SHIMADZU

Application News

Liquid Chromatography Mass Spectrometry No.C68

Analysis of Drug Degradants by LC/MS

This Application News introduces an example of high-speed analysis of drug degradants by LC/MS.
The ultra fast features of the LCMS-2020, scan rates up to 15,000 u/sec and positive/negative polarity switching as fast as 15 msec, were used in combination with in-source CID (Collision Induced Dissociation) to produce experimental results. In addition, formula prediction was conducted using the LCMS-IT-TOF, and separation of the sample components was carried out using the Prominence UFLCXR.

■ Analysis of Penicillin G Using the LCMS-2020

Fig. 1 shows a UV chromatogram and TIC chromatogram of penicillin G (1 mg/mL aqueous solution). We conducted a total of 6 measurements, all using different MS conditions consisting of positive or negative mode in conjunction with a range of 3 different DL and Q-array DC voltages (Fig. 2). At a scan speed of 15,000 u/sec, each mass spectrum was acquired within 0.05 sec. A complete cycle of voltage and polarity tests took 0.3 sec, and approximately 20 mass spectra were obtained for one UFLC peak under each set of MS conditions. Fragmentation ions of penicillin G were generated as a result of in-source CID. However, reduced sensitivity was seen at the high voltage settings of (c) 70 V and (f) -70 V of the Q-array due to the loss of normal ion focusing at these values. Fig. 3 shows the fragmentation ion assignments.

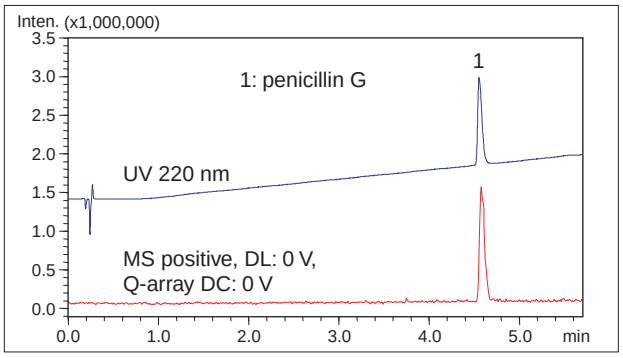

Fig. 1 Chromatograms of Penicillin G Standard Solution

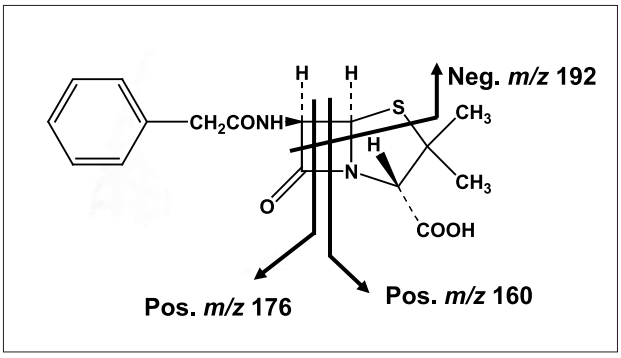

Fig. 3 Fragmentation of Penicillin G

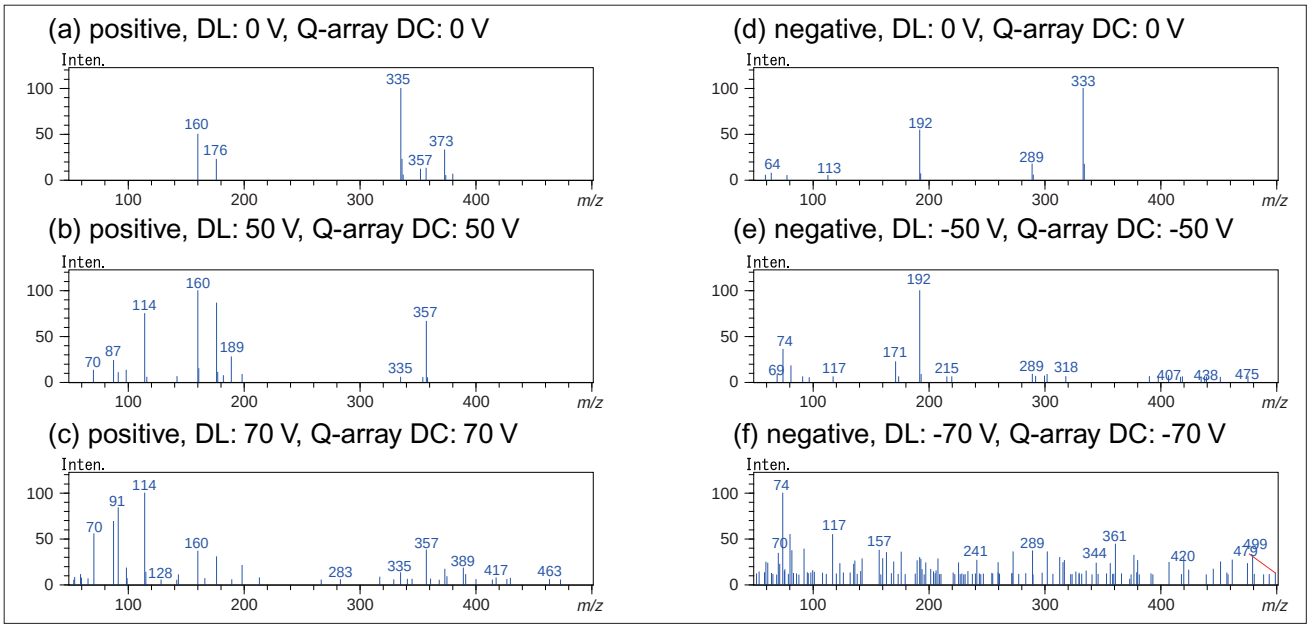

Fig. 2 Mass Spectra of Penicillin G

Mass Spectra of Penicillin G ((a) positive, DL: 0 V, Q-array DC: 0 V, (b) positive, DL: 50 V, Q-array DC: 50 V, (c) positive, DL: 70 V, Q-array DC: 70 V, (d) negative, DL: 0 V, Q-array DC: 0 V, (e) negative, DL: -50 V, Q-array DC: -50 V, (f) negative, DL: -70 V, Q-array DC: -70 V)

■ Analysis of Penicillin G Degradants Using LCMS-2020

A penicillin G standard solution (10 mg/mL aqueous solution) was heated at 60 ˚C for 40 hours to carry out decomposition. As shown in the chromatogram of Fig. 4, the penicillin G peak almost completely disappeared. It was observed that the mass spectral patterns of peaks 1 and 2 are very similar to each other. The mass spectra of peak 1 are shown in Fig. 5. The molecular weight is thought to be 308, and the fragmentation is different from that of penicillin G, so the structure is presumed to be different.

■ Formula Prediction Using LCMS-IT-TOF

We also conducted MS/MS measurement using the LCMS-IT-TOF in order to determine the likely composition of the degradants. Using both positive and negative detection, $C_{15}H_{20}N_2O_3S$ was the top-ranked prediction. Benzylpenilloic acids (Fig. 6) are known to be penicillin G impurities. Considering the formula prediction results and the existence of the 2 similar degradant peaks, it is supposed that these compounds were present in the degradation sample.

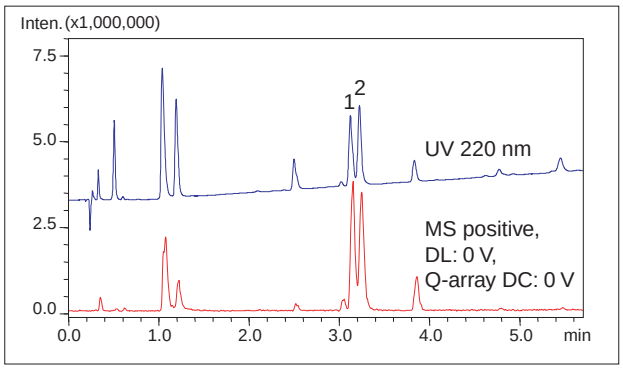

Fig. 4 Chromatograms of Degradation Products of Penicillin G

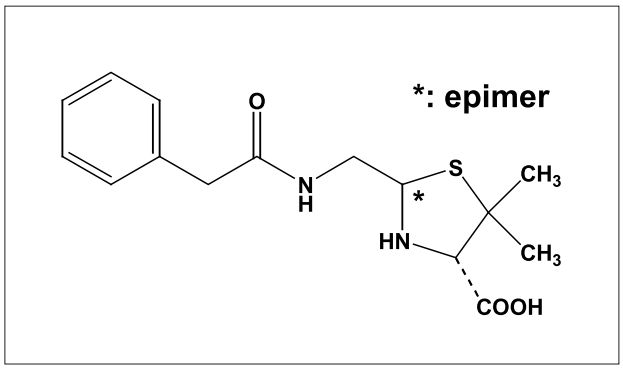

Fig. 6 Structures of Benzylpenilloic Acids

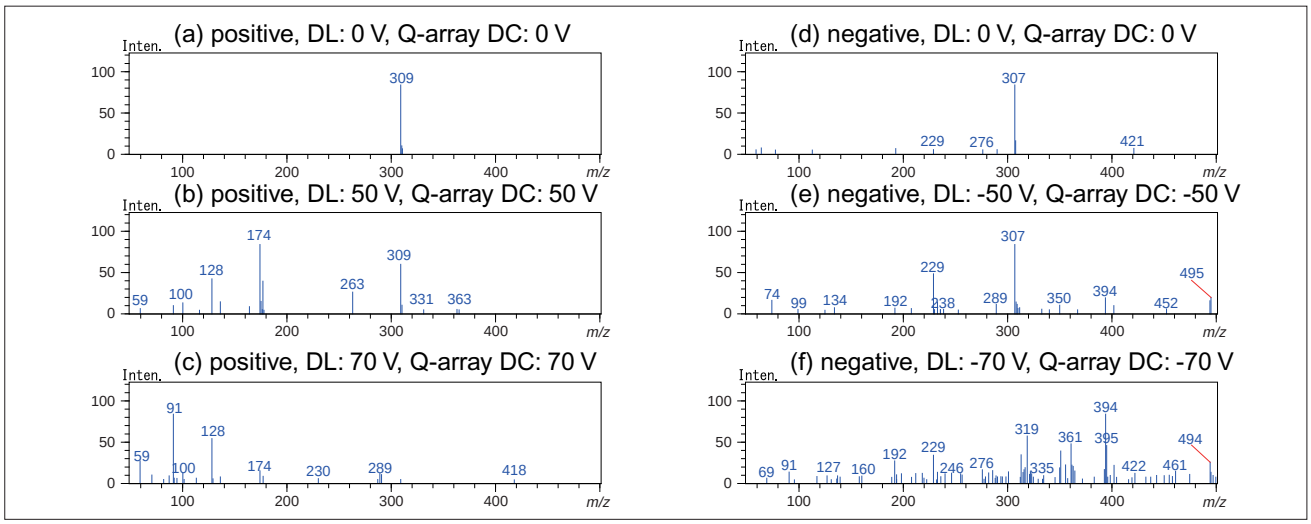

Fig. 5 Mass Spectra of Degradation Product of Penicillin G (peak 1)

Mass Spectra of Degradation Product of Penicillin G (peak 1) ((a) positive, DL: 0 V, Q-array DC: 0 V, (b) positive, DL: 50 V, Q-array DC: 50 V, (c) positive, DL: 70 V, Q-array DC: 70 V, (d) negative, DL: 0 V, Q-array DC: 0 V, (e) negative, DL: -50 V, Q-array DC: -50 V, (f) negative, DL: -70 V, Q-array DC: -70 V)

Table 1 Analytical Conditions

Column	: Shim-pack XR-ODS II (75 mm L. × 2.0 mm I.D., 2.2 µm)
Mobile Phase	: A: 50 mmol/L (ammonium) formate buffer (pH 3.9) B: 100 mmol/L (ammomium) formate buffer (pH 3.9)/ acetonitrile= 1/1
Time Program	: 20 %B (0 min) - 50 %B (5 min) - 20 %B (5.01 min) - STOP (10 min)
Flow Rate	: 0.8 mL/min
Column Temp.	: 40 ˚C
Injection Volume	: 0.5 µL

Detection	
PDA	
Wavelength	: 220 nm
MS (LCMS-2020)	
Probe Voltage	: +4.5 kV(ESI-Positive mode), -3.5 kV (ESI-Negative mode)
Nebulizing Gas Flow	: 1.5 L/min
Drying Gas Flow	: 20.0 L/min
DL Temp.	: 300 ˚C
Block Heater Temp.	: 450 ˚C
DL, Q-array Voltages	: (a) DL: 0 V, Q-array DC: 0 V, (b) DL: 50 V, Q-array DC: 50 V, (c) DL: 70 V, Q-array DC: 70 V, (d) DL: 0 V, Q-array DC: 0 V, (e) DL: -50 V, Q-array DC: -50 V, (f) DL: -70 V, Q-array DC: -70 V
Event Time	: 0.05 sec
Scan Range	: *m/z* 50 - 500

SHIMADZU

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