Natural Product Sciences
14(3) : 152-155 (2008)

Determination of Alisol B 23-acetate and Alisol C 23-acetate in Alismatis Rhizoma by HPLC–ESI-MS

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Abstract – An HPLC–ESI-MS method has been developed to identify and quantify two main tetracyclic triterpenes, alisol B 23-acetate and alisol C 23-acetate in the Alismatis Rhizoma (Taeg-Sa). The relative distribution of the two triterpenes in the methanolic extract of commercially available Alismatis Rhizoma was established by selective ion monitoring (SIM) mode via electrospray ionization (ESI) source. Regression equations revealed good linear relationship, and the correlation coefficients were 0.999 and 0.998 for alisol B 23-acetate and alisol C 23-acetate, respectively, between the peak areas of the components and their concentration in a range of 0.06 – 2.0 μg/mL. It was found that there were significant differences in the amount of alisol B 23-acetate and alisol C 23-acetate between Korean and Chinese origins. The results showed that this method could be used to identify the two components in Alismatis Rhizoma with high sensitivity and selectivity.
Keywords – HPLC–ESI-MS, Alismatis Rhizoma, tetracyclic triterpenes, alisol B 23-acetate, alisol C 23-acetate

Introduction

Alismatis Rhizoma (Taeg-Sa, 澤瀉) is the corm of *Alisma orientale* (G. Samuelsson) Juz. or *A. plantago-aquatica* L. (Alismataceae), of which lateral roots and epidermis are removed before drying (Toh, 1995).

It has been reported that the main secondary metabolites of Alismatis Rhizoma are sesquiterpenes, diterpenes, and triterpenes such as alisol A, alisol A 24-acetate, alisol B, alisol B 23-acetate, alisol C and alisol C 23-acetate (Yamaguchi *et al*., 1994; Fukuyama *et al*., 1988).

Alismatis Rhizoma has been used for several oriental herbal preparations such as Taeg-Sa-San, O-Ryeong-San, and is known to have various biological activities including diuretic, cholesterol lowering, anti-allergic, anti-inflammatory and antibacterial activity. Especially, the main component, alisol B 23-acetate, is known to participate in these biological effects (Makino *et al*., 2002; Matsuda *et al*., 1999; Matsuda *et al*., 1998; Kubo *et al*., 1997).

In Korea, the import of Alismatis Rhizoma is under the control of the government to promote the domestic cultivation of this herbal medicine. Therefore, it is necessary to evaluate the quality of Korean Alismatis Rhizoma for differentiation with imported ones and for the further quality improvement. Up to now, several researchers have reported on the individual quantification of alisol A 24-acetate, alisol B 23-acetate, and alismol by HPLC-UV technique (Park *et al*., 2005; Lee *et al*., 2004; Wen *et al*., 1998; Yoshikawa *et al*., 1994). However, the quantitative analysis of tetracyclic triterpenes with UV detector has some limits because these compounds are usually lack of chromophores. Especially, with regard to herbal preparation products, UV chromatograms show high matrix effect and peak overlap. In this study, an analytical method was developed to determine alisol B 23-acetate and alisol C 23-acetate by HPLC–ESI-MS with high selectivity and sensitivity.

Experimental

Plant materials – Two samples (K1 and K2), the rhizomes of *A. plantago-aquatica* L. (Alismataceae), had been collected in Soonchun, Chonnam and Sangju, Kyeongbook in Korea, respectively. Five Korean Alismatis Rhizoma (K3-K7) were purchased from local herbal store as listed in the Table 1 during 2004-2005. Four Chinese Alismatis Rhizoma (C1-C4) were provided by Ms. Jin Zhang in the College of Traditional Chinese Materia

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Table 1. Contents of alisol B 23-acetate and alisol C 23-acetate in Korean and Chinese Alismatis Rhizoma

Samples	Alisol B 23-acetate		Alisol C 23-acetate	
	mean (mg/g)[a]	RSD[b]	mean (mg/g)	RSD
Sangju[c] (K1)	2.60	8.4	0.42	9.8
Sooncheon[c] (K2)	2.52	2.1	0.38	5.4
Busan (K3)	2.65	1.0	0.34	3.1
Kwangju (K4)	3.08	3.2	0.36	1.6
Taegu (K5)	2.46	7.0	0.37	9.1
Seoul (K6)	1.98	6.2	0.39	15.4
Ahnyang (K7)	2.36	7.5	0.23	1.8
Shenyang-I (C1)	0.08	6.4	0.17	4.8
Shenyang-II (C2)	1.66	3.5	0.24	5.7
Shenyang-III (C3)	1.49	2.1	0.22	5.8
HongKong (C4)	1.44	5.9	0.20	0.6

[a] Data are expressed as mean of three independent experiments.
[b] RSD: relative standard deviation (%) = (the standard deviation value (SD) / the average value of content (mg/g)) × 100
[c] The province where the rhizomes were cultivated. The others are the province where the samples were purchased.

Medica, Shenyang Pharmaceutical University, Liaoning, China, in 2005.

Solvents and reagents – Solvents used in this experiment were HPLC-grade. Methanol (MeOH) and water was purchased from Fischer (USA) and Mallinckrodt (USA), respectively. A membrane filter (MF3-13 PTFE, diameter - 13 mm, pore size - 0.50 μm, Advantec, CA, USA) was used to filter each sample.

Standards and samples – Pure alisol B 23-acetate and alisol C 23-acetate were isolated from the dried Alismatis Rhizoma in our laboratory, and identified by comparing their NMR and MS data with the published values (Lee *et al*., 2001). In a 10 ml-volumetric flask, the standard compounds (approx. 1 mg) were accurately weighed and dissolved in HPLC grade methanol to make a stock solution. Working calibration solutions were prepared to be ranged from 7.8 to 5000 ng/mL by successive two-fold serial dilution of the stock solution with methanol. The rhizomes were lyophilized and finely pulverized. The powder (3 g) was extracted with 100 ml of methanol by ultrasonication twice at 37 °C for 90 min. The supernatants were filtered through a membrane filter and stored at –20 °C for HPLC analysis.

HPLC analysis – A Hewlett-Packard 1100 series HPLC system equipped with an autosampler, a column oven and a binary pump (Hewlett-Packard, Avondale, CA, USA) was used. A 5 μl volume of standard or sample solutions was directly injected on a XTerra RP 18 column (4.6 × 150 mm, 5 μm, Waters) using a gradient MeOH-water solvent system at 37 °C. The running condition (or initial condition) was kept at 75% MeOH for the first 20 min, increased to 90% MeOH for the later 30 min. The flow rate was 0.3 ml/min. The Chemstation software (Hewlett-Packard, Avondale, CA, USA) was used to operate this HPLC system.

ESI-mass spectrometry – All ESI-MS spectra were acquired using a Finnigan MAT LCQ ion-trap mass spectrometer (San Jose, CA, USA) equipped with a Finnigan electrospray source and capable of analyzing ions up to *m/z* 2000. Mass spectrometer conditions were optimized in order to achieve maximum sensitivity. The source voltage was set to +36.5 V and the capillary temperature to 300 °C. The other conditions were as follows: capillary voltage, +36.5 V; inter-octapole lens voltage, –94 V; sheath gas flow, 80 arbitrary units; auxiliary gas flow, 20 arbitrary units. Nitrogen (> 99.999%) and He (> 99.999%) were used as sheath and damping gas, respectively. The sodium cationized molecular ions were isolated with an isolation width of 2 *m/z* units and fragmented using collision energy of 50% for MS^2 experiments. The MS data were acquired in Selective ion monitoring (SIM) mode to quantify the triterpenes. The mass scale was calibrated in the positive-ion mode using a solution consisting of caffeine, the tetra-peptide MRFA, and Ultramark 1621 (Sigma, St. Louis, MO, USA) solution. The Xcalibur software (Finnigan MAT) was used for the operation.

Results and Discussion

Two prostane triterpenes, alisol B 23-acetate (**1**) and alisol C 23-acetate (**2**), were selected as standard

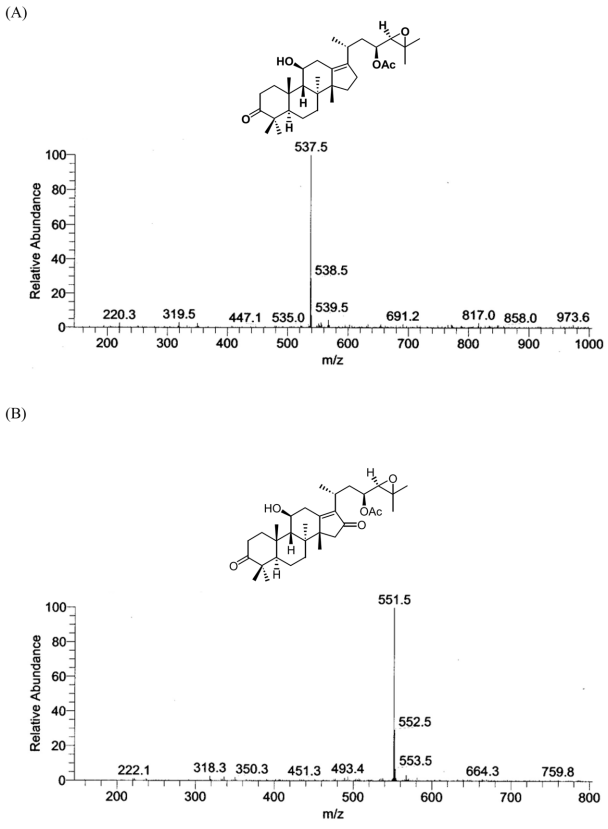

Fig. 1. Chemical structures and ESI-MS spectra of standard compounds. (A) alisol B 23-acetate. (B) alisol C 23-acetate.

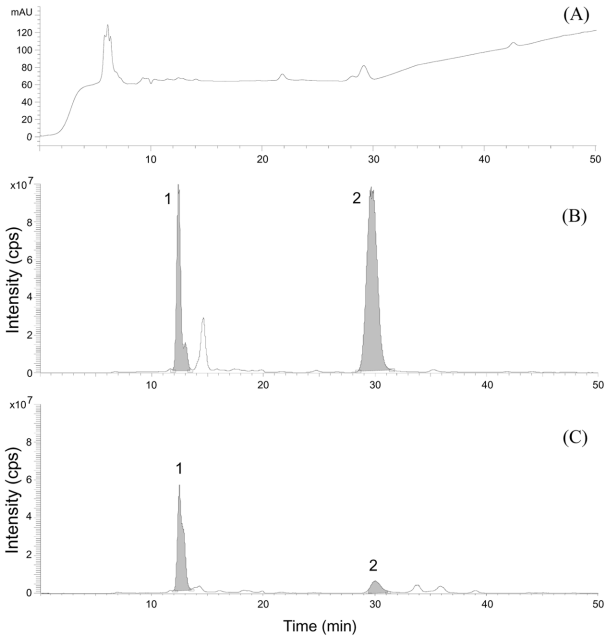

Fig. 2. Chromatograms of the methanol extract of Alismatis Rhizoma. (A) UV chromatogram of the methanol extract of commercial Alismatis Rhizoma (K1). (B) ESI-MS chromatogram of the extract of Korean Alismatis Rhizoma (K1) in SIM mode. (C) ESI-MS chromatogram of the extract of Chinese Alismatis Rhizoma (C1) in SIM mode. Two segments were set for ESI-MS as follows: the ion at *m/z* 551 was selected for the first 20 min period and the ion at *m/z* 537 was selected for the latter period. In this condition, the peaks 1 and 2 corresponded to alisol C 23-acetate (t_R 12.4 min) and alisol B 23-acetate (t_R 29.7 min), respectively.

compounds (Fig. 1). They displayed clear quasimolecular ion peaks at *m/z* 537 $[M + Na]^+$ and *m/z* 551 $[M + Na]^+$, respectively (Fig. 1). These parent ions were selected for quantification of these compounds in selected ion mode (SIM) because intensity of their fragment ions was much low.

Calibration curves were constructed for each of the reference standards in concentrations of 7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000, 5000 ng/mL in duplicates. The calibration curves showed good linearity and the correlation coefficients were found to be 0.999 and 0.998 for alisol B 23-acetate and alisol C 23-acetate, respectively, over the concentration range of 0.06 - 2.0 μg/mL. The limit of detection (LOD) was about 5 ng/mL for both alisol B 23-acetate and alisol C 23-acetate, based on a signal-to-noise (*S/N*) ratio of 3 : 1.

As shown in Fig. 2, while the peak intensity and peak selectivity were not enough to determine the alisol C 23-acetate by UV detector, at 210 nm (Fig. 2A), the standard compounds (**1** and **2**) were clearly identified in the extracts of the commercial Alismatis Rhizoma by ESI-MS in SIM mode (Fig. 2B and 2C).

The method reproducibility was evaluated by the intra-day and inter-day variability for three injections of standard solutions and three replicates analysis of sample solutions, respectively. The coefficient of variance (CV) was less than 15.4%, which demonstrated good precision of this method (Table 1).

The recovery was assessed by spiking sample with two concentrations of each reference compound, namely: 2.5 μg/g and 0.63 μg/g. The average recoveries were between 90.5 ± 1.2% (mean ± CV, $n = 3$) and 103.2 ± 2.7% ($n = 3$).

The HPLC–ESI-MS method developed in this research has been applied to the determination of these components in the extracts of Alismatis Rhizoma, and made it possible to determine the two prostane triterpenes (compounds **1** and **2**). The two provinces, Sangju and Sooncheon are two major regions for cultivation of Alismatis Rhizoma in South Korea.

The results showed that the content of alisol B 23-acetate was between 1.98 mg/g and 3.08 mg/g for Korean Alismatis Rhizoma, while the value was between 0.08 mg/g and 1.66 mg/g for Chinese ones. The amount of alisol C 23-acetate was between 0.23 mg/g and 0.42 mg/g for Korean Alismatis Rhizoma. The value was between

0.17 mg/g and 0.24 mg/g for Chinese ones (Table 1 and Fig. 2). These results are consistent with the previous report that the Korean Alismatis Rhizoma showed higher content of alisol B 23-acetate than Chinese ones (Lee *et al*., 2004).

Notably, the sample C1 showed the lowest content of the two components. Actually, Chinese samples (C1-C4, especially C1) were dark yellow or yellowish brown color and hardly broken, while Korean ones showed yellowish white color and easily broken into powder. Considering the fact that Korean and Chinese Alismatis Rhizoma have identical plant origin, it could be suggested that these differences in the content of prostane triterpenes and the appearance come from different conditions in drying process. It has been reported that Alismatis Rhizoma dried at 50 °C or above this temperature showed deep color and lost luster (Hyun *et al*., 2006). In addition, it has been reported that the chemical change of triterpene constituents in this herbal medicine occurred during the drying process (Yoshikawa *et al*., 1994).

In the present investigation, HPLC/ESI/MS method was first applied to quantify tetracyclic triterpenes in Alismatis Rhizoma. The results showed that this method could be used to identify the prostane triterpenes in herbal preparation products and to differentiate Korean and Chinese Alismatis Rhizoma with high selectivity and sensitivity.

Acknowledgement

This work was supported by Jinju National University Grant 2007. We wish to thank Hee Jin Kim, Kye Jun Jang, Byung-Jae Kim and Hyo Eun Lee at Korea Science Academy, Pusan, Korea for purchasing the Korean commercial Alismatis Rhizoma.

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(Accept July 21, 2008)