeia of People's Republic of China 2010* (Pharmacopoeia Committee of P. R. China, 2010).

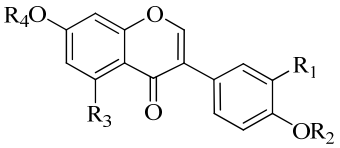
In this paper, we used QAMS method to determine eight components in the decoction pieces of *Astragali Radix*, as well as its two different dosage forms including granule and oral liquid products. By using calycosin as the internal reference substance, RCFs of other components, such as campanulin, genistin, ononin, genistein, formononetin, methylnissolin, and astragaloside IV, were calculated with the good reproducibility in different chromatographic conditions. The quantitative analysis results of the eight compounds in *Astragali Radix*, obtained by UPLC-DAD, showed that the quantification of multi-compounds in *Astragali Radix* by QAMS method could be feasible.

Materials and methods

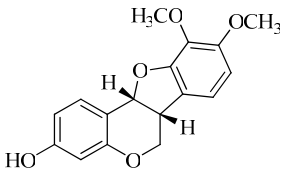
Materials

Acetonitrile of HPLC grade was purchased from Fisher Scientific (USA). Formic acid of analytical grade was purchased from Meridian Medical Technologies (MREDA, USA). Water for UPLC analysis was purified by Milli-Q Water Purification System (Millipore, USA).

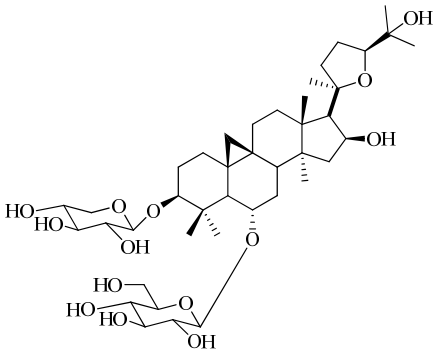
The reference compounds, campanulin, ononin, calycosin, formononetin, and methylnissolin were isolated and their molecular structures were characterized by spectroscopic techniques (MS, ^{1}H-NMR, and ^{13}C-NMR). Astragaloside IV was purchased from National Institute for Food and Drug Control (Beijing, China); genistin and genistein were obtained from Tianjin Zhongxin Pharmaceutical Group Co., Ltd. (Tianjin, China). The chemical structures of components are displayed in Fig. 1. The purities of the standards were all above 98%.



1 R_1=OH	R_2=CH_3	R_3=H	R_4=β-*D*-Glc
2 R_1=H	R_2=H	R_3=OH	R_4=β-*D*-Glc
3 R_1=H	R_2=CH_3	R_3=H	R_4=β-*D*-Glc
4 R_1=OH	R_2=CH_3	R_3=H	R_4=H
5 R_1=H	R_2=H	R_3=OH	R_4=H
6 R_1=H	R_2=CH_3	R_3=H	R_4=H



7



8

Fig. 1 Structures of eight major components in *Astragali Radix*

Decoction pieces (D-1, D-2, and D-3) of *Astragali Radix* and its two different dosage forms including granule (G-1, G-2, G-3, G-4, G-5, and G-6) and oral liquid (OL-1, OL-2, and OL-3) products, used in the

research were purchased from different pharmacy stores in Tianjin.

Chromatographic conditions

Chromatographic analyses were performed on Waters Acquity UPLC equipped with binary solvent manager, sample manager, column oven, and DAD detector. Chromatographic separation was carried out on Acquity UPLC BEH-C_{18} column (100 mm × 2.1 mm, 1.7 μm) at 50 ℃. The mobile phase was composed of 0.1% formic acid-water (A) and acetonitrile (B). The gradient program was as follows: 0—4 min, 15%—20% B; 4—6 min, 20%—25% B; 6—8 min, 25%—28% B; 8—13 min, 28%—50% B; and 13—14 min, 50%—90% B. The flow rate of mobile phase was set at 0.4 mL/min. The injection volume was 2 μL. The UV wavelength was set at 254 nm in 0—10.5 min to detect the flavonoids (campanulin, genistin, ononin, calycosin, genistein, formononetin, and methylnissolin) and at 203 nm in 10.5 — 14.0 min to detect the saponin (astragaloside IV).

UPLC-DAD-MS/MS analysis

Waters Acquity UPLC Tandem Quattro Premier XE™ Mass Spectrometer with software version of Mass Lynx V4.1 (Waters, USA) was used for the qualitative analysis. UPLC conditions were the same as those of the analysis by UPLC-DAD. Nitrogen was used as desolvation gas for the MS analysis at the flow rate of 600 L/h for ESI (+). The flow rate of cone gas was set at 50 L/h. The desolvation temperature was fixed at 300 ℃, and the source temperature was set at 100 ℃. Capillary voltage was 3200 V for ESI (+). For the full scan, the spectra were recorded in the range of *m/z* 100—1000.

Preparation of reference solution

Individual stock solutions of the reference substances were prepared by dissolving the reference substances in 70% methanol to obtain campanulin 2.004 mg/mL, genistin 1.002 mg/mL, ononin 1.002 mg/mL, calycosin 2.002 mg/mL, genistein 1.002 mg/mL, formononein 2.010 mg/mL, methylnissolin 1.002 mg/mL, and astragaloside IV 2.010 mg/mL. A mixed solution containing all the reference substances was prepared and diluted in series with 70% methanol to obtain eight different concentration. The different concentration of the mixed solution was used for constructing the reference curve. In order to evaluate the limits of quantification (LOQ) and the limits of detection (LOD) of the compounds, the mixed solution with the lowest concentration was serially diluted to obtain a series of reference solutions. All the solutions were stored at 4 ℃ until use.

Sample preparation

The decoction pieces and granule products of *Astragali Radix* were pulverized into fine powder. The powdered sample (0.5 g) was then extracted by ultrasonic extraction method with 25 mL of 70% methanol for 30 min at room temperature.

The oral liquid products from different batches were diluted to different multiples, according to the requirement for detection.

All the samples were centrifuged at 15 000 r/min for 10 min before analysis.

Method validation

Samples of decoction pieces were used to validate the UPLC method by investigating the linearity, LOD, LOQ, intra- and inter-day precisions, repeatability, stability, and recovery. All the compounds were identified by checking their retention time and MS data against those of corresponding reference compounds. Each concentration was analyzed for three times for plotting the standard curves. The LOD and LOQ were computed using three times and ten times of the signal-to-noise (S/N) ratios, respectively. Intra- and inter-day variations which determined the precision of the method were investigated by repetitively injecting for six times in the same day for three successive days, respectively. Six samples were prepared independently for checking the repeatability. The sample solution was stored at room temperature for investigating its stability by repeatedly injecting the sample solution at 0, 1, 2, 4, 6, 8, 12, 16, and 24 h. Recovery test was carried out by adding certain amounts of eight reference substance solutions to 0.25 g powder of sample in sextuplicate. Samples were prepared by the sample preparation method stated above.

Results and discussion

Optimization of extraction conditions

To ensure the efficient extraction of the main components in decoction pieces of *Astragali Radix*, the extraction conditions were optimized by varying several parameters, including extraction solvent (50%,

70% methanol aqueous solution and pure methanol), sample-solvent ratio (25, 50, and 100 mL/g of sample), and ultrasonication time (15, 30, and 45 min). Eventually, a combination of 70% methanol aqueous solution, 50 mL/g of sample, and 30 min of ultrasonication time was found to afford the best extraction efficiency.

Methodological validation of UPLC system

The QAMS method established in our work was validated in terms of linearity, LOD, LOQ, precision, repeatability, stability, and recovery tests. The results are respectively shown in Tables 1 and 2. The linearity test indicated good linear correlations by showing correlation coefficient values above 0.9997. For the quantified compounds, LODs and LOQs were 0.01—0.42 and 0.02—1.26 μg/mL, respectively. The relative standard deviation (RSD) values of intra- and inter-day precisions were below 3.33%, and the repeatability was less than 2.76%. Stability test showed that the RSD values were lower than 3.55%, indicating that the sample solution was stable at room temperature for at least 24 h. The recovery rates ranged from 93.94% to 104.20% with RSD values lower than 4.65%. The UPLC-DAD chromatograms of the reference solution and sample solutions of three different *Astragali Radix* products were shown in Fig. 2. All these values are reliable, indicating that the UPLC method is appropriate for evaluating the quality of *Astragali Radix*.

Location of target chromatographic peaks

The quantitative analysis with QAMS method is accomplished by using the RCF rather than reference substances, and there is certainly a question of how to locate the chromatographic peaks of the objective substances. In this paper, the difference between the internal reference substance and the test substances and the ratio of the retention time between the internal reference substance and the test substances were investtigated among three BEH-C_{18} columns of different lots, and the results were shown in Table 3. These data revealed that both parameters were not much affected by using different columns. UPLC-DAD-MS/MS method was further used to validate the position of chromatographic peaks of the eight target compounds. Through accurate mass measurements of their respective molecular ions and fragments, these compounds were unequivocally identified as campanulin (**1**), genistin (**2**), ononin (**3**), calycosin (**4**), genistein (**5**),

Table 1 Linear regression, LODs, and LOQs for eight compounds (*n* = 3)

Compounds	Regression equations[a]	r^2	Linear ranges / (μg·mL^{-1})	LOD[b] / (μg·mL^{-1})	LOQ[c]/ (μg·mL^{-1})
campanulin	$Y = 286.2\ X + 7.2090$	0.9999	0.31—20.04	0.02	0.06
genistin	$Y = 154.5\ X - 0.4373$	0.9999	0.04— 2.50	0.01	0.04
ononin	$Y = 341.5\ X + 3.3164$	0.9999	0.19—12.02	0.01	0.02
calycosin	$Y = 430.2\ X + 6.4873$	0.9999	0.38—24.02	0.01	0.04
genistein	$Y = 575.3\ X + 1.0232$	0.9999	0.02— 1.20	0.01	0.02
formononetin	$Y = 489.9\ X + 9.8249$	0.9999	0.19—12.06	0.01	0.04
methylnissolin	$Y = 14.5\ X - 0.2160$	0.9999	0.19—12.02	0.06	0.19
astragaloside IV	$Y = 4.8\ X + 0.2313$	0.9997	1.26—40.20	0.42	1.26

[a] *Y* is the peak area, *X* is the concentration of reference solutions

[b] LOD refers to the limit of detection, S/N=3

[c] LOQ refers to the limit of quantity, S/N=10

Table 2 Intra- and inter-day precisions, repeatability, stability, and recovery for eight compounds (*n* = 6)

Compounds	Intra-day RSD / %	Inter-day RSD / %	Repeatability RSD / %	Stability RSD / %	Recovery / %	RSD / %
campanulin	0.32	1.97	1.29	2.88	100.06	2.85
genistin	1.19	1.67	1.86	3.55	98.48	2.90
ononin	0.35	2.19	1.21	2.90	97.87	2.91
calycosin	0.36	1.92	1.09	2.96	100.59	2.71
genistein	0.46	1.95	1.82	2.20	93.94	2.64
formononetin	0.33	2.05	0.88	2.74	104.20	1.61
methylnissolin	3.33	3.08	2.76	2.90	95.46	4.65
astragaloside IV	3.12	3.20	2.45	2.44	100.23	3.29

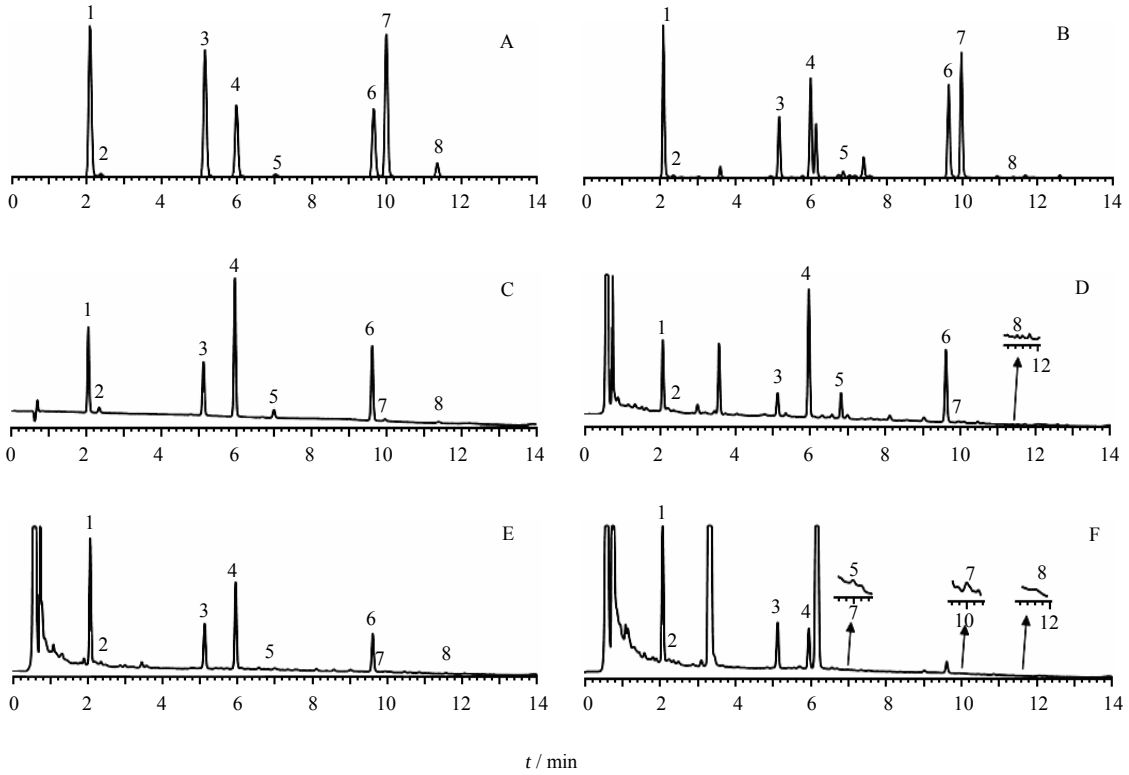


Fig. 2 UPLC-MS total ion current chromatograms of mixed reference solution (A), sample solution from decoction pieces under positive ion mode (B), and UPLC-UV chromatograms of standard solution of eight compounds (C), sample solutions of decoction pieces (D), granule (E), and oral liquid (F)

1: campanulin 2: genistin 3: ononin 4: calycosin 5: genistein 6: formononetin 7: methylnissolin 8: astragaloside IV

Table 3 Retention time and MS data of eight components in *Astragali Radix* under positive ion mode ($\bar{x} \pm s$)

Peak No.	Retention time[a]		MS Data (*m/z*)		Identification
	Differences	Ratios			
1	–3.910 ± 0.017	0.345 ± 0.002	447$[M+H]^+$	285$[M+H–Glc]^+$, 253$[M+H–Glc–CH_3OH]^+$, 225$[M+H–Glc–CH_3OH–CO]^+$	campanulin
2	–3.617 ± 0.011	0.394 ± 0.003	433$[M+H]^+$	271$[M+H–Glc]^+$, 253$[M+H–Glc–H_2O]^+$, 153[M+H–Glc–*p*-ethynylphenol]$^+$, 243$[M+H–Glc–CO]^+$	genistin
3	–0.847 ± 0.012	0.858 ± 0.003	431$[M+H]^+$	269$[M+H–Glc]^+$, 237$[M+H–Glc–CH_3OH]^+$, 209$[M+H–Glc–CH_3OH–CO]^+$	ononin
4[b]	—	—	285$[M+H]^+$	253$[M+H–CH_3OH]^+$, 225$[M+H–CH_3OH–CO]^+$	calycosin
5	1.037 ± 0.006	1.174 ± 0.002	271$[M+H]^+$	253$[M+H–H_2O]^+$, 153[M+H–*p*-ethynylphenol]$^+$, 243$[M+H–CO]^+$, 253$[M+H–H_2O]^+$, 225$[M+H–H_2O–CO]^+$	genistein
6	3.667 ± 0.006	1.614 ± 0.004	269$[M+H]^+$	237$[M+H–CH_3OH]^+$, 209$[M+H–CH_3OH–CO]^+$	formononetin
7	4.013 ± 0.011	1.672 ± 0.005	301$[M+H]^+$	269$[M+H–CH_3OH]^+$, 241$[M+H–CH_3OH–CO]^+$, 211$[M+H–CH_3OH–CO–CH_2O]^+$, 167$[M+H–CH_3OH–CO–CH_2O–CO_2]^+$	methylnissolin
8	5.327 ± 0.025	1.892 ± 0.009	807$[M+Na]^+$	645$[M+Na–Glc]^+$, 627$[M+Na–Glc–H_2O]^+$	astragaloside IV

[a] Difference = retention time of target substance – retention time of internal reference substance

Ratio = retention time of target substance / retention time of internal reference substance

[b] "4" in "Peak No." corresponding to internal reference substance

formononetin (**6**), methylnissolin (**7**), and astragaloside IV (**8**) (Table 3). The total ion current chromatograms of the mixed reference solutions and sample solutions from decoction pieces under positive ion mode are displayed in Fig. 2, which reveal that the peak location by UPLC-DAD-MS/MS method is reliable.

Robustness of QAMS

The QAMS method was applied in the quality control of *Astragali Radix* by using the RCF. In this study, calycosin was chosen as the internal reference substance given its extensive distribution in *Astragali Radix*, as well as that it is cheap and commercially available. The content of calycosin was determined by using an external standard. The RCF (f_{si}) is a constant ratio in a computational formula and could be calculated as follows:

$$f_{si}=\frac{f_s}{f_i}=\frac{C_s/A_s}{C_i/A_i}(i=1,2,3...n)$$

Where A_s is the peak area of internal reference substance; C_s is the concentration of internal reference substance; A_i is the peak area of under tested substance; and C_i is the concentration of test substance (Wang *et al*, 2006)

The value of f_{si} was calculated at different concentration of the reference substance (n = 3 for each concentration), and the average value ($\overline{f_{si}}$) was utilized to calculate the content of the analyte as follows:

$$C_i'=\frac{C_s A_i}{A_s \overline{f_{si}}}$$

Since RCF is a key parameter in the application of QAMS method for the quality control, to understand the influence of chromatographic conditions on RCF values, several chromatographic parameters were varied on three batches of columns. These parameters are column temperature (45, 50, and 55 ℃), flow rate (0.35, 0.40, and 0.45 mL/min), and the concentration (0.05%, 0.10%, and 0.20%) of the formic acid used in the mobile phase. As indicated in Table 4, regardless of the chromatographic conditions, the RCF values were in good agreement with each other. We could therefore exclude the chromatographic influence on RCF values.

Table 4 RCFs in robustness test for QAMS method ($\overline{x} \pm s$)

Compounds	RCFs			
	S_1[a]	S_2[b]	S_3[c]	S_4[d]
campanulin	0.666 ± 0.005	0.666 ± 0.005	0.668 ± 0.004	0.667 ± 0.004
genistin	0.356 ± 0.010	0.353 ± 0.005	0.352 ± 0.005	0.356 ± 0.009
ononin	0.793 ± 0.004	0.792 ± 0.005	0.791 ± 0.005	0.793 ± 0.005
genistein	1.315 ± 0.061	1.340 ± 0.015	1.353 ± 0.007	1.338 ± 0.016
formononetin	1.141 ± 0.017	1.141 ± 0.017	1.143 ± 0.015	1.144 ± 0.014
methylnissolin	0.033 ± 0.001	0.033 ± 0.001	0.034 ± 0.001	0.033 ± 0.001
astragaloside IV	0.011 ± 0.001	0.012 ± 0.001	0.011 ± 0.001	0.012 ± 0.001

[a] S_1 was performed on Column 1 with 0.10% formic acid and the flow rate of mobile phase of 0.40 mL/min

[b] S_2 was performed on Column 1 with the column temperature of 50 ℃ and the flow rate of mobile phase of 0.40 mL/min

[c] S_3 was performed on three batches (175300772, 189310381, and 189310271) of Acquity UPLC BEH-C_{18} column (100 mm × 2.1 mm, 1.7 μm) with 0.10% formic acid, the column temperature of 50 ℃, and the flow rate of mobile phase of 0.40 mL/min

[d] S_4 was performed on Column 1 with 0.10% formic acid and the column temperature of 50 ℃

Analysis of *Astragali Radix* products

To quantify the contents of the chemical components, a total of twelve samples (decoction pieces of *Astragali Radix* and its two different dosage forms including granule and oral liquid products) of commercial *Astragali Radix* products were analyzed by both UPLC-DAD and QAMS methods. The results are summarized in Table 5. The RSD values obtained by comparing the data derived from both methods were below 3.98%, which decreased within an acceptable range.

The contents of the eight compounds were more or less distinguishable in the decoction pieces of *Astragali Radix* and its two different dosage forms, which may contribute to the difference in curative effects. Even when the samples were in the same dosage form, this phenomenon also existed among different batches. For instance, the total contents of calycosin, formononetin, methylnissolin, and astragaloside IV were not consistent among different producing areas in the decoction pieces with Max/Min above 4. Several factors, such as country of origin, harvesting period, processing, and manufacturing process, can result in variations of the chemical composition of *Astragali Radix*.

Table 5 Contents of eight components in twelve *Astragali Radix* samples ($\bar{x} \pm s$, n = 3)

Method	Dosage form[c]	Compound							
		Calycosin	Campanulin	Genistin	Ononin	Genistein	Formononetin	Methylnissolin	Astragaloside IV
EMS[a]	D-1	77.41 ± 0.22	149.65 ± 0.82	5.69 ± 0.10	65.38 ± 0.24	1.06 ± 0.01	58.13 ± 0.64	27.43 ± 0.19	BLOQ[d]
	D-2	320.20 ± 1.43	174.84 ± 0.92	4.11 ± 0.08	55.27 ± 0.42	4.84 ± 0.06	172.46 ± 1.07	93.48 ± 2.10	142.24 ± 3.79
	D-3	253.26 ± 1.34	181.09 ± 0.62	4.98 ± 0.07	57.70 ± 0.28	5.43 ± 0.02	132.73 ± 0.48	75.75 ± 3.15	199.47 ± 6.57
	G-1	401.67 ± 0.61	765.23 ± 0.77	31.51 ± 0.01	273.36± 0.38	9.71 ± 0.08	166.47 ± 0.19	111.46 ± 2.00	470.03 ± 4.17
	G-2	23.05 ± 0.27	25.82 ± 0.43	BLOQ	12.54 ± 0.11	BLOQ	14.22 ± 0.08	BLOQ	218.48 ± 7.92
	G-3	177.01 ± 1.09	452.19 ± 2.26	17.28 ± 0.04	147.39 ± 0.46	2.22 ± 0.02	57.28 ± 0.30	34.62 ± 0.64	385.09 ± 3.44
	G-4	24.60 ± 0.11	100.96 ± 0.48	3.44 ± 0.09	38.02 ± 0.14	BLOQ	BLOQ	BLOQ	310.09 ± 4.45
	G-5	184.23 ± 0.30	185.21 ± 0.53	5.37 ± 0.06	61.31 ± 0.34	1.68 ± 0.02	85.93 ± 0.29	40.80 ± 0.84	295.67 ± 3.49
	G-6	111.74 ± 0.42	103.37 ± 0.37	5.45 ± 0.07	48.51 ± 0.15	1.32 ± 0.02	34.47 ± 0.16	20.36 ± 0.73	421.87 ± 11.57
	OL-1	5.19 ± 0.04	0.98 ± 0.01	0.26 ± 0.01	0.36 ± 0.01	BLOQ	0.60 ± 0.01	1.10 ± 0.04	38.39 ± 0.66
	OL-2	5.22 ± 0.01	BLOQ	0.15 ± 0.01	BLOQ	0.03 ± 0.01	0.55 ± 0.01	1.09 ± 0.04	38.26 ± 0.86
	OL-3	28.38 ± 0.10	139.28 ± 0.57	3.47 ± 0.04	42.76 ± 0.18	0.12 ± 0.01	7.57 ± 0.01	5.31 ± 0.04	148.57 ± 1.63
QAMS[b]	D-1	—	148.06 ± 0.80	5.70 ± 0.10	64.98 ± 0.24	1.12 ± 0.01	57.49 ± 0.63	27.45 ± 0.19	BLOQ
	D-2	—	174.05 ± 0.92	4.10 ± 0.08	55.42 ± 0.42	4.85 ± 0.05	169.87 ± 1.05	96.07 ± 2.17	142.29 ± 3.73
	D-3	—	180.11 ± 0.62	5.00 ± 0.07	57.79 ± 0.28	5.43 ± 0.02	130.88 ± 0.47	77.65 ± 3.26	198.46 ± 6.46
	G-1	—	757.93 ± 0.76	32.47 ± 0.01	272.30 ± 0.38	9.64 ± 0.08	164.08 ± 0.18	114.75 ± 2.08	464.92 ± 4.11
	G-2	—	25.97 ± 0.42	BLOQ	12.56 ± 0.11	BLOQ	14.47 ± 0.09	BLOQ	210.90 ± 7.63
	G-3	—	447.32 ± 2.24	17.69 ± 0.04	146.69 ± 0.45	2.26 ± 0.02	56.97 ± 0.30	35.02 ± 0.66	380.43 ± 3.38
	G-4	—	98.25 ± 0.48	3.32 ± 0.09	37.22 ± 0.14	BLOQ	BLOQ	BLOQ	298.95 ± 4.28
	G-5	—	183.98 ± 0.54	5.40 ± 0.06	61.31 ± 0.34	1.74 ± 0.02	84.99 ± 0.28	41.42 ± 0.87	292.70 ± 3.42
	G-6	—	102.96 ± 0.37	5.47 ± 0.07	48.48 ± 0.15	1.38 ± 0.02	34.59 ± 0.15	20.23 ± 0.76	415.51 ± 11.33
	OL-1	—	0.99 ± 0.01	0.27 ± 0.01	0.37 ± 0.01	BLOQ	0.61 ± 0.01	1.13 ± 0.05	37.25 ± 0.63
	OL-2	—	BLOQ	0.16 ± 0.01	BLOQ	0.03 ± 0.01	0.56 ± 0.01	1.11 ± 0.05	37.13 ± 0.82
	OL-3	—	137.50 ± 0.57	3.56 ± 0.04	42.47 ± 0.18	0.12 ± 0.01	7.45 ± 0.01	5.49 ± 0.04	146.18 ± 1.60

[a] ESM-external standard method

[b] QAMS-quantitative analysis of multi-components by single marker

[c] Samples of D (decoction pieces) were obtained from three provinces, G (granule) from six manufacturers, and OL (oral liquid) from three batches of two manufacturers. The content unit of decoction pieces (D-1—D-3) and granule (G-1—G-6) was expressed as $\mu g \cdot g^{-1}$, and the content unit of oral liquid (OL-1—OL-3) was expressed as $\mu g \cdot mL^{-1}$

[d] BLOQ refers to below the limits of quantity

Conclusion

By quantitatively analyzing eight major components in the decoction pieces of *Astragali Radix*, as well as its two different dosage forms including granule and oral liquid products, we have shown that, compared to UPLC-DAD method, QAMS is a sensitive, simple, and reliable method for multi-component quality control. Moreover, QAMS method is advantageous in reducing cost and overcoming the availability issue of reference substances as well, which is promising to be widely applied in the field of quality evaluation of CMM.

References

Cheng XD, Hou CH, Zhang XJ, Xie HY, Zhou WY, Yang L, Zhang SB, Qian RL, 2004. Effects of Huang-qi (Hex) on inducing cell differentiation and cell death in K562 and HEL cells. *Acta Biochem Biophys Sin* 36(3): 211-217.

Gu YC, Wang GJ, Fawcett JP, 2004. Determination of astragaloside IV in rat plasma by liquid chromatography electrospray ionization mass spectrometry. *J Chromatogr B* 801: 285-288.

Hao JJ, Wu WY, Da J, Yao S, Long HL, Yang Z, Cai LY, Yang M, Liu X, Jiang BH, Guo DA, 2011. Ruggedness and robustness of conversion factors in method of simultaneous determination of multi-components with single reference standard. *J Chromatogr B* 1218: 5618-5627.

He B, Liu Y, Yang SY, Zhang Y, 2013. Simultaneous determination of 10 constituents in Shuangqing Yanhou Tablets by HPLC-QAMS. *Chin Tradit Herb Drugs* 44(8): 974-981.

Liu J, Zhao ZZ, Chen HB, 2011. Review of *Astragali Radix*. *Chin Herb Med* 3(2): 90-105.

Liu YH, Chen B, Zhao YY, Wang B, Zhang QY, Zhang L, Tu PF, 2006. Quantification and stability studies on the flavonoids of *Radix hedysari*. *J Agric Food Chem* 54: 6634-6639.

Lu S, Chen KJ, Yang QY, Sun HR, 2011. Progress in the research of *Radix Astragali* in treating chronic heart failure: effective ingredients, dose-effect relationship and adverse reaction. *Chin J Integr Med* 17(6): 473-477.

Pharmacopoeia Committee of P. R. China, 2010. *Pharmacopoeia of the People's Republic of China*, Vol. I. China Medical Science and

Technology Press: Beijing.

Song JZ, Yiu HHW, Qiao CF, Han QB, Xu HX, 2008. Chemical comparison and classification of *Radix Astragali* by determination of isoflavonoids and astragalosides. *J Pharm Biomed Anal* 47: 399-406.

Wang ZM, Gao HM, Fu XT, Wang WH, 2006. Multi-components quantitation by one marker new method for quality evaluation of Chinese herbal medicine. *China J Chin Mater Med* 31(23): 1925-1928.

Wu T, Bligh SWA, Gu LH, Wang ZT, Liu HP, Cheng XM, Branford-White CJ, Hu ZB, 2005. Simultaneous determination of six isoflavonoids in commercial *Radix Astragali* by HPLC-UV. *Fitoterapia* 76: 157-165.

Xiao HB, Krucker M, Albert K, Liang XM, 2004. Determination and identification of isoflavonoids in *Radix Astragali* by matrix solid-phase dispersion extraction and high-performance liquid chromatography with photodiode array and mass spectrometric detection. *J Chromatogr A* 1032: 117-124.

Xie W, Du L, 2011. Diabetes is an inflammatory disease: Evidence from traditional Chinese medicines. *Diabetes Obes Metab* 13: 289-301.

Yu DH, Bao YM, Wei CL, An LJ, 2005. Studies of chemical constituents and their antioxidant activities from *Astragalus mongholicus* Bunge. *Biomed Environ Sci* 18: 297-301.

Yu QT, Qi LW, Li P, Yi L, Zhao J, Bi ZM, 2007. Determination of seventeen main flavonoids and saponins in the medicinal plant Huang-qi (*Radix Astragali*) by HPLC-DAD-ELSD. *J Sep Sci* 30: 1292-1299.

Yu X, Song J, Xiong ZL, Li FM, 2010. Simultaneous assay of epimedin A, epimedin B, epimedin C and icariin in herba epimedii by QAMS. *China J Chin Mater Med* 35(24): 3310-3313.

Zhang RP, Zhang XP, Ruan YF, Ye SY, Zhao HC, Cheng QH, Wu DJ, 2009. Protective effect of *Radix Astragali* injection on immune organs of rats with obstructive jaundice and its mechanism. *World J Gastroenterol* 15(23): 2862-2869.

Zhang WD, Zhang C, Liu RH, Li HL, Zhang JT, Mao C, Chen CL, 2005. Quantitative determination of astragaloside IV, a natural product with cardioprotective activity, in plasma, urine and other biological samples by HPLC coupled with tandem mass spectrometry. *J Chromatogr B* 822: 170-177.

Zou GX, You XM, Zhang Y, Wang GH, Jiang H, 2008. New method of multi-components quantitation by one marker for quality evaluation of Guan mai kang capsula. *China J Chin Mater Med* 33(15): 1828-1831.