

## Application Note

# Cannabinoid Isomer Identification and Quantitation by UPLC-MS/MS Analysis in Forensic Urine Samples

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Este é um Resumo de aplicações e, por isso, não inclui uma seção de experimento detalhada.

*For forensic toxicology use only.*

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## Abstract

In forensic toxicology, detecting use of cannabis by urine confirmation testing has been traditionally limited to analysis of the major  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) metabolite, 11-nor-9-carboxy- $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -cTHC). However, in the United States legalization of hemp has led to increased production and sale of psychoactive cannabidiol (CBD) derivatives including  $\Delta^8$ -THC and  $\Delta^{10}$ -THC isomers. The increasing availability and growing use of these derivatives necessitates an expansion of cannabinoid confirmation test protocols. An isomer-selective definitive method is presented for the quantitative confirmation of  $\Delta^8$ -THC,  $\Delta^9$ -THC,  $\Delta^{10}$ -THC,  $\Delta^8$ -cTHC,  $\Delta^9$ -cTHC, and CBD by liquid chromatography/tandem mass spectrometry (UPLC-MS/MS).

## Benefits

- Simple sample preparation

- Baseline separation between  $\Delta^8$  and  $\Delta^9$  isomers of THC and cTHC
  - Accurate quantification of  $\Delta^8$ -THC,  $\Delta^9$ -THC,  $\Delta^{10}$ -THC,  $\Delta^8$ -cTHC,  $\Delta^9$ -cTHC, and CBD in urinary samples
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## Introduction

Cannabis continues to be one of the widest used recreational drugs in the United States with over 50 million Americans (about 18%) reporting its use in 2021.<sup>1</sup> Detection of  $\Delta^9$ -THC use is typically accomplished by monitoring the major  $\Delta^9$ -THC excreted metabolite,  $\Delta^9$ -cTHC. Recently, however, formulations claiming to contain CBD derivatives such as  $\Delta^8$  and  $\Delta^{10}$  THC have gained in popularity, with recent studies demonstrating an increasing prevalence of these isomers in casework. The presence of isomers can interfere with the quantification of the traditional  $\Delta^9$  isomers as they share MRM transitions and are often not fully resolved from each other by traditional methods. Therefore, an expanded scope of cannabinoid testing protocols is needed to both accurately quantify these isomers as well as the traditional  $\Delta^9$  isomers. This application summarizes a rapid UPLC-MS/MS method for accurately identifying and quantifying  $\Delta^9$ -THC,  $\Delta^8$ -THC,  $\Delta^{10}$ -THC,  $\Delta^9$ -cTHC,  $\Delta^8$ -cTHC, and CBD. Baseline separation is achieved between isomers allowing accurate quantification and detection in authentic case samples.

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## Experimental

### Sample Preparation

All cannabinoid reference material and internal standards were from Millipore Sigma (Round Rock, TX) with the exception of  $\Delta^8$ -THC, which was from Cayman Chemical (Ann Arbor, MI). IMCSzyme glucuronidase and buffer were from Integrated Micro Chromatography Systems (Irmo, SC).

Urine samples were prepared for testing by combining 20  $\mu$ L of urine with 20  $\mu$ L of internal standard mix ( $\Delta^9$ -THC-D3,  $\Delta^9$ -cTHC-D3, CBD-D3) and 20  $\mu$ L of hydrolysis reagent. Samples were incubated for 1 hr at room temperature followed by the addition of 150  $\mu$ L of 70% methanol containing 0.1% formic acid.

### UPLC-MS/MS Analysis

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Samples were analyzed using an ACQUITY™ UPLC™ I-Class (FTN) System interfaced with a Xevo™ TQD Mass Spectrometer. The UPLC system parameters are listed in Table 1 and chromatographic gradient in Table 2.

## Method Conditions

LC system:	ACQUITY UPLC I-Class PLUS FTN System
Detection:	Xevo TQD Mass Spectrometer
Column:	CORTECS UPLC C <sub>18</sub> +; 1.6 µm; 2.1 x 50 mm p/n: 186007114
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	10 µL
Flow rate:	Refer to Gradient Table
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	Acetonitrile
Needle wash:	95:5 acetonitrile/water
Run time:	5.5 minutes

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*Table 1. UPLC Parameters for the Isomer Selective Method*

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Time (min)	Flow rate (mL/min)	%A	%B	Curve
Initial	0.400	45	55	Initial
1.9	0.400	33	67	11
4.4	0.400	5	95	11
4.6	0.400	45	55	11
5.5	0.400	45	55	11

Table 2. UPLC Gradient Table

Table 3 describes the Xevo TQD Mass Spectrometer parameters used for all analyses and MRM parameters are shown in Table 4. All THC isomers and CBD were acquired under positive ESI and the cTHC isomers were acquired under negative ESI. Quantifier and qualifier MRM transitions were monitored for each analyte.

## Method Conditions

MS system:	Xevo TQD
Ionization mode:	ESI+/ESI-
Capillary voltage:	2.5 kV
Desolvation temperature:	500 °C
Source temperature:	150 °C
Desolvation gas:	800 L/Hr
Cone gas:	100 L/Hr
MS1 resolution:	Unit

MS2 resolution:

Unit

Table 3. Mass Spectrometry Parameters

Analyte	Precursor Ion ( <i>m/z</i> )	Product Ion ( <i>m/z</i> )	Cone voltage (V)	Collision energy (eV)	Acquisition time (min)	Polarity
$\Delta^8$ -THC	315.2	193.1 (quan) 123.1 (qual)	32 32	22 32	4.1–5.0	+
$\Delta^9$ -THC	315.2	193.1 (quan) 123.1 (qual)	32 32	22 32		
$\Delta^{10}$ -THC	315.2	193.1 (quan) 123.1 (qual)	32 32	22 32		
$\Delta^8$ -cTHC	343.2	299.2 (quan) 245.1 (qual)	45 45	20 30	1.0–2.7	-
$\Delta^9$ -cTHC	343.2	299.2 (quan) 245.1 (qual)	45 45	20 30		
CBD	315.2	193.1 (quan) 123.1 (qual)	32 32	22 32	2.1–3.6	+
$\Delta^9$ -THC-D3	318.2	196.1	40	23	4.1–5.0	+
$\Delta^9$ -cTHC-D3	346.2	302.2	45	20	1.0–2.7	-
CBD-D3	318.2	196.1	35	20	2.1–3.6	+

Table 4. MRM parameters for cannabinoid isomer method.

Data were acquired and analyzed using MassLynx™ Software (V4.1). Quantification was performed using TargetLynx™.

## Results and Discussion

A more complete description and discussion of this assay validation, performance, and application to casework is detailed elsewhere.<sup>2</sup>

## Chromatography

Chromatographic resolution is a function of column selectivity and efficiency. To this end, multiple column

chemistries were evaluated to achieve the separation between the isomers of THC and cTHC. Figure 1 shows the chromatography of  $\Delta^8$ -cTHC,  $\Delta^9$ -cTHC, CBD,  $\Delta^8$ -THC,  $\Delta^9$ -THC, and  $\Delta^{10}$ -THC. The chromatographic system provided near baseline separation of  $\Delta^8$ -cTHC and  $\Delta^9$ -cTHC as well as the  $\Delta^8$ ,  $\Delta^9$ , and  $\Delta^{10}$ -THC isomers. The CORTECS™ C<sub>18</sub>+ Column (p/n: 186007114 <<https://www.waters.com/nextgen/us/en/shop/columns/186007114-cortecs-c18-column-90a-16--m-21-mm-x-50-mm-1-pk.html>> ) that was used has a solid core construction resulting in a narrow particle size distribution enabling maximum efficiency. This, combined with a small particle diameter and the column's unique selectivity, resulted in excellent resolution in a time frame which is compatible with a routine confirmation workflow.

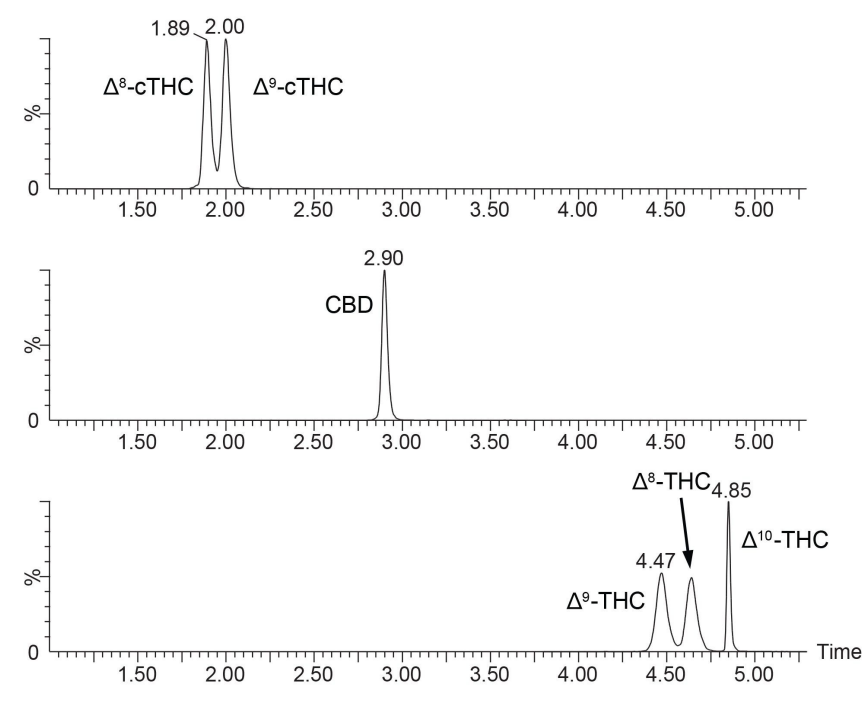


Figure 1. MRM chromatograms (overlaid). Upper panel  $\Delta^8$ -cTHC (1.89 min) and  $\Delta^9$ -cTHC (2.00 min); middle panel CBD (2.90 min); bottom lower panel  $\Delta^9$ -THC (4.47 min),  $\Delta^8$ -THC (4.64 min), and  $\Delta^{10}$ -THC (4.85 min).

## Quantitative Results

The analytical range for each analyte spanned from 10–1000 ng/mL with an LOD of 4 ng/mL. Calibration curves were linear with  $R^2$  values >0.99 for all analytes. All calibration points were within 10% of target values.

Representative calibration curves for  $\Delta^8$ -cTHC and  $\Delta^9$ -cTHC are shown in Figure 2. Precision and accuracy were evaluated both within (N=5) and between (N=6) analytical batches. These data are presented in Table 5. The coefficient of variation (%CV) of the LOD was under 17.3% for all compounds, meeting the detection and quantification criteria of <20%. Low and high controls were within 15% and assay bias for all QCs and the LOD was  $<\pm 15\%$ . Carryover was 0.03% for all analytes, meeting criteria. No interferences were seen with 12 analyte negative urines or in response to 102 drugs routinely tested for by NTC.

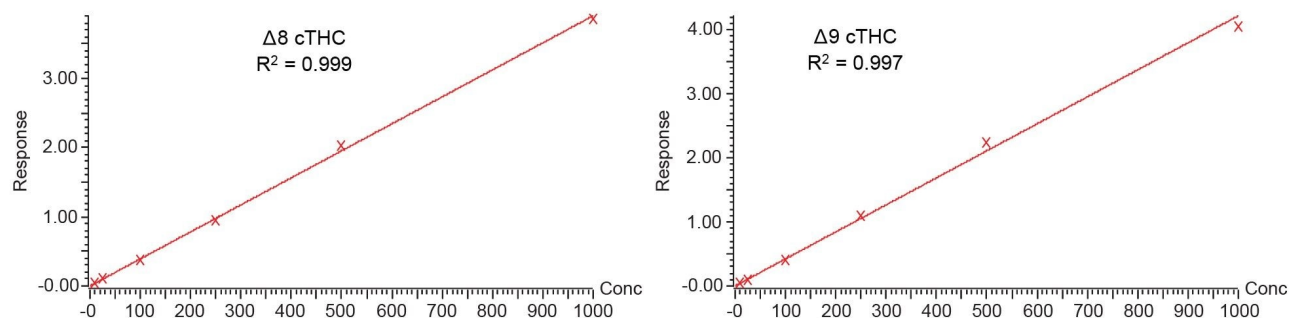


Figure 2. Representative calibration curves for  $\Delta^8$ -cTHC (left graph) and  $\Delta^9$ -cTHC (right graph) are shown. Calibration curved ranged from 10–1000 ng/mL for each isomer.

Analyte	Bias (%CV)				
	LOD (4 ng/mL)	15 ng/mL	40 ng/mL	400 ng/mL	800 ng/mL
$\Delta^8$ -THC	-1.6% (15.3)	-3.4% (12.8)	-5.7% (9.6)	-2.6% (4.6)	-4.9% (4.0)
$\Delta^9$ -THC	-5.7% (9.3)	-7.3% (7.1)	-5.2% (5.4)	0.5% (3.4)	-4.1% (3.7)
$\Delta^{10}$ -THC	-6.8% (17.3)	-4.4% (14.5)	-10.2% (10.0)	-1.5% (9.1)	-6.0% (9.2)
$\Delta^8$ -cTHC	-3.4% (13.8)	-7.3% (10.1)	-3.8% (5.0)	0.5% (6.9)	-3.9% (2.1)
$\Delta^9$ -cTHC	1.6% (14.2)	-9.6% (12.0)	-3.2% (4.8)	1.2% (5.3)	-2.7% (3.1)
CBD	-8.9% (6.1)	-11.5% (5.1)	-9.0% (4.0)	-0.6% (3.6)	-6.1% (2.1)

Table 5. Method precision and bias.

There were sample-to-sample variations in the values of the matrix effects for the individual analytes. However, these were paralleled by the internal standards so that these effects were well normalized and did not impact the sensitivity, accuracy, or precision of the method.

Method comparisons were performed using a previous method and 36 samples that were positive for  $\Delta^9$ -cTHC only, with no detected  $\Delta^8$ -cTHC. Regression analysis revealed a correlation with a slope of 1.004 and an  $R^2$  value of 0.991 indicating excellent agreement between the two methods without any bias.

The method was applied to de-identified urine samples that had initially screened positive for cannabinoids by immunoassay.  $\Delta^9$ -cTHC was detected in >98% of cases.  $\Delta^8$ -cTHC was detected in 14% of samples. Parent drug was not detectable in any of the cases. This aligns well with the prevalence of  $\Delta^8$ -cTHC seen in vaping products in NY State.<sup>3</sup>

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## Conclusion

A method for the rapid and accurate determination of  $\Delta^8$ -THC,  $\Delta^9$ -THC,  $\Delta^{10}$ -THC, CBD,  $\Delta^8$ -cTHC, and  $\Delta^9$ -cTHC was developed, validated, and applied to authentic cannabinoid positive case samples. The high efficiency CORTECS C<sub>18</sub>+ Column enabled the baseline separation of the individual cannabinoid isomers from each other, allowing the accurate quantification of  $\Delta^8$ -cTHC and  $\Delta^9$ -cTHC. Comparison to a previously validated method revealed excellent agreement for  $\Delta^9$ -cTHC. Application to case samples revealed significant concomitant use of  $\Delta^8$ -THC and  $\Delta^9$ -THC, with the prevalence of  $\Delta^8$ -THC matching closely with its prevalence in vaping products in NY State.

## Acknowledgement

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nsduh-annual-national-report <<https://www.samhsa.gov/data/report/2021-nsduh-annual-national-report>>  
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## Featured Products

ACQUITY UPLC I-Class PLUS System <

<https://www.waters.com/nextgen/us/en/products/chromatography/chromatography-systems/acquity-uplc-i-class-plus-system.html>>

Xevo TQD Triple Quadrupole Mass Spectrometry <[https://www.waters.com/waters/en\\_US/Xevo-TQD-Triple-Quadrupole-Mass-Spectrometry/nav.htm?cid=134608730](https://www.waters.com/waters/en_US/Xevo-TQD-Triple-Quadrupole-Mass-Spectrometry/nav.htm?cid=134608730)>

MassLynx Mass Spectrometry Software <<https://www.waters.com/513662>>

TargetLynx <<https://www.waters.com/nextgen/us/en/products/informatics-and-software/mass-spectrometry-software/masslynx-mass-spectrometry-software/masslynx-quantitation-applications.html>>

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