












**Worklist: 4301**

| <u>LAB_CASE</u> | <u>ITEM</u> | <u>ITEM_TYPE</u> | <u>DESCRIPTION</u>                              |   |
|-----------------|-------------|------------------|---|---|
| C2020-0959      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ                 |  |
| C2020-0982      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ                 |  |
| C2020-0994      | 1           | UCK              | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |
| C2020-0995      | 1           | UCK              | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |  |
| C2020-1038      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ                 |  |
| C2020-1039      | 4           | BCK              | AM 27 Blood THC Quant by LC-QQQ                 |  |
| C2020-1047      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ                 |  |
| C2020-1076      | 1           | BCK              | AM 27 Blood THC Quant by LC-QQQ                 |  |
| C2020-1084      | 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: 6/10/2020  
Plate lot#: 200303

Analyst: Anne Nord  
Plate Expiration: 09-03-2020

**Mobile phase A:** 0.1% Formic Acid in LCMS Water MTBE  
**Mobile phase B:** 0.1% Formic acid in Acetonitrile Hexane  
LCMS Methanol

**Blank Blood Lot:** 20A52255 **Urine Blank:** 6920 **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 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.
- 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: *THC curve 3-100*  
*THC-OH not evaluated for urine samples.*



**Toxicology AM method 27/26 external prep information**

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, 75 ul THC-OH in 9767.5 ul meOH

Ppd 2/13/20 Exp: 8/13/20 lot 21320 by AMN

| Drug   | lot         | expiration |
|--------|-------------|------------|
| C-THC  | FE07171501  | 9/1/2020   |
| THC-OH | FE07721601  | 7/1/2021   |
| THC    | FE001041701 | 3/1/2022   |

AM 27/26 blood control 100 ul working solution lot (91319) in 9900 ul blood lot (20A52255)

ppd 02/13/20 Exp 08/13/20 lot b81320 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-THC by AMN

AM 27/26 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (6920)

|                        |             |   |        |            |
|------------------------|-------------|---|--------|------------|
| ppd 4/17/20 Exp 9120   | lot u101720 | Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC | by BAW | out of use |
| ppd 6/9/20 exp 8/13/20 | lot 6920    | Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC | by amn | 6/8/2020   |

# AM #27 Cannabinoids



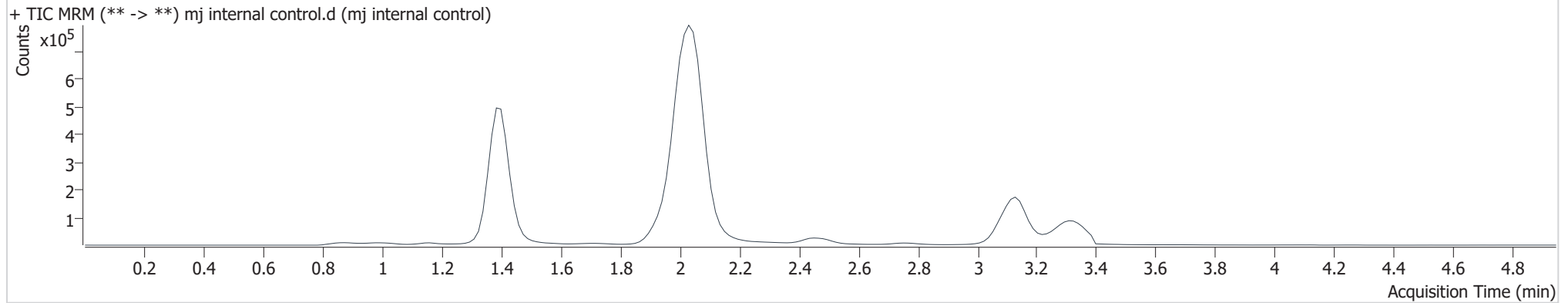
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin  
6/11/2020 8:34:00 AM

**Instrument** 69679  
**Type** QC  
**Acq. Method** AM 27 THC quant.m  
**Sample Position** P3-H1  
**Injection Volume** 10  
**Acq. Date-Time** 6/10/2020 12:38:09 PM  
**Sample Info.**

**Data File** mj internal control.d  
**Sample** mj internal control  
**Operator** Anne Nord  
**Comment**

**Sample Chromatogram**



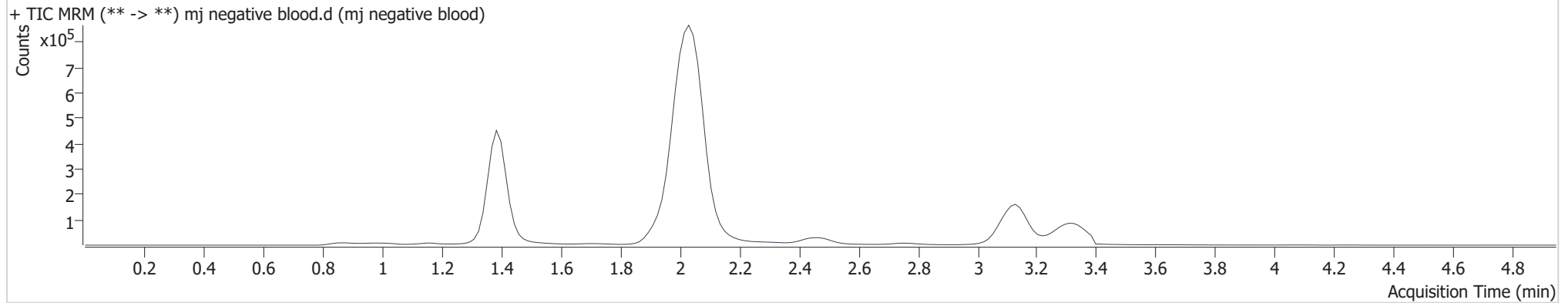
| Name     | RT    | Resp.  | S/N   | Ratio | S/N    | ISTD Resp. | Final Conc.  |
|----------|-------|--------|-------|-------|--------|------------|--------------|
| THC-OH   | 1.390 | 113407 | ∞     | 9.7   | ∞      | 1186669    | 4.985 ng/ml  |
| THC-COOH | 1.415 | 133206 | 665.1 | 37.9  | 1684.1 | 773839     | 15.311 ng/ml |
| THC      | 3.153 | 30160  | ∞     | 23.4  | ∞      | 809359     | 4.518 ng/ml  |



# AM #27 Cannabinoids

|                                |   |                  |                     |
|--------------------------------|---|------------------|---------------------|
| <b>Batch results</b>           | D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin |                  |                     |
| <b>Calibration Last Update</b> | 6/11/2020 8:34:00 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>          | 6/10/2020 12:45:51 PM   |                  |                     |
| <b>Sample Info.</b>            |   |                  |                     |

## Sample Chromatogram



# AM #27 Cannabinoids

**Batch results**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin

**Calibration Last Update**

6/11/2020 8:34:00 AM

**Instrument**

69679

**Type**

Sample

**Acq. Method**

AM 27 THC quant.m

**Sample Position**

Vial 4

**Injection Volume**

10

**Acq. Date-Time**

6/10/2020 2:41:15 PM

**Sample Info.****Data File**

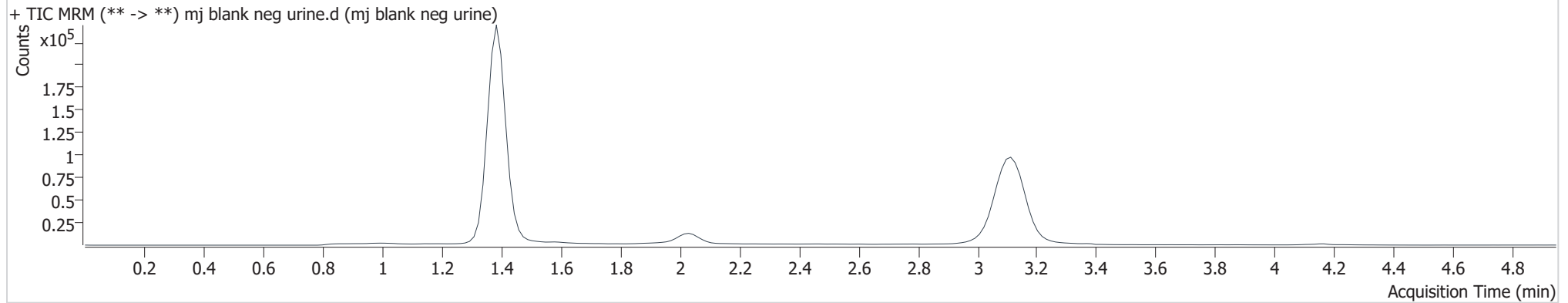
mj blank neg urine.d

**Sample**

mj blank neg urine

**Operator**

Anne Nord

**Comment****Sample Chromatogram**

# AM #27 Cannabinoids

**Batch results**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin

**Calibration Last Update**

6/11/2020 8:34:00 AM

**Instrument**

69679

**Type**

Sample

**Acq. Method**

AM 27 THC quant.m

**Sample Position**

P3-A3

**Injection Volume**

10

**Acq. Date-Time**

6/10/2020 2:48:53 PM

**Sample Info.****Data File**

mj neg urine.d

**Sample**

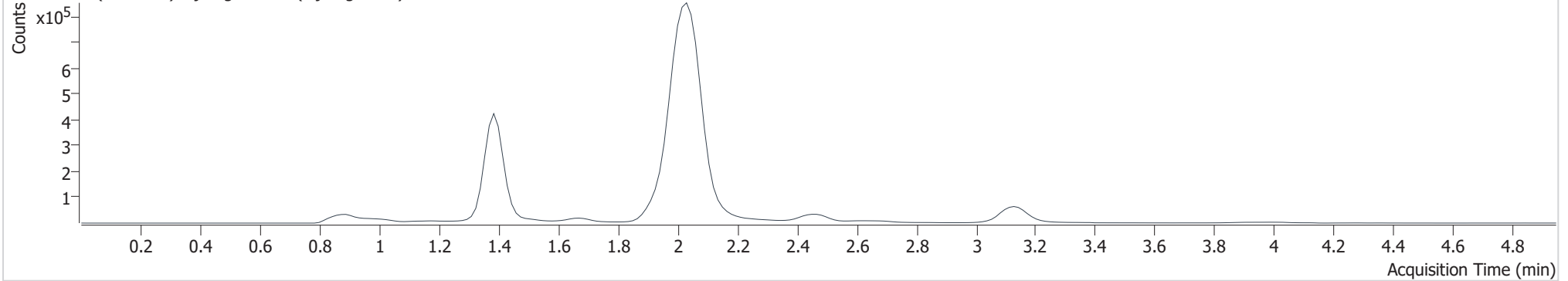
mj neg urine

**Operator**

Anne Nord

**Comment****Sample Chromatogram**

+ TIC MRM (\*\* -&gt; \*\*) mj neg urine.d (mj neg urine)



# AM #27 Cannabinoids



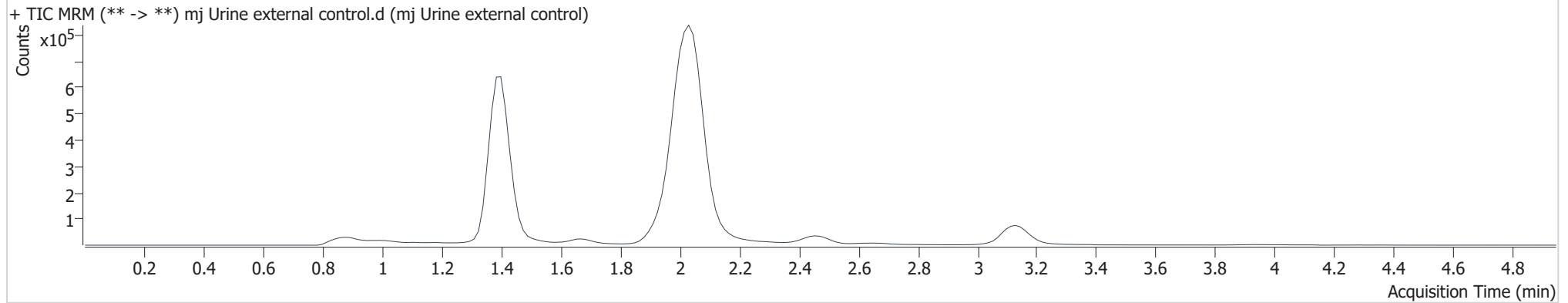
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin  
6/11/2020 8:34:00 AM

**Instrument** 69679  
**Type** Sample  
**Acq. Method** AM 27 THC quant.m  
**Sample Position** P3-B3  
**Injection Volume** 10  
**Acq. Date-Time** 6/10/2020 2:56:38 PM  
**Sample Info.**

**Data File** mj Urine external control.d  
**Sample** mj Urine external control  
**Operator** Anne Nord  
**Comment**

**Sample Chromatogram**



| Name     | RT    | Resp.  | S/N   | Ratio            | S/N   | ISTD Resp. | Final Conc.  |
|----------|-------|--------|-------|------------------|-------|------------|--------------|
| THC-OH   | 1.390 | 398815 | ∞     | 56.8 <b>High</b> | ∞     | 1265314    | 16.874 ng/ml |
| THC-COOH | 1.415 | 272270 | 320.5 | 37.2             | 654.1 | 563156     | 42.026 ng/ml |
| THC      | 3.153 | 57209  | ∞     | 24.5             | ∞     | 430973     | 14.220 ng/ml |

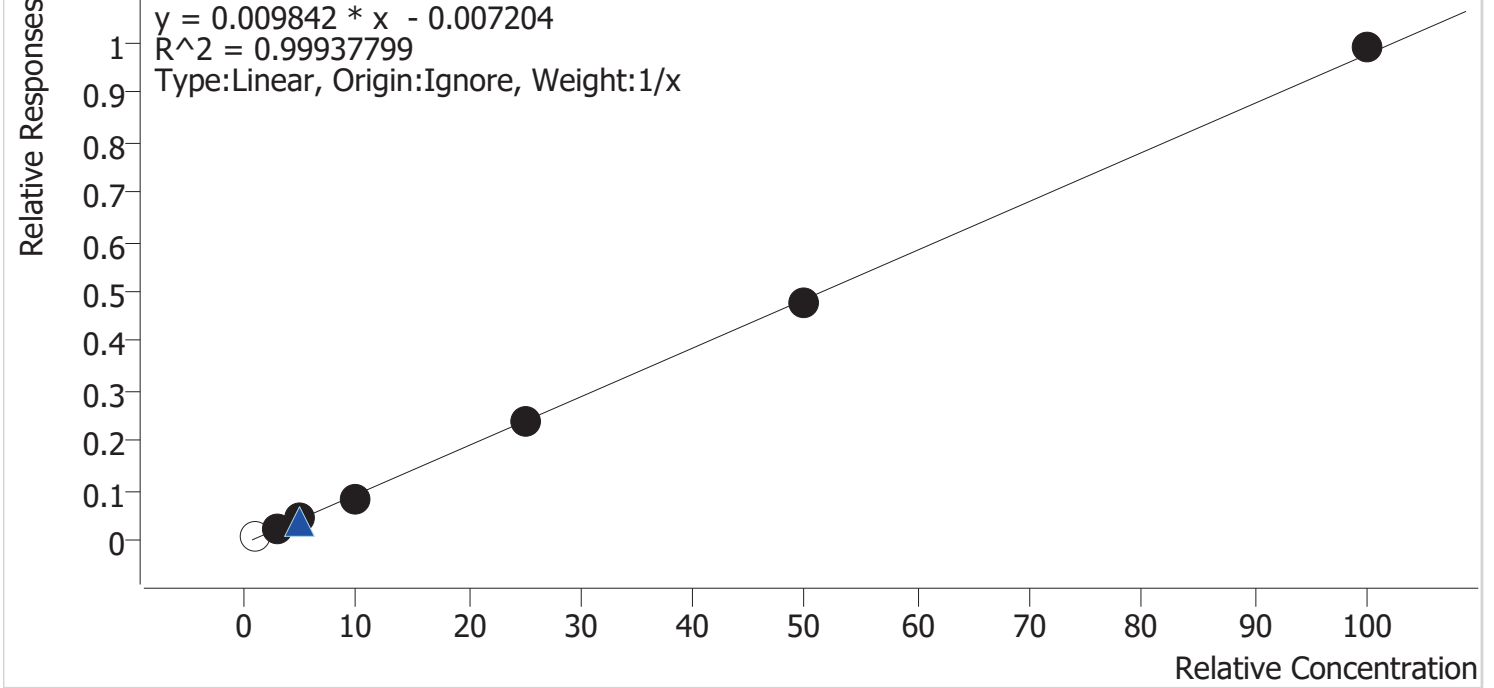
Urine evaluated for THC and THC-COOH only.



Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\lam 27-28 6-10-20\QuantResults\mj.batch.bin  
Last Cal. Update 6/11/2020 8:34 AM  
Analyst Name ISP\datastor  
Analyte THC Internal Standard THC-d3

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

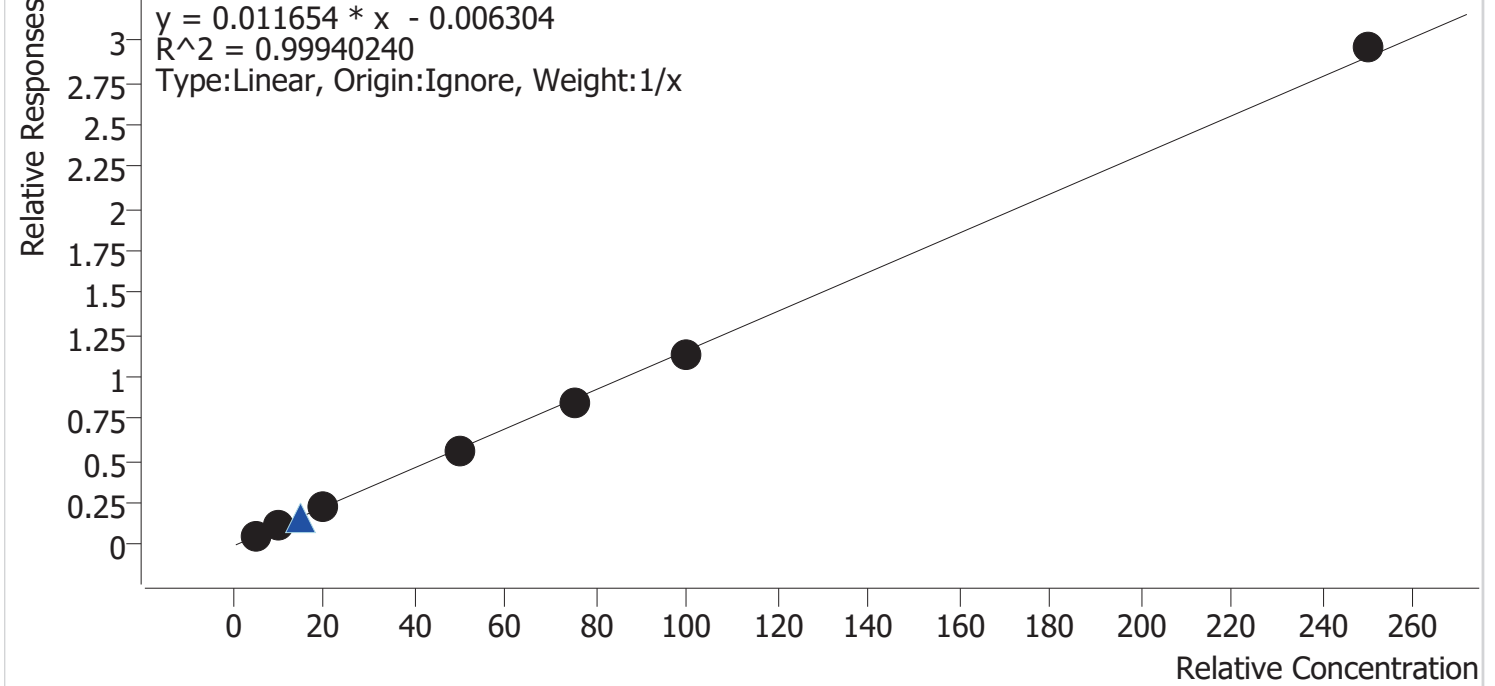


| Sample   | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj qc1   | 1     | x       | 1.0                    | 1.6                 | 160.0    |
| mj cal2  | 2     | ✓       | 3.0                    | 3.2                 | 105.1    |
| mj cal 3 | 3     | ✓       | 5.0                    | 5.1                 | 102.4    |
| mj cal 4 | 4     | ✓       | 10.0                   | 9.3                 | 93.5     |
| mj cal 5 | 5     | ✓       | 25.0                   | 24.8                | 99.4     |
| mj cal 6 | 6     | ✓       | 50.0                   | 49.1                | 98.3     |
| mj cal 7 | 7     | ✓       | 100.0                  | 101.4               | 101.4    |

# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin  
**Last Cal. Update** 6/11/2020 8:34 AM  
**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

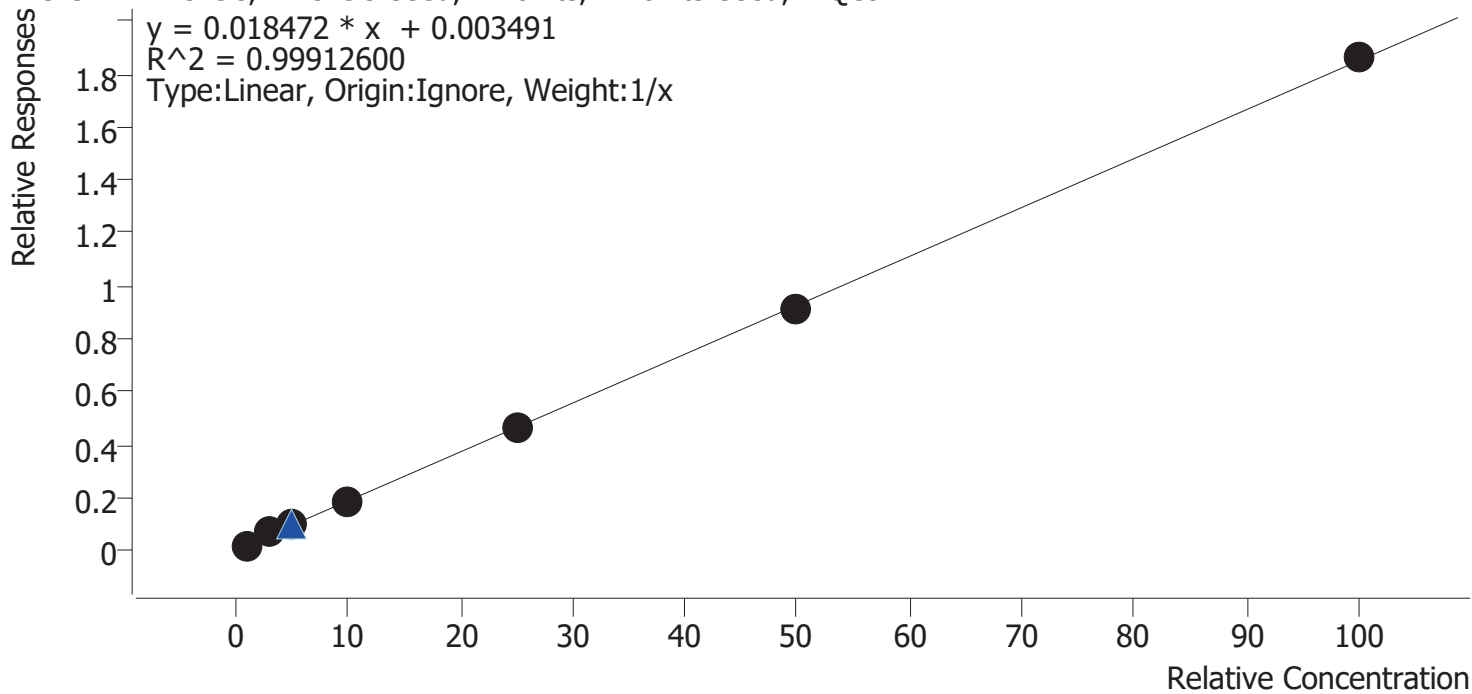


| Sample   | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj qc1   | 1     | ✓       | 5.0                    | 5.2                 | 104.8    |
| mj cal2  | 2     | ✓       | 10.0                   | 10.0                | 99.7     |
| mj cal 3 | 3     | ✓       | 20.0                   | 20.2                | 100.9    |
| mj cal 4 | 4     | ✓       | 50.0                   | 48.6                | 97.2     |
| mj cal 5 | 5     | ✓       | 75.0                   | 72.9                | 97.2     |
| mj cal 6 | 6     | ✓       | 100.0                  | 98.4                | 98.4     |
| mj cal 7 | 7     | ✓       | 250.0                  | 254.8               | 101.9    |

# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin  
**Last Cal. Update** 6/11/2020 8:34 AM  
**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

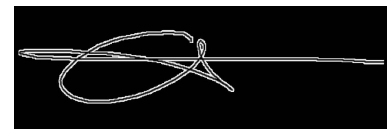


| Sample   | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|------------------------|---------------------|----------|
| mj qc1   | 1     | ✓       | 1.0                    | 0.8                 | 81.5     |
| mj cal2  | 2     | ✓       | 3.0                    | 3.4                 | 113.7    |
| mj cal 3 | 3     | ✓       | 5.0                    | 5.4                 | 107.2    |
| mj cal 4 | 4     | ✓       | 10.0                   | 9.9                 | 99.3     |
| mj cal 5 | 5     | ✓       | 25.0                   | 25.0                | 99.8     |
| mj cal 6 | 6     | ✓       | 50.0                   | 48.9                | 97.8     |
| mj cal 7 | 7     | ✓       | 100.0                  | 100.6               | 100.6    |





# AM #27 Cannabinoids



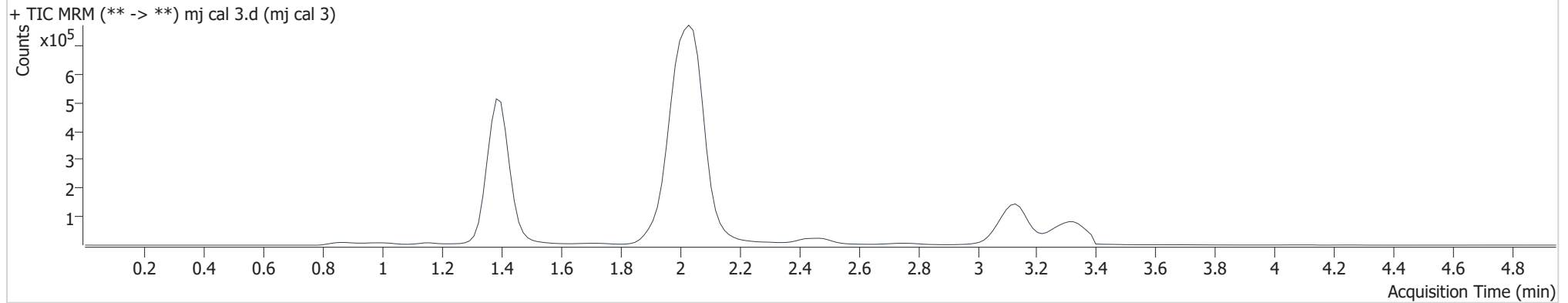
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin  
6/11/2020 8:34:00 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** AM 27 THC quant.m  
**Sample Position** P3-C1  
**Injection Volume** 10  
**Acq. Date-Time** 6/10/2020 11:59:30 AM  
**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   | 1.390 | 129249 | ∞        | 9.9   | ∞                    | 1261272    | 5.359 ng/ml  |
| THC-COOH | 1.415 | 180802 | 226466.2 | 37.0  | 565.0                | 789667     | 20.187 ng/ml |
| THC      | 3.153 | 31206  | ∞        | 27.4  | 22082445<br>295712.0 | 722937     | 5.118 ng/ml  |

# AM #27 Cannabinoids

**Batch results**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin

**Calibration Last Update**

6/11/2020 8:34:00 AM

**Instrument**

69679

**Type**

Cal

**Acq. Method**

AM 27 THC quant.m

**Sample Position**

P3-D1

**Injection Volume**

10

**Acq. Date-Time**

6/10/2020 12:07:14 PM

**Sample Info.****Data File**

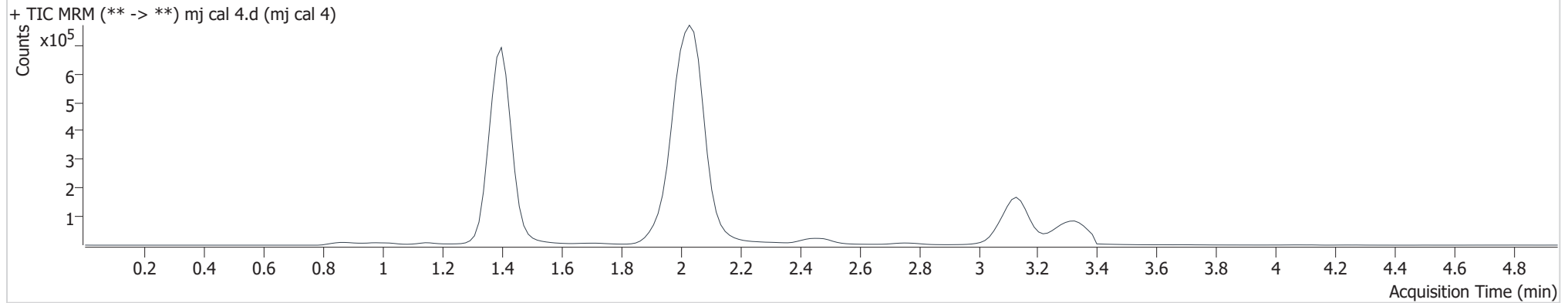
mj cal 4.d

**Sample**

mj cal 4

**Operator**

Anne Nord

**Comment****Sample Chromatogram**

| Name     | RT    | Resp.  | S/N    | Ratio | S/N    | ISTD Resp. | Final Conc.  |
|----------|-------|--------|--------|-------|--------|------------|--------------|
| THC-OH   | 1.390 | 235140 | ∞      | 10.1  | ∞      | 1257780    | 9.932 ng/ml  |
| THC-COOH | 1.415 | 445147 | 9956.5 | 38.6  | 1765.8 | 795089     | 48.581 ng/ml |
| THC      | 3.153 | 65649  | ∞      | 26.4  | ∞      | 774319     | 9.347 ng/ml  |

# AM #27 Cannabinoids



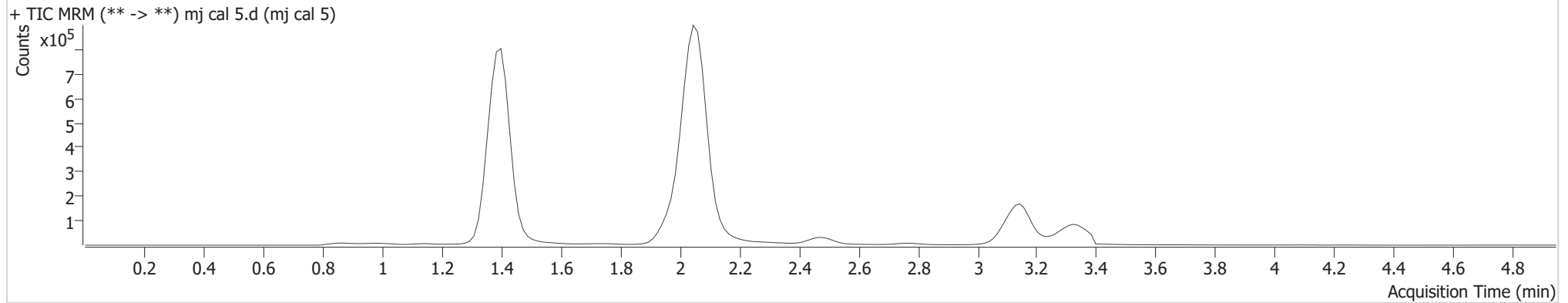
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin  
6/11/2020 8:34:00 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** AM 27 THC quant.m  
**Sample Position** P3-E1  
**Injection Volume** 10  
**Acq. Date-Time** 6/10/2020 12:14:58 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   | 1.390 | 537889 | ∞      | 11.0  | ∞                    | 1158262    | 24.952 ng/ml |
| THC-COOH | 1.400 | 584958 | 1215.4 | 38.9  | 2940.7               | 693969     | 72.868 ng/ml |
| THC      | 3.153 | 150718 | ∞      | 25.1  | 20470718<br>283739.9 | 635216     | 24.841 ng/ml |



# AM #27 Cannabinoids



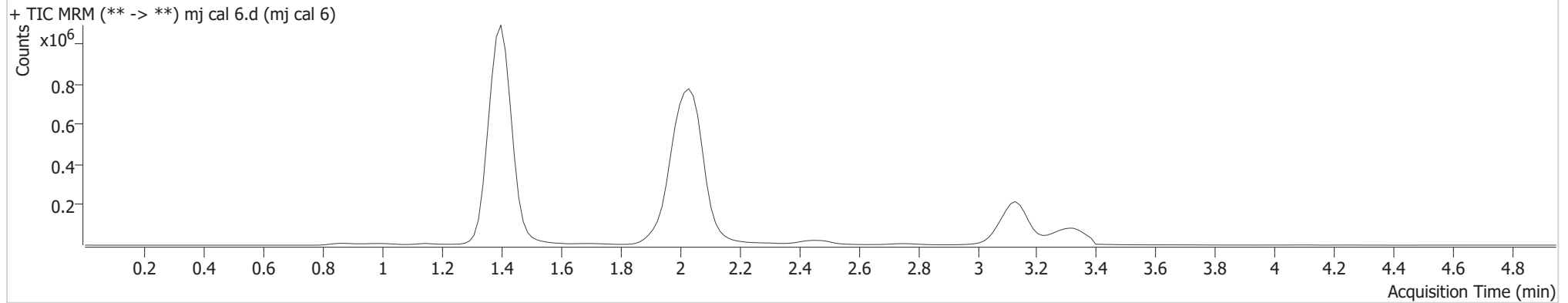
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin  
6/11/2020 8:34:00 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** AM 27 THC quant.m  
**Sample Position** P3-F1  
**Injection Volume** 10  
**Acq. Date-Time** 6/10/2020 12:22:42 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   | 1.390 | 1123282 | ∞      | 11.6  | ∞      | 1238414    | 48.915 ng/ml |
| THC-COOH | 1.415 | 880879  | 1325.8 | 39.3  | 4631.2 | 772478     | 98.388 ng/ml |
| THC      | 3.153 | 355600  | ∞      | 25.1  | ∞      | 746273     | 49.148 ng/ml |

# AM #27 Cannabinoids



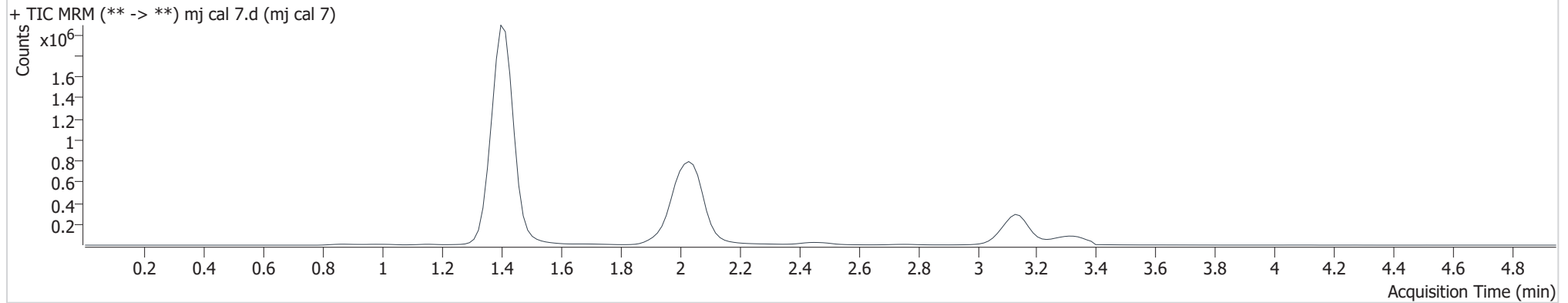
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin  
6/11/2020 8:34:00 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** AM 27 THC quant.m  
**Sample Position** P3-G1  
**Injection Volume** 10  
**Acq. Date-Time** 6/10/2020 12:30:27 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   | 1.390 | 2278680 | ∞       | 11.7  | ∞      | 1223760    | 100.615 ng/ml |
| THC-COOH | 1.415 | 2128815 | 14260.5 | 39.7  | 4084.8 | 718505     | 254.770 ng/ml |
| THC      | 3.153 | 735859  | ∞       | 26.1  | ∞      | 742785     | 101.393 ng/ml |