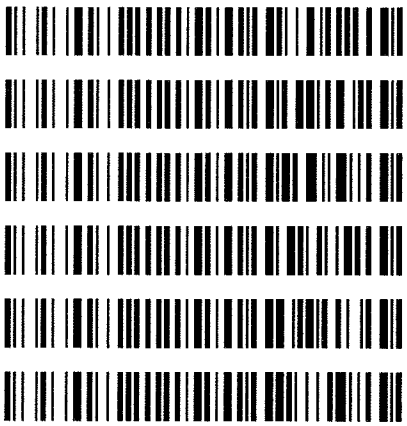
**REVIEWED**
*By Anne Nord at 1:17 pm, Nov 06, 2018*

10/31/2018

**Worklist: 2766**

| LAB_CASE | ITEM | TASK_ID | DESCRIPTION |
|---|---|---|---|
| C2018-2060 | 1 | 130703 | AM 27 Blood THC Quant by LC-QQQ |
| C2018-2089 | 1 | 130699 | AM 27 Blood THC Quant by LC-QQQ |
| C2018-2101 | 1 | 130700 | AM 27 Blood THC Quant by LC-QQQ |
| C2018-2113 | 1 | 130701 | AM 27 Blood THC Quant by LC-QQQ |
| C2018-2163 | 1 | 130702 | AM 27 Blood THC Quant by LC-QQQ |
| C2018-2172 | 1 | 130704 | AM 27 Blood THC Quant by LC-QQQ |



# AM# 27: Quantitation of THC and Metabolites in Blood by LC-MS/MS

Extraction Date: 10/31/18 Analyst: Britany Wylie
Plate lot#: 0539904 Plate Expiration: 09/10/2019

**Mobile phase A**: 0.1% Formic Acid in LCMS Water **Mobile phase B:** 0.1% Formic acid in Acetonitrile
MTBE LCMS Methanol Hexane
**Blank Blood Lot**: 18G207D7 **Column**: UCT Selectra DA 100 x 2.1mm 3um
**LCMS-QQQ ID**: 62340

## Pre-Analytic:

- [x] 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- [x] 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.
- [x] 3. Create worklist:

## Analytic:

- [x] 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- [x] 2. Pipette **1000µL blood (calibrated pipette) Pipette ID: k52558g** in wells of analytical (standards) plate.
- [x] 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- [x] 4. Pipette **500µL 0.1% formic acid in water** in wells of analytical plate.
- [x] 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- [x] 6. Transfer **800µL of blood+acid** mixture to corresponding wells of SLE+ plate.
- [x] 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent). ***(Load at 85-100 PSI- Selector to the right)*** Manifold ID: 66792
- [x] 8. Wait 5 minutes.
- [x] 9. Add **2.25mL MTBE. (*Add in 3 increments of 750uL)***
- [x] 10. Wait 5 minutes.
- [x] 11. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
- [x] 12. Add **2.25mL Hexane. (*Add in 3 increments of 750uL)***
- [x] 13. Wait 5 minutes.
- [x] 14. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
- [x] 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. *SPE Dry ID: 66819*
- [x] 16. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

## Post-Analytic

- [x] 1. Create batch and process data.
  Batch path: cann quant Batch Name: _____
- [x] 2. Make any necessary integration changes, Curve weighting of Linear 1/x with $r^2$ values ≥0.98 for each analyte
- [x] 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less). Ion ratios must be within +/- 20% of the averaged calibrators
- [x] 4. Case sample response for THC and OH-THC 3ng/mL (quantitative), Carboxy-THC: 10ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
- [x] 5. Did all QCs pass for each analyte? (Y) N
- [x] 6 Enter QCs into control charting.
- [x] 7 Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:

Toxicology AM method 27 external prep information
working solution 1 ug/ml in meoh C-THC, THC-OH, THC
Stock solution 1mg/ml 10 ul each THC, THC-OH 100 ug/ml 100 ul C-THC in 9890 ul meOH
Ppd 6/5/18 Exp: 4/1/19 lot 6518 by AMN

| Drug | lot (cerilliant) | expiration |
|---|---|---|
| C-THC | FE03121501 | 3/1/2020 |
| THC-OH | FE01141502 | 1/1/2020 |
| THC | FE04231406 | 4/1/2019 |

AM 27 control 50 ul working solution lot (6518) in 4950 ul blood lot (17J20718)

| | | | |
|---|---|---|---|
| ppd 6/5/18 Exp 4/1/19 | lot 6518 | Concentration 10 ng/ml each | by AMN |
| ppd 9/20/18 Exp 4/1/19 | lot 92018 | Concentration 10 ng/ml each | by AMN |

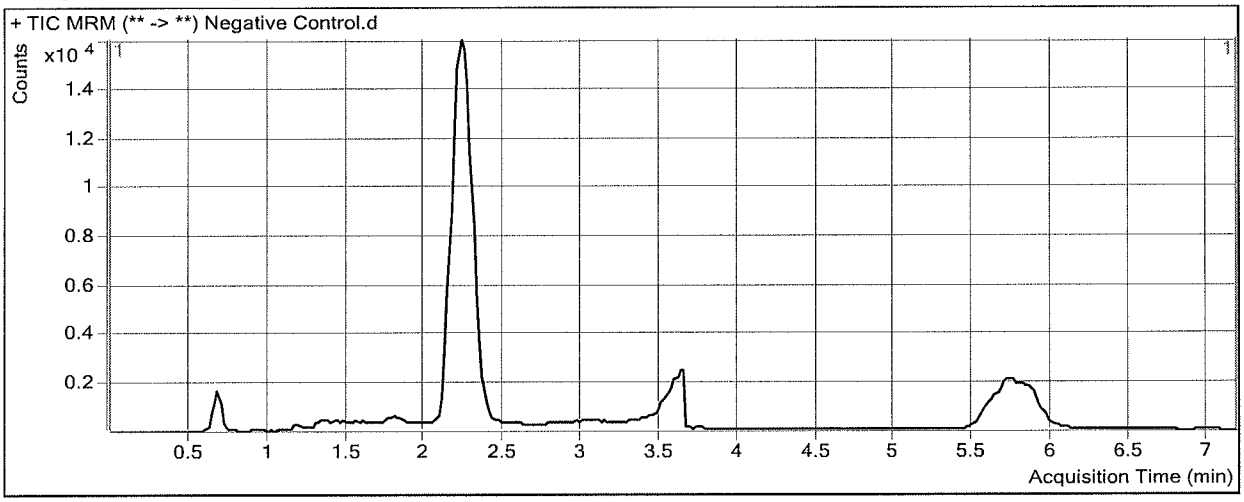
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 11:29 | **Data File** | Negative Control.d |
| **Sample Type** | Sample | **Sample Name** | Negative Control |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.m |
| **Position** | P1-H2 | **Sample Info** | |
| **Inj Vol** | -1 | **Comment** | AM 27 Cannabinoid Confirmation |

## Sample Chromatogram



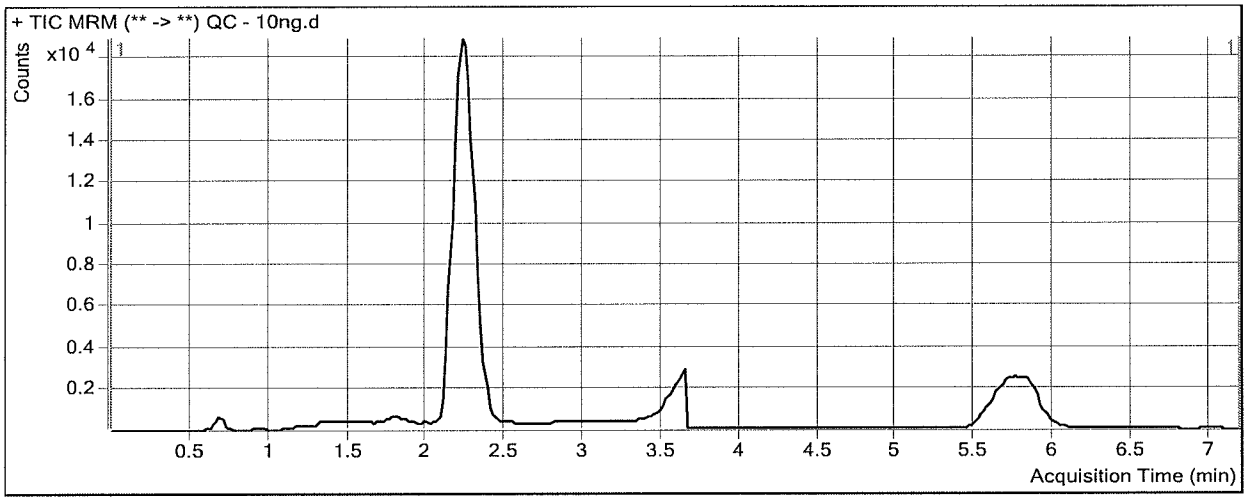
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 11:41 | **Data File** | QC - 10ng.d |
| **Sample Type** | QC | **Sample Name** | QC - 10ng |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.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 | 2.256 | 4669 | 115678 | 0.0404 | 5.0459 |
| THC-COOH | THC-COOH-d9 | 2.306 | 7340 | 46027 | 0.1595 | 9.2502 |
| THC | THC-d3 | 5.833 | 2395 | 45457 | 0.0527 | 4.8300 |

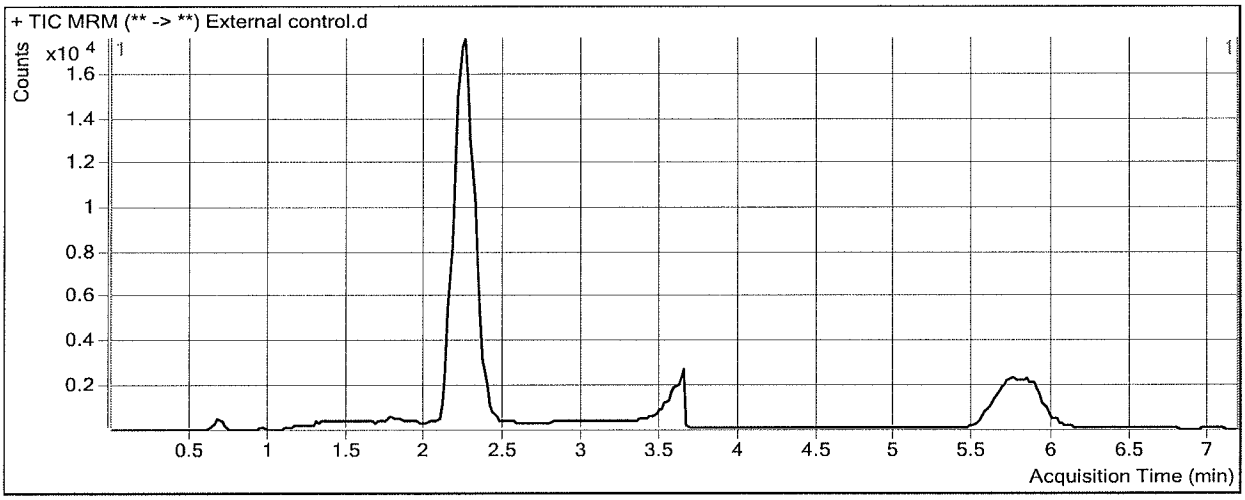
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 11:53 | **Data File** | External control.d |
| **Sample Type** | Sample | **Sample Name** | External control |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.m |
| **Position** | P1-G2 | **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 | 2.236 | 8131 | 100891 | 0.0806 | 9.8323 |
| THC-COOH | THC-COOH-d9 | 2.326 | 6231 | 41071 | 0.1517 | 8.7483 |
| THC | THC-d3 | 5.833 | 4844 | 38574 | 0.1256 | 12.0675 |

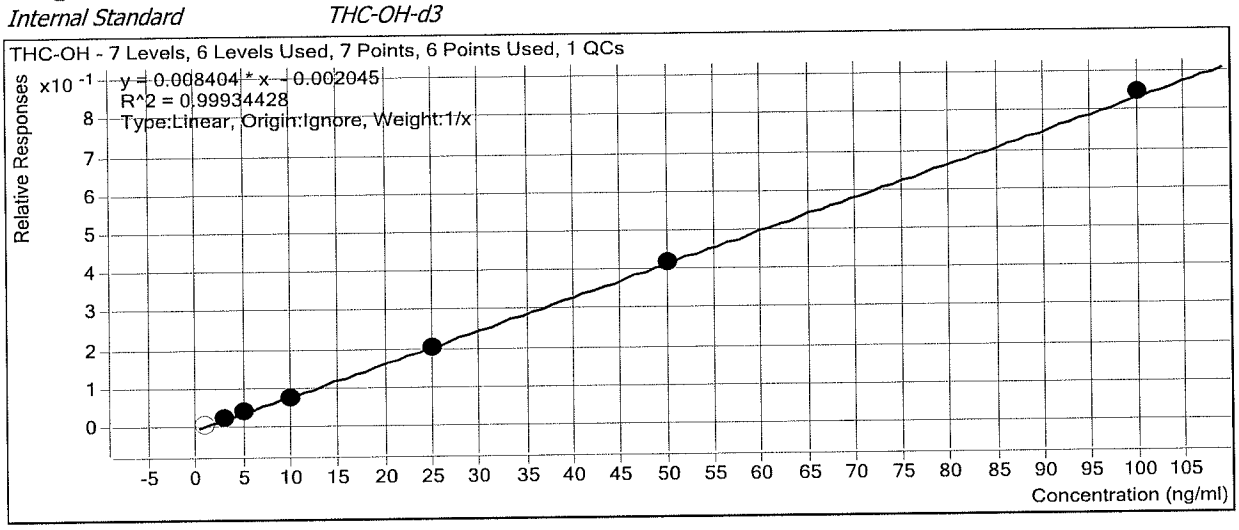
# ISP Forensics
# Calibration Curve Report

**Batch Data Path** D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin

**Last Calib Update** 11/5/2018 8:30 AM **Analyst Name** ISP TOX

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

*Internal Standard* *THC-OH-d3*




| Sample | Level | Enabled | Exp Conc | Final Conc | Accuracy |
|---|---|---|---|---|---|
| Cal 1 - 1ng | 1 | ☐ | 1 | 1.2 | 117.6 |
| Cal 2 - 3ng | 2 | ☑ | 3 | 3.3 | 109.2 |
| Cal 3 - 5ng | 3 | ☑ | 5 | 4.9 | 98.7 |
| QC - 10ng | 3 | ☑ | 5 | 5.0 | 100.9 |
| Cal 4 - 10ng | 4 | ☑ | 10 | 9.3 | 93.5 |
| Cal 5 - 25ng | 5 | ☑ | 25 | 24.4 | 97.6 |
| Cal 6 - 50ng | 6 | ☑ | 50 | 50.1 | 100.1 |
| Cal 7 - 100ng | 7 | ☑ | 100 | 101.0 | 101.0 |

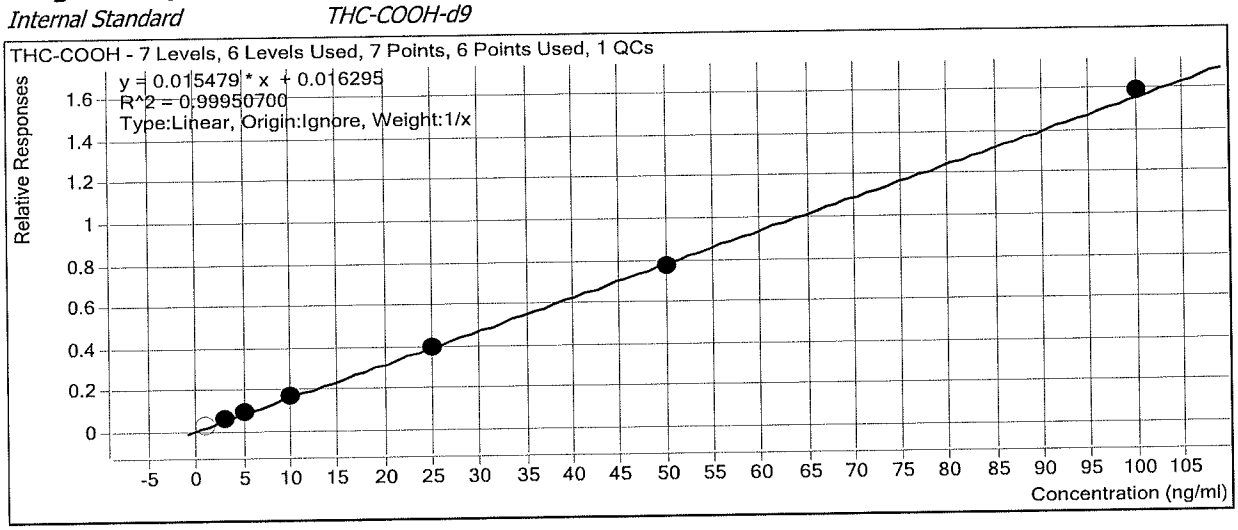
# ISP Forensics
# Calibration Curve Report

**Batch Data Path** D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin

**Last Calib Update** 11/5/2018 8:30 AM **Analyst Name** ISP TOX

***Target Compound*** *THC-COOH*

*Internal Standard* *THC-COOH-d9*




| Sample | Level | Enabled | Exp Conc | Final Conc | Accuracy |
|---|---|---|---|---|---|
| Cal 1 - 1ng | 1 | ☐ | 1 | 1.2 | 122.0 |
| Cal 2 - 3ng | 2 | ☑ | 3 | 3.2 | 106.8 |
| Cal 3 - 5ng | 3 | ☑ | 5 | 4.8 | 95.9 |
| QC - 10ng | 3 | ☑ | 5 | 9.3 | 185.0 |
| Cal 4 - 10ng | 4 | ☑ | 10 | 9.9 | 99.1 |
| Cal 5 - 25ng | 5 | ☑ | 25 | 24.7 | 98.9 |
| Cal 6 - 50ng | 6 | ☑ | 50 | 48.9 | 97.9 |
| Cal 7 - 100ng | 7 | ☑ | 100 | 101.4 | 101.4 |

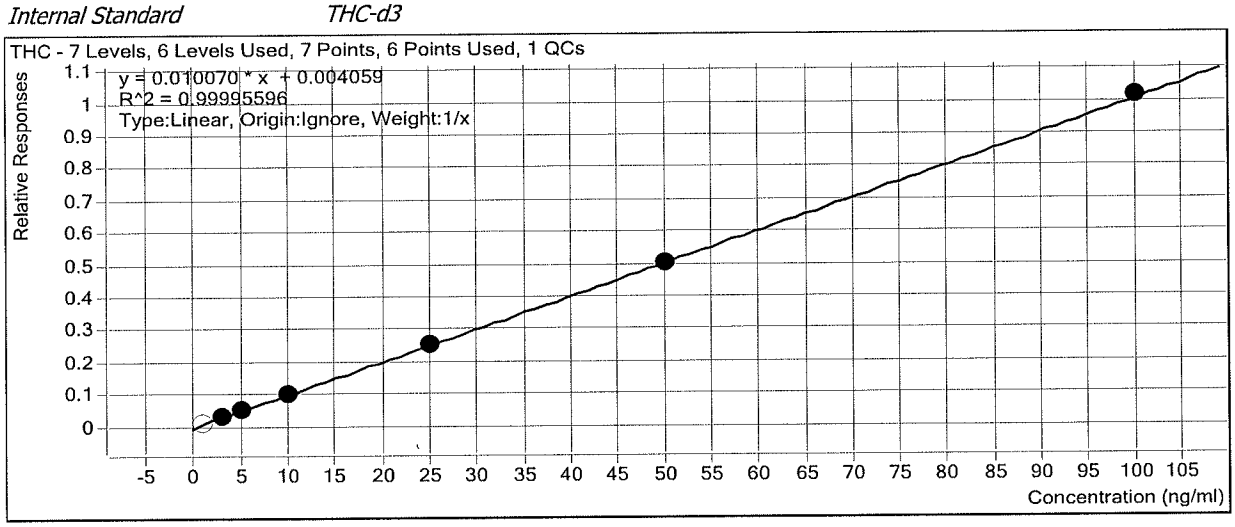
# ISP Forensics
# Calibration Curve Report

**Batch Data Path** D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin

**Last Calib Update** 11/5/2018 8:30 AM **Analyst Name** ISP TOX

***Target Compound*** *THC*

*Internal Standard* *THC-d3*




| Sample | Level | Enabled | Exp Conc | Final Conc | Accuracy |
|---|---|---|---|---|---|
| Cal 1 - 1ng | 1 | ☐ | 1 | 0.9 | 93.0 |
| Cal 2 - 3ng | 2 | ☑ | 3 | 3.0 | 100.6 |
| Cal 3 - 5ng | 3 | ☑ | 5 | 5.1 | 101.3 |
| QC - 10ng | 3 | ☑ | 5 | 4.8 | 96.6 |
| Cal 4 - 10ng | 4 | ☑ | 10 | 9.9 | 98.8 |
| Cal 5 - 25ng | 5 | ☑ | 25 | 24.8 | 99.4 |
| Cal 6 - 50ng | 6 | ☑ | 50 | 49.8 | 99.5 |
| Cal 7 - 100ng | 7 | ☑ | 100 | 100.4 | 100.4 |

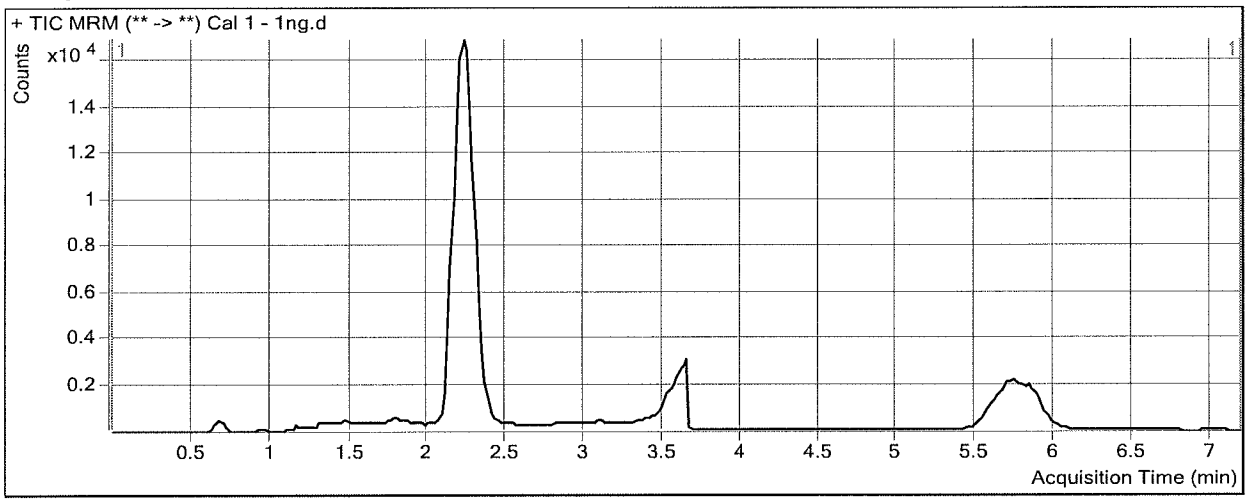
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 09:54 | **Data File** | Cal 1 - 1ng.d |
| **Sample Type** | Calibration | **Sample Name** | Cal 1 - 1ng |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.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 | 2.236 | 853 | 108735 | 0.0078 | 1.1762 |
| THC-COOH | THC-COOH-d9 | 2.306 | 1519 | 43171 | 0.0352 | 1.2198 |
| THC | THC-d3 | 5.773 | 543 | 40456 | 0.0134 | 0.9304 |

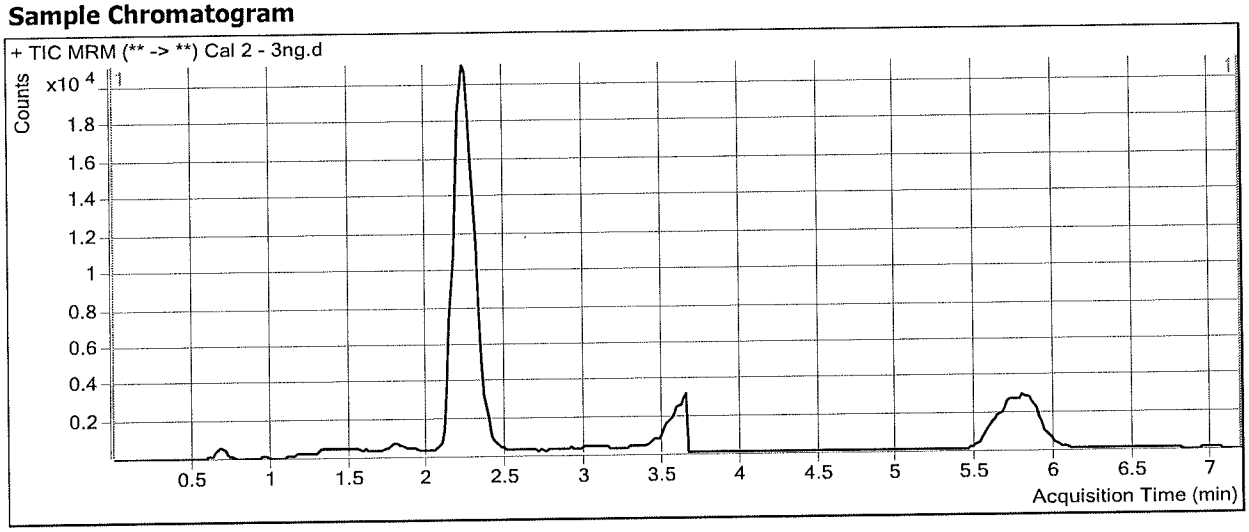
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 10:06 | **Data File** | Cal 2 - 3ng.d |
| **Sample Type** | Calibration | **Sample Name** | Cal 2 - 3ng |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.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 | 2.236 | 3372 | 132315 | 0.0255 | 3.2752 |
| THC-COOH | THC-COOH-d9 | 2.326 | 3482 | 52839 | 0.0659 | 3.2045 |
| THC | THC-d3 | 5.833 | 1807 | 52462 | 0.0344 | 3.0177 |

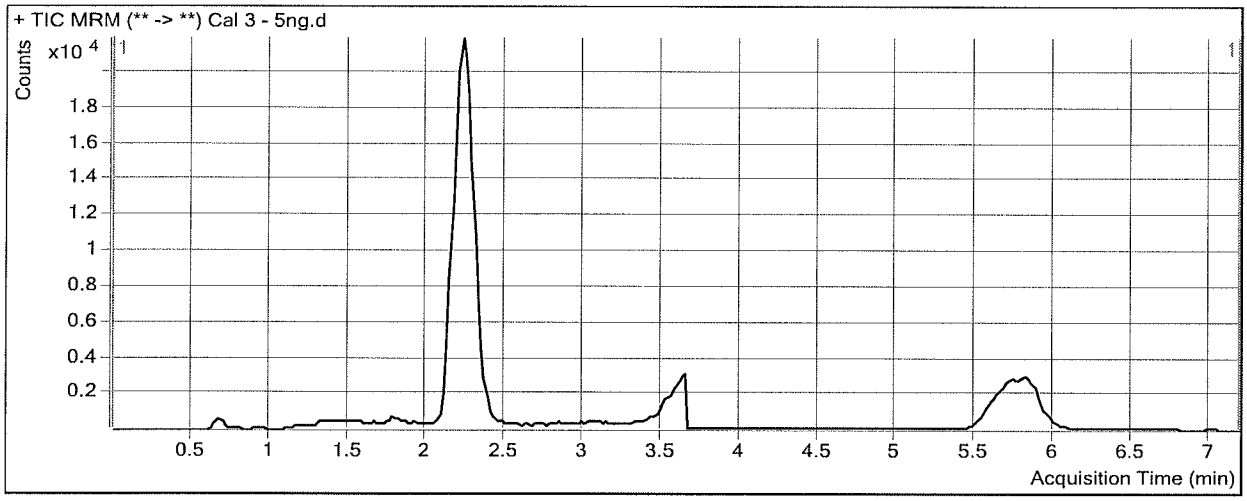
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 10:18 | **Data File** | Cal 3 - 5ng.d |
| **Sample Type** | Calibration | **Sample Name** | Cal 3 - 5ng |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.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 | 2.216 | 5287 | 134154 | 0.0394 | 4.9328 |
| THC-COOH | THC-COOH-d9 | 2.306 | 4633 | 51162 | 0.0905 | 4.7971 |
| THC | THC-d3 | 5.813 | 2823 | 51288 | 0.0550 | 5.0631 |

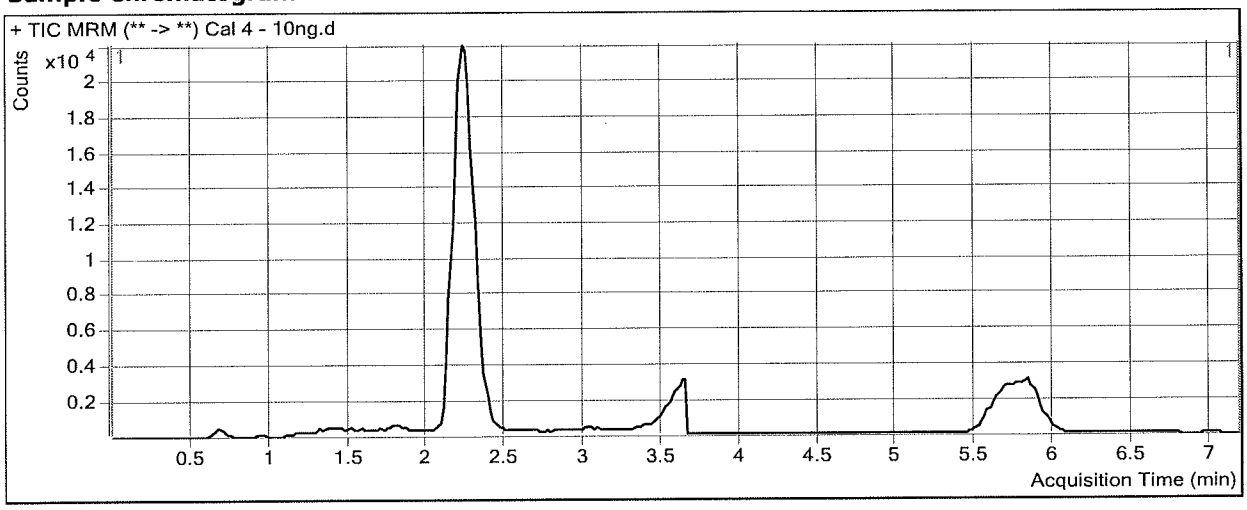
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 10:30 | **Data File** | Cal 4 - 10ng.d |
| **Sample Type** | Calibration | **Sample Name** | Cal 4 - 10ng |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.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 | 2.236 | 9993 | 130607 | 0.0765 | 9.3467 |
| THC-COOH | THC-COOH-d9 | 2.326 | 8614 | 50780 | 0.1696 | 9.9062 |
| THC | THC-d3 | 5.833 | 5365 | 51799 | 0.1036 | 9.8820 |

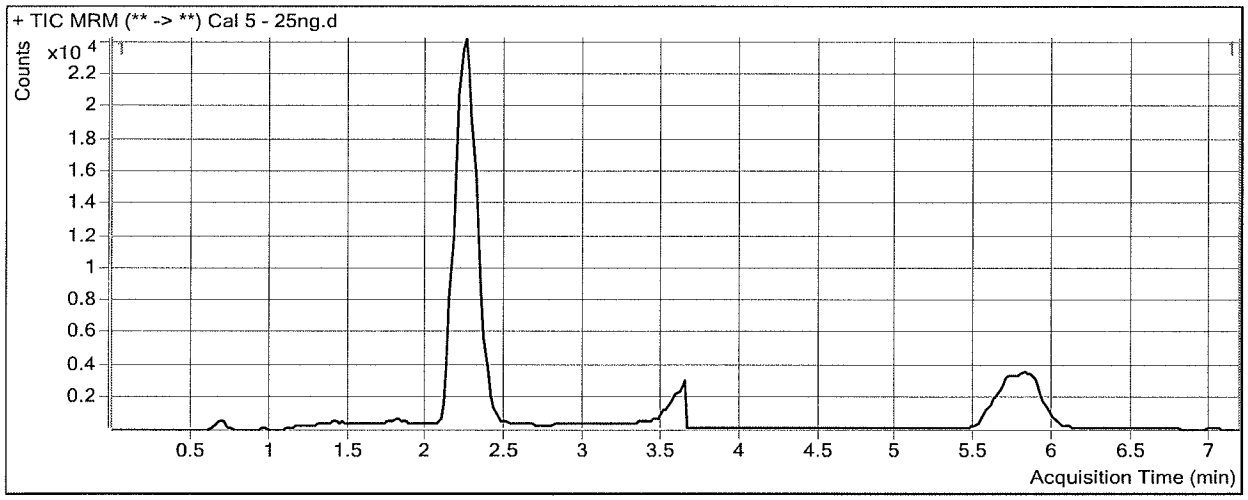
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 10:42 | **Data File** | Cal 5 - 25ng.d |
| **Sample Type** | Calibration | **Sample Name** | Cal 5 - 25ng |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.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 | 2.236 | 25967 | 127904 | 0.2030 | 24.3994 |
| THC-COOH | THC-COOH-d9 | 2.326 | 20064 | 50296 | 0.3989 | 24.7192 |
| THC | THC-d3 | 5.853 | 12935 | 50880 | 0.2542 | 24.8423 |

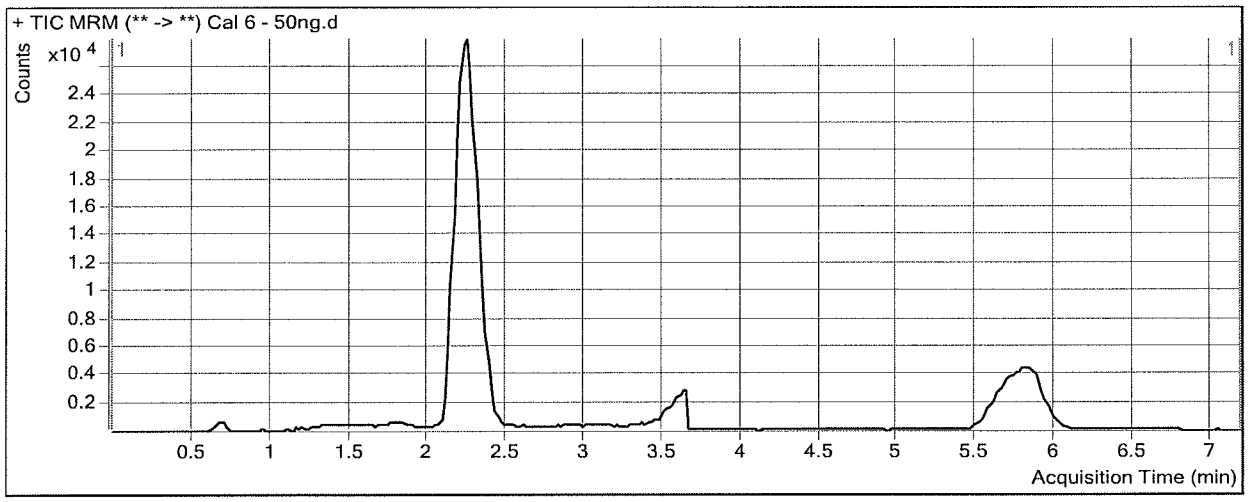
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 10:54 | **Data File** | Cal 6 - 50ng.d |
| **Sample Type** | Calibration | **Sample Name** | Cal 6 - 50ng |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.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 | 2.236 | 51633 | 123323 | 0.4187 | 50.0603 |
| THC-COOH | THC-COOH-d9 | 2.326 | 36500 | 47177 | 0.7737 | 48.9298 |
| THC | THC-d3 | 5.833 | 25332 | 50145 | 0.5052 | 49.7647 |

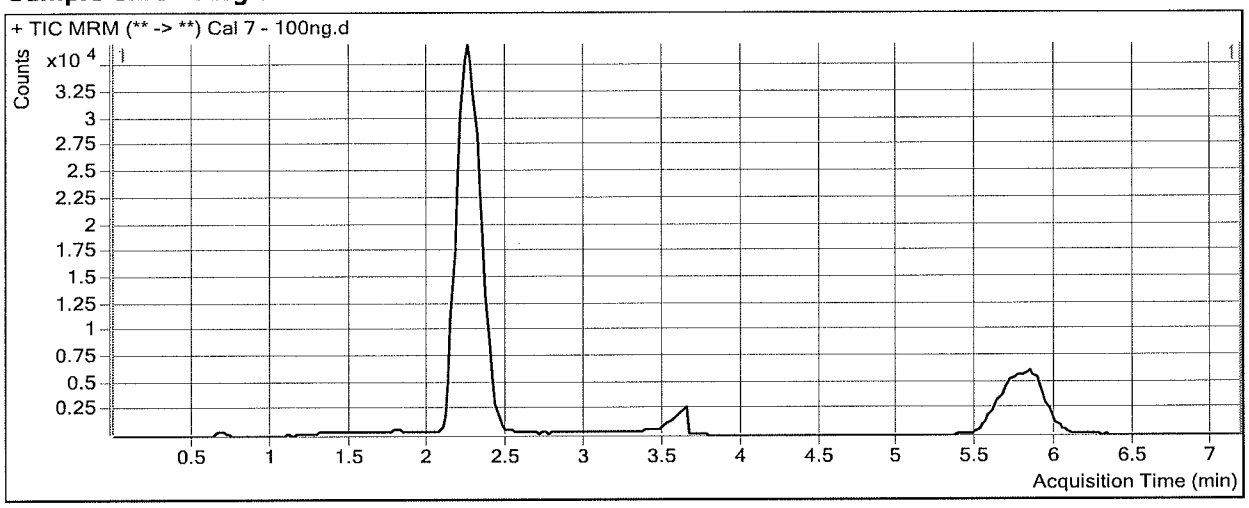
# ISP FORENSICS - Cd'A Instrument # 62340
# Cannabinoids Analysis Report

| | | | |
|---|---|---|---|
| **Batch Data Path** | D:\2018 Data\10312018 cann quant\QuantResults\cann quant.batch.bin | | |
| **Analysis Time** | 11/5/2018 8:30 AM | **Analyst Name** | ISP Tox |
| **Report Time** | 11/5/2018 8:31 AM | **Reporter Name** | ISP Tox |
| **Last Calib Update** | 11/5/2018 8:30 AM | **Batch State** | Processed |

## Analysis Info

| | | | |
|---|---|---|---|
| **Acq Time** | 2018-11-02 11:06 | **Data File** | Cal 7 - 100ng.d |
| **Sample Type** | Calibration | **Sample Name** | Cal 7 - 100ng |
| **Dilution** | 1 | **Acq Method** | AM 27 Quant THC 7-2017.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 | 2.236 | 103226 | 121919 | 0.8467 | 100.9857 |
| THC-COOH | THC-COOH-d9 | 2.326 | 73133 | 46096 | 1.5865 | 101.4433 |
| THC | THC-d3 | 5.833 | 48477 | 47744 | 1.0154 | 100.4302 |