SHIMADZU

Application News

No.C83

Liquid Chromatography Mass Spectrometry

Qualitative Analysis of Tochu Tea Using a Triple Quadrupole LC/MS/MS [LCMS-8030]

The LCMS-8030 triple quadrupole mass spectrometer is used in qualitative analysis to conduct precursor ion scans to search for precursor ions that produce common product ions, and to conduct neutral loss scans to detect ions that dissociate to common neutral fragments. Here, we describe the precursor ion scan in the search for active ingredients in Tochu tea using the LCMS-8030.

In the precursor ion scan, scanning is conducted by the first quadrupole, or Q1, and the product ions formed through collision induced dissociation (CID) are selectively analyzed by Q3. This permits examination of the precursor ions that produce common product ions, and is therefore utilized for screening of substances having a common partial structure.

Tochu tea, used here as the target sample, contains abundant amounts of geniposidic acid (an iridoid glycoside) and chlorogenic acid (3-caffeoylquinic acid). According to Application News No. C82 and the cited reference below[1], the major product ions of geniposidic acid, chlorogenic acid, and asperuloside are *m/z* 123, 191, and 147, respectively. Therefore we searched for related compounds by conducting a precursor ion scan using these as product ions. Using Tochu tea as an actual sample, we conducted a precursor ion scan by LC/MS measurement using a Q1 scan range of *m/z* 200 to 500 (Table 1). The measurement results, presented as TIC chromatograms, are shown in Fig. 1. The 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, then diluted 10 to 1 with water. Finally it was filtered through a 0.2 µm filter. Peak 1 of event 1 was eluted at 3.4 minutes, peaks 2 and 3 of event 2 were eluted at 7.6 and 10.2 minutes, respectively, and peak 4 of event 3 was eluted at 8.6 minutes (Fig. 1).

Table 1 Precursor Ion Scan Parameters

Event No.	Scan Type	Prec.of *m/z*	Start *m/z*	End *m/z*	CE V	Scan Speed u/sec
1	Prec.	123	200	500	30	3333
2	Prec.	191	200	500	30	3333
3	Prec.	147	200	500	30	3333

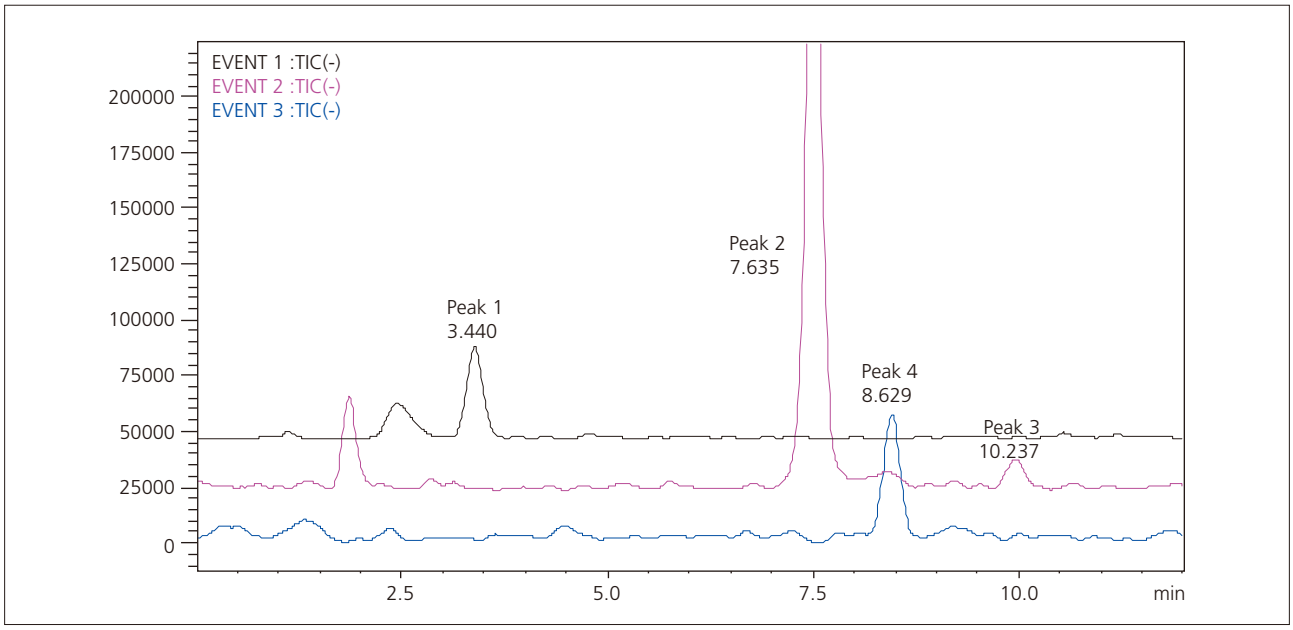

Fig. 1 TIC Chromatograms of Tochu Tea (Precursor Ion Scan)

[Reference]
1) Shuhan Tang, Zhigang Wang, Chaomei Ma, Masao Hattori, J.Trad.Med. 25, 112-118, 2008

The spectra obtained as a result of the precursor ion scan of peak 1 of event 1 (3.4 min), peaks 2 (7.6 min) and 3 (10.2 min) of event 2, and peak 4 of event 3 (8.6 min) are shown in Fig. 2.

Peak 1 of event 1 is identified as geniposidic acid with a mass of 374 when the product ion is set to *m/z* 123. The *m/z* 373 base peak is a deprotonated molecular ion $[M-H]^-$ and *m/z* 419 is a formic acid adduct ion $[M+HCOO]^-$.

It is determined that peaks 2 and 3 of event 2 are both chlorogenic acid with a mass of 354 using the set product ion *m/z* 191. The *m/z* 353 base peak is a deprotonated molecular ion $[M-H]^-$. Peaks 2 and 3 are substances in which the product ion and precursor ion consist of the same components. From the results of analysis of the standard substance described in Application News C82, it is presumed that peaks 2 and 3 are the *trans*- and *cis*-isomers, respectively, of chlorogenic acid.

Peak 4 of event 3 is identified as asperuloside with a mass of 414 using the product ion *m/z* 147. The *m/z* 459 base peak is a formic acid adduct ion $[M+HCOO]^-$, *m/z* 413 is a deprotonated molecular ion $[M-H]^-$, and *m/z* 450 is a chlorine adduct ion $[M+Cl]^-$.

The precursor ion information associated with these 4 eluted peaks is summarized in Table 2. Thus, the LC/MS/MS precursor ion scan mode provides the power to search for related substances with components having a similar structure.

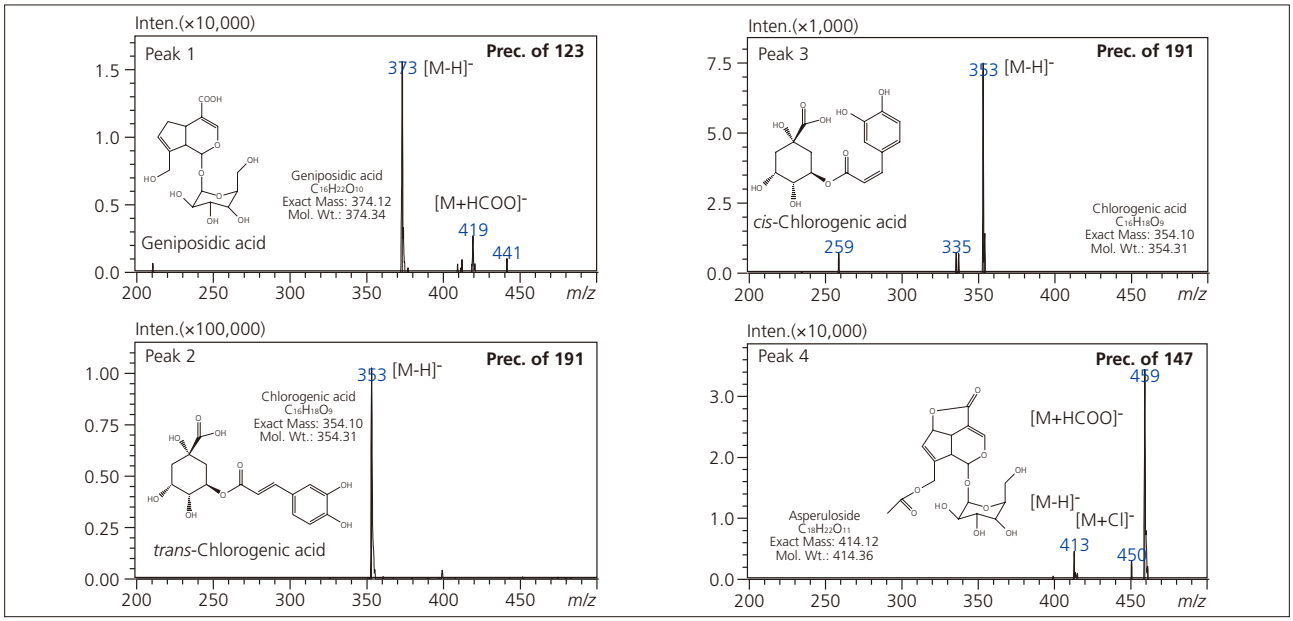

Fig. 2 MS Spectra of Peaks Obtained Using Precursor Ion Scan

Table 2 List of Compounds in Tochu Tea Obtained by Precursor Ion Scan

Event	Precursor of *m/z*	Geniposidic Acid Peak 1	*Trans*-Chlorogenic Acid Peak 2	*Cis*-Chlorogenic Acid Peak 3	Asperuloside Peak 4
1	123	373 $[M-H]^-$ 419 $[M+HCOO]^-$			
2	191		353 $[M-H]^-$	353 $[M-H]^-$	
3	147				413 $[M-H]^-$ 450 $[M+Cl]^-$ 459 $[M+HCOO]^-$

[Explanation of Terms]
· Precursor ion: Specific ion selected from Q1 (first MS step)
· Product ion: Ion originating from collision induced dissociation (CID) of precursor ion

Table 3 Analytical Conditions

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

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