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Journal of Chromatography B, 865 (2008) 159–166
JOURNAL OF CHROMATOGRAPHY B
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Short communication

# Simultaneous determination of two Amadori compounds in Korean red ginseng (*Panax ginseng*) extracts and rat plasma by high-performance anion-exchange chromatography with pulsed amperometric detection

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Received 6 August 2007; accepted 19 February 2008
Available online 23 February 2008

**Abstract**

A new simple, rapid and sensitive high-performance anion-exchange chromatography method with pulsed amperometric detection (HPAEC–PAD) was developed and validated for the simultaneous determination of two Amadori compounds, arginyl-fructose and arginyl-fructosyl-glucose in Korean red ginseng (*Panax ginseng*) extracts, rat plasma. Separation of the two target analytes was efficiently undertaken on CarboPac PA1 anion-exchange column with isocratic elution (400 mM sodium hydroxide and deionized water (90:10, v/v)) at flow rate 0.7 mL/min within 15 min of single chromatographic run. Under optimized conditions, the detection limits (signal-to-noise ratio equal to 3) were 20 and 25 ng/mL for arginyl-fructose and arginyl-fructosyl-glucose, respectively. Calibration curves of peak area for the two analytes were linear over three orders of magnitude with a correlation coefficients greater than 0.999. The accuracy of the method was tested by recovery measurement of the spiked samples which yielded good results of 94.15–102.62%. This method was successfully applied to the quantification of arginyl-fructose and arginyl-fructosyl-glucose in herbal extracts and in the plasma samples from rat.
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*Keywords:* High-performance anion-exchange chromatography; Pulsed amperometric detection; Amadori compounds; Red ginseng; Rat plasma

## 1. Introduction

*Panax ginseng*, one of the most well known herbal medicines in oriental countries has been widely used as alternative medicine, health food and tonic owing to the belief of its effectiveness in maintaining youth and prolongation of the life span. Major ingredients of ginseng are saponins, ginsenoside, which has been reported to be effective on the cardiovascular and central nervous systems [1–3] and also as an anti-oxidant [4–6]. Other than ginsenosides, amino acid derivatives, arginyl-fructose (Arg-Fru) and arginyl-fructosyl-glucose (Arg-Fru-Glc) have been identified in Korean red ginseng, a steamed ginseng [7], along with their various pharmacological activities such as anti-oxidant, anti-diabetes, anti-obesity and the modulation of glucose transport [8].

Due to the high enrichment of arginine in crude ginseng [9], Arg-Fru and Arg-Fru-Glc are the major Amadori compounds formed by the reaction of maltose, arginine and glucose, arginine respectively during the steaming and heat-drying processes of Korean red ginseng preparation. Amadori compounds are generated by a non-enzymatic glycation reaction between amino acids and reducing sugars, which contributes to the aroma, flavor, taste and color of food and also influences their nutritional and toxicological properties [10]. With the increasing recognition of the various therapeutic effects of Korean red ginseng [11–14], application of Arg-Fru and Arg-Fru-Glc as health food or alternative medicine is being actively sought. In this regard, the development of a reliable analytical method is required for their accurate quantification in herbal extracts and plasma.

The analysis of Amadori compounds, however, is not an easy task, especially in the samples of complex systems such

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1570-0232/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.jchromb.2008.02.012

as food or biological fluids. Various column chromatographic techniques have been tried for the separation and quantification of Amadori compounds. Previously, a classical amino acid analyzer method with post-column reaction using ninhydrin was applied to the analysis of Amadori compounds [15,16]. This method, however, had several disadvantages such as insufficient separation, poor sensitivity and long analysis time. Derivatization of carbohydrate moiety using such as trimethylsilyation in gas chromatography (GC) was also tried but it still required time-consuming derivatization procedures and complex separation steps due to the generation of tautomeric form [17]. Additionally, HPLC method with a refractive index detector has been tried for analyzing Amadori compounds, but it also has problems such as poor sensitivity and insufficient separation [18]. The method of pre- or post-column derivatization and UV or fluorometric detection by HPLC has been widely used for sugar analysis [19–22] and it has been applied to analyze Amadori compounds. For example, Ryu et al. tried to analyze for Arg-Fru in aged garlic extract by HPLC and post-column reaction with triphenyltetrazolium chloride [23]. However, the method still requires time-consuming clean-up procedures to separate Amadori compounds from the complex matrix prior to the chromatographic step. TLC method using ninhydrin and anisaldehyde reagent has been also reported for sugar and amino acid detection to analyze Amadori compounds [24]. But this method is applied to the qualitative detection of Amadori compounds, not to quantitative.

Recently, a new method using high-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAEC–PAD) [25–30] or high-performance anion-exchange chromatography with mass spectrometer (HPAEC–MS) [31,32] has been developed for the determination of sugars. HPAEC–PAD method employs alkaline condition where affinity between sugar and quaternary amine stationary phase occurs. Under alkaline conditions, weak-acidic sugars are present as anions, which can be separated and detected by anion-exchange column with PAD. This method yielded good resolution, high sensitivity and selectivity for the analysis of sugar [33,34], which can be extended to the analytical method for Amadori compounds. Davidek et al. [35] developed a method for the separation and simultaneous determination of Maillard precursors and Amadori compounds in Maillard reaction model system. This analytical method, however, has a limited application in much simpler model systems. Up to now, there is no report of the simultaneous determination of hexose-derived and di-hexose-derived Amadori compounds in complex matrix such as natural herbal extracts or biological fluids by high-performance anion-exchange chromatography with pulsed amperometric detection.

In the present study, we developed a new analytical method which can determine the Amadori compounds, Arg-Fru and Arg-Fru-Glc simultaneously using HPAEC–PAD. This method can be applied for the simple, rapid, sensitive and simultaneous determination of Arg-Fru and Arg-Fru-Glc in the two complex matrices, herbal extracts and biological samples such as plasma, at the same time. We believe that with the present study, a useful and versatile analytical method has been provided for the study of Arg-Fru and Arg-Fru-Glc, which can be extended to the research for other Amadori compounds.

## 2. Experimental

### 2.1. Chemicals and reagents

L-Arginine, maltose monohydrate, D-glucose, *N*-acetyl neuraminic acid, perchloric acid, ninhydrin and trichloroacetic acid were from Sigma (St. Louis, MO). Formic acid was purchased from Fluka (Burchs, Switzerland). Ammonia solution (28%) and butanol were from Junsei (Junsei Chemical Co., Ltd, Japan). Methanol and acetonitrile were HPLC grade from Burdick & Jackson (Honeywell International Inc., Muskegon, MI). 50% (w/w) sodium hydroxide solution was from Fisher Scientific (Fair Lawn, NJ) and the water used was ultra-pure deionized water (18.2 MΩ cm) produced from Millipore Milli-Q Gradient system (Millipore, Bedford, MA). All other reagents used were of highest grade available.

### 2.2. Synthesis of arginyl-fructose and arginyl-fructosyl-glucose

Arg-Fru (**1**), Arg-Fru-Glc (**2**) (Fig. 1) was prepared according to the previous report [36]. Mixture of maltose, L-arginine and glucose, L-arginine were dissolved in glacial acetic acid, respectively, and stirred for 1 h at 70–80 °C. After cooling to room temperature, the resultant slurry was centrifuged at 1000 × *g* for 10 min and the supernatant was concentrated to dryness. The dried sample was dissolved in 50 mL of water and applied to a cation exchange column (CG-Amberlite IR-120-1, Fluka, Burchs, Switzerland). After washing with 200 mL of water, 300 mL of 0.5% ammonia solution was eluted and the effluent was freeze-dried. Then the dried sample was purified using silica gel column (Kieselgel 160, 70–230 mesh, Merck, Germany) with butanol–acetic acid–water (2:1:1) solution. The fractions were identified by TLC with ninhydrin reagent. Resultant compounds **1** and **2** were obtained as off-white powder. Structures of synthesized **1** and **2** were confirmed using NMR and LC–MS by which purities were confirmed to be over 95%. The NMR spectral data of the synthesized **1** and **2** were identical with the previously reported data and mass of **1** and **2** was analyzed to be *m/z* 337 and *m/z* 449 ([M+H]), respectively, in LC–MS analysis (Fig. 2). The LC–MS analysis was performed using a Finnigan LCQ Deca XP ion trap mass spectrometer with ESI positive mode and surveyor HPLC system (ThermoQuest, San Jose, CA).

### 2.3. High-performance anion-exchange chromatography coupled with pulsed amperometric detection

The analysis was performed by Dionex ion chromatography system (ICS2500, Dionex, Sunnyvale, CA) composed of autosampler (AS 50 with a 25 μL sample loop), gradient pump (GP-50) with on line degasser and electrochemical detector (ED-50). The chromatographic separation was

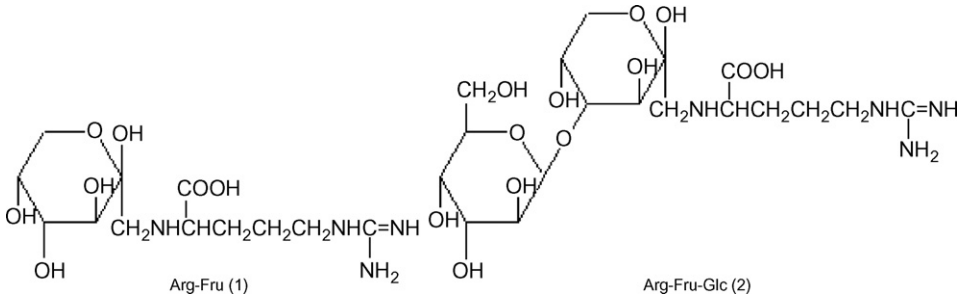


Fig. 1. Chemical structures of arginyl-fructose (Arg-Fru, **1**) and arginyl-fructosyl-glucose (Arg-Fru-Glc, **2**).

accomplished on a CarboPac PA1 anion-exchange column (250 mm × 4 mm, Dionex) and a CarboPac PA1 guard column (50 mm × 4 mm, Dionex). The column temperature was 30 °C. 400 mM sodium hydroxide was prepared by diluting 50% (w/w) sodium hydroxide solution. The elution was performed with water (A) and 400 mM sodium hydroxide (B) (10:90, v/v) using binary gradient elution. The flow rate was 0.7 mL/min and injection volume was 25 μL. The Arg-Fru (**1**), Arg-Fru-Glc (**2**) were detected by ECD equipped with a gold working electrode and Ag/AgCl reference electrode operating quadruple potential waveform. The potential waveform was as follow. 0–0.4 s, $E = 0.10$ V, 0.2 s Integration = Begin, 0.4 s Integration = End; 0.41–0.42 s, $E = -2.00$ V; 0.43 s, $E = 0.60$ V; 0.44–0.50 s, $E = -0.10$ V. We controlled the data acquiring and process with Chromeleon 6.5 software of Dionex.

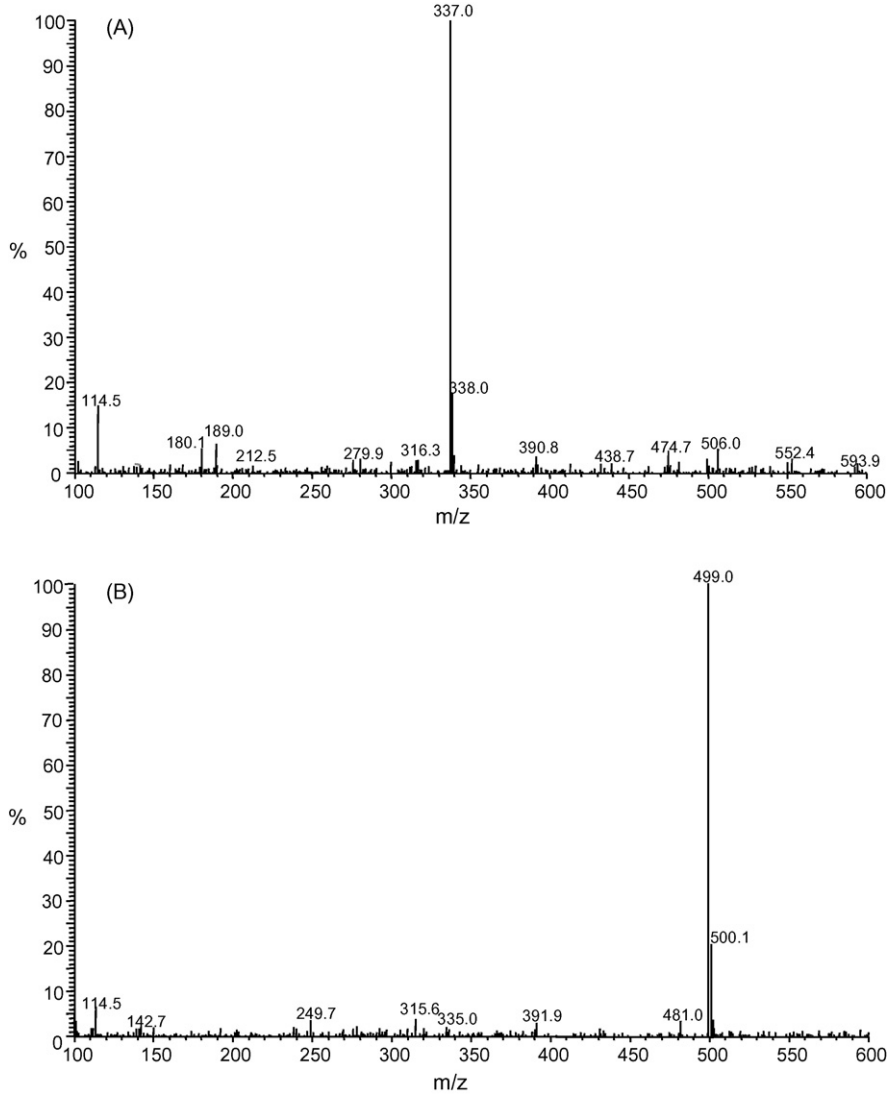


Fig. 2. Mass spectra of Arg-Fru (A) and Arg-Fru-Glc (B).

### 2.4. Standard preparation

Standard stock solutions of **1**, **2** were prepared in water at concentration level of 1 mg/mL, respectively, stored at 4 °C. Two standard stock solutions were mixed to make standard working solutions and serially diluted with water to obtain concentrations for calibration curve standards. *N*-acetylneuraminic acid was chose as an internal standard (I.S.) after several aminosugars were tested for recovery and interferences. Standard stock solution of *N*-acetylneuraminic acid (I.S.) was prepared at 1 mg/mL in water. This solution was diluted with water to get a concentration of 100 μg/mL. This solution was added to standard working solutions to a final concentration of 5 μg/mL.

### 2.5. Sample preparation

Eight different kinds of red ginseng products, commercially available in Korea were purchased from public market. About 20 mg of powder or paste samples were weighed and dissolved with 10 mL of water. The sample solutions were diluted with water to be within standard calibration range and *N*-acetylneuraminic acid (as I.S.) was added to the sample solutions with a final concentration of 5 μg/mL. After sonication for 15 min without any sample clean-up, the supernatant was filtered with 0.2 μm PVDF membrane filter and injected (25 μL) into the HPAEC–PAD.

Rat blood samples were collected into the heparinized tubes and were centrifuged at 3000 rpm for 5 min at 4 °C. Plasma obtained was stored at −20 °C until analysis. 10 μL of 100 μg/mL *N*-acetylneuraminic acid (as internal standard, final concentration of 5 μg/mL) was added to 100 μL of plasma sample. And then 90 μL of 10% (w/v) of TCA (trichloroacetic acid) was added to each sample for deproteinization. After vortexing for 5 min, the samples were centrifuged at 14,000 rpm, 4 °C for 10 min. The supernatant was separated and filtered through 0.2 μm PVDF membrane filter and 25 μL of the sample was injected into the HPAEC–PAD.

### 2.6. Assay validation

#### 2.6.1. Validation procedure for red ginseng extracts

The calibration curves (0.05–20 μg/mL) for **1** and **2** were constructed by plotting the peak area ratios of standard/I.S. against the concentrations. The precision and accuracy of the method were determined by analyzing **1** and **2** in red ginseng extract products spiked with four QC samples (0.2, 1, 10, 16 μg/mL). The spiked red ginseng samples were analyzed in replicate experiments ($n = 5$) for the intra-assay evaluations. The LOD was determined when the ratio of signal-to-noise was 3. The LOQ in red ginseng extract products was determined when signal-to-noise was 10.

#### 2.6.2. Validation procedure for rat plasma

The spiked samples in the rat plasma were prepared by adding and mixing 10 μL standard working solution with 490 μL of blank plasma. 10 μL of 100 μg/mL *N*-acetylneuraminic acid (I.S., final concentration of 5 μg/mL) was added to 100 μL of spiked plasma samples (containing **1** and **2**). The control plasma samples were spiked with **1** and **2** at five concentrations over range of 0.1–10 μg/mL. The LOD was determined when the ratio of signal-to-noise was 3 and the LOQ was determined when signal-to-noise was 10. The precision and accuracy in the plasma samples spiked with **1** and **2** were evaluated with four concentrations of 0.5, 1, 5, 10 μg/mL. The intra-day precision was determined within one day by analyzing five replicates, while the inter-day precision was assayed on five consecutive days. The intra-day and inter-day precision was defined as %R.S.D. and accuracy was determined by comparing the measured concentration with its theoretical value. The recovery of **1** and **2** from plasma was determined by spiking with the standards at 0.5, 1 and 5 μg/mL. The extraction recovery was assessed by comparing the peak area ratios obtained from three spiked plasma samples with the peak area ratios of the same amount of the analytes and internal standard directly dissolved in the water. Stability of three freeze–thaw cycles and room temperature in plasma for 4 h on the compound stability were checked by analyzing of QC samples at three concentrations.

### 2.7. Application of the assay

The developed method was applied for the pharmacokinetic study after oral administration of **1** and **2** to male Sprague-Dawley rats, weighing 220–250 g supplied by Sam-Tako, Inc. (Gyeonggi-do, Korea). Animal care and study protocol employed were in accordance with Institutional Ethics Committee for Animal Care and Use (IECACU) at Amorepacific R&D center. The rats were housed under controlled environmental conditions (temperature, 23 ± 1 °C, humidity, 60 ± 5%). Animals were fasted for 12 h prior to administration and received a single oral gavage of **1** or **2** (100 mg/kg in saline solution), respectively. Heparinized blood samples (300 μL) were collected from a caudal vein at various times over the following 24 h (0, 10, 30, 60, 120, 240, 420 and 1440 min) after dosing. Immediately after each collection, plasmas were separated from blood cells by centrifugation and 100 μL aliquots were stored at −20 °C until the analysis was performed. Three rats were used for the each time points. Pharmacokinetic data and parameters were calculated and prepared using MS Excel® and WinNonlin® Professional Edition ver 5.2 (Pharsight, Mountain View, CA).

## 3. Results and discussion

### 3.1. Protein precipitation solutions

Various solutions such as PCA (perchloric acid), TCA (trichloroacetic acid), methanol and acetonitrile were tested to get an optimal condition for deproteinization of rat plasma. Finally, PA (6%, v/v), TCA (10%, w/v) solution, CAN (3:1, v/v) and methanol (5:1, v/v) were compared for the efficiency of deproteinization. As a result, 10% (w/v) TCA solution was selected since it has the least interference from matrix endogenous compounds and displays the best Arg-Fru, Arg-Fru-Glc recovery from rat plasma at the same time.

Table 1
Detection limit, linear range for Arg-Fru (**1**), Arg-Fru-Glc (**2**)

| Compound | Detection limit (μg/mL) | Linear range (μg/mL) | $R^2$ value | %R.S.D. for peak area ($n = 5$) |
|---|---|---|---|---|
| Arg-Fru (**1**) | 0.020 | 0.05–20 | 0.9999 | 2.20 |
| In spiked plasma | 0.1 | 0.1–10 | 0.9982 | 2.54 |
| Arg-Fru-Glc (**2**) | 0.025 | 0.05–20 | 0.9998 | 1.98 |
| In spiked plasma | 0.1 | 0.1–10 | 0.9973 | 2.76 |

### 3.2. Chromatographic separation of Arg-Fru (*1*) and Arg-Fru-Glc (*2*)

Carbohydrate targeted HPAEC coupled with pulsed amperometric detection was selected for the analysis of two analytes. Utilizing weak acidic nature of carbohydrate, CarboPac PA1 column method was employed to separate these hexose-derived and di-hexose-derived compounds **1** and **2** selectively. *N*-acetylneuraminic acid, an amino sugar of which structure has amino and acid group, was selected for the internal standard. In search for the condition for the simultaneous determination and separation of these compounds from complex matrix interference of rat plasma and red ginseng extracts, sodium hydroxide solution and deionized water was chosen as mobile phase. In preliminary test, gradient condition using sodium acetate solutions and sodium hydroxide ranging from 100 to 500 mM was tried but it was found to be inappropriate for the complete resolution of **2** from endogenous compounds in rat plasma and internal standard, *N*-acetylneuraminic acid. To increase resolution, sodium hydroxide concentration and elution speed was optimized to gradient elution of 400 mM sodium hydroxide and water 90:10 at 0.7 mL/min. Without a need to add sodium acetate, a simple gradient condition using water and sodium hydroxide solution was enough to successfully separate both analytes and internal standard within 15 min. Use of 400 mM sodium hydroxide solution substantially improved the resolution and retention time. And it was very helpful in eliminating some interference present at high concentrations before another sample injection. In the first 15 min, isocratic elution of binary mixture of 90:10 was undertaken for 15 min and then, if necessary 15 min of isocratic elution was repeated for the column conditioning to remove remaining interferences and regenerate column before next sample injection. Integrated amperometry with positive potential was used to detect **1** and **2**. This detection method is a PAD (pulsed amperometric detection) type electrochemical detector where a gold electrode oxidizes sugar. With this detection method, we could determine two compounds simultaneously without any pre-treatment. Also, we have tested the long-term stability of PAD detector using standard solution of 5 μg/mL. %R.S.D. of area ($n = 50$) for **1** and **2** were 1.12 and 1.10, respectively. In the optimized chromatographic condition, we could completely separate **1** and **2** at 5.3 and 7.3 min, respectively. They could be separated within 15 min of chromatographic running time with good peak shapes (Fig. 3). Conspicuously, even though compound **1** has a fructose sugar moiety, which can be substantially interfered by various endogenous monosaccharides in plasma, **1** was completely separated from other monosaccharides through our method. Compound **2** and I.S. (*N*-acetylneuraminic acid) were also separated well from other disaccharides and endogenous compounds from sample matrix. In addition, target analytes were separated well from other carbohydrates of matrix of red ginseng extracts (Fig. 4). There was no interference with I.S. from the sample matrix.

In conclusion, this method showed good chromatographic separation in red ginseng extracts or rat plasma. Fig. 5 shows

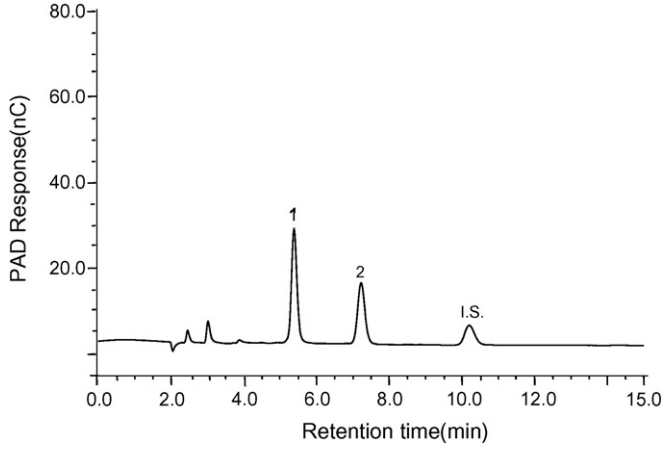


Fig. 3. HPAEC–PAD chromatogram obtained for a standard mixture of Arg-Fru (5 μg/mL), Arg-Fru-Glc (5 μg/mL) and I.S. (*N*-acetylneuraminic acid, 5 μg/mL). Chromatographic conditions: 400 mM NaOH/water = 90/10; flow rate of mobile phase: 0.7 mL/min, column temperature: 30 °C, injection volume: 25 μL. Peak: 1, Arg-Fru; 2, Arg-Fru-Glc; I.S., *N*-acetylneuraminic acid.

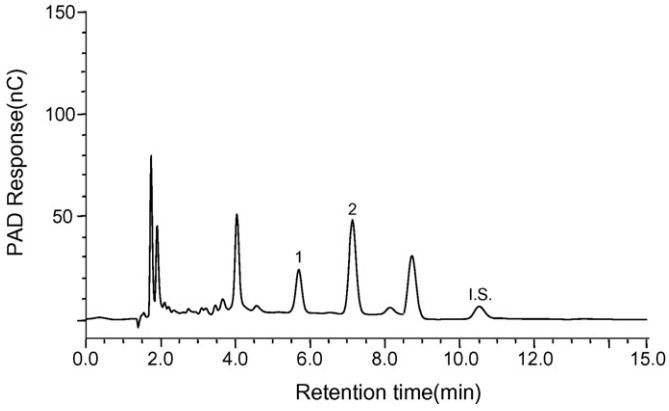


Fig. 4. Separation of (**1**) Arg-Fru, (**2**) Arg-Fru-Glc and internal standard (*N*-acetylneuraminic acid) in red ginseng product. Chromatographic conditions: 400 mM NaOH/water = 90/10. Other conditions are same as those in Fig. 3.

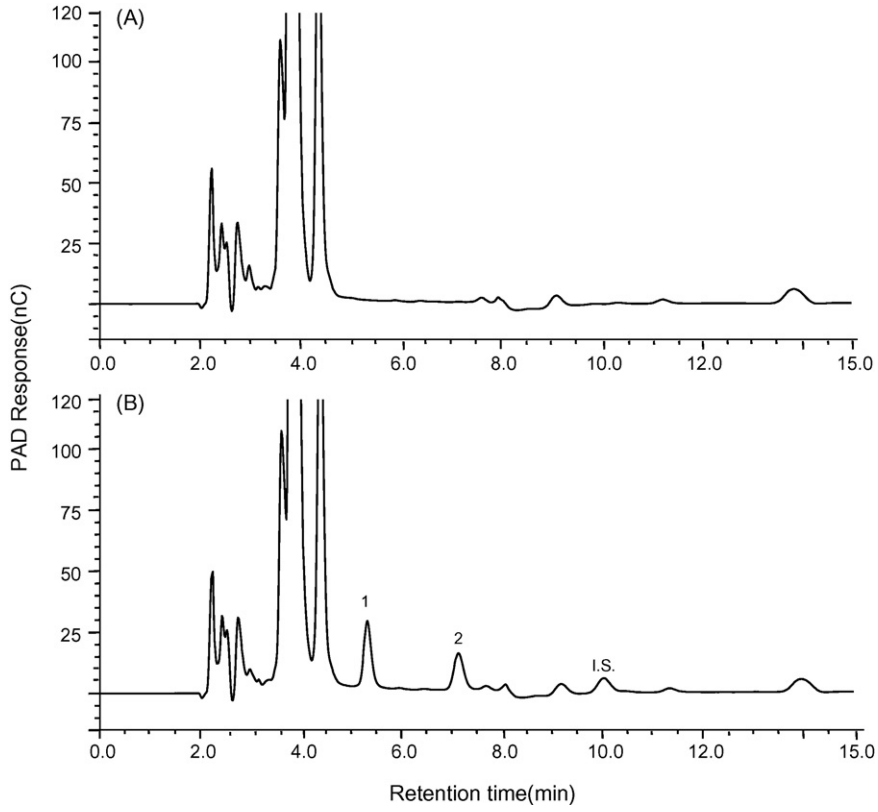


Fig. 5. HPAEC–PAD chromatograms of Arg-Fru and Arg-Fru-Glc from rat plasma. Chromatographic conditions: 400 mM NaOH/water = 90/10; flow rate of mobile phase: 0.7 mL/min, column temperature: 30 °C, injection volume: 25 μL. Blank plasma (A). Plasma sample spiked with I.S. (*N*-acetylneuraminic acid), Arg-Fru and Arg-Fru-Glc (B). Peak: 1, Arg-Fru (5 μg/mL); 2, Arg-Fru-Glc (5 μg/mL); I.S., *N*-acetylneuraminic acid (5 μg/mL).

the representative HPAEC chromatogram of **1** and **2** in rat plasma and the absence of any interference.

### 3.3. Linearity and detection limits

Standard calibration curve was obtained with the concentration range between 0.05 and 20 μg/mL. In Table 1, the result was summarized and %R.S.D. of peak area was calculated from 3 sets of standard solution. Linearity between the peak area and concentration of **1**, **2** was shown to be excellent in this concentration range and the correlation coefficients were more than 0.9998 for **1** and **2**. The detection limits were obtained to be 20 ng/mL for **1** and 25 ng/mL for **2** for standard solution, respectively. LOQ was measured to be 50 ng/mL for both **1** and **2**.

In spiked rat plasma samples, linearity between the peak area and concentration of **1**, **2** was shown to be good from 0.1 to 10 μg/mL and the correlation coefficients were more than 0.9973 for **1** and **2**. The detection limits were obtained to be 0.1 μg/mL for **1** and **2**. LOQ was measured to be 0.5 μg/mL for both **1** and **2**.

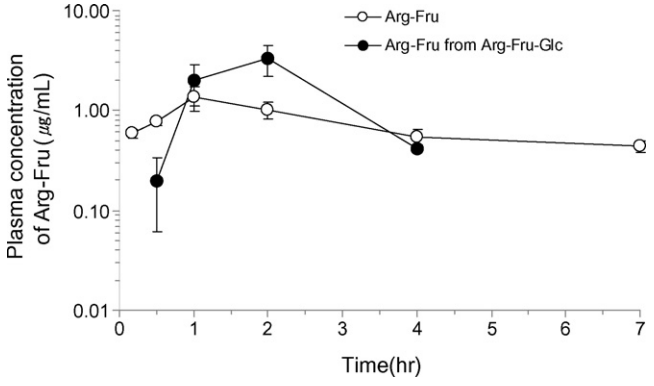


Fig. 6. Plasma concentration–time profile of Arg-Fru and Arg-Fru-Glc in rat plasma after oral administration.

### 3.4. Precision and accuracy

The accuracy for **1** and **2** were determined by adding the known amount of **1**, **2** standards to the commercial red ginseng products and the result was shown in Table 2. (The accuracy was calculated based on the difference of the total concentrations between the spiked samples and the original samples.) Mean accuracy was from 94.68 to 102.11% for **1** and 94.15 to 101.46% for **2**. Precision (as relative standard deviation, R.S.D.) was examined with four concentrations and result was from 0.51 to 2.68% for **1** and from 1.84 to 2.35% for **2**. LOQ of **1** and **2** were 0.1 μg/mL for red ginseng extract products.

Precision and accuracy of **1** from rat plasma was summarized in Table 3. When 4 concentrations (0.5, 1, 5 and 10 μg/mL) were assayed, intra-day precision for **1** was 1.37, 2.50, 2.87 and 2.80%, respectively, and accuracy was from 96.39 to 102.62%.

Table 2
Recovery and precision of Arg-Fru (**1**), Arg-Fru-Glc (**2**) in red ginseng extract product

| Compound | Added concentration (μg/mL) | Measured concentration (mean ± S.D.) (μg/mL) | Recovery (%) (mean ± S.D., $n = 5$) | Precision R.S.D. (%) |
|---|---|---|---|---|
| **1** | 16 | 16.34 ± 0.08 | 102.11 ± 0.52 | 0.51 |
| | 10 | 9.98 ± 0.13 | 99.76 ± 1.27 | 1.28 |
| | 1 | 1.02 ± 0.02 | 101.46 ± 1.47 | 1.44 |
| | 0.2 | 0.19 ± 0.01 | 94.68 ± 2.54 | 2.68 |
| **2** | 16 | 15.90 ± 0.37 | 99.40 ± 2.34 | 2.35 |
| | 10 | 10.06 ± 0.21 | 100.60 ± 2.12 | 2.11 |
| | 1 | 1.02 ± 0.02 | 101.46 ± 1.87 | 1.84 |
| | 0.2 | 0.19 ± 0.02 | 94.47 ± 2.50 | 2.65 |

Table 3
Recovery and precision of Arg-Fru (**1**) in rat plasma

| Measurement | Spiked concentration (μg/mL) | Measured concentration (mean ± S.D.) (μg/mL) | Recovery (mean ± S.D., $n = 5$) (%) | Precision R.S.D. (%) |
|---|---|---|---|---|
| Intra-day | 10 | 10.21 ± 0.14 | 102.01 ± 1.40 | 1.37 |
| | 5 | 4.97 ± 0.12 | 99.42 ± 2.48 | 2.50 |
| | 1 | 1.03 ± 0.03 | 102.62 ± 2.94 | 2.87 |
| | 0.5 | 0.48 ± 0.02 | 96.39 ± 2.70 | 2.80 |
| Inter-day | 10 | 10.24 ± 0.19 | 102.35 ± 1.85 | 1.81 |
| | 5 | 5.02 ± 0.13 | 100.30 ± 2.58 | 2.57 |
| | 1 | 1.02 ± 0.02 | 101.50 ± 2.33 | 2.30 |
| | 0.5 | 0.48 ± 0.02 | 95.52 ± 3.35 | 3.50 |

Table 4
Recovery and precision of Arg-Fru-Glc (**2**) in rat plasma

| Measurement | Spiked concentration (μg/mL) | Measured concentration (mean ± S.D.) (μg/mL) | Recovery (mean ± S.D., $n = 5$) (%) | Precision R.S.D. (%) |
|---|---|---|---|---|
| Intra-day | 10 | 9.88 ± 0.15 | 98.81 ± 1.49 | 1.51 |
| | 5 | 4.80 ± 0.10 | 96.06 ± 2.01 | 2.09 |
| | 1 | 1.02 ± 0.03 | 101.65 ± 3.36 | 3.30 |
| | 0.5 | 0.49 ± 0.02 | 97.58 ± 2.92 | 2.99 |
| Inter-day | 10 | 9.95 ± 0.19 | 99.53 ± 1.85 | 1.86 |
| | 5 | 4.94 ± 0.13 | 98.72 ± 2.51 | 2.54 |
| | 1 | 1.01 ± 0.03 | 100.77 ± 2.81 | 2.79 |
| | 0.5 | 0.48 ± 0.02 | 95.38 ± 3.68 | 3.85 |

Inter-day precision was tested for 5 days and the result was shown in Table 3. Inter-day precision ranged from 1.81 to 3.50% and accuracy was from 95.52 to 102.35% at examined concentrations. In case of **2** (Table 4), intra-day precision ranged from 1.51 to 3.30% and accuracy was from 96.06 to 101.65%. Inter-day precision was from 1.86 to 3.85% and accuracy ranged from 95.38 to 100.77%. These data show that the method we developed has satisfactory reproducibility and good analytical performance.

### *3.5. Determination of Arg-Fru (1) and Arg-Fru-Glc (2) in red ginseng extract products*

To test the applicability and the accuracy of the developed method, it was applied to the simultaneous analysis of **1** and **2** in 8 red ginseng products (or health foods), commercially available in Korean market. The quantitation of the analytes was performed using *N*-acetylneuraminic acid as internal standard which was not present in the red ginseng extract. **1** and **2** were completely resolved from sample matrix in most cases. Although **1** had a minor interference from adjacent peak in some samples, this method has produced good results.

Content of **1** in the tested samples ranged from 0.35 to 2.51% and **2** was determined to be from 0.25 to 2.64% (Table 5). This result indicated that the present method could be applied to the herbal extracts without any problem.

Table 5
Arg-Fru (**1**) and Arg-Fru-Glc (**2**) concentrations of red ginseng samples on the market

| Sample | **1** Conc. (%) | **2** Conc. (%) |
|---|---|---|
| G-1 | 0.95 | 2.64 |
| G-2 | 1.51 | 2.33 |
| JL | 2.51 | 1.32 |
| JG | 1.80 | 0.96 |
| LTJS | 1.62 | 1.05 |
| DH | 0.35 | 0.29 |
| NH | 1.57 | 0.25 |
| RGHK | 0.85 | 0.77 |

### 3.6. Application of the analytical method to assess time course of plasma concentrations of Arg-Fru and Arg-Fru-Glc after oral administration in rats

The described method was applied to the analysis of rat plasma after oral administration (100 mg/kg in saline) of **1** and **2**. Fig. 6 shows the plasma concentration profiles of **1** and Arg-Fru produced from **2** ($n = 3$). Arg-Fru-Glc (**2**) appeared to be changed to Arg-Fru (**1**) by separation of glucose during absorption and was not detected in plasma itself.

## 4. Conclusion

In this study, we established a new simple, rapid and sensitive high-performance anion-exchange chromatography method for the simultaneous determination of arginyl-fructose (**1**) and arginyl-fructosyl-glucose (**2**) in Korean red ginseng (*P. ginseng*) extracts and rat plasma. It is the first analytical study for the simultaneous determination of **1** and **2** using HPAEC–PAD method. With this method, two Amadori compounds produced during the preparation of steaming and heat-drying process of ginseng could be easily analyzed within 15 min of single chromatographic run, excluding any complex derivatization steps. This method was proved to give a good resolution, a high sensitivity and a wide applicability to the complex samples such as natural herbal extract products and biological fluids, which was successfully applied to the assessment of time course of plasma concentrations of Arg-Fru and Arg-Fru-Glc after oral administration in rats. We believe that the further *in vivo* pharmacokinetic/pharmacodynamic study or *in vitro* metabolism study for **1** and **2** would be feasible with this new method. In addition, a valuable insight has been provided for the analytical method development for other Amadori compounds.

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