High Performance Liquid Chromatograph Mass Spectrometer   LCMS-9050

Application News

Qualitative Analysis of Drug Metabolites Using LCMS-9050

Yutaka Umakoshi

User Benefits

◆ The LCMS-9050 quadrupole time-of-flight (Q-TOF) mass spectrometer and LabSolutions Insight Explore™ provide qualitative analysis of drug metabolites.

◆ Analyze and Target Screening function in LabSolutions Insight Explore enables peak extraction of candidate drug metabolites.

■ Introduction

After a drug is ingested into the body, it is metabolized by various reactions, including oxidation, reduction, hydrolysis, and conjugation. The prediction of drug metabolites is important because the metabolites may differ from the original drug in terms of efficacy and toxicity.

This Application News introduces an example of qualitative analysis of drug metabolites using the LCMS-9050 quadrupole time-of-flight (Q-TOF) mass spectrometer. Lenvatinib, a known anticancer drug, was administered orally to rats, and the metabolites in the liver were analyzed. Drug metabolite peaks were extracted using the Analyze function in LabSolutions Insight Explore. The Target Screening function narrowed down the candidate peaks based on a list of drug metabolites, and the MS and MS/MS spectra were analyzed in detail.
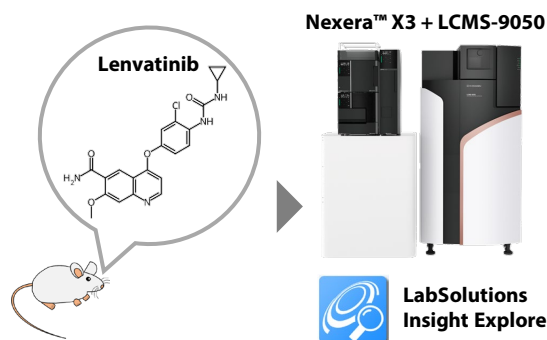

Nexera™ X3 + LCMS-9050

Lenvatinib

LabSolutions Insight Explore

Fig. 1 Overview

■ Sample Pretreatments and Analytical Conditions

Lenvatinib was administered (4 mg/kg/day) orally to male SD rats aged 10 weeks for 5 days, and their livers were then removed. Sample preparation was performed as follows.

1. Approximately 60 mg of rat liver was weighed, and methanol was added to give a concentration of 50 mg/mL.

2. Three zirconia beads 3 mm were added and crushed with Bead Smash 12 (Cooling, 30 sec, 4,000 rpm).

3. After centrifugation (4 ℃, 5 min, 13,000 rpm), 500 µL of the supernatant was collected.

4. After centrifugal concentration, it was redissolved in 50 µL of methanol.

Table 1 shows the analysis conditions. LCMS-9050 was used for the measurement, and the data was acquired in Data Dependent Acquisition (DDA) mode. In DDA mode, MS/MS spectrum of ions with high intensity in MS measurement are automatically acquired. In this mode, MS spectrum useful for molecular composition analysis and MS/MS spectrum useful for structural analysis can be acquired simultaneously.

Table 1 Analytical Conditions

[HPLC] Nexera X3	
Column:	Shim-pack Scepter™ C18-120 (100 mm × 2.1 mm I.D., 3 µm)*¹
Column Temp.:	40 ℃
Solvent A:	Water + 0.1 % Formic acid
Solvent B:	Acetonitrile + 0.1 % Formic acid
Rinse:	Acetonitrile + 0.1 % Formic acid
Gradient:	B conc. 5 % (0 min) → 40 % (9.0 min) → 98 % (10.0-12.0 min) → 5 % (12.1-15.0 min)
Flowrate:	0.25 mL/min
Injection Volume:	5 µL
[MS] LCMS-9050	
Ionization:	ESI positive
Nebulizing Gas:	3.0 L/min
Drying Gas:	10.0 L/min
Heating Gas:	10.0 L/min
DL Temp.:	250 ℃
Heat Block Temp.:	400 ℃
Interface Temp.:	300 ℃
CID Gas Pressure:	230 kPa
Mode:	DDA
MS Scan Range:	m/z 50-1,000 (0.100 sec)
MS/MS Scan Range:	m/z 50-1,000 (0.100 sec × 10)
CE:	35 ± 17 kV
DDA Threshold:	3,000

*1 P/N: 227-31014-05

■ Data Analysis

Fig. 2 shows the workflow for metabolite qualitative analysis. First, a list of molecular formulas of metabolites that may be produced by lenvatinib was prepared. The list of molecular formulas was made using previous research and metabolic reaction prediction software. Next, the peak extraction was performed using the Analyze function of LabSolutions Insight Explore. After that, the Target Screening function imported the prepared list and narrowed down the candidate peaks.

Name	Formula
Lenvatinib	C21H19ClN4O4
Demethylation	C20H17ClN4O4
Demethylation, Oxidation	C20H17ClN4O5
Hydrolysis	C17H14ClN3O3
Oxidation	C21H19ClN4O5
·	·
·	·
·	·

#	RT	m/z	Response	Target Name	Target Formula	Target m/z	Mass Error (mDa)
675	8.113	443.11239	1865758	Oxidation	C21H19ClN4O5	443.11167	0.72
417	6.309	443.11238	432537	Oxidation	C21H19ClN4O5	443.11167	0.71
122	3.855	219.07671	188734	O-dearylation2	C11H10N2O3	219.07642	0.29
303	5.392	403.09096	791444	O-dearylation1 OH-gL...	C16H19ClN2O8	403.09027	0.69
584	7.497	227.05843	2397710	O-dearylation1	C10H11ClN2O2	227.05818	0.25
551	7.269	427.11738	9222297	Lenvatinib	C21H19ClN4O4	427.11676	0.62
435	6.439	344.08019	3490408	Hydrolysis	C17H14ClN3O3	344.07965	0.54
581	7.364	413.10186	108585	Demethylation	C20H17ClN4O4	413.10111	0.75
295	5.359	399.12836	138870	----	----	----	----
294	5.364	397.18096	149472	----	----	----	----
293	5.359	396.17704	584142	----	----	----	----
292	5.364	379.15186	147977	----	----	----	----
291	5.359	378.14937	1134566	----	----	----	----
290	5.359	377.14632	5864459	----	----	----	----
289	5.338	368.15571	253334	----	----	----	----
288	5.319	415.13070	594592	----	----	----	----
287	5.315	402.23407	514954	----	----	----	----
297	5.373	418.15952	120751	----	----	----	----

Analyze

Predict

Group

Spectrum

Chromatogram

Advanced

Screen

Fig. 2 Workflow of Qualitative Analysis

■ Detected Compounds

The workflow described above detected several metabolites that were likely derived from lenvatinib. The chromatograms of the detected compounds are shown in Fig. 3. In addition to lenvatinib (1), compounds thought to be derived from demethylation (2), hydrolysis (3), dearylation (4), (5), (6), and oxidation (7) reactions were detected.
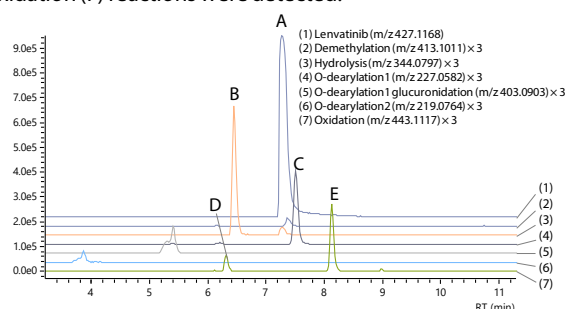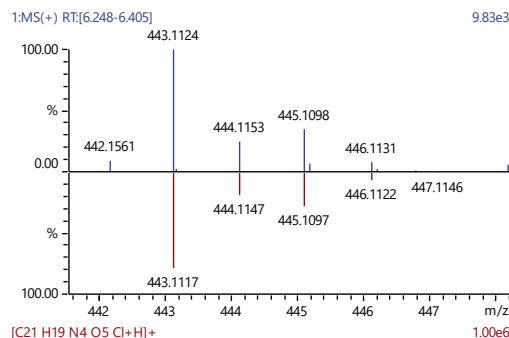
(1) Lenvatinib (m/z 427.1168)
(2) Demethylation (m/z 413.1011) × 3
(3) Hydrolysis (m/z 344.0797) × 3
(4) O-dearylation1 (m/z 227.0582) × 3
(5) O-dearylation1 glucuronidation (m/z 403.0903) × 3
(6) O-dearylation2 (m/z 219.0764) × 3
(7) Oxidation (m/z 443.1117) × 3

Fig. 3 Chromatogram
(All peaks except for lenvatinib are shown at 3-fold magnification.)

Below is a more detailed analysis of peak A for lenvatinib and peaks B to E for metabolites with higher intensities.

■ Comparison of Measured and Theoretical MS Spectra

Fig. 4 shows the measured and theoretical MS spectra. The isotopic ratio of ^{35}Cl to ^{37}Cl was approximately 3:1, and all peaks A to E were confirmed to reflect the natural isotopic ratio of chlorine in the MS spectrum. It was also confirmed that the differences between the measured and theoretical monoisotopic mass were all within 1 mDa.

A: Lenvatinib (C21H19ClN4O4) Mass Error: 0.63 mDa
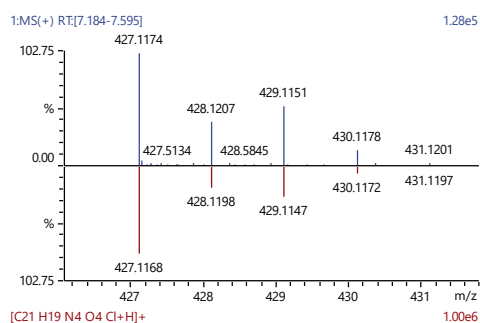
B: Hydrolysis (C17H14ClN3O3) Mass Error: 0.54 mDa
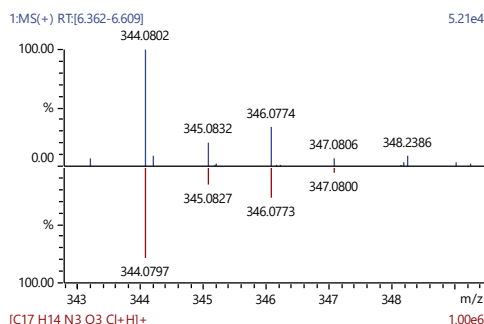
C: O-dearylation1 (C10H11ClN2O2) Mass Error: 0.24 mDa

D: Oxidation (C21H19ClN4O5) Mass Error: 0.72 mDa

E: Oxidation (C21H19ClN4O5) Mass Error: 0.72 mDa

Fig. 4 MS Spectrum
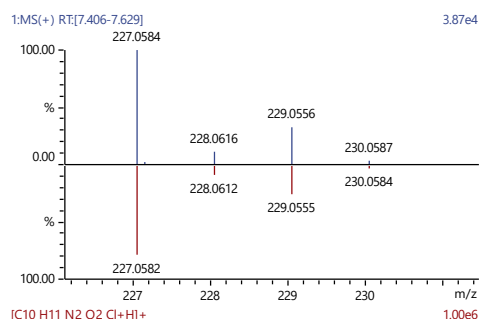(Top: measured MS spectrum, bottom: theoretical MS spectrum)

■ MS/MS Spectrum

Fig. 5 shows the MS/MS spectrum acquired in DDA mode. The cleavage positions predicted using the Assign function in LabSolutions Insight Explore are also shown. For more information about the Assign function, see the previous Application News.[1,2]
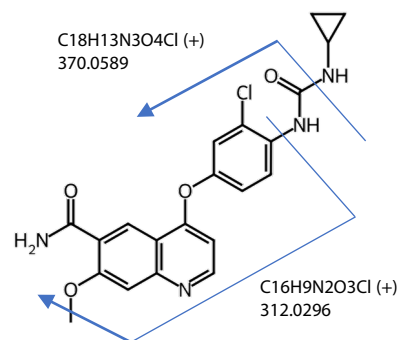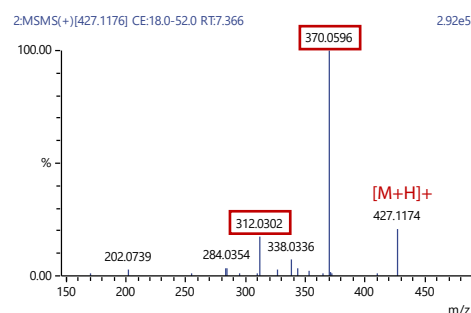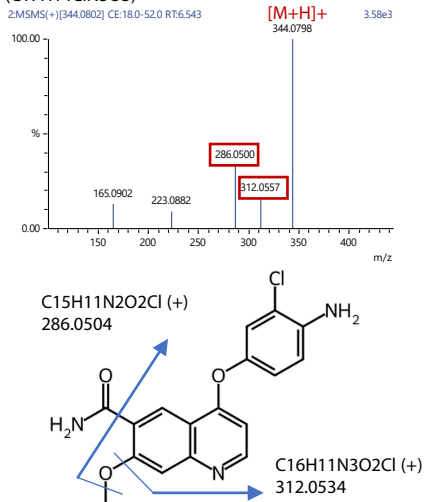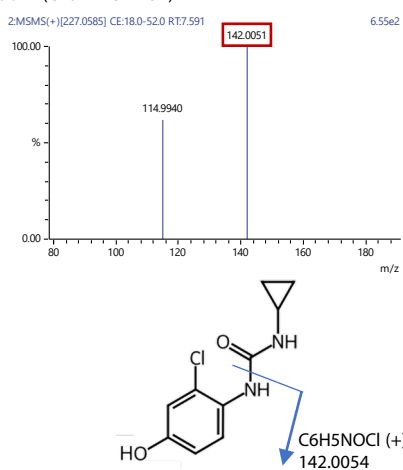
A: Lenvatinib (C21H19ClN4O4)

C18H13N3O4Cl (+)
370.0589

C16H9N2O3Cl (+)
312.0296

Fig 5 MS/MS Spectrum and Estimated Cleavage Positions

B: Hydrolysis (C17H14ClN3O3)

2:MSMS(+)[344.0802] CE:18.0-52.0 RT:6.543

[M+H]+ 3.58e3
344.0798

100.00

%

286.0500
312.0557
165.0902   223.0882

0.00
150   200   250   300   350   400   m/z

C15H11N2O2Cl (+)
286.0504

C16H11N3O2Cl (+)
312.0534

C: O-dearylation1 (C10H11ClN2O2)

2:MSMS(+)[227.0585] CE:18.0-52.0 RT:7.591

142.0051   6.55e2

100.00

%

114.9940

0.00
80   100   120   140   160   180   m/z

C6H5NOCl (+)
142.0054

D: Oxidation (C21H19ClN4O5)

2:MSMS(+)[443.1118] CE:18.0-52.0 RT:6.269   4.24e3

370.0595
100.00

%

387.0852
[M+H]+
443.1126

0.00
350   375   400   425   450   475   m/z

C18H13N3O4Cl (+)
370.0589

C18H16N4O4Cl ([+H]+)
387.0855

E: Oxidation (C21H19ClN4O5)

4:MSMS(+)[443.1112] CE:18.0-52.0 RT:8.059   1.97e3

386.0535
100.00

%

295.0274
323.0239
[M+H]+
443.1149

0.00
250   300   350   400   450   500   m/z

C18H13N3O5Cl (+)
386.0538

Fig 5 MS/MS Spectrum and Estimated Cleavage Positions (Continued)
Since the detailed oxidation positions of peaks D and E could not be
determined from the MS/MS spectrum, the estimated structures are shown.

Two metabolites produced by oxidation, peaks D and E, were
detected. The product ion with the highest intensity, peak D,
was C18H13N3O4Cl (+), consistent with lenvatinib. On the other
hand, the product ion with the highest intensity, peak E, was
C18H13N3O5Cl (+), which was different from lenvatinib.
Therefore, it was assumed that peaks D and E had different
oxidation positions.

■ Conclusion

Several metabolites derived from lenvatinib were detected by
the LCMS-9050 quadrupole time-of-flight mass spectrometer
and LabSolutions Insight Explore. By preparing a list of drug
metabolites, it was possible to easily extract candidate
metabolite peaks. This workflow can also be applied to the
metabolite analysis of drugs other than lenvatinib.

<Related Applications>

1. Analysis of Impurities in Pharmaceuticals Using LCMS-9030
    Quadrupole Time-of-Flight Liquid Chromatograph-Mass
    Spectrometer 01-00017-EN
2. Screening Analysis of Metabolites in Red Wine 01-00329-EN

LabSolutions Insight Explore, Nexera, and Shim-pack Scepter are trademarks of Shimadzu Corporation or its affiliated companies in Japan and/or other countries.

Related Products
Some products may be updated to newer models.

LCMS-9050
Quadrupole Time-of-Flight Liquid Chromatograph Mas…

Shim-pack Scepter LC Columns
HPLC Column

Related Solutions

Small Molecule Pharmaceutical

Life Science

Metabolomics

Pharmaceutical and Biopharmaceutical

Price Inquiry

Product Inquiry

Technical Service / Support Inquiry

Other Inquiry