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Quality Evaluation of Alismatis Rhizoma by High Performance Liquid Chromatography

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The quality of Alismatis Rhizoma was evaluated by reversed-phase high performance liquid chromatographic method. Alisol B 23-acetate was used as a standard marker for evaluation. This component was fully separated from the other components in the plant extracts on a ODS column. Identification of alisol B 23-acetate was carried out by comparing the LC/MS spectrum of separated peak from the extract with that of standard. Alisol B 23-acetate contents in Alismatis Rhizoma obtained from several herbal markets were varied from 0.15% to 0.56%.

Key words: Alismatis Rhizoma, Alisol B 23-acetate, HPLC, Quality evaluation

INTRODUCTION

Alismatis Rhizoma is the tuber of *Alisma plantago-aquatica* var. *orientale* (Alismataceae), from which periderm has been usually removed. The external appearance of this herbal drug is a spherical or conical, 3-8 cm in length, 3-5 cm in diameter, showing sometimes a 2- to 4-branched irregular tuber. This plant has been used in Korea and China as a diuretic agent (Bae, 2000). Protostane type triterpens (Murata *et al.*, 1970, Fukuyama *et al.*, 1998), guaiane type sesquiterpenes (Yoshikawa *et al.*, 1992) and kaurane type diterpenes (Yamaguchi *et al.*, 1994) have been reported as the main components from this plant. Orientalol A, -C, guaiane sesquiterpenes showed diuretic activity (Yoshikawa *et al.*, 1992, 1994a) and alisols, protostane type triterpenes were characterized to possess anti-hypertentive (Yamaguchi *et al.*, 1994), anti-complement activity (Matsuda *et al.*, 1998; Lee *et al.*, 2003), and repairing action to cholinergic acetyl transferase (Yamahara *et al.*, 1989).

In the previous paper, we reported the isolation and cytotoxic activity of four protostane-type triterpenes, alisol B 23-acetate, alisol C 23-acetate, alisol B, and alisol A 24-acetate, from this plant. It had been reported that alisol B 23-acetate as main component of some Alismatis species, showed cytotoxic activity against L1210, K562, A549, SK-OV3, B16-F10, and HT1080 tumor cell lines (Lee *et al.*, 2001). The importance of alisol B 23-acetate is increased for the development of new lead compound in some biological activities. For the purpose of the effective utilization of natural resources we tried to evaluate the quality of the Alismatis Rhizoma by means of determining alisol B 23-acetate. There were only a few reports for the evaluation of this plant (Yoshikawa *et al.*, 1994[b]). In this report a modified HPLC method for the dertermination of alisol B 23-acetate was described and applied to some Alismatis Rhizome samples.

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MATERIALS AND METHODS

Instruments and chemicals

The chromatographic system for quantitative analysis consisted of a SCL-10A system controller, an LC-10AD pump, a SPD-10MVP diode array detector (Shimadzu, Japan), column temperature controller (Waters, CA, USA) and a Rheodyne 7725 injector with a 20 μL sample loop. For qualitative analysis a 5989B LC/MSD system (Hewlett-Packard, USA) with a 59987A electrospray interface, G1312A binary pump and G1315A photo diode array detector were used. Methanol (Tedia, OH, USA), acetonitrile

(J. T. Baker, NJ, USA.) used in this work were of HPLC grade and other reagents were of analytical grade. Milli-Q (Millipore, MA, USA) treated water (with resistivity more than 18 MΩ cm) was used throughout the experiment.

Preparation of alisol B 23-acetate standard

Alisol B 23-acetate was not commercially available, hence it was isolated from the Alismatis Rhizoma as reported previously (Lee *et al.*, 2001) and identified by ^{1}H-NMR, ^{13}C-NMR and MS. The purity confirmed by HPLC was more than 99.5%.

Materials and sample preparation

The samples of Alismatis Rhizoma (Alis-01~Alis-08) were collected from 8 different herbal markets in Korea. They were identified by one of us (K. Bae) in the College of Pharmacy, Chungnam National University (CNU), Korea. The voucher specimens were deposited at the herbarium in the College of Pharmacy, CNU.

The air dried and coarsely powdered sample (0.5 g) was extracted with 10 mL of acetonitrile under ultrasonic bath for 30 min, 2 times at 50°C. The extract was evaporated to dryness under vacuum and the residue was dissolved in 2 mL methanol. The solution was centrifuged at 13,000 rpm for 10 min and the supernatant was injected to HPLC.

Chromatography and identification

The HPLC separation of alisol B 23-acetate for qualitative and quantitative analysis was performed using a reverse phase system. A Zorbax 300SB C18 (4.6×150 mm, Hewlett-Packard Co., CA, USA) column with 75% acetonitrile at a flow rate 1.0 mL/min was used. Detection was carried out at UV 215 nm. The identification of the components in the standard solution and sample was carried out by analyzing the mass spectrum of peak corresponding to alisol B 23-acetate in the HPLC effluent.

RESULTS AND DISCUSSION

Separation and identification of alisol B 23-acetate

To select an optimal mobile phase composition for the analysis of alisol B 23-acetate from Alismatis Rhizoma, several HPLC runs with various ratio of acetonitrile in water as mobile phase were performed. A solution of 75% acetonitrile in water was selected for mobile phase based upon the capacity factor of alisol B 23-acetate and resolution between alisol B 23-acetate and neighboring components in Alismatis Rhizoma (Fig. 2). A chromatogram of authentic alisol B 23-acetate and an extract of Alismatis Rhizoma obtained by the selected condition is shown in Fig. 2, which indicates the base line separation of alisol B 23-acetate and neighboring components in Alismatis

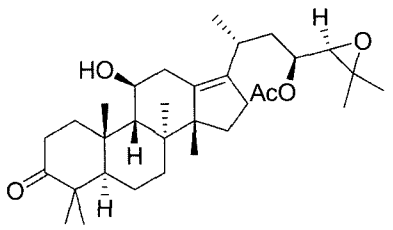

Fig. 1. Chemical structure of alisol B 23-acetate

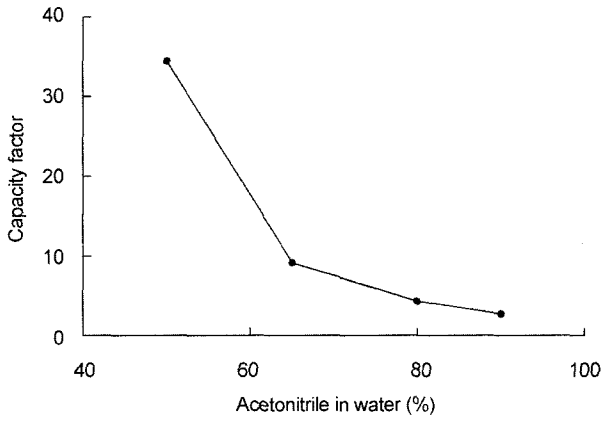

Fig. 2. Capacity factor of alisol B 23-acetate with various concentration of acetonitrile in water on Zorbax 300SB C_{18} column

Rhizoma. The peak, appeared in about 4.3 min, on the chromatogram of Alismatis Rhizoma extract (Fig. 3b) could be confirmed as alisol B 23-acetate by comparing retention times and mass spectra with those of authentic sample (Fig. 4). The base peak was found at m/z 537.3, supposed to be a sodium ion adduct of alisol B 23-acetate. The characteristic peaks, such as $[M+H]^+$ (m/z 515.2), $[M-OH]^+$ (m/z 497.2) and $[M+K]^+$ (m/z 553.2) were also found.

Extraction efficiency of Alisol B 23-acetate

The coarsely powdered Alismatis Rhizoma was extracted with several solvents, namely, chloroform, ethyl acetate, acetonitrile, methanol and 50% methanol in water. Among these solvents acetonitrile showed the best extraction efficiency (Fig. 5), hence acetonitrile was selected as standard extraction solvent. To optimize the extraction temperature and time, alisol B 23-acetate was extracted at different temperature and time span. As shown in Fig. 6, alisol B 23-acetate could not fully extracted in acetonitrile at 25°C even more than 2 h of extraction time. The extraction efficiency at 50°C was better than that at 25°C, while the extraction efficiency was decreased at 50°C when extracted longer than 30 min. That means alisol B 23-acetate in Alismatis Rhizoma could be extracted at the highest extent in acetonitrile for 30 min at 50°C.

Linearity, precision and accuracy of the method

The calibration function of alisol B 23-acetate standard

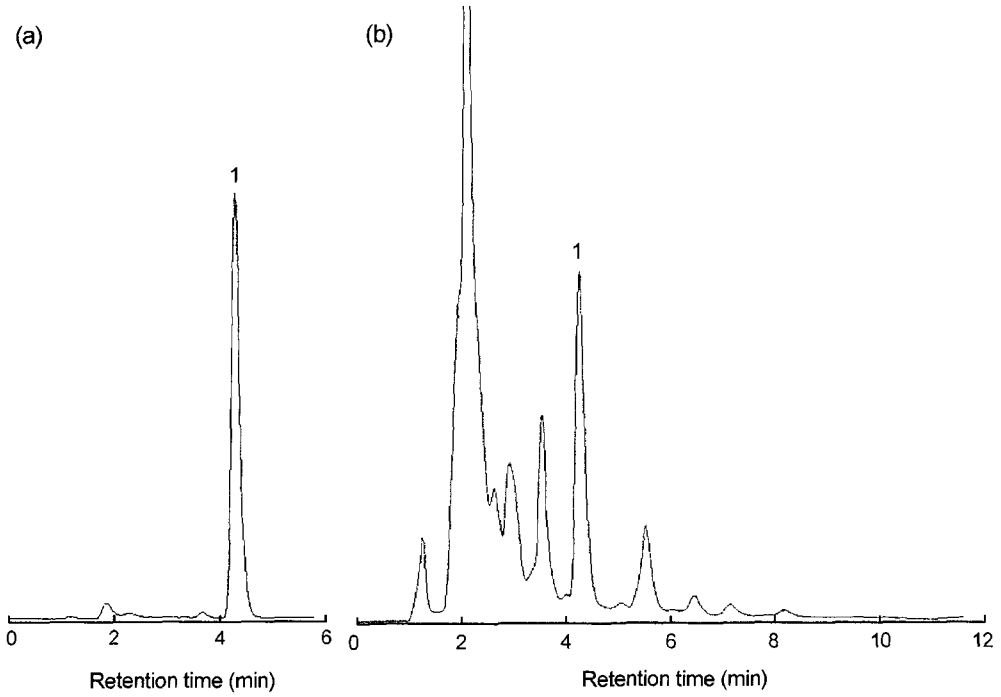

Fig. 3. Chromatograms of (a) a standard solution and (b) an extract of Alismatis Rhizoma separated on Zorbax 300SB C_{18} column with 75% acetonitrile. Peak 1 : Alisol B 23-acetate.

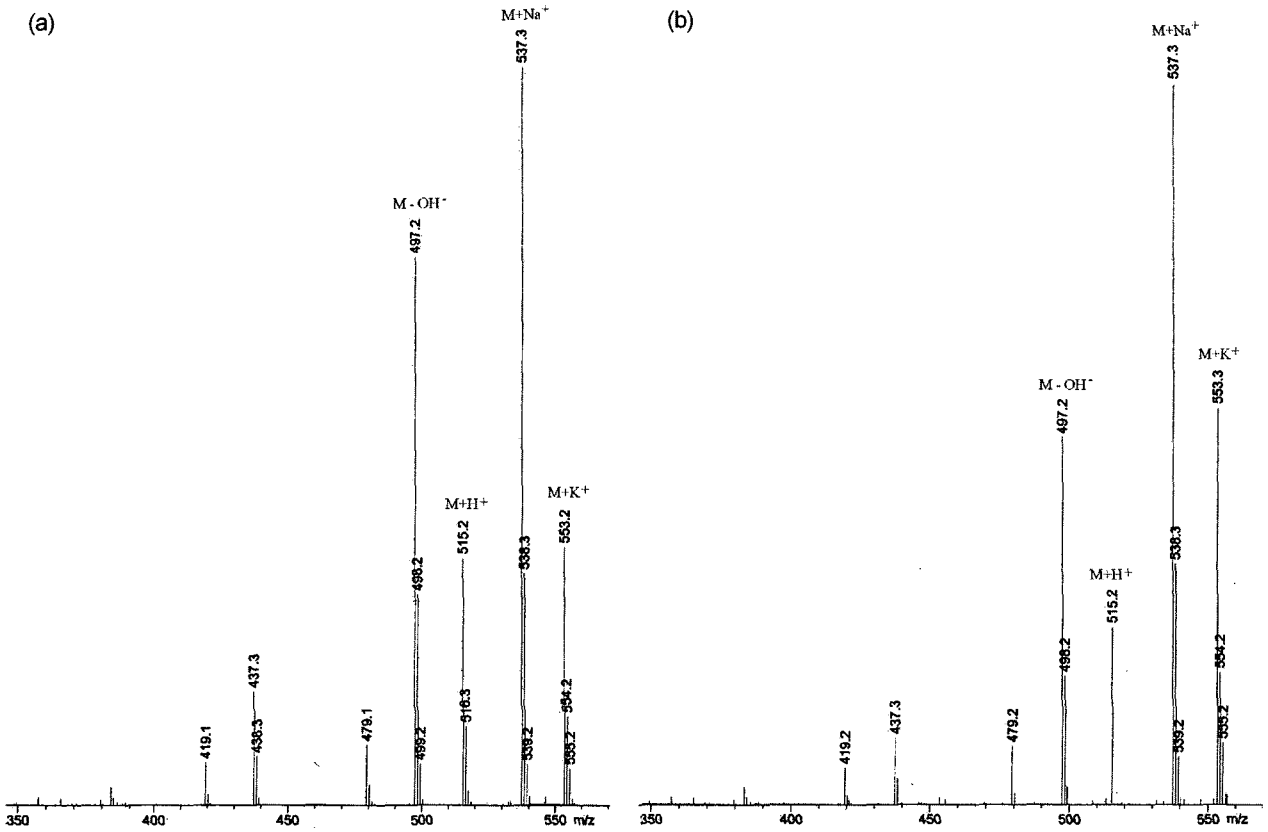

Fig. 4. Mass spectra of separated peaks (R_t~4.3 min) of (a) a standard solution and (b) an extract of Alismatis Rhizoma. MS conditions: Ionization; ESI-Positive, Quadrupole temp.; 99°C, Fragment voltage; 80 V, Nebulizer; nitrogen 9 L/min, 345°C, 40 psi.

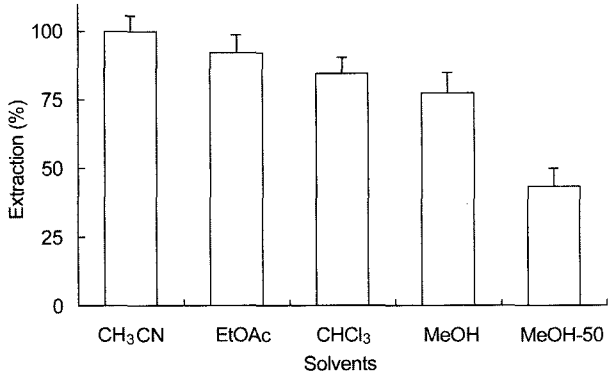

Fig. 5. Effects of solvent on the extraction efficiency of alisol B 23-acetate from Alismatis Rhizoma. The relative extraction efficiency was presented in compare with that of acetonitrile. EtOAc; ethyl acetate, MeOH; methanol, MeOH-50; 50% methanol.

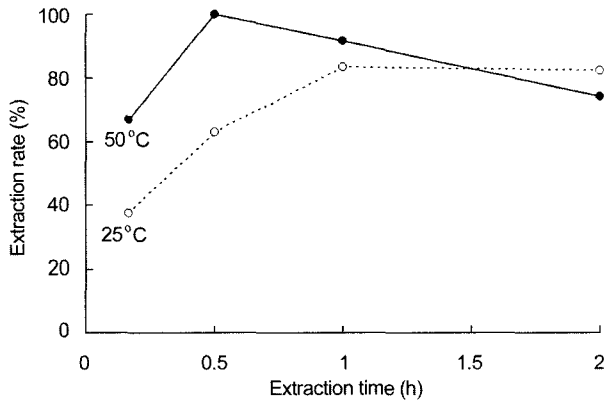

Fig. 6. Effects of extraction time and temperature on the extraction efficiency of alisol B 23-acetate at 25°C (-○-) and 50°C (-●-) in acetonitrile as a solvent from Alismatis Rhizoma. The relative extraction efficiency was calculated in compare with that at 50°C for 30 min.

calculated with peak area (y, mAU) and concentration (x, mg/mL) was $y = 2.32x + 0.01$ ($r^2 = 0.9996$), over the concentration range 0.05 to 1 mg/mL. The detection limit of alisol B 23-acetate was 0.5 μg/mL at a signal to noise ratio of 3. Table I showed the precision and accuracy of this method. The intraday precision of the analysis was obtained from the separated injections of four different concentrations of standard (0.05, 0.1, 0.5 and 1.0 mg/mL). Inter day precision was also calculated from the analysis results of the standards in 5 consecutive days. The mean values of the intraday and inter day precisions were 1.53% and 2.98%, respectively. The mean accuracy of this method was 98.4% over the range of 0.05 to 1.0 mg/mL.

Analysis of alisol B 23-acetate in Alismatis Rhizoma

Alisol B 23-acetate in Alismatis Rhizoma obtained from several herbal markets in Korea was analyzed using the reversed-phase HPLC method. The variation in the contents of alisol B 23-acetate in Alismatis Rhizoma is presented in Table II. The content of alisol B 23-acetate in Alismatis Rhizoma ranged from 0.15% to 0.56% with mean value of 0.313%. As shown in the box plot (Fig. 7), the alisol B 23-acetate in Alis-08 was somewhat high in comparison with that in the other sample indicating that Alis-08 was not typical of the rest of the samples. The mean content of alisol B 23-acetate in Alismatis Rhizoma except Alis-08 was 0.277%.

Table I. The intraday and inter day precisions of the peak area of alisol B 23-acetate analyzed by HPLC method

Standard (mg/ml)	Intraday		Inter day		Accuracy (%)
	Mean	CV (%)	Mean	CV (%)	
0.05	0.55	2.11	0.05	2.31	93.6
0.10	0.09	1.80	0.10	5.63	94.0
0.50	0.52	1.21	0.53	2.41	104.4
1.00	1.02	1.00	1.02	1.56	101.7

The results from 3 experiments. CV: Coefficence of variation.

Table II. Content of alisol B 23-acetate in Alisma Rhizoma

Sample No.	Herbal maket	Content (%)
Alis-01	KD	0.461±0.046
Alis-02	DS	0.317±0.093
Alis-03	BJD	0.203±0.019
Alis-04	SJ	0.150±0.016
Alis-05	SK	0.282±0.026
Alis-06	SO	0.277±0.013
Alis-07	SSD	0.250±0.043
Alis-08	SJ	0.560±0.073

Data were given as mean ± S.D. (n = 3-4) in % based on dried sample.

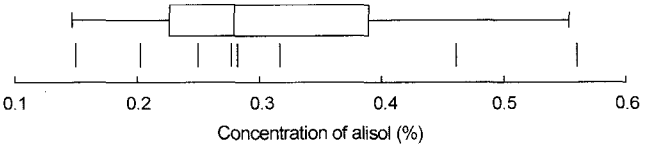

Fig. 7. One-way scatter plot and box plot for the distribution of alisol B 23-acetate from Alismatis Rhizoma.

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