

CS

REVIEWED
By Sarah Pickle at 11:57 am, Aug 27, 2020

Worklist: 4455

| <u>LAB CASE</u> | <u>ITEM</u> | <u>ITEM TYPE</u> | <u>DESCRIPTION</u> | |
|-----------------|-------------|------------------|---------------------------------|---|
| M2020-2857 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| M2020-2958 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| M2020-3055 | 2 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| M2020-3115 | 3 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2109 | 2 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2388 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2403 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2406 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2418 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2419 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2422 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2423 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| P2020-2424 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |

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

Extraction Date: 08/21/2020

Analyst: Celena Shrum

Plate lot#: IDP-108-2-200303

Plate Expiration: 09/30/2020

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: 445283-4

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 069901

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. Using a calibrated pipette, add **1000µl blood (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: #42**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **500µL 0.1% formic acid in water blood sample** 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: 067104
- 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: 067103**
- 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: 5ng/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, 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 curve range: 1-100, Carboxy-THC curve range: 5-250, THC-OH curve range: 5-100 (qualitative only)

CS

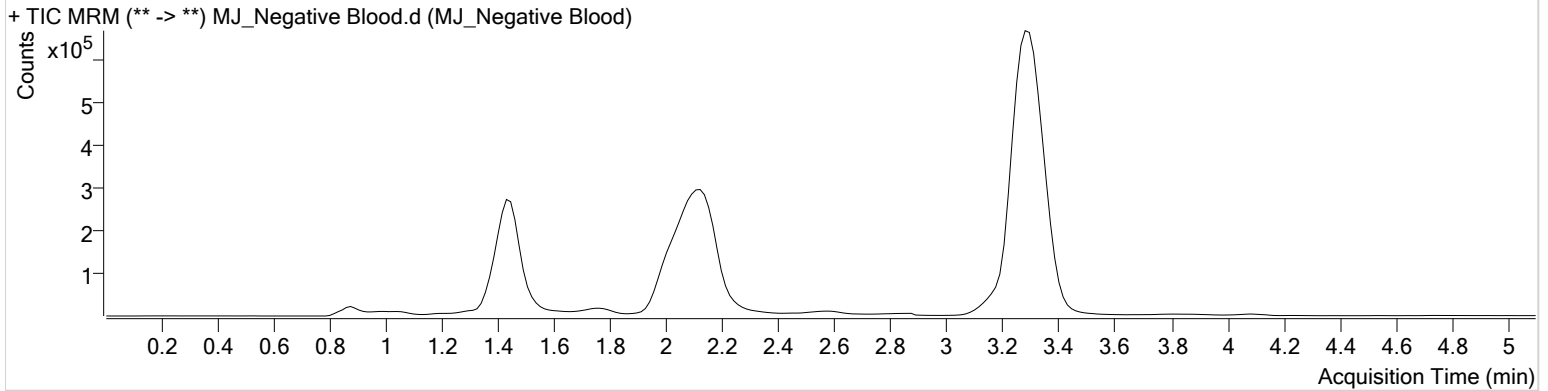


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

| | | | |
|-------------------------|----------------------|------------------|---------------------|
| Instrument | Falco | Data File | MJ_Negative Blood.d |
| Type | Sample | Sample | MJ_Negative Blood |
| Acq. Method | AM 27 THC quant.m | Operator | Celena Shrum |
| Sample Position | P3-A2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/21/2020 5:17:11 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|--------|-------|--------|-----|----------------|-------|------------|-------------------------|
| THC-OH | 1.513 | 100527 | ∞ | 3.3 Low | 19.32 | 1226663 | 0.4748 ng/ml Low |

AM #27 Cannabinoid Quant. Results

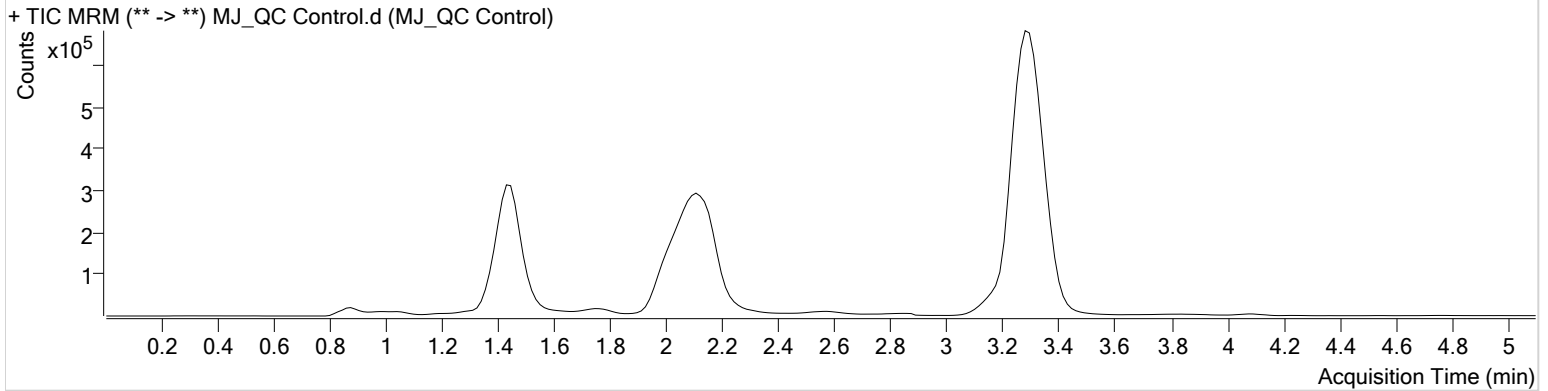


Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

| | | | |
|-------------------------|----------------------|------------------|-----------------|
| Instrument | Falco | Data File | MJ_QC Control.d |
| Type | Sample | Sample | MJ_QC Control |
| Acq. Method | AM 27 THC quant.m | Operator | Celena Shrum |
| Sample Position | P3-H1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/21/2020 5:02:00 PM | | |

Sample Info.

Sample Chromatogram

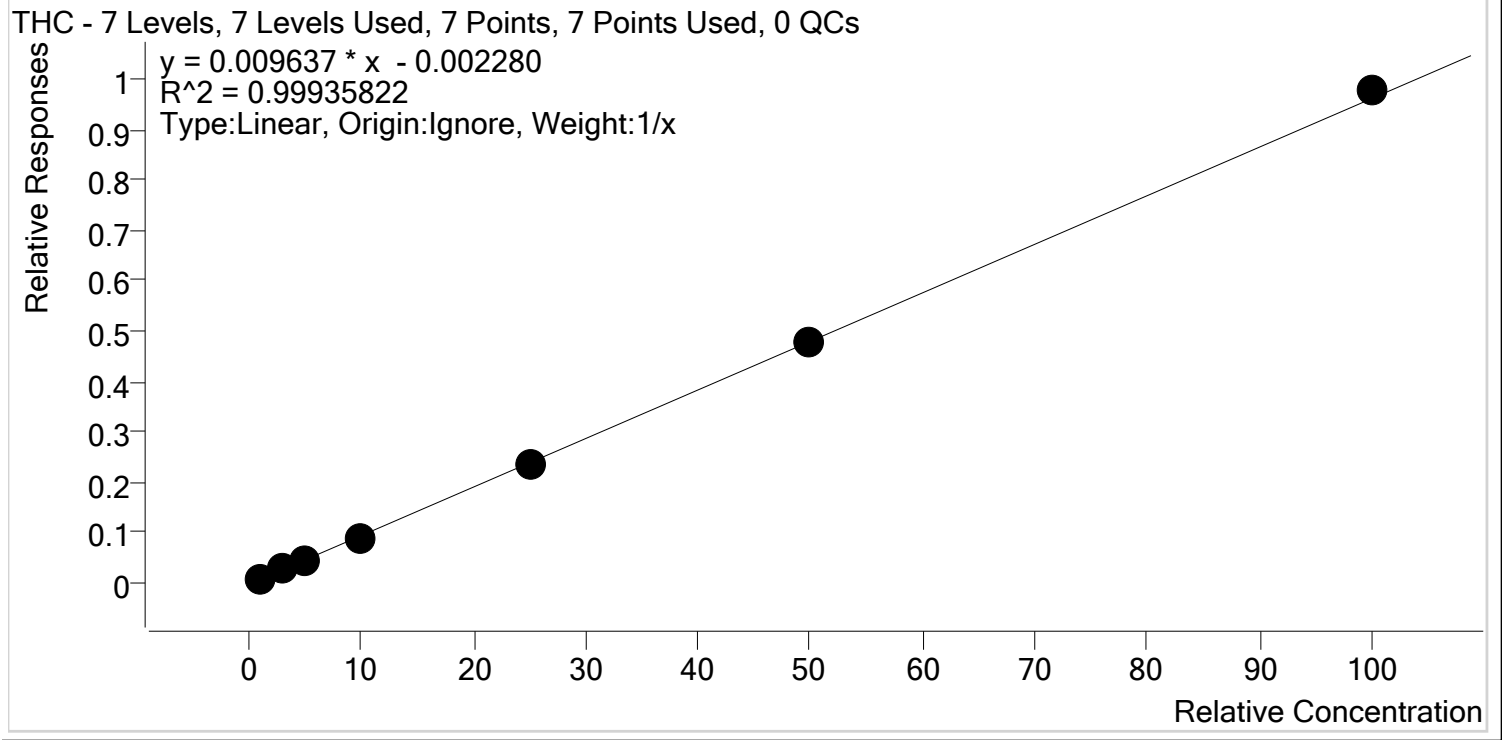


| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|--------|-------|--------|------------|---------------|
| THC-COOH | 1.474 | 132908 | ∞ | 57.0 | 433.82 | 356555 | 15.6918 ng/ml |
| THC-OH | 1.453 | 188117 | ∞ | 9.1 | ∞ | 1220700 | 4.4899 ng/ml |
| THC | 3.300 | 209329 | 267.72 | 29.7 | 901.08 | 5380145 | 4.2741 ng/ml |



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Last Cal. Update 8/26/2020 7:05 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

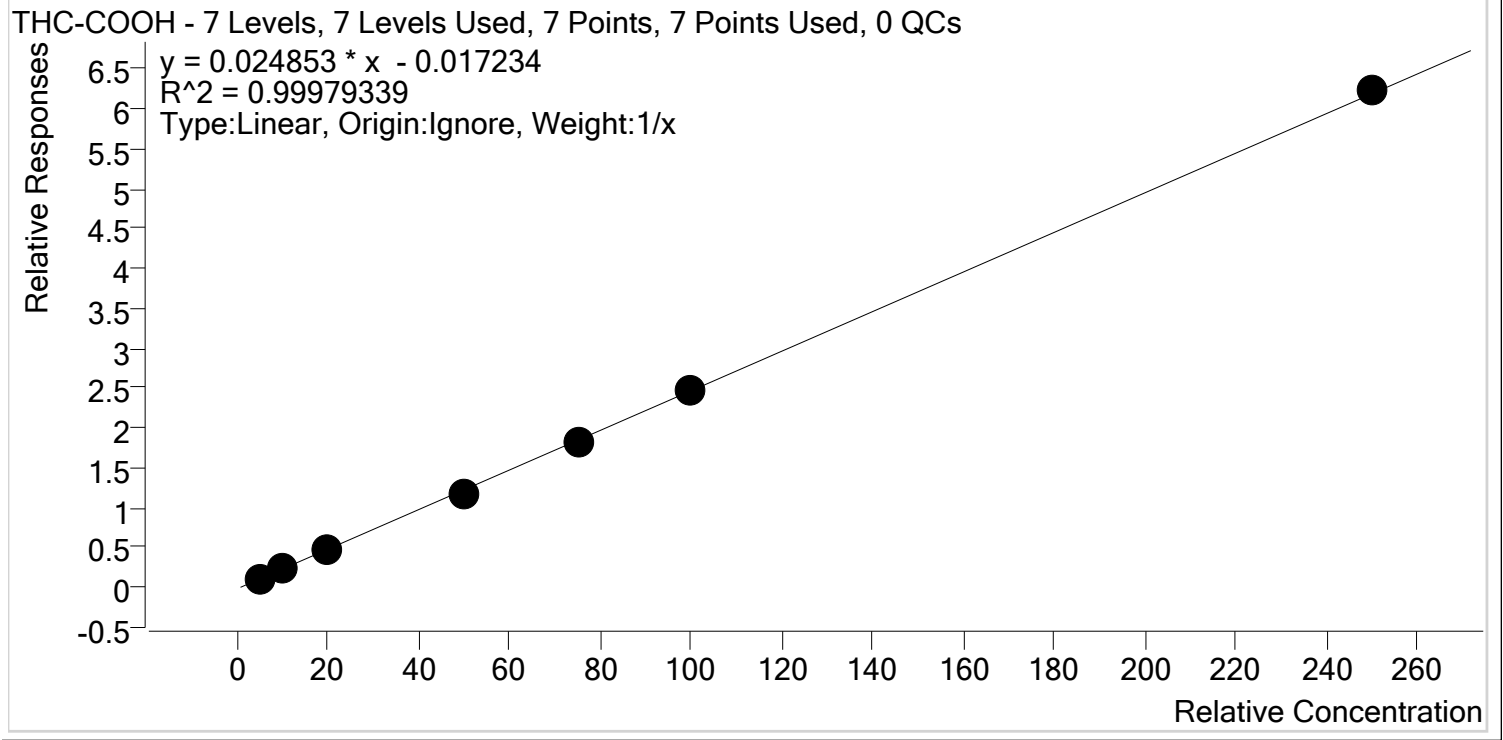


| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| MJ_Cal 1 | 1 | ✓ | 1.0 | 1.1 | 113.9 |
| MJ_Cal 2 | 2 | ✓ | 3.0 | 2.9 | 97.8 |
| MJ_Cal 3 | 3 | ✓ | 5.0 | 4.8 | 95.4 |
| MJ_Cal 4 | 4 | ✓ | 10.0 | 9.4 | 93.7 |
| MJ_Cal 5 | 5 | ✓ | 25.0 | 24.6 | 98.4 |
| MJ_Cal 6 | 6 | ✓ | 50.0 | 49.6 | 99.2 |
| MJ_Cal 7 | 7 | ✓ | 100.0 | 101.6 | 101.6 |



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Last Cal. Update 8/26/2020 7:05 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

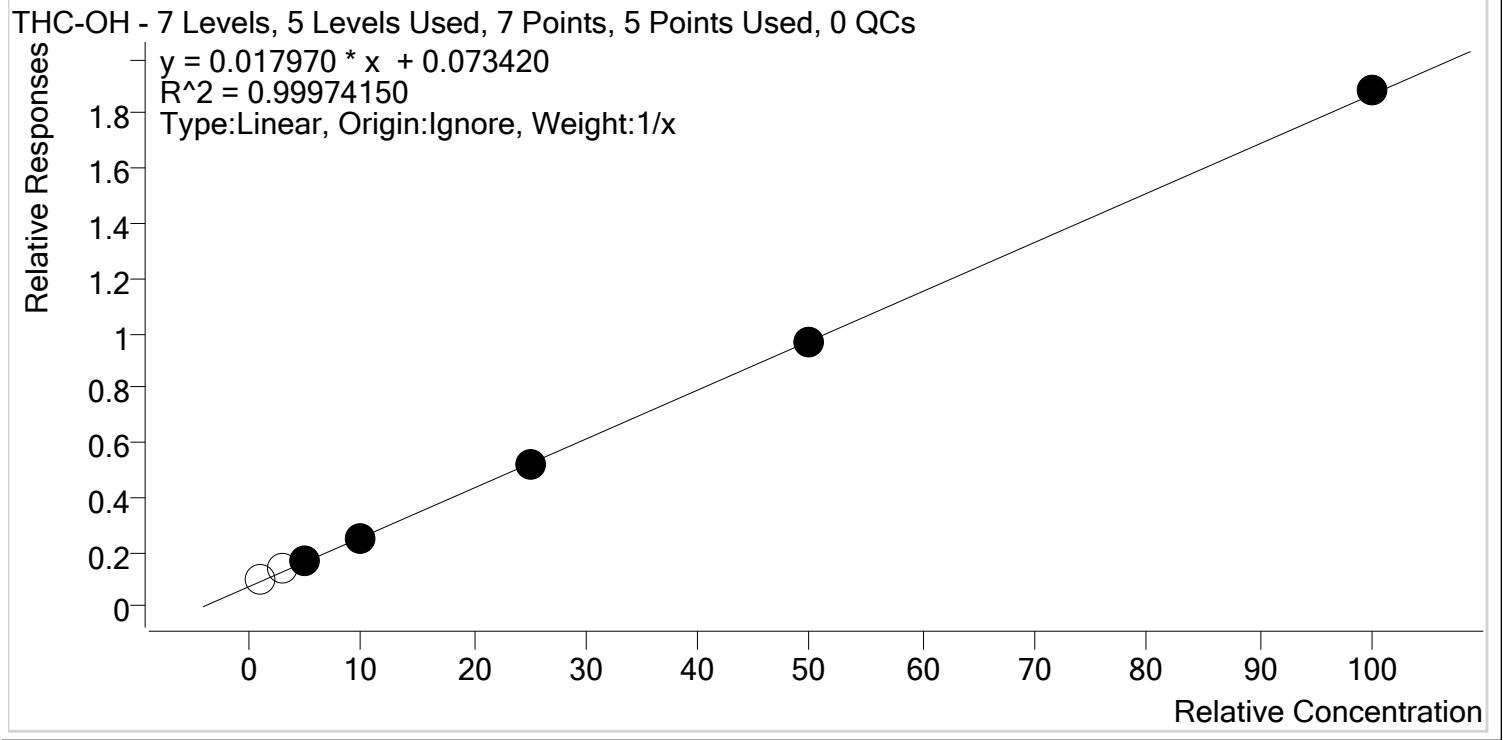


| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| MJ_Cal 1 | 1 | ✓ | 5.0 | 5.1 | 101.1 |
| MJ_Cal 2 | 2 | ✓ | 10.0 | 10.0 | 100.0 |
| MJ_Cal 3 | 3 | ✓ | 20.0 | 20.4 | 102.2 |
| MJ_Cal 4 | 4 | ✓ | 50.0 | 48.4 | 96.8 |
| MJ_Cal 5 | 5 | ✓ | 75.0 | 74.3 | 99.0 |
| MJ_Cal 6 | 6 | ✓ | 100.0 | 100.3 | 100.3 |
| MJ_Cal 7 | 7 | ✓ | 250.0 | 251.6 | 100.6 |



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Last Cal. Update 8/26/2020 7:05 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| MJ_Cal 1 | 1 | x | 1.0 | 1.6 | 163.1 |
| MJ_Cal 2 | 2 | x | 3.0 | 3.4 | 113.2 |
| MJ_Cal 3 | 3 | ✓ | 5.0 | 5.2 | 104.2 |
| MJ_Cal 4 | 4 | ✓ | 10.0 | 9.7 | 97.0 |
| MJ_Cal 5 | 5 | ✓ | 25.0 | 24.7 | 98.9 |
| MJ_Cal 6 | 6 | ✓ | 50.0 | 49.6 | 99.1 |
| MJ_Cal 7 | 7 | ✓ | 100.0 | 100.8 | 100.8 |

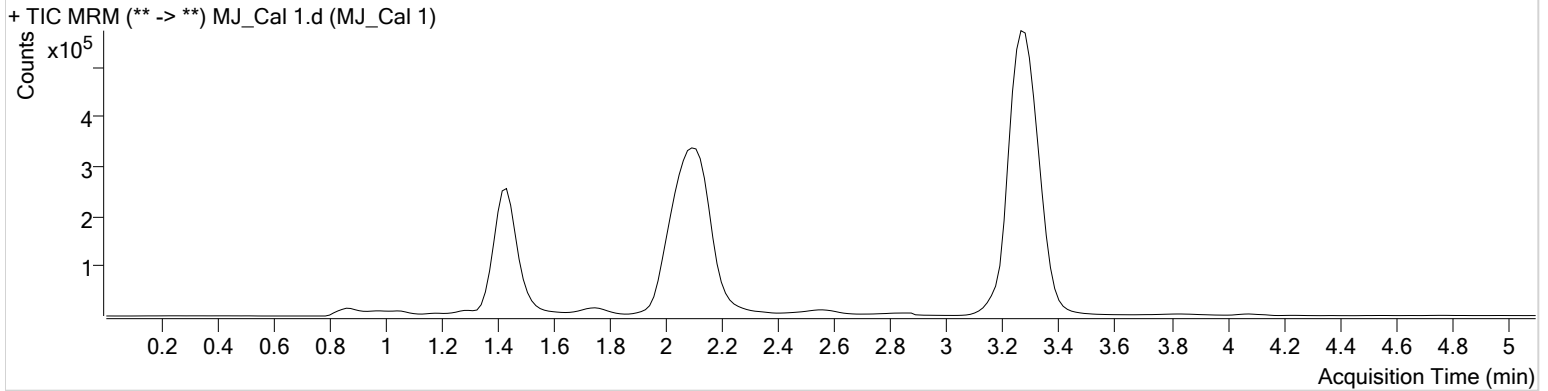


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

Instrument Falco **Data File** MJ_Cal 1.d
Type Cal **Sample** MJ_Cal 1
Acq. Method AM 27 THC quant.m **Operator** Celena Shrum
Sample Position P3-G3 **Comment**
Injection Volume 10
Acq. Date-Time 8/21/2020 4:01:12 PM
Sample Info.

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|-------|----------------|-------|------------|-------------------------|
| THC-COOH | 1.459 | 34926 | ∞ | 57.1 | ∞ | 322264 | 5.0542 ng/ml |
| THC-OH | 1.483 | 100758 | ∞ | 4.8 Low | 21.73 | 980823 | 1.6309 ng/ml Low |
| THC | 3.285 | 37600 | 95.36 | 32.8 | ∞ | 4322394 | 1.1393 ng/ml Low |

CS

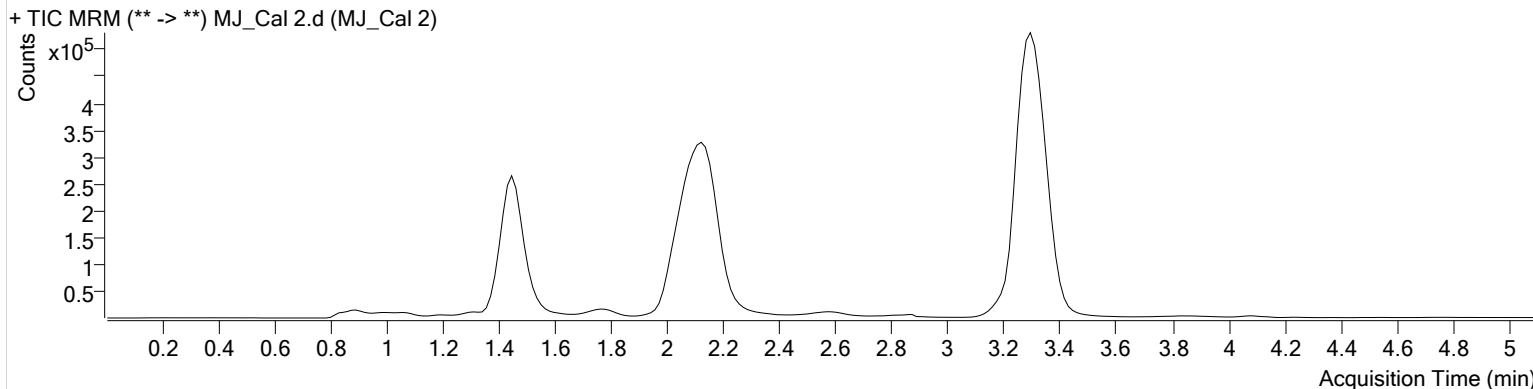


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

| | | | |
|-------------------------|----------------------|------------------|--------------|
| Instrument | Falco | Data File | MJ_Cal 2.d |
| Type | Cal | Sample | MJ_Cal 2 |
| Acq. Method | AM 27 THC quant.m | Operator | Celena Shrum |
| Sample Position | P3-H3 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/21/2020 4:08:56 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|--------|----------------|--------|------------|-------------------------|
| THC-COOH | 1.474 | 71661 | ∞ | 59.4 | ∞ | 309898 | 9.9977 ng/ml |
| THC-OH | 1.498 | 125213 | ∞ | 7.0 Low | 49.46 | 931149 | 3.3974 ng/ml |
| THC | 3.300 | 100472 | 500.53 | 31.0 | 241.52 | 3867061 | 2.9327 ng/ml Low |

AM #27 Cannabinoid Quant. Results

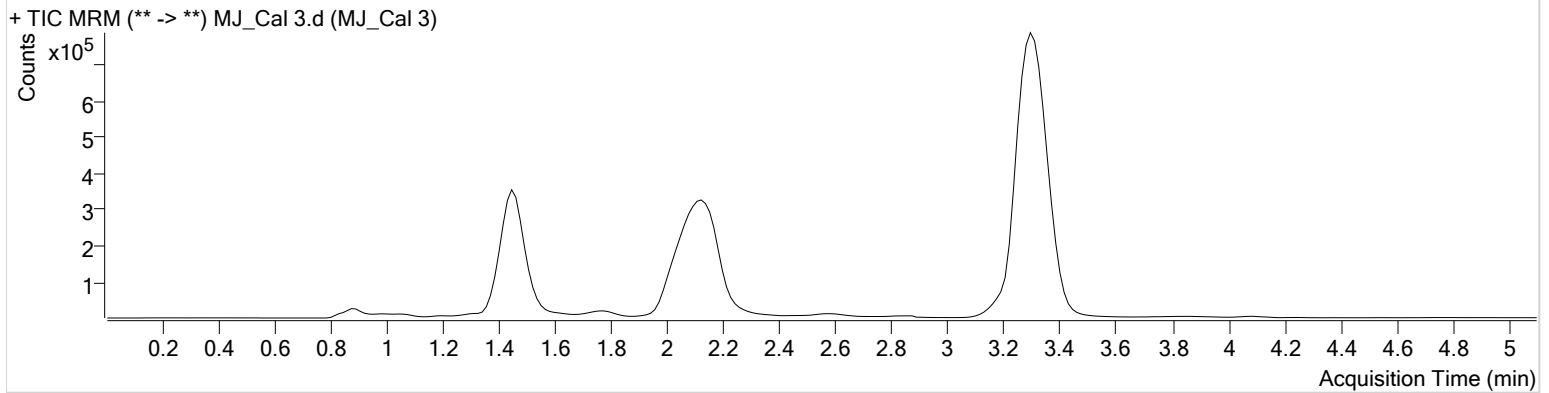


Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

| | | | |
|-------------------------|----------------------|------------------|--------------|
| Instrument | Falco | Data File | MJ_Cal 3.d |
| Type | Cal | Sample | MJ_Cal 3 |
| Acq. Method | AM 27 THC quant.m | Operator | Celena Shrum |
| Sample Position | P3-C1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/21/2020 4:16:31 PM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|--------|-------|--------|------------|---------------|
| THC-COOH | 1.474 | 175597 | ∞ | 56.9 | ∞ | 357725 | 20.4443 ng/ml |
| THC-OH | 1.468 | 208175 | ∞ | 8.9 | 100.58 | 1246039 | 5.2113 ng/ml |
| THC | 3.315 | 257772 | 653.16 | 28.5 | ∞ | 5900906 | 4.7697 ng/ml |

CS

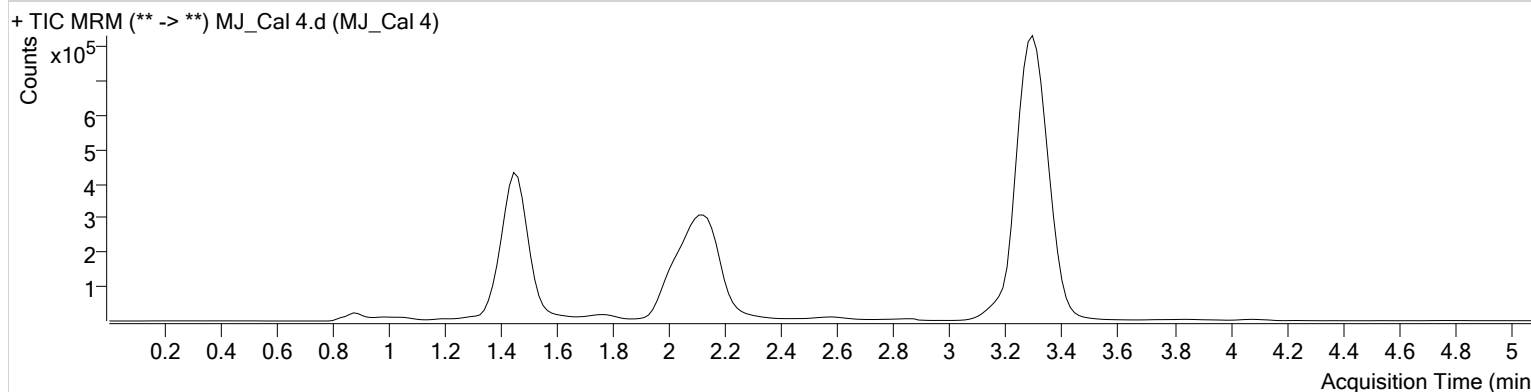


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

| | | | |
|-------------------------|----------------------|------------------|--------------|
| Instrument | Falco | Data File | MJ_Cal 4.d |
| Type | Cal | Sample | MJ_Cal 4 |
| Acq. Method | AM 27 THC quant.m | Operator | Celena Shrum |
| Sample Position | P3-D1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/21/2020 4:24:06 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|--------|-----|-------|--------|------------|---------------|
| THC-COOH | 1.474 | 440885 | ∞ | 61.0 | ∞ | 371920 | 48.3910 ng/ml |
| THC-OH | 1.453 | 321234 | ∞ | 10.0 | ∞ | 1297003 | 9.6968 ng/ml |
| THC | 3.300 | 535274 | ∞ | 25.1 | 261.56 | 6078723 | 9.3743 ng/ml |

CS

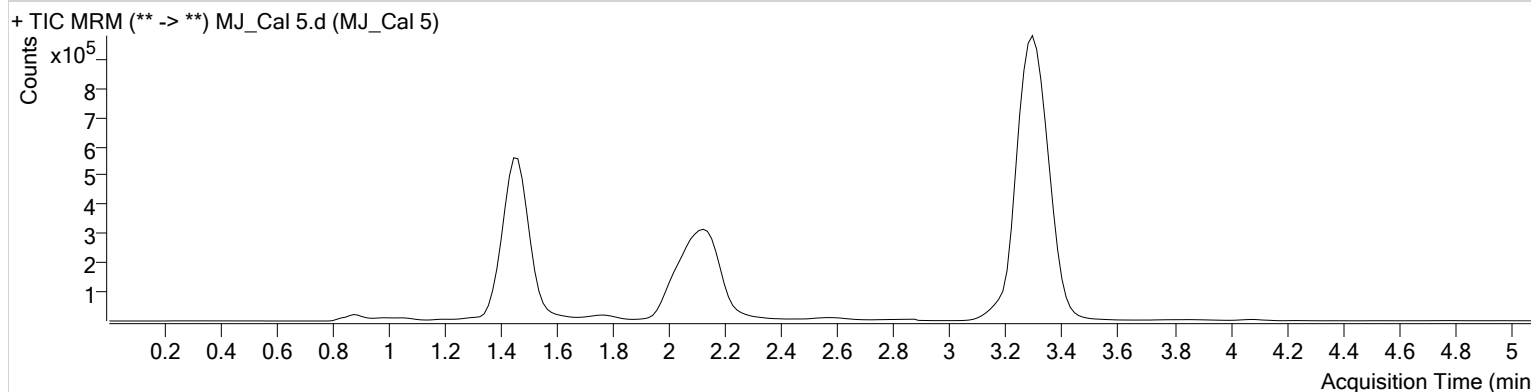


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

| | | | |
|-------------------------|----------------------|------------------|--------------|
| Instrument | Falco | Data File | MJ_Cal 5.d |
| Type | Cal | Sample | MJ_Cal 5 |
| Acq. Method | AM 27 THC quant.m | Operator | Celena Shrum |
| Sample Position | P3-E1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/21/2020 4:31:40 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|---------|---------|-------|---------|------------|---------------|
| THC-COOH | 1.474 | 667245 | ∞ | 61.3 | ∞ | 364906 | 74.2673 ng/ml |
| THC-OH | 1.453 | 676001 | ∞ | 11.7 | ∞ | 1306113 | 24.7155 ng/ml |
| THC | 3.300 | 1430255 | 3037.90 | 25.8 | 1929.89 | 6090614 | 24.6048 ng/ml |

AM #27 Cannabinoid Quant. Results

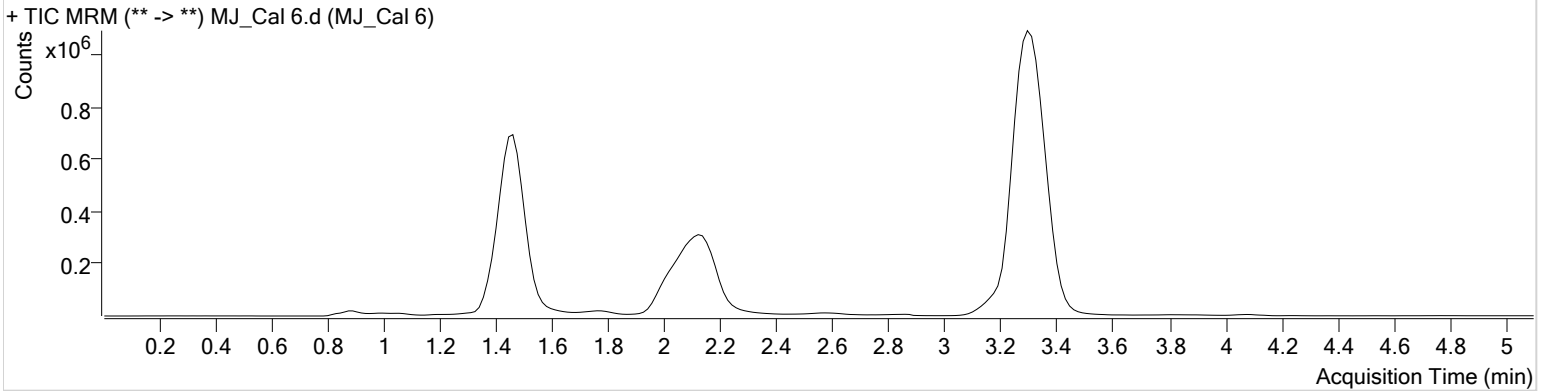


Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

| | | | |
|-------------------------|----------------------|------------------|--------------|
| Instrument | Falco | Data File | MJ_Cal 6.d |
| Type | Cal | Sample | MJ_Cal 6 |
| Acq. Method | AM 27 THC quant.m | Operator | Celena Shrum |
| Sample Position | P3-F1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/21/2020 4:39:14 PM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|---------|----------|-------|---------|------------|----------------|
| THC-COOH | 1.474 | 870235 | ∞ | 60.1 | ∞ | 351566 | 100.2911 ng/ml |
| THC-OH | 1.453 | 1238962 | ∞ | 11.8 | 756.67 | 1285014 | 49.5673 ng/ml |
| THC | 3.315 | 2711682 | 16785.04 | 25.8 | 1752.48 | 5702918 | 49.5782 ng/ml |

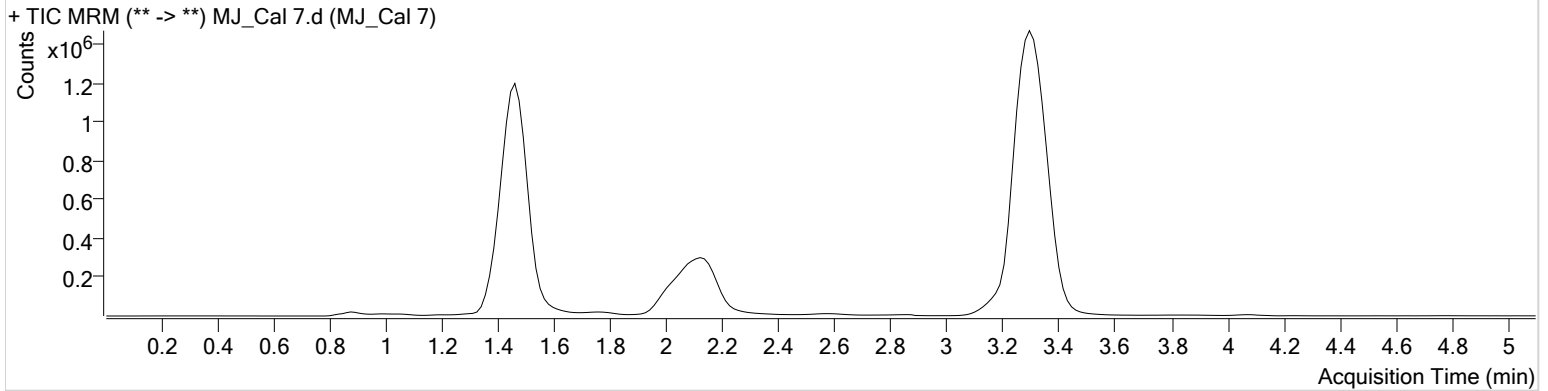
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\AM 27-28 082120 CS\QuantResults\THCQ.batch.bin
Calibration Last Update 8/26/2020 7:05:54 AM

| | | | |
|-------------------------|----------------------|------------------|--------------|
| Instrument | Falco | Data File | MJ_Cal 7.d |
| Type | Cal | Sample | MJ_Cal 7 |
| Acq. Method | AM 27 THC quant.m | Operator | Celena Shrum |
| Sample Position | P3-G1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 8/21/2020 4:46:49 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|----------|-------|---------|-----|-------|---------|------------|----------------|
| THC-COOH | 1.474 | 2056865 | ∞ | 61.3 | ∞ | 329908 | 251.5544 ng/ml |
| THC-OH | 1.453 | 2325730 | ∞ | 13.0 | 10496.3 | 1233811 | 100.8091 ng/ml |
| THC | 3.300 | 5375300 | ∞ | 26.2 | ∞ | 5502864 | 101.6010 ng/ml |