High Performance Liquid Chromatographic Assay of Saikosaponins from *Radix Bupleuri* in China

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In this study, the quantitative analysis of saikosaponins from *Radix Bupleuri* in China was performed by high performance liquid chromatography. Saikosaponin-a and -d were converted completely into saikosaponin-b₁ and -b₂ by mild acid treatment. Distinctive measuring of these converted diene-saponins provided a rapid and selective method for the determination of saikosaponin-a and -d in commercial samples of *Radix Bupleuri*. The conditions of extraction and conversion of saikosaponins were optimized using orthogonal design L₉(3⁴). The HPLC analysis was performed on ODS-C₁₈ column with a flow rate of 1.0 ml/min and detection wavelength of 250 nm. Well resolved chromatograms of saikosaponin-b₁ and -b₂ were obtained with an isocratic elution of acetonitrile:1% formic acid water (37.5:62.5). Calibration curves of saikosaponin-b₁ and -b₂ were linear in the range of 4.9—98.0 µg/ml and 3.5—71.0 µg/ml, respectively. The average recovery of saikosaponin-b₁ and -b₂ were 98.3% (RSD=3.1%) and 96.4% (RSD=1.8%), respectively. Seventeen samples of different species and habitats of *Radix Bupleuri* were analyzed by the developed HPLC method.

Key words *Radix Bupleuri*; saikosaponin; conversion; orthogonal design; HPLC

Radix Bupleuri (Chinese name, Chaihu) is a well-known traditional Chinese medicine, which mainly grows in the areas of north subtropics. Forty species, seventeen varieties and seven forms grow wide in China and among them seventeen species, six varieties and one forms are considered as medicinal herbs.¹⁾ According to the Chinese pharmacopoeia,²⁾ *Radix Bupleuri* is derived from the dried root of *Bupleurum Chinense* DC. and *Bupleurum scorzonifolium* WILD. In China, it has been used for over 2000 years to treat cold fever, chill and fever in turn, the feeling of oppression and illness in the chest and hypochondria.³⁾ Three major oleanane-saponins named saikosaponin-a (1), -c (2), -d (3) and many minor saponins are isolated from the roots of this plant.^{4—6)} Their structures are shown in Fig. 1. These saikosaponins have been extensively studied in the field of pharmacology and immunology.^{7,8)} The following activities are reported for saikosaponin-a and -d: Anti-cancer,⁹⁾ anti-inflammation,¹⁰⁾ corticosterone secreting,¹¹⁾ plasma-cholesterol lowering action,¹²⁾ hemolytic activity,¹³⁾ effects on membrane fluidity,¹⁴⁾ and protective action against hepatic damage.¹⁵⁾ However, no such biological activities are observed for saikosaponin-c.

Recently, because of the increasing demand as well as a shortage of quality product, many other kinds *Bupleurum* spp. roots, have been used as medicine in different areas of China. Chemical analyses of this crude drug have been reported by thin-layer chromatography (TLC)¹⁶⁾ and high performance liquid chromatography (HPLC).^{17,18)} However, the accuracy and reproducibility of TLC method were not satisfactory. Due to saikosaponin-a (1), -c (2) and -d (3) do not exhibit any absorption maximum in the ultraviolet (UV) region above 220 nm, they were usually analyzed below 210 nm in the most reported HPLC methods. Most of the genuine saikosaponins contain the very unstable allyloxy linkage, therefore are converted into artificial diene-saponins by mild acid treatment¹⁹⁾; saikosaponin-b₁ (4) from saikosaponin-a (1) and saikosaponin-b₂ (6) from saikosaponin-d (3). It has been reported that saikosaponin-b₁ and -b₂ are not the natural components of *Radix Bupleuri* but formed during

the extraction process.²⁰⁾ These converted diene-saponins show characteristic and strong absorption maxima (at 250 nm) and could be easily detected by HPLC-UV with high sensitivity. Therefore, it provides a possible method that can determine the contents of saikosaponin-a and -d by the analysis of their conversion products. This conversion had been applied to analyze the saikosaponin-a and -d,¹⁹⁾ but the reported conversion reaction was not proved to be complete and quantitative. In this study, the conversing conditions were optimized and LC-MS and LC-PDA were applied to prove that saikosaponin-a and -d were converted into saikosaponin-b₁ and -b₂ completely and quantitatively. This optimized method was applied to evaluate the quality of 17 commercial samples.

MATERIALS AND METHODS

Chemical and Reagent Saikosaponin-a, -d, -b₁ and -b₂ were purchased from National institute of China for the Control of Pharmaceutical and Biological Products (with the purity higher than 98.0%). Acetonitrile (Yuwang Co., China) was of HPLC grade. Hydrochloric acid, sodium hydroxide, methanol and ammonia (Yuwang Co., China) were of analytical grade.

Medicinal Materials The seventeen samples of *Radix Bupleuri* were purchased from Liaoning, Jilin, Tianjin, Liaoyuan, Hebei, Shanxi, Shandong, Sichuan, Anhui, Neimeng, Ningxia and Qinghai provinces.

Apparatus Shimadzu LC-10AD high performance liquid chromatograph and a stainless-steel column (4.6 mm×250 mm, ID) packed with Hypersil ODS (5 µm) were used. Data were acquired by SPD-10A and SPD-10AVP with CKChromTM and Shimadzu CLASS VP workstation, respectively.

The LC-MS systems consisted of the LC-10ADvp pump, SIL-HTc autosampler, CTO-10Avp column oven and LCMS-2010A mass spectrometer with an atmospheric pressure chemical ionization (APCI) interface in positive mode (Shimadzu, Kyoto, Japan). Chromatographic separation was

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Fig. 1. Structures of Saikosaponins

(1) Saikosaponin-a; (2) saikosaponin-c; (3) saikosaponin-d; (4) saikosaponin-b₁; (5) saikosaponin-h; (6) saikosaponin-b₂.

achieved on a 150×4.6 mm, 5 μm Diamonsil C₁₈ column (Dikma, Beijing, China). Data acquisition was performed with Shimadzu LCMSsolution 3.0 software.

Chromatographic Conditions Isocratic elution of the HPLC system was performed with acetonitrile–water–formic eluents of different ratio (50 : 50; 40 : 60; 37.5 : 62.5; 35 : 65). In these cases, the column temperature was maintained at 25 °C; the mobile phase contained acetonitrile : 1% formic acid water (37.5 : 62.5); the flow rate was 1.0 ml/min. The injection volume and the detection wavelength were 20 μl and 250 nm, respectively.

For LC-PDA, the chromatographic conditions were the same as described above.

For LC-MS, the mobile phase contained acetonitrile : 1% formic acid water (37.5 : 62.5) and the flow rate was 0.8 ml/min. The conditions of mass spectrometer were as follows: APCI interface temperature 400 °C, CDL temperature 250 °C, Heat Block temperature 200 °C, detect voltage 1.5 kV. Total Ion Chromatogram (TIC) mode was used and the scan range of *m/z* was 200–1000. N₂ was used as nebulizing gas and drying gas with a flow rate of 2.5 l/min and 2 l/min, respectively.

Extraction Procedure About 0.5 g of the pulverized *Radix Bupleuri* was weighed and mixed with 15 ml of methanol. The mixture was extracted by ultrasonic for 3 times, 1 h each time. The extracted solution was filtered and the filtrate was evaporated to dryness under the reduced pressure at 60 °C.

Conversion Procedure The extracted samples were treated with 8% HCl in 50% methanol solution, then incubated at 25 °C for 14 h. After 14 h, the solution was adjusted to pH 7 with 8% NaOH solution and dissolved to 25 ml with

methanol. The solution was filtered through 0.45 μm organic membrane filters. A 20 μl aliquot of sample was injected into the HPLC.

Saikosaponin-a 10.1 mg and saikosaponin-d 9.9 mg were converted into corresponding saikosaponin-b₁ and -b₂ under the described above conditions.

Standard Solutions Saikosaponin-a and saikosaponin-d were dissolved with methanol to make a solution containing 1.01 mg/ml saikosaponin-a and 0.45 mg/ml saikosaponin-d, respectively.

Saikosaponin-b₁ and saikosaponin-b₂ were added into a 10 ml measuring flask and dissolved with methanol to make a stock solution containing 0.98 mg/ml saikosaponin-b₁ and 1.03 mg/ml saikosaponin-b₂, respectively.

Then 0.1 ml saikosaponin-b₁ and -b₂ were added into a 5 ml measuring flask and dissolved with methanol to make standard solutions of 0.02 mg/ml and 0.021 mg/ml, respectively.

Method Validation Calibration standards for saikosaponin-b₁ and -b₂ (5, 20, 40, 60, 80, 100 μl) were prepared from stock solutions. Calibration curves were calculated by plotting the peak area of analyte *versus* analyte concentration. Stability of the derivatives was examined for 2 d room temperature. The assay precision was determined by 6 replicate analysis of the sample, while the recovery was evaluated simultaneously.

RESULTS AND DISCUSSION

Optimization of Conversion Conditions Generally, conversion methods involve the investigation of many variables, which may affect the efficiency of conversion. Selec-

tion of these variables and their levels is critical. Several statistical techniques, such as simplex optimization and factorial design, were employed for the optimization of experiment methods. Factorial design has some advantages over simplex optimization in that global optimum can be provided, large amounts of quantitative information can be extracted and both discrete and continuous factors can be estimated. One obvious disadvantage of the factorial design is the large number of experiments required when several variables are examined. However, the number of the experiments can be considerably reduced by the use of orthogonal array design. In our study, $L_9(3^4)$ orthogonal design was selected. $L_9(3^4)$ means that 4 factors and 3 levels can be investigated in the orthogonal tests. But it was not necessary that every factors was choose, we can only choose three or two factors, and the others rows were considered as the error item. Error item was necessary to do analysis-of-variance analysis. Conversion temperature, time and the concentration of hydrochloric acid could impact the conversion results. In order to obtain the best conditions, the above factors were optimized. The three factors of the concentration of HCl (A), the reaction temperature (B) and reaction time (C) were arranged in an orthogonal design $L_9(3^4)$ and the fourth row was considered as the error item. Each factor had three levels: factor A (A_1 , 1%; A_2 , 4%; and A_3 , 8%); factor B (b_1 , 30 °C; B_2 , 20 °C; and B_3 , 25 °C); factor C (C_1 , 14 h; C_2 , 24 h; and C_3 , 8 h). In our study, before sample solutions were analyzed, the standard solution of saikosaponin- b_1 and - b_2 were analyzed first. The A_i represents the peak area of saikosaponin- b_1 and - b_2 in sample solutions; The A_s represents the peak area of saikosaponin- b_1 and - b_2 in standard solutions; In order to predigest the calculation progress, the ratio of peak area (A_i/A_s) was applied. Therefore, A_i/A_s does not have unit, and it is just a ratio. So, all of the data in our Table 1 does not have unit too. The ratio of peak area (A_i/A_s) of saikosaponin- b_1 and that of saikosaponin- b_2 were selected as the assessment indices of conversion conditions. Nine experiments were performed. The results and details of each procedure are shown in Table 1-1. From results of the intuitionistic analysis (Table 1-2) and analysis-of-variance analysis (Table 1-3), it could be found that the concentration of HCl played more important role than the reaction time and reaction temperature. $A_3B_3C_1$ (8% HCl, 25 °C and 14 h) was selected as the optimal condition. No hydrolysis of the sugar moiety took place under these conditions, whereas the formation of the prosapogenins was observed at elevated temperature (over 30 °C) or under stronger acidic conditions. In order to investigate whether saikosaponin-a and -d had been converted completely into the corresponding diene-saikosaponins, the contents of b_1 and b_2 were determined. The results showed that the amounts of saikosaponin- b_1 and saikosaponin- b_2 were equivalent to those of saikosaponin-a and -d before being converted, which means that, saikosaponin-a and -d had been completely converted under above conditions.

Optimization of Extraction Conditions Various extraction procedures were compared by measuring the amounts of saikosaponin- b_1 and - b_2 extracted from Radix Bupleuri. Saikosaponin-a and -d are extremely unstable, being partly converted into the diene-saponins along with other secondary products during the process of the extraction of the crude drug with boiling methanol. Therefore, extraction at low

Table 1-1. Arrangement and Results of Orthogonal Test for Conversion Reaction

Test No.	A	B	C	Error	SS- b_2 (A_i/A_s)	SS- b_1 (A_i/A_s)
1	1 (1%)	1 (30 °C)	1 (14 h)	1	0.811	1.058
2	1 (1%)	2 (20 °C)	2 (24 h)	2	0.779	0.641
3	1 (1%)	3 (25 °C)	3 (8 h)	3	0.803	0.465
4	2 (4%)	1 (30 °C)	2 (24 h)	3	0.893	1.583
5	2 (4%)	2 (20 °C)	3 (8 h)	1	0.830	1.155
6	2 (4%)	3 (25 °C)	1 (14 h)	2	0.982	1.722
7	3 (8%)	1 (30 °C)	3 (8 h)	2	0.949	1.583
8	3 (8%)	2 (20 °C)	1 (14 h)	3	1.102	1.783
9	3 (8%)	3 (25 °C)	2 (24 h)	1	1.096	2.172

A=the concentration of HCl; B=the reaction temperature; C=reaction time; SS- b_2 =saikosaponin- b_2 ; SS- b_1 =saikosaponin- b_1 ; A_i =peak area of saikosaponins of samples solution; A_s =peak area of saikosaponins of standard solution; A_i/A_s =ratio of peak area.

Table 1-2. Intuitionistic Analysis Results of Orthogonal Test for Conversion Reaction

Index	Level	Factors			Optimal condition
		A	B	C	
SS- b_2	I	2.393	2.653	2.895	$A_3B_3C_1$
	II	2.706	2.712	2.769	
	III	3.147	2.883	2.583	
	R	0.753	0.231	0.312	
SS- b_1	I	2.163	4.224	4.563	$A_3B_3C_1$
	II	4.461	3.579	4.395	
	III	5.538	4.359	3.204	
	R	3.375	0.78	1.359	

I=the sum of 1 level of each factor; II=the sum of 2 level of each factor; III=the sum of 3 level of each factor; R=the range of each factor.

Table 1-3. ANOVA Results of Orthogonal Test for Conversion Reaction

	Source of variance	Sum of squares	Degrees of freedom	Mean square	F value	p value
SS- b_2	A	0.096	2	0.048	73.77	$p < 0.05$
	B	0.009	2	0.005	7.23	
	C	0.017	2	0.008	12.69	
	Error	0.001	2	0.001		
SS- b_1	A	1.978	2	0.989	34.77	$p < 0.05$
	B	0.116	2	0.058	2.04	
	C	0.367	2	0.183	6.44	
	Error	0.057	2	0.028		

$F_{0.01}(2, 2)=99$; $F_{0.05}(2, 2)=19$.

temperature was bound to minimize the formation of other products. The ultrasonic extraction was selected to extract the saikosaponins Radix Bupleuri due to the low temperature. The repeated orthogonal design $L_9(3^4)$ was adopted to optimize the ultrasonic extraction methods of saikosaponins. In our study, before sample solutions were analyzed, the standard solution of saikosaponin- b_1 and - b_2 were analyzed first. The A_i represents the peak area of saikosaponin- b_1 and - b_2 in sample solutions; The A_s represents the peak area of saikosaponin- b_1 and - b_2 in standard solutions; In order to predigest the calculation progress, the ratio of peak area (A_i/A_s) was applied. Therefore, A_i/A_s does not have unit, and it is just a ratio. So, all of the data in our Table 2 does not have unit too. The ratio of peak area (A_i/A_s) of saikosaponin- b_1 and that of

Table 2-1. Arrangement and Results of Orthogonal Test for Extraction Procedures (Repeated Test)

Test No.	A	B	C	D	SS-b ₂ (A ₁ /A _s)		SS-b ₁ (A ₁ /A _s)	
1	1 (10 ml)	1 (1 h)	1 (3%)	1 (1 time)	2.870	2.855	6.759	6.750
2	1 (10 ml)	2 (2 h)	2 (0%)	2 (2 times)	3.283	3.427	7.290	7.443
3	1 (10 ml)	3 (0.5 h)	3 (6%)	3 (3 times)	3.221	2.784	7.102	6.254
4	2 (15 ml)	1 (1 h)	2 (0%)	3 (3 times)	3.452	3.592	7.979	7.504
5	2 (15 ml)	2 (2 h)	3 (6%)	1 (1 time)	2.826	2.903	6.377	6.644
6	2 (15 ml)	3 (0.5 h)	1 (3%)	2 (2 times)	2.710	3.006	6.284	6.912
7	3 (20 ml)	1 (1 h)	3 (6%)	2 (2 times)	3.246	3.341	7.060	6.913
8	3 (20 ml)	2 (2 h)	1 (3%)	3 (3 times)	3.016	3.037	6.652	7.165
9	3 (20 ml)	3 (0.5 h)	2 (0%)	1 (1 time)	2.420	2.340	6.102	5.809

A=the amount of methanol; B=extraction time; C=concentration of ammonia in methanol; D=extraction times; SS-b₂=saikosaponin-b₂; SS-b₁=saikosaponin-b₁; A₁=peak area of saikosaponins of samples solution; A_s=peak area of saikosaponins of standard solution; A₁/A_s=ratio of peak area.

Table 2-2. Intuitionistic Results of Orthogonal Test for Extraction Procedures

Index	Level	Factor				Optimal condition
		A	B	C	D	
SS-b ₂	I	18.439	19.356	17.494	16.214	A ₂ B ₁ C ₂ D ₃
	II	18.489	18.491	18.513	19.012	
	III	17.401	16.481	18.321	19.102	
	R	0.363	0.958	0.340	0.963	
SS-b ₁	I	41.598	42.965	40.523	38.440	A ₂ B ₁ C ₂ D ₃
	II	41.700	41.570	42.127	41.901	
	III	39.701	38.464	40.350	42.657	
	R	0.666	1.501	0.592	1.406	

I=the sum of 1 level of each factor; II=the sum of 2 level of each factor; III=the sum of 3 level of each factor; R= the range of each factor.

Table 2-3. ANOVA Results of Orthogonal Test for Extraction Procedures

	Source of variance	Sum of squares	Degrees of freedom	Mean square	F value	p value
SS-b ₂	A	0.126	2	0.063	3.31	p<0.01
	B	0.725	2	0.363	19.11	
	C	0.098	2	0.049	2.57	p<0.01
	D	0.899	2	0.450	23.69	
	Error	0.171	9	0.019		
SS-b ₁	A	0.422	2	0.211	2.11	p<0.01
	B	1.770	2	0.885	8.83	
	C	0.320	2	0.160	1.60	p<0.01
	D	1.685	2	0.843	8.41	
	Error	0.903	9	0.100		

$F_{0.01}(2, 9)=8.02$; $F_{0.05}(2, 9)=4.26$.

saikosaponin-b₂ were selected as the assessment indices of extraction conditions. The amount of methanol (A), extraction time (B), concentration of ammonia in methanol (C) and extraction times (D) were studied, respectively. Each factor had three levels: factor A (A₁, 10 ml; A₂, 15 ml; and A₃, 20 ml); factor B (B₁, 1 h; B₂, 2 h; and B₃, 0.5 h); factor C (C₁, 3%; C₂, 0%; and C₃, 6%); and factor D (D₁, 1; D₂, 2; and D₃, 3). The orthogonal table was full filled. The repeated orthogonal test was applied to obtain the error item. Eighteen experiments were performed. Results and details of each procedure are shown in Table 2-1. From intuitionistic analysis (Table 2-2) and analysis-of-variance analysis (Table 2-3), a

Fig. 2. (a) Chromatogram of a Standard Mixture Containing Saikosaponin-b₁ and -b₂ at 250 nm and (b) Chromatogram of Samples of Radix Bupleuri Which Was not Treated by Mild Acid at 250 nm

conclusion was made that extraction duration and times played more important roles than other factors in the extraction procedure. The following optimal condition was selected: 15 ml methanol, 0% consumption of ammonia in methanol, and 3 times of extraction, 1 h each time (A₂B₁C₂D₃).

Chromatographic Analysis Saikosaponin-b₁ and -b₂ were not the natural components of Radix Bupleuri but were formed during the extraction process. The ultrasonic extraction method was chosen to extract the saikosaponins. The saikosaponin-b₁ and -b₂ were not detected when the extract was analyzed directly by HPLC before it was treated by mild acid. Figure 2 shows that natural saikosaponins of Radix Bupleuri did not contain saikosaponin-b₁ and -b₂ and the ultrasonic extraction could prevent the production of -b₁ and -b₂ during the extraction process.

In order to identify the conversion products, diene-saikosaponins, LC-MS was applied to analyze standard the solution of saikosaponin-b₁ and -b₂, and conversion products. The results are shown in Fig. 3. Mass spectrums of the conversion products were similar to those of saikosaponin-b₁ and -b₂. It was shown that saikosaponin-a and -d were converted into saikosaponin-b₁ and -b₂, respectively.

The standard solution of saikosaponin-a, -d, -b₁, -b₂ and converted -a and -d were also analyzed by LC-PDA. The maximal absorbance wavelength of -b₁ and -b₂ were 250 nm, and saikosaponin-a and -d were 208 nm, respectively. After saikosaponin-a and -d were treated under the optimization conversion condition, saikosaponin-a and -d disappeared and -b₁ and -b₂ were detected at the 208 nm chromatogram. The Fig. 4 show the results. It confirmed that conversion reaction was complete and no hydrolysis of the sugar moiety took place under the conversion conditions.

To assay saikosaponins in Radix Bupleuri with good resolution and a reasonable elution time, various isocratic elution using acetonitrile–water were performed as described in Experimental. The optimal analysis condition found was acetonitrile:1% formic acid water (37.5:62.5). The chromatograms are shown in Fig. 5.

Linearity, Accuracy, Precision, and Limits of Quantification Using the method described above, a validation

Fig. 3. Mass Spectrum of Saikosaponins

(E) Saikosaponin- b_1 ; (F) saikosaponin-a which was treated by mild acid; (G) saikosaponin- b_2 ; (H) saikosaponin-d which was treated by mild acid.

Fig. 4. Chromatograms of SS-a, SS-d and SS- b_1 , SS- b_2 at 208 nm and 250 nm

(a) Chromatogram of SS-a at 208 nm; (b) chromatogram of SS- b_1 converted from SS-a at 250 nm; (c) chromatogram of SS- b_1 (4) converted from SS-a (1) at 208 nm; (d) chromatogram of SS-d at 208 nm; (e) chromatogram of SS- b_2 converted from SS-d at 250 nm; (f) chromatogram of SS- b_2 (4) converted from SS-d (1) at 208 nm.

study on the analysis of diene-saponins was carried out. Linearity was assessed by analyzing a series of concentrations of saikosaponin- b_1 and - b_2 . The calibration curves were linear in the range of 4.9–98.0 $\mu\text{g/ml}$ and 3.5–71 $\mu\text{g/ml}$ with the correlation coefficients of 0.9996 and 0.9999 for saikosaponin- b_1 and - b_2 , respectively.

The lowest acceptable level of the calibration curve was regarded as the limit of quantification (LOQ). In this study, the LOQ of saikosaponin- b_1 and - b_2 was 4.9 and 3.5 $\mu\text{g/ml}$, respectively. The intra-day variability of the contents of saikosaponins was determined using the same sample solution. The relative standard deviations (R.S.D.) were 2.0% for - b_1 and 1.7% for - b_2 . The reproducibility of the total procedure for - b_1 and - b_2 was tested and the R.S.D. ($n=6$) were 1.7% for - b_1 and 2.2% for - b_2 . The results showed excellent stability and reproducibility, respectively. The average recovery of - b_1 and - b_2 was 98.3% (R.S.D.=3.1%) and 96.4% (R.S.D.=1.8%), respectively.

Estimation of Habitats and Species for Radix Bupleuri

Recently, due to the increasing demand for this herb, many kinds of Radix Bupleuri, which were collected from different habitats, have been collected as medicine. As we know that the diversity of growing environment induces the differences of herbal quality. Therefore, we collected 17 commercial samples from the main habitats of Radix Bupleuri which be-

Fig. 5. Chromatogram of Sample of Radix Bupleuri Which Was Treated by Mild Acid

Table 3. Contents of Saikosaponins in 17 Radix Bupleuri Samples from Different Habitats

Sample	Origin	Scientific name	Content (mg/g)	
			SS-a	SS-d
1	Liaoning	<i>B. chinense</i> DC.	0.674	0.570
2	Dongbei	<i>B. scorzonrifolium</i> WILLD.	0.046	0.106
3	Jilin	<i>B. scorzonrifolium</i> WILLD.	1.252	0.934
4	Hebei	<i>B. chinense</i> DC.	1.134	1.093
5	Liaoyuan	<i>B. scorzonrifolium</i> WILLD.	0.451	0.552
6	Shandong	<i>B. scorzonrifolium</i> WILLD.	1.521	1.656
7	Shanxi	<i>B. chinense</i> DC.	1.724	1.785
8	Guangxi	<i>B. chinense</i> DC.	1.413	1.916
9	Sichuan	<i>B. malconense</i> SHAN et Y. LI	—	—
10	Qinghai	<i>B. longicaule</i> var. <i>qiraldii</i>	0.249	0.372
11	Henan	<i>B. chinense</i> DC.	3.904	4.581
12	Neimeng	<i>B. bicaule</i> HELM	1.093	0.534
13	Shanxi	<i>B. yinchowense</i> SHAN et Y. LI	0.520	0.704
14	Yinchuan	<i>B. yinchowense</i> SHAN et Y. LI	1.879	1.704
15	Tianjin	<i>B. chinense</i> DC.	1.306	1.600
16	Shanxi	<i>B. bicaule</i> HELM	1.729	1.912
17	Neimeng	<i>B. smithii</i> WOLFF var. <i>parvifolium</i>	0.431	0.844

SS-a=saikosaponin-a; SS-d=saikosaponin-d.

long to 7 species and evaluated their qualities. The results were showed in Table 3. The saikosaponins contents of *B. chinense* DC. were the highest in all the species of Radix Bupleuri. Both *B. scorzonrifolium* WILLD and *B. bicaule* HELM contained the higher saikosaponins than the others. But no saikosaponin-a and -d were detected in the *B. malconense* SHAN et Y. LI from Sichuan province. The contents of saikosaponins of six *B. chinense* DC. samples, which were collected from different habitats, were evaluated by the HPLC method established in the present study. The saikosaponin's contents of *B. chinense* DC. from Henan province reached a highest value of 3.904 mg/g (SS-a) and 4.581 mg/g (SS-d), respectively. On the other hand, the contents of Saikosaponins from four *B. scorzonrifolium* WILLD samples were detected by the same method. The contents of saikosaponins of *B. scorzonrifolium* WILLD from Shandong province reached 1.521 mg/g (SS-a) and 1.656 mg/g (SS-d), respectively. The content of saikosaponins in *B. chinense* DC. were generally higher than that of in *B. scorzonrifolium* WILLD. This conclusion was accordant with the previous reports.¹⁾ In conclusion, the contents of saikosaponins depended on not

only the species but also the habitats. Therefore, it is necessary to control the quality of the Radix Bupleuri from different species and habitats.

CONCLUSION

In the present study, the conversion method of saikosaponin-a and -d was optimized and the conversion reaction was proved to be completed using LC-MS and LC-PDA. The HPLC method was developed to determine the conversion products, saikosaponin-b₁ and -b₂. The results showed that a good separation (concerning resolution and analysis time) is obtained by isocratic elution employing acetonitrile:1% formic acid water (37.5:62.5). The HPLC method established in the present study was presumed to be a reliable method for analysis of the contents of saikosaponins in different species and habitats of Radix Bupleuri. It was confirmed that this method could be used as a routine analytical tool for the quantitative analysis of saikosaponin-a and -d in Radix Bupleuri.

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