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Quantitative Evaluation of Twelve Major Components of *Astragali Radix* Sulfur-Fumigated with Different Durations by UPLC-MS

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Abstract: In this study, an improved UPLC-MS method for simultaneously quantifying twelve major components belonging to two chemical types was developed and validated, and was applied to quantitatively compare the quality of *Astragali Radix* sulfur-fumigated with different durations and the fresh reference sample. The results showed that the contents of triterpenes Astragaloside III and Astragaloside IV decreased moderately, while the flavonoids calycosin, formononetin, and 7,2'-dihydroxy-3',4'-dimethoxyisoflavane decreased significantly, and its corresponding flavonoid glycosides increased accordingly, which indicatied that the happening of chemical transformation of flavonoids and glycosides in the sulfur-fumigated process. These transformations were further confirmed by the the synthesis of flavonoid glycosides under the simulated sulphur-fumigation circumstances. Furthermore, the sulfur-fumigated duration had a proportional relationship with the contents of compounds **7**, **11**, and **12**. All these results suggested that the established method was precise, accurate and sensitive enough for the global quality evaluation of sulfur-fumigated *Astragali Radix*, and sulfur-fumigation can not only change the proportions of bioactive components, but also cause the chemical transformation in the Astragali Radix.

Keywords: *Astragali Radix*; Sulfur-fumigation duration; quantification; synthesis.

1. Introduction

Astragali Radix (AR), the dry roots of *Astragalus membranaceus* (Fisch.) Bunge. or *Astragalus mongholicus* Bunge., is one of the most widely used Qi-tonifying Chinese herbal medicines. In theory of traditional Chinese medicines (TCM), AR has good effects, including tonify Qi of the kidney [1], strengthening exterior and reducing sweat, tonifying Qi and lifting yang, inducing diuresis to alleviate edema, sweet and warm tastes removing fever, promoting wound healing and tissue regeneration [2]. Combining with pharmacological studies, AR has been used in clinic to treat diabetic and reduce the risk of diabetic complications [3], cardio-cerebrovascular disease, respiratory disease, and digestive system disease [4], due to its immunomodulation [5], anti-inflammation [6], anti-tumor [7], nerve cell protecting and recovery [8], anti-aging, and cardioprotective effects [9]. Previous researches found that the main active ingredients of AR includes flavonoids and isoflavones, saponins, polysaccharide, and others [10]. Traditionally, the post-harvest processing of the roots of AR is sun-dry the whole fresh root after cleaning. Because of mildew prone, AR was recently reported as being sulfur-fumigated during post-harvesting handled to storage. So it's necessary to compare its chemical profiles variations after the sulfur-fumigation. Sulfur-fumigation,

which is low-cost and easy operation, has been commonly used to prevent medicinal herbs from pest infestation, mold, and bacterial contamination [11]. But recent studies demonstrated that this method could cause the residue of hazardous substances such as sulfur dioxide and heavy metals, which posed a threat to human health [12]. Furthermore, sulfur-fumigation was reported to reduce the content of active ingredient in herbs, and influence the chemical transformation of bioactive components, even alleviate the pharmacological activities of edible herbs [13-15]. In 2004, the State Food and Drug Administration of China pointed out that sulfur fumigated medicinal herbs are inferior [16]. However, the method of sulfur fumigation handled medicinal herbs and foods still prevails all over the world, which exerts a negative impact on the safe application of edible herbs. To the best of our knowledge, few systemic studies were reported on quantitative evaluation of sulfur-fumigated AR, in particular the effects of sulfur-fumigation durations on the proportions of bioactive components in AR has not been quantitatively evaluated.

In this study, an improved UPLC-MS method for simultaneously quantifying twelve major components (including eight flavonoids calycosin (**1**), Calycosin-7-glucoside (**2**), formononetin (**3**), Ononin (**4**), methylnissolin (**5**), astraisoflavan-7-O-β-D-glucoside (**6**), 7,2'-dihydroxy-3',4'-dimethoxy -isoflavane (**7**), and 7,2'-dihydroxy-3',4'-dimethoxy isoflavan-7-O-β-D-glucopyranoside (**8**), four triterpenoid saponins Astragaloside I (**9**), Astragaloside II (**10**), Astragaloside III (**11**), and Astragaloside IV (**12**) (**Figure 1**) of AR was developed and validated, and applied for quantitative evaluation of AR samples sulfur-fumigated with different durations.

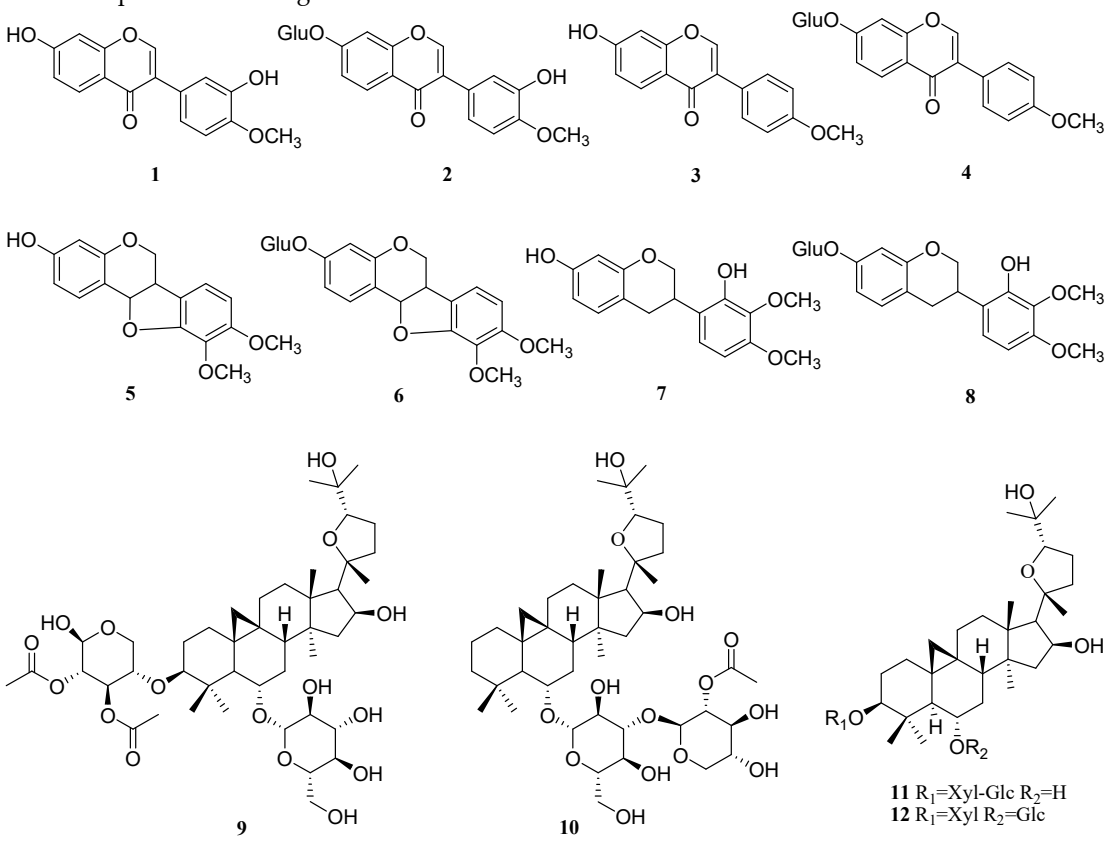

Figure 1. The structures of reference compounds **1-12**

2. Experimental

2.1. Chemicals and reagents

Twelve reference compounds with purities of > 98.0%, including calycosin (**1**), Calycosin-7-glucoside (**2**), formononetin (**3**), Ononin (**4**), methylnissolin (**5**), astraisoflavan-7-O-β-D-glucoside (**6**),

7,2'-dihydroxy-3',4'-dimethoxyisoflavane (7), 7,2'-dihydroxy-3',4'-dimethoxy isoflavan-7-O-β-D-glucopyranoside (**8**), Astragaloside I (**9**), Astragaloside II (**10**), Astragaloside III (**11**), and Astragaloside IV (**12**), were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). These compounds showed high stability in methanol solution, of which their structures are shown in **Figure. 1**. Methanol and acetonitrile were of high performance liquid chromatography (HPLC) grade (Tedia, USA). Distilled water was further purified by Milli-Q system (Millipore, Milford, MA, USA); formic acid was purchased from the first chemical company of Nanjing (Jiangsu, China); other chemicals were of analytical grade. All solvents and samples were filtered through 0.22 μm membrane filters before injecting into UHPLC.

The fresh reference *Astragali Radix* sample was collected from Inner Mongolia Autonomous region, the indigenous cultivating region of *Astragali Radix* and authenticated by Prof. Rong-Tao Li. The voucher specimen (AM171114-1) was deposited at the Institute of Medicinal Plant Development, Beijing, China.

2.2. Sulphur-fumigation of AR

The sulphur-fumigated AR samples were self-prepared in our lab from the non-fumigated reference AR sample (AM171114-1) following the modified procedures similar to that by herbal farmers or wholesalers: 50 g AR slices were moistened with 4 mL of water, and left for 0.5 h. Two grams of sulphur powder was heated until burning, then the burning sulphur and the moistened AR slices were carefully put into the lower and upper layer of a desiccator respectively. Seven portions (50 g each portion) were prepared to study of the sulfur-fumigation extent at different collection points of 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, and 72 h, respectively. After fumigation, the AR slices were dried at 40 ℃and ground into fine powder.

2.3. Instrument

Analyses were carried out using an Agilent 1290 Infinity II RRLC system consisting of a quaternary delivery system, a degasser and an auto-sampler (Agilent, Malaysia). Chromatographic separation was achieved on an ACQUITY UPLC™ HSS T3 (100 mm×2.1 mm, 1.8 μm, Waters, USA). The column was maintained at 40°C and eluted at a flowing rate of 0.25 mL/min, The mobile phase consisted of 0.2% formic acid water (A) and acetonitrile (B) using a gradient elution of 1–20% B at 0–5 min, 20–25% B at 5–8 min, 30–40% B at 15–18 min, 40–60% B at 185–20 min, 60–90% B at 20–23 min, and 99–1% B at 23–25 min.

An Applied Biosystems 3200 Q-Trap system (AB SCIEX, Singapore) equipped with an electrospray ionization (ESI) source was used and the system was operated in positive and negative mode. Optimization of multiple reaction monitoring (MRM) conditions were carried out with a followed source-dependent parameters: Gas 1 and gas 2 were set at 50 psi. The optimized ion spray voltages were set at 5500 V and -4500V in positive and negative ion mode, respectively. The optimized ion spray voltage and temperature were set at 5500V and 700 ◦C, respectively. The operating vaporizer temperature, 500 °C. Nitrogen gas was used in all analyses, and data acquisition and processing were performed using Analyst software version 1.6.2. The MRM parameters are outlined in **Table 1**.

Table 1. MRM transitions and parameters for the detection of the 12 analytes.

No.	Analyte	Precursor ion (m/z)	Product ion (m/z)	DP (V)	CE (V)
1	Calycosin	285.2	213.1	70	45
2	Calycosin-7-glucoside	447.3	285.4	60	20

3	Formononetin	269.0	167.2	70	47
4	Ononin	431.1	269.1	65	20
5	Methylnissolin	301.2	167.2	54	20
6	Astraisoflavan-7-O-β-D-glucoside	463.3	167.4	60	40
7	7,2'-dihydroxy-3',4'-dimethoxyisoflavane	303.0	167.2	55	19
8	7,2'-dihydroxy-3',4'-dimethoxy Isoflavan-7-O-β-D-glucopyranoside	463.3	301.0	-74	-24
9	Astragaloside I	867.9	59.1	-250	-83
10	Astragaloside II	825.7	59.1	-110	-70
11	Astragaloside III	783.8	160.9	-150	-47
12	Astragaloside IV	783.8	101.0	-115	-57

2.4. Standard solutions preparation.

Standard stock solution 1 and 2 consisted of 7 (**1** - **7**) and 5 (**8** - **12**) accurately weighed reference compounds were directly prepared in methanol, respectively. The final concentrations of these twelve reference compounds in stock solutions were prepared to be 11.88 ug/ml for formononetin, 21.6 ug/ml for calycosin, 17.41 ug/ml for methylnissolin, 15.5ug/ml for 7,2'-dihydroxy-3',4'-dimethoxyisoflavane, 17.44 ug/ml for calycosin-7-glucoside, 38.064 ug/ml for Calycosin-7-glucoside, 6.427 ug/ml for astraisoflavan-7-O-β-D-glucoside, 62.4ug/ml for 7,2'-dihydroxy-3',4'-dimethoxyisoflavan-7-O-β-D-glucopyranoside, 214 ug/ml for astragaloside I, 61.2 ug/ml for astragaloside II, 28.2 ug/ml for astragaloside III and 114.6 ug/ml for astragaloside IV, respectively. The working standard solutions were prepared by diluting the stock solutions with methanol to a series of proper concentrations. The solutions were brought to room temperature and filtered through 0.22 μm membrane filters, and an aliquot of 5 μL was injected into UPLC-MS for the followed analysis.

2.5. Sample preparation

Methanol extracts: Each AR was accurately weighed (approximately 1.0 g) and heat relux with 50.0 mL of methanol for 4 h. The extract was then filtered by a 0.22 μm PTFE syringe filter before LC-MS analysis.

2.6. Method validation

Method validation assays were carried out according to currently accepted Food and Drug Administration (FDA) guidance.

2.6.1. Calibration curves, limits of detection and quantification.

The calibration curves for 12 reference compounds were established by plotting peak area ratios of each analyte using the linear regression analysis using $1/X^2$ as a weighting factor. Calibration curves had to have a correlation coefficient (r) of 0.995 or better. The limit of detection (LOD) was determined as signal-to-noise ratio >3 and the limit of quantification (LOQ) was measured as signal-to-noise ratio >10 (**Table 2**).

Table 2. Validation with respect to linearity, LOQ, LOD, precision, repeatability and stability

Analytes	Regression equation (μg/mL)	Linear range (μg/mL)	Correlation coefficient (R2)	LOQ (ng/ml)	LOD (ng/ml)	Precision RSD (%)		Repeatability RSD (%)	Stability RSD (%)
						Intra-day	Inter-day		
Calycosin	y=446765x+322603	0.4752~11.88	0.9989	0.59	0.06	2.06%	3.16%	4.47%	3.93%
Calycosin-7-glucoside	y=202558x+365088	0.864~21.6	0.9992	1.08	0.11	1.31%	2.09%	3.43%	2.95%
Formononetin	y=469388x+141426	0.3482~17.41	0.9986	0.87	0.35	1.65%	1.69%	4.09%	3.37%
Ononin	y=51545x+4316.5	0.31~15.5	0.9994	3.10	1.55	1.42%	3.86%	4.63%	4.05%
Methylnissolin	y=329224x+84783	0.6976~17.44	0.9993	0.09	0.02	1.46%	2.53%	4.31%	2.99%
Astraisoflavan-7-O-β-D-glucoside	y=130675x+208291	1.5226~38.064	0.9991	0.08	0.04	2.03%	2.78%	4.49%	3.73%
7,2'-dihydroxy-3',4'-dimethoxyisoflavane	y=57437x+11250	0.2571~6.427	0.9990	0.32	0.35	1.96%	3.53%	4.92%	3.77%
7,2'-dihydroxy-3',4'-dimethoxy isoflavan-7-O-β-D-glucopyranoside	y=127060x+236161	2.496~62.4	0.9988	0.01	0.001	1.25%	2.37%	3.68%	1.50%
Astragaloside I	y=1009.3x-10607	8.56~214	0.9991	0.09	0.04	2.36%	3.18%	4.84%	2.52%
Astragaloside II	y=10750x+25435	1.028~25.2	0.9993	0.03	0.002	1.65%	3.78%	2.66%	2.03%
Astragaloside III	y=12640x+30515	1.128~28.2	0.9993	0.03	0.01	1.41%	2.19%	2.65%	1.87%
Astragaloside IV	y=6748.6x+164720	4.584~114.6	0.9989	0.06	0.01	2.18%	1.98%	4.67%	2.06%

2.6.2. Precision, repeatability and stability

The intra- and inter-day precisions were determined by analyzing 12 analytes from standard stock solution in six replicates during a single day and by duplicating the experiments on three successive days. To further evaluate the repeatability of the developed assays, samples were analyzed in six replicates. Their criteria for acceptability of data were within ± 15% relative error (R.E.) from the nominal values and a precision of within ±1 5% relative standard deviation (R.S.D.). Stability of AR sample was tested at room temperature and analyzed at 0, 2, 4, 6, 8, 10, 12 and 24 h. The contents of the corresponding compounds were calculated from the corresponding calibration curves.

2.6.3. Recovery test

The measured recoveries of the compounds were determined by the method of standard addition. Three concentration levels (low, medium, high) of the mixed standard solutions were spiked with a sample of AR, which was analyzed previously using the above described method and the concentration of each component was calculated according to the calibration curves (**Table 3**).

Table 3. Results of recovery.

Analytes	Recovery (150%)	Recovery (100%)	Recovery (50%)
Calycosin	95.8%	101.5%	103.5%
Calycosin-7-glucoside	10.3.2%	97.1%	96.7%
Formononetin	99.2%	105.2%	95.4%
Ononin	104.7%	101.4%	99.6%
Methylnissolin	105.5%	102.3%	103.9%
Astraisoflavan-7-O-β-D-glucoside	98.9%	99.6%	104.1%
7,2'-dihydroxy-3',4'-dimethoxyisoflavane	101.6%	102.3%	104.5%
7,2'-dihydroxy-3',4'-dimethoxy	101.8%	98.8%	102.7%
isoflavan-7-O-β-D-glucopyranoside	95.5%	98.4%	101.7%
Astragaloside I	98.9%	99.6%	103.1%
Astragaloside II	103.5%	104.3%	104.5%
Astragaloside III	99.1%	102.9%	98.6%
Astragaloside IV	103.8%	97.3%	104.1%

3. Results and Discussion

3.1. Optimization of suitable LC-MS conditions

We initially attempted to optimize one suitable LC-MS method to simultaneously determine all 12 chemical marker compounds in AR. However, we could not obtain acceptable results using one method, where the tested compounds could simultaneously achieve well ion response in single ion mode. Thus, separated two batches of analysis were performed under different ion modes. Compounds **1** - **7** and **8** - **12** were performed in positive and negative ion mode, respectively. **Figure.**

2 presented the typical positive base peak intensity (BPI) chromatograms of plasma samples from all the experimental groups.

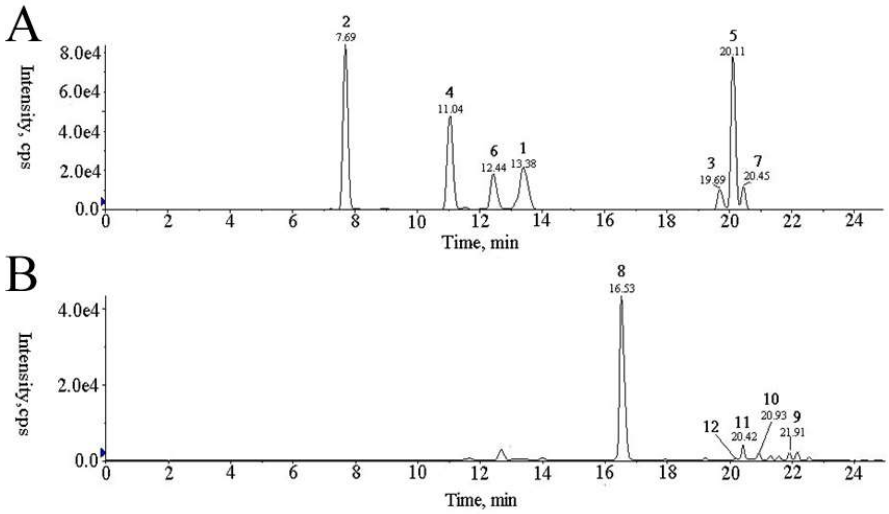

Figure. 2. MRM chromatograms in (A) positive and (B) negative modes. Identification-1, Calycosin; 2, Calycosin-7-glucoside; 3, Formononetin; 4, Ononin; 5, Methylnissolin; 6, Astraisoflavan-7-O-β-D-glucoside; 7, 7,2'-dihydroxy-3',4'-dimethoxyisoflavane; 8, 7,2'-dihydroxy-3',4'-dimethoxy; 8, Isoflavan-7-O-β-D-glucopyranoside; 9, Astragaloside I; 10, Astragaloside II; 11, Astragaloside III; 12, Astragaloside IV.

Meanwhile, two different columns, different mobile phases and detecting ion modes were tested during method development. The selection of UPLC columns with high separation efficiency is a prerequisite. Here, two chromatographic columns, BEH (ethylene bridged hybrid) C18 column (2.1 mm × 100 mm, 1.7 μm, Waters) and HSS (high strength silica) T3 column (2.1 mm × 100 mm, 1.8 μm, Waters), were utilized to investigate for the comprehensive metabolome. The BEH C18 column is the universal column choice for UPLC separations. While HSS T3 column with 100% silica particle, is used to retain and separate smaller, more water-soluble polar organic compounds than the BEH C18 column (Zhao et al., 2013). The result showed that HSS T3 column could gain a more extensive retention and a better chromatographic separation for the 12 tested analysts.

Mobile phases including acetonitrile-water and methanol-water with modifiers such as acetic acid, formic acid, and different gradient elution modes were all investigated. The results showed that the mobile phase consisted of water (0.2% formic acid) and acetonitrile (0.2% formic acid) gave the best separation and peak shape.

3.2. Method validation

3.2.1. Calibration curves, limits of detection and quantification.

Standard stock solutions were prepared as described in the section 'Preparation of standard solutions' and diluted to appropriate concentrations to establish the calibration curves. At least six different concentrations were analyzed in triplicate, and the calibration curves were then constructed by plotting the peak areas vs. the concentration of each analyte. As showed in **Table 2**, all the analytes showed good linearity ($R^2 \geq 0.9986$) in a relatively wide concentration range. The analysis of LOD and LOQ also showed a well quantification, which were ranged from 0.001-1.55 ng/ml and 0.01-3.10 ng/ml, respectively.

3.2.2. Precision, repeatability and stability

The precisions were determined by analyzing known concentrations of the 12 analytes from two standard stock solutions in six replicates during a single day and by duplicating the experiments. To further evaluate the repeatability of the developed assays, samples were analyzed in six replicates as described above. Stability of AR sample was tested at room temperature and analyzed at different time points within one day. The contents of the 12 analytes were calculated from the corresponding calibration curves. **Table 2** indicated that the RSD values for measurement precision, repeatability and stability of the 12 compounds were all less than 5.0%, which demonstrates good precision, repeatability and stability of the developed method.

3.2.2.Accuracy

Accuracy of the analytical method was evaluated by measuring percentage recovery of 12 analytes. The results of the recovery test are shown in **Table 3**, which were all ranged from 95%-105% at three spiked concentrations.

3.3 Quantification of the major components in AR with and without sulphur-fumigation

The validated LC-MS method was applied for quantitative determination of the 12 components with and without sulphur-fumigation. The contents of eight flavonoids and four triterpenoid saponins were summarized in **Table 4**. From the results, it can be found that compared with the non-fumigated sample, the contents of two flavonoids calycosin (**1**) and formononetin (**3**) decreased significantly ranging from 39.2% to 45.4% and 35.5% to 40.5%, respectively; 7,2'-dihydroxy -3',4'-dimethoxyisoflavane (**7**) had a large fluctuation ranging from 6.5% to 39.8%; the content of methylnissolin (**5**) had no obvious change in the sulfur-fumigated samples; while the contents of four flavonoid glycosides (compounds **2**, **4**, **6**, and **8**) all increased remarkably which suggested that the happening of chemical transformation of flavonoids and glycosides in the sulfur-fumigated samples. In addition, the contents of Astragaloside III (**11**) and Astragaloside IV (**12**) decreased moderately ranging from 11.5% to 40.0% and 15.5% to 47.7%, respectively, when compared with the non-fumigated sample; the content of Astragaloside I (**9**) also displayed no obvious change in the sulfur-fumigated sample; the content of Astragaloside II (**10**) was not detected because of the limited detection. Furthermore, the analyses of the detected compounds' contents in different sulfur-fumigated time suggested that the reduction proportions of compounds **7**, **11**, and **12** had a proportional relationship with sulfur-fumigated time. All above results indicated that sulphur-fumigation can decrease the contents of partial aglycones and triterpenoid saponins and increase the contents of flavonoid glycosides in AR significantly. Therefore, it could be concluded that sulphur-fumigation can significantly influence the inherent quality of raw materials of AR.

3.4 General procedure for the synthesis of flavonoid glycosides

The variation of flavonoids and glycosides contents in the sulphur-fumigation of AR compared with the reference sample suggested the flavonoids may have a reaction with glucoses under high temperature and acidic conditions during the sulphur-fumigation process. In order to confirm the deduction, we further designed the procedure for the synthesis of flavonoid glycosides which was similar to the sulphur-fumigation circumstances.

Calycosin (**1**), formononetin (**3**), and 7,2'-dihydroxy-3',4'-dimethoxyisoflavane (**7**) (10 mg each) were dissolved with mixed solvent DMSO and H_2O (2 mL each) in the sealing tubes, respectively. Then 1 mL of concentrated hydrochloric acid and D-glucose (10 mg, 0.055 mmoL) were added. The reaction mixture was heated to 80◦C. After 12 hours, the four reaction mixtures were analyzed by HPLC, respectively. The results showed that calycosin-7-glucoside (**2**), ononin (**4**), astraiso-flavan--7-O-β-D -glucoside (**6**), and 7,2'-dihydroxy-3',4'-dimethoxy isoflavan-7-O-β-D-glucopy- ranoside (**8**) were generated by comparison with the standard materials (**Figure. 3**). This experiment further confirmed that sulfur-fumigation can increase the extent of transformation of flavonoids to flavonoid glycosides.

Table 4. The contents of twelve reference compounds in AR with and without sulphur-fumigation (mg/g, n=3).

Compounds	AR with and without sulphur-fumigation.												
	1 h	2 h	4 h	6 h	8 h	12 h	16 h	24 h	36 h	48 h	60 h	72 h	non-fumigated
1	7.49 ± 0.03	5.04 ± 0.04	6.40 ± 0.02	7.36 ± 0.07	4.91 ± 0.00	5.97 ± 0.01	7.44 ± 0.10	5.37 ± 0.02	5.42 ± 0.05	4.86 ± 0.01	5.28 ± 0.06	6.03 ± 0.03	13.55 ± 0.68
2	4.24 ± 0.05	5.45 ± 0.02	5.10 ± 0.00	4.83 ± 0.01	4.61 ± 0.04	4.27 ± 0.02	4.07 ± 0.05	4.39 ± 0.03	4.33 ± 0.04	4.48 ± 0.01	3.92 ± 0.02	3.87 ± 0.02	3.21 ± 0.01
3	0.59 ± 0.01	0.59 ± 0.03	0.75 ± 0.02	0.78 ± 0.00	0.50 ± 0.02	0.64 ± 0.01	0.50 ± 0.03	0.62 ± 0.00	0.60 ± 0.02	0.56 ± 0.01	0.61 ± 0.02	0.63 ± 0.01	1.37 ± 0.04
4	0.81 ± 0.02	1.19 ± 0.09	1.04 ± 0.01	1.05 ± 0.04	0.88 ± 0.05	0.80 ± 0.07	1.06 ± 0.02	0.89 ± 0.00	0.91 ± 0.02	0.95 ± 0.01	0.76 ± 0.02	0.75 ± 0.01	0.40 ± 0.02
5	0.30 ± 0.01	0.25 ± 0.00	0.27 ± 0.02	0.27 ± 0.00	0.21 ± 0.00	0.22 ± 0.01	0.35 ± 0.02	0.22 ± 0.05	0.23 ± 0.01	0.22 ± 0.02	0.23 ± 0.00	0.22 ± 0.01	0.24 ± 0.01
6	0.55 ± 0.00	0.90 ± 0.01	0.69 ± 0.01	0.64 ± 0.02	0.59 ± 0.00	0.58 ± 0.05	0.53 ± 0.07	0.58 ± 0.00	0.52 ± 0.02	0.67 ± 0.00	0.47 ± 0.00	0.44 ± 0.01	0.32 ± 0.02
7	4.18 ± 0.06	2.61 ± 0.01	2.99 ± 0.05	3.44 ± 0.00	2.93 ± 0.02	3.29 ± 0.04	3.06 ± 0.01	2.51 ± 0.00	3.17 ± 0.02	2.60 ± 0.00	2.54 ± 0.00	3.24 ± 0.01	4.47 ± 0.10
8	0.86 ± 0.00	1.59 ± 0.00	1.38 ± 0.05	1.20 ± 0.07	1.13 ± 0.06	1.16 ± 0.02	0.95 ± 0.01	1.27 ± 0.00	1.08 ± 0.04	1.42 ± 0.06	0.97 ± 0.00	0.75 ± 0.00	0.68 ± 0.00
9	0.71 ± 0.01	0.74 ± 0.02	0.70 ± 0.00	0.71 ± 0.05	0.70 ± 0.00	0.70 ± 0.02	0.74 ± 0.04	0.69 ± 0.07	0.70 ± 0.00	0.68 ± 0.01	0.67 ± 0.02	0.70 ± 0.04	0.73 ± 0.04
10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	0.78 ± 0.06	0.84 ± 0.02	0.79 ± 0.01	0.70 ± 0.04	0.71 ± 0.00	0.73 ± 0.01	0.75 ± 0.00	0.59 ± 0.02	0.60 ± 0.00	0.64 ± 0.01	0.57 ± 0.02	0.58 ± 0.01	0.95 ± 0.03
12	3.97 ± 0.00	4.17 ± 0.02	3.73 ± 0.01	3.38 ± 0.01	3.59 ± 0.00	3.53 ± 0.02	3.61 ± 0.01	2.68 ± 0.05	2.81 ± 0.02	3.03 ± 0.04	2.62 ± 0.00	2.58 ± 0.01	4.94 ± 0.02

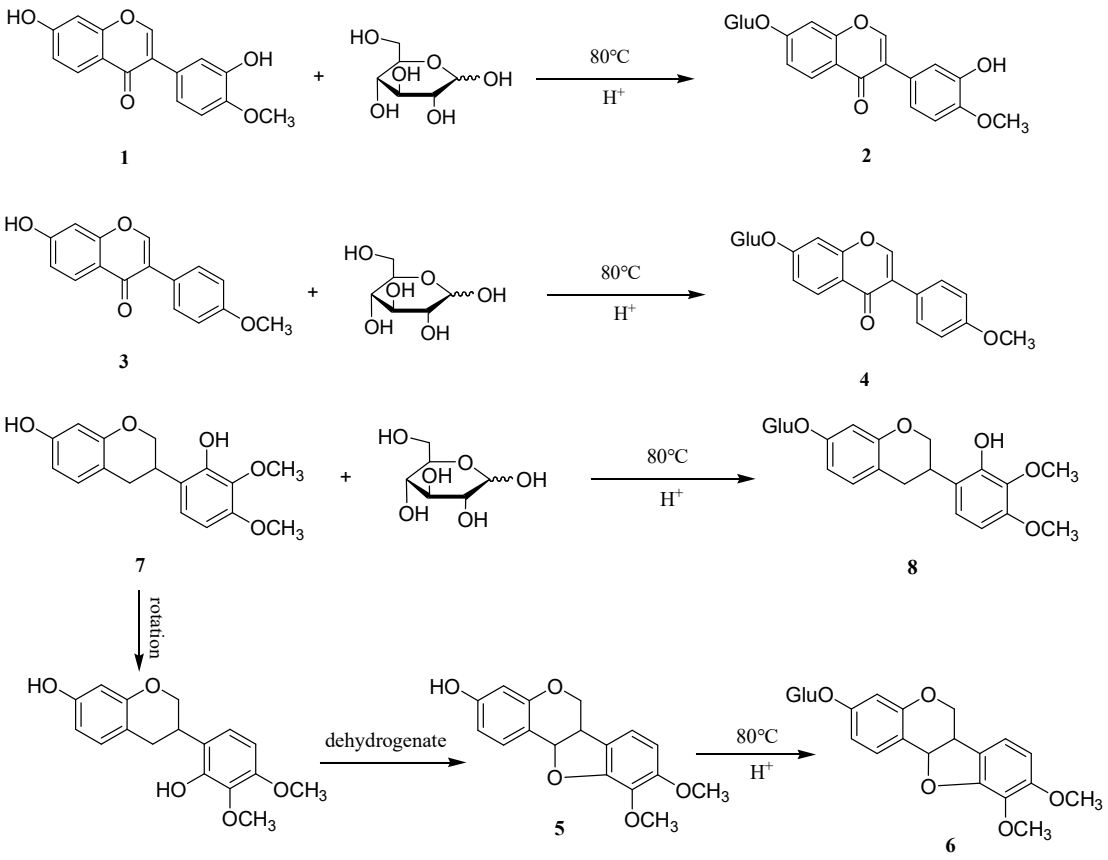

Figure 3. The synthesis of flavonoid glycosides

4. Conclusions

In the present study, a LC-MS method was established for simultaneous quantification of twelve major components in AR, and successfully applied for quantitatively evaluating the effects of sulfur-fumigation on the quality of AR. Compared with previously reported methods, the newly developed method used MRM mode of LC-MS which was the first application to simultaneously detect flavonoids and triterpenoid saponins in A. mongholicus.

On the other hand, the contents of the major flavonoids decreased significantly, while its corresponding glycosides increased accordingly when compared with non-fumigated AR. The contents of the major triterpene glycosides also decreased in the sulfur-fumigation samples, but the degree of reductions were limited. sulphur-fumigation can influence not only the contents of components in AR, but also the chemical transformation of flavonoids and glycosides. It was suggested that sulphur-fumigation should be forbidden for processing and conservation of Chinese medicinal herbs before the efficacy and safety of sulphur-fumigated herbs are systematically investigated. Alternatives to sulphur-fumigation for processing and conservation of AR should also be further developed.

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Sample Availability: Samples of the compounds ..... are available from the authors