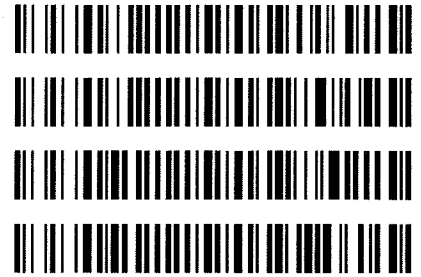


REVIEWED
By tsalazar at 9:19 am, Dec 28, 2018

12/27/2018

Worklist: 2858

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>
C2018-2442	1	135381	AM 27 Blood THC Quant by LC-QQQ
C2018-2445	2	135382	AM 27 Blood THC Quant by LC-QQQ
M2018-6026	1	135383	AM 27 Blood THC Quant by LC-QQQ
M2018-6075	1	135384	AM 27 Blood THC Quant by LC-QQQ



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

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

Extraction Date: 12/20/18

Analyst: Anne Nord

Plate lot#: 0539904

Plate Expiration: 09/10/2019

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

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: 122118 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

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

ISP FORENSICS - Cd'A Instrument # 62340

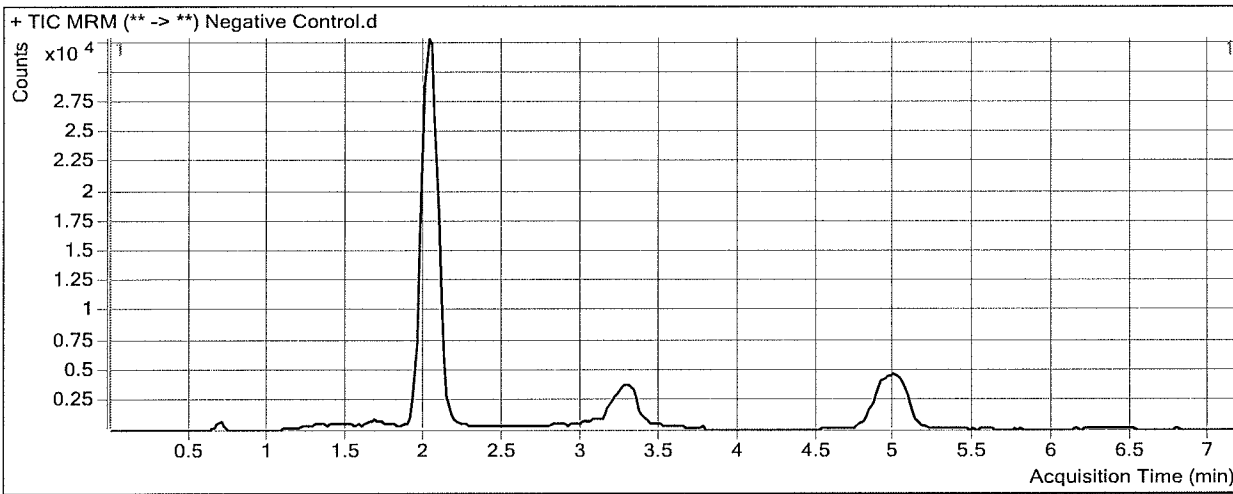
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 14:21 **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-COOH	THC-COOH-d9	2.045	1654	65787	0.0251	0.6152

ISP FORENSICS - Cd'A Instrument # 62340

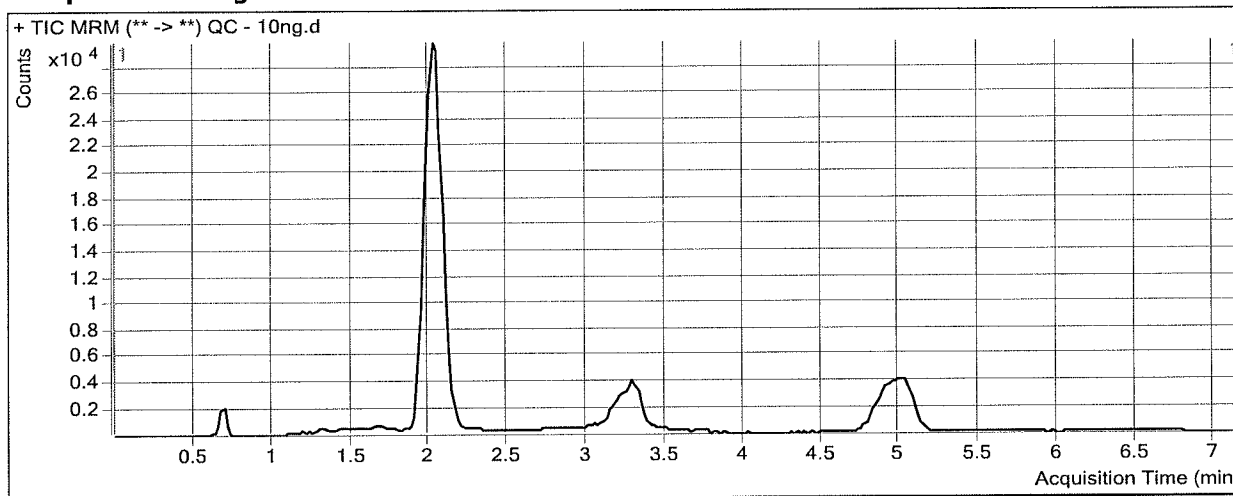
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 14:33 **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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.035	6163	155598	0.0396	4.9182
THC-COOH	THC-COOH-d9	2.085	10534	57699	0.1826	10.6127
THC	THC-d3	5.012	2919	57616	0.0507	4.7789

ISP FORENSICS - Cd'A Instrument # 62340

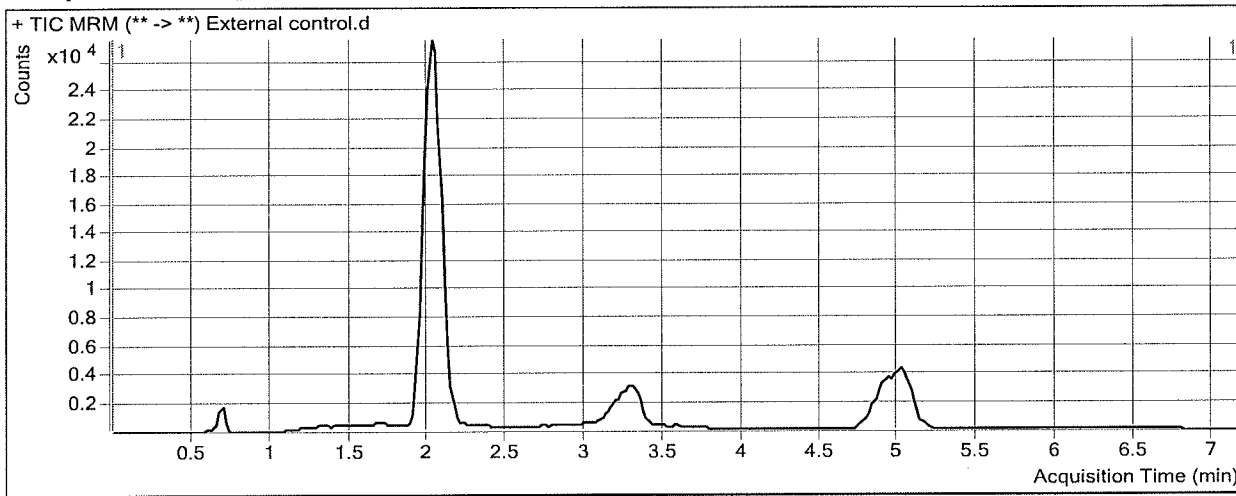
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 14:45 **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

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.055	12398	137925	0.0899	11.0157
THC-COOH	THC-COOH-d9	2.105	9616	51587	0.1864	10.8572
THC	THC-d3	5.012	6935	52074	0.1332	12.8562

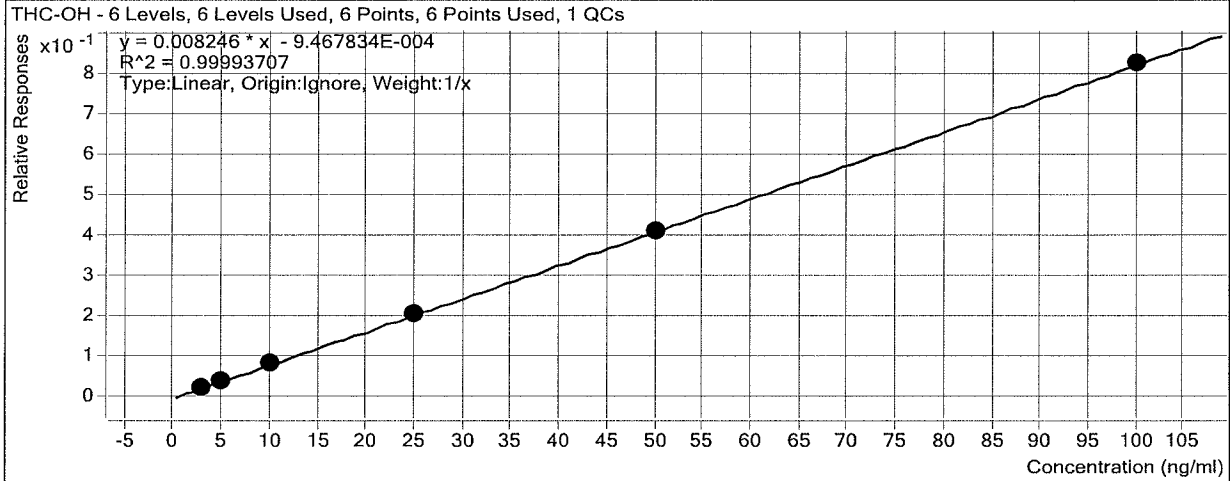
ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
12/27/2018 8:51 AM

Analyst Name ISP TOX

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



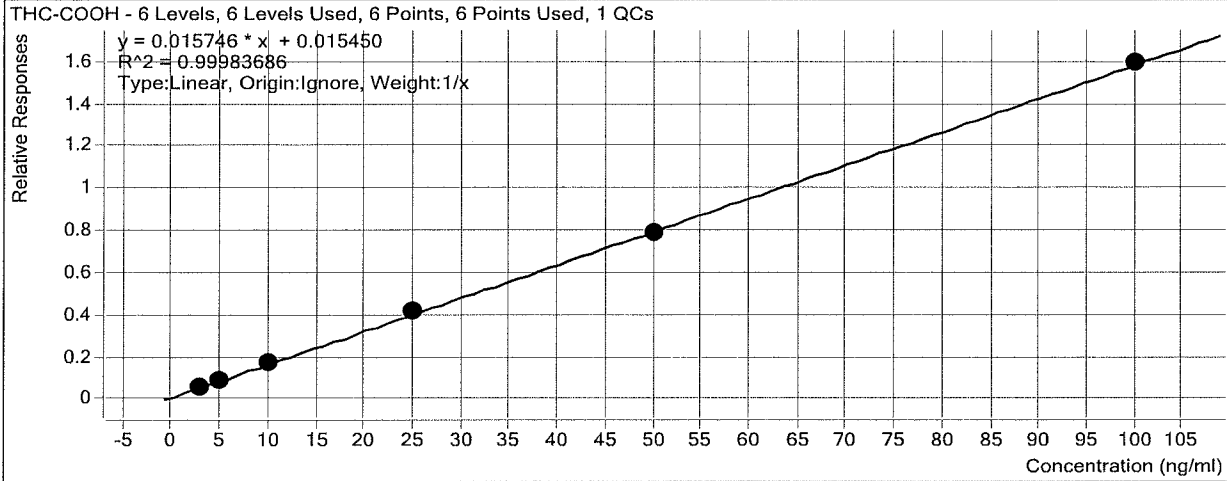
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Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.0	101.1
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	4.9	97.3
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.1	101.3
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	25.2	100.9
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.7	99.4
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	100.0	100.0
QC - 10ng	7	<input checked="" type="checkbox"/>	5	4.9	98.4

ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
12/27/2018 8:51 AM **Analyst Name** ISP TOX

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



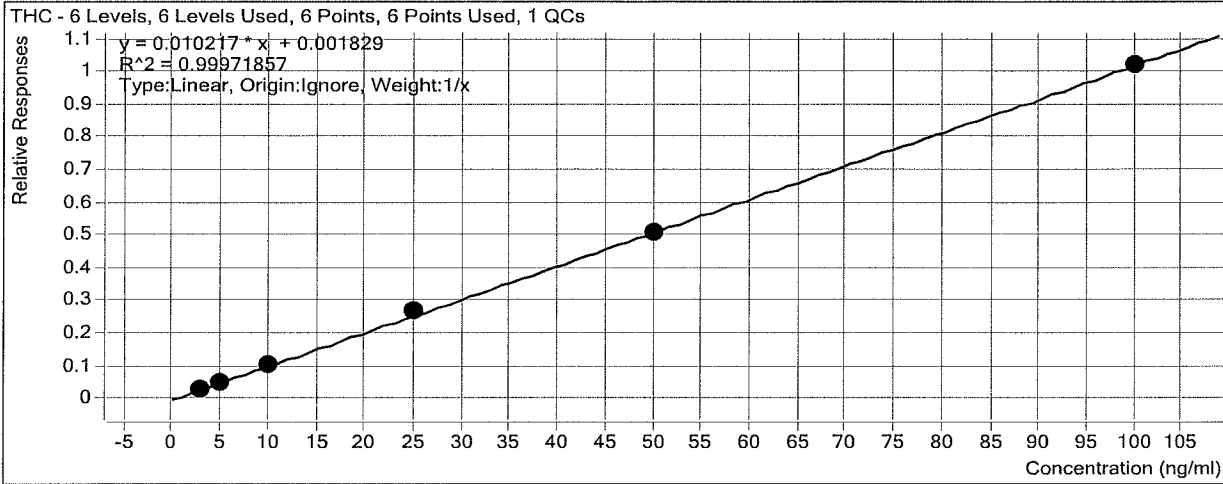
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	2.9	98.1
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.0	100.4
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.1	100.7
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	25.5	102.0
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.3	98.5
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	100.2	100.2
QC - 10ng	7	<input checked="" type="checkbox"/>	10	10.6	106.1

ISP Forensics Calibration Curve Report

Batch Data Path
Last Calib Update

D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
12/27/2018 8:51 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	3.0	98.9
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	4.9	98.6
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.0	100.1
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	25.9	103.7
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.6	99.1
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	99.6	99.6
QC - 10ng	7	<input checked="" type="checkbox"/>	5	4.8	95.6

ISP FORENSICS - Cd'A Instrument # 62340

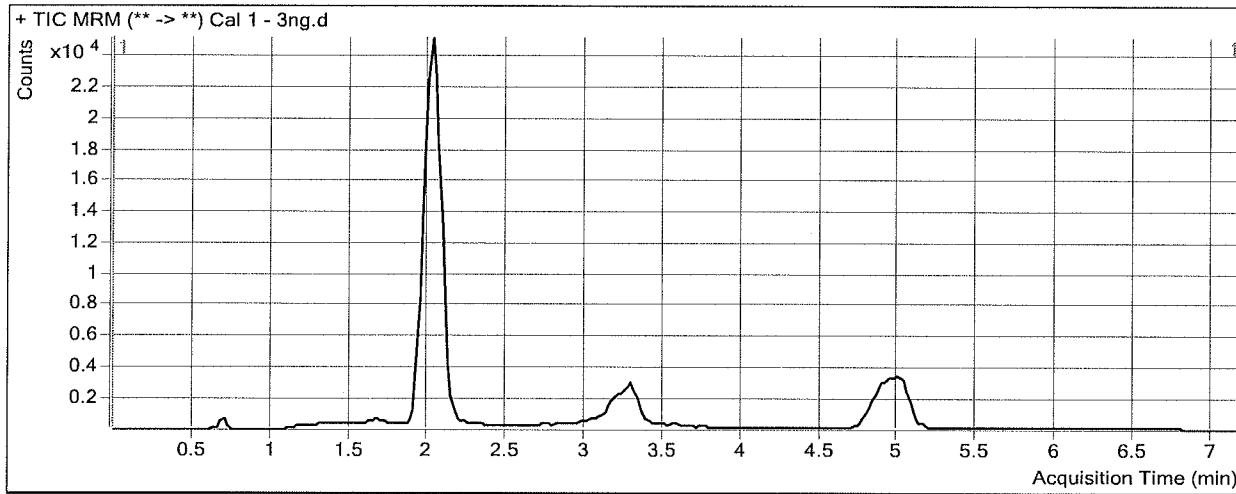
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 12:58 **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	3181	132240	0.0241	3.0323
THC-COOH	THC-COOH-d9	2.125	3193	51681	0.0618	2.9424
THC	THC-d3	4.972	1594	49595	0.0321	2.9671

ISP FORENSICS - Cd'A Instrument # 62340

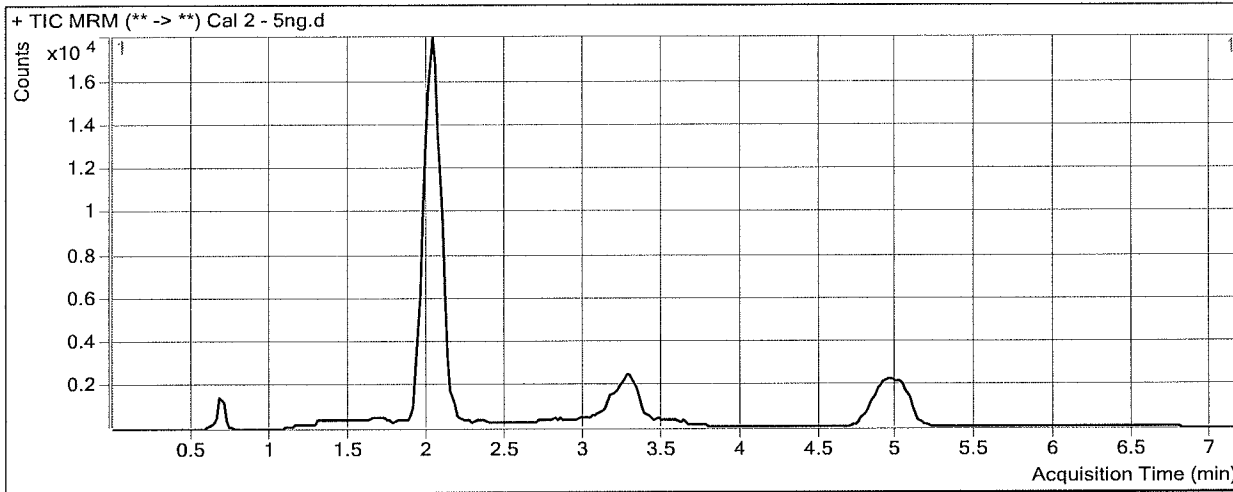
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 13:10 **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	3637	92846	0.0392	4.8656
THC-COOH	THC-COOH-d9	2.105	3412	36092	0.0945	5.0222
THC	THC-d3	4.992	1703	32623	0.0522	4.9317

ISP FORENSICS - Cd'A Instrument # 62340

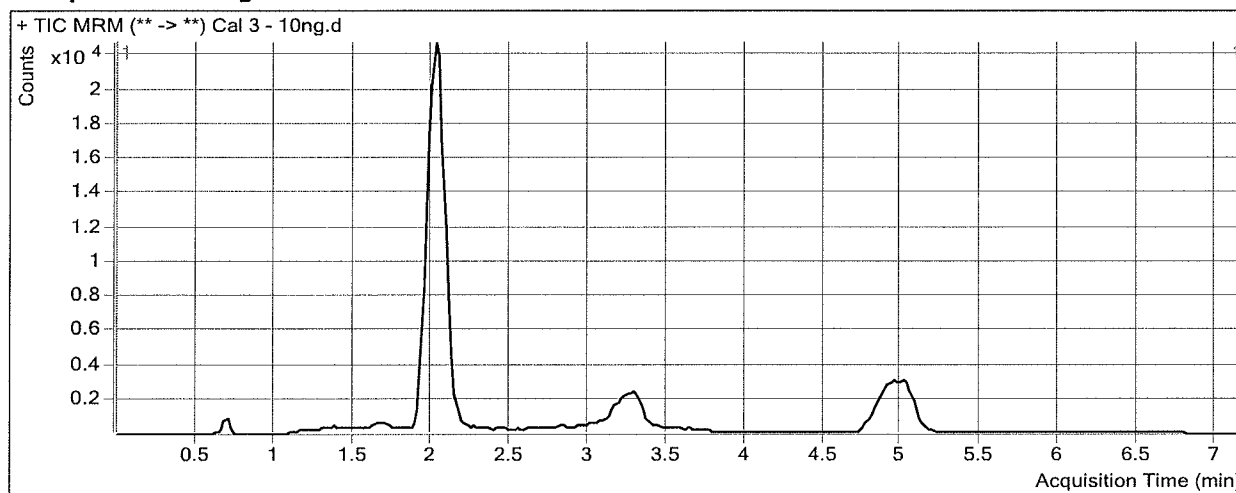
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 13:22 **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	2.055	9432	114241	0.0826	10.1271
THC-COOH	THC-COOH-d9	2.085	7667	44047	0.1741	10.0730
THC	THC-d3	4.992	4357	41868	0.1041	10.0070

ISP FORENSICS - Cd'A Instrument # 62340

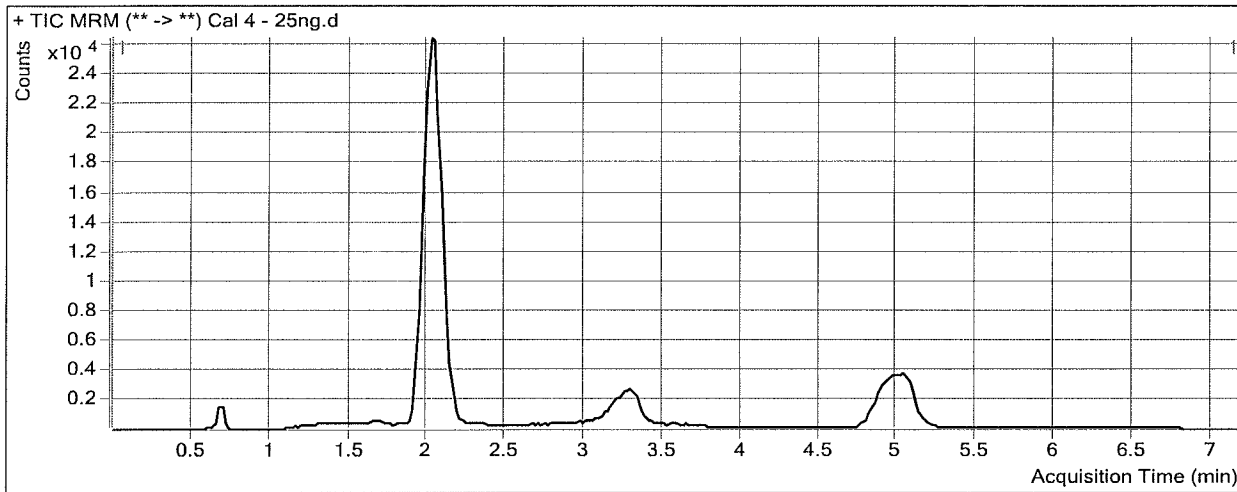
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 13:34 **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	24409	117934	0.2070	25.2134
THC-COOH	THC-COOH-d9	2.105	18387	44085	0.4171	25.5059
THC	THC-d3	5.012	11293	42361	0.2666	25.9127

ISP FORENSICS - Cd'A Instrument # 62340

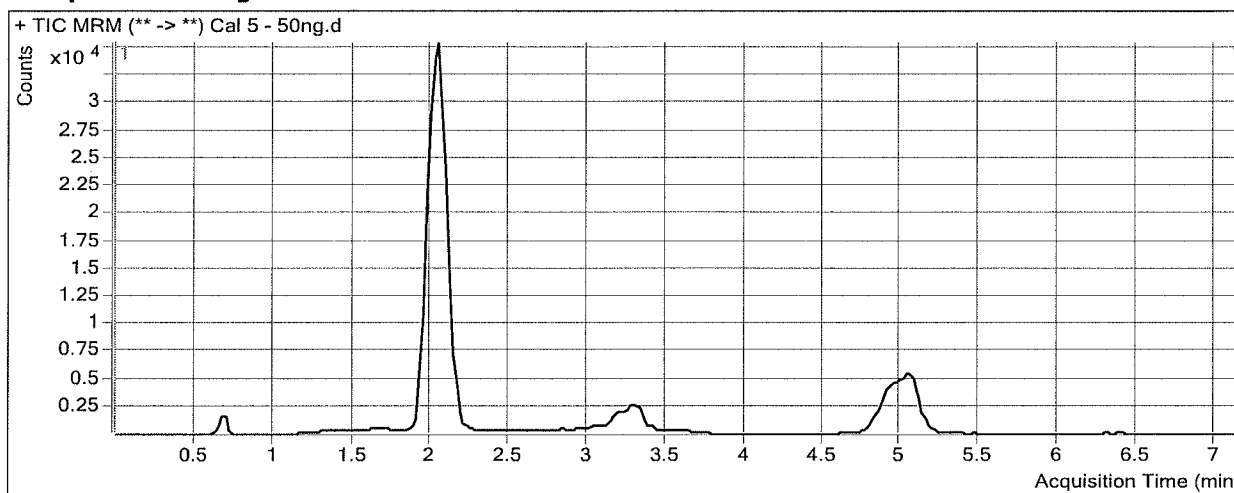
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 13:45 **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	54321	132783	0.4091	49.7246
THC-COOH	THC-COOH-d9	2.105	38877	49137	0.7912	49.2652
THC	THC-d3	5.032	24258	47734	0.5082	49.5599

ISP FORENSICS - Cd'A Instrument # 62340

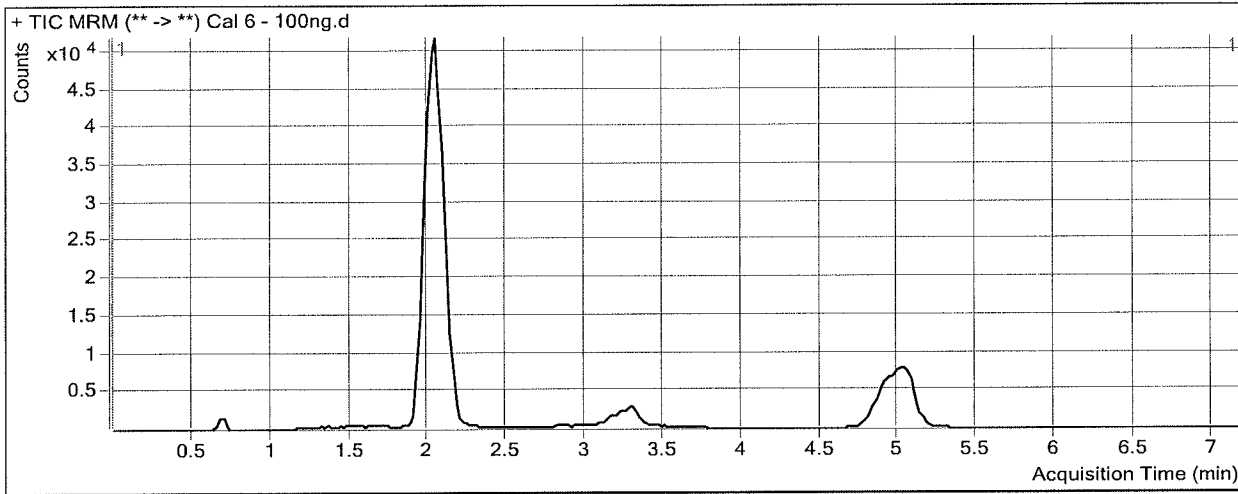
Cannabinoids Analysis Report

Batch Data Path D:\2018 Data\122118 cann quant\QuantResults\cann quant.batch.bin
Analysis Time 12/27/2018 8:51 AM **Analyst Name** ISP Tox
Report Time 12/27/2018 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 12/27/2018 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2018-12-21 13:57 **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	2.035	118122	143354	0.8240	100.0370
THC-COOH	THC-COOH-d9	2.105	81267	51012	1.5931	100.1912
THC	THC-d3	5.012	50325	49353	1.0197	99.6216