

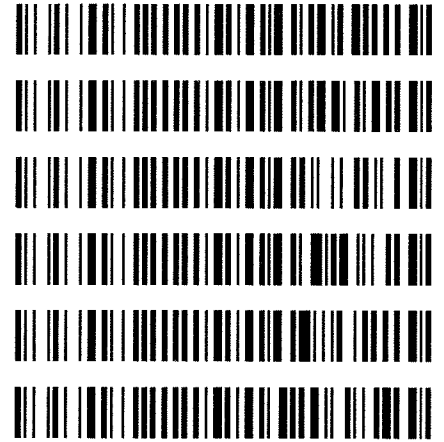
REVIEWED

By Britany Wylie at 1:38 pm, Apr 09, 2019

4/8/2019

Worklist: 3245

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>
C2019-0499	1	147719	AM 27 Blood THC Quant by LC-QQQ
C2019-0501	1	147720	AM 27 Blood THC Quant by LC-QQQ
C2019-0550	1	147721	AM 27 Blood THC Quant by LC-QQQ
C2019-0555	1	147722	AM 27 Blood THC Quant by LC-QQQ
C2019-0588	1	147723	AM 27 Blood THC Quant by LC-QQQ
C2019-0626	1	147724	AM 27 Blood THC Quant by LC-QQQ



A handwritten signature in black ink, consisting of a stylized, cursive letter 'A' with a horizontal line extending to the right.

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

Extraction Date: 04/08/19
Plate lot#: 0539904

Analyst: Anne Nord
Plate Expiration: 09/10/19

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

Blank Blood Lot: 19A207P3
LCMS-QQQ ID: 62340
Column: UCT Selectra DA 100 x 2.1mm 3um

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: 2019 Data\AM 27\040819 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 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 75 ul THC-OH in 9767.5 ul meOH
Ppd 3/13/19 Exp: 9/13/19 lot 91319 by AMN

Drug	lot	expiration
C-THC	FE01061702 cerillient	3/1/2022
THC-OH	318.1b18.1L1a	12/1/2021
THC	135.1b71.0L6	11/1/2021

AM 27 control 100 ul working solution lot (91319) in 9900 ul blood lot (19A207p3)
ppd 3/13/19 Exp 9/13/19 lot 31319 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-TH by AMN



ISP FORENSICS - Cd'A Instrument # 62340

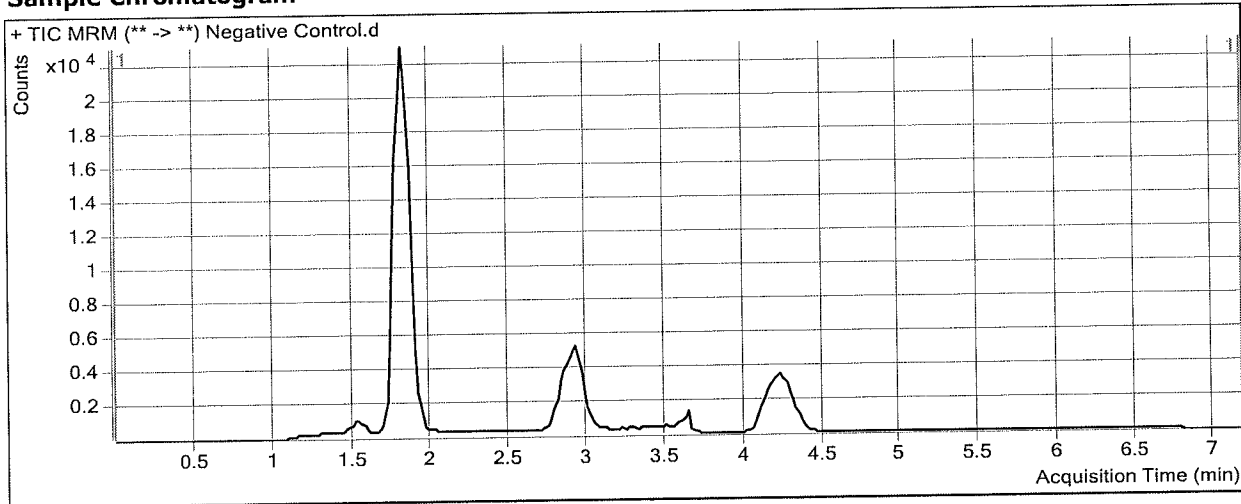
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
Analysis Time 4/9/2019 8:41 AM **Analyst Name** ISP Tox
Report Time 4/9/2019 8:42 AM **Reporter Name** ISP Tox
Last Calib Update 4/9/2019 8:41 AM **Batch State** Processed

Analysis Info

Acq Time 2019-04-08 18:34 **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



ISP FORENSICS - Cd'A Instrument # 62340

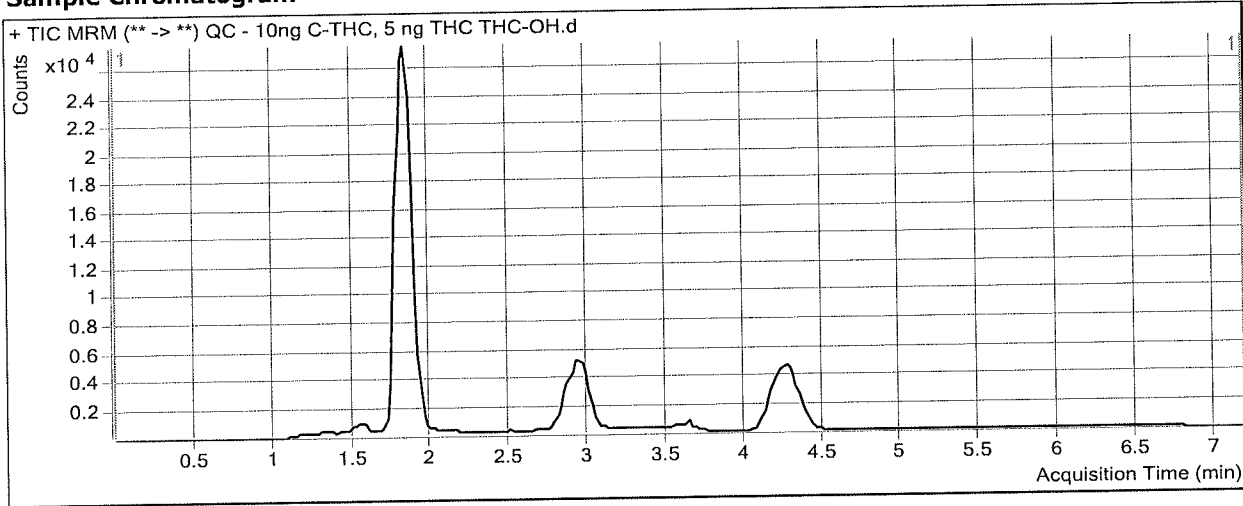
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
Analysis Time 4/9/2019 8:41 AM **Analyst Name** ISP Tox
Report Time 4/9/2019 8:42 AM **Reporter Name** ISP Tox
Last Calib Update 4/9/2019 8:41 AM **Batch State** Processed

Analysis Info

Acq Time 2019-04-08 18:46 **Data File** QC - 10ng C-THC, 5 ng THC THC-OH.d
Sample Type QC **Sample Name** QC - 10ng C-THC, 5 ng THC THC-OH
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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	1.835	5224	131325	0.0398	4.9912
THC-COOH	THC-COOH-d9	1.905	8300	50505	0.1643	10.0012
THC	THC-d3	4.292	2694	55655	0.0484	4.8127

ISP FORENSICS - Cd'A Instrument # 62340

Cannabinoids Analysis Report

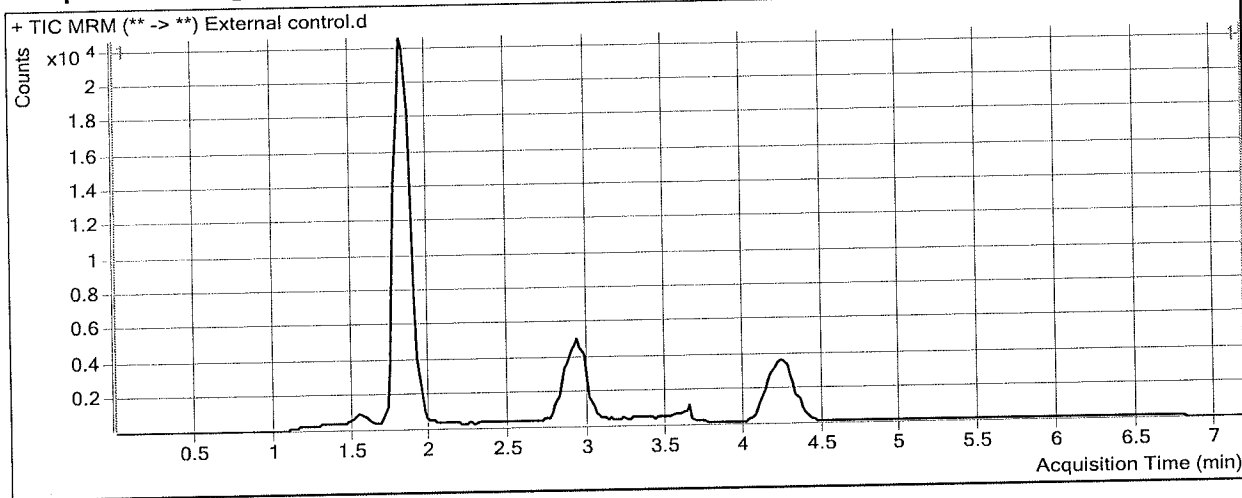
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Analysis Time 4/9/2019 8:41 AM **Analyst Name** ISP Tox
Report Time 4/9/2019 8:42 AM **Reporter Name** ISP Tox
Last Calib Update 4/9/2019 8:41 AM **Batch State** Processed

Analysis Info

Acq Time 2019-04-08 18:58 **Data File** External control.d
Sample Type Sample **Sample Name** External control
Dilution 1 **Acq Method** AM 27 Quant THC 7-2017.m
Position P1-B2 **Sample Info**
Inj Vol -1 **Comment** AM 27 Cannabinoid Confirmation

7.5 THC, THC-OH, IS-C-THC

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	1.835	5579	99765	0.0559	6.9260 ✓
THC-COOH	THC-COOH-d9	1.885	9004	42198	0.2134	13.1808 ✓
THC	THC-d3	4.252	3185	39410	0.0808	8.0334 ✓

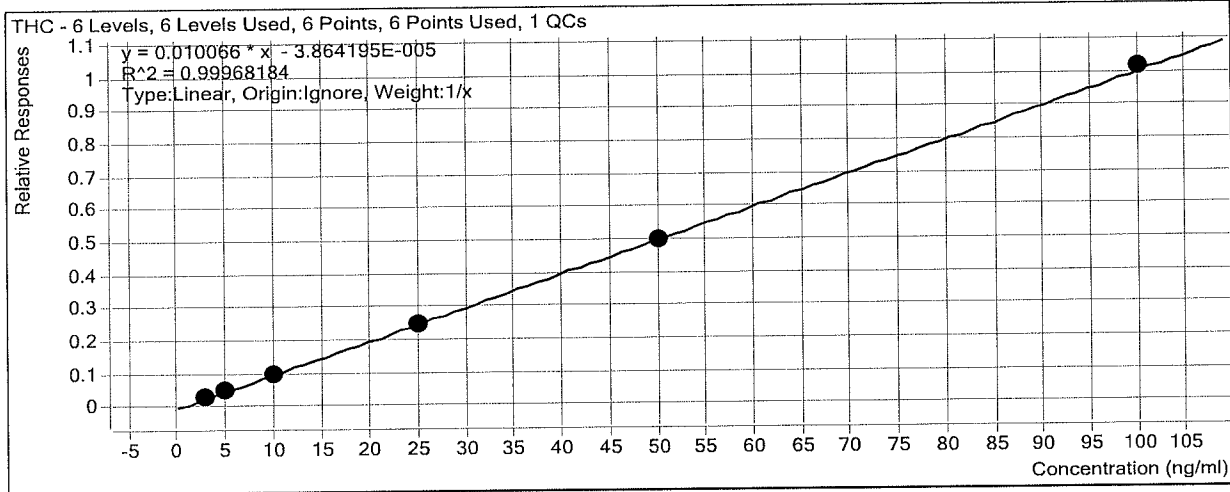
ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
4/9/2019 8:41 AM

Analyst Name ISP TOX

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



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.2	105.6
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.0	99.1
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	9.7	97.3
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	24.3	97.2
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.9	99.8
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	100.9	100.9
QC - 10ng C-THC, 5 ng THC THC-OH	7	<input checked="" type="checkbox"/>	5	4.8	96.3

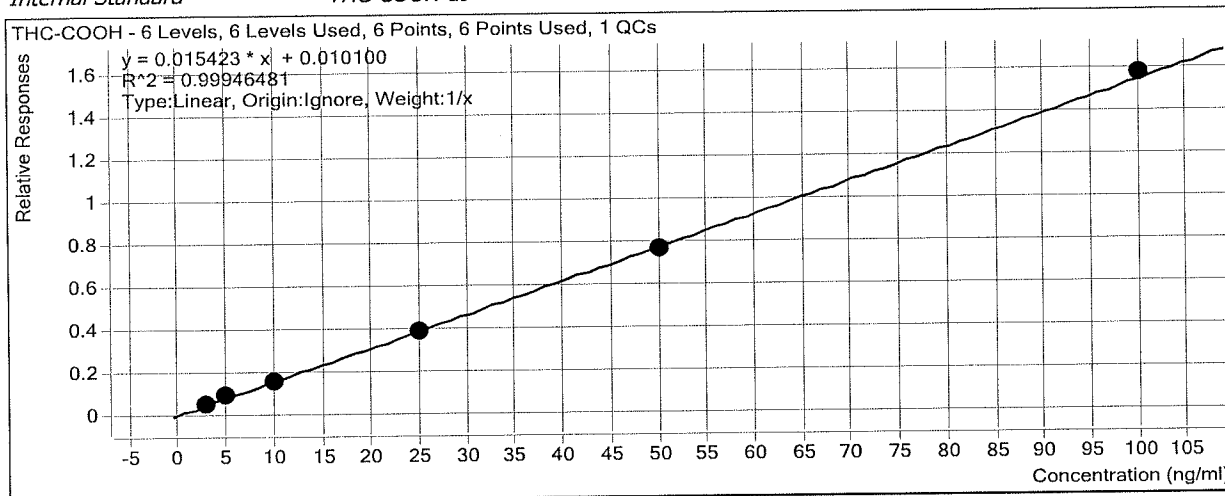
ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
4/9/2019 8:41 AM

Analyst Name ISP TOX

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



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.0	99.1
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.3	106.7
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	9.7	96.7
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	24.4	97.4
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.3	98.7
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	101.3	101.3
QC - 10ng C-THC, 5 ng THC THC-OH	7	<input checked="" type="checkbox"/>	10	10.0	100.0

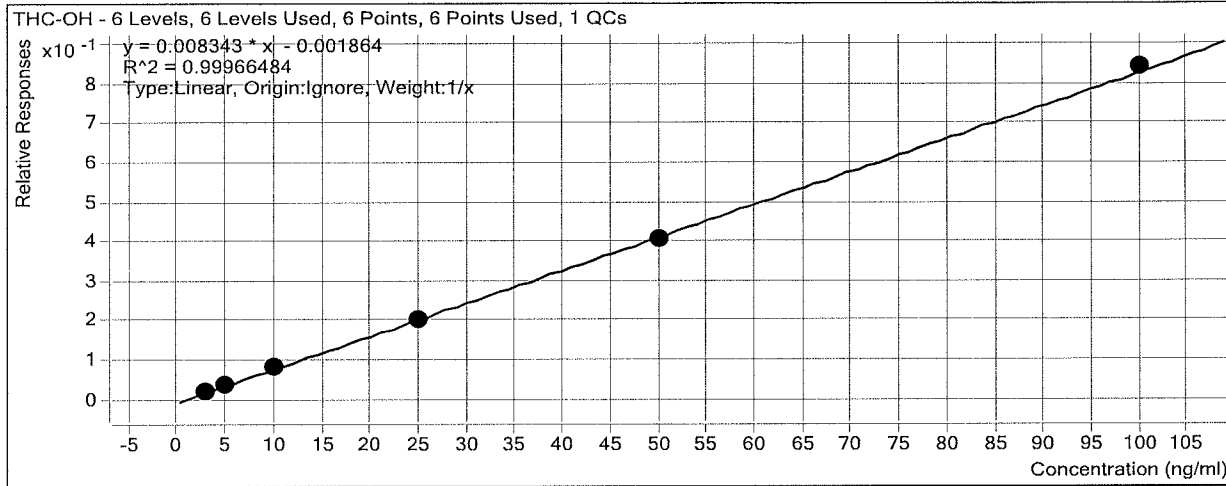
ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
4/9/2019 8:41 AM

Analyst Name ISP TOX

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



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.0	100.2
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.0	100.5
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.2	102.4
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	24.3	97.4
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.2	98.5
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	101.1	101.1
QC - 10ng C-THC, 5 ng THC THC-OH	7	<input checked="" type="checkbox"/>	5	5.0	99.8

ISP FORENSICS - Cd'A Instrument # 62340

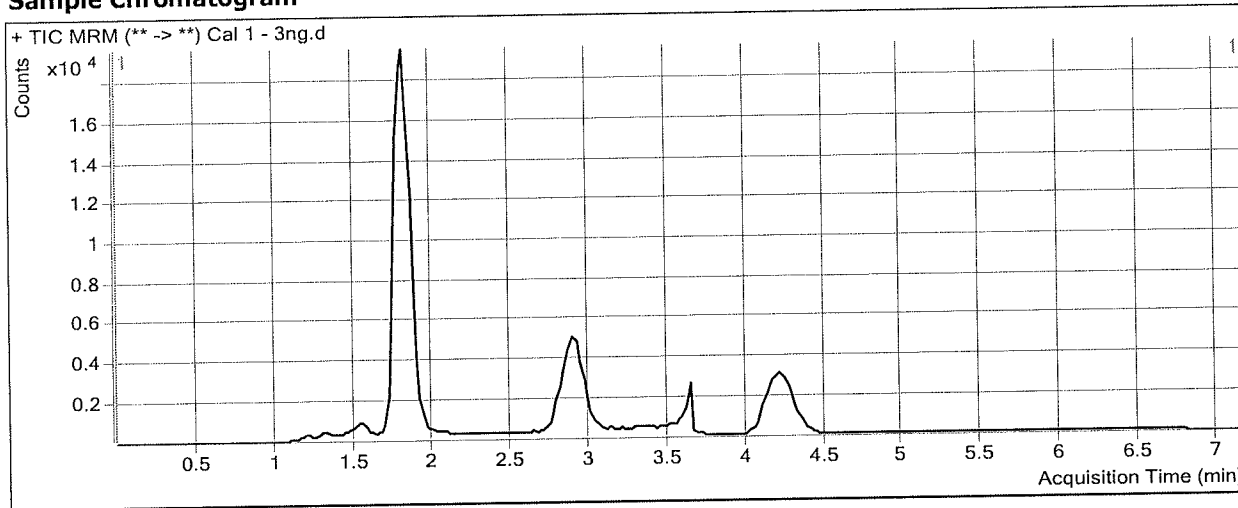
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
Analysis Time 4/9/2019 8:41 AM **Analyst Name** ISP Tox
Report Time 4/9/2019 8:42 AM **Reporter Name** ISP Tox
Last Calib Update 4/9/2019 8:41 AM **Batch State** Processed

Analysis Info

Acq Time 2019-04-08 17:11 **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-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	1.815	2100	90463	0.0232	3.0054
THC-COOH	THC-COOH-d9	1.865	2242	40064	0.0560	2.9733
THC	THC-d3	4.232	1159	36388	0.0319	3.1693

ISP FORENSICS - Cd'A Instrument # 62340

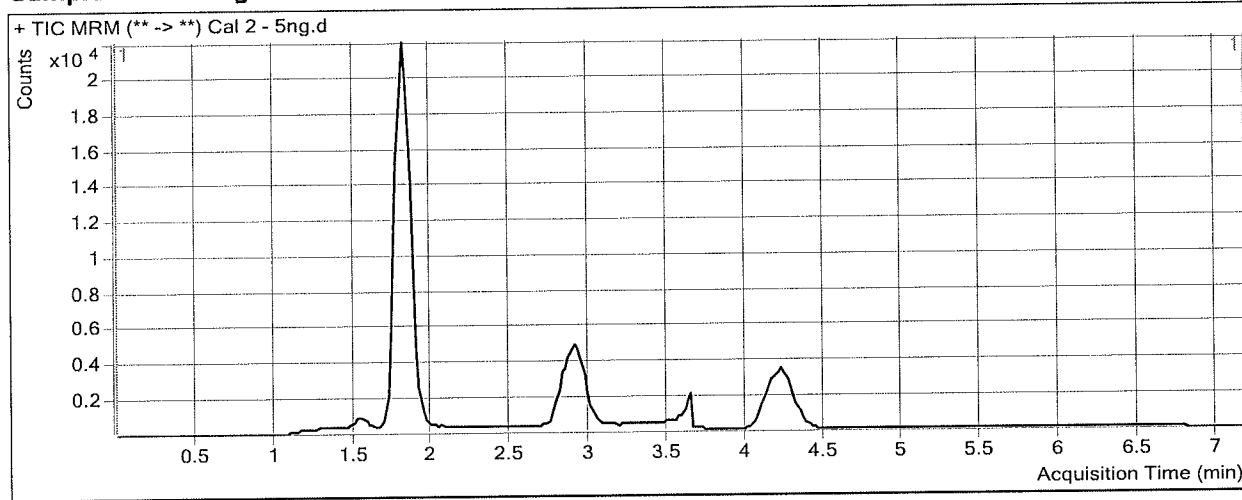
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
Analysis Time 4/9/2019 8:41 AM **Analyst Name** ISP Tox
Report Time 4/9/2019 8:42 AM **Reporter Name** ISP Tox
Last Calib Update 4/9/2019 8:41 AM **Batch State** Processed

Analysis Info

Acq Time 2019-04-08 17:23 **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-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	1.855	3973	99230	0.0400	5.0227
THC-COOH	THC-COOH-d9	1.885	3853	41707	0.0924	5.3355
THC	THC-d3	4.252	1943	38993	0.0498	4.9534

ISP FORENSICS - Cd'A Instrument # 62340

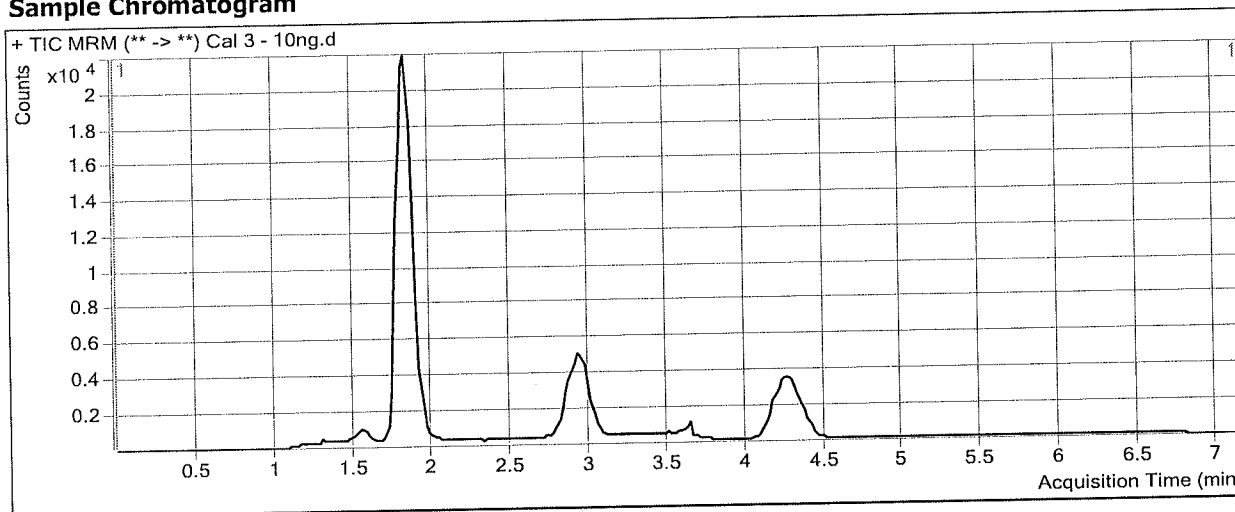
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin		
Analysis Time	4/9/2019 8:41 AM	Analyst Name	ISP Tox
Report Time	4/9/2019 8:42 AM	Reporter Name	ISP Tox
Last Calib Update	4/9/2019 8:41 AM	Batch State	Processed

Analysis Info

Acq Time	2019-04-08 17:35	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-D1	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	1.835	7952	95203	0.0835	10.2355
THC-COOH	THC-COOH-d9	1.885	6601	41440	0.1593	9.6741
THC	THC-d3	4.292	3838	39197	0.0979	9.7306

ISP FORENSICS - Cd'A Instrument # 62340

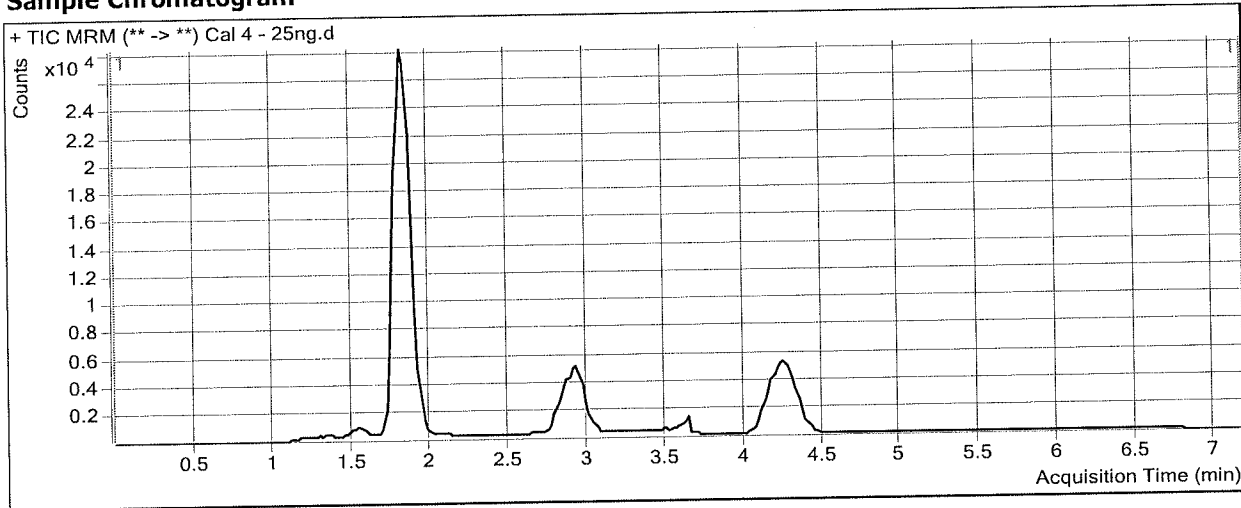
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
Analysis Time 4/9/2019 8:41 AM **Analyst Name** ISP Tox
Report Time 4/9/2019 8:42 AM **Reporter Name** ISP Tox
Last Calib Update 4/9/2019 8:41 AM **Batch State** Processed

Analysis Info

Acq Time 2019-04-08 17:47 **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-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	1.835	22559	112101	0.2012	24.3448
THC-COOH	THC-COOH-d9	1.885	17966	46565	0.3858	24.3621
THC	THC-d3	4.252	12509	51123	0.2447	24.3125

ISP FORENSICS - Cd'A Instrument # 62340

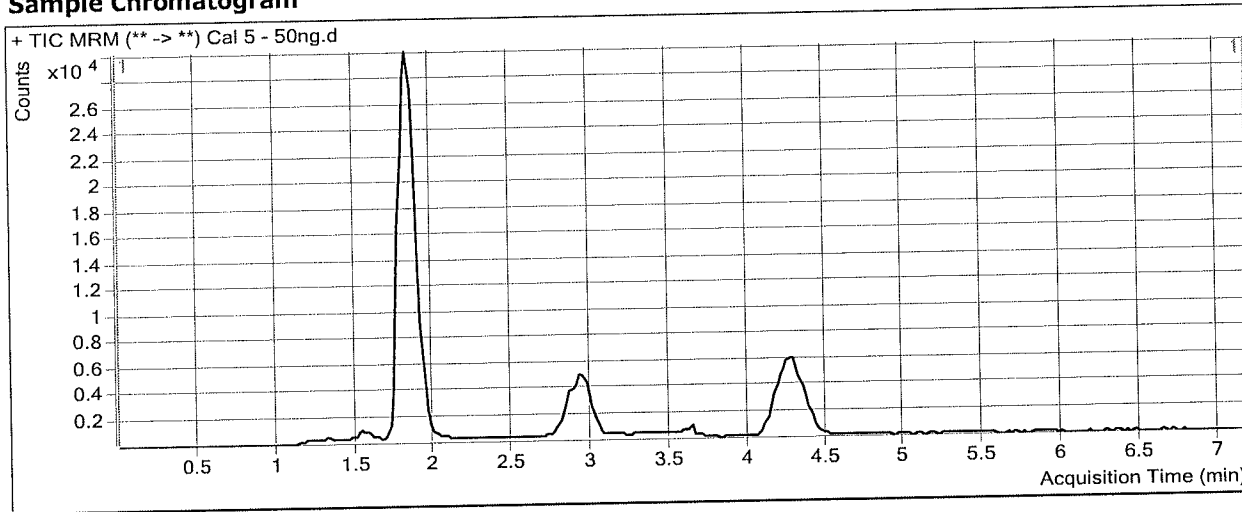
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin		
Analysis Time	4/9/2019 8:41 AM	Analyst Name	ISP Tox
Report Time	4/9/2019 8:42 AM	Reporter Name	ISP Tox
Last Calib Update	4/9/2019 8:41 AM	Batch State	Processed

Analysis Info

Acq Time	2019-04-08 17:58	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-F1	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	1.855	39469	96512	0.4090	49.2418
THC-COOH	THC-COOH-d9	1.885	31271	40560	0.7710	49.3347
THC	THC-d3	4.292	22740	45278	0.5022	49.8977

ISP FORENSICS - Cd'A Instrument # 62340

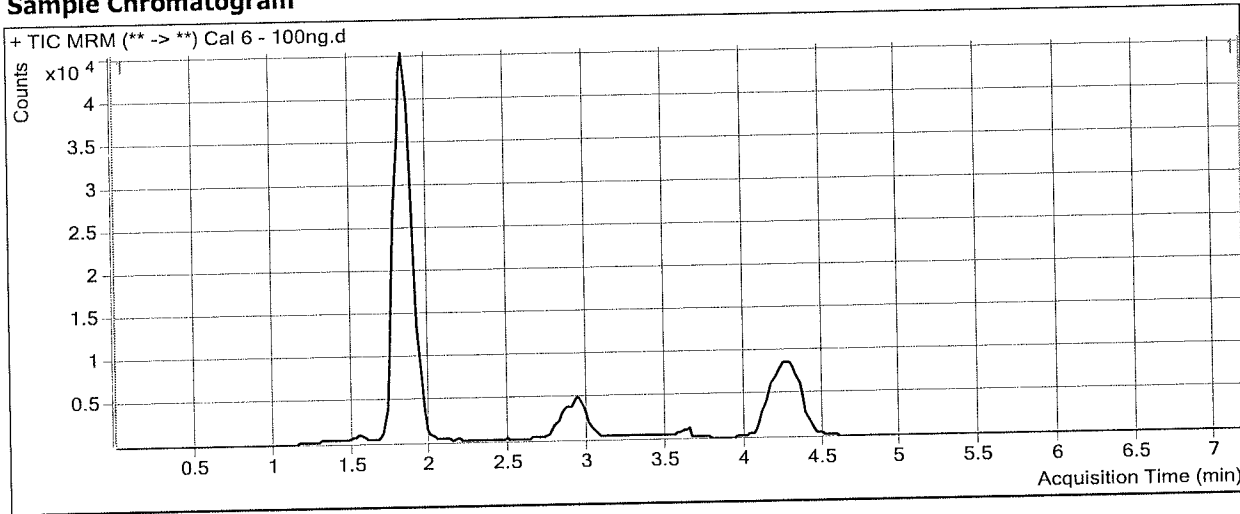
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\040819\QuantResults\cann quant.batch.bin
Analysis Time 4/9/2019 8:41 AM **Analyst Name** ISP Tox
Report Time 4/9/2019 8:42 AM **Reporter Name** ISP Tox
Last Calib Update 4/9/2019 8:41 AM **Batch State** Processed

Analysis Info

Acq Time 2019-04-08 18:10 **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-G1 **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	1.835	92284	109600	0.8420	101.1498
THC-COOH	THC-COOH-d9	1.885	69143	43964	1.5727	101.3203
THC	THC-d3	4.272	52300	51478	1.0160	100.9365