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Determination of astragalin and astragaloside content in Radix Astragali using high-performance liquid chromatography coupled with pulsed amperometric detection

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ABSTRACT

Astragalin and astragalosides were measured in Radix Astragali using reversed-phase chromatography coupled with pulsed amperometric detection. Because the target compounds showed poor stability in aqueous solutions, they were extracted in 100% methanol under reflux. All compounds were detected with high sensitivity under highly alkaline conditions using sodium hydroxide as a post-column eluent. The limits of detection and quantification of target compounds were 0.02–0.36 μg/mL and 0.06–1.09 μg/mL, respectively, and the linear regression coefficients were 0.9982–1.0000. The intra- and inter-day precisions were <0.92% in retention time and <4.78% in calculated contents. Average recoveries were >91.33%. Astragalin and astragaloside contents between Radix Astragali at different ages and in different parts were successfully determined without sample purification or concentration.

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1. Introduction

Radix Astragali is the root of *Astragalus membranaceus*, a member of the Leguminosae family [1]. Radix Astragali (RA) is widely used as a health food supplement to increase energy or as a component in traditional Chinese medicine (TCM). The compounds contained in Radix Astragali exert pharmaceutical effects that mediate processes such as hematopoietic functions [2], anti-hyperglycemic activity [3–6], anti-inflammatory activity [7,8], renoprotection [9], and angiogenesis [10].

The contents of astragalosides and isoflavonoids are used as standards to assess the quality of Radix Astragali. Astragalosides, triterpenoid-type glycosides, can be indicators of the quality of Radix Astragali. Astragaloside I (AST-I), astragaloside II (AST-II), astragaloside III (AST-III) and astragaloside IV (AST-IV) are the primary astragalosides. In particular, AST-IV is known to have various beneficial effects on processes such as osteogenesis, neuroprotection, angiogenesis, anti-inflammatory activity and cardioprotective activity [11–16]. Pharmacokinetic studies of AST-IV using chromatography are commonly reported in the literature [16–20]. Astragalin, a flavonoid-type glycoside that has anti-inflammatory activity, is also present in various medicinal plants including Radix Astragali [21].

Generally, 4–6 year-old Radix Astragali are available on the Korean market, and the price per gram increases by 1.5–2-fold per year of the age of the plant. Therefore, the bioactive compound content needs to be calculated according to the age of the root or the part from which the extract is taken. In the present study, the content of the four major astragalosides and astragalin in Radix Astragali was analyzed using chromatographic methods. The chemical structures of these compounds are shown in Fig. 1.

In some of the literature, the bioactive compounds of Radix Astragali were analyzed by HPLC-DAD-ELSD. Flavonoids can be detected by diode array detector (DAD) with high sensitively because they have strong chromophores that can absorb wavelengths of approximately 280 nm [22,23]. On the other hand, evaporative light scattering detector (ELSD) is more suitable than DAD for the detection of astragalosides because they have poor UV chromophores. Therefore, DAD and ELSD are used together to analyze flavonoid and triterpenoid-type compounds simultaneously. Mass spectrometry is also used for the identification [24,25] and measurement [26–29] of bioactive compounds in Radix Astragali.

In this study, astragalin and astragalosides in Radix Astragali were examined using reversed-phase HPLC coupled with pulsed amperometric detection (PAD), a type of electrochemical detection [30,31]. The principle of pulsed amperometric detection is as follows: amperometric detection utilizes a flowing current to initiate a chemical conversion of electro-active analytes which undergo an oxidation reaction according to the potential applied. However, when applying constant potential, electrode is contaminated by products of oxidation, and the foul electrodes reduce signal.

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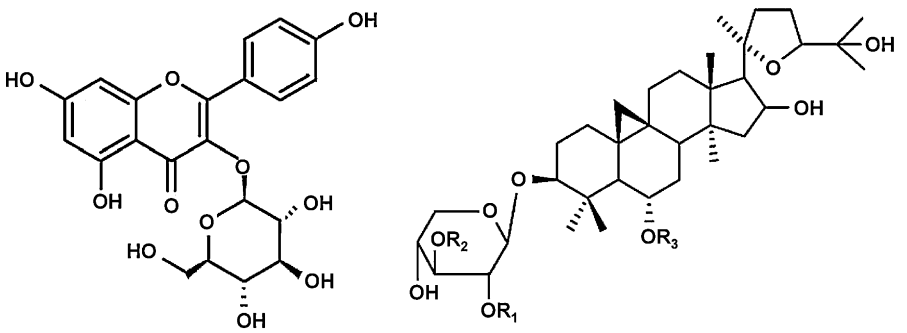

Astragalin

	R_1	R_2	R_3
AST I	Ac	Ac	Glc
AST II	Ac	H	Glc
AST III	Glc	H	H
AST IV	H	H	Glc

Fig. 1. Chemical structures of astragalin and astragalosides.

To solve this problem, pulsed amperometric detection (PAD) was developed by applying higher or lower potentials that are used for cleaning the electrode. In PAD, a working potential is applied for a short time, followed by cleaning potential. The potential waveforms are various according to the target compounds. Carbohydrates or amino acids can be sensitively analyzed on gold electrode by the amperometric detection because these compounds become anion, electro-active form, under strong alkaline condition. Therefore, PAD is most often used for detection of carbohydrates after an anion exchange separation. Astragalin and astragalosides can be detected by PAD because they has sugar moiety in their chemical structures. We separated the astragalin and astragalosides using reversed-phase chromatographic method, and detected by PAD under alkaline condition [32]. Therefore, this technique requires the application of a strong alkaline solution (NaOH) after column separation. The high sensitivity of this method makes it possible to perform direct analysis without additional concentration or purification processes and thus can reduce experimental time, efforts and errors.

Using this analytical method, the stability of astragalin and astragalosides was tested and the optimal extraction method was determined. Moreover, the contents of astragalin and astragalosides were assessed based on the age and area of Radix Astragali from which they were extracted.

2. Materials and methods

2.1. Materials

Astragalin and astragaloside standards were purchased from ChromaDex (Santa Ana, CA, USA). Digitoxin was purchased from Wako (Tokyo, Japan). HPLC-grade acetonitrile and 50% sodium hydroxide (NaOH) were purchased from Fisher Scientific (Fairlawn, NJ, USA). Absolute methanol was purchased from Duksan Pure Chemicals (Ansan, South Korea). All other reagents and solvents used were of guaranteed or analytical grade. A Millipore membrane filter (type HA, pore size: 0.45 mm) was used for solvent filtration. All samples were filtered through disposable syringe filters (hydrophobic PTFE; pore size: 0.20 mm; Advantec MFS, Tokyo, Japan) before injection. Standard solutions, sample solutions and the mobile phase were prepared with 18 MΩ deionized water produced by an Automatic Aquarius AW-1001 (Top Trading, Seoul, South Korea) purification system. The weight of each sample was measured on a Mettler Toledo AX 105 Delta-Range (Greifensee, Switzerland). Whole roots of *A. membranaceus* (six-year-old, five-year-old, and four-year-old) were cultivated in Jecheon, South Korea. For method validation and extraction optimization, a package of Radix Astragali slices (KFDA standardized product) cultivated in Jecheon and packaged under nitrogen was purchased from a local drugstore.

2.2. High-performance liquid chromatography

HPLC equipment, consisting of a Model Nanospace SI-2/3201 pump, 3002 UV detector and a 3004 column oven, was purchased from Shiseido (Tokyo, Japan). The PAD system from the ICS-3000 series Dionex (Sunnyvale, CA, USA) was equipped with an Au-Flowcell containing a gold working electrode and a solvent-compatible cell containing an Ag/AgCl reference electrode. Chromatographic separation was performed using a Kinetex C18 column (100 mm × 2.1 mm id; 2.6 μm particle size; Phenomenex, Torrance, CA, USA) protected by a KrudKatcher ultra in-line filter (Phenomenex). The potential waveform was as follows: E1 = −0.2 V (from 0.00 to 0.04 s); E2 = 0 V (from 0.05 to 0.21 s); E3 = +0.22 V (from 0.22 to 0.46 s); E4 = 0 V (from 0.47 to 0.56 s); E5 = −2 V (from 0.57 to 0.58 s); and E6 = +0.6 V (0.59 s). The gradient program of mobile phase is as follows: linear gradient elution from 19% to 20% acetonitrile for 7 min, linear gradient elution from 20% to 32.5% acetonitrile from 7 to 8 min, linear gradient elution from 32.5 to 45% acetonitrile from 8 to 20 min, isocratic elution with 45% acetonitrile from 20 to 25 min, and finally, reconditioning of the column with A:B 19% acetonitrile for 5 min. The flow rate was 0.2 mL/min and the separation temperature was 30 °C. The injection volume was 10 μL. A post-column delivery system of 200 mM sodium hydroxide with a flow rate of 0.8 mL/min was added as a post-column reagent. The mobile phase was created by degassing with vacuum filtration after the mixture of water with acetonitrile on a daily basis, followed by sonication for 20 min before use. A post-column delivery system was purged with helium throughout the experiment to remove carbonate from the water. The data were controlled on a computer running the Chromeleon client program supplied by Dionex.

2.3. Standard preparation

Stock solutions were prepared by dissolving 1 mg of each standard in 1 mL of methanol in an Eppendorf tube. Each stock solution

was diluted to create calibration points (0.05, 0.1, 0.5, 1, 5, 10, 20 and 40 μg/mL). The concentration of the internal standard (Digitoxin) for all analytes was 10 μg/mL.

2.4. Sample preparation

Five whole roots of *A. membranaceus* of different ages (four, five and six-years-old) were prepared. Each root was separated into the head, main root and lateral root. Each part was powdered, and 1.00 g of Radix Astragali powder was extracted in 40 mL of methanol under reflux for 10 min and then filtered. The internal standard and deionized water were added to the extracted solution to obtain a final concentration of 10 mg/mL Radix Astragali and 10 μg/mL of internal standard.

2.5. Method validation

The five linear calibration curves were made for each concentration (0.05, 0.1, 0.5, 1, 5, 10, 20, and 40 μg/mL). The regression equation was $y = ax + b$, where y and x were the ratio of the peak area (analytes/internal standard) and sample concentration, respectively.

The limits of detection (LOD) and quantification (LOQ) were determined according to ICH guideline Q2B [33]. LOD was defined as $3.3\sigma/S$ and LOQ was $10\sigma/S$. The standard deviation of the y-intercepts of the regression lines was used as σ (standard deviation of the response) and the slope of calibration curve was used as S.

The precision of the analysis was evaluated by performing intra- and inter-day assays of Radix Astragali and then checking the relative standard deviation (%RSD) of the retention times (min) and contents (μg/mL). Five injections were performed each day and repeated for three consecutive days. The accuracy was evaluated by recovery test. Fortified samples were prepared by adding known amounts of standards to the Radix Astragali extracts, which were then analyzed by HPLC. Three injections of each sample were performed to measure recovery.

3. Results and discussion

3.1. Stability and extraction optimization

3.1.1. Stability test

To determine the efficiency of the sample storage method and extraction of Radix Astragali, the chemical stability of the compounds needed to be examined. To do this, the decomposition rates of extracts stored in 20% methanol solution and 100% methanol solution at 5 °C were measured and correlated to the storage time. When dissolved in 20% methanol for five weeks, over 97% of astragalin and AST-IV were preserved, while only 73% of AST-III, 60% of AST-II and 30% of AST-IV were preserved (Fig. 2). When dissolved in 100% methanol, all compounds were preserved. The compounds were also stable when they were frozen. Therefore, AST-I, AST-II and AST-III are unstable when stored in an aqueous solution.

When the compounds were dissolved in 100% methanol, the peaks were broad because the solvent strength of 100% methanol is much stronger than that of the mobile phase. However, once the stock solutions were diluted in water, the compounds began to decompose. Therefore, the stock solution and samples were diluted with water just prior to HPLC injection. When the samples were dissolved in 20% methanol, all compounds showed over 98% preservation for 24 h.

These results indicate that the astragaloside contents in Radix Astragali are easily influenced by moisture. Therefore, further study is needed to determine how soil humidity and storage conditions affect the quality of Radix Astragali extracts.

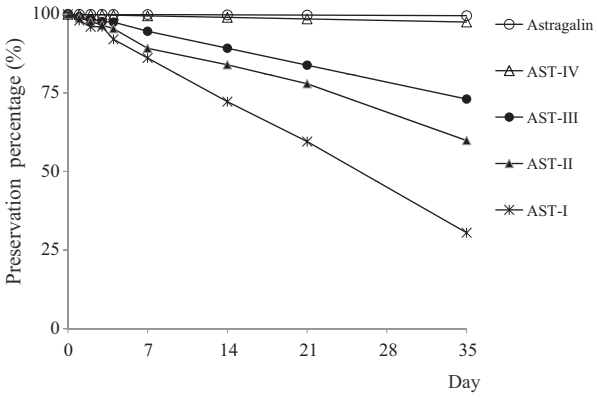

Fig. 2. Preservation percentage (%) of astragalin and astragalosides in a 20% methanol solution at 5 °C according to the storage time.

3.1.2. Optimization of the extraction method

Studies designed to determine the optimal method for extraction have been published previously [26,34]. Zu et al. compared the efficiency of the Soxhlet extraction, reflux extraction, ultrasonic extraction and microwave-assisted extraction using 80% ethanol [26]. The results showed that micro-assisted extraction showed the best extraction efficiency, while Soxhlet extraction and reflux extraction, which were performed at 90 °C, showed lower extraction efficiency compared to other extraction methods performed at room temperature. Conversely, Song et al. compared the extraction efficiency of the same four extraction methods using 100% methanol [34]. The results suggested that the extraction efficiencies were highest with microwave-assisted extraction, followed by reflux extraction, Soxhlet extraction and ultrasonic extraction.

In this study, the extraction efficiency of astragalin and astragalosides were compared between reflux extraction and ultrasonic extraction using water, 20% methanol, 40% methanol, 60% methanol, 80% methanol and 100% methanol as solvents. Ultrasonic extraction was performed at room temperature, while reflux extraction was performed at the boiling point of the solvent used. The results are shown in Fig. 3. Extraction using 100% methanol showed the best extraction efficiency in both reflux and ultrasonic extraction. On average, reflux extraction showed better extraction efficiency than ultrasonic extraction with 100% methanol, but when the compounds were extracted with 60–80% methanol, ultrasonic extraction showed better extraction efficiency. These results

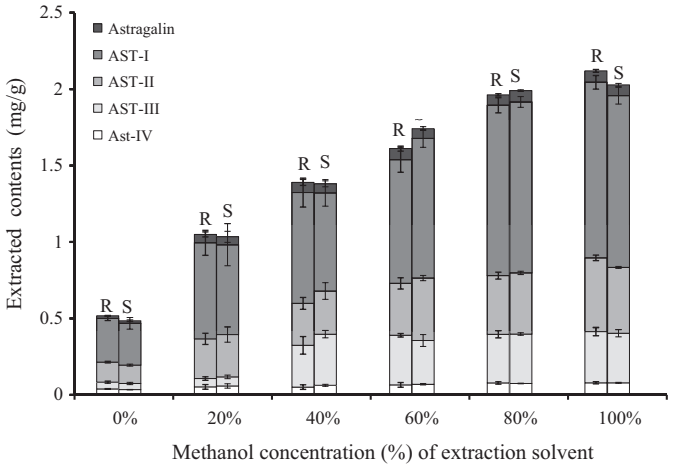

Fig. 3. Comparison of the extraction efficiency of astragalin and astragalosides from Radix Astragali between reflux (*R*) and sonication (*S*) according to the methanol percentage.

Table 1
Investigated linear range, linear equation, correlation coefficient, limits of detection (LOD), and limits of quantification (LOQ).

	Linear range	Linear equation	r^2	LOD (ng)	LOQ (ng)
Astragalin	1.09–40	$y = 0.0745x + 0.0319$	0.9993	3.6	10.9
Ast-IV	0.06–40	$y = 0.1561x + 0.0091$	1.0000	0.2	0.6
AST-III	0.08–40	$y = 0.1481x - 0.0013$	0.9982	0.3	0.8
AST-II	0.18–40	$y = 0.0950x + 0.0099$	0.9996	0.6	1.8
AST-I	0.47–40	$y = 0.0665x + 0.0136$	0.9996	1.6	4.7

agree with those of previous reports because ultrasonic extraction was more efficient when using 80% methanol [26] and reflux extraction was more efficient when using 100% methanol [34]. When extracting the compounds in aqueous solution at high temperatures, decomposition might be accelerated, resulting in low extraction efficiency. Each extraction was performed five times per method and per solvent, and there was no statistically significant difference ($p > 0.05$) between reflux extraction and the ultrasonic method when 100% methanol was used (Suppl. Table 1). Therefore, both extraction methods can be used.

To confirm the stability of the standards in anhydrous solvent at high temperature, standards were added to 100% methanol, refluxed for 1 h, and then diluted and injected into the HPLC system. The compounds were stable at boiling point when extracted using 100% methanol (recovery >94%). As the recovery rate shows, compounds dissolved in anhydrous solvents are almost preserved. It seems like, the immediate cause of decomposition is water, and heat can also promote hydrolysis in aqueous solution. In this study, reflux extraction in 100% methanol was used for Radix Astragali extraction.

3.2. Method validation

3.2.1. Linearity, sensitivity

The linearity of the PAD response for each compound was examined using eight standard stock solutions (0.05, 0.1, 0.5, 1, 5, 10, 20 and 40 μg/mL). A calibration curve was constructed by linear regression of the peak area ratio (analyte/I.S.) versus the analyte concentration. The equations and linear ranges are summarized in Table 1. The linear regression coefficients of PAD were 0.9982–1.0000. According to the previous reports, the linear regression coefficients of ELSD were 0.9983–0.9991 by Yu et al. [22], and was 0.9994–0.9997 by Qi et al. [23]. The linearity was similar between PAD and ELSD, but linear range showed significant differences. The linear range of AST-IV detected by ELSD was 0.17–6.9 μg by Yu et al. [22], and was 0.26–5.20 μg by Qi et al. [23]. Meanwhile, the linear range of AST-IV detected by PAD was 0.0006–0.4 μg. Therefore, ELSD method seem to more convenient for routine analysis because it has wider linear range than PAD. On the other hand, PAD can determine up to the nano-gram levels. Therefore, PAD method is more suitable for micro-analysis.

The LODs and LOQs obtained for astragalin were 3.6 ng and 10.9 ng, respectively, and those of astragalosides were 0.2–1.6 ng and 0.6–4.7 ng, respectively. In a previous report, Qi et al. investigated the sensitivities of using UV detection and ELSD detection for isoflavonoids and astragalosides in Radix Astragali [23]. According to the study, the LODs and LOQs of isoflavonoids were 1.2–5.2 ng and 4.2–23 ng, respectively, at UV 280 nm. Therefore, the sensitivity of PAD for isoflavonoid detection was not significantly increased compared to UV detection because the LOD and LOQ of astragalin for the PAD method were 3.6 ng and 10.9 ng, respectively. For the detection of astragalosides, the LODs and LOQs were 70–110 ng and 145–270 ng, respectively, using the ELSD method. In other words, PAD can detect or determine 50–300 times lower concentration of astragalosides than ELSD. Therefore, sensitivity of PAD for astragalosides was higher than those of ELSD when analyzing astragalosides. The LODs and LOQs of astragalosides detected by MS were 0.03–0.06 ng and 0.10–0.15 ng [26]. Although the sensitivity of PAD was not higher than that of MS, PAD showed enough sensitivity to be sufficient for microanalysis.

3.2.2. Accuracy and precision

The chromatograms of standard compounds and Radix Astragali are shown in Fig. 4. Intra- and inter-day precision was determined by analyzing samples five times during a single day and on three different days, respectively. The relative standard deviation (%RSD) was <0.92% for retention time (min) and <4.78% for the calculated contents (μg/g). The data are shown in Table 2. A recovery test was used to evaluate the accuracy of this method (Table 3). The mean recoveries and RSD ranges were 91.33–98.55% and 1.16–6.47%, respectively.

To validate the method, the Radix Astragali was analyzed using HPLC-UV at 203 nm. Astragalin with strong absorptivity showed good sensitivity, but astragalosides and internal standard showed lower sensitivity than the PAD system because they have poor chromophores. According to UV detection, the astragalin and AST IV contents in Radix Astragali were calculated as 72.46 μg/g and 1083.14 μg/g, respectively. The data obtained by UV detection generally agreed with those obtained by PAD (astragalin: 70.20 μg/g, AST-IV: 1123.72). Other compounds were difficult to determine, because they were contained below the LOQ in Radix Astragali (Suppl. Fig. 1).

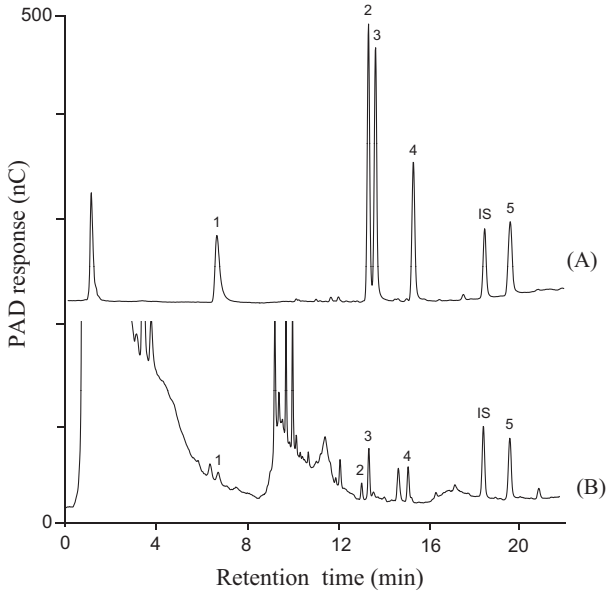

Fig. 4. Chromatograms of 20 μg/mL standard compounds (A) and 10 mg/mL Radix Astragali (B). Peaks: 1, astragalosides; 2, AST-IV; 3, AST-III; 4, AST-II; 5, AST-I; I.S., Digitoxin.

Table 2
Intra- and inter-day validations for the retention time (min) and calculated contents (μg/mL) in Radix Astragali.

Analyte	Intra-day precision ($n = 5$, mean)												Inter-day precision (n = 15, mean)			
	Day 1				Day 2				Day 3				RT (min)	RSD (%)	Content (μg/g)	RSD (%)
	RT (min)	RSD (%)	Content (μg/g)	RSD (%)	RT (min)	RSD (%)	Content (μg/g)	RSD (%)	RT (min)	RSD (%)	Content (μg/g)	RSD (%)				
Astragalin	6.41	0.57	70.48	4.78	6.41	0.50	69.56	3.80	6.37	0.43	70.56	4.30	6.40	0.55	70.20	4.05
AST IV	13.41	0.19	76.98	4.44	13.44	0.27	77.10	3.49	13.35	0.42	77.98	4.40	13.40	0.41	77.35	3.87
AST III	13.73	0.20	319.02	1.71	13.74	0.43	320.61	2.07	13.70	0.92	322.41	1.80	13.72	0.57	320.68	1.78
AST III	15.43	0.11	422.38	1.85	15.49	0.90	424.44	1.48	15.42	0.83	427.03	1.43	15.45	0.69	424.62	1.55
AST I	19.77	0.10	1122.95	2.35	19.88	0.79	1111.34	1.06	19.84	0.55	1136.86	4.26	19.83	0.56	1123.72	2.85

Table 3
Recovery test for the determination of astragalin and astragalosides in Radix Astragali ($n = 3$).

Compound	Added (mg)	Recovery (%)	RSD (%)
Astragalin	0.10	92.81 ± 6.01	6.47
	1.00	95.11 ± 1.91	2.01
AST-IV	0.10	91.33 ± 3.69	4.04
	1.00	96.19 ± 1.68	1.75
AST-III	0.10	97.60 ± 2.73	2.80
	1.00	98.55 ± 1.15	1.16
AST-II	0.10	96.58 ± 1.51	1.56
	1.00	97.08 ± 3.62	3.73
AST-I	0.10	96.96 ± 3.15	3.25
	1.00	97.91 ± 1.90	1.94

3.3. Application

To compare the astragalin and astragaloside contents according to the age and part of Radix Astragali, whole roots of four-, five- and six-year old plants cultivated on the same farm in Jecheon, South Korea, were prepared. Each root was separated into the head, main root and lateral root, and each part was powdered, extracted and analyzed using the optimized conditions. The results are shown in Fig. 5 and Table 4.

In South Korea, the price per gram of Radix Astragali increases by 1.5–2-fold per year of the age of the plant. However, there were no significant differences in the astragalin and astragaloside contents between four-, five- and six-year-old Radix Astragali. Five individual roots of the same age from the same farm showed up to 40%RSD in compound contents.

When comparing the compound content from different parts of the root, the head portion contained smaller amounts of astragalin, AST-I and AST-II than other parts ($p < 0.05$). However, there were no significant differences between the compound contents of the main root and the lateral root. AST-III was found more abundantly in main root than other parts ($p < 0.05$). Although a previous study by Yu et al. reported that the astragaloside contents of lateral roots were higher than those in the main roots [22], these differences could not be confirmed in the present study. The statistical results are shown in Table 5.

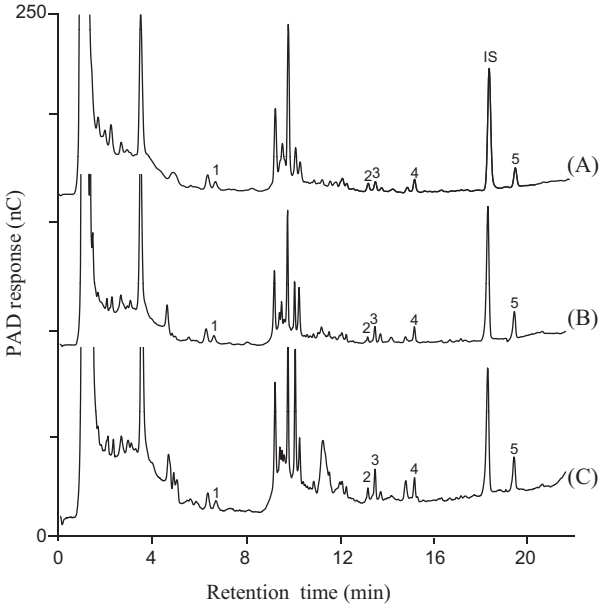

Fig. 5. Chromatograms of four-year-old Radix Astragali head (A), main root (B) and lateral root (C). Peaks: 1, astragalosides; 2, AST-IV; 3, AST-III; 4, AST-II, 5, AST-I; I.S., Digitoxin.

The overall saponin content in four-, five- and six-year-old whole roots was 0.3–0.7 mg/g (Table 4), while the total saponin content in individual slices of Radix Astragali was approximately 1.946 mg/g (Table 2). The Radix Astragali slices were cultivated at the same farm as the whole roots and were stored with nitrogen packing, while the whole roots were stored in a warehouse without any vacuum package. Therefore, it is likely that the storage conditions caused the difference in saponin content.

Table 4
The contents (μg/g) of astragalin and astragalosides of different age and parts of Radix Astragali ($n = 5$).

	Astragalin	AST-I	AST-II	AST-III	AST-IV	Total
4-Year-old						
Head	54.13 ± 14.82	138.89 ± 42.15	53.57 ± 4.092	64.02 ± 0.33	37.73 ± 17.73	348.33 ± 50.85
Main Root	74.67 ± 15.07	443.76 ± 29.84	126.52 ± 34.43	77.05 ± 17.24	43.72 ± 18.45	765.72 ± 64.39
Leteral Root	68.63 ± 8.47	396.66 ± 57.10	100.71 ± 7.55	38.74 ± 5.30	43.73 ± 16.31	648.47 ± 55.39
5-Year-old						
Head	37.39 ± 12.10	144.54 ± 25.84	44.33 ± 3.92	50.77 ± 6.03	28.48 ± 11.46	305.52 ± 41.45
Main Root	65.97 ± 19.51	412.39 ± 19.92	141.08 ± 33.34	107.85 ± 23.02	37.85 ± 9.60	765.14 ± 54.51
Leteral Root	50.52 ± 22.57	503.44 ± 106.99	145.23 ± 43.65	71.38 ± 21.78	29.48 ± 10.51	800.06 ± 151.37
6-Year-old						
Head	37.92 ± 11.98	106.53 ± 23.97	52.23 ± 10.14	48.23 ± 11.85	29.62 ± 10.63	274.53 ± 50.55
Main Root	53.21 ± 18.89	391.29 ± 38.08	109.00 ± 37.54	94.37 ± 17.11	34.82 ± 11.90	682.69 ± 92.41
Leteral Root	65.90 ± 17.99	382.70 ± 67.15	116.67 ± 16.68	54.50 ± 9.66	39.04 ± 15.16	658.81 ± 81.27

Table 5
The mean contents of astragalin and astragalosides of each part and *p*-value calculated with a pairwise *t*-test (two-sided, alpha level = 0.05) ($n = 15$).

	Head	Main Root	Leteral Root
Astragalin			
Mean ± SD	43.15 ± 14.50	64.62 ± 18.94	61.68 ± 18.07
p-Value[a]	–	<0.001	<0.001
p-Value[b]	–	–	0.616
AST-I			
Mean ± SD	129.99 ± 34.10	415.81 ± 35.78	427.60 ± 92.77
p-Value[a]	–	<0.001	<0.001
p-Value[b]	–	–	0.628
AST-II			
Mean ± SD	54.04 ± 7.51	125.53 ± 35.26	120.87 ± 31.68
p-Value[a]	–	<0.001	<0.001
p-Value[b]	–	–	0.592
AST-III			
Mean ± SD	54.34 ± 14.83	93.09 ± 22.14	54.87 ± 18.99
p-Value[a]	–	<0.001	0.941
p-Value[b]	–	–	<0.001
AST-IV			
Mean ± SD	31.94 ± 13.33	38.80 ± 13.37	37.42 ± 14.52
p-Value[a]	–	0.002	0.115
p-Value[b]	–	–	0.723

[a] *p*-Value with the contents of head.
[b] *p*-Value with the contents of main root.

According to Yu et al., the main and lateral roots of two species of Radix Astragali contain about 0.8–1.7 mg/g total saponin [22], and according to Zu et al., the total saponin content of Radix Astragali was 1.584 mg/g [26]. Song et al. reported that the AST-II content was 0.5–2.9 mg/g, AST-II was 0.15–0.70 mg/g, and AST-IV was 0.04–0.22 mg/g in ten different species of Radix Astragali [34]. Therefore, there were many differences in the contents between each sample.

The contents of Radix Astragali extracts can be influenced by storage, soil and climatic conditions. In particular, the astragaloside contents may be more sensitive to these factors than other compounds because they are unstable in moist environments. Therefore, the standardization of cultivation and storage techniques is essential for producing high quality Radix Astragali extracts rich in bioactive compounds.

4. Conclusion

We developed a chromatographic method coupled with pulsed amperometric detection for determining the astragalin and astragaloside content in Radix Astragali extracts by determining the stability and optimal extraction conditions. Because the target compounds decomposed in aqueous solutions, Radix Astragali was extracted in 100% methanol under reflux. The precision and accuracy of the analytical method were confirmed through intra- and inter-day validation and the results of a recovery test. Using this method, astragalin and astragaloside contents were determined in the head, main root and lateral roots of four-, five- and six-year-old Radix Astragali. There were no significant differences between the contents of four-, five- and six-year-old Radix Astragali extracts, although extracts from the head contained smaller amount of astragalin, AST-I and AST-II than the other parts. According to these results, the storage condition appears to be a more important factor for the quality of the extract than the age of the plant or the part of the root that is extracted.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.12.035.

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