REVIEWED
By Anne Nord at 4:17 pm, Sep 14, 2020

9/14/2020 BW

**Worklist: 4512**

| LAB_CASE | ITEM | ITEM_TYPE | DESCRIPTION | |
|---|---|---|---|---|
| C2020-1650 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1691 | 1 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |
| C2020-1697 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1698 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1725 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1728 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1734 | 1 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |
| C2020-1747 | 2 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1750 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1774 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1783 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |  |
| C2020-1800 | 1 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |
| C2020-1801 | 1 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |

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

Extraction Date: 8/12/20 Analyst: Britany Wylie
Plate lot#: 200723 Plate Expiration: 1-23-2021
**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**: 20G20792 **Urine Blank:** 73020 **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 250ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
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.
☒ 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 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 and OH-THC 3ng/mL (quantitative blood), 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? (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: Original injection started 9-11-20, due to inconsistent pressure curves and retention time shifting, run was stopped. analytical plate placed in freezer until 9/12/20 when troubleshooting in the lab could be performed. The guard column was clogged and removed, all samples including cals & controls were reinjected. Only data from reinjection was evaluated.
BW 9-14-20

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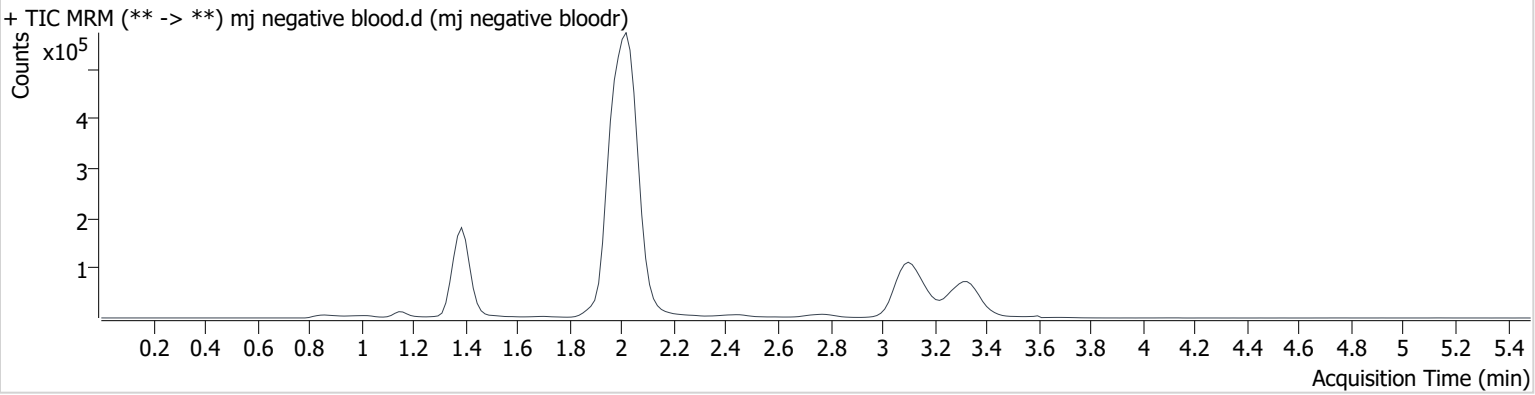
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj negative blood.d |
| **Type** | Sample | **Sample** | mj negative bloodr |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-A2 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 4:37:58 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**

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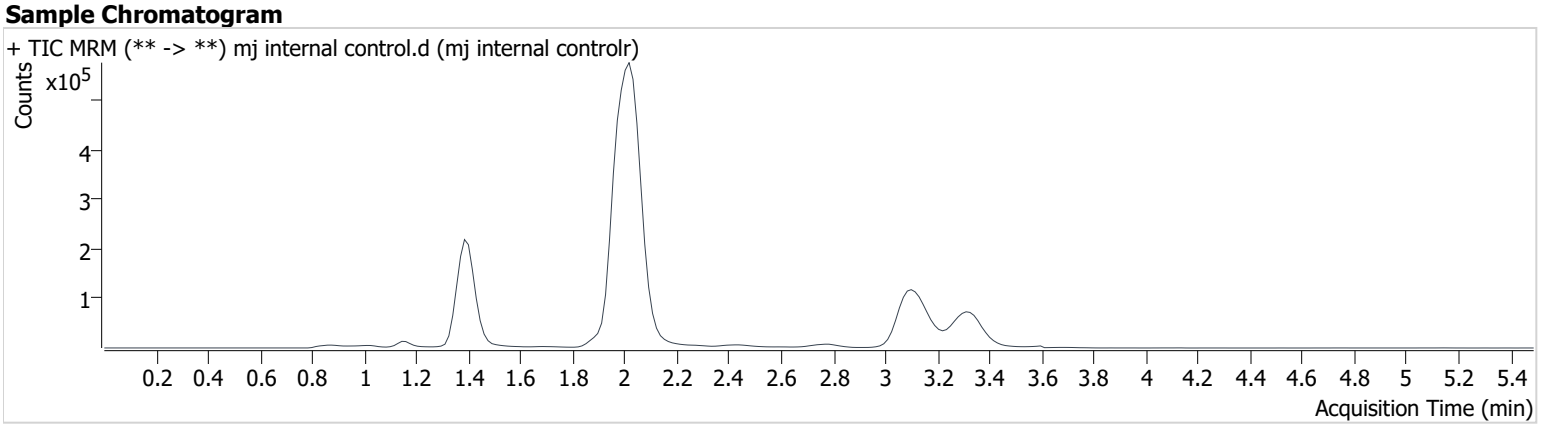
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj internal control.d |
| **Type** | QC | **Sample** | mj internal controlr |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-H1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 4:30:16 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.391 | 62268 | ∞ | 14.8 | ∞ | 566676 | 4.164 | ng/ml |
| THC-COOH | 1.416 | 55500 | 151073.9 | 34.6 | 25854.1 | 273158 | 15.083 | ng/ml |
| THC | 3.152 | 38114 | ∞ | 26.7 | ∞ | 352261 | 4.311 | ng/ml |

THC-

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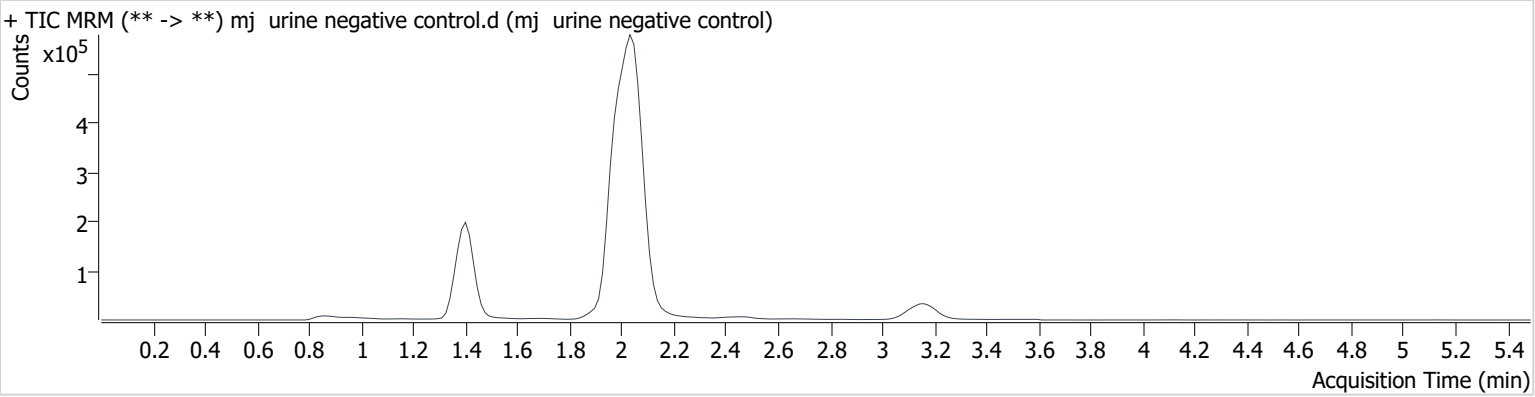
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj urine negative control.d |
| **Type** | Sample | **Sample** | mj urine negative control |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-C3 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 7:19:18 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



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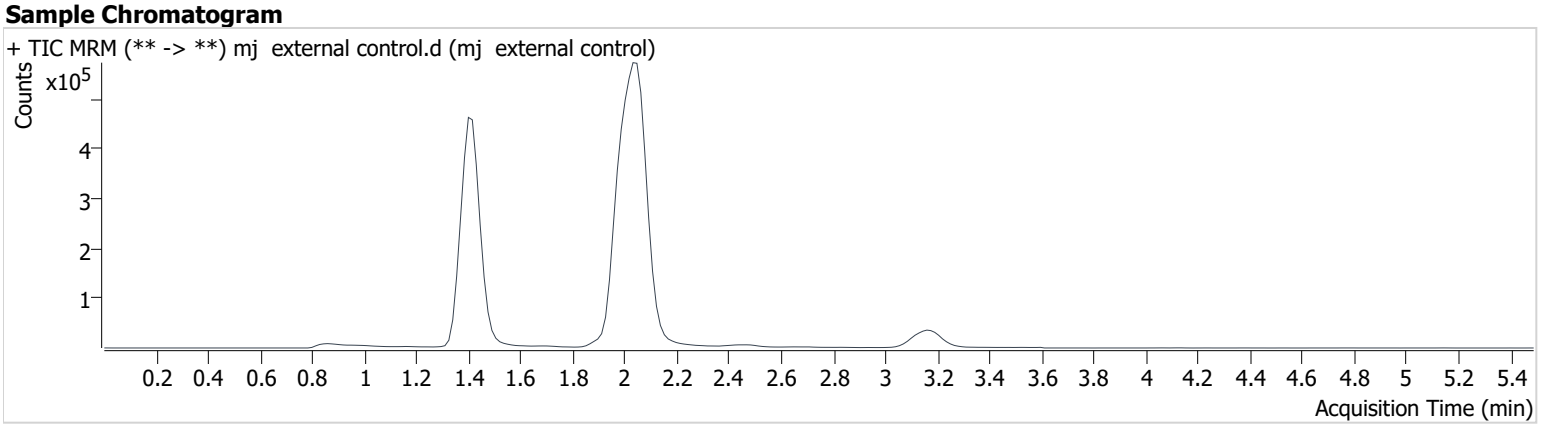
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj external control.d |
| **Type** | Sample | **Sample** | mj external control |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-D3 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 7:34:40 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.406 | 844956 | ∞ | 12.5 | ∞ | 719725 | 40.902 | ng/ml |
| THC-COOH | 1.431 | 173545 | 681.4 | 31.4 | 370.1 | 296124 | 41.060 | ng/ml |
| THC | 3.182 | 57809 | ∞ | 25.1 | ∞ | 200319 | 11.068 | ng/ml |

# Toxicology AM method 27/26 external prep information

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working solution 1 ug/ml in meoh C-THC, THC-OH, THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 150 ul THC-OH in 9692.5 ul meOH
Ppd 8/26/20 Exp: 7/1/21 lot 82620 by AMN

| Drug | lot | expiration |
|---|---|---|
| C-THC | FE01061702 | 3/1/2022 |
| THC-OH | FE07221601 | 7/1/2021 |
| THC | FE01041701 | 3/1/2022 |

**AM 27/26 blood control 100 ul working solution lot () in 9900 ul blood lot ()**

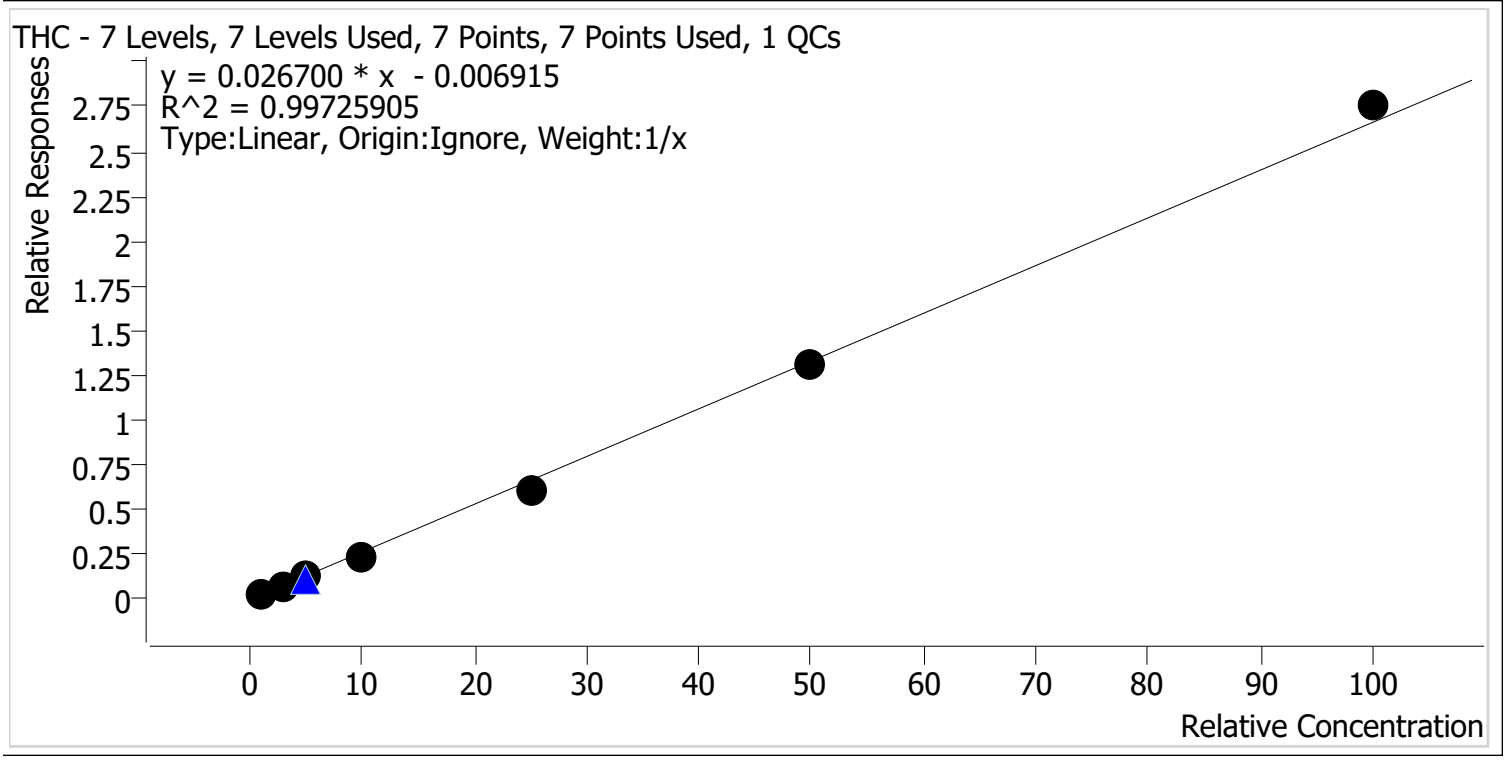
| | | | |
|---|---|---|---|
| | | Concentration 7.5 ng/ml THC,<br>15 ng/ml C-THC, THC-OH | |

**AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine lot (73020)** **out of use**

| | | | | |
|---|---|---|---|---|
| ppd 8/26/20 Exp 7/1/21 | lot u82620 | Concentration 30 ng/ml THC, and<br>60 ng/ml C-THC, THC-OH | by amn | |
| | | | | |
| | | | | |
| | | | | |

# Compound Calibration Report

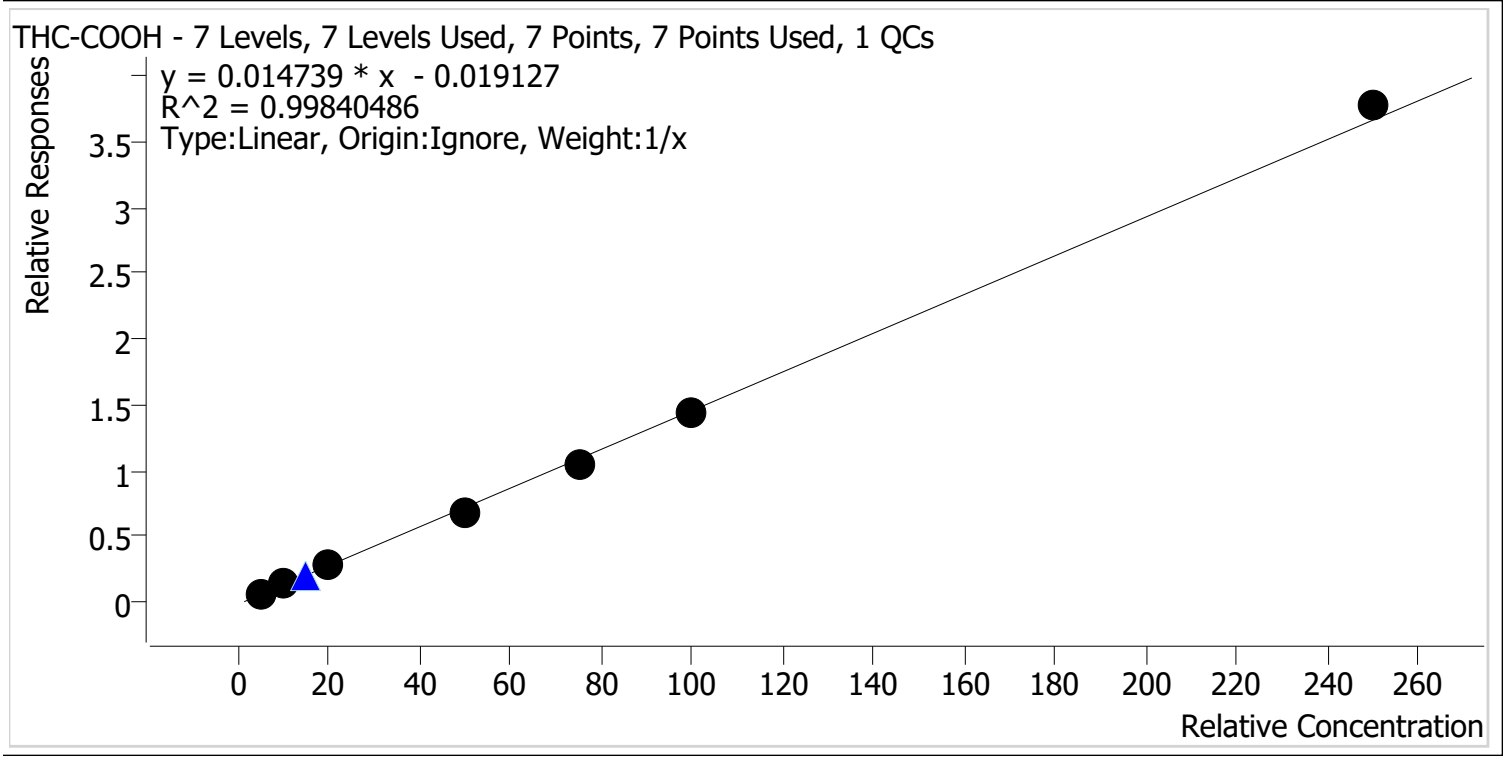
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin |
| **Last Cal. Update** | 9/12/2020 9:44 PM |
| **Analyst Name** | ISP\datastor |
| **Analyte** | THC |
| **Internal Standard** | THC-d3 |




| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|---|---|---|---|---|---|
| mj qc1r | 1 | ✔ | 1.0 | 1.3 | 125.5 |
| mj cal2r | 2 | ✔ | 3.0 | 2.9 | 95.5 |
| mj cal 3r | 3 | ✔ | 5.0 | 4.7 | 94.1 |
| mj cal 4r | 4 | ✔ | 10.0 | 8.9 | 89.5 |
| mj cal 5r | 5 | ✔ | 25.0 | 23.2 | 92.8 |
| mj cal 6r | 6 | ✔ | 50.0 | 49.6 | 99.1 |
| mj cal 7 | 7 | ✔ | 100.0 | 103.5 | 103.5 |

# Compound Calibration Report

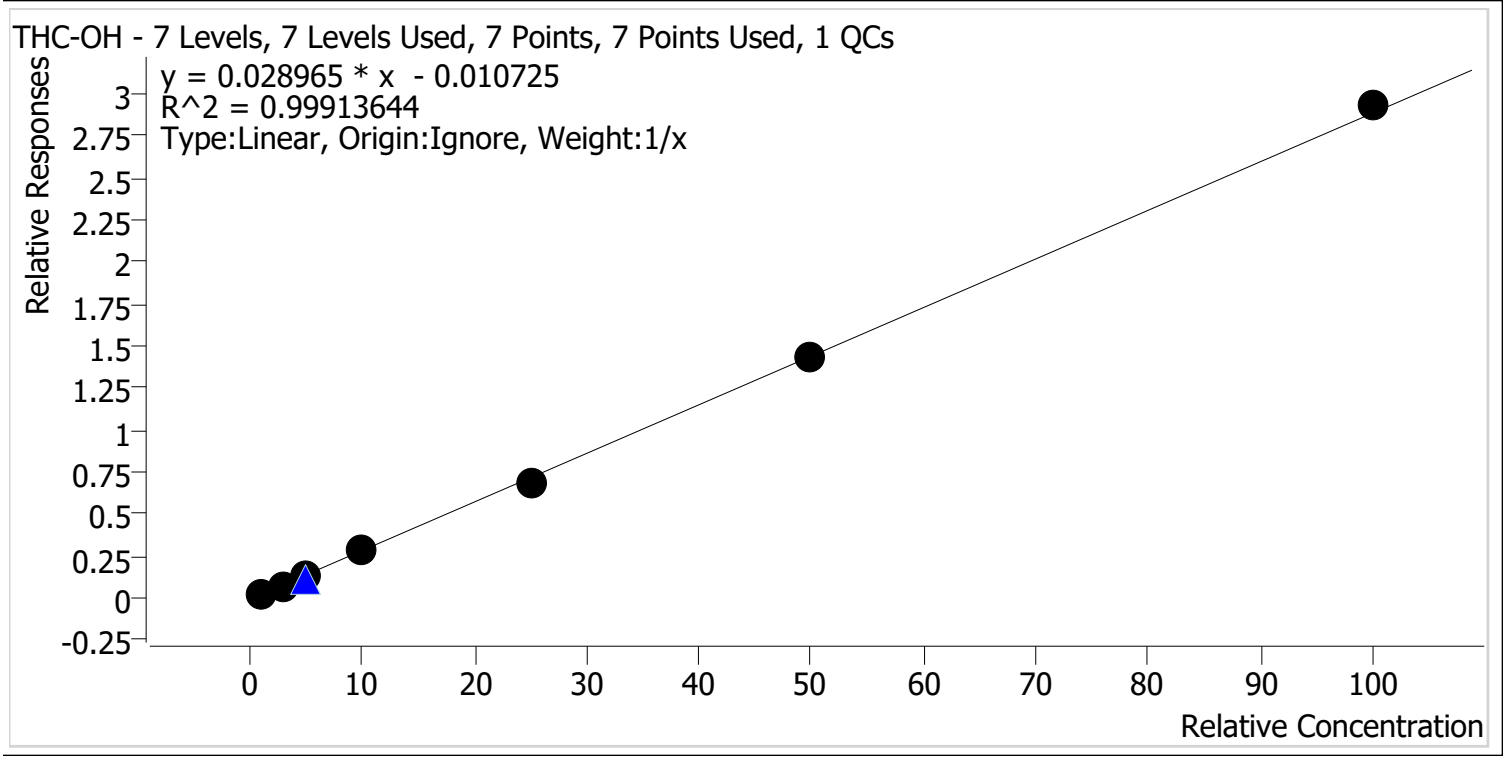
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin |
| **Last Cal. Update** | 9/12/2020 9:44 PM |
| **Analyst Name** | ISP\datastor |
| **Analyte** | THC-COOH |
| **Internal Standard** | THC-COOH-d9 |




| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|---|---|---|---|---|---|
| mj qc1r | 1 | ✔ | 5.0 | 5.4 | 108.2 |
| mj cal2r | 2 | ✔ | 10.0 | 10.1 | 101.2 |
| mj cal 3r | 3 | ✔ | 20.0 | 19.9 | 99.6 |
| mj cal 4r | 4 | ✔ | 50.0 | 46.9 | 93.8 |
| mj cal 5r | 5 | ✔ | 75.0 | 71.7 | 95.6 |
| mj cal 6r | 6 | ✔ | 100.0 | 98.5 | 98.5 |
| mj cal 7 | 7 | ✔ | 250.0 | 257.5 | 103.0 |

# Compound Calibration Report

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin |
| **Last Cal. Update** | 9/12/2020 9:44 PM |
| **Analyst Name** | ISP\datastor |
| **Analyte** | THC-OH |
| **Internal Standard** | THC-OH-d3 |




| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|---|---|---|---|---|---|
| mj qc1r | 1 | ✔ | 1.0 | 1.1 | 113.2 |
| mj cal2r | 2 | ✔ | 3.0 | 2.7 | 90.1 |
| mj cal 3r | 3 | ✔ | 5.0 | 4.9 | 98.8 |
| mj cal 4r | 4 | ✔ | 10.0 | 10.2 | 102.0 |
| mj cal 5r | 5 | ✔ | 25.0 | 23.7 | 94.8 |
| mj cal 6r | 6 | ✔ | 50.0 | 49.8 | 99.7 |
| mj cal 7 | 7 | ✔ | 100.0 | 101.5 | 101.5 |

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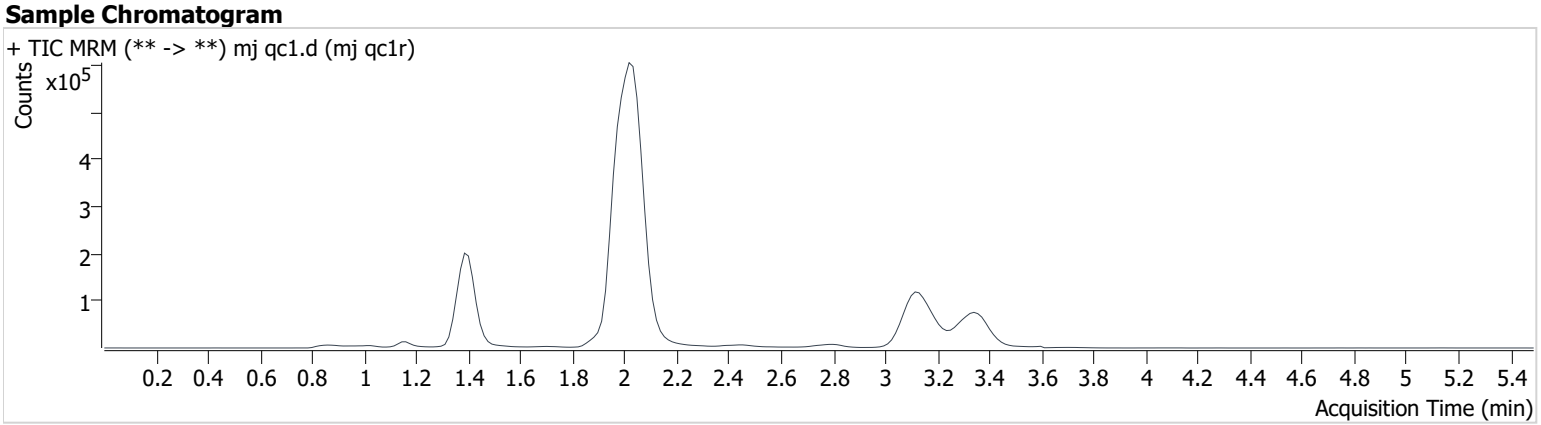
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj qc1.d |
| **Type** | Cal | **Sample** | mj qc1r |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-A1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 3:36:15 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.391 | 13853 | ∞ | 14.9 | ∞ | 628082 | 1.132 | ng/ml Low |
| THC-COOH | 1.416 | 18033 | 74.2 | 36.3 | 30.5 | 297372 | 5.412 | ng/ml |
| THC | 3.197 | 10326 | ∞ | 26.8 | ∞ | 388125 | 1.255 | ng/ml Low |

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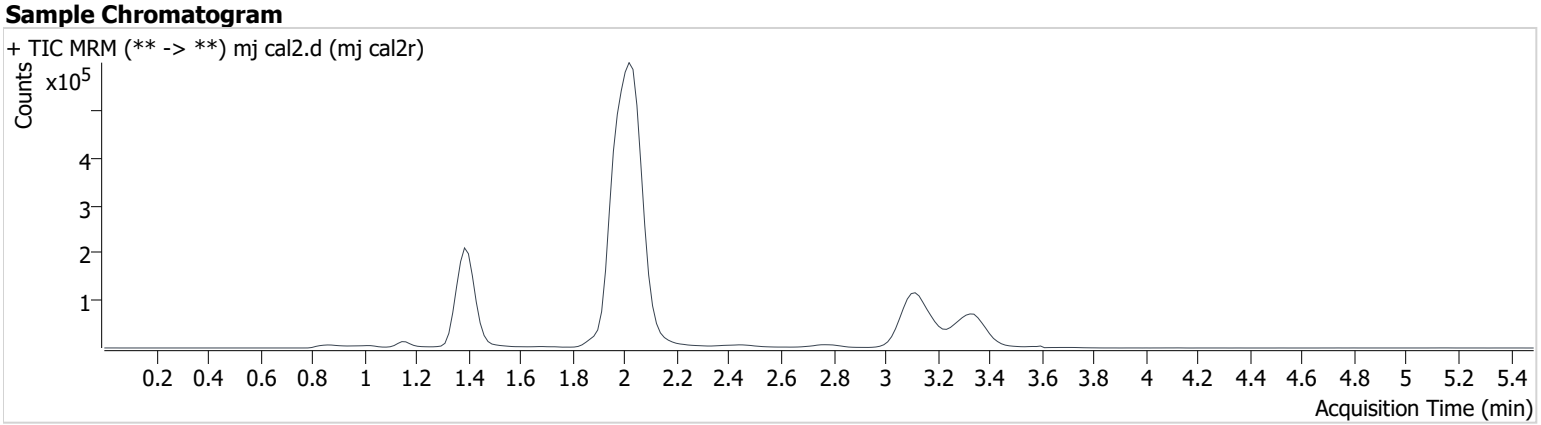
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal2.d |
| **Type** | Cal | **Sample** | mj cal2r |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-B1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 3:44:00 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.391 | 41159 | ∞ | 14.5 | ∞ | 609311 | 2.702 | ng/ml Low |
| THC-COOH | 1.416 | 38730 | 49845.2 | 32.4 | 17628.9 | 297701 | 10.124 | ng/ml |
| THC | 3.182 | 25780 | ∞ | 28.1 | ∞ | 370503 | 2.865 | ng/ml Low |

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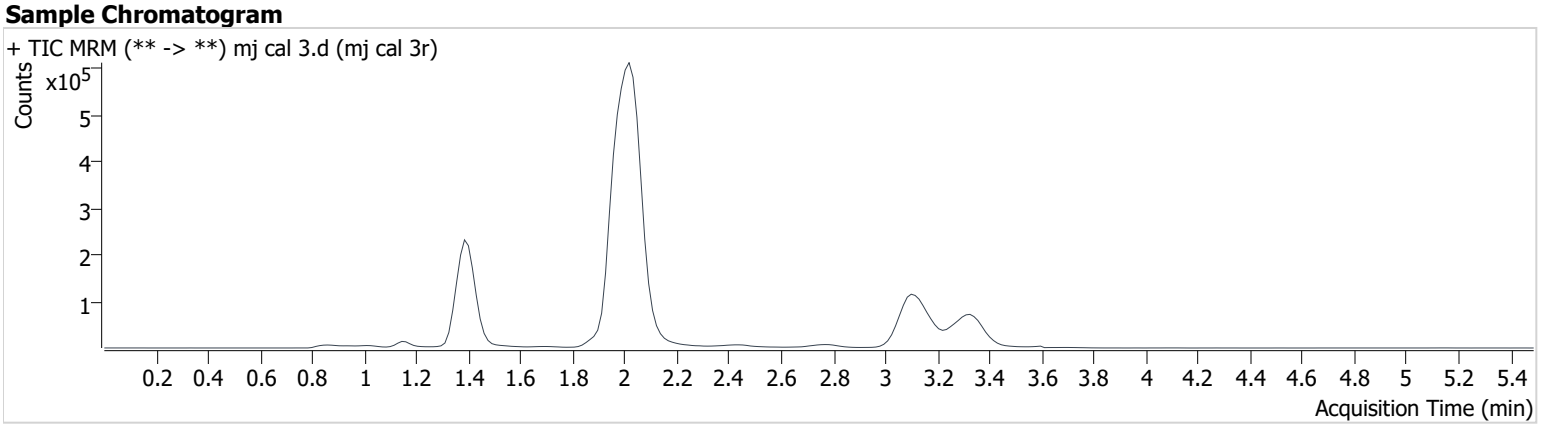
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 3.d |
| **Type** | Cal | **Sample** | mj cal 3r |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-C1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 3:51:43 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.391 | 78431 | ∞ | 13.6 | ∞ | 592735 | 4.939 | ng/ml |
| THC-COOH | 1.416 | 79219 | 495.7 | 32.8 | 55649.5 | 288467 | 19.930 | ng/ml |
| THC | 3.182 | 42675 | ∞ | 27.6 | ∞ | 359474 | 4.705 | ng/ml |

THC-OH not evaluated in urine samples

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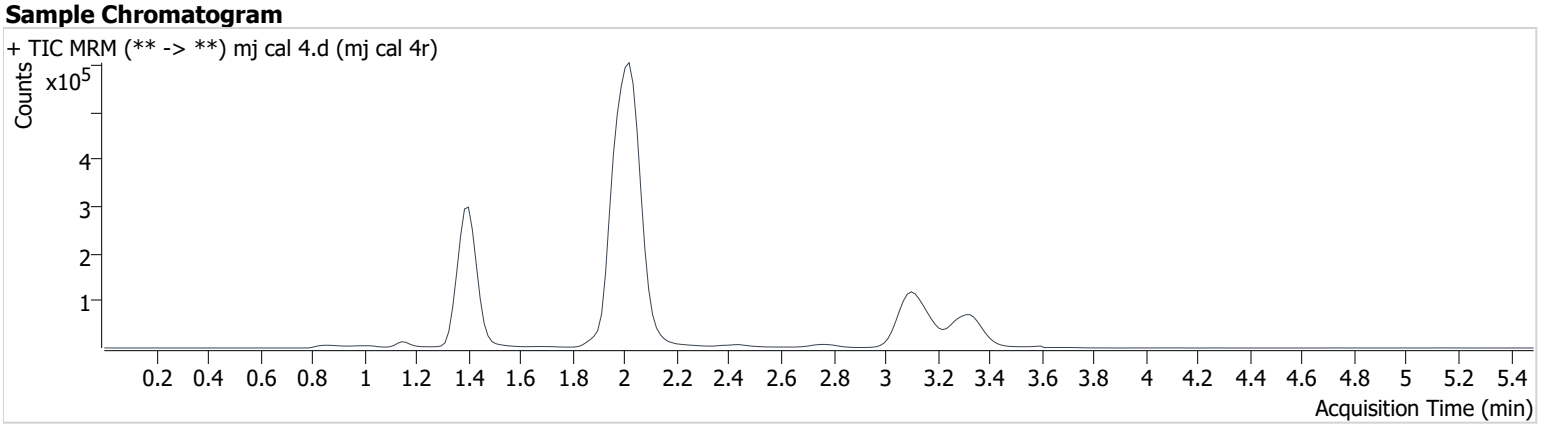
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 4.d |
| **Type** | Cal | **Sample** | mj cal 4r |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-D1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 3:59:27 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.391 | 164322 | ∞ | 11.5 | ∞ | 576947 | 10.203 | ng/ml |
| THC-COOH | 1.416 | 191106 | 946.3 | 33.8 | 630.4 | 284252 | 46.912 | ng/ml |
| THC | 3.167 | 81827 | ∞ | 25.8 | ∞ | 352737 | 8.947 | ng/ml |

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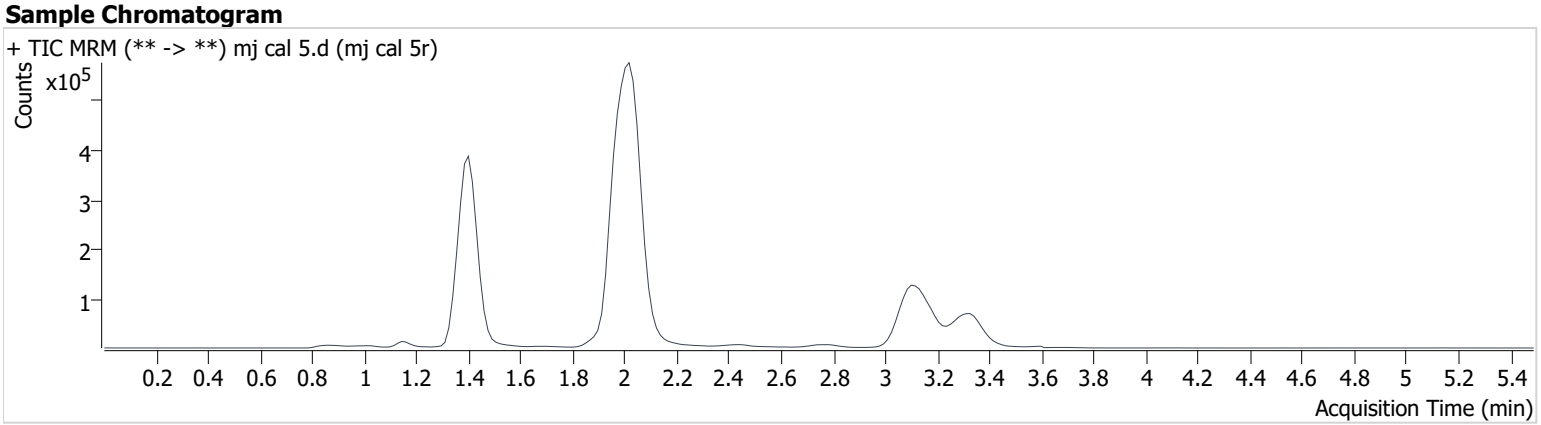
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 5.d |
| **Type** | Cal | **Sample** | mj cal 5r |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-E1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 4:07:10 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.391 | 368604 | ∞ | 12.7 | ∞ | 545538 | 23.697 | ng/ml |
| THC-COOH | 1.416 | 281883 | 539346.6 | 34.5 | 269.7 | 271623 | 71.707 | ng/ml |
| THC | 3.152 | 205762 | ∞ | 24.8 | ∞ | 335969 | 23.197 | ng/ml |

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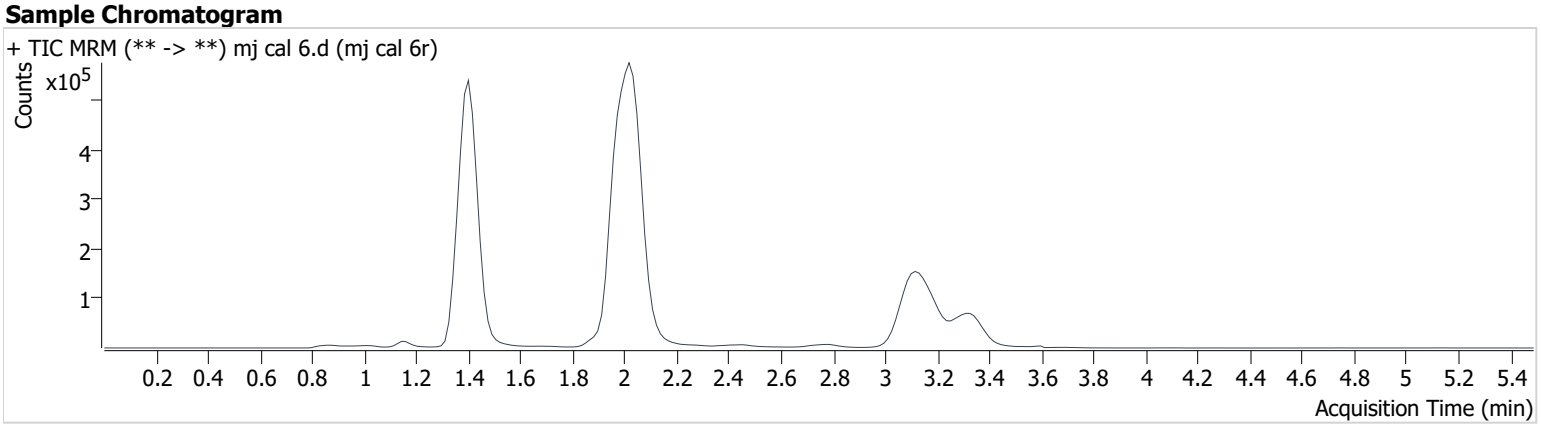
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal 6.d |
| **Type** | Cal | **Sample** | mj cal 6r |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-F1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 4:14:52 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.391 | 828244 | ∞ | 12.2 | ∞ | 578189 | 49.826 | ng/ml |
| THC-COOH | 1.416 | 392599 | 2346.2 | 33.6 | 6728.5 | 274164 | 98.453 | ng/ml |
| THC | 3.152 | 435061 | ∞ | 24.3 | ∞ | 330539 | 49.556 | ng/ml |

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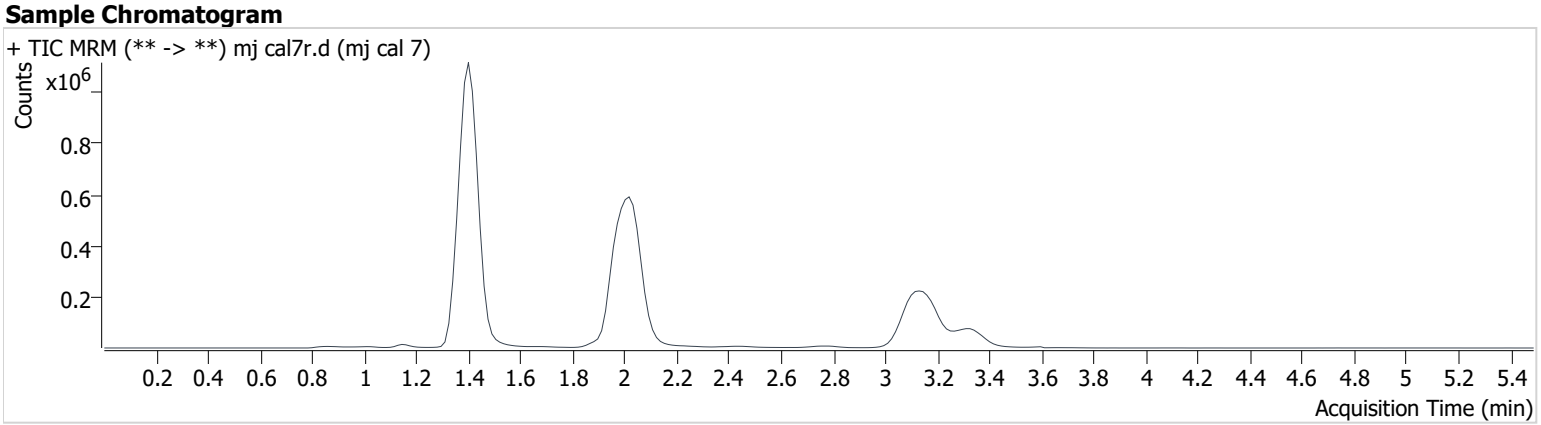
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 9-11-2020 reinject\QuantResults\thcq.batch.bin
**Calibration Last Update** 9/12/2020 9:44:18 PM

| | | | |
|---|---|---|---|
| **Instrument** | 69679 | **Data File** | mj cal7r.d |
| **Type** | Cal | **Sample** | mj cal 7 |
| **Acq. Method** | AM 27 THC quant.m | **Operator** | Britany Wylie |
| **Sample Position** | P3-G1 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 9/12/2020 4:45:40 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. | |
|---|---|---|---|---|---|---|---|---|
| THC-OH | 1.391 | 1855396 | ∞ | 12.5 | ∞ | 633397 | 101.501 | ng/ml |
| THC-COOH | 1.416 | 1044338 | 1021.4 | 34.5 | 1711.1 | 276597 | 257.463 | ng/ml |
| THC | 3.167 | 936682 | ∞ | 24.2 | ∞ | 339895 | 103.474 | ng/ml |

9/11/20 THC Q

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| a | cal 1 ng ✓ | NEG ✓ | 1774 ✓ | | | QC 1 |
| b | cal 3 ng ✓ | 1650 ✓ | 9-11-20 1758-1 ✓ BW | | | cal 100 ng |
| c | cal 5 ng ✓ | 1697 ✓ | neg urine ✓ | | | cal 50 ng |
| d | cal 10ng ✓ | 1698 ✓ | u-ext, B | | | cal 25 ng |
| e | cal 25 ng ✓ | 1725 ✓ | 1691, C | | | cal 10ng |
| f | cal 50 ng ✓ | 1728 ✓ | 1734, D | | | cal 5 ng |
| g | cal 100 ng ✓ | 1747-2 ✓ | 1800, E | | | cal 3 ng |
| h | QC 1 ✓ | 1750 ✓ | 1801, F | | NEG | cal 1ng |

Case # Prefix:
C2020-

BW