

















Worklist: 2892

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>	
C2018-2560	1	137481	AM 27 Blood THC Quant by LC-QQQ	
C2018-2570	1	137482	AM 27 Blood THC Quant by LC-QQQ	
C2018-2577	1	137483	AM 27 Blood THC Quant by LC-QQQ	
C2018-2604	1	137484	AM 27 Blood THC Quant by LC-QQQ	
C2018-2606	1	137485	AM 27 Blood THC Quant by LC-QQQ	
C2018-2607	1	137486	AM 27 Blood THC Quant by LC-QQQ	
C2018-2608	1	137487	AM 27 Blood THC Quant by LC-QQQ	
C2018-2610	1	137488	AM 27 Blood THC Quant by LC-QQQ	
C2019-0004	1	137489	AM 27 Blood THC Quant by LC-QQQ	
C2019-0007	1	137490	AM 27 Blood THC Quant by LC-QQQ	
C2019-0015	1	137491	AM 27 Blood THC Quant by LC-QQQ	
C2019-0016	1	137492	AM 27 Blood THC Quant by LC-QQQ	
C2019-0054	1	137493	AM 27 Blood THC Quant by LC-QQQ	
C2019-0064	2	137494	AM 27 Blood THC Quant by LC-QQQ	
C2019-0087	1	137495	AM 27 Blood THC Quant by LC-QQQ	
C2019-0101	1	137496	AM 27 Blood THC Quant by LC-QQQ	



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

Extraction Date: 1/15/19
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
Hexane
Blank Blood Lot: 18G207D7
LCMS-QQQ ID: 62340
Column: UCT Selectra DA 100 x 2.1mm 3um
LCMS Methanol

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\011519 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: Negative control dried out, it was re-constituted and injected.



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
 ppd 9/20/18 Exp 4/1/19 lot 92018 Concentration 10 ng/ml each by AMN
 ppd 01/14/18 Exp 4/1/19 lot 011419 Concentration 10 ng/ml each by AMN

ISP FORENSICS - Cd'A Instrument # 62340

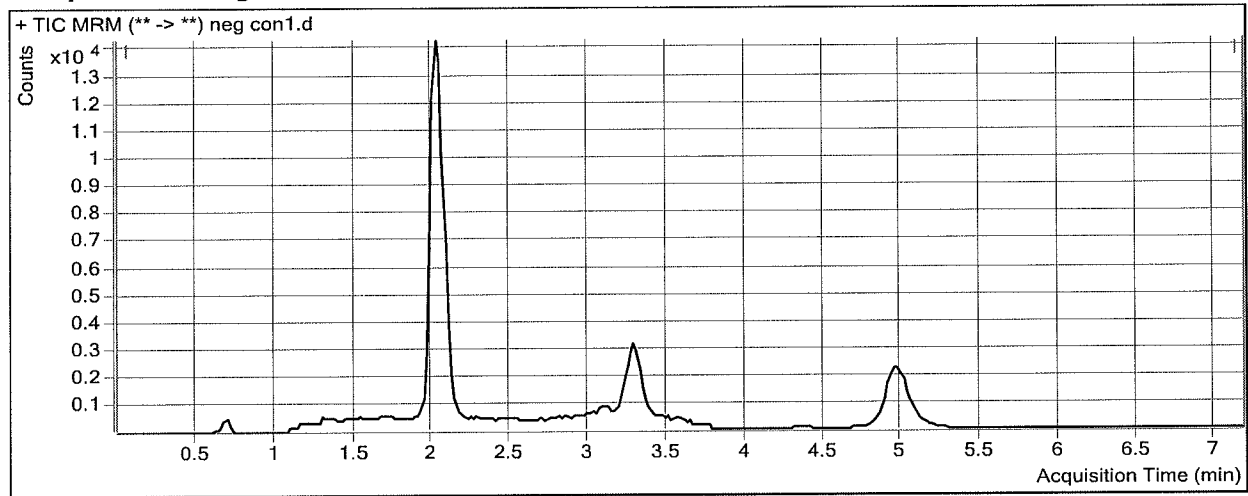
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 17:25 **Data File** neg con1.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

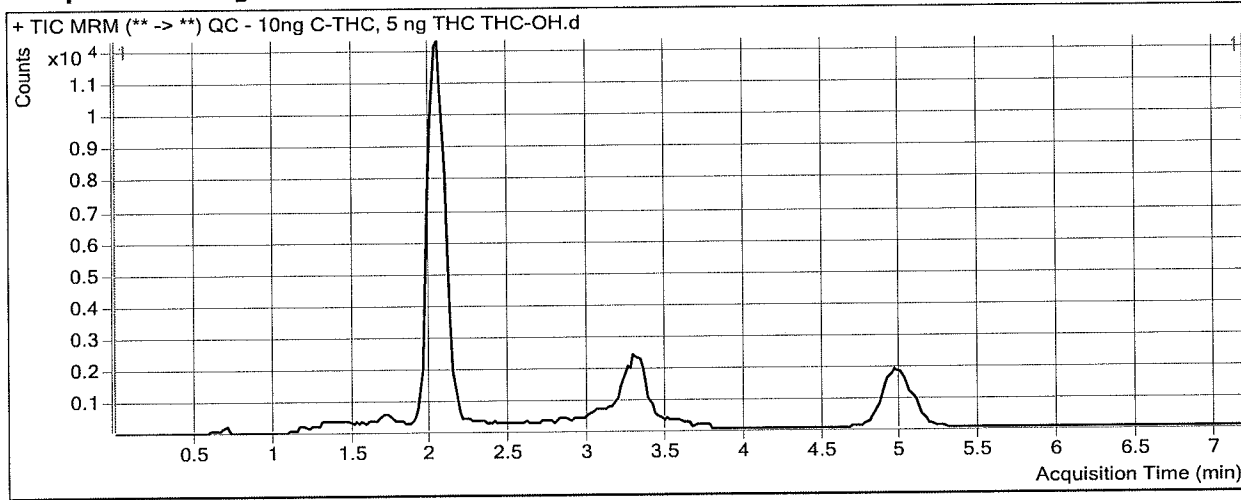
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 15:34 **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	2.035	2240	58785	0.0381	4.7797
THC-COOH	THC-COOH-d9	2.125	4229	26000	0.1627	9.9744
THC	THC-d3	4.992	1068	22975	0.0465	4.5831

ISP FORENSICS - Cd'A Instrument # 62340

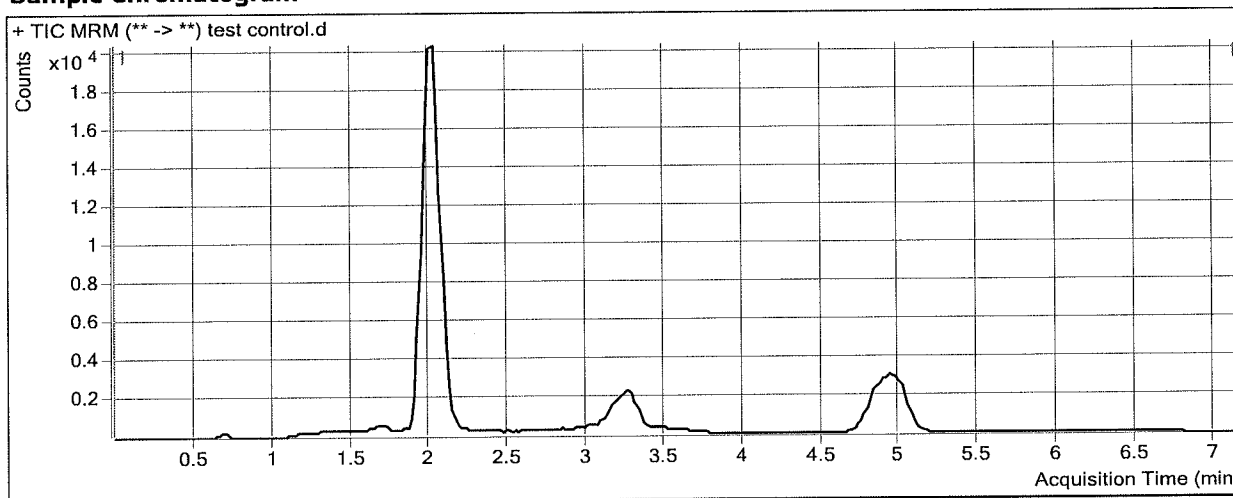
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 22:33 **Data File** test control.d *External control lot 011419*
Sample Type Sample **Sample Name** test control
Dilution 1 **Acq Method** AM 27 Quant THC 7-2017.m
Position P1-C4 **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	2.015	9165	98848	0.0927	11.4586
THC-COOH	THC-COOH-d9	2.065	6785	39524	0.1717	10.5624
THC	THC-d3	4.972	5175	40799	0.1268	12.6867

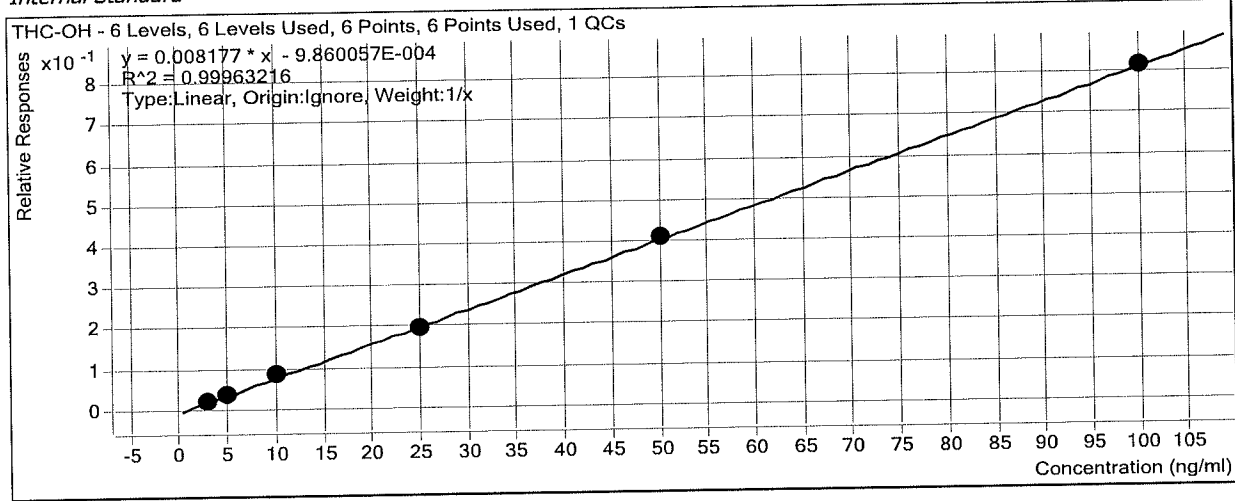
ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
1/17/2019 9:50 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	2.9	97.6
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	4.9	98.8
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.6	105.6
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	24.4	97.5
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	50.3	100.6
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	99.9	99.9
QC - 10ng C-THC, 5 ng THC THC-OH	7	<input checked="" type="checkbox"/>	5	4.8	95.6

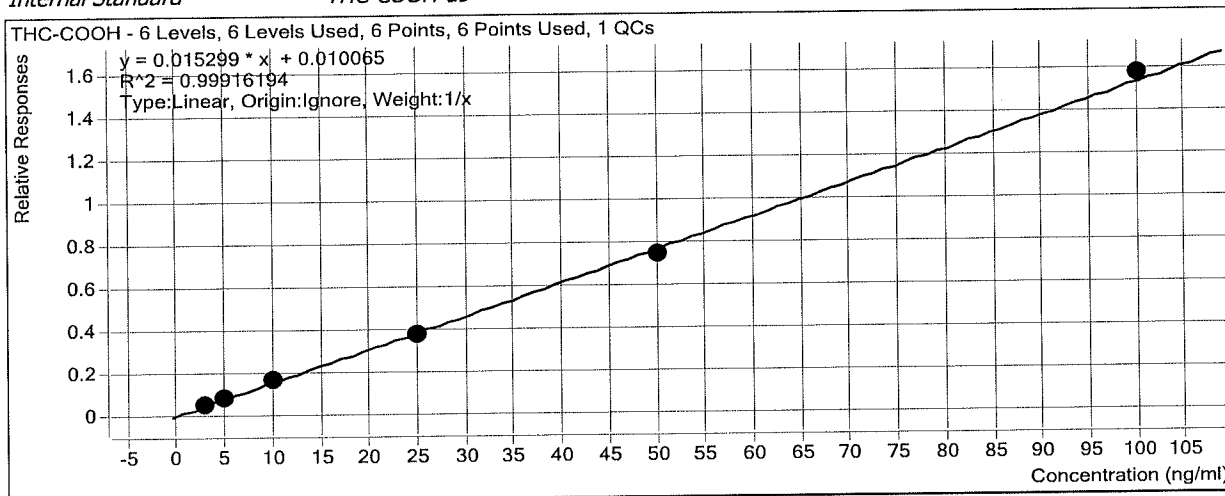
ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
1/17/2019 9:50 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	100.1
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.1	102.1
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.2	101.8
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	24.3	97.2
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	48.4	96.8
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	102.0	102.0
QC - 10ng C-THC, 5 ng THC THC-OH	7	<input checked="" type="checkbox"/>	10	10.0	99.7

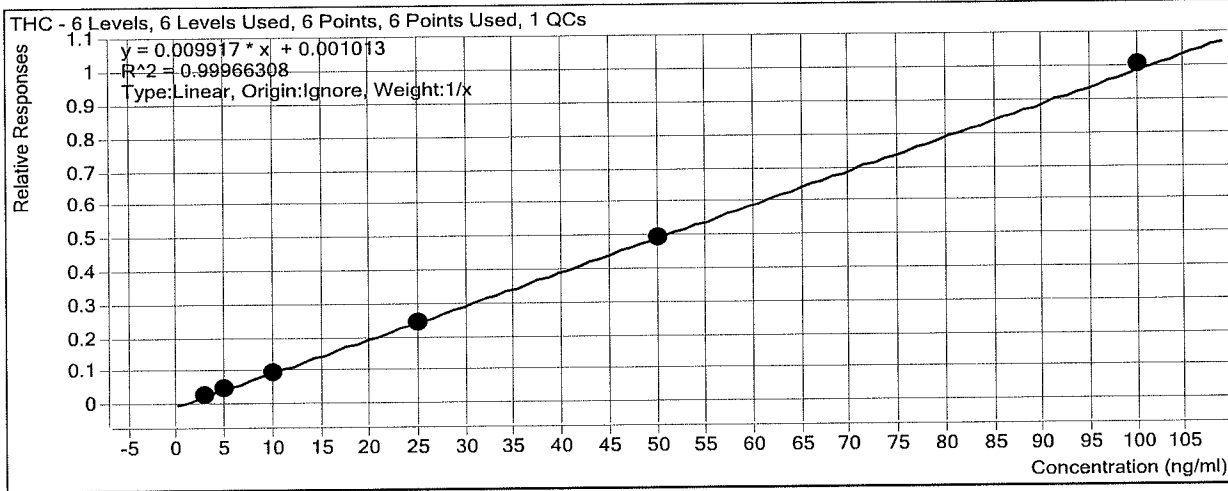
ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
1/17/2019 9:50 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	3.1	102.4
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.1	102.5
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	9.8	97.6
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	24.3	97.3
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.5	99.0
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	101.2	101.2
QC - 10ng C-THC, 5 ng THC THC-OH	7	<input checked="" type="checkbox"/>	5	4.6	91.7

ISP FORENSICS - Cd'A Instrument # 62340

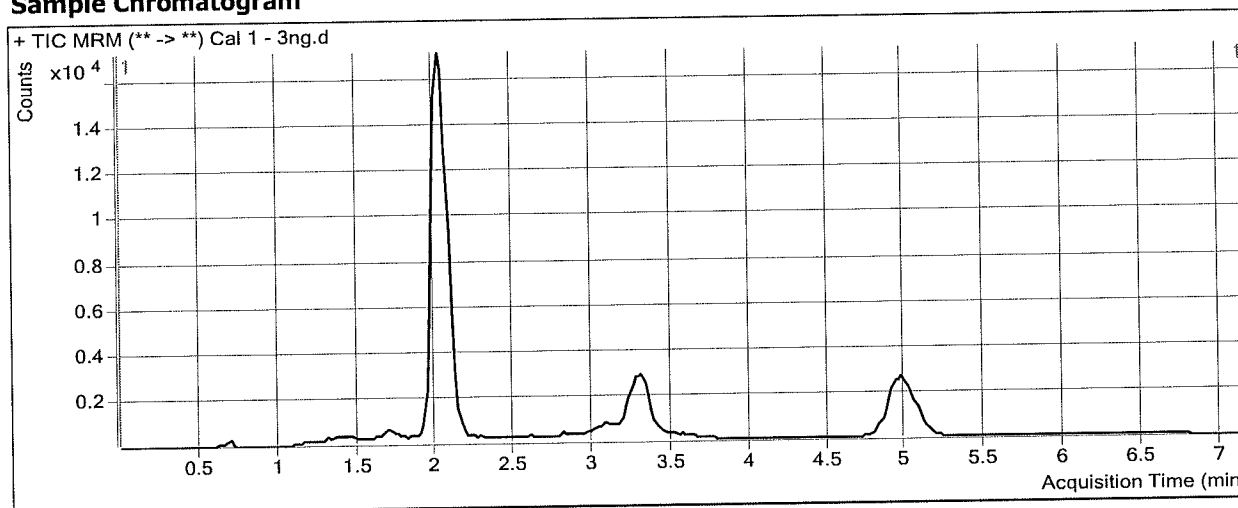
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 13:59 **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	2.015	1844	80341	0.0230	2.9273
THC-COOH	THC-COOH-d9	2.105	1938	34600	0.0560	3.0036
THC	THC-d3	4.992	980	31134	0.0315	3.0722

ISP FORENSICS - Cd'A Instrument # 62340

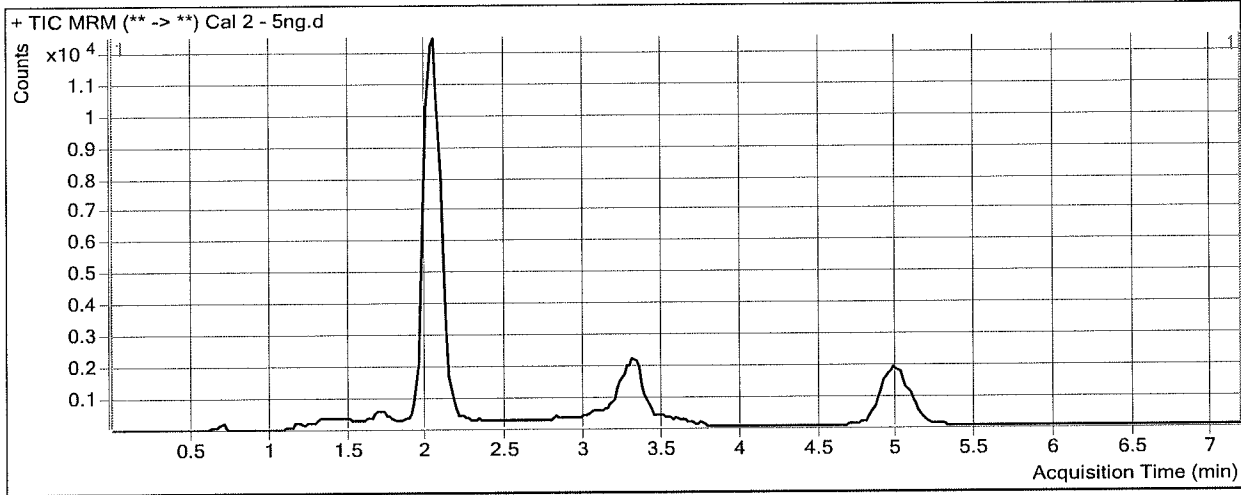
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 14:11 **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	2.035	2369	60088	0.0394	4.9411
THC-COOH	THC-COOH-d9	2.105	2331	26428	0.0882	5.1069
THC	THC-d3	5.012	1185	22867	0.0518	5.1228

ISP FORENSICS - Cd'A Instrument # 62340

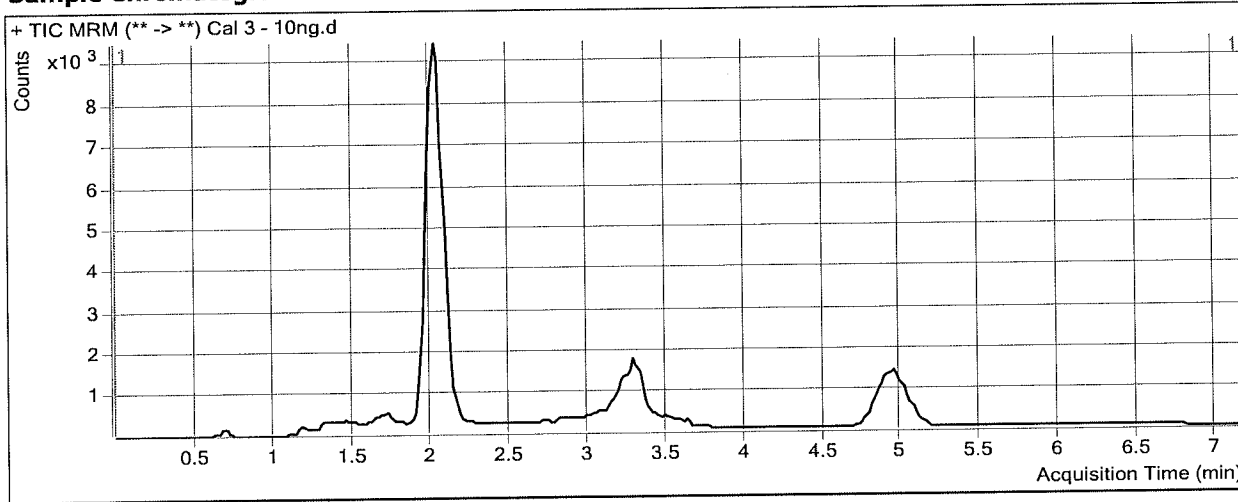
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 14:23 **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	2.035	3767	44119	0.0854	10.5618
THC-COOH	THC-COOH-d9	2.105	2998	18084	0.1658	10.1772
THC	THC-d3	4.972	1610	16459	0.0978	9.7585

ISP FORENSICS - Cd'A Instrument # 62340

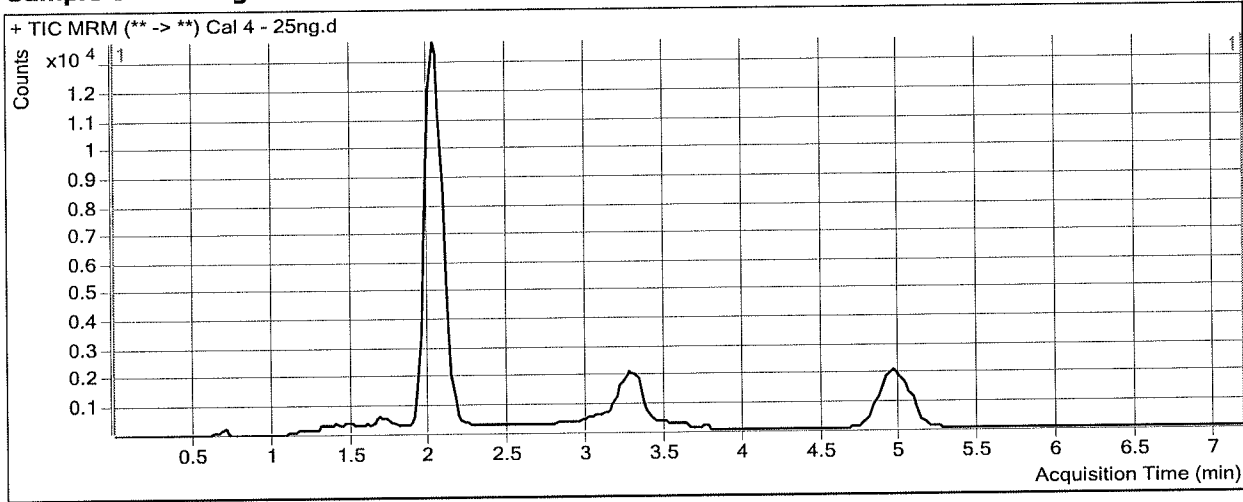
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 14:35 **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	2.035	11227	56626	0.1983	24.3662
THC-COOH	THC-COOH-d9	2.105	9433	24714	0.3817	24.2890
THC	THC-d3	4.972	5153	21263	0.2423	24.3323

ISP FORENSICS - Cd'A Instrument # 62340

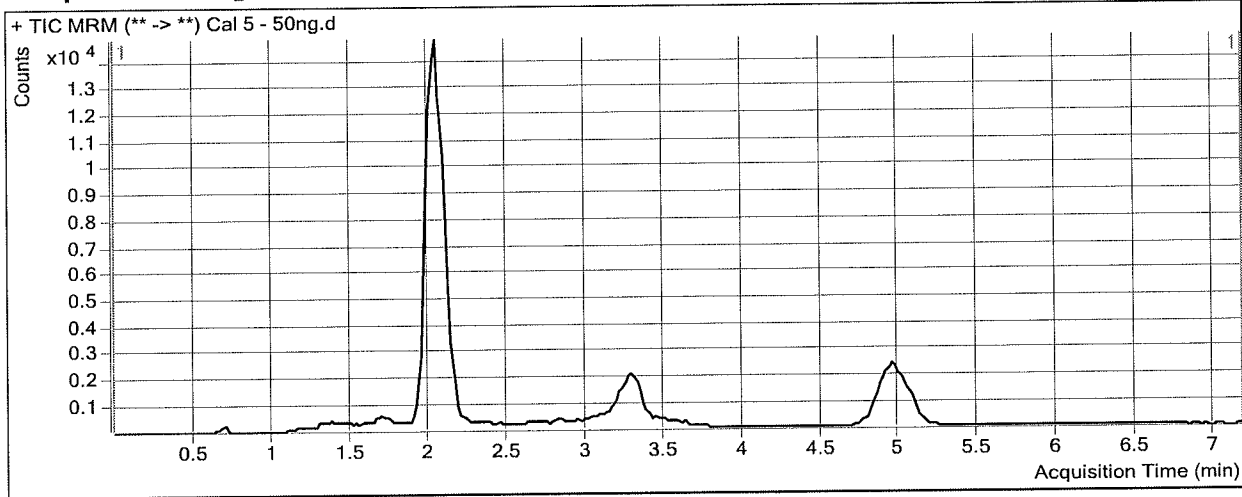
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 14:47 **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	2.035	20488	49916	0.4104	50.3141
THC-COOH	THC-COOH-d9	2.105	16467	21944	0.7504	48.3889
THC	THC-d3	4.992	9278	18857	0.4920	49.5082

ISP FORENSICS - Cd'A Instrument # 62340

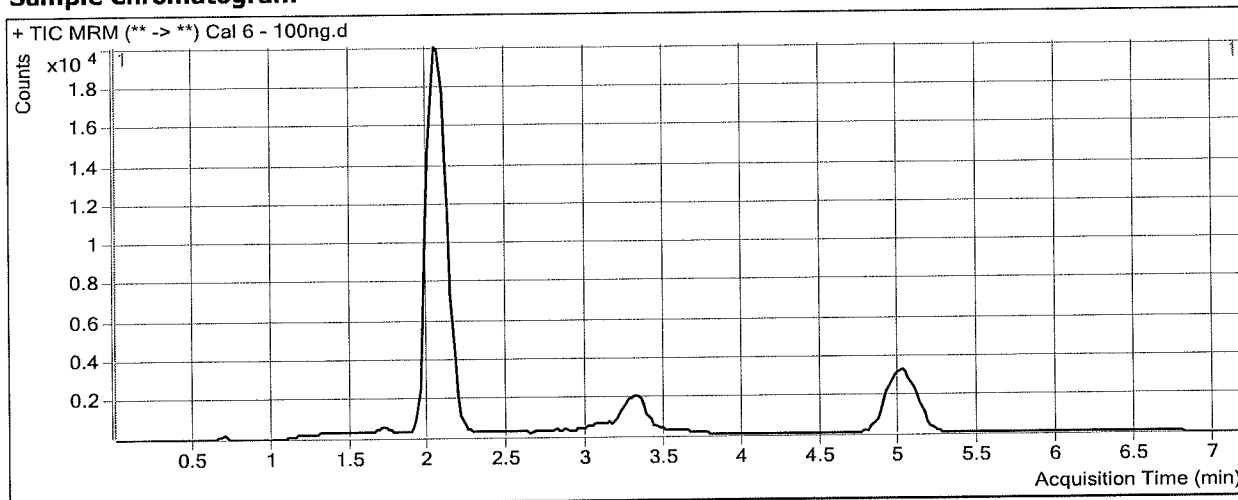
Cannabinoids Analysis Report

Batch Data Path D:\2019 Data\AM 27\011519\QuantResults\cann quant.batch.bin
Analysis Time 1/17/2019 9:50 AM **Analyst Name** datastor
Report Time 1/17/2019 10:35 AM **Reporter Name** ISP Tox
Last Calib Update 1/17/2019 9:50 AM **Batch State** Processed

Analysis Info

Acq Time 2019-01-16 14:58 **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	2.055	42980	52682	0.8158	99.8894
THC-COOH	THC-COOH-d9	2.105	35972	22896	1.5711	102.0343
THC	THC-d3	5.032	19433	19342	1.0047	101.2059