

Application News

No. C82

Liquid Chromatography Mass Spectrometry

Analysis of Geniposidic Acid and Chlorogenic Acid in Tochu Tea Using a Triple Quadrupole LC/MS/MS [LCMS-8030]

Tochu tea made using tochu (*Eucommia ulmoides*) leaves is well known as one of the five great traditional Chinese medicines. Tochu tea leaves contain abundant amounts of geniposidic acid (an iridoid glucoside) and chlorogenic acid (3-caffeoylquinic acid). Geniposidic acid is known to possess several pharmacological functions, one of which is the ability to reduce blood pressure. LC/MS is widely used for the analysis of these types of polyphenols in plant extracts. Here, we introduce an analysis of geniposidic acid and chlorogenic acid in tochu tea using a triple quadrupole mass spectrometer.

Fig. 1 shows the MS/MS spectra of geniposidic acid and chlorogenic acid (*trans isomer*) in standard solution. The deprotonated ions $[M-H]^-$, m/z 373 and m/z 353, were used as the precursor ions for electrospray ionization (ESI) negative ion mode. In quantitative analysis by MRM (Multiple Reaction Monitoring), the product ions m/z 123 and m/z 191 were monitored for geniposidic acid and chlorogenic acid, respectively. Fig. 2 shows the MRM chromatograms of the standards and the various MRM parameters that were used.

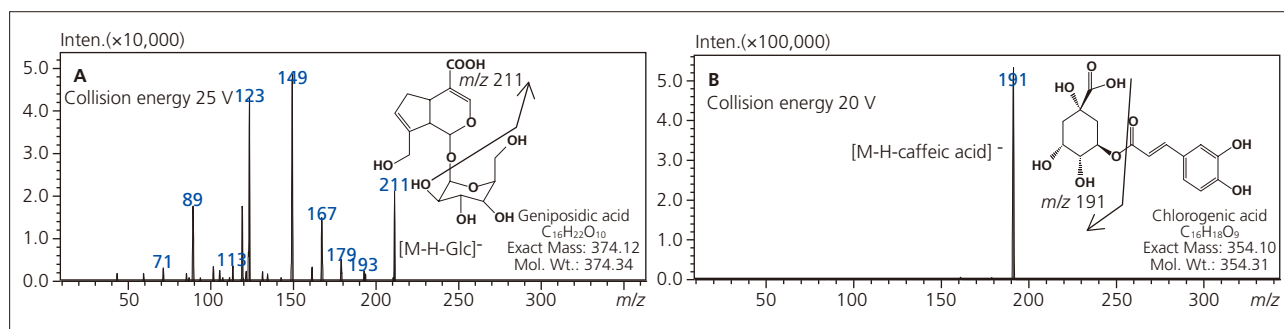


Fig. 1 MS/MS Spectra of the Standard Solutions (A: Geniposidic Acid, B: Chlorogenic Acid)

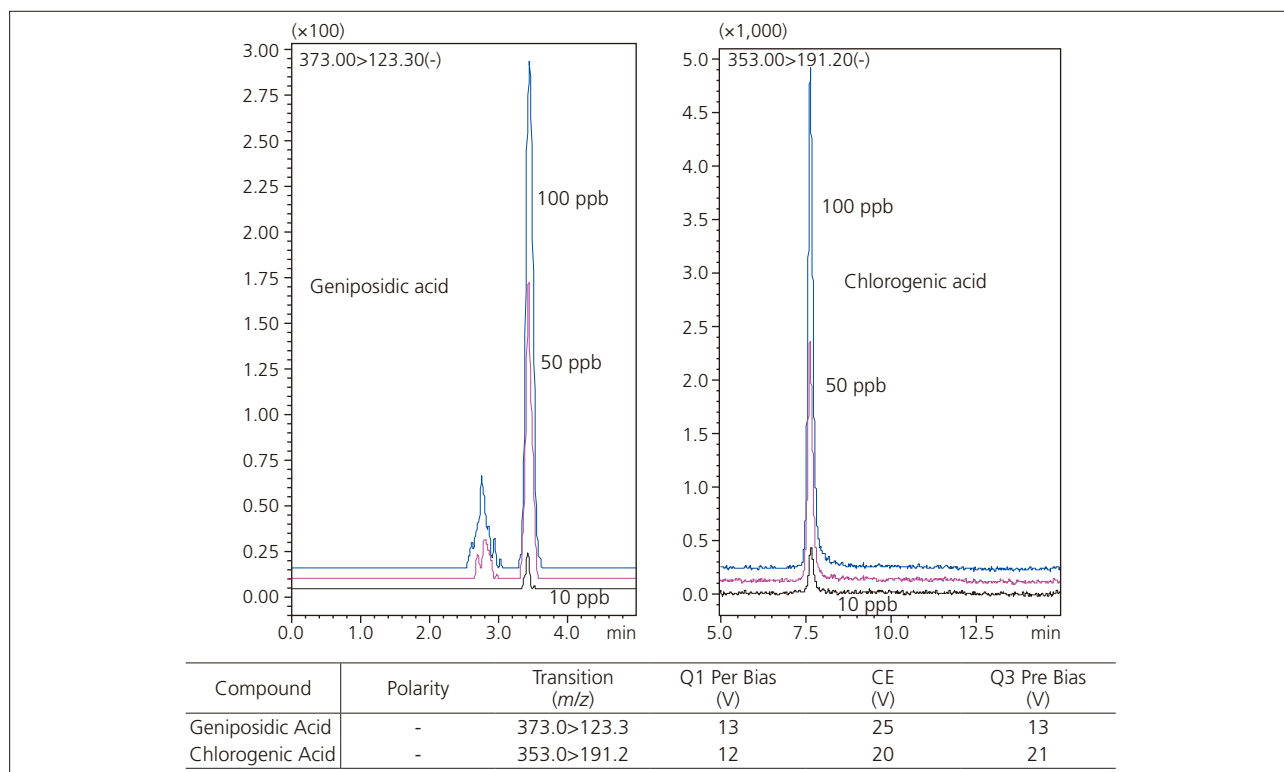


Fig. 2 MRM Chromatograms of the Standard Solutions and Respective MRM Mode Parameters

Fig. 3 shows the calibration curves for geniposidic acid and chlorogenic acid. Both calibration curves show wide dynamic range and excellent linearity with a correlation coefficient (R^2) value greater than 0.999 ($n = 5$).

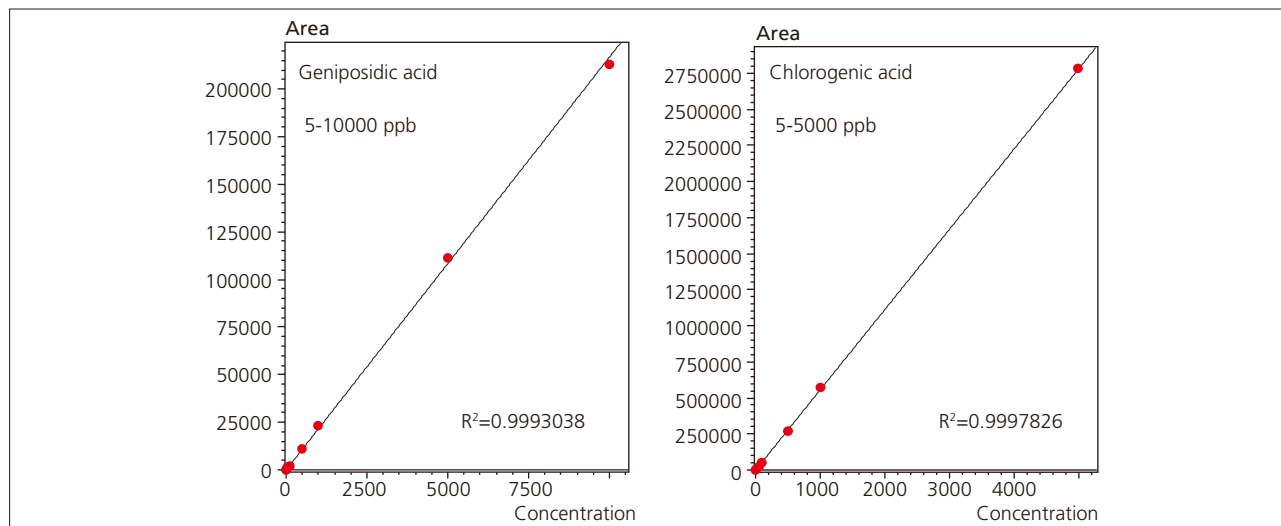


Fig. 3 Calibration Curves of Geniposidic Acid and Chlorogenic Acid ($n = 5$)

Tochu tea sample was prepared by extraction through steeping 20 g of tochu leaves in 300 mL of boiling hot water for 10 minutes and repeating this process 3 times. The volume was then adjusted to 1 L with water and then diluted 1000 to 1 with water. Finally, it was filtered through a 0.2 μm filter and analyzed by LC/MS/MS. Fig. 4 shows the MRM chromatograms of

geniposidic acid and chlorogenic acid extracted from tochu tea. Analysis was conducted without significant interference from contaminating substances. The 1000 to 1 dilution of tochu tea contained 600–700 ppb of geniposidic acid and chlorogenic acid. Thus, the undiluted tochu tea was determined to contain about 600–700 ppm of geniposidic acid and chlorogenic acid.

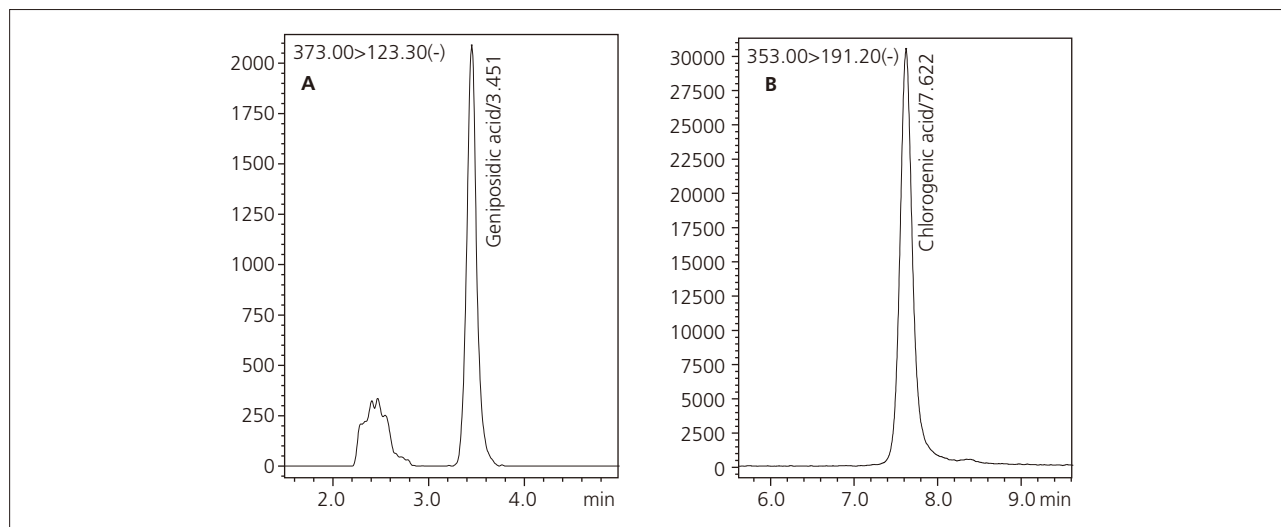


Fig. 4 MRM Chromatograms of Geniposidic Acid (A) and Chlorogenic Acid (B) in Tochu Tea

Table 1 Analytical Conditions

| | | | |
|----------------------------|---|--------------------------|--------------------------|
| Column | : Shim-pack VP-ODS (150 mmL. \times 2.0 mmI.D., 5 μm) | Column Temperature | : 40 $^{\circ}\text{C}$ |
| Mobile Phase A | : 0.1 % Formic acid in water | Nenulizing Gas Flow | : 1.5 L/min |
| Mobile Phase B | : Acetonitrile with 0.1 % formic acid | Block Heater Temperature | : 500 $^{\circ}\text{C}$ |
| Time Program | : 10 %B (0 min) \rightarrow 20 %B (10 min) \rightarrow 10 %B (10.01-20 min) | Drying Gas Flow | : 20 L/min |
| Flow Rate | : 0.2 mL/min | | |
| Injection Volume | : 2 μL | | |
| Probe Voltage | : -3.5 kV (ESI-negative mode) | | |
| DL Temperature | : 300 $^{\circ}\text{C}$ | | |
| DL Voltage/Q-array Voltage | : Using default values | | |