SHIMADZU
Excellence in Science

Gas Chromatograph-Mass Spectrometer GCMS-TQ™ 8040 NX

Application News

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

Yuki Sakamoto[1], Maiko Kusano[2], Masaya Fujishiro[2] and Taka-aki Matsuyama[2]
1: Shimadzu Corporation, 2: Department of Legal Medicine, Showa University School of Medicine

User Benefits

- MRM in GC-MS/MS uses two-stage mass filtering which allows for the detection of THC and its metabolites without interference from impurities in urine.
- Using MRM creates a method sensitive enough to detect THC and its metabolites in urine at 1 ng/mL that can be used to quantify trace levels of cannabis metabolites in urine.

■ Introduction

Cannabis is a long-abused drug with pharmacological effects that include hallucinations, pain relief, and sedation. While the main psychoactive component of cannabis, delta-9-tetrahydrocannabinol (Δ^9-THC), is subject to restrictions in many countries worldwide, another active component called cannabidiol (CBD) is not regulated in many countries. Cannabidiol has no major side effects and is starting to be used as a seizure medication, in cosmetics, and in foods.

Restrictions on cannabis are undergoing continuous reforms with some countries tightening restrictions and others relaxing them. Japan has recently revised its laws to tighten regulations on cannabis use and a portion of these laws went into effect in December 2024.

Δ^9-THC in cannabis 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.

This Application News investigated four methods of sample pretreatment that could be used to measure THC and its metabolites in urine and presents results obtained using the sample pretreatment method selected by this investigation in a quantitative analysis by GC-MS/MS.

■ Sample Pretreatment

The compounds targeted for quantitation were Δ^9-THC, Δ^8-THC, their main hydroxy-metabolites Δ^9-OH-THC and Δ^8-OH-THC, and the 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.

Four sample pretreatment methods were investigated: ISOLUTE SLE+ (Biotage), MonoSpin C18-CX (GL Sciences Inc.), Micro Volume QuEChERS Kit (Shimadzu Corporation), and liquid-liquid extraction.

200 µL of blank urine was spiked with the target compounds and internal standards at 50 ng/mL, this mixture was reacted with β-glucuronidase or subjected to alkaline hydrolysis, and the resulting sample was acidified. The acidified sample was then subjected to one of the four methods of sample pretreatment, dried under a nitrogen gas stream, reconstituted in 25 µL of acetonitrile, combined with 25 µL of BSTFA (w/1 % TCMS), and heated at 70 °C for 30 minutes for TMS derivatization.

■ Analytical Conditions

Analytical conditions are shown in Table 1. MRM transitions for each target compound were optimized by selecting for precursor ions at two *m/z* values.

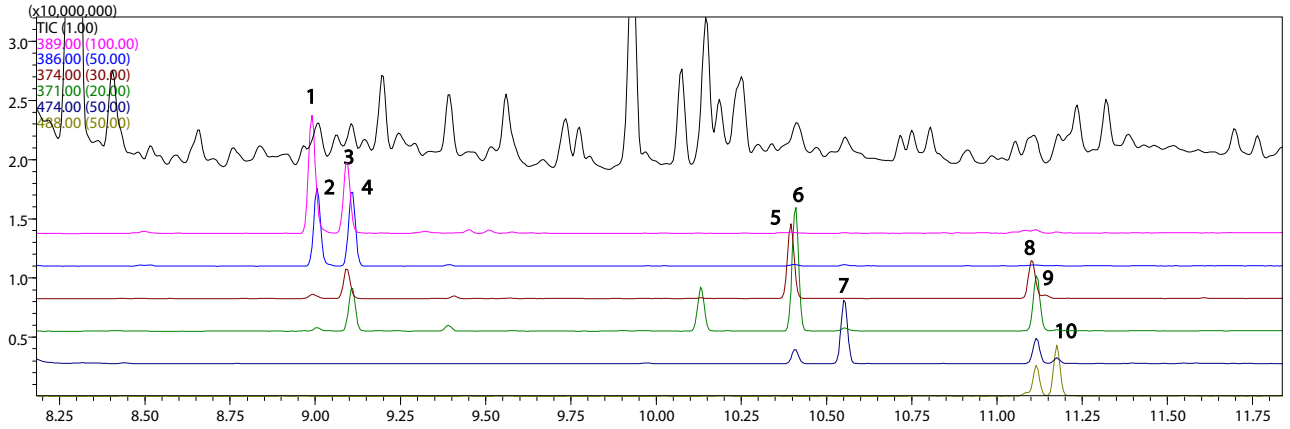
Table 1 Analytical Conditions

GC-MS:	GCMS-TQ8040 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)
Carrier Gas Control:	Constant linear velocity (45.6 cm/sec)
Column Flow rate:	1.44 mL/min
Injection Mode:	Splitless
High-Pressure Injection:	250 kPa (1 min)
Injection Volume:	2 µL
[MS]	
Ion Source Temp.:	230 °C
IF Temp.:	280 °C
Data Acquisition Mode:	MRM
Event Time:	0.3 sec

MRM Transitions

	Quantitation ion	CE	Confirmation ion 1	CE	Confirmation ion 2	CE
Δ^8-THC-d3-TMS	389.3>306.2	24	306.2>234.2	25		
Δ^8-THC-TMS	386.3>303.2	24	303.2>231.2	25		
Δ^9-THC-d3-TMS	389.3>374.3	12	389.3>318.2	15	374.3>292.2	15
Δ^9-THC-TMS	386.3>371.3	12	386.3>315.2	15	371.3>289.2	15
Δ^9-OH-THC-d3-2TMS	374.3>292.2	18	374.3>268.2	15	477.3>374.3	12
Δ^9-OH-THC-2TMS	371.3>289.2	18	371.3>265.2	15	474.3>371.3	12
Δ^8-OH-THC-2TMS	303.2>246.2	27	474.3>303.2	24	474.3>369.3	15
Δ^9-THC-COOH-d3-2TMS	476.3>358.3	24	491.3>374.3	10	491.3>401.3	10
Δ^9-THC-COOH-2TMS	473.3>355.3	24	488.3>371.3	10	488.3>398.3	10
Δ^8-THC-COOH-2TMS	488.3>432.3	12	488.3>303.2	24	432.3>314.2	12

Fig. 1 GCMS-TQ™8040 NX Triple Quadrupole Gas Chromatograph Mass Spectrometer



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

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

■ Results

• Investigation of Sample Pretreatment

A chromatographic separation pattern was obtained by analyzing a spiked urine sample (100 ng/mL) in scan mode 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 separated by mass.

Table 2 shows target compound recoveries for each of the four tested sample pretreatment methods. Target compounds were added to urine before hydrolysis (pre-hydrolysis sample) or after hydrolysis (post-hydrolysis sample), and recovery was calculated using the peak area of each target compound in the pre-hydrolysis sample as a percentage of the peak area in the post-hydrolysis sample.

Recovery was low across all target compounds regardless of the extraction method used. Next, the hydrolysis step was investigated for its effect on recovery. Target compounds were added to water or a urine sample before hydrolysis and recoveries were calculated with or without the hydrolysis step (alkaline hydrolysis) (Table 3).

Hydrolysis reduced the recovery rate of most target compounds from both water and urine by around 20 to 30 %. Enzymatic hydrolysis by β-glucuronidase also produced similar results. Given that the main metabolites of THC are excreted in urine as glucuronic acid conjugates, hydrolysis cannot be eliminated from sample pretreatment and this level of target compound loss was deemed unavoidable.

Table 2 Recoveries of the Four Tested Extraction Methods

	ISOLUTE SLE+	MonoSpin C18-CX	Micro Volume QuEChERS	Liquid-liquid extraction
Δ^8-THC-TMS	22 %	48 %	59 %	76 %
Δ^9-THC-TMS	20 %	45 %	57 %	76 %
Δ^9-OH-THC-2TMS	20 %	68 %	53 %	81 %
Δ^8-OH-THC-2TMS	23 %	70 %	56 %	81 %
Δ^9-THC-COOH-2TMS	21 %	54 %	40 %	71 %
Δ^8-THC-COOH-2TMS	24 %	61 %	38 %	72 %

Table 3 Target Compound Recoveries With and Without Hydrolysis

	Urine		Water	
	With hydrolysis	No hydrolysis	With hydrolysis	No hydrolysis
Δ^8-THC-TMS	54 %	96 %	63 %	87 %
Δ^9-THC-TMS	47 %	74 %	57 %	83 %
Δ^9-OH-THC-2TMS	57 %	93 %	69 %	95 %
Δ^8-OH-THC-2TMS	63 %	98 %	74 %	97 %
Δ^9-THC-COOH-2TMS	59 %	98 %	82 %	93 %
Δ^8-THC-COOH-2TMS	53 %	88 %	92 %	101 %

Once it was accepted that hydrolysis would reduce recovery to some extent, it was determined that liquid-liquid extraction provided the best recovery and was therefore selected from the four sample pretreatment methods tested. The resulting sample preparation workflow used is shown in Fig. 3.

Internal standards were added to 200 µL of urine to a final concentration of 50 ng/mL. The pH of the mixture was adjusted with 10N sodium hydroxide solution, then the mixture was heated at 50 °C for 15 minutes to perform alkaline hydrolysis. Next, liquid-liquid extraction was performed by adding 800 µL of a hexane/ethyl acetate mixture (7:1, v/v), vortex mixing, and centrifuging the mixture (this process was performed twice). Hydrochloric acid was then added to lower the pH of the mixture to between 2 and 2.5 and the same liquid-liquid extraction process was performed two more times. The organic layers collected during this this procedure were combined, dried under a stream of nitrogen, and reconstituted with acetonitrile. Next, 25 µL of BSTFA with 1 % TMCS was added and the mixture was heated at 70 °C for 30 minutes to perform TMS derivatization.

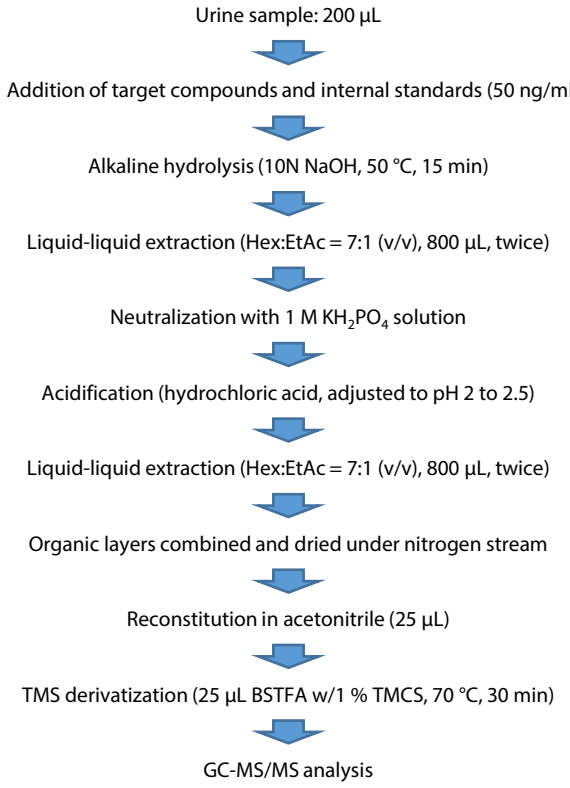


Fig. 3 Sample Pretreatment Workflow with Liquid-Liquid Extraction

• Method Validation

MRM chromatograms of THC and its metabolites in urine at 1 ng/mL are shown in Fig. 4. The results show that all target compounds were detected with adequate sensitivity. Fig. 5 shows the calibration curves for THC and its metabolites in urine in the range of 1-500 ng/mL. Linearity was demonstrated by the correlation coefficient (R) of every calibration curve being 0.997 or higher.

Table 4 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 102 to 106 % and intra-day repeatability (%RSD) was within 2.3 % for all target compounds. Accuracy at high concentrations ranged from 97 to 102 % and repeatability (%RSD) was within 3.2 % for all target compounds, showing that the quantitative accuracy of the method is acceptable.

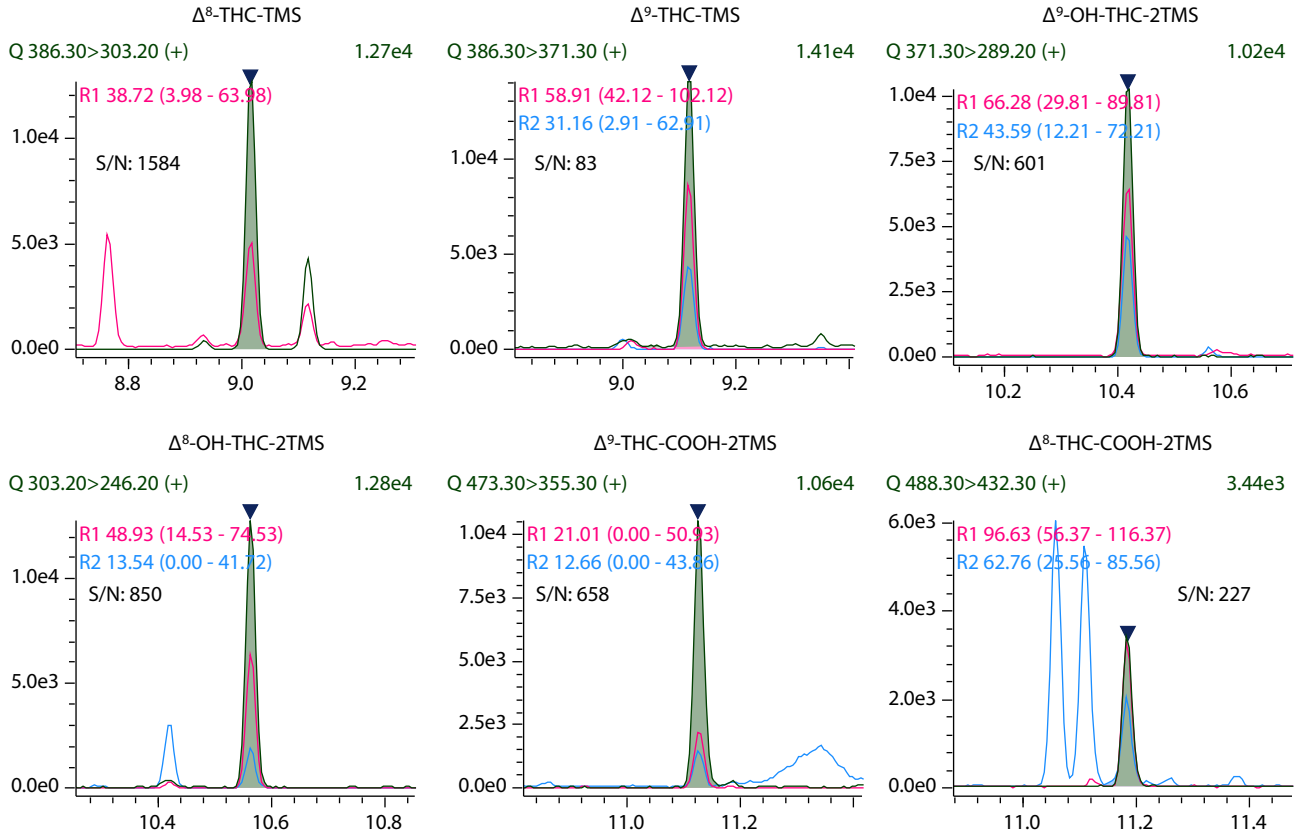


Fig. 4 MRM Chromatograms for 1 ng/mL in Urine

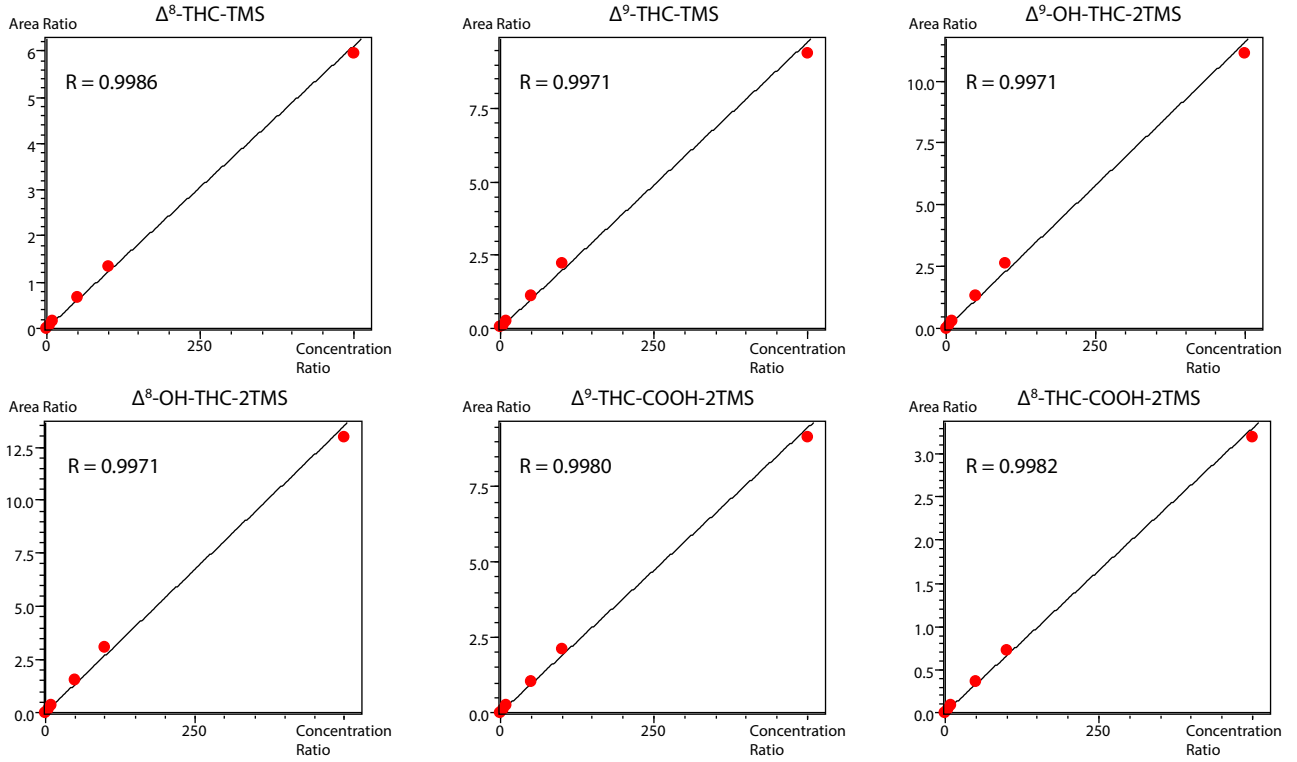


Fig. 5 Calibration Curves for THC and THC Metabolites (Concentration in Urine: 1 to 500 ng/mL)

Table 4 Intra-Day Repeatability for Low Concentrations (15 ng/mL) and High Concentrations (250 ng/mL)

Low (15 ng/mL)	Concentration (ng/mL)						Accuracy (%)	SD	%RSD
	No. 1	No. 2	No. 3	No. 4	No. 5	Mean			
Δ^8-THC-TMS	15.6	15.6	15.1	14.9	15.4	15.3	102.2	0.343	2.2
Δ^9-THC-TMS	16.3	16.3	16.0	15.4	15.8	16.0	106.5	0.374	2.3
Δ^9-OH-THC-2TMS	16.2	16.2	15.9	15.6	16.0	16.0	106.6	0.276	1.7
Δ^8-OH-THC-2TMS	16.2	16.3	15.8	15.6	15.9	16.0	106.4	0.271	1.7
Δ^9-THC-COOH-2TMS	15.7	15.6	15.3	14.9	15.3	15.4	102.4	0.342	2.2
Δ^8-THC-COOH-2TMS	15.5	15.3	15.3	15.3	15.5	15.4	102.6	0.117	0.8

High (250 ng/mL)	Concentration (ng/mL)						Accuracy (%)	SD	%RSD
	No. 1	No. 2	No. 3	No. 4	No. 5	Mean			
Δ^8-THC-TMS	244.7	239.2	235.9	248.4	245.9	242.8	97.1	5.136	2.1
Δ^9-THC-TMS	250.0	246.2	240.9	254.1	252.4	248.7	99.5	5.256	2.1
Δ^9-OH-THC-2TMS	253.0	245.9	243.1	256.3	255.3	250.7	100.3	5.903	2.4
Δ^8-OH-THC-2TMS	251.7	242.1	240.8	260.4	252.3	249.5	99.8	8.105	3.2
Δ^9-THC-COOH-2TMS	251.0	241.5	242.6	249.6	250.0	246.9	98.8	4.509	1.8
Δ^8-THC-COOH-2TMS	251.6	239.9	242.0	250.8	247.8	246.4	98.6	5.237	2.1

■ Conclusion

This Application News investigated the simultaneous analysis of cannabis constituents Δ^8-THC and Δ^9-THC, and their metabolites Δ^8-OH-THC, Δ^9-OH-THC, Δ^8-THC-COOH, and Δ^9-THC-COOH in urine.

Four different extraction methods were tested for the sample pretreatment step. A liquid-liquid extraction method provided the best recovery among the four extraction methods tested and the optimized sample pretreatment workflow resulted in an analytical method with adequate sensitivity and quantitative accuracy.

GC-MS/MS was used to create a method sensitive enough to readily detect target compounds at 1 ng/mL in urine that is suitable for the quantitative analysis of trace amounts of cannabis metabolites in urine.

<References>

1) II-5 Cannabis Test Methods, Ed. By The Pharmaceutical Society of Japan: Toxicological Test Methods and Annotations 2017

01-00860-EN First Edition: Feb. 2025

SHIMADZU
Shimadzu Corporation
www.shimadzu.com/an/

Related Products Some products may be updated to newer models.

GCMS-TQ™8040 NX

Triple Quadrupole Gas Chromatograph Mass Spectrome···

SH Series

SH-I-5Sil MS

Related Solutions

Clinical Research and Forensics

Forensic Analysis

Price Inquiry

Product Inquiry

Technical Service / Support Inquiry

Other Inquiry