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High Performance Liquid Chromatograph Mass Spectrometer LCMS-9050

Application News

Qualitative Analysis of Drug Metabolites Using LCMS-9050

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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.

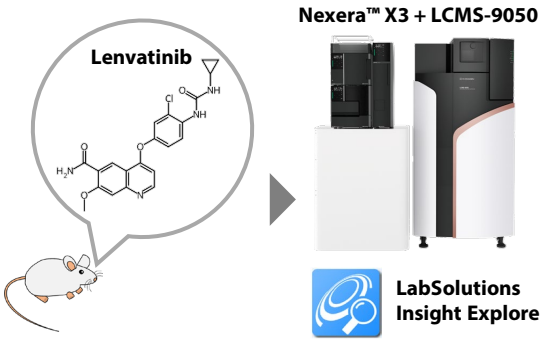


Fig. 1 Overview

■ 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 °C, 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 °C
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 °C
Heat Block Temp.:	400 °C
Interface Temp.:	300 °C
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.

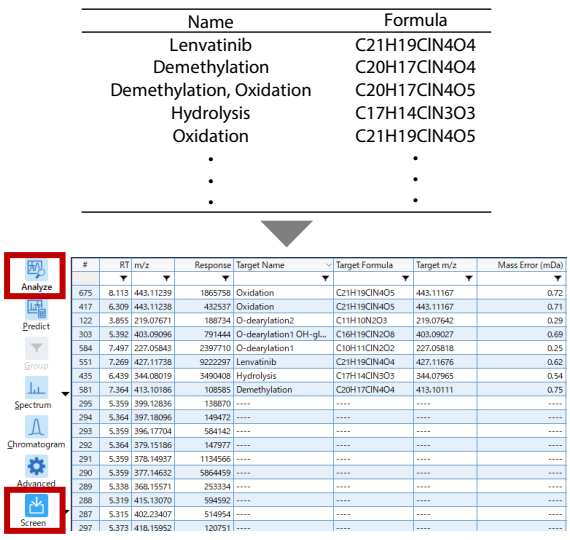


Fig. 2 Workflow of Qualitative Analysis

■ 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.

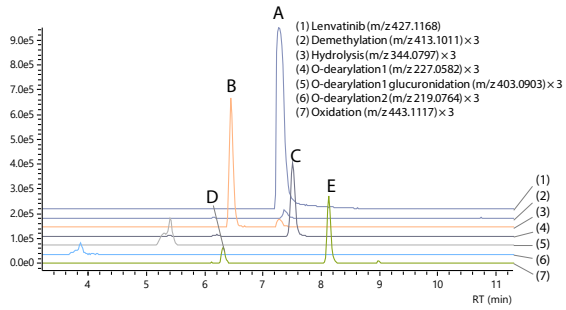


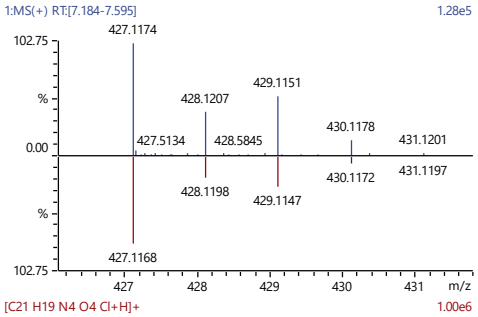
Fig. 3 Chromatogram
(All peaks except for lenvatinib are shown at 3-fold magnification.)

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

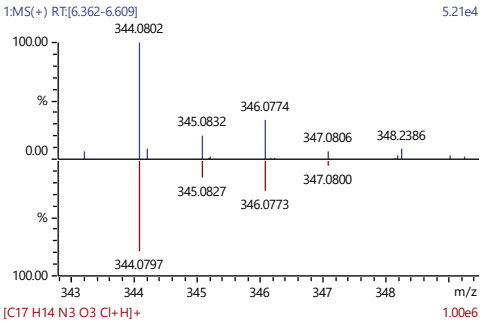
■ Comparison of Measured and Theoretical MS Spectra

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.

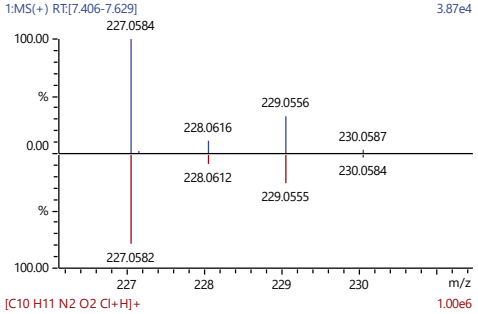
A: Lenvatinib (C21H19ClN4O4) Mass Error: 0.63 mDa



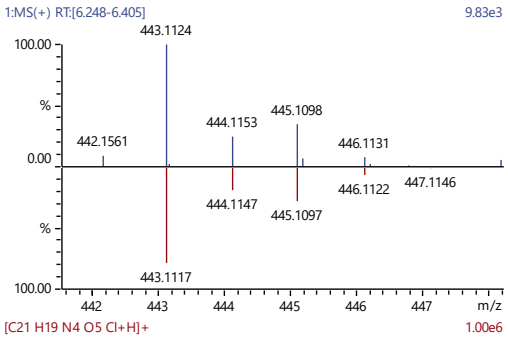
B: Hydrolysis (C17H14ClN3O3) Mass Error: 0.54 mDa



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

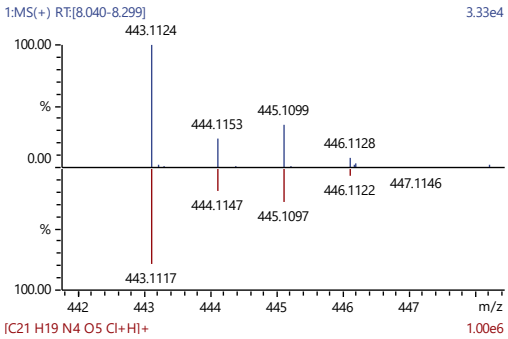


Fig. 4 MS Spectrum
(Top: measured MS spectrum, bottom: theoretical MS spectrum)

■ 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)

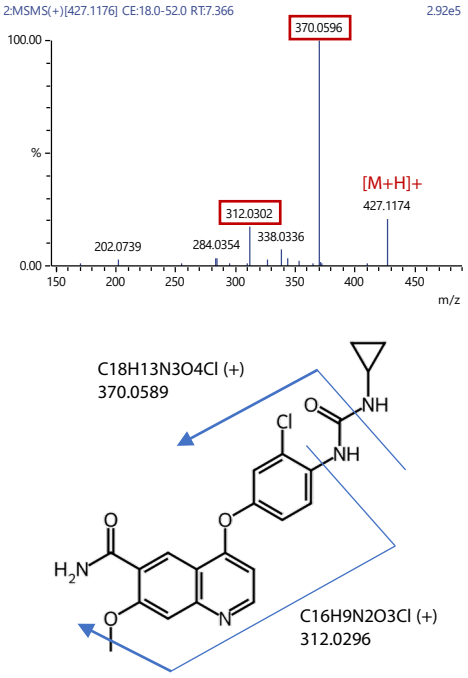
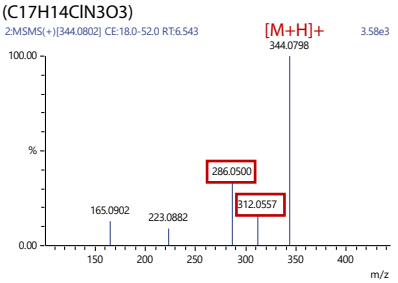
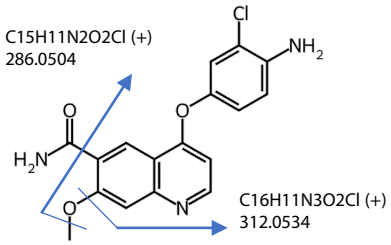


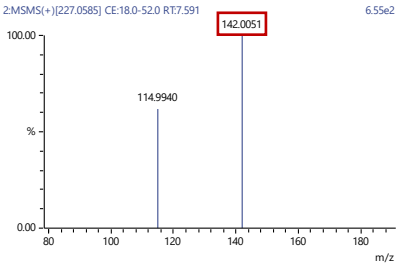
Fig 5 MS/MS Spectrum and Estimated Cleavage Positions

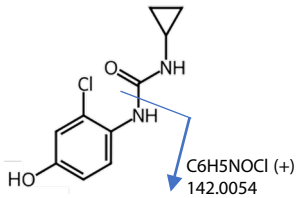
B: Hydrolysis (C17H14ClN3O3)



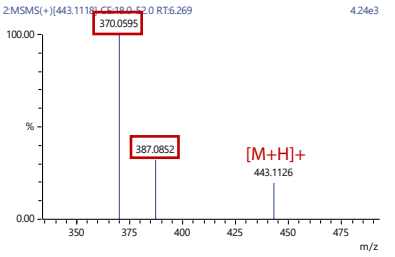


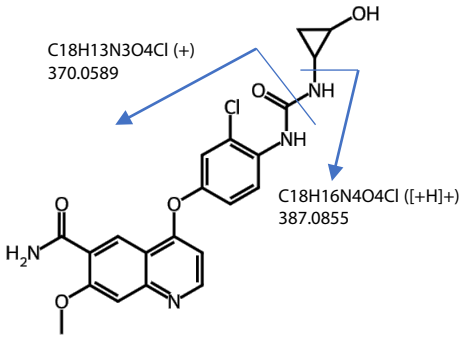
C: O-dearylation1 (C10H11ClN2O2)



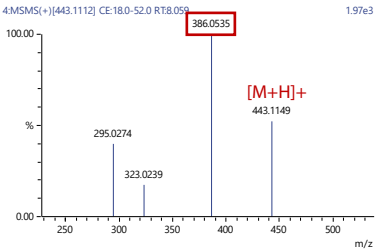


D: Oxidation (C21H19ClN4O5)





E: Oxidation (C21H19ClN4O5)



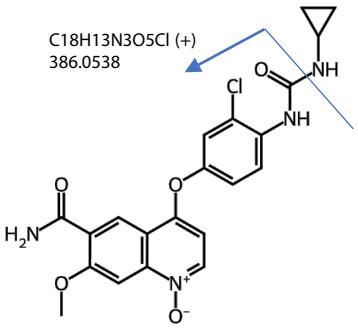


Fig 5 MS/MS Spectrum and Estimated Cleavage Positions (Continued)
Since the detailed oxidation positions of peaks D and E could not be determined from the MS/MS spectrum, the estimated structures are shown.

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.

<Acknowledgements>

We would like to express our sincere thanks to Dr. Satoshi Yamauchi of Nara Medical University for providing us with samples for this application.

<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

01-00757-EN First Edition: Jun. 2024

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