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Research article

Quantitative aspects of the hydrolysis of ginseng saponins: Application in HPLC-MS analysis of herbal products

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ABSTRACT

Background: Ginseng is one of the most valuable herbal supplements. It is challenging to perform quality control of ginseng products due to the diversity of bioactive saponins in their composition. Acid or alkaline hydrolysis is often used for the structural elucidation of these saponins and sugars in their side chains. Complete transformation of the original ginsenosides into their aglycones during the hydrolysis is one of the ways to determine a total saponin group content. The main hurdle of this approach is the formation of various by-products that was reported by many authors.

Methods: Separate HPLC assessment of the total protopanaxadiol, protopanaxatriol and ocotillol ginsenoside contents is a viable alternative to the determination of characteristic biomarkers of these saponin groups, such as ginsenoside Rf and pseudoginsenoside F_{11}, which are commonly used for authentication of *P. ginseng* Meyer and *P. quinquefolius* L. samples respectively. Moreover, total ginsenoside content is an ideal aggregated parameter for standardization and quality control of ginseng-based medicines, because it can be directly applied for saponin dosage calculation.

Results: Different hydrolysis conditions were tested to develop accurate quantification method for the elucidation of total ginsenoside contents in herbal products. Linearity, limits of quantification, limits of detection, accuracy and precision were evaluated for the developed HPLC-MS method.

Conclusion: Alkaline hydrolysis results in fewer by-products than sugar elimination in acidic conditions. An equimolar response, as a key parameter for quantification, was established for several major ginsenosides. The developed approach has shown acceptable results in the analysis of several different herbal products.

1. Introduction

Ginseng is one of the most revered medicinal plant in traditional oriental medicine [1]. Different medicinal applications of ginseng, including anti-Alzheimer disease activity [2], anticancer properties [3] and treatment of the skin diseases, e.g. atopic dermatitis [4], are often associated with its active components – ginsenosides. The ginsenoside molecule consists of two parts – the scaffold (sapogenin) and the sugar side chain(s). Currently, more than 600 ginsenosides have been tentatively characterized, and the most common sapogenins are ocotillol (OT), protopanaxadiol (PPD), and protopanaxatriol (PPT) [5]. For quality control of raw and processed ginseng roots via quantification of several major ginsenosides as quality markers, fast, cheap and reliable high-performance chromatography (HPLC) methods with UV [6] and evaporative light scattering detection (ELSD) [7] have been introduced. HPLC with a mass spectrometric (MS) detection can be employed for the determination of a higher number of co-eluting ginsenosides [8–10]. To elucidate the type and authenticity of ginseng materials either targeted determination of several specific markers, such as 24(R)-pseudoginsenoside F_{11} for *P. quinquefolius* and ginsenoside Rf for *P. ginseng* [10], or multi-targeted ginsenoside profiling followed by chemometric data analysis [6–9] can be conducted. Another way to perform quality control of ginseng containing products is to calculate the total ginsenoside content. Direct HPLC determination of all saponins is unattainable task, because it is nearly impossible

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to obtain all the individual standards of ginsenosides due to the huge chemical diversity of these compounds. Moreover, one also has to elucidate the presence of 'acidic' acetyl- and malonyl-ginsenosides [8,11,12]. As a solution to these issues, at the first step hydrolysis may be conducted to transform the original ginsenosides, including the acidic ones, into corresponding sapogenins [13–15]. Next, quantification of the separated sapogenins can be performed to estimate total saponin group contents.

There are three most commonly used ways to obtain ginsenoside sapogenins: acid hydrolysis, alkaline hydrolysis, and enzymatic hydrolysis. Acid hydrolysis of ginsenosides is usually conducted in aqueous environment (1–6M hydrochloric acid in water or a mixture of dioxane-water) at temperatures of 80–120°C for several hours. In case of PPD and PPT aglycones, the presence of a hydroxyl group at the tertiary C-20(S) carbon atom may cause epimerization during acid hydrolysis. The presence of by-products, namely 20(R)-protopanaxadiol and 25-hydroxy-20(S)-protopanaxadiol, after acid hydrolysis of the saponin fraction separated from ginseng extracts has been reported [14]. Earlier, a GC-MS method for quantitative determination of PPD, PPT, and oleanolic acid aglycones after oxidative sugar chain cleavage during alkaline hydrolysis was developed and tested using standards of ginsenosides Rg_1, Re, Rd, Rb_1, Rc, and Ro [13]. Currently, the only limitation of such GC methods is the complexity of sample preparation and derivatization. Enzymatic hydrolysis is often used to increase ginsenoside contents in the extract [16] and leads to the formation of less polar ginsenosides, such as Rg_2, F_1, and Rh_1 [17].

It is evident that in order to measure the total ginsenoside content, ginsenosides of different types should be quantitatively converted into their corresponding aglycones. Therefore, a hydrolysis should be conducted with constant and high yield of target sapogenin(s) as well as with formation of a minimum possible number of by-products, including intermediate less polar ginsenosides. Complete transformation of the ginsenosides was not achieved in the previous studies at different pH conditions [14,15]. The goal of our research was to develop a method for elucidation the total saponin content in ginseng based herbal products via preliminary sugar chain elimination and direct HPLC-MS quantification of the sapogenins in the hydrolysate solution.

2. Materials and methods

2.1. Materials

Pseudoginsenosides RT_5, F_{11} and ginsenosides Re, Rg_1, Rh_1, Rf, Rd, Rb_1, Rb_3, standards (>98%) together with the sapogenins panaxatriol (PT), panaxadiol (PD), protopanaxatriol (PPT), protopanaxadiol (PPD) standards (>98%) were purchased from PhytoLab GmbH (Germany). HPLC-grade methanol was obtained from Burdick & Jackson (Muskegon, Ml, USA), HPLC-grade acetonitrile was purchased from Panreac (Barcelona, Spain). HPLC grade 2-propanol, pyridine, 1-butanol 99.8%, dimethyl sulfoxide (DMSO) ≥99.9%, formic acid 99.9+%, sodium methoxide (MeONa) 95% and potassium hydroxide (KOH) 99.99% were obtained from Sigma-Aldrich (Steinheim, Germany). The Milli-Q system water purification system (Watford, USA) was used.

2.2. Preparation of standards

The individual stock solutions of 9 ginsenosides and 4 sapogenins were prepared by dissolving approximately 2 mg of their standards in 2 mL methanol, and then sonicated for 1 minute. Solutions were stored at −30°C until analysis. By diluting the stock solutions with methanol, several working solutions containing appropriate amounts of ginsenosides Rg_1 (PPT-type), Rb_1 (PPD-type) and F_{11} (OT-type) were prepared. Calibration solutions were prepared in concentrations of 20, 50, 100, 250, 500, 750, 1000, 2000, 5000 ng/mL.

2.3. Sample preparation

Small cultivated American ginseng (100%, 5 years old, WI, USA) dry root slices were from Leiyunshang Pharmaceutical Co., Ltd. (Shanghai, China). A sample of ginseng food supplement (KRKA (Novo mesto, Slovenia) was purchased in the local pharmacy. Commercially available Korean red ginseng Sliced Gold (100%, 6 years old, red ginseng root slices immersed in honey syrup) and Korean ginseng tea purchased from Korean Ginseng MFG., Ltd. (Pyeongtaek, South Korea) were used. At the first step, root samples were crushed to approximately 2 mm long particles using a FP2200 laboratory blender (Waring, USA). Then a 0.2 g of the particles were immersed in a 15 mL vial with 5 mL of 70% aqueous methanol solution and incubated for 10 min. The mixture was then sonicated (280 W, 35 kHz) for 40 min in an ultrasonic bath (SAPHIRE; Saphire Ltd., Russia). The residue was separated after 10 min of centrifugation at 4000 rpm. After that, 5 mL of fresh solvent was added and the extraction was repeated. Prior to hydrolysis, the extracts were diluted with methanol 500 times (American ginseng dry roots), 50 times (Korean red ginseng), 80 times (Korean ginseng tea), 2000 times (ginseng-based food supplement). Then an aliquot of 100 μL was 10 times diluted with deionized water and applied to an Oasis Hydrophilic-Lipophilic Balance (HLB) cartridge (1 mL, 30 mg, Waters, USA). Highly polar constituents were removed by washing with 2 × 0.5 mL of 5% aqueous methanol, then saponins were eluted by pure methanol.

2.4. Acid hydrolysis conditions

An aliquot of 100 μL of each individual ginsenoside (Rg_1, Rb_1, RT_5) standard solution (5 μg/mL) in methanol was added to 200 μL of 0.12M and 1.2M hydrochloric acid in methanol. The samples were heated at 80°C for varying time intervals (0.5 − 3 hours), then cooled to room temperature. Then solvent was evaporated to dryness under a stream of nitrogen. Then the residue was re-dissolved in 750 μL of deionized water. Saponin fraction was extracted with 750 μL of chloroform under stirring on an orbital shaker for 10 min. Organic layer was separated after centrifugation (10 min, 4000 rpm), evaporated to dryness under a gentle stream of nitrogen and re-suspended in 100 μL of deionized water/acetonitrile/formic acid (95:5:0.1, v/v/v).

2.5. Alkaline hydrolysis and clean-up procedure

The extracts and the aliquots of 100 μl of individual ginsenosides Rg_1, Rf, Rh_1, Re, Rb_1, Rd, Rb_3, RT_5, F_{11} (5 μg/mL) were dried under a stream of nitrogen, then CH_3ONa (1.5, 3, or 12 mg) and 3 mL of solvent (methanol, acetonitrile, DMSO, pyridine) were added. Alternatively, the hydrolysis was held in 3 mL of 5% KOH aqueous solution. The samples were heated for 3 hours (or 24 hours for KOH) and cooled to the room temperature. Then solvent was evaporated under a gentle stream of nitrogen. Dry residue was re-dissolved in 3 mL of 5% aqueous methanol and the aqueous layer was neutralized with 1% aqueous formic acid.

Solid-phase extraction (SPE) was used to purify the hydrolysis products. Neutralized solution (pH = 7.0) was applied to an Oasis HLB cartridge (30 mg, 1 mL) preconditioned with 1 mL of methanol and 1 mL of deionized water. The cartridge was washed with 0.5 mL of a 5% aqueous methanol, then target compounds were eluted with two portions (0.5 mL) of pure methanol. The eluate was dried

under the stream of nitrogen and re-dissolved in 0.1 mL of 5% aqueous methanol.

2.6. HPLC-MS conditions

For the HPLC analysis, a Dionex Ultimate 3000 system (Thermo Scientific, USA) was used. A Hypersil Gold aQ column (150 mm × 2.1 mm, 3 μm, Thermo Scientific, USA) with a Hypersil Gold aQ guard column (10 × 2.1 mm, Thermo Scientific, USA) at a flow rate of 0.5 mL/min at 30°C was employed. Sample injection volume was 3 μL. The eluent consisted of deionized water/acetonitrile (95/5, v/v) with 0.1% formic acid (mobile phase A) and HPLC-grade acetonitrile with 0.1% formic acid (mobile phase B). The elution was performed in the gradient conditions. The gradient program started with 5% B and held for 2 min. Then phase B increased linearly to 95% during 13 minutes and kept constant for 3 minutes. After that the initial level of 5% was reached in 1 minute and the system equilibrated for 4 minutes prior to the subsequent chromatographic run. The mass spectra were acquired using a Q Exactive Orbitrap (Thermo Scientific, USA) equipped with heated electrospray ionization probe (HESI II). Ion source parameters were set as follows: capillary temperature, 270°C; aux gas heater temperature, 280°C; sheath gas (N_2) flow rate, 40 arbitrary units (a.u.); auxiliary gas (N_2) flow rate, 10 a.u.; sweep gas (N_2) flow rate, 5 a.u; ion spray voltage, 4.0 kV. The instrument operated in full spectrum acquisition mode from 90 to 1350 m/z, with a resolution of 35,000 at 400 m/z. Mass spectra were handled in the XCalibur 2.2 software (Thermo Scientific). For the selective detection of saponins and the corresponding sapogenins three intensive ion signals were summed [18,19] (m/z 405.3518, 423.3623, 441.3729 for PPT and PT; m/z 407.3674, 425.3781, 443.3881 for PPD and PD; m/z 421.3645, 439.3717, 457.3912 for OT). To calculate the molar responses the following equation was used:

$$Response\left(\frac{cps}{nmol\ mL^{-1}}\right) = \frac{S \times M}{C \times 1000} \quad (1)$$

where C is the concentration of a standard solution (5 μg/mL), S – sapogenin peak area (counts per second (cps) and M is a molar mass (g/mol) of a standard compound.

2.7. Linearity, precision and accuracy

After the HPLC-MS analysis of the hydrolysates of the calibration solutions, the linear ranges and correlation coefficients were established. Limit of detection (LOD) and the limit of quantification (LOQ) were defined as the analyte amounts that can be detected with a S/N of 3 and 10, respectively. Three replicates of the standard solutions containing ginsenosides Rh_1 (PPT-type), Rd (PPD-type) and F_{11} (OT-type) at three different concentrations (200, 500, and 2000 ng/mL) were hydrolyzed and analyzed during the same day to elucidate the intra-day precision. The inter-day precision was measured in the same way on three consecutive days. To determine the accuracy, the calculated concentration for standard solution samples were compared with the theoretical values.

3. Results and discussion

3.1. Acid hydrolysis of ginseng saponins

According to the previous report [14], in the acidic conditions, diglycosides and 25,26-hydrated aglycones prevail as hydrolysis products of PPT and PPD ginsenosides. Thus, to build up a quantitative approach for total ginsenoside content evaluation, it was decided to achieve the stable yields of the end-products for three ginsenosides with different aglycones: Rg_1 (PPT-type), Rb_1 (PPD-type), and RT_5 (OT-type), by varying the hydrolysis conditions. The hydrolysis was carried out in the 0.12M and 1.2M hydrochloric acid solutions. After the reaction, ginsenoside Rg_1 was not observed in the mass chromatograms; instead, partially hydrolyzed species and reaction by-products appeared. Regardless of the reaction duration and acid concentration, the prevailing product was the one corresponding to simultaneous hydration and methylation of the sapogenin with a molecular formula of $C_{31}H_{56}O_5$ (calculated from $[M+H]^+$ at m/z 509.4198, Δ = 0.5 ppm). Two other reaction products have been attributed to separate hydration ($C_{30}H_{54}O_5$, $[M+H]^+$ at m/z 495.4041, Δ = 0.5 ppm) and methylation (m/z $C_{31}H_{54}O_4$, $[M+H]^+$ at 491.4095, Δ = 0.03 ppm) of sapogenin. Formation of the PT during the acid hydrolysis was confirmed by the reference standard (Fig. 1). Peak area for PT calculated using total ion current (TIC) signal was about 0.6% (Table 1). Partially hydrolyzed products presumably corresponding to the monoglycosylated saponin (m/z 638.4381, $C_{36}H_{62}O_9$, Δ = 1.2 ppm) and its methylated derivate (m/z 653.4620, $C_{37}H_{65}O_9$, Δ = 0.5 ppm) were formed only in the 0.12M hydrochloric acid.

The hydrolysis of PPD ginsenosides in 1.2M hydrochloric acid results in the formation of several by-products. Similarly to the PPT ginsenoside hydrolysis, the main product is formed via simultaneous hydration and methylation of the PPD sapogenin with a molecular formula of $C_{31}H_{56}O_4$ (calculated from $[M+H]^+$ at m/z 493.4251, Δ = 0.07 ppm). The detected cleavage products are shown in Table 1. Similar products were observed in several previous studies [14,15], where the yields of the aglycones after acid hydrolysis were below 15%.

Acid hydrolysis is more suitable for ginsenosides with OT aglycone. Due to absence of double bonds in the aglycone side chain, no hydration by-products were observed after the acid hydrolysis of pseudoginsenoside RT_5. The reaction in 1.2M hydrochloric acid solution ensures complete transformation of RT_5 into OT after 1.5 hours, but further increasing of the hydrolysis duration results in the formation of an unknown OT isomer (Table 1). The epimerization that takes place during the hydrolysis in acidic conditions is an adverse process, because some herbal products already contain 20(R)-ginsenosides [20], which ideally should be measured separately from the original saponins.

3.2. Alkaline hydrolysis of ginseng saponins

Alkaline hydrolysis is known to give fewer by-products and to produce genuine PPT and PPD aglycones [13]. Equal portions of ginsenosides were subjected to the alkaline hydrolysis with 5% potassium hydroxide aqueous solution. The elimination of all sugar substituents was not achieved even after 24 h of hydrolysis at a high temperature (100°C). Increasing of the potassium hydroxide concentration up to 20% has improved the reaction yield, however complete transformation of monoglucosides (intermediate products) into their sapogenins was not achieved. Moreover, the increase of the alkaline concentration resulted in the appearance of numerous reaction by-products.

When sodium methoxide was used as an alkaline reagent, PPT and PPD aglycones have been obtained as main reaction products for ginsenosides Rg_1, Rf, Rh_1, Re and Rb_1, Rd, Rb_3, correspondingly (Fig. 1). Neither epimerization nor formation of methylated and hydrated by-products was observed. The hydrolysis was conducted by employing 3 different amounts of sodium methoxide (1.5, 3, 8, and 12 mg) in 4 different solvents (methanol, acetonitrile, dimethyl sulfoxide and pyridine). The reaction was held at 80 – 110°C for 3 – 8 hours. Only in case of using acetonitrile as a solvent, sapogenins were obtained free of any by-products, except for intermediate products. For all studied PPT

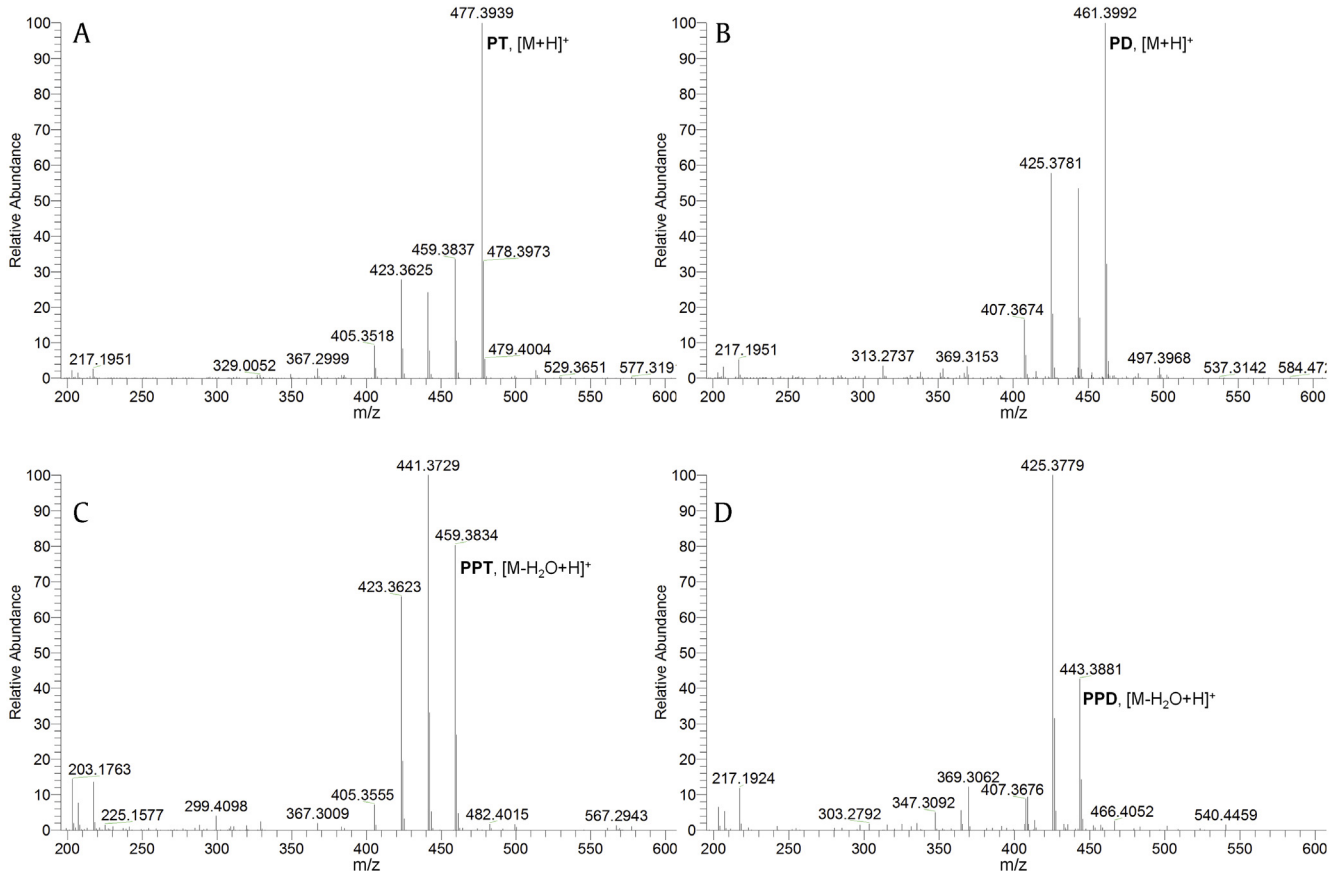

Fig. 1. The mass spectra of the sapogenins obtained after acid (A, B) and alkaline (C, D) hydrolysis of ginseng saponins with protopanaxatriol (PPT) and protopanaxadiol PPD aglycones, correspondingly. PT – panaxatriol; PD – panaxadiol.

ginsenosides, the residual content of the saponin did not exceed 6% (2.9% for Rg_1, 5.3% for Rf, and 6.1% for Rh_1), while for PPD ginsenosides no residual peaks were observed (Fig. 2). The optimal conditions are as follows: 8 mg of sodium methoxide, 3 hours, 100°C. High reaction yields were observed only for hydrolysis of the reference standard solutions, while on the chromatograms of hydrolysates of herbal extracts, abundant peaks of monoglucosides were detected. A substantial amount of intermediate products was observed for herbal extracts even after changing reagents ratio (diluting extracts or increasing amount of sodium methoxide to 12°mg), applying higher temperature (120°C) and increasing reaction time (12°hours). However, it should be noted that total peak areas of PPT and PPD ginsenosides in diluted herbal extracts did not exceed the peak areas of the most concentrated ginsenoside standards used (5°μg/mL). This means that the added amount of reagent should be sufficient for complete transformation of ginsenosides in herbal extracts into their corresponding aglycones, but the reagent is consumed on the hydrolysis of interfering matrix components. To remove these components, the extract pre-purification procedure was developed using Oasis HLB cartridge for SPE. The preliminary SPE allowed increasing of the reaction yields in all tested herbal

Table 1
HPLC data on cleavage products of ginsenosides obtained after 2 hours of acid hydrolysis with 1.2M hydrochloric acid in methanol

Ginsenoside	Peak N№	Retention, min	Peak area, %	Annotation
Rg_1	1	9.58	27.56	Hydrated PT-type sapogenin
	2	10.66	56.7	Methylated and hydrated PT-type sapogenin
	3	11.53	15.15	Methylated PT-type sapogenin
	4	12.76	0.59	Panaxatriol (PT)
RT_5	1	5.36	0.10	Pseudoginsenoside RT_5
	2	10.76	93.95	Ocotillol (OT)
	3	11.12	2.09	Ocotillol, isomer
	4	13.17	3.71	Dehydrated OT-type sapogenin
	5	16.28	0.08	Unknown by-product
Rb_1	1	8.53	0.17	PD-type monoglucoside
	2	11.13	35.87	Hydrated PD-type sapogenin
	3	12.14	37.94	Methylated and hydrated PD-type sapogenin
	4	13.20	24.21	Methylated PD-type sapogenin
	5	17.51	1.44	Panaxadiol (PD)

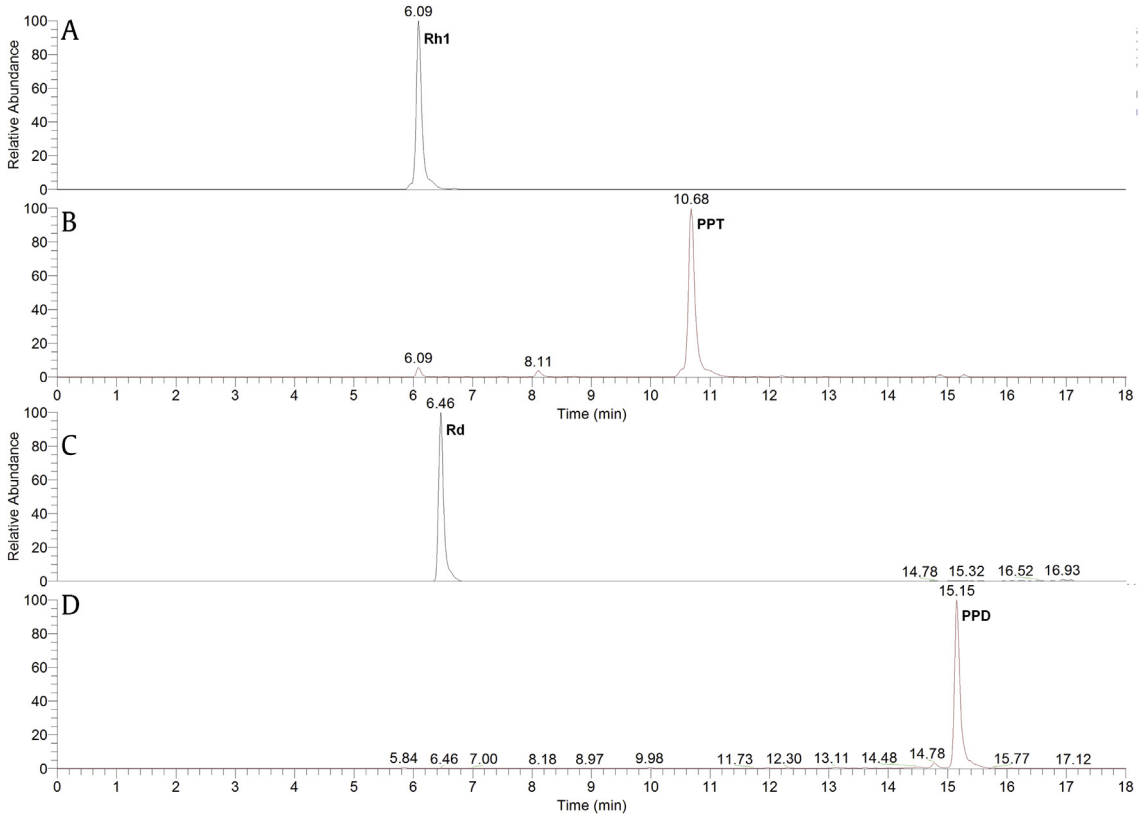

Fig. 2. Mass chromatograms reconstructed with isolated sapogenin fragmentation ions (m/z 405.4, 423.4, 441.4 for protopanaxatriol (PPT), m/z 407.4, 425.4, 443.4 for protopanaxadiol (PPD) for Rh_1 (A, B), and Rd (C, D) before and after the reaction with sodium methoxide in acetonitrile, correspondingly.

samples. As a result, no abundant peaks corresponding to the intermediate monoglycosylated ginsenosides were detected, while several additional abundant peaks corresponding to the 20(R)-PPT/PPD and hydrated aglycones were observed for ginseng tea and red ginseng samples (Fig. 3). These peaks may be attributed to the ginsenosides with modified C-17 side chains that are found in processed ginseng samples [13,20].

3.3. Method validation

3.3.1. Calculation of the responses for major ginsenosides

To develop an accurate assay method for PPT, OT, and PPD ginsenosides in raw plant materials and herbal products, two essential conditions should be fulfilled: The equimolar hydrolysis reaction yield should be achieved for ginsenosides with different sugar chains, and the peak areas for target sapogenins should be

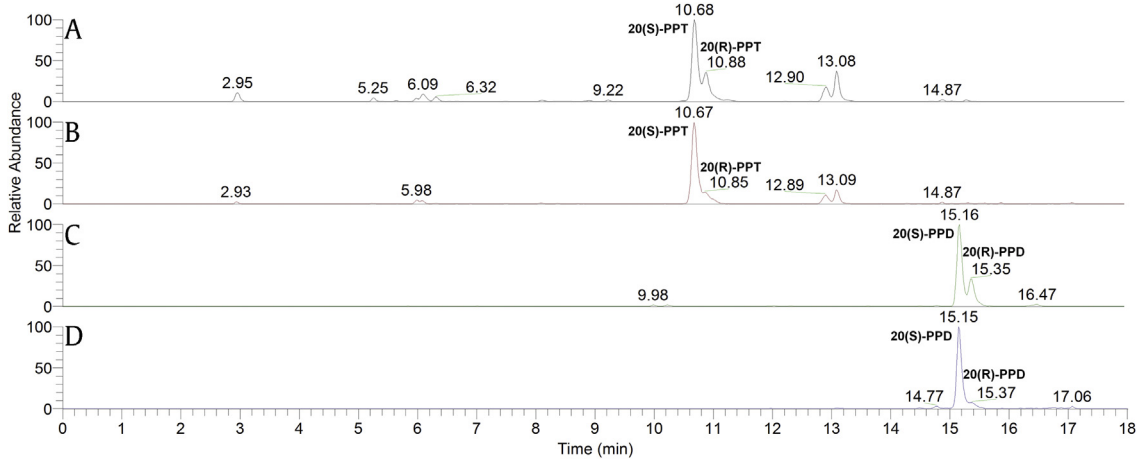

Fig. 3. Mass chromatograms reconstructed with isolated protopanaxatriol (PPT) fragmentation ions (m/z 405.4, 423.4, 441.4) and with isolated protopanaxadiol (PPD) fragmentation ions (m/z 407.4, 425.4, 443.4) for Korean red ginseng (A, C) and Korean ginseng tea (B, D), correspondingly.

Table 2
Responses calculated for ginsenosides with different sugar substituents and aglycone types (n = 2)

Ginsenoside	Fragmentary formula	Molecular weight, Da	Molar concentration, nmol/mL	Average peak area, $\times 10^8$, cps	Response, $\times 10^7$, cps × mL/nmol
Rg_1	PPT−20−Glc−6−Glc	801.01	6.24	2.47	3.84
Re	PPT−20−Glc−6−GlcRha	947.15	5.28	2.11	3.99
Rf	PPT−20−H−6−GlcGlc	801.01	6.24	2.54	4.07
Rh_1	PPT−20−H−6−Glc	638.87	7.83	2.99	3.82
Rb_1	PPD−20−GlcGlc−3−GlcGlc	1109.29	4.51	2.44	5.41
Rb_3	PPD−20−GlcXyl−3−GlcGlc	1079.27	4.63	2.44	5.27
Rd	PPD−20−Glc−3−GlcGlc	947.15	5.28	3.04	5.76
RT_5	OT−6−Glc	654.87	7.64	0.13	0.17
F_{11}	OT−6−GlcRha	801.01	6.24	0.94	0.15

* All solutions were prepared at a concentration of 5°μg/mL.

reproducible. To evaluate the molar response calculated by the Eq. (1), we performed the hydrolysis of several ginsenosides with different sugar chains and aglycones (Table 2). The responses calculated by Eq. (1) were similar for ginsenosides that possess the same aglycone, which gives an opportunity to measure the total contents of PPT-, PPD- and OT-type saponins by using group reference standards, i.e. most readily available ginsenosides of each type. In this way a molar total saponin content (mmol/g) can be measured for each group. Optionally, these total saponin contents can also be expressed in mg/g, but the value will depend on the molar mass of the reference compound selected for each group. For PPT-type saponins, simultaneous presence of bigger molecules, such as malonyl derivatives and PPT-type ginsenosides with three sugar moieties (Re, R_1), and smaller monoglycosylated saponins (Rh_1, F_1) leads to the idea of using a compound bearing two sugars (e.g. Rg_1) as the reference standard for PPT-type saponins to estimate the average weight content in a product. A large portion of PPD-type ginsenosides is composed of Rb_1, Rb_2, Rb_3, and Rc that have similar molecular weights. Pseudoginsenoside F_{11} is the most abundant OT-type saponin in American ginseng [21]. Therefore, using these compounds (Rg_1, Rb_1, and F_{11}) as reference standards provides more accurate evaluation of total saponin contents (mg/g).

Table 3
Analytical characteristics of the developed method for determination of the total PPT, PPD, and OT ginsenoside contents

Sapogenin	Linear range (ng/mL)	Correlation coefficient, R^2	LOD[1], ng/mL	LOQ[2], ng/mL
PPT	50-5000	0.9988	20	50
PPD	50-5000	0.9972	20	50
OT	250-5000	0.9970	80	250

[1] LOD − limit of detection.
[2] LOQ − limit of quantification.

3.3.2. Linearity and quantification limits

To check the linearity of the calibration curve during hydrolysis, for a mixture of group reference standards containing Rg_1 (PPT), Rb_1 (PPD) and F_{11} (OT) ginsenosides, calibration curves were plotted over a concentration range of 50−5000°ng/mL. Calculated values of the correlation coefficients, LODs and LOQs, are presented in Table 3.

Good linearity (correlation coefficient ≥0.99) in the wide concentration range allows determining of the total ginsenoside content up to 5°μg/mL, and also allows performing hydrolysis of diluted extracts from plant material, thus keeping the reaction yield on a high level.

3.3.3. Accuracy and precision

To assess accuracy and precision of the method, mixed standard solutions at three levels were analyzed in triplicate in the selected hydrolysis conditions. The average values determined for low, medium, and high standard solution were in agreement with the calculated concentrations, thus the accuracy was between 95.5% and 105.5%. Relative standard deviation for the low standard solution samples was below 12% level and was less than 8.5% for the medium and high standard solution samples. The data listed in Table 4 show that the precision and accuracy of the proposed method are adequate for the determination of ginsenosides of three studied sapogenin-types after alkaline hydrolysis with sodium methoxide.

3.4. Application of the developed method in the analysis of herbal products

The developed hydrolysis method was tested during the analysis of four different types of ginseng containing products. Total amounts of PPT, PPT, and OT ginsenosides determined after the hydrolysis were compared with the results obtained by

Table 4
The precision and the accuracy of the developed method

Sapogenin	Concentration added, ng/mL	Intra-day (n = 3)			Inter-day (n = 9)		
		Concentration found, ng/mL (mean ± SD*)	Accuracy, %	Precision, %	Concentration found, ng/mL (mean ± SD)	Accuracy, %	Precision, %
PPT	200	205.80 ± 15.93	102.90	7.74	200.28 ± 16.68	100.14	8.33
	500	505.68 ± 28.22	101.14	5.58	493.70 ± 27.42	98.74	5.55
	2000	2016.77 ± 73.19	100.84	3.63	1980.32 ± 79.09	99.02	3.99
PPD	200	200.37 ± 9.62	100.18	4.80	199.04 ± 11.41	99.52	5.73
	500	514.76 ± 36.31	102.95	7.05	502.58 ± 35.83	100.52	7.13
	2000	2059.35 ± 136.39	102.97	6.62	2021.39 ± 122.47	101.07	6.06
OT	200	211.26 ± 22.73	105.63	10.75	191.91 ± 21.94	95.95	11.43
	500	527.25 ± 33.46	105.45	6.34	501.17 ± 36.66	100.23	7.32
	2000	2095.34 ± 133.91	104.77	6.39	1956.25 ± 162.07	97.81	8.28

* SD − standard deviation.

Table 5
Total contents of PPT, PPD, and OT ginsenosides assayed after the alkaline hydrolysis (n = 3) and by the external standard method (ESM)

Ginsenoside content, μg/g		Ginseng food supplement	American dry ginseng root slices	Korean red ginseng root slices	Korean ginseng tea
PPT	Hydrolysis	31.83 ± 3.82	16.90 ± 1.77	0.50 ± 0.06	1.15 ± 0.13
	ESM	10.79	10.47	0.27	0.79
PPD	Hydrolysis	33.74 ± 3.71	51.75 ± 5.69	0.38 ± 0.05	2.42 ± 0.18
	ESM	38.15	34.71	0.36	2.91
OT	Hydrolysis	< LOD*	1.25 ± 0.14	< LOD	< LOD
	ESM	< LOD	1.19	< LOD	0.008

* LOD – limit of detection.

external calibration (Table 5). To perform this comparison, concentrations of two OT-type ginsenosides (pseudoginsenosides RT_5, F_{11}), nine PPD-type ginsenosides (Rc, Rd, Rb_1, Rb_2, Rb_3, Rg_3, C-K, Rh_2, F_2), and seven PPT-type ginsenosides (R_1, Rg_1, Re, Rf, Rh_1, Rg_2, F_1) determined in the previous study [18] using external standard method (ESM) were summed. The data in Table 5 show that in most cases total ginseng contents determined by the developed hydrolysis-based approach are significantly higher than those attributed to the major ginsenosides that are typically used for quantitative assessment of ginseng extracts. For PPD ginsenosides, the calculated content was equal or insignificantly smaller than the one determined using a sum of nine major ginsenosides comprising the largest part of the PPD-type saponins content. Therefore, employing only three ginsenosides as group reference standards, the developed approach could be regarded as inexpensive way to evaluate PPT, PPD, and OT total saponin contents and has a potential for the implementation in the quality control system for ginseng based products. Additionally, one can rely on the developed approach when calculating the safe dosage for the intake of ginseng preparations [21].

4. Conclusions

The determination of total saponin group contents can be conducted by the use of the hydrolysis in alkaline media followed by highly selective HPLC-MS detection of the resulting sapogenins. Epimerization and by-products formation observed in case of acid hydrolysis was not an issue in the optimized alkaline hydrolysis conditions, which gives an opportunity to separately detect 20(R)-PPT and 20(R)-PPD in samples containing modified ginsenosides, such as red ginseng and Korean ginseng tea. The completeness of the hydrolysis was achieved by employing sodium methoxide. American and Asian ginseng samples can be discerned by the presence of pseudo ginsenosides, which are also subjected to the hydrolysis in selected conditions resulting in the formation of OT. The developed rapid and straightforward total ginsenoside content quantification method has shown acceptable precision and accuracy in a wide concentration range, which makes it applicable for quality control of ginseng based products.

Declaration of competing interest

The authors confirm that there were no conflicts of interest in performing this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2020.07.001.

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