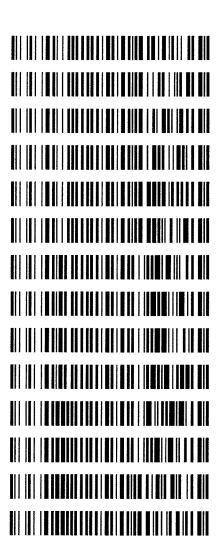


Worklist: 2670

LAB CASE	<u>ITEM</u>	TASK ID	DESCRIPTION
C2018-1575	1	125706	AM 27 Blood THC Quant by LC-QQQ
C2018-1619	1	125707	AM 27 Blood THC Quant by LC-QQQ
C2018-1642	1	125708	AM 27 Blood THC Quant by LC-QQQ
C2018-1715	2	125709	AM 27 Blood THC Quant by LC-QQQ
C2018-1723	1	125710	AM 27 Blood THC Quant by LC-QQQ
C2018-1724	1	125711	AM 27 Blood THC Quant by LC-QQQ
M2018-3766	2	125712	AM 27 Blood THC Quant by LC-QQQ
M2018-3822	5	125713	AM 27 Blood THC Quant by LC-QQQ
M2018-3849	2	125715	AM 27 Blood THC Quant by LC-QQQ
M2018-3871	2	125714	AM 27 Blood THC Quant by LC-QQQ
P2018-2159	1	125719	AM 27 Blood THC Quant by LC-QQQ
P2018-2160	1	125718	AM 27 Blood THC Quant by LC-QQQ
P2018-2218	1	125717	AM 27 Blood THC Quant by LC-QQQ
P2018-2264	1	125716	AM 27 Blood THC Quant by LC-QQQ



# AM# 27: Quantitation of THC and Metabolites in Blood by LC-MS/MS

Extraction Date: 8/28/18 Run Date: 8/29/18 Analyst: Britany Wylie

Plate lot#: 0515037 Plate Expiration: 09/28/2018

Mobile phase A: 0.1% Formic Acid in LCMS Water Mobile phase B: 0.1% Formic acid in Acetonitrile

MTBE LCMS Methanol Hexane

Blank Blood Lot: 18G207D7 Column: UCT Selectra DA 100 x 2.1mm 3um

**LCMS-QQQ ID**: 62340

## **Pre-Analytic:**

- □ 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- ☑ 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

## **Analytic:**

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Pipette 1000μL blood (calibrated pipette) Pipette ID: k52558g in wells of analytical (standards) plate.
- Place on shaking incubator at ambient temp., 900rpm for 15 minutes. Shaker ID: 66759
- 24. Pipette 500μL 0.1% formic acid in water in wells of analytical plate.
- \(\mathbb{P}\) 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- (Σ) Transfer **800μL of blood+acid** mixture to corresponding wells of SLE+ plate.
- 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent). (Load at 85-100 PSI- Selector to the right) Manifold ID: 66792
- №8. Wait 5 minutes.
- 9. Add 2.25mL MTBE. (Add in 3 increments of 750uL)
- 11. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- 12. Add 2.25mL Hexane. (Add in 3 increments of 750uL)
- №13. Wait 5 minutes.
- 2 14. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. SPE Dry ID: 66819
- 16. Reconstitute in 100μL 100% MeOH and heat seal plate with foil. Place in autosampler and run worklist.

#### Post-Analytic

- 1. Create batch and process data.
  - Worklist path: 08292018 Cann quant reinsect Batch Name: 8292018 the
- $\not P$  2. Make any necessary integration changes, Curve weighting of Linear 1/x with  $r^2$  values  $\geq 0.98$  for each analyte
- 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less). Ion ratios must be within +/- 20% of the averaged calibrators
- 4. Case sample response for THC and OH-THC 3ng/mL (quantitative), Carboxy-THC: 10ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
- ( 5. Did all QCs pass for each analyte? Y N
- ∠ 6 Enter QCs into control charting.
- ☑ 7 Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

comments: all cal & samples | ac's were reconstituted & reinjected.

part way through initial run it was noted that there was no internal standard in the sample blank, a new blank was made & the batch rest reinjected.

Batch Data Path D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

 Analysis Time
 9/4/2018 11:39 AM
 Analyst Name
 ISP Tox

 Report Time
 9/4/2018 11:40 AM
 Reporter Name
 ISP Tox

 Last Calib Update
 9/4/2018 11:39 AM
 Batch State
 Processed

**Analysis Info** 

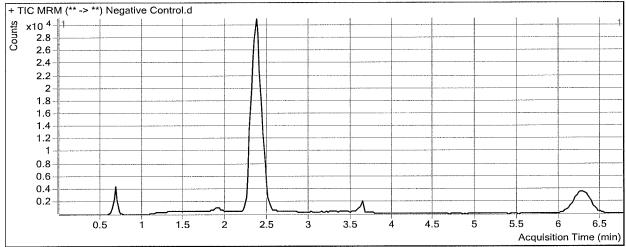
Acq Time2018-08-29 21:38Data FileNegative Control.dSample TypeSampleSample NameNegative Control

Dilution 1 Acq Method AM 27 Quant THC 7-2017.m

**Position** P1-E3 **Sample Info** 

Inj Vol -1 Comment AM 27 Cannabinoid Confirmation

## **Sample Chromatogram**



Results	
---------	--

CompoundISTD CompoundRTResponseISTD RespResp RatioFinal ConcTHC-COOHTHC-COOH-d92.5062803693050.04041.5604

Batch Data Path D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

Analysis Time9/4/2018 11:39 AMAnalyst NameISP ToxReport Time9/4/2018 11:40 AMReporter NameISP ToxLast Calib Update9/4/2018 11:39 AMBatch StateProcessed

**Analysis Info** 

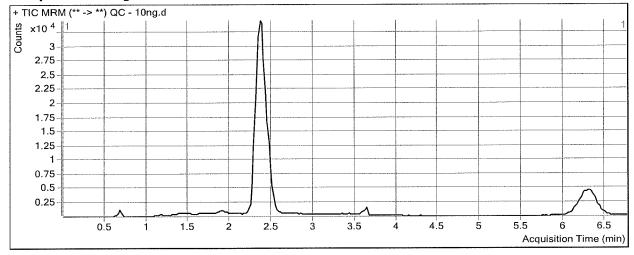
 Acq Time
 2018-08-29 21:50
 Data File
 QC - 10ng.d

 Sample Type
 QC
 Sample Name
 QC - 10ng

Dilution 1 Acq Method AM 27 Quant THC 7-2017.m

**Position** P1-H1 **Sample Info** 

Inj Vol -1 Comment AM 27 Cannabinoid Confirmation



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.396	19379	199900	0.0969	9.7981
THC-COOH	THC-COOH-d9	2.466	15531	69452	0.2236	10.1409
THC	THC-d3	6.353	6797	59684	0.1139	10.0453



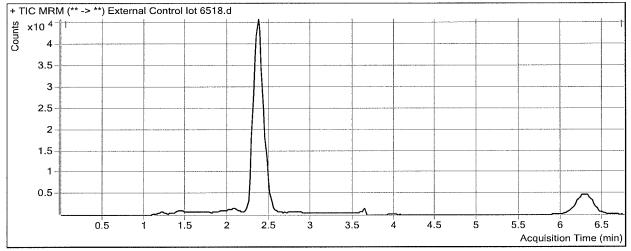
Batch Data Path D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

Analysis Time9/4/2018 11:39 AMAnalyst NameISP ToxReport Time9/4/2018 11:40 AMReporter NameISP ToxLast Calib Update9/4/2018 11:39 AMBatch StateProcessed

**Analysis Info** 

Acq Time2018-08-29 22:13Data FileExternal Control lot 6518.dSample TypeSampleSample NameExternal Control lot 6518Dilution1Acq MethodAM 27 Quant THC 7-2017.mPositionP1-H3Sample Info

Inj Vol -1 Comment AM 27 Cannabinoid Confirmation



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	15680	276891	0.0566	5.9715
THC-COOH	THC-COOH-d9	2.486	10854	84077	0.1291	5.7132
THC	THC-d3	6.313	5183	65356	0.0793	7.0550



# ISP Forensics Calibration Curve Report

**Batch Data Path** 

D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

**Last Calib Update** 

9/4/2018 11:39 AM

**Analyst Name** 

160

180

200

220

240

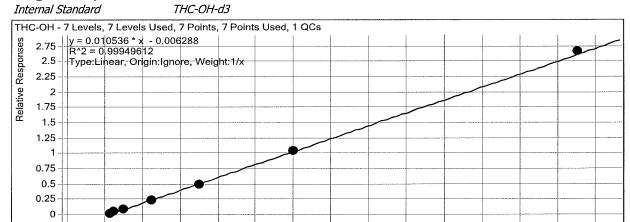
Concentration (ng/ml)

260

ISP TOX

Target Compound

THC-OH



120

140

Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	$\square$	3	3.2	106.2
Cal 2 - 5ng	2	$\square$	5	5.1	101.9
Cal 3 - 10ng	3	$\square$	10	10.0	99.6
QC - 10ng	3	$\square$	10	9.8	98.0
Cal 4 - 25ng	4	$\square$	25	23.8	95.2
Cal 5 - 50ng	5	$\square$	50	48.3	96.6
Cal 6 - 100ng	6	$\square$	100	99.0	99.0
Cal 7 - 250ng	7	$\square$	250	253.6	101.4

60

40

80

100



# ISP Forensics Calibration Curve Report

**Batch Data Path** 

D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

**Last Calib Update** 

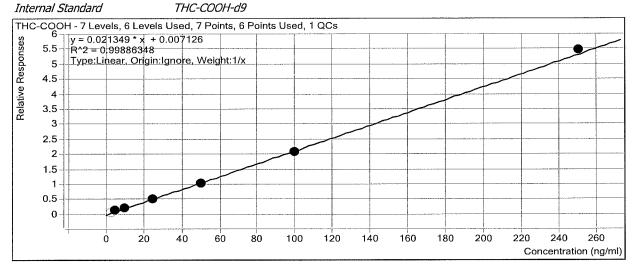
9/4/2018 11:39 AM

**Analyst Name** 

ISP TOX

Target Compound

THC-COOH



Sample	Levei	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1		3	3.4	114.2
Cal 2 - 5ng	2	$\square$	5	5.5	110.9
Cal 3 - 10ng	3	$\square$	10	9.8	97.9
QC - 10ng	3	$\square$	10	10.1	101.4
Cal 4 - 25ng	4	$\square$	25	23.7	95.0
Cal 5 - 50ng	5	$\square$	50	48.6	97.2
Cal 6 - 100ng	6	☑	100	96.8	96.8
Cal 7 - 250ng	7	$\square$	250	255.5	102.2



# **ISP Forensics Calibration Curve Report**

**Batch Data Path** 

D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

**Last Calib Update** 

9/4/2018 11:39 AM

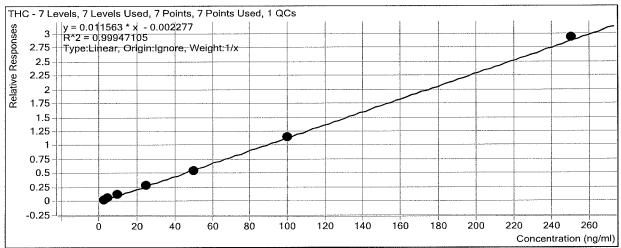
**Analyst Name** 

ISP TOX

Target Compound

THC THC-d3

Internal Standard



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.0	100.8
Cal 2 - 5ng	2	☑	5	5.2	103.6
Cal 3 - 10ng	3	☑	10	10.2	102.4
QC - 10ng	3	☑	10	10.0	100.5
Cal 4 - 25ng	4	☑	25	24.3	97.4
Cal 5 - 50ng	5	$\square$	50	47.7	95.4
Cal 6 - 100ng	6	☑	100	98.9	98.9
Cal 7 - 250ng	7	☑	250	253.6	101.4



Batch Data Path D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

Analysis Time9/4/2018 11:39 AMAnalyst NameISP ToxReport Time9/4/2018 11:40 AMReporter NameISP ToxLast Calib Update9/4/2018 11:39 AMBatch StateProcessed

**Analysis Info** 

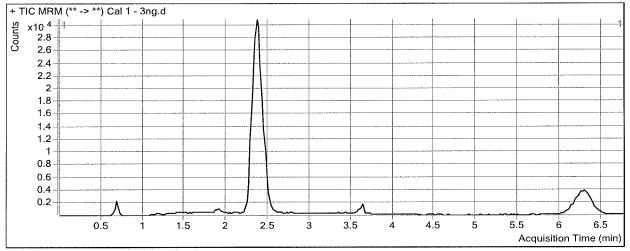
 Acq Time
 2018-08-29 20:03
 Data File
 Cal 1 - 3ng.d

 Sample Type
 Calibration
 Sample Name
 Cal 1 - 3ng

**Dilution** 1 **Acq Method** AM 27 Quant THC 7-2017.m

Position P1-A1 Sample Info

Inj Vol -1 Comment AM 27 Cannabinoid Confirmation



Resu	ılts
------	------

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	5083	186422	0.0273	3.1846
THC-COOH	THC-COOH-d9	2.486	5445	67827	0.0803	3.4267
THC	THC-d3	6.293	1764	53974	0.0327	3.0238



Batch Data Path D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

Analysis Time9/4/2018 11:39 AMAnalyst NameISP ToxReport Time9/4/2018 11:40 AMReporter NameISP ToxLast Calib Update9/4/2018 11:39 AMBatch StateProcessed

**Analysis Info** 

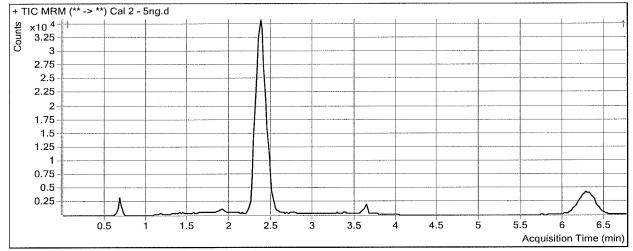
 Acq Time
 2018-08-29 20:15
 Data File
 Cal 2 - 5ng.d

 Sample Type
 Calibration
 Sample Name
 Cal 2 - 5ng

**Dilution** 1 **Acq Method** AM 27 Quant THC 7-2017.m

**Position** P1-B1 **Sample Info** 

Inj Vol -1 Comment AM 27 Cannabinoid Confirmation



R	es	u	lts

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	10097	213087	0.0474	5.0942
THC-COOH	THC-COOH-d9	2.486	9636	76808	0.1255	5.5427
THC	THC-d3	6.313	3368	58437	0.0576	5.1817



Batch Data Path D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

Analysis Time9/4/2018 11:39 AMAnalyst NameISP ToxReport Time9/4/2018 11:40 AMReporter NameISP ToxLast Calib Update9/4/2018 11:39 AMBatch StateProcessed

**Analysis Info** 

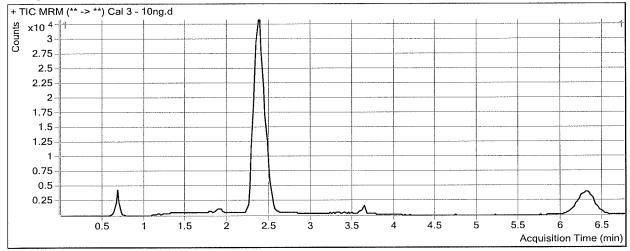
 Acq Time
 2018-08-29 20:27
 Data File
 Cal 3 - 10ng.d

 Sample Type
 Calibration
 Sample Name
 Cal 3 - 10ng

**Dilution** 1 **Acq Method** AM 27 Quant THC 7-2017.m

**Position** P1-C1 **Sample Info** 

Inj Vol -1 Comment AM 27 Cannabinoid Confirmation



R	es	ul	ts

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.396	18945	191950	0.0987	9.9645
THC-COOH	THC-COOH-d9	2.486	14978	69277	0.2162	9.7935
THC	THC-d3	6.333	5960	51303	0.1162	10.2434



D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin **Batch Data Path** 

**Analyst Name** ISP Tox **Analysis Time** 9/4/2018 11:39 AM 9/4/2018 11:40 AM **Report Time** Reporter Name ISP Tox **Batch State** Processed **Last Calib Update** 9/4/2018 11:39 AM

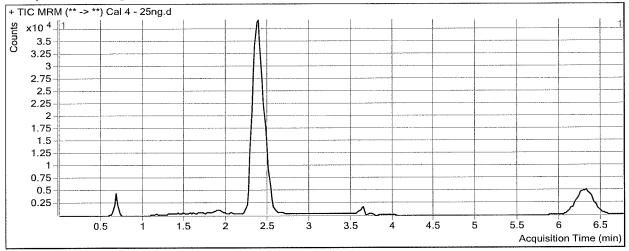
**Analysis Info** 

**Acq Time** 2018-08-29 20:39 **Data File** Cal 4 - 25ng.d Sample Type Calibration Sample Name Cal 4 - 25ng

AM 27 Quant THC 7-2017.m Acq Method Dilution 1

P1-D1 Sample Info **Position** 

Comment AM 27 Cannabinoid Confirmation Inj Vol -1



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	48677	199111	0.2445	23.8003
THC-COOH	THC-COOH-d9	2.486	36531	71053	0.5141	23.7486
THC	THC-d3	6,313	15480	55448	0.2792	24.3410



**Batch Data Path** 

D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

**Analysis Time** Report Time

9/4/2018 11:39 AM 9/4/2018 11:40 AM Analyst Name ISP Tox Reporter Name ISP Tox

**Last Calib Update** 

9/4/2018 11:39 AM

**Batch State** Processed

**Analysis Info** 

**Acq Time** 

2018-08-29 20:50

**Data File** 

Cal 5 - 50ng.d

Sample Type Dilution

Calibration

Sample Name

Cal 5 - 50ng

**Position** 

P1-E1

**Acq Method** 

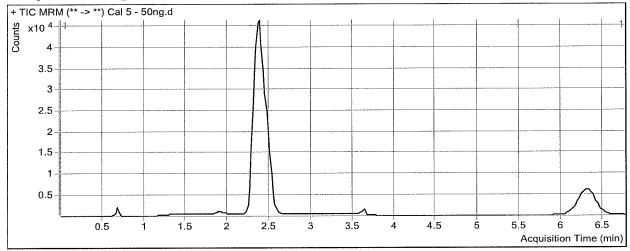
AM 27 Quant THC 7-2017.m

Inj Vol

-1

Sample Info Comment

AM 27 Cannabinoid Confirmation



Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	97641	194186	0.5028	48.3206
THC-COOH	THC-COOH-d9	2.486	71868	68763	1.0451	48.6217
THC	THC-d3	6.313	29596	53868	0.5494	47.7106



Batch Data Path D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

Analysis Time9/4/2018 11:39 AMAnalyst NameISP ToxReport Time9/4/2018 11:40 AMReporter NameISP ToxLast Calib Update9/4/2018 11:39 AMBatch StateProcessed

**Analysis Info** 

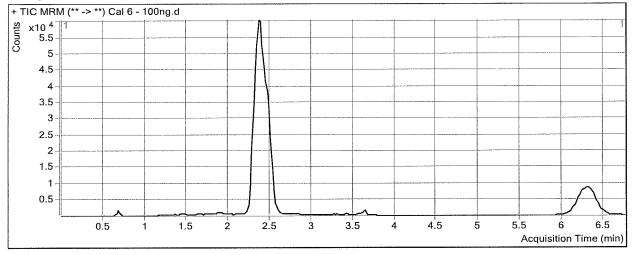
 Acq Time
 2018-08-29 21:02
 Data File
 Cal 6 - 100ng.d

 Sample Type
 Calibration
 Sample Name
 Cal 6 - 100ng

**Dilution** 1 **Acq Method** AM 27 Quant THC 7-2017.m

**Position** P1-F1 **Sample Info** 

Inj Vol -1 Comment AM 27 Cannabinoid Confirmation



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	190547	183722	1.0371	99.0350
THC-COOH	THC-COOH-d9	2.466	132151	63753	2.0729	96.7612
THC	THC-d3	6.313	60721	53191	1.1416	98.9211



Batch Data Path D:\2018 Data\08292018 cann quant reinject\QuantResults\8292018 the.batch.bin

 Analysis Time
 9/4/2018 11:39 AM
 Analyst Name
 ISP Tox

 Report Time
 9/4/2018 11:40 AM
 Reporter Name
 ISP Tox

 Last Calib Update
 9/4/2018 11:39 AM
 Batch State
 Processed

**Analysis Info** 

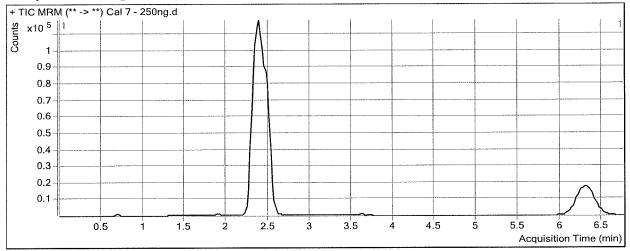
 Acq Time
 2018-08-29 21:14
 Data File
 Cal 7 - 250ng.d

 Sample Type
 Calibration
 Sample Name
 Cal 7 - 250ng

**Dilution** 1 **Acq Method** AM 27 Quant THC 7-2017.m

**Position** P1-G1 **Sample Info** 

Inj Vol -1 Comment AM 27 Cannabinoid Confirmation



Results						
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	539156	202260	2.6657	253.6007
THC-COOH	THC-COOH-d9	2.486	354221	64846	5.4625	255.5323
THC	THC-d3	6.333	162158	55346	2.9299	253.5784



#### Request for Departure from an Analytical Method

Date of Request

9/4/18

Forensic Scientist

Britany Wylie

#### Analytical Method

Toxicology AM #27: Quantitative Analysis of THC and Metabolites in Blood by LCMS-QQQ

4.3.2.4 For calibrators and controls 10 ng and below the accuracy must be within 30%, for calibrators and controls greater than 10 ng/mL the accuracy must be within 20%. If the control over 10 ng falls outside the accuracy range at the analyst's discretion the compound may be reported qualitatively.

## Request

I am requesting a deviation to report the results for THC-OH from worklist 2670 qualitatively due to the external control concentrations of THC being outside the accuracy range of 30% with a value of 5.97 ng/ml.

Discipline Leader Review		
Departure approved Comments:		
Departure Not Approved Comments:		

Date: 09/06/2018

Celena Shrum Toxicology Discipline Lead

Celena Shrum