








Boyle

Worklist: 1795

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>	
C2017-1221	1	88998	AM 27 Blood THC Quant by LC	
C2017-1257	1	88999	AM 27 Blood THC Quant by LC	
C2017-1258	1	89000	AM 27 Blood THC Quant by LC	
C2017-1267	1	89001	AM 27 Blood THC Quant by LC	
C2017-1268	1	89002	AM 27 Blood THC Quant by LC	
C2017-1277	2	89003	AM 27 Blood THC Quant by LC	
P2017-1258	1	89004	AM 27 Blood THC Quant by LC	

A

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

Extraction Date: 6-28-17

Analyst: Anne Nord

PRE-ANALYTIC

Plate Lot# Custom - 0490364 Plate Exp. 9-21-2017 External QC Lot 91317, exp 9-13-17

- ✓ 1. Ensure all solutions are within expiration date.
- Mobile Phase A: **0.1% Formic Acid in LCMS Water** • **0.1% Formic Acid in water**
 - Mobile Phase B: **0.1% Formic Acid in LCMS Acetonitrile** • **MTBE**
 - **LCMS Methanol** • **Hexane**
 - **Blank/Negative Blood: Lot 321632-1**
- ✓ 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.
- ✓ 4. Create worklist. Data path name: 62817 THC Quant

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. Place cover on Plate
Blank blood for locations containing standards/QCs and internal standards
Sample blood for locations containing only internal standards
- ✓ 3. Place on shaking incubator at ambient temp., **900rpm for 15 minutes**. *Shaker ID 66759*
- ✓ 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 66819*
- ✓ 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: 62817 Cann Quant
- ✓ 2. Make any necessary integration changes. Limit curves based on validated linear ranges (3-50ng/mL).
- ✓ 3. Were all appropriate standards used in the curve for each analyte? Y/N
Are r^2 values ≥ 0.98 for each analyte? Y/N
- ✓ 4. Did all QCs pass for each analyte? Y/N Were QCs entered into QC charting? Y/N
- ✓ 5. Central File Packet to include: ✓ LIMS Worklist: ✓ Method Checklist ✓ Calibration and Control Reports

COMMENTS



ISP FORENSICS - Cd'A Instrument # 62340

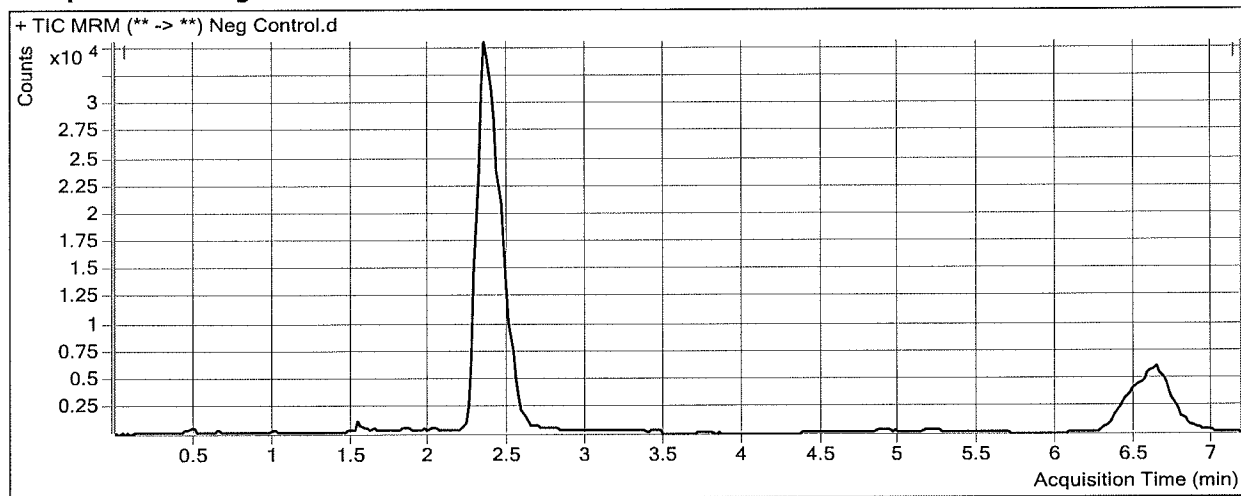
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 15:10 **Data File** Neg Control.d
Sample Type Sample **Sample Name** Neg Control
Dilution 1 **Acq Method** Quant THC 2017.m
Position P2-a2 **Sample Info**
Inj Vol -1 **Comment** AM 27 cannabinoid confirmation

Sample Chromatogram



ISP FORENSICS - Cd'A Instrument # 62340

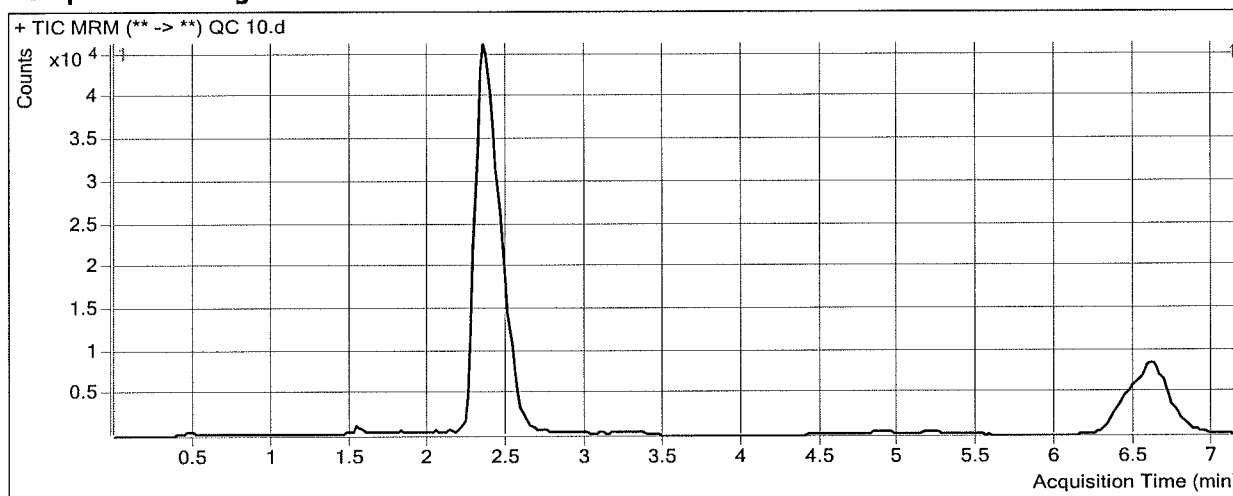
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 15:21 **Data File** QC 10.d
Sample Type QC **Sample Name** QC 10
Dilution 1 **Acq Method** Quant THC 2017.m
Position P2-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	2.356	27726	340847	0.0813	9.7006
THC-COOH	THC-COOH-d9	2.506	19150	107030	0.1789	9.6338
THC	THC-d3	6.593	16041	139567	0.1149	10.2740

ISP FORENSICS - Cd'A Instrument # 62340

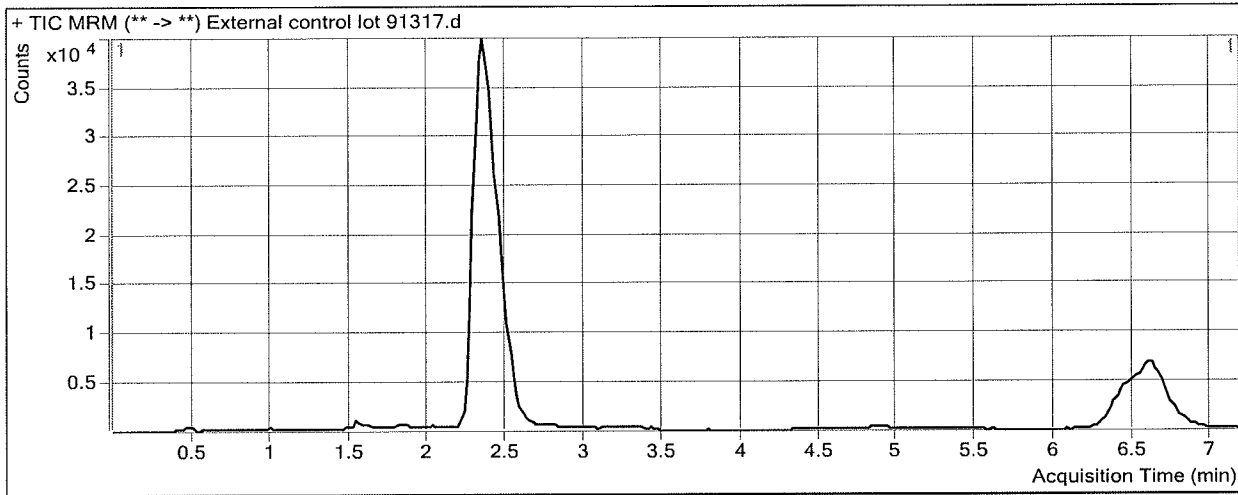
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 15:33 **Data File** External control lot 91317.d
Sample Type Sample **Sample Name** External control lot 91317
Dilution 1 **Acq Method** Quant THC 2017.m
Position p2b2 **Sample Info**
Inj Vol -1 **Comment** AM 27 cannabinoid confirmation 10 ng

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.356	24898	295804	0.0842	10.0295
THC-COOH	THC-COOH-d9	2.486	13937	94604	0.1473	7.7849
THC	THC-d3	6.613	10875	122849	0.0885	7.9376

ISP FORENSICS - Cd'A Instrument # 62340

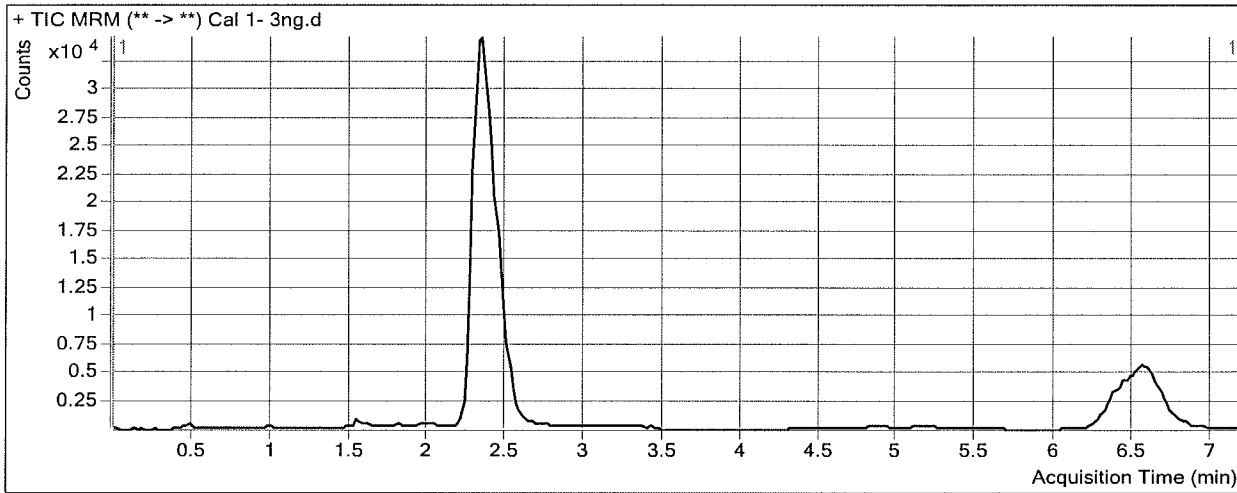
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 13:35 **Data File** Cal 1- 3ng.d
Sample Type Calibration **Sample Name** Cal 1- 3ng
Dilution 1 **Acq Method** Quant THC 2017.m
Position P2-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	2.356	6791	272036	0.0250	3.1352
THC-COOH	THC-COOH-d9	2.486	5264	84356	0.0624	2.8166
THC	THC-d3	6.593	3871	109631	0.0353	3.2299

ISP FORENSICS - Cd'A Instrument # 62340

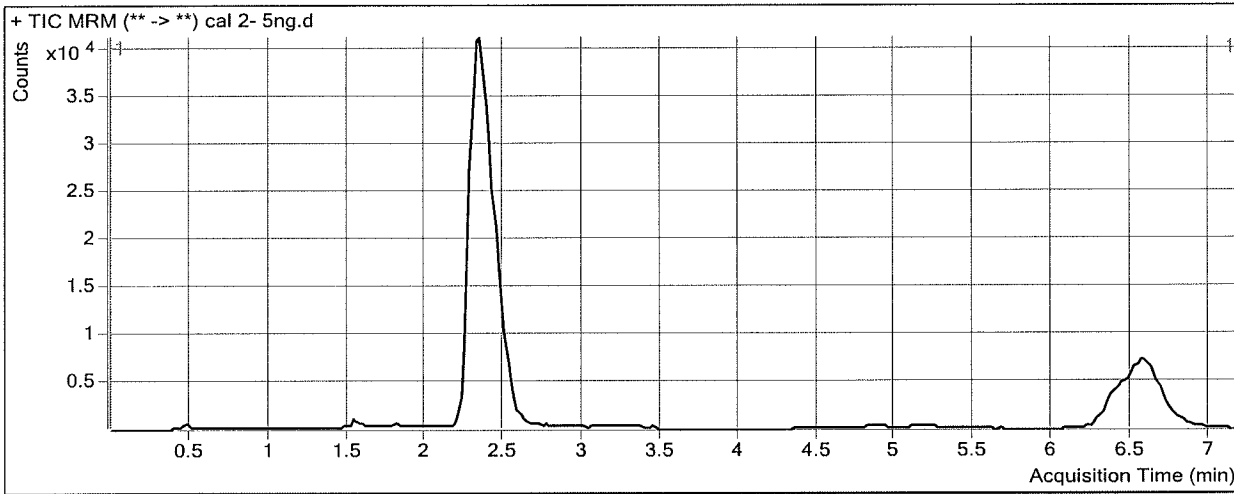
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 13:47 **Data File** cal 2- 5ng.d
Sample Type Calibration **Sample Name** cal 2- 5ng
Dilution 1 **Acq Method** Quant THC 2017.m
Position P2-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	2.356	13616	323737	0.0421	5.1260
THC-COOH	THC-COOH-d9	2.486	11037	100371	0.1100	5.5994
THC	THC-d3	6.573	6884	133242	0.0517	4.6767

ISP FORENSICS - Cd'A Instrument # 62340

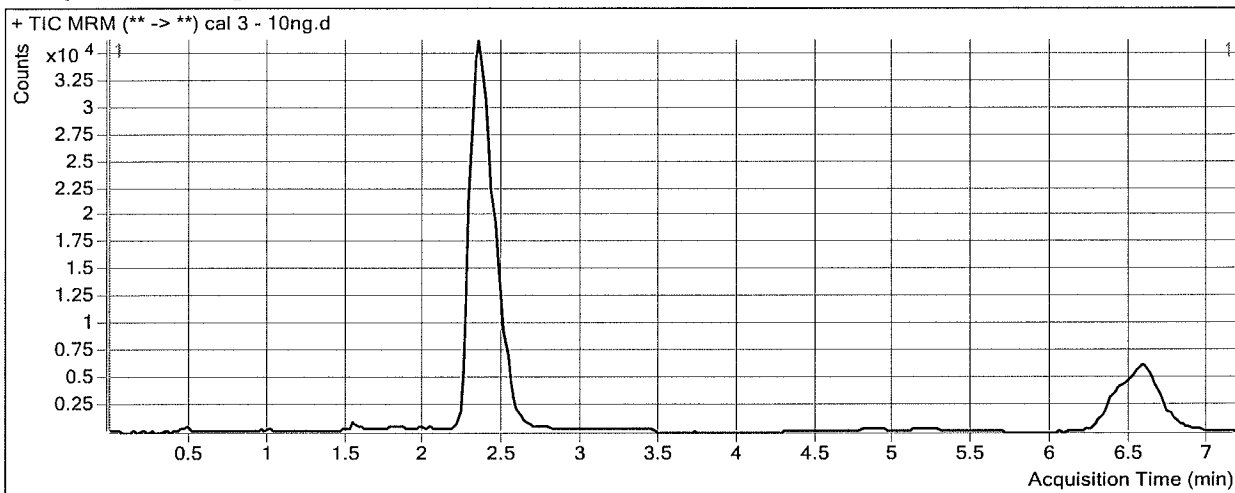
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 13:58 **Data File** cal 3 - 10ng.d
Sample Type Calibration **Sample Name** cal 3 - 10ng
Dilution 1 **Acq Method** Quant THC 2017.m
Position P2-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	2.356	21676	264145	0.0821	9.7842
THC-COOH	THC-COOH-d9	2.486	14571	81221	0.1794	9.6621
THC	THC-d3	6.573	11927	106518	0.1120	10.0125

ISP FORENSICS - Cd'A Instrument # 62340

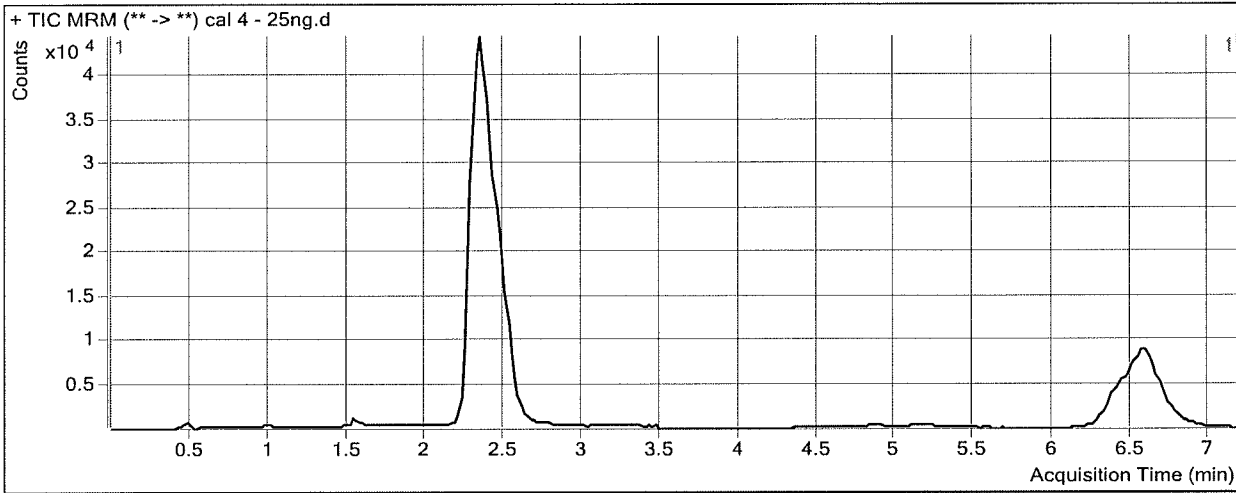
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 14:10 **Data File** cal 4 - 25ng.d
Sample Type Calibration **Sample Name** cal 4 - 25ng
Dilution 1 **Acq Method** Quant THC 2017.m
Position P2-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	2.356	61393	292931	0.2096	24.6328
THC-COOH	THC-COOH-d9	2.486	43488	94471	0.4603	26.0987
THC	THC-d3	6.593	35151	122884	0.2860	25.4128

ISP FORENSICS - Cd'A Instrument # 62340

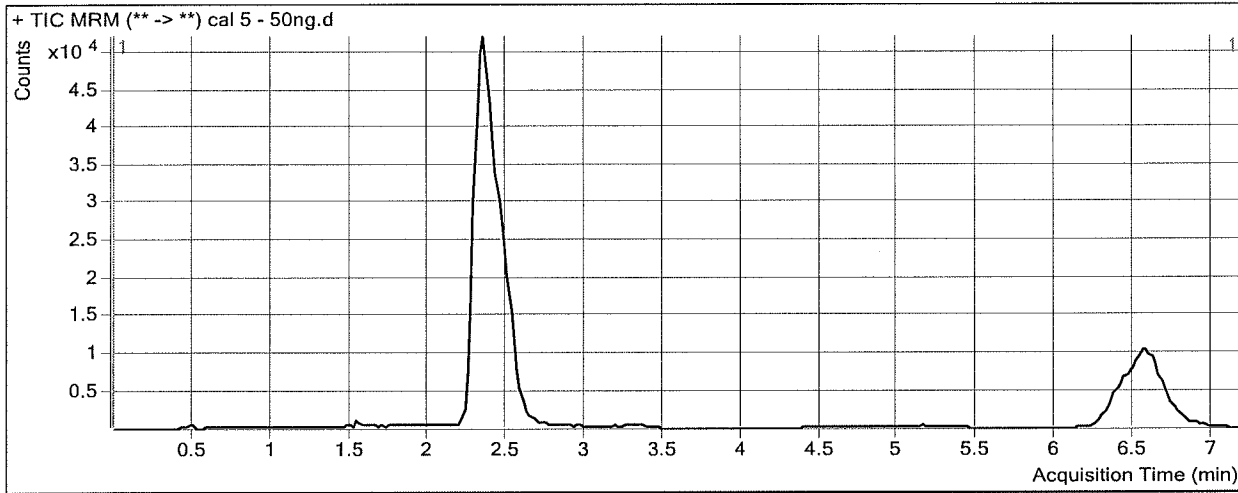
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 14:22 **Data File** cal 5 - 50ng.d
Sample Type Calibration **Sample Name** cal 5 - 50ng
Dilution 1 **Acq Method** Quant THC 2017.m
Position P2-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	2.356	119929	284145	0.4221	49.3758
THC-COOH	THC-COOH-d9	2.486	69428	83388	0.8326	47.8789
THC	THC-d3	6.553	63897	114077	0.5601	49.6605

ISP FORENSICS - Cd'A Instrument # 62340

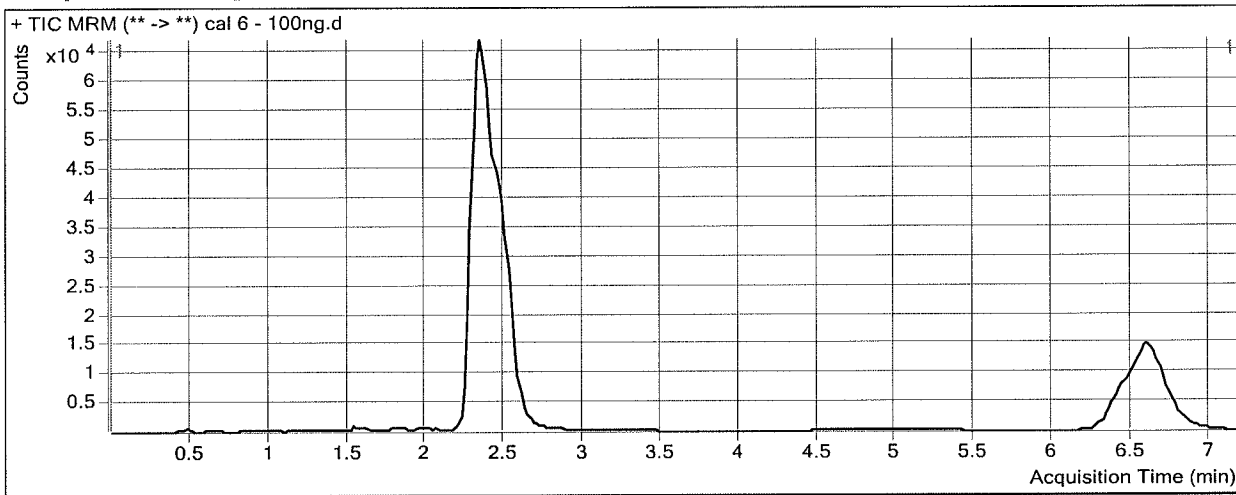
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin		
Analysis Time	7/5/2017 8:51 AM	Analyst Name	ISP Tox
Report Time	7/5/2017 8:52 AM	Reporter Name	ISP Tox
Last Calib Update	7/5/2017 8:51 AM	Batch State	Processed

Analysis Info

Acq Time	2017-06-30 14:34	Data File	cal 6 - 100ng.d
Sample Type	Calibration	Sample Name	cal 6 - 100ng
Dilution	1	Acq Method	Quant THC 2017.m
Position	P2-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	2.356	235072	286463	0.8206	95.7822
THC-COOH	THC-COOH-d9	2.486	142474	87024	1.6372	94.9549
THC	THC-d3	6.593	124339	114506	1.0859	96.1738

ISP FORENSICS - Cd'A Instrument # 62340

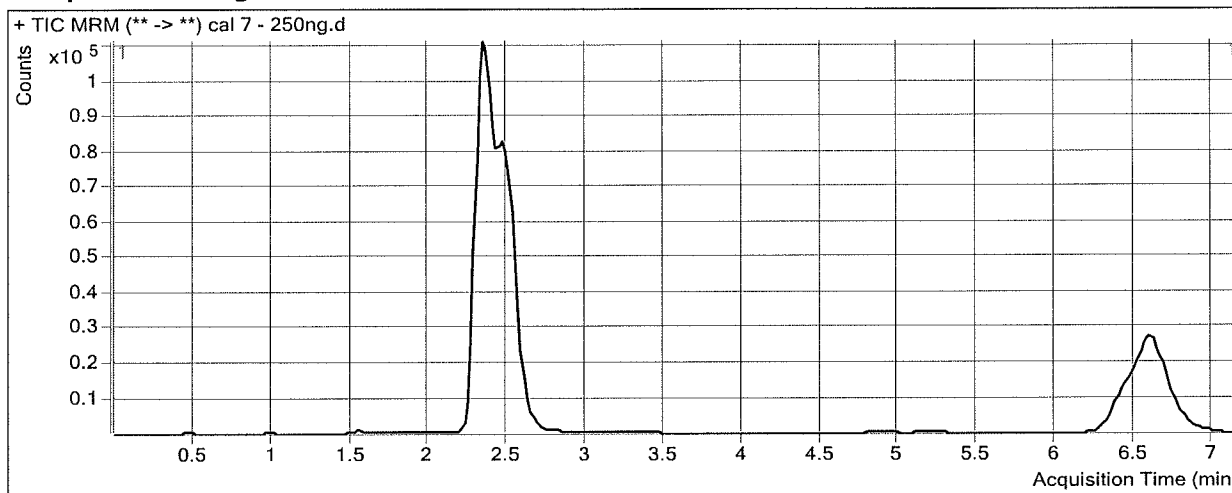
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin
Analysis Time 7/5/2017 8:51 AM **Analyst Name** ISP Tox
Report Time 7/5/2017 8:52 AM **Reporter Name** ISP Tox
Last Calib Update 7/5/2017 8:51 AM **Batch State** Processed

Analysis Info

Acq Time 2017-06-30 14:46 **Data File** cal 7 - 250ng.d
Sample Type Calibration **Sample Name** cal 7 - 250ng
Dilution 1 **Acq Method** Quant THC 2017.m
Position P2-G1 **Sample Info**
Inj Vol -1 **Comment** AM 27 cannabinoid confirmation

Sample Chromatogram



Results

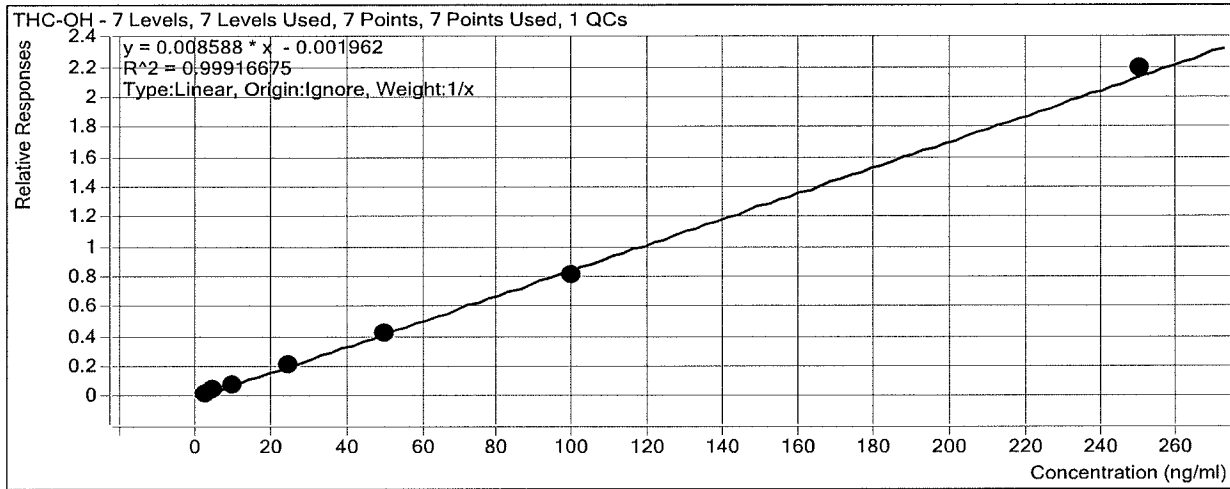
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.356	577537	263794	2.1893	255.1637
THC-COOH	THC-COOH-d9	2.486	361715	82405	4.3895	255.9894
THC	THC-d3	6.593	303051	105669	2.8679	253.8339

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin

Last Calib Update 7/5/2017 8:51 AM **Analyst Name** ISP TOX

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



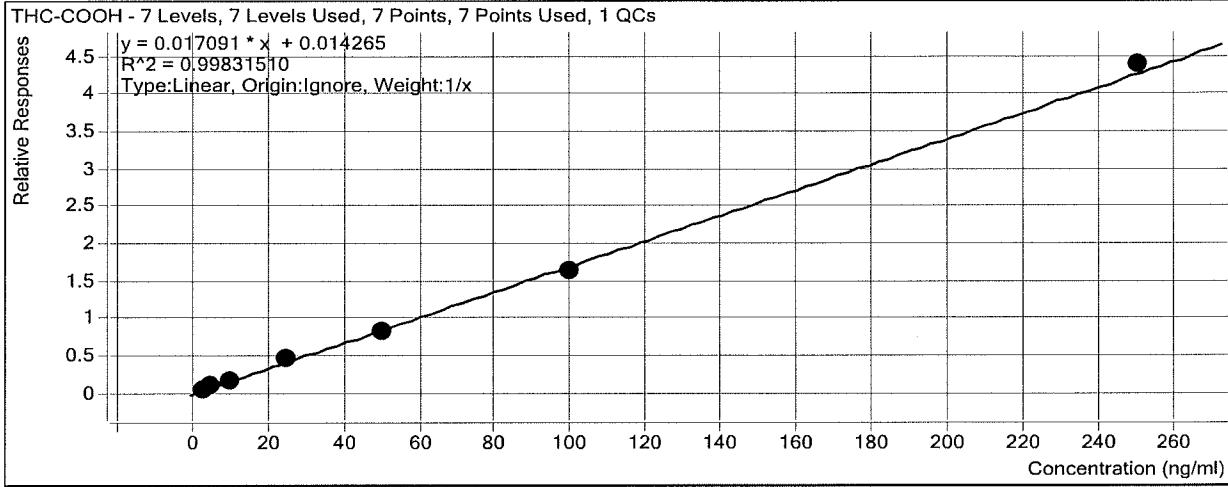
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1- 3ng	1	<input checked="" type="checkbox"/>	3	3.1	104.5
cal 2- 5ng	2	<input checked="" type="checkbox"/>	5	5.1	102.5
cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	9.8	97.8
QC 10	3	<input checked="" type="checkbox"/>	10	9.7	97.0
cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	24.6	98.5
cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.4	98.8
cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	95.8	95.8
cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	255.2	102.1

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin

Last Calib Update 7/5/2017 8:51 AM **Analyst Name** ISP TOX

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



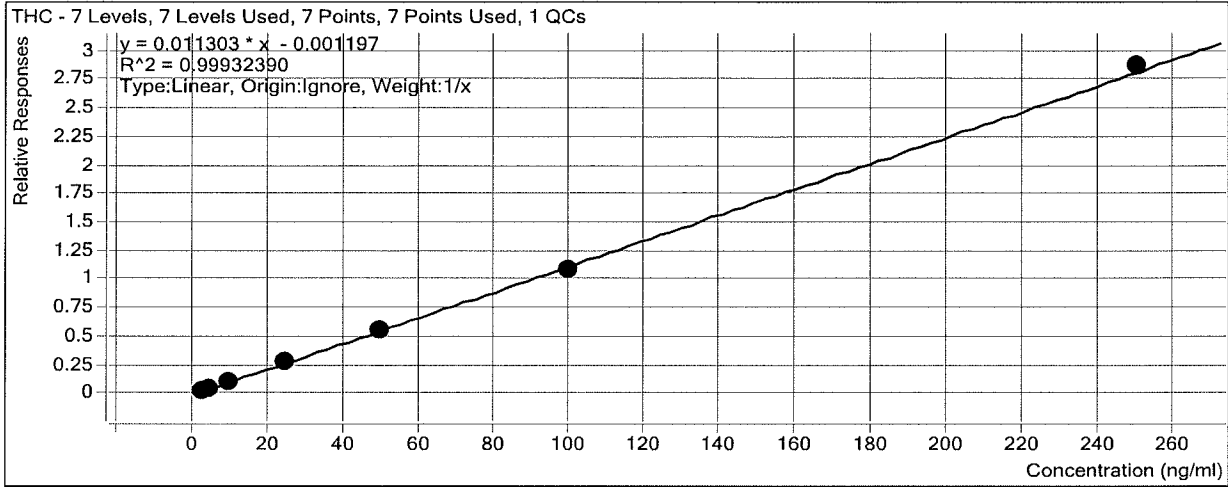
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1- 3ng	1	<input checked="" type="checkbox"/>	3	2.8	93.9
cal 2- 5ng	2	<input checked="" type="checkbox"/>	5	5.6	112.0
cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	9.7	96.6
QC 10	3	<input checked="" type="checkbox"/>	10	9.6	96.3
cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	26.1	104.4
cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	47.9	95.8
cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	95.0	95.0
cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	256.0	102.4

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\62817 THC Quant\QuantResults\68217 cann quant.batch.bin

Last Calib Update 7/5/2017 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	3.2	107.7
cal 2- 5ng	2	<input checked="" type="checkbox"/>	5	4.7	93.5
cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.0	100.1
QC 10	3	<input checked="" type="checkbox"/>	10	10.3	102.7
cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	25.4	101.7
cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	49.7	99.3
cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	96.2	96.2
cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	253.8	101.5