A Sensitive and Fast Assay to Quantify Cannabidiol and Tetrahydrocannabinol in Human Urine Using Microelution SPE Coupled With UPLC-MS/MS Analysis

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INTRODUCTION

Cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) are two major components (**Figure 1**) of hemp plants. CBD and THC are widely used for medical treatments like anxiety, inflammatory and neuropathic pain, Dravet syndrome, and Lennox-Gastaut syndrome. Since CBD and THC have high lipophilicity, poor aqueous solubility, and low oral bioavailability, development of a sensitive analytical assay to quantify these compounds in biological matrices becomes very challenging. In this presentation, we report a sensitive, fast, and robust assay to quantify CBD and THC in human urine using microelution solid-phase extraction (SPE) followed by ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) analysis.

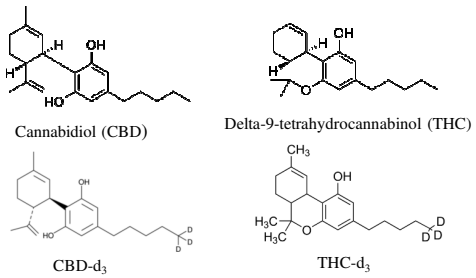

Figure 1: The chemical structures of CBD, THC, and their internal standards (CBD-d_3 and THC-d_3)

METHOD

Sample Preparation

Human urine (treated with 2% bovine serum albumin [BSA]) samples containing CBD, THC, and their stable isotope-labeled internal standards (ISs) were extracted using a Waters Oasis HLB 96-well µElution Plate with formic acid in water as a wash solvent and acetonitrile (ACN) as an eluent.

LC-MS Conditions

UPLC: Agilent 1290 Infinity II LC Systems
Columns: Apex Scientific HALO® 90 Å C18, 2.7 µm, 3.0 x 150 mm
Column temperature: 40°C

METHOD (cont.)

LC-MS Conditions (cont.)

Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in acetonitrile
Flow rate: 0.6 mL/min
HPLC gradient profile:

Time (min)	Events	Value
0.0	B.conc.	40%
0.5	B.conc.	40%
2.5	B.conc.	95%
3.5	B.conc.	95%
3.6	B.conc.	40%
4.0	Stop	

SCIEX API 6500+ triple quadruple mass spectrometer was used in electrospray ionization (ESI) mode with positive ions to monitor CBD, THC, CBD-d_3, and THC-d_3 at ion transitions 315.4→193.2, 315.3→193.1, 318.4→196.3 and 318.3→196.4, respectively. A diversion valve was incorporated to reduce MS system contamination.

RESULTS & DISCUSSION

In order to develop a sensitive, fast, and robust assay, the experimental conditions were thoroughly optimized for stabling sample storage, reducing nonspecific binding (NSB), enhancing extraction efficiency, and increasing chromatographic separation. The results of stability test showed that CBD and THC are unstable when subjected to high temperatures, which indicates that samples should be processed at a lower temperature (**Figure 2**). The results of NSB test (**Figure 3**) showed that both CBD and THC in human urine appear severe NSB to the extraction tubes; therefore, different additives like BSA, zwitterionic detergent CHAPS, and nonionic detergent Triton X-100 for reducing NSB were tested. The best solution for resolving NSB issue in this study was to add BSA in human urine (2%, w/v) when collecting the sample. In order to achieve high sensitivity, sample preparation techniques including protein precipitation (PPE), supported liquid extraction (SLE), and SPE with different sorbents were also compared. The results demonstrated that SPE (µ-elution) provided the highest recoveries for both CDB and THC (**Figure 4**).

A quantitative assay was successfully developed and qualified within a range of 0.100 to 100 ng/mL (**Figures 5** and **6**). The ranges of intra-day accuracy (%Bias) for the lower limit of quantification (LLOQ) and other quality controls (QCs) for both CBD and THC were within 20% and within 15%, respectively.

RESULTS & DISCUSSION (cont.)

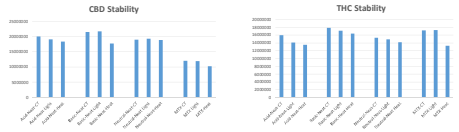

Figure 2: The results of stability test for CBD and THC

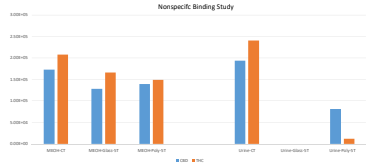

Figure 3: The results of non-specific test for CBD and THC

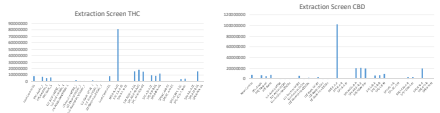

Figure 4: The results of extraction efficiency test

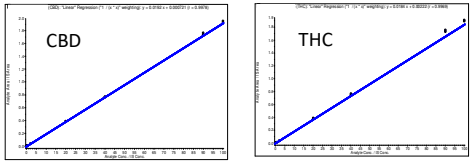

Figure 5: The typical calibration curves

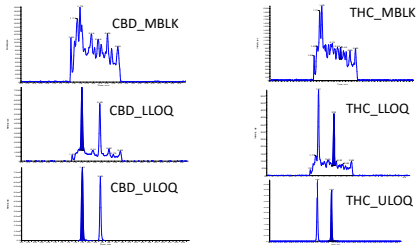

Figure 6: The typical chromatograms

RESULTS & DISCUSSION (cont.)

Table 1: The inter-day precision and accuracy

THC-QC (ng/mL)	*n*	Mean	S.D.	%CV	Accuracy
0.1	18	0.0953	0.00894	9.4	107
0.3	18	0.275	0.00514	1.9	91.3
10	18	10.13	0.069	0.7	98.8
80	18	84.1	1.393	1.7	106.1
1000	18	1020	32.7	3.2	102

CBD-QC (ng/mL)	*n*	Mean	S.D.	%CV	Accuracy
0.1	18	0.107	0.00384	3.6	107
0.3	18	0.274	0.00555	2.0	91.3
10	18	9.88	0.112	1.1	98.8
80	18	84.9	0.881	1.0	106.1
1000	18	1030	26.8	2.6	103

Table 2: The parameters of standard curves

Analyte:	CBD	THC
Range of standard curve	0.100 - 100 ng/mL	0.100 - 100 ng/mL
Inter-assay precision for STDs (%CV)	0.7 to 10.4	0.8 to 11.1
Inter-assay accuracy for STDs (%CV)	92.0 to 106.0	93.0 to 104.8

Table 3: The internal standard response

Internal Standard:	CBD-d_3	THC-d_3
IS response for accepted STDs and QCs (%CV)	10.2 to 15.3	10.2 to 12.9

CONCLUSIONS

- A sensitive, fast, and robust LC-MS/MS method for the quantification of cannabidiol (CBD) and tetrahydrocannabinol (THC) in human urine was developed by thoroughly optimizing the experimental conditions.
- Microelution SPE was successfully used to remove complex interferences in matrix and enrich the analyte of interest.
- The method was qualified as linear, accurate, precise, and reproducible. It can be used to determine the concentration of CBD and THC as low as 0.100 ng/mL using only 100 µL of sample.