Technical Note: 10306

Confirmation and Quantitation of THC in Oral Fluid Using an MRM Method on a GC-Triple Quadrupole MS

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Key Words

- TSQ Quantum XLS
- ToxLab Forms 2.5 Software
- Drugs of Abuse
- Oral Fluid Drug Testing
- THC
- Toxicology

Overview

Analysis of oral fluids is becoming an increasingly popular biological fluid as an alternative to blood and urine to analyze for drugs of abuse. As opposed to urine, the collection of oral fluid can be monitored without embarrassment. Also, it has the advantages over blood in its ability to be collected without pain or the need for extensive training.

The marijuana plant (*Cannabis sativa*) contains Δ9-tetrahydrocannabinol (THC), a pharmacologically active compound known to have mind-altering properties. Since THC (Figure 1) is one of the most common illegally used drugs, its analysis in oral fluid is gaining increased importance for drug testing. This analysis can be extremely challenging due to the low concentrations of THC typically found in oral fluid, and due to the low volume of oral fluid that is typically available for analysis. Also, as with many biological fluids, the chemical background from the matrix can limit the low detection levels required for this assay.

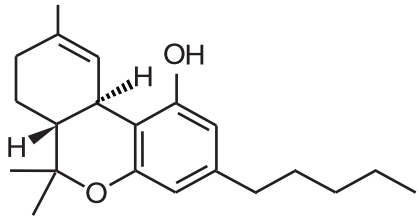


Figure 1: Chemical Structure of Δ9-THC

The methodology presented here focuses on the use of the Thermo Scientific TSQ Quantum XLS for the confirmation and quantitation of THC in an oral fluid matrix. For this assay, THC-D3 was used as the deuterated internal standard. Samples were first extracted using solid phase extraction. After extraction, the samples were derivatized with bis (trimethylsilyl) trifluoroacetamide (BSTFA). The silylated reaction products were analyzed using the TSQ Quantum XLS™ triple stage quadrupole GC-MS/MS system using multiple reaction monitoring (MRM). Known negative oral fluid calibration standard was spiked and extracted at 0.2, 2, and 20 ng/mL for use as calibrators. Using a 200 µL oral fluid sample size reconstituted in 50 µL of toluene after extraction, the resulting method demonstrated precision of 6% or less for the coefficient of variation at QC levels of 0.8 and 2.5 ng/mL. Quantitative accuracy across a range of 0.2 to 20 ng/mL was demonstrated, with the 0.2 ng/mL sample level quantitating within 2% on average from its actual value.

Introduction

Marijuana represents the largest proportion of positive drug screens as reported by one laboratory system.[1] Marijuana is said to produce euphoria and has a sedative effect.[2] When THC enters the body, it is rapidly metabolized to, among others, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, which is the metabolite typically analyzed in urine, blood and hair. However, in saliva there is still a large amount of parent THC remaining, so its presence in oral fluid can be measured as an indication of use. Because THC contains a hydroxyl functional group that does not lend itself well to gas chromatography, samples for THC confirmation are typically derivatized.

The TSQ Quantum XLS triple stage quadrupole was chosen over a single quadrupole due to its ability detect analytes to very low quantitation limits in complex biological matrices. This is accomplished without the need for a multi-dimensional GC approach, which can be difficult to set up and maintain, and can make development of additional assays on the instrument more challenging. The benchtop TSQ Quantum XLS further improves upon the time-tested TSQ 7000 GC-triple quadrupole technology, which represents the standard in MRM confirmatory analyses of drug use. Thermo Scientific ToxLab Forms 2.5.1 software provided automated sample analysis and quantitation, and was used for method validation, including assessments of precision and linearity.

This method describes a productive procedure for high-throughput GC-MS/MS confirmation and quantitation of Δ9-THC in oral fluids. The method utilizes BSTFA for derivatization, which caps labile hydrogens with trimethylsilyl ($-Si(CH_3)_3$) groups, creating the TMS derivative of THC. BSTFA was selected because of its ease of use in derivatization and the high molecular weight of BSTFA derivatized THC, giving intense precursor ions for MRM transitions.

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Methods

To provide a comprehensive view of method development and validation, the details 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 was used due to its ease of use and the cleanliness of the resultant extracts.

Sample Preparation

Negative Calibrator Oral Fluid (Orasure, Bethlehem, PA) was used for sample preparation. A sample size of 200 µL was selected. Calibrators, quality controls, and linearity samples were spiked with appropriate amounts of THC (Cerilliant, Round Rock, TX). Three point calibration at 0.2, 2, 20 ng/mL was used for calculation of all quantitative amounts. A 40% (0.8 ng/mL) and a 125% control (2.5 ng/mL) were prepared from a 20 ng/mL THC working solution. Batches contained a negative control, the three calibrators, and a 40% control and 125% control. THC-D3 (Cerilliant) was used as the deuterated internal standard, and was added to each sample at a final concentration of 2 ng/mL.

Prior to extraction, the samples were brought to an approximate pH of 6 by adding 2 mL of pH 6 phosphate buffer. Each sample was extracted by solid phase extraction on Thermo Scientific HyperSep Verify AX columns (P/N 60108-764). The extraction columns were conditioned with sequential rinses of the following: 2 mL methanol, 2 mL DI water, and 1 mL 0.1 M HCl. Between each conditioning step, the columns were not allowed to dry. The pH-adjusted samples were then loaded onto the column and extracted under low vacuum. 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, and the sample eluents were collected in clean tubes under low vacuum with 3 mL of elution solvent (hexane:ethyl acetate, 75:25 v:v).

200 µL Oral Fluid sample size
Add 2 mL of pH 6 phosphate buffer
Add THC-D3 internal standard
HyperSep™ Verify™ AX columns (p/n 60108-764)
Conditioning
2 mL methanol
2 mL DI water
1 mL 0.1 M HCl
Sample load
Wash
2 mL DI water
2 mL 0.1 M HCl: acetonitrile, (70:30 V:V)
Elution with 3 mL hexane: ethyl acetate (75:25, v:v)
Blown down under nitrogen @ 40 °C
Derivatized with 30 µL BSTFA @ 80 °C for 20 min
Blown down under nitrogen @ 40 °C
Reconstituted with 50 µL toluene

Table 1: Summary of sample preparation steps

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 30 µL of BSTFA at 80 °C for 20 minutes, after which the access BSTFA was evaporated to dryness at 40 °C under nitrogen. For analysis, 50 µL of toluene was added to the derivatized extracts, and the resulting samples were transferred to autosampler vials with glass inserts and loaded onto the Thermo Scientific AS 3000 II autosampler for GC/MS analysis. Table 1 summarizes sample prep, extraction, and derivatization steps.

Instrumental Analysis

The Thermo Scientific TRACE GC Ultra was equipped with a standard split/splitless injector. A 5 mm ID Thermo Scientific deactivated glass liner (p/n 45350033) was used in the injector with a glass wool plug. The split/splitless injector temperature was set to 250 °C. A 2 µL injection volume was programmed on the AS 3000 II autosampler, and a splitless injection was used. The analytical column was a TRACE™ TR-5MS 15 m x 0.25 mm ID x 0.25 µm film (p/n 260F130P), which was installed 64 mm into the injection port (Figure 2).

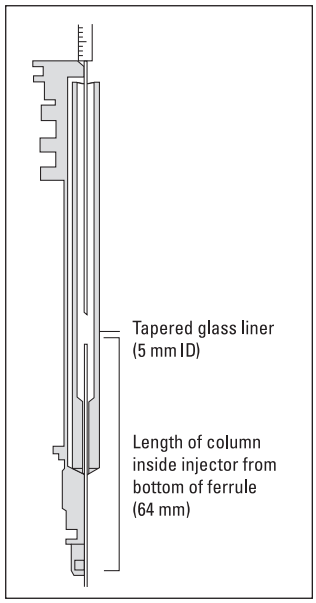


Figure 2: Column installation in GC split/splitless injection port (not to scale)

Carrier gas flow was set to a constant rate of 1.2 mL/min of helium. The initial temperature on the TRACE GC Ultra™ was set to 60 °C. Upon injection of the sample, the oven temperature was immediately ramped at 35 °C/min to a final temperature of 320 °C with no final hold, for a total run time of 7.43 minutes and a THC retention time of 5.77 minutes. The TSQ Quantum XLS source temperature was set to 200 °C, and the mass spectrometer was tuned using default *AutoTune* parameters. These tune settings were used for acquisition, with the default detector gain for the MRM mode left at 2 x 10^6.

For initial mass spectrometer method development, high concentrations of derivatized THC and THC-D3 were injected and analyzed in electron ionization (EI) full scan to determine precursor masses for EI MRM. Methods were then created to measure each precursor ion's product ion scan at various collision energies and collision pressures. From these product ion scans, the most intense ions were selected for each MRM transition at the optimum collision energy and collision cell pressure.

The set of MRM transitions, dwell times, collision energies, and the collision cell pressure used to detect THC and its deuterated internal standard are shown in Table 2. The transition from mass 386 to mass 303 was used as the quantitative transition for THC, with the transition from mass 371 to mass 289 as the confirming transition. For THC-D3 the quantitative and confirming transitions were mass 389 to 306 and mass 374 to 292 respectively. Table 2 summarizes instrument parameters for the method.

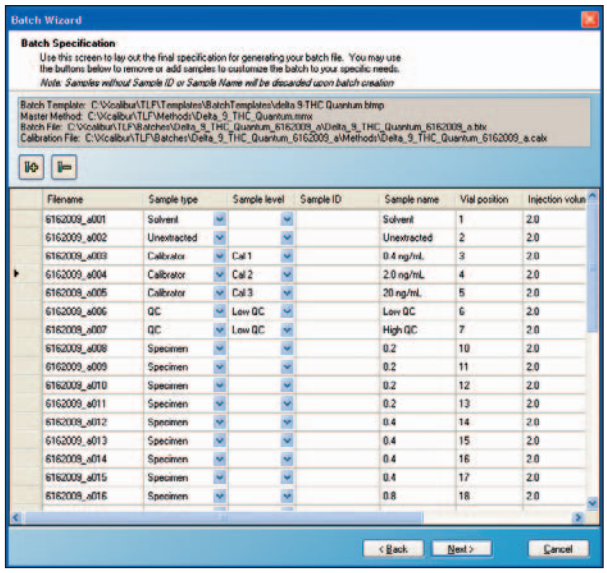


Figure 3: ToxLab Forms 2.5 Batch Wizard

Sample Processing and Result Derivation

For sample acquisition, peak detection and quantitation, ToxLab™ Forms 2.5.1 software was utilized. By incorporating all of the vital components of analysis into a unified workflow oriented application, ToxLab Forms provides an integrated solution to THC GC-MS/MS confirmation. To make use of ToxLab Forms for method validation, a Thermo Scientific Xcalibur instrument method was first created for the mass spectrometer, autosampler, and GC. Next, a Master Method was created within ToxLab Forms, including processing parameters for component identification and quantitation, and QC criteria specific to the method.

Batch creation was performed through the *Batch Wizard* function of ToxLab Forms, 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.

Concentration calculations were based on a three-point calibration at 0.2, 2 and 20 ng/mL, using THC-D3 as the internal standard. 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 and calibration 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 THC less than the method limit of detection (LOD), which in this case was 0.2 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 (0.1 ng/mL) was used as a second

TRACE GC Ultra with AS 3000 II Autosampler

Column	TR-5 MS
SSL injector	250 °C, 2 µL split injection, 1.2 mL/min, 3 kPa surge
Splitless liner	ID 5 mm with glass wool
Oven program	
Start	60 °C, no hold
Ramp1	35 °C/min to 320 °C, no hold
Transfer line	280 °C

TSQ Quantum XLS

EI ionization	Closed Exit EI Ion Volume. 70 eV, 50 µA emission
Tuning	Autotune
Collision energy	25 eV

MRM Transitions Monitored

	Precursor	Product	Width	Time	Collision Energy
THC Quant. Transition	386.24	303.20	0.40	0.40	25
THC Qual. Transition	371.24	289.19	0.40	0.40	25
THC-D3 Quant. Transition	389.26	306.20	0.40	0.40	25
THC-D3 Qual. Transition	374.26	292.19	0.40	0.40	25

Table 2: Instrument method parameters

criterion, and all negative controls were evaluated for compliance to both criteria. Qualitative criteria included ion ratio and retention time target ranges based on an average of the calibrators, along with peak shape considerations. These criteria were applied to all sample types.

Ratios were defined as follows: Ratios were calculated for THC-D3 (292:306) and THC (289:303), and for each ratio, an acceptable range of ± 20% was established. Similarly, the target retention time for THC and THC-D3 was set using a ± 2% retention time window based on an average of the calibrators' retention times. Each validation batch was reviewed for compliance with these criteria, and for a batch to be accepted, it had to comply with all of these QC criteria.

Results

The analysis of THC in oral fluids using the TSQ Quantum XLS system was validated through determination of linear range, carryover, and precision. Three separate batches were prepared and analyzed: one for linearity/carryover and two for precision. 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.

Linear Range Determination

The determination of assay linearity was performed at concentrations across a broad dynamic range. The linearity batch included an unextracted standard, a negative control, the 0.2, 2 and 20 ng/mL calibrator, a 40% control sample (8 ng/mL) and a 125% control sample (2.5 ng/mL). To evaluate method linearity, samples at 0.2, 0.4, 0.8, 2, 4, 8, 20 and 40 ng/mL were prepared and extracted, along with the calibrator and controls. These samples were then injected 4 times each, and the resulting 32 data points were quantified based on the three point calibration. All quantitative values were within ± 20% of their target concentrations, and a least squares fit analysis comparing the average quantitative value for each level to its expected value was found to have a correlation coefficient of 0.997 (Figure 4). The correlation coefficient of the three point calibration used for all quantitation was found to be 1.0000. Chromatography for the quantitation ions and all qualifiers was exceptional, as shown in Figure 5.

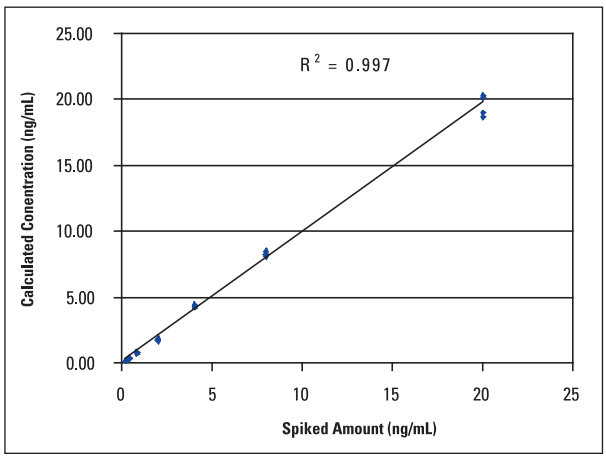


Figure 4: Assay linearity demonstrated between 0.2 and 20 ng/mL THC in oral fluid

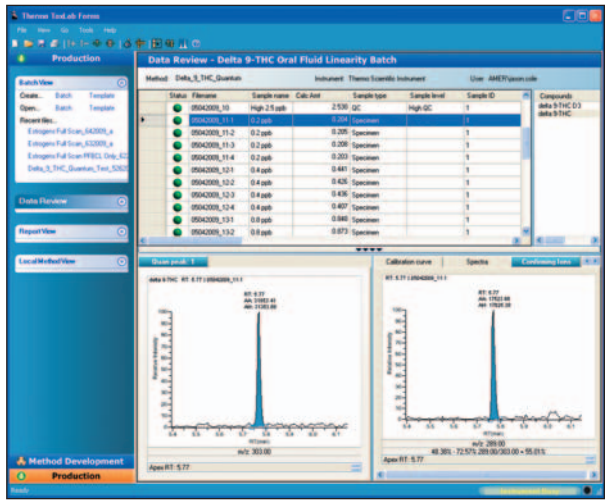


Figure 5: Example of extracted ion profiles of THC quantitative and qualifier ions monitored at 0.2 ng/mL

An additional component of the linearity study was to determine whether carryover was significant over the linearity range for the method. To do so, a negative control was injected following the 40 ng/mL level. This negative was evaluated for acceptability according to the batch criteria described above. Under these constraints, there was no significant carryover even following the four injections of the 40 ng/mL level.

For this method, the LOD and LOQ were both determined to be at 0.2 ng/mL or lower, with each of four injections at 0.2 ng/mL quantitating at less than 5% deviation from the theoretical amount and at an average deviation of 2%. The upper limit of linearity was determined to be 20 ng/mL. All of the 40 ng/mL level calculated just under the ± 20% allowable error for this concentration. However, since this concentration was consistently close to failing the QC criteria for quantitative accuracy, it was decided not to include this in the linear range of the assay because of a high probability of eventual failure. The reason this concentration consistently quantitates near its allowable QC limit is due to contribution of an isotope from the quantitative ion of the analyte to the quantitative ion of the internal standard. Table 3 includes a summary of the linearity/carryover study for THC on the TSQ Quantum XLS.

Expected Concentration (ng/mL)	Average Calculated Concentration (ng/mL)	% Deviation from Actual
0.2	0.21	2%
0.4	0.43	6%
0.8	0.84	4%
2	1.8	-8%
4	4.4	9%
8	8.3	4%
20	20	2%
40	34	-17%
Negative	0	n/a

Table 3: Results of linearity/carryover study. Calculated concentrations representing points on the linearity curve were obtained by averaging four injections made at that concentration.

Concentration	CV for Batch 1	CV for Batch 2	Inter-batch CV
0.8 ng/mL	3%	5%	6%
2.5 ng/mL	5%	3%	4%

Table 4: Results of precision study showing intra-day coefficients of variations less than 6% and inter-day coefficients of variation for interbatch calculated amounts less than 7%

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 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. The method described above provides excellent quantitative precision, with CVs all 6% or less. Also, quantitation of all samples were within 20% of their spiked amount. Table 4 includes a summary of the precision results for parent THC on the TSQ Quantum XLS.

Conclusion

The TSQ Quantum XLS operated in selected reaction monitoring mode proved to be both selective and sensitive enough to routinely measure THC in oral fluid at a 2 ng/mL cutoff level. This was exemplified by the excellent accuracy at the 0.2 ng/mL sample level analyzed during the linearity study, where all four injections at the level quantitated within 5% of the actual amount. The linearity study also demonstrated ample linear range for the assay, determined to be between 0.2 to 20 ng/mL. Across this range, all samples also gave ion ratios which were within 20% of the ion ratios of the calibrator. Furthermore, the intra- and inter-day precision studies showed that the coefficients of variation for the assay at 0.8 and 2.5 ng/mL were well under the 10% value required by many regulatory bodies. Because instrument method development and validation were performed in an extracted oral fluid calibrating solution, the results demonstrate performance of the TSQ Quantum XLS system for method validation as they would be performed within a working laboratory.

The TSQ Quantum XLS was chosen for this assay not only because its performance exceeds that required for the analysis, but also because of its ease of use and speed of analysis relative to alternative approaches. Setup and daily use of this method was as easy as for a typical single quadrupole confirmation method, without requiring a complex multi-dimensional GC approach. Also, the ease of developing an MRM confirmation method on the TSQ Quantum XLS allows the user more flexibility in expanding to other confirmation assays that prove difficult to analyze on a GC single quadrupole instrument. Finally, at a retention time of less than 6 minutes, the methodology described offers a productive means for high-throughput laboratories to confirm and quantitate the use of THC through oral fluid sampling.

Acknowledgement

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References

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2. Baselt, Randell C. *Disposition of Toxic Drugs and Chemicals in Man. Eighth Edition*. Biomedical Publications. Foster City, California. 2008. pp 1513–1518.

Original data acquired using the Thermo Scientific TSQ Quantum GC. Performance of the Thermo Scientific Quantum XLS typically meets or exceeds these results.

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