reviewed 6/20/17

6/19/2017

**Worklist: 1768**

| LAB_CASE | ITEM | TASK_ID | DESCRIPTION |
|---|---|---|---|
| C2017-0951 | 1 | 87394 | AM 27 Blood THC Quant by LC |
| C2017-0952 | 1 | 87395 | AM 27 Blood THC Quant by LC |
| C2017-1077 | 1 | 87396 | AM 27 Blood THC Quant by LC |
| C2017-1078 | 1 | 87399 | AM 27 Blood THC Quant by LC |
| C2017-1087 | 1 | 87400 | AM 27 Blood THC Quant by LC |
| M2017-1668 | 1 | 87402 | AM 27 Blood THC Quant by LC |
| M2017-2289 | 1 | 87397 | AM 27 Blood THC Quant by LC |
| P2017-1066 | 1 | 87401 | AM 27 Blood THC Quant by LC |
| P2017-1127 | 1 | 87398 | AM 27 Blood THC Quant by LC |

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

**Extraction Date:** 6-14-17 **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***
- Mobile Phase B: ***0.1% Formic Acid in LCMS Acetonitrile***
- ***LCMS Methanol***
- **Blank/Negative Blood: Lot *321632-1***
- ***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: 61717 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: 61417 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

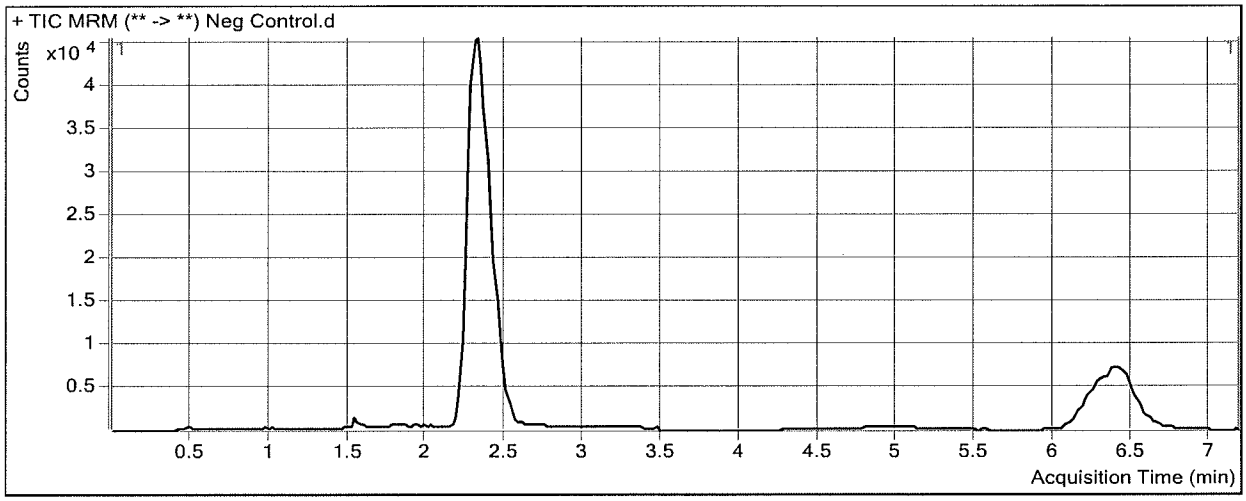
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 10:52 | **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



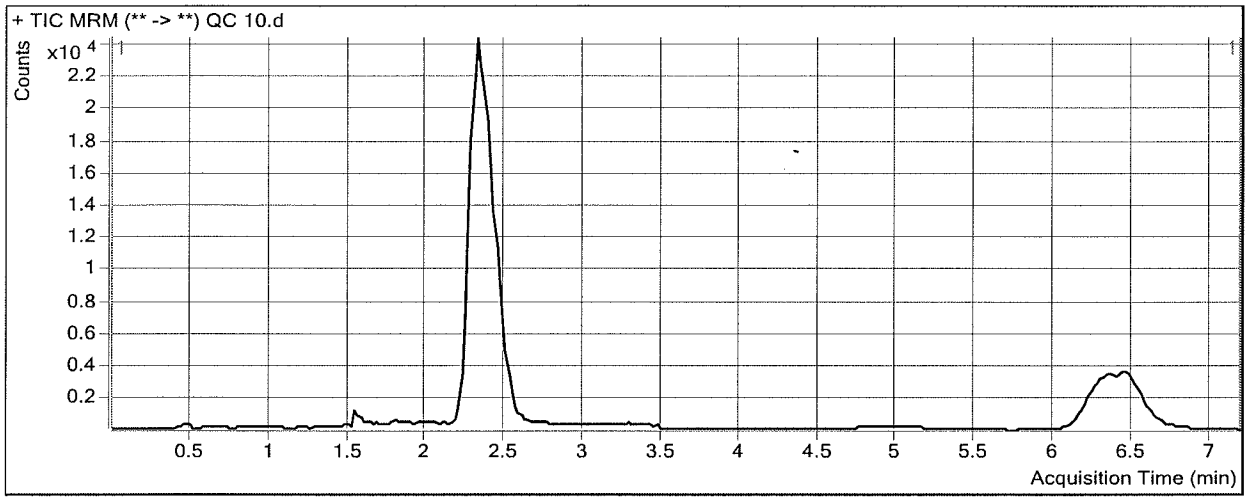
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 11:04 | **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 | 2.336 | 14901 | 168192 | 0.0886 | 10.2200 |
| THC-COOH | THC-COOH-d9 | 2.446 | 11514 | 58295 | 0.1975 | 10.0970 |
| THC | THC-d3 | 6.473 | 8198 | 68257 | 0.1201 | 10.2351 |

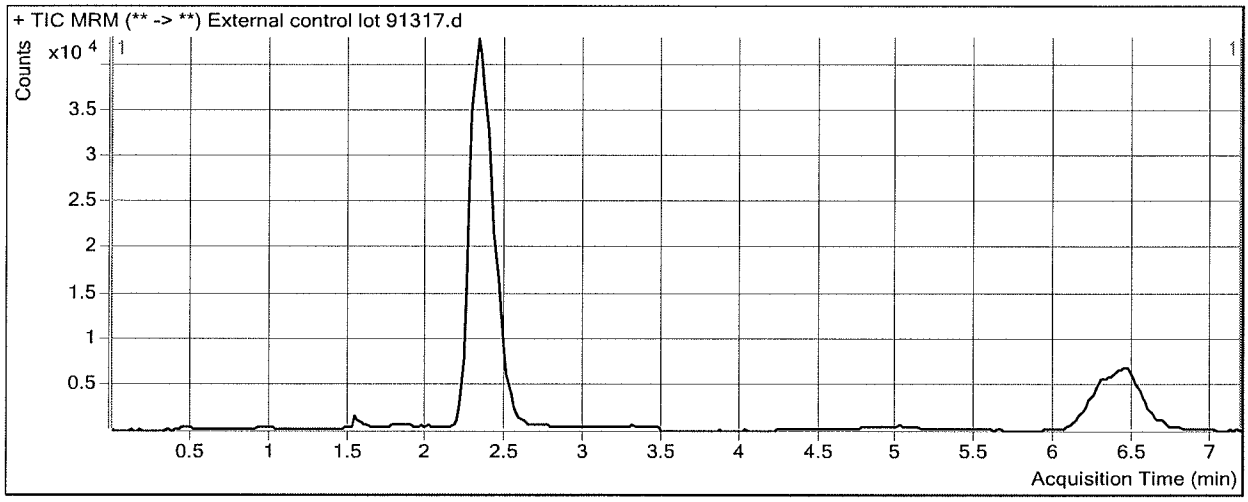
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 11:15 | **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 | 2.336 | 26236 | 304339 | 0.0862 | 9.9568 |
| THC-COOH | THC-COOH-d9 | 2.446 | 14642 | 102293 | 0.1431 | 7.1935 |
| THC | THC-d3 | 6.453 | 11382 | 127506 | 0.0893 | 7.6223 |

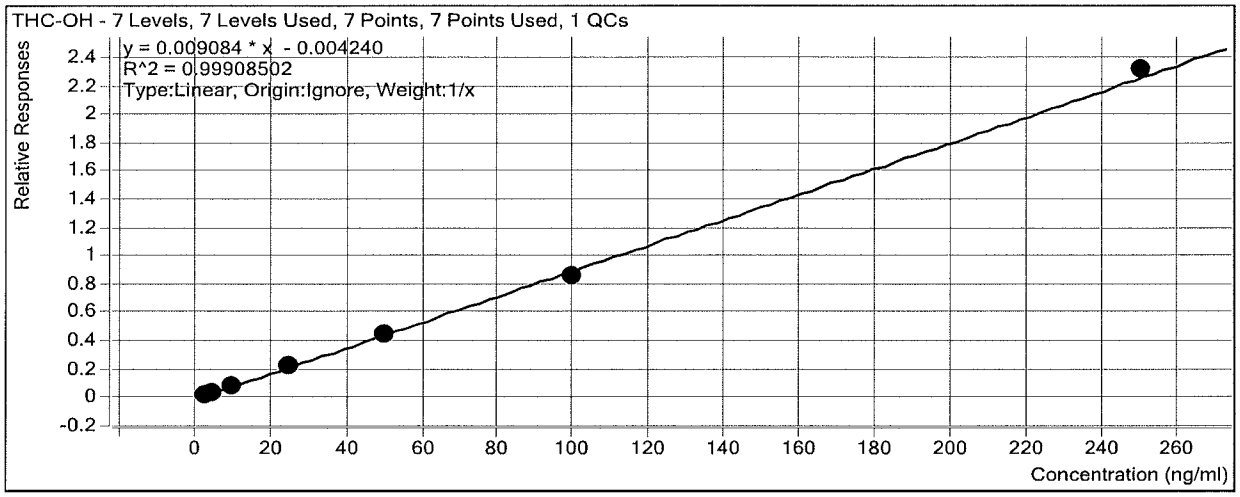
# ISP Forensics
# Calibration Curve Report

**Batch Data Path** D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin

**Last Calib Update** 6/15/2017 3:51 PM **Analyst Name** ISP TOX

***Target Compound*** *THC-OH*

*Internal Standard* *THC-OH-d3*



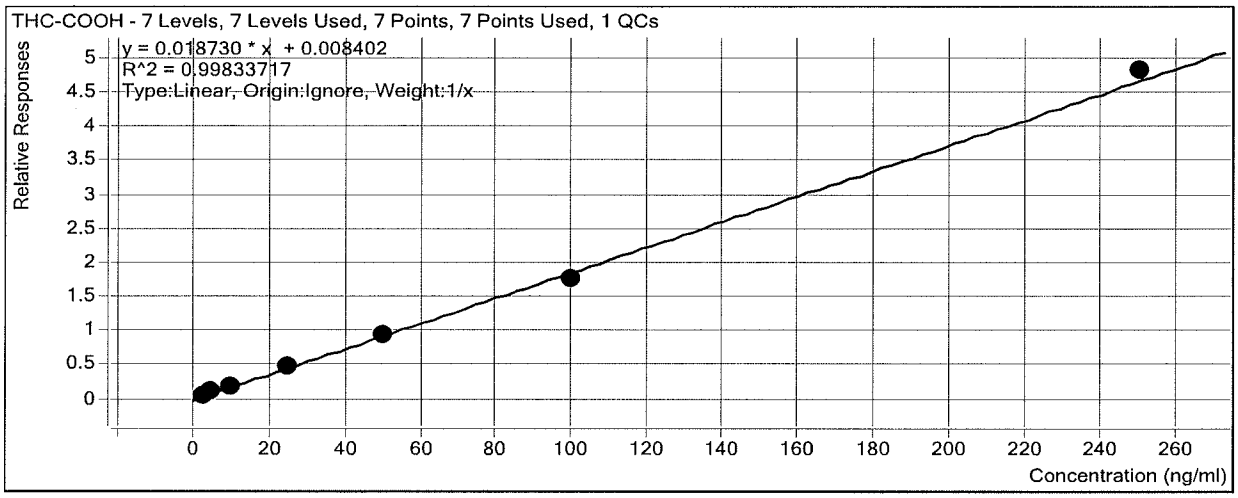

| Sample | Level | Enabled | Exp Conc | Final Conc | Accuracy |
|---|---|---|---|---|---|
| Cal 1- 3ng | 1 | ☑ | 3 | 3.2 | 106.3 |
| cal 2- 5ng | 2 | ☑ | 5 | 5.0 | 100.9 |
| cal 3 - 10ng | 3 | ☑ | 10 | 9.6 | 95.8 |
| QC 10 | 3 | ☑ | 10 | 10.2 | 102.2 |
| cal 4 - 25ng | 4 | ☑ | 25 | 25.1 | 100.3 |
| cal 5 - 50ng | 5 | ☑ | 50 | 49.6 | 99.3 |
| cal 6 - 100ng | 6 | ☑ | 100 | 95.4 | 95.4 |
| cal 7 - 250ng | 7 | ☑ | 250 | 255.0 | 102.0 |

# ISP Forensics
# Calibration Curve Report

**Batch Data Path** D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin

**Last Calib Update** 6/15/2017 3:51 PM **Analyst Name** ISP TOX

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



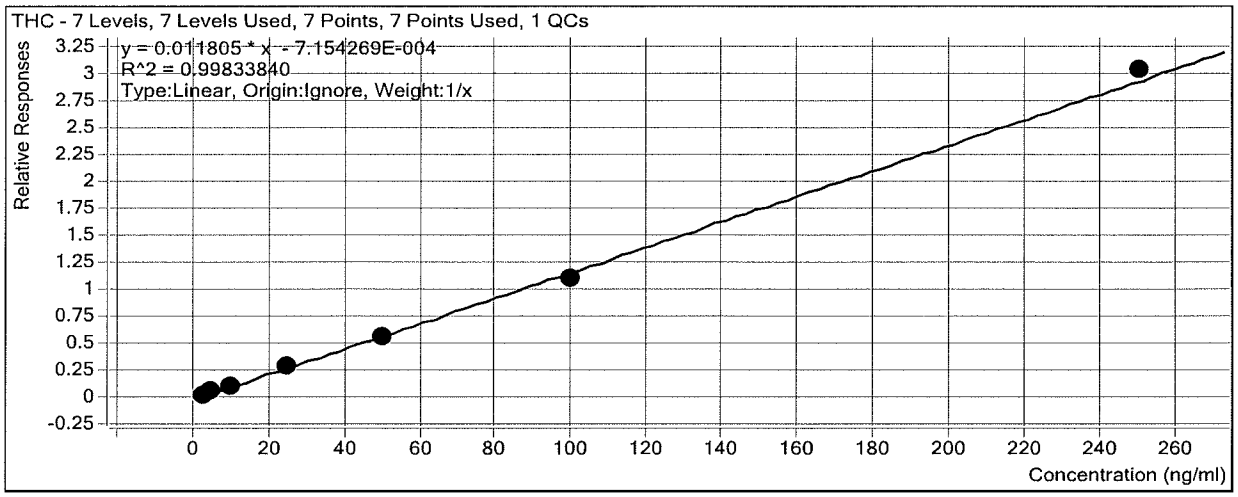

| Sample | Level | Enabled | Exp Conc | Final Conc | Accuracy |
|---|---|---|---|---|---|
| Cal 1- 3ng | 1 | ☑ | 3 | 3.1 | 102.3 |
| cal 2- 5ng | 2 | ☑ | 5 | 5.0 | 100.5 |
| cal 3 - 10ng | 3 | ☑ | 10 | 10.2 | 101.7 |
| QC 10 | 3 | ☑ | 10 | 10.1 | 101.0 |
| cal 4 - 25ng | 4 | ☑ | 25 | 25.3 | 101.0 |
| cal 5 - 50ng | 5 | ☑ | 50 | 49.0 | 98.1 |
| cal 6 - 100ng | 6 | ☑ | 100 | 93.6 | 93.6 |
| cal 7 - 250ng | 7 | ☑ | 250 | 256.8 | 102.7 |

# ISP Forensics
# Calibration Curve Report

**Batch Data Path** D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin

**Last Calib Update** 6/15/2017 3:51 PM **Analyst Name** ISP TOX

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




| Sample | Level | Enabled | Exp Conc | Final Conc | Accuracy |
|---|---|---|---|---|---|
| Cal 1- 3ng | 1 | ☑ | 3 | 3.2 | 105.7 |
| cal 2- 5ng | 2 | ☑ | 5 | 5.1 | 102.6 |
| cal 3 - 10ng | 3 | ☑ | 10 | 9.7 | 97.5 |
| QC 10 | 3 | ☑ | 10 | 10.2 | 102.4 |
| cal 4 - 25ng | 4 | ☑ | 25 | 25.2 | 101.0 |
| cal 5 - 50ng | 5 | ☑ | 50 | 47.8 | 95.7 |
| cal 6 - 100ng | 6 | ☑ | 100 | 94.6 | 94.6 |
| cal 7 - 250ng | 7 | ☑ | 250 | 257.3 | 102.9 |

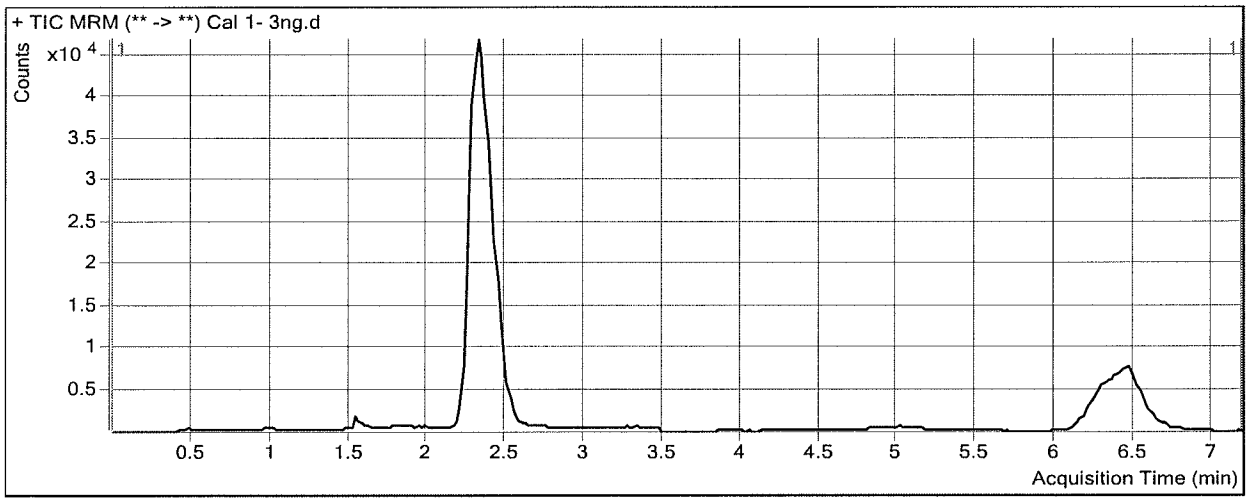
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 09:17 | **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 | 2.336 | 8759 | 354253 | 0.0247 | 3.1887 |
| THC-COOH | THC-COOH-d9 | 2.446 | 7284 | 110552 | 0.0659 | 3.0691 |
| THC | THC-d3 | 6.453 | 5245 | 142796 | 0.0367 | 3.1723 |

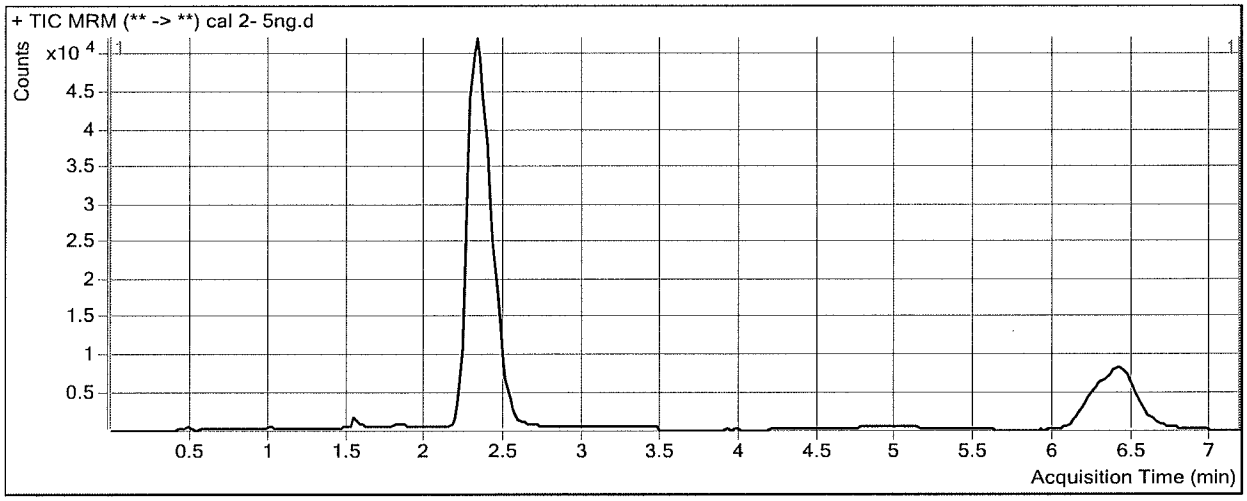
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 09:29 | **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 | 2.336 | 16409 | 394473 | 0.0416 | 5.0459 |
| THC-COOH | THC-COOH-d9 | 2.466 | 12248 | 119416 | 0.1026 | 5.0275 |
| THC | THC-d3 | 6.433 | 9257 | 154641 | 0.0599 | 5.1314 |

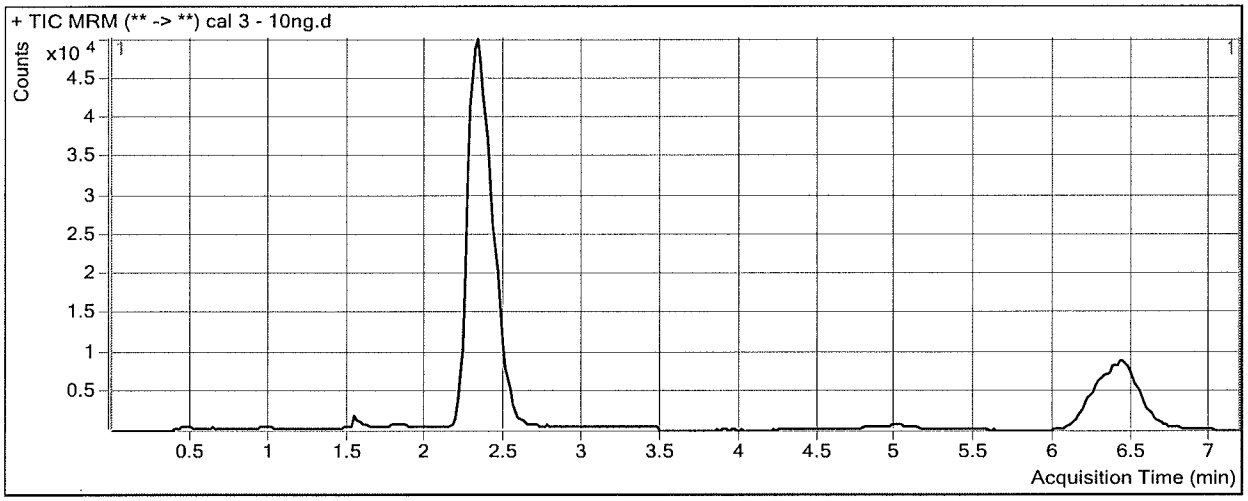
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 09:41 | **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 | 2.336 | 29866 | 360914 | 0.0828 | 9.5764 |
| THC-COOH | THC-COOH-d9 | 2.466 | 23308 | 117205 | 0.1989 | 10.1690 |
| THC | THC-d3 | 6.433 | 17708 | 154821 | 0.1144 | 9.7496 |

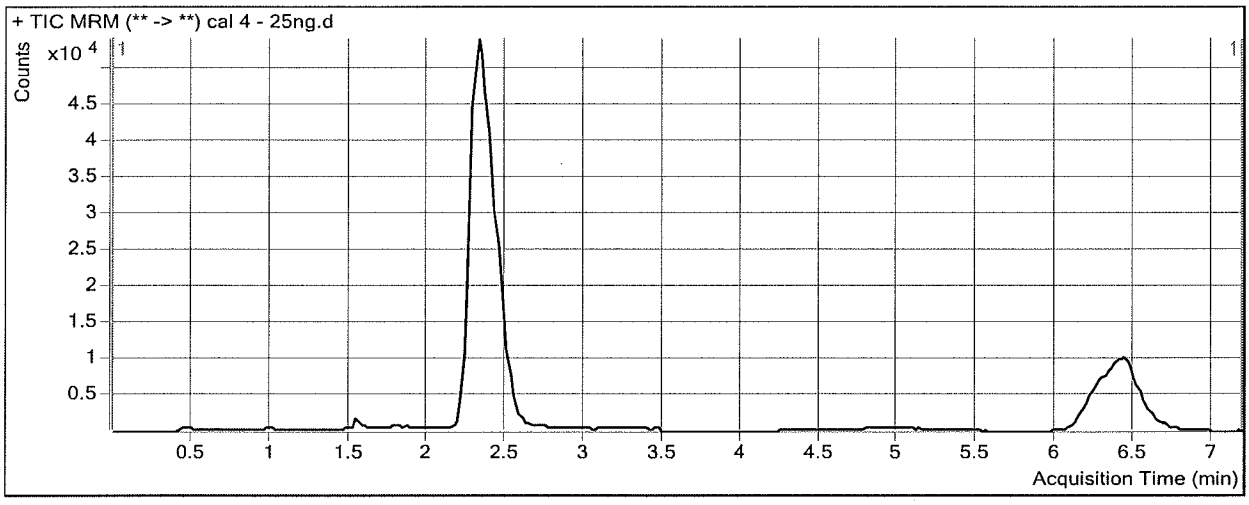
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 09:52 | **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 | 2.336 | 76185 | 340830 | 0.2235 | 25.0740 |
| THC-COOH | THC-COOH-d9 | 2.466 | 52932 | 109919 | 0.4816 | 25.2620 |
| THC | THC-d3 | 6.433 | 42547 | 143153 | 0.2972 | 25.2384 |

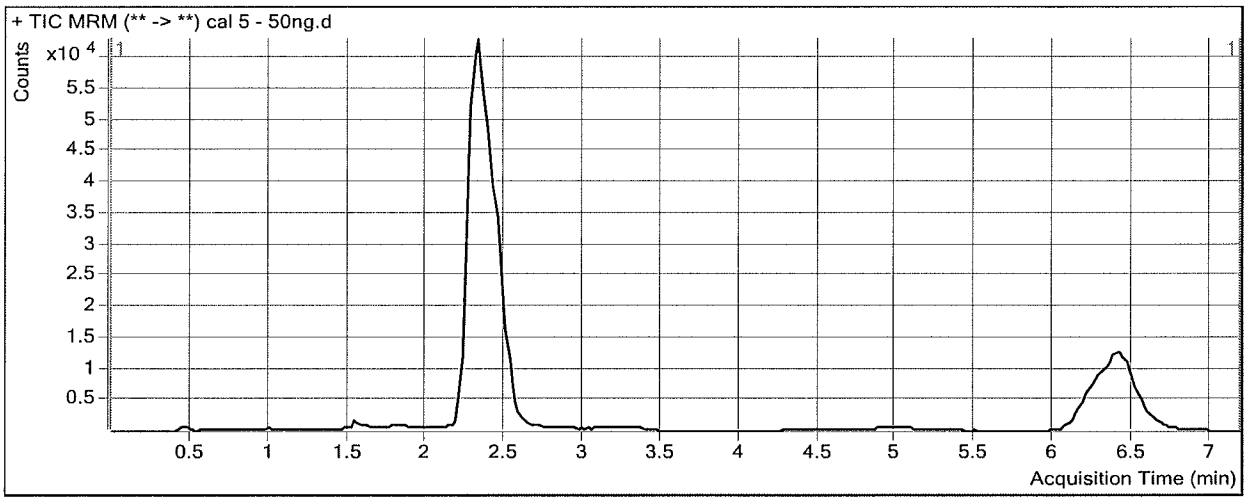
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 10:04 | **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 | 2.336 | 147153 | 329454 | 0.4467 | 49.6374 |
| THC-COOH | THC-COOH-d9 | 2.446 | 98359 | 106118 | 0.9269 | 49.0381 |
| THC | THC-d3 | 6.393 | 79585 | 141090 | 0.5641 | 47.8446 |

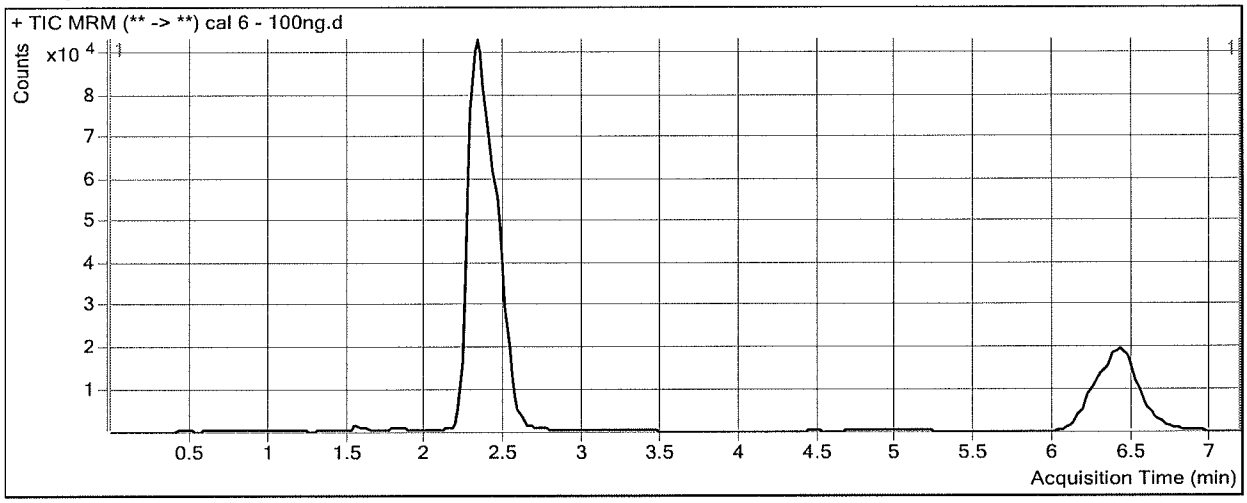
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 10:16 | **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 | 2.336 | 333069 | 386059 | 0.8627 | 95.4420 |
| THC-COOH | THC-COOH-d9 | 2.446 | 195376 | 110914 | 1.7615 | 93.5989 |
| THC | THC-d3 | 6.413 | 171689 | 153888 | 1.1157 | 94.5726 |

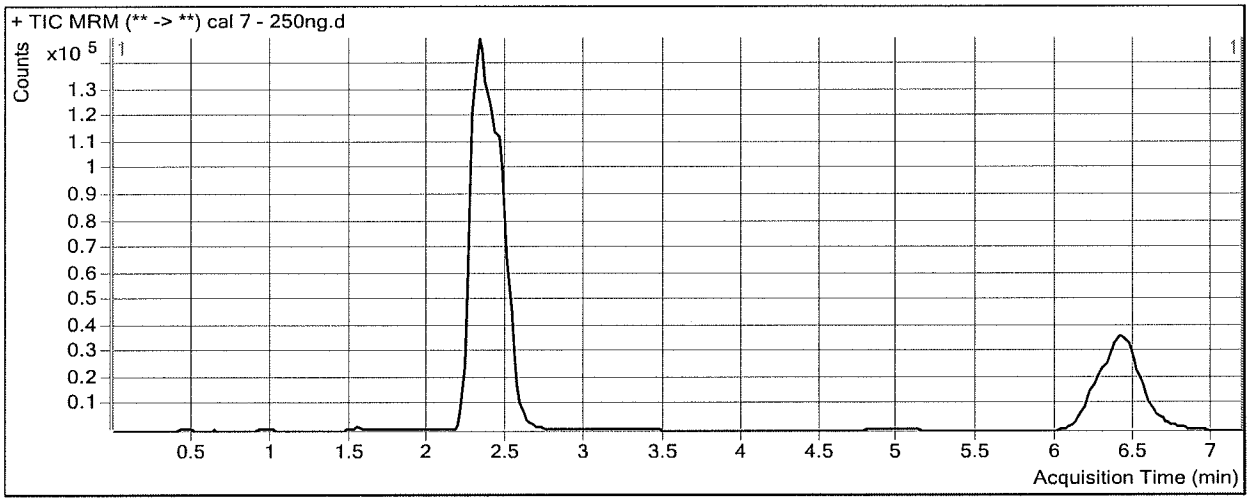
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2017 Data\61417 THC Quant\QuantResults\61417 cann quant.batch.bin | | |
| **Analysis Time** | 6/15/2017 3:51 PM | **Analyst Name** | ISP Tox |
| **Report Time** | 6/15/2017 3:52 PM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 6/15/2017 3:51 PM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2017-06-15 10:28 | **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 | 2.336 | 793299 | 343054 | 2.3125 | 255.0355 |
| THC-COOH | THC-COOH-d9 | 2.466 | 479439 | 99491 | 4.8189 | 256.8354 |
| THC | THC-d3 | 6.413 | 420476 | 138474 | 3.0365 | 257.2911 |