












Worklist: 1585

central data reviewed by B. Wylie on 3/3/17

B. Wylie

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>	
C2017-0126	1	78193	AM 27 Blood THC Quant by LC	
C2017-0148	1	78194	AM 27 Blood THC Quant by LC	
C2017-0181	1	78195	AM 27 Blood THC Quant by LC	
C2017-0225	2	78196	AM 27 Blood THC Quant by LC	
M2017-0400	1	78203	AM 27 Blood THC Quant by LC	
M2017-0449	2	78197	AM 27 Blood THC Quant by LC	
M2017-0561	3	78198	AM 27 Blood THC Quant by LC	
P2017-0149	1	78199	AM 27 Blood THC Quant by LC	
P2017-0182	1	78200	AM 27 Blood THC Quant by LC	
P2017-0197	1	78201	AM 27 Blood THC Quant by LC	
P2017-0348	1	78202	AM 27 Blood THC Quant by LC	



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

Extraction Date: 2-28-17 Analyst: Anne Nord

PRE-ANALYTIC

Plate Lot# Custom - 0490364 Plate Exp. 9-21-2017 External QC Lot 31317, exp 3-13-17

- ✓ 1. Ensure all solutions are within expiration date.
- Mobile Phase A: **0.1% Formic Acid in LCMS Water**
 - Mobile Phase B: **0.1% Formic Acid in LCMS Acetonitrile**
 - **LCMS Methanol**
 - **Blank/Negative Blood: Lot 321632**
 - **0.1% Formic Acid in water**
 - **MTBE**
 - **Hexane**
- 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: 2-28-17 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. Mix via aspirate and dispense. 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: 022817 Quant cannabinoid
- ✓ 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

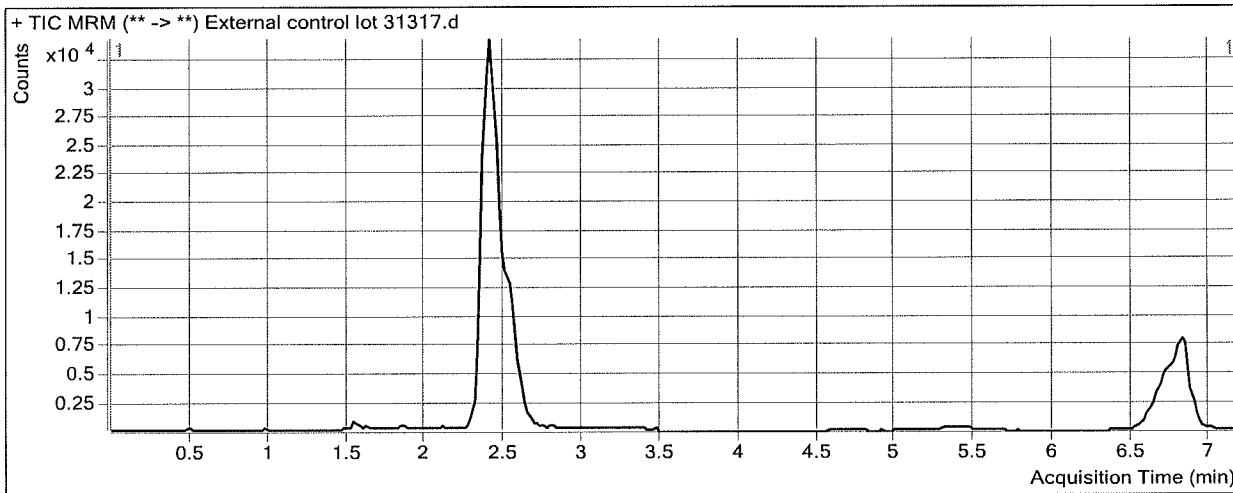
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\2-28-17 THC Quant\QuantResults\022817 quant cannabiniod.batch.bin
Analysis Time 3/1/2017 2:03 PM **Analyst Name** ISP Tox
Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 15:27 **Data File** External control lot 31317.d
Sample Type Sample **Sample Name** External control lot 31317
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	2.436	19943	213075	0.0936	9.4753
THC-COOH	THC-COOH-d9	2.566	9801	65666	0.1492	7.8024
THC	THC-d3	6.813	7825	86057	0.0909	8.5483

ISP FORENSICS - Cd'A Instrument # 62340

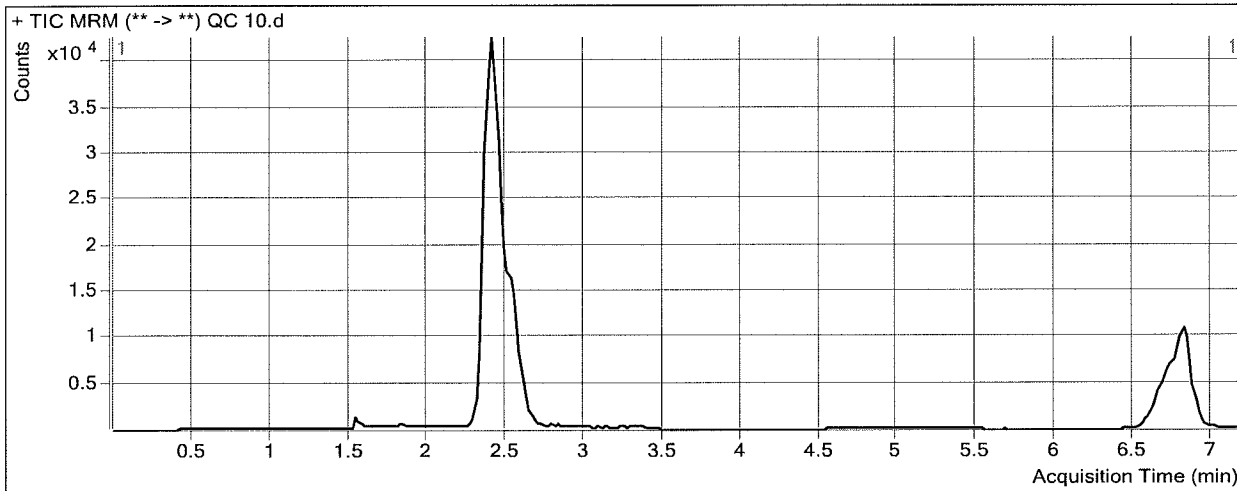
Cannabinoids Analysis Report

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Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 15:15 **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	2.436	24949	267239	0.0934	9.4548
THC-COOH	THC-COOH-d9	2.566	12744	80552	0.1582	8.2198
THC	THC-d3	6.833	11284	107734	0.1047	9.6921

ISP FORENSICS - Cd'A Instrument # 62340

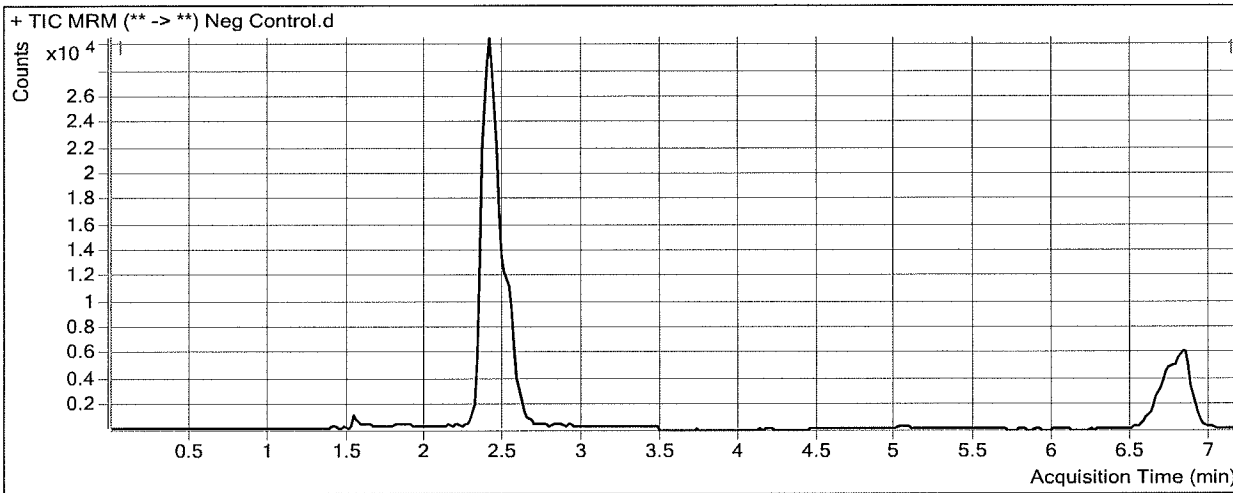
Cannabinoids Analysis Report

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Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 15:03 **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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.676	310	208302	0.0015	1.5290

ISP FORENSICS - Cd'A Instrument # 62340

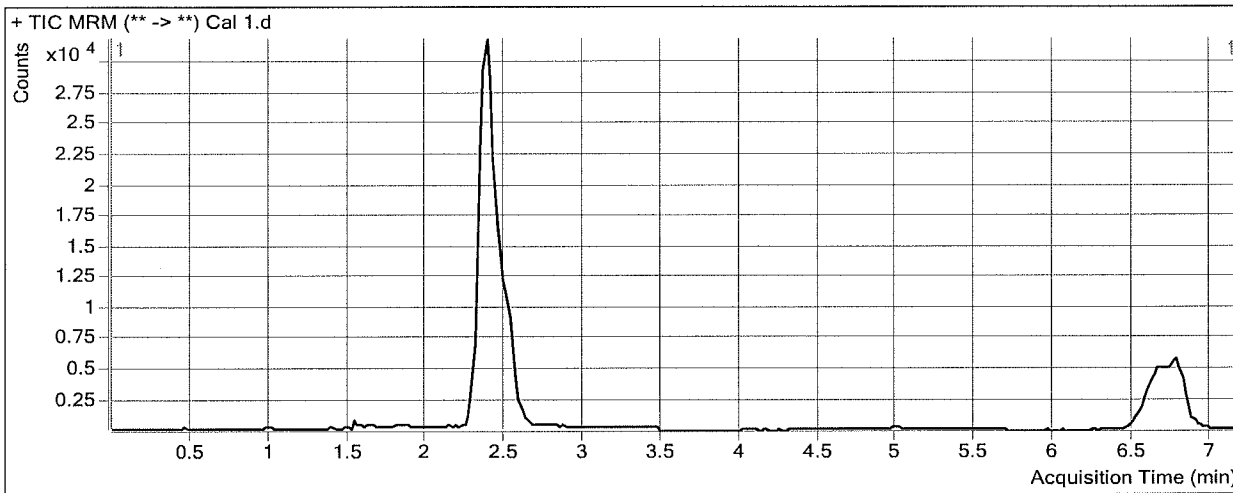
Cannabinoids Analysis Report

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Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 13:28 **Data File** Cal 1.d
Sample Type Calibration **Sample Name** Cal 1
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	2.396	4937	210427	0.0235	3.4247
THC-COOH	THC-COOH-d9	2.546	3542	63227	0.0560	3.4621
THC	THC-d3	6.753	2224	87583	0.0254	3.1214

ISP FORENSICS - Cd'A Instrument # 62340

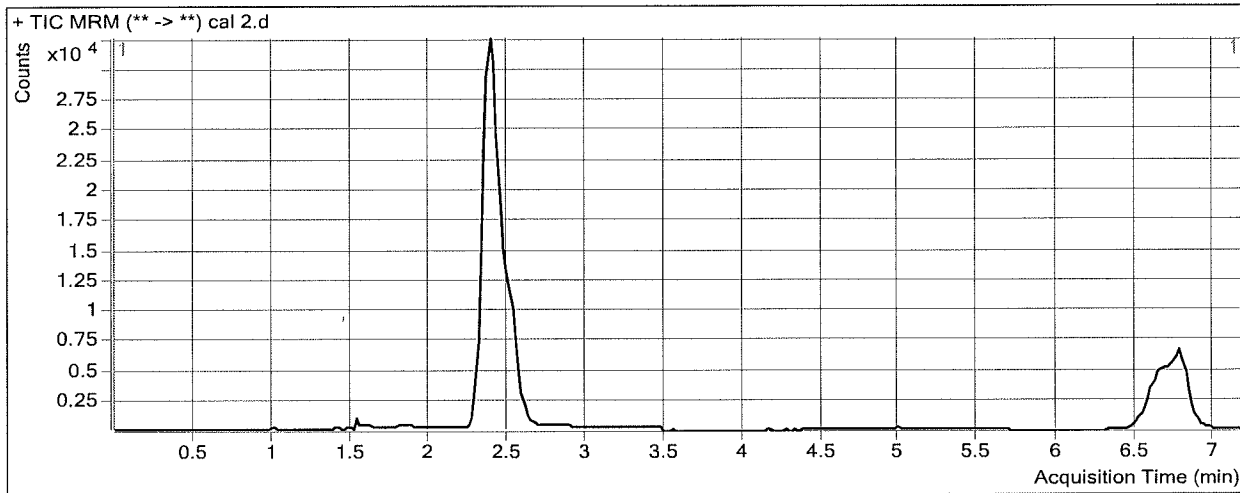
Cannabinoids Analysis Report

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Analysis Time 3/1/2017 2:03 PM **Analyst Name** ISP Tox
Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 13:40 **Data File** cal 2.d
Sample Type Calibration **Sample Name** cal 2
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	2.396	9142	218083	0.0419	5.0172
THC-COOH	THC-COOH-d9	2.546	5707	63751	0.0895	5.0217
THC	THC-d3	6.773	4241	87273	0.0486	5.0421

ISP FORENSICS - Cd'A Instrument # 62340

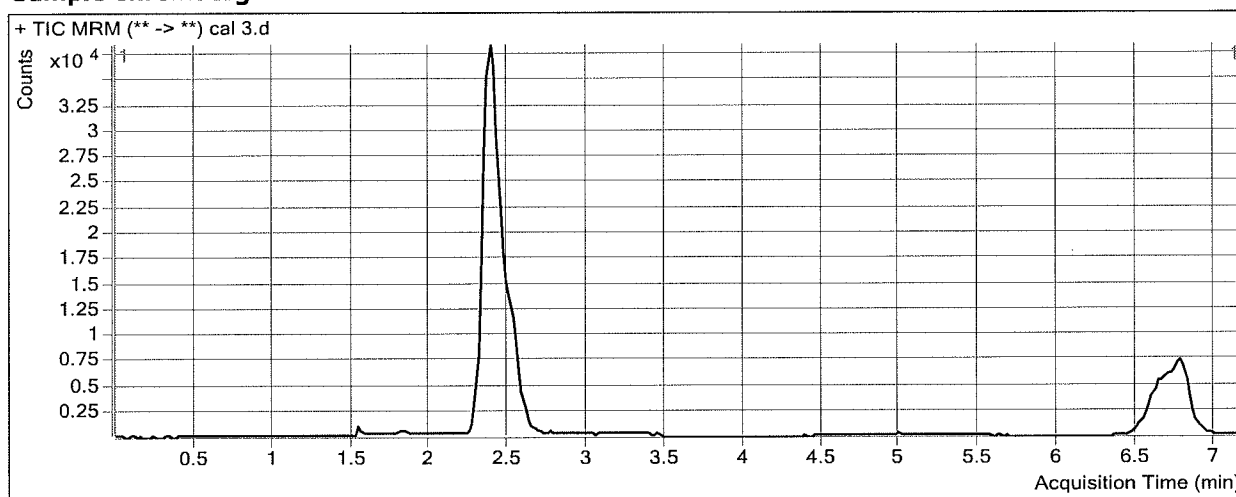
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\2-28-17 THC Quant\QuantResults\022817 quant cannabiniod.batch.bin
Analysis Time 3/1/2017 2:03 PM **Analyst Name** ISP Tox
Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 13:52 **Data File** cal 3.d
Sample Type Calibration **Sample Name** cal 3
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	2.416	21142	249085	0.0849	8.7234
THC-COOH	THC-COOH-d9	2.546	11534	66758	0.1728	8.8975
THC	THC-d3	6.773	9398	99517	0.0944	8.8389

ISP FORENSICS - Cd'A Instrument # 62340

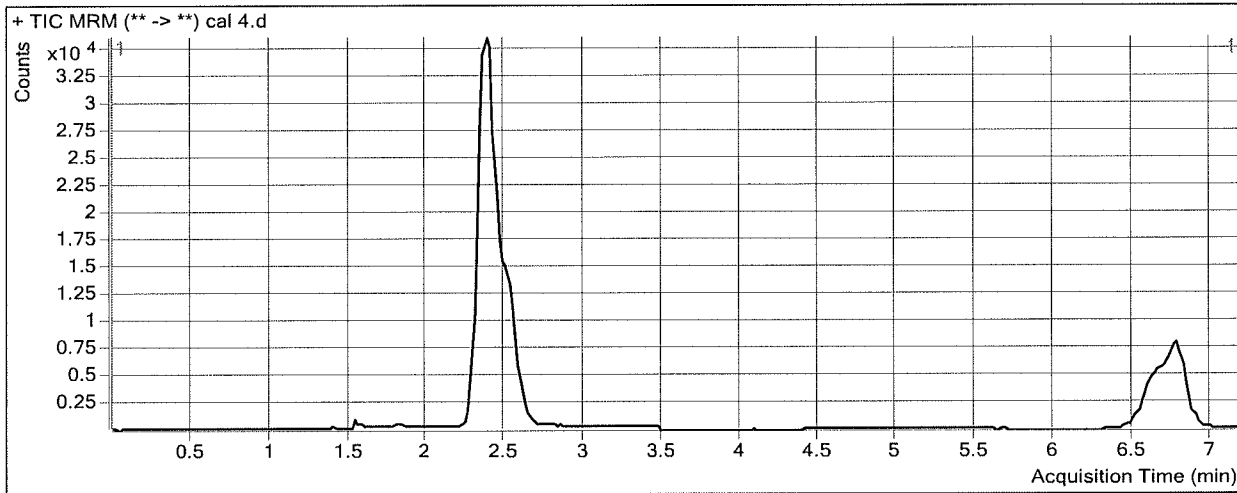
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\2-28-17 THC Quant\QuantResults\022817 quant cannabiniod.batch.bin
Analysis Time 3/1/2017 2:03 PM **Analyst Name** ISP Tox
Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 14:04 **Data File** cal 4.d
Sample Type Calibration **Sample Name** cal 4
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	2.396	46876	214851	0.2182	20.2233
THC-COOH	THC-COOH-d9	2.546	25953	62078	0.4181	20.3168
THC	THC-d3	6.773	20719	88876	0.2331	20.3249

ISP FORENSICS - Cd'A Instrument # 62340

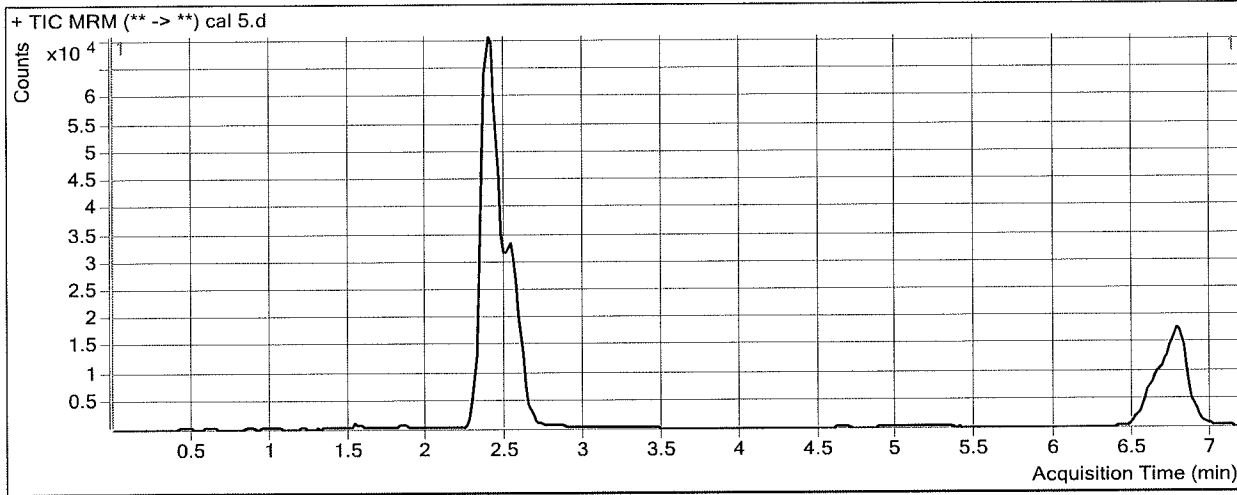
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\2-28-17 THC Quant\QuantResults\022817 quant cannabiniod.batch.bin
Analysis Time 3/1/2017 2:03 PM **Analyst Name** ISP Tox
Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 14:16 **Data File** cal 5.d
Sample Type Calibration **Sample Name** cal 5
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	2.416	197131	298294	0.6609	58.4138
THC-COOH	THC-COOH-d9	2.546	104042	85127	1.2222	57.7521
THC	THC-d3	6.793	85042	124068	0.6854	57.7847

ISP FORENSICS - Cd'A Instrument # 62340

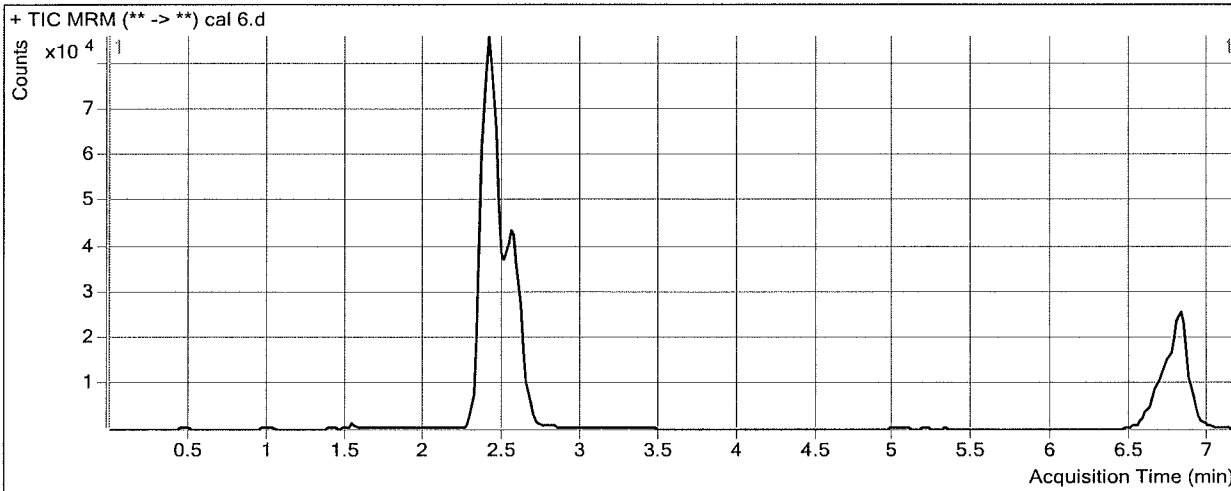
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\2-28-17 THC Quant\QuantResults\022817 quant cannabiniod.batch.bin
Analysis Time 3/1/2017 2:03 PM **Analyst Name** ISP Tox
Report Time 3/2/2017 4:58 PM **Reporter Name** ISP Tox
Last Calib Update 3/1/2017 2:03 PM **Batch State** Processed

Analysis Info

Acq Time 2017-02-28 14:28 **Data File** cal 6.d
Sample Type Calibration **Sample Name** cal 6
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	2.416	315570	268545	1.1751	102.7783
THC-COOH	THC-COOH-d9	2.566	156568	74268	2.1081	98.9957
THC	THC-d3	6.813	132249	103513	1.2776	106.8250

ISP FORENSICS - Cd'A Instrument # 62340

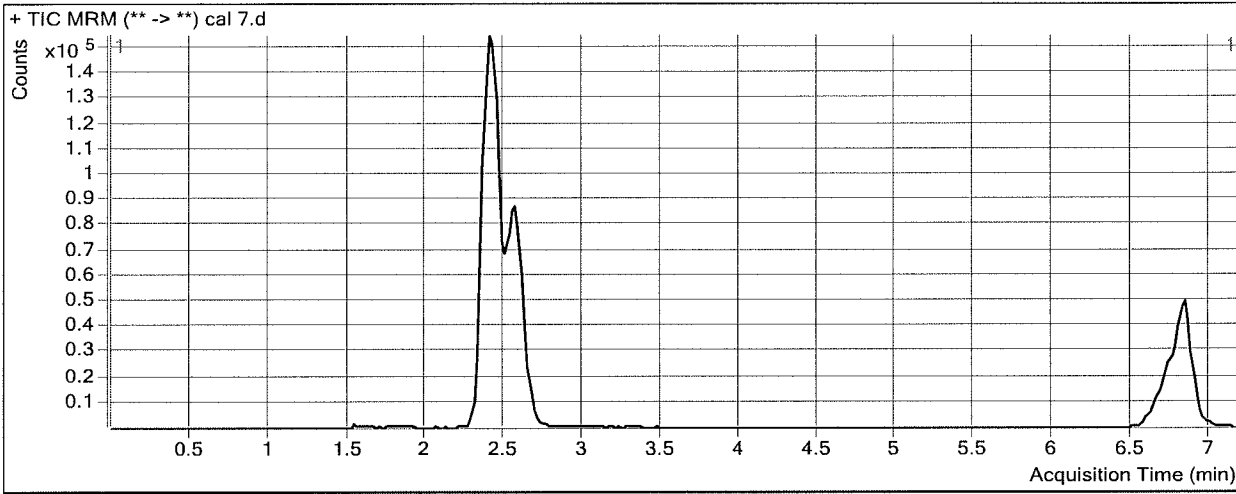
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\2-28-17 THC Quant\QuantResults\022817 quant cannabiniod.batch.bin		
Analysis Time	3/1/2017 2:03 PM	Analyst Name	ISP Tox
Report Time	3/2/2017 4:58 PM	Reporter Name	ISP Tox
Last Calib Update	3/1/2017 2:03 PM	Batch State	Processed

Analysis Info

Acq Time	2017-02-28 14:40	Data File	cal 7.d
Sample Type	Calibration	Sample Name	cal 7
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	2.436	781217	277329	2.8169	244.4194
THC-COOH	THC-COOH-d9	2.566	364800	68562	5.3207	248.5541
THC	THC-d3	6.833	309043	99762	3.0978	257.5686