REVIEWED
By Tamara Salazar at 11:31 am, Dec 08, 2022

12/7/2022

REVIEWED
By Britany Wylie at 10:34 am, Dec 09, 2022

**Worklist: 6179**

| LAB_CASE | ITEM | ITEM_TYPE | DESCRIPTION |
|---|---|---|---|
| C2022-2571 | 2 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2586 | | BCK | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2646 | | BCK | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2647 | | BCK | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2650 | | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |
| C2022-2664 | | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |

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

Extraction Date 12/6/22 Analyst: Anne Nord
Plate lot#: 220802 Plate re-test: 2/2/23

**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**: 22B52016-1 **Urine Blank:** 12522 **Column**: UCT Selectra DA 100 x 2.1mm 3um
**LCMS-QQQ ID**: 69679

## Pre-Analytic:

☒ 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
☒ 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

## Analytic:

☒ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
☒ 2. Urine hydrolysis: add 1.5 ml urine to blank plate, add 250 ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
Pipette **1000µL blood (calibrated pipette) Pipette ID: I41142J** in wells of analytical (standards) plate.
☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 4. Pipette **500µL 0.1% formic acid in water blood sample**, **500 ul saturated phosphate buffer in urine** in wells of analytical plate.
☒ 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 6. Transfer **800µL of blood+acid or urine 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).
***(Load at 85-100 PSI- Selector to the right)*** Manifold ID: 66792
☒ 8. Wait 5 minutes.
☒ 9. Add **2.25mL MTBE. (*Add in 3 increments of 750uL)***
☒ 10. Wait 5 minutes.
☒ 11. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 12. Add **2.25mL Hexane. (*Add in 3 increments of 750uL)***
☒ 13. Wait 5 minutes.
☒ 14. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
*SPE Dry ID: 66819*
☒ 16. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

## Post-Analytic

☒ 1. Create batch and process data.
☒ 2. Make any necessary integration changes, Curve weighting of Linear 1/x with $r^2$ values ≥0.98 for each analyte
☒ 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
☒ 4. Case sample response for THC 1ng/ml, OH-THC 3ng/mL (quantitative blood), Carboxy-THC: 5 ng/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.
☒ 5. Did all QCs pass for each analyte? (if not is it describe in comments section)
☒ 6. Enter QCs into control charting.
☒ 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

12-9/22

COMMENTS: *The negative urine, C2022-~~2264~~2664-1 and the end of run blood control evaporated and did not inject. The samples were reconstituted and re-injected. Those injections were evaluated.*

THC limit of quantitation 3 ng, limit of detection response of 1 ng or greater.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| a | cal 1 | Internal control urine | 2650-1 | | | |
| b | cal 2 | negative blood | | | | |
| c | cal 3 | 2571-2 | | | | |
| d | cal 4 | 2586-1 | | | | |
| e | Cal 5 | 2646-1 | | | | |
| f | cal 6 | 2647-1 | | | | |
| g | cal 7 | negative urine | | | | |
| h | Internal control (blood) | 2664-1 | | | | |

Plate position 3

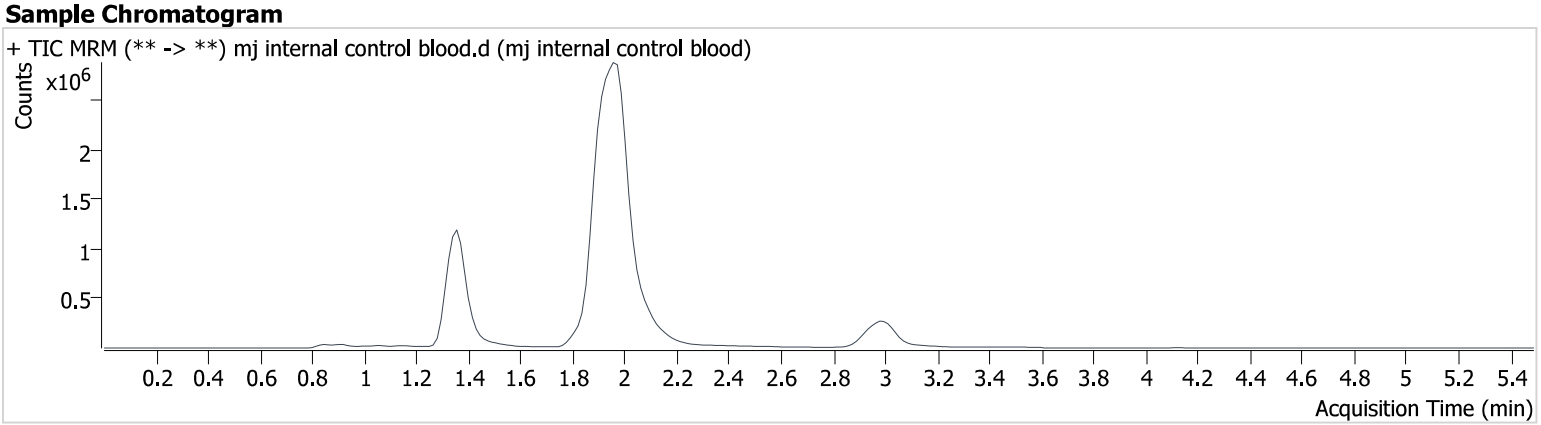
c2022-____-_

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj internal control blood.d |
| **Type** | QC | **Sample** | mj internal control blood |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-H1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 4:28:49 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



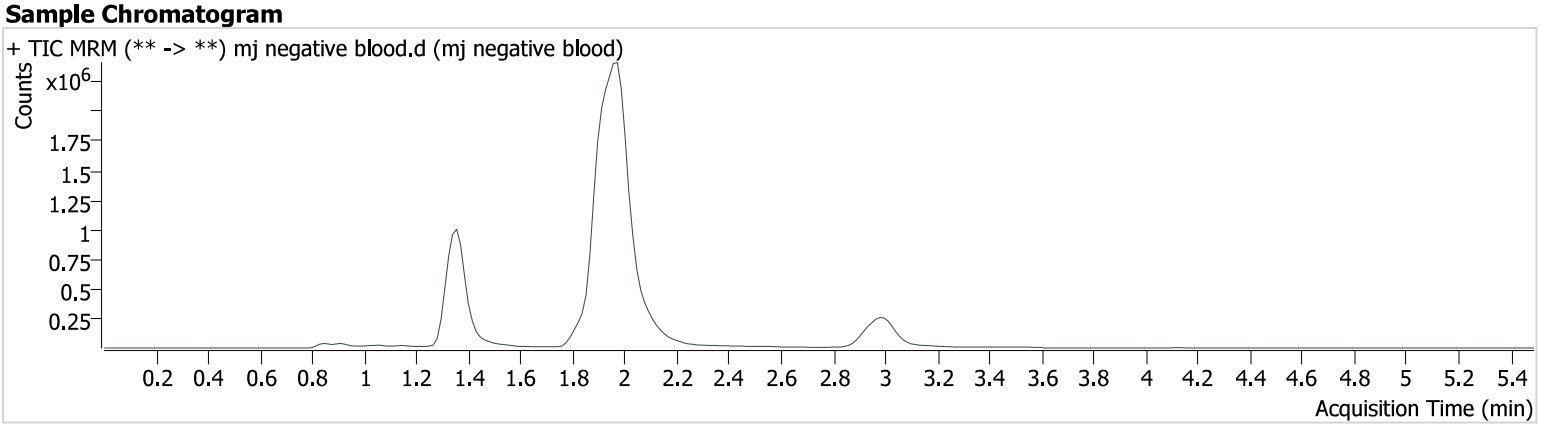

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 58311 | ∞ | 994.44 | ∞ | 3649948 | 4.442 ng/ml |
| THC-COOH | 1.388 | 85715 | ∞ | 275.29 | ∞ | 1266078 | 13.515 ng/ml |
| THC | 3.001 | 225327 | 35476.7 | 25.46 | 559.9 | 1821588 | 4.740 ng/ml |

# AM #27 Cannabinoids

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin |
| **Calibration Last Update** | 12/7/2022 12:09:45 PM |

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj negative blood.d |
| **Type** | Sample | **Sample** | mj negative blood |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-B2 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 4:35:34 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



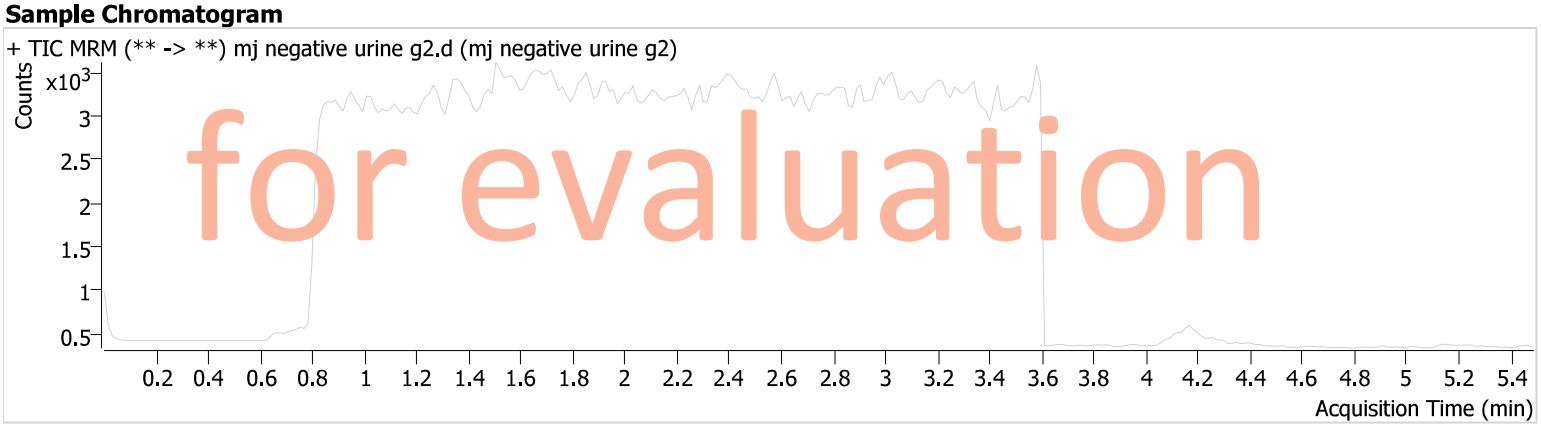
# AM #27 Cannabinoids

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin |
| **Calibration Last Update** | 12/7/2022 12:09:45 PM |

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj negative urine g2.d |
| **Type** | Sample | **Sample** | mj negative urine g2 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-G2 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 5:35:50 PM | | |
| **Sample Info.** | | | |



**Sample Chromatogram**



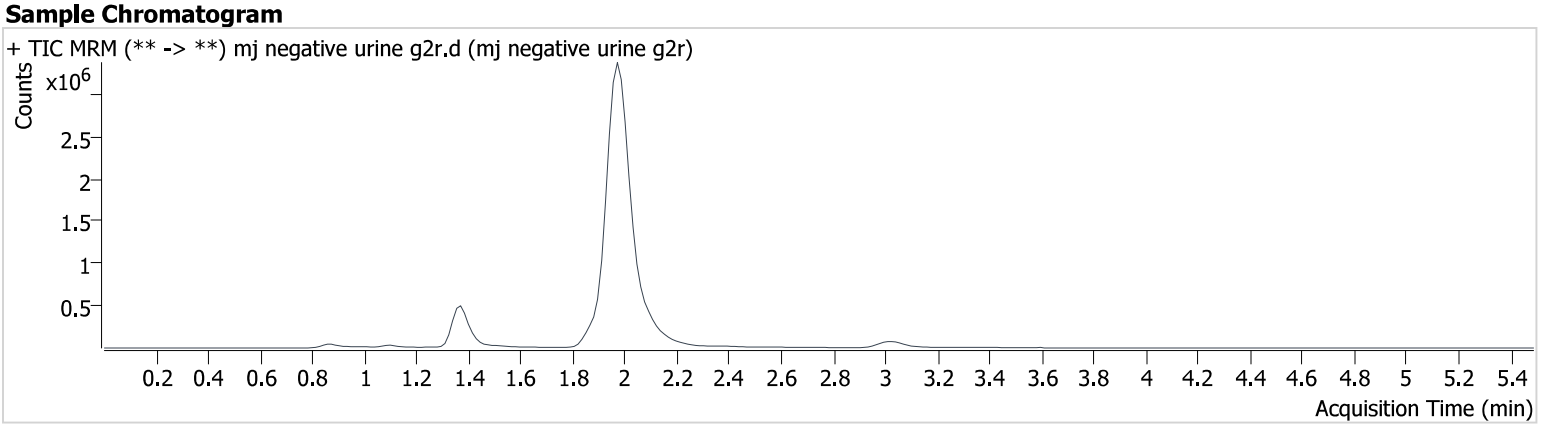

Sample did not inject, sample was reconstituted and injected that injection was evaluated.

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj negative urine g2r.d |
| **Type** | Sample | **Sample** | mj negative urine g2r |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-G2 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/7/2022 11:07:49 AM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**

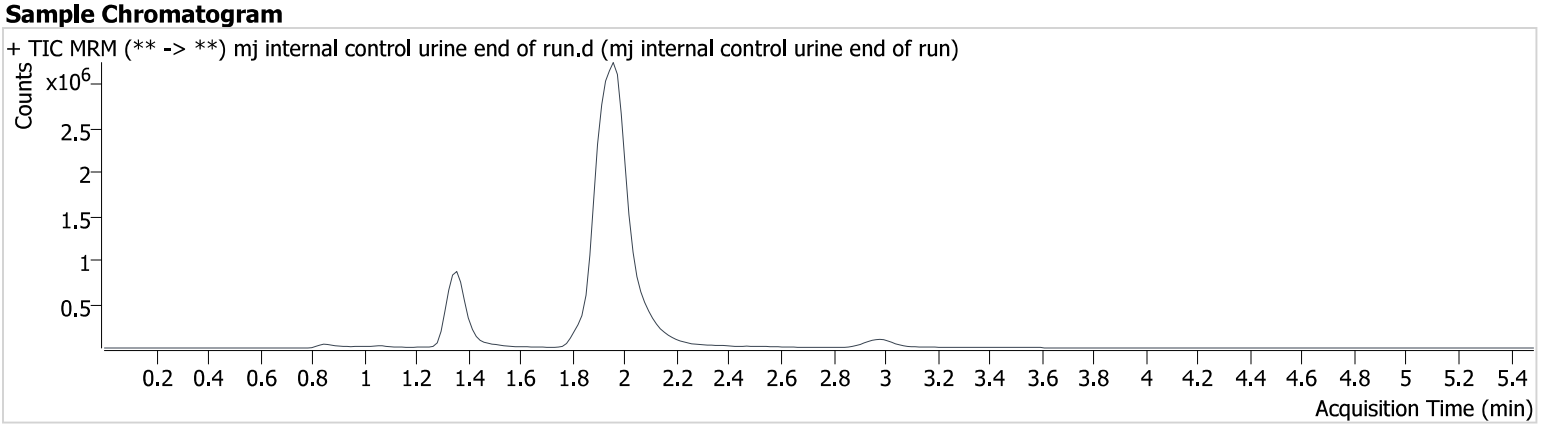


# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj internal control urine end of run.d |
| **Type** | Sample | **Sample** | mj internal control urine end of run |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-A2 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 6:15:59 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



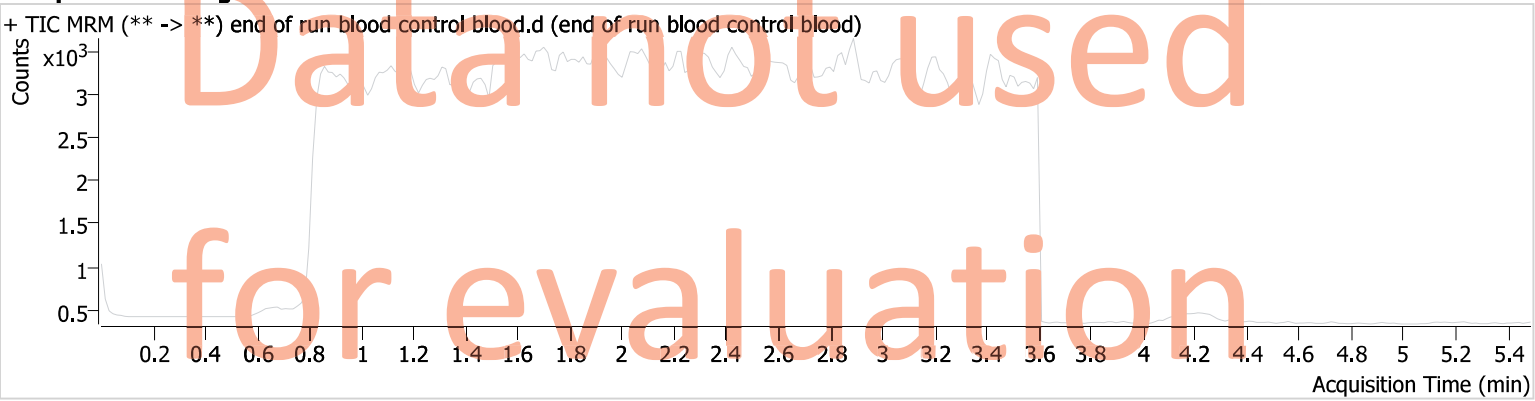

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 45084 | ∞ | 819.19 | ∞ | 2562733 | 4.857 ng/ml |
| THC-COOH | 1.373 | 63257 | ∞ | 272.82 | ∞ | 872580 | 14.377 ng/ml |
| THC | 3.001 | 77040 | 1027.5 | 24.63 | ∞ | 631877 | 4.679 ng/ml |

# AM #27 Cannabinoids

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin |
| **Calibration Last Update** | 12/7/2022 12:09:45 PM |

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | end of run blood control blood.d |
| **Type** | Sample | **Sample** | end of run blood control blood |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-H1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 6:22:44 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**





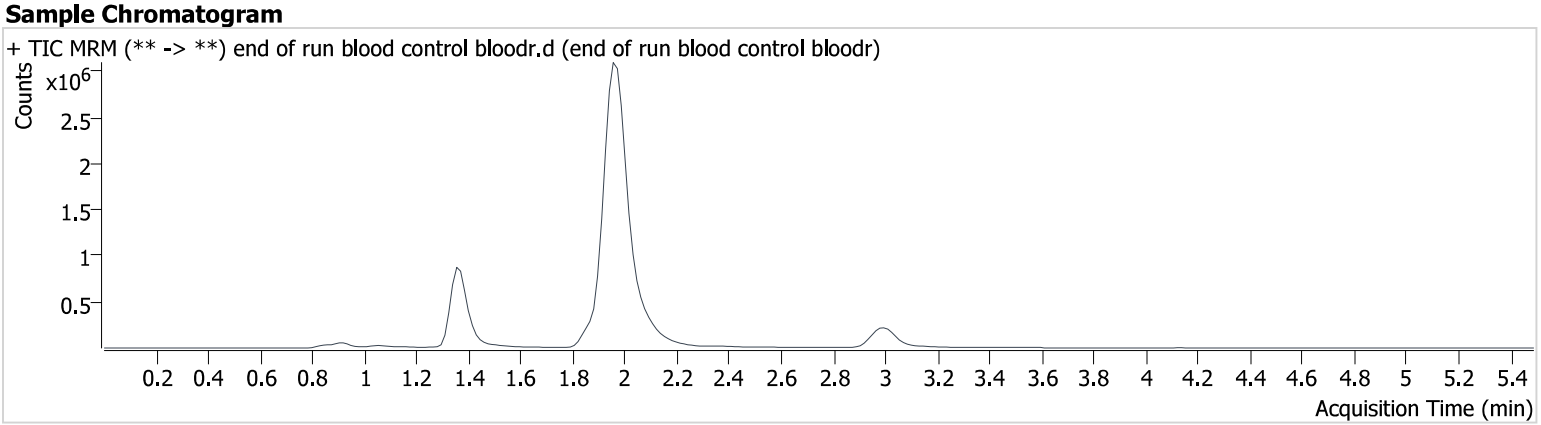

sample did not inject, the sample was reconstituted and injected that injection was evaluated.

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | end of run blood control bloodr.d |
| **Type** | Sample | **Sample** | end of run blood control bloodr |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-H1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/7/2022 11:27:57 AM | | |
| **Sample Info.** | | | |

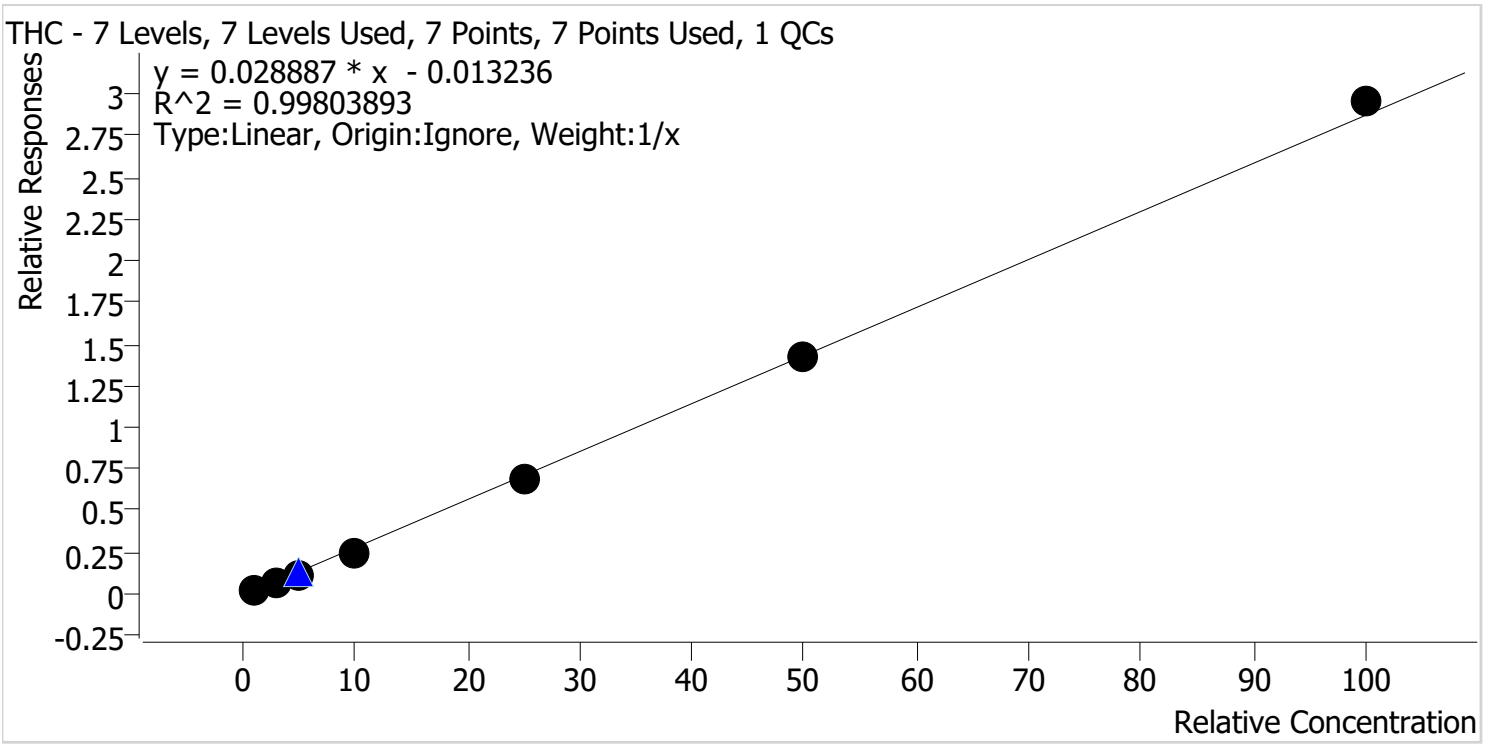
**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 37478 | ∞ | 865.06 | ∞ | 2224162 | 4.666 ng/ml |
| THC-COOH | 1.388 | 59091 | 144.2 | 263.24 | ∞ | 822320 | 14.262 ng/ml |
| THC | 3.016 | 146526 | 3744.8 | 25.96 | 280.7 | 1229657 | 4.583 ng/ml |

# Compound Calibration Report

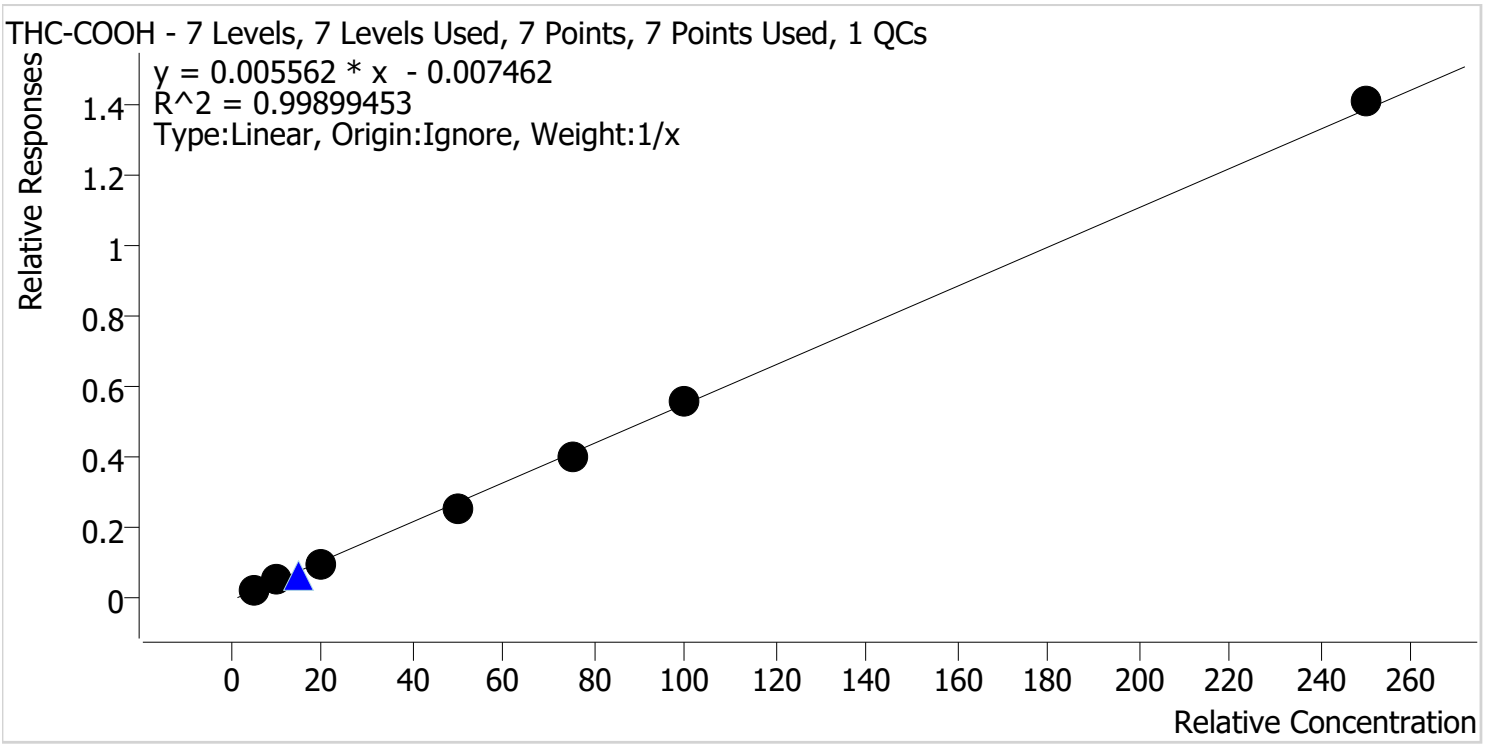
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin |
| **Last Cal. Update** | 12/7/2022 12:09 PM |
| **Analyst Name** | ISP\datastor |
| **Analyte** | THC |
| **Internal Standard** | THC-d3 |




| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|---|---|---|---|---|---|
| mj cal 1 | 1 | ✔ | 1.0 | 1.3 | 125.5 |
| mj cal 2 | 2 | ✔ | 3.0 | 2.9 | 95.3 |
| mj cal 3 | 3 | ✔ | 5.0 | 4.5 | 90.7 |
| mj cal 4 | 4 | ✔ | 10.0 | 9.1 | 91.0 |
| mj cal 5 | 5 | ✔ | 25.0 | 23.9 | 95.8 |
| mj cal 6 | 6 | ✔ | 50.0 | 49.5 | 99.0 |
| mj cal 7 | 7 | ✔ | 100.0 | 102.8 | 102.8 |

# Compound Calibration Report

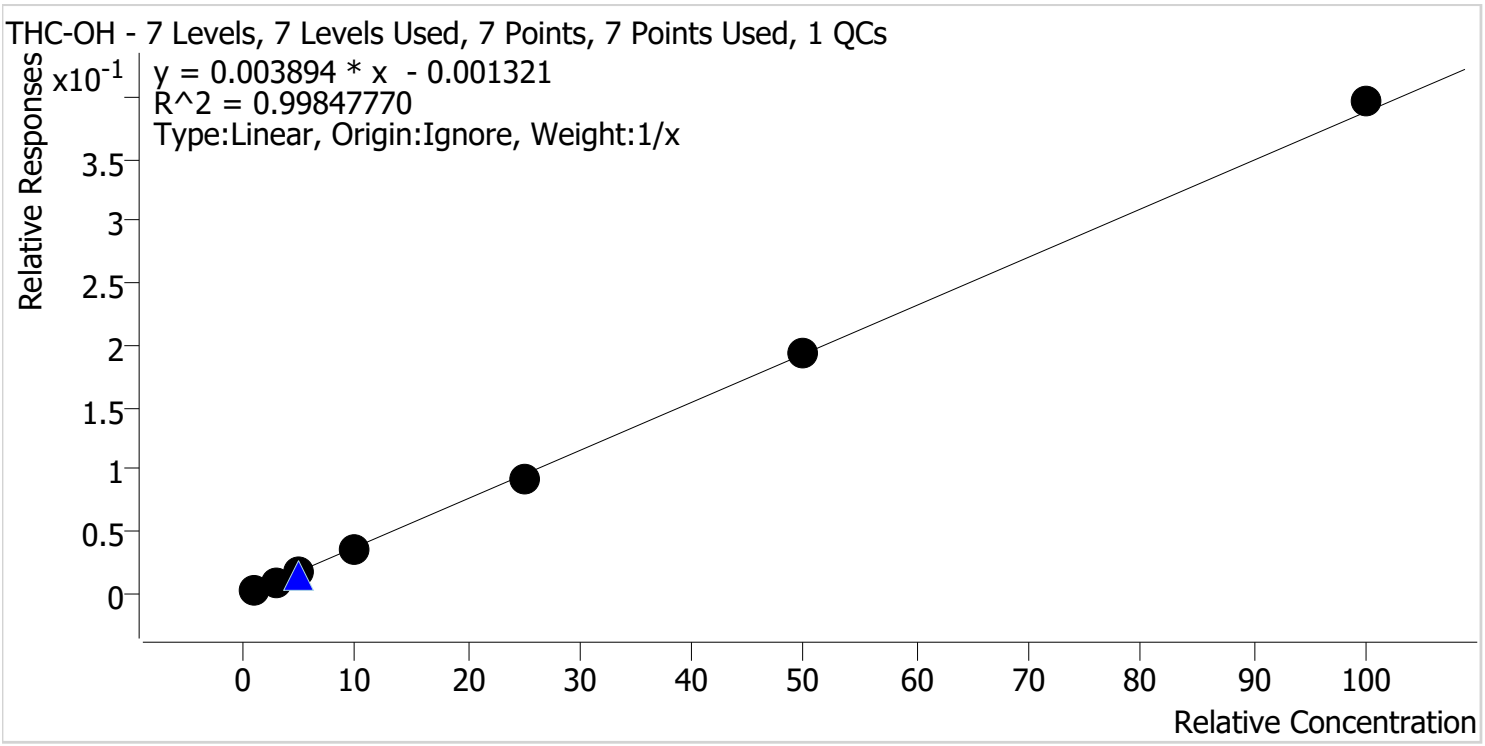
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin |
| **Last Cal. Update** | 12/7/2022 12:09 PM |
| **Analyst Name** | ISP\datastor |
| **Analyte** | THC-COOH |
| **Internal Standard** | THC-COOH-d9 |




| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|---|---|---|---|---|---|
| mj cal 1 | 1 | ✔ | 5.0 | 5.4 | 108.0 |
| mj cal 2 | 2 | ✔ | 10.0 | 10.2 | 102.0 |
| mj cal 3 | 3 | ✔ | 20.0 | 19.2 | 96.0 |
| mj cal 4 | 4 | ✔ | 50.0 | 46.8 | 93.5 |
| mj cal 5 | 5 | ✔ | 75.0 | 73.3 | 97.8 |
| mj cal 6 | 6 | ✔ | 100.0 | 101.1 | 101.1 |
| mj cal 7 | 7 | ✔ | 250.0 | 254.0 | 101.6 |

# Compound Calibration Report

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin |
| **Last Cal. Update** | 12/7/2022 12:09 PM |
| **Analyst Name** | ISP\datastor |
| **Analyte** | THC-OH |
| **Internal Standard** | THC-OH-d3 |



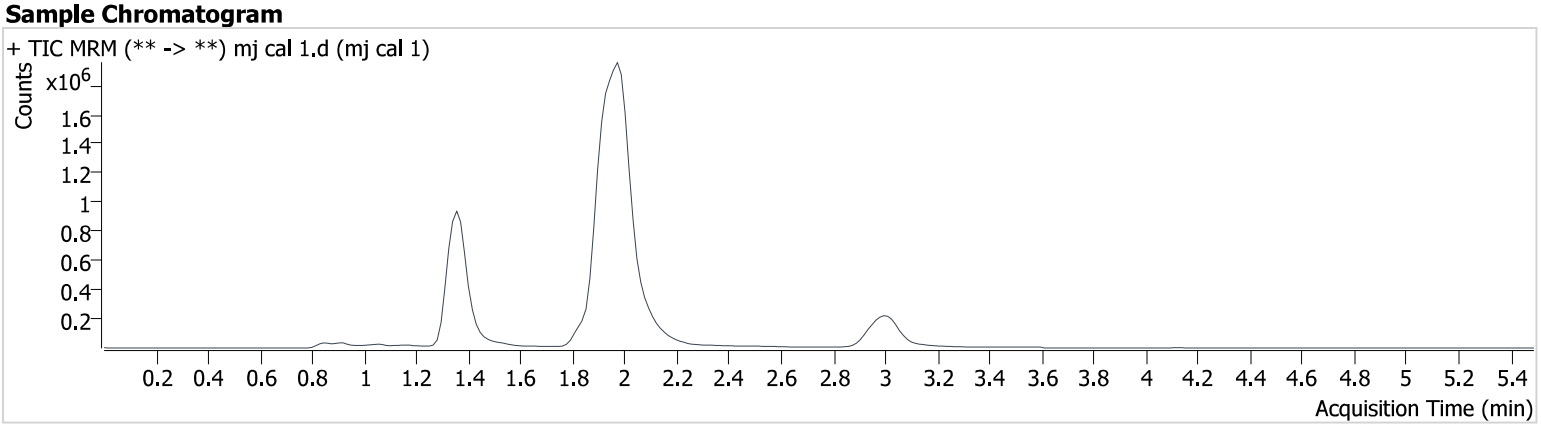

| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|---|---|---|---|---|---|
| mj cal 1 | 1 | ✔ | 1.0 | 1.2 | 122.1 |
| mj cal 2 | 2 | ✔ | 3.0 | 2.9 | 96.8 |
| mj cal 3 | 3 | ✔ | 5.0 | 4.6 | 91.2 |
| mj cal 4 | 4 | ✔ | 10.0 | 9.2 | 92.2 |
| mj cal 5 | 5 | ✔ | 25.0 | 23.8 | 95.3 |
| mj cal 6 | 6 | ✔ | 50.0 | 50.1 | 100.3 |
| mj cal 7 | 7 | ✔ | 100.0 | 102.1 | 102.1 |

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 1.d |
| **Type** | Cal | **Sample** | mj cal 1 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-A1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 3:35:01 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



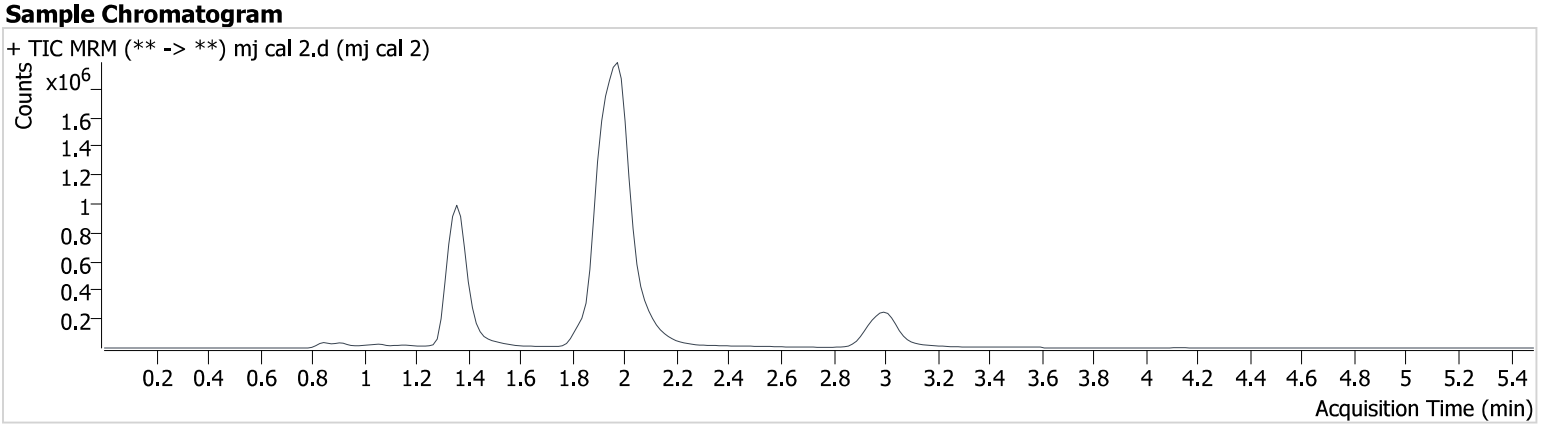

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.349 | 12050 | ∞ | 967.44 | ∞ | 3509925 | 1.221 ng/ml | Low |
| THC-COOH | 1.388 | 23253 | ∞ | 261.69 | 93.5 | 1030063 | 5.401 ng/ml | |
| THC | 3.016 | 38547 | 533.4 | 24.65 | 165.7 | 1675538 | 1.255 ng/ml | |

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 2.d |
| **Type** | Cal | **Sample** | mj cal 2 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-B1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 3:41:45 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



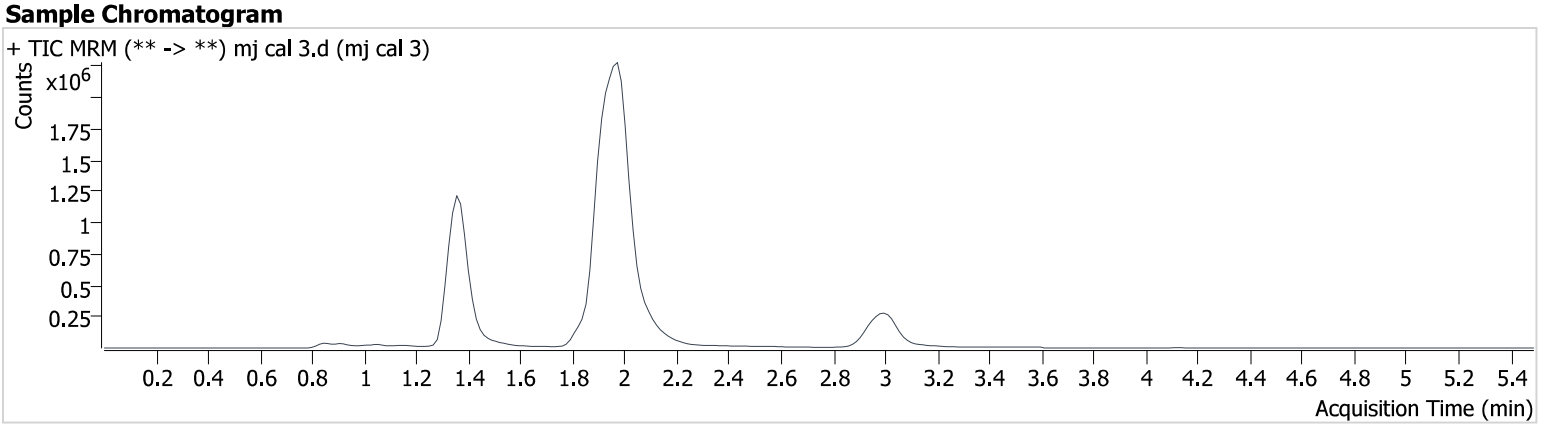

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 33748 | 383.7 | 918.51 | ∞ | 3378506 | 2.905 ng/ml | Low |
| THC-COOH | 1.388 | 51918 | ∞ | 255.41 | 385.2 | 1054384 | 10.195 ng/ml | |
| THC | 3.016 | 124062 | 3758.4 | 23.97 | 247.1 | 1789472 | 2.858 ng/ml | |

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 3.d |
| **Type** | Cal | **Sample** | mj cal 3 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-C1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 3:48:29 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



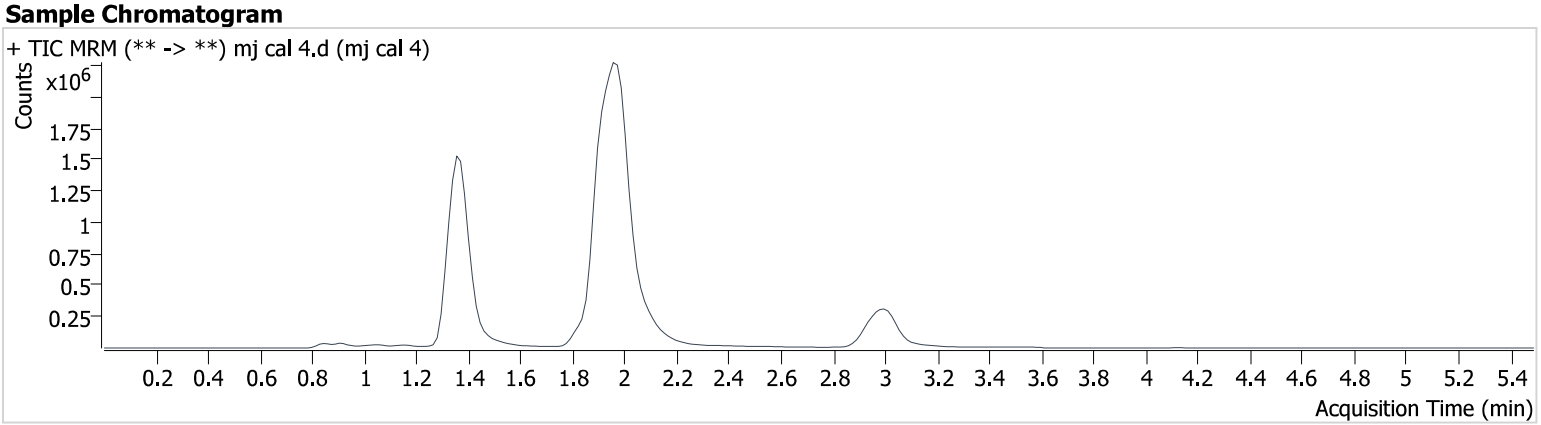

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 59899 | ∞ | 978.27 | ∞ | 3646043 | 4.558 ng/ml |
| THC-COOH | 1.388 | 120003 | 1432.3 | 263.22 | 1089.9 | 1207790 | 19.207 ng/ml |
| THC | 3.016 | 223127 | 1777.6 | 24.68 | 498.0 | 1894267 | 4.536 ng/ml |

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 4.d |
| **Type** | Cal | **Sample** | mj cal 4 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-D1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 3:55:14 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



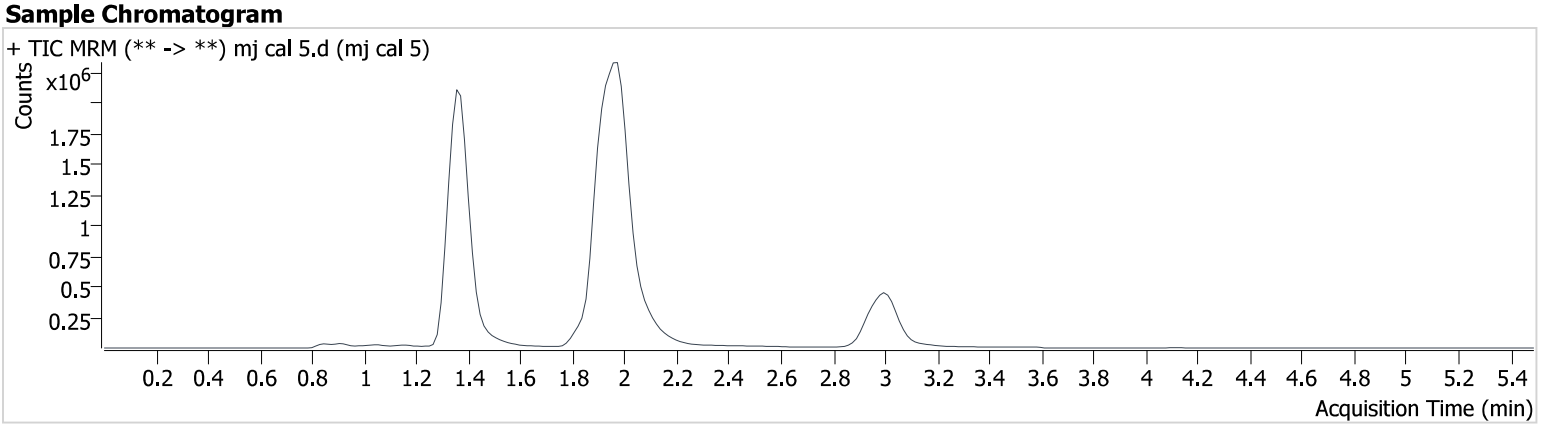

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 124684 | 873.2 | 916.90 | ∞ | 3605891 | 9.219 ng/ml |
| THC-COOH | 1.388 | 300980 | 186.6 | 269.28 | ∞ | 1191428 | 46.764 ng/ml |
| THC | 3.016 | 470666 | ∞ | 24.36 | 709.1 | 1886356 | 9.096 ng/ml |

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 5.d |
| **Type** | Cal | **Sample** | mj cal 5 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-E1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 4:01:58 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



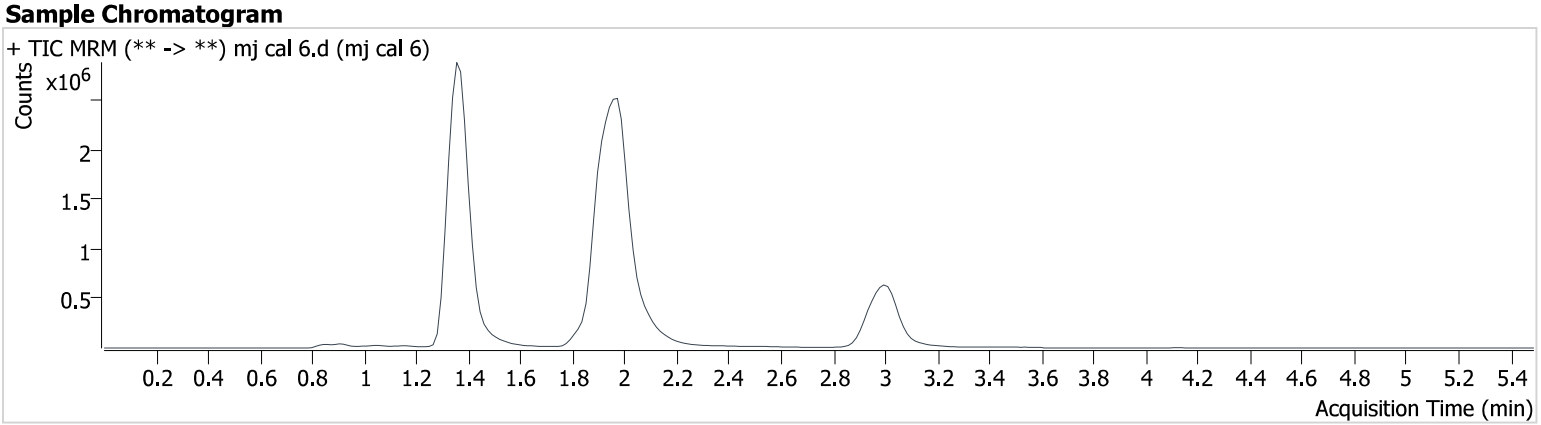

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 337741 | ∞ | 873.80 | ∞ | 3691825 | 23.832 ng/ml |
| THC-COOH | 1.388 | 472632 | ∞ | 260.40 | 5045.3 | 1180201 | 73.347 ng/ml |
| THC | 3.001 | 1322902 | 11855.8 | 23.87 | 882.1 | 1950453 | 23.938 ng/ml |

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 6.d |
| **Type** | Cal | **Sample** | mj cal 6 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-F1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 4:08:42 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



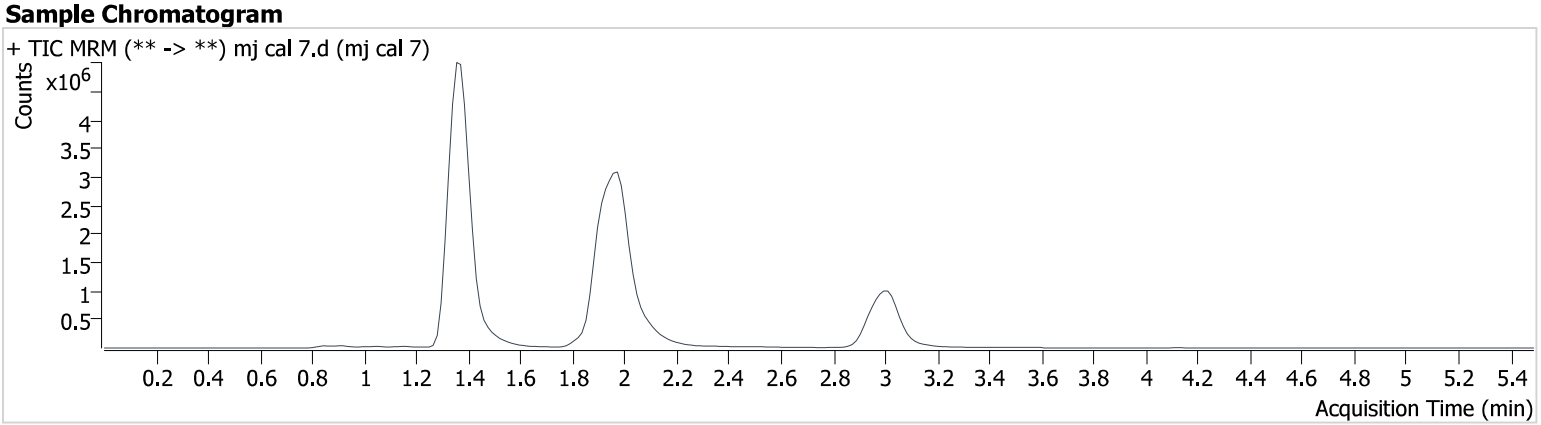

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 710804 | 15748.0 | 826.79 | ∞ | 3664944 | 50.144 ng/ml |
| THC-COOH | 1.388 | 631581 | ∞ | 255.36 | ∞ | 1138680 | 101.072 ng/ml |
| THC | 3.001 | 2644641 | 37376.7 | 24.15 | 1878.2 | 1866227 | 49.516 ng/ml |

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\120622\QuantResults\cann.batch.bin
**Calibration Last Update** 12/7/2022 12:09:45 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 7.d |
| **Type** | Cal | **Sample** | mj cal 7 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Anne Nord |
| **Sample Position** | P3-G1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 12/6/2022 4:15:27 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.364 | 1447644 | 72604.8 | 816.69 | ∞ | 3652409 | 102.121 ng/ml |
| THC-COOH | 1.388 | 1503454 | 386.3 | 251.93 | ∞ | 1069870 | 254.014 ng/ml |
| THC | 3.016 | 5197568 | 1278794.5 | 24.67 | 5659.8 | 1758096 | 102.802 ng/ml |