LC/MS/MS Analysis of THCA and THCA Glucuronide in Urine

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ABSTRACT

A fast, quantitative method eliminating the hydrolysis, extraction and derivatization performed in traditional gas chromatography mass spectrometry (GC/MS) analysis of 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCA) was developed. Glucuronidated THCA is a major urinary metabolite of Δ^9-tetrahydrocannabinol (THC), the most commonly used illicit drug worldwide. GC/MS THCA analysis requires hydrolysis to convert the conjugated THCA to unbound THCA for reliable detection and measurement of total metabolite. Ultra high pressure liquid chromatography tandem mass spectrometry (UHPLC/MSMS) facilitates monitoring of intact glucuronides in short run times and avoids extraction and derivatization for sample analysis.

Urine samples were diluted tenfold in water and analyzed on an Agilent 1290 Infinity LC and 6490 Triple Quadrupole in a 3 minute cycle time. Two transitions were monitored for THCA, THCA Glucuronide and internal standard, THCA D9 by positive electrospray ionization in multiple reaction monitoring mode following gradient separation. The method was validated and applied to 30 positive authentic urine samples to evaluate overall concordance with the original 4.5 minutes GC/MS method.

The limits of detection and quantification were 4 ng/mL and the upper limits of linearity and carryover were 1000 ng/mL for both THCA and THCA Glucuronide. Calibration curve correlation coefficients exceeded 0.99. Inter/intra-assay precision did not exceed 9% coefficient of variation and accuracy was within 16% of the target concentrations for the two compounds. Matrix effect was 36.7% for THCA and 19.5% for THCA Glucuronide. As expected, THCA Glucuronide was the more dominant species in most samples. The LC/MS/MS and GC/MS data correlated well with correlation coefficient of 0.92 and 86.6% agreement. Discordance occurred at the 10 ng/mL cutoff level or due to interference. Interferences preventing GC/MS confirmation were absent under LC/MS/MS in 2 of 3 instances.

The LC/MS/MS method for Total THCA Analysis accommodates low detection limits and fast turn-around times with minimal sample preparation. It demonstrated good correlation with the GC/MS method, resolving some GC/MS peak interferences.

INTRODUCTION

•Marijuana is the most commonly used illicit drug worldwide and is frequently detected in drug testing (1).
•Δ^9-Tetrahydrocannabinol (THC) is the most psychoactive, principle constituent of marijuana (2).
•11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCA) and its conjugate THCA Glucuronide are inactive, major urinary metabolites of THC (2,3).

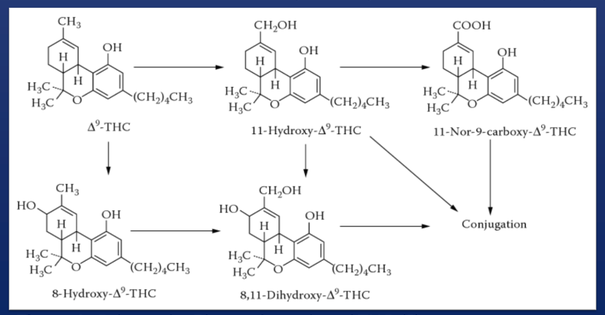

Figure 1: Scheme for Biotransformation of THC (3)

INTRODUCTION

•Conventional analysis for Total THCA utilizes gas chromatography mass spectrometry (GC/MS) following initial sample hydrolysis (enzyme or alkaline) to convert THCA Glucuronide back to unbound THC.
•Hydrolysis can be costly and time-consuming and enzyme hydrolysis is susceptible to variability, which affects the veracity of the reported result (4).
•THCA analysis by GC/MS also requires sample extraction and derivatization.
•Analysis by liquid chromatography tandem mass spectrometry (LC/MS/MS) can be viable with dilution as the only sample pretreatment, precluding extraction and derivatization.
•Direct measurement of THCA and intact THCA glucuronide with the availability of a THCA Glucuronide reference standard has been demonstrated via application of LC/MS/MS with electrospray ionization (ESI) (4).
•The elimination of hydrolysis, extraction and derivatization tests in Total THCA urinalysis affords time and cost savings.
•Ultra high pressure (UHP) LC/MS/MS also facilitates desired shorter run times due to less LC column back pressure allowing higher flow rates.
•A novel UHPLC/MS/MS application for Total THCA analysis is presented.

MATERIALS AND METHODS

•The UPLC method consisted of a gradient from 40% A (0.05% formic acid in water)/60% B (0.05% formic acid in methanol:acetonitrile (50:50)) to 5% A/ 95% B in 1.4 minutes using a 0.5 mL/min flow rate.
•Mass spectral data on the Agilent 6490 Triple Quadrupole was acquired in positive ESI mode.
•Two transition ions each for THCA, THCA Glucuronide and internal standard, THCA D9 were selected after optimization using loop injection of each compound on the 6490 with the aid of MassHunter Optimizer.
•Urine samples (50 µL) were diluted 10X with acetonitrile:water (50:50) and centrifuged.
•Prepared samples were injected (10 µL) on a Phenomenex Kinetex C18 column and analyzed on the Agilent 1290 Infinity LC and 6490 Triple Quadrupole.
•The LC/MS/MS method was validated and applied to authentic urine samples previously found positive under a 4.5 min GC/MS method.

RESULTS

ANALYTE	CONCENTRATION (ng/mL) LOD	LOQ	ULOL	ULOC	CUTOFF	R^2	MATRIX EFFECT
THCA	4	4	1,000	1,000	10	>0.99	+36.7%
THCA Glucuronide	4	4	1,000	1,000	10	>0.99	+19.5%

Analyte	[ ] (ng/mL)	Accuracy (%)	Interday precision (N=30) (%)	Intraday Precision (n=10) (%CV)
THCA	10	93.5	9.4	7.5
	20	96.4	6.8	5.3
	75	88.4	5.2	5.2
THCA Glucuronide	10	98.6	7.5	5.9
	20	105.3	6.2	5.0
	75	96.2	9.5	7.0

Table 1: Validation Summary for THCA and THCA Glucuronide

RESULTS

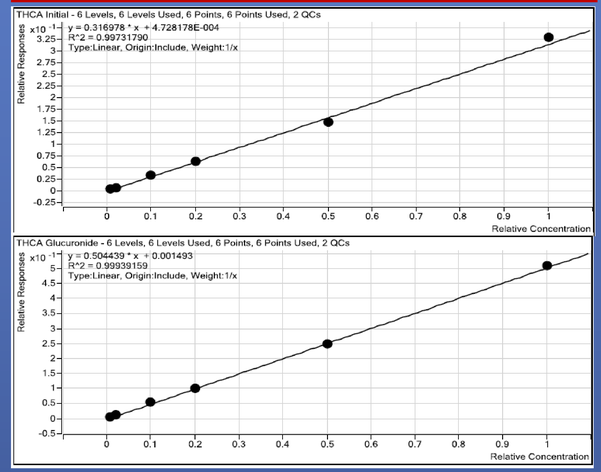

Figure 2: Representative Calibration Curves for THCA and THCA Glucuronide

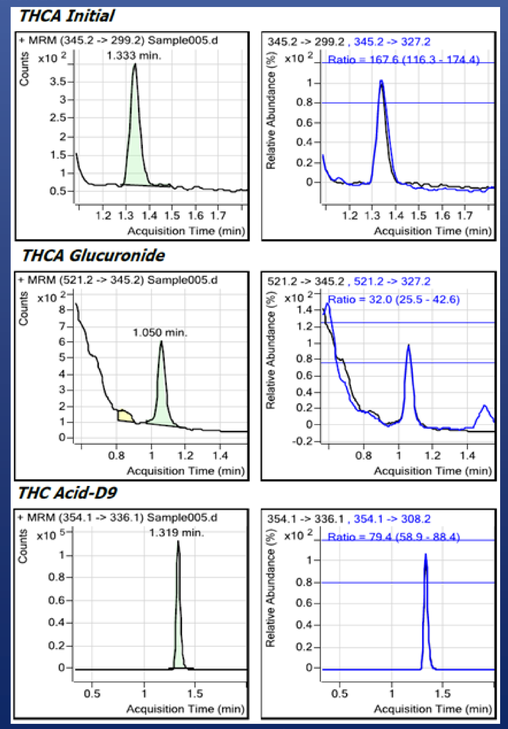

Figure 3: Representative MRM Chromatogram at 4 ng/mL

RESULTS

	[THCA]	[THCA Glucuronide]
Mean (ng/mL)	107.2	249.7
Min (ng/mL)	0	0
Max (ng/mL)	>1000	>1000
Median (ng/mL)	62.6	58.4
Species Present		**Frequency (%)**
THCA only		13.3
THCA Glucuronide only		13.3
THCA + THCA Glucuronide		73.3

Table 2: Summary of Results of the Total THCA Analysis of 30 Authentic Urine Specimens

- LC/MS/MS-GC/MS Correlation Coefficient= 0.92.
- % LC/MS/MS-GC/MS Positivity Agreement= 86.6%

DISCUSSION

- THCA undergoes extensive glucuronidation prior to elimination in urine, so as expected THCA Glucuronide was present at higher concentrations than THCA in 73% of the authentic samples.
- LC/MS/MS patient results were reported as Total THCA, the sum of THCA and THCA bound to glucuronide, in keeping with traditional reporting.
- The LC/MS/MS and GC/MS data for Total THCA correlated well with discordance only occurring at the cutoff level or in the presence of interference.
- Interferences preventing GC/MS confirmation were absent under LC/MS/MS in 2 of 3 instances for the authentic sample analysis.

CONCLUSION

•The UHPLC/MS/MS method for Total THCA Analysis has low detection and quantification limits and a fast turn-around time with minimal sample preparation and can be applied to high-throughput urinalysis.
• The method demonstrated good correlation with the GC/MS method, resolving some GC/MS peak interferences.
•THCA glucuronide was generally the more dominant species in authentic samples.

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