

*B. Wylie*

2/3/2017

**Worklist: 1524**

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>	
M2016-3887	1	75950	AM 27 Blood THC Quant by LC	
M2016-4546	1	75967	AM 27 Blood THC Quant by LC	
M2016-4783	1	75951	AM 27 Blood THC Quant by LC	
M2016-4961	3	75952	AM 27 Blood THC Quant by LC	
M2016-5244	2	75953	AM 27 Blood THC Quant by LC	
M2016-5346	1	75954	AM 27 Blood THC Quant by LC	
M2017-0066	1	75955	AM 27 Blood THC Quant by LC	
P2016-2424	1	75956	AM 27 Blood THC Quant by LC	
P2016-2487	1	75957	AM 27 Blood THC Quant by LC	
P2016-2628	1	75958	AM 27 Blood THC Quant by LC	
P2016-2760	1	75966	AM 27 Blood THC Quant by LC	
P2016-2790	1	75959	AM 27 Blood THC Quant by LC	
P2016-2855	1	75960	AM 27 Blood THC Quant by LC	
P2016-2866	1	75961	AM 27 Blood THC Quant by LC	
P2016-2934	1	75962	AM 27 Blood THC Quant by LC	
P2017-0033	1	75963	AM 27 Blood THC Quant by LC	
P2017-0073	1	75964	AM 27 Blood THC Quant by LC	
P2017-0097	1	75965	AM 27 Blood THC Quant by LC	



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

Extraction Date: 1-31-17

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** • **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: 1-31-2017 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: 1-31-17 amn can 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<sup>2</sup> 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 Calibration Curve Report

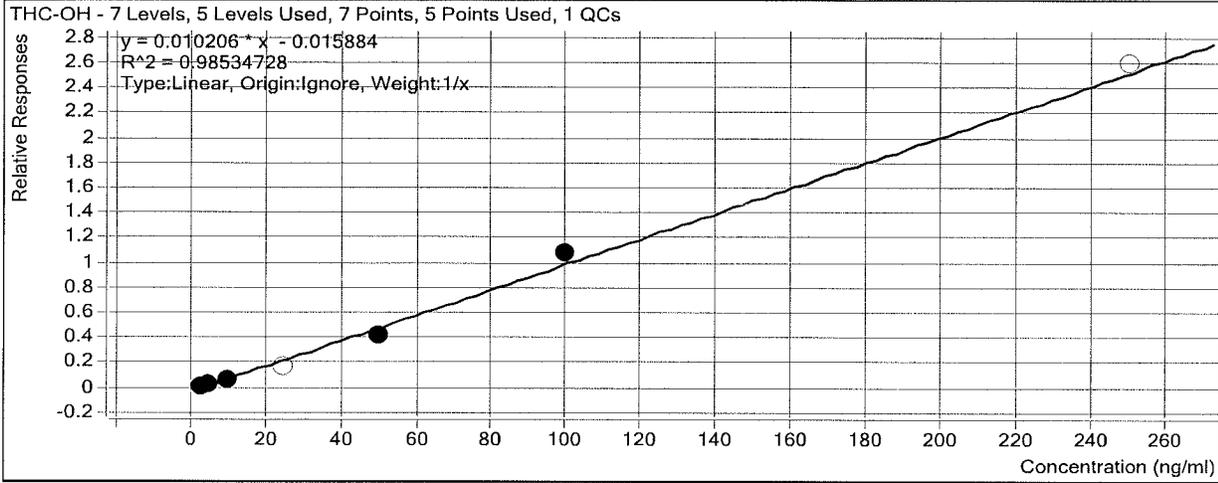
**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin

**Last Calib Update** 2/1/2017 6:02 PM

**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	3.5	116.6
cal 2	2	<input checked="" type="checkbox"/>	5	5.2	104.7
cal 3	3	<input checked="" type="checkbox"/>	10	8.5	84.9
QC 10	3	<input checked="" type="checkbox"/>	10	8.5	85.0
cal 4	4	<input type="checkbox"/>	25	18.5	74.2
cal 5	5	<input checked="" type="checkbox"/>	50	42.9	85.9
cal 6	6	<input checked="" type="checkbox"/>	100	107.8	107.8
cal 7	7	<input type="checkbox"/>	250	255.7	102.3

# ISP Forensics Calibration Curve Report

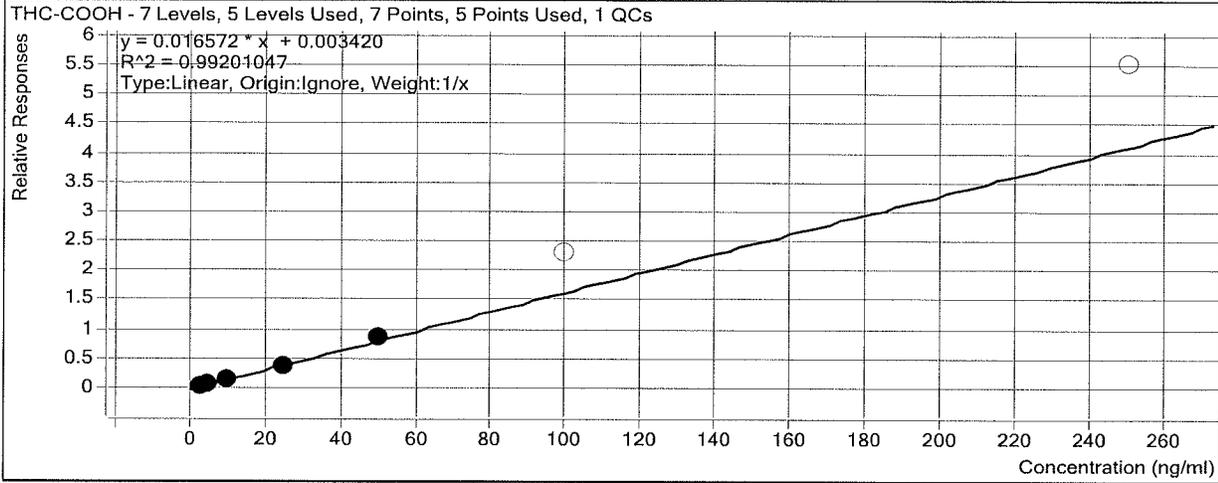
**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin

**Last Calib Update** 2/1/2017 6:02 PM

**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	3.1	102.0
cal 2	2	<input checked="" type="checkbox"/>	5	5.2	103.2
cal 3	3	<input checked="" type="checkbox"/>	10	10.1	101.2
QC 10	3	<input checked="" type="checkbox"/>	10	8.8	87.7
cal 4	4	<input checked="" type="checkbox"/>	25	22.1	88.6
cal 5	5	<input checked="" type="checkbox"/>	50	52.5	105.0
cal 6	6	<input type="checkbox"/>	100	139.0	139.0
cal 7	7	<input type="checkbox"/>	250	333.3	133.3

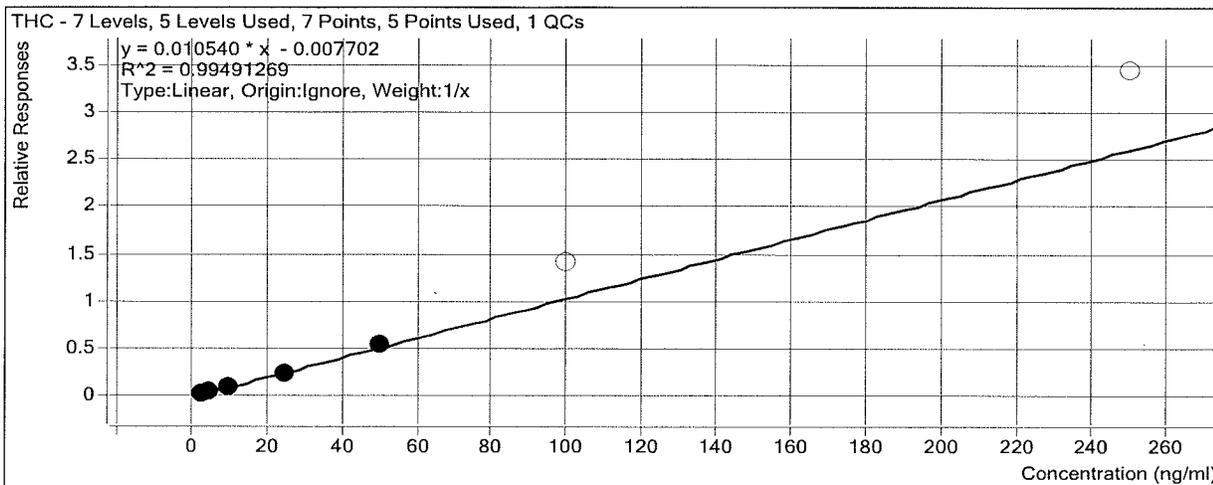
# ISP Forensics Calibration Curve Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin

**Last Calib Update** 2/1/2017 6:02 PM

**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	3.2	106.6
cal 2	2	<input checked="" type="checkbox"/>	5	5.0	100.9
cal 3	3	<input checked="" type="checkbox"/>	10	9.6	96.4
QC 10	3	<input checked="" type="checkbox"/>	10	9.4	93.8
cal 4	4	<input checked="" type="checkbox"/>	25	22.9	91.8
cal 5	5	<input checked="" type="checkbox"/>	50	52.2	104.4
cal 6	6	<input type="checkbox"/>	100	135.4	135.4
cal 7	7	<input type="checkbox"/>	250	328.0	131.2

# ISP FORENSICS - Cd'A Instrument # 62340

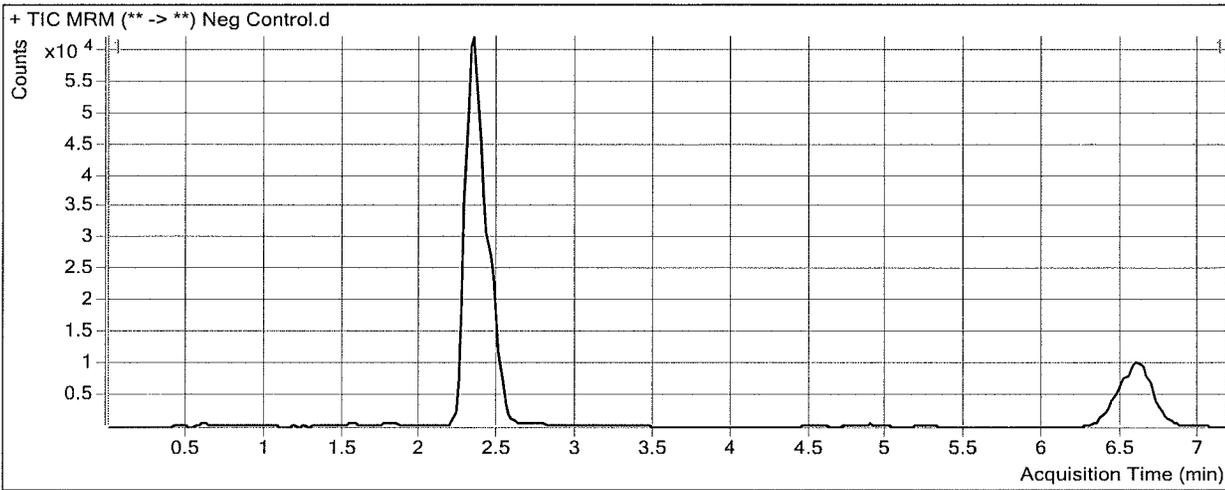
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 03:56 **Data File** Neg Control.d  
**Sample Type** Sample **Sample Name** Neg Control  
**Dilution** 1 **Acq Method** Quant THC 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-OH	THC-OH-d3	2.596	448	448313	0.0010	1.6543
THC-COOH	THC-COOH-d9	2.346	2114	147836	0.0143	0.6565

# ISP FORENSICS - Cd'A Instrument # 62340

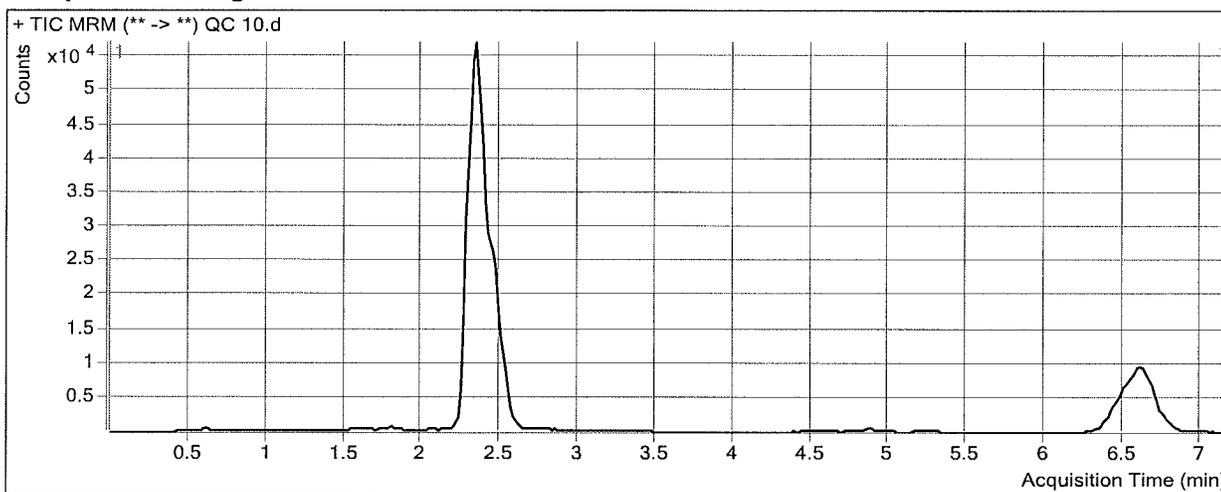
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 04:08 **Data File** QC 10.d  
**Sample Type** QC **Sample Name** QC 10  
**Dilution** 1 **Acq Method** Quant THC 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	2.356	26820	378435	0.0709	8.5008
THC-COOH	THC-COOH-d9	2.506	18531	124506	0.1488	8.7748
THC	THC-d3	6.633	12286	134716	0.0912	9.3836

# ISP FORENSICS - Cd'A Instrument # 62340

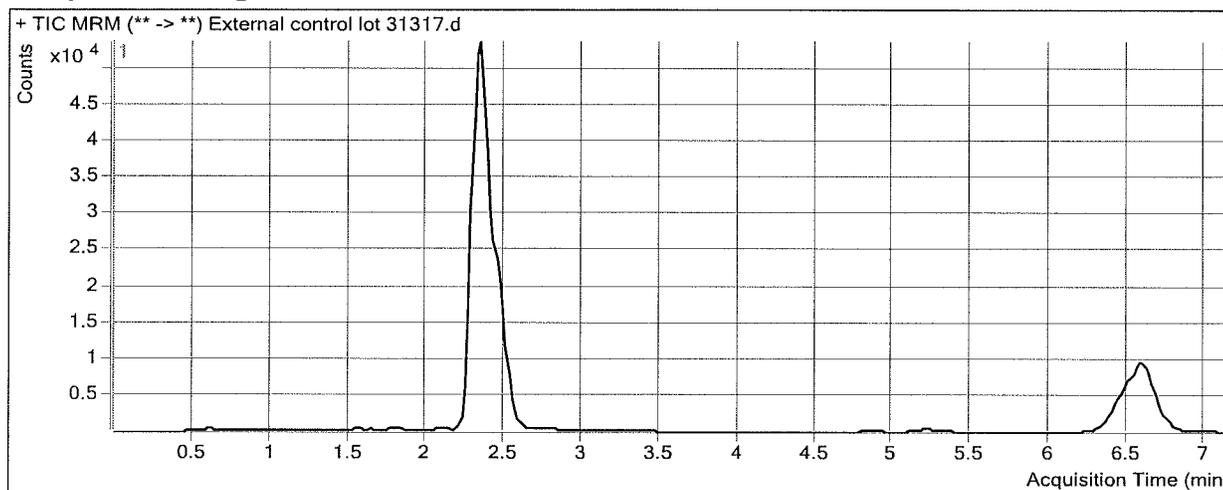
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 04:20 **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** p1b2 **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	28077	346686	0.0810	9.4919
THC-COOH	THC-COOH-d9	2.486	15104	118686	0.1273	7.4729
THC	THC-d3	6.613	10520	133782	0.0786	8.1913

# ISP FORENSICS - Cd'A Instrument # 62340

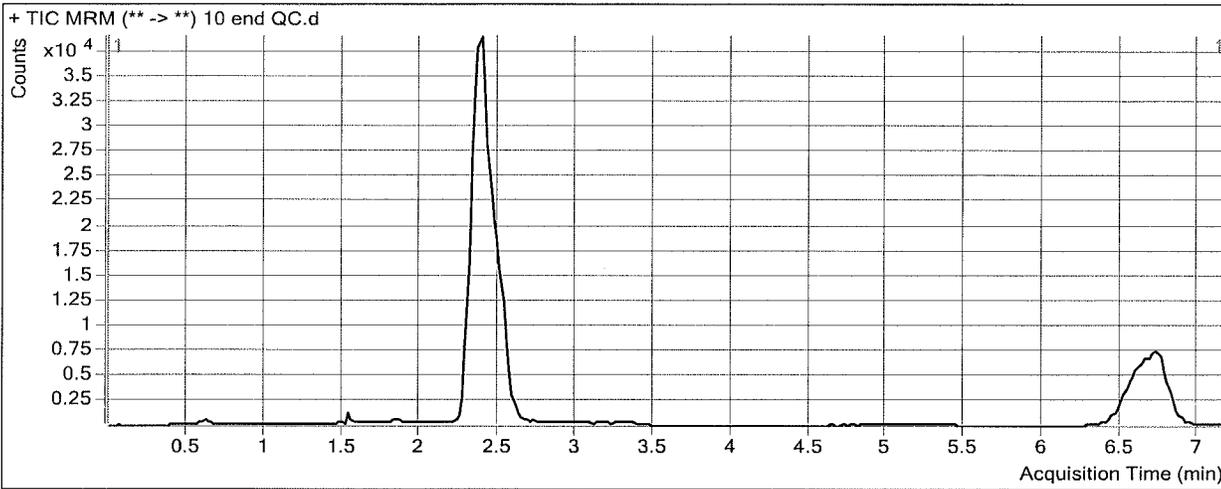
## Cannabinoids Analysis Report

<b>Batch Data Path</b>	D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin		
<b>Analysis Time</b>	2/1/2017 6:02 PM	<b>Analyst Name</b>	ISP Tox
<b>Report Time</b>	2/1/2017 6:03 PM	<b>Reporter Name</b>	ISP Tox
<b>Last Calib Update</b>	2/1/2017 6:02 PM	<b>Batch State</b>	Processed

### Analysis Info

<b>Acq Time</b>	2017-02-01 13:25	<b>Data File</b>	10 end QC.d
<b>Sample Type</b>	Sample	<b>Sample Name</b>	10 end control
<b>Dilution</b>	1	<b>Acq Method</b>	Quant THC 2017.m
<b>Position</b>	P1-A6	<b>Sample Info</b>	
<b>Inj Vol</b>	-1	<b>Comment</b>	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	21668	255588	0.0848	9.8634
THC-COOH	THC-COOH-d9	2.526	15925	96097	0.1657	9.7936
THC	THC-d3	6.753	10506	110292	0.0953	9.7682

# ISP FORENSICS - Cd'A Instrument # 62340

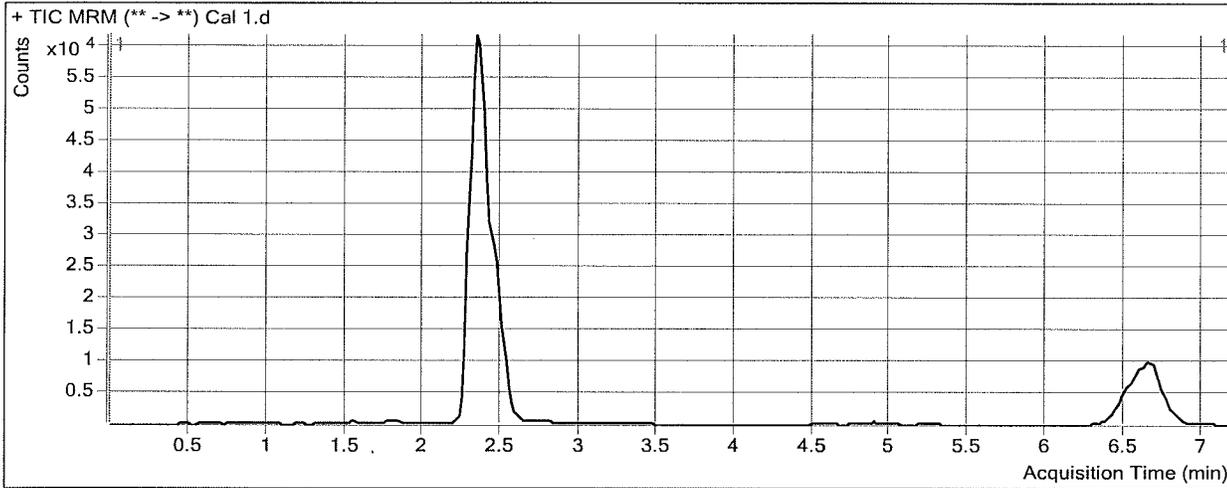
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 02:21 **Data File** Cal 1.d  
**Sample Type** Calibration **Sample Name** Cal 1  
**Dilution** 1 **Acq Method** Quant THC 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	2.376	8562	431771	0.0198	3.4994
THC-COOH	THC-COOH-d9	2.506	7688	142036	0.0541	3.0600
THC	THC-d3	6.693	4020	154581	0.0260	3.1983

# ISP FORENSICS - Cd'A Instrument # 62340

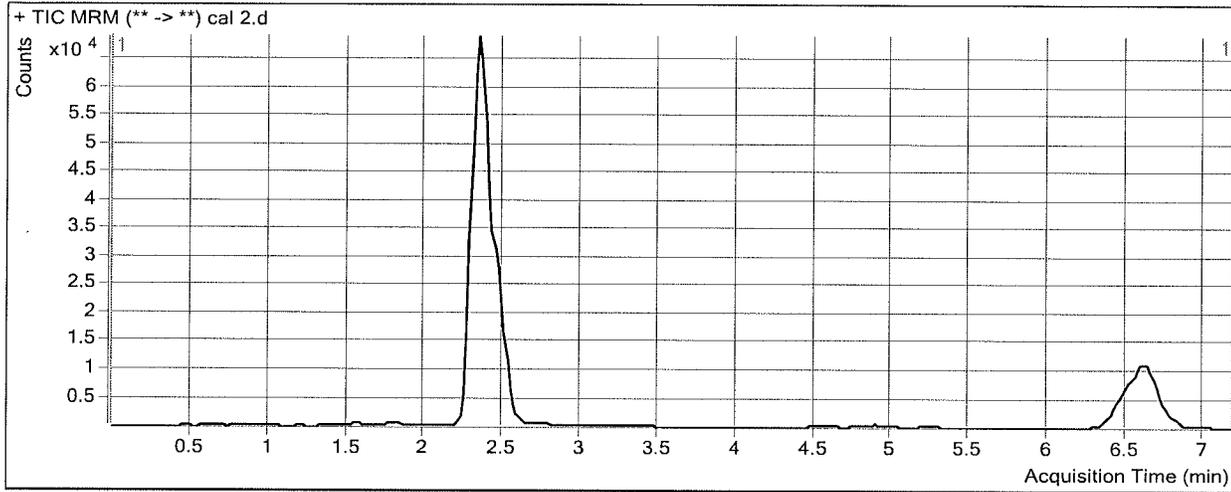
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 02:33 **Data File** cal 2.d  
**Sample Type** Calibration **Sample Name** cal 2  
**Dilution** 1 **Acq Method** Quant THC 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	2.376	17888	476206	0.0376	5.2371
THC-COOH	THC-COOH-d9	2.506	13567	152612	0.0889	5.1581
THC	THC-d3	6.613	7677	168857	0.0455	5.0441

# ISP FORENSICS - Cd'A Instrument # 62340

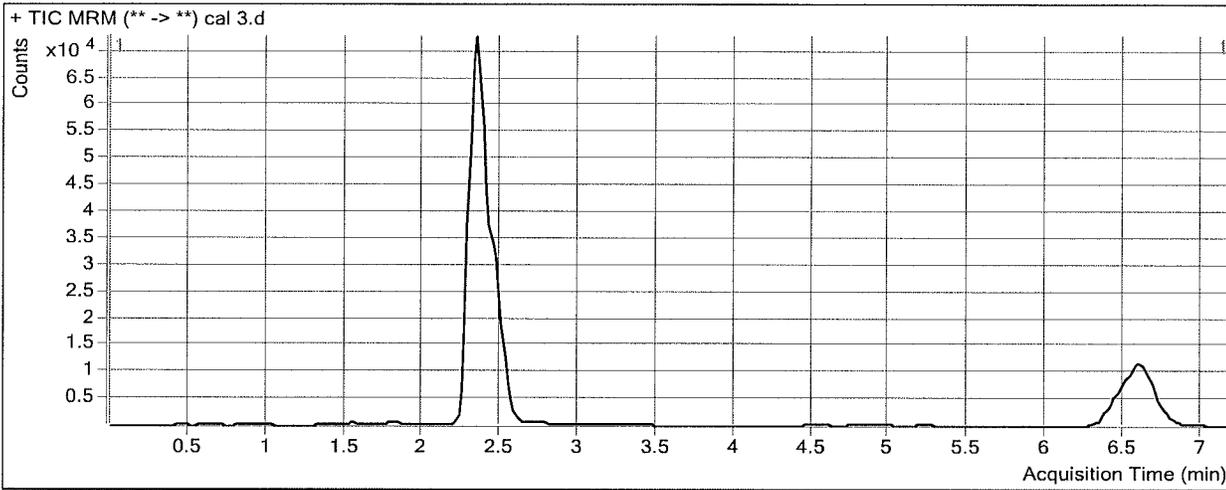
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 02:45 **Data File** cal 3.d  
**Sample Type** Calibration **Sample Name** cal 3  
**Dilution** 1 **Acq Method** Quant THC 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	2.356	34303	484686	0.0708	8.4912
THC-COOH	THC-COOH-d9	2.506	26683	155900	0.1712	10.1217
THC	THC-d3	6.613	15805	168332	0.0939	9.6392

# ISP FORENSICS - Cd'A Instrument # 62340

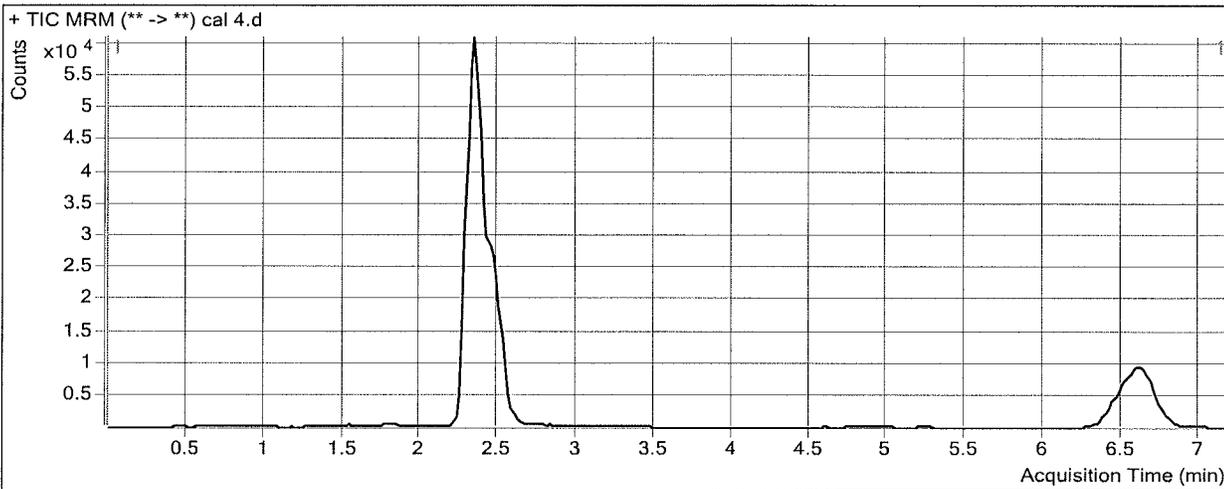
## Cannabinoids Analysis Report

<b>Batch Data Path</b>	D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin		
<b>Analysis Time</b>	2/1/2017 6:02 PM	<b>Analyst Name</b>	ISP Tox
<b>Report Time</b>	2/1/2017 6:03 PM	<b>Reporter Name</b>	ISP Tox
<b>Last Calib Update</b>	2/1/2017 6:02 PM	<b>Batch State</b>	Processed

### Analysis Info

<b>Acq Time</b>	2017-02-01 02:57	<b>Data File</b>	cal 4.d
<b>Sample Type</b>	Calibration	<b>Sample Name</b>	cal 4
<b>Dilution</b>	1	<b>Acq Method</b>	Quant THC 2017.m
<b>Position</b>	P1-D1	<b>Sample Info</b>	
<b>Inj Vol</b>	-1	<b>Comment</b>	AM 27 cannabinoid confirmation

### Sample Chromatogram



### Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.376	61673	355746	0.1734	18.5434
THC-COOH	THC-COOH-d9	2.506	42740	115363	0.3705	22.1500
THC	THC-d3	6.653	28169	120335	0.2341	22.9400

*Not included in curve*

# ISP FORENSICS - Cd'A Instrument # 62340

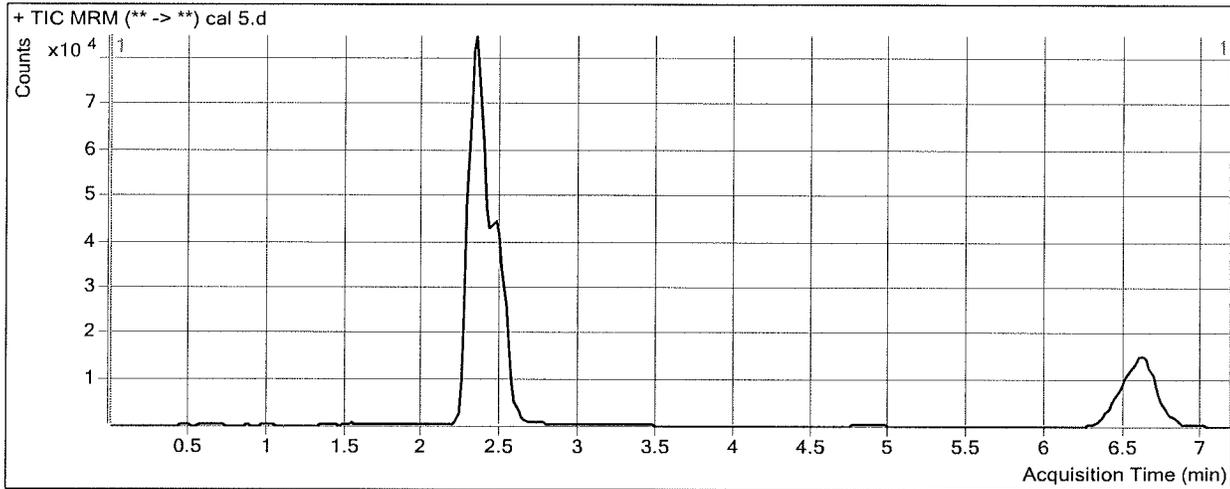
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 03:09 **Data File** cal 5.d  
**Sample Type** Calibration **Sample Name** cal 5  
**Dilution** 1 **Acq Method** Quant THC 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	2.356	174063	412257	0.4222	42.9276
THC-COOH	THC-COOH-d9	2.486	116626	133499	0.8736	52.5102
THC	THC-d3	6.613	77241	142445	0.5423	52.1784

# ISP FORENSICS - Cd'A Instrument # 62340

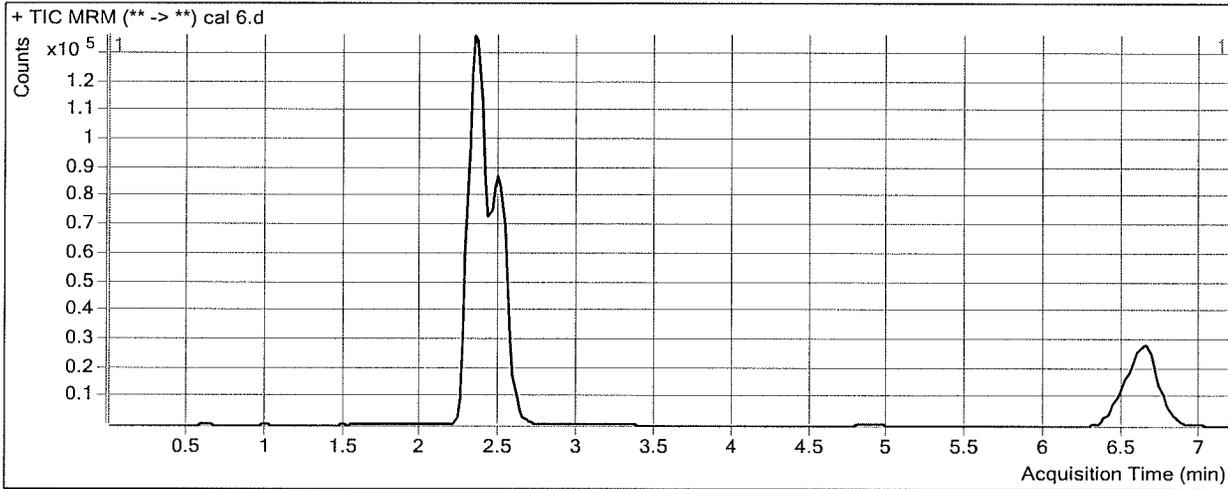
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 03:20 **Data File** cal 6.d  
**Sample Type** Calibration **Sample Name** cal 6  
**Dilution** 1 **Acq Method** Quant THC 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	2.376	488628	450454	1.0847	107.8448
THC-COOH	THC-COOH-d9	2.506	313713	136035	2.3061	138.9522
THC	THC-d3	6.653	215939	152166	1.4191	135.3714

*Not included in curve*

# ISP FORENSICS - Cd'A Instrument # 62340

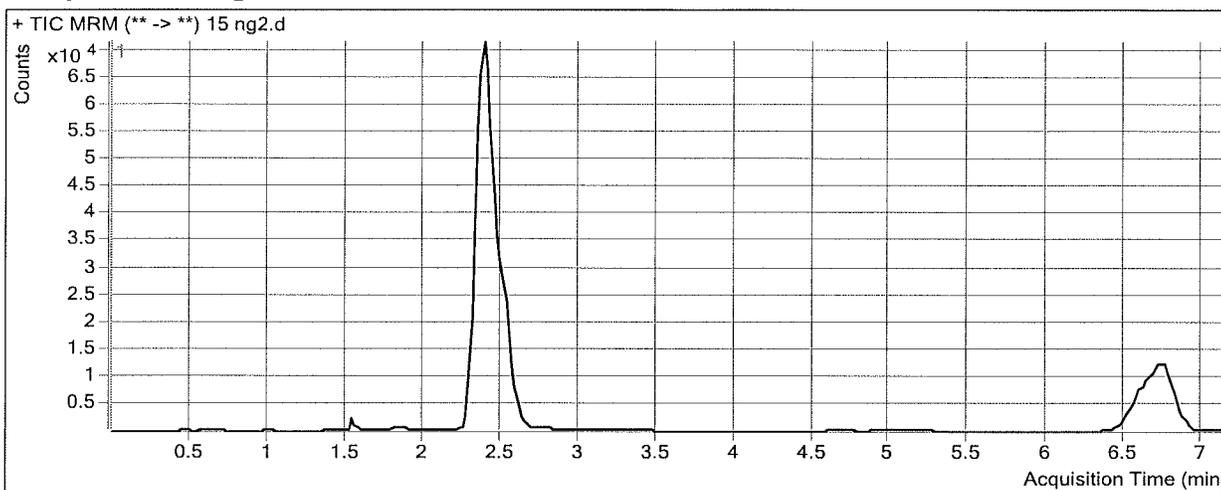
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 12:02 **Data File** 15 ng2.d  
**Sample Type** Sample **Sample Name** 15 ng2  
**Dilution** 1 **Acq Method** Quant THC 2017.m  
**Position** p1e4 **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	72206	444215	0.1625	17.4837
THC-COOH	THC-COOH-d9	2.546	27371	141854	0.1930	11.4370
THC	THC-d3	6.733	24855	163586	0.1519	15.1464

*Not evaluated quantitatively*

# ISP FORENSICS - Cd'A Instrument # 62340

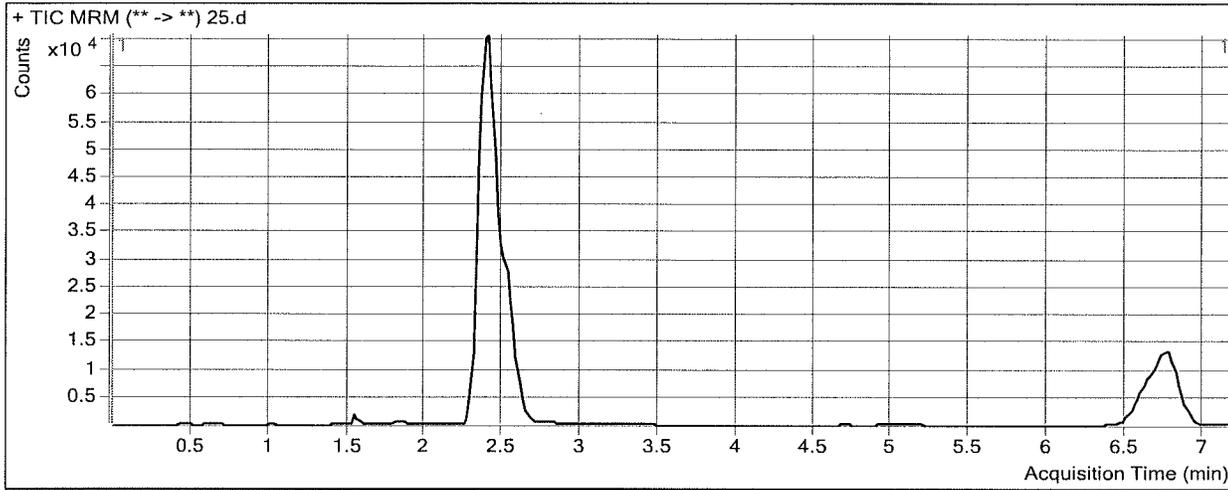
## Cannabinoids Analysis Report

**Batch Data Path** D:\2017 Data\1-31-17 THC Quant\QuantResults\1-31-17 amn cann quant.batch.bin  
**Analysis Time** 2/1/2017 6:02 PM      **Analyst Name** ISP Tox  
**Report Time** 2/1/2017 6:03 PM      **Reporter Name** ISP Tox  
**Last Calib Update** 2/1/2017 6:02 PM      **Batch State** Processed

### Analysis Info

**Acq Time** 2017-02-01 12:26      **Data File** 25.d  
**Sample Type** Sample      **Sample Name** 25  
**Dilution** 1      **Acq Method** Quant THC 2017.m  
**Position** P1-F4      **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	112467	399501	0.2815	29.1408
THC-COOH	THC-COOH-d9	2.546	42083	129398	0.3252	19.4186 <i>not evaluated for quant</i>
THC	THC-d3	6.773	37528	147462	0.2545	24.8762 <i>A</i>