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ORIGINAL ARTICLE

Simultaneous Determination of Four Compounds, Campesterol, Emodin8-O-β-D-Glucopyranoside, Quercetin, and Isoquercitrin in *Reynoutria sachalinensis* by High-performance Liquid Chromatography-Diode Array Detector

Min Rye Eom[1], Jin Bae Weon[1], Youn Sik Jung[1], Ga Hee Ryu[1], Woo Seung Yang[1], Choong Je Ma[1,2]

[1]Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, [2]Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon 200-701, Republic of Korea

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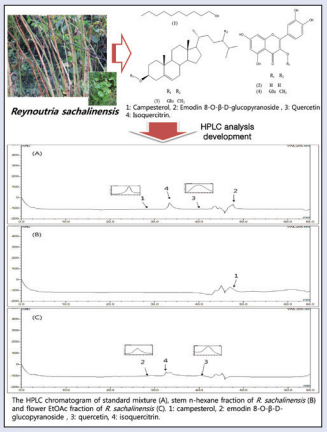
ABSTRACT

Background: *Reynoutria sachalinensis* is a well-known and used herbal medicine to treatment of arthralgia, jaundice, amenorrhea, coughs, carbuncles, and sores. **Objective:** We have developed high-performance liquid chromatography analysis method for simultaneous determination of isolated four compounds, campesterol, emodin8-O-β-D-glucopyranoside, quercetin, and isoquercitrin from *R. sachalinensis* is. **Materials and Methods:** The four compounds were separated on Shiseido C_{18} column (S-5 μm, 4.6 mm I.D. ×250 mm) at a column temperature of 25°C. The mobile phase composed of water and methanol with gradient elution system, and flow rate is 1.0 ml/min. The detection wavelength was set at 205 nm. **Results:** Validation of this analytical method was evaluated by linearity, precision, and accuracy test. This established method had good linearity (R^2 > 0.997). The relative standard deviation values of intra- and inter-day testing were indicated that <2%, and accuracy is 91.66%–103.31% at intraday and 91.69%–103.31% at intraday. The results of recovery test were 92.60%–108.99%. **Conclusion:** In these results, developed method was accurate and reliable to the quality evaluation of campesterol, emodin 8-O-β-D-glucopyranoside, quercetin, and isoquercitrin isolated from *R. sachalinensis*.

Key words: High-performance liquid chromatography, quality control, *Reynoutria sachalinensis,* simultaneous determination, validation

SUMMARY

- We have developed high-performance liquid analysis method for simultaneous determination of 4 compounds of *Reynoutria sachalinensis*.



The HPLC chromatogram of standard mixture (A), stem n-hexane fraction of *R. sachalinensis* (B) and flower EtOAc fraction of *R. sachalinensis* (C). 1: campesterol, 2: emodin 8-O-β-D-glucopyranoside , 3: quercetin, 4: isoquercitrin.

Abbreviations used: HPLC: High-performance liquid chromatography, DAD: Diode array detector, LOD: Limit of detection, LOQ: Limit of quantitation, ICH: International Conference on Harmonisation.

Correspondence:

Prof. Choong Je Ma,
Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, Hyoja-2 Dong, Chuncheon 200-701, Republic of Korea.
E-mail: cjma@kangwon.ac.kr

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INTRODUCTION

Reynoutria sachalinensis belongs to Polygonaceae family, is a well-known and used herbal medicine from Northeastern Asia and Russia. *R. sachalinensis* is has been traditionally used to treatment of arthralgia, jaundice, amenorrhea, coughs, scalds, burns, traumatic injuries, carbuncles, and sores.[1] The previous study showed that anthraquinones and flavonoids in flower of *R. sachalinensis* are extracts have antioxidant activity and vanicoside A and B of rhizomes of *R. sachalinensis* are exhibited β-glucosidase inhibitory activity.[2-4] In addition, resveratrol in stem and root of *R. sachalinensis* is has the anti-cancer effects, and *R. sachalinensis* leaves extracts can be used as a plant fungicide against powdery mildew.[5,6]

Phytochemical studies revealed that *R. sachalinensis* contain phenolic compounds, anthraquinones, stilbenes, and flavonoids.[7-14]

Recently, the use of herbal medicines has increased as food and medicines. Several compounds in natural products increase the therapeutic effect due to the synergistic effect. However, mixed with a lot

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of compound materials have difficulty in quality control. In addition, the content of the bioactive compound is affected by the area, temperature, and other factors. Therefore, it is desirable to establish a method for the quantitative analysis of compounds in natural products and many researchers developed a quality control analysis of natural products. The simple and reliable analysis method has been used for quality control of a wide range of medicinal herbs.[15,16]

In general, high-performance liquid chromatography (HPLC) is one of the chromatography and relative techniques and is used for analysis of compounds in herbal medicine.[17] HPLC is a simple and accurate analysis method for herbal medicine and can separate complex compounds mixture. Thus, HPLC was applied for quality control of herbal medicine over the past decades.[18]

In this study, we established to simultaneous determination method for effective qualitative and quantitative analysis of four compounds, campesterol, emodin 8-O-β-D-glucopyranoside, quercetin, and isoquercitrin.

MATERIALS AND METHODS

Plants and analysis regents

The stem and flower of *R. sachalinensis* were collected from medicinal herb field in Kangwon National University in Chuncheon, Korea. The voucher specimen (No. CJ152M) has been deposited in the natural products laboratory, the Kangwon National University in Chuncheon, Korea. HPLC grade solvents, water, and acetonitrile were purchased from J. T. Baker (USA). Trifluoroacetic acid was purchased from Dae Jung in Korea.

High-performance liquid chromatography equipment and conditions

Four compounds were analyzed using the HPLC (Dionex Ultimate 3000 System, Germany). The HPLC was coupled with a pump (LPG 3X00), an auto sampler (ACC-3000), a column oven (TCC-3000SD), and diode array ultraviolet (UV)/visible detector (DAD-3000RS). Separation of *R. sachalinensis* was performed with Shiseido C_{18} column (S-5 μm, 4.6 mm I.D. ×250 mm), and the column temperature was maintained at room temperature. The mobile phase was composed of water (A) and methanol (B) at a flow rate of 1.0 ml/min, and the sample injection volume was 20 μl. The gradient flow was as follows: 0–5 min 0% B, 5–30 min 0%–50% B, 30–35 min 50% B, 35–40 min 95% B, 40–50 min 95% B, 50–60 min 95%–100% B, and 60–65 min 100%–0% B. The diode array detector (DAD) was set at 205, 254, 280, and 330 nm.

Preparation of standard and sample solutions

Compounds were dissolved in methanol to prepare the stock solution of campesterol (4) (2000 μg/ml), emodin 8-O-β-D-glucopyranoside (7) (50 μg/ml), quercetin (8) (100 μg/ml), and isoquercitrin (9) (500 μg/ml), respectively. Chemical structures of compounds were presented in Figure 1. The stock solution was diluted appropriately with methanol for use as a working standard solution. The working solutions were used for calibration curve and method validation. Stock solution and working solutions were stored 4°C before HPLC analysis. The dried stem and flower of *R. sachalinensis* were extracted by ultrasonication extraction in 80% methanol. The solvents were removed at 40°C using vacuum evaporator and obtained the residues were freeze-dried. All solutions were filtered through a 0.45 μm membrane filter before HPLC analysis.

Validation of the high-performance liquid chromatography method

The reproducibility and accuracy of the HPLC established method were confirmed by performing the linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, and accuracy test in accordance with the guidelines of the International Conference on Harmonisation. The stock solution was diluted to the appropriate concentration with methanol for the construction of the calibration curve. The correlation of coefficient (R^2) was used as measure of linearity. The LOD and LOQ data were obtained based on signal-to-noise ratios of about 3.3 and 10, respectively. The precision of the HPLC method was carried out though intra- and inter-day test and expressed and assessed as the relative standard deviation (RSD) (%). Accuracy was expressed using a spike recovery test. The spike recoveries were calculated by following equation: Spike recovery (%) = (total amount detected − amount original)/amount spiked × 100%.

RESULTS AND DISCUSSION

Optimization of high-performance liquid chromatography chromatographic conditions

To obtain optimal analysis conditions, different wavelength and solvent composition were tested repeatedly. Mobile phase consisted with water and methanol and the maximum absorption wavelength of four compounds set at 205 nm. The peaks were identified by comparing the retention time of each compound in HPLC chromatogram and UV spectrum [Figure 2].

Linearity, limit of detection, and limit of quantitation

Calibration curves were constructed by plotting the peak area (Y) and

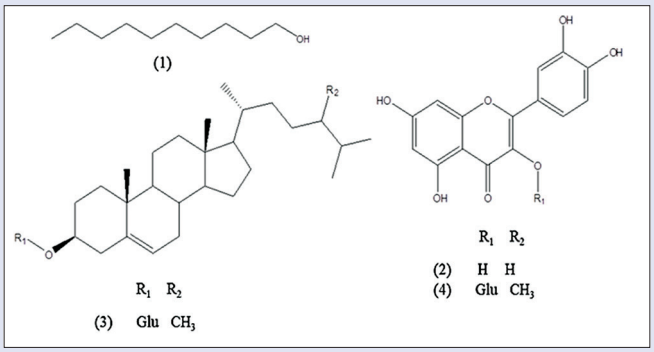

Figure 1: Chemical structures of standard compounds. 1: Campesterol, 2: Emodin 8-O-β-D-glucopyranoside, 3: Quercetin, 4: Isoquercitrin

Table 1: The regression data, limit of detection, and limit of quantitations four isolated compounds analyzed by high-performance liquid chromatography-diode array detector

Compounds	Regression equation	R^2	Linear range (μg/ml)	LOD (μg/ml)	LOQ (μg/ml)
1	y=0.034x+0.022	0.998	125.00-1000.00	1.13	3.44
2	y=0.099x−0.089	0.997	1.56-12.50	0.03	0.08
3	y=0.038x−0.036	0.997	3.13-25.00	0.47	1.41
4	y=0.160x−0.714	0.999	31.25-250.00	0.11	0.33

y: Peak area; x: Amount (μg). LOD: Limit of detection; LOQ: Limit of quantitation; 1: Campesterol; 2: Emodin 8-O-β-D-glucopyranoside; 3: Quercetin; 4: Isoquercitrin

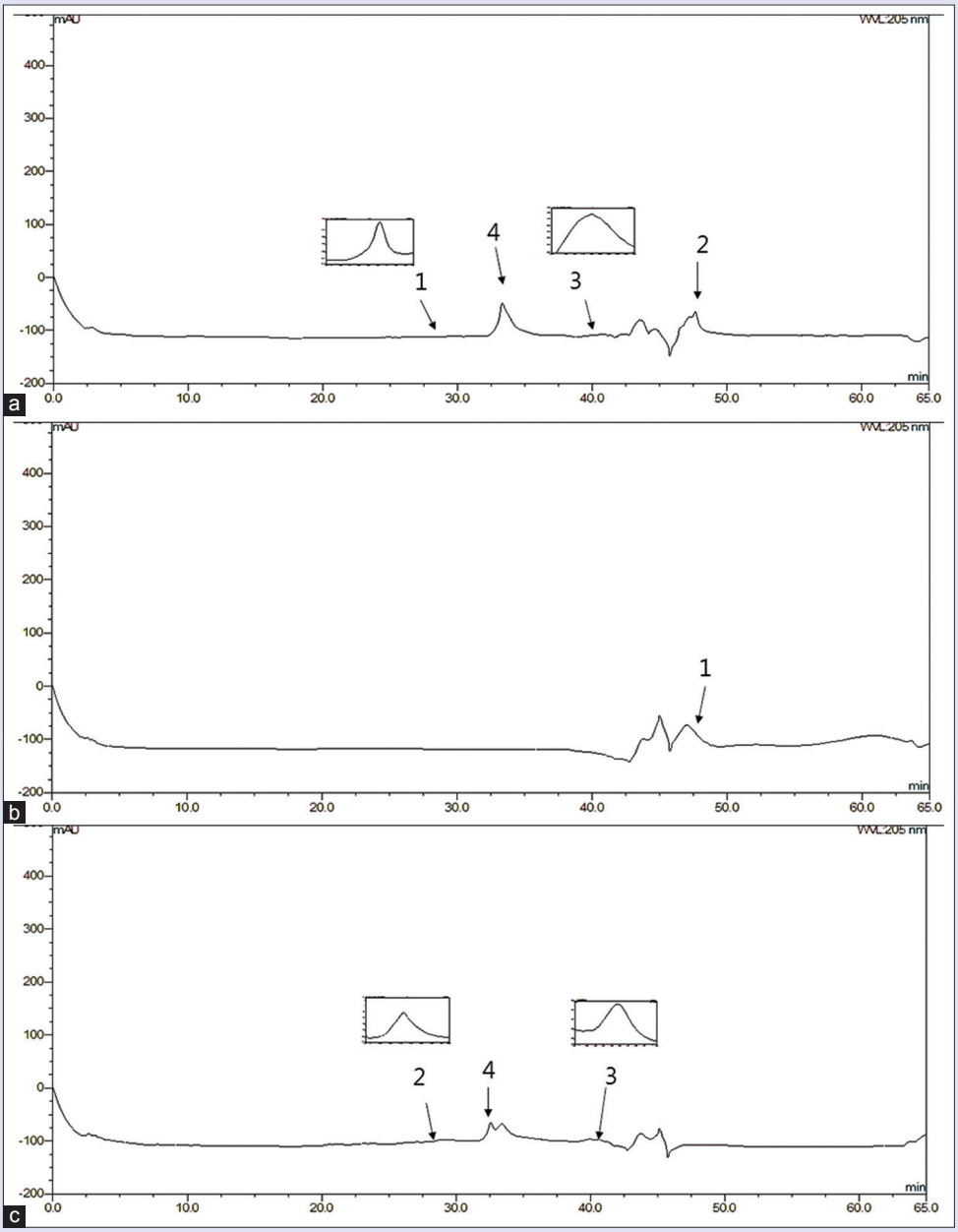

Figure 2: The high-performance liquid chromatography chromatogram of standard mixture (a) stem n-hexane fraction of *Reynoutria sachalinensis* (b) and flower effects of ethyl acetate fraction of *Reynoutria sachalinensis* (c). 1: campesterol, 2: emodin 8-O-β-D-glucopyranoside, 3: quercetin, 4: isoquercitrin

each concentration of four compounds (x, μg/ml). The correlation coefficient (R^2) value showed a very good linearity ($R^2 > 0.997$) in the linear range. The LOD and LOQ values were in the range 1.13 μg/ml and 3.44 μg/ml, respectively [Table 1]. These results showed high sensitivity of this established HPLC-DAD method.

Precision and accuracy

The precision of developed HPLC analysis method was investigated by intra- and inter-day test, and then, precision was evaluated by the RSD values. As results, RSD values were showed that 0.06%–0.74% at intraday, 0.07%–0.52% at interday, respectively. In addition, accuracy of intra- and inter-day was ranged with 91.66–103.31 and 91.69–103.31%, respectively. These results are shown in Table 2. In addition, the recovery test was carried out to verify the accuracy of the method. Recovery of compounds was ranged with 92.60%–108.99% [Table 3].

Analysis of *Reynoutria sachalinensis* sample

The content of the four compounds analyzed in stem and flower of *R. sachalinensis* extract using the established HPLC method. The peaks of each compound were separated successfully without interference of other compounds within 60 min. The amount of compound contained in each fraction was calculated by the calibration curves of standard. The result is shown in Table 4.

CONCLUSION

In this study, we have developed a HPLC analysis method for simultaneous determination of four compounds campesterol, emodin 8-O-β-D-glucopyranoside, quercetin, and isoquercitrin in *R. sachalinensis*. The developed method showed good linearity, precision, and recovery. In addition, this method was demonstrated

Table 2: Intra- and inter-day precision data of four compounds

Compound	Concentration (μg/ml)	Intraday			Interday		
		Mean (μg/ml)	RSD (%)	Accuracy (%)	Mean (μg/ml)	RSD (%)	Accuracy (%)
1	500	516.57±1.00	0.19	103.31	516.57±0.34	0.07	103.31
	250	248.04±0.64	0.26	99.22	247.91±0.45	0.18	99.17
	125	126.75±0.08	0.06	101.40	127.22±0.48	0.37	101.78
2	12.5	12.70±0.09	0.74	101.57	12.67±0.01	0.07	101.33
	6.25	6.30±0.02	0.37	100.85	6.18±0.01	0.15	98.85
	3.25	2.86±0.01	0.07	91.66	2.87±0.01	0.52	91.69
3	25	25.21±0.07	0.28	100.85	25.30±0.06	0.25	101.18
	12.5	11.92±0.06	0.48	95.39	11.89±0.04	0.34	95.08
	6.25	6.15±0.02	0.31	98.43	6.05±0.03	0.44	96.72
4	125	124.92±0.14	0.11	99.94	124.95±0.10	0.08	99.96
	62.5	63.48±0.13	0.21	101.56	63.37±0.05	0.08	101.40
	31.25	29.50±0.16	0.53	94.39	29.54±0.05	0.18	94.51

Concentration injected of compounds. 1: Campesterol; 2: Emodin 8-O-β-D-glucopyranoside; 3: Quercetin; 4: Isoquercitrin; RSD: Relative standard deviation

Table 3: Recovery of the four compounds from *Reynoutria sachalinensis*

Compound	Spiked (μg/ml)	Found (μg/ml)	RSD (%)	Recovery (%)
1	500	260.19±0.28	0.11	104.08
	250	115.76±0.45	0.39	92.60
	125	62.38±0.27	0.44	99.80
2	12.5	6.05±0.06	0.95	96.76
	6.25	3.17±0.06	1.93	101.41
	3.125	1.65±0.02	1.47	105.31
3	25	12.44±0.06	0.50	99.49
	12.5	6.75±0.04	0.58	107.92
	6.25	3.19±0.01	0.24	102.02
4	125	64.10±0.14	0.22	102.56
	62.5	32.61±0.09	0.30	104.35
	31.25	17.03±0.14	0.82	108.99

Recovery (%) = (amount found-original amount)/amount spiked × 100%. 1: Campesterol; 2: Emodin 8-O-β-D-glucopyranoside; 3: Quercetin; 4: Isoquercitrin; RSD: Relative standard deviation

Table 4: Contents of four compounds in *Reynoutria sachalinensis*

Compounds	Content (μg/mg)
1	612.34±0.29
2	19.27±0.11
3	16.71±0.03
4	240.74±0.11

1: Campesterol; 2: Emodin 8-O-β-D-glucopyranoside; 3: Quercetin; 4: Isoquercitrin

sensitive, reliable, and reproducible for simultaneous determination of *R. sachalinensis*. Therefore, the present study provided an example for quality evaluation and identification of medicinal effect of *R. sachalinensis* using the HPLC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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