생맥산의 구성 생약 중 오미자의 schizandrin과 gomisin A의 정량 분석

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Quantitative Analysis of Schizandrin and Gomisin A in Traditional Herbal Formula, Saengmaek-san

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Abstract

Saengmaek-san (SMS) comprises three herbal medicines Liriopis seu Ophiopogonis Tuber, Ginseng Radix, and Schisandrae Fructus. SMS has been traditionally used for the treatment of ischemic disease in Asia. In this study, an analytical method using high-performance liquid chromatography (HPLC) and a photodiode array detector (PDA) (HPLC－PDA) was used for the quantitative analysis of schizandrin and gomisin A in SMS, a traditional herbal formula. Chromatographic separation of the two compounds was carried out using a Gemini C18 column and two mobile phases, distilled water and acetonitrile, with gradient elution. The coefficient of determination of the calibration curve for the quantitative analysis of the marker components of Schisandrae Fructus in an SMS sample, schizandrin and gomisin A, was 1.0000. The limit of detection of the marker components was 3.28 mg/mL and 3.19 mg/mL, and the limit of quantification was 9.93 mg/mL and 9.67 mg/mL. Recovery and intra- and interday precisions were 95.44-103.58% and <1.5%, respectively. The quantities of schizandrin and gomisin A were 0.244 and 0.028 mg/freeze-dried g, respectively. The optimized HPLC－PDA method could be expected to provide basic data for the quality control of SMS and other related herbal formulas.

Keywords: Quantitative Analysis, Saengmaek-san, HPLC-PDA

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Introduction

Saengmaek-san (SMS) is an oriental herbal prescription consisting of three medicinal herbs, Liriopis seu Ophiopogonis Tuber (Liliaceae), Ginseng Radix (Araliaceae), and Schisandrae Fructus (Schisandraceae), in the ratio of 2:1:1 based on dry weight[1]. SMS was first recorded in Li's *Nei Wai Shang Bian Huo Luen* in the Keum Dynasty. Since then, it has also been recorded in Heo's *Dongeuibogam* in the Joseon Dynasty in Korea[1,2]. It has been demonstrated to have various pharmacological activities, including anti-inflammatory, anti-oxidant, gastrointestinal motility, and anti-atopic dermatitis effects[1,3-7]. Phytochemical ingredients in an SMS sample consisting of three herbal medicines—Liriopis seu Ophiopogonis Tuber (LOT), Ginseng Radix (GR), and Schisandrae Fructus (SF)—have been isolated and are reported on, specifically: spicatoside A (steroid saponin, from LOT)[8], ginsenoside Rb1 and ginsenoside Rg1 (triterpenoid saponins, from GR)[9], and schizandrin, gomisin A, and gomisin N (lignans, from SF)[10]. Wu et al.[11] have reported on the analysis of SMS using ultra-performance liquid chromatography with tandem high-definition mass spectrometry (UPLC-HDMS). However, this study focuses on the phytochemical profiling of three raw medicines (LOT, GR, and SF). The objective of this study is to develop and validate an analytical method for the quantitative analysis of schizandrin and gomisin A in SMS extracts using high-performance liquid chromatography (HPLC) and photodiode array detector (PDA) (HPLC-PDA).

Discussion

1) Materials and methods

(1) Plant material

Three raw herbal medicines—LOT, GR, and SF—consisting SMS were purchased from Kwangmyungdang Medicinal Herbs (Ulsan, Korea) in March 2012. The botanical origins of the three raw herbs were identified by Professor Jung-Hoon Kim, School of Korean Medicine of Pusan National University (Yangsan, Korea) according to *The Dispensatory on the Visual and Organoleptic Examination of Herbal Medicine*[12]. Voucher specimens (2012-KE40-1 ~ KE40-3) have been deposited at the Herbal Medicine Research Division, Korea Institute of Oriental Medicine (KIOM).

(2) Chemicals and reagents

Two reference standard compounds, schizandrin (PubChem CID: 23915, purity 99.9%) and gomisin A (PubChem CID: 3001662, purity 98.0%) were purchased from Wako Chemicals (Osaka, Japan). The methyl alcohol (PubChem CID: 887, purity ≥99.9%), acetonitrile (PubChem CID: 6342, purity ≥99.9%), and water (PubChem CID: 962) used in our experiments were all liquid

chromatography and spectrophotometry grade solvents, purchased from J.T. Baker (Phillipsburg, NJ, USA).

(3) Preparation of SMS water extract

The SMS water extract consisting of three medicinal herbs—LOT, GR, and SF—were formulated as shown in Table 1 and 50 L of water was added. The formulated sample was extracted for 2 h at 100°C under 0.98 bar pressure, using an electric extractor (COSMOS-660; Kyungseo Machine Co., Incheon, Korea). The extract was filtered using a standard sieve (no. 270, 53 mm, 203 Æ; Chung Gye Sang Gong Sa, Seoul, Korea). The filtered solution was lyophilized using a LP100R freeze dryer (IlShinBioBase, Yangju, Korea). The quantity of powdered SMS water extract was 1.33 kg (26.58%).

Table 1. Composition of SMS

Herbal medicine	Scientific name	Origin	Amount
Liriopis seu Ophiopogonis Tuber	*Liriope platyphylla* Wang et Tang	Miryang, Korea	2.50 kg
Ginseng Radix	*Panax ginseng* C. A. Meyer	Yeongju, Korea	1.25 kg
Schisandrae Fructus	*Schisandra chinensis* (Turcz.) Baillon	Samcheok, Korea	1.25 kg
		Total amount	5.00 kg

(4) HPLC conditions

Quantitative analysis of the two marker compounds in an SMS sample (schizandrin and gomisin A) was conducted using a Shimadzu Prominence LC-20A series HPLC system (Kyoto, Japan); it consisted of pumps, a column oven, autosampler, and PDA detector. All chromatographic data were acquired and processed using Shimadzu LC solution software (Version 1.24 SP1; Kyoto, Japan). The parameters for HPLC analysis of the two lignans (schizandrin and gomisin A) are given in Table 2.

Table 2. Chromatographic parameters for HPLC analysis of two lignans in SMS: schizandrin and gomisin A

Chromatographic parameter			
Column	Gemini C_{18} analytical column (250 × 4.6 mm, 5 μm)		
Flow rate (mL/min)	1.0		
Injection volume (μL)	10.0		
Column temperature (°C)	40		
Mobile phase	A: Distilled water, B: Acetonitrile		
Gradient elution	Time (min)	A (%)	B (%)
	0	60	40
	30	15	85
	35	15	85
	40	60	40
	50	60	40

(5) Mass spectrometry (MS) conditions

MS analysis of the two lignans in SMS was performed using a Waters triple quadrupole mass spectrometer (Milford, MA, USA) equipped with an electrospray ionization source. All data were processed by Waters MassLynx software (version 4.1; Milford, MA, USA). The parameters for MS analysis were as Table 3.

Table 3. MS parameters for identification of schizandrin and gomisin A

MS parameter	
Ion mode	Positive
Capillary voltage (kV)	3.3
Extract voltage (V)	3.0
Source temperature (°C)	120
RF lens voltage (V)	0.3
De-solvation temperature (°C)	300
De-solvation gas (L/h)	600
Cone gas (L/h)	50
Collision gas (mL/min)	0.14

(6) Method validation

The established and applied HPLC-PDA method was verified by analysis of linearity, precision, and recovery, according to the International Conference on Harmonisation (ICH) guidelines and a previous study[13,14].

2) Results and discussion

(1) Optimization of chromatographic separation

Optimal conditions for the quantitative analysis of the two lignan components (schizandrin and gomisin A) in SMS extract were established using a Gemini C_{18} analytical column (250 × 4.6 mm, 5 mm; Phenomenex, Torrance, CA, USA) and gradient elution with distilled water and acetonitrile. The two marker compounds were separated within 20 min at wavelength 250 nm with 9.69 resolution (Fig. 1).

(2) Regression equation and linearity

The regression equations (y = ax + b) of schizandrin and gomisin A were prepared by measuring the peak areas (y) versus the concentrations (x, mg/mL) three times, using standard solutions. The concentrations of schizandrin and gomisin A for the regression equation was 0.78-50.00 and 0.16-10.00 mg/mL, respectively. The linearity of the calibration curve was evaluated by the value of the coefficient of determination (r^2). The two components showed excellent

linearity (r^2 = 1.0000) in the concentration range tested. These data are summarized in Table 4.

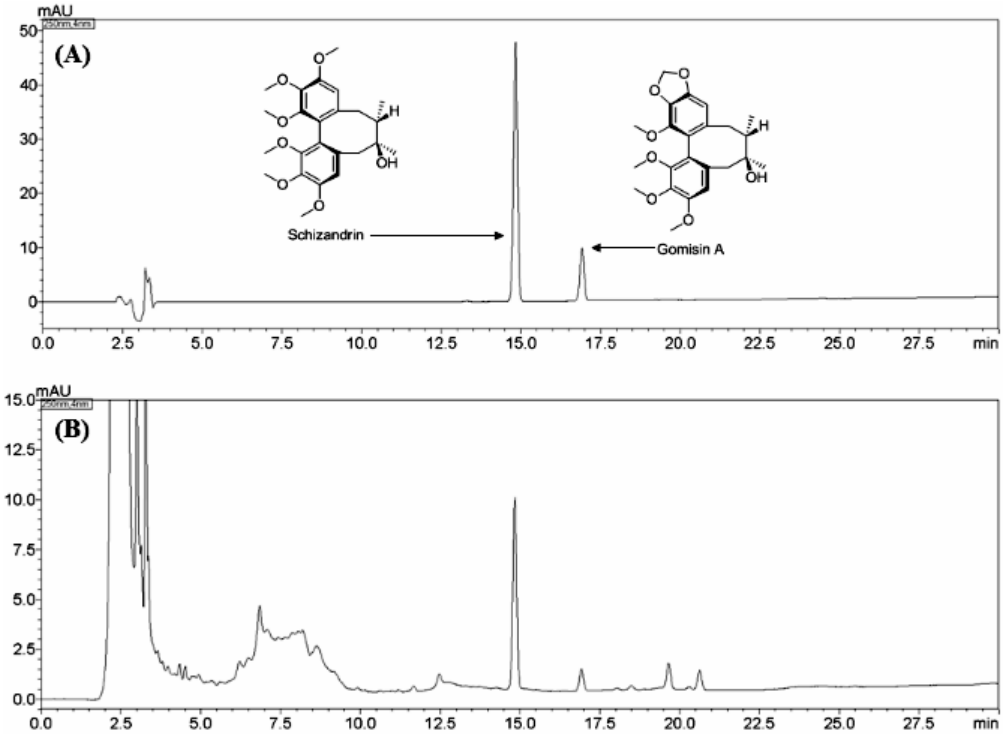

Figure 1. HPLC chromatograms of the standard solution (A) and Saengmaek-san sample (B) at 250 nm.

(3) Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined as follows: LOD = 3.3 × s/S and LOQ = 10 × s/S (s: standard deviation of the y-intercept in each calibration curve, S: slope of the calibration curve). The LOD and LOQ of schizandrin were calculated to be 3.28 mg/mL and 9.93 ng/mL, respectively. The LOD and LOQ of gomisin A were calculated to be 3.19 mg/mL and 9.67 ng/mL, respectively (see Table 4).

Table 4. Linear range, regression equation, coefficient of determination (r^2), LOD, and LOQ for schizandrin and gomisin A

Compound	Linear range (mg/mL)	Regression equation (y = ax + b)*	r^2	LOD† (ng/mL)	LOQ‡ (ng/mL)
Schizandrin	0.78–50.00	y = 17214.11x – 2254.79	1.0000	3.28	9.93
Gomisin A	0.16–10.00	y = 176740.09x – 455.88	1.0000	3.19	9.67

*y: peak area (mAU) of compounds; x: concentration (mg/mL) of compounds; †LOD: 3.3 × σ/S; ‡LOQ: 10 × σ/S. (σ: standard deviation of the y-intercept and S is the slope of the calibration curve)

(4) Accuracy and precision

Accuracy was verified by the recovery test. The recoveries of schizandrin and gomisin A were 95.44-103.58% and the relative standard deviation (RSD) values of the two compounds were within 1.5%. In order to verify reproducibility, the standard solution was measured six times and the reproducibility was evaluated by the RSD values of peak area and retention time. Subsequently, the two marker compounds showed good reproducibility; RSD values were 0.25-0.53%. The intra- and interday precisions of schizandrin and gomisin A were assessed as RSD values, which were 0.16-1.22%. These results indicated that the established method was indeed appropriate for the quantitative analysis of schizandrin and gomisin A from an SMS extract (Table 5).

Table 5. Recovery and precision data for the assay of schizandrin and gomisin A

Compound	Spike con (mg/mL)	Accuracy		Precision		Repeatability (n = 6)	
		Recovery (%)	RSD (%)	Intraday RSD (%)	Interday RSD (%)	RSD (%) of peak area	RSD (%) of retention time
Schizandrin	1.00	102.17	1.15	0.96	0.90	0.25	0.37
	2.00	101.22	0.81	0.82	1.04		
	4.00	95.44	0.38	0.16	0.22		
Gomisin A	0.25	100.60	1.38	0.93	0.85	0.25	0.53
	0.50	100.95	1.11	1.01	1.22		
	1.00	103.58	1.02	0.25	0.30		

(5) MS confirmation

MS spectra confirming the two lignans, schizandrin and gomisin A, are shown in Fig. 2. They were detected at *m/z* 433.4 and 417.4, respectively, as the $[M+H]^+$ form in the positive ion mode.

(6) Determination of two lignin compounds in SMS sample

The established and optimized HPLC method was then used for the quantitative analysis of schizandrin and gomisin A, the marker components of Schisandrae Fructus in an SMS sample. The quantities of schizandrin and gomisin A were 0.244 and 0.028 mg/freeze-dried g, respectively (Table 6).

Table 6. The amounts of two marker compounds in SMS extract (n = 3)

Compound	Mean	SD (10^{-1})	RSD (%)
Schizandrin	0.244	0.004	0.184
Gomisin A	0.028	0.002	0.791

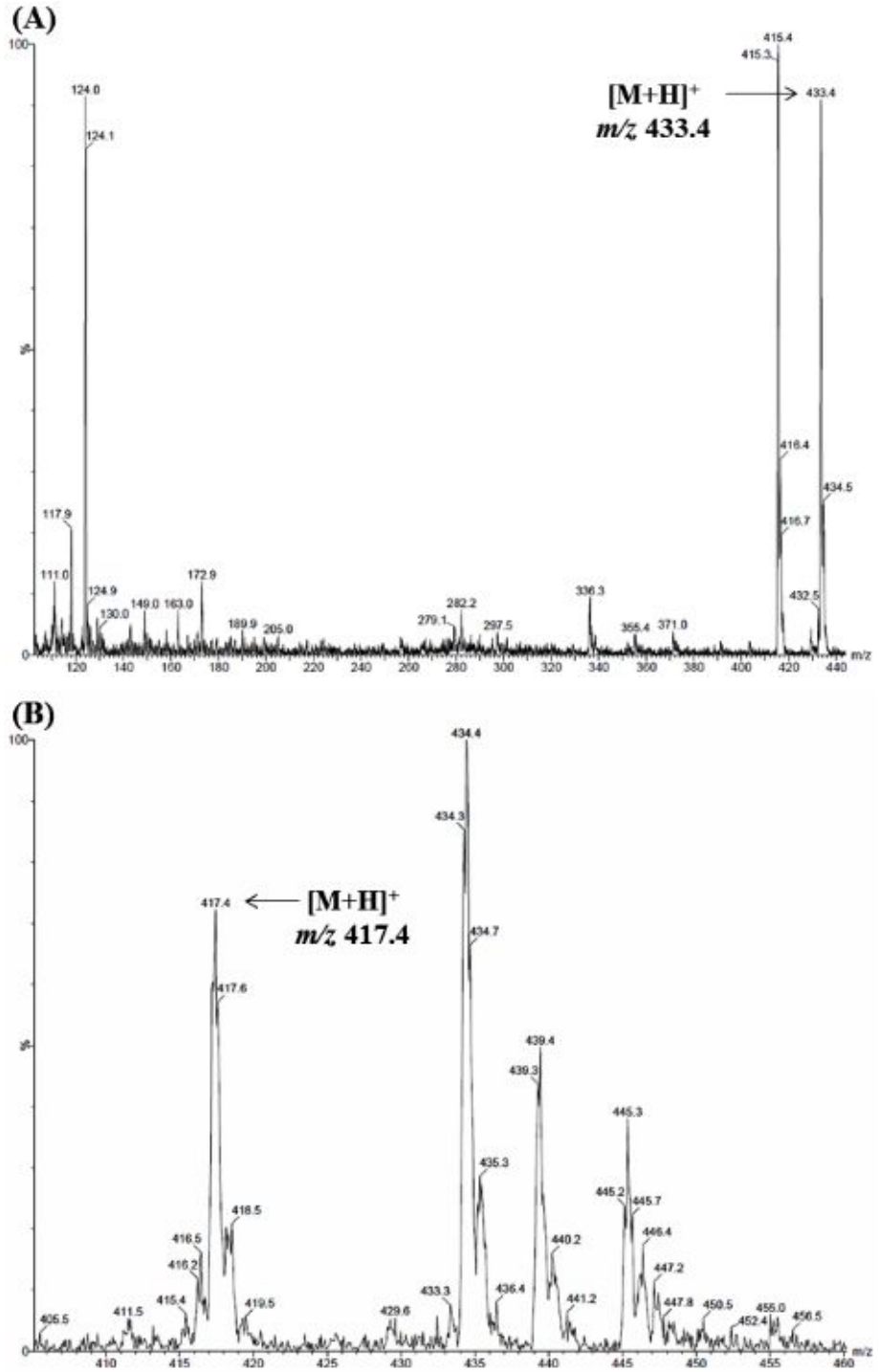

Figure 2. Mass spectra of two lignans, schizandrin (A) and gomisin A (B).

Conclusion

This study presents a simple and convenient HPLC-PDA method applicable for the quantitative analysis of the marker components of Schisandrae Fructus —schizandrin and gomisin A— in the oriental herbal prescription SMS. This optimized HPLC-PDA method could be expected to provide basic data for the quality control of SMS and other related herbal formulas.

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