






Worklist: 1418

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>	
C2016-1754	1	72058	AM 27 Blood THC Quant by LC	
C2016-1781	1	72059	AM 27 Blood THC Quant by LC	
C2016-2132	1	72060	AM 27 Blood THC Quant by LC	
M2016-2899	1	72061	AM 27 Blood THC Quant by LC	
M2016-3017	1	72062	AM 27 Blood THC Quant by LC	
M2016-3056	1	72063	AM 27 Blood THC Quant by LC	
M2016-3383	1	72064	AM 27 Blood THC Quant by LC	
M2016-3637	2	72065	AM 27 Blood THC Quant by LC	
M2016-3805	1	72066	AM 27 Blood THC Quant by LC	
M2016-3832	2	72067	AM 27 Blood THC Quant by LC	
M2016-3956	1	72068	AM 27 Blood THC Quant by LC	
M2016-4125	1	72069	AM 27 Blood THC Quant by LC	
M2016-4622	1	72070	AM 27 Blood THC Quant by LC	
P2016-1778	1	72071	AM 27 Blood THC Quant by LC	
P2016-1832	1	72072	AM 27 Blood THC Quant by LC	
P2016-1880	1	72073	AM 27 Blood THC Quant by LC	
P2016-1889	1	72074	AM 27 Blood THC Quant by LC	
P2016-1979	1	72075	AM 27 Blood THC Quant by LC	
P2016-2118	1	72076	AM 27 Blood THC Quant by LC	
P2016-2143	1	72077	AM 27 Blood THC Quant by LC	
P2016-2161	1	72078	AM 27 Blood THC Quant by LC	
P2016-2171	3	72079	AM 27 Blood THC Quant by LC	
P2016-2246	1	72080	AM 27 Blood THC Quant by LC	

Worklist: 1418

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>	
P2016-2356	1	72081	AM 27 Blood THC Quant by LC	
P2016-2489	1	72082	AM 27 Blood THC Quant by LC	
P2016-2490	1	72083	AM 27 Blood THC Quant by LC	
P2016-2631	1	72084	AM 27 Blood THC Quant by LC	
P2016-2634	1	72085	AM 27 Blood THC Quant by LC	



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

Date: 12-13-16 Analyst: Anne Nord

PRE-ANALYTIC

Plate Lot# 0490364 Plate Exp. 9-21-17

- ✓ 1. Ensure all solutions are within expiration date.

Mobile Phase A: <i>0.1% Formic Acid in LCMS Water</i>	Prep Date: <u>NA</u>	<i>0.1% Formic Acid in water</i>	Prep Date: <u>NA</u>
Mobile Phase B: <i>0.1% Formic Acid in LCMS Acetonitrile</i>	Prep Date: <u>NA</u>	<i>MTBE</i>	Prep Date: <u>NA</u>
<i>LCMS Methanol</i>	Open Date: <u>NA</u>	<i>Hexane</i>	Open Date: <u>NA</u>

Column: UCT Selectra DA 100 x 2.1 mm 3um

- ✓ 2. Check levels of mobile phases and needle wash and refill as necessary. Ensure waste is not full.
- ✓ 3. Purge Pump and Load appropriate Acq. Method, allow system to equilibrate for approx. 30 min. Is pressure stable? Y / N
- ✓ 4. Was maintenance performed on the instrument? Y / N If yes, explain: _____
5. Create worklist. Data path name: 121316 Quant.cann

ANALYTIC

- ✓ 1. Remove standards plate, blood, and samples from cold storage. Allow to reach room temperature.
- ✓ 2. Add **1000 µL blood** to wells of analytical (standards) plate. Mix via aspirate and dispense. Place cover on Plate
Blank blood for locations containing standards/QCs and internal standards (Blank Blood Used _____)
Sample blood for locations containing only internal standards
- ✓ 3. Place on shaking incubator at ambient temp., **900rpm for 15 minutes**. Shaker ID _____
- ✓ 4. Pipette **500µL 0.1% formic acid** to all wells of standards 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). Wait 5 min.
(Load blood samples at 85- 100 PSI- Selector to Right)
- ✓ 8. Add **2.25mL MTBE** and allow to flow under gravity for 5 minutes. **(add in 3 increments of 750uL)**
- ✓ 9. Apply positive pressure for approx. 15 seconds **(10-15 PSI- Selector to left -)**
- ✓ 10. Add **2.25mL Hexane** and allow to flow under gravity for 5 minutes. **(add in 3 increments of 750uL)**
- ✓ 11. Apply positive pressure for approx. 15 seconds. **(10-15 PSI Selector to the left)**
- ✓ 12. Remove collection plate containing eluate.
- ✓ 13. Place collection plate on SPE Dry and evaporate to dryness at approx. 35°C. SPE Dry ID _____
- ✓ 14. Reconstitute in **100 µL MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

POST-ANALYTIC

- ✓ 1. Open quantitation software and create a new quantitation batch. Batch name: 121316 quant.cann
- ✓ 2. Make any necessary integration changes. Limit curves based on validated linear ranges (3-250ng/mL).
- ✓ 3. Were all appropriate standards used in the curve for each analyte? Y / N
Are r² values ≥ 0.98 for each analyte? Y / N
- ✓ 4. For unknown samples, are the response ratios for each analyte within ± 20% of the average ratios of the standards and QC's? Y / N - positive samples
- ✓ 5. Did all QCs pass for each analyte? Y / N Were QCs entered into QC charting? Y / N
6. Central File Packet to include: LIMS Worklist: Method Checklist Calibration and Control Reports

COMMENTS



ISP FORENSICS - Cd'A Instrument # 62340

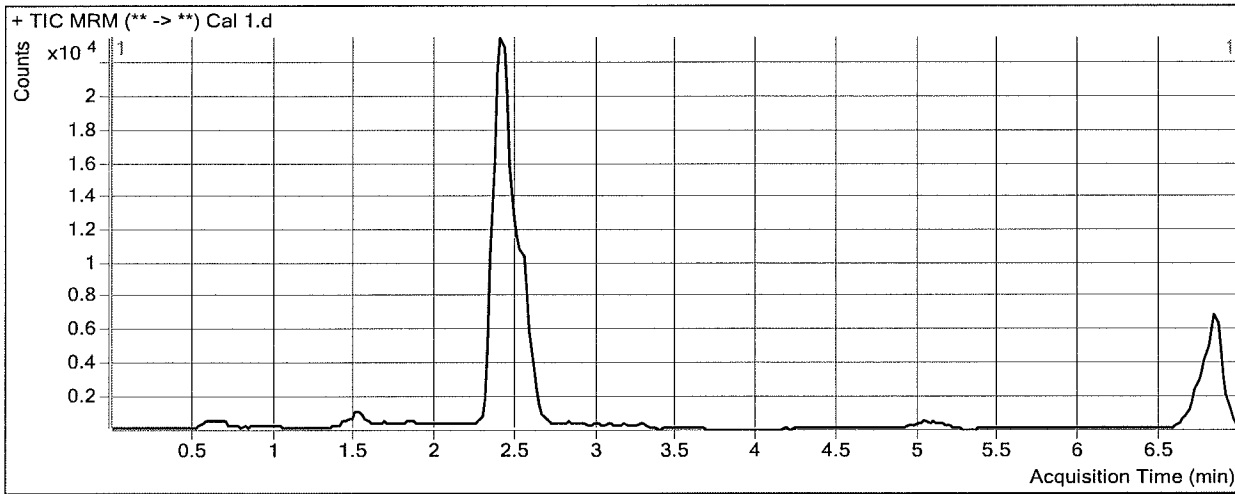
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 09:11 **Data File** Cal 1.d
Sample Type Calibration **Sample Name** Cal 1
Dilution 1 **Acq Method** Quant THC.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	2.436	4010	167721	0.0239	3.5718
THC-COOH	THC-COOH-d9	2.586	3006	63076	0.0477	3.4660
THC	THC-d3	6.853	1418	62077	0.0228	3.5154

ISP FORENSICS - Cd'A Instrument # 62340

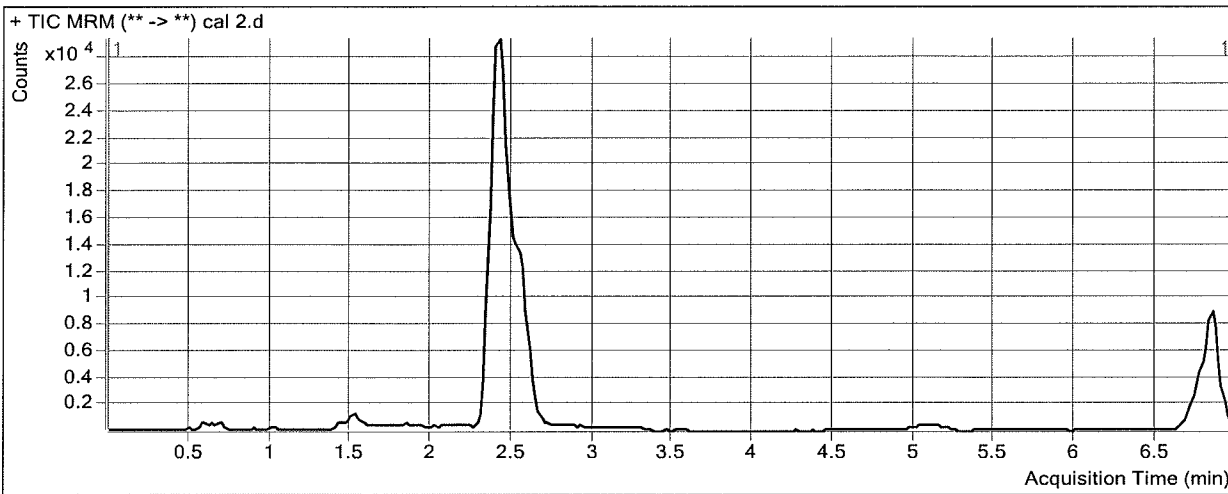
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 09:23 **Data File** cal 2.d
Sample Type Calibration **Sample Name** cal 2
Dilution 1 **Acq Method** Quant THC.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	2.436	8528	204579	0.0417	4.8408
THC-COOH	THC-COOH-d9	2.606	6785	74852	0.0906	5.1631
THC	THC-d3	6.853	3183	72216	0.0441	4.9589

ISP FORENSICS - Cd'A Instrument # 62340

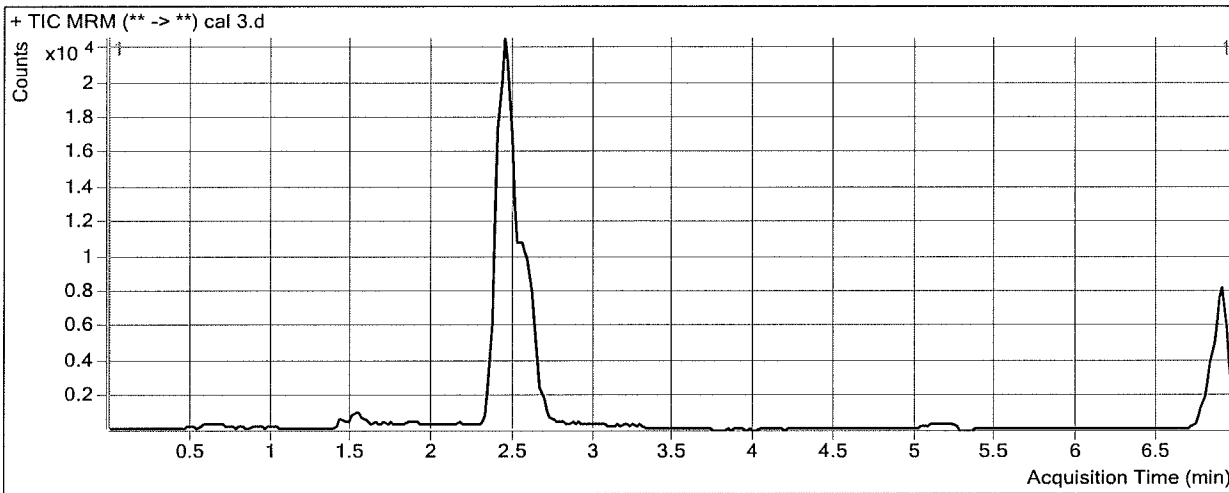
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 09:34 **Data File** cal 3.d
Sample Type Calibration **Sample Name** cal 3
Dilution 1 **Acq Method** Quant THC.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	2.456	14493	149080	0.0972	8.8053
THC-COOH	THC-COOH-d9	2.626	10141	55088	0.1841	8.8524
THC	THC-d3	6.914	5395	53779	0.1003	8.7828

ISP FORENSICS - Cd'A Instrument # 62340

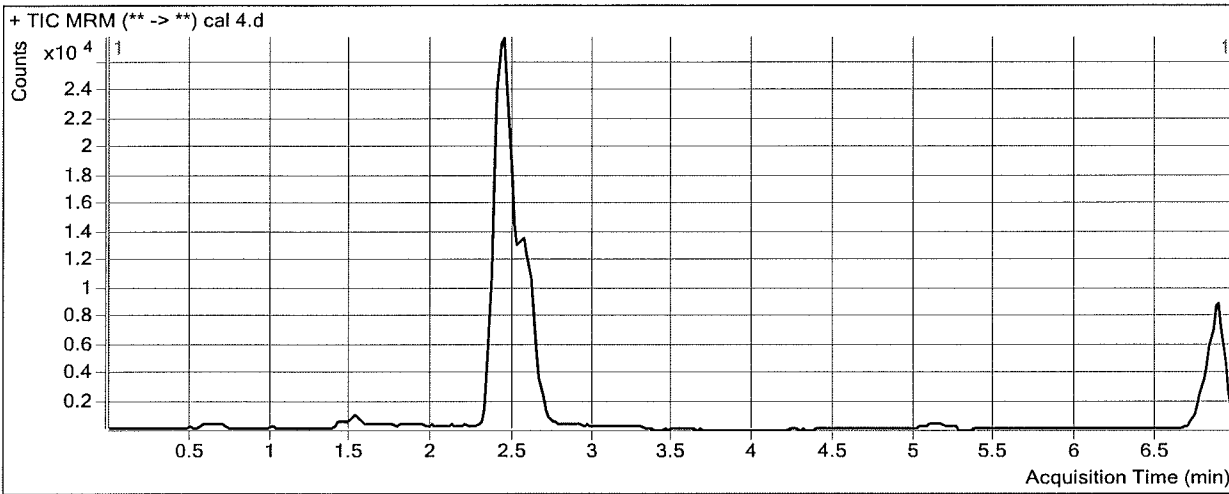
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 09:46 **Data File** cal 4.d
Sample Type Calibration **Sample Name** cal 4
Dilution 1 **Acq Method** Quant THC.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	2.456	35128	164038	0.2141	17.1527
THC-COOH	THC-COOH-d9	2.606	22717	60081	0.3781	16.5120
THC	THC-d3	6.894	13100	55687	0.2352	17.9543

ISP FORENSICS - Cd'A Instrument # 62340

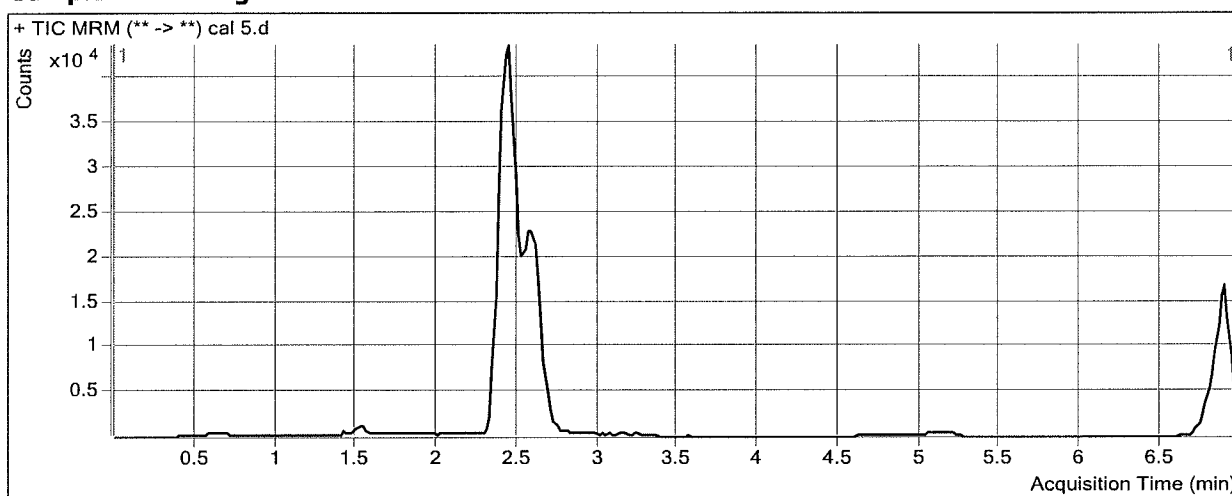
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 09:58 **Data File** cal 5.d
Sample Type Calibration **Sample Name** cal 5
Dilution 1 **Acq Method** Quant THC.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	2.456	111901	193881	0.5772	43.0694
THC-COOH	THC-COOH-d9	2.606	70243	69622	1.0089	41.4166
THC	THC-d3	6.894	42158	70275	0.5999	42.7432

ISP FORENSICS - Cd'A Instrument # 62340

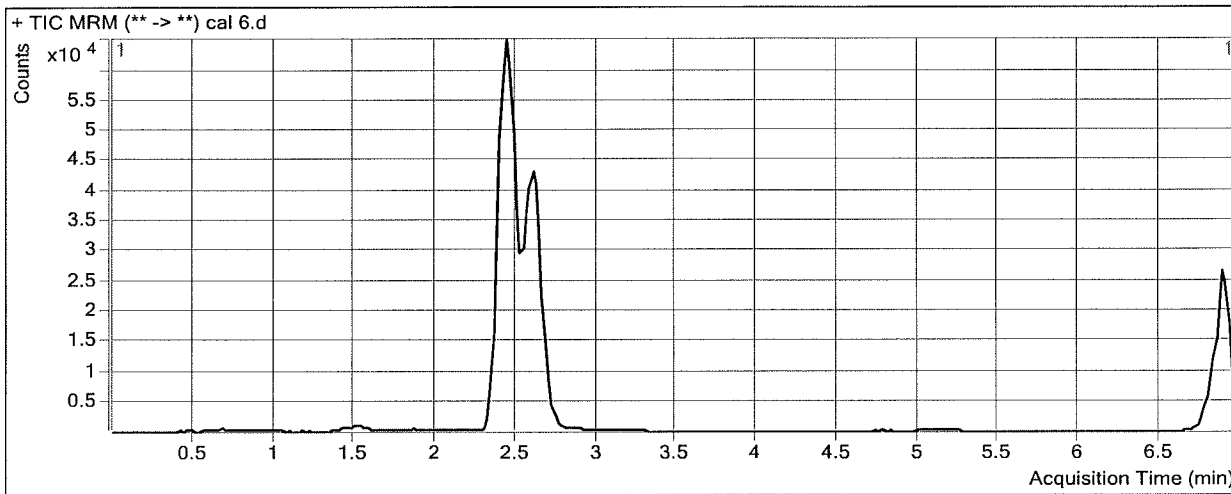
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 10:09 **Data File** cal 6.d
Sample Type Calibration **Sample Name** cal 6
Dilution 1 **Acq Method** Quant THC.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	2.456	272350	177483	1.5345	111.4159
THC-COOH	THC-COOH-d9	2.626	173558	63003	2.7548	110.3428
THC	THC-d3	6.894	94907	58694	1.6170	111.8824

ISP FORENSICS - Cd'A Instrument # 62340

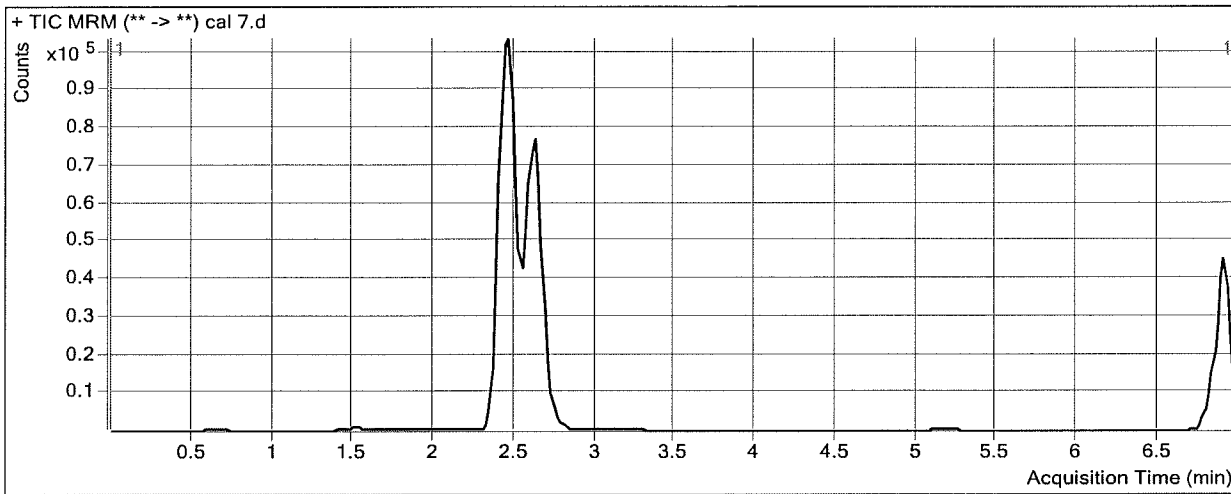
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 10:21 **Data File** cal 7.d
Sample Type Calibration **Sample Name** cal 7
Dilution 1 **Acq Method** Quant THC.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	2.476	540835	157962	3.4238	246.2969
THC-COOH	THC-COOH-d3	2.626	340597	54402	6.2608	248.7591
THC	THC-d3	6.914	177514	49424	3.5916	246.1174

ISP FORENSICS - Cd'A Instrument # 62340

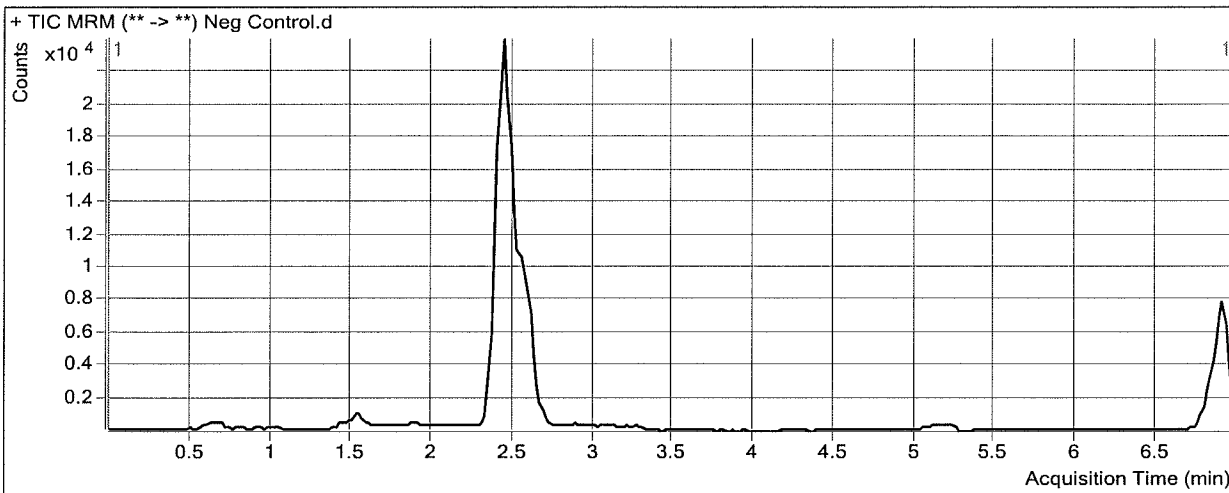
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 10:44 **Data File** Neg Control.d
Sample Type Sample **Sample Name** Neg Control
Dilution 1 **Acq Method** Quant THC.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	THC-d3	6.914	84	56811	0.0015	2.0631

ratio also out of range

ISP FORENSICS - Cd'A Instrument # 62340

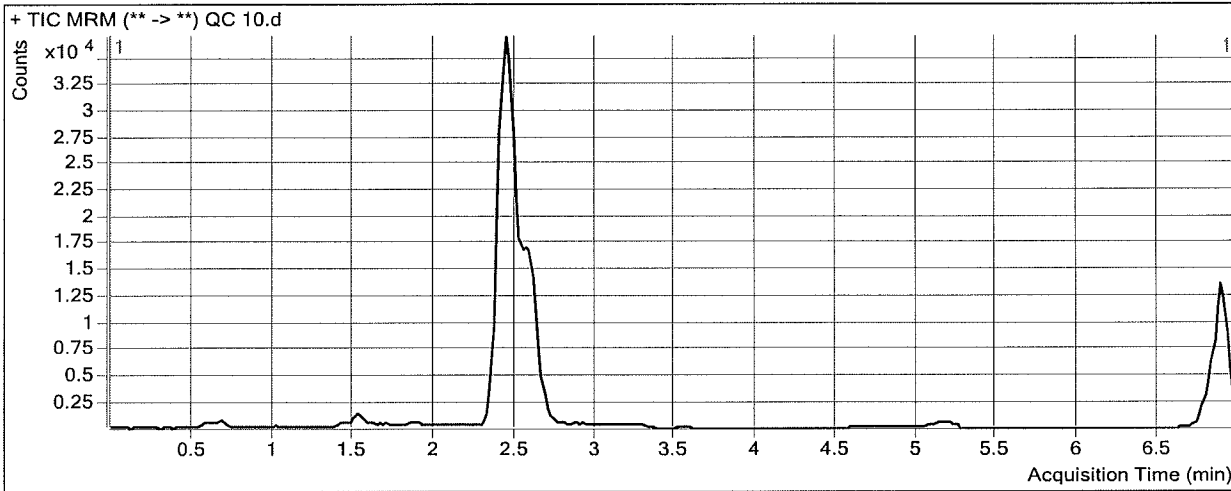
Cannabinoids Analysis Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 10:56 **Data File** QC 10.d
Sample Type QC **Sample Name** QC 10
Dilution 1 **Acq Method** Quant THC.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	2.476	32813	233583	0.1405	11.8937 ✓
THC-COOH	THC-COOH-d9	2.626	22212	84544	0.2627	11.9566 ✓
THC	THC-d3	6.894	12707	80367	0.1581	12.7113 ✓

ISP FORENSICS - Cd'A Instrument # 62340

Cannabinoids Analysis Report

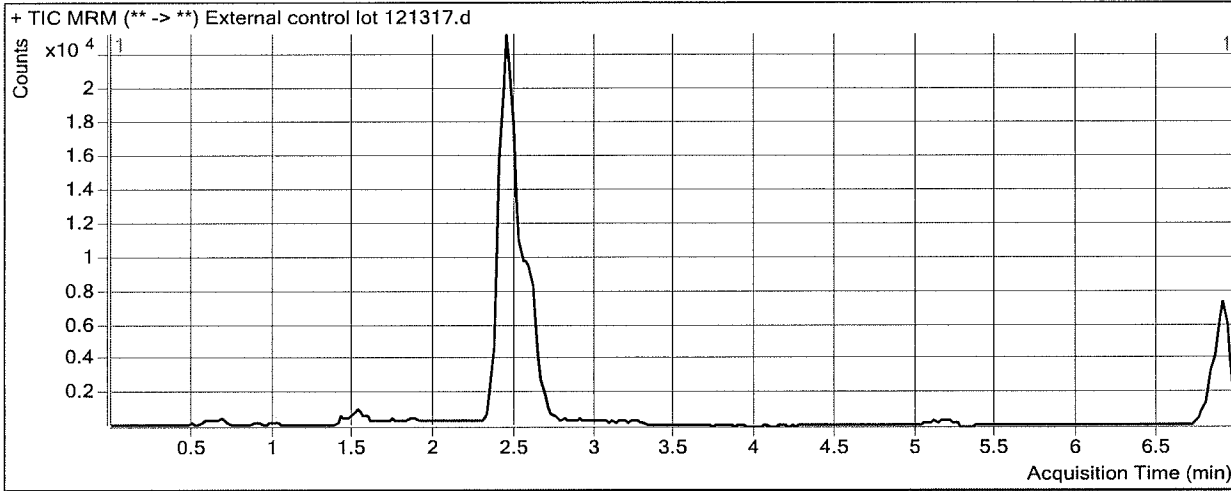
Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin
Analysis Time 12/15/2016 8:07 AM **Analyst Name** ISP Tox
Report Time 12/15/2016 8:10 AM **Reporter Name** ISP Tox
Last Calib Update 12/15/2016 8:07 AM **Batch State** Processed

Analysis Info

Acq Time 2016-12-14 11:08 **Data File** External control lot 121317.d
Sample Type Sample **Sample Name** External control lot 121317
Dilution 1 **Acq Method** Quant THC.m
Position p1b2 **Sample Info**
Inj Vol -1 **Comment** AM 27 cannabinoid confirmation

Sample Chromatogram

10 ng/ml A



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.476	15353	143468	0.1070	9.5046 ✓
THC-COOH	THC-COOH-d9	2.626	8856	53353	0.1660	8.1376 ✓
THC	THC-d3	6.914	4132	48399	0.0854	7.7669 ✓

ISP Forensics Calibration Curve Report

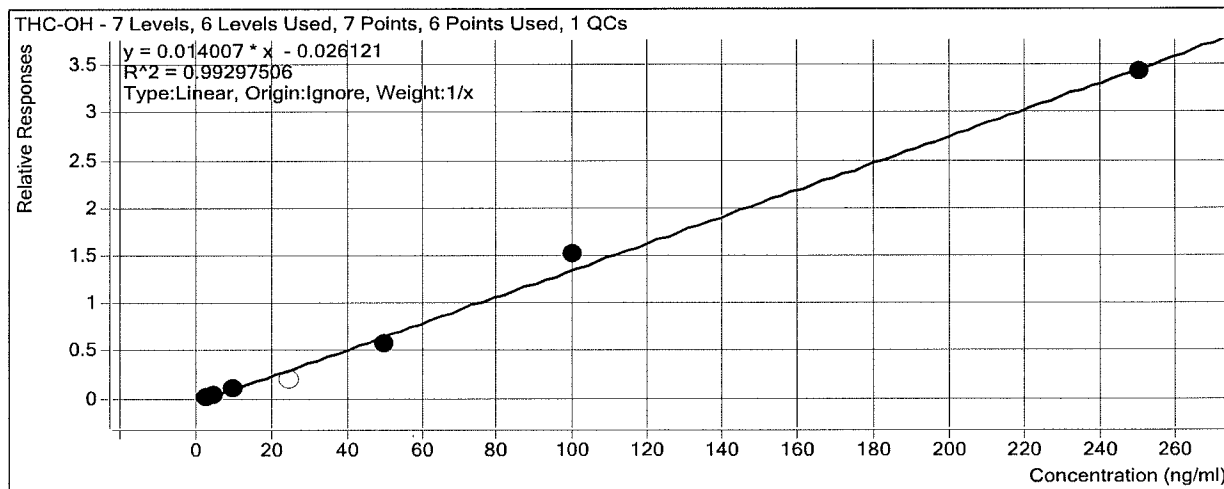
Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin

Last Calib Update 12/15/2016 8:07 AM

Analyst Name ISP TOX

Target Compound *THC-OH*

Internal Standard *THC-OH-d3*



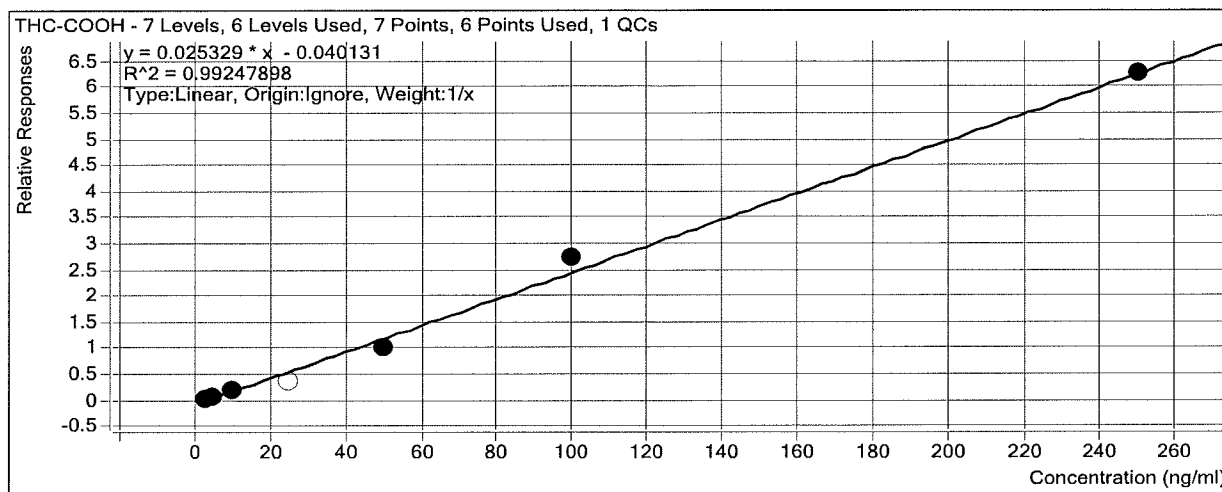
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	<input checked="" type="checkbox"/>	3	3.6	119.1
cal 2	2	<input checked="" type="checkbox"/>	5	4.8	96.8
cal 3	3	<input checked="" type="checkbox"/>	10	8.8	88.1
QC 10	3	<input checked="" type="checkbox"/>	10	11.9	118.9
cal 4	4	<input type="checkbox"/>	25	17.2	68.6
cal 5	5	<input checked="" type="checkbox"/>	50	43.1	86.1
cal 6	6	<input checked="" type="checkbox"/>	100	111.4	111.4
cal 7	7	<input checked="" type="checkbox"/>	250	246.3	98.5

ISP Forensics Calibration Curve Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin

Last Calib Update 12/15/2016 8:07 AM **Analyst Name** ISP TOX

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



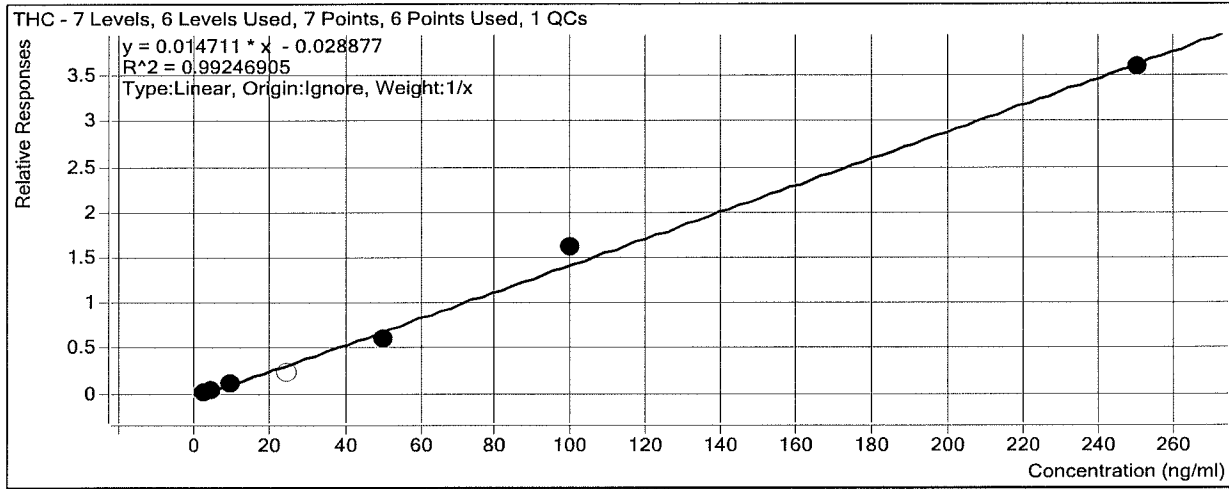
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	<input checked="" type="checkbox"/>	3	3.5	115.5
cal 2	2	<input checked="" type="checkbox"/>	5	5.2	103.3
cal 3	3	<input checked="" type="checkbox"/>	10	8.9	88.5
QC 10	3	<input checked="" type="checkbox"/>	10	12.0	119.6
cal 4	4	<input type="checkbox"/>	25	16.5	66.0
cal 5	5	<input checked="" type="checkbox"/>	50	41.4	82.8
cal 6	6	<input checked="" type="checkbox"/>	100	110.3	110.3
cal 7	7	<input checked="" type="checkbox"/>	250	248.8	99.5

ISP Forensics Calibration Curve Report

Batch Data Path D:\2016 Data\121316 quant cann\QuantResults\121316 quant cann.batch.bin

Last Calib Update 12/15/2016 8:07 AM **Analyst Name** ISP TOX

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



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	<input checked="" type="checkbox"/>	3	3.5	117.2
cal 2	2	<input checked="" type="checkbox"/>	5	5.0	99.2
cal 3	3	<input checked="" type="checkbox"/>	10	8.8	87.8
QC 10	3	<input checked="" type="checkbox"/>	10	12.7	127.1
cal 4	4	<input type="checkbox"/>	25	18.0	71.8
cal 5	5	<input checked="" type="checkbox"/>	50	42.7	85.5
cal 6	6	<input checked="" type="checkbox"/>	100	111.9	111.9
cal 7	7	<input checked="" type="checkbox"/>	250	246.1	98.4