Mongolian Journal of Chemistry

Institute of Chemistry & Chemical Technology

Mongolian Academy of Sciences

Simultaneous analysis of major ingredients of *Gardenia* fruit by HPLC-MS/TQMS method

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ARTICLE INFO: Received: 2 Feb, 2016; Revised: 22 Nov, 2016; Accepted: 7 Dec, 2016

Abstract: An efficient, accurate HPLC-MS/TQMS method was introduced for the quantitative/qualitative simultaneous analysis of main ingredients, namely geniposide and genipingentiobioside, in the *Gardenia* fruit. The separation was successfully obtained using a C_8 (100mm×2.1mm, 5μm, 30°C) column by gradient elution with ultrapure water as mobile phase, where flow rate was set to 0.2 ml/min and detection wavelength at 240 nm. The analytical method was validated and the quantification of active compounds, namely genipingentiobioside and gardenoside, was performed. Linearity, precision, repeatability, stability and recovery were also reported. The quantitative analysis revealed that both main ingredients as geniposide and genipingentiobioside have performed a good linear relationship in 0.1-100 mg/ml concentration range (r=1.00000 and r =0.99998). The average content was measured to be 4.842% with RSD 0.96% for geniposide and 1.1976% with RSD 0.47% for genipingentiobioside in the *Gardenia* fruit. Accordingly, this method would be feasible for the quantity and quality control of crude drugs.

Keywords: *HPLC-MS/TQMS, geniposide, genipingentiobioside, quantity control*

INTRODUCTION

The dried ripe fruit of *Gardenia jasminoides* Ellis, a Rubiaceae family, has been wildly used in Mongolian, Chinese and Kampo medicine. Now it becomes a hot topic due to its numerous good effects, such as on the treatment of irritability in febrile diseases, jaundice, acute conjunctivitis, epitasis, haematuria, pyogenic infections and ulcers of the skin, sprains and painful swellings due to blood stasis [1-7]. It contains mainly iridoid, such as geniposide, genipingentiobioside, gardenoside and geniposidic acid, etc. [8]. These ingredients were believed to be the pharmaceutically active components of this fruit.

HPLC-MS/TQMS technology has unique advantages in the research of chemical composition, quality standard and fingerprint of traditional Chinese medicine [9, 10]. Recently, many researchers have focused on the HPLC measurement of the amount of Gardenia acid, genipin glycoside geniposide, crocin and geniposide in the Gardenia [11-14] and qualitative analysis of Gardenia chemical compositions [15-17].

However, the research results on liquid chromatography and mass spectrometry simultaneous determination of geniposide, genipin melibiose and gardenoside in the Gardenia for qualitative and quantitative analysis are not been reported.

The authors, using a high performance liquid chromatography and mass spectrometry (HPLC-MS/MS), have determined simultaneously the active chemical components in *Gardenia* fruit. Having analyzed the retention time of the chromatographic peaks, primary/secondarymass spectra and comparison with the reference standards, both quality and quantity of two active components, namely, geniposide and genipingentiobioside have been analyzed. A comprehensive validation study was also carried out on linearity, precision, repeatability, stability and recovery. The developed HPLC-MS/TQMS method would be helpful for the quality control of crude drugs.

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DOI: http://dx.doi.org/10.5564/mjc.v17i43.744

EXPERIMENTAL

Sample preparation: The dry fruits of *Gardenia* purchased from Inner Mongolia were ground to powder and passed through a 60-mesh sieve. For extracting the active components, 0.5 g powders was added in 10 ml ultrapure water and treated with ultrasonic homogenizer (70 W, 20 kHz) for 30 min. The mixture was centrifuged (3000 rpm) and the supernatant was filtered with 0.45 μm porous membrane for analysis. After that, the solution was diluted 1000 times for HPLC measurement.

Preparation of reference solutions: Three reference solutions containing 1 mg/ml of geniposide (code No. 00007070-212, Wako, Japan) or 1 mg/ml of genipingentiobioside (code No. 130527, Sichuan Wei Keqi Biotechnology Co., Ltd., China) and their equal mixture (1:1) in ultrapure water were also prepared. The solutions were diluted 100 times again before HPLC measurement.

HPLC analysis: Prepared solutions were analyzed on a Prominence LC-20A (SHIMADZU, Japan), a triple quadrupole mass spectrometry (TQ-MS) combined with a liquid Chromatography Mass Spectrometer system (API2000 LC-MS; AB SCIEX, America). HPLC conditions were as following: the chromatography column is ACE C_8 (100 mm×2.1 mm, 5 μm); it was adapted gradient with methanol-water solution for mobile phase; the flow

rate is 0.2 ml/min; the detection wavelength is 240 nm; the column temperature is 30°C; the injected volume of sample is 0.5 µl. Conditions for the MS spectrum was as following: the ionization source is electrospray ionization (ESI); the scan patterns is positive ion mode; the scanned area is 10-1100 *m/z*; capillary voltage is 5500 V for LC-MS and 4500 V for MS/MS; the nebulizer pressure is 50 psi for LC-MS and 17 psi for MS/MS; the gas temperature is 300°C; the voltage of pieces: 120 V. Experimental data was processed by Analyst 1.4.2 (AB SCIEX, America).

RESULTS AND DISCUSSIONS

Qualitative analysis of MS determination: The extract of *Gardenia jasminoides* fruits was injected into API2000 for the MS measurement. Figure 1 shows that the mass-charge ratio of main peaks which are 410.9 *m/z*, amu (peak 1), 572.7 m/z, amu (peak 2) and 426.9 *m/z*, amu (peak 3).

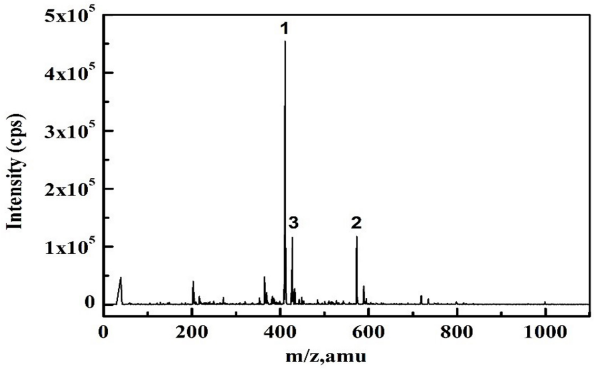

Fig.1. Qualitative analysis of extract of Gardenia fruit

Reference solutions of geniposide and genipingentiobioside were injected into API2000 separately for MS measurement. Geniposide (Mm=388.1) and genipingentiobioside (Mm=550.2) are 410.8 *m/z*, amu (M+X=388.1+22.7) and 572.9 *m/z*, amu (M+X=550.2+22.7), respectively, as shown in Figure 2A and 2B.

In the MS/MS measurement of quasi-molecular ion peak of reference solution, an ion with mass to charge ratio of 22.8±0.5 m/z, amu was detected in the secondary mass spectrometry fragment ions for both references, suggesting X is estimated to be Na^+.

Quantitative analysis by HPLC: The extract of Gardenia *jasminoides* fruits and reference solution of the mixture of geniposide and genipingentiobioside (0.5µl each) were injected into the instrument for LC analysis. Retention times of the detected peaks 1, 2 and 3 for the extract were 15.23 min, 14.16 min and 11.71 min, respectively, as shown in Figure 3B. Among them, the retention times for peak 1 and peak 2 were in consistent with that of peak 1 (geniposide) and peak 2 (genipingentiobioside) of the reference solution (Figure 3A), suggesting the two components detected in *Gardenia jasminoides* fruits should be geniposide and genipingentiobioside. The extract and references mixture 0.5µl were injected six times into the HPLC instrument and the area of chromatographic peaks were determined for calculate the content of them by using external standard method. The average content of geniposide was 4.842% with relative standard deviation (RSD) 0.96% and the genipingentiobioside was 1.1976% with RSD 0.47%, respectively.

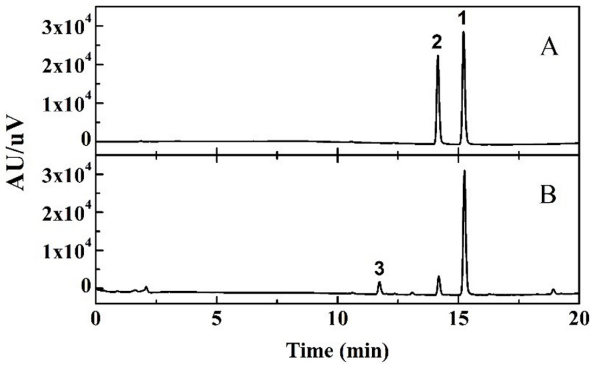

Fig. 3. HPLC chromatograms of extract of Gardenia fruit and reference solution. A: Extract of Gardenia fruit; B: Mixture of geniposide (peak1) and genipingentiobioside (peak2)

Method validation: *Linearity*

Linearity range of response was determined for the mixture of geniposide and genipingentiobioside reference solution. Linearity was determined on five level of concentrations as 0.1, 1, 5, 50 and 100 mg/ml with three parallel injections for each level by using ultrapure water.

A standard curve was drawn with concentration of reference solution on the x axis, the intensity of chromatographic peak area as vertical coordinate (y).

The linear regression equation of geniposide and genipingentiobioside and its correlation coefficients (r) were obtained as following:

y = 22863x - 1045.2, r =1.00000 for geniposide;
y = 18471x - 3431.9, r =0.99998 for genipingentiobioside;

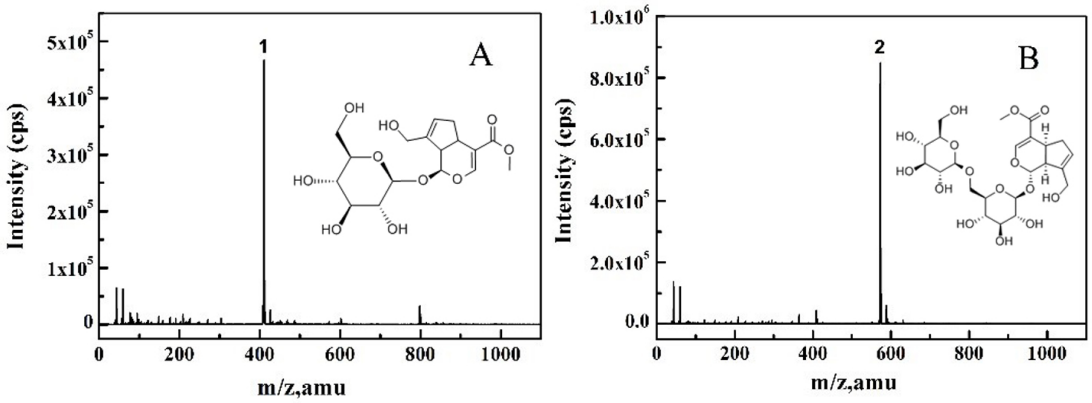

Fig. 2. Mass Spectra of reference solutions, A: TIC of geniposide, peak 1: 410.9 *m/z*, amu; B: TIC of genipingentiobioside, peak 2: 572.7 *m/z*, amu

It can be seen that both geniposide and genipingentiobioside have showed good linear relationship in the range from 0.1 to 100 mg/ml, which indicated that analytical method performance indicators meet the verification requirements of the quantitative analysis method.

Extract Stability: Extract of gardenia fruits (0.5µl) were solubilized in ultrapure water before the analysis and the stability was evaluated at room temperature every 2h, up to 12h. The average peak area of geniposide was 68500 with RSD of 0.21%, and genipingentiobioside was 7417 with RS.D of 1.22%, which indicate that the extract were found to be stable in water at room temperature for at least 12h.

Precision of the sample preparation: To evaluate the precision of the sample preparation, five solutions at 0.5µl in ultrapure water were prepared. The estimated RSD of average peak area was 0.18 % for geniposide and 0.70 % for genipingentiobioside, which indicated the well precision of the sample preparation.

Repeatability of the method: In order to evaluate the repeatability of the method, six solutions (0.5µl) prepared from the extract of Gardenia fruits injected and the chromatographic peak areas were determined. Then the amount of each injection was calculated in order to estimate the RSD, Geniposide (average content (n=6) (4.84% and R.S.D. 0.96%) and genipingentiobioside (1.20%, R.S.D. 0.47%), respectively.

Recovery: In order to evaluate the percentage recovery of reference solution into extract, 1 ppm of the mixture of geniposide and genipingentiobioside were added into the prepared extract of Gardenia respectively, and then extracted 0.5 µl from it to analyze 5 times. The recovery results showed that average spike recoveries and RSD was 109.13% and 0.30% for geniposide, and 94.49% and 0.31% for genipingentiobioside.

DISCUSSION

The content of extract with water is the highest among three samples which were extracted with different solvents of ultrapure water. The samples were scanned in the range of 200-650 nm wavelength by using a spectrophotometer, and the maximum absorption peak of reference solution of geniposide and genipingentiobioside were observed at 238 nm and 239 nm, respectively. Therefore, these two compounds were detected at 240 nm wavelength simultaneously in the follow-up tests. The active ingredients of samples can be measured by both C_{18} and C_8 columns which were used in the liquid chromatography. It was found that the C_8 column can more improve the efficiency of tests than C_{18}, therefore, the C_8 column was selected. Crude drug extract is an extremely complex mixture with multi chemical compositions. It was difficult to quantify each chemical composition simultaneously by only liquid chromatography, while, liquid chromatography with the mass spectrometer detector can well solve the problem. This method is capable for detecting variety of ingredients in crude drugs with good correspondence.

CONCLUSIONS

In the present study, we applied HPLC-MS/TQMS method to determine the content of geniposide and genipingentiobioside in *Gardenia jasminoides* fruits. This determination method is an efficient, accurate and comprehensive evaluation of the active ingredients. Accordingly, this is a feasible method in the crude drug research and quality control.

ACKNOWLEDGEMENTS

This work is partially supported by International Research Funding of Muroran Institute of Technology.

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