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High Performance Liquid Chromatograph Mass Spectrometer LCMS™-2050

Application News

Using a Single Quadrupole Mass Spectrometer to Check Peptide Synthesis and Analyze Impurities

Miho Kawashima and Tomoko Kuriki

User Benefits

- A single quadrupole mass spectrometer can be used in combination with an HPLC system to check for known compounds or quickly predict the molecular weight of unknown impurities.
- Designed for easy maintenance, the LCMS-2050 high performance liquid chromatograph mass spectrometer helps shorten instrument downtimes and reduce the time and labor required for maintenance.

■ Introduction

When analyzing peptides, mobile phases containing trifluoroacetic acid (TFA) are commonly used for analyzing purity levels by HPLC. This article describes an example of measuring a commercial peptide standard sample to check molecular weights and analyze impurities by MS using TFA-based mobile phase conditions and optional deconvolution software. The LCMS-2050 is a compact single quadrupole mass spectrometer (Fig. 1), which was developed specifically for easy operability and is a powerful tool for easily obtaining mass information that uses the same operating methods as HPLC systems. The LCMS-2050 is also designed for easy maintenance, which helps reduce instrument downtime and the time and labor required for maintenance.

Fig. 1 Nexera™ and LCMS™-2050 Systems

■ Sample Pretreatment and Analysis Conditions

The target peptides measured are indicated in Table 1.

Samples for analysis were prepared by dissolving and diluting commercial standard samples of those peptides in ultrapure water.

Table 1 Peptides Measured

#	Compounds	Formula	Molecular Weight
1	Somatostatin	$C_{76}H_{104}N_{18}O_{19}S_2$	1637.88
2	ACTH1-17 fragment	$C_{95}H_{145}N_{29}O_{23}S$	2093.41

Analytical conditions are indicated in Tables 2 and 3.

Table 2 LC Analytical Conditions (Nexera™ X3)

Column:	Shim-pack Scepter™ C18-120*1 (50 mm × 2.1 mm I.D., 1.9 µm)
Temperature:	40 °C
Mobile Phase:	Conditions for Using TFA A) 0.1 % TFA in H_2O B) 0.1 % TFA in acetonitrile Conditions for Using Formic Acid A) 0.1 % Formic acid in H_2O B) 0.1 % Formic acid in acetonitrile
Gradient:	B. Conc 10 % (0.00 to 1.50 min) → 80 % (5.00 min) → 10 % (5.01 to 8.00 min)
Flowrate:	0.4 mL/min

*1 P/N: 227-31012-03

Table 3 MS Analysis Conditions (LCMS-2050)

Ionization:	ESI/APCI (DUIS™), Positive mode
Mode:	Scan (*m/z* 250-2000)
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
DL Temperature:	450 °C
Desolvation Temperature:	200 °C
Interface Voltage:	+3.0 kV
Qarray Voltage:	+20 V

■ Comparison of TFA and Formic Acid Mobile Phases

When analyzing peptides, mobile phases containing TFA are commonly used for analyzing purity levels by an HPLC. However, considering the influence residual TFA can have when using an MS, using an instrument specialized for peptide analysis or changing to an acid other than TFA is recommended. On the other hand, changing the acid added to the mobile phase can change chromatogram peak separation patterns, which can require reassessing the analytical conditions.

In this example, the results from measuring somatostatin and ACTH1-17 fragment standard solutions using mobile phases containing either formic acid or TFA were compared. The resulting chromatograms obtained with a PDA detector are shown in Figs. 2 and 3. For the somatostatin solution, both mobile phases provided relatively good peak shapes. For the ACTH1-17 fragment solution, however, the formic acid resulted in more peak fronting than the TFA, which prevented detecting some of the impurity peaks that were detected with the TFA-based mobile phase.

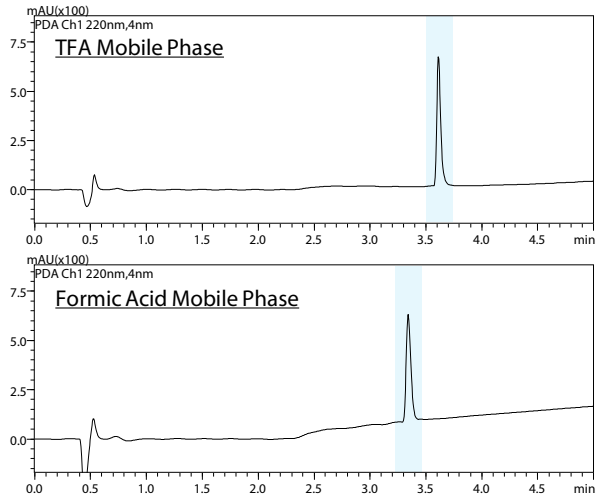

Fig. 2 Chromatogram from 0.1 mg/mL Somatostatin Standard Solution (5 µL Injected)

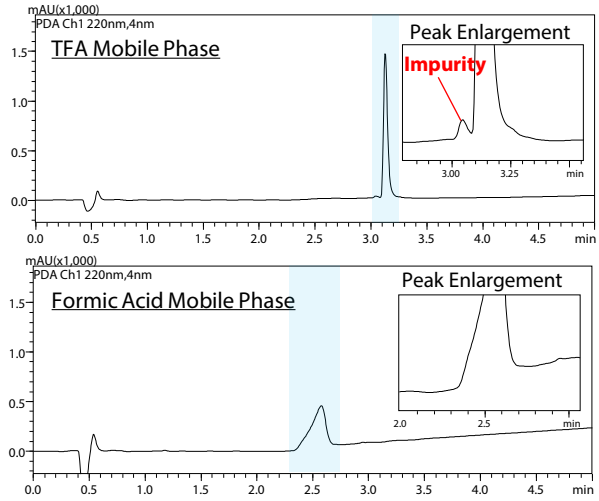

Fig. 3 Chromatogram from 2 mg/mL ACTH1-17 Standard Solution (10 µL Injected)

Note: This analysis serves as a single example. Actual results will vary depending on the compound measured.
It does not mean that TFA-based mobile phases will always result in better peak shapes than formic acid-based mobile phases.

For the ACTH1-17 fragment solution, the ion at *m/z* 704.1 was detected as the base peak in the mass spectrum for the impurity peak using the TFA-based mobile phase. The mass spectrum and corresponding mass chromatogram at *m/z* 704.1 are shown in Fig. 4. The mass chromatogram shows a peak in the position that matches the impurity peak shown in the PDA detector chromatogram.

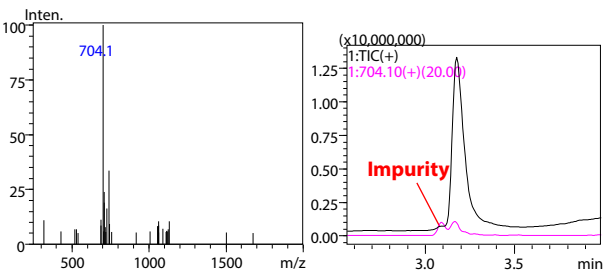

Fig. 4 Mass Spectrum and Mass Chromatogram of Impurity Peak Detected from 2 mg/mL ACTH1-17 Fragment Standard Solution (10 µL Injected)

■ Scan Analysis of Standard Peptide Samples

Somatostatin and ACTH1-17 fragment standard solutions were scanned using a mobile phase containing TFA. The TIC and mass spectrum for the somatostatin solution are shown in Fig. 5 and for the ACTH1-17 fragment solution in Fig. 6. Ions attributed to the respective multiply charged protonated molecules were detected.

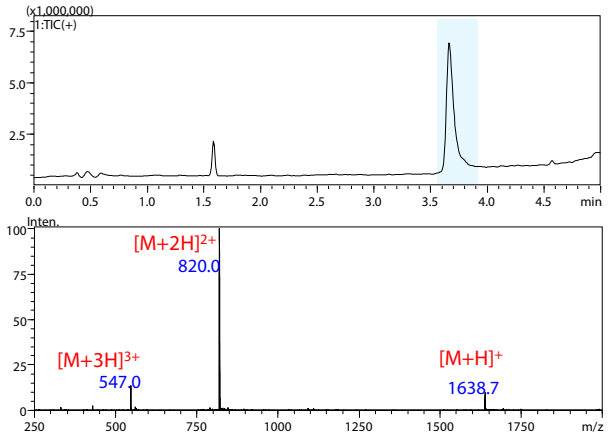

Fig. 5 TIC and Mass Spectrum from 0.1 mg/mL Standard Somatostatin Solution (5 µL Injected)

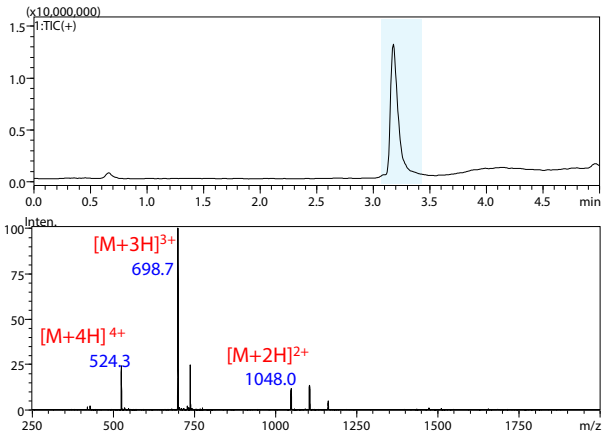

Fig. 6 TIC and Mass Spectrum from 2 mg/mL ACTH1-17 Fragment Standard Solution (10 µL Injected)

The results in Fig. 5 were entered in the deconvolution software to calculate the molecular weights in Fig. 7. The resulting calculated molecular weight of 1637.6 is consistent with the theoretical molecular weight (1637.88).

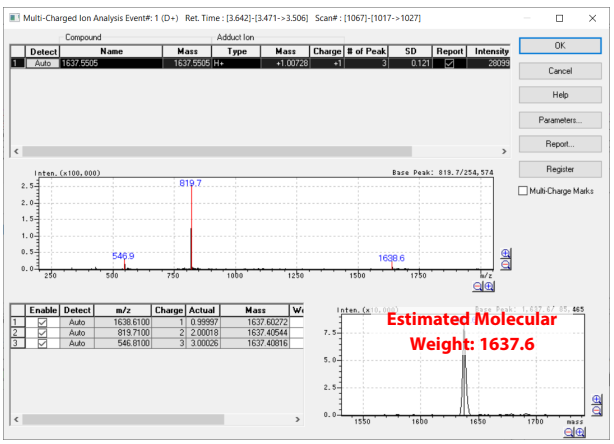

Fig. 7 Somatostatin Deconvolution Results

The results in Fig. 6 were entered in the deconvolution software to calculate the molecular weights in Fig. 8. The resulting calculated molecular weight of 2093.4 is consistent with the theoretical molecular weight (2093.41).

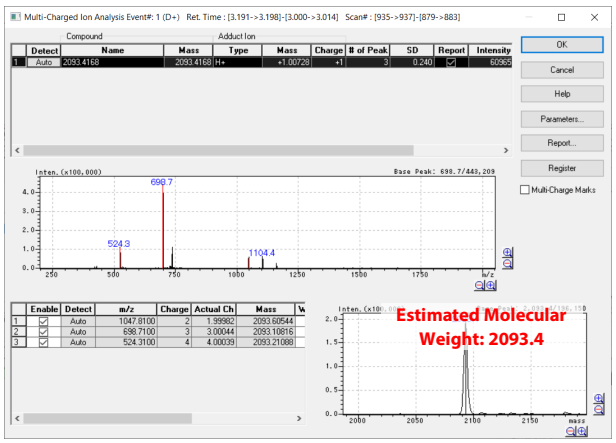

Fig. 8 ACTH1-17 Fragment Deconvolution Results

■ Easy Maintenance

If a mobile phase containing TFA is used, TFA will remain in the system even after the mobile phase is changed. So particular care is required due to higher background levels, inhibition of ionization, and other consequences from ionization of the TFA itself and also the possible effects on subsequent analyses.

In order to eliminate effects from TFA-derived ions, flow channels need to be adequately cleaned, and parts that contact the mobile phase need to be replaced in the HPLC unit, and parts also need to be replaced and the ion transport unit (lens system) cleaned in the MS unit.

The LCMS-2050 is designed so the area around the interface can be easily maintained, and the desolvation line (DL) used to load samples into the vacuum chamber can be replaced without tools and without stopping evacuation (Fig. 9). The ion transport unit can be removed easily from the front of the instrument and placed in a beaker for ultrasonic cleaning (Fig. 10). The LCMS-2050 reduces instrument downtime and the time and labor required for maintenance.

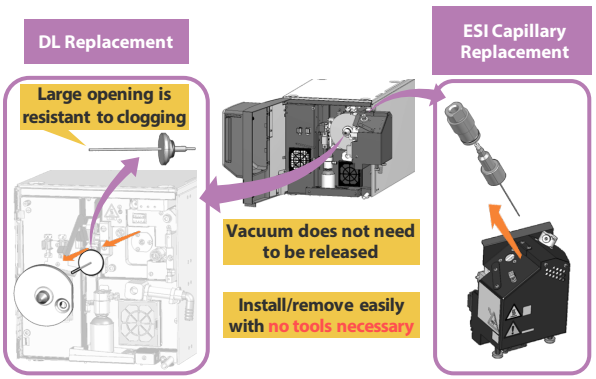

Fig. 9 Illustration of DL and ESI Capillary Replacement

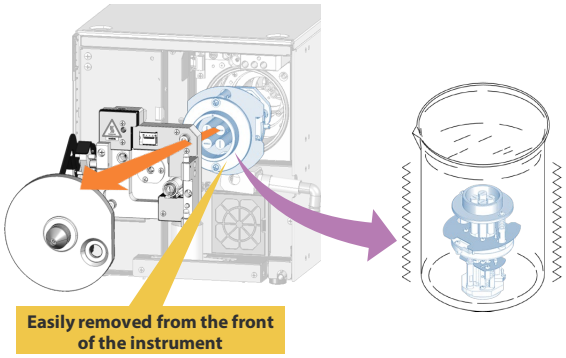

Fig. 10 Illustration of Ion Transport Unit (Lens System) Maintenance

■ Conclusion

Commercial peptide standard samples were analyzed using a mobile phase containing TFA, and deconvolution software was used to check molecular weights and mass spectra for impurities. The results showed peaks from multiply charged ions derived from target peptides and provided calculated molecular weight values that were consistent with theoretical molecular weight values. The test confirmed that the LCMS-2050 single quadrupole mass spectrometer can be used to quickly check the molecular weights of peptides.

Cleaning and maintenance are normally required after using TFA-based mobile phases in HPLC and MS systems, but the LCMS-2050 is designed to be especially easy to maintain. Consequently, it can be expected to shorten instrument downtimes and reduce the time and labor required for maintenance.

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