

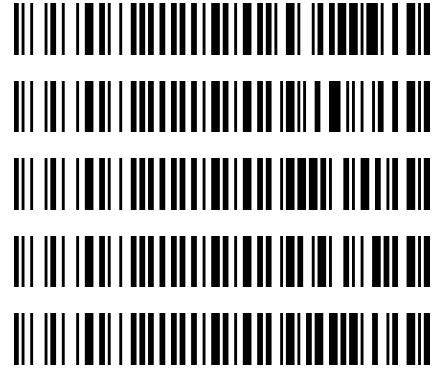


**Worklist: 3746**

**REVIEWED**

*By Sarah Pickle at 9:38 am, Oct 10, 2019*

| <u>LAB CASE</u> | <u>ITEM</u> | <u>ITEM TYPE</u> | <u>DESCRIPTION</u>              |
|-----------------|-------------|------------------|---------------------------------|
| C2019-1587      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ |
| C2019-1801      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ |
| C2019-1893      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ |
| C2019-1906      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ |
| C2019-1935      | 1           | 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: 10/08/19  
Plate lot#: 190716

Analyst: Anne Nord  
Plate Expiration: 1-16-2020

**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:** 19H52275-1 **Urine Blank:** 8919  
**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: 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 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  $\geq 0.98$  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: *Blood samples only in this run.*

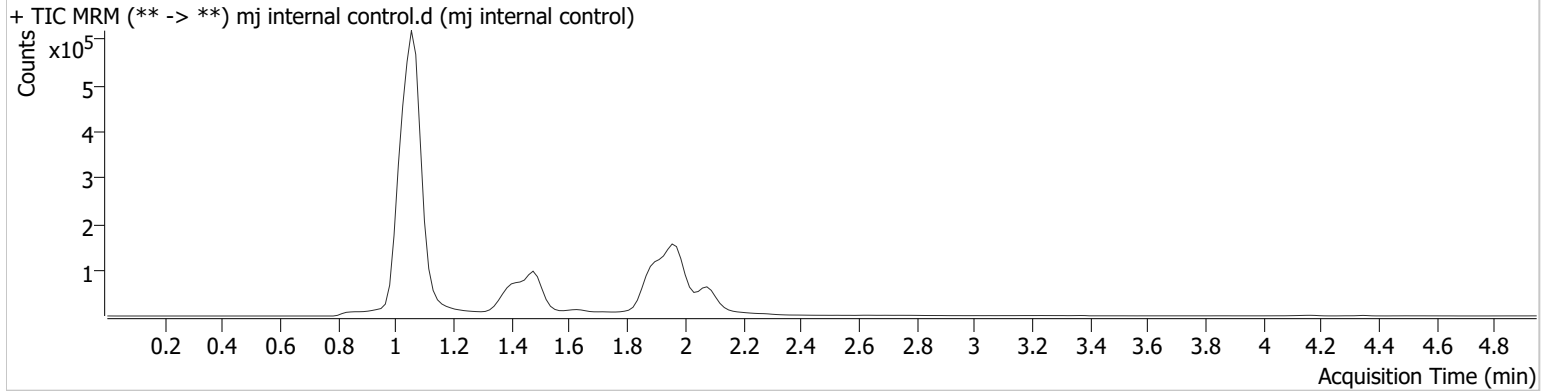
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 10/9/2019 8:45:08 AM

|                         |                      |                  |                       |
|-------------------------|----------------------|------------------|-----------------------|
| <b>Instrument</b>       | 69679                | <b>Data File</b> | mj internal control.d |
| <b>Type</b>             | QC                   | <b>Sample</b>    | mj internal control   |
| <b>Acq. Method</b>      | AM 27 THC quant.m    | <b>Operator</b>  | Anne Nord             |
| <b>Sample Position</b>  | P3-H1                | <b>Comment</b>   |                       |
| <b>Injection Volume</b> | 10                   |                  |                       |
| <b>Acq. Date-Time</b>   | 10/8/2019 3:59:42 PM |                  |                       |

**Sample Info.**

## Sample Chromatogram



| Name     | RT    | Resp.  | S/N   | Ratio | S/N    | ISTD Resp. | Final Conc.  |
|----------|-------|--------|-------|-------|--------|------------|--------------|
| THC-OH   | 1.059 | 139348 | ∞     | 10.1  | 133.5  | 1886613    | 5.042 ng/ml  |
| THC-COOH | 1.070 | 133141 | 576.1 | 37.5  | 3041.7 | 789982     | 13.892 ng/ml |
| THC      | 1.965 | 25641  | ∞     | 25.4  | 188.1  | 860581     | 4.469 ng/ml  |

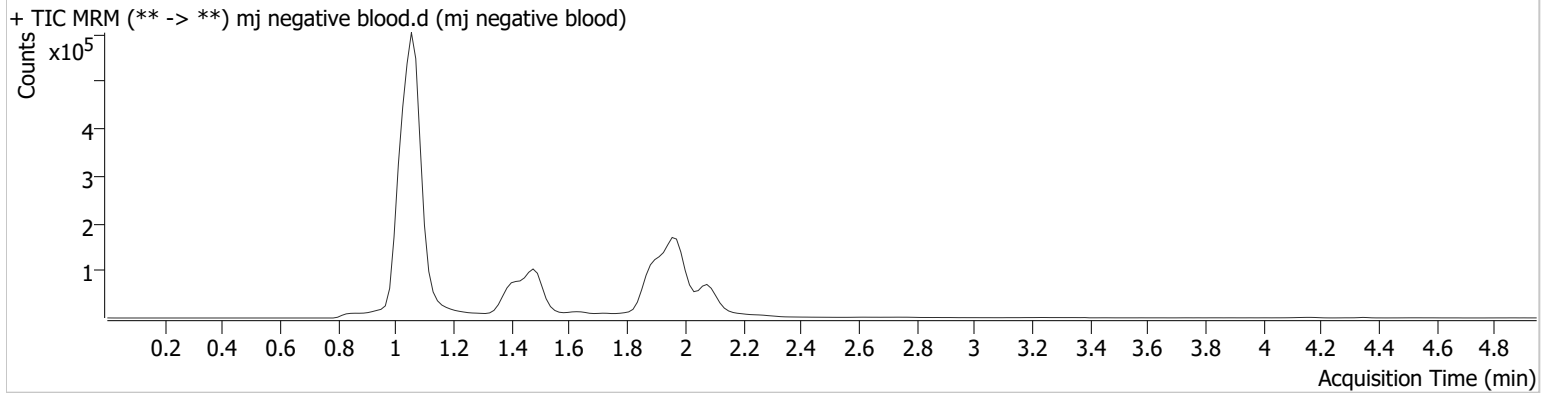
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 10/9/2019 8:45:08 AM

|                         |                      |                  |                     |
|-------------------------|----------------------|------------------|---------------------|
| <b>Instrument</b>       | 69679                | <b>Data File</b> | mj negative blood.d |
| <b>Type</b>             | Sample               | <b>Sample</b>    | mj negative blood   |
| <b>Acq. Method</b>      | AM 27 THC quant.m    | <b>Operator</b>  | Anne Nord           |
| <b>Sample Position</b>  | P3-A2                | <b>Comment</b>   |                     |
| <b>Injection Volume</b> | 10                   |                  |                     |
| <b>Acq. Date-Time</b>   | 10/8/2019 4:07:24 PM |                  |                     |

**Sample Info.**

## Sample Chromatogram



| Name   | RT    | Resp. | S/N | Ratio   | S/N | ISTD Resp. | Final Conc.     |
|--------|-------|-------|-----|---------|-----|------------|-----------------|
| THC-OH | 0.957 | 54302 | ∞   | 2.0 Low | ∞   | 2171058    | 1.544 ng/ml Low |

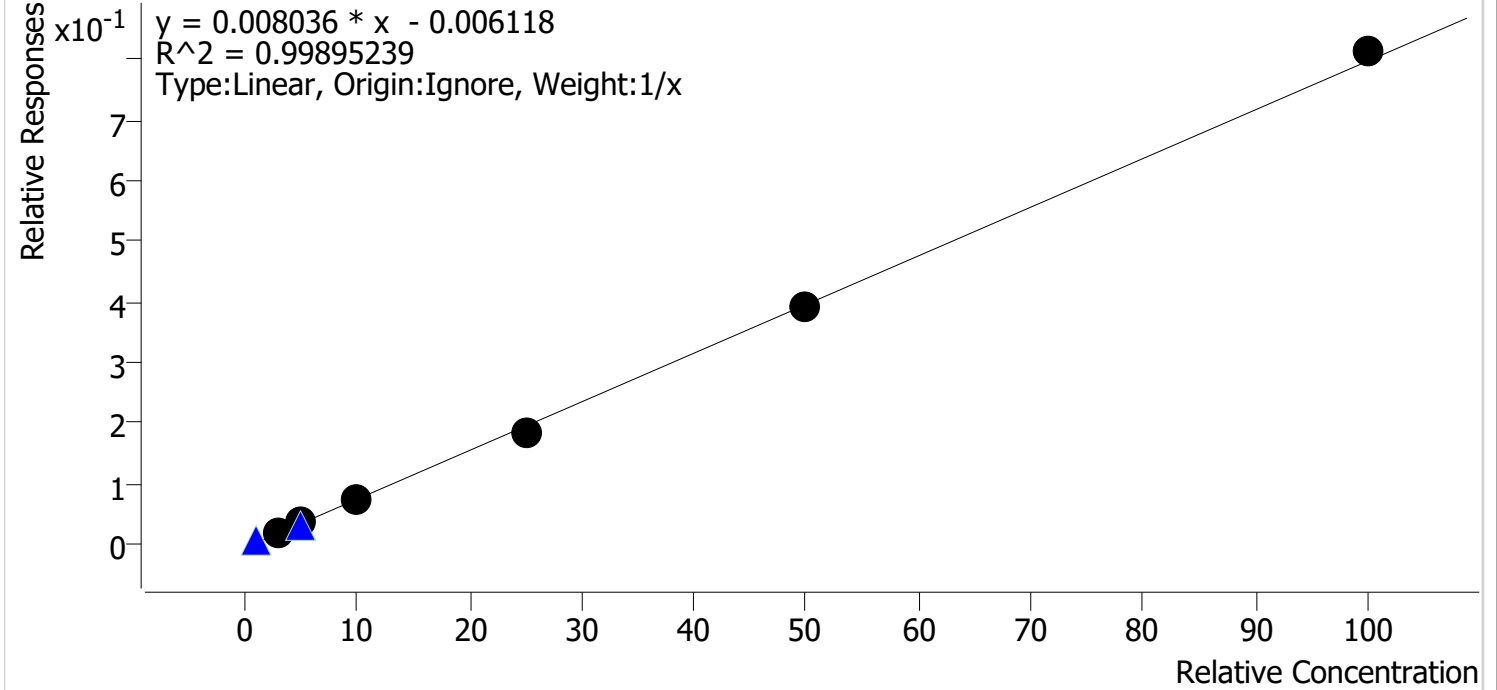
# Compound Calibration Report



**Batch results**      D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Last Cal. Update**    10/9/2019 8:45 AM  
**Analyst Name**        ISP\datastor  
**Analyte**                THC

**Internal Standard**      THC-d3

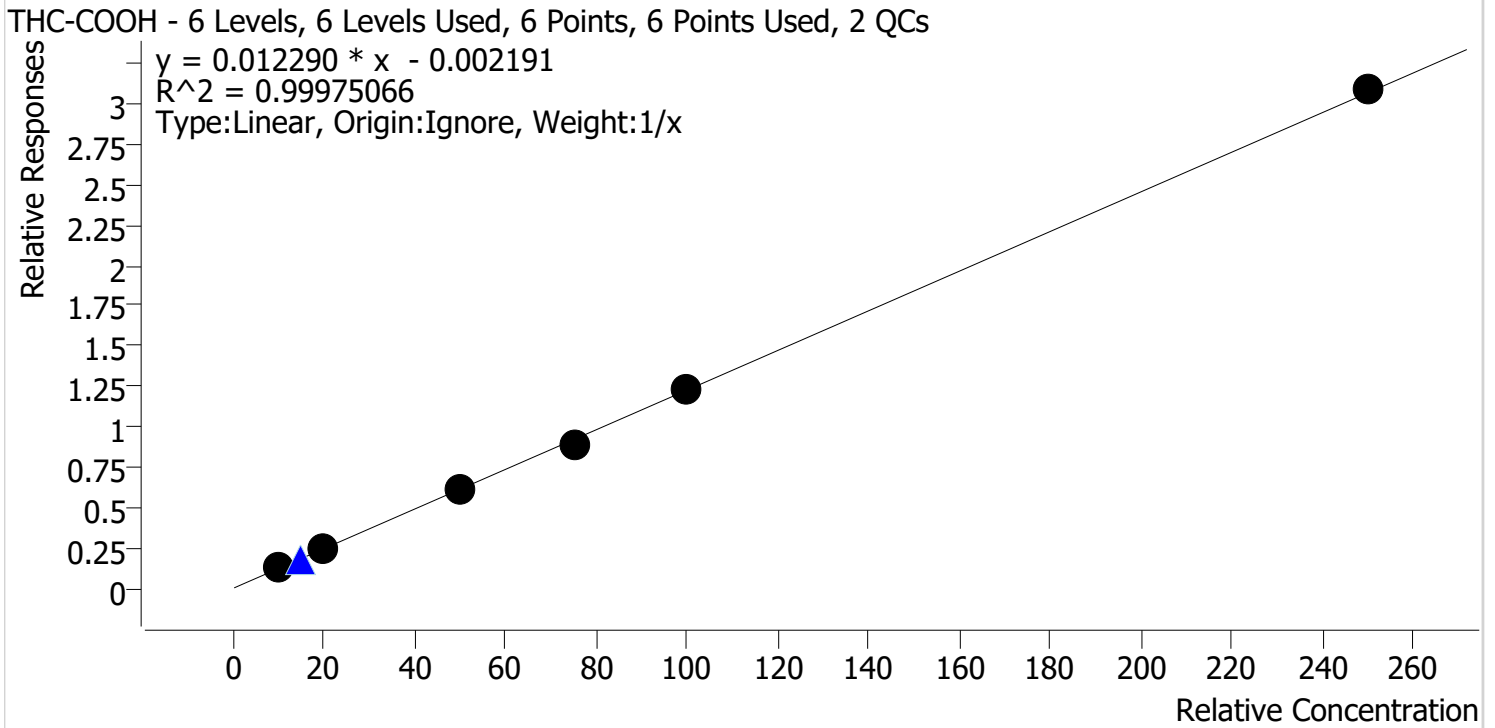
THC - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 2 QCs



| Sample   | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal2  | 2     | ✓       | 3.0                    | 3.2                 | 107.3    |
| mj cal 3 | 3     | ✓       | 5.0                    | 5.0                 | 101.0    |
| mj cal 4 | 4     | ✓       | 10.0                   | 9.6                 | 96.1     |
| mj cal 5 | 5     | ✓       | 25.0                   | 23.6                | 94.5     |
| mj cal 6 | 6     | ✓       | 50.0                   | 49.6                | 99.2     |
| mj cal 7 | 7     | ✓       | 100.0                  | 101.9               | 101.9    |

# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Last Cal. Update** 10/9/2019 8:45 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-d9

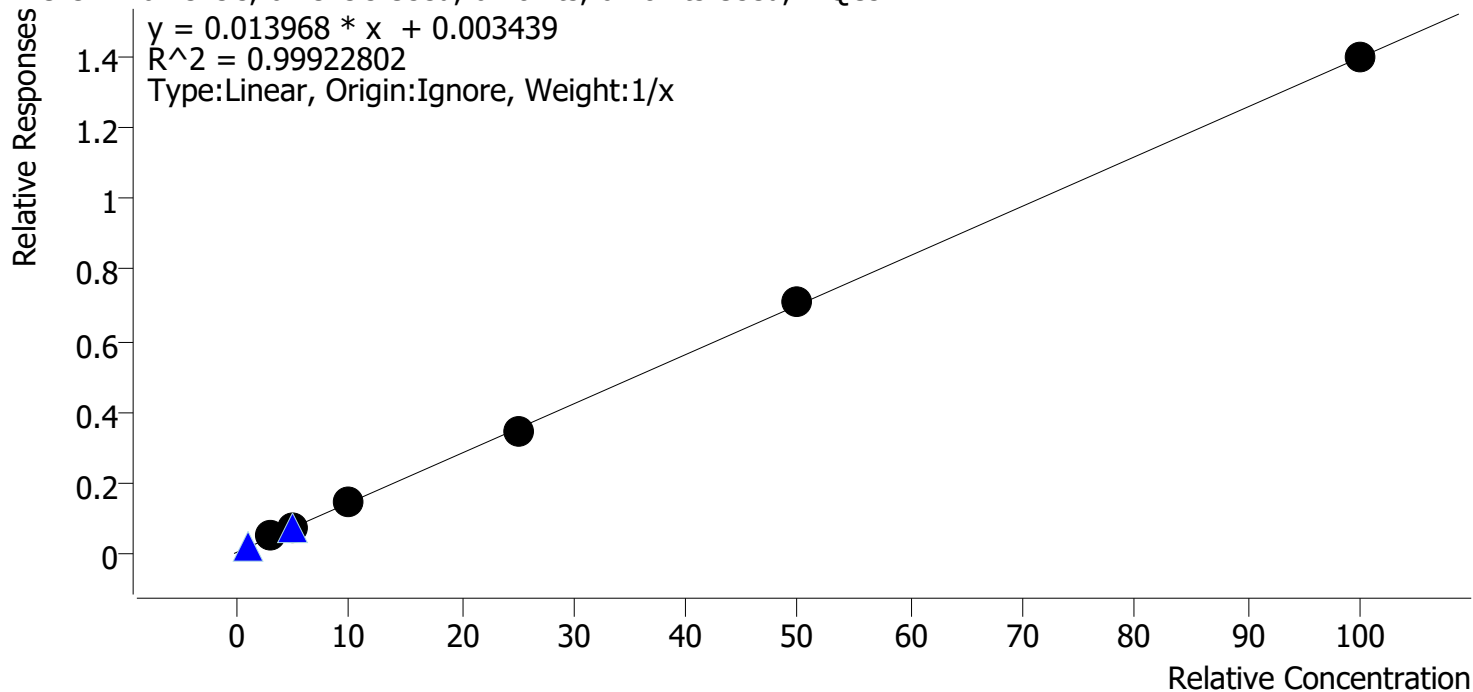


| Sample   | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal2  | 2     | ✓       | 10.0                   | 10.2                | 101.5    |
| mj cal 3 | 3     | ✓       | 20.0                   | 20.2                | 100.9    |
| mj cal 4 | 4     | ✓       | 50.0                   | 49.5                | 99.0     |
| mj cal 5 | 5     | ✓       | 75.0                   | 72.9                | 97.2     |
| mj cal 6 | 6     | ✓       | 100.0                  | 100.7               | 100.7    |
| mj cal 7 | 7     | ✓       | 250.0                  | 251.5               | 100.6    |

# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Last Cal. Update** 10/9/2019 8:45 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-d3

THC-OH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 2 QCs



| Sample   | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj cal2  | 2     | ✓       | 3.0                    | 3.3                 | 109.6    |
| mj cal 3 | 3     | ✓       | 5.0                    | 4.6                 | 92.0     |
| mj cal 4 | 4     | ✓       | 10.0                   | 10.0                | 99.9     |
| mj cal 5 | 5     | ✓       | 25.0                   | 24.1                | 96.5     |
| mj cal 6 | 6     | ✓       | 50.0                   | 51.0                | 102.0    |
| mj cal 7 | 7     | ✓       | 100.0                  | 100.0               | 100.0    |

# AM #27 Cannabinoids

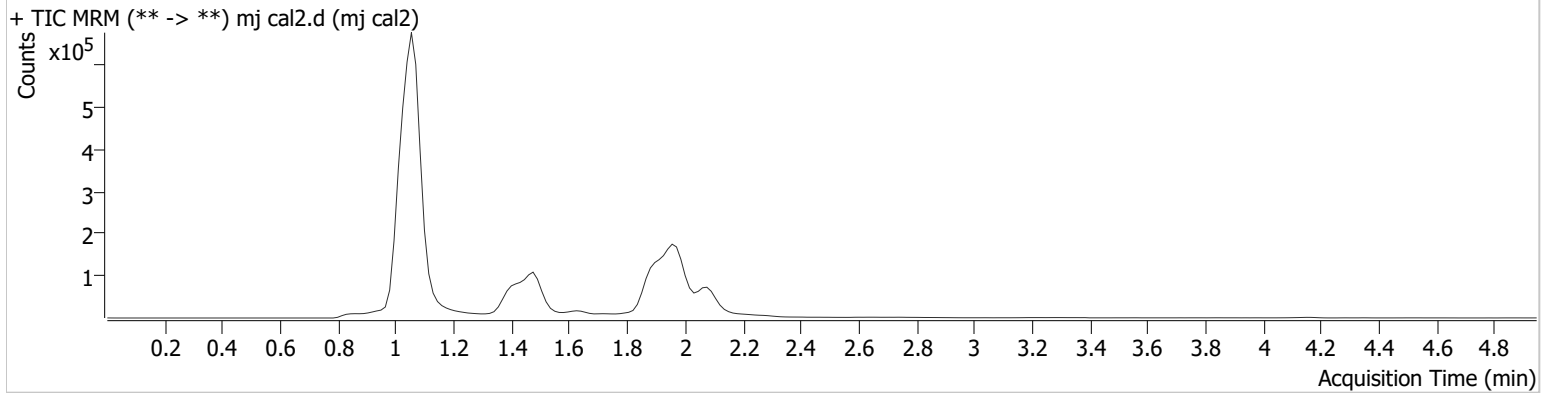


**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 10/9/2019 8:45:08 AM

|                         |                      |                  |           |
|-------------------------|----------------------|------------------|-----------|
| <b>Instrument</b>       | 69679                | <b>Data File</b> | mj cal2.d |
| <b>Type</b>             | Cal                  | <b>Sample</b>    | mj cal2   |
| <b>Acq. Method</b>      | AM 27 THC quant.m    | <b>Operator</b>  | Anne Nord |
| <b>Sample Position</b>  | P3-B1                | <b>Comment</b>   |           |
| <b>Injection Volume</b> | 10                   |                  |           |
| <b>Acq. Date-Time</b>   | 10/8/2019 3:13:29 PM |                  |           |

**Sample Info.**

## Sample Chromatogram



| Name     | RT    | Resp.  | S/N    | Ratio | S/N    | ISTD Resp. | Final Conc.  |
|----------|-------|--------|--------|-------|--------|------------|--------------|
| THC-OH   | 1.059 | 106429 | 15.2   | 8.9   | ∞      | 2156469    | 3.287 ng/ml  |
| THC-COOH | 1.055 | 105184 | 3741.9 | 35.0  | 1029.4 | 858240     | 10.151 ng/ml |
| THC      | 1.980 | 18903  | ∞      | 25.9  | 127.7  | 956608     | 3.220 ng/ml  |



# AM #27 Cannabinoids

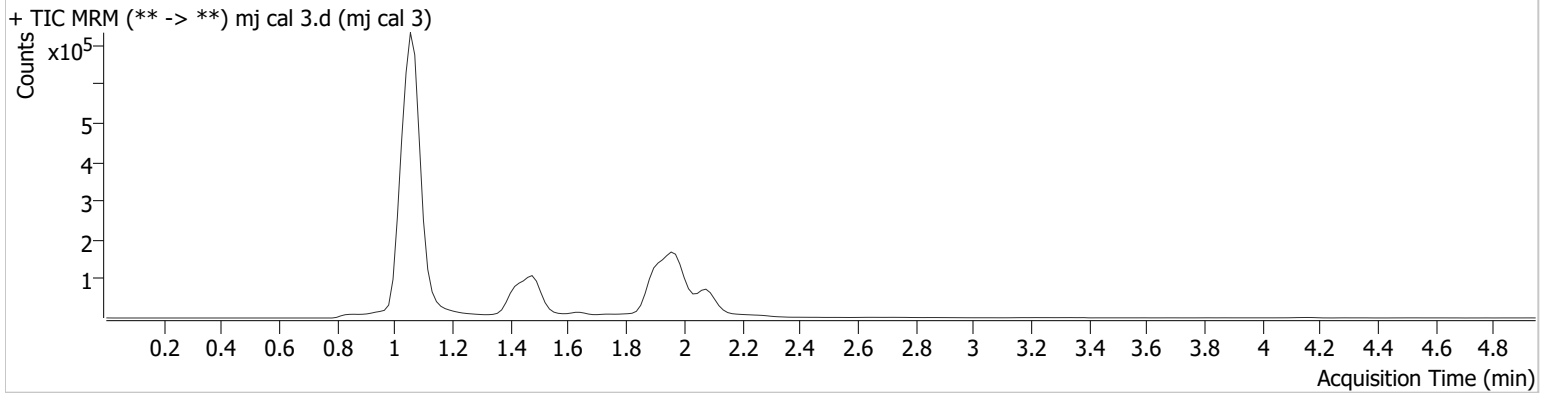


**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 10/9/2019 8:45:08 AM

|                         |                      |                  |            |
|-------------------------|----------------------|------------------|------------|
| <b>Instrument</b>       | 69679                | <b>Data File</b> | mj cal 3.d |
| <b>Type</b>             | Cal                  | <b>Sample</b>    | mj cal 3   |
| <b>Acq. Method</b>      | AM 27 THC quant.m    | <b>Operator</b>  | Anne Nord  |
| <b>Sample Position</b>  | P3-C1                | <b>Comment</b>   |            |
| <b>Injection Volume</b> | 10                   |                  |            |
| <b>Acq. Date-Time</b>   | 10/8/2019 3:21:11 PM |                  |            |

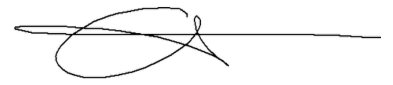
**Sample Info.**

## Sample Chromatogram



| Name     | RT    | Resp.  | S/N      | Ratio | S/N   | ISTD Resp. | Final Conc.  |
|----------|-------|--------|----------|-------|-------|------------|--------------|
| THC-OH   | 1.059 | 132032 | ∞        | 8.6   | ∞     | 1949881    | 4.601 ng/ml  |
| THC-COOH | 1.070 | 194128 | 233447.4 | 33.6  | 741.7 | 789366     | 20.189 ng/ml |
| THC      | 1.980 | 30506  | 437.3    | 26.3  | ∞     | 885260     | 5.049 ng/ml  |

# AM #27 Cannabinoids

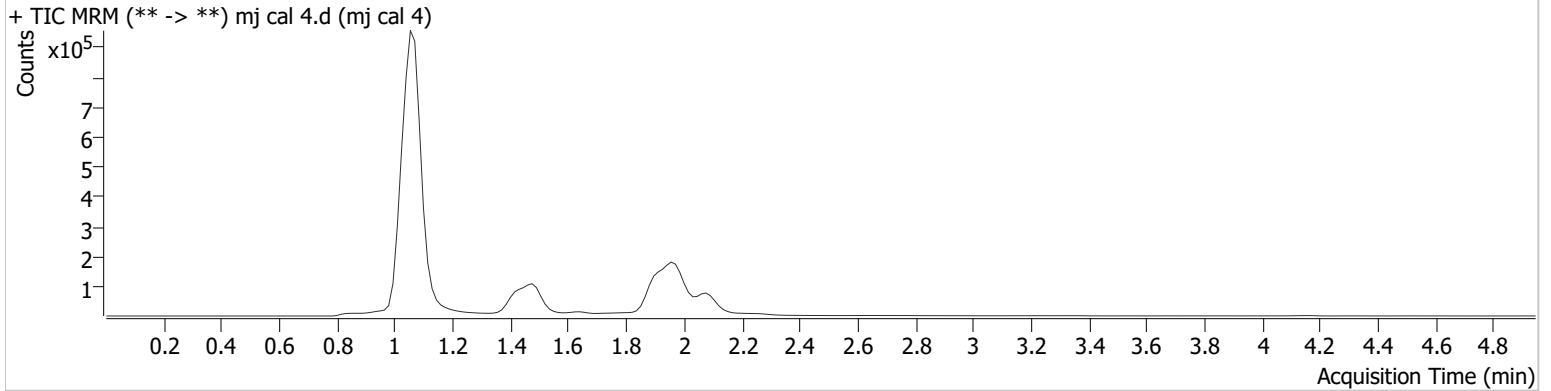


**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 10/9/2019 8:45:08 AM

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|-------------------------|----------------------|------------------|------------|
| <b>Instrument</b>       | 69679                | <b>Data File</b> | mj cal 4.d |
| <b>Type</b>             | Cal                  | <b>Sample</b>    | mj cal 4   |
| <b>Acq. Method</b>      | AM 27 THC quant.m    | <b>Operator</b>  | Anne Nord  |
| <b>Sample Position</b>  | P3-D1                | <b>Comment</b>   |            |
| <b>Injection Volume</b> | 10                   |                  |            |
| <b>Acq. Date-Time</b>   | 10/8/2019 3:28:53 PM |                  |            |


**Sample Info.**

## Sample Chromatogram



| Name     | RT    | Resp.  | S/N       | Ratio | S/N    | ISTD Resp. | Final Conc.  |
|----------|-------|--------|-----------|-------|--------|------------|--------------|
| THC-OH   | 1.059 | 285917 | 4556484.7 | 10.3  | ∞      | 2000576    | 9.985 ng/ml  |
| THC-COOH | 1.070 | 480681 | 952.9     | 38.2  | 4127.7 | 793035     | 49.497 ng/ml |
| THC      | 1.980 | 64671  | 3048.1    | 25.2  | 166.1  | 909226     | 9.612 ng/ml  |

# AM #27 Cannabinoids

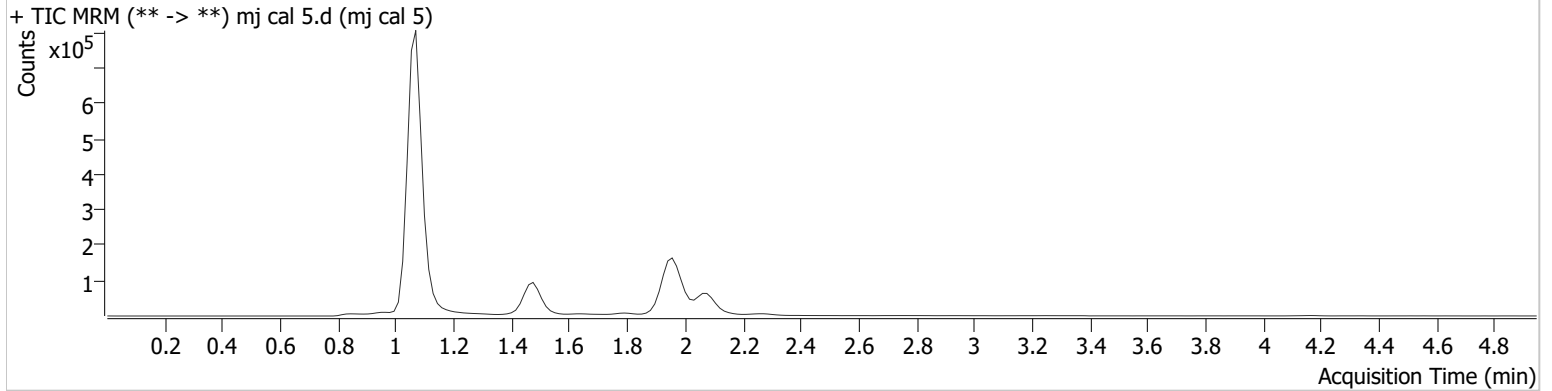


**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 10/9/2019 8:45:08 AM

|                         |                      |                  |            |
|-------------------------|----------------------|------------------|------------|
| <b>Instrument</b>       | 69679                | <b>Data File</b> | mj cal 5.d |
| <b>Type</b>             | Cal                  | <b>Sample</b>    | mj cal 5   |
| <b>Acq. Method</b>      | AM 27 THC quant.m    | <b>Operator</b>  | Anne Nord  |
| <b>Sample Position</b>  | P3-E1                | <b>Comment</b>   |            |
| <b>Injection Volume</b> | 10                   |                  |            |
| <b>Acq. Date-Time</b>   | 10/8/2019 3:36:36 PM |                  |            |

**Sample Info.**

## Sample Chromatogram



| Name     | RT    | Resp.  | S/N    | Ratio | S/N    | ISTD Resp. | Final Conc.  |
|----------|-------|--------|--------|-------|--------|------------|--------------|
| THC-OH   | 1.074 | 334158 | ∞      | 11.4  | ∞      | 981127     | 24.137 ng/ml |
| THC-COOH | 1.070 | 382788 | 2940.3 | 37.9  | 7038.8 | 428112     | 72.931 ng/ml |
| THC      | 1.965 | 90824  | 3882.5 | 24.8  | ∞      | 494537     | 23.615 ng/ml |

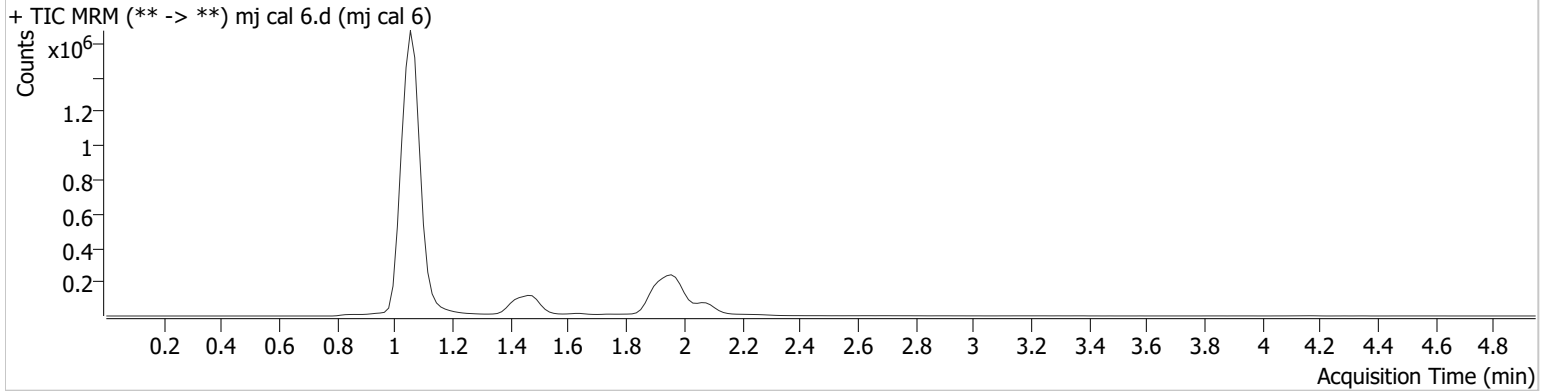
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 10/9/2019 8:45:08 AM

|                         |                      |                  |            |
|-------------------------|----------------------|------------------|------------|
| <b>Instrument</b>       | 69679                | <b>Data File</b> | mj cal 6.d |
| <b>Type</b>             | Cal                  | <b>Sample</b>    | mj cal 6   |
| <b>Acq. Method</b>      | AM 27 THC quant.m    | <b>Operator</b>  | Anne Nord  |
| <b>Sample Position</b>  | P3-F1                | <b>Comment</b>   |            |
| <b>Injection Volume</b> | 10                   |                  |            |
| <b>Acq. Date-Time</b>   | 10/8/2019 3:44:19 PM |                  |            |

**Sample Info.**

## Sample Chromatogram



| Name     | RT    | Resp.   | S/N        | Ratio | S/N    | ISTD Resp. | Final Conc.   |
|----------|-------|---------|------------|-------|--------|------------|---------------|
| THC-OH   | 1.059 | 1593205 | 61091146.0 | 11.8  | ∞      | 2225189    | 51.012 ng/ml  |
| THC-COOH | 1.055 | 968275  | 8527.8     | 38.4  | 4977.7 | 783761     | 100.701 ng/ml |
| THC      | 1.965 | 380319  | 3767.3     | 24.5  | 3497.4 | 969336     | 49.584 ng/ml  |

# AM #27 Cannabinoids

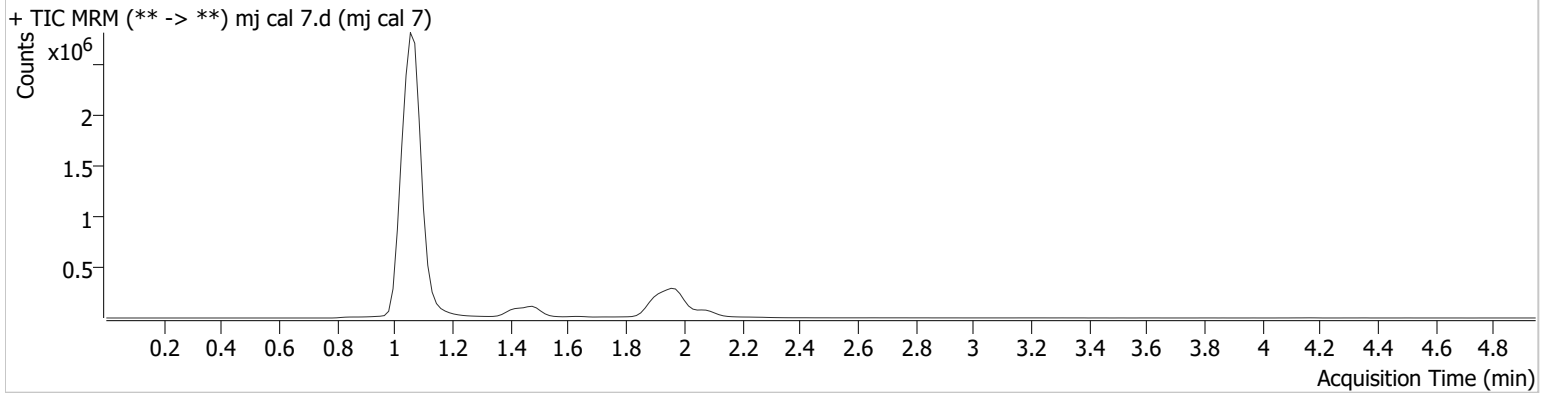


**Batch results** D:\MassHunter\Data\2019\am28-27\100819\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 10/9/2019 8:45:08 AM

|                         |                      |                  |            |
|-------------------------|----------------------|------------------|------------|
| <b>Instrument</b>       | 69679                | <b>Data File</b> | mj cal 7.d |
| <b>Type</b>             | Cal                  | <b>Sample</b>    | mj cal 7   |
| <b>Acq. Method</b>      | AM 27 THC quant.m    | <b>Operator</b>  | Anne Nord  |
| <b>Sample Position</b>  | P3-G1                | <b>Comment</b>   |            |
| <b>Injection Volume</b> | 10                   |                  |            |
| <b>Acq. Date-Time</b>   | 10/8/2019 3:52:01 PM |                  |            |

**Sample Info.**

## Sample Chromatogram



| Name     | RT    | Resp.   | S/N        | Ratio | S/N    | ISTD Resp. | Final Conc.   |
|----------|-------|---------|------------|-------|--------|------------|---------------|
| THC-OH   | 1.059 | 2955209 | 67311801.0 | 11.9  | ∞      | 2110964    | 99.977 ng/ml  |
| THC-COOH | 1.055 | 2388262 | 12527.7    | 39.0  | 7685.7 | 773126     | 251.530 ng/ml |
| THC      | 1.980 | 752871  | 67430.9    | 24.9  | 2004.6 | 926130     | 101.919 ng/ml |