



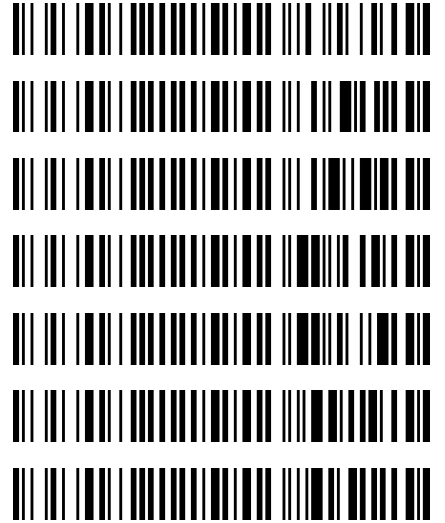
REVIEWED

By Sarah Collins at 12:38 pm, Nov 07, 2022

1/3/2022

Worklist: 6153

| <u>LAB_CASE</u> | <u>ITEM</u> | <u>ITEM_TYPE</u> | <u>DESCRIPTION</u> |
|-----------------|-------------|------------------|---------------------------------|
| C2022-2380 | BCK | | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2391 | BCK | | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2393 | BCK | | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2408 | BCK | | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2410 | BCK | | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2429 | BCK | | AM 27 Blood THC Quant by LC-QQQ |
| C2022-2440 | BCK | | AM 27 Blood THC Quant by LC-QQQ |



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

Extraction Date 11/2/22
Plate lot#: 220802

Analyst: Anne Nord
Plate re-test: 2/2/23

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE LCMS Methanol

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 22B52016-1 **Urine Blank:** blood only **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

COMMENTS: *THC samples between 1 and 3ng reported as < 3ng.*



| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|-----------------------------|-------------------------------|--------|---|---|---|
| a | cal 1 | Internal control blood 2nd | 2440-1 | | | |
| b | cal 2 | negative blood | | | | |
| c | cal 3 | 2380-1 | | | | |
| d | cal 4 | 2391-1 | | | | |
| e | Cal 5 | 2393-1 | | | | |
| f | cal 6 | 2408-1 | | | | |
| g | cal 7 | 2410-1 | | | | |
| h | Internal control (blood) | 2429-1 | | | | |

Plate position 3

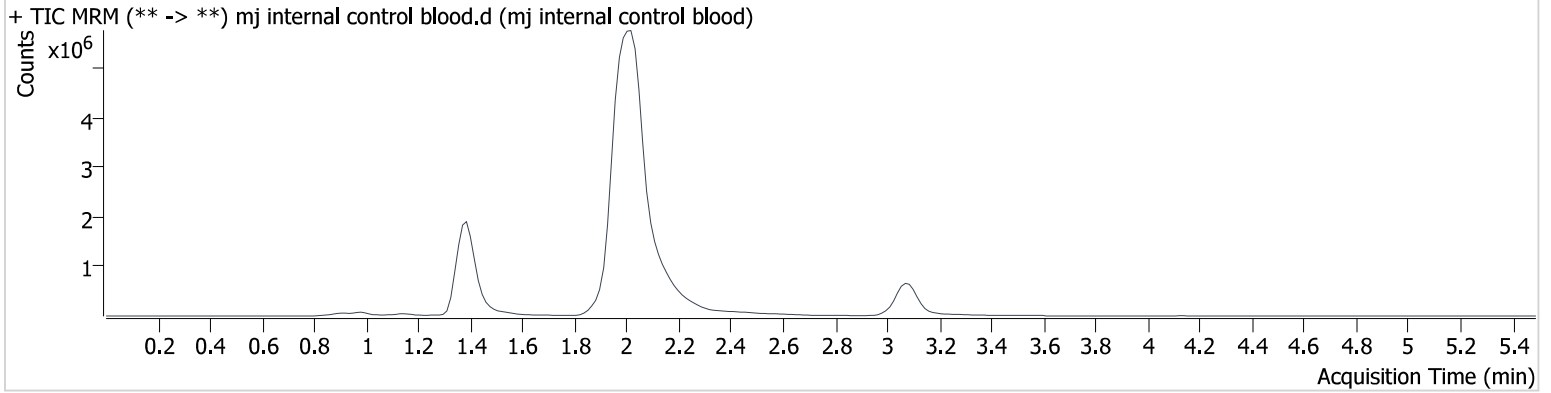
c2022-____-__

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

| | | | |
|-------------------------|-----------------------|------------------|-----------------------------|
| 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 | 11/2/2022 11:07:55 PM | | |
| Sample Info. | | | |

Sample Chromatogram



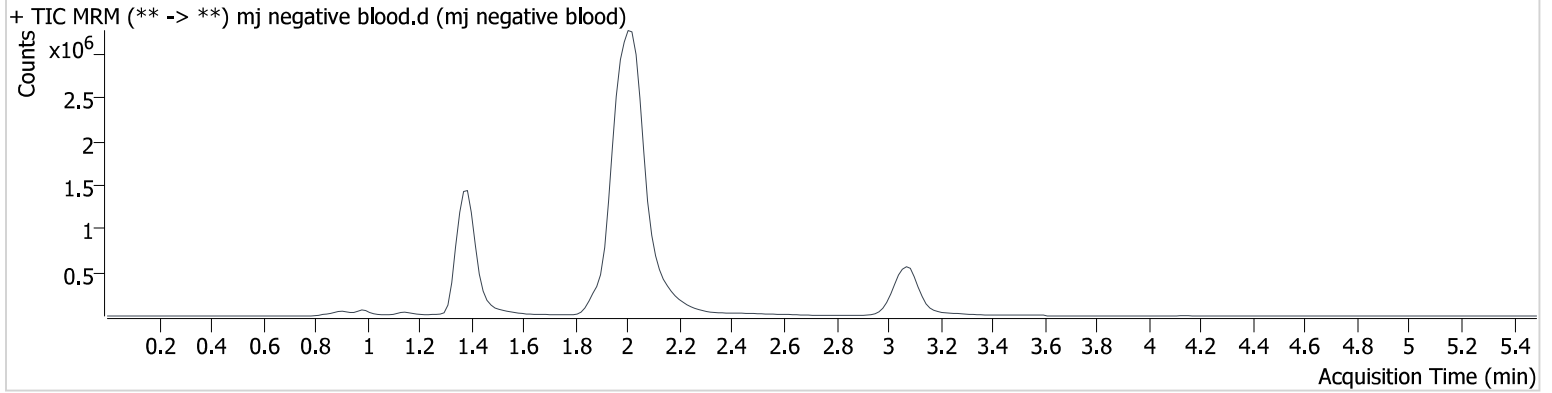
| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|--------|-------|-------|------------|--------------|
| THC-OH | 1.379 | 99660 | 1604.9 | 935.3 | ∞ | 6061136 | 4.499 ng/ml |
| THC-COOH | 1.403 | 120809 | ∞ | 275.4 | ∞ | 1579050 | 14.064 ng/ml |
| THC | 3.092 | 448177 | ∞ | 23.7 | 452.5 | 3421452 | 4.856 ng/ml |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

| | | | |
|-------------------------|-----------------------|------------------|---------------------|
| 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 | 11/2/2022 11:14:39 PM | | |
| Sample Info. | | | |

Sample Chromatogram

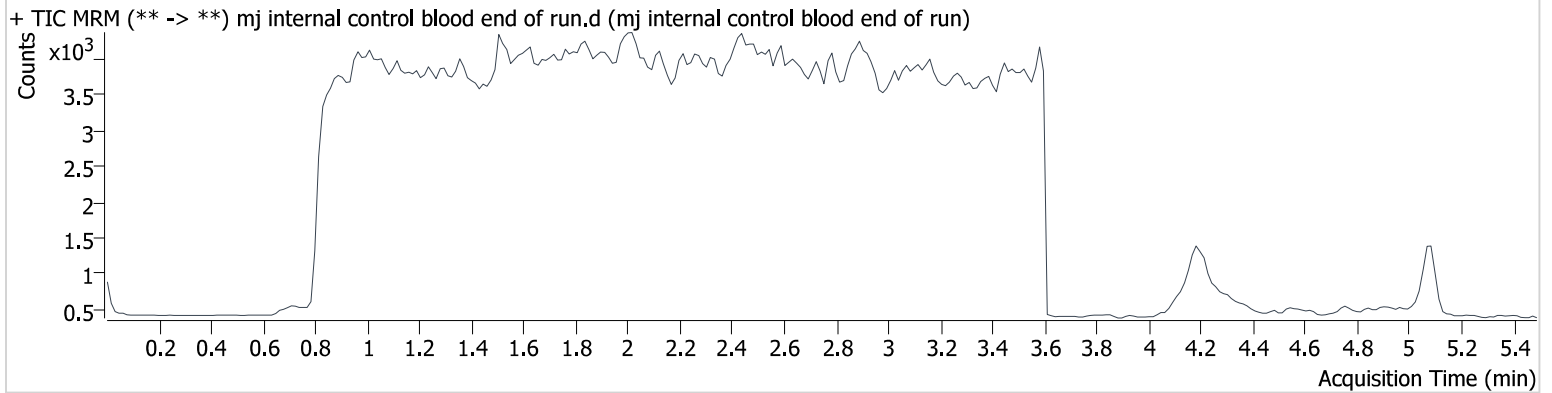


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

| | | | |
|-------------------------|----------------------|------------------|--|
| Instrument | 69679 | Data File | mj internal control blood end of run.d |
| Type | Sample | Sample | mj internal control blood end of run |
| Acq. Method | AM 27 THC quant.m | Operator | Anne Nord |
| Sample Position | P3-A2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 11/3/2022 1:01:45 AM | | |
| Sample Info. | | | |

Sample Chromatogram



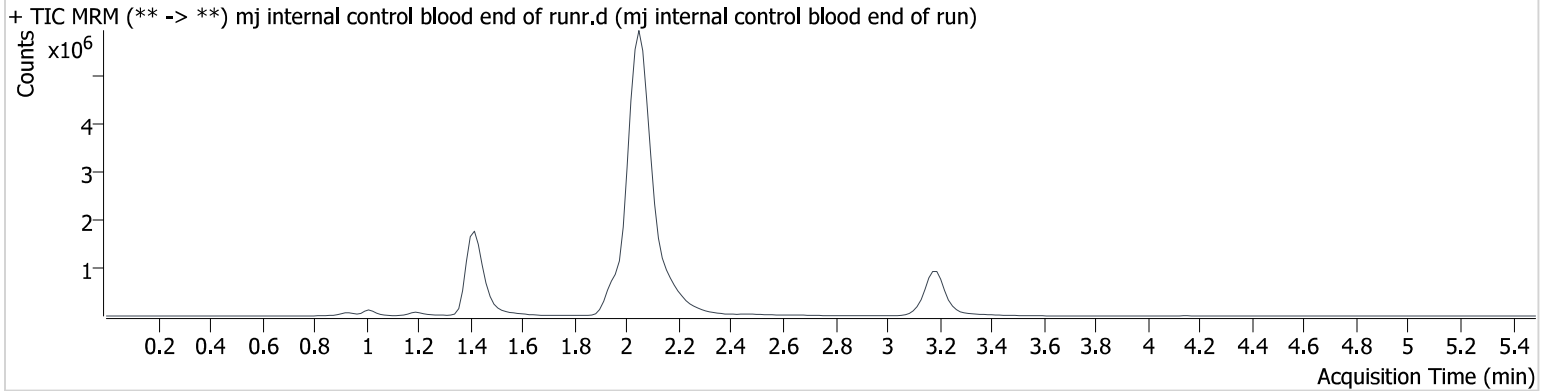
Sample evaporated and did not inject.

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

| | | | |
|-------------------------|----------------------|------------------|--|
| Instrument | 69679 | Data File | mj internal control blood end of run.r.d |
| Type | Sample | Sample | mj internal control blood end of run |
| Acq. Method | AM 27 THC quant.m | Operator | Anne Nord |
| Sample Position | P3-A2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 11/3/2022 7:48:44 AM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|------------------------------|--------|-----------|-------|-------|------------|--------------|
| THC-OH | 1.409 | 93126 | ∞ | 813.6 | ∞ | 4964221 | 5.103 ng/ml |
| THC-COOH | 1.448 | 121880 | 3654.2 | 259.5 | ∞ | 1503458 | 14.830 ng/ml |
| THC | 3.197 Hig h | 564376 | 1722765.5 | 22.8 | 948.4 | 4582932 | 4.595 ng/ml |

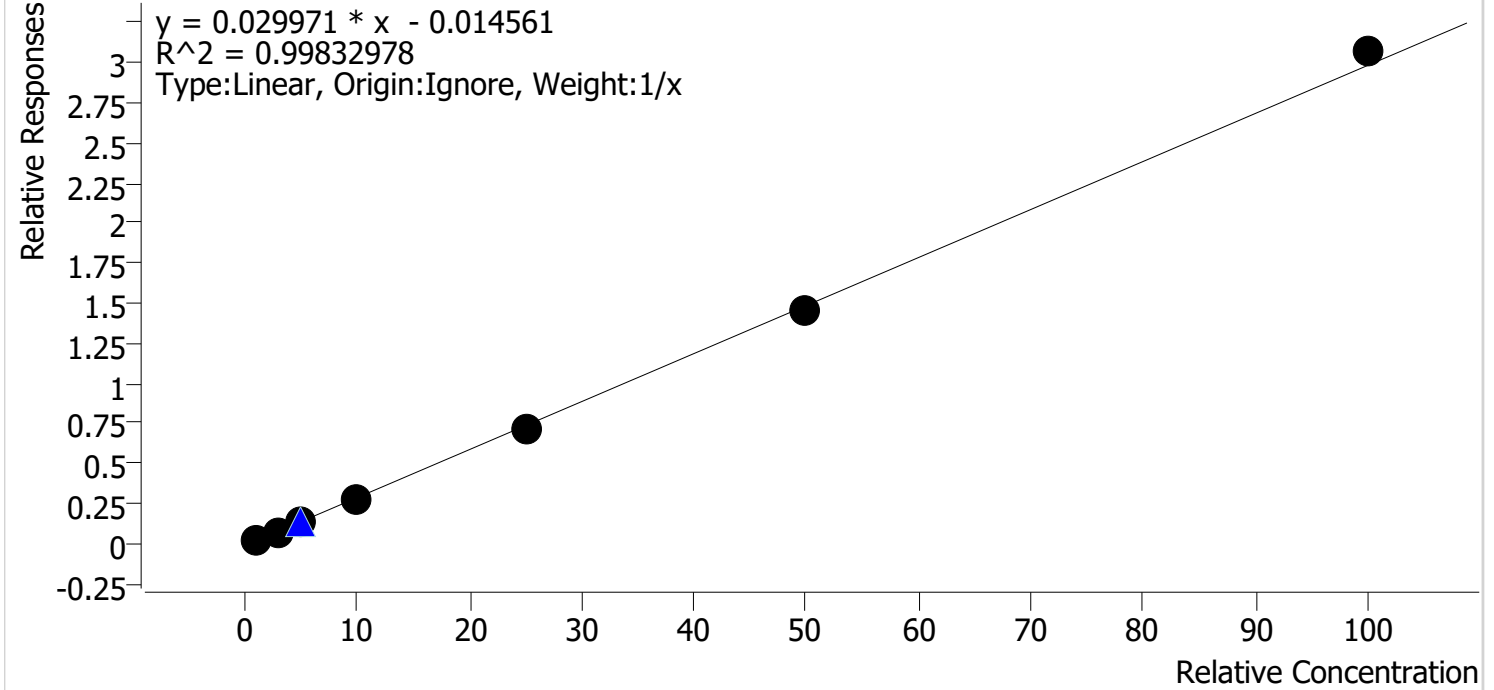
Evaporated sample was reconstituted and injected.

The retention time shifted similarly for both the THC and the THC internal standard. The THC is will be evaluated as positive.

Compound Calibration Report

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Last Cal. Update 11/3/2022 8:22 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs

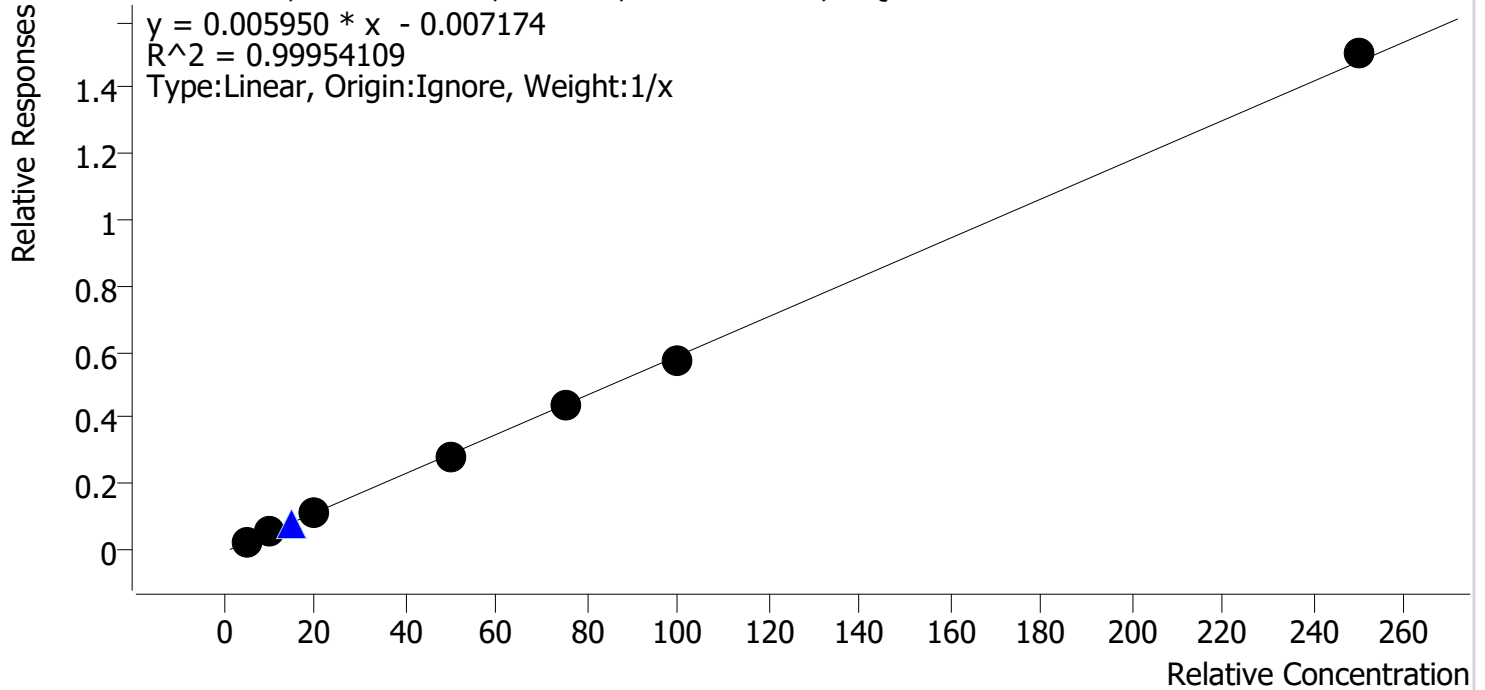


| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal 1 | 1 | ✓ | 1.0 | 1.2 | 124.2 |
| mj cal 2 | 2 | ✓ | 3.0 | 2.8 | 92.5 |
| mj cal 3 | 3 | ✓ | 5.0 | 4.6 | 91.9 |
| mj cal 4 | 4 | ✓ | 10.0 | 9.4 | 94.3 |
| mj cal 5 | 5 | ✓ | 25.0 | 24.0 | 96.0 |
| mj cal 6 | 6 | ✓ | 50.0 | 49.1 | 98.2 |
| mj cal 7 | 7 | ✓ | 100.0 | 102.8 | 102.8 |

Compound Calibration Report

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Last Cal. Update 11/3/2022 8:22 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-d9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs

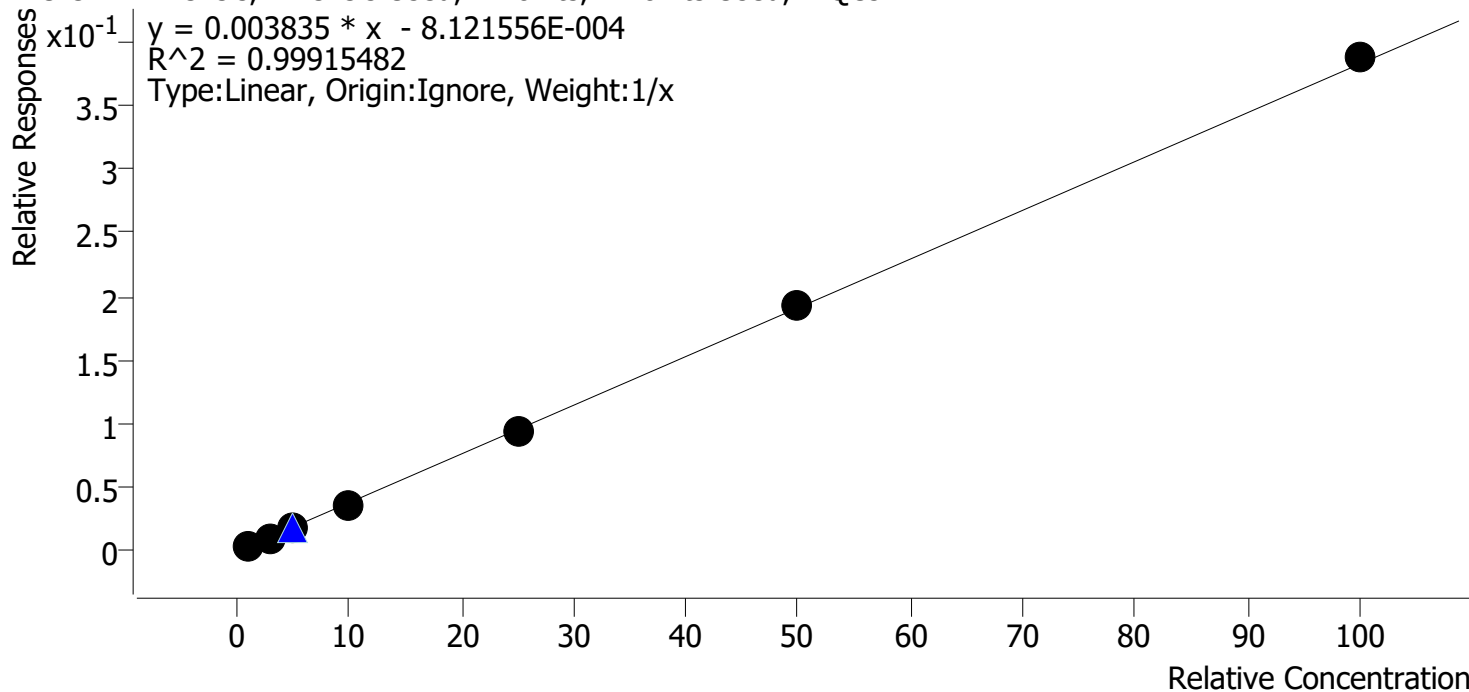


| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal 1 | 1 | ✓ | 5.0 | 5.2 | 103.8 |
| mj cal 2 | 2 | ✓ | 10.0 | 10.3 | 102.7 |
| mj cal 3 | 3 | ✓ | 20.0 | 19.5 | 97.4 |
| mj cal 4 | 4 | ✓ | 50.0 | 48.6 | 97.2 |
| mj cal 5 | 5 | ✓ | 75.0 | 74.4 | 99.2 |
| mj cal 6 | 6 | ✓ | 100.0 | 98.0 | 98.0 |
| mj cal 7 | 7 | ✓ | 250.0 | 254.0 | 101.6 |

Compound Calibration Report

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Last Cal. Update 11/3/2022 8:22 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



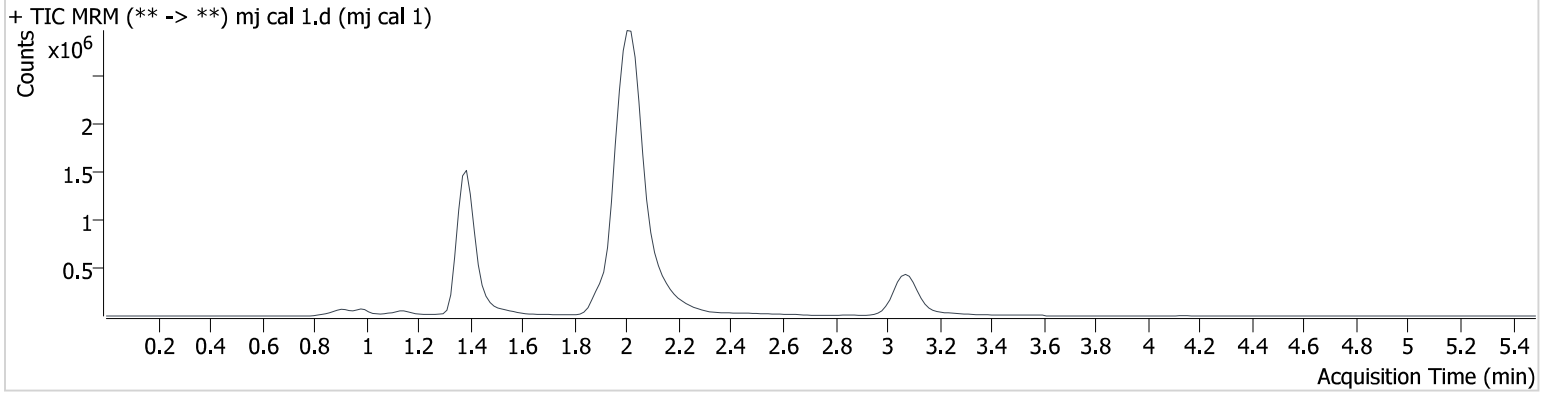
| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal 1 | 1 | ✓ | 1.0 | 1.2 | 118.0 |
| mj cal 2 | 2 | ✓ | 3.0 | 2.9 | 97.9 |
| mj cal 3 | 3 | ✓ | 5.0 | 4.5 | 90.2 |
| mj cal 4 | 4 | ✓ | 10.0 | 9.4 | 94.1 |
| mj cal 5 | 5 | ✓ | 25.0 | 24.6 | 98.2 |
| mj cal 6 | 6 | ✓ | 50.0 | 50.1 | 100.2 |
| mj cal 7 | 7 | ✓ | 100.0 | 101.3 | 101.3 |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

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 11/2/2022 10:14:05 PM
Sample Info.

Sample Chromatogram



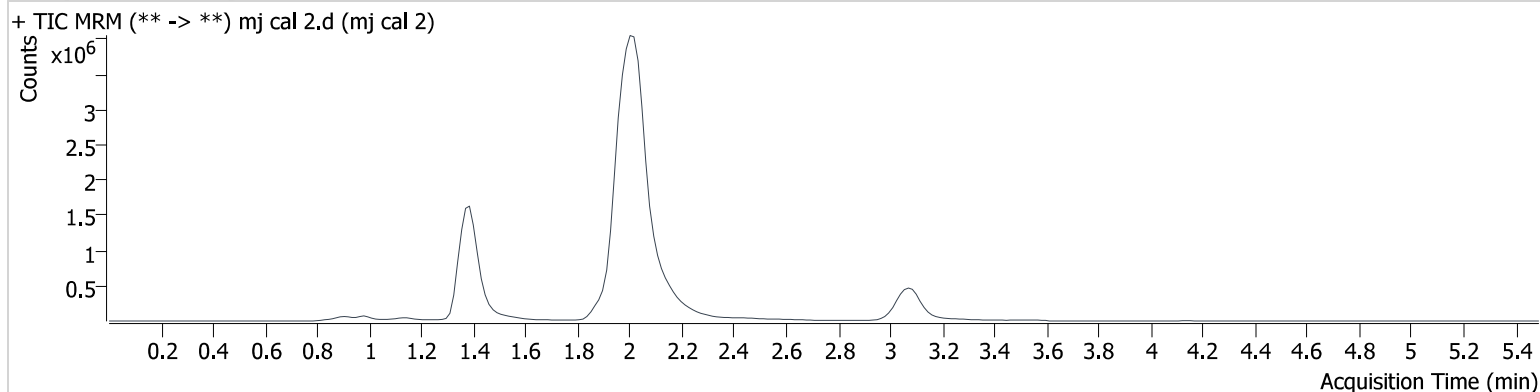
| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|----------|-------|-------|-------|-------|-------|------------|-------------|-----|
| THC-OH | 1.379 | 20695 | 229.7 | 977.8 | ∞ | 5576059 | 1.180 ng/ml | Low |
| THC-COOH | 1.418 | 29375 | 430.5 | 272.2 | 130.7 | 1238576 | 5.192 ng/ml | |
| THC | 3.077 | 63833 | 323.2 | 25.2 | 46.1 | 2815873 | 1.242 ng/ml | |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

| | | | |
|-------------------------|-----------------------|------------------|------------|
| 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 | 11/2/2022 10:20:50 PM | | |
| Sample Info. | | | |

Sample Chromatogram



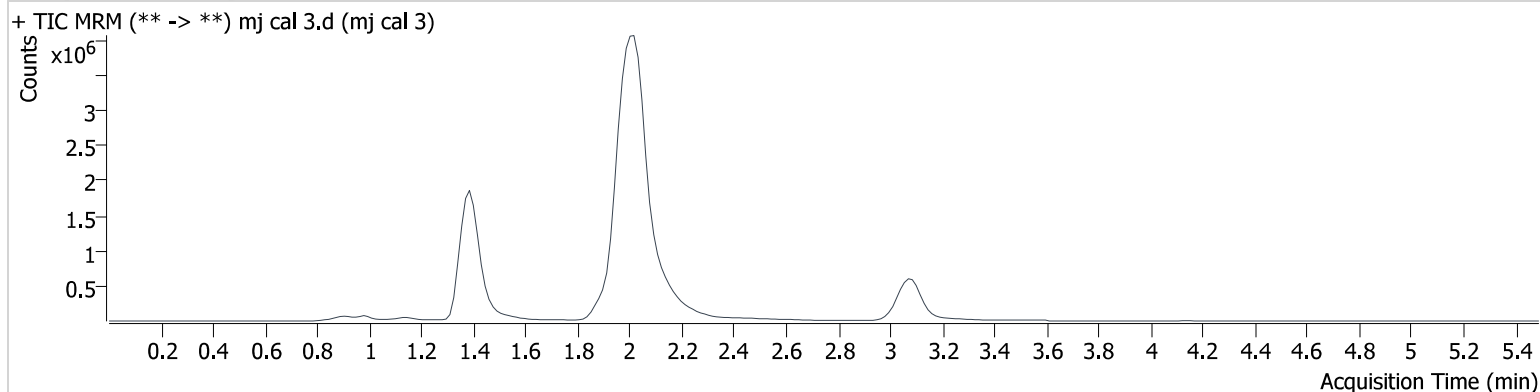
| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|----------|-------|--------|------------------------|-------|--------|------------|--------------|------------|
| THC-OH | 1.379 | 59609 | ∞ | 986.7 | 6352.9 | 5703305 | 2.937 ng/ml | Low |
| THC-COOH | 1.403 | 73618 | 530.9 | 253.2 | ∞ | 1364665 | 10.272 ng/ml | |
| THC | 3.092 | 196966 | 2441732244 058020.0 | 25.1 | 175.4 | 2871398 | 2.775 ng/ml | |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

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 11/2/2022 10:27:34 PM
Sample Info.

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|--------|-------|-------|------------|--------------|
| THC-OH | 1.394 | 96969 | 666.3 | 957.1 | ∞ | 5880391 | 4.512 ng/ml |
| THC-COOH | 1.418 | 161463 | ∞ | 264.1 | ∞ | 1484908 | 19.480 ng/ml |
| THC | 3.092 | 417651 | 5851.0 | 23.7 | 426.8 | 3392148 | 4.594 ng/ml |

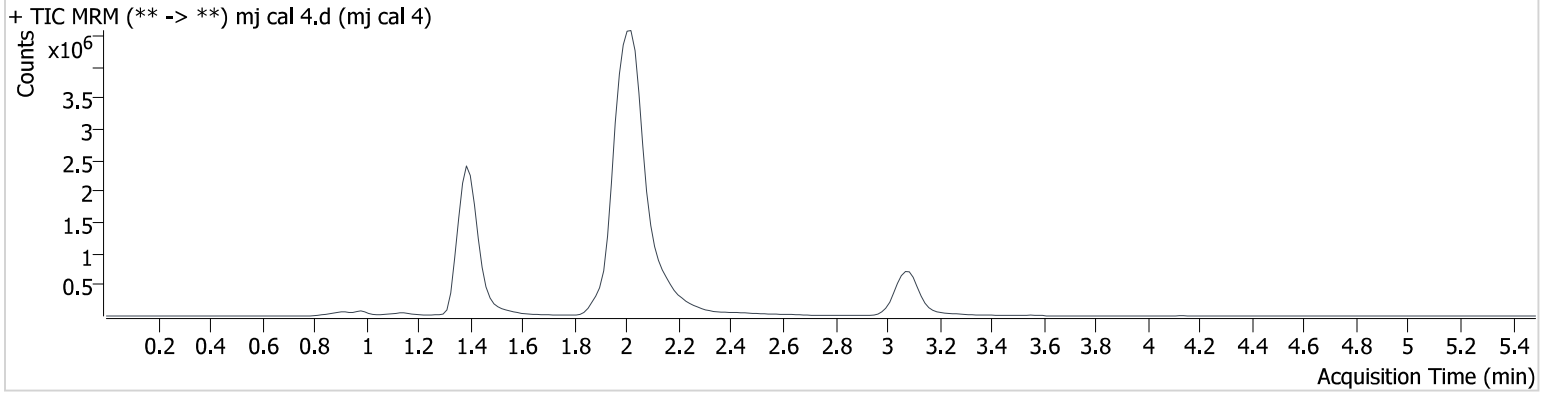
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-D1
Injection Volume 10
Acq. Date-Time 11/2/2022 10:34:19 PM
Sample Info.

Data File mj cal 4.d
Sample mj cal 4
Operator Anne Nord
Comment

Sample Chromatogram



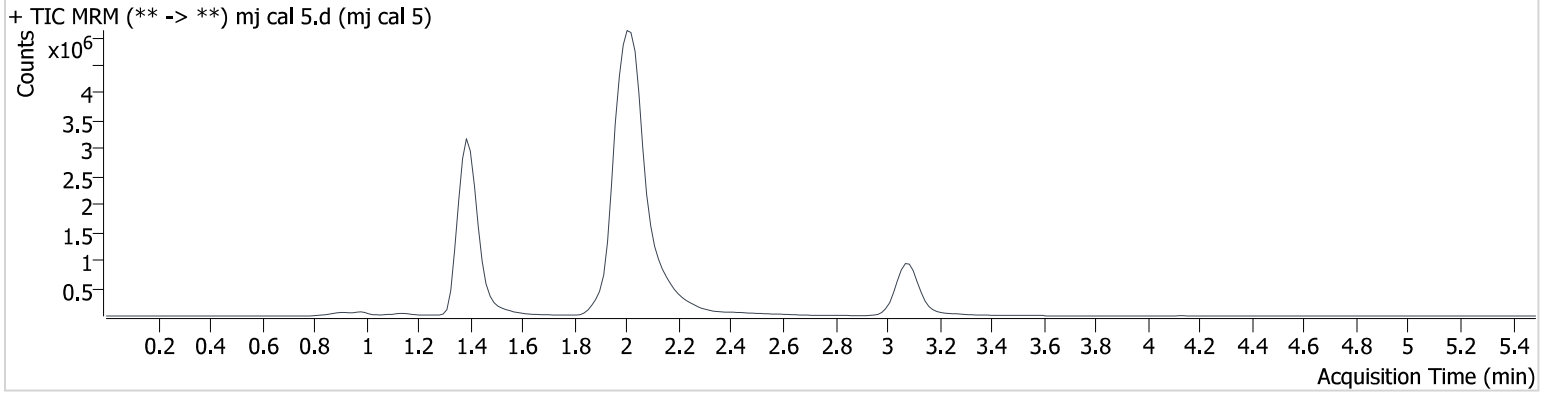
| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|--------|-------|--------|------------|--------------|
| THC-OH | 1.394 | 208877 | ∞ | 884.5 | ∞ | 5918496 | 9.415 ng/ml |
| THC-COOH | 1.418 | 419517 | 7074.7 | 269.2 | ∞ | 1487601 | 48.601 ng/ml |
| THC | 3.092 | 945283 | 7863.7 | 23.7 | 1618.2 | 3524799 | 9.434 ng/ml |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

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 11/2/2022 10:41:04 PM
Sample Info.

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|---------|---------|-------|--------|------------|--------------|
| THC-OH | 1.379 | 526930 | 11924.3 | 836.0 | ∞ | 5642953 | 24.561 ng/ml |
| THC-COOH | 1.418 | 623481 | ∞ | 262.0 | 3517.3 | 1431053 | 74.428 ng/ml |
| THC | 3.092 | 2262062 | 22168.6 | 24.4 | 8628.2 | 3209961 | 23.999 ng/ml |

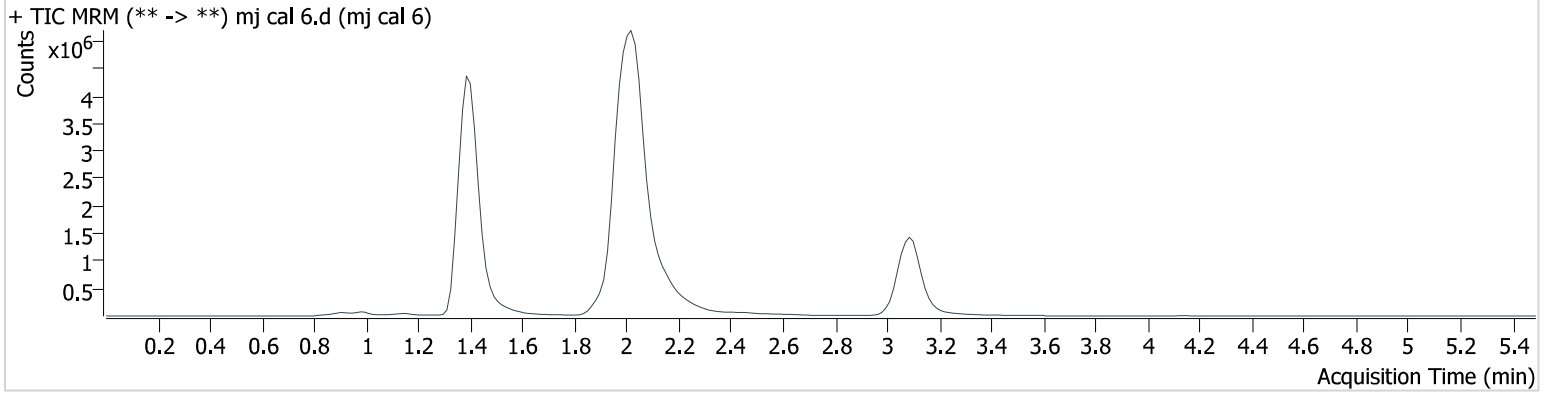
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-F1
Injection Volume 10
Acq. Date-Time 11/2/2022 10:47:49 PM
Sample Info.

Data File mj cal 6.d
Sample mj cal 6
Operator Anne Nord
Comment

Sample Chromatogram



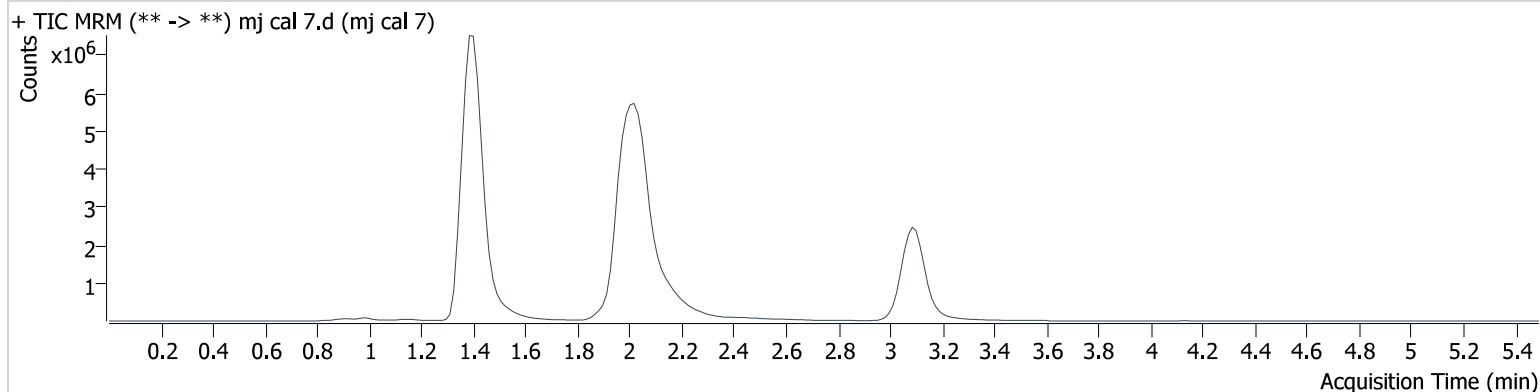
| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|---------|---------|-------|--------|------------|--------------|
| THC-OH | 1.394 | 1103059 | ∞ | 807.7 | ∞ | 5763104 | 50.121 ng/ml |
| THC-COOH | 1.418 | 807448 | 5406.4 | 272.0 | ∞ | 1402155 | 97.987 ng/ml |
| THC | 3.092 | 4755327 | 63083.1 | 24.5 | 8474.7 | 3262286 | 49.122 ng/ml |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2022\am 27-28\110222r\QuantResults\cann.batch.bin
Calibration Last Update 11/3/2022 8:22:37 AM

| | | | |
|-------------------------|-----------------------|------------------|------------|
| 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 | 11/2/2022 10:54:33 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|----------|---------|-------|---------|------------|---------------|
| THC-OH | 1.394 | 2234577 | 17395.2 | 813.2 | ∞ | 5765537 | 101.275 ng/ml |
| THC-COOH | 1.418 | 2038933 | ∞ | 258.7 | 24756.5 | 1355320 | 254.039 ng/ml |
| THC | 3.092 | 10070424 | 98267.2 | 24.8 | 9293.5 | 3283000 | 102.834 ng/ml |