













REVIEWED
By Sarah Pickle at 11:39 am, Aug 26, 2019



8/20/2019

Worklist: 3616

| <u>LAB_CASE</u> | <u>ITEM</u> | <u>TASK_ID</u> | <u>DESCRIPTION</u> | |
|-----------------|-------------|----------------|---------------------------------|---|
| C2019-1225 | 1 | 160838 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1297 | 1 | 160839 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1390 | 1 | 160840 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1439 | 1 | 160841 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1440 | 1 | 160842 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1485 | 1 | 160843 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1486 | 1 | 160844 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1518 | 1 | 160845 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1530 | 1 | 160846 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1552 | 1 | 160847 | AM 27 Blood THC Quant by LC-QQQ |  |
| C2019-1564 | 1 | 160848 | AM 27 Blood THC Quant by LC-QQQ |  |
| P2019-2079 | 1 | 160849 | AM 27 Blood THC Quant by LC-QQQ |  |



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

Extraction Date: 08-16-19
Plate lot#: 0539904

Analyst: Anne Nord
Plate Expiration: 09/10/19

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

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 445283-2 Hemostat
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.
- 3. Create worklist:

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Pipette **1000µL blood (calibrated pipette) Pipette ID: k52558g** in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- 4. Pipette **500µL 0.1% formic acid in water** in wells of analytical 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).
(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 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.
- 5. Did all QCs pass for each analyte? Y / N
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

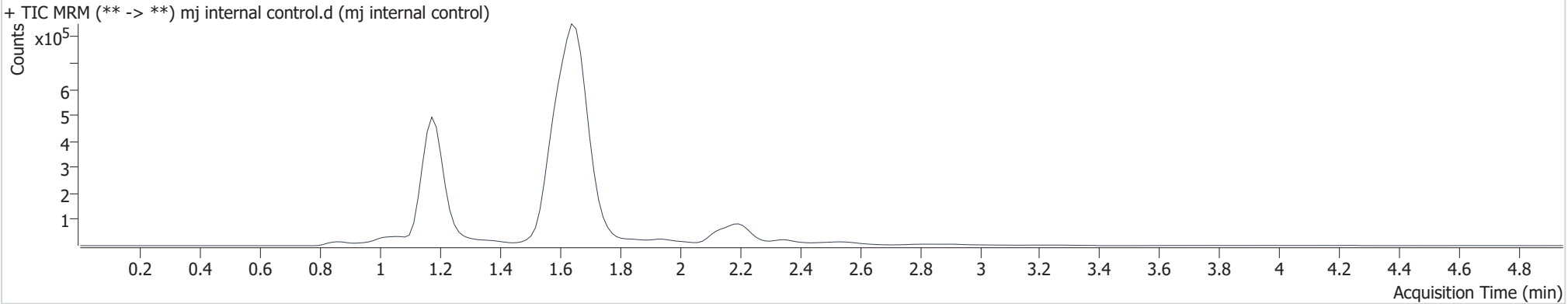
COMMENTS:

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin
Calibration Last Update 8/19/2019 2:59:25 PM

| | | | |
|-------------------------|----------------------|------------------|-----------------------|
| Instrument | 69679 | Data File | mj internal control.d |
| Type | QC | Sample | mj internal control |
| Acq. Method | AM 27 THC quant.m | Operator | Anne Nord |
| Sample Position | P3-H1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/16/2019 4:36:56 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|------|-------|-------|------------|------------------------|
| THC-COOH | 1.190 | 59834 | 97.7 | 35.5 | 159.2 | 604636 | 9.592 ng/ml Low |
| THC-OH | 1.194 | 195722 | ∞ | 7.4 | ∞ | 1563579 | 5.266 ng/ml |
| THC | 2.206 | 15760 | ∞ | 21.2 | 28.7 | 508671 | 4.758 ng/ml |

AM #27 Cannabinoids

Batch results

D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin

Calibration Last Update

8/19/2019 2:59:25 PM

Instrument

69679

Type

Sample

Acq. Method

AM 27 THC quant.m

Sample Position

P3-A2

Injection Volume

10

Acq. Date-Time

8/16/2019 4:44:39 PM

Sample Info.**Data File**

mj negative blood.d

Sample

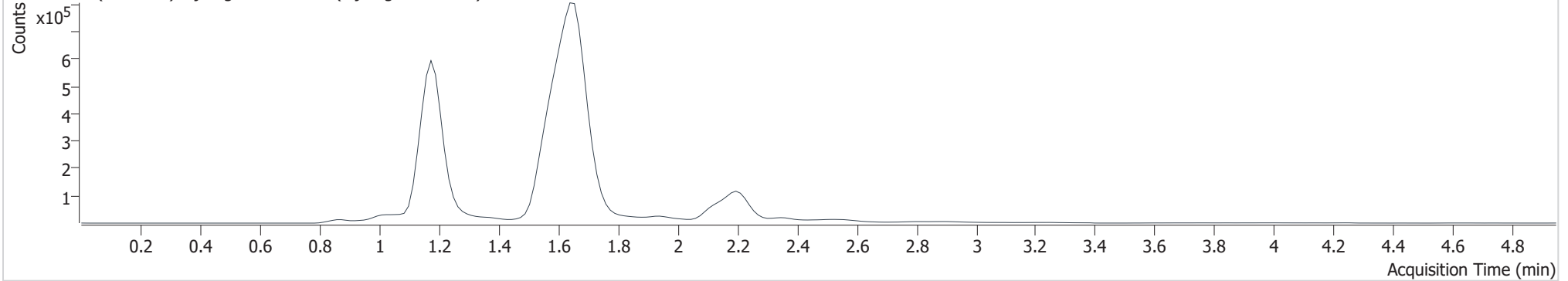
mj negative blood

Operator

Anne Nord

Comment**Sample Chromatogram**

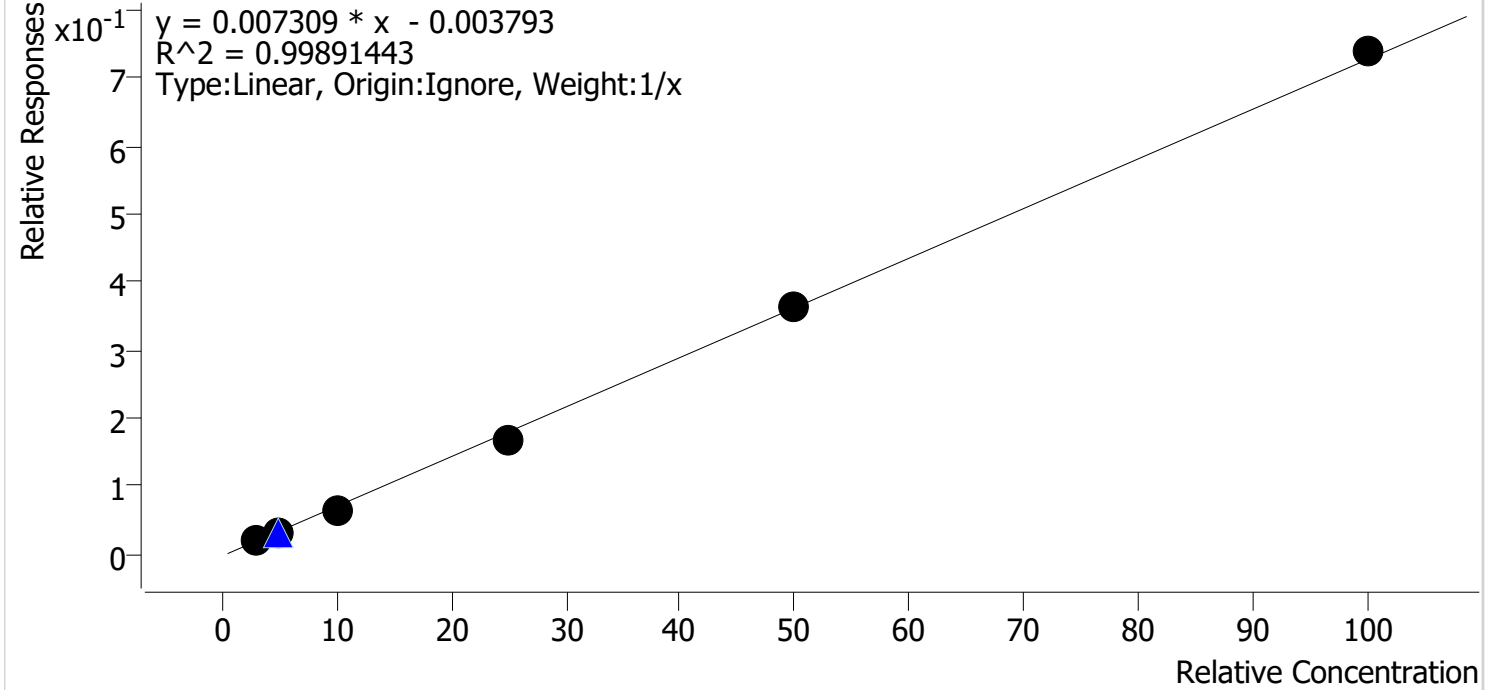
+ TIC MRM (** -> **) mj negative blood.d (mj negative blood)



Compound Calibration Report

Batch results D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin
Last Cal. Update 8/19/2019 2:59 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

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

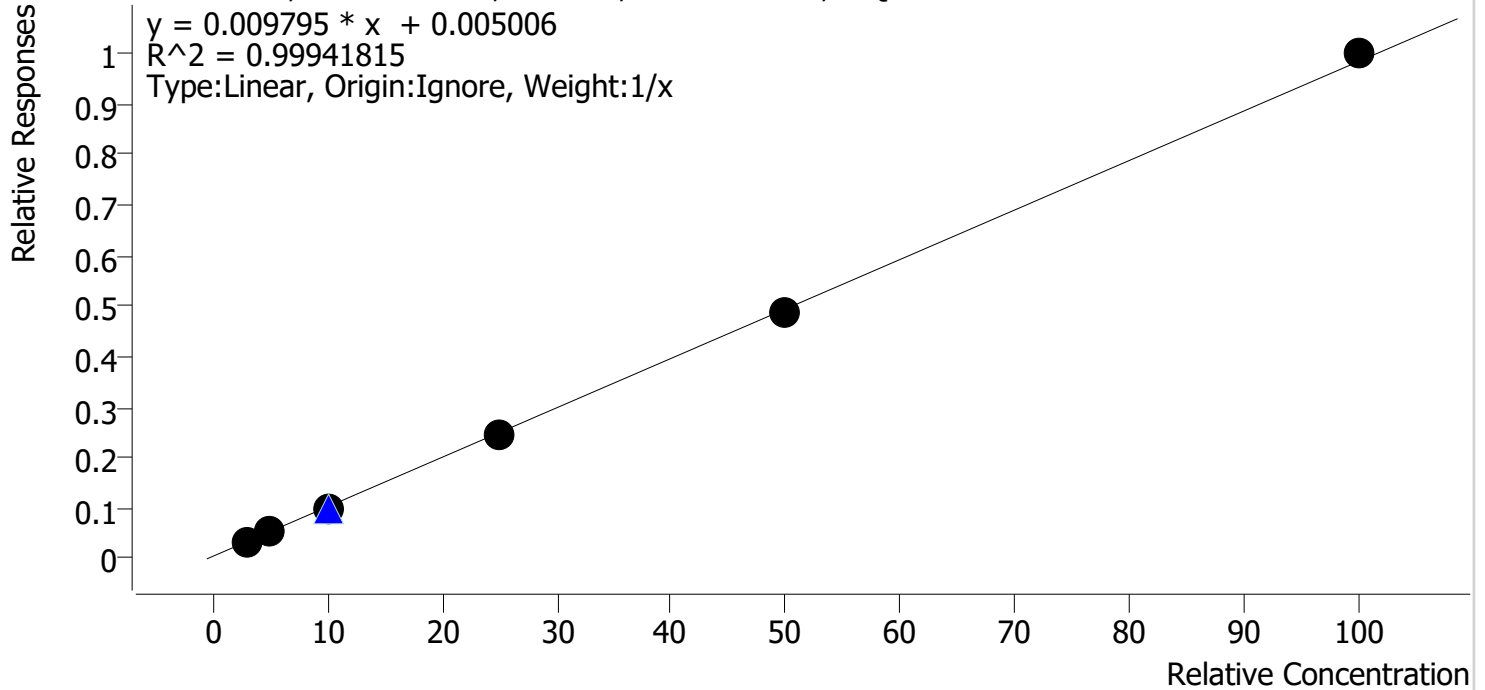


| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal2 | 2 | ✓ | 3.0 | 3.3 | 110.7 |
| mj cal 3 | 3 | ✓ | 5.0 | 4.9 | 97.7 |
| mj cal 4 | 4 | ✓ | 10.0 | 9.5 | 95.3 |
| mj cal 5 | 5 | ✓ | 25.0 | 23.7 | 94.7 |
| mj cal 6 | 6 | ✓ | 50.0 | 50.1 | 100.1 |
| mj cal 7 | 7 | ✓ | 100.0 | 101.5 | 101.5 |

Compound Calibration Report

Batch results D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin
Last Cal. Update 8/19/2019 2:59 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-d9

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



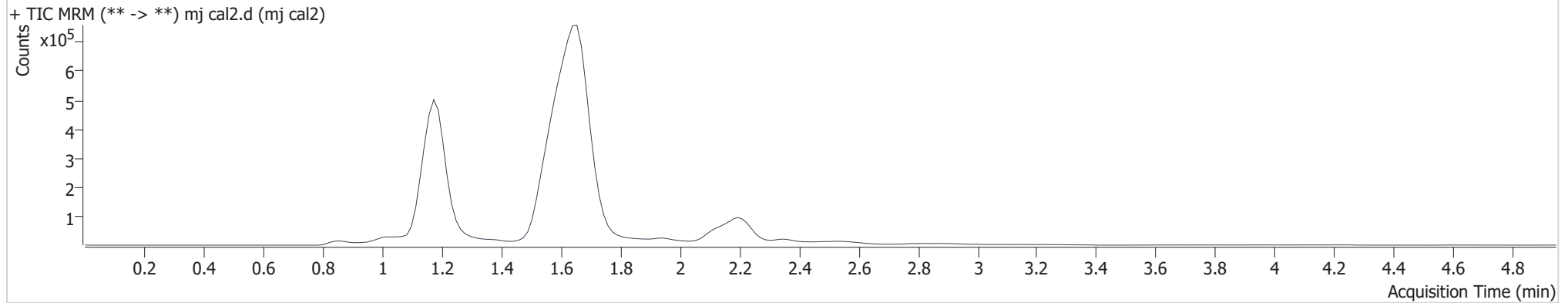
| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal2 | 2 | ✓ | 3.0 | 3.0 | 99.0 |
| mj cal 3 | 3 | ✓ | 5.0 | 5.3 | 106.2 |
| mj cal 4 | 4 | ✓ | 10.0 | 9.8 | 97.8 |
| mj cal 5 | 5 | ✓ | 25.0 | 24.3 | 97.3 |
| mj cal 6 | 6 | ✓ | 50.0 | 49.1 | 98.1 |
| mj cal 7 | 7 | ✓ | 100.0 | 101.5 | 101.5 |

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin
Calibration Last Update 8/19/2019 2:59:25 PM

| | | | |
|-------------------------|----------------------|------------------|-----------|
| Instrument | 69679 | Data File | mj cal2.d |
| Type | Cal | Sample | mj cal2 |
| Acq. Method | AM 27 THC quant.m | Operator | Anne Nord |
| Sample Position | P3-B1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/16/2019 3:50:35 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|-------|-------|-------|------------|------------------------|
| THC-COOH | 1.190 | 23637 | 285.2 | 34.2 | 73.3 | 693491 | 2.969 ng/ml Low |
| THC-OH | 1.194 | 169797 | ∞ | 7.8 | ∞ | 1854013 | 2.952 ng/ml Low |
| THC | 2.221 | 13065 | ∞ | 22.1 | 119.9 | 637806 | 3.322 ng/ml |

AM #27 Cannabinoids

**Batch results**

D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin

Calibration Last Update

8/19/2019 2:59:25 PM

Instrument

69679

Type

Cal

Acq. Method

AM 27 THC quant.m

Sample Position

P3-C1

Injection Volume

10

Acq. Date-Time

8/16/2019 3:58:20 PM

Sample Info.**Data File**

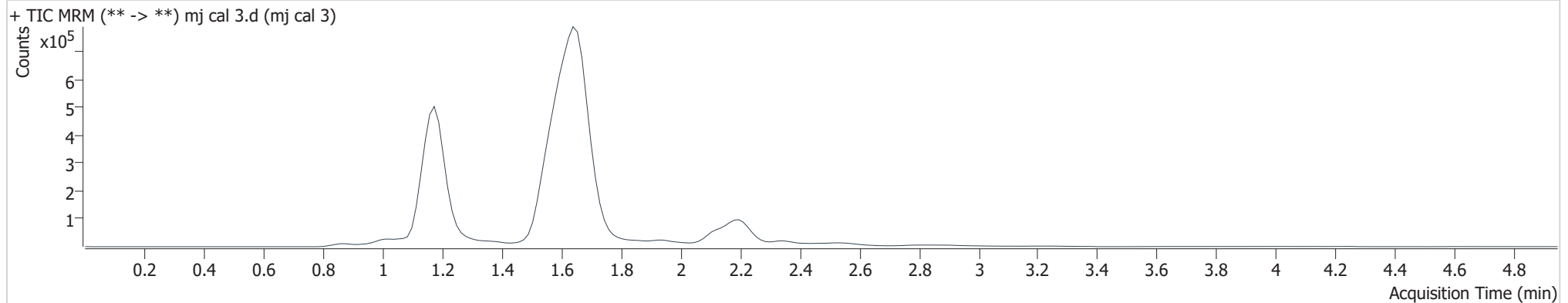
mj cal 3.d

Sample

mj cal 3

Operator

Anne Nord

Comment**Sample Chromatogram**

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|------|-------|-------|------------|------------------------|
| THC-COOH | 1.190 | 37733 | 85.2 | 31.7 | 169.3 | 661891 | 5.309 ng/ml Low |
| THC-OH | 1.194 | 220409 | ∞ | 8.3 | ∞ | 1779359 | 5.176 ng/ml |
| THC | 2.206 | 20241 | ∞ | 23.2 | ∞ | 634538 | 4.884 ng/ml |

AM #27 Cannabinoids

**Batch results**

D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin

Calibration Last Update

8/19/2019 2:59:25 PM

Instrument

69679

Type

Cal

Acq. Method

AM 27 THC quant.m

Sample Position

P3-D1

Injection Volume

10

Acq. Date-Time

8/16/2019 4:06:04 PM

Sample Info.**Data File**

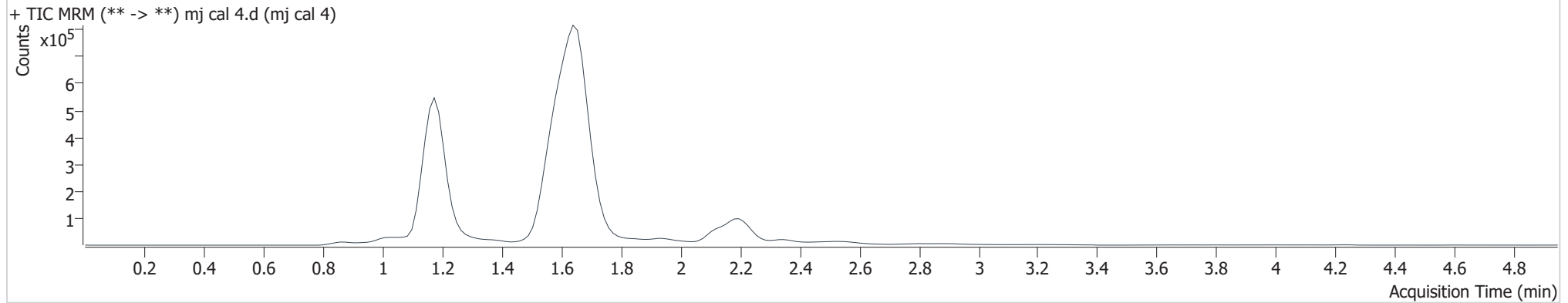
mj cal 4.d

Sample

mj cal 4

Operator

Anne Nord

Comment**Sample Chromatogram**

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|-------|-------|------|------------|------------------------|
| THC-COOH | 1.190 | 66652 | 112.8 | 31.8 | 27.3 | 660954 | 9.784 ng/ml Low |
| THC-OH | 1.179 | 335976 | ∞ | 8.7 | ∞ | 1733854 | 9.992 ng/ml |
| THC | 2.191 | 40220 | ∞ | 28.6 | ∞ | 611036 | 9.525 ng/ml |

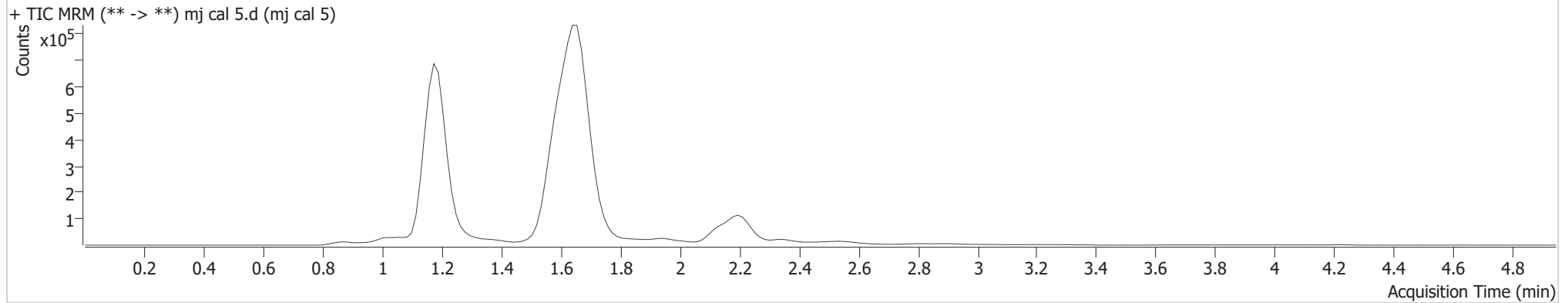
AM #27 Cannabinoids



Batch results D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin
Calibration Last Update 8/19/2019 2:59:25 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 | 8/16/2019 4:13:46 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|-------|-------|---------|------------|--------------|
| THC-COOH | 1.190 | 161371 | 255.9 | 34.1 | 475.7 | 663056 | 24.336 ng/ml |
| THC-OH | 1.179 | 688983 | ∞ | 9.9 | 10992.8 | 1750387 | 23.758 ng/ml |
| THC | 2.221 | 103620 | ∞ | 24.4 | ∞ | 612434 | 23.669 ng/ml |

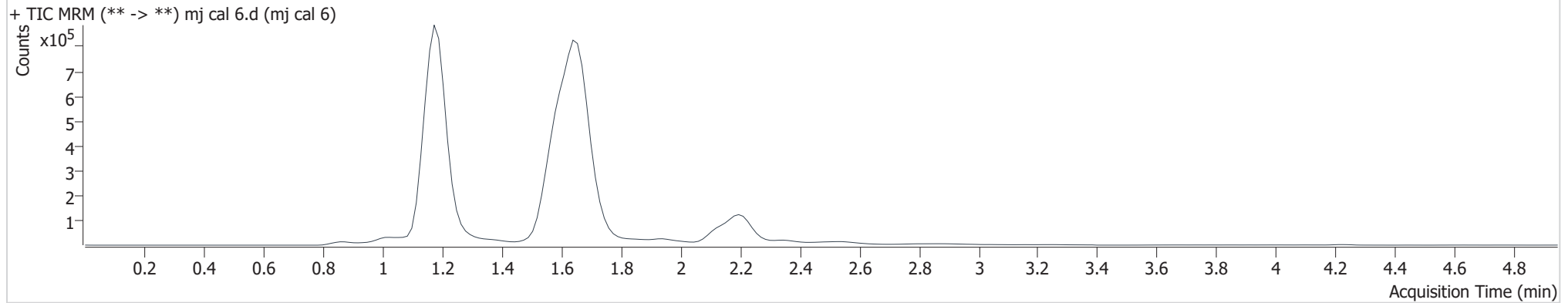
AM #27 Cannabinoids



Batch results D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin
Calibration Last Update 8/19/2019 2:59:25 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 | 8/16/2019 4:21:30 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|---------|-------|-------|--------|------------|--------------|
| THC-COOH | 1.190 | 317369 | 270.7 | 36.4 | 3351.9 | 653526 | 49.069 ng/ml |
| THC-OH | 1.179 | 1394913 | ∞ | 9.4 | ∞ | 1736047 | 51.994 ng/ml |
| THC | 2.206 | 209348 | ∞ | 23.3 | ∞ | 578056 | 50.071 ng/ml |

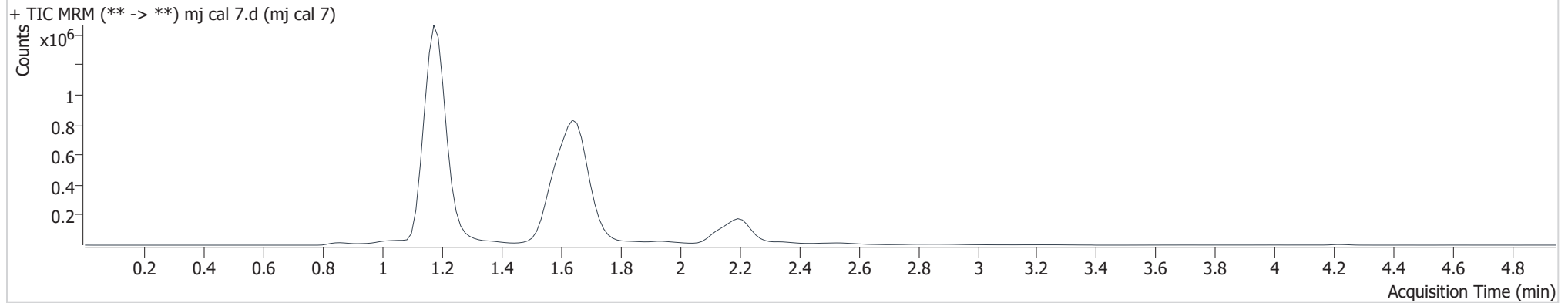
AM #27 Cannabinoids



Batch results D:\MassHunter\Data\2019\am27 081619\QuantResults\cann quant.batch.bin
Calibration Last Update 8/19/2019 2:59:25 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 | 8/16/2019 4:29:14 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|---------|--------|-------|-------|------------|---------------|
| THC-COOH | 1.190 | 683822 | 1271.3 | 38.5 | 693.1 | 684160 | 101.533 ng/ml |
| THC-OH | 1.179 | 2842905 | ∞ | 10.9 | ∞ | 1910918 | 99.127 ng/ml |
| THC | 2.206 | 473378 | ∞ | 25.2 | ∞ | 641224 | 101.529 ng/ml |