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Application News

Analysis of THC Metabolites in Urine by GC/MS-Scan

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User Benefits

- GC/MS-scan analysis can obtain mass spectral data, which improves the reliability of drug testing.
- GC/MS-scan offers quantitative accuracy at ppb concentration levels sufficient for confirmation testing of initial screening results.

■ Introduction

Cannabis contains over 100 different cannabinoids. The main psychoactive component of cannabis, delta-9-tetrahydrocannabinol (Δ^9-THC), affects the central nervous system causing symptoms such as hallucinations, pain relief, and sedation. Conversely, cannabidiol (CBD) has no hallucinatory effects, is not regulated in many countries, and is starting to be used in a variety of applications, including seizure medication, cosmetics, and supplements. Restrictions on cannabis are undergoing continuous reforms with some countries tightening restrictions and others relaxing them. In Japan, restrictions on cannabis for medical use are being relaxed, while laws were recently amended to tighten regulations on cannabis use and a portion of these laws went into effect in December 2024. Cannabis for recreational use is still prohibited in more than half of the countries in the world.

The main restricted component of cannabis is Δ^9-THC, which is metabolized in the body and principally excreted in urine and feces as the metabolites Δ^9-OH-THC, Δ^9-THC-COOH, and their glucuronide conjugates.[1)] Based on this, urine must be analyzed for these metabolites to prove cannabis use. Although LC-MS/MS offers the advantage of relatively simple sample pretreatment and is the prevailing technique used to test for cannabis metabolites in urine, GC-MS methods can record the full mass spectrum, which can be used to confirm the initial screening results.

This Application News describes the analysis of cannabis metabolites in urine using a GC/MS-scan method. GC/MS-scan analysis can identify cannabis metabolites at sub-ppb levels in urine. GC/MS-scan analysis also produces highly repeatable and consistent quantitative data with low intra-day variability.

■ Analytical Conditions

Analytical conditions are shown in Table 1. This Application News used GCMS-TQ8040 NX, but GCMS-QP2020 NX and GCMS-QP2050 can also be used for the same analysis.

Table 1 Analytical Conditions

GC-MS:	GCMS-TQ™ 8040 NX
Auto-injector:	AOC-30i
Column:	SH-I-5Sil MS P/N: 221-75954-30 (30 m, 0.25 mm, I.D. 0.25 µm)
[GC]	
Injection Temp.:	280 °C
Column Oven Temp.:	150 °C (1 min) → (15 °C/min) → 320 °C (3 min)
Injection Mode:	Splitless
Carrier Gas Control:	Linear velocity (45.6 cm/sec)
High-Pressure Injection:	250 kPa (1.5 min)
Injection Volume:	2 µL
[MS]	
Ion Source Temp.:	230 °C
IF Temp.:	280 °C
Data Acquisition Mode:	Scan (*m/z* 45 to 600)
Event Time:	0.3 sec

■ Sample Pretreatment

Investigation into sample pretreatment methods for the analysis of cannabis metabolites in urine can be found in Application News 01-00860.[2)] The sample pretreatment method used in the present analysis was liquid-liquid extraction. The sample pretreatment workflow is shown in Fig. 1. The compounds targeted for quantification were Δ^9-THC, Δ^8-THC, their main hydroxy-metabolites Δ^9-OH-THC and Δ^8-OH-THC, and their main carboxy-metabolites Δ^9-THC-COOH and Δ^8-THC-COOH. Δ^8-THC-d3, Δ^9-THC-d3, Δ^9-OH-THC-d3, and Δ^9-THC-COOH-d3 were used as internal standards.

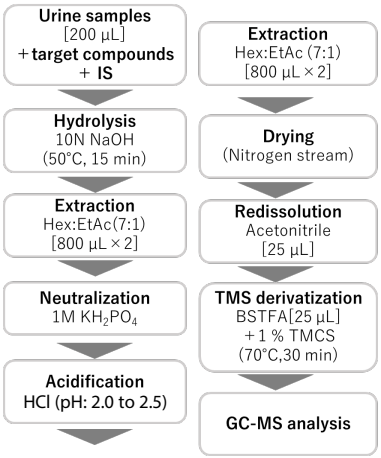


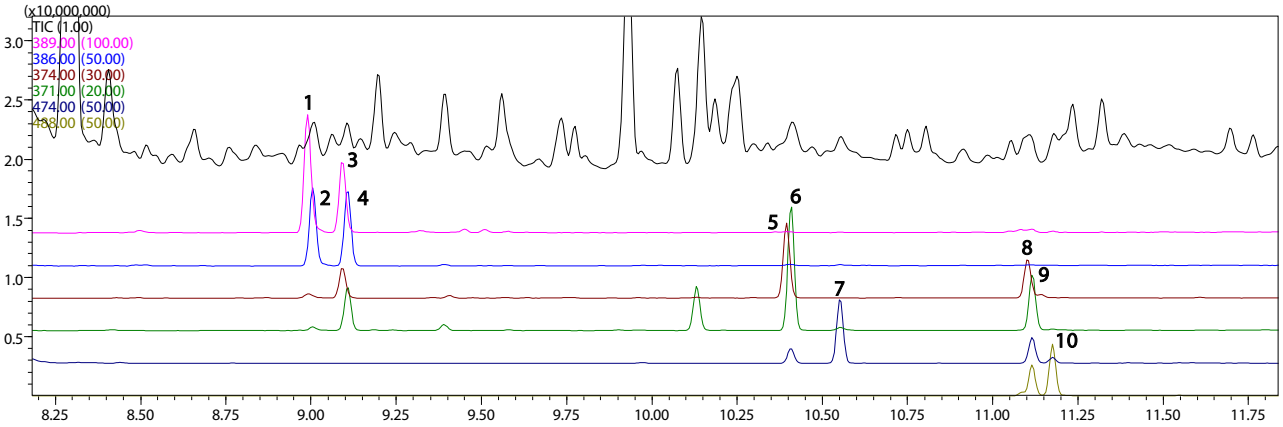
Fig. 1 Sample Pretreatment Workflow

■ Results

Representative chromatographic separation pattern was obtained by analyzing a spiked urine sample (100 ng/mL) to obtain the total ion current chromatogram (TICC) and mass chromatograms as shown in Fig. 2. Although the target compounds and the internal standards are in close proximity on the chromatogram, they can be readily separated by mass.

• Sensitive Enough for Mass Spectrum-Based Confirmation

Fig. 3 shows the mass spectra and mass chromatograms for each target compound in urine samples spiked at 15 ng/mL. The S/N of all mass chromatograms was 68.2 or higher, demonstrating sufficient sensitivity for all target compounds. Fig. 3 compares the mass spectra obtained from a urine sample containing target compounds at 15 ng/mL with mass spectra obtained from standard solutions at 100 ng/mL. Although overlap between internal standards and sample impurities prevents mass spectrum subtraction from being used with some compounds, in this analysis, ion ratios were determined for ions characteristic to every target compound and spectrum-based cross-referencing was possible.



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

Fig. 2 Total Ion Current Chromatogram of Spiked Urine (100 ng/mL) and Mass Chromatograms of Each Target Compound

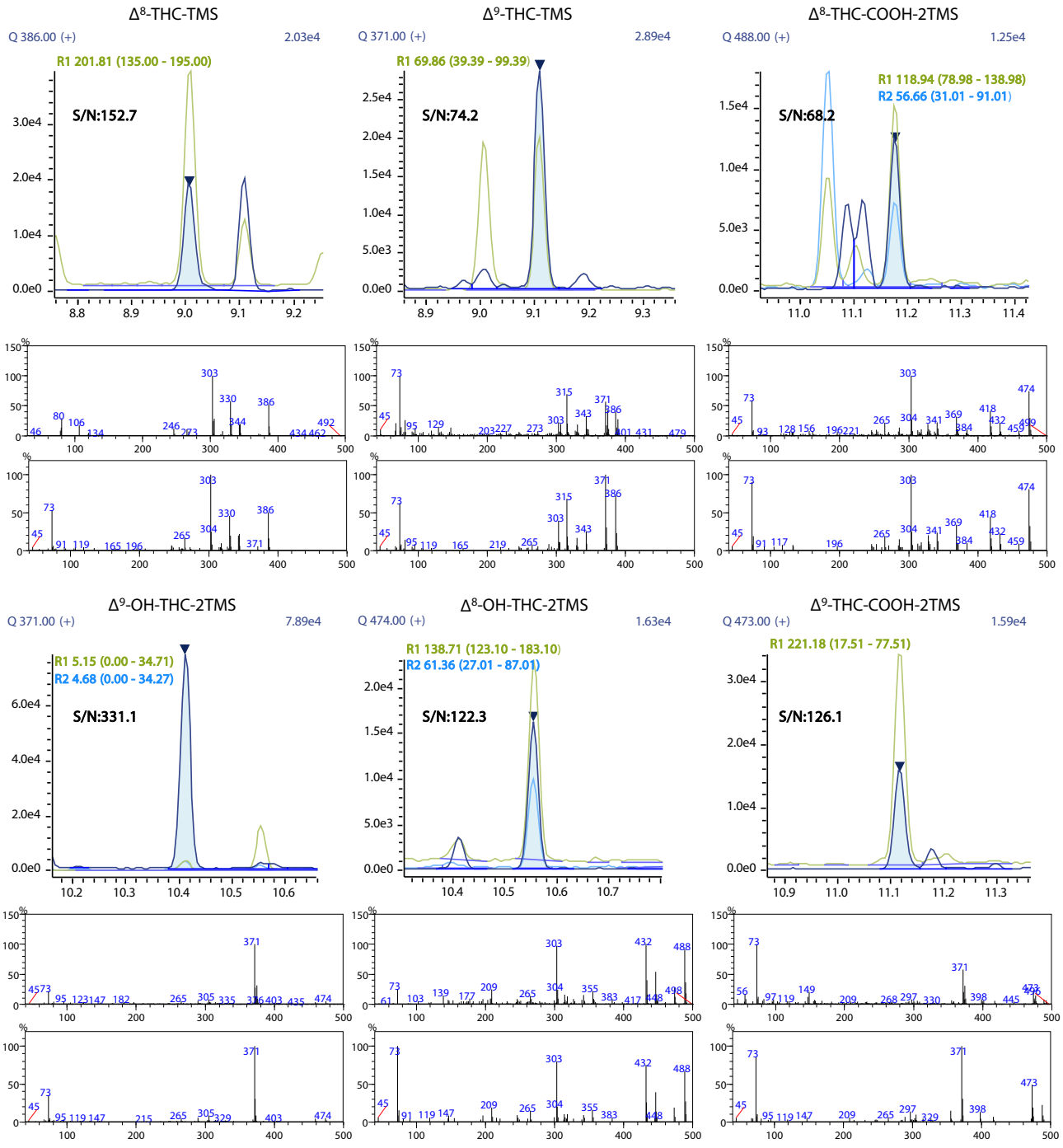


Fig. 3 Mass Chromatograms and Mass Spectra of Target Compounds
(Top: Mass Spectra from Spiked Urine Sample, Bottom: Mass Spectra from Standard Samples)

• Calibration Curve Linearity

Fig. 4 shows the calibration curves for THC and its metabolites in urine over the range of 1 to 100 µg/mL. Linearity was demonstrated by the correlation coefficient (R) of every calibration curve being 0.995 or higher.

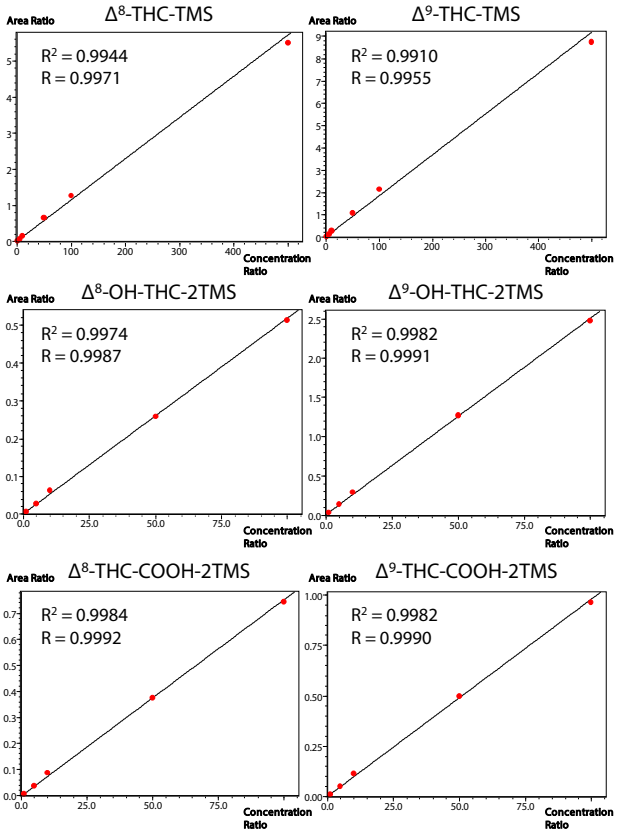


Fig. 4 Calibration Curves for Target Compounds

• Accuracy of Repeated Analysis (Intra-Day Variability)

Table 2 shows the results for intra-day variability (n = 5) for low concentration (15 ng/mL) and high concentration (250 ng/mL) samples. Accuracy at low concentrations ranged from 95 to 113 % and intra-day repeatability (%RSD) was within 5 % for all target compounds. Accuracy at high concentrations (250 ng/mL) ranged from 86 to 101 % and repeatability (%RSD) was within 5 % for all target compounds, showing that the quantitative accuracy of the method is acceptable.

■ Conclusion

This Application News confirms that GC/MS-scan analysis can be used for mass spectrum-based confirmation and offers quantitative accuracy sufficient to measure target compounds in urine at ppb concentration levels. Although the quantitative sensitivity of GC/MS-scan analysis is inferior to that of LC-MS/MS or GC-MS/MS methods, GC/MS-scan analysis records a full mass spectrum that can be cross-referenced to confirm initial screening results.

<References>

1) II-5 Cannabis Test Methods, Ed. By The Pharmaceutical Society of Japan: Toxicological Test Methods and Annotations 2017
2) Analysis of THC Metabolites in Urine by GC-MS/MS (01-00860)

Table 2 Intra-Day Repeatability for Low Concentrations (15 ng/mL) and High Concentrations (250 ng/mL)

15 ng/mL	No.1	No.2	No.3	No.4	No.5	Mean	Accuracy (%)	%RSD
Δ8-THC-TMS	16.1	16.1	16.3	15.8	15.7	16.0	106.7	1.5
Δ9-THC-TMS	17.9	16.9	16.4	16.7	16.5	16.9	112.6	3.7
Δ9-OH-THC-2TMS	14.8	15.3	14.8	14.3	14.5	14.8	98.4	2.6
Δ8-OH-THC-2TMS	15.8	14.5	14.2	14.2	14.3	14.6	97.3	4.7
Δ9-THC-COOH-2TMS	14.4	14.6	14.2	14.5	13.9	14.3	95.5	1.8
Δ8-THC-COOH-2TMS	14.2	14.3	14.5	14.6	13.8	14.3	95.0	2.2
250 ng/mL	No.1	No.2	No.3	No.4	No.5	Mean	Accuracy (%)	%RSD
Δ8-THC-TMS	248.7	236.2	228.0	244.7	249.9	241.5	96.6	3.8
Δ9-THC-TMS	257.0	247.8	247.9	255.5	253.0	252.2	100.9	1.7
Δ9-OH-THC-2TMS	218.0	208.5	206.4	217.2	212.2	212.5	85.0	2.4
Δ8-OH-THC-2TMS	226.3	219.4	216.8	227.6	226.5	223.3	89.3	2.2
Δ9-THC-COOH-2TMS	202.5	216.0	218.0	227.2	222.3	217.2	86.9	4.3
Δ8-THC-COOH-2TMS	206.4	219.2	226.5	227.1	223.5	220.6	88.2	3.9

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