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Application News

Liquid Chromatograph-Mass Spectrometer LCMS™-9030

Analysis of Impurities in Pharmaceuticals Using LCMS-9030 Quadrupole Time-of-Flight Liquid Chromatograph-Mass Spectrometer

J. Nakazono, T. Iida

User Benefits

- Highly accurate qualitative analysis for impurities in pharmaceuticals is possible by using this system.
- The analysis software LabSolutions Insight Explore™ can be used for composition estimation, database search for the structural formula, and fragment assignment analysis.

■ Introduction

It is extremely important to identify trace impurities in pharmaceuticals to guarantee their quality and safety. The structural formulas of impurities in pharmaceuticals are described in official documents such as the Japanese Pharmacopoeia (JP), the European Pharmacopoeia, and the United States Pharmacopeia. In general, the HPLC-UV method is widely used for analysis of impurities. In contrast, structural analysis using a mass spectrometer such as LC/MS/MS is attracting attention as a useful means for identifying detected impurities.

This application news introduces an example of structural analysis of the impurities in montelukast sodium using the quadrupole time-of-flight (QTOF) mass spectrometer LCMS-9030 (Fig. 1) and the software LabSolutions Insight Explore. Montelukast sodium is listed in the 17th edition of the JP and is used as a therapeutic drug for bronchial asthma and allergic rhinitis.

Fig. 1 Exterior of Nexera™ X3 and LCMS™-9030

■ Analysis of Montelukast Sodium

According to the preparation procedure for montelukast sodium (Fig. 2) described in the Japanese Pharmacopoeia (JP), solution A (1 mg/mL) was prepared using the montelukast standard for system suitability test. Table 1 lists the analytical conditions.

Fig. 3 shows the obtained UV chromatogram. Montelukast, the principal component, was eluted at the retention time of approximately 10 min, and impurity peaks ① to ⑤ were detected before and after that.

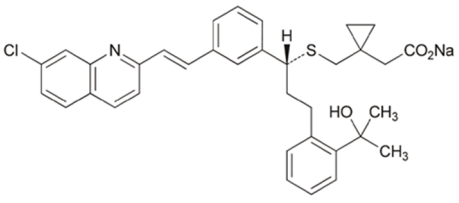


Fig. 2 Structural Formula of Montelukast Sodium

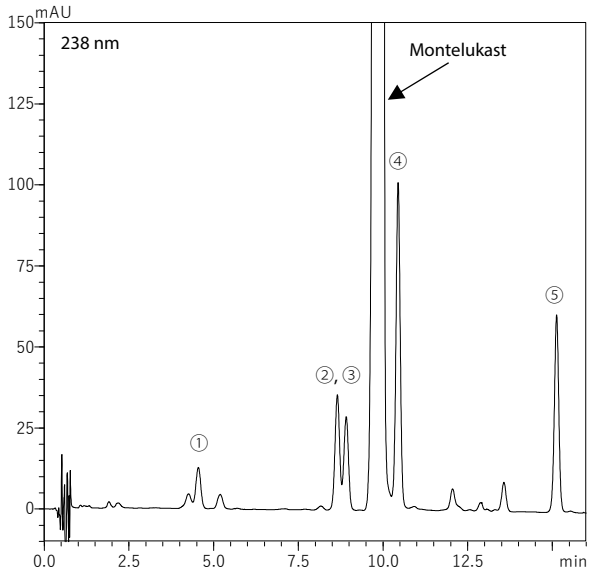


Fig. 3 UV Chromatogram of Solution A

Table 1 Analytical Conditions

[UHPLC conditions (Nexera X3)]	
Column	: Shim-pack Scepter™ Phenyl-120, 50 mm×2.1 mm I.D., 1.9 µm*1
Mobile phase A	: Water/Formic acid = 2000/3
Mobile phase B	: Acetonitrile/Formic acid = 2000/3
Flow rate	: 0.25 mL/min
Time program (%B)	: 45% (0–3 min) → 65% (16 min) → 45% (16.1–25 min)
Column temp.	: 30 ℃
Injection volume	: 10 µL
Detection	: UV 238 nm
[MS conditions (LCMS-9030)]	
Ionization	: ESI positive
Mode	: MS, MS/MS scan
Nebulizing gas flow	: 3.0 L/min
Drying gas flow	: 10.0 L/min
Heating gas flow	: 10.0 L/min
DL temp.	: 250 ℃
BH temp.	: 400 ℃
Interface temp.	: 300 ℃

*1: P/N 227-31063-03

■ Composition Estimation for Impurities

Peaks for impurities, corresponding respectively to the retention time detected by UV, were observed on the extracted ion chromatogram (XIC) at *m/z* 602.2126, 732.2579, 570.1864 and 568.2072 by mass spectrometry. Fig. 4 shows the total ion chromatogram (TIC) of solution A and the XIC of each impurity.

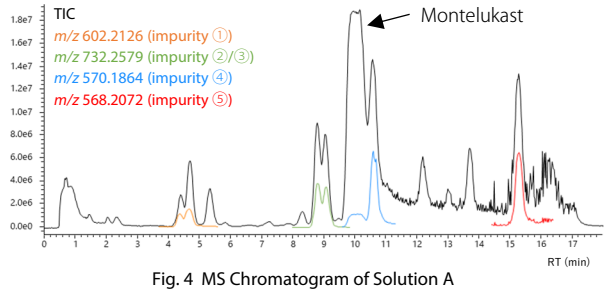
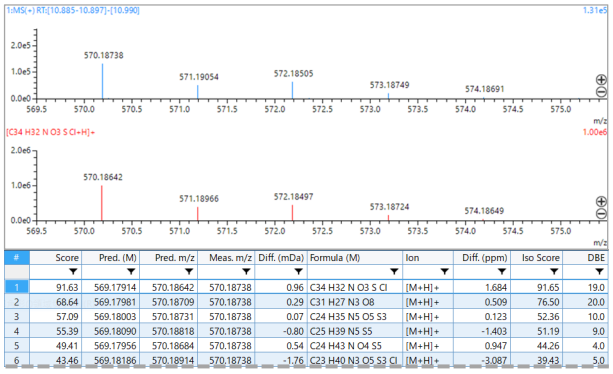


Fig. 4 MS Chromatogram of Solution A

Based on the obtained MS spectra, composition estimation was performed using Insight Explore. As an example, Fig. 5 shows the composition estimation results for the impurity peak ④ (*m/z* 570.1864). It was found that the compositional formula with the highest score is $C_{34}H_{32}NO_3SCl$.



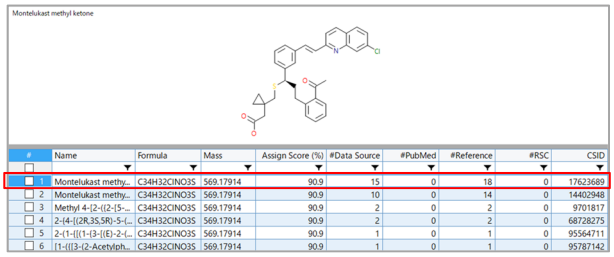
#	Score	Pred. (M)	Pred. m/z	Meas. m/z	Diff. (mDa)	Formula (M)	Ion	Diff. (ppm)	Iso Score	DBE
1	91.63	569.17914	570.18642	570.18738	0.96	C34 H32 N O3 S Cl	[M+H]+	1.684	91.65	19.0
2	68.64	569.17981	570.18709	570.18738	0.29	C31 H27 N3 O8	[M+H]+	0.509	76.50	20.0
3	57.09	569.18003	570.18731	570.18738	0.07	C24 H35 N5 O5 S3	[M+H]+	0.123	52.36	10.0
4	55.39	569.18090	570.18818	570.18738	-0.80	C25 H39 N5 S5	[M+H]+	-1.403	51.19	9.0
5	49.41	569.17956	570.18684	570.18738	0.54	C24 H43 N O4 S5	[M+H]+	0.947	44.26	4.0
6	43.46	569.18186	570.18914	570.18738	-1.76	C23 H40 N3 O5 S3 Cl	[M+H]+	-3.087	39.43	5.0

Fig. 5 Results of Composition Estimation for Impurity ④
(Top: Observed MS Spectrum, Middle: Theoretical Spectrum, Bottom: Candidates of Compositional Formula)

■ Compound Search and Fragment Assignment

An analysis was carried out using the "Assign" function of Insight Explore to confirm the structural formula and the compound name of impurity peak ④. This software makes it possible to list compounds from the online ChemSpider™ database based on compositional formula information. Then, by conducting the assignment for the obtained compounds, the degree of coincidence (assign score) between the product ions obtained via fragment prediction and those observed in the measured MS/MS spectrum is calculated.

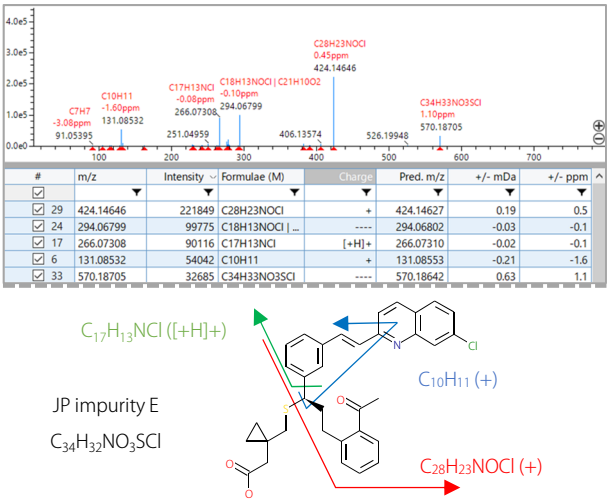
An online database search for the compositional formula $C_{34}H_{32}NO_3SCl$ found six candidate compounds (Fig. 6). The top-ranked compound was Montelukast methyl ketone (ChemSpider ID 17623689). This compound was found to be same as the impurity E described in the JP: [1-({[(1*R*)-3-(2-Acetylphenyl)-1-{3-[(*E*)-2-(7-chloro-2-quinolinyl)vinyl] phenyl}propyl]sulfanyl}methyl)cyclopropyl]acetic acid.



#	Name	Formula	Mass	Assign Score (%)	#Data Source	#PubMed	#Reference	#RSC	CSID
1	Montelukast methy...	C34H32ClNO3S	569.17914	90.9	15	0	18	0	17623689
2	Montelukast methy...	C34H32ClNO3S	569.17914	90.9	10	0	14	0	14402948
3	Methyl 4-[2-({2-[5-...	C34H32ClNO3S	569.17914	90.9	2	0	2	0	9701817
4	2-[4-[(2R,3S,5R)-5-(...	C34H32ClNO3S	569.17914	90.9	2	0	2	0	68728275
5	2-(1-({[1-(3-{(E)-2-(...	C34H32ClNO3S	569.17914	90.9	1	0	1	0	95564711
6	[1-({[3-(2-Acetylph...	C34H32ClNO3S	569.17914	90.9	1	0	1	0	95787142

Fig. 6 Results of ChemSpider Database Search for $C_{34}H_{32}NO_3SCl$

Next, Fig. 7 shows an example of automatic assignment for fragment ions by the "Assign" function.



#	m/z	Intensity	Formulae (M)	Charge	Pred. m/z	+/- mDa	+/- ppm
29	424.14646	221849	C28H23NOCl	+	424.14627	0.19	0.5
24	294.06799	99775	C18H13NOCl | ...	----	294.06802	-0.03	-0.1
17	266.07308	90116	C17H13NCl	[+H]+	266.07310	-0.02	-0.1
6	131.08532	54042	C10H11	+	131.08553	-0.21	-1.6
33	570.18705	32685	C34H33NO3SCl	----	570.18642	0.63	1.1

Fig. 7 Results of Fragment Assignment for Impurity E

Finally, the LCMS-9030 analytical results for each impurity are summarized in Table 2. It was also found that each of the impurity peaks other than ④ corresponds to the impurities described in the JP. Moreover, it was possible to analyze the impurities with high mass accuracy within 1 mDa of mass error compared to the theoretical mass value.

Table 2 LCMS-9030 Analytical Results for Each Impurity

Impurity peak	HPLC RT (min)	JP listed impurities	$[M+H]^+$ theoretical	$[M+H]^+$ observed	Error (mDa)
①	4.55	A	602.2126	602.2132	0.59
②/③	8.65/8.92	C/D	732.2579	732.2584	0.52
-	9.97	Montelukast	586.2177	586.2179	0.15
④	10.45	E	570.1864	570.1874	0.96
⑤	15.13	F	568.2072	568.2079	0.70

■ Conclusion

By using a Shimadzu LCMS-9030 quadrupole time-of-flight liquid chromatograph-mass spectrometer and an analysis software LabSolutions Insight Explore, it is possible to analyze the structure of impurities contained in pharmaceuticals. This procedure is expected to contribute to the analysis of trace impurities in products in other fields such like food and chemical industries.

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