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Application News

High Performance Liquid Chromatograph Mass Spectrometer LCMS™-2050

Analysis of Impurities in Montelukast Using Single Quadrupole Mass Spectrometer

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User Benefits

- Easy to obtain molecular weight information of APIs about main components and impurities contained in pharmaceuticals.
- Compound structures can be deduced by acquiring a pseudo-MS/MS spectrum through in-source CID .

■ Introduction

Official documents such as the Japanese Pharmacopoeia (JP), the European Pharmacopoeia (EP), and the United States Pharmacopeia (USP) describe the structural formulae of impurities in pharmaceuticals, and the identification of trace impurities in pharmaceuticals is important for quality assurance.

The HPLC-UV method is widely used to evaluate the purity, and if any impurity is detected, it must be identified. In such analyses, the standard approach is then to connect a mass spectrometer (LC-MS) and obtain molecular weight information to help with identification.

This report presents an example of the analysis of impurities in montelukast sodium using the Nexera™ series high-performance liquid chromatograph and the LCMS-2050 high-performance liquid chromatography-mass spectrometer. Montelukast sodium is listed in the 17th edition of the Japanese Pharmacopoeia and is used to treat bronchial asthma and allergic rhinitis.

This article shows 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

Test solution A (1 mg/mL) was prepared by dissolving montelukast sodium (Fig. 1), the reference standard for system suitability test according to the preparation procedure for montelukast sodium described in the JP. The analysis conditions are shown in Table 1 and Table 2. The LCMS-2050 is the same size as the LC unit and can be incorporated into existing LC systems such as the Nexera series and i-Series.

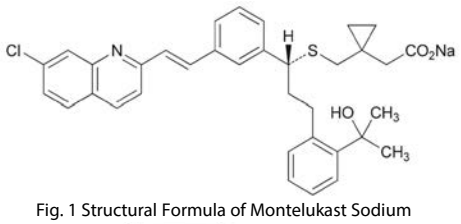

Fig. 1 Structural Formula of Montelukast Sodium

Fig. 2 Nexera™ and LCMS™-2050 System

Table 1 LC Analytical Conditions

System:	Nexera XR
Column:	Shim-pack Scepter™ Phenyl-120*1 (50 mm × 2.1 mm I.D., 1.9 µm)
Mobile Phases:	A: Water/formic acid = 2000:3 B: Acetonitrile/formic acid = 2000:3
Flowrate:	0.25 mL/min
Time Program:	B Conc. 45 % (0-3 min) →65 % (16 min) →45 % (16.1-25 min)
Column Temp.:	30 °C
Injection Volume:	10 µL
Vial:	SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
Detection:	238 nm (SPD-M40)

*1 P/N: 227-31063-03 *2 P/N: 227-34001-01

Table 2 MS Analytical Conditions

Ionization:	ESI/APCI (DUIS™), Positive mode
Mode:	MS scan (*m/z* 100-1000)
Nebulizing Gas Flow:	3.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
DL Temperature:	250 °C
Desolvation Temperature:	400 °C
Interface Voltage:	3.0 kV
Qarray Voltage:	80 V

■ LC Analysis Results

A montelukast sodium solution was analyzed, and the UV chromatogram obtained from the analysis is shown in Fig. 3. The main component, montelukast, was eluted at a retention time of about 9.7 min, and multiple impurity peaks were detected before and after the main peak.

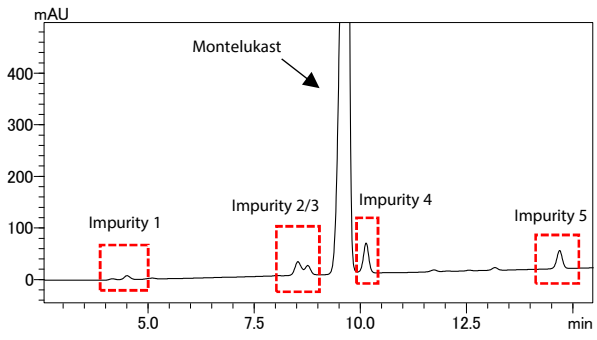

Fig. 3 UV Chromatogram for Montelukast Sodium

■ MS Analysis Results

The following is an example of confirming the molecular weight of impurity components based on the data obtained by the LCMS-2050. Fig. 4 shows the total ion chromatogram (TIC) for MS scan measurements in the same analysis as Fig. 3. Significant peaks for impurities 1 to 5 were also detected in the TIC chromatogram (Fig. 4).

JP lists six substances closely related to montelukast and reports their retention times relative to montelukast. Table 3 shows a list of the related substances whose relative retention times roughly match those of impurities 1 to 5. Fig. 5 suggests that impurity 4 and 5 are *m/z* 570 and *m/z* 568, respectively, which is consistent with the molecular formula information for related substances E and F described in the JP. Impurities 1 to 3 are also consistent with the JP information.

Table 3 Montelukast and Related Substances

Related substances	Impurity	Relative retention time	Molecular formula
A	1	0.4	$C_{35}H_{36}NO_4SCl$
C/D	2/3	0.9	$C_{41}H_{46}NO_5S_2Cl$
Montelukast	-	-	$C_{35}H_{36}NO_3SCl$
E	4	1.2	$C_{34}H_{32}NO_3SCl$
F	5	1.9	$C_{35}H_{34}NO_2SCl$

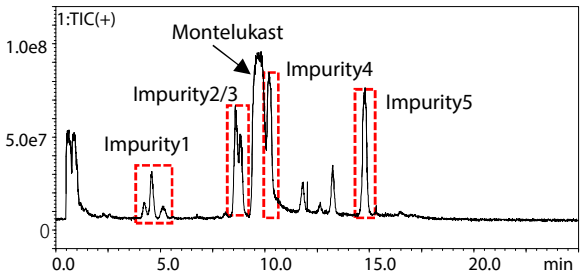

Fig. 4 TIC Chromatogram of Montelukast Sodium

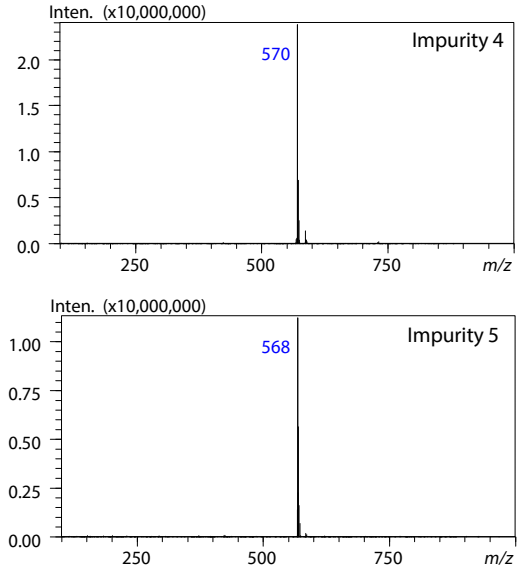

Fig. 5 Mass Spectra of Impurities 4 and 5 in Montelukast

■ Structural Analysis by In-Source CID

In addition to molecular weight information, the LCMS-2050 can also obtain structural information about molecules by setting a high Qarray voltage level to intentionally dissociate molecules within the ion source (in-source CID). Such ions formed by dissociation are referred to as fragment ions.

The mass spectrum for impurity 4 obtained by in-source CID is shown in Fig. 6. Based on the structural formula for related substance E (Fig. 7) described in JP, fragmentation positions were predicted. It was confirmed that there was no difference between the theoretical and measured molecular/ion weights.

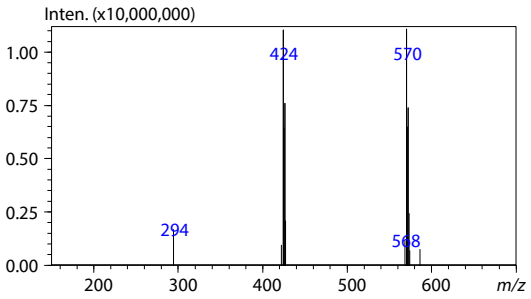

Fig. 6 Mass Spectrum of Impurity 4 after In-Source CID

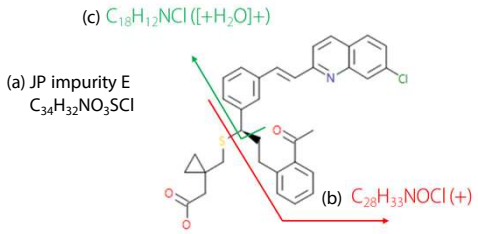

Fig. 7 Structural Formula and Predicted Fragments of Related Substance E

Table 4 Theoretical and Measured Values for Proton-Adduct Molecular and Fragment Ions of Related Substance E

Molecular formula of predicted ion	Theoretical value	Measured value
(a) Proton-adduct molecular: $C_{34}H_{32}NO_3SCl$	570	570
(b) Fragment ion: $C_{28}H_{23}NOCl$ (+)	424	424
(c) Fragment ion: $C_{18}H_{12}NCl$ ([+H_2O]+)	294	294

■ Conclusion

The LCMS-2050 liquid chromatograph mass spectrometer enables confirmation of mass information about the main components and impurities contained in pharmaceuticals. The structure of impurities can be predicted by using in-source CID to measure fragment ions. This method is expected to contribute to the structural analysis of trace impurities in other fields.

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