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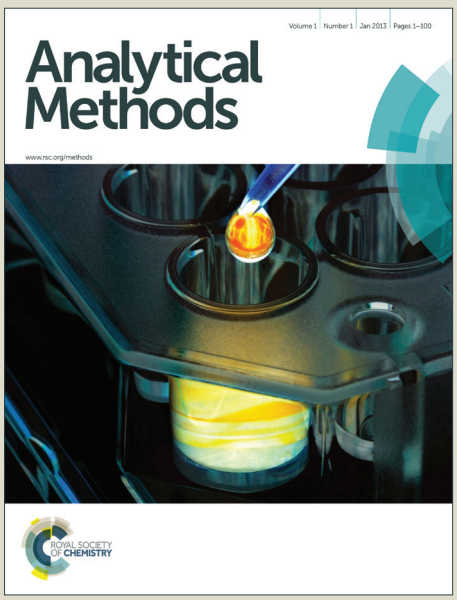

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Rapid and simultaneous quantification of seven bioactive components in Radix Astragali based on pressurized liquid extraction combined with HPLC-ESI-MS/MS analysis

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ABSTRACT

A simple, rapid and sensitive pressurized liquid extraction (PLE) and high-performance liquid chromatography tandem mass spectrometric (HPLC-MS/MS) method has been developed for the simultaneous quantification of seven main bioactive components (calycosin, calycosin-7-*O*-β-D-glycoside, formononetin, formononetin-7-*O*-glycoside, astragaloside IV, astragaloside II and astragaloside III) in Radix Astragali. A gradient elution program was developed using a mobile phase consisting of water containing 0.1% formic acid and acetonitrile containing 0.1% formic acid. Chromatographic separation was successfully achieved on an Agilent Zorbax XDB C_{18} column (2.1 mm × 50 mm, 3.5 μm) with a flow rate of 0.50 mL/min. Detection was based on a triple quadrupole mass spectrometer using a multiple reaction monitoring (MRM) mode with an electrospray ionization source (ESI). The assay was fully validated to demonstrate the specificity, linearity, recovery, matrix effect, accuracy, precision and stability. The limits of detection and limits of quantification of seven bioactive components were in the range of 0.03-0.60 and 0.10-2.00 ng/mL, respectively. The intra- and inter-day precisions were all less than 15%. Most importantly, the running time of analyzing one sample is only 4.5 min. The established PLE and HPLC-ESI-MS/MS method could serve as a simple, rapid and sensitive method for quality evaluation of Radix Astragali.

Key Words: Radix Astragali; bioactive components; pressurized liquid extraction; HPLC-ESI-MS/MS

1. Introduction

Radix Astragali (Huang-Qi in Chinese), derived from the medicinal plant *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge., is one of the most widely used Chinese herbs present either as a single herb or as a collection of herbs in a complex prescription [1]. Many pharmacological and clinical studies have demonstrated that Radix Astragali possesses many biological activities, including hepatoprotective, neuroprotective effect against ischemic brain injury, immunological properties, cardiotonic and antiaging activities, strengthening the superficial resistance, promoting the discharge of pus and the growth of new tissues [2–5]. It has been used in traditional Chinese medicines (TCMs) for the treatment of nephritis, diabetes, cancer, etc. for centuries as an important tonic, an atonic, a diuretic, and an adjunctive medicine [6, 7]. Most importantly, Radix Astragali is also a kind of nutritious food usually added in many cooking dishes all over the world.

It is well know that currently demand for the medicinal and edible plant from which Radix Astragali is obtained is outstripping supply as a consequence of increased interest in natural herbal medicines and foods. However, there are different origins of Radix Astragali, such as *Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao or *A. membranaceus* (Fisch.) Bge., and there are also different places of origin. In some areas of China, such as Heilongjiang Province, the leaves and flowers of Radix Astragali were even taken as natural health food and deemed to have the same activities as Radix Astragali [1]. Obviously, the contents of bioactive components in these plants and products, may vary significantly even in different batches, and therefore affect the therapeutic effects and safety. Therefore, it is of great importance to study the variation of the contents of bioactive flavonoids and saponins in different batches of Radix Astragali, in order to provide clear evidence and scientific support for efficient utilization of Radix Astragali.

Chemical and pharmacological investigations on Radix Astragali revealed that flavonoids and saponins are two kinds of main bioactive components responsible for pharmacological activities and therapeutic efficacy [1, 8], and therefore these two kinds of bioactive components should be chosen as the markers for the quality control of Radix Astragali. Flavonoids in Radix Astragali mainly include calycosin, formononetin, calycosin-7-*O*-β-D-glycoside, and formononetin-7-*O*-glycoside, etc. And saponins in Radix Astragali mainly include astragaloside IV, astragaloside II, astragaloside III, etc. These flavonoids and

saponins are all those components with relatively higher contents in Radix Astragali and with significant pharmacological effects.

All kinds of analytical methods have been reported for the quality evaluation of Radix Astragali using one or several components as marker(s), either through analysis of flavonoids by HPLC-UV [9] and LC-MS [10, 11], or through analysis of saponins by TLC [12], HPLC-ELSD [13, 14], and LC-MS [15–17]. However, the markers of these published methods for the quality control of Radix Astragali are only one or several components, not the accepted comprehensive markers "flavonoids and saponins". Yu QT *et al* reported an HPLC-DAD-ELSD method for the simultaneous determination of flavonoids and saponins in Radix Astragali [1]. However, the LC running time in this method was up to 80 min, which is too long to be utilized in high throughput analysis in the field of medicinal and food-stuff industry. An LC-ESI-ToF MS method had been developed for the determination of seven astragalosides in Radix Astragali and related preparations, but the running time was still up to 65 min [20]. In addition, the sensitivities of the analytes in these two methods were not enough to meet the increasing analytical requirements nowadays.

In present study, a simple, rapid and sensitive pressurized liquid extraction (PLE) and high-performance liquid chromatography tandem mass spectrometric (HPLC-MS/MS) method has been developed for the simultaneous quantification of seven main bioactive components (calycosin, calycosin-7-*O*-β-D-glycoside, formononetin, formononetin-7-*O*-glycoside, astragaloside IV, astragaloside II and astragaloside III) in Radix Astragali. HPLC-ESI-MS/MS was considered as an attractive alternative to HPLC-UV technique in routine quality control of Radix Astragali, especially in situations where high sample throughput and fast analytical speed are needed.

2. Experimental

2.1 Chemicals and reagents

Reference standards of calycosin, calycosin-7-*O*-β-D-glycoside, formononetin, formononetin-7-*O*-glycoside, astragaloside IV, astragaloside II and astragaloside III (HPLC purities ≥ 98%) were all purchased from Shanghai Jingke Chemicals Co., Ltd (Shanghai, China). The chemical structures of seven compounds are shown in Fig. 1. Buspirone (Internal standard, IS, batch No. 039K1325) was purchased from Sigma (St. Louis, MO, USA). Acetonitrile (HPLC grade) was obtained from Merck (Darmstadt, Germany). DMSO (Batch No. 806077) was purchased from Tedia (Fairfield, OH, USA). Ultrapure water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA). All other

chemicals were of analytical grade.

Eighteen (18) batches of Radix Astragali samples were purchased from different origins of China. All these samples were sealed in plastic bags and stored at -20°C until analysis.

2.2 Preparation of standard solutions

The reference standard of each compound was accurately weighed and dissolved by adding appropriate volume of dimethyl sulfoxide (DMSO) respectively, and the individual solution was separately prepared at a concentration at 1.00 mg/mL. All stock solutions (10 μL per solution) were added in one Eppendorf tube and diluted with MeOH to make the final volume of 1 mL as standard mixture solution (10 μg/mL for each compound). A series of working standard solutions simultaneously containing seven compounds were then prepared at concentrations of 1.0, 2.0, 5.0, 10, 20, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL for each compound. Stock solution of internal standard (buspirone) was prepared in MeOH with a concentration of 1.00 mg/mL, and added appropriate water in order to dilute to a final concentration of 10 ng/mL. All the stock and working standard solutions were stored at 4°C prior to use.

2.3 Preparation of samples solutions

Pressurized liquid extraction (PLE) is an effective and rapid sample extraction system for medicinal herbs, which has advantages of short operation time, high extraction efficiency, less solvent consumption and good repeatability [18]. In this study, sample preparation was performed using PLE on a Dionex ASE 350 system (Dionex, Sunnyvale, CA, USA) under the optimized conditions. In brief, dried powders (powdered by a pulveriser and sieved through a 40-mesh sieve) of sample (1.0 g) were mixed with diatomaceous earth (Merck, Darmstadt, Germany) in a proportion of 1:1 (w/w), and then placed into 11 mL stainless steel extraction cell. The conditions of PLE method were as follows: solvent, 70% ethanol; temperature, 100°C; static extraction time, 15 min; static cycle, 3; pressure, 1500 psi; and 60% of flush volume. Once completed, the PLE extractions were transferred into separate 50 mL volumetric flasks, diluted to volume with the same solvent, mix well. The resulting solutions were further filtered through 0.22 μm Millipore nylon membranes and the filtrates were stored at 4°C prior to use.

Aliquots of 100 μL filtrates were mixed with 10 μL of methanol (or standard or QC solution) and 100 μL of IS solution (10 ng/mL buspirone). After vortex for 30 s, all the solutions were then transferred to HPLC vials. A volume of 10 μL of this solution was then injected onto the column.

2.4 Instrumentation and analytical conditions

The HPLC system consisted of an LC-20AD pump, a DGU-20 A_3 degasser, an SIL-20AC auto-sampler and a CTO-20A column oven (Shimadzu, Japan). An Agilent Zorbax XDB C_{18} column (2.1 mm × 50 mm, 3.5 μm) was used for separation, and maintained at room temperature. The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B), and was performed using a linear gradient at a flow rate of 0.50 mL/min: 5% B at 0-0.80 min; 5-98% B at 0.80-2.00 min; 98% B at 2.00-3.00 min; 98-2% B at 3.00-3.01 min; 2% B at 3.01-4.50 min. All the samples were filtered through 0.45 μm membranes, and aliquots of 10 μL filtrates were injected for LC-MS/MS analysis.

The HPLC system was coupled with an API 4000 Qtrap mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) via a Turbo IonSpray ionization interface. Following optimization of the setting parameters, the ESI source was operated in positive mode with the curtain, nebulizer and turbo-gas (all nitrogen) set at 20, 50 and 50 psi, respectively. The source temperature was 500ºC and the ion spray needle voltage was 5500 V. The mass spectrometer was operated at unit resolution for Q1 and low resolution for Q3 in the multiple reaction monitoring mode, with a dwell time of 150 ms per multiple reaction monitoring channel. The collision energy (CE), declustering potential (DP) and precursor/product ion pairs of seven components and buspirone (IS) were shown in Table 1. Data were collected and analyzed by the Analyst Data Acquisition and Processing software (Version 1.5.2, Applied Biosystems/MDS Sciex, Concord, ON, Canada).

2.5 Method validation

The method was validated in terms of specificity, linearity, limits of detection (LOD), limits of quantification (LOQ), precision, repeatability, stability and recovery.

The specificity was assessed by comparing the chromatograms obtained from the samples with those obtained from solvent and different standard solutions.

All calibration curves were constructed by plotting the peak area ratio of each compound with IS versus the concentration of the corresponding working standard solution. Analysis at each working standard concentration was performed in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to the concentration of the diluted standard solution when the signal-to-noise ratio was approximately 3 and 10, respectively.

Intra- and inter-day variation was selected to determine the precision of the established method. For the intra-day variation, mixed standard solutions at three different concentration levels (high, medium and low)

of seven analytes were injected in one day. For the inter-day variation, the three mixed solutions were injected over three consecutive days. Variations were expressed as the relative standard deviation (RSD) of the replicates.

For repeatability, six independently prepared solutions of sample HQ-01 were analysed in triplicate. The content (mg/g) of each compound in the sample was determined from the corresponding calibration curve, and the RSD value of the content was calculated as a measurement of the repeatability of the established method.

To assess the stability of seven target compounds in the prepared samples, the same sample (HQ-01) solution was stored at 4 °C and analysed at 0, 4, 8, 12, 24, 36, and 48 h. The RSD value of the peak area was determined for this investigation.

The test for recovery was carried out to examine the accuracy of the method. Accurate amounts of each standard solution at three different concentrations (80%, 100% and 120% of the original content of each compound) were added to 1.0 g of sample HQ-01. Triplicate experiments were performed at each level. The mixture was extracted and analyzed as described. The extraction recovery of each analyte was calculated using the following equation:

$$\text{Recovery (\%)} = (\text{found amount-original amount})/\text{spiked amount} \times 100$$

2.6 Statistics

All the contents of seven main bioactive components in Radix Astragali were presented as mean ± SD. Within group comparisons were performed by IBM SPSS.19.0 software (IBM, Chicago, IL, USA) to identify the genetic relationship between the various samples from different places of origin.

3. Results and discussion

3.1 Mass spectrometry and chromatography

In the present study, the full-scan product ion mass spectra of seven compounds and buspirone (Internal standard) are shown in Fig. 1. In the full-scan Q1 mass spectra of calycosin, calycosin-7-*O*-β-D-glycoside, formononetin, formononetin-7-*O*-glycoside and astragaloside II, the most abundant peaks were all their protonated molecular ion $[M+H]^+$ found at *m/z* = 285.1, 447.0, 269.1, 431.0 and 827.4, respectively. However, for astragaloside III and astragaloside IV, the most abundant peaks were both sodium adduct ion $[M+N_a]^+$ found at *m/z* =807.4 and *m/z* =807.3, respectively. For these seven compounds, the abundances of those ion peaks ($[M+H]^+$ and $[M+N_a]^+$) were stable enough and sufficient for the accurate quantification.

For buspirone, the most abundant peak was the protonated molecular ion $[M+H]^+$ found at m/z = 386.3. Several product ions with similar abundances usually existed in the full-scan product ion mass spectra. For each analyte of interest, one most stable characteristic product ion, but not the most abundant product ion will be chosen to be the product ion for quantification, while one or two other most abundant and characteristic product ions will be chosen to be the product ions for identification in order to avoid false positive results (see Table 1 and Fig. 1). Other MS conditions such as ion spray voltage, curtain gas pressure, nebulizer gas pressure, heater gas pressure, source temperature and collision energy were further optimized to improve the sensitivity and response stability of these seven compounds.

During the optimization of chromatographic conditions, in order to achieve symmetric peak shapes and short chromatographic running times, the mobile phase consisting of acetonitrile with 0.1% formic acid and water with 0.1% formic acid was used on an Agilent Zorbax XDB C18 column (2.1 mm × 50 mm, 3.5 μm) depending on the analytical experiences in our laboratory. In addition, a sharper gradient elution program was utilized in order to achieve short running time and rapid re-equilibrium. Under the present chromatographic conditions, symmetric peak shapes of each analyte of interest and buspirone were obtained.

An internal standard is usually required in LC-MS/MS analysis in order to eliminate the effects from matrix and the extraction efficiency. In this study, buspirone, a readily available chemical compound, was selected as the IS, which displays similar chromatographic retention behavior (t_R = 2.43 min) with seven analytes of interest. Most importantly, as a chemically synthesized compound, buspirone will not exist in traditional Chinese medicines and animal feed. Thus, there were no interferences of IS from seven bioactive compounds and solvents.

3.2 Method validation

Typical HPLC-MS/MS chromatograms of mixed standards (the concentration of each compound: 1.0 ng/mL), and extract of actual sample HQ-01 are shown in Fig. 2. Calycosin, calycosin-7-*O*-β-D-glycoside, formononetin, formononetin-7-*O*-glycoside, astragaloside IV, astragaloside II, astragaloside III and buspirone were eluted at 2.59, 2.36, 2.75, 2.47, 2.54, 2.63, 2.56 and 2.43 min, respectively. The whole chromatographic running time for one sample is only 4.5 min. No peaks interfering with each analyte of interest were observed in the period of analysis, demonstrating the specificity of developed LC-ESI-MS/MS.

The results of linearities, ranges, limits of detection (LODs), limits of quantification (LOQs) of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS were shown in Table 2. Linearity was determined on a series of work solutions with different concentration levels, and the calibration data showed good linearities (R>0.995) in the corresponding concentration ranges. The LODs and LOQs of seven bioactive components were in the range of 0.03-0.60 and 0.10-2.00 ng/mL, respectively.

Table 3 summarizes the intra and inter-day precision and accuracy values for the QC samples. The intra and inter-day precisions for each compound were less than 13.6%, while accuracy was within ± 11.0%. The accuracy and precision results demonstrated that the values were all within the acceptable range and the method was proved to be accurate and precise.

Multiple injections of independently prepared solutions of sample HQ-01 showed that repeatability variations (% RSD) were less than 3.21% (n=6). All the RSD values in the stability test were lower than 4.53%, indicating the good stabilities of seven bioactive components.

LC-MS/MS results may be adversely affected by lack of specificity and selectivity due to ion suppression caused by the sample matrix effect [19]. In present assay, matrix effects of each analyte of interest were simultaneously evaluated in the field of recovery test. Table 4 summarizes the recoveries of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS (n=6) using sample HQ-01. The recoveries were all in the range of 95.1-104.7%, which indicated that the method had a good accuracy for the quantitative determination of samples, and that ion suppression or enhancement from sample matrix was acceptable for this current analytical method.

3.3 Sample determination and hierarchical cluster analysis

The developed method was applied for quantitative determination of seven bioactive components (calycosin, calycosin-7-*O*-β-D-glycoside, formononetin, formononetin-7-*O*-glycoside, astragaloside IV, astragaloside II and astragaloside III) in various Radix Astragali samples from different places of origin, including Shanxi, Heilongjiang, Inner Mongolia and Gansu province. The contents of seven main bioactive components in Radix Astragali were showed in Table 5. It was found that the contents of calycosin, calycosin-7-*O*-β-D-glycoside and astragaloside IV were relative higher than those reported in literatures ($P < 0.05$) [20, 21], which was attributed to the different sample preparation method. In addition, the contents of three saponins and four flavonoids in Radix Astragali from Inner Mongolia are all relatively higher than those from three other places of origin ($P < 0.05$).

Hierarchical cluster analysis (HCA) is a statistical method for finding relatively homogeneous clusters of cases based on measured characteristics and has been widely used for species authentication, origin discrimination, and quality evaluation of traditional Chinese medicines [22]. The hierarchical clustering process can be represented as a tree or dendrogram, in which each step in the clustering process is illustrated by a connection of the tree [23]. In the present study, the multivariate statistical analysis of data using the HCA technique was performed with SPSS version 19.0, and differences between these classes were tested with average Euclidean distances by using the ward method. Fig. 3 presents a dendrogram of 18 batches of samples based on the contents of seven selected variables (i.e. seven bioactive components). The clustering results illustrated that all the tested samples could be mainly divided into three clusters: batches 17, 18, 15, 16, 2, 1 and 6 were in cluster A; batches 7, 8, 5, 3 and 4 were in cluster B; and batches 9–14 were in cluster C. These results were highly consistent with the places of origin, in which batches 9–14 were from Inner Mongolia, batches 5-8 were from Heilongjiang, batches 1-4 were from Shanxi, and batches 15-18 were from Gansu.

The contents of three saponins and four flavonoids in Radix Astragali from different places of origin show significant differences ($P < 0.05$). It is indicated that environment is an important factor in the formation and variation of chemical constituents, including climate, geology, and soil, especially the composition, content and existing form of various elements in the soil. As a result, it is very important to evaluate the variation of the contents of bioactive flavonoids and saponins in Radix Astragali from different places of origin in order to ensure the safe and efficient utilization of Radix Astragali.

4. Conclusions

A simple, rapid and sensitive pressurized liquid extraction (PLE) and high-performance liquid chromatography tandem mass spectrometric (HPLC-MS/MS) method has been developed for the simultaneous quantification of seven main bioactive components (calycosin, calycosin-7-*O*-β-D-glycoside, formononetin, formononetin-7-*O*-glycoside, astragaloside IV, astragaloside II and astragaloside III) in Radix Astragali. The running time of analyzing one sample is only 4.5 min, and more than 150 samples could be assayed daily, including sample preparation, data acquisition and processing. The analytical method described for seven main bioactive components' quantification may be an alternative for routine analyses, which was shown to provide comprehensive information including the source and quality evaluation combined with content determination of flavonoids and saponins in Radix Astragali.

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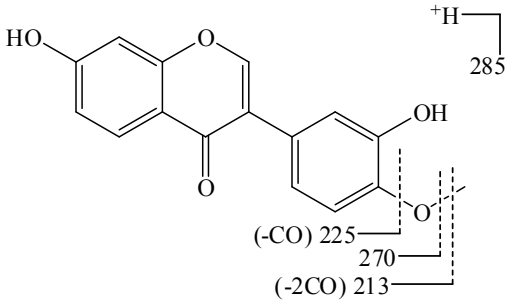
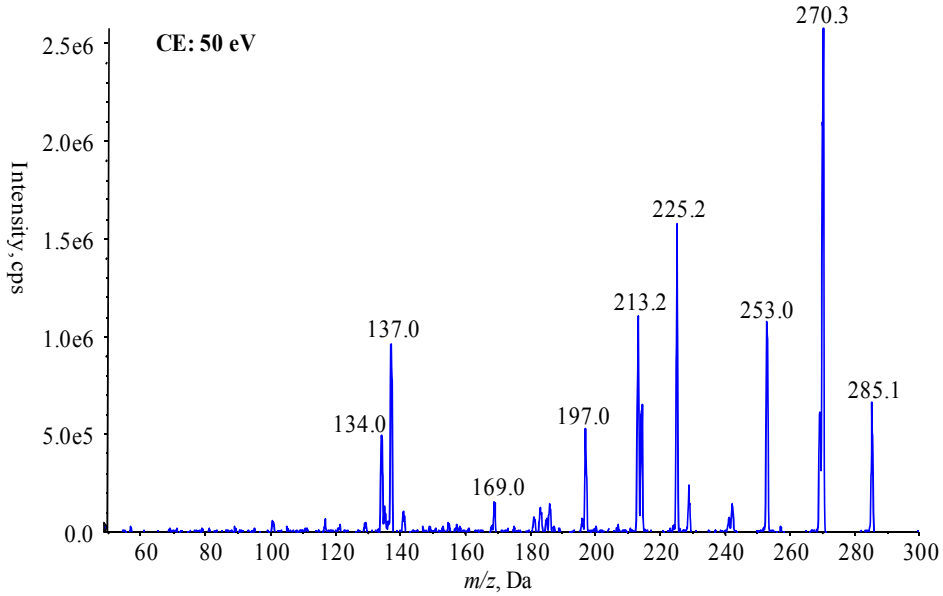
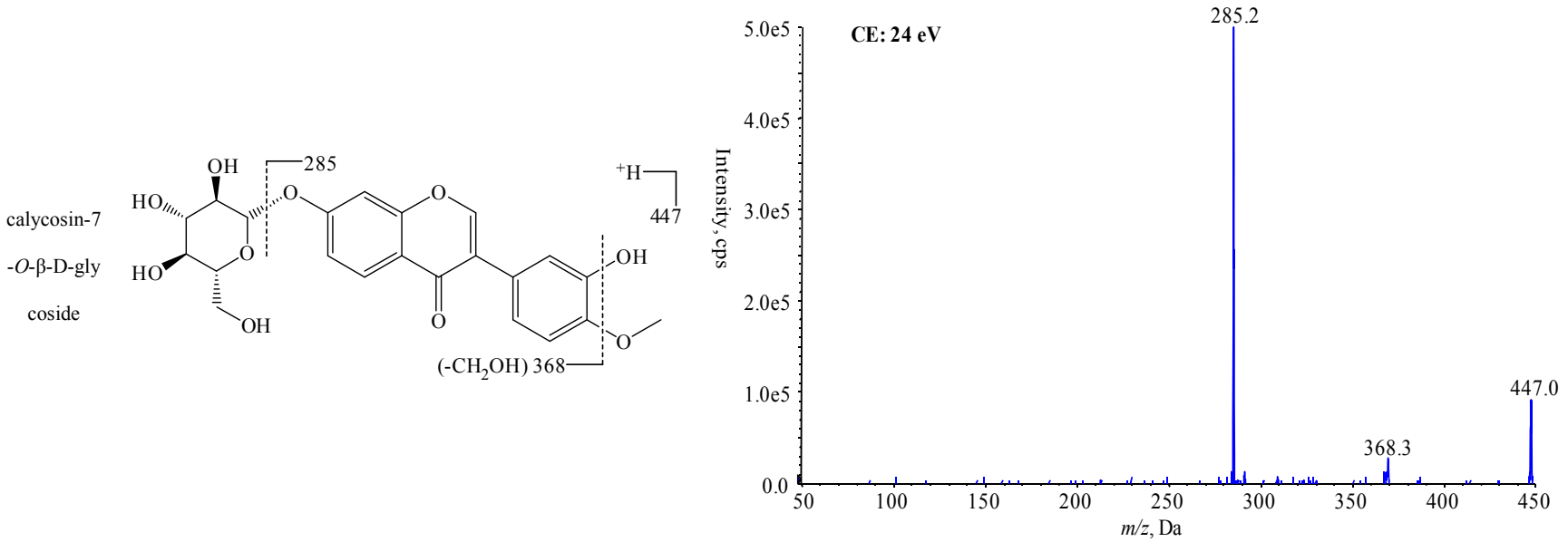
Figure captions:

Fig. 1. Chemical structures, fragmentation schemes and full-scan product ion spectra of $[M+H]^+$ ions of seven bioactive components in Radix Astragali and internal standard (buspirone).

Fig. 2. Typical HPLC-MS/MS chromatograms of mixed standards (the concentration of each compound: 1.0 ng/mL) (A), and extract of actual sample HQ-01 (B).

Fig. 3. Dendrogram of HCA for 18 batches of samples of Radix Astragali. The hierarchical clustering was performed by SPSS 19.0 software. Ward method was applied, and squared Euclidean distance was selected as the measurement. Dendrogram resulted from the contents of seven bioactive components in the tested samples.

Compound	Chemical structure	Full-scan product ion spectra
calycosin		



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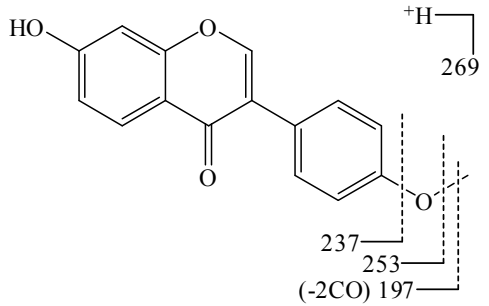

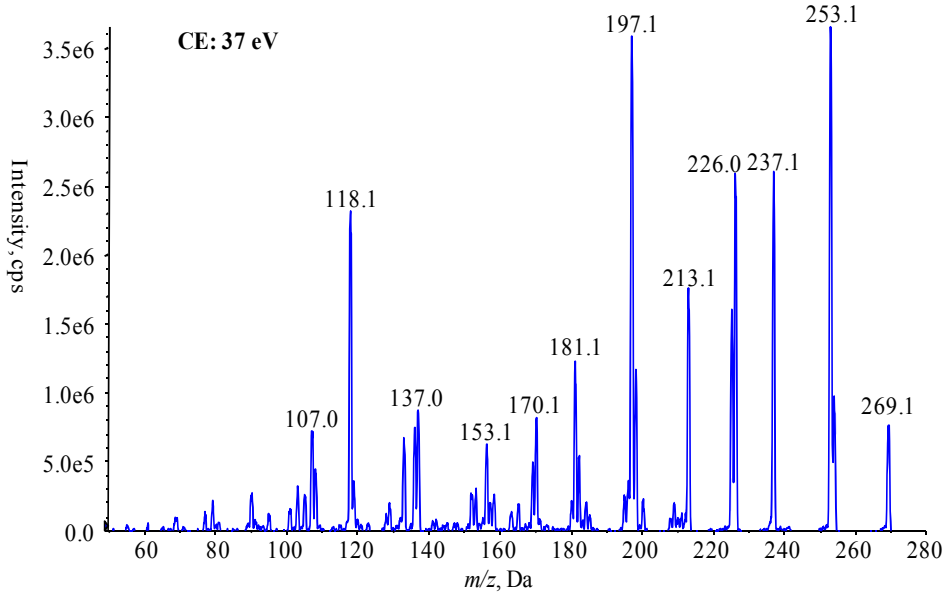

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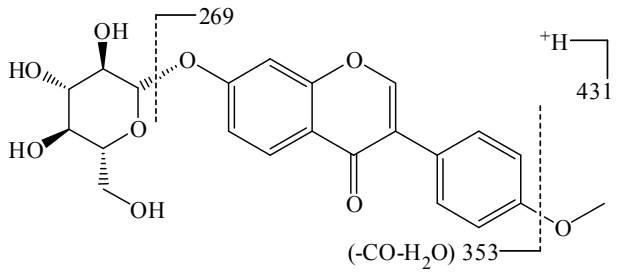

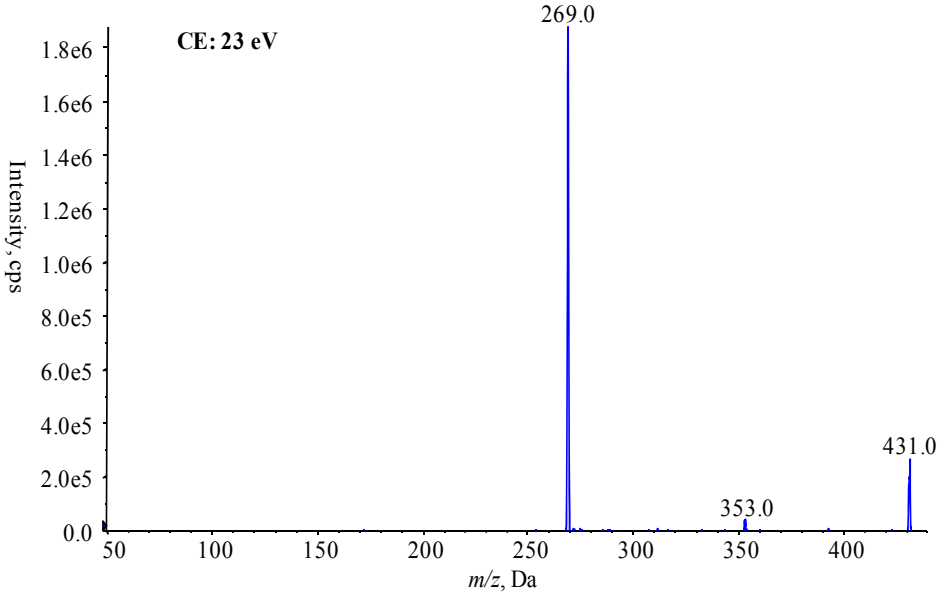

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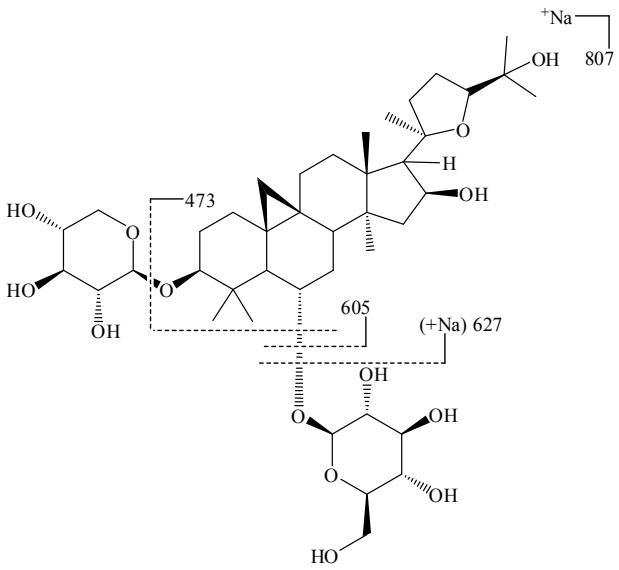

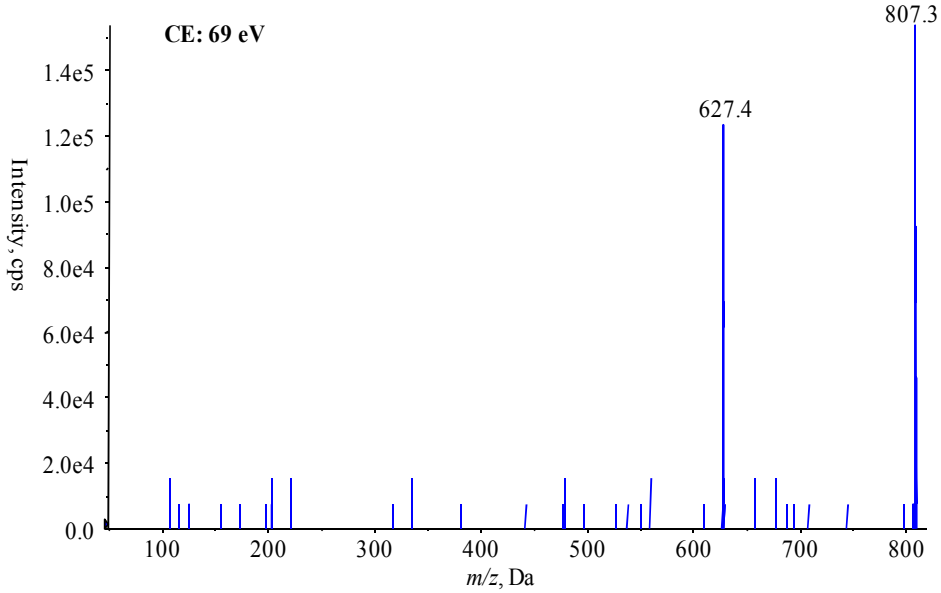

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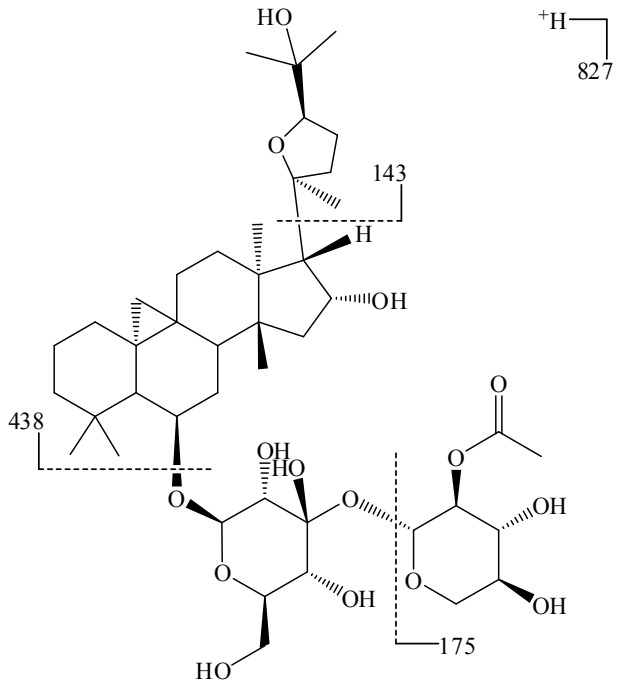

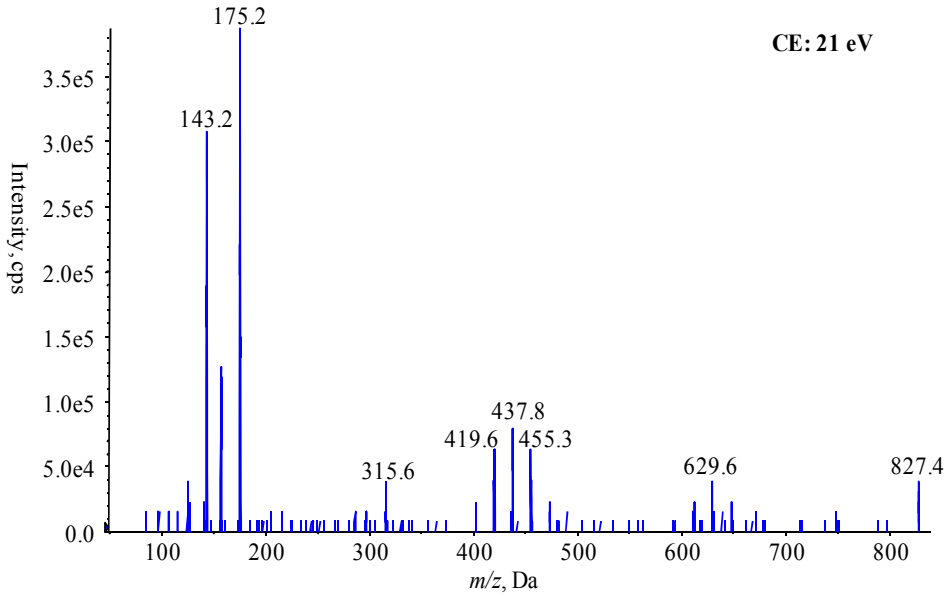

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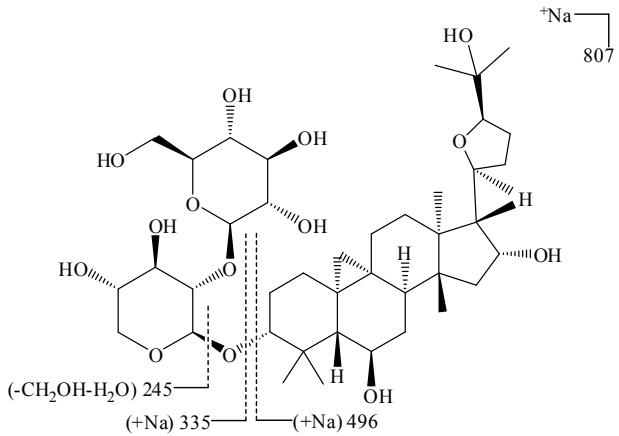
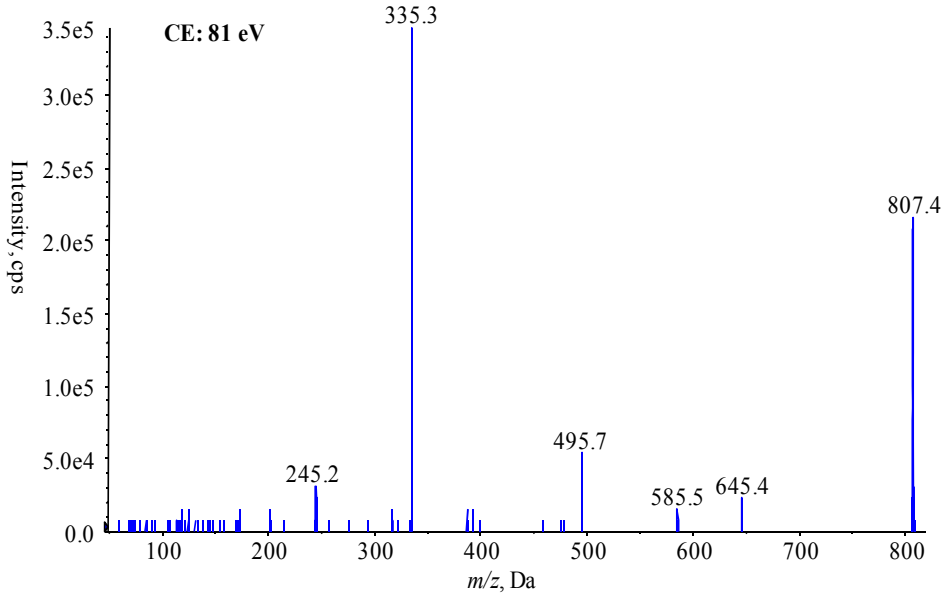




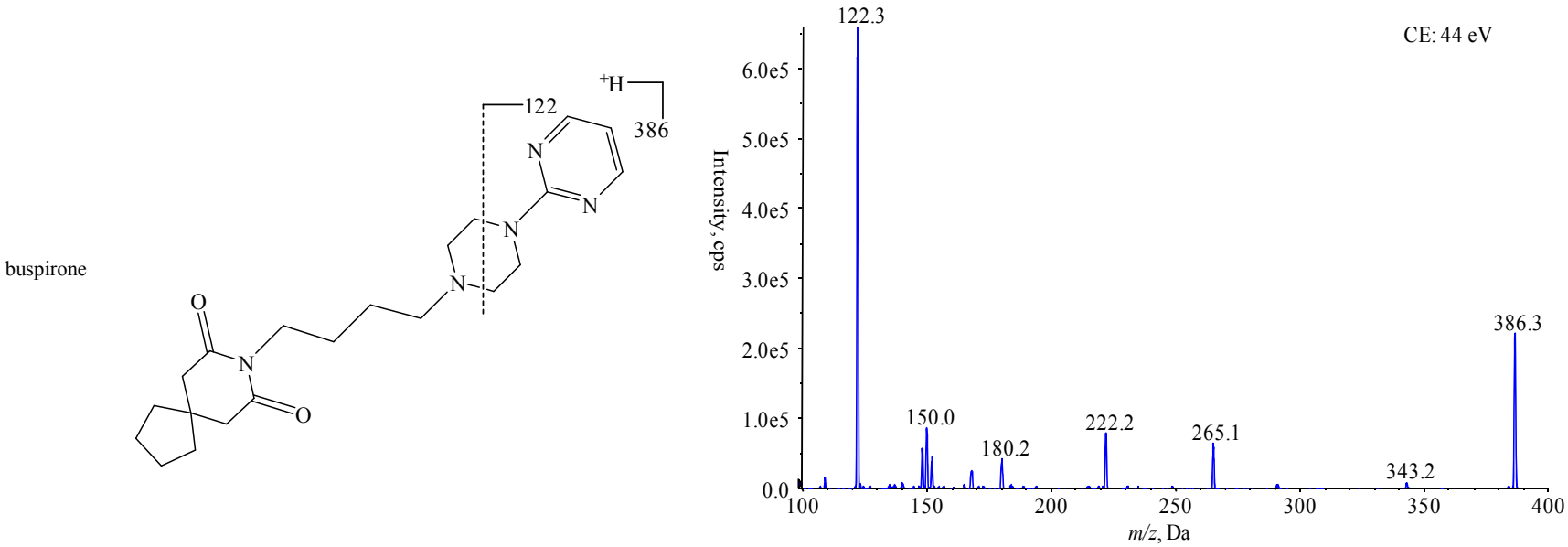
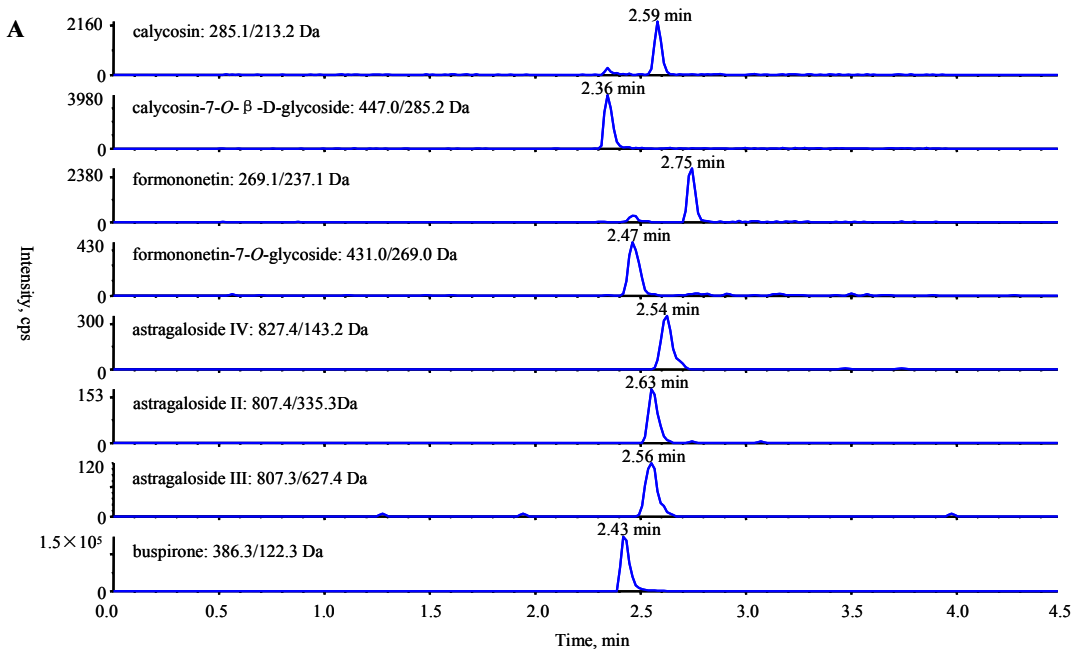


Fig. 1. Chemical structures, fragmentation schemes and full-scan product ion spectra of $[M+H]^+$ ions of seven bioactive components in Radix Astragali and internal standard (buspirone).



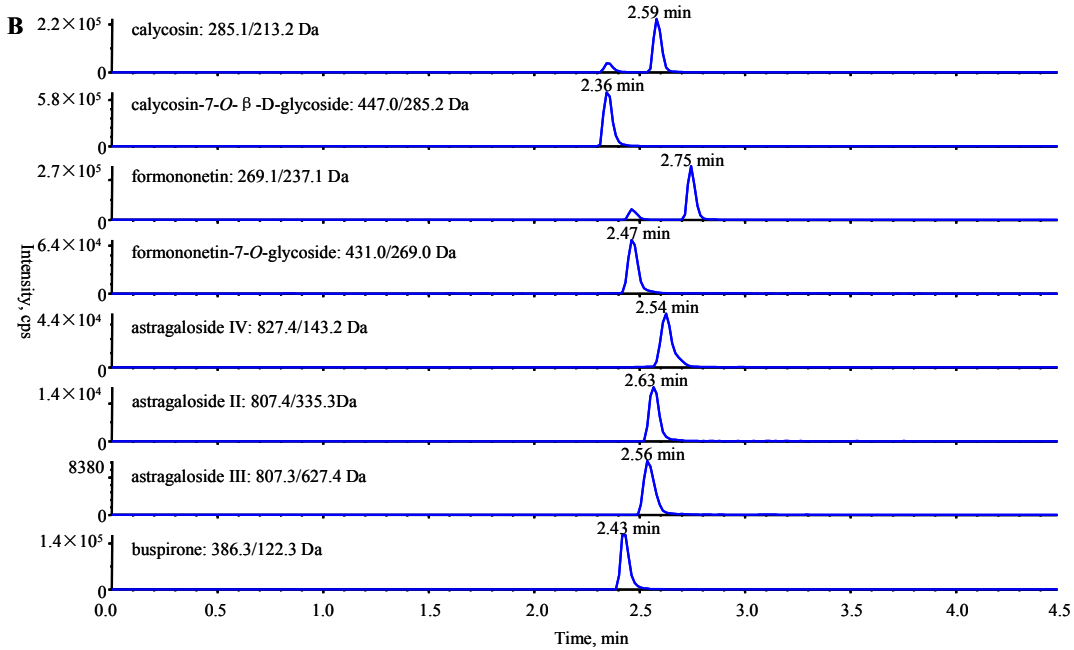


Fig. 2. Typical HPLC-MS/MS chromatograms of mixed standards (the concentration of each compound: 1.0 ng/mL) (A), and extract of actual sample HQ-01 (B).

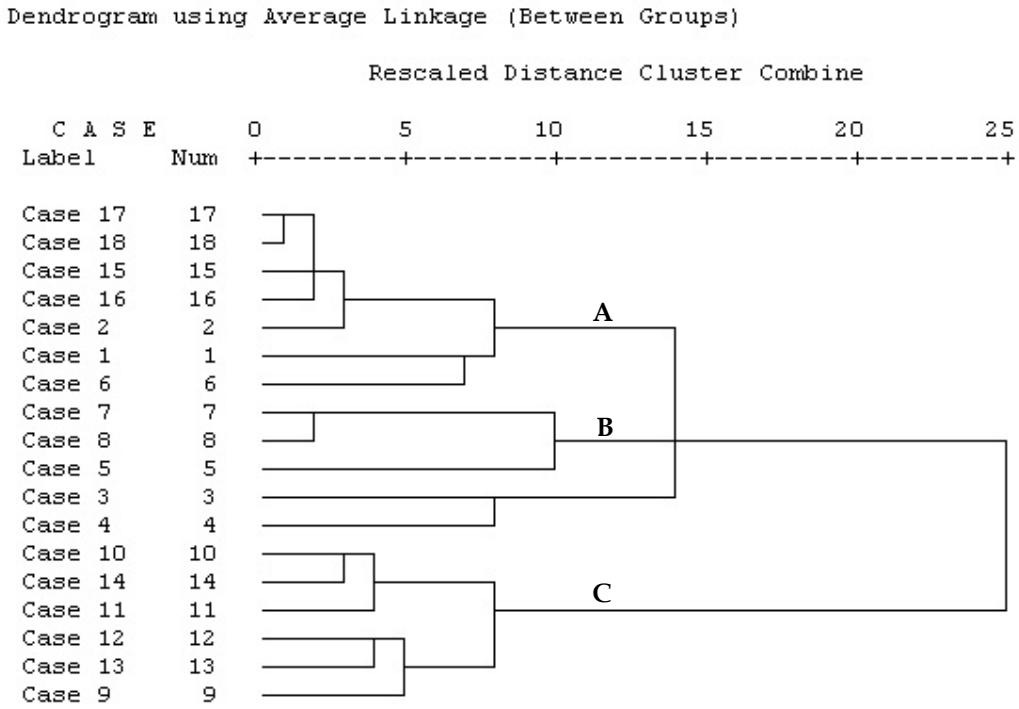


Fig. 3. Dendrogram of HCA for 18 batches of samples of Radix Astragali. The hierarchical clustering was performed by SPSS 19.0 software. Ward method was applied, and squared Euclidean distance was selected as the measurement. Dendrogram resulted from the contents of seven bioactive components in the tested samples.

Table captions:

Table 1 MS parameters of seven bioactive components in Radix Astragali and buspirone (IS) determined by LC-ESI-MS/MS.

Table 2 Linearities, ranges, LODs and LOQs of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS.

Table 3 Intra- and inter-day accuracy and precision of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS (n=6).

Table 4 Recoveries of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS (n=6).

Table 5 Contents (mg/g) of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS (n=3).

Table 1 MS parameters of seven bioactive components in Radix Astragali and buspirone (IS) determined by LC-ESI-MS/MS.

MS Parameters	Parent ion (m/z)	Product ion for quantification (m/z)	Product ions for identification (m/z)	DP (V)	CE (eV)	CXP (V)	CAD
calycosin	285.1	213.2	270.3, 225.2	100	50	10	High
calycosin-7-O-β-D-glycoside	447.0	285.2	368.3	80	24	10	High
formononetin	269.1	237.1	253.1, 197.1	115	37	10	High
formononetin-7-O-glycoside	431.0	269.0	353.0	121	23	10	High
astragaloside IV	807.3*	627.4	605.4, 473.4	271	69	10	High
astragaloside II	827.4	143.2	175.2, 437.8	146	21	10	High
astragaloside III	807.4*	335.3	495.7, 245.2	261	81	10	High
IS	386.3	122.3	NA	100	44	10	High

NA: Not available.

*: Sodium adduct ion $[M+Na]^+$.

Table 2 Linearities, ranges, LODs and LOQs of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS.

Analyte	Calibration curves	Correlation coefficient (R)	Range (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)
calycosin	y = 1.04E-02 x + 6.20E-04	0.9965	0.10-500	0.03	0.10
calycosin-7-O-β-D-glycoside	y = 2.55E-02 x + 5.84E-04	0.9972	0.10-500	0.03	0.10
formononetin	y = 1.27E-02 x + 8.42E-05	0.9952	0.10-200	0.03	0.10
formononetin-7-O-glycoside	y = 2.93E-03 x + 4.52E-05	0.9987	0.50-500	0.20	0.50
astragaloside IV	y = 6.87E-05 x - 7.42E-06	0.9967	2.00-500	0.60	2.00
astragaloside II	y = 2.20E-04 x + 1.39E-04	0.9957	2.00-500	0.60	2.00
astragaloside III	y = 1.10E-04 x - 5.23E-05	0.9981	1.00-500	0.30	1.00

y: the peak area ratio of each compound with IS.

x: the concentration of the corresponding working standard solution.

Table 3 Intra- and inter-day accuracy and precision of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS (n=6).

Analyte	Added concentration (ng/mL)	Intra-day (n = 6)			Inter-day (n = 6)		
		Found concentration (ng/mL)	RE (%)	RSD (%)	Found concentration (ng/mL)	RE (%)	RSD (%)
calycosin	0.20	0.213	6.50	11.6	0.208	4.00	6.99
	20.0	21.4	7.00	5.87	20.9	4.50	12.0
	400	416	4.00	6.19	421	5.25	4.88
calycosin-7-O-β-D-glycoside	0.20	0.189	-5.50	9.46	0.193	-3.50	6.56
	20.0	21.5	7.50	3.55	20.7	3.50	10.2
	400	379	-5.25	6.47	388	-3.00	3.83
formononetin	0.20	0.192	-4.00	12.6	0.198	-1.00	12.5
	10.0	9.79	-2.10	6.51	10.9	9.00	13.6
	160	168	5.00	4.59	172	7.50	7.03
formononetin-7-O-glycoside	1.00	1.11	11.0	8.78	1.08	8.00	4.96
	20.0	19.4	-3.00	6.39	20.4	2.00	9.48
	400	386	-3.50	4.52	393	-1.75	6.90
astragaloside IV	5.00	4.75	-5.00	7.99	4.83	-3.40	4.98
	50.0	51.6	3.20	6.19	50.8	1.60	4.60
	400	414	3.50	8.54	407	1.75	6.34
astragaloside II	5.00	4.88	-2.40	11.1	4.91	-1.80	6.69
	50.0	49.1	-1.80	6.07	50.5	1.00	7.73
	400	391	-2.25	4.61	394	-1.50	5.46
astragaloside III	2.00	1.89	-5.50	4.26	1.95	-2.50	8.63
	20.0	21.3	6.50	7.16	21.4	7.00	6.69
	400	392	-2.00	5.06	387	-3.25	9.22

Table 4 Recoveries of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS (n=6).

Analyte	Original amount (mg/g)	Added amount (mg/g)	Found amount (mg/g)	Recovery (%)	RSD (%)
calycosin	1.61	1.290	2.958	103.4	4.88
		1.613	3.189	97.7	2.24
		1.935	3.505	97.3	3.38
calycosin-7-O-β-D-glycoside	2.38	1.902	4.237	98.3	4.95
		2.377	4.787	101.4	1.97
		2.852	5.341	104.7	1.92
formononetin	0.36	0.289	0.645	98.3	2.72
		0.362	0.715	97.6	5.00
		0.434	0.788	97.8	4.99
formononetin-7-O-glycoside	1.31	1.050	2.314	96.3	3.48
		1.312	2.649	101.8	1.19
		1.575	2.841	96.5	2.45
astragaloside IV	0.50	0.397	0.888	98.8	2.69
		0.497	0.969	95.1	3.87
		0.596	1.112	103.9	4.79
astragaloside II	2.04	1.634	3.694	100.8	1.28
		2.043	3.989	95.3	2.09
		2.451	4.393	95.1	1.64
astragaloside III	0.21	0.169	0.385	102.2	1.41
		0.211	0.418	97.8	4.35
		0.254	0.459	97.2	1.70

Table 5 Contents (mg/g) of seven bioactive components in Radix Astragali determined by LC-ESI-MS/MS (n=3).

No.	Origin	calycosin	calycosin-7-O-β-D-glycoside	formononetin	formononetin-7-O-glycoside	astragaloside IV	astragaloside II	astragaloside III
HQ-01	Shanxi	1.61 ± 0.23	2.38 ± 0.36	0.36 ± 0.07	1.31 ± 0.18	0.50 ± 0.11	2.04 ± 0.28	0.21 ± 0.03
HQ-02	Shanxi	1.56 ± 0.33	2.22 ± 0.31	0.33 ± 0.08	1.24 ± 0.16	0.49 ± 0.18	2.15 ± 0.37	0.33 ± 0.08
HQ-03	Shanxi	1.66 ± 0.18	2.26 ± 0.40	0.45 ± 0.05	1.46 ± 0.13	0.55 ± 0.14	2.37 ± 0.31	0.26 ± 0.07
HQ-04	Shanxi	1.72 ± 0.41	2.36 ± 0.22	0.37 ± 0.02	1.35 ± 0.09	0.53 ± 0.16	2.58 ± 0.47	0.39 ± 0.11
HQ-05	Heilongjiang	1.07 ± 0.16	1.75 ± 0.45	0.47 ± 0.14	1.66 ± 0.47	0.69 ± 0.15	2.17 ± 0.22	0.33 ± 0.02
HQ-06	Heilongjiang	1.53 ± 0.55	2.03 ± 0.49	0.41 ± 0.18	1.33 ± 0.18	0.63 ± 0.14	2.08 ± 0.37	0.28 ± 0.03
HQ-07	Heilongjiang	1.21 ± 0.52	1.97 ± 0.67	0.37 ± 0.10	1.51 ± 0.26	0.48 ± 0.19	2.25 ± 0.29	0.31 ± 0.05
HQ-08	Heilongjiang	1.17 ± 0.23	2.14 ± 0.33	0.40 ± 0.09	1.62 ± 0.41	0.50 ± 0.15	2.17 ± 0.38	0.32 ± 0.03
HQ-09	Inner Mongolia	1.89 ± 0.35	2.54 ± 0.30	0.64 ± 0.12	1.48 ± 0.22	0.87 ± 0.23	2.58 ± 0.52	0.58 ± 0.02
HQ-10	Inner Mongolia	1.96 ± 0.26	2.62 ± 0.44	0.58 ± 0.10	1.56 ± 0.15	0.73 ± 0.08	2.34 ± 0.16	0.50 ± 0.05
HQ-11	Inner Mongolia	1.92 ± 0.39	2.37 ± 0.17	0.65 ± 0.15	1.62 ± 0.24	0.68 ± 0.16	2.45 ± 0.35	0.52 ± 0.06
HQ-12	Inner Mongolia	2.11 ± 0.64	2.44 ± 0.32	0.54 ± 0.14	1.55 ± 0.29	0.81 ± 0.17	2.57 ± 0.33	0.54 ± 0.01
HQ-13	Inner Mongolia	2.07 ± 0.58	2.29 ± 0.45	0.60 ± 0.13	1.63 ± 0.09	0.85 ± 0.15	2.62 ± 0.29	0.57 ± 0.05
HQ-14	Inner Mongolia	2.02 ± 0.29	2.48 ± 0.24	0.57 ± 0.11	1.71 ± 0.30	0.77 ± 0.16	2.44 ± 0.20	0.49 ± 0.03
HQ-15	Gansu	1.64 ± 0.18	2.16 ± 0.24	0.27 ± 0.08	1.05 ± 0.34	0.51 ± 0.17	2.04 ± 0.22	0.32 ± 0.01
HQ-16	Gansu	1.43 ± 0.26	2.05 ± 0.21	0.31 ± 0.05	1.14 ± 0.34	0.44 ± 0.08	2.07 ± 0.12	0.36 ± 0.05
HQ-17	Gansu	1.56 ± 0.21	2.12 ± 0.17	0.32 ± 0.04	1.08 ± 0.20	0.53 ± 0.11	2.11 ± 0.12	0.35 ± 0.03
HQ-18	Gansu	1.50 ± 0.31	2.13 ± 0.15	0.34 ± 0.06	1.00 ± 0.23	0.48 ± 0.22	2.08 ± 0.16	0.38 ± 0.07