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# Simultaneous Analysis of Bioactive Metabolites from *Rehmannia glutinosa* by HPLC-DAD-MS/MS

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**Abstract** – A high-performance liquid chromatography (HPLC) with diode array detector (DAD) and electrospray ionization mass spectrometry (ESI-MS) was established for the simultaneous determination of five representative metabolites of the iridoid and phenolic classes from *Rehmannia glutinosa*. The optimal chromatographic conditions were obtained on an ODS column (5 mm, 4.6 × 250 mm) with the column temperature at 25°C. The mobile phase was composed of water and acetonitrile using a gradient elution with the flow rate 0.3 mL/min. Detection wavelength was set at 205 nm. All calibration curves showed good linear regression ($r^2 > 0.997$) within test ranges. Limits of detection (LOD) and quantitation (LOQ) values were lower than 0.123 and 0.373 μg/mL, respectively. The developed method provided satisfactory precision and accuracy with overall intra-day and inter-day variations of 0.09 - 0.76% and 0.16 - 1.41%, respectively, and the overall recoveries of 99.03 - 102.67% for all of the compounds analyzed. In addition, effectiveness of diverse extraction methods was compared to each other for the development of standard analytic method. The verified method was successfully applied to the quantitative determination of five representative metabolites in twenty-one commercial *Rehmannia glutinosa* samples from different markets in Korea and China. The analytical results showed that the contents of the five analytes vary significantly with sources.
**Keywords**– HPLC-DAD-MS, *Rehmannia glutinosa*, iridoids, phenolic compounds

## Introduction

Herbal medicines have been practiced to maintain good health and treat diseases in the Asia communities and recently in worldwide. Increment of worldwide attention and concomitant pharmaceutical research has made it essential to carry out the quality control measurement for the herbal medicines. However, serious hindrance has been attributed to the lack of recognition and regulation of profession, qualified practitioners, quality-controlled herbal medicines, and evidence-based clinical studies (Normile, 2003; Chan, 2005). Thus it is urgently needed to establish a comprehensive qualified evaluation method based on analysis of the whole bioactive compounds in order to accurately reflect the quality of herbal medicines.

*Rehmannia glutinosa. (Gaertn.)* Libosch. ex Fisch. & C.A. Mey., a comprehensive traditional herbal medicine, belongs to the family of Scrophulariaceae (Zhang *et al.,* 1996). It is one of the most widely used herbal medicine with the effect of removing pathogenic heat from blood, nourishing *Yin* and promotion the production of body fluids (Jiangsu Mew Medical College, 1975). In recent years there have been many reports of not only the pharmacological functions and activities on the blood, immune, endocrine, cardiovascular, and nervous systems (Chang *et al*., 1998), but also a wide variety of clinical applications such as hemostasis (Liang *et al*., 1999), anti-tumor treatment (Chao *et al*., 2006), immune-enhancement (Gao and Wu, 1990), anti-hypertension (Chen *et al*., 1996, 1997), bone metabolism (Oh *et al.,* 2003), as well as treatment for concretion in the urinary tract and ulcerative stomatitis (Zhang *et al*., 2008). Up to date a number of constituents such as iridoids, phenolics, glycosides, and norcarotenoids have been reported from this plant (Tomoda *et al*., 1971; Morota *et al.,* 1989). Due to the presence of multiple compounds that might be associated with the therapeutic functions (Xue and Roy *et al*., 2003; Qian *et al*., 2007), however, a single or a few compounds could not be responsible for the overall pharmacological activities of *R. glutinosa*.

Based upon the prior researches, among the components of *R. glutinosa* selected for analysis were five representative compounds with significant bioactivities and relatively large contents: catalpol (**1**) (Kitagawa *et al*., 1971),

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aucubin (**2**) (Oshio *et al*., 1981; Oshio *et al*., 1982; Toshiko *et al*., 1982), and ajugol (**3**) (Oshio *et al*., 1981; Oshio *et al*., 1982; Toshiko *et al*., 1982) of iridoids and forsythiaside (**4**) (Shoyama *et al*., 1986) and acteoside (**5**) (Wu *et al*., 1984; Chun *et al*., 2002) of phenolics (Fig. 1). The standard methods have been developed for the simultaneous identification and quantitative analysis of these compounds. Using these methods, the contents of bioactive compounds in twenty-one commercial *R. glutinosa* from China and Korea were analyzed and compared to each other.

## Experimental

**Plant materials** – Twenty one samples of *Rehmannia glutinosa* grown in different regions were provided by the National Center for Standardization of Herbal Medicine, such as J-1 (Andong, Korea), J-2 (Andong, Korea), J-3 (Jechon, Korea), J-4 (Uiseong, Korea), J-5 (Uiseong, Korea), J-6 (Gunwi, Korea), J-7 (Gunwi, Korea), J-8 (Andong, Korea), J-9 (Hanam, China), J-10 (Hanam, China), J-11 (unknown area, China), J-12 (unknown area, China), J-13 (Kijoo, China), J-14 (unknown area, China), J-15 (Hanam, China), J-16 (unknown area, China), J-17 (unknown area, China), J-18 (unknown area, China), J-19 (unknown area, China), J-20 (unknown area, China), and J-21 (Andong, Korea).

**Reagents, chemicals and samples** – catalpol (**1**), aucubin (**2**), ajugol (**3**), forsythiaside (**4**) and acteoside (**5**) isolated and purified from *Rehmannia glutinosa* by a series of chromatography procedures were provided from National Center for Standardization of Herbal Medicine, and the structures were elucidated by comparison of spectral data (UV, IR, MS, $^{1}$H-NMR, $^{13}$C-NMR) with the literature data. The purities of these compounds were determined to be higher than 96% by normalization of the peak area detection by HPLC analysis. *p*-Hydroxybenzaldehyde as an internal standard compound was obtained from Sigma-Aldrich (USA).

Acetonitrile, methanol, and the de-ionized water (J. T. baker, USA) used in this work were of HPLC grade and ethanol (Duksan, Korea) was analytical grade.

**Instrumentation and chromatographic conditions** – The HPLC system was consisted of Agilent 1200 series equipped with an autosampler, a column oven, a binary pump, diode array detector (Agilent Technologies, Waldbronn, Germany) and a degasser (Agilent Technologies, Tokyo, Japan). The Chemstation software (Agilent Technologies, Avondale, CA, USA) was used to operate this HPLC-DAD system. Separation was performed on a Waters Sunfire$^{TM}$ $C_{18}$ (5 mm. 4.6 mm × 250 mm) analytical column. A gradient elution of A(water) and B (acetonitrile) was used (0 min, 10% B; 10 min, 10% B; 30 min, 60% B; 40 min, 60% B; 45 min, 10% B in A *v/v*). Standard or sample solutions of 10 mL were directly injected to the HPLC system and the mobile phase flow rate was 0.3 mL/min and the column temperature was set at 25°C.

All ESI-MS and ESI-$MS^n$ spectra were acquired using a Finnigan MAT LCQ ion-trap mass spectrometer (San

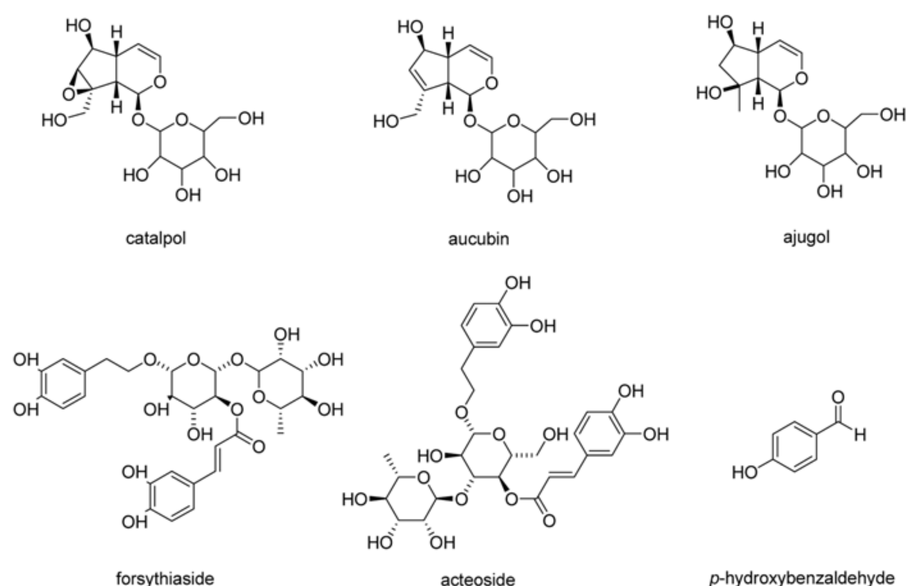


**Fig. 1.** Chemical structures of representative compounds in *Rehmannia glutinosa.*

Jose, CA, USA) equipped with a Finnigan electrospray source and capable of analyzing ions up to *m/z* 2000. Mass spectrometer conditions were firstly optimized using flow injection analysis of the standards without the HPLC column. The conditions were as follows: a sheath gas flow rate 60 arbitrary units, aux gas flow rate 0 arbitrary units, capillary temperature 250°C, spray voltage 5 kV, capillary voltage 39 V, tube lens offset 55 V.

**Preparation of test sample** – In order to achieve quantitative extraction method, variables involved in the procedure such as extraction method, solvent, and extraction time were optimized. Ultrasonication, vortex and reflux methods were compared to each other for the selection of the optimal extraction method (Table 1) at 30% aqueous methanol solvent for 30 min. Aqueous methanol or ethanol solutions were tried as the extraction solvent (Table 2) using ultrasonication method at 30 min. Yields were also compared for the extraction times of 10, 20, 30, 60, and 90 min to determine the optimal extraction time (Table 3) using ultrasonication method at 30% aqueous methanol. As a result, 30% aqueous methanol for 30 min using ultrasonication was chosen for the best extraction condition.

Accurately weighed *R. glutinosa* powder of 100 mg was extracted with 10 mL of 70% aqueous methanol, by means of sonication at room temperature for 30 min. After the filtration through 0.2 µm membrane filter, to an aliquot of 1 mL of the filtrate was added 5 mg of internal standard compound, evaporated under vacuum, and then dissolved in 90% aqueous acetonitrile prior to analysis.

**Table 1.** Comparison of effectiveness by extraction method (µg/g, n = 3)

| Compounds | Ultrasonication | Vortex | Reflux |
|---|---|---|---|
| 1 | 15149.91 ± 39.16 | 13760.72 ± 5.66 | 14156.92 ± 53.99 |
| 2 | 123.60 ± 0.79 | 96.16 ± 3.54 | 141.21 ± 0.91 |
| 3 | 1153.25 ± 19.76 | 992.49 ± 11.85 | 1100.23 ± 9.09 |
| 4 | 162.87 ± 6.22 | 151.91 ± 3.48 | 140.71 ± 0.48 |
| 5 | 4622.37 ± 19.89 | 4291.82 ± 23.44 | 4219.71 ± 16.73 |

**Calibration** – Stock solution (1 mg/mL) of the catalpol (**1**), aucubin (**2**), ajugol (**3**), forsythiaside (**4**) and acteoside (**5**) isolated from *R. glutinosa* were prepared individually in methanol, and different concentration (1, 2, 4, 8, 15, and 20 µg/mL) of these were loaded onto an HPLC for the preparation of the calibration function. The calibration function of individual compound was calculated with peak area ($y$), concentration ($x$, µg/mL), and mean values (n = 3) ± standard deviation.

## Result and discussion

For the simultaneous determination of *Rehmannia glutinosa*, the chromatographic condition was firstly investigated. Various mixtures of water and acetonitrile were tested as a mobile phase. The optimal wavelength for detection was tested at 205, 220, 230, 250, and 280 nm, respectively. Considering the significantly different UV maxima between the two types of analytes, optimum detection wavelength was decided at 205 nm where the five compounds showed a relatively good absorption. The temperature for detection was tested at 25, 30, 35, 40, and 45°C. Optimum detection temperature was decided at 25°C where the baseline was stable and all of the five compounds had good theoretical plate, capacity factor, separation factor, and resolution (Table 4). The presences of catalpol (**1**), aucubin (**2**), ajugol (**3**), forsythiaside (**4**) and acteoside (**5**) in *R. glutinosa* were verified by comparing each retention time and UV spectrum with those of each

**Table 2.** Comparison of effectiveness by extraction solvent (µg/g, n = 3)

| Compounds | 70% Methanol | 50% Methanol | 70% Ethanol | 50% Ethanol |
|---|---|---|---|---|
| 1 | 15149.89 ± 22.05 | 14356.62 ± 37.28 | 15807.80 ± 18.93 | 15459.18 ± 51.16 |
| 2 | 123.60 ± 0.79 | 107.34 ± 3.03 | 119.19 ± 5.07 | 135.06 ± 3.46 |
| 3 | 1250.70 ± 22.72 | 929.15 ± 2.63 | 1169.56 ± 5.37 | 1007.76 ± 40.21 |
| 4 | 162.87 ± 6.22 | 140.18 ± 1.47 | 153.62 ± 5.92 | 172.97 ± 4.78 |
| 5 | 4622.41 ± 29.44 | 4140.16 ± 35.14 | 3936.16 ± 27.10 | 3223.41 ± 1.37 |

**Table 3.** Comparison of effectiveness by extraction time (µg/g, n = 3)

| Compounds | 10 min | 20 min | 30 min | 60 min | 90 min |
|---|---|---|---|---|---|
| 1 | 13015.89 ± 39.71 | 14291.66 ± 17.75 | 15149.89 ± 22.05 | 15425.79 ± 65.93 | 14823.26 ± 43.26 |
| 2 | 75.91 ± 4.36 | 121.60 ± 4.05 | 123.60 ± 0.79 | 89.08 ± 1.27 | 128.05 ± 0.91 |
| 3 | 931.80 ± 15.74 | 1097.99 ± 13.53 | 1153.72 ± 23.09 | 1018.93 ± 4.98 | 1014.03 ± 5.74 |
| 4 | 106.62 ± 2.57 | 150.18 ± 2.71 | 162.87 ± 6.22 | 181.35 ± 4.81 | 199.56 ± 1.92 |
| 5 | 3858.77 ± 25.42 | 4284.75 ± 18.03 | 4622.41 ± 29.44 | 4398.46 ± 17.60 | 4518.55 ± 19.79 |

**Table 4.** compared with temperature of column oven.

| Temperature (°C) | Analytes | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| Theoretical plate (N) | | | | | |
| 25 | 18856.00 ± 22.72 | 15356.33 ± 2743.61 | 21331.67 ± 668.65 | 165748.00 ± 4303.65 | 234840.33 ± 3661.90 |
| 30 | 19140.73 ± 305.18 | 14006.33 ± 586.60 | 22270.67 ± 410.55 | 144132.33 ± 2887.80 | 223839.33 ± 37955.98 |
| 35 | 18520.67 ± 270.05 | 16177.67 ± 1025.29 | 22101.67 ± 752.55 | 39055.67 ± 1113.49 | 212890.00 ± 32486.15 |
| 40 | 18576.33 ± 284.04 | 16216.00 ± 714.83 | 21961.33 ± 270.48 | 32714.33 ± 6655.00 | 193682.00 ± 17748.88 |
| 45 | 19104.33 ± 227.14 | 17116.67 ± 533.24 | 21786.00 ± 848.04 | 39123.33 ± 6756.27 | 129392.33 ± 10275.29 |
| Capacity factor (k') | | | | | |
| 25 | 10.70 ± 0.01 | 13.05 ± 0.01 | 19.57 ± 0.06 | 41.24 ± 0.02 | 42.13 ± 0.03 |
| 30 | 10.59 ± 0.02 | 12.75 ± 0.02 | 19.01 ± 0.04 | 40.83 ± 0.02 | 41.71 ± 0.02 |
| 35 | 10.49 ± 0.01 | 12.52 ± 0.01 | 18.67 ± 0.05 | 40.53 ± 0.01 | 41.37 ± 0.00 |
| 40 | 10.40 ± 0.01 | 12.30 ± 0.01 | 18.25 ± 0.05 | 40.31 ± 0.02 | 41.08 ± 0.02 |
| 45 | 10.35 ± 0.02 | 12.13 ± 0.03 | 17.91 ± 0.05 | 40.01 ± 0.03 | 40.76 ± 0.03 |
| Separation factor (á) | | | | | |
| 25 | - | 1.22 ± 0.00 | 1.50 ± 0.01 | 2.11 ± 0.01 | 1.11 ± 0.00 |
| 30 | - | 1.20 ± 0.00 | 1.49 ± 0.00 | 2.15 ± 0.01 | 1.09 ± 0.00 |
| 35 | - | 1.19 ± 0.00 | 1.49 ± 0.00 | 2.17 ± 0.01 | 1.08 ± 0.00 |
| 40 | - | 1.18 ± 0.00 | 1.48 ± 0.01 | 2.21 ± 0.01 | 1.07 ± 0.00 |
| 45 | - | 1.17 ± 0.00 | 1.48 ± 0.00 | 2.23 ± 0.01 | 1.06 ± 0.00 |
| Resolution (Rs) | | | | | |
| 25 | - | 5.88 ± 0.31 | 12.77 ± 0.58 | 44.22 ± 0.53 | 2.30 ± 0.02 |
| 30 | - | 5.40 ± 0.09 | 12.47 ± 0.23 | 44.61 ± 0.24 | 2.17 ± 0.09 |
| 35 | - | 5.31 ± 0.11 | 12.86 ± 0.23 | 31.85 ± 0.26 | 1.38 ± 0.03 |
| 40 | - | 5.03 ± 0.06 | 12.66 ± 0.05 | 30.61 ± 2.04 | 1.18 ± 0.08 |
| 45 | - | 4.87 ± 0.03 | 12.64 ± 0.13 | 32.77 ± 1.76 | 1.16 ± 0.07 |

standard compound and spiking with authentic standards. As a result, a gradient acetonitrile-water solvent system at 205 nm and 25 °C gave the desired separation within the running time of 45 min (Fig. 2).

A methanol stock solution, containing five standard compounds, was prepared and diluted to a series of appropriate concentrations for the construction of calibration curves. The curves showed good linearity and the correlation coefficients were found to be in the range of 0.997-0.999 for all the compounds, over the concentration ranges 1 - 20 μg/mL. Limits of detection and limits of quantitation were determined by means of serial dilution based on a signal-to-noise (S/N) ratio of 3 : 1 and 10 : 1, respectively. LOD and LOQ were less than 0.123 and

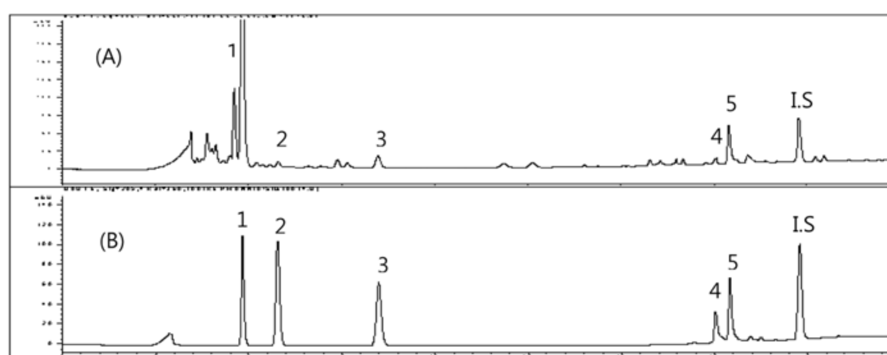


**Fig. 2**. HPLC chromatograms of standard extract of *Rehmannia glutinosa* (A) and standard mixture (B).

0.369 μg/mL, respectively (Table 5).

The accuracy and precision tests were carried out by measurement of the intra-day and inter-day variability and recovery of these constituents. The measurement of intra-day and inter-day variability was utilized to determine the precision of this method. The intra-day variation was determined by analyzing in the triplicate same mixed standard methanol solution for three times within 1 day. While for the inter-day variability test, the solution was examined in triplicate for consecutive 3 days. The average percentage recoveries were evaluated by calculating the ratio of detected amount versus the added amount and the mean recovery of each compound was 99.03 - 102.32%. The relative standard deviation (RSD) was taken as a tool of precision. The RSD of intra-day and inter-day variability was less than 1.41% (Table 6).

Since the chromatographic peaks could not be identified unambiguously only by retention time and UV spectra in HPLC, HPLC-DAD-MS/MS was used as a supplement for confirmation of peak identification, by comparing the retention time, molecular ion and fragment pattern. In this experiment, mass spectral conditions were optimized in positive-ion mode and five compounds exhibited distinct quasi-molecular ions $[M+Na]^+$ in this mode (Table 7). In HPLC-ESI-MS spectra, molecular ion and fragmentation pattern of each compound were well matched with chemical structures (Fig. 3). Throughout these results, five standard compounds were identified in the extract of *Rehmannia glutinsa* and the specificity of each peak for **1**-**5** was clearly demonstrated.

**Table 5.** Calibration curves, LOD and LOQ of five standard compounds

| Compounds | Range (μg/mL) | regression equation[a] | $r^2$ | LOD[b] (μg/mL) | LOQ[c] (μg/mL) |
|---|---|---|---|---|---|
| 1 | 1 ~ 20 | $y = 0.03707x + 0.00206$ | 0.997 | 0.070 | 0.211 |
| 2 | 1 ~ 20 | $y = 0.05259x - 0.00169$ | 0.999 | 0.057 | 0.172 |
| 3 | 1 ~ 20 | $y = 0.02665x - 0.00085$ | 0.999 | 0.083 | 0.251 |
| 4 | 1 ~ 20 | $y = 0.03390x - 0.00049$ | 0.999 | 0.123 | 0.373 |
| 5 | 1 ~ 20 | $y = 0.04505x + 0.03309$ | 0.998 | 0.089 | 0.271 |

a) y: peak area, x: concentration of the analyte (μg/mL).
b) LOD, limit of detection. c) LOQ, limit of quantification.

**Table 6.** Precision and accuracy of the five standard compounds

| Compounds | Spiked amount (μg) | Intra-day (n = 5) | | Inter-day (n = 5) | |
|---|---|---|---|---|---|
| | | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| 1 | 4 | 100.31 | 0.31 | 99.47 | 0.51 |
| | 8 | 99.30 | 0.29 | 99.73 | 0.33 |
| | 12 | 101.12 | 0.21 | 101.20 | 0.69 |
| 2 | 2 | 99.94 | 0.76 | 100.42 | 0.54 |
| | 4 | 102.32 | 0.19 | 102.67 | 0.28 |
| | 8 | 99.39 | 0.09 | 100.21 | 0.16 |
| 3 | 2 | 100.63 | 0.59 | 101.53 | 1.41 |
| | 4 | 101.49 | 0.21 | 101.31 | 0.54 |
| | 8 | 99.32 | 0.19 | 99.36 | 0.23 |
| 4 | 2 | 99.36 | 0.44 | 100.35 | 0.81 |
| | 4 | 99.94 | 0.26 | 99.93 | 0.49 |
| | 8 | 100.91 | 0.14 | 100.47 | 0.34 |
| 5 | 4 | 99.68 | 0.62 | 99.21 | 0.40 |
| | 8 | 101.94 | 0.14 | 101.63 | 0.16 |
| | 12 | 99.03 | 0.12 | 99.69 | 0.17 |

**Table 7.** HPLC-DAD-MS/MS Data of the five standard compounds.

| Peak No. | MW[a] | MS (*m/z*) | MS/MS (*m/z*) | C.E[b] | Ion mode | Identification |
|---|---|---|---|---|---|---|
| 1 | 362 | 385 | 203 | 29 | Positive | Catalpol |
| 2 | 346 | 369 | 203 | 29 | Positive | Aucubin |
| 3 | 348 | 371 | 271 | 29 | Positive | Ajugol |
| 4 | 624 | 647 | 501 | 33 | Positive | Forsythiaside |
| 5 | 624 | 647 | 501 | 33 | Positive | Acteoside |

a) molecular weight.
b) collision energy.

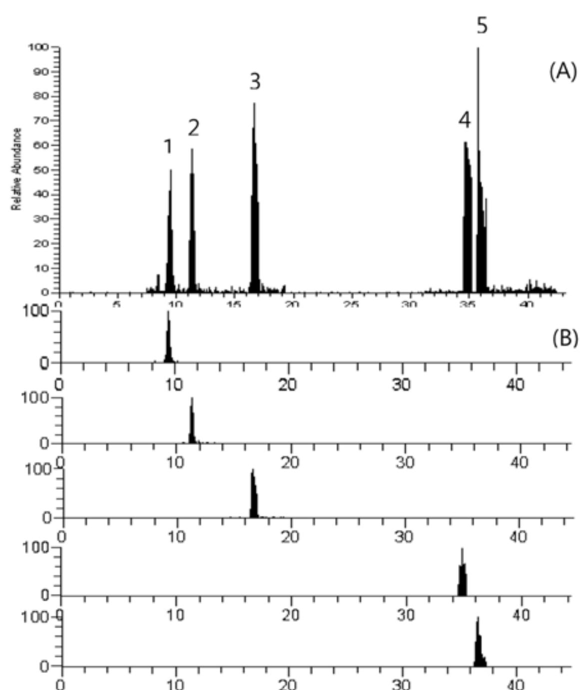


**Fig. 3.** Total ion chromatograms of *Rehmannia glutinosa* in SIM mode (A) and SRM mode (B).

The developed method was applied for the measurement of concentration of standard components in commercial *Rehmannia glutinosa*. Twenty-one commercial products from various areas of Korea and China were analyzed. The test samples were prepared as described for the development of analytical method and injected in triplicate. The results are summarized in Table 8. It was found that contents of standard compounds in various regional samples vary significantly by sources. Among the five components, catalpol, ajugol and acteoside had remarkably higher concentrations than aucubin and forsythiaside (catalpol, 9125.41 ± 5571.25 μg/g; aucubin, 209.98 ± 131.61 μg/g; ajugol, 721.21 ± 444.92 μg/g; forsythia-side; 99.66 ± 74.41 μg/g; acteoside, 742.33 ± 914.52 μg/g). Among the various regional samples, J-16 (unknown area, China) had the lowest iridoids contents. There were no significant differences in concentrations between two different origins (Korea and China) samples, but Chinese samples showed larger standard variation than those of Korea.

## Conclusion

The standard analytical methods using HPLC-DAD-MS/MS have been developed for simultaneous identification and quantification of catalpol, aucubin, ajugol, forsythiaside and acteoside in the traditional medicinal

**Table 8.** Content of five compounds in samples of twenty-one commercial products from diverse sources (μg/g)

| Samples | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| J-1 | 15,984.89 | 376.99 | 994.98 | 132.17 | 1,600.95 |
| J-2 | 14,683.11 | 125.59 | 87.69 | 167.82 | 3,882.35 |
| J-3 | 19,449.64 | 253.66 | 636.28 | 74.92 | 160.03 |
| J-4 | 12,400.24 | 171.52 | 488.29 | 86.06 | 688.65 |
| J-5 | 13,856.75 | 221.45 | 636.28 | 74.92 | 160.03 |
| J-6 | 10,985.99 | 177.87 | 704.37 | 184.01 | 670.04 |
| J-7 | 15,320.27 | 227.73 | 821.94 | 122.65 | 539.09 |
| J-8 | 7,554.87 | 398.65 | 1,659.75 | 139.95 | 191.95 |
| J-9 | 3,237.66 | 159.32 | 477.48 | - | 271.80 |
| J-10 | 1,499.14 | 78.13 | 265.85 | 81.00 | 424.38 |
| J-11 | 4,118.37 | 131.47 | 425.30 | - | 441.57 |
| J-12 | 4,695.87 | 120.04 | 568.74 | 59.71 | 276.37 |
| J-13 | 3,503.39 | 124.16 | 723.27 | 86.52 | 206.82 |
| J-14 | 1,666.36 | 73.99 | 220.62 | 78.35 | 102.79 |
| J-15 | 5,635.70 | 90.08 | 871.69 | 66.62 | - |
| J-16 | 757.41 | - | - | - | 1,042.29 |
| J-17 | 14,235.40 | 540.36 | 1,100.06 | 149.06 | 833.64 |
| J-18 | 12,483.10 | 311.21 | 1,246.36 | 127.91 | 2,064.90 |
| J-19 | 7,210.51 | 181.86 | 782.77 | 89.48 | 111.24 |
| J-20 | 12,296.52 | 380.97 | 1,766.84 | 171.65 | 295.95 |
| J-21 | 10,058.54 | 264.53 | 366.90 | - | 1,624.04 |
| Mean | 9125.41 | 209.98 | 721.21 | 99.66 | 742.33 |
| Standard deviation | 5571.75 | 131.61 | 444.92 | 74.41 | 914.52 |

plant *Rehmannia glutinosa*. Various validation parameters such as specificity, linearity, detection limit, quantitation limit, accuracy and precision were successfully obtained and individually validated. In addition, effectiveness of diverse extraction methods were compared to each other. This method was successfully applied for the measurement of concentrations of standard components from diverse sources, thus demonstrating its potential for the quality control of *R. glutinosa*.

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## References

Chan K., Chinese medicinal materials and their interface with Western medical concepts. *J. Ethnopharmacol.* **96**, 1-18 (2005).

Chao, J.C-J., Chiang, S.W., Wang, C.C., Tsai, Y.H., Wu, M.s., Hot water-extracted *Lycium barbarum* and *Rehmannia glutinosa* inhibit proliferation and induce apoptosis of hepatocellular carcinoma cells. *World. J. Gastroentero.* **12**, 4478-4484 (2006).

Chen, D.D., Dai, D.Z., Zhang, T., Rehmanniae suppresses the elevated activity of rat cerebral $Ca^{2+}$, $Mg^{2+}$-ATPase induced by isoprenaline. *Pharmacol. Clin. Chin. Mater. Med.* **12**, 23-24 (1996).

Chen, D.D., Dai, D.Z., Zhang, T., Rehmanniae regresses the cardiac hypertrophy and the elevated activity of rat cardium and cerebrum mitochondria $Ca^{2+}$, $Mg^{2+}$-ATPase induced by L-thyroxine. *Pharmacol. Clin. Chin. Mater. Med.* **13**, 27-28 (1997).

Chun, J.C., Kim, J.C., Hawng, I.T., Kim. S.E., Acteoside from *Rehmannia glutinosa* nullifies paraquat activity in *Cucumis sativus*. *Pest. Biochem. Physiol.* **72,** 153-159 (2002).

Gao, X.D., Wu, W.T., Biological function of five Chinese traditional drugs on proliferation and IL-2 production of the mice lymphocytes. *Chin. Pharm. J.* **21**, 43-45 (1990).

Jiangsu Mew Medical college, *Lexicon of Traditional Chinese Drugs*. Shanghai People's press, Shanghai, pp.74-76 (1975).

Kitagawa, I., Nishimura, T., Furubayashi, A., Yosioka, I., On the constituents of rhizoma of *Rehmannia glutinosa* Libosch. forma *hueichingensis* Hsiao. *Yakugaku Zasshi* **9,** 593-596 (1971).

Liang, A.H., Xue, B.Y., Wang, J.H., Hao, J.D., Yang, H., Yi, H., A study on hemostatic and immunologic actions of fresh- and dry Dihuang. *Chin. J. Chin. Mater. Med.* **24**, 663-666 (1999).

Morota, T., Sasaki, H., Nishimura, H., Sugama, K., Masao, C.C.Z, Mitsuhashi, H., Two iridoid glycosides from *Rehmannia glutinosa*. *Phytochemistry* **28,** 2149-2153 (1989).

Oh, K.O., Kim, S.W., Kim, J.Y., Ko, S.Y., Kim, H.M., Baek, J.H., Ryoo, H.M., Kim, J.K., Effect of *Rehmannia glutinosa* Libosch. extracts on bone metabolism. *Clin. Chim. Acta.* **334,** 185-195 (2003).

Normile, M., The new face of traditional Chinese medicine. *Science* **299,** 188-190 (2003).

Oshio, H., Inouye, H., Iridoid glycosides of *Rehmannia glutinosa*. *Phytochemistry* **21,** 133-138 (1982).

Oshio, H., Naruse, Y., Inouye, H., Quantitative analysis of iridoid glycosides of *Rehmannia glutinosa*. *Phytochemistry* **21,** 133-138 (1981).

Qian, Z.-M., Li, H.-J., Li, P., Ren, M.-T., Tang, D., Simultaneous qualitation and quantification of thirteen bioactive compounds in *Flos Lonicerae* by high-performance liquid chromatography with diode array detector and mass spectrometry. *Chem. Pharm. Bull.* **55,** 1073-1076 (2007).

Shoyama, Y., Matsumoto, M., Nishioka, I., Four caffeoyl glycosides form callus tissue of *Rehmannia glutinosa*. *Phytochemistry* **25,** 1633-1636 (1986).

Tomoda, M., Kato, S., Onuma, M., Water-soluble constituents of Rehmanniae radix. On the consistents of roots of *Rehmannia glutinosa* var.purpurea. *Chem. Pharm. Bull.* **19,** 2411-2416 (1971).

Toshiko, H., Katsuya, K., Shuhichi, T., Utako, A., Constituents of leaves and root of kaikei jio. *Shoyakugaku Zashi* **36,** 1-5 (1982).

Wu, S.J., Xu, S.M., Li, Y.C., Study of chemical constituents from *Rehmannia glutinosa* f. hueichingensis. *Chin. Trad. Herb Drugs.* **15,** 6-8 (1984).

Xue, T.H. and Roy, R., Studying traditional Chinese medicine. *Science* **300**, 740-741 (2003).

Zhang, G.J., Ji, J.Y., Liu, Q., Zhang, L.H., Gong, G.Y., Jin, Z.X., Ren, S.z., *Collected Edition of Identification and Assessment of Common TCD*. Heilongjiang Science and Technique Press, Harbin, China, pp.301-302 (1993).

Zhang, R.X., Li, M.X., Jia, Z.P., *Rehmannia glutinosa*: Review of botany, chemistry and pharmacology. *J. Ethnopharmacol.* **117,** 199-214 (2008).

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