Worklist: 1805

LAB CASE	ITEM	TASK ID	DESCRIPTION	
C2017-1238	1	89728	AM 27 Blood THC Quant by LC	
C2017-1263	1	89729	AM 27 Blood THC Quant by LC	
C2017-1309	1	89730	AM 27 Blood THC Quant by LC	
C2017-1313	1	89731	AM 27 Blood THC Quant by LC	
M2017-2821	2	89732	AM 27 Blood THC Quant by LC	
M2017-3046	2	89733	AM 27 Blood THC Quant by LC	

Quantitation of THC and Metabolites in Blood by LC-MS/MS

Extraction Date: 7-11-17   **Analyst:** Anne Nord

PRE-ANALYTIC

Plate Lot# Custom - 0490364   Plate Exp.   9-21-2017   **External QC** Lot 91317, exp 9-13-17

✓ 1. Ensure all solutions are within expiration date.

- Mobile Phase A: *0.1% Formic Acid in LCMS Water*
- Mobile Phase B: *0.1% Formic Acid in LCMS Acetonitrile*
- *LCMS Methanol*
- **Blank/Negative Blood: Lot *321632-1***

- *0.1% Formic Acid in water*
- *MTBE*
- *Hexane*

Column: *UCT Selectra DA 100 x 2.1 mm 3um*

✓ 2. Check levels of mobile phases and needle wash and refill as necessary. Ensure waste is not full.

✓ 3. Purge Pump and Load appropriate Acq. Method, allow system to equilibrate for approx. 30 min.

✓ 4. Create worklist. Data path name: 7/11/17 THC Quant

ANALYTIC

✓ 1. Remove standards plate, blood, and samples from cold storage. Allow to reach room temperature.

✓ 2. Add *1000 μL blood* to wells of analytical (standards) plate. Place cover on Plate
   Blank blood for locations containing standards/QCs and internal standards
   Sample blood for locations containing only internal standards

✓ 3. Place on shaking incubator at ambient temp., **900rpm for 15 minutes**. *Shaker ID 66759*

✓ 4. Pipette *500μL 0.1% formic acid* to all wells of standards plate.

✓ 5. Place on shaking incubator at ambient temp., **900rpm for 15 minutes**.

✓ 6. 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). Wait 5 min.
   (*Load blood samples at 85- 100 PSI- Selector to Right*)

✓ 8. Add *2.25mL MTBE* and allow to flow under gravity for 5 minutes. *(add in 3 increments of 750uL)*

✓ 9. Apply positive pressure for approx. 15 seconds *(10-15 PSI- Selector to left -.*

✓ 10. Add *2.25mL Hexane* and allow to flow under gravity for 5 minutes. . *(add in 3 increments of 750uL)*

✓ 11. Apply positive pressure for approx. 15 seconds. *(10-15 PSI Selector to the left)*

✓ 12. Remove collection plate containing eluate.

✓ 13. Place collection plate on SPE Dry and evaporate to dryness at approx. 35°C. *SPE Dry ID 66819*

✓ 14. Reconstitute in *100 μL MeOH* and heat seal plate with foil. Place in autosampler and run worklist.

POST-ANALYTIC

✓ 1. Open quantitation software and create a new quantitation batch.
   Batch name: 7/11/7 cann quant

✓ 2. Make any necessary integration changes. Limit curves based on validated linear ranges (3-50ng/mL).

✓ 3. Were all appropriate standards used in the curve for each analyte? (Y)/ N
   Are r² values ≥0.98 for each analyte? (Y) / N

✓ 4. Did all QCs pass for each analyte? (Y) / N   Were QCs entered into QC charting? (Y)/ N

✓ 5. Central File Packet to include: ✓ LIMS Worklist: ✓ Method Checklist ✓ Calibration and
   Control Reports

COMMENTS

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 12:12	**Data File**	Neg Control.d
Sample Type	Sample	**Sample Name**	Neg Control
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-a2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
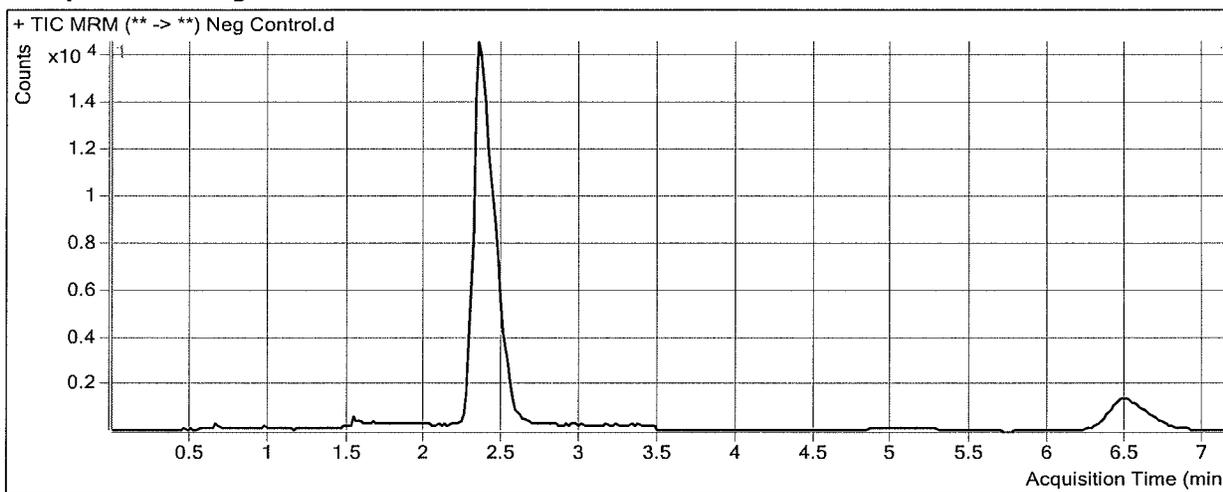
Sample Chromatogram

Samples Report Cannabinoids.xlsx

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 12:24	**Data File**	QC 10.d
Sample Type	QC	**Sample Name**	QC 10
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-H1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
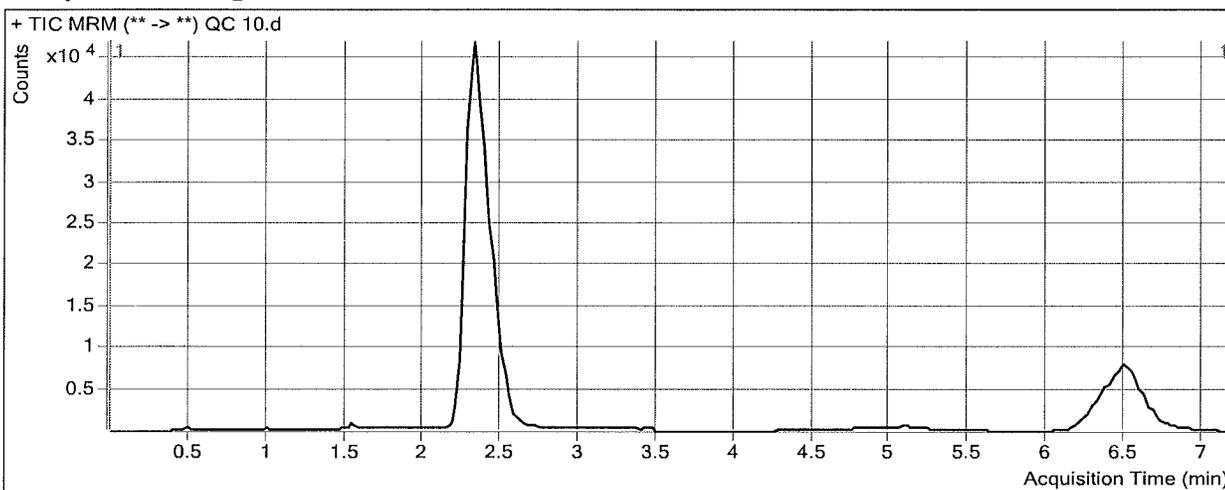
Sample Chromatogram

+ TIC MRM (** -> **) QC 10.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.336	28585	342610	0.0834	9.6590
THC-COOH	THC-COOH-d9	2.486	20666	104303	0.1981	10.0967
THC	THC-d3	6.493	16165	132360	0.1221	10.4661

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 12:36	**Data File**	External control lot 91317.d
Sample Type	Sample	**Sample Name**	External control lot 91317
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	p2b2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation 10 ng

Sample Chromatogram
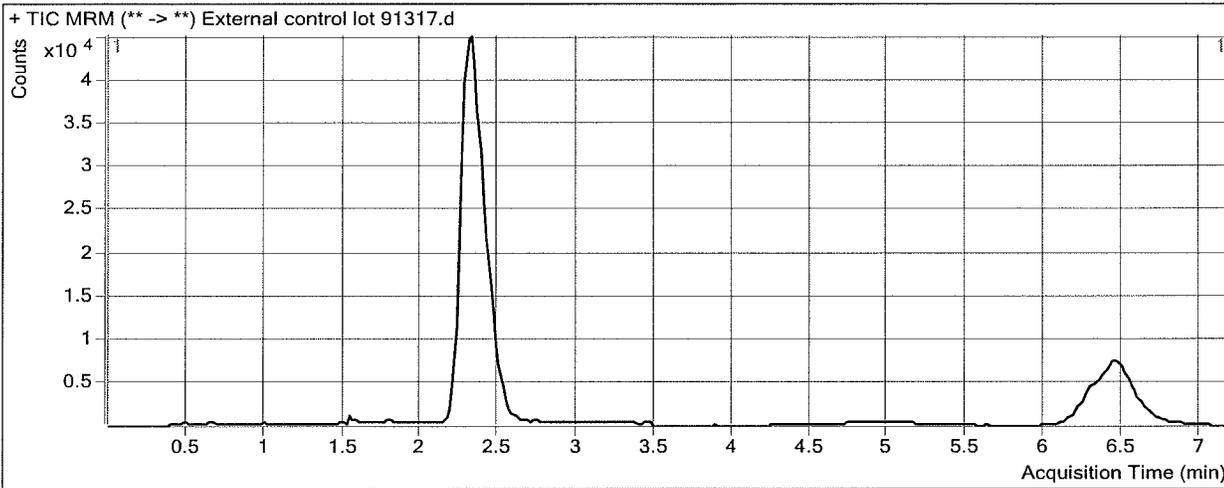
+ TIC MRM (** -> **) External control lot 91317.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.336	29795	337049	0.0884	10.2285
THC-COOH	THC-COOH-d9	2.466	16324	102008	0.1600	7.9902
THC	THC-d3	6.433	11786	103655	0.1137	9.7437

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 10:37	**Data File**	Cal 1- 3ng.d
Sample Type	Calibration	**Sample Name**	Cal 1- 3ng
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-A1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
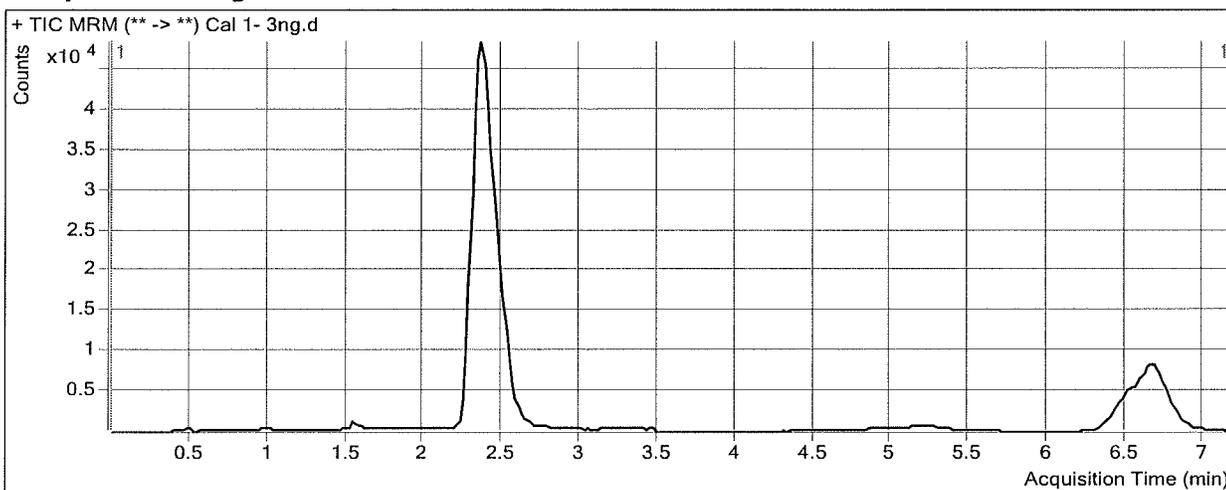
Sample Chromatogram

+ TIC MRM (** -> **) Cal 1- 3ng.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	9682	385914	0.0251	2.9716
THC-COOH	THC-COOH-d9	2.526	7975	116503	0.0685	2.9281
THC	THC-d3	6.693	4834	149246	0.0324	2.7696

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 10:49	**Data File**	cal 2- 5ng.d
Sample Type	Calibration	**Sample Name**	cal 2- 5ng
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-B1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
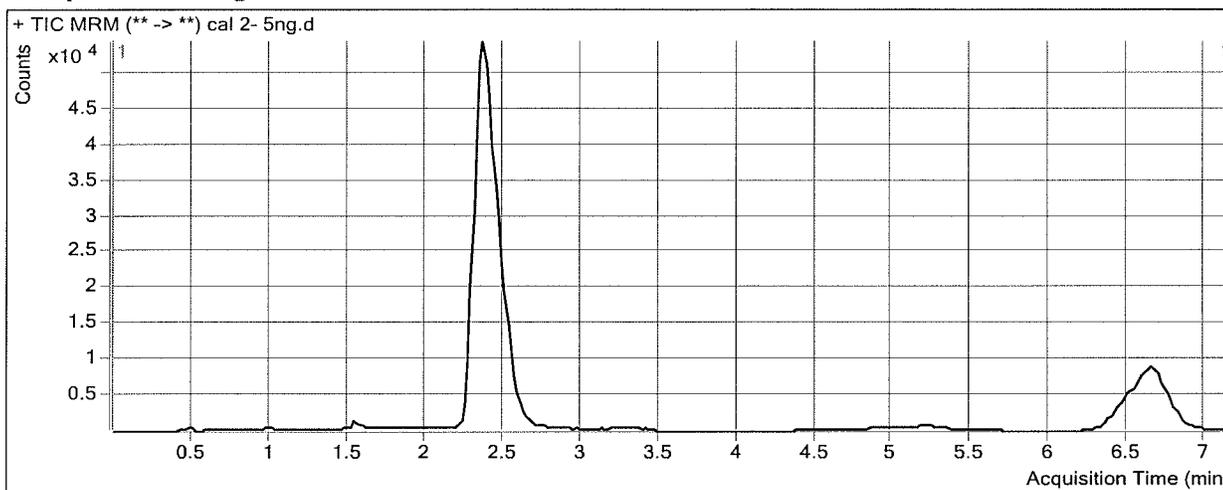
Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.396	17973	432274	0.0416	4.8616
THC-COOH	THC-COOH-d9	2.526	12906	123475	0.1045	4.9220
THC	THC-d3	6.653	9166	154997	0.0591	5.0633

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 11:01	**Data File**	cal 3 - 10ng.d
Sample Type	Calibration	**Sample Name**	cal 3 - 10ng
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-C1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
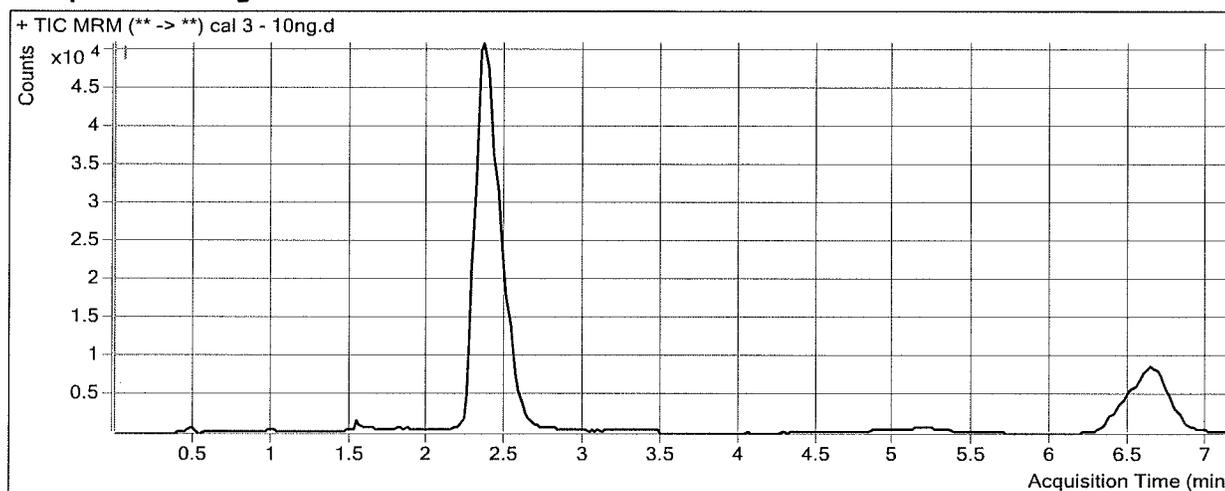
Sample Chromatogram

+ TIC MRM (** -> **) cal 3 - 10ng.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	32754	385051	0.0851	9.8462
THC-COOH	THC-COOH-d9	2.506	22399	113451	0.1974	10.0579
THC	THC-d3	6.633	16861	143263	0.1177	10.0855

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 11:13	**Data File**	cal 4 - 25ng.d
Sample Type	Calibration	**Sample Name**	cal 4 - 25ng
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-D1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
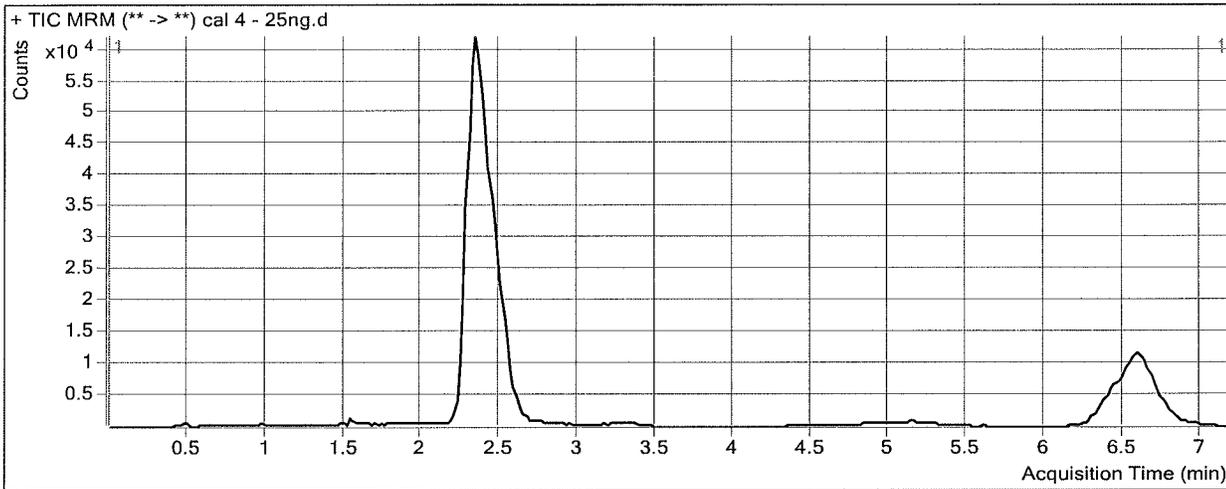
Sample Chromatogram

+ TIC MRM (** -> **) cal 4 - 25ng.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.356	88638	412568	0.2148	24.7218
THC-COOH	THC-COOH-d9	2.506	57270	124327	0.4606	24.6076
THC	THC-d3	6.593	46882	159796	0.2934	25.1549

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 11:25	**Data File**	cal 5 - 50ng.d
Sample Type	Calibration	**Sample Name**	cal 5 - 50ng
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-E1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
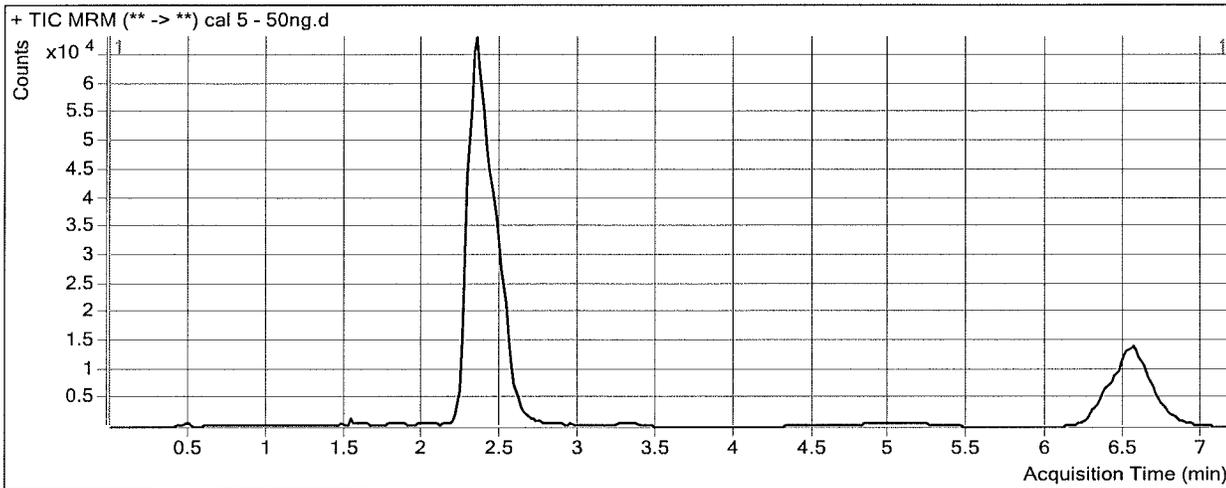
Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.356	175095	364386	0.4805	55.1743
THC-COOH	THC-COOH-d9	2.486	111217	109721	1.0136	55.1760
THC	THC-d3	6.553	89864	140601	0.6391	54.8099

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-12 11:37	**Data File**	cal 6 - 100ng.d
Sample Type	Calibration	**Sample Name**	cal 6 - 100ng
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-F1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
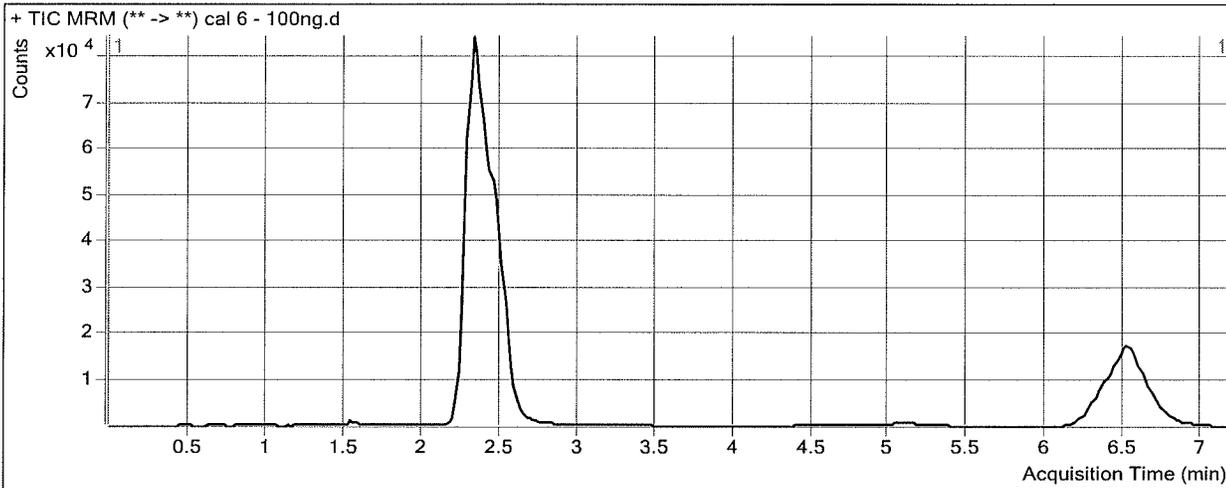
Sample Chromatogram

+ TIC MRM (** -> **) cal 6 - 100ng.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.336	300287	357366	0.8403	96.4102
THC-COOH	THC-COOH-d9	2.466	181663	105732	1.7181	94.1206
THC	THC-d3	6.513	148401	133329	1.1130	95.4556

Batch Data Path	D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin		
Analysis Time	7/12/2017 3:38 PM	**Analyst Name**	ISP Tox
Report Time	7/12/2017 3:40 PM	**Reporter Name**	ISP Tox
Last Calib Update	7/12/2017 3:38 PM	**Batch State**	Processed

Analysis Info

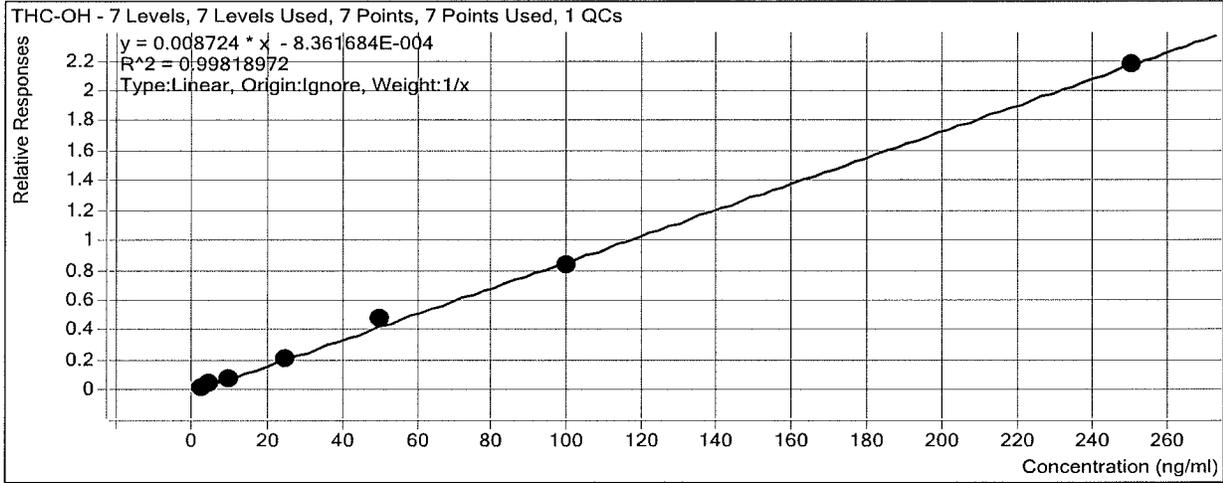|			
Acq Time	2017-07-12 11:48	**Data File**	cal 7 - 250ng.d
Sample Type	Calibration	**Sample Name**	cal 7 - 250ng
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-G1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
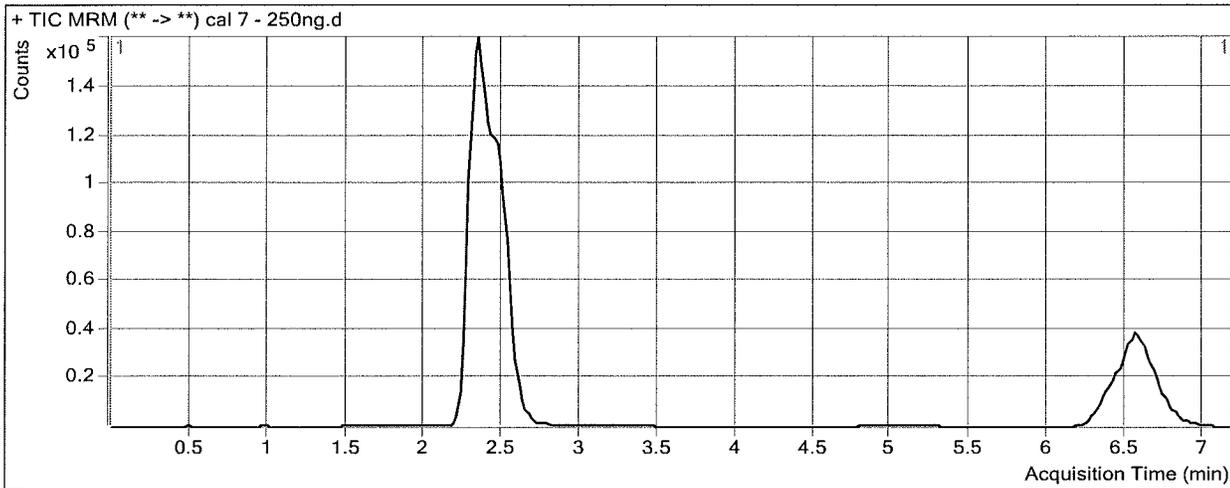
Sample Chromatogram

+ TIC MRM (** -> **) cal 7 - 250ng.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.356	859525	395794	2.1716	249.0144
THC-COOH	THC-COOH-d9	2.486	508829	111597	4.5595	251.1878
THC	THC-d3	6.553	427941	147010	2.9110	249.6612

ISP Forensics
Calibration Curve Report

Batch Data Path   D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin

Last Calib Update   7/12/2017 3:38 PM   **Analyst Name**   ISP TOX

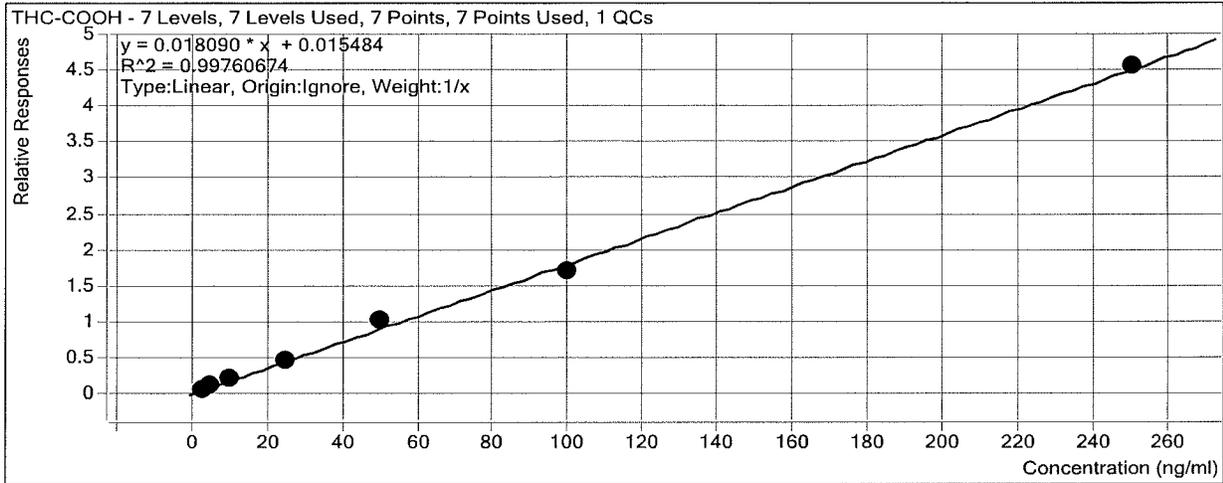## Target Compound   *THC-OH*
Internal Standard   *THC-OH-d3*

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs

$y = 0.008724 * x - 8.361684E-004$
$R^2 = 0.99818972$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1- 3ng	1	☑	3	3.0	99.1
cal 2- 5ng	2	☑	5	4.9	97.2
cal 3 - 10ng	3	☑	10	9.8	98.5
QC 10	3	☑	10	9.7	96.6
cal 4 - 25ng	4	☑	25	24.7	98.9
cal 5 - 50ng	5	☑	50	55.2	110.3
cal 6 - 100ng	6	☑	100	96.4	96.4
cal 7 - 250ng	7	☑	250	249.0	99.6

ISP Forensics
Calibration Curve Report

Batch Data Path   D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin

Last Calib Update   7/12/2017 3:38 PM   **Analyst Name**   ISP TOX

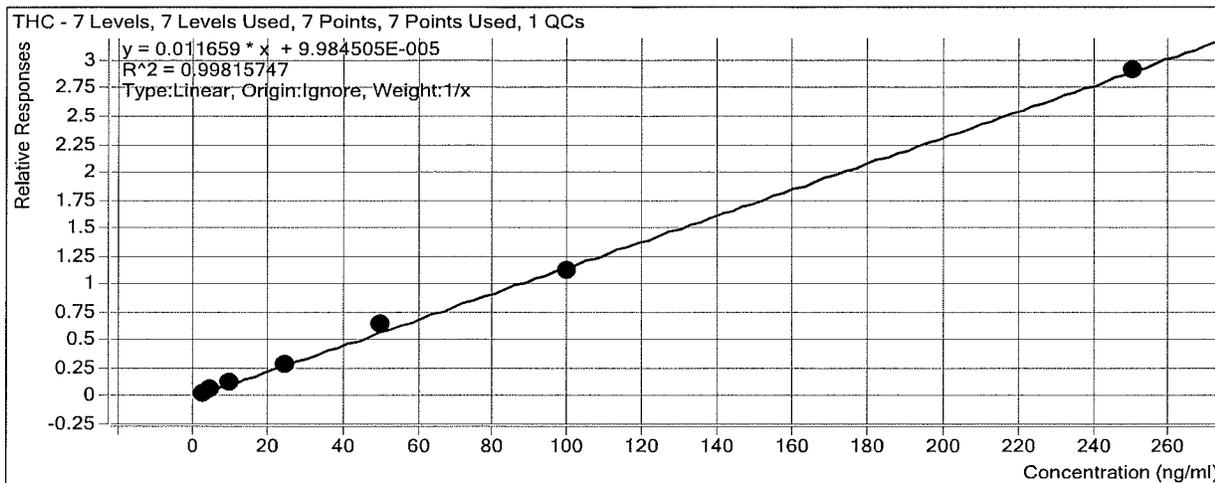*Target Compound*   *THC-COOH*
Internal Standard   *THC-COOH-d9*

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs

$y = 0.018090 * x + 0.015484$
$R^2 = 0.99760674$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1- 3ng	1	☑	3	2.9	97.6
cal 2- 5ng	2	☑	5	4.9	98.4
cal 3 - 10ng	3	☑	10	10.1	100.6
QC 10	3	☑	10	10.1	101.0
cal 4 - 25ng	4	☑	25	24.6	98.4
cal 5 - 50ng	5	☑	50	55.2	110.4
cal 6 - 100ng	6	☑	100	94.1	94.1
cal 7 - 250ng	7	☑	250	251.2	100.5

ISP Forensics
Calibration Curve Report

Batch Data Path   D:\2017 Data\71117 THC Quant\QuantResults\71117 cann quant.batch.bin

Last Calib Update   7/12/2017 3:38 PM   **Analyst Name**   ISP TOX

Target Compound   *THC*
Internal Standard   *THC-d3*

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs

$y = 0.011659 * x + 9.984505E\text{-}005$
$R^2 = 0.99815747$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1- 3ng	1	☑	3	2.8	92.3
cal 2- 5ng	2	☑	5	5.1	101.3
cal 3 - 10ng	3	☑	10	10.1	100.9
QC 10	3	☑	10	10.5	104.7
cal 4 - 25ng	4	☑	25	25.2	100.6
cal 5 - 50ng	5	☑	50	54.8	109.6
cal 6 - 100ng	6	☑	100	95.5	95.5
cal 7 - 250ng	7	☑	250	249.7	99.9