Application News

Liquid Chromatograph Mass Spectrometer

MRM and Product Ion Scan Analysis of THC Metabolites in Urine Using LC-MS/MS

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User Benefits

- THC and its metabolites can be detected at urine concentrations of 1 ng/mL without derivatization.
- Product ion scan provides mass spectral information, improving the reliability of the test for application certification.
- It uses the same mobile phase as the LC-MS/MS Forensic Toxicology Database, Shimadzu's method package for drug and toxin screening. THC metabolites analysis and the screening can be changed with just column switching.

■ Introduction

Cannabis regulation is evolving through both deregulation and enforcement measures. While some countries are legalizing cannabis for medical and recreational use, there are also efforts to tighten regulations against illicit use and illegal trade. Japan recently revised its cannabis laws to tighten regulations on use, with some of these amendments taking effect in December 2024.

Δ9-tetrahydrocannabinol (Δ9-THC), the primary psychoactive compound in cannabis, is metabolized in the body to Δ9-THC-COOH and Δ9-THC-OH, which are subsequently excreted in urine and feces. To validate the use of cannabis products containing these regulated compounds, it is essential to measure these metabolites in urine. This analysis requires not only high sensitivity and accurate quantification but also confirmation through mass spectral patterns to establish clear evidence.

In liquid chromatography-tandem mass spectrometry (LC-MS/MS), spectral patterns obtained from product ion scan enhance the accuracy of cannabis use verification. This application demonstrates the analysis of THC and its metabolites in urine using the LCMS-8050 (Figure 1). Both multiple reaction monitoring (MRM) for sensitive quantification and product ion scan for qualitative analysis were employed.

Fig. 1 LCMS-8050

■ Sample Pretreatment

The target compounds are listed in Table 1. Reference standards were spiked into urine to evaluate the validity of the analysis. Referring to the results of the study in Application News (No.01-00860), the samples were pretreated using liquid-liquid extraction (Fig. 2). Finally, samples were reconstituted in a 1:1 = water : methanol mixture and used for analysis.

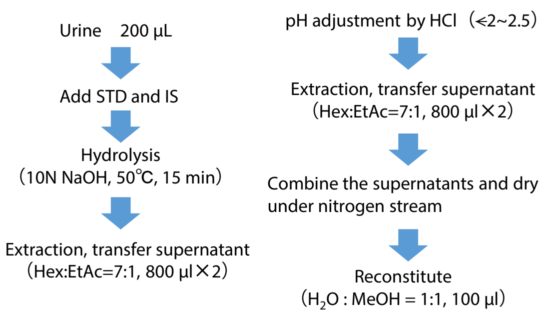


Fig. 2 Sample pretreatment workflow

Table 1 Target Compounds List

#	Type	Compounds	Formula
1	I.S.	Δ9-THC-OH-d3	C21H27D3O3
2	Target	Δ9-THC-OH	C21H30O3
3	Target	Δ8-THC-OH	C21H30O3
4	Target	Δ8-THC-COOH	C21H28O4
5	Target	Δ9-THC-COOH	C21H28O4
6	I.S.	Δ9-THC-COOH-d3	C21H25D3O3
7	I.S.	Δ9-THC-d3	C21H27D3O2
8	Target	Δ9-THC	C21H30O2
9	I.S.	Δ8-THC-d3	C21H27D3O2
10	Target	Δ8-THC	C21H30O2

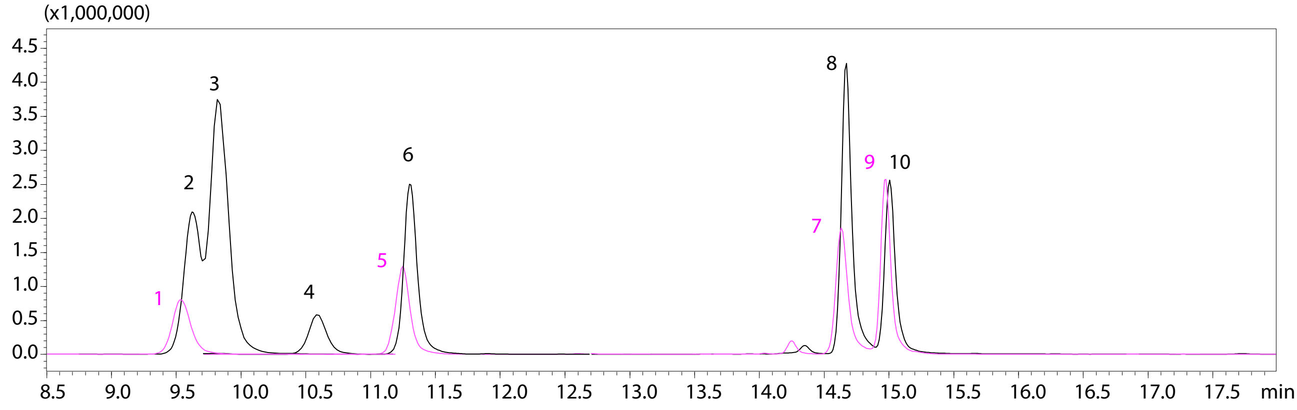
■ Analytical Conditions

Table 2 shows the analytical conditions of LC-MS/MS. Fig. 3 shows the resulting chromatograms under these conditions.

Table 2 Nexera™ & LCMS-8050 Condition

System	: Nexera XS
Column	: Raptor ARC-18 (100 mm × 3.0 mm I.D., 1.8 µm, P/N : 931421E*) * Catalog number of Restek
Temperature	: 40°C
Injection volume	: 10 µL
Mobile phases	: 10 mM ammonium formate + 0.1 % Formic acid water 10 mM ammonium formate + 0.1 % Formic acid MeOH
Flow rate	: 0.4 mL/min
Time program (%B)	: 70 % (0-9 min)→85 % (12-18 min)→ 70 % (18-23 min)
System	: LCMS-8050 (ESI Positive)
Nebulizing gas	: 3 L/min
Drying gas	: 5 L/min
Heating gas	: 15 L/min
DL temp.	: 150°C
Heat block temp.	: 400°C
Interface temp.	: 400°C
MRM transitions	

Compounds	Target Ion	
Δ9-THC-OH-d3	334.15>196.15 (CE -25 V)	334.15>201.05 (CE -24 V) 334.15>105.20 (CE -37 V)
Δ9-THC-OH	331.15>193.15 (CE -25 V)	331.15>201.05 (CE -26 V) 331.15>271.05 (CE -20 V)
Δ8-THC-OH	331.15>201.05 (CE -26 V)	331.15>271.05 (CE -20 V) 331.15>193.15 (CE -25 V)
Δ8-THC-COOH	345.30>327.10 (CE -16 V)	345.30>299.20 (CE -18 V) 345.30>193.10 (CE -28 V)
Δ9-THC-COOH	345.30>327.10 (CE -16 V)	345.30>299.20 (CE -18 V) 345.30>193.10 (CE -28 V)
Δ9-THC-COOH-d3	347.95>302.10 (CE -20 V)	347.95>196.15 (CE -26 V) 347.95>119.20 (CE -28 V)
Δ9-THC-d3	318.15>196.15 (CE -22 V)	318.15>123.00 (CE -32 V) 318.15>162.10 (CE -20 V)
Δ9-THC	315.15>193.15 (CE -23 V)	315.15>259.05 (CE -20 V) 315.15>135.20 (CE -20 V)
Δ8-THC-d3	318.15>196.15 (CE -22 V)	318.15>123.00 (CE -32 V) 318.15>162.10 (CE -20 V)
Δ8-THC	315.15>193.15 (CE -23 V)	315.15>259.05 (CE -20 V) 315.15>135.20 (CE -20 V)



1: Δ9-THC-OH-d3, 2: Δ9-THC-OH, 3: Δ8-THC-OH, 4: Δ8-THC-COOH 5: Δ9-THC-COOH-d3, 6: Δ9-THC-COOH, 7: Δ9-THC-d3, 8: Δ9-THC, 9: Δ8-THC-d3, 10: Δ8-THC

Fig. 3 MRM Chromatogram of the Standard Mixture

■ Validation of the MRM method

Fig. 4 shows the MRM chromatogram obtained when the standard was spiked into urine at a concentration of 1 ng/mL. All compounds were detected with sufficient sensitivity. Fig. 5 summarizes the calibration curves generated over a urine concentration range of 1-500 ng/mL, demonstrating excellent linearity with R=0.9995 or higher.

Table 3 presents the results of the evaluation of diurnal variation (n=5) at low (15 ng/mL) and high (250 ng/mL) concentrations. At the low concentration, accuracy range was 103~108%, with repeatability (RSD%) within 4.7%. At the high concentration, accuracy range was 104~110%, with repeatability within 6.6%, indicating sufficient accuracy and precision for quantification.

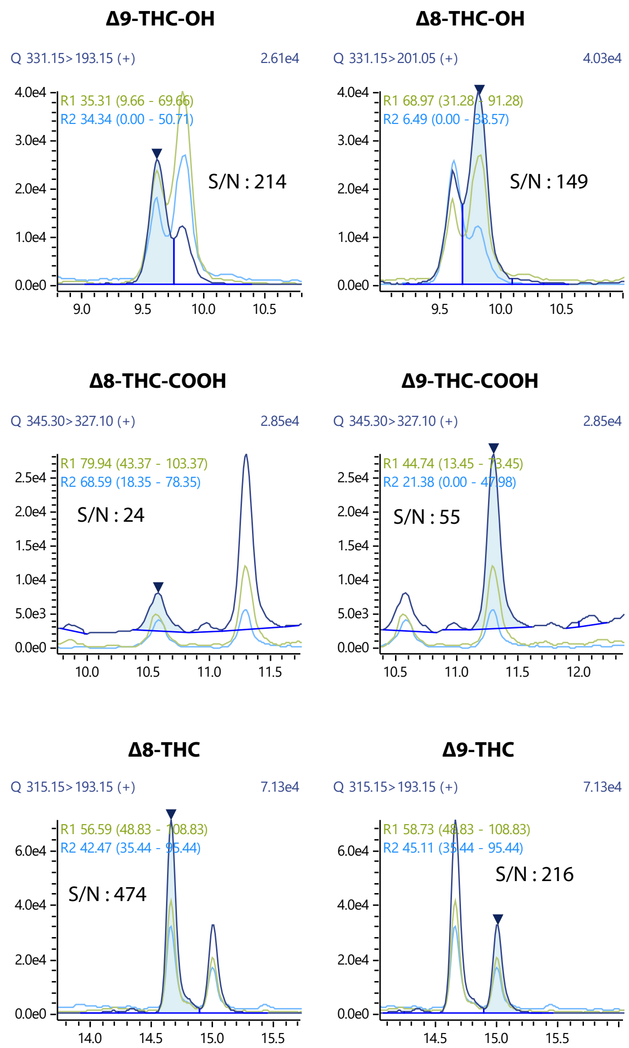


Fig. 4 MRM Chromatograms (1 ng/mL in urine)

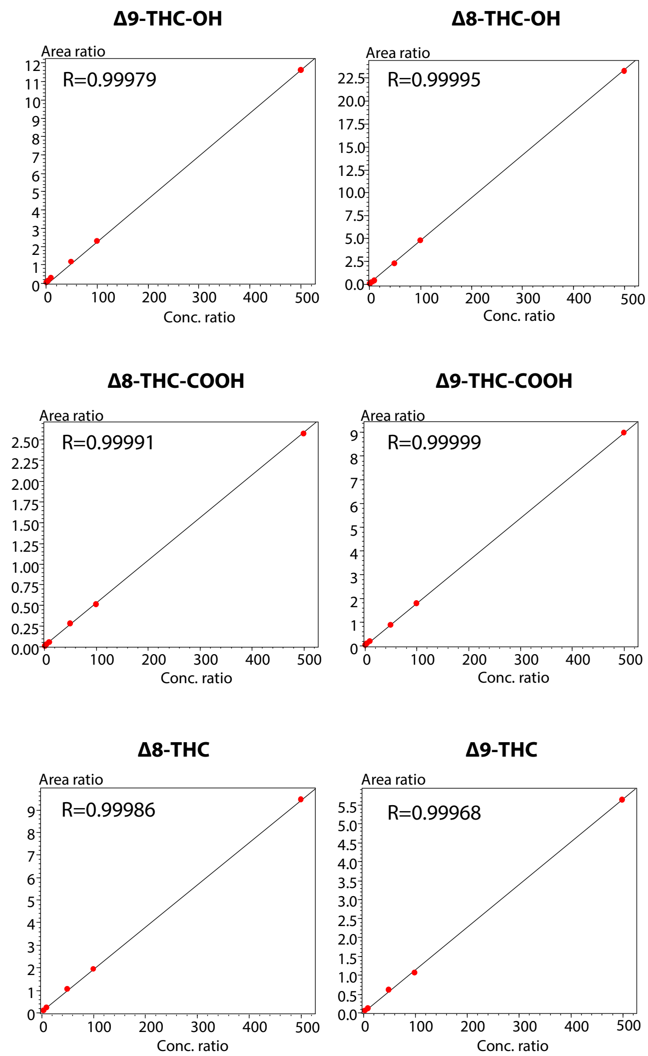


Fig. 5 Calibration Curves

Table 3 Intra-day reproducibility (Top : Low concentration, Bottom: High concentration)

		Conc. (ng/mL) 1	2	3	4	5	Average	Accuracy (%)	RSD%
15 ng/mL	Δ9-THC-OH	15.9	16.1	15.7	15.7	16.5	16.1	107.2	2.4
	Δ8-THC-OH	16.9	15.1	15.4	15.6	16.1	15.7	104.7	4.7
	Δ8-THC-COOH	14.9	14.6	15.8	16.5	15.5	15.6	103.8	4.7
	Δ9-THC-COOH	16.0	15.7	15.8	16.7	16.3	16.1	107.6	2.1
	Δ9-THC	15.1	15.2	15.4	14.7	16.4	15.5	103.4	4.4
	Δ8-THC	16.0	16.0	15.9	15.3	17.2	16.2	108.0	4.0
		1	2	3	4	5	Average	Accuracy (%)	RSD%
250 ng/mL	Δ9-THC-OH	263.8	275.0	283.0	278.2	259.5	271.9	108.8	3.6
	Δ8-THC-OH	245.9	277.7	271.0	284.6	288.3	273.5	109.4	6.1
	Δ8-THC-COOH	241.2	259.0	256.6	274.4	269.2	260.1	104.0	4.9
	Δ9-THC-COOH	240.8	272.9	260.8	287.8	273.1	267.1	106.8	6.6
	Δ9-THC	259.5	286.2	273.6	283.6	277.5	276.1	110.4	4.0
	Δ8-THC	250.7	277.6	268.5	281.7	267.6	269.2	107.7	4.5

■ Product Ion Scan for identification

Fig. 6 shows the product ion scan results for a sample spiked with standards at a urine concentration of 15 ng/mL. Peaks corresponding to both quantitative and confirmatory ions were detected. A comparison of the product ion mass spectra is shown on the right side of the chromatogram. The upper spectra shows the mass spectra obtained from this analysis, and the lower spectra shows the mass spectra obtained by analyzing the standard. The spectra for all compounds closely matched those of the standard. The CID conditions and product ions used in the analysis are shown in Table 4.

Table 4 Parameters for Product Ion Scan

Compounds	CE	Precursor	Target Ions
Δ9-THC-OH	-25 V	331.15	201.00, 193.10, 313.20
Δ8-THC-OH	-25 V	331.15	201.00, 193.10, 271.20
Δ8-THC-COOH	-25 V	345.30	299.20, 193.10, 119.10
Δ9-THC-COOH	-25 V	345.30	193.10, 299.20, 257.10
Δ9-THC	-20 V	315.15	193.10, 259.10, 135.15
Δ8-THC	-20 V	315.15	193.10, 259.10, 135.15

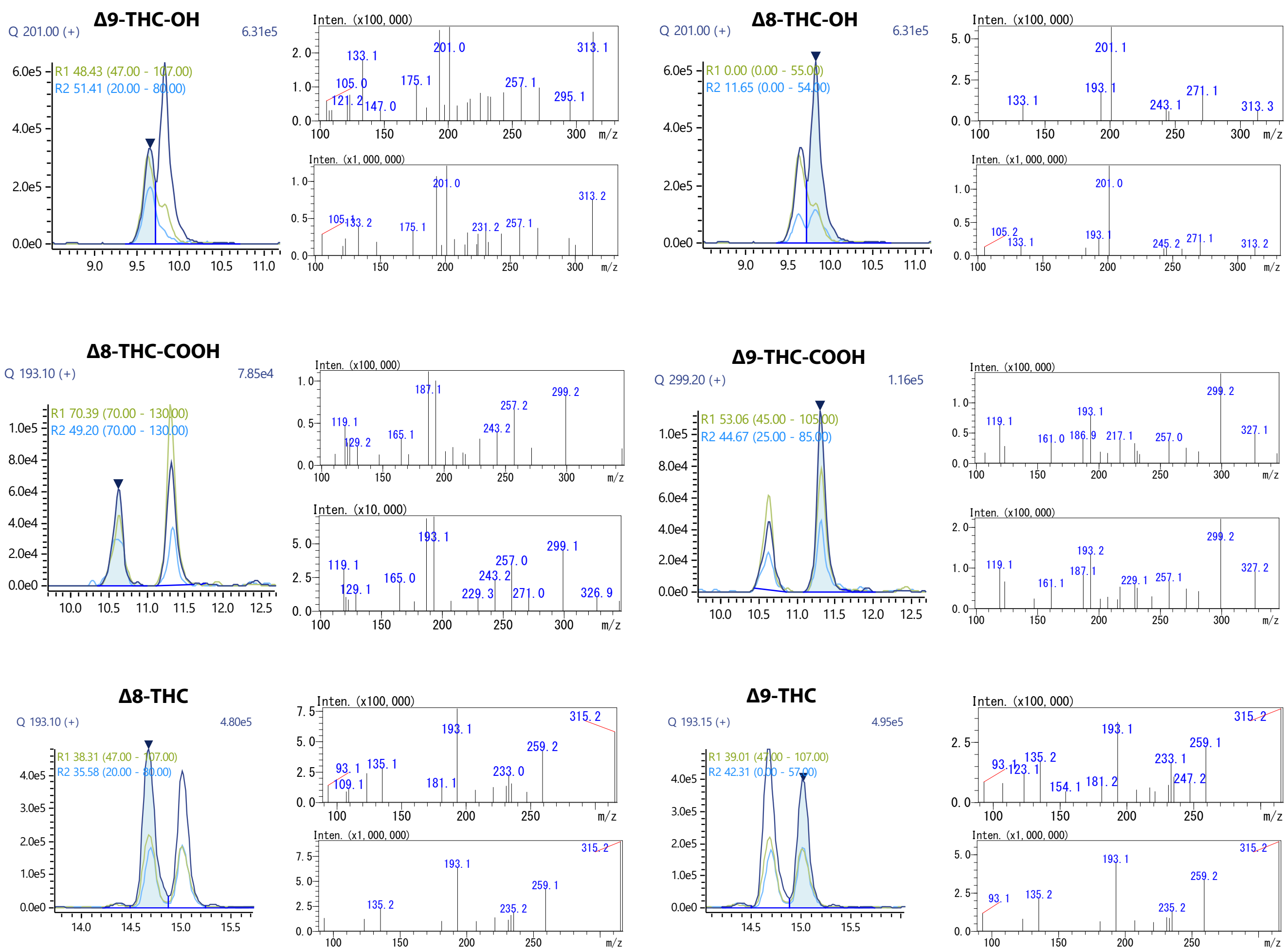


Fig. 6 Comparison of MS chromatograms and mass spectra of product ion scans

■ Conclusion

The LCMS-8050 was used to quantify THC and its metabolites in urine. MRM analysis demonstrated sufficient sensitivity, even at concentrations as low as 1 ng/mL. Matching of mass spectra using product ion scanning can also improve the accuracy of compound identification.

This application uses the same mobile phases as method package products such as the LC-MS/MS Forensic Toxicology Database, Shimadzu's method package for drugs and toxins screening. This system can be easily adapted for both THC metabolites analysis and toxicant screening by simply switching columns.

<Related Applications>

1. Analysis of THC Metabolites in Urine by GC-MS/MS, No. 01-00860

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