Quantification of maltol in Korean ginseng (*Panax ginseng*) products by high-performance liquid chromatography-diode array detector

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ABSTRACT

Background: Maltol, as a type of phenolic compounds, is produced by the browning reaction during the high-temperature treatment of ginseng. Thus, maltol can be used as a marker for the quality control of various ginseng products manufactured by high-temperature treatment including red ginseng. For the quantification of maltol in Korean ginseng products, an effective high-performance liquid chromatography-diode array detector (HPLC-DAD) method was developed. **Materials and Methods:** The HPLC-DAD method for maltol quantification coupled with a liquid-liquid extraction (LLE) method was developed and validated in terms of linearity, precision, and accuracy. An HPLC separation was performed on a C18 column. **Results:** The LLE methods and HPLC running conditions for maltol quantification were optimized. The calibration curve of the maltol exhibited good linearity (R^2 = 1.00). The limit of detection value of maltol was 0.26 μg/mL, and the limit of quantification value was 0.79 μg/mL. The relative standard deviations (RSDs) of the data of the intra- and inter-day experiments were <1.27% and 0.61%, respectively. The results of the recovery test were 101.35–101.75% with an RSD value of 0.21–1.65%. The developed method was applied successfully to quantify the maltol in three ginseng products manufactured by different methods. **Conclusion:** The results of validation demonstrated that the proposed HPLC-DAD method was useful for the quantification of maltol in various ginseng products.

Key words: High-performance liquid chromatography-diode array detector, maltol, *Panax ginseng,* quantification, validation

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INTRODUCTION

Korean ginseng (*Panax ginseng* Meyer), a traditional herbal medicine, belongs to the family Araliaceae within the genus *Panax*.[1] It is mainly cultivated in Korea, China, and Eastern Siberia. Currently, ginseng products in the market can be largely classified as fresh ginseng and processed products, dried ginseng (DG) and red ginseng (RG).[2] DG is manufactured by simple drying process of fresh ginseng, but RG is manufactured by steaming and drying process of fresh ginseng.[3] Various Korean ginseng products have been reported to have many beneficial effects such as anti-tumor, anti-diabetic, immunostimulatory, anti-oxidant, and protection against gastric damage.[4-7] The major bioactive substances of ginseng are ginsenosides, polyacetylenes, phenolic compounds, alkaloids, acidic polysaccharides, and amino acids.[8]

Among major bioactive substances of ginseng, maltol, which is type of phenolic compounds, exists in a very small amount in fresh ginseng or DG but in high concentrations in RG.[9] Maltol has been reported to have anti-oxidative effects and to inhibit aging-related lipid peroxidation.[10] Maltol is produced by the browning reaction during the high-temperature treatment of ginseng. Therefore, maltol tends to increase in ginseng products processed by high-temperature and high-pressure (HTHP), including RG processed by hot steam.[11] Thus, maltol can be used as a marker compound for the quality control of various ginseng products manufactured by high-temperature treatment including RG. Several analytical methods for the detection of maltol in ginseng have been reported to date. Selective ion monitoring mode for gas chromatography/mass spectrometry was used for

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maltol analysis in RG.[11] The most common method for the identification and quantification of maltol in RG involves a high-performance liquid chromatography (HPLC) system combined with diode array detector (DAD).[12,13] In case of HTHP-RG, however, HTHP process resulted in various unidentified compounds production. Therefore, it is very hard to separate and quantify maltol exactly in HTHP-RG.

This study was conducted to develop improved and validated HPLC-DAD method for the quantification of maltol in various ginseng products, especially in HTHP ginseng product.

MATERIALS AND METHODS

Materials and chemicals

A 4-year-old fresh *P. ginseng* was obtained from a ginseng market in Korea (Seoul, Korea). HPLC-grade water, acetonitrile, and methanol were obtained from J. T. Baker (Phillipsburg, NJ, USA), and other solvents and reagents of analytical grade were procured. Maltol standard was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of ginseng products

All of the ginseng products were prepared after washing and removing the rootlets of fresh ginseng. The DG was prepared by hot air-drying at 50°C for 5 days. The RG was prepared by steaming the ginseng at 95°C for 3 h, followed by hot air-drying at 50°C for 48 h. HTHP-RG was made by drying at 50°C for 36 h followed by autoclaving at 140°C (3 kg/cm^2) for 20 min, and then hot air-drying at 50°C. Ginseng products prepared by different manufacturing methods (DG, RG, and HTHP-RG) are presented in Figure 1. The moisture content of the final products was reduced to <15%, which is within the inspection criterion that is defined in the domestic ginseng-related regulations.[14]

Sample preparation for high-performance liquid chromatography analysis

Direct extraction

One gram of HTHP-RG powder was extracted using 20 mL of 80% methanol at 80°C for 1 h. The samples were re-extracted by the same method and then filtered with an 8 μm filter paper (Whatman, Maidstone, UK) for vacuum concentration. After the sample was completely concentrated, it was dissolved in 5 mL of HPLC methanol and filtered using a 0.2 μm polyvinyl difluoride (PVDF) syringe filter (Waters, Milford, MA, USA) for HPLC analysis.

Figure 1: Ginseng products prepared by different preparation methods. Dried ginseng (a), red ginseng (RG) (b), and high-temperature and high-pressure-RG (c)

Liquid-liquid extraction

Dried ginseng, RG, and HTHP-RG powder (1 g each) were extracted using 20 mL of 80% methanol at 80°C for 1 h. The samples were re-extracted by the same method and then filtered with an 8 μm filter paper (Whatman) for vacuum concentration. After the samples were concentrated until only the water was left, 10 mL of water and 10 mL of ethyl acetate were added. The samples were then separated as layers, and the supernatant (ethyl acetate layer) was decanted into the evaporation flask. Another 10 mL of ethyl acetate was added to the water layer, after which the extraction was repeated. The ethyl acetate layer from the two extractions was then combined and subjected to vacuum concentration. The DG extract was dissolved in 5 mL of HPLC methanol and filtered using a 0.2 μm PVDF syringe filter (Waters) for HPLC analysis.

Preparation of standard stock solution

Standard stock solutions of maltol were prepared by dissolving 1 mg of maltol standard in 1 mL methanol. The analytical working solutions were prepared by diluting this stock solution with methanol. These working solutions were used for the calibration curves and validation of the proposed method. The stock solutions were stored at 4°C.

High-performance liquid chromatography analysis

Analysis of the maltol was conducted in a Jasco (Tokyo, Japan) HPLC system with PU-2089 Plus gradient pump equipped with a degasser, an AS-2075 Plus autosampler, and a MD-2010 Plus DAD. Data were collected with the Jasco Chrompass Software. Comparative analysis was carried out using a SunFire (Waters) C18 column (particle size: 5 μm, id: 4.6 mm, length: 250 mm). The mobile phase consisting of eluent of A (2% acetic acid in water) and B (0.5% acetic acid in acetonitrile) was run at 1.2 mL/min. The linear gradient elution program was set as follows: 100% A at 0–20 min, 100–97% A at 20–24 min, and 10% A at 24–30 min. The eluted maltol was detected at 274 nm. The injection volume was 10 μL, and the column temperature was maintained at 40°C.

Method validation

The HPLC method was validated in terms of linearity, precision, and accuracy according to the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use.[15,16]

Linearity, limit of detection and limit of quantification

To obtain a calibration curve for the linearity evaluation, the maltol standard material was diluted stepwise to five concentrations using methanol, and HPLC analysis was performed 12 times. A linear regression equation (y = ax + b; a = slope, b = y-intercept, x = sample concentration, y = peak area) was calculated, and linearity was confirmed through the correlation coefficient (R^2).

The limit of detection (LOD) and limit of quantification (LOQ) were measured to examine the lowest concentration of analytic samples for possible detection and quantification. The LOD and LOQ were calculated through the calibration curve and measured at signal to noise ratio of 3 and 10, respectively.

Precision

The precision of the method was evaluated by repetitive testing, intra-day and inter-day. The intra-day variability test was performed to check the degree of changes in results caused by a varying experimental environment in the same sample. Intra-day variability was evaluated by obtaining the relative standard deviation (RSD) through five repetitive measurements of samples with three different concentrations within the concentration with confirmed linearity. The inter-day variability was evaluated by calculating the RSD through five repetitive experiments and by changing the experiment dates-on the 1st, 3rd, and 5th day-based on three different concentrations within the concentration with confirmed linearity. The RSD was taken as the measurement of precision: RSD (%) = (standard deviation/mean

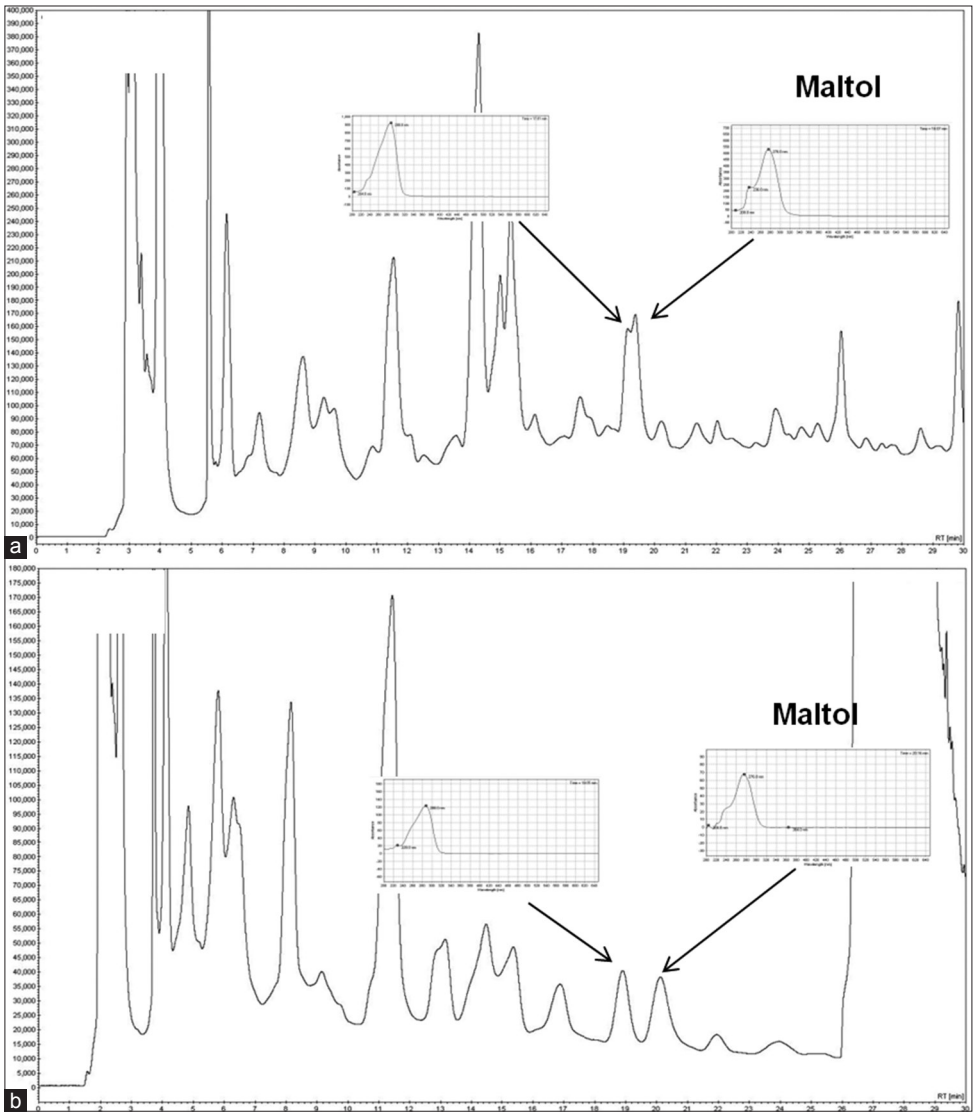

Figure 2: High-performance liquid chromatography (HPLC) chromatograms of high-temperature and high-pressure red ginseng sample assayed by the previous HPLC method (a), and the modified HPLC method for maltol analysis (b). An HPLC separation was performed on a C18 column

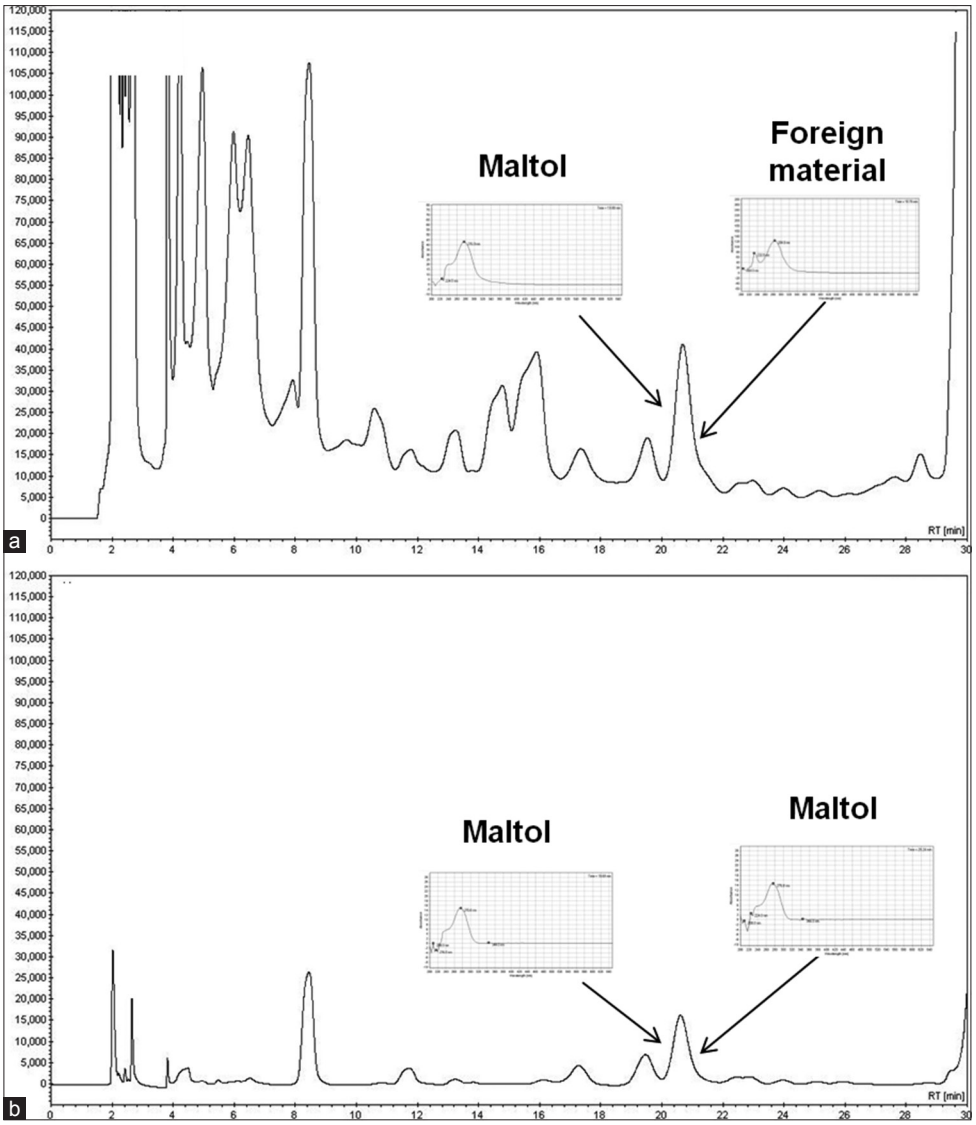

Figure 3: High-performance liquid chromatography (HPLC) elution profiles obtained using two different sample preparation methods. High-temperature and high-pressure red ginseng sample for HPLC analysis was prepared by direct extraction method (a), and liquid-liquid extraction method (b). An HPLC separation was performed on a C18 column

Table 1: Calibration curve, LOD, LOQ of maltol

Compound	Linear range (μg/mL)	Regressione quation[a]	R^2 (n=12)	LOD[b] (μg/mL)	LOQ[b] (μg/mL)
Maltol	0.39-100.00	y=558.1x-81.5	1.00	0.26	0.79

[a]y: Peak area; x: Concentration (μg/mL); [b]Values were calculated using intra-day (n=12) analyses. LOD: Limit of detection; LOQ: Limit of quantification

measured amount) ×100. The appropriate RSD value for precision is within 3%.

Accuracy

To evaluate the accuracy, the DG samples with known concentration were spiked with different concentrations of maltol standard solution and subsequently extracted, and the recovery was calculated. The recovery of the added standard was calculated by the following equation: Recovery (%) = (amount found – original amount)/amount spiked × 100. The ideal range of recovery was 90–110%.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22.0 (SPSS Inc., Chicago, IL, USA). Statistical comparison was performed using a One-way analysis of variance test followed by Duncan's multiple range tests. $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Optimization of high-performance liquid chromatography condition

For the analysis of maltol in HTHP-RG, an HTHP-RG extract was analyzed by the previous HPLC method

Table 2: Precision data of maltol

Compound	Concentration (μg/mL)	Intra-day			Inter-day		
		Mean±SD (μg/mL)	RSD (%)	Accuracy[a] (%)	Mean±SD (μg/mL)	RSD (%)	Accuracy[b] (%)
Maltol	25.00	25.00±0.32	1.27	99.99	24.98±0.02	0.08	99.92
	12.50	12.51±0.09	0.72	100.04	12.56±0.06	0.45	100.46
	6.25	6.25±0.03	0.49	99.93	6.21±0.04	0.61	99.38

[a]Values were calculated using intra-day (n=5) analyses; [b]Values were calculated using inter-day (n=3) analyses. SD: Standard deviation; RSD: Relative standard deviation

Table 3: Recovery test of maltol[a]

Compound	Spiked concentration (μg/g)	Measured concentration (μg/g)	RSD (%)	Recovery[b] (%)
Maltol	25.00	25.44±0.05	0.21	101.75
	12.50	12.67±0.21	1.65	101.35
	6.25	6.34±0.05	0.84	101.47

[a]Recovery test was conducted by adding three different concentrations of standard solution to a known quantity of DG sample; [b]Values were calculated using intra-day (n=5) analyses. DG: Dried ginseng; RSD: Relative standard deviation

Table 4: Maltol content in different ginseng products

	Content (μg/g)[a]
DG	0.50±0.04
RG	8.00±0.08
HTHP-RG	32.82±1.24

[a]Values represent the mean of the intra-day (n=3) analyses±SD. SD: Standard deviation; DG: dried ginseng; RG: Red ginseng; HTHP-RG: High-temperature and high-pressure red ginseng

developed for the analysis of maltol in RG.[13] As exhibited in Figure 2a, maltol (Retention time: 19.4 min) was not completely separated from the previous peak (19.1 min). To obtain chromatograms with good separation of maltol in HTHP-RG, we modified the previous HPLC method in terms of the elution condition and flow rate. As exhibited in Figure 2b, the maltol (20.1 min) peak was well separated from the peak that appeared at 18.8 min. This result suggests that the developed HPLC-DAD method separates maltol without any interference.

Optimization of the extraction method

The chromatograms obtained by HPLC using the two different sample preparation methods under the selected chromatographic conditions are presented in Figure 3. As displayed in the results, foreign material was confirmed to have been mixed in the maltol peak in the direct extraction (DE) method [Figure 3a], but such foreign material was eliminated in the liquid-liquid extraction (LLE) method [Figure 3b]. The DE method is considered to be a simple, less time-consuming method than LLE.[17] However, this co-extraction with other components of the matrix can occur during the DE process, thus lowering its selectivity.[18] As such, this study decided to use the LLE method as an extraction method for maltol analysis.

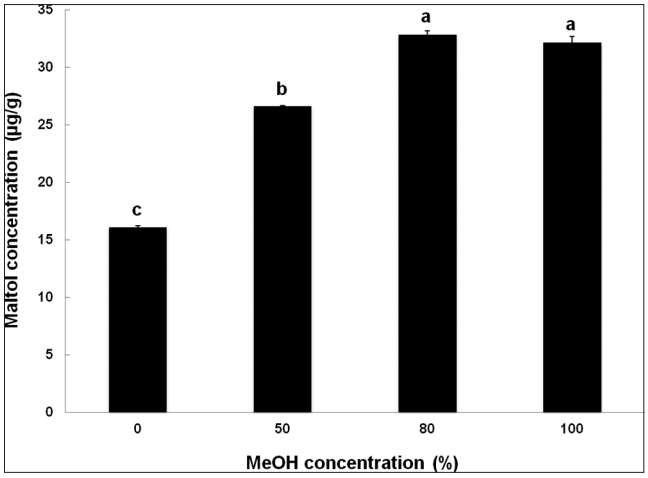

Figure 4: Extraction efficiencies of maltol as a function of extraction solvent concentration in high-temperature and high-pressure red ginseng (HTHP-RG) sample. HTHP-RG sample for high-performance liquid chromatography (HPLC) analysis was prepared by liquid-liquid extraction method. An HPLC separation was performed on a C18 column. Values represent the mean of the intra-day (n = 3) analyses ± standard deviation. Different letters indicate significant differences (P < 0.05)

The experiments were performed using various concentrations of methanol (0, 50, 80, and 100%) for the optimization of extraction solvent. The results indicated that 80% methanol extraction demonstrated the highest maltol content [Figure 4]. Thus, 80% methanol was selected for the optimal solvent for LLE extraction.

Method validation

Linearity, limit of detection, and limit of quantification

The stock solution of the maltol was diluted with methanol to come up with five concentrations. To establish the calibration curve, standard solutions of five different concentrations were analyzed 12 times. The correlation coefficient values of the maltol exhibited good linearity (R^2 = 1.00). The LOD value of the maltol was 0.26 μg/mL, and its LOQ value was 0.79 μg/mL [Table 1].

Precision

The RSD values of the intra-day and inter-day tests for maltol were 0.49–1.27% and 0.08–0.61%, respectively, with accuracy ranges of 99.93–100.04% for the intra-day test and 99.38–100.46% for the inter-day test [Table 2].

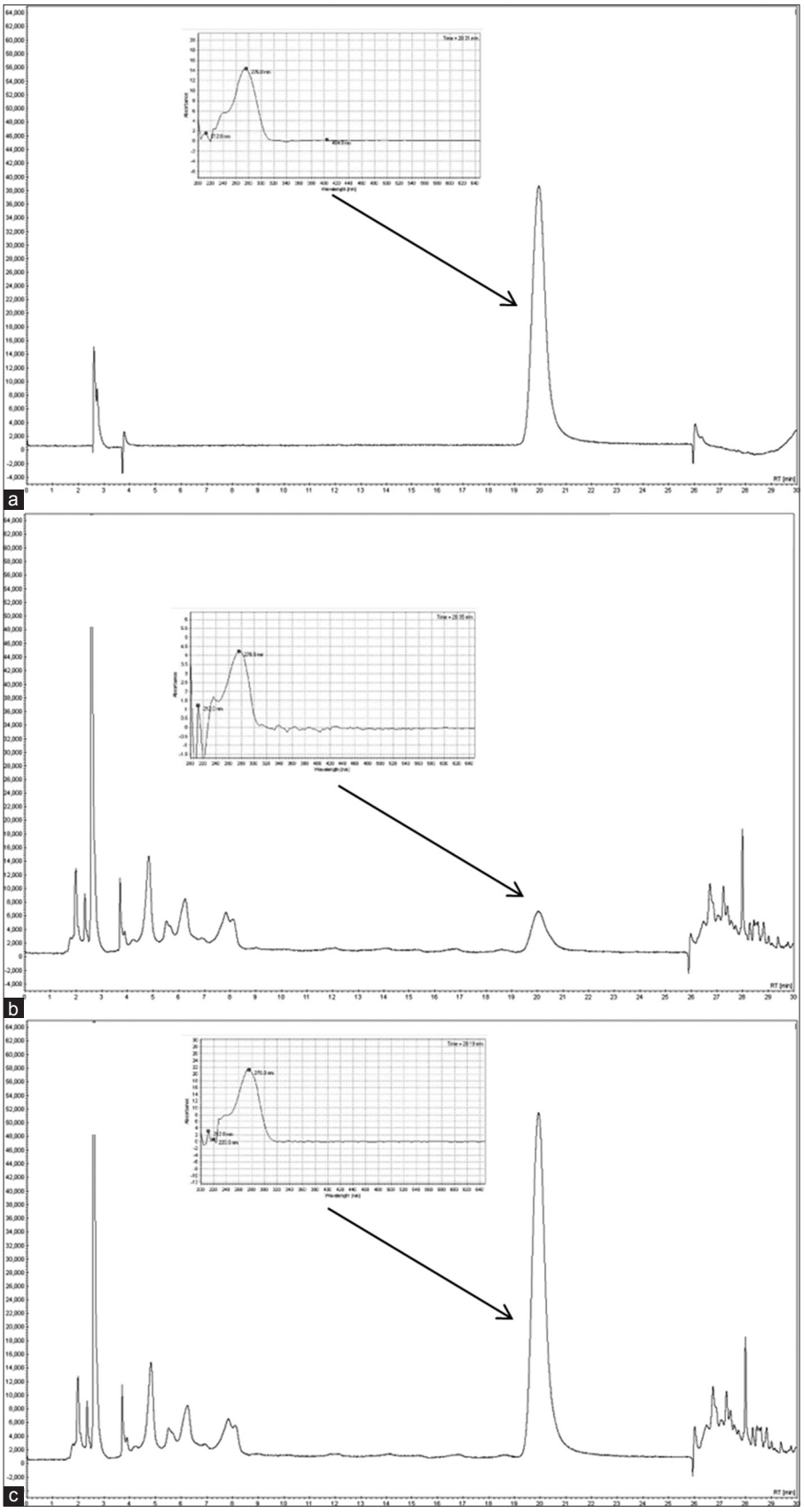

Figure 5: High-performance liquid chromatography (HPLC) chromatograms for the determination of maltol in dried ginseng (DG) sample. Standard solution (a), blank DG sample (b), and spiked DG sample (c). Blank DG and spiked DG samples for HPLC analysis were prepared by liquid-liquid extraction method. An HPLC separation was performed on a C18 column

These results indicate that this method demonstrates good precision.

Accuracy

In the results of the recovery rate, maltol ranged from 101.35% to 101.75%, RSD 0.21–1.65%. Based on the results of the recovery test, the analysis method used for the maltol of the ginseng sample exhibited excellent accuracy [Table 3]. Figure 4 presents the difference in the chromatograms between the standard [Figure 5a], blank DG sample [Figure 5b], and spiked DG sample [Figure 5c].

Analysis of maltol in various ginseng products

The three ginseng products prepared by different manufacturing methods were analyzed using the developed HPLC-DAD method. The maltol content was calculated from the calibration curve of the standards. The amounts of maltol in the three ginseng products are displayed in Table 4. The maltol contents in the assayed samples were found to be significantly different. In particular, the HTHP-RG exhibited the highest maltol content. This result suggests that the amount of maltol in ginseng was influenced by the HTHP treatment. Maltol is formed by thermal degradation of starch or pyrolysis of sucrose.[11] During the heating process of ginseng, amino acid compounds take browning reaction with maltose to produce 4-O-α-D-glucosyl-1-deo xy-2,3-diketosaccharide. Since this compound is unstable, 2-ketone group and C-6-hydroxyl group condensate to be glycoside B. After that, glycoside B takes further hydrolysis of glucose and rearrangement to be maltol.[19] Therefore, it was considered that extensive heating treatment for HTHP-RG may accelerate the increase of maltol than DG or RG. This was consistent with the finding in the previous study wherein the changes in the maltol content in ginseng treated by HTHP (puffed RG) were analyzed using a gas chromatography-mass selective detector. In that study, the maltol content of HTHP-RG was higher compared to that of the general RG.[11]

CONCLUSION

Health functional foods based on ginseng contain various active compounds, and these active compounds compositions are changed by processing methods. Thus, it is important to conduct regular quality control to show its physiological efficacy and effects in health functional foods consistently.[20] In this study, we developed the HPLC-DAD method for the detection of maltol in various ginseng products. Optimization of the extraction method and HPLC conditions was performed. Validation of the method was accomplished in terms of linearity, precision, and accuracy. The results of the validation demonstrated that this method offers good linearity, precision, and accuracy. The developed assay method was applied successfully to quantify the maltol in three ginseng products manufactured by different methods. Thus, this analytical method is considered to be usable when maltol is used as a marker for the quality control of various ginseng products such as RG manufactured under the HTHP treatment condition. It may also be used in studies on the correlation between the maltol content in ginseng products and physiological activities.

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