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Application News

LCMS-2050 High Performance Liquid Chromatograph Mass Spectrometer

Analysis of Impurities in Atorvastatin Using Single Quadrupole Mass Spectrometer

K. Koterasawa, K. Mukaibatake, and A. Toyama

User Benefits

- Easy to obtain molecular weight information of APIs and impurities contained in pharmaceuticals.
- Detected masses of APIs and impurities can be viewed directly on UV chromatograms for easy interpretation.
- Compound structures can be deduced by acquiring a pseudo-MS/MS spectrum through in-source CID.

■ Introduction

In addition to the API, drug substances contain trace amounts of impurities such as side reaction products, unreacted residues, and degradation products. When impurities are contained above the threshold specified in ICH-Q3, it is necessary to confirm their structure and safety.

The HPLC-UV method is generally used to evaluate the purity, and if an impurity is detected, it is required to confirm its identity. In this analysis, it is a standard approach to further connect a mass detector (LC-MS) to obtain molecular weight information that helps with impurity identification. Using the LC-MS system, it is possible to further deduce the molecular structure by performing MS/MS analysis.

In this report, we present an example of an analysis of a pharmaceutical product containing impurities using the LCMS-2050 high-performance liquid chromatography-mass spectrometer. We will show how mass information can be automatically added to absorbance detector data for easy peak identification, and furthermore, how impurities can be analyzed and identified using in-source CID.

■ Analytical Conditions and Samples

As a pharmaceutical sample, a solution of 1 mg/mL was prepared by dissolving commercially available atorvastatin calcium (purity ≥98%, Fig. 1). The impurity information for atorvastatin was referred to EP10.4.

For the analysis, we used a LC-MS system combining Nexera™ HPLC and LCMS-2050 as shown in Fig. 2. Analytical conditions are shown in Table 1 and Table 2.

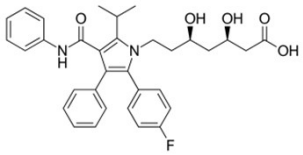


Fig. 1 Structural Formula of Atorvastatin

Fig. 2 Nexera™ and LCMS™-2050 System

Table 1 LC Analytical Conditions

System:	Nexera XR
Column:	Shim-pack™ XR-ODS*1 (50 mm x 2.0 mm I.D., 2.2 µm)
Mobile Phases:	A: 10 mM ammonium acetate aq. B: Acetonitrile
Flowrate:	0.3 mL/min
Gradient	B.Conc 10 % (0-2 min) →30 % (4-6 min) →100 % (20 min)
Colum Temperature:	40 °C
Injection Volume:	1 µL
Detection:	PDA 190-800 nm

*1 P/N：S228-41605-92

Table 2 MS Analytical Conditions

Ionization:	ESI/APCI (DUIS™)
Mode:	SCAN (*m/z* 100-1000)
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
DL Temperature:	200 °C
Desolvation Temperature:	450 °C
Interface Voltage:	3.0 kV
Qarray Voltage:	20/120 V

■ LC Analysis Results

A calcium atorvastatin solution was analyzed with simultaneous UV and mass detection, and the obtained UV chromatogram is shown in Fig. 3. The API, atorvastatin, eluted at 10.5 min, and several impurity peaks were detected before and after the API elution. The mass information obtained from the LCMS-2050 is overlaid onto the UV chromatogram along the retention time (Mass-it™ function). In this view, mass information of the main components and impurities can be intuitively understood, and it is easy to check for the presence of hidden components with low UV absorption.

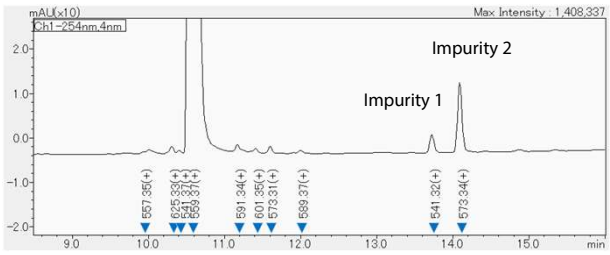


Fig. 3 UV Chromatogram and Mass Information for Atorvastatin

■ MS Analysis Results

In the UV chromatogram, two peaks with areas greater than 0.10% relative to the API were labeled as Impurities 1 and 2 (Impurity 1: 0.20%, Impurity 2: 0.78%). These were also detected as prominent peaks in the TIC chromatogram obtained by LCMS-2050 (Figure 4).

The average mass spectra obtained for the elution times of Impurities 1 and 2 are shown in Figure 5. From the results of these mass spectra, it is possible to estimate the molecular weight of each impurity and the presence or absence of co-elution.

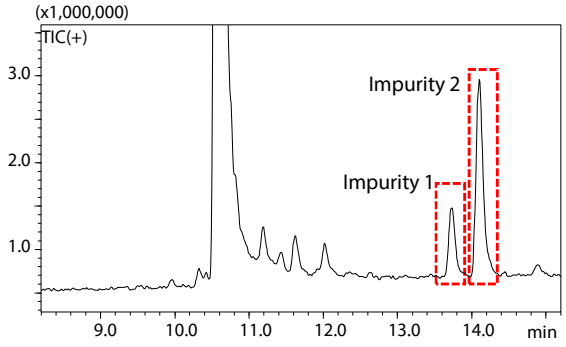


Fig. 4 TIC Chromatogram of Atorvastatin

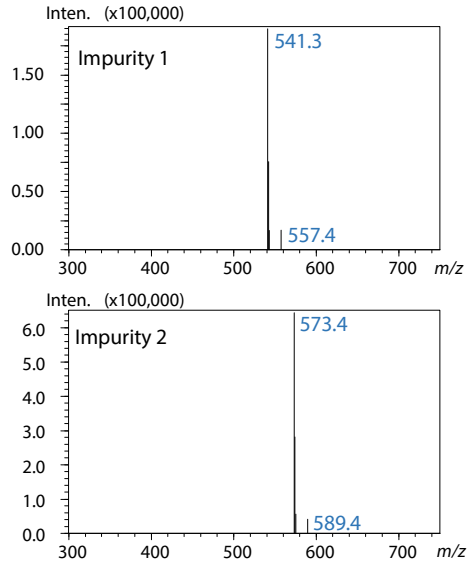


Fig. 5 Mass Spectra of Impurities 1 and 2 in Atorvastatin

■ Structural Analysis by In-Source CID

The LCMS-2050 can measure a pseudo-MS/MS spectrum by inducing collisional dissociation of analyte molecules before entering the mass analyzer. This technique for measuring ion fragments for structural elucidation is known as in-source CID.

The mass spectra obtained with in-source CID for the above two impurities are shown in Figures 6 and 8. To elucidate the structure, the m/z of detected fragments were matched against fragmentation of known impurities generated in-silico by ACD/Labs MS Workbook Suite software. The results suggested that Impurities 1 and 2 were Impurities H and G listed in EP10.4, respectively (Figure 7,9, Table 4,5).

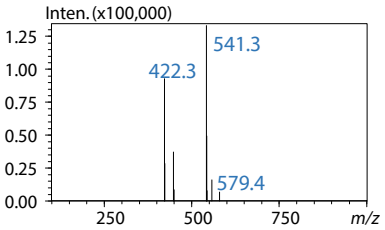
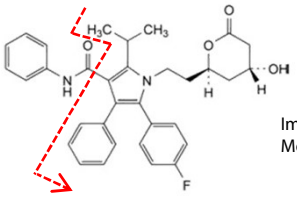


Fig. 6 Mass Spectrum of Impurity 1 with In-Source CID



Impurity H
Monoisotopic mass: 540.2

Fragment portion (*m/z* 422.2)

Fig. 7 Structural Formula and Predicted Fragments of Impurity H

Table 4 Theoretical and Measured Values for Molecular and Fragment Ions of Impurity H

Molecular Formula of Measured Ion	Theoretical Value	Measured Value
Protonated molecule: C33H34FN2O4 (+)	541.3	541.3
Fragment ion: C26H28FNO3 (+)	422.2	422.3

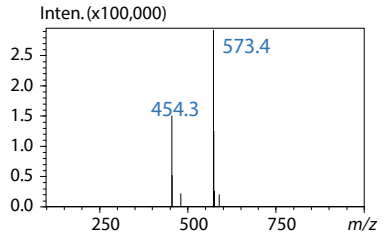
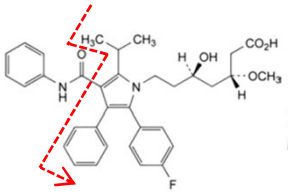


Fig. 8 Mass Spectrum of Impurity 2 with In-Source CID



Impurity G
Monoisotopic mass: 572.3

Fragment portion (*m/z* 454.2)

Fig. 9 Structural Formula and Predicted Fragments of Impurity G

Table 5 Theoretical and Measured Values for Molecular and Fragment Ions of Impurity G

Molecular Formula of Measured Ion	Theoretical Value	Measured Value
Protonated molecule ion: C34H37FN2O5 (+)	573.3	573.4
Fragment ion: C27H32FNO4 (+)	454.2	454.3

■ Conclusion

The benefit of adding a mass detector to Nexera HPLC for the analysis of API and impurities was demonstrated.

Molecular weight information could be easily acquired, and the signature mass signals were overlaid directly onto a standard UV chromatogram, allowing the operator to interpret the whole picture of the analysis at a glance. Furthermore, the structure of the impurities could be estimated by in-source CID.

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