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Short communication

Determination of betaine in *Lycium chinense* fruits by liquid chromatography–electrospray ionization mass spectrometry

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Abstract

A rapid and sensitive high-performance liquid chromatography–electrospray mass spectrometric method has been developed for the determination of betaine in *Lycium chinense* fruits. Betaine was analyzed on a system consisting of a NH_2 stationary phase and a mobile phase of water–acetonitrile (25:75) by isocratic elution for 40 min. Betaine was identified and quantitated by electrospray ionization mass spectrometry with selected ion monitoring of the protonated ion $[Betaine+H]^+$ and clustered ions $[n\text{Betaines}+H]^+$. The limit of detection for betaine by this method was ca. 0.2 ng/ml and the relative standard deviations of the assay (intra- and inter-day) were less than 8.1%.

Keywords: *Lycium chinense*; Betaine

1. Introduction

The dried ripe fruit of *Lycium chinense* Miller (Solanaceae) has been widely used in folk medicine as a tonic and is still used in Korea as a ingredient of herbal drugs and functional foods. Several volatile, steroidal and alkaloidal compounds in this plant are known to have various bioactivities [1–5]. Among them, one of the major constituents of *L. chinense* is betaine (Fig. 1), a zwitterionic compound.

Several analytical approaches for the determination of betaine in plants or biological tissues have been published, such as liquid chromatography (LC) [6–9] and gas chromatography–mass spectrometry (GC–MS) [10]. Because it has a poor UV-chromophore, betaine needs low-wavelength UV detection. If there are substances such as vitamins and amino acids in the sample matrix, the direct detection of betaine by low-wavelength UV may be difficult. Pre- or post-derivatization of betaine with a labeling method can overcome this problem but the step for sample preparation should be complicated. Fast atom bombardment (FAB) MS [11] analyses may give good sensitivities for this kind of compounds, how-

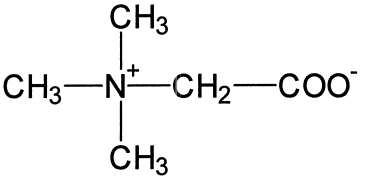

Fig. 1. The structure of betaine.

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ever, the drawback of these approaches also need derivatization steps using esterification of the carboxylic acid of betaine with *n*-alkylalcohol for the good sensitivity. Apart from methods using MS, which can gain in specificity on recording selected ions, other detection methods also suffer from interfering compounds when the samples are determined.

Therefore, we have developed a new method that can determine betaine in *L. chinense* by LC–electrospray-MS. In this paper, an LC–MS method to enhance both selectivity and sensitivity of betaine in *L. chinense* with selected-ion monitoring (SIM) is described. This study is also the first application of electrospray-MS to analyze betaine, one of the zwitter compounds.

2. Experimental

2.1. Material

The dried ripe fruits of *L. chinense* were purchased from the herbal drug market in Seoul and the boucher specimen was deposited in the herbarium of College of Pharmacy, Seoul National University. Betaine hydrochloride was purchased from Aldrich (Milwaukee, WI, USA). Acetonitrile (HPLC grade) and glacial acetic acid were obtained from Fischer Scientific (Fair Lawn, NJ, USA). All solvents used during the extraction process were of analytical-reagent grade and water was purified using a Milli-Q system (Waters Chromatography Division, Millipore, Milford, MA, USA).

2.2. Preparation of sample and standard solutions

The dried fruits of *L. chinense* (0.1 g) were sonicated with 10 ml of methanol for 30 min at room temperature and centrifuged for 3 min (3000 rpm) and then the supernatant was collected. This procedure was repeated three times and the supernatants were combined and evaporated to dryness in a stream of N_2. The residue was dissolved in 1 ml of water and this solution was filtered through a 0.2-μm membrane, and 5-μl aliquots of the filtrates were injected directly into the LC column connected to electrospray-MS system. A stock solution of betaine hydrochloride was prepared in water (2.5 mg/ml) and standard solutions of betaine were prepared by diluting the stock standard with mobile phase to give concentrations of 1, 0.5, 0.1 and 0.02 mg/ml, respectively.

2.3. Chromatography

LC separation of MeOH extracts of *L. chinense* were performed on a Hitachi HPLC system (Hitachi, Japan) equipped with a variable-wavelength detector (L-7400), autoinjector (L-7250), quaternary pump (L-7100) and controller module (D-7000). The column used in this study was a YMC NH_2-phase column (250×4.6 mm) with 5 μm packing material (Wilmington, NC, USA). The flow-rate was set at 1.03 ml/min. The UV detector was set to 210 nm to determine the detection limit of betaine. The mobile phase was water–acetonitrile (25:75), isocratic elution and the column effluent was mixed with 0.2% acetic acid (methanol–water–acetic acid, 90:9.8:0.2, flow-rate=0.03 ml/min) solution to enhance the ionization of the compound in the MS ion chamber.

2.4. Electrospray ionization mass spectrometry

The system used is a Hewlett-Packard electrospray system consisting of a 59987A electrospray and a 5989B mass spectrometer (Wilmington, DE, USA). The quadrupole temperature was 120°C and the spectra were acquired in the positive mode. The temperature of drying N_2 gas was 350°C and flow-rate of drying N_2 gas was 40 ml/min. Nebulizing N_2 gas in ion chamber was maintained 80 p.s.i. (1 p.s.i.=6894.76 Pa). Ions were accelerated from the electrospray ion source into the mass spectrometer and focused through hexapoles and skimmers. Voltages applied for these electrical elements were optimized while constantly infusing betaine standard solution into the electrospray ion source. The entrance lens and capillary exit voltage were set 18 V and 78 V, respectively. Entrance lens and capillary exit were also optimized by monitoring the ion abundance of direct flow injection of betaine.

2.5. Calibration curve and recovery

Calibration curve was generated by plotting the ion intensities vs. concentrations of standard solu-

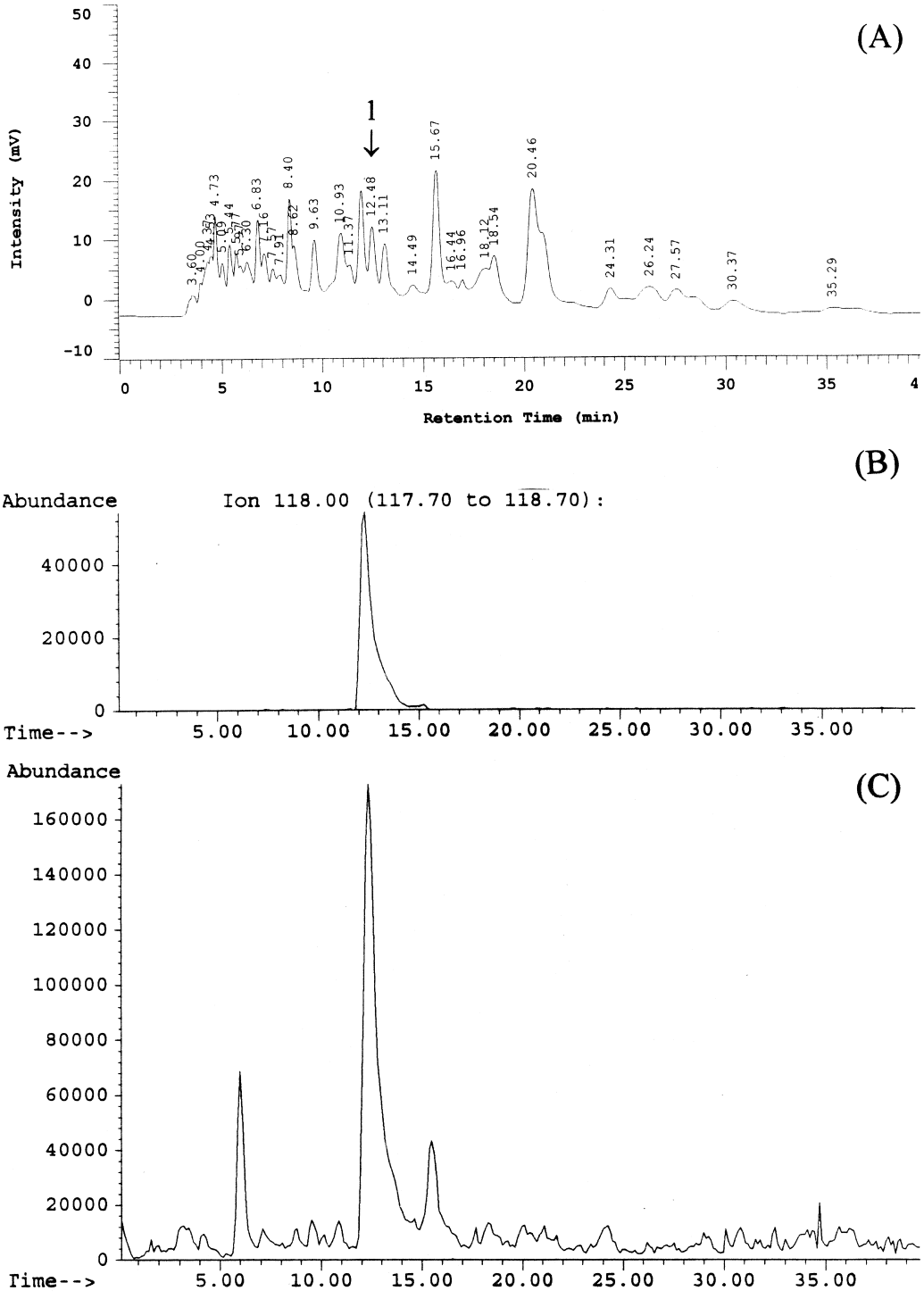

Fig. 2. Chromatograms of MeOH extract of *Lycium chinense* obtained by (A) LC–UV, (B) LC–electrospray-MS (selected ion chromatogram at *m*/*z* 118) and (C) LC–electrospray-MS (total ion chromatogram). Concentration of betaine is ca. 0.02 mg/ml. Peak 1=betaine.

tions. Each standard was analyzed in triplicate, and the average set of triplicated analyses was taken as the ion intensities of standards. The intra- and inter-day relative standard deviations (RSDs) were measured by comparing the ion intensities of betaine standard solution obtained on the three different days. Recovery was determined by spiking samples with betaine, followed by extraction with same procedure as the samples.

3. Results and discussion

Various columns and solvents were studied to enhance the resolution and sensitivity for betaine in *L. chinense*. Due to the polarity of betaine and its compatibility with electrospray-MS, an aminopropyl (NH_2)-silica LC column using acetonitrile–water without buffer was selected as column and eluent, respectively. The LC and mass chromatogram (selected ion chromatogram at m/z 118 and total ion chromatogram) of MeOH extract of *L. chinense* are shown in Fig. 2.

In order to optimize electrospray parameters for the quantification of betaine, flow injection analyses were also performed. Both positive and negative electrospray ionization conditions were applied for betaine in mobile phase, and only positive ion mass spectra could be obtained during flow injection analyses. In the positive electrospray ionization mode, the protonated molecule $[M+H]^+$ of betaine at m/z 118 was observed with strong intensity as well as some cluster ions (Fig. 3). These cluster ions were found at m/z 235, 352, 469, and were identified as $[nM+H]+$ ions ($n=2$, 3 and 4), which were characteristic ions of betaine in electrospray ionization and could be used for identification of beatine in analysis. These phenomena were also observed in FD (field desorption) mass spectra of betaine, which seemed to be generated by the intra-molecular reaction of betaine in ion chamber [12]. In the negative electrospray ionization mode, neither deprotonated betaine ion $[M-H]^-$ nor any cluster ions were detected in spite of optimization of ion voltage of entrance lens and capillary exit. Even though betaine has both carboxyl and amine groups in its

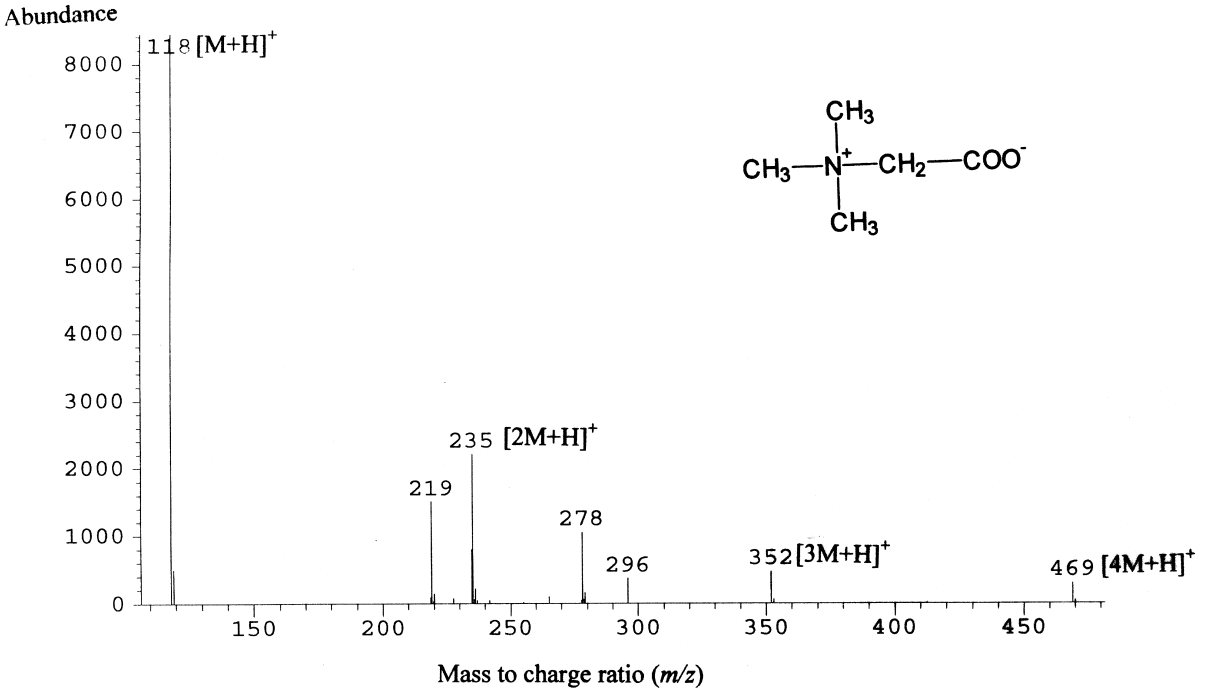

Fig. 3. The positive ion electrospray mass spectrum of betaine.

structure, it was very interesting that betaine was detected in only the positive ion mode. Since the strongest protonated molecule of betaine [Betaine+H]$^+$ was observed at m/z 118, this ion was used for the determination of betaine in *L. chinense*. Other cluster ions such as m/z 235, 352 and 469 were used to confirm betaine in samples.

In the LC chromatogram (Fig. 2A), the resolution of betaine was less than 0.8 under optimal conditions and some interference was found in the chromatogram. However, no interference was observed when samples were monitored using the selected ion mode at m/z 118 (Fig. 2B), which corresponds to the protonated ion of betaine. The retention time for betaine was approximately 12.5 min. The calibration curve was linear from 0.02 to 1 mg/ml with a correlation coefficient greater than 0.999.

To determine the detection limit of betaine, serial dilutions of betaine in mobile phase were prepared, injected and monitored at m/z 118. Because all *L. chinense* samples contain high concentrations of betaine, no blank sample was available for preparation of standards or controls. Therefore, all standards were prepared in mobile phase. To investigate whether any signals at m/z 118 could be detected in the solvent used for the preparation of standards, a solvent blank was analyzed by LC–MS and no peaks at m/z 118 were observed in the blank. The limit of detection of LC–electrospray-MS for betaine was approximately 0.2 ng/ml based on a signal-to-noise ratio of 3, whereas that of UV detection was 0.1 μg/ml. The precision of the assay method was obtained by triplicate betaine standard samples (20 μg/ml). The mean intra-day RSD of betaine in these samples was 6.1% (range 4.3–7.8%) whereas the mean inter-day RSD for analysis of the same samples on the three consecutive days was 8.1%. The amount of betaine in the seed of *L. chinense* varied from 0.15 to 0.21% (0.15 to 0.21 mg/ml) and these values are consistent with the results of LC–UV method.

In conclusion, this method would be useful in the quantitative and qualitative analyses of betaine in *L. chinense*. It also would be useful for the quality control of *L. chinense* in pharmaceutical or health food products.

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