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Optimization of the Separation and Validation Method for Ginsenoside Re and Ginsenoside Rg1 Compounds in Panax Ginseng Powder Extract Using the HPLC Method: A Systematic Literature Review

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Abstract: The analytical methods used to analyze ginsenoside compounds include TLC, HPTLC, HPLC, UPLC, GC and MS. The HPLC method is the most widely used analytical technique with various combinations of UV detection or with ELSD. The HPLC method is a method of separating and purifying chemical compounds or medicinal compounds. To study new information from optimization for the separation of ginsenoside Re and ginsenoside Rg1 compounds, then validate the analytical method to show that the method provides accurate results in accordance with the requirements. The data and information used in this review article come from national and international journals. The data and information search method was carried out using the literature research method. keywords: "Optimization of Separation and Method Validation of Ginsenoside Compounds using the HPLC method" with a journal publication period 2019-2023. Of the 35 research articles found, only 9 journals met the inclusion and exclusion criteria. Studies on ginsenoside compounds Re and Rg1 in ginseng plants are still not well separated. So it's still necessary to optimize the analytical method using the HPLC method and then validate the analytical method to show that the method provides accurate results in accordance with the requirements.

Keywords: Gingseng; Ginsenoside Re; Ginsenoside Rg1; method validation; HPLC

Introduction

Herbal plants are plants that have been found tocontain compounds that have the ability to prevent, heal and perform biological functions. Especially, the ginseng plant (*Panax ginseng*) which is a herbal plant used for medicine. The uses of the ginseng plant in medicines include antioxidant, anti-inflammatory, antibacterial, antiviral and antifungal (Ratan, Haidere, Hong, Park, Lee, Lee, & Cho. 2021). Based on the United States Department of Agriculture (USDA), the classification of ginseng (Panax ginseng) is as follows:

: Plantae
: Tracheophyta
: Magnoliopsida
: Apiales
: Araliaceae
: Panax
ginseng

The ginseng plant comes from the Araliaceae family, has around 30 different species based on the region of origin, examples include Panax ginseng C. A. Meyer which comes from Korea, *Panax quinquefolius* L. which comes from America, *Panax japoniicus* C.A. Meyer

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which comes from Japan and *Panax notoginseng* which comes from China (Yun, 2001).

Panax ginseng C.A. Meyer has been used as a herbal medicine in East Asia, especially this herb originating from Korea and China. The name *Panax ginseng* comes from the genus *Panax* and the species ginseng. The genus name "Panax" is derived from the Greece, pan meaning all and axos meaning medicine, which can be interpreted as a cure for all diseases (Shahrajabian, Sun & Qi Cheng, 2019; Yun, 2001). The part of Panax ginseng used as a medicinal ingredient is the root. Ginseng root in Korea is widely consumed in the form of capsules, tablets, powder, concentrated extract, soft capsules and drinks. In general, ginseng is planted in fertile soil and requires a harvest time of around 4-6 years (Baeg & So, 2013). At 6 years of age, the ginseng plant is considered mature ginseng because it has the best shape and has the highest amount of secondary metabolites that become its active substances (Kim et al., 2016).

Ginseng plants contain saponins, phenolic compounds, flavonoids and polysaccharides (Choi, Shin, Kim, & Baik, 2022). Glycoside saponin compounds consist of ocotillo, oleanolic acid and ginsenoside (Makky, 2018). Basically, ginsenoside compounds have the same structure in the form of a steroid nucleus with 17 carbon atoms arranged in four rings the main component contained in ginseng is the triterpenoid saponin compound, namely ginsenoside (Ratan, et al., 2021). Ginsenoside compounds have benefits for antianti-diabetes, anti-inflammatory, cancer, hepatoprotection, anti-aging and anti-oxidative (Zhang et al., 2020).

There are 3 types of ginsenoside, including protopanaxadiol (PPD), protopanaxatriol (PPT) and oleanolic acid (OA) which are grouped based on the number and position of sugar in the glycoside (Hsu, Jen, Inbaraj & Chen, 2022). Ginsenoside can be categorised into major ginsenoside and minor ginsenoside. Major ginsenoside compounds include Rb1, Rb2, Rc, Rd, Re, Rf and Rg1, while the minor ginsenoside compounds are Rg3, Rg2, Rg5, Rh1, Rh3, Rh4 and F1 (Wei et al., 2011). Ginsenoside content (Re, Rg1, Rb1, and Rd) increased when ginseng was 5 years old (Lee, Lee, Cho, Kim, Kim, Yoon Park, Yang, & Lee, 2022). Ginseng faces many challenges during its growth period, for example, at the age of 1 to 2 years, ginseng can experience stress conditions and cannot grow, lack a defense system, and can inhibit growth until harvest time.

Currently, analytical methods are needed to identify and quantify ginsenoside compounds contained in ginseng plants (Fuzzati, 2004). Several analytical methods used to analyze ginsenoside compounds include: TLC, HPTLC, HPLC, UPLC, GC and MS (Baek, Bae, & Park, 2012). Among several methods, the HPLC method is the most widely used analytical technique with various combinations of ultraviolet (UV) detection or with evaporative light scattering detection (ELSD)(Sun, Gu, Fang, Wang, Wang, Lee, Li, Li, & Sung, 2009). The HPLC method is a method used to separate and purify chemical compounds or medicinal compounds.

HPLC can also be used to test the purity of active ingredients, maintain the synthesis process and monitor the quality of an ingredient (Permata, Yade Metri. et al. 2019). The principle of KCKT is the adsorption of the stationary phase and the difference in polarity of the mobile phase. Based on the principle of HPLC there are 2, namely reverse phase and normal phase. Reverse phase is when the mobile phase used is polar and the stationary phase is non-polar with the quality of the separation results decreasing as the solvent polarity increases, while the Normal phase is when the mobile phase used is non-polar and the *stationary phase* used is polar used for semipolar to polar analytes with the quality of the separation results increasing as the solvent polarity increases (Gandjar, et al. 2007). Elution on the mobile phase is divided into two types, namely isocratic elution and gradient elution. Isocratic elution consists of the composition of the mobile phase carried out constantly during the separation process, while in gradient elution the composition of the mobile phase changes during separation (can be polar to more nonpolar and nonpolar more to polar) (A Practical Guide to High Performance Liquid Chromatography, 2021).

Validation is an activity that is carried out to authorise and document a procedure, the process is running effectively and fulfils quality requirements (EMEA, 2001). Validation is necessary when there are changes to facilities, equipment and systems. The method of analysis here refers to on how to do the analysis, this must be explained in detail regarding the stages that need to be carried out for each test (NATA, 2012).

This systematic review aims to examine new information from optimization for the separation of ginsenoside Re and ginsenoside Rg1 compounds, then validate the analytical method to show that the method provides accurate results in accordance with the requirements.

Method

This research design is a systematic review of scientific journals, research reports on optimization and method validation of ginsenoside Re and ginsenoside Rg1 compounds in ginseng powder extract using HPLC method. The search for information sources was carried out electronically in March 2024. The database used to

search for literature is Google Scholar. The data and information used in this article review came from national and international journals. The search for articles published in Indonesia as the main literature was searched using the keywords "Optimisation of Separation and Method Validation of Ginsenoside Compounds using the HPLC method" with the journal publishing period between 2019-2023. The search results from Google Scholar contained 35 research articles according to the predetermined research time span, but only 9 selected journals were reviewed based on inclusion and exclusion criteria and 25 other journals were eliminated.

Journals found in the database were then screened based on inclusion and exclusion criteria. The inclusion criteria are study design in the form of articles with predetermined keywords, articles obtained from Google Scholar, articles published in the last 5 years, research including data on the results of optimization of ginsenoside Re compounds and ginsenoside Rg1 compounds using HPLC methods and validation of analytical methods. Exclusion criteria, articles are not full text, articles with data that do not include the results of optimisation of ginsenoside Re and ginsenoside Rg1 compounds, the language used in the article is not English or Indonesian. Journals were selected if they met the inclusion and exclusion criteria.

The article search flow is organised according to the guidelines *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) (see figure 1).

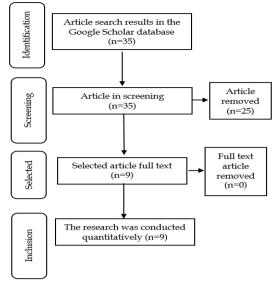


Figure 1. The article Search Flow

Result and Discussion

The systematic literature review used groups similar data according to the results assessed to answer the objectives using the narrative method. Articles that are appropriate for inclusion are collected into one and summarized including the name of the researcher, year of publication, title, methods and research results as Table 1.

Reference	Article Title	Method	Result
Zhou, Huang, He,	Green and Efficient Extraction of	Using HPLC brand (Agilent 1100),	This study proposes a
Zhao, Liu, Zhang, &	Polysaccharide and Ginsenoside	vacuum degasser, quaternary gradient	more efficient and more
Huang, (2022)	from American Ginseng (Panax	pump and autosampler, scattering	efficient deep eutectic
	quinquefolius L.) by Deep	detector (ELSD-LT), column	solvent (DES) aqueous
	Eutectic Solvent Extraction and	temperature 400C, mobile phase	two-phase extraction
	Aqueous Two-Phase System	consists of acetonitrile: water: 5% acetic	system for the extraction
		acid solution (10: 85: 5, $v / v / v$) (A) and	of ginsenoside and
		acetonitrile: water (80:20, v/v) (B) $\rightarrow 0$	polysaccharides from
		minutes (0% B), 0-10 minutes (30 B), 10-	American ginseng. This
		25 minutes (50% B), 25-40 minutes (100%	method avoids the use of
		B), 40-50 minutes (100% B), 50-53	organic reagents and is
		minutes (0% B), and 53-60 minutes (0%	more environmentally
		B), flow rate 1.5 ml/minute, injection	friendly.
		volume 10 µL and wavelength 203 nm.	
		Validation of the analytical method was	
		carried out in the form of linearity (r2	
		0.99), precision, accuracy and stability,	
		with a requirement of less than 3%.	
Yang Xiu, Xue Li,	Simultaneous determination	Using the HPLC brand, Dionex	The developed HPLC-
Xiuli Sun, Dan Xiao,	and difference evaluation of 14	Ultimate 3000, Thermo Scientific	MRM/MS method was
Rui Miao, Huanxi	ginsenosides in Panax ginseng	Syncronis C18 column (diameter 100	validated to identify 14
Zhao, Shuying Liu	roots cultivated in different	mm × 2.1 mm, 1.7 mm). TSQ Endura	ginsenosides precisely,
(2019)	areas and ages by high-	triple quadrupole mass spectrometer	accurately, stably and

Tabel 1. Screening Results of Article used in the systematic Literatur Review

Reference	Article Title	Method	Result
	performance liquid chromatography coupled with triple quadrupole mass spectrometer in the multiple reaction-monitoring mode combined with multivariate statistical analysis	(Thermo Fisher Scientific Inc.), temperature 35°C, flow rate 0.25 mL/min, injection volume 1 μ L, using the mobile phase Solvent A (0.1% formic acid solution, v/v) and solvent B (acetonitrile) with a gradient elution of 0–5 minutes (25–30% B); 5–8 minutes (30–32% B); 8–9 minutes (32–36% B); 9– 16 minutes (36–37% B); 16–18 minutes (37–70% B) and 18–20 minutes (70–95% B). Validation of analytical methods was carried out in the form of linearity, LOD, LOQ, precision, recovery,	sensitively. Also in this study the HPLC- MRM/MS method with multivariate statistical analysis provides insight into the characteristics of ginsenoside accumulation and can be used to differentiate ginseng cultivated in various regions and ages.
Xu, L., Xu, J., Shi, G., Xiao, S., Dai, R., Wu, S., Sun, B., Zhang, X., & Zhao, Y. (2020).	Optimization of flash extraction, separation of ginsenosides, identification by HPLC-FT-ICR- MS and determination of rare ginsenosides in mountain cultivated ginseng	repeatability and stability. Using HPLC-FT-ICR-MS (Agilent 1260), column 5 HC-C18 (250 4.6 mm), wavelength 203 nm, temperature $25 \circ C$, flow rate 0.8 mL/min, injection volume 10 µL and mobile phase Water (A) and acetonitrile (B) with gradient elution (28–35% B at 0–5 min, 35–50% B at 5–15 min, 50–68% B at 15–25 min, 68–90% B at 25 minutes, –28 minutes, 90% B at 28–40 minutes).	This study used flash extraction method (FEM) to isolate ginsenoside from mountain-cultivated ginseng (MCG). The research results showed that MCG had twice the ginsenoside content of garden-cultivated ginseng (CG).
Yu, J., Xu, T., Lin, H., Lin, Y., Zhou, J., & Zhang, Y. (2021).	Comprehensive quality evaluation of American ginseng for different parts and abnormal trait based on the major ginsenoside contents and morphological characteristics	Using HPLC, equipped with a binary pump (Shimadzu, LC-2030C 3D), reverse phase C18 column (4:6 mm × 250 mm, 5°µm), wavelength 203 nm, temperature 40°C, flow rate 1.0 mL /min and mobile phase Acetonitrile and water containing 0.05% phosphoric acid (V/V) with a gradient: 0-35°min, 19% acetonitrile; 35-55°min, 19-29% acetonitrile; 55-70°min, 29% acetonitrile and 70-100°min, 29~40% acetonitrile. Validation of analytical methods was carried out in the form of linearity, LOD, LOQ, precision, repeatability, stability and recovery.	This research explains that the 5 main ginsenosides are found in various parts of the American ginseng plant, each part has different contents.
Song, H., Song, K. W., & Hong, S. P. (2020)	Simultaneous quantification of six nonpolar ginsenosides in white ginseng by reverse-phase high-performance liquid chromatography coupled with integrated pulsed amperometric detection	Using HPLC, Dual Pump model and iPad Systems Nanospace SI-2/3201 pump (ICS-5000 series, Dionex), Unison UK C-18 Column (ID 150.0 × 2.00 mm; 3.0 µm, temperature 450C, flow rate 0.20 mL/min, injection volume 10 µL and mobile phase pure water: acetonitrile (9:1) (solvent A) and pure water: acetonitrile (15:85) (solvent B) with a gradient of 37% B (0 –5 minutes), 37– 51% B (5–6 minutes), 51% B (6–23 minutes), 51–43% B (23–24 minutes), 43– 37% B (24–51 minutes) and maintenance at 37% B (51–55 minutes). Validation of the linearity and sensitivity, accuracy	Gradient elution system all target components are separated within 50 minutes. The rhizome head (RH), main root (MR), lateral root and hairy root (HR) of a six year old white ginseng sample were found to contain nonpolar ginsenoside.
Zhang, L., Wang, S., Qu, B., Chi, H., Quan, Y., & Wu, X. (2019)	Efficient separation determination of protopanaxatriol ginsenosides Rg1, Re, Rf, Rh1, Rg2 by HPLC	and precision analysis methods. Using the Agilent 1100 HPLC brand with a quaternary pump and G1314AVWD detector, Shiseido UG 80 Capcell Pak NH2 column (4.6 mm ID ×	A new protocol has been developed to identify ginsenosides Rg1, Re, Rf, Rh1 and Rg2. By using an

Jurnal Penelitian Pendidikan IPA (JPPIPA)

Reference	Article Title	Method	Result
		250 mm, 5 m), temperature 250C, flow rate 0.80 mg mL-1, wavelength 203nm, mobile phase using isocratic elution carried out with Acetonitrile (A) and water (B) = 76:24 (v/v) with elution gradient 0-3 minutes, 89% A, 3-25 minutes, 89-84% A, 25- 30 minutes, 84- 82% A, 30-35 minutes, 82-76% A, and other conditions are carried out as for isocratic elution. Validation methods were carried out in the form of linearity, LOD, LOQ, precision and accuracy, recovery. There is the addition of MS analysis.	efficient and flexible liquid chromatography method to detect ginsenoside in ginseng extract. This method has been validated and demonstrated efficient recovery in the analysis of white and red ginseng.
Thi Thu, D., Thi Kieu Anh, N., Thi Thanh Phuong, N., Thi Hong Hanh, N., & Thanh Đat, N. (2021).	Simultaneous determination of notoginsenoside R1 and ginsenosides Rg1, Re, Rb1 in dietary supplements by HPLC- DAD	Using HPLC, DAD with an InertSustain C18 column (250 mm × 4.6 mm i.d.; particle size 5 µm), injection volume 20 µL, flow rate 1.6 mL/minute, with mobile phase: Acetonitrile (A) and Water (B), with a gradient of 0 - 20 minutes (20% (A) 80% (B)); 20 - 40 minutes (20 \rightarrow 45% (A) 80 \rightarrow 55% (B)); 40 - 75 minutes (45 \rightarrow 55% (A) 55 \rightarrow 45% (B)); 75 - 80 minutes (20% (A) 80% (B)) and wavelength: 203 nm. Validation of the specificity, LOD, LOQ, linearity and accuracy analysis methods.	To measure notoginsenoside R1 and the three ginsenosides Rg1, Re and Rb1 in dietary supplements. The method used solid phase extraction (SPE) and high- performance liquid chromatography with a diode array detector (HPLC-DAD) has been optimized. Detection limits and quantitation and recovery of compounds were determined. This method was applied to 20 samples of food supplements containing Ginseng and Prowdogingeng
Hsu, B. Y., Jen, C. te, Inbaraj, B. S., & Chen, B. H. (2022).	A Comparative Study on Analysis of Ginsenosides in American Ginseng Root Residue by HPLC-DAD-ESI-MS and UPLC-HRMS-MS/MS	Using HPLC-DAD brand (1200), degasser (G1379B), quaternary pump (BIN pump G1312B), autosampler (1260 Infinity G1329B 1260 ALS), column temperature controller (G1316B TCC SL), photodiode array detector (DAD, G1315C DADSL), Single quadrupole mass spectrometer (6130) with multi- mode ion source (ESI and APCI), with Supelco Ascentis Express C18 and Waters Cortecs T3C18 columns, the agents used are deionized water (A) and acetonitrile (B) : 75% A and 25% B, maintained for 1.5 minutes, flow rate 1mL/minute, wavelength 205 nm, temperature 50°C. Validation of LOD, LOQ, recovery and precision analysis methods was carried out.	Pseudoginseng. This study shows that by using an appropriate chromatography column and mobile phase, 10 types of ginsenosides, including saikosaponin A, can be analyzed in a short time. Two analytical methods, HPLC-DAD- ESI-MS and UPLC- HRMS-MS/MS, were demonstrated in this study to produce accurate results and high precision.
Abashev, M., Stekolshchikova, E., & Stavrianidi, A. (2021)	Quantitative aspects of the hydrolysis of ginseng saponins: Application in HPLC-MS analysis of herbal products	Using HPLC, Dionex Ultimate 3000 system, with Hypersil Gold aQ column (150 mm × 2.1 mm, 3 μ m), with flow rate 0.5 mL/min, temperature 30°C, injection volume 3 μ L and mobile phase deionized water / acetonitrile (95/5, v/v) with 0.1% formic acid (mobile	For the developed HPLC- MS method, linearity, limit of quantification, limit of detection, accuracy, and precision were evaluated. Different hydrolysis conditions

Reference	Article Title	Method	Result
		phase A) and HPLC grade acetonitrile	were tested to develop an
		with 0.1% formic acid (mobile phase B)	accurate quantification
		with a 5% gradient system B and held	method to describe the
		for 2 minutes, then phase B was	total ginsenoside content
		increased linearly to 95% over 13 min	in herbal products.
		and held constant for 3 min, after which	-
		an initial concentration of 5% was	
		reached in 1 min and the system was	
		equilibrated for 4 min before the next	
		chromatography run. Validation of	
		linearity, precision and accuracy	
		analysis methods was carried out.	

In several of these review articles, validation of ginsenoside compounds using the HPLC method is discussed. From the results of research conducted (Zhou, et al., 2022) it was concluded that this research investigated the extraction method of polysaccharides and ginsenoside from American ginseng using a twophase watering system and DES (deep eutectic solvent). This method produces crude polysaccharides and ginsenosides in 30 minutes faster than the conventional method which requires two hours of heat reflux. The green solvent DES and EOPO are recovered, so they can be used again for subsequent extractions. This extraction method can be used to extract natural plants because it has the advantages of being environmentally friendly, efficient and easy to operate. In addition, the antioxidant activity and cytotoxicity of ginseng polysaccharides were also tested. Results from validation parameters that match the requirements (Zhou et al., 2022):

Parameter	Result
Liniarity	0.99
Accuracy	0.82%, 0.61%, 0.65%, 1.35%,
-	1.30% and 1.31%)
Precision	0.78%, 0.56%, 0.65%, 1.01%,
	1.22% and 1.33%
Stability for 12 hours	1.35%, 1.21%, 0.79%, 0.88%,
-	1.25%, and 1.01%)

Research using the HPLC-MRM/MS method combined with multivariate statistical analysis provides deep insight into the characteristics of ginsenosides accumulation and can be used to differentiate ginseng cultivated in various regions and ages. Validation results showed a good linear correlation between concentration and integrated peak area, LOD and LOQ that were sensitive enough to identify and quantify ginsenoside, good precision, acceptable recovery of the analytical method and stability of the sample solution for at least 24 hours. Repeatability is also high, thus demonstrating the accuracy of this method for measuring ginsenoside (Xiu et al., 2019). Whereas, results of another study using the FEM protocol to extract ginsenoside from MCG. The ginsenoside content in MCG is much higher than CG. These findings provide an important theoretical basis for the clinical application of MCG (Xu et al. 2020).

Research conducted by (Yu et al., 2021) shows that American ginseng has strong qualities related to the ginsenoside content (Rg1, Re, Rb1, Rc and Rd). In addition, this study also suggests the effective and economical use of all parts of the American ginseng plant, so that there is no significant difference between the ginsenoside content in normal and abnormal ginseng parts. The validation results of the analytical method showed a strong relationship between the measured ginsenoside content and the test peak area. LOD and LOQ have been determined using the signal to noise ratio. The LOD and LOQ have been determined specifically for each ginsenoside evaluated. The results show that this analysis technique is considered accurate and can be used to identify ginsenoside content in samples. When the samples were added to the standard mixture, their precision, repeatability and stability were assessed and the results showed that the recovery rate was high for each ginsenoside (Yu et al., 2021).

The use of the RP-HPLC-IPAD method was attempted for the first time in identifying nonpolar ginsenosides found in white ginseng. Nonpolar ginsenoside as much as 37.8-56.8% was found in HR white ginseng. As a result, white ginseng HR is expected to be widely used as a medicine. The results of the analytical validation method explain that the linearity value shows 0.9992 or close to 1, sensitivity is measured based on LOD and LOQ, with LOD ranging from 0.06 to 0.2 ng and LOQ ranging from 0.25 to 2 ng, while the accuracy value and precision indicates intraday, interday and recovery testing. The RSD % of intraday and interday tests are 0.21-2.36% and 0.24-4.92% respectively. All values show satisfactory results within 5%. For the recovery test, the average recovery and RSD were 96.72-102.02% and 0.48-6.89%, respectively (Song et al., 2020).

Thi Thumet al., (2021) succeeded in developing a fast, efficient and accurate ginseng analysis method. In this method, four types of ginsenosides (Rg1, Re, Rg2, and Rh1) can be separated well within 10 minutes using

isocratic HPLC conditions. Even for ginsenosides Rg1 and Re which are difficult to separate, this method was able to produce excellent resolution. The analysis time for ginsenoside Re is two and a half times shorter, while for ginsenoside Rg1 it is four times shorter, and for ginsenosides Rh1 and Rg2 it is eight times shorter compared to the previous method. This method also does not require complicated gradient elution or solid phase extraction. Additionally, it appears that the retention time of ginsenoside is related to its molecular weight, with a higher molecular weight providing a longer retention time. This method has been tested for accuracy, linearity and precision. Using QTOF-MS, additional confirmation of the five ginsenosides was performed. Analysis of raw ginseng, white ginseng, and red ginseng extracts showed satisfactory recovery for all five components (Thi Thu, et al., 2021).

The development of a method to measure R1, Rg1, Rb1, and Re simultaneously with the HPLC-DAD method has been validated and meets the analytical method requirements according to AOAC guidelines. The results of applying this method to 20 commercial samples show that the method developed is appropriate and feasible. The results of this study can provide a reference procedure for laboratories to save costs when simultaneously measuring active ingredients in health supplements containing Ginseng and Pseudoginseng. Matrix sample chromatogram results This method shows good linearity, with low detection limits and quantitation limits, compound recovery values are also in a good range and this method also shows good precision (Thi Thu et al., 2021).

A study comparing the HPLC-DAD-ESI-MS method with UPLC-HRMS-MS/MS was carried out to assess the separation efficiency of nine ginsenosides in ginseng root residue plus the internal standard saikosaponin A, with a retention time of 18 minutes for the former method and six minutes for the latter. Both methods showed high accuracy and precision with similar total ginsenoside content. However, the UPLC-HRMS-MS/MS method shows much higher sensitivity than the HPLC-DAD-ESI-MS method, as evidenced by the low LOD and LOQ values obtained in the previous method (Hsu et al., 2022).

Alkaline hydrolysis conditions produce fewer byproducts than sugar elimination under acidic conditions. 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 (Abashev, Stekolshchikova, & Starvrianidi, 2021) which carried out tests to evaluate the separation results on ginsenoside compounds, especially ginsenoside Re and ginsenoside Rg1. Based on the data previously described, the compounds ginsenoside Re and ginsenoside Rg1 have not separated properly. This can be proven by optimizing the separation of ginsenoside Re and ginsenoside Rg1 compounds, so that these compounds can be separated properly (Abashev et al., 2021).

Conclusion

Based on this study, it is concluded that the study of the ginsenoside compounds Re and Rg1 in ginseng plants is still not well separated, so it is still

necessary to optimize the analytical method using the HPLC method and then validate the analytical method to show that the method provides accurate results in accordance with condition.

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Author Contributions

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Conflicts of Interest

The authors declare no conflict of interest.

References

- Abashev, M., Stekolshchikova, E., & Stavrianidi, A. (2021). Quantitative aspects of the hydrolysis of ginseng saponins: Application in HPLC-MS analysis of herbal products. *Journal of Ginseng Research*, 45(2), 246–253. https://doi.org/10.1016/j.jgr.2020.07.001.
- Baeg, I. H., & So, S. H. (2013). The world ginseng market and the ginseng (Korea). In *Journal of Ginseng Research* (Vol. 37, Issue 1, pp. 1–7). Elsevier B.V. https://doi.org/10.5142/jgr.2013.37.1.
- Baek, S. H., Bae, O. N., & Park, J. H. (2012). Recent methodology in Ginseng analysis. In *Journal of Ginseng Research* (Vol. 36, Issue 2, pp. 119-134). https://doi.org/10.5142/jgr.2012.36.2.119.
- Choi, G. S., Shin, J. S., Kim, W., & Baik, M. Y. (2022). Increases in Ginsenoside Rg3, Compound K, and Antioxidant Activity of Cultivated Wild Panax Ginseng (CWPG) by Puffing. *Foods*, *11*(19). https://doi.org/10.3390/foods11192936.
- Emea. (2001). Note for Guidance on process validation. *October, March.*

- Fuzzati, N. (2004). Analysis methods of ginsenosides. In Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences (Vol. 812, Issues 1-2 SPEC. ISS., pp. 119–133). https://doi.org/10.1016/j.jchromb.2004.07.039.
- Gandjar, I. G., & Rohman, A. (2007). Kimia Farmasi Analisis. Pustaka Pelajar. http://library.fmipa.uny.ac.id/opac/index.php?p =show_detail&id=4406&keywords
- Hsu, B. Y., Jen, C. te, Inbaraj, B. S., & Chen, B. H. (2022). A Comparative Study on Analysis of Ginsenosides in American Ginseng Root Residue by HPLC-DAD-ESI-MS and UPLC-HRMS-MS/MS. *Molecules*, 27(10).

https://doi.org/10.3390/molecules27103071.

- Kemenkes RI (2020) Farmakope Indonesia edisi VI, Departemen Kesehatan Republik Indonesia.
- Kim, Y. J., Jang, M. G., Zhu, L., Silva, J., Zhu, X., Sukweenadhi, J., Kwon, W. S., Yang, D. C., & Zhang, D. (2016). Cytological characterization of anther development in Panax ginseng Meyer. *Protoplasma*, 253(4), 1111–1124. https://doi.org/10.1007/s00709-015-0869-3.
- Kumar Verma, N. (2017). An Overview on Panax ginseng. www.ijpacr.com.
- Lee, T. K., Lee, J. Y., Cho, Y. J., Kim, J. E., Kim, S. Y., Yoon Park, J. H., Yang, H., & Lee, K. W. (2022). Optimization of the extraction process of high levels of chlorogenic acid and ginsenosides from shortterm hydroponic-cultured ginseng and evaluation of the extract for the prevention of atopic dermatitis. *Journal of Ginseng Research*, 46(3), 367– 375.https://doi.org/10.1016/j.jgr.2021.10.006.
- Makky, E. A. (2018). Korean Red Ginseng: Benefits Versus Precautions. *INNOSC Theranostics and Pharmacological Sciences*, 1(1), 10–13. https://doi.org/10.26689/itps.
- Merck. (2021). A Practical Guide to High Performance Liquid Chromatography.
- NATA. (2012). Guidelines for the validation and verification of quantitative and qualitative test methods. *National Association of Testing Authorities, December* 2006, 1–32. http://www.demarcheiso17025.com/document/ Guidelines for the validation and verification of quantitative and qualitative test methods.pdf.
- Permata, Y. M., Bachri, M., Reveny, J., & Sibuea, F. M. (2019). Formulation and Quantitative Analysis of Betamethasone Valerate and Neomycin Sulfate Cream by High Performance Liquid Chromatography and Spectrophotometry. *Open* access Macedonian journal of medical sciences, 7(22), 3841–3846.

https://doi.org/10.3889/oamjms.2019.516

- Ratan, Z. A., Haidere, M. F., Hong, Y. H., Park, S. H., Lee, J. O., Lee, J., & Cho, J. Y. (2021). Pharmacological potential of ginseng and its major component ginsenosides. In *Journal of Ginseng Research* (Vol. 45, Issue 2, pp. 199–210). Elsevier B.V. https://doi.org/10.1016/j.jgr.2020.02.004.
- Shahrajabian, M. H., S. W. and C. Q. (2019). A review of Ginseng species in different regions as a multipurpose herb in traditional Chinese medicine, modern herbology and pharmacological science. https://doi.org/10.5897/JMPR2019.6731
- Song, H., Song, K. W., & Hong, S. P. (2020). Simultaneous quantification of six nonpolar ginsenosides in white ginseng by reverse-phase high-performance liquid chromatography coupled with integrated pulsed amperometric detection. *Journal of Ginseng Research*, 44(4), 563–569. https://doi.org/10.1016/j.jar.2019.07.002

https://doi.org/10.1016/j.jgr.2019.07.002.

- Sun, B. S., Gu, L. J., Fang, Z. M., Wang, C. yan, Wang, Z., Lee, M. R., Li, Z., Li, J. J., & Sung, C. K. (2009). Simultaneous quantification of 19 ginsenosides in black ginseng developed from Panax ginseng by HPLC-ELSD. *Journal of Pharmaceutical and Biomedical Analysis*, 50(1), 15–22. https://doi.org/10.1016/j.jpba.2009.03.025.
- Thi Thu, D., Thi Kieu Anh, N., Thi Thanh Phuong, N., Thi Hong Hanh, N., & Thanh Đat, N. (n.d.). (2021). Simultaneous determination of notoginsenoside R1 and ginsenosides Rg1, Re, Rb1 in dietary supplements by HPLC-DAD. In *Vietnamese Journal* of Food Control (Vol. 4, Issue 2).
- U.S. Department of Agriculture. Panax ginseng. Available at: https://acir.aphis.usda.gov/s/cirdtaxon/a0ut000000mK3gAAE/panax-ginseng (Accessed: 20 April 2024).
- Wei, Y., Zhao, W., Zhang, Q., Zhao, Y., & Zhang, Y. (2011). Purification and characterization of a novel and unique ginsenoside Rg 1-hydrolyzing β-D-Glucosidase from Penicillium sclerotiorum. *Acta Biochimica et Biophysica Sinica*, 43(3), 226–231. https://doi.org/10.1093/abbs/gmr001
- Xiu, Y., Li, X., Sun, X., Xiao, D., Miao, R., Zhao, H., & Liu, S. (2019). Simultaneous determination and difference evaluation of 14 ginsenosides in Panax ginseng roots cultivated in different areas and ages by high-performance liquid chromatography coupled with triple quadrupole mass spectrometer in the multiple reaction-monitoring mode combined with multivariate statistical analysis. *Journal of Ginseng Research*, 43(4), 508–516. https://doi.org/10.1016/j.jgr.2017.12.001.
- Xu, L., Xu, J., Shi, G., Xiao, S., Dai, R., Wu, S., Sun, B., Zhang, X., & Zhao, Y. (2020). Optimization of flash extraction, separation of ginsenosides,

identification by HPLC-FT-ICR-MS and determination of rare ginsenosides in mountain cultivated ginseng. *RSC Advances*, *10*(72), 44050-44057. https://doi.org/10.1039/d0ra07517e.

- Yu, J., Xu, T., Lin, H., Lin, Y., Zhou, J., & Zhang, Y. (2021). Comprehensive quality evaluation of American ginseng for different parts and abnormal trait based on the major ginsenoside contents and morphological characteristics. *BioMed Research International*, 2021. https://doi.org/10.1155/2021/8831080.
- Yun, T.K. (2001) 'Brief Introduction of Panax ginseng C.A. Meyer', *J Korean Med Sci*, 16(Suppl), pp. S3–S5. Available at:

https://doi.org/10.3346/jkms.2001.16.S.S3.

- Zhang, H., Abid, S., Ahn, J. C., Mathiyalagan, R., Kim, Y. J., Yang, D. C., & Wang, Y. (2020). Characteristics of Panax ginseng cultivars in Korea and China. In *Molecules* (Vol. 25, Issue 11). MDPI AG. https://doi.org/10.3390/molecules25112635
- Zhang, L., Wang, S., Qu, B., Chi, H., Quan, Y., & Wu, X. (2019). Efficient separation determination of protopanaxatriol ginsenosides Rg1, Re, Rf, Rh1, Rg2 by HPLC. *Journal of Pharmaceutical and Biomedical Analysis*, 170, 48–53. https://doi.org/10.1016/j.jpba.2019.03.025.
- Zhou, R. R., Huang, J. H., He, D., Yi, Z. Y., Zhao, D., Liu, Z., Zhang, S. H., & Huang, L. Q. (2022). Green and Efficient Extraction of Polysaccharide and Ginsenoside from American Ginseng (Panax quinquefolius L.) by Deep Eutectic Solvent Extraction and Aqueous Two-Phase System. *Molecules*, 27(10). https://doi.org/10.3390/molecules27103132.