








Worklist: 6271**REVIEWED**

By Britany Wylie at 8:12 am, Mar 14, 2023

| <u>LAB_CASE</u> | <u>ITEM</u> | <u>ITEM_TYPE</u> | <u>DESCRIPTION</u> | |
|-----------------|-------------|------------------|---|---|
| C2023-0383 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2023-0385 | 1 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |
| C2023-0393 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2023-0418 | 1 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |
| C2023-0441 | 2 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |
| C2023-0443 | 2 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2023-0461 | 3 | 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 3-9-23
Plate lot#: 230113

Analyst: Anne Nord
Plate re-test: 7/13/23

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:** 12522
LCMS-QQQ ID: 69679

Column: UCT Selectra DA 100 x 2.1mm 3um

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. SN > 10
- 4. Case sample response for THC 1ng/ml LOD 3ng/ml LOQ, OH-THC 3ng/mL LOD and LOQ, Carboxy-THC: 5 ng/mL (qualitative only). 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. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *Curve limits THC 3-100 dropped cal 1 due to poor response for qualifier ion*

C2023-0383-1 was not injected on 3-9-23 injected 3-10-23 and evaluated.

C2023-0441-2 was not injected (the wrong position was injected) on 3-9-23 the correct location was injected and evaluated 3-10-23.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|-----------------------------|----------------|-------|---|---|---|
| a | cal 1 | Internal urine | 441-2 | | | |
| b | cal 2 | negative blood | 461-3 | | | |
| c | cal 3 | 383-1 | | | | |
| d | cal 4 | 393-1 | | | | |
| e | Cal 5 | 443-2 | | | | |
| f | cal 6 | negative urine | | | | |
| g | cal 7 | 385-1 | | | | |
| h | Internal control (blood) | 418-1 | | | | |

Plate position 3

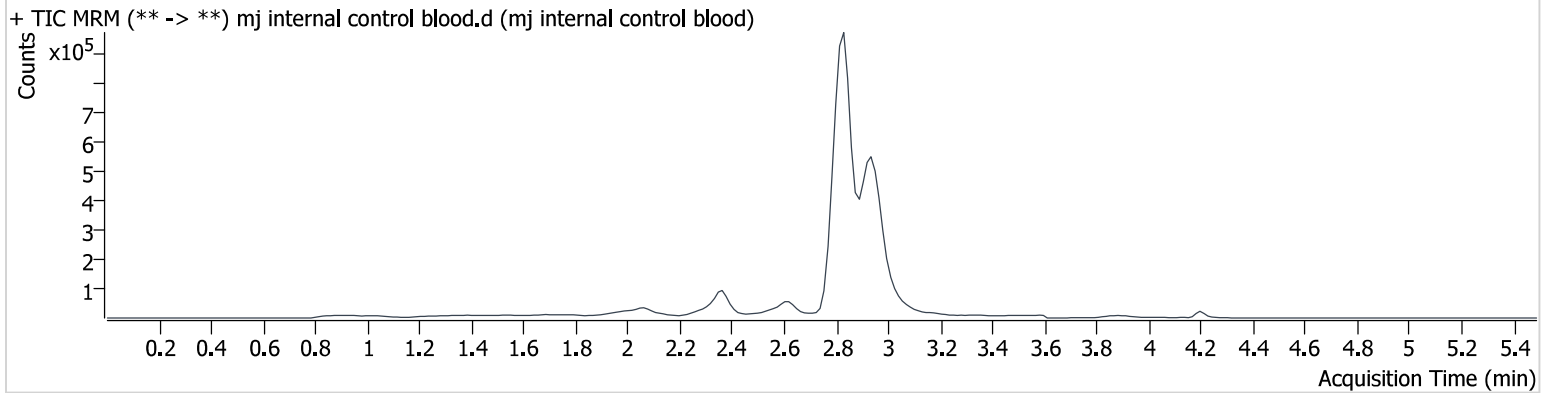
c2023-0 ____-

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

| | | | |
|-------------------------|---------------------|------------------|-----------------------------|
| Instrument | 69679 | Data File | mj internal control blood.d |
| Type | QC | Sample | mj internal control blood |
| Acq. Method | thc quant 60 40.m | Operator | Anne Nord |
| Sample Position | P3-H1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 3/9/2023 5:52:39 PM | | |
| Sample Info. | | | |

Sample Chromatogram



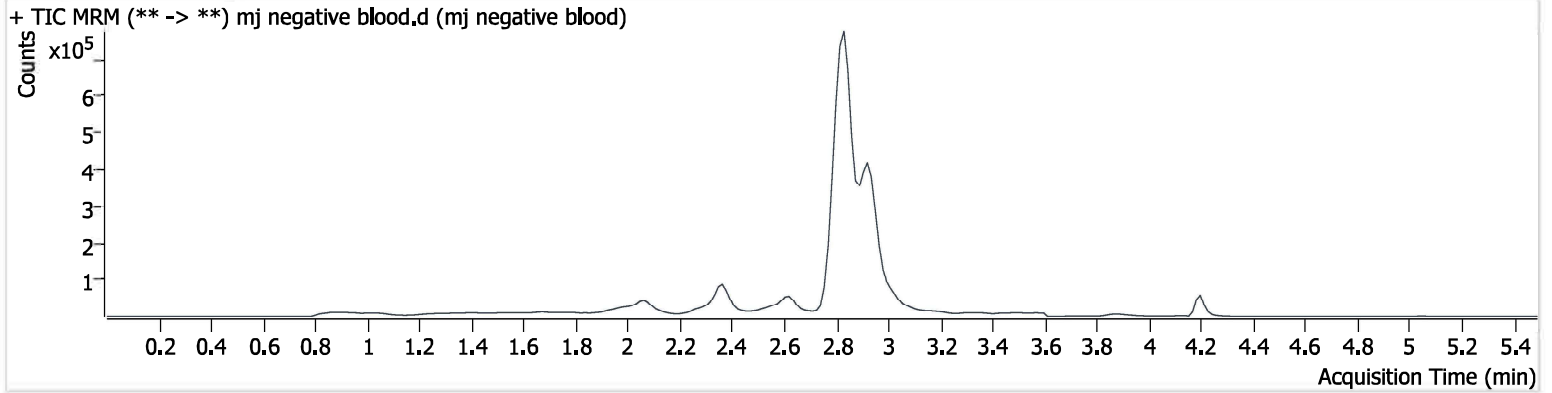
| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|-------|--------|------|------------|--------------|
| THC-OH | 2.833 | 76252 | ∞ | 963.28 | ∞ | 3864957 | 4.349 ng/ml |
| THC-COOH | 2.950 | 130497 | 387.9 | 269.86 | ∞ | 1650417 | 15.205 ng/ml |
| THC | 4.197 | 5597 | 115.1 | 40.53 | 78.2 | 54840 | 4.415 ng/ml |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

| | | | |
|-------------------------|---------------------|------------------|---------------------|
| Instrument | 69679 | Data File | mj negative blood.d |
| Type | Sample | Sample | mj negative blood |
| Acq. Method | thc quant 60 40.m | Operator | Anne Nord |
| Sample Position | P3-B2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 3/9/2023 5:59:15 PM | | |
| Sample Info. | | | |

Sample Chromatogram

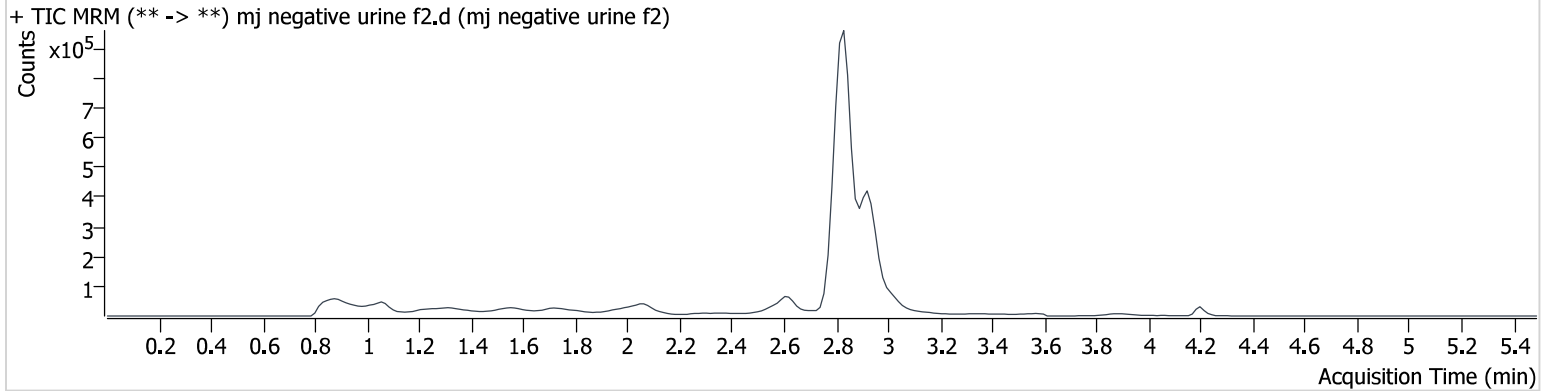


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

| | | | |
|-------------------------|---------------------|------------------|------------------------|
| Instrument | 69679 | Data File | mj negative urine f2.d |
| Type | Sample | Sample | mj negative urine f2 |
| Acq. Method | thc quant 60 40.m | Operator | Anne Nord |
| Sample Position | P3-F2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 3/9/2023 6:32:15 PM | | |
| Sample Info. | | | |

Sample Chromatogram

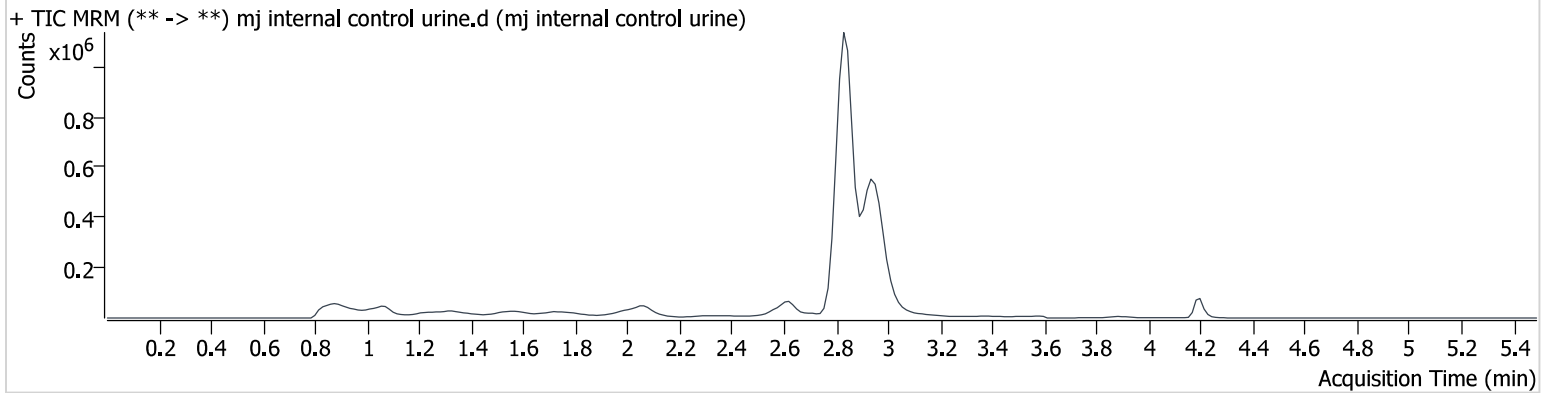


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

| | | | |
|-------------------------|---------------------|------------------|-----------------------------|
| Instrument | 69679 | Data File | mj internal control urine.d |
| Type | Sample | Sample | mj internal control urine |
| Acq. Method | thc quant 60 40.m | Operator | Anne Nord |
| Sample Position | P3-A2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 3/9/2023 7:31:39 PM | | |
| Sample Info. | | | |

Sample Chromatogram



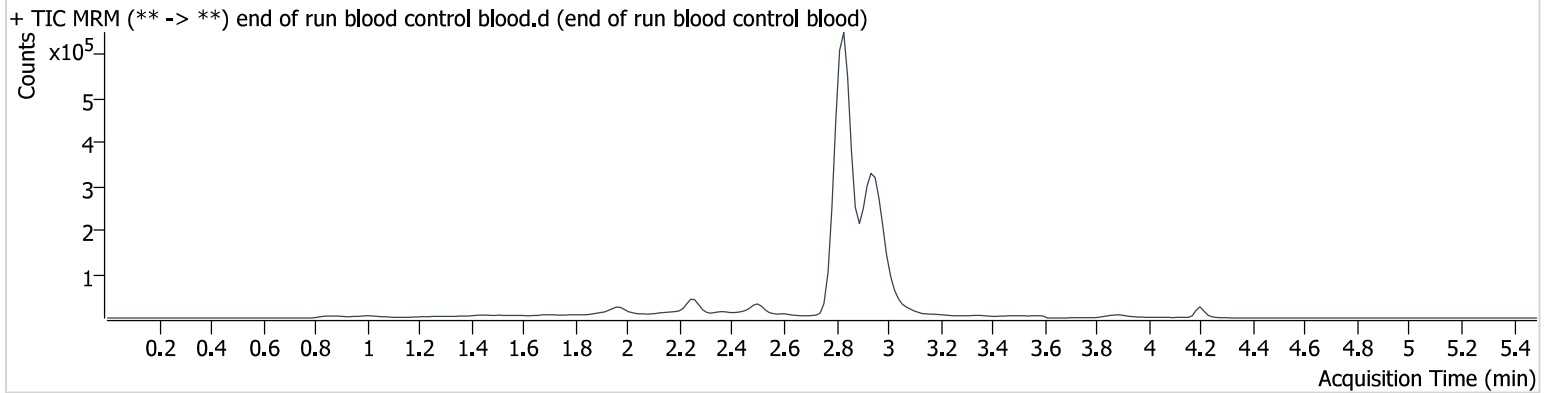
| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|--------|--------|-------|------------|--------------|
| THC-OH | 2.848 | 87563 | ∞ | 918.05 | ∞ | 4276381 | 4.495 ng/ml |
| THC-COOH | 2.965 | 130675 | 3090.1 | 271.57 | 440.3 | 1669552 | 15.066 ng/ml |
| THC | 4.197 | 22511 | 564.2 | 33.23 | 873.4 | 177644 | 5.363 ng/ml |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

| | | | |
|-------------------------|-----------------------|------------------|----------------------------------|
| Instrument | 69679 | Data File | end of run blood control blood.d |
| Type | Sample | Sample | end of run blood control blood |
| Acq. Method | thc quant 60 40.m | Operator | Anne Nord |
| Sample Position | P3-H1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 3/10/2023 12:14:05 PM | | |
| Sample Info. | | | |

Sample Chromatogram

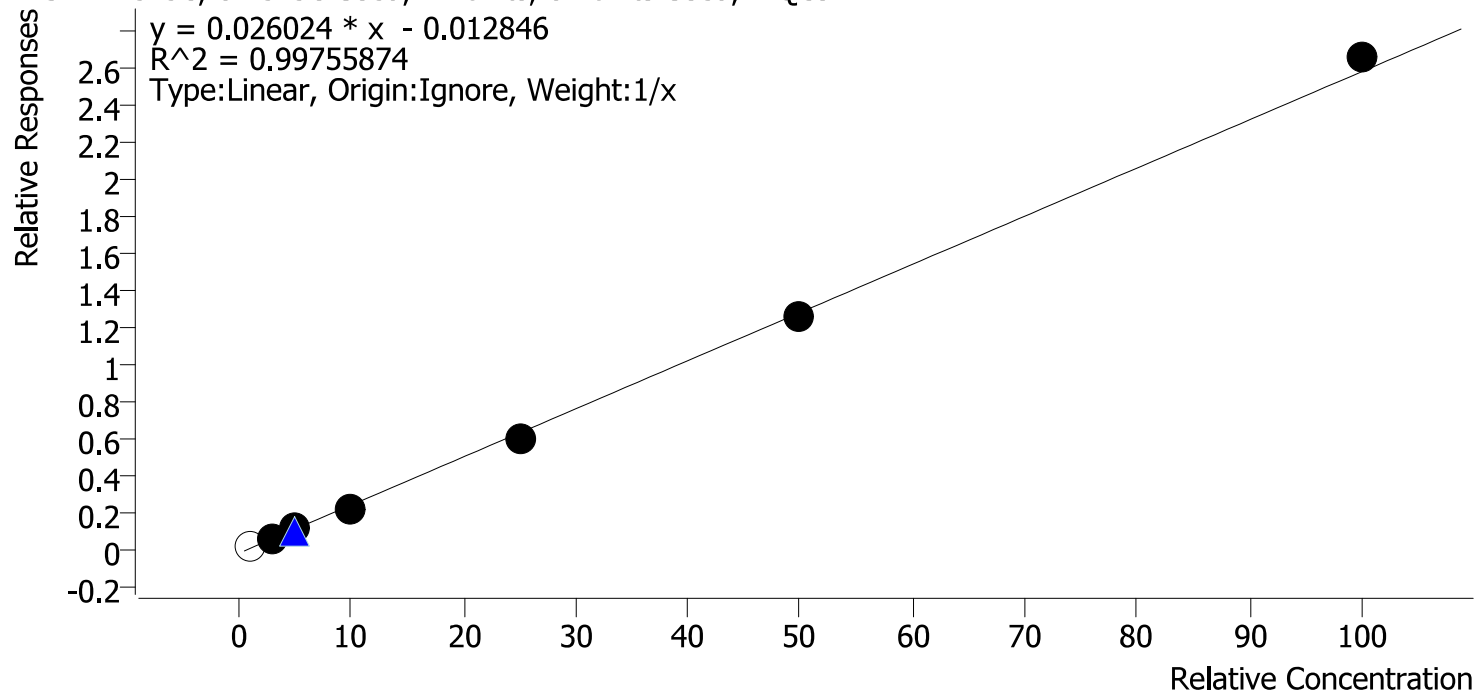


| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|-------|-------|--------|------|------------|--------------|
| THC-OH | 2.833 | 51649 | ∞ | 829.33 | ∞ | 2480556 | 4.563 ng/ml |
| THC-COOH | 2.965 | 77290 | 664.7 | 259.55 | ∞ | 995988 | 14.950 ng/ml |
| THC | 4.197 | 6195 | 174.6 | 41.59 | 63.3 | 57734 | 4.617 ng/ml |

Compound Calibration Report

Batch results D:\MassHunter\Data\2023\lam 27-28\030923\QuantResults\cann add.batch.bin
Last Cal. Update 3/10/2023 1:00 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs

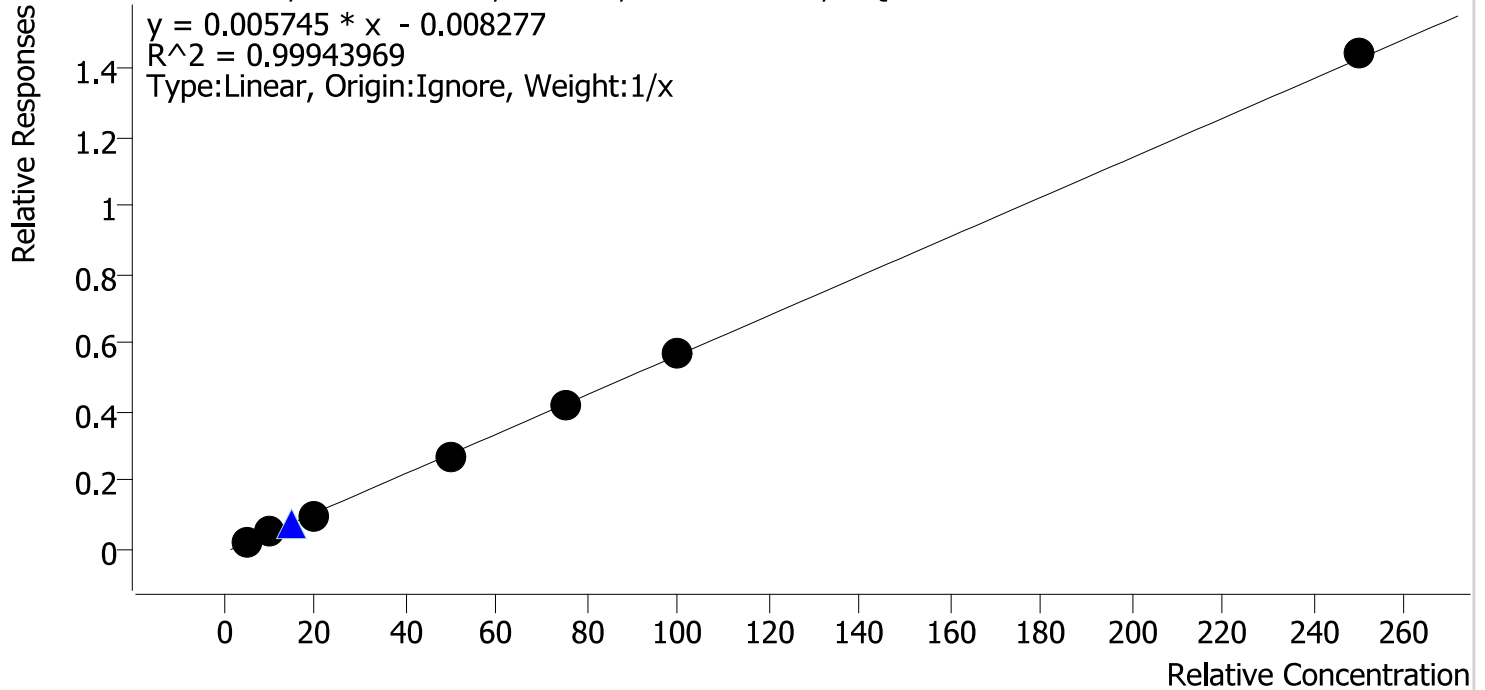


| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal 1 | 1 | × | 1.0 | 1.6 | 157.0 |
| mj cal 2 | 2 | ✓ | 3.0 | 3.3 | 109.9 |
| mj cal 3 | 3 | ✓ | 5.0 | 5.3 | 105.5 |
| mj cal 4 | 4 | ✓ | 10.0 | 9.0 | 89.6 |
| mj cal 5 | 5 | ✓ | 25.0 | 23.4 | 93.8 |
| mj cal 6 | 6 | ✓ | 50.0 | 49.1 | 98.3 |
| mj cal 7 | 7 | ✓ | 100.0 | 102.9 | 102.9 |

Compound Calibration Report

Batch results D:\MassHunter\Data\2023\lam 27-28\030923\QuantResults\cann add.batch.bin
Last Cal. Update 3/10/2023 1:00 PM
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

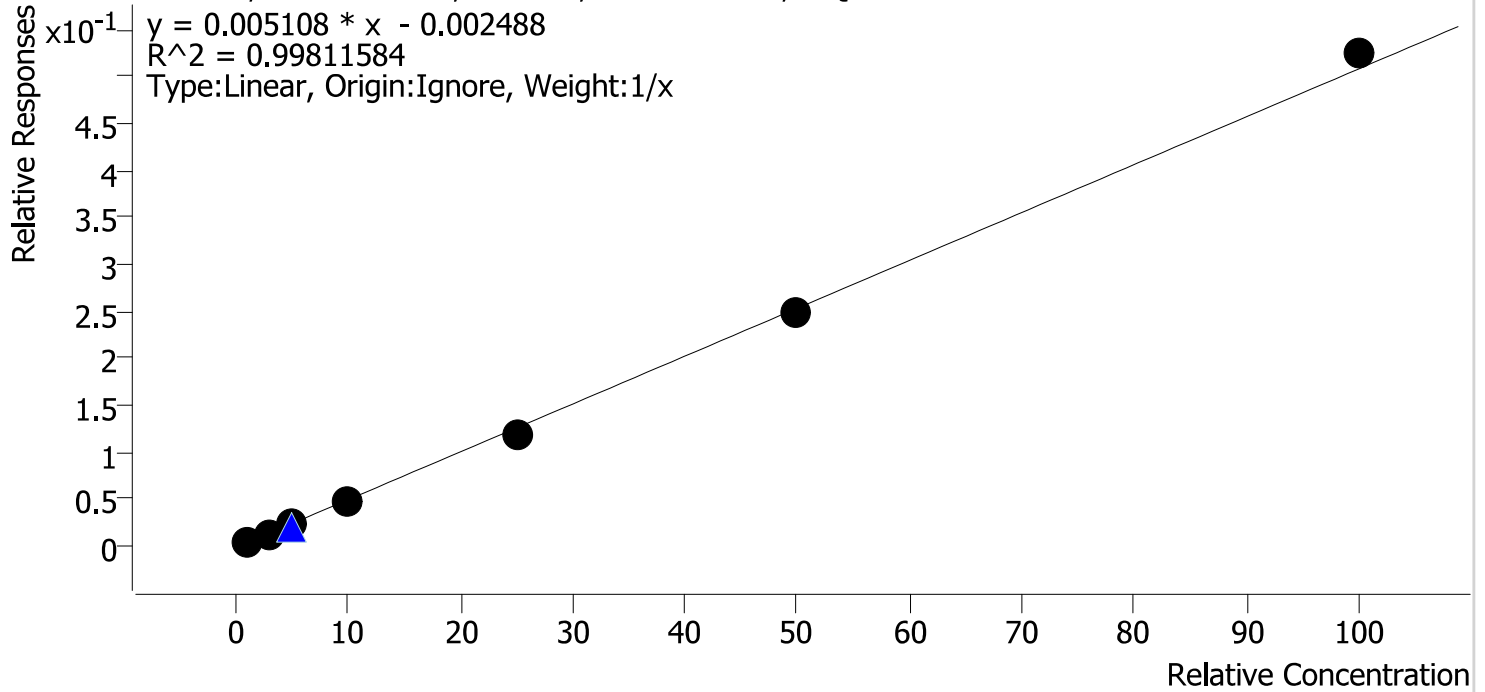


| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal 1 | 1 | ✓ | 5.0 | 5.4 | 108.9 |
| mj cal 2 | 2 | ✓ | 10.0 | 9.9 | 98.7 |
| mj cal 3 | 3 | ✓ | 20.0 | 19.2 | 95.9 |
| mj cal 4 | 4 | ✓ | 50.0 | 47.6 | 95.3 |
| mj cal 5 | 5 | ✓ | 75.0 | 74.9 | 99.8 |
| mj cal 6 | 6 | ✓ | 100.0 | 100.3 | 100.3 |
| mj cal 7 | 7 | ✓ | 250.0 | 252.7 | 101.1 |

Compound Calibration Report

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Last Cal. Update 3/10/2023 1:00 PM
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 | 123.0 |
| mj cal 2 | 2 | ✓ | 3.0 | 2.9 | 95.1 |
| mj cal 3 | 3 | ✓ | 5.0 | 4.6 | 92.7 |
| mj cal 4 | 4 | ✓ | 10.0 | 9.3 | 92.5 |
| mj cal 5 | 5 | ✓ | 25.0 | 23.9 | 95.5 |
| mj cal 6 | 6 | ✓ | 50.0 | 49.0 | 98.0 |
| mj cal 7 | 7 | ✓ | 100.0 | 103.2 | 103.2 |

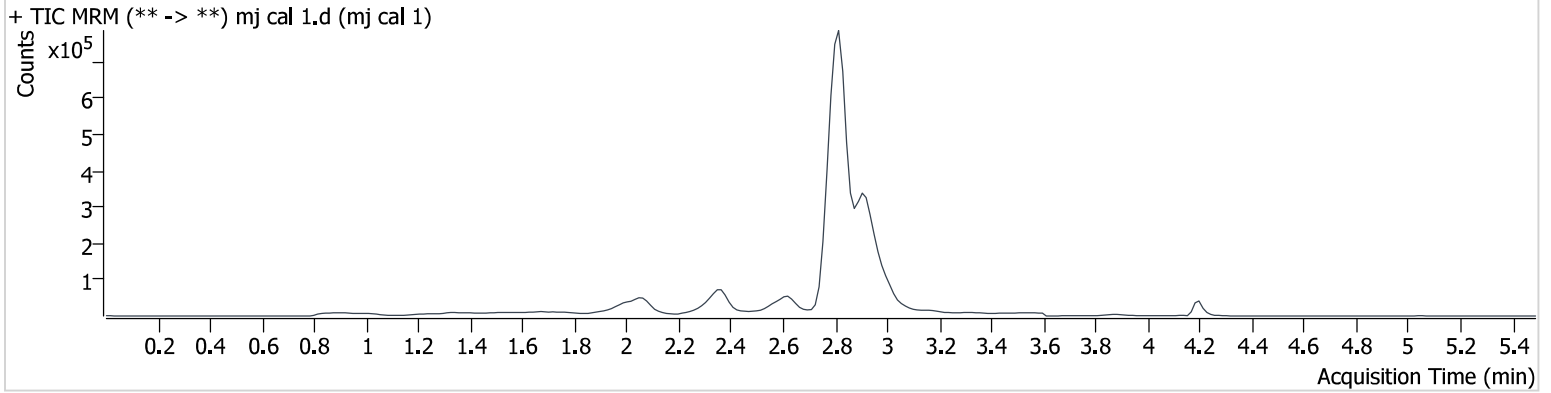
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

Instrument 69679
Type Cal
Acq. Method thc quant 60 40.m
Sample Position P3-A1
Injection Volume 10
Acq. Date-Time 3/9/2023 5:06:19 PM
Sample Info.

Data File mj cal 1.d
Sample mj cal 1
Operator Anne Nord
Comment

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|----------|-------|-------|-------|------------|------|------------|-------------|-----|
| THC-OH | 2.818 | 13952 | ∞ | 953.31 | ∞ | 3678120 | 1.230 ng/ml | Low |
| THC-COOH | 2.950 | 27660 | ∞ | 317.07 | ∞ | 1202569 | 5.445 ng/ml | |
| THC | 4.197 | 3067 | 235.1 | 51.92 High | 29.6 | 109468 | 1.570 ng/ml | |

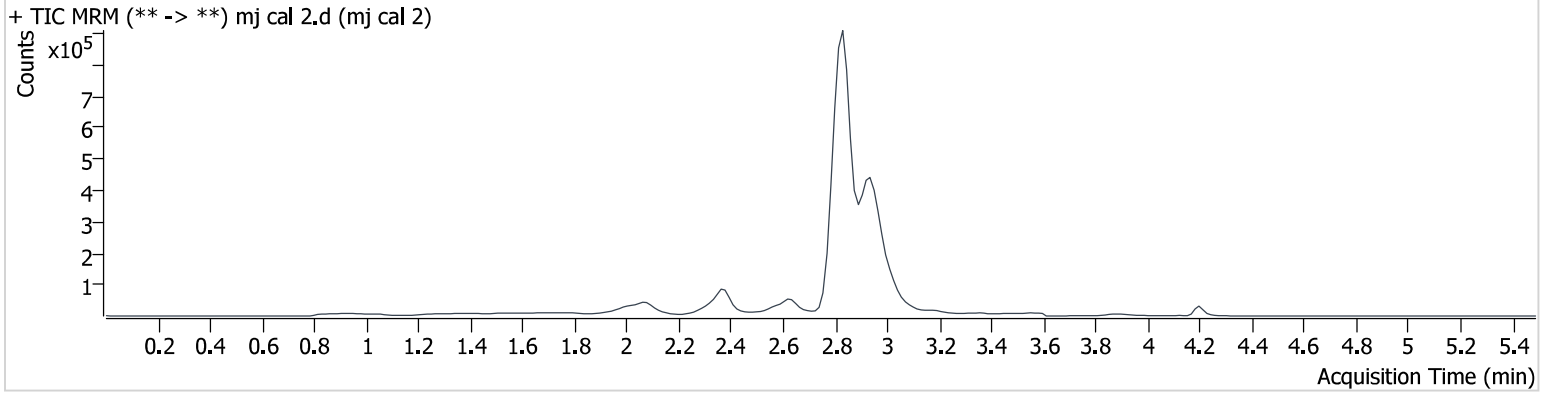
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

Instrument 69679
Type Cal
Acq. Method thc quant 60 40.m
Sample Position P3-B1
Injection Volume 10
Acq. Date-Time 3/9/2023 5:13:05 PM
Sample Info.

Data File mj cal 2.d
Sample mj cal 2
Operator Anne Nord
Comment

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|-------|--------|--------|------|------------|------------------------|
| THC-OH | 2.833 | 46528 | ∞ | 992.90 | ∞ | 3848585 | 2.854 ng/ml Low |
| THC-COOH | 2.965 | 70058 | 1225.2 | 280.67 | ∞ | 1446519 | 9.872 ng/ml |
| THC | 4.197 | 5597 | 481.5 | 41.93 | 44.7 | 76700 | 3.297 ng/ml |

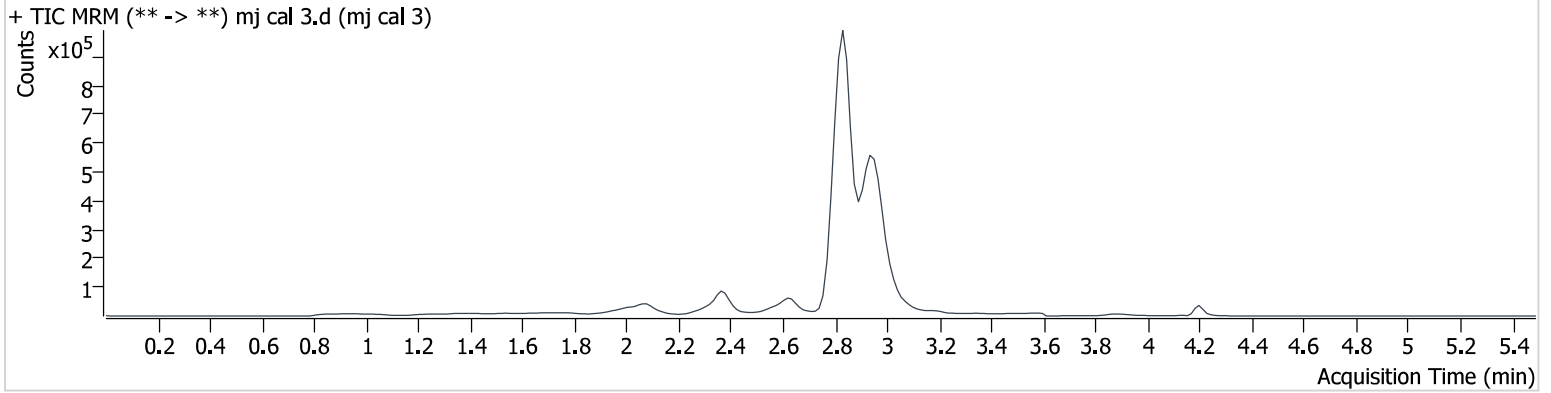
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

Instrument 69679
Type Cal
Acq. Method thc quant 60 40.m
Sample Position P3-C1
Injection Volume 10
Acq. Date-Time 3/9/2023 5:19:41 PM
Sample Info.

Data File mj cal 3.d
Sample mj cal 3
Operator Anne Nord
Comment

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|-------|--------|-------|------------|--------------|
| THC-OH | 2.833 | 81974 | ∞ | 953.24 | ∞ | 3867279 | 4.636 ng/ml |
| THC-COOH | 2.965 | 155863 | 497.6 | 260.87 | ∞ | 1529654 | 19.179 ng/ml |
| THC | 4.197 | 10460 | 288.8 | 38.61 | 164.7 | 84051 | 5.276 ng/ml |

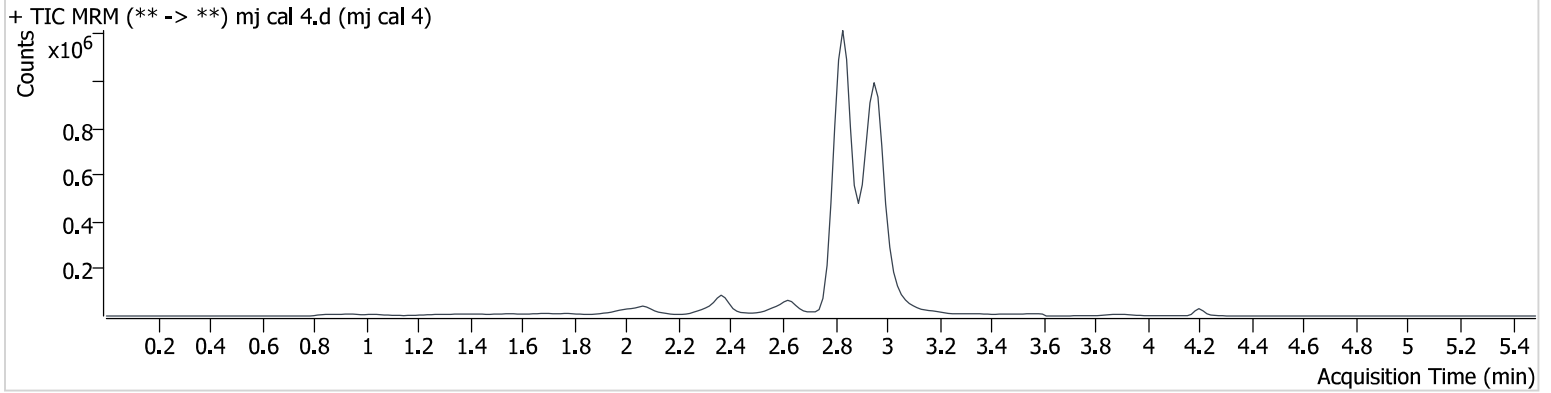
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

Instrument 69679
Type Cal
Acq. Method thc quant 60 40.m
Sample Position P3-D1
Injection Volume 10
Acq. Date-Time 3/9/2023 5:26:17 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 | 2.833 | 179503 | ∞ | 913.04 | ∞ | 4007830 | 9.255 ng/ml |
| THC-COOH | 2.965 | 426754 | ∞ | 253.11 | 6896.0 | 1608203 | 47.634 ng/ml |
| THC | 4.197 | 14966 | 417.1 | 32.85 | 119.4 | 67889 | 8.965 ng/ml |

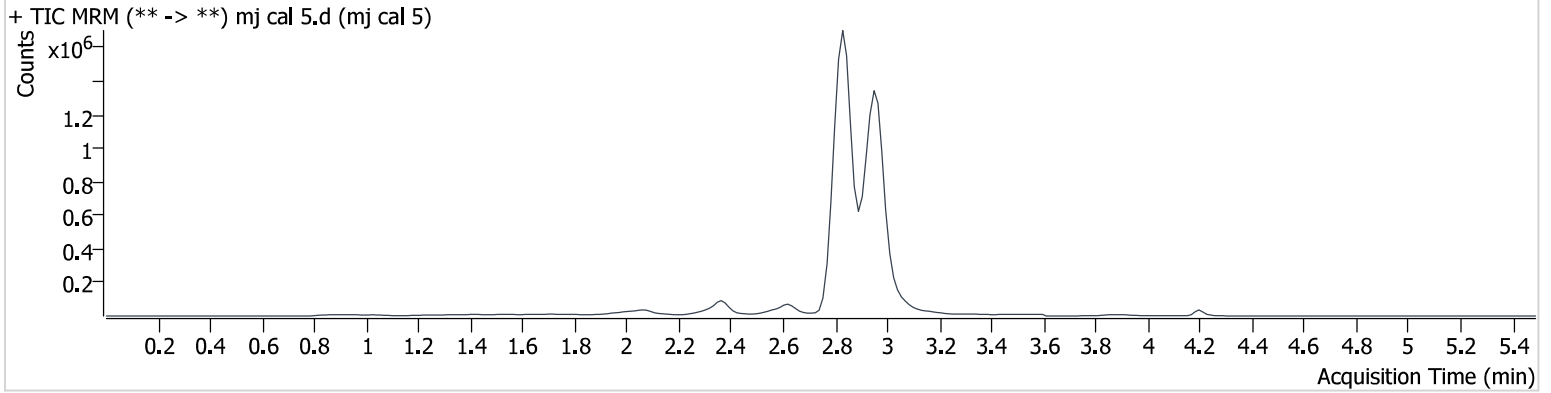
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

Instrument 69679
Type Cal
Acq. Method thc quant 60 40.m
Sample Position P3-E1
Injection Volume 10
Acq. Date-Time 3/9/2023 5:32:53 PM
Sample Info.

Data File mj cal 5.d
Sample mj cal 5
Operator Anne Nord
Comment

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|----------|--------|----------|------------|--------------|
| THC-OH | 2.833 | 460481 | ∞ | 884.18 | ∞ | 3854215 | 23.875 ng/ml |
| THC-COOH | 2.950 | 651519 | 4443.2 | 251.17 | ∞ | 1544211 | 74.886 ng/ml |
| THC | 4.197 | 33541 | 879.0 | 35.20 | 100.8 | 56157 | 23.444 ng/ml |

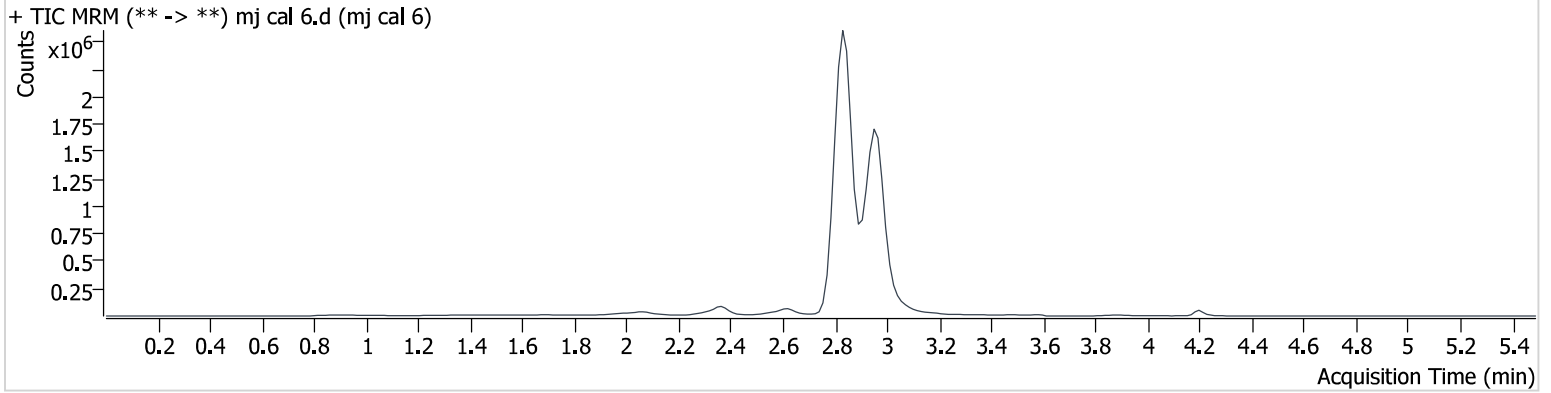
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

Instrument 69679
Type Cal
Acq. Method thc quant 60 40.m
Sample Position P3-F1
Injection Volume 10
Acq. Date-Time 3/9/2023 5:39:29 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 | 2.833 | 931758 | ∞ | 868.80 | ∞ | 3760016 | 48.998 ng/ml |
| THC-COOH | 2.950 | 865952 | 3552.8 | 246.46 | 18641.9 | 1524837 | 100.300 ng/ml |
| THC | 4.197 | 71388 | 2141.9 | 35.93 | 1431.0 | 56399 | 49.132 ng/ml |

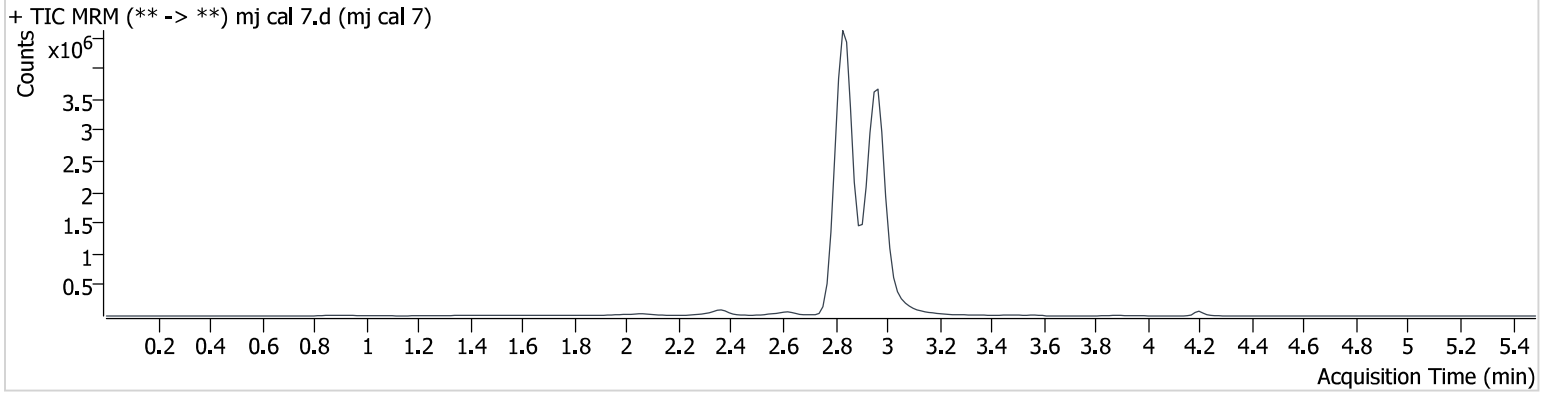
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\030923\QuantResults\cann add.batch.bin
Calibration Last Update 3/10/2023 1:00:11 PM

Instrument 69679
Type Cal
Acq. Method thc quant 60 40.m
Sample Position P3-G1
Injection Volume 10
Acq. Date-Time 3/9/2023 5:46:03 PM
Sample Info.

Data File mj cal 7.d
Sample mj cal 7
Operator Anne Nord
Comment

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|---------|--------|--------|-------|------------|---------------|
| THC-OH | 2.833 | 1987813 | ∞ | 854.64 | ∞ | 3790291 | 103.153 ng/ml |
| THC-COOH | 2.965 | 2115910 | ∞ | 244.67 | ∞ | 1466043 | 252.684 ng/ml |
| THC | 4.197 | 127533 | 1680.5 | 31.58 | 453.9 | 47861 | 102.886 ng/ml |