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Application News

Liquid Chromatograph Mass Spectrometer LCMS™-8050

Detection of THC Metabolites in Urine by LCMS-8050 with Supported Liquid Extraction Method

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User Benefits

- SLE provides effective extraction and clean up of THC metabolites in urine samples after enzymatic hydrolysis
- The developed method meets the UNODC (United Nations Office on Drugs and Crime)'s criteria for MS-based detection of THC metabolites in biological specimens
- LCMS-8050 exhibits remarkable LOQ (5 ng/mL) for detection of THC metabolites in urine

■ Introduction

Despite its role as recreational and medicinal drug, cannabis can potentially lead to dependence and behavior disturbances affecting a person's daily duties as long-term effects. This concern has prompted authorities to consider advanced technique to trace the track of cannabis intake especially in biological fluids. Urine is among the biological fluid frequently used for routine cannabis tracing.

The parent drug, Δ9-tetrahydrocannabinol (THC), has a clearance half-life of less than 30 minutes and is not traceable in urine. THC will be converted into its primary metabolites, 11-hydroxy-Δ9-tetrahydrocannabinol (11-OH-THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), during Phase I metabolism in the liver. These two metabolites are further metabolized to produce glucuronide conjugates of 11-OH-THC and THC-COOH in Phase II metabolism and are excreted in the urine. The glucuronate conjugates typically appear in the urine within 60 minutes but can take up to 4 hours upon ingestion/inhalation. The half-life of THC-COOH is extensive and can be detected in the urine up to 7 days after a single use. It is therefore feasible to trace cannabis intake in the urine sample.

Mass spectrometry is the gold standard in forensic and clinical analysis for its sensitivity and selectivity. In this report, liquid chromatography mass spectrometry-triple quadrupole system (LCMS-8050) was employed for measuring THC metabolites in urine samples. Enzymatic hydrolysis is essential step to hydrolyze glucuronide moiety from THC metabolites. Consequently, sample cleanup is prerequisite in urine sample preparation. Supported liquid extraction (Biotage ISOLUTE® SLE+) was selected for both extraction and sample clean up.

Table 1 Analytical conditions for detection of THC metabolites in urine

Column	Biphenyl column (100 mm x 2.1 mm x 2.6 µm)
Mobile phase	A : 0.2% formic acid in water B : 0.2% formic acid in methanol
Gradient program	0-1 min (5%B), 1.01-2 min (40%B), 2.01-7 min (100%B), 7.01-10 min (100%B), 10.01-14 min (5%B)
Flow rate	0-8 min (0.3 mL/min), 8.01-11 min (0.5 mL/min), 11.01-14 min (0.3 mL/min)
Oven temperature	40˚C
Injection volume	1 µL

Interface	Heated ESI
MS mode	MRM, positive mode
Heat block temperature	400˚C
DL temperature	250˚C
Interface temperature	300˚C
Nebulizing gas	N_2, 3 L/min
Drying gas	N_2, 10 L/min
Heating gas	Zero air, 10 L/min

Table 2 MRM transitions of analytes and deuterated IS

Analyte	Transitions	Analyte	Transitions	Analyte	Transitions	Analyte	Transitions
THC-COOH	345.21>327.20*	THC-COOH-D3	348.20>330.15	11-OH-THC	331.23> 193.20*	11-OH-THC-D3	334.30>316.35
	345.21>193.15		348.20>302.15		331.23>201.15		334.30>196.15
	345.21>119.2		348.20>90.90				334.30>201.15

*quantifier ion

Table 3. Matrix effect and recovery (n=6)

Analyte	Matrix effect (%)			Recovery (%)		
	15 ng/mL	50 ng/mL	100 ng/mL	15 ng/mL	50 ng/mL	100 ng/mL
THC-COOH	101.1	89.8	91.5	104.9	92.0	103.7
11-OH-THC	71.0	87.5	94.6	106.2	99.8	105.2

Table 4. Accuracy and precision (intraday and interday repeatability, n=3)

Analyte	Accuracy (%)			Precision (%)		
	15 ng/mL	50 ng/mL	100 ng/mL	15 ng/mL	50 ng/mL	100 ng/mL
THC-COOH	101.3	92.1	90.4	2.9	9.0	3.1
11-OH-THC	106.4	96.5	97.1	2.5	8.3	1.1

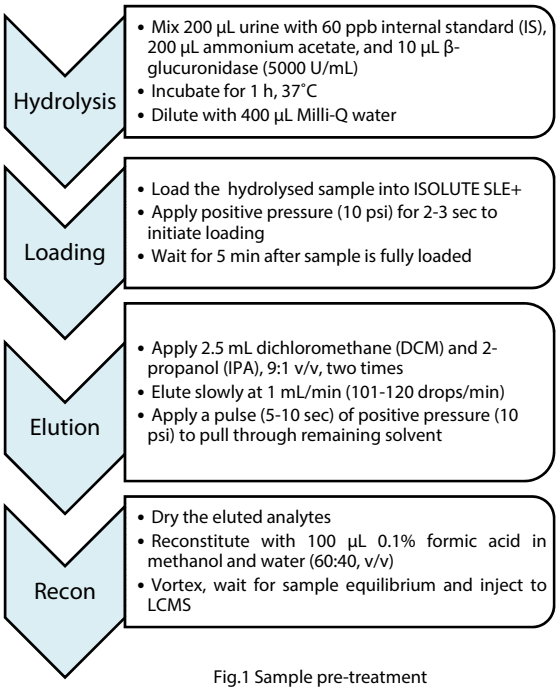


Fig.1 Sample pre-treatment

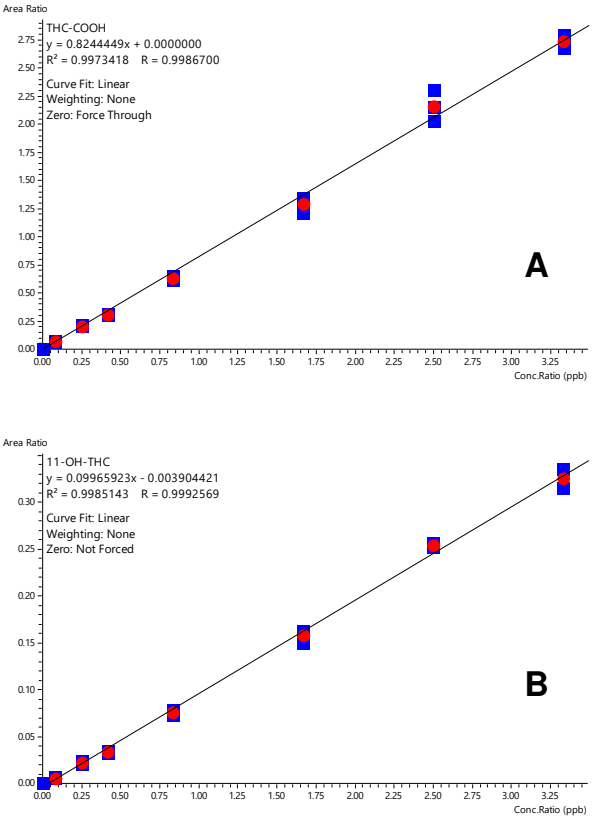


Fig. 2 Representative of matrix-based calibration curve for (A) THC-COOH and (B) 11-OH-THC.

■ Measurement Conditions and Samples

Sample preparation

Sample pre-treatment was performed in four steps (hydrolysis, sample loading, elution, reconstitution) by using Biotage ISOLUTE® SLE+ 1 mL cartridge (P/N: 820-0140-C) as shown in Fig. 1. Measurement was carried out on LCMS-8050 with schedule MRM program. Analytical conditions (liquid chromatography, mass spectrometry, and MRM transitions) are described in Table 1 and 2.

■ Results and Discussion

SLE works based on the ability of analytes to partition from the SLE phase into organic elution solvent. The more hydrophobic the analytes, the more likely analytes will partition. Since THC-COOH and 11-OH-THC are hydrophobic compounds (logP 5.24 and 5.78, respectively), it will be easy to partition these analytes from SLE phase by eluting organic solvent (DCM and IPA). It is therefore, SLE was utilized for sample extraction as well as clean up.

Two to three MRM transitions were used for detection and quantitation of THC metabolites and IS. MRM transitions are based on Shimadzu LC/MS/MS Forensic Toxicology Database (P/N: 225-31175-92) or auto MRM optimization. This ready-to-use database is very effective to reduce running cost and time in forensic or clinical analysis. Auto MRM optimization function is available in LabSolutions™ software and will assist user to obtain optimized parameters (MRM, collision energy) of each analyte.

There are seven criteria set by UNODC for MS-based detection of THC metabolites in biological specimens: linearity, LOQ (limit of quantitation), extraction recovery, matrix effect, accuracy, precision, and specificity/selectivity.

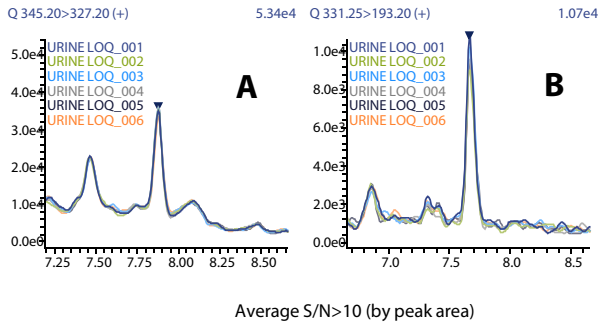
Linearity and LOQ

Method validation was carried out by following the United Nations Office on Drugs and Crime (UNODC) guideline. Calibration curve was established based on urine blank matrix containing seven different concentrations (5, 15, 25, 50, 100, 150, 200 ng/mL). Each point was measured in triplicate (n=3). Matrix-based calibration was repeated in six different days to investigate interday repeatability. Linearity of calibration curves was 0.992 or more (Fig. 2).

The limit of quantitation (LOQ) is determined based on the lowest concentration at which signal-to-noise ratio is greater than 10 (S/N>10). Injection was repeated six times for LOQ measurement. The LOQ was 5 ng/mL which is well below the requirement for cannabinoids in urine, 15 ng/mL (Fig. 3) (Ministry of Health Malaysia).

Recovery and matrix effect

Table 3 shows measurement of extraction recovery and matrix effect. Area ratio of analytes in neat and post-extraction spiked blank matrix was compared to determine matrix effect. Matrix effect ranged from 71 to 101%.



Average S/N>10 (by peak area)

Fig. 3 Overlay chromatograms of THC-COOH (A) and 11-OH-THC (B) from six injections (n=6) in blank matrix at LOQ (5 ng/mL).

Recovery was calculated based on analysis of analytes at three different concentrations. For each concentration, six extract replicates were measured by LCMS-8050. Extraction recovery was observed between 92% to 106%. Taken together, this demonstrates the efficiency of extraction and clean up by SLE method.

Accuracy and precision

Blank urine was spiked with THC metabolites at three different concentrations (15, 50, and 100 ng/mL) and analyzed in replicate (intraday repeatability, n=3) on three consecutive days (interday repeatability, n=3) to measure accuracy. Percent accuracy of both metabolites ranged between 90.4 to 106.4% (Table 4). It is in accordance with the guideline stating all results should fall within ±20% of the expected values at lower concentrations and within ±15% at the higher concentrations.

Based on the accuracy measurement, the precision RSD was determined. The RSD should be lower than 20% at lower concentrations and better than 15% at higher concentrations. The RSDs of THC-COOH and 11-OH-THC at three different concentrations were observed at 9% or less (Table 4).

Specificity/Selectivity

Specificity/Selectivity was investigated based on substance interference under the following analysis:

- Standard solutions of drugs of interest (THC metabolites)
- Standard solutions of drugs from other groups such as amphetamine, morphine, diazepam, alprazolam, and dextromethorphan
- Blank urine from five different sources
- Blank urine spiked with drugs of interest (THC metabolites)

Standard and spiking concentration was carried out at 15 ng/mL.

There was no interfering substances at the retention times of THC metabolites based on injection of spiked blank urine samples (Fig. 4). Additionally, retention times of drugs from other groups were distant from retention times of THC metabolites.

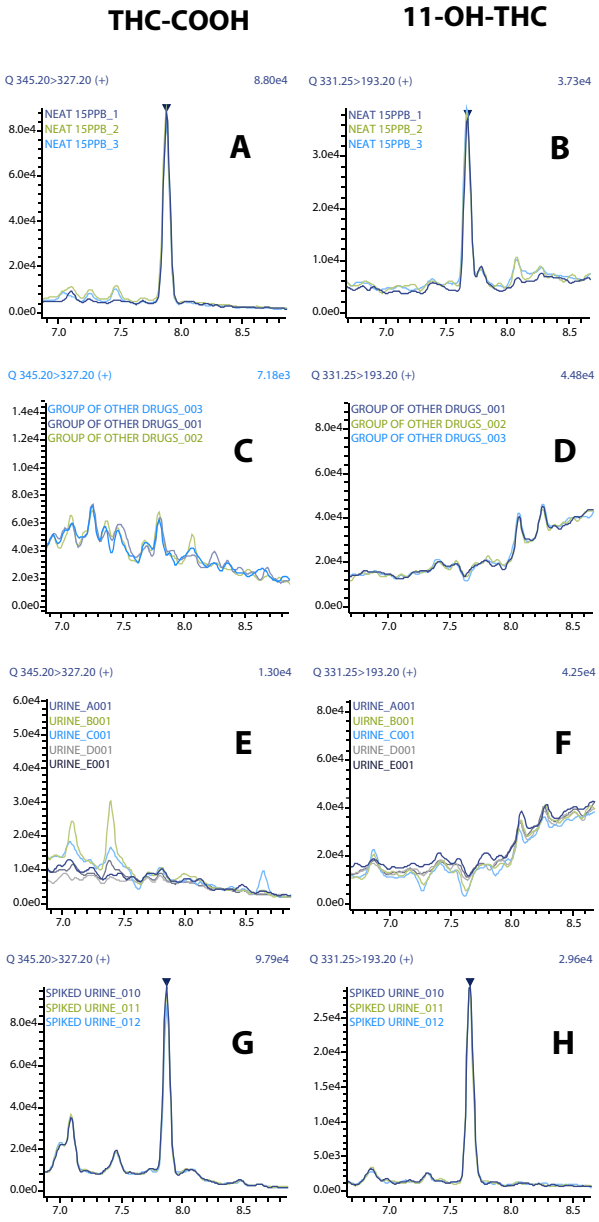


Fig. 4 Overlay chromatograms of (A & B) 15 ng/mL THC metabolites in neat/solvent (n=3), (C & D) group of other drugs in RT window of THC metabolites (n=3), (E & F) blank urine specimens from five sources (n=5), and (G & H) 15 ng/mL THC metabolites spiked in blank urine specimens (n=3).

■ Conclusion

LCMS-based method for detection of THC metabolites, THC-COOH and 11-OH-THC, in urine was validated using Shimadzu LCMS-8050 following guideline from the UNODC. SLE method by using Biotage ISOLUTE® SLE+ was employed to extract and clean up urine samples. The method satisfyingly meets all criteria (calibration linearity, LOQ, recovery, accuracy, precision, specificity/selectivity) set by the UNODC for MS-based detection method of THC metabolites in biological specimens. Owing to remarkable sensitivity of LCMS-8050 and effectiveness of SLE, the method is expected to be a substantial support for routine screening of THC metabolites in biological samples.

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