Simultaneous Determination of Seven Compounds in *Samsoeum* by HPLC-PDA

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ABSTRACT

Objectives : To develop and validate HPLC-PDA methods for simultaneous determination of seven constituents in *Samsoeum*(SSE).

Methods : Reverse-phase chromatography using a Gemini C18 column operating at 40°C, and photodiode array(PDA) detection at 254 and 280 nm, were used for quantification of the seven marker components of SSE. The mobile phase using a gradient flow consisted of two solvent systems. Solvent A was 1.0% (v/v) aqueous acetic acid and solvent B was acetonitrile with 1.0% (v/v) acetic acid.

Results : Calibration curves were acquired with r^2>0.9997, and the relative standard deviation (RSD) values (%) for intra- and inter-day precision were less than 3.0%. The recovery rate of each compound was in the range of 100.07-112.65%, with an RSD less than 4.0%. The contents of seven compounds in SSE were 1.24-10.53 mg/g.

Conclusions : The established method will be helpful to improve quality control of SSE.

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Ⅰ. Introduction

Many traditional herbal medicines were used for prevention and therapy of diseases. These had generally no side effects and powerful efficacy, so the interest of the people and use of traditional herbal medicines increased lately[1-3].

Samsoeum (SSE, *Shensuyin* in Chinese) is one of traditional herbal medicine and was first described in the Song dynasty (China, about AD1107)[4]. It has been widely used for the treatment of headache, fever, cough, rhinorrhea, analgesia, andantipyresis[4,5]. In addition, SSE also has been used for the symptoms caused by internal factors such as congestion with phlegm, tidal fever, and emesis[4]. A composite traditional Korean medicine, SSE is a basic prescription consisting of herbal plants of 12 species (Table1). Until now, pharmacological studies of SSE have been reported anti-allergic and anti-asthma effect[6-9], and it prevents pulmonary damage from ozone[5]. However no previous work has investigated the High-performance liquid chromatography (HPLC) simultaneous analysis on components of SSE. Therefore, we needed to develop the simultaneous determination for quality control of traditional herbal medicine.

High-performance liquid chromatography (HPLC), coupled with other techniques, particularly photodiode array (PDA) detection, is a convenient, widely used, and powerful approach for the rapid identification of constituents in botanical extracts and plants important in traditional Chinese medicine[10]. Hence, in this study, we focused on quantitative determination of main components in SSE, and explored HPLC-PDA coupled methods for simultaneous determination of seven constituents of SSE(Fig. 1).

Table 1. Composition of *Samsoeum*

Composition	Amount(g)	Purchase place	Source
Perillae Herba	3.75	Omniherb	Geochang, Korea
Puerariae Radix	3.75	Omniherb	Jecheon, Korea
Pinelliae Tuber	3.75	HMAX	China
Anthrisci Radix	3.75	HMAX	China
Ginseng Radix	3.75	Omniherb	Geumsan, Korea
Poria Sclerotium	3.75	Omniherb	Yeongcheon, Korea
Aurantii Fructus	2.8125	HMAX	China
Platycodonis Radix	2.8125	Omniherb	Yeongcheon, Korea
Glycyrrhizae Radix	2.8125	HMAX	China
Citri Unshius Pericarpium	2.8125	Omniherb	Jeju, Korea
Zingiberis Rhizoma	3.75	Omniherb	Yeongcheon, Korea
Zizyphi Fructus	3.75	Omniherb	Yeongcheon, Korea
Total	41.25		
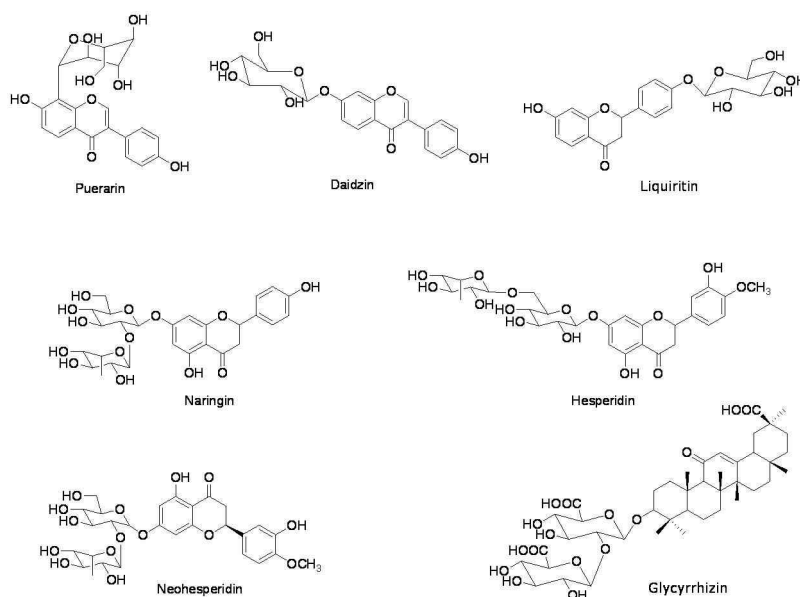
Fig. 1. Chemical structures of seven constituents in *Samsoeum*.

II. Materials and Methods

1. Chromatographic system

Analysis was performed using a Shimadzu LC-20A HPLC system (Shimadzu Co., Kyoto, Japan), consisting of a solvent delivery unit, an on-line degasser, a column oven, an autosampler, and a PDA detector. The data processor employed LCsolution software (Version 1.24). The analytical column used was a Gemini C18 (250×4.6 mm;

particle size 5 μm, Phenomenex, Torrance, CA, USA). The mobile phases were solvent A (1.0% v/v aqueous acetic acid) and solvent B (acetonitrile with 1.0% v/v acetic acid). The gradient flow was as follows: (A)/(B)=95/5 (0 min) → (A)/(B)=30/70 (40 min) → (A)/(B)= 0/100 (45 min; hold for 5 min) → (A)/(B)= 95/5 (55 min; hold for 15 min). The column temperature was maintained at 40°C. The analysis was carried out at a flow-rate of 1.0 mL/min with PDA detection from 190-400 nm. The injection volume was 10 μL.

2. Reagents and materials

Puerarin, daidzin, liquiritin, naringin, glycyrrhizin (all purity ≥98.0%) and hesperidin (purity ≥ 92.0%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Neohesperidin (purity ≥99.0%) was obtained from ChromaDex (Irvine, CA, USA). HPLC-grade reagents, methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ, USA). Glacial acetic acid was analytical reagent grade, procured from Merck KGaA (Darmstadt, Germany). The materials of SSE were purchased from Omniherb (Yeongcheon, Korea) and HMAX (Jecheon, Korea), respectively. It was taxonomically confirmed by Professor Je-Hyun Lee, Dongguk University, Gyeongju, Republic of Korea. Each voucher specimen (2008-KE28-1~KE28-12) was deposited at the Herbal Medicine EBM Research Center, Korea Institute of Oriental Medicine.

3. Preparation of standard solutions and calibration curves

Standard stock solutions of seven compounds (all 1,000 μg/mL) were prepared in methanol and held below 4°C. Working standard solutions were prepared by serial dilution of stock solutions with methanol. All calibration curves were obtained from assessment of peak areas of standard solutions in the concentration ranges: puerarin, 1.56-200.00 μg/mL; daidzin, 0.78-100.00 μg/mL; liquiritin, 0.78-100.00 μg/mL; naringin, 1.95-250.00 μg/mL; hesperidin, 1.56-200.00 μg/mL; neohesperidin, 1.56-200.00 μg/mL; and glycyrrhizin, 3.13-100.00 μg/mL

4. Preparation of sample solutions

A decoction of SSE was prepared in our laboratory from a mixture of chopped crude herbs purchased from Omniherb (Yeongcheon, Korea) and HMAX (Jecheon, Korea). SSE was prepared as described in Table 1 and extracted in distilled water at 100°C for 2 hr. The extract solution was evaporated to dryness and then freeze dry (yield; 12.6%). The lyophilized SSE extract was weighed (200 mg) into a 20 mL flask and distilled water was added to the volumetric mark. And then the sample was filtered through a 0.2 um syringe filter. Injection volume for HPLC analysis was 10 μL.

5. Limits of detection (LOD) and quantification (LOQ)

Stock solutions of reference compounds were further diluted with methanol to assess LOD and LOQ values. The LOD and LOQ data obtained under the chromatographic conditions used in this report were determined using signal-to-noise (S/N) ratios of 3 and 10, respectively.

6. Precision and accuracy

Repeatability was assessed by analysis of seven independently prepared standard solutions. The

relative standard deviation (RSD) of peak areas of analytes, and peak retention times for each standard, were calculated.

Intra- and inter-day precision was determined using a standard addition method to prepare spiked samples, employing both standards and controls.

Recovery tests were performed by adding known amounts of reference standards to SSE samples before extraction. An average recovery was calculated using the formula: Recovery (%) = (Amount$_{determined}$ - Amount$_{original}$)/Amount$_{spiked}$ × 100.

III. Results and Discussion

1. Optimization of the chromatographic conditions

In our method, satisfactory separation was obtained using mobile phases consisting of (A) 1.0% (v/v) aqueous acetic acid, and (B) acetonitrile with 1.0% (v/v) acetic acid, with a gradient flow of (A)/(B) =95/5 (0 min) → (A)/(B)=30/70 (40 min) → (A)/(B)= 0/100 (45 min; hold for 5 min) → (A)/(B)= 95/5 (55 min; hold for 15 min). Quantitation was achieved by PDA detection from 190-400 nm, based on peak area. The selectivity of the HPLC is illustrated in Fig. 2, where good separation of marker compounds from other components of the extract can be noted. Using optimized chromatography conditions, all analytes were eluted before 40 min, and showed resolution better than 1.15 and good specificity in sample analysis. Representative HPLC chromatograms of standard and extract are shown in Fig. 2.
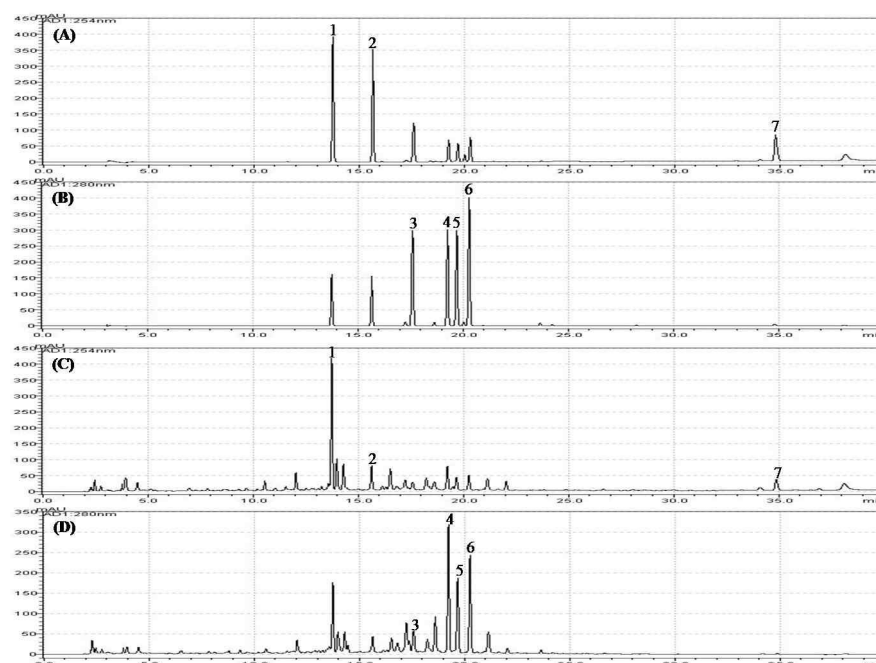

Fig. 2. HPLC chromatogram of the standard mixture of seven major compounds with detection at 254 nm (A) and 280 nm (B), *Samsoeum* sample at 254 nm (C) and 280 nm (D). Puerarin (1), daidzin (2), liquiritin (3), naringin (4), hesperidin (5), neohesperidin (6), and glycyrrhizin (7)

2. Recovery

A recovery test was performed by addition of known amounts of seven marker compounds such as puerarin, daidzin, liquiritin, naringin, hesperidin, neohesperidin and glycyrrhizin. Standard compounds, at each of two different levels, were mixed with sample powder, and extracted. The recovery of each standard ranged from 100.07-112.65%, and the RSD range was 0.23-3.46%(Table 2).

Table 2. Recovery of Seven Marker Compounds (n=3)

Compounds	Original mean (ug/mL)	Spiked (ug/mL)	Detected mean (ug/mL)	Recovery mean (%)	SD	RSD (%)
Puerarin	53.42	10.00	64.36	109.40	0.39	0.36
		25.00	78.44	100.07	0.64	0.64
Daidzin	12.66	2.00	14.92	112.65	1.50	1.33
		5.00	17.93	105.23	1.83	1.74
Liquiritin	24.32	4.00	28.43	102.76	0.24	0.23
		10.00	35.00	106.76	2.31	2.16
Naringin	106.22	20.00	126.60	101.87	1.31	1.29
		50.00	156.41	100.38	1.30	1.29
Hesperidin	56.71	10.00	67.69	109.72	1.35	1.23
		25.00	82.67	103.82	1.09	1.05
Neohesperidin	60.01	10.00	10.90	108.90	2.82	2.59
		25.00	85.90	103.54	0.58	0.56
Glycyrrhizin	40.34	8.00	49.16	110.35	3.81	3.46
		20.00	60.86	102.61	1.80	1.76

3. Linearity, range, LOD and LOQ

Calibration curves were obtained using standard solutions containing 1.56-200.00 μg/mL of puerarin, 0.78-100.00 μg/mL of daidzin, 0.78-100.00 μg/mL of liquiritin, 1.95-250.00 μg/mL of naringin, 1.56-200.00 μg/mL of hesperidin, 1.56-200.00 μg/mL of neohesperidin, and 3.13-100.00 μg/mL of glycyrrhizin as marker compounds. Line equations representing calibration curves and their correlation coefficients are summarized in Table 3.

Ranges of the LODs and LOQs were 0.041-0.522 μg/mL and 0.137-1.739 μg/mL, respectively. These data are shown in Table 3.

Table 3. Regression Data, Linear Ranges, LODs and LOQs for Marker Compounds (n=3)

Compounds	Linear range (ug/mL)	Slope	Intercept	Correlation coefficient	LOD (ug/mL)	LOQ (ug/mL)
Puerarin	1.56 - 200.00	40369.09	-4248.84	0.9997	0.047	0.158
Daidzin	0.78 - 100.00	37991.95	2862.21	0.9998	0.052	0.172
Liquiritin	0.78 - 100.00	18338.85	-978.63	1.0000	0.056	0.186
Naringin	1.95 - 250.00	17333.29	-2201.40	0.9999	0.055	0.184
Hesperidin	1.56 - 200.00	18669.49	-2370.68	1.0000	0.055	0.184
Neohesperidin	1.56 - 200.00	23891.33	-3109.41	0.9999	0.041	0.137
Glycyrrhizin	3.13 - 100.00	8064.35	1345.30	0.9999	0.522	1.739

4. Accuracy and precision

Repeatability or intra-assay precision was assessed by repeatedly measuring retention times and peak areas for three independently prepared samples of analytes. Measurement precision was below RSD 1.0% for peak responses and below RSD 0.2% for retention times, with all analytes. Thus, the HPLC assay offered good repeatability under optimized conditions.

To test the accuracy and precision of the analytical method, the intra- and inter-day variations for measurement of seven marker compounds were determined, and are summarized in Table 4. The precision of the method in simultaneous determination of the seven marker compounds was acceptable as the RSD did not exceed 3.0% at any concentration. The intra-day accuracy ranged from 97.56-106.16%, and the inter-day accuracy ranged from 98.86-106.39%.

Table 4. Precision and Accuracy of Analytical Results (n=3)

Compounds	Spiked Conc. (ug/mL)	Intra-day			Inter-day		
		Observed Conc. (ug/mL)	Precision (RSD %)	Accuracy (%)	Observed Conc. (ug/mL)	Precision (RSD %)	Accuracy (%)
Puerarin	10.00	10.62	0.21	106.16	10.64	1.12	106.39
	25.00	24.75	0.04	99.01	24.74	0.09	98.98
Daidzin	2.00	2.09	1.82	104.68	1.98	0.61	98.86
	5.00	4.96	0.31	99.25	5.01	0.10	100.18
Liquiritin	4.00	3.90	1.43	97.56	4.09	2.27	102.22
	10.00	10.04	0.22	100.39	9.96	0.37	99.65
Naringin	20.00	20.20	0.71	100.98	20.30	0.70	101.48
	50.00	49.92	0.11	99.84	49.88	0.11	99.76
Hesperidin	10.00	10.38	1.40	103.76	10.41	1.80	104.11
	25.00	24.85	0.23	99.40	24.84	0.30	99.34
Neohesperidin	10.00	10.34	1.48	103.42	10.33	0.58	103.31
	25.00	24.86	0.25	99.45	24.87	0.10	99.47
Glycyrrhizin	8.00	8.40	1.15	104.97	8.35	0.27	104.44
	20.00	19.84	0.19	99.20	19.86	0.05	99.29

5. Determination of main constituents in SSE

Our assay was subsequently applied to the simultaneous determination of seven compounds, puerarin, daidzin, liquiritin, naringin, hesperidin, neohesperidin, and glycyrrhizin, in SSE. Fig. 2 show chromatograms of reference compounds and water extract of SSE, with detection of eluents at 254 nm and 280 nm. The analytical results for each compound identified are summarized in Table 5.

Table 5. Analytical Results for Marker Compounds in Samsoeum (n=3)

Batch (#)	Compounds											
	Puerarin			Daidzin			Liquiritin			Naringin		
	Mean (mg/g)	SD	RSD (%)	Mean (mg/g)	SD	RSD (%)	Mean (mg/g)	SD	RSD (%)	Mean (mg/g)	SD	RSD (%)
1	5.29	0.012	0.224	1.26	0.015	1.154	2.27	0.027	1.201	10.38	0.051	0.487
2	5.33	0.009	0.166	1.24	0.001	0.057	2.31	0.043	1.880	10.53	0.088	0.834
3	5.29	0.057	1.085	1.24	0.003	0.252	2.31	0.028	1.231	10.43	0.067	0.642

Batch (#)	Compounds								
	Hesperidin			Neohesperidin			Glycyrrhizin		
	Mean (mg/g)	SD	RSD (%)	Mean (mg/g)	SD	RSD (%)	Mean (mg/g)	SD	RSD (%)
1	5.64	0.049	0.868	6.01	0.108	1.850	3.96	0.039	0.973
2	5.78	0.071	1.236	5.99	0.890	0.890	4.04	0.004	0.096
3	5.64	0.044	0.777	5.95	0.827	0.827	4.01	0.023	0.579

IV. Conclusion

A HPLC method was developed for simultaneous determination of puerarin, daidzin, liquiritin, naringin, hesperidin, neohesperidin, and glycyrrhizin, to evaluate the quality of SSE. In the present work, simultaneous determination of the seven marker compounds in SSE was validated with respect to linearity, precision, and accuracy. The method will be helpful to improve quality control of SSE.

Acknowledgements

References

1. Jiang WY. Therapeutic wisdom in traditional Chinese medicine: a perspective from modern science. Trends Pharmacol Sci. 2005;26:558-63.

2. Liu S, Yi LZ, Liang YZ. Traditional Chinese medicine and separation science. J Sep Sci. 2008;31:2113-37.

3. Won JB, Ma JY, Ma CJ. Simultaneous determination of four marker components in yukmijihwang tang by high performance liquid chromatography/diode array detector. Arch Pharm Res. 2010;33:619-23.

4. Hwang DY. Bang Yak Hap Pyeon. Seoul: Namsandang. 1992:152.

5. Choi SB, Rhee HK. A study of effects of Samsouem on the analgesia, antipyresis and O_3 poisoned lung in rats and mice. J KyungHee OMC. 1985;8:139-48.

6. Ryu NH, Park SM, Kang H, Shim BS, Kim SH, Choi SH, Ahn KS. Effedt of Samsoeum on cytokine regulation of mouse T cell. Korean J Orintal Physiology & Pathology. 2008;22(4): 856-62.

7. Nam BS, Kim YB. The experimental study on the anti-allergic effects on Samsoeum and Samsoeumgamibang. J Korean Oriental Medical Surgery, Ophthalmology & Otolaryngology. 2002;

15(1):343-55.

8. Cho SJ, Kim HW, Kim BY, Cho SI. Sam So Eum, a herb extract, as the remedy for allergen -induced asthma in mice. Pulm Pharmacol Ther. 2008;21:578-83.

9. Kim HW, Cho SJ, Kim BY, Baek JW, Jeong HW, Cho SI, Effects of Samso-Eum on cytokine productions in asthmatic mice. Kor J Herbology. 2007;22(2):175-80.

10. Zhang H, Shen P, Cheng Y. Identification and determination of the major constituents in traditional Chinese medicine Si-Wu-Tang by HPLC coupled with DAD and ESI-MS. J Pharm Biomed Anal. 2004;34:705-13.