Technical Note: 10161

High-Throughput Confirmation and Quantitation of a THC Metabolite in Urine Using the DSQ II GC/MS

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Key Words

- DSQ II GC/MS
- ToxLab 2.0 Software
- THC
- Toxicology
- Urine Drug Testing

Overview

The marijuana plant (*Cannabis sativa*) contains tetrahydrocannabinol (THC), a pharmacologically active compound known to have mind-altering properties. In the United States and elsewhere, the use of either the plant and/or the THC active component is regulated or prohibited. In the realm of workplace drug testing, regulations stipulate testing for THC and/or its metabolites in urine, while alternate matrices, such as hair, sweat, oral fluids, and blood are also used to confirm THC use. Toxicologists in other disciplines may also test for THC use, for such diverse applications as driving impairment analyses, postmortem investigations, and clinical toxicology. Analytical methods for THC and its metabolites range from immunoassay techniques to gas chromatography/mass spectrometry (GC/MS). The methodology presented here focuses on the use of the DSQ™ II GC/MS system for the confirmation and quantitation of the primary urinary metabolite of THC, 11-nor-9-carboxy-Δ-9-THC, or THCA (Figure 1).

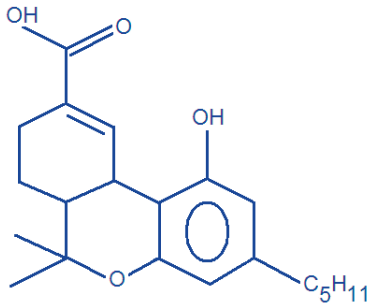

Figure 1: Chemical Structure of 11-nor-9-carboxy-Δ-9-THC

For this assay, a 3 mL urine sample size was used, with THCA-D9 as the deuterated internal standard. Because THCA is excreted in the form of a conjugated glucuronide, a hydrolysis step to remove the glucuronide and allow for quantitation of THCA is required. Samples were subjected to basic hydrolysis and then extracted using solid phase extraction. After extraction, the samples were derivatized with bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% TMCS. The final reaction products were analyzed using a DSQ II single stage quadrupole GC/MS system. A calibrator at 15 ng/mL was used for single point calibration. The resulting method demonstrated excellent precision, no interference for a number of tested compounds and provided linearity from 1.5 to 1000 ng/mL, with a limit of detection and limit of quantitation of 1.5 ng/mL.

Introduction

In the United States, marijuana use as determined by workplace drug testing programs has been on a continual decline; however, marijuana still represents the largest proportion of positive drug screens as reported by one laboratory system.[1] Marijuana use produces euphoria and has a sedative effect.[2] When THC enters the body, it is rapidly metabolized to, among others, 11-nor-9-carboxy-Δ-9-tetrahydrocannabinol (THCA), with little parent drug remaining. The presence of THCA in a urine sample identifies the donor as one who has been exposed to some form of THC, either through consumption (oral or smoked) or via indirect contact.[2] Because THCA contains a carboxyl functional group that does not lend itself well to gas chromatography, samples for THCA confirmation are typically derivatized, which allows laboratories to realize increased productivity and take full advantage of the speed and ease of use that is afforded by GC/MS.

The DSQ II, a single stage quadrupole mass spectrometer with a curved prefilter that minimizes background noise derived from excited neutrals, was used for this analysis. Coupled to a TRACE GC Ultra™ gas chromatograph and an AS3000 autosampler, this GC/MS system represents the standard for confirmatory analyses of drug use. ToxLab™ 2.0 software provided automated sample analysis and quantitation, and the method was fully validated, including assessments of precision, interference, and linearity. This method describes the GC/MS confirmation and quantitation of THCA in urine, and does not include other matrices or other THC metabolites, nor does it encompass analysis of parent THC. The method utilizes BSTFA + 1% TMCS for derivatization, which caps labile hydrogens with trimethylsilyl (-$Si(CH_3)_3$ groups, creating the di-TMS derivative of THCA. Other derivatives may be equally suitable, but BSTFA was selected due to the high molecular weight of the derivatized THCA, the volatility of BSTFA, and the ease of use afforded by not having to evaporate the excess derivative prior to analysis.

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Methods

To provide a comprehensive view of method development and validation, methods for sample preparation, acquisition, and analysis are described in detail below. Sample preparation plays a critical role in method validation since many certifying bodies recommend or require method validation performed in matrix. In this case, solid phase extraction is used due to its ease of use and the cleanliness of the resultant extracts.[3]

Sample Preparation

Known negative urine was collected and used for sample preparation. A sample size of 3 mL was selected. Calibrators, quality controls, and linearity samples were spiked with appropriate amounts of THCA (Cerilliant, Round Rock, TX). Single point calibration at 15 ng/mL was used for calculation of all quantitative amounts. A commercial control (Medical Analysis Systems, Level G3, Freemont, CA) calibrated to represent 125% of 15 ng/mL (18.75 ng/mL) was used as the positive control for the batch, and the 40% control (6 ng/mL) was prepared from THCA source material from an alternate source (Alltech Associates, Deerfield, IL). All batches contained an unextracted standard, the calibrator, a negative control, a 40% control and the 125% control. THCA-D9 (Cerilliant) was used as the deuterated internal standard, and was added to each sample at a final concentration of 15 ng/mL. An unextracted standard was prepared by adding 100 µL of 450 ng/mL THCA standard solution and 100 µL of 450 ng/mL THCA-D9 internal standard solution to a labeled tube, yielding the equivalent of a 15 ng/mL sample. The purpose of the unextracted standard is to demonstrate recovery, to prep the GC/MS system, and to demonstrate ion ratios. The unextracted standard is not subjected to the hydrolysis or extraction steps but instead proceeds directly to the dry-down step, at which point it rejoins the rest of the samples for derivatization and analysis.

Prior to extraction, the samples were hydrolyzed by adding 100 µL of 10 M KOH, followed by heating at 60 °C for 20 minutes. After hydrolysis, samples were made acidic using 1 mL of glacial acetic acid, bringing the pH to 3.5 ± 0.5. Each sample was extracted by solid phase extraction on Thermo Scientific HyperSep™ Verify™ CX columns (P/N 60108-742). The extraction columns were conditioned with sequential rinses of the following: 3 mL methanol, 3 mL DI water, and 1 mL 0.1 M HCl. Between each conditioning step, the columns were not allowed to dry. The hydrolyzed, pH-adjusted samples were then loaded onto the column and extracted under low vacuum (″ 3 in. Hg). The columns were then washed sequentially with 2 mL of DI water and 2 mL of 0.1 M HCl: acetonitrile solution (70:30 v:v). The columns were then dried under high vacuum for five minutes. A final rinse of 200 µL of hexane was then pulled through the columns, and sample eluents were collected in clean tubes under low vacuum (″ 1 in. Hg) with 3 mL of elution solvent (hexane:ethyl acetate, 50:50 v:v).[3]

The extracts were evaporated to dryness at 40 °C under nitrogen. Caution was taken to prevent excessive drying of the extracts. Next, the dried samples were derivatized with 50 µL of BSTFA with 1% TMCS at 60 °C for 20 minutes. For analysis, 75 µL of ethyl acetate were added to the derivatized extracts, and the resulting samples were transferred to autosampler vials with glass inserts and loaded onto the AS 3000 autosampler for GC/MS analysis. Table 1 summarizes sample prep, extraction, and derivatization steps.

Instrumental Analysis

The DSQ II mass spectrometer used for this analysis was configured with a 250 L/s turbomolecular pump, and the TRACE GC Ultra was equipped with a standard split/splitless injector. A 5 mm i.d. deactivated glass liner was used in the injector and glass wool was used in the liner. The split/splitless injector temperature was set to 270 °C. A 2 µL injection volume was programmed on the AS 3000 autosampler, and a 10:1 split injection was used. The analytical column was a TRACE™ TR-35MS 15 m x 0.2 mm i.d. x 0.33 µm film, which was installed 64 mm into the injection port (Figure 2).

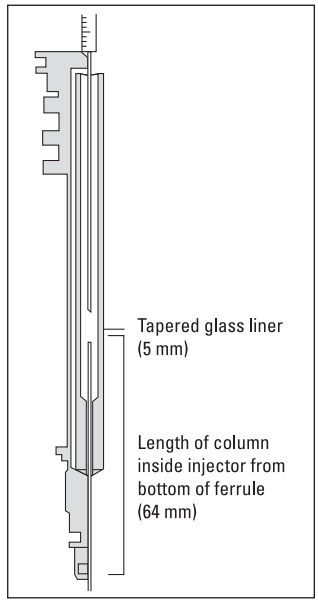

Figure 2: Column installation in GC split/splitless injection port (not to scale)

Sample Preparation and Hydrolysis
1. Label 13 x 100 mm screw top culture tubes
2. Add 3 mL of blank urine, QC or donor specimen
3. Spike calibrator and low QC with THCA
4. Add 100 µL of working THCA-D9 internal standard to each tube
5. Add 100 µL 10 M KOH to each urine sample
6. Vortex gently
7. Cap and heat at 60 °C for 20 minutes
8. Remove from heat, let cool and add 1 mL glacial acetic acid
9. Vortex gently
10. Prepare vacuum manifold for sample extraction

Extraction
1. Condition SPE columns sequentially with a. 3 mL methanol b. 3 mL DI water c. 1 mL 0.1 M HCl
2. Apply samples at low vacuum
3. Rinse SPE columns sequentially with a. 2 mL DI water b. 2 mL 70:30 0.1 M HCl: Acetonitrile
4. Dry columns at high vacuum for 5 minutes
5. Rinse with 200 µL hexane
6. Elute THCA with 3 mL 50:50 ethyl acetate: hexane; collect in labeled culture tubes

Concentration and Derivatization
1. Evaporate samples at < 40 °C under N_2 stream until dry
2. Add 50 µL BSTFA w/ 1% TMCS
3. Cap culture tubes, vortex and heat at 60 °C for 20 minutes.
4. Remove from heat, let cool and add 75 µL of ethyl acetate; vortex
5. Transfer resulting extracts to autosampler vials with inserts for GC/MS analysis

Table 1: Sample Prep, Extraction and Derivatization Summary

Programmed carrier gas flow started with an initial flow rate of 2.5 mL/min of helium. At 1.95 minutes, the flow was ramped to 12.5 mL/min to get the heavy matrix compounds through the column as quickly as possible. The initial temperature on the TRACE GC Ultra was set to 230 °C. The high temperature at the beginning of the analytical run allowed the THCA to elute from the column as quickly as possible. Due to the high boiling point of derivatized THCA, it is not necessary to use the solvent to recondense the sample at the head of the column. The initial oven hold time was 0.1 minutes, after which the GC temperature ramped at 60 °C/min to a final temperature of 320 °C for 1.8 minutes, for a total run time of 3.40 minutes and a THCA retention time of 1.73 minutes.

The DSQ II source temperature was set to 300 °C, and the mass spectrometer was tuned using default *AutoTune* parameters. These tune settings were used for acquisition, with a detector gain of 3 x 10^5. For initial mass spectrometer method development, high concentrations of derivatized THCA and THCA-D9 were injected and analyzed in electron impact (EI) full scan to determine masses for EI selected ion monitoring (SIM). The set of SIM masses and dwell times used to detect THCA and its deuterated internal standard are shown in Table 2. Mass 371 was used as the quantitation mass for THCA, and mass 380 was the quantitation mass for internal standard, THCA-D9. A narrow SIM width enhances sensitivity and builds on the mass stability and resolution of the DSQ II, while a short dwell time provides quantitative precision across the narrow GC peak that results from the use of fast GC. Table 2 summarizes instrument parameters for the validated method.

Sample Processing and Result Derivation

For sample acquisition, peak detection and quantitation, ToxLab 2.0 software was utilized. By incorporating all of the vital components of analyses into a unified workflow-oriented application, ToxLab 2.0 provides an integrated solution to THCA GC/MS confirmation. To make use of ToxLab 2.0 for method validation, an instrument method was created for the mass spectrometer, autosampler, and GC. A processing method for component identification and quantitation was developed. In ToxLab 2.0, these methods were integrated into a single master method, which also allows the user to establish criteria specific to the method. Batch creation was performed through the *Batch Wizard* function of ToxLab 2.0, which greatly simplified and streamlined sample entry, particularly for the longer validation batches (Figure 3). This highlights the applicability of this software to routine analysis of toxicological samples.[4]

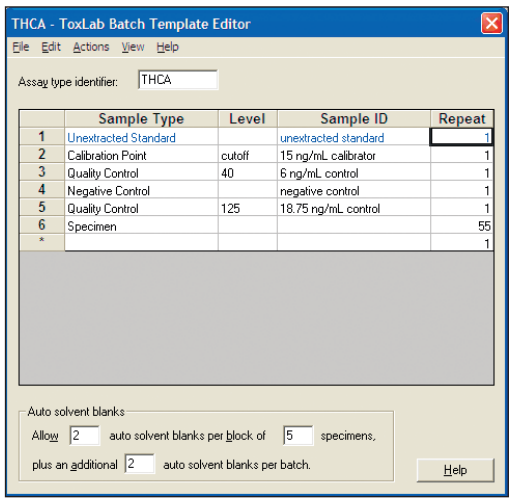

Figure 3: ToxLab 2.0 Batch Template Editor, showing framework for THCA batches

DSQ II	
Source Temp (°C):	300
Acquisition Time (min):	1.85
Detector Gain:	3 x 10^5
Start Time (min):	1.6
THCA Mass (m/z):	371.2 473.2 488.2
THCA-D9 Mass (m/z):	380.2 497.2
Width (amu):	0.5
Dwell Time (ms):	10

TRACE GC Ultra	
Oven Method	
Initial Temp (°C):	230
Initial Time (min):	0.1
Rate (°C/min):	60
Final Temperature (°C):	320
Final Hold Time (min):	1.8
SSL Method	
Temperature (°C):	270
Mode:	Split
Split Ratio:	10:1
Split Flow:	25
Constant Septum Purge:	on
Carrier Method	
Initial Value (mL/min):	2.5
Initial Time:	1.95
Rate #1 (mL/min2):	999.9
Final Flow (mL/min):	12.5
Hold Time #1 (min):	5
Gas Saver:	on
Gas Saver Flow (mL/min):	100
Gas Saver Time (min):	0.5
Vacuum Compensation:	on
Transferline Temp (°C):	280

AS 3000	
Sample Volume (µL):	2
Plunger Strokes:	5
Viscous Sample:	Yes
Sampling Depth in Vial:	Bottom
Injection Depth:	Standard
Pre-Inj Dwell Time (sec):	0
Post-Inj Dwell Time (sec):	0
Sample Rinses:	0
Pre-Injection Solvent Rinses	0
Post-Inj Solvent Rinses	
Solvent A (50:50 EtOAc:$MeCl_2$):	3
Solvent B (50:50 EtOAc:$MeCl_2$):	3

Table 2: Instrument method summary for the SIM analysis of THCA on the DSQ II

Concentration calculations were based on a single point calibrator at 15 ng/mL, using THCA-D9 as the internal standard. Linear calibration including the origin created the calibration curve, and calculated amounts were based on this curve. All validation batches had to conform to quality control (QC) criteria, including quantitative and qualitative bounds checking.

Quantitative criteria for the batch included acceptable quantitation ranges for all samples in each batch. All calculated amounts for QC samples and study samples had to fall within ± 20% of the expected concentration in order to accept the sample. Failure of a QC sample within a batch would mean the entire batch would need to be repeated. In addition to this quantitative window, negative controls were evaluated based on two additional criteria. One means of assessing a negative control is a quantitative value for THCA less than the method limit of detection (LOD), which in this case was 1.5 ng/mL. An alternate criterion for negative controls is that the calculated amount must be less than a pre-determined percentage of the method cutoff. For this method, a level of 5% of the cutoff (0.75 ng/mL) was used as a second criterion, and all negative controls were evaluated for compliance to both criteria.

Qualitative criteria included ion ratio and retention time target ranges based on the calibrator, along with peak shape considerations. These criteria were applied to all sample types. Ion ratio ranges for the batch were developed based on the appropriate ratios from the 15 ng/mL calibrator. Ratios were defined as follows:

$$ion\ ratio = \frac{area\ of\ qual\ ion}{area\ of\ quant\ ion} \times 100$$

Ratios were calculated for THCA-D9 (497:380) and THCA (473:371 and 488:371), and for each ratio, an acceptable range of ± 20% was established. Similarly, the target retention time for THCA and THCA-D9 was set using a ± 2% retention time window based on the calibrator retention time. Peak symmetry requirements required the peaks to be > 90% symmetrical at 50% peak height.

Each validation batch was reviewed for compliance with these criteria, and for a study batch to be accepted, it had to comply with all of these QC criteria.

Results

The analysis of THCA in urine using the DSQ II GC/MS system was thoroughly validated through determination of linear range, carryover, precision, and specificity. Four separate batches were prepared and analyzed: one for linearity/carryover, one for specificity, and two for precision. Each batch included the appropriate quality controls and calibration standards, along with validation samples prepared according to Table 3. Batch acceptability was determined by applying the QC criteria described above. Carryover was assessed during the course of the linearity study. Precision analyses were performed on two separate batches analyzed on two separate days, while specificity assessed potential interference from a number of compounds. Limits of detection and quantitation were determined both analytically and statistically. The DSQ II demonstrated excellent intra- and inter-day precision, linearity from 1.5 to 1,000 ng/mL, with carryover below the QC limits following 1,000 ng/mL, and no interference was seen for this assay for the compounds tested. With 5.5 minute inject-to-inject times, the method also provides a productive means of performing this confirmation.

Linearity
1. Unextracted (15 ng/mL)
2. 40% Control (Alltech)
3. Calibrator (15 ng/mL)
4. Negative
5. 125% Control (MAS)
6. 1.5 ng/mL x 7
7. 2.25 ng/mL x 7
8. 3 ng/mL x 7
9. 6 ng/mL x 7
10. 15 ng/mL x 7
11. 30 ng/mL x 7
12. 60 ng/mL x 7
13. 167 ng/mL x 7
14. 333 ng/mL x 7
15. 500 ng/mL x 7
16. 1000 ng/mL x 7

Precision
Batch 1
1. Unextracted (15 ng/mL)
2. 40% Control (Alltech)
3. Calibrator (15 ng/mL)
4. Negative
5. 125% Control (MAS)
6. 6 ng/mL x 7
7. 15 ng/mL x 7
8. 18.75 ng/mL x 7
Batch 2
1. Unextracted (15 ng/mL)
2. 40% Control (Alltech)
3. Calibrator (15 ng/mL)
4. Negative
5. 125% Control (MAS)
6. 6 ng/mL x 7
7. 15 ng/mL x 7
8. 18.75 ng/mL x 7

Interference
1. Unextracted (15 ng/mL)
2. 40% Control (Alltech)
3. Calibrator (15 ng/mL)
4. Negative
5. 125% Control (MAS)
6. Negative w/ Interference #1
7. 6 ng/mL w/ Interference #1
8. 18.75 ng/mL w/ Interference #1
9. Negative w/ Interference #2
10. 6 ng/mL w/ Interference #2
11. 18.75 ng/mL w/ Interference #2
12. etc…

Table 3: Validation study sample preparation guide for THCA confirmation in urine

Linear Range Determination

The determination of assay linearity was performed at concentrations across a broad dynamic range. The linearity batch, as with every validation batch, included an unextracted standard, a negative control, the 15 ng/mL calibrator, a 40% control sample (6 ng/mL) and a 125% commercial control sample (18.75 ng/mL). To evaluate method linearity, samples at 1.5, 2.25, 3, 6, 15, 30, 60, 167, 333, 500 and 1,000 ng/mL were prepared and extracted, along with the calibrator and controls. These samples were then injected 7 times each, and the resulting 77 data points were quantified based on the 15 ng/mL calibrator. All 77 quantitative values were within ± 20% of their target concentrations, and a regression analysis comparing the average quantitative value for each level to its expected value was found to have a correlation coefficient of 0.9989 (Figure 4). At the lowest level, 1.5 ng/mL, the coefficient of variation (CV) of the calculated amount was 3.6%, with an average concentration of 1.46 ng/mL. Chromatography for the quantitation ion and all qualifiers was exceptional, as shown in Figure 5.

In addition to evaluating quantitative performance, the ratios of the qualifier ions to the quantitation ion for both THCA and THCA-D9 were also evaluated across the concentration range. For THCA, *m/z* 371 served as the quantitation mass, while *m/z* 473 and 488 were used for confirmation. *m/z* 380 was used as the quantitation mass for the internal standard, with *m/z* 497 used as the THCA-D9 confirmatory ion. The acceptable ion ratio ranges were calculated based on the appropriate ratios from the 15 ng/mL calibrator, and a relative range of 20% was used as evaluation criteria for the 77 linearity injections. For each of these injections, the ion ratios were calculated and all were found to be within the acceptable range, indicating excellent linearity of ion ratios across the concentration range.

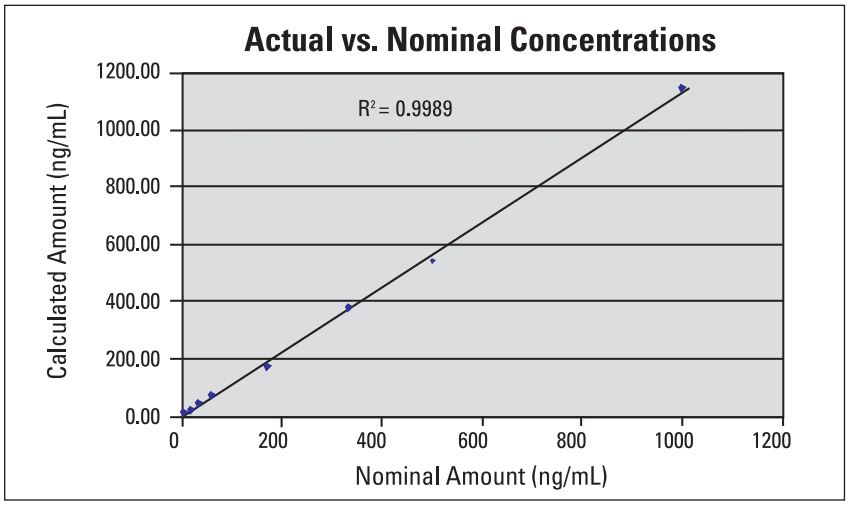

Figure 4: Linearity study results, comparing average concentrations for replicates at 11 different levels to the nominal amounts at each level. The regression analysis for this study gave a correlation coefficient of 0.9989 across all 11 levels.

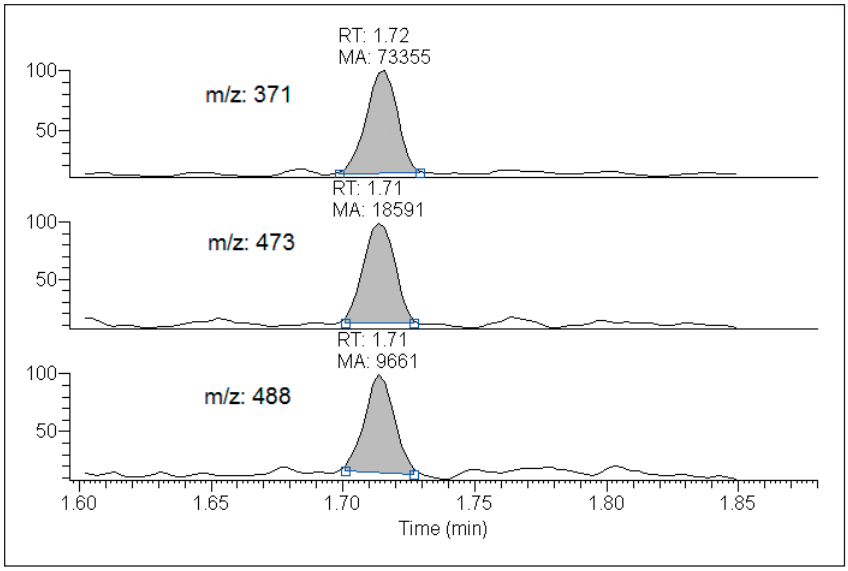

Figure 5: *m/z* 371, 473, and 488 from the 1.5 ng/mL level, showing good chromatography and signal intensity at the limit of detection for this method.

An additional component of the linearity study included a determination of the carryover limit for the method. To do so, a negative control was injected following each set of linearity samples. These negatives were evaluated for acceptability according to the batch criteria described above. Under these constraints, there was no significant carryover even following the 7 injections of the 1,000 ng/mL level. The use of a gas-tight syringe coupled with syringe rinse steps ensures minimal carryover.

Finally, for this batch to be considered acceptable, the quality controls for the batch had to meet QC criteria described above. For the 40% control, the calculated value was 6.5 ng/mL, an 8% deviation from the target and well within the ± 20% quantitation range, and the ion ratios were also within the ± 20% target range. The calculated amount of the 125% control was 19.9 ng/mL, a 6% deviation from nominal and within ± 20%; the ion ratios also met their criteria. The negative control was negative by two different criteria. For this method, the LOD is 1.5 ng/mL, and no trace of THCA was seen in the negative control. As such, the negative control was acceptable and the linearity batch was accepted. Table 4 includes a summary of the linearity/carryover study for THCA on the DSQ II.

Expected Concentration (ng/mL)	Average Calculated Concentration (ng/mL)
1.5	1.46
Negative	0
2.25	2.10
Negative	0
3	2.91
Negative	0
6	5.91
Negative	0
15	15.1
Negative	0
30	30.5
Negative	0
60	62.8
Negative	0
167	165
Negative	0
333	371
Negative	0
500	535
Negative	0.3
1000	1150
Negative	0.3

Table 4: Results of linearity/carryover study. Calculated concentrations representing points on the linearity curve were obtained by averaging seven injections made at that concentration

Intra- and Inter-day Precision

Instrument precision and method precision were measured by extracting two separate precision batches and running these batches on two different days. The precision study was designed to indicate precision at the 40% level, at the cutoff of 15 ng/mL and at the 125% level. Coefficients of variation (CV) were calculated for the average concentrations at each level, and these CVs were to be less than 10% for each concentration. As with the linearity batch, the precision batches must comply with the QC criteria, and all controls were acceptable. To gauge inter-day precision, the percent difference in the average quantitation amounts at each level were to be less than 10%.

The method described above provides excellent quantitative precision, with CVs all less than 2%, and percent differences all less than 2%. Table 5 includes a summary of the precision results for THCA on the DSQ II.

Specificity

To determine assay specificity, an interference study was also performed. A number of compounds with potential to interfere with the immunoassay screening test for THCA were included in this test, as were a range of other drugs. Table 6 describes the drugs and their respective concentrations. THC parent and analogs were assessed individually, as was ibuprofen. The remaining drugs were analyzed as a mixture. For each interference test, the potential interferent was spiked at the concentration specified in Table 6 into a blank urine sample, a 6 ng/mL sample and an 18.75 ng/mL sample. All negatives met the negative control criteria for THCA, and each 40% and 125% control quantified within 20% of the target concentration, showing that none of the potential interferents tested affected quantitation. Also, all ion ratios were checked against the ion ratios of the calibrator and each were within 20% of the calibrator ion ratios, showing no interference with the confirming ions. Retention times fell within the specified window of ± 2% of the calibrator retention time. The interference batch also complied with all applicable QC criteria, and the results of the specificity batch were accepted as demonstrating the assay to be free of interference from the tested compounds.

Concentration	CV for Batch 1	CV for Batch 2	Inter-batch Percent Difference
6 ng/mL	1.6%	1.8%	1.3%
15 ng/mL	1.0%	1.4%	1.5%
18.75 ng/mL	1.2%	1.0%	0.4%

Table 5: Results of precision study showing intra-day coefficients of variations of 2% or less and percent differences for inter-batch calculated amounts of 1.5% or less

Drug	Concentration (ng/mL)
delta-9-THC	500
11-hydroxy-delta-9-THC	500
Cannabidiol	500
delta-8-THC	500
Ibuprofen	10,000
Cannabinol	500
Ethosuximide	1000
alpha-Methyl-alpha-propylsuccinimide	1000
Metharbital	1000
Barbital	1000
Methsuximide	1000
Phensuximide	1000
Normethsuximide	1000
Mephenytoin	1000
Ethotoin	1000
Mephobarbital	1000
PEMA	1000
Phenobarbital	1000
Methyl PEMA	1000
10,11-Dihydrocarbamazepine	1000
Primidone	1000
Phenytoin	1000
Carbamazepine	1000
4-Methylprimidone	1000
Caffeine	667
Methadone	500
Cocaine	500
Codeine	833
6-Monoacetylmorphine	1250
Diacetylmorphine	1250

Table 6: List of compounds tested for potential interference, along with concentrations tested

Conclusion

The analysis of THCA on the DSQ II was completed with a THCA retention time of less than two minutes. The validated method shown describes one that is very sensitive, with a wide dynamic range, from 1.5 to 1000 ng/mL. All samples tested in this range gave calculated amounts that were within 20% of the nominal values, based on a one-point calibration curve at 15 ng/mL. Across this range, all samples also gave ion ratios which were within 20% of the ion ratios of the calibrator. A series of replicate injections at the reported LOD of 1.5 ng/mL gave a coefficient of variation of 3.6% and an average calculated value of 1.46 ng/mL, demonstrating remarkable sensitivity even when using a split injection technique. Method precision and specificity were also excellent, with intra-day coefficients of variation all less than 2% at three different concentrations. Because all method development and validation was performed in extracted urine matrix, the results demonstrate performance of the DSQ II system based on actual samples. These results also accurately reflect method development and validation as they would be performed within a working laboratory.

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