

reviewed 11/22/17

11/22/2017

BWylee

Worklist: 2041

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>	
C2017-2148	1	100789	AM 27 Blood THC Quant by LC	
C2017-2174	1	100788	AM 27 Blood THC Quant by LC	
C2017-2242	1	100790	AM 27 Blood THC Quant by LC	
C2017-2255	1	100791	AM 27 Blood THC Quant by LC	
C2017-2308	1	100787	AM 27 Blood THC Quant by LC	
M2017-4559	1	100792	AM 27 Blood THC Quant by LC	
M2017-4595	1	100793	AM 27 Blood THC Quant by LC	
M2017-4757	1	100794	AM 27 Blood THC Quant by LC	



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

Extraction Date: 11-21-17

Analyst: Anne Norz

Plate lot#: 0515037

Plate Expiration: 9/28/18

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 17J20718

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: 2609543** 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: 2017 Data\ 112117 can quant Batch Name: 112117 can quant
- 2. Make any necessary integration changes, r^2 values ≥ 0.98 for each analyte.
- 3. Did all QCs pass for each analyte? Y/N Enter QCs into control charting?
- 4. 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 9880 ul meOH lot (Fisher 168427)
Ppd 8/17/17 Exp: 2/17/18 lot 21718 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 100 ul working solution lot (21717) in 9990 ul blood lot (321632)
ppd 8/17/17 Exp 2/17/18 by AMN Concentration 10 ng/ml each



ISP FORENSICS - Cd'A Instrument # 62340

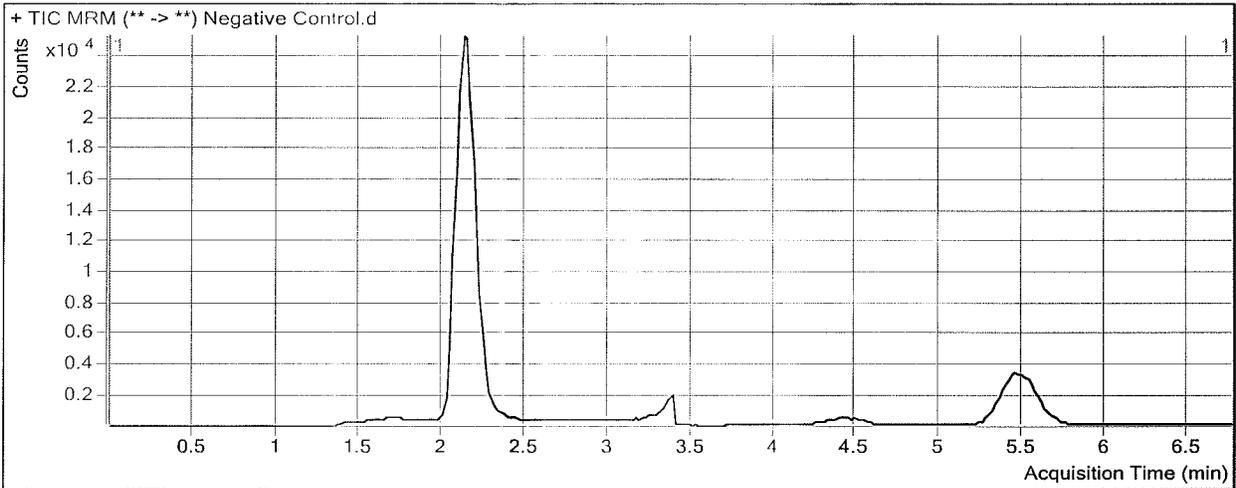
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 16:39 **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

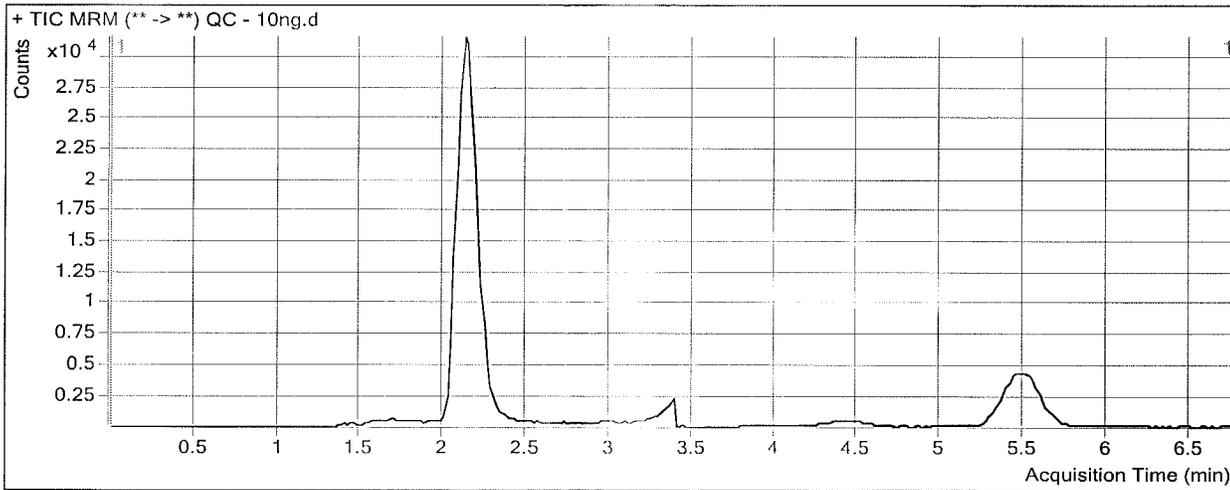
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 16:51 **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	2.135	15024	172584	0.0871	9.3605
THC-COOH	THC-COOH-d9	2.225	10227	64719	0.1580	9.0742
THC	THC-d3	5.532	6765	61761	0.1095	9.9518

ISP FORENSICS - Cd'A Instrument # 62340

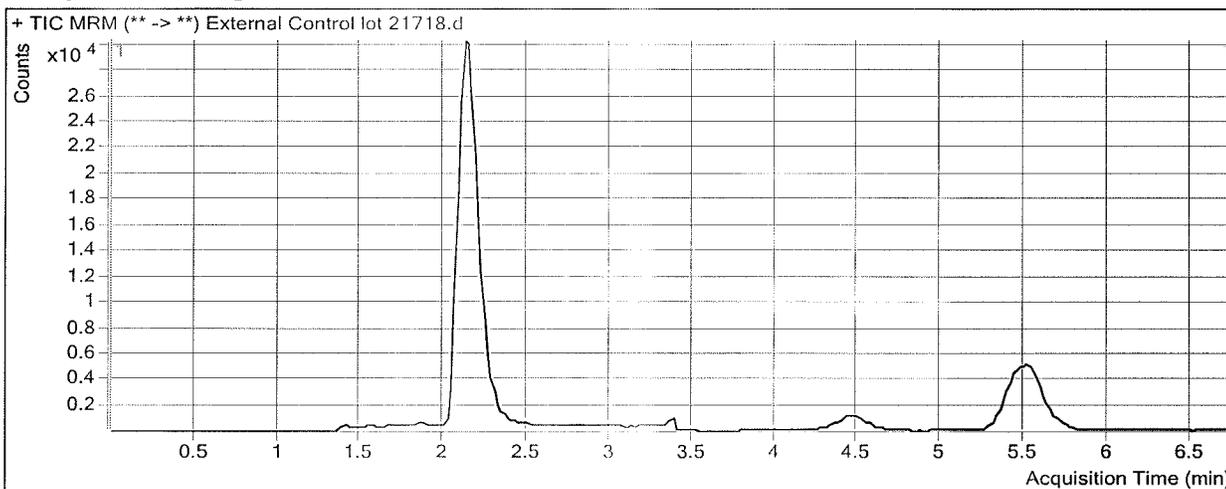
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 17:03 **Data File** External Control lot 21718.d
Sample Type Sample **Sample Name** External Control lot 21718
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

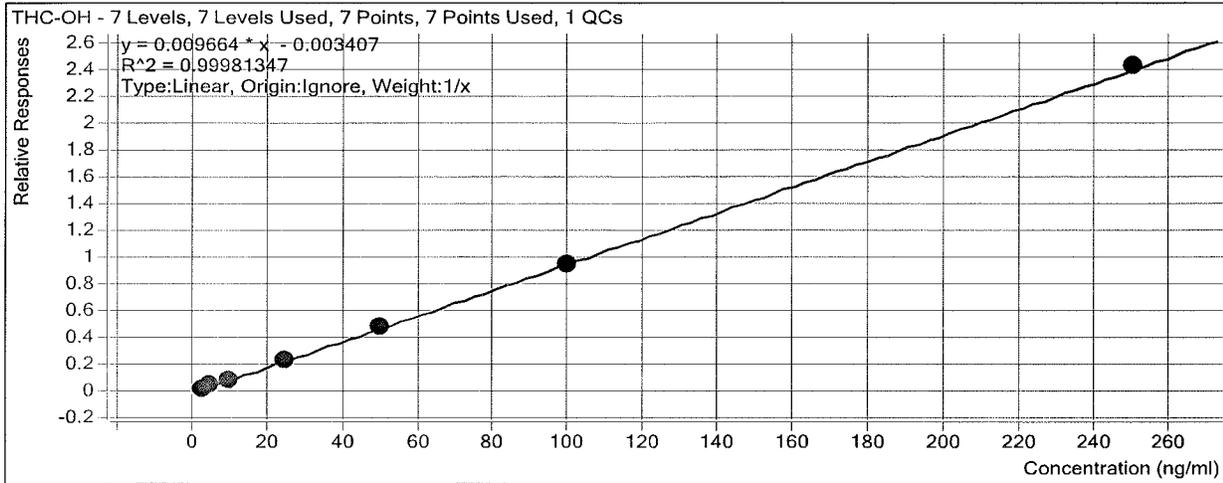
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.135	12475	161353	0.0773	8.3530
THC-COOH	THC-COOH-d9	2.225	14286	60040	0.2379	13.4338
THC	THC-d3	5.552	7334	66231	0.1107	10.0598

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin

Last Calib Update 11/22/2017 9:31 AM **Analyst Name** ISP TOX

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



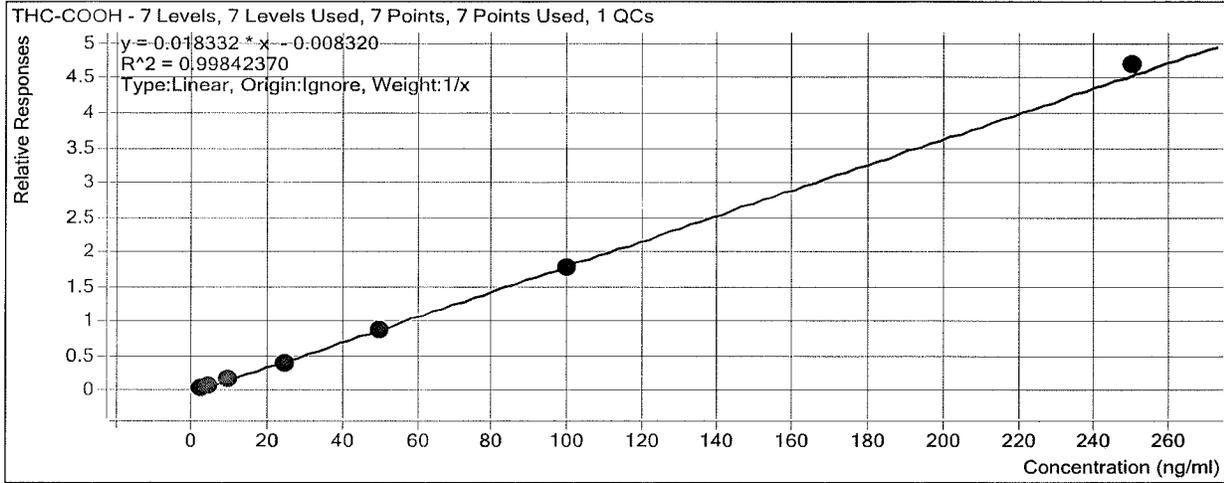
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.1	102.1
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.1	101.7
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.0	100.1
QC - 10ng	3	<input checked="" type="checkbox"/>	10	9.4	93.6
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	23.9	95.8
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	50.3	100.7
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	99.0	99.0
Cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	251.6	100.6

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin

Last Calib Update 11/22/2017 9:31 AM **Analyst Name** ISP TOX

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



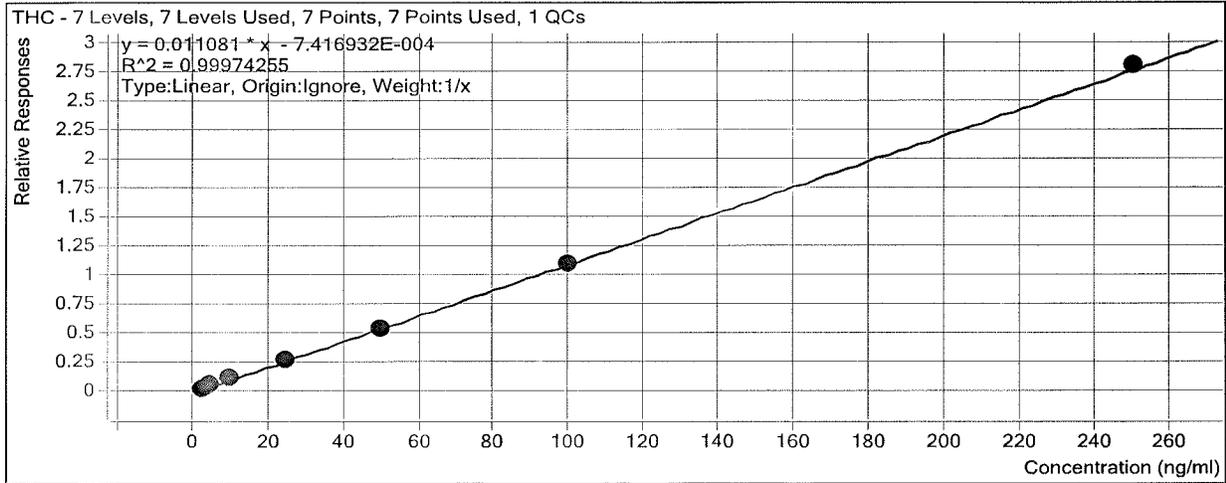
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.5	115.8
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.0	100.2
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	9.6	95.9
QC - 10ng	3	<input checked="" type="checkbox"/>	10	9.1	90.7
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	22.7	90.9
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.0	97.9
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	96.8	96.8
Cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	256.5	102.6

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin

Last Calib Update 11/22/2017 9:31 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	99.9
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.2	103.1
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.2	102.5
QC - 10ng	3	<input checked="" type="checkbox"/>	10	10.0	99.5
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	24.0	95.9
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.4	98.9
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	98.8	98.8
Cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	252.4	100.9

ISP FORENSICS - Cd'A Instrument # 62340

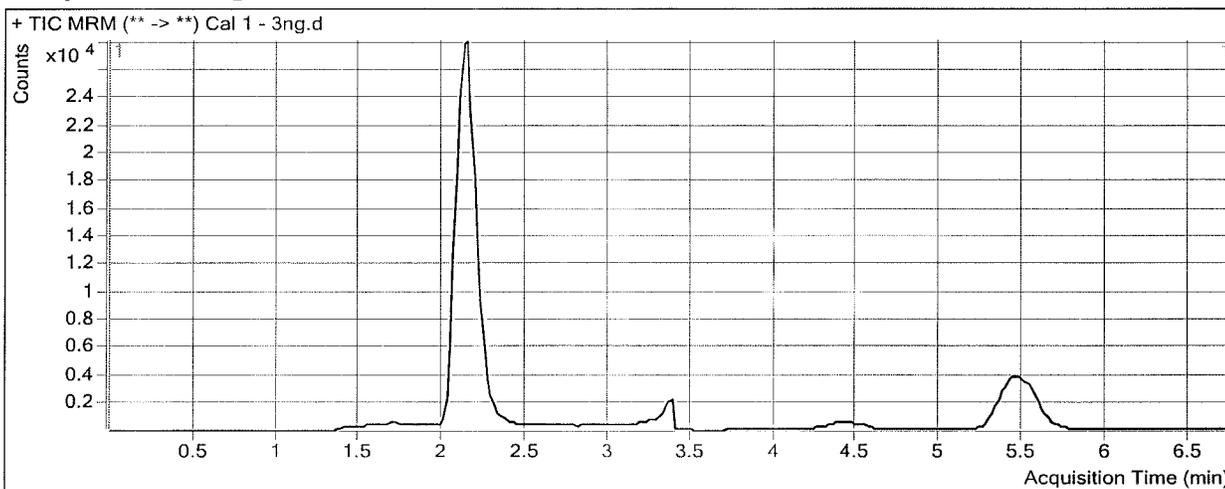
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 15:04 **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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.135	4323	165019	0.0262	3.0635
THC-COOH	THC-COOH-d9	2.225	3242	58572	0.0554	3.4737
THC	THC-d3	5.532	1933	59565	0.0325	2.9958

ISP FORENSICS - Cd'A Instrument # 62340

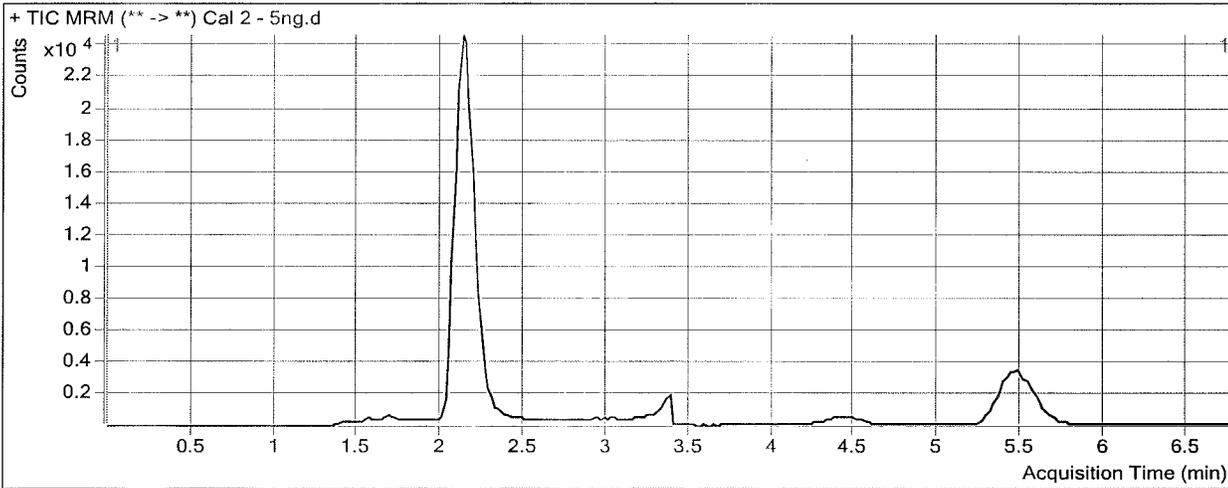
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 15:16 **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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.155	6272	137070	0.0458	5.0871
THC-COOH	THC-COOH-d9	2.225	4212	50451	0.0835	5.0079
THC	THC-d3	5.552	2746	48681	0.0564	5.1569

ISP FORENSICS - Cd'A Instrument # 62340

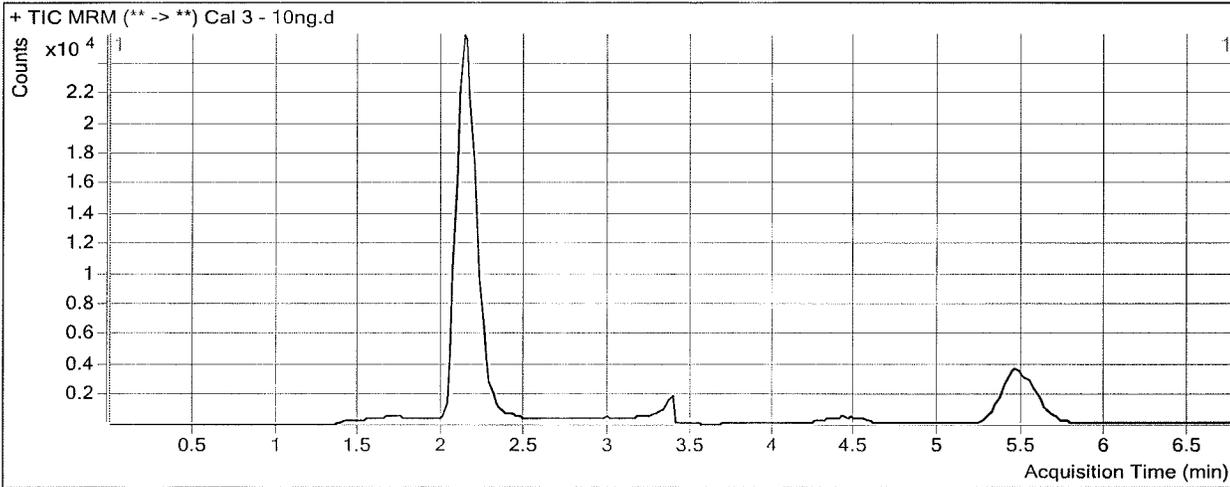
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 15:28 **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	2.135	12802	137209	0.0933	10.0071
THC-COOH	THC-COOH-d9	2.225	8587	51297	0.1674	9.5851
THC	THC-d3	5.532	5320	47147	0.1128	10.2493

ISP FORENSICS - Cd'A Instrument # 62340

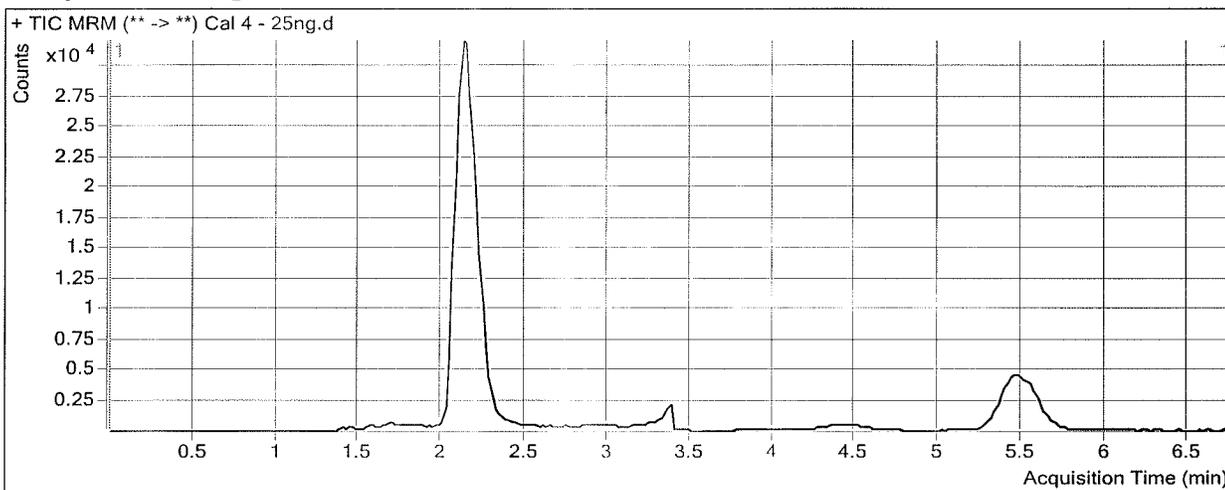
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 15:40 **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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.135	35040	153664	0.2280	23.9483
THC-COOH	THC-COOH-d9	2.225	23405	57313	0.4084	22.7303
THC	THC-d3	5.552	14253	53825	0.2648	23.9639

ISP FORENSICS - Cd'A Instrument # 62340

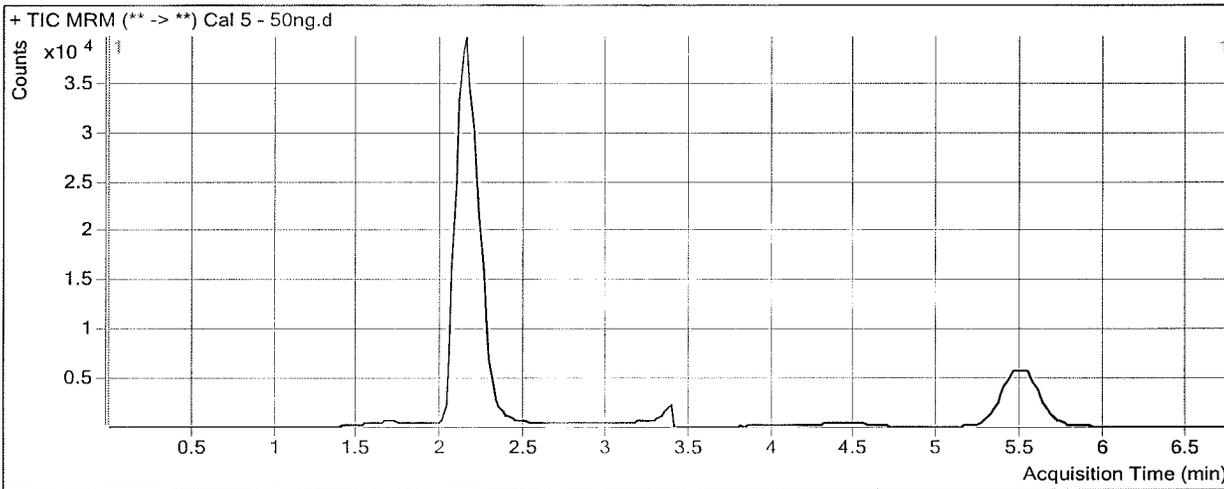
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 15:52 **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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.135	75524	156359	0.4830	50.3331
THC-COOH	THC-COOH-d9	2.225	51042	57410	0.8891	48.9533
THC	THC-d3	5.532	29172	53324	0.5471	49.4372

ISP FORENSICS - Cd'A Instrument # 62340

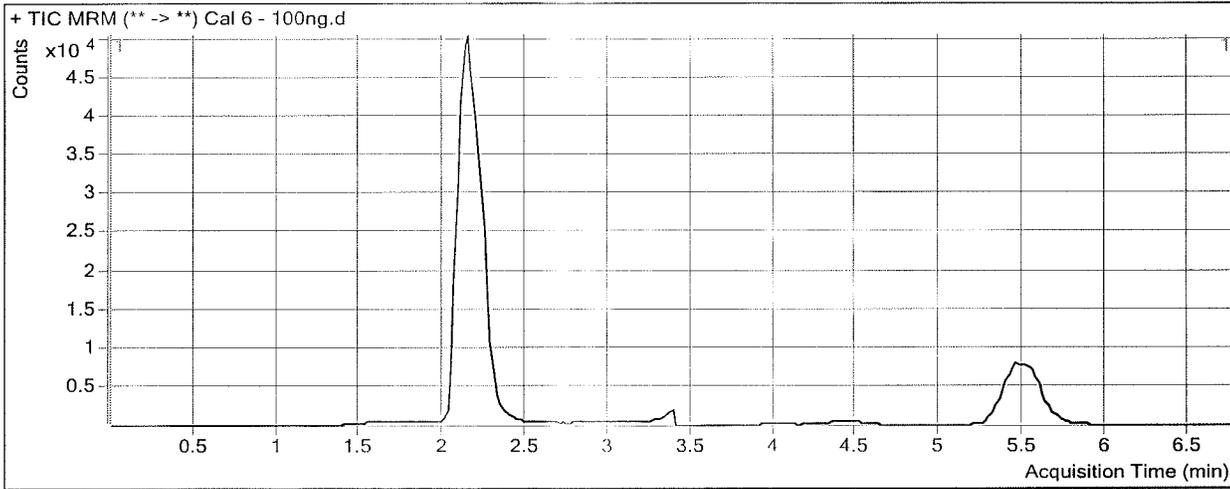
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 16:04 **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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.135	141550	148509	0.9531	98.9802
THC-COOH	THC-COOH-d9	2.225	94378	53442	1.7660	96.7893
THC	THC-d3	5.532	57659	52682	1.0945	98.8366

ISP FORENSICS - Cd'A Instrument # 62340

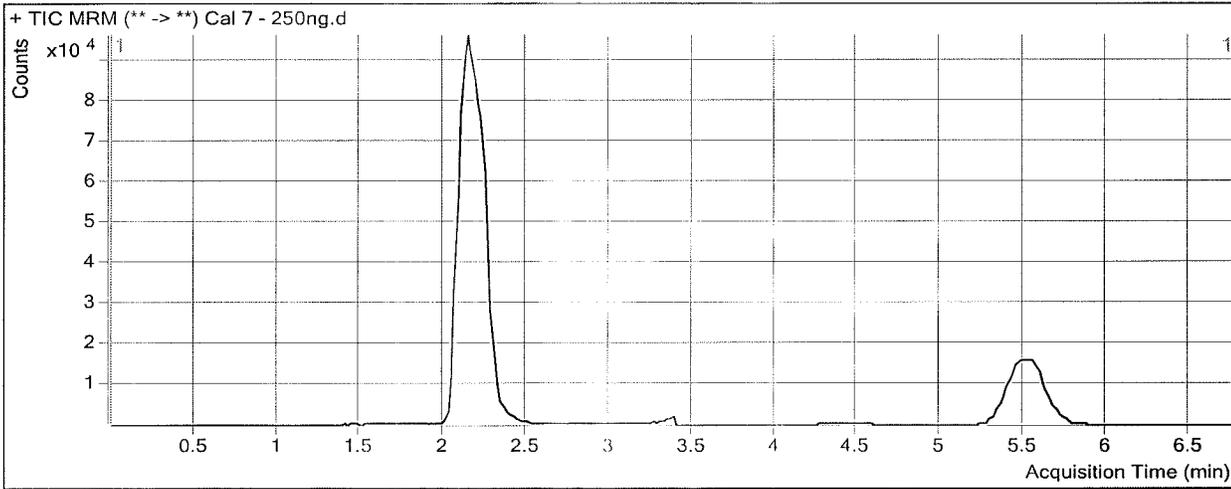
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\112117 cann quant\QuantResults\112117 cann quant.batch.bin
Analysis Time 11/22/2017 9:31 AM **Analyst Name** ISP Tox
Report Time 11/22/2017 9:32 AM **Reporter Name** ISP Tox
Last Calib Update 11/22/2017 9:31 AM **Batch State** Processed

Analysis Info

Acq Time 2017-11-21 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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.135	395539	162915	2.4279	251.5808
THC-COOH	THC-COOH-d9	2.225	255878	54523	4.6930	256.4604
THC	THC-d3	5.532	156749	56068	2.7957	252.3604