reviewed 7/23/18 BAW

Worklist: 2585

LAB CASE	ITEM	TASK ID	DESCRIPTION
C2018-1243	1	121980	AM 27 Blood THC Quant by LC-QQQ
C2018-1299	1	121981	AM 27 Blood THC Quant by LC-QQQ
C2018-1305	1	121982	AM 27 Blood THC Quant by LC-QQQ
C2018-1329	1	121983	AM 27 Blood THC Quant by LC-QQQ
C2018-1339	2	121984	AM 27 Blood THC Quant by LC-QQQ
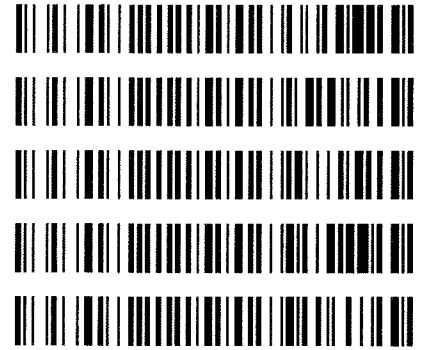
AM# 27: Quantitation of THC and Metabolites in Blood by LC-MS/MS

Extraction Date: _7-19-18_   Analyst: _Anne Nord_

Plate lot#: 0515037   Plate Expiration: 9/28/2018

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: 17J0718   **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.
- ☒ 3. Create worklist:

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.
- ☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- ☒ 4. Pipette **500µL 0.1% formic acid in water** in wells of analytical 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). *(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)*
- ☒ 10. Wait 5 minutes.
- ☒ 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.
- ☒ 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: _O7/19 2018 Cann quant_   Batch Name: _Cann quant_
- ☒ 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥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:

Toxicology AM method 27 external prep information

working solution 1 ug/ml in meoh C-THC, THC-OH, THC

Stock solution 1mg/ml 10 ul each THC, THC-OH 100 ug/ml 100 ul C-THC in 9890 ul meOH

Ppd 6/5/18 Exp: 4/1/19 lot 6518   by AMN

Drug	lot (cerilliant)	expiration
C-THC	FE03121501	3/1/2020
THC-OH	FE01141502	1/1/2020
THC	FE04231406	4/1/2019

AM 27 control 50 ul working solution lot (6518) in 4950 ul blood lot (17J20718)

ppd 6/5/18 Exp 4/1/19   lot 6518   Concentration 10 ng/ml each   by AMN
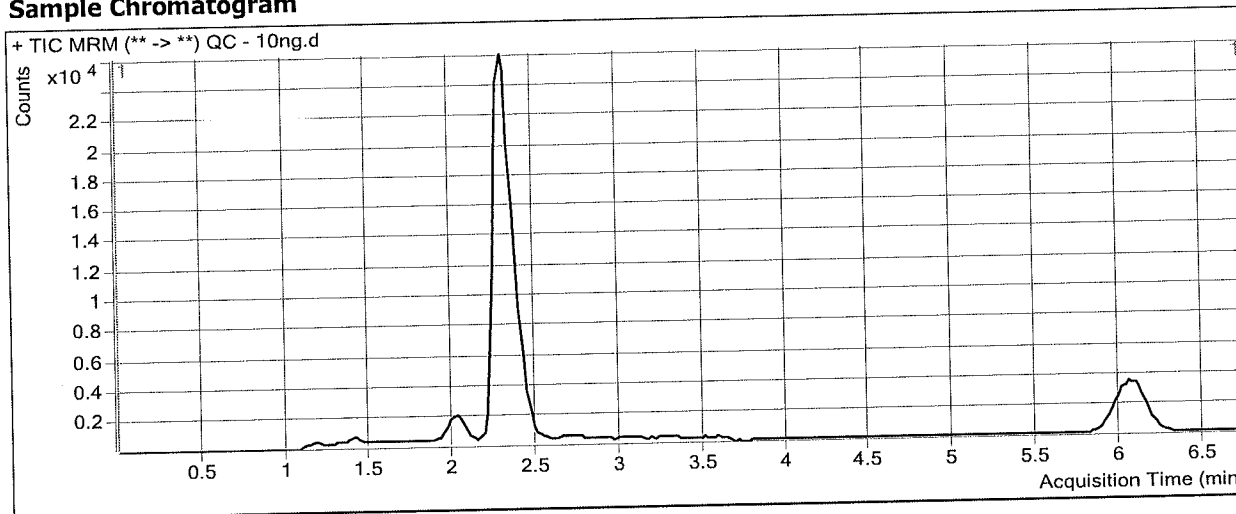
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 18:14	**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

Sample Chromatogram

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

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	14831	138468	0.1071	9.9669
THC-COOH	THC-COOH-d9	2.406	9534	47638	0.2001	9.9725
THC	THC-d3	6.093	4586	38796	0.1182	10.2144

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 18:03	**Data File**	Negative Control.d
Sample Type	Sample	**Sample Name**	Negative Control
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-A2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 Cannabinoid Confirmation
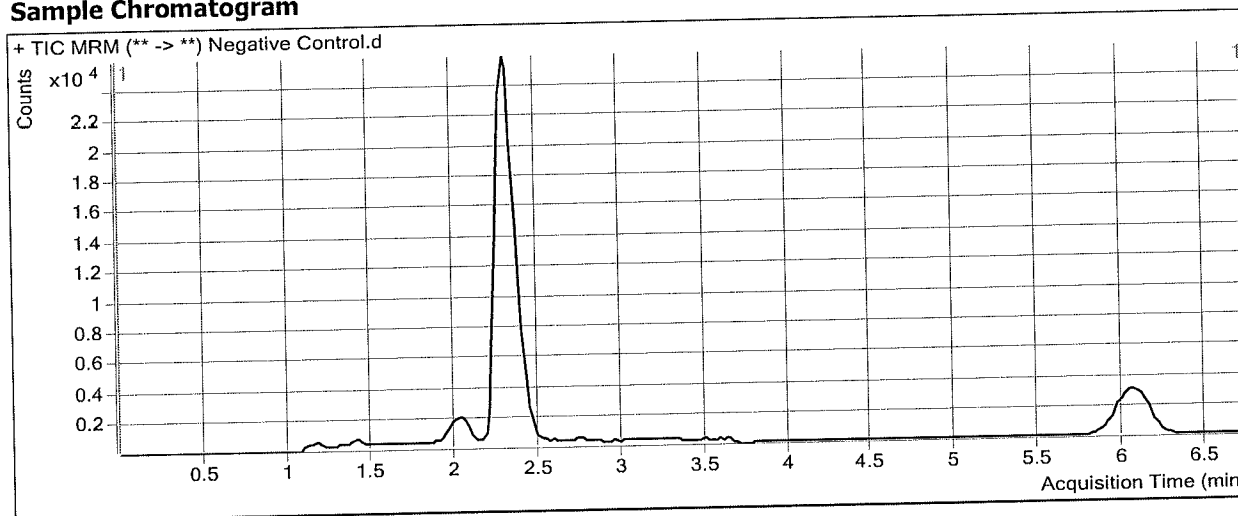
Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.416	1270	156370	0.0081	0.2373
THC-COOH	THC-COOH-d9	2.065	1083	53403	0.0203	0.6075

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

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 18:26	**Data File**	External Control lot 6518.d
Sample Type	Sample	**Sample Name**	External Control lot 6518
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-B2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 Cannabinoid Confirmation
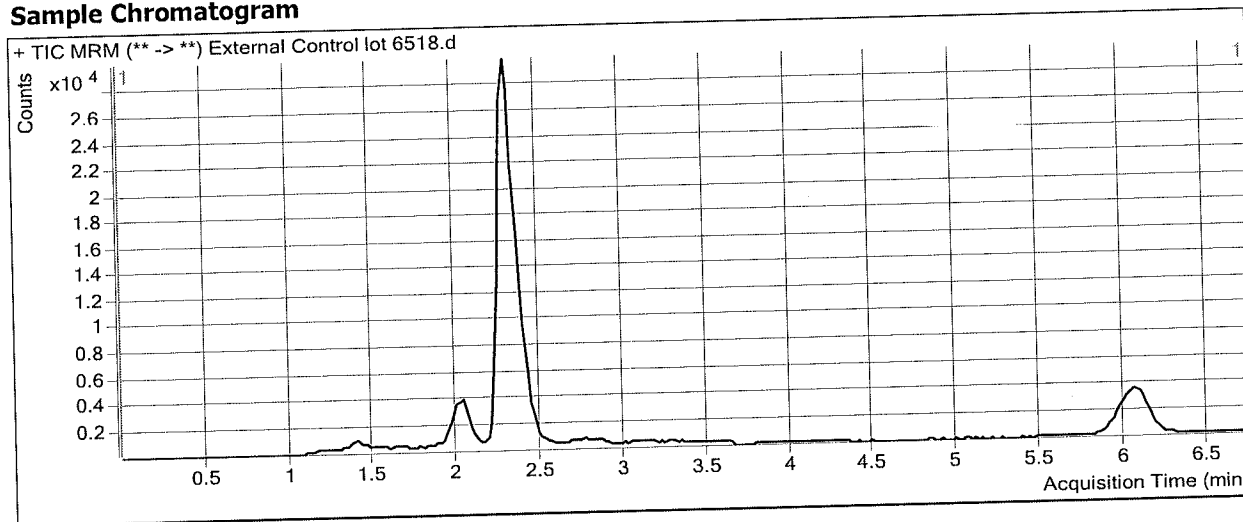
Sample Chromatogram

+ TIC MRM (** -> **) External Control lot 6518.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	12840	152975	0.0839	7.6891
THC-COOH	THC-COOH-d9	2.406	9985	52669	0.1896	9.4233
THC	THC-d3	6.093	4467	38363	0.1164	10.0651

ISP Forensics
Calibration Curve Report

Batch Data Path   D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update   7/20/2018 7:02 AM   **Analyst Name**   ISP TOX

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

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

$y = 0.011859 * x - 0.002931$
$R^2 = 0.99983326$
Type:Linear, Origin:Ignore, Weight:1/x
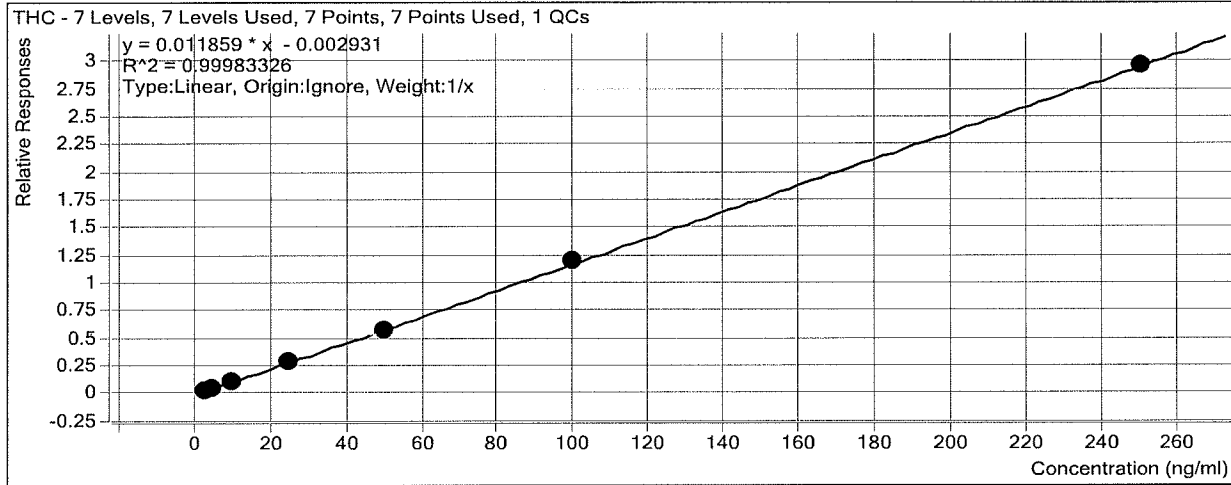
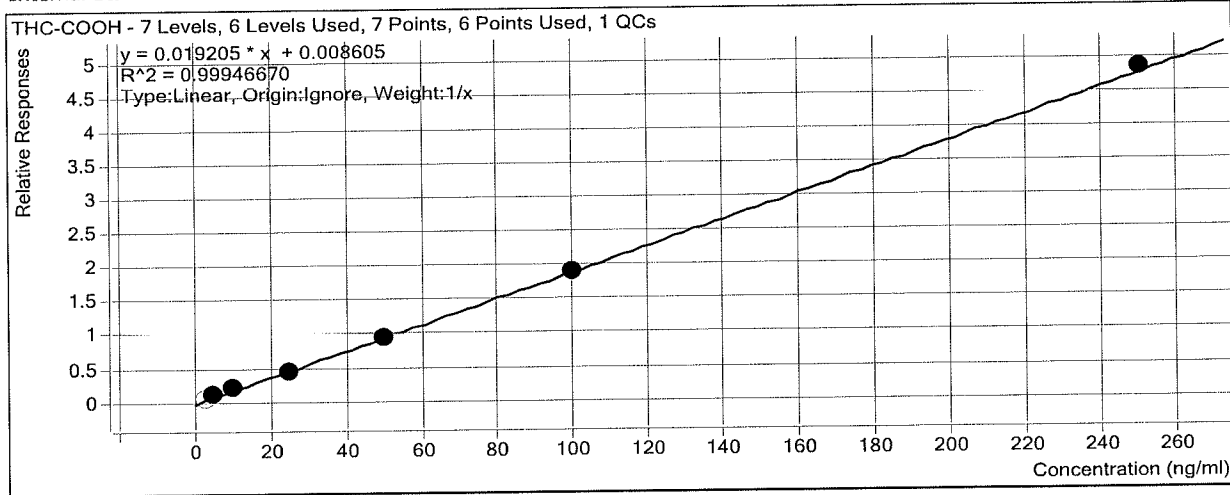| Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.2	106.1
Cal 2 - 5ng	2	☑	5	4.9	97.5
Cal 3 - 10ng	3	☑	10	9.7	96.9
QC - 10ng	3	☑	10	10.2	102.1
Cal 4 - 25ng	4	☑	25	24.8	99.3
Cal 5 - 50ng	5	☑	50	49.3	98.7
Cal 6 - 100ng	6	☑	100	101.7	101.7
Cal 7 - 250ng	7	☑	250	249.4	99.8

ISP Forensics
Calibration Curve Report

Batch Data Path   D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update   7/20/2018 7:02 AM   **Analyst Name**   ISP TOX

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

THC-COOH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs

$y = 0.019205 * x + 0.008605$
$R^2 = 0.99946670$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☐	3	3.0	98.4
Cal 2 - 5ng	2	☑	5	5.1	102.1
Cal 3 - 10ng	3	☑	10	10.6	105.7
QC - 10ng	3	☑	10	10.0	99.7
Cal 4 - 25ng	4	☑	25	23.5	94.0
Cal 5 - 50ng	5	☑	50	49.0	98.1
Cal 6 - 100ng	6	☑	100	99.1	99.1
Cal 7 - 250ng	7	☑	250	252.7	101.1

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update 7/20/2018 7:02 AM   **Analyst Name**   ISP TOX

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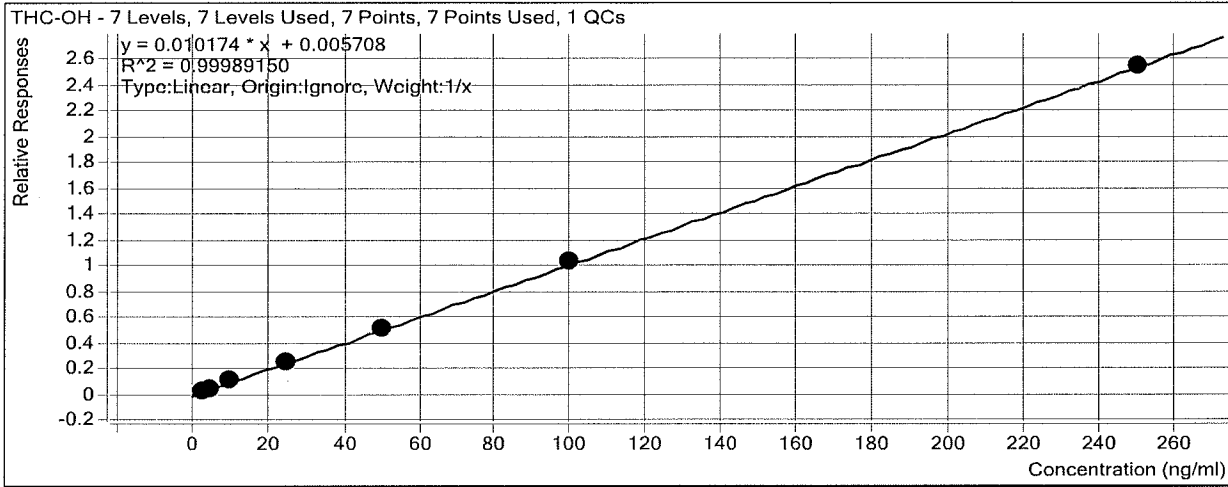*Internal Standard*   *THC-OH-d3*

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

y = 0.010174 * x + 0.005708
R^2 = 0.99989150
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.1	102.7
Cal 2 - 5ng	2	☑	5	4.9	98.3
Cal 3 - 10ng	3	☑	10	10.1	101.2
QC - 10ng	3	☑	10	10.0	99.7
Cal 4 - 25ng	4	☑	25	24.3	97.3
Cal 5 - 50ng	5	☑	50	49.8	99.5
Cal 6 - 100ng	6	☑	100	101.3	101.3
Cal 7 - 250ng	7	☑	250	249.5	99.8

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 16:39	**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
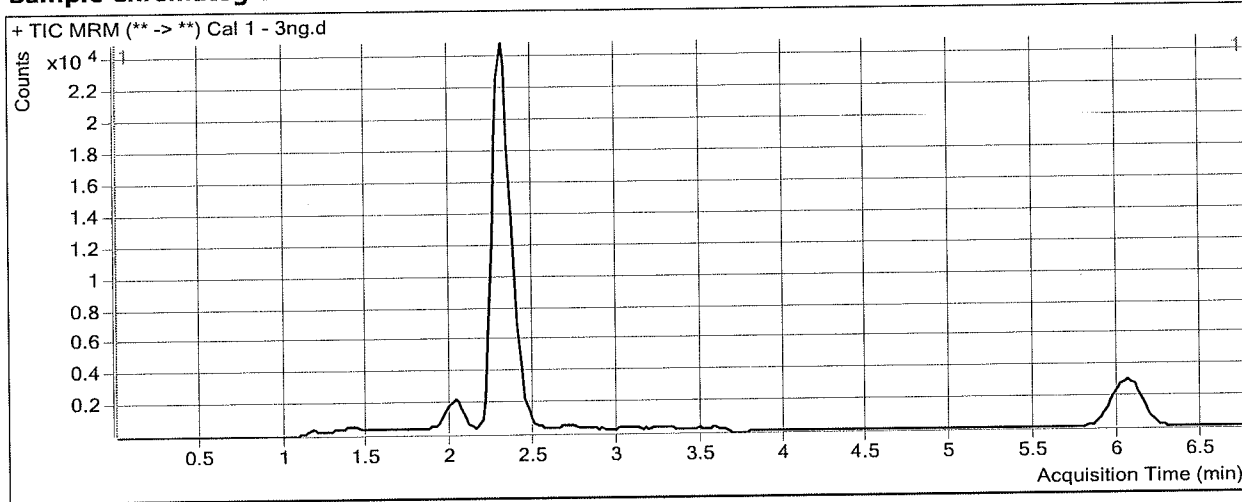
Sample Chromatogram

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

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	5199	140361	0.0370	3.0797
THC-COOH	THC-COOH-d9	2.406	3038	46538	0.0653	2.9512
THC	THC-d3	6.073	1313	37691	0.0348	3.1844

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 16:51	**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
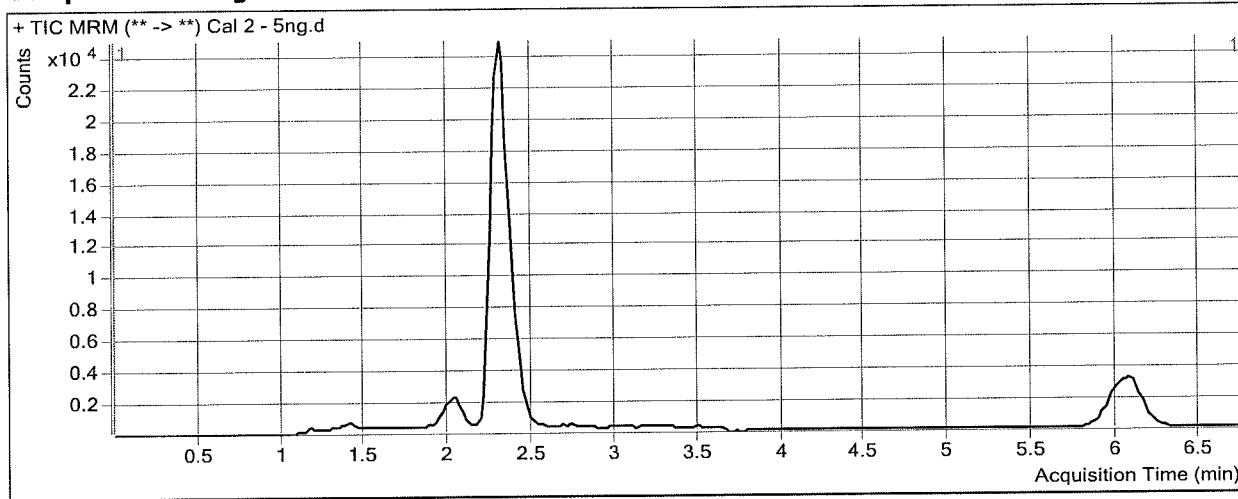
Sample Chromatogram

+ TIC MRM (** -> **) Cal 2 - 5ng.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	7729	138799	0.0557	4.9126
THC-COOH	THC-COOH-d9	2.406	5020	47078	0.1066	5.1044
THC	THC-d3	6.093	2197	40049	0.0549	4.8740

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 17:03	**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
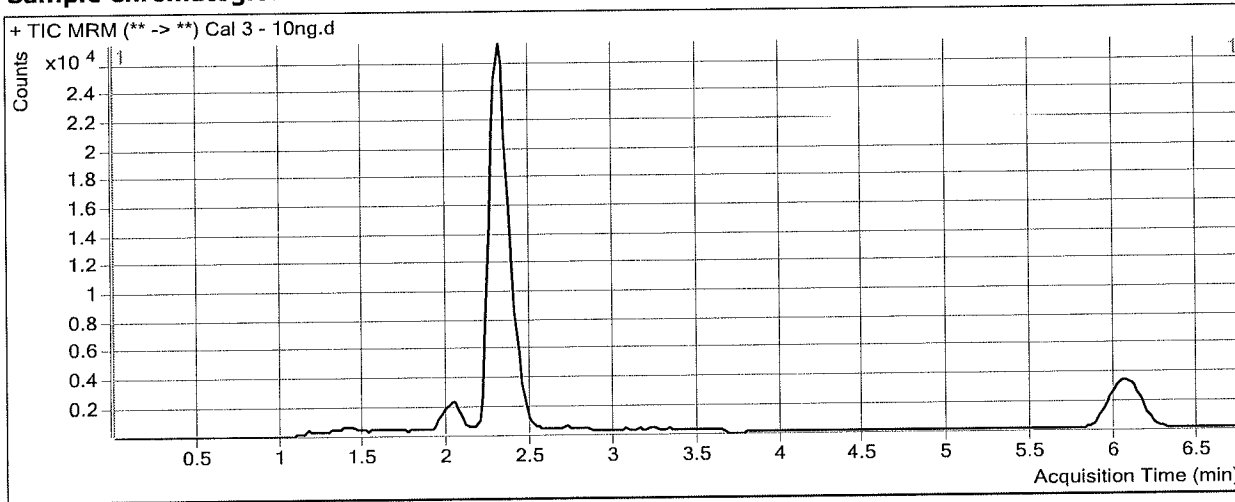
Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	15979	147081	0.1086	10.1174
THC-COOH	THC-COOH-d9	2.406	10308	48705	0.2116	10.5722
THC	THC-d3	6.093	4517	40332	0.1120	9.6925

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

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 17:15	**Data File**	Cal 4 - 25ng.d
Sample Type	Calibration	**Sample Name**	Cal 4 - 25ng
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-D1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 Cannabinoid Confirmation
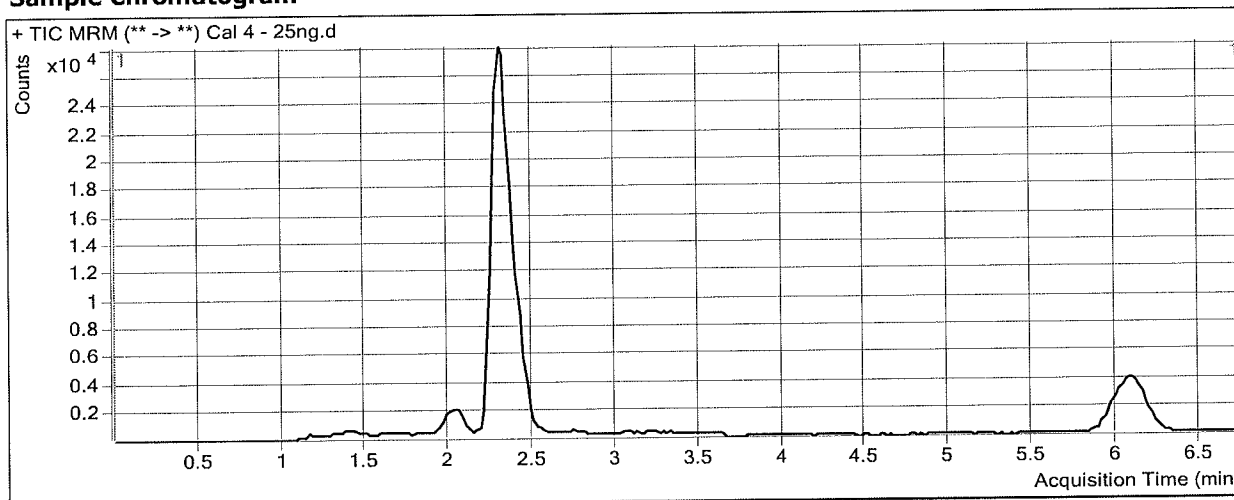
Sample Chromatogram

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

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	33427	131974	0.2533	24.3342
THC-COOH	THC-COOH-d9	2.426	20490	44570	0.4597	23.4893
THC	THC-d3	6.113	11304	38764	0.2916	24.8370

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 17:27	**Data File**	Cal 5 - 50ng.d
Sample Type	Calibration	**Sample Name**	Cal 5 - 50ng
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-E1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 Cannabinoid Confirmation
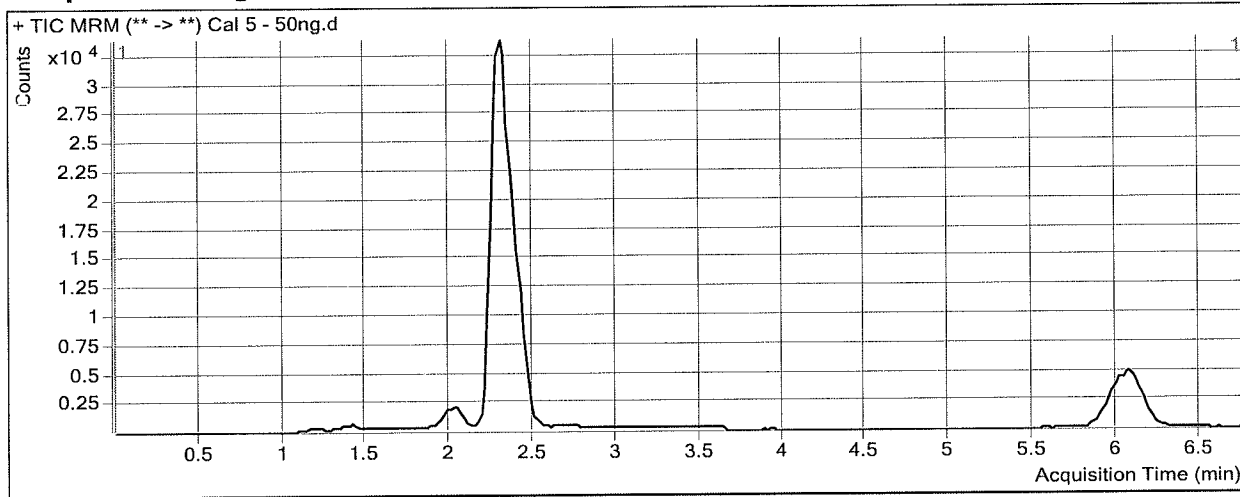
Sample Chromatogram

+ TIC MRM (** -> **) Cal 5 - 50ng.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	68348	133520	0.5119	49.7534
THC-COOH	THC-COOH-d9	2.406	42578	44798	0.9504	49.0408
THC	THC-d3	6.073	21548	37013	0.5822	49.3406

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

Batch Data Path	D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin
Analysis Time	7/20/2018 7:02 AM	**Analyst Name** ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name** ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State** Processed

Analysis Info

Acq Time	2018-07-19 17:39	**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
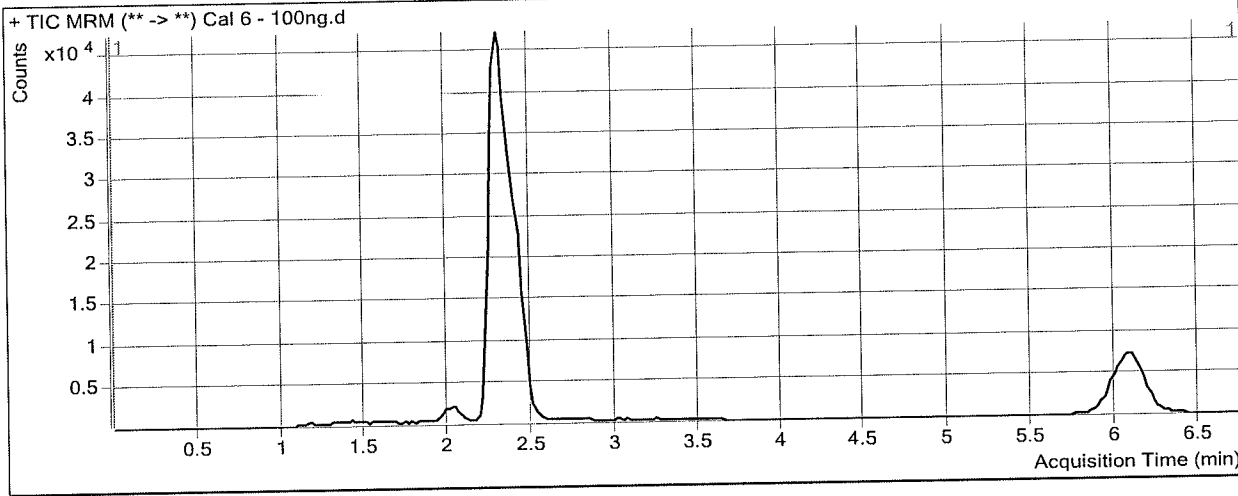
Sample Chromatogram

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

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	140525	135659	1.0359	101.2554
THC-COOH	THC-COOH-d9	2.406	84401	44166	1.9110	99.0578
THC	THC-d3	6.093	44896	37336	1.2025	101.6511

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

Batch Data Path   D:\2018 Data\07192018 cann quant\QuantResults\cann quant.batch.bin

Analysis Time	7/20/2018 7:02 AM	**Analyst Name**	ISP Tox
Report Time	7/20/2018 7:04 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/20/2018 7:02 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-07-19 16:16	**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
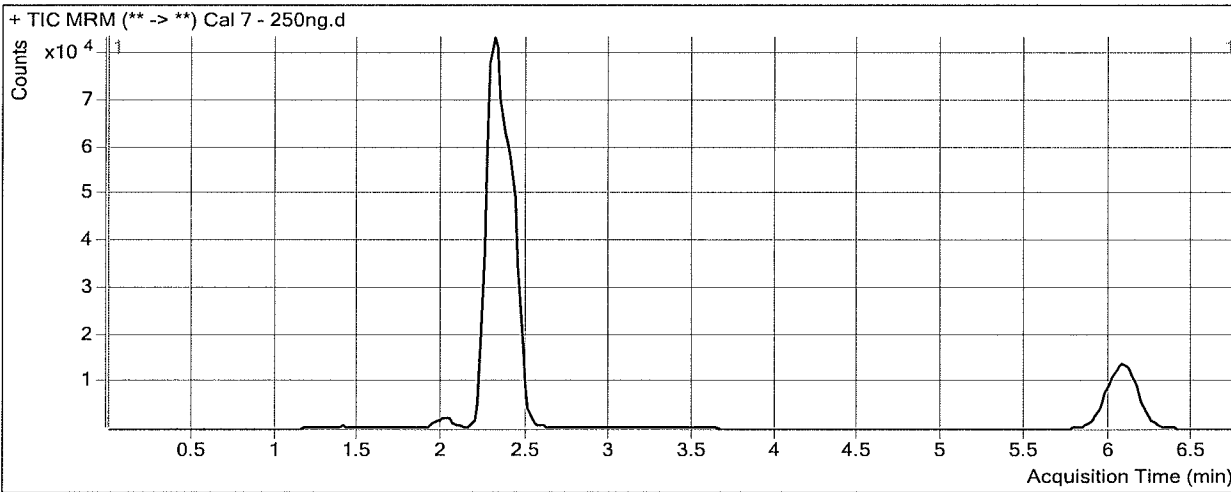
Sample Chromatogram

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

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.316	347738	136658	2.5446	249.5472
THC-COOH	THC-COOH-d9	2.406	209879	43164	4.8624	252.7356
THC	THC-d3	6.073	109548	37074	2.9548	249.4204

Idaho State Police
Forensic Services
Toxicology Discipline

Request for Departure from an Analytical Method

<u>Date of Request</u>
06/28/18

<u>Forensic Scientist</u>
Anne Nord

<u>Analytical Method</u>
Method 26

<u>Type of Deviation</u>
☒ Minor   ☐ Major

<u>Departure</u>

<u>Allow the response from 5ng/ml for Carboxy-THC to be evaluated as positive at the analyst's discretion.</u>

4.3 Evaluation of Results
4.3.1 Minimum Criteria:
4.3.1.1
Analyte Administrative Threshold
THC 3 ng/mL
Carboxy-THC 10 ng/mL
THC-OH 3 ng/mL
Calculated sample concentration of 3 ng/mL or greater for THC and THC-OH, a calculated
sample concentration of 10 ng/mL or greater for Carboxy-THC.
Allow for the positive control to be prepared in house.

<u>Rationale for Departure</u>
AM 26 is a screening method for cannabinoids, the function of the method is to indicate if cannabinoids are present. There is a potential for a positive sample to give a response in this range in the screen.

Discipline Leader Review
☒ Departure approved- this deviation will remain in effect until the next method revision at which time the change will be made in the analytical method.

☐ Departure Not Approved
        Comments:

Celena Shrum   Date: 06/28/18
Celena Shrum
Toxicology Program Discipline Leader