REVIEWED
By Anne Nord at 2:18 pm, Oct 21, 2019

TS $ 10/18/2019

**Worklist: 3765**

| LAB_CASE | ITEM | ITEM_TYPE | DESCRIPTION |
|---|---|---|---|
| M2019-4285 | 2 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| M2019-4386 | 3 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| M2019-4438 | 8 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-2690 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-2907 | 2 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3026 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3027 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3039 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3047 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3048 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3054 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3056 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3058 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3060 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |
| P2019-3083 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |

TS 9

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

Extraction Date: 10/17/19 Analyst: Sarah Pickle
Plate lot#: Lot # 190716 Item # IDP-108 Plate Expiration: 1/16/20

**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**: 445283-3 **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. Pipette **1000 µL blood (calibrated pipette)** in wells of analytical (standards) plate. **Pipette ID: #3**
☒ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 067105*
☒ 4. Pipette **500 µL 0.1% formic acid in LCMS water** in wells of analytical plate.
☒ 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 6. Transfer **800 µL 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 85-95 PSI- Selector to the right)*** Manifold ID: 067104
☒ 8. Wait 5 minutes.
☒ 9. Add **2.25 mL MTBE. (*Add in 3 increments of 750 µL*)**
☒ 10. Wait 5 minutes.
☒ 11. Apply positive pressure for approx. 15 seconds. ***(12-15 PSI- Selector to the left).***
☒ 12. Add **2.25 mL Hexane. (*Add in 3 increments of 750 µL*)**
☒ 13. Wait 5 minutes.
☒ 14. Apply positive pressure for approx. 15 seconds. ***(12-15 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 **100 µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

## Post-Analytic

☒ 1. Create batch and process data.
Worklist path: D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP Batch Name: THCQ SP
☒ 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: *Curve Ranges: THC: 1-100, THC-COOH 5-250, THC-OH 3-100*
*Samples were extracted on 10/17/19. Due to the pressure maxing out and stopping the run in the middle of the night, a new column was installed and all samples that were ran were reinjected on 10/18/19. Calibrator 7, m2019-4386-3, m2019-4438-8, and p2019-3026-1 were also reconstituted and reinjected on 10/18/19.*

Case sample m2019-4169-2 from worklist 3753 was ran with this worklist with Sarah Pickle acting as the primary analyst, performing steps 3-16. I,Tamara Salazar, approved of all steps used in this method.

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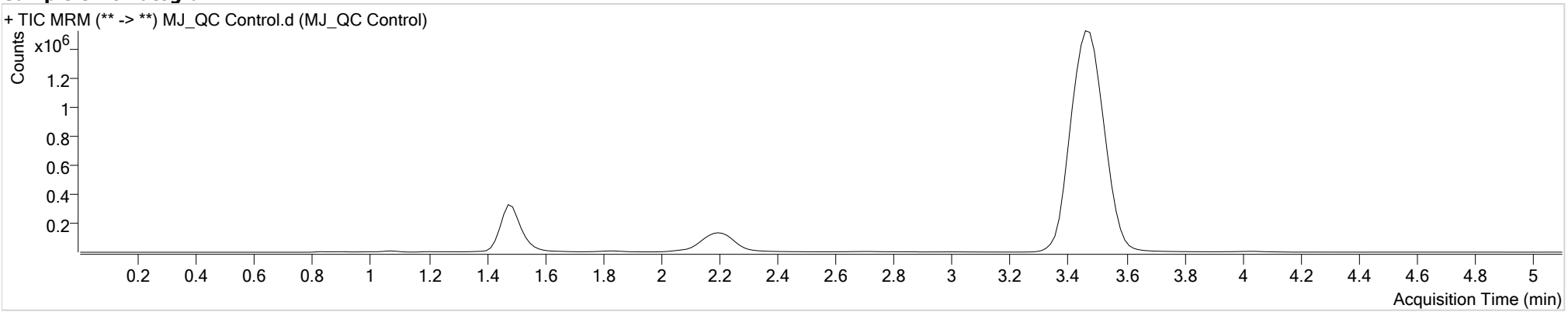
# AM #27 Cannabinoids Quant. Results

TS $

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Calibration Last Update** | 10/21/2019 8:15:22 AM |

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_QC Control.d |
| **Type** | Sample | **Sample** | MJ_QC Control |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-A6 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 12:35:22 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



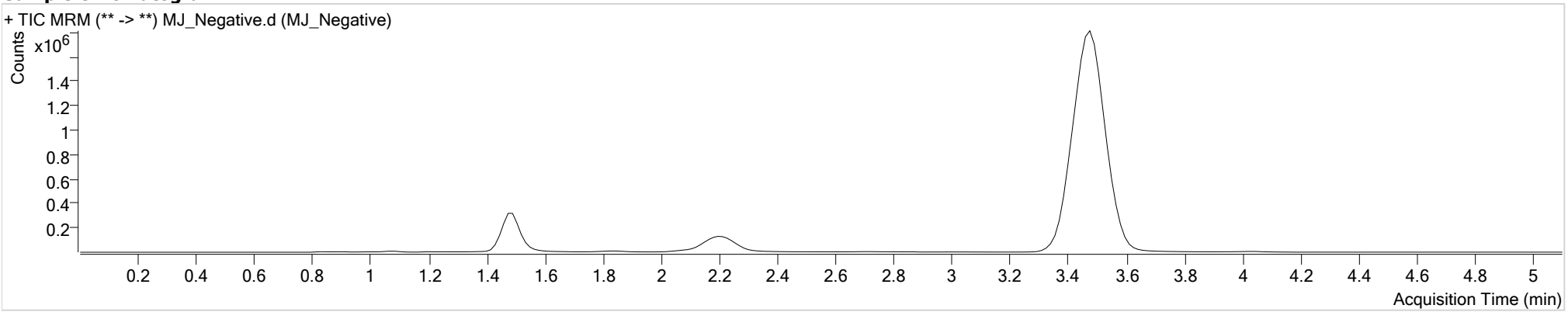

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC | 3.480 | 416397 | 2877.22 | 27.6 | 481.35 | 11669578 | 4.3663 ng/ml |
| THC-COOH | 1.519 | 86776 | ∞ | 62.6 | 508.22 | 268899 | 14.1260 ng/ml |
| THC-OH | 1.483 | 78611 | ∞ | 12.3 | ∞ | 1119332 | 4.6716 ng/ml |

# AM #27 Cannabinoids Quant. Results

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Calibration Last Update** | 10/21/2019 8:15:22 AM |

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_Negative.d |
| **Type** | Sample | **Sample** | MJ_Negative |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-H5 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 12:58:08 PM | | |
| **Sample Info.** | | | |

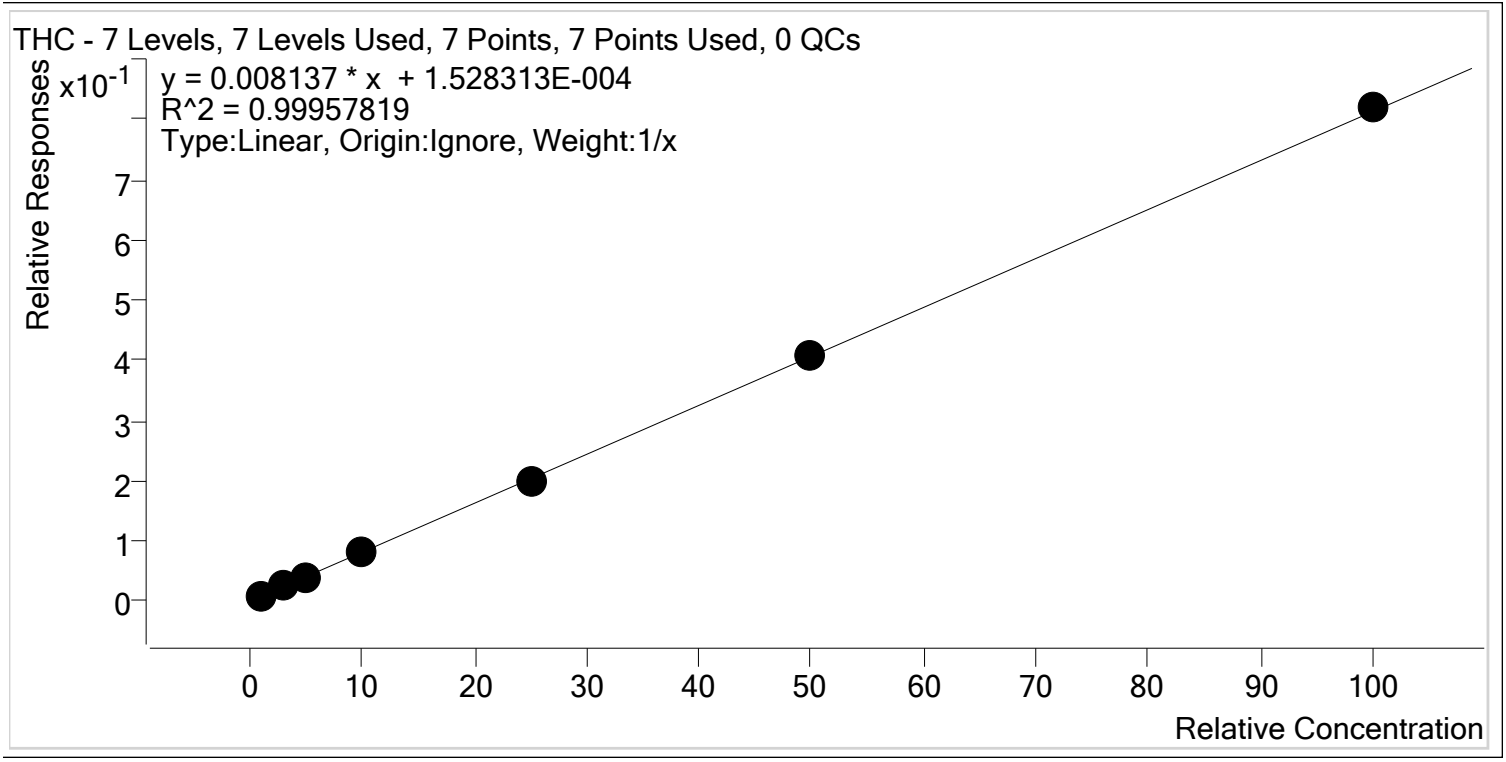
**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC-OH | 1.558 **High** | 18409 | ∞ | | | 1200848 | 0.2465 ng/ml |

# AM #27 Cannabinoids Quant. Calibration Curve Report

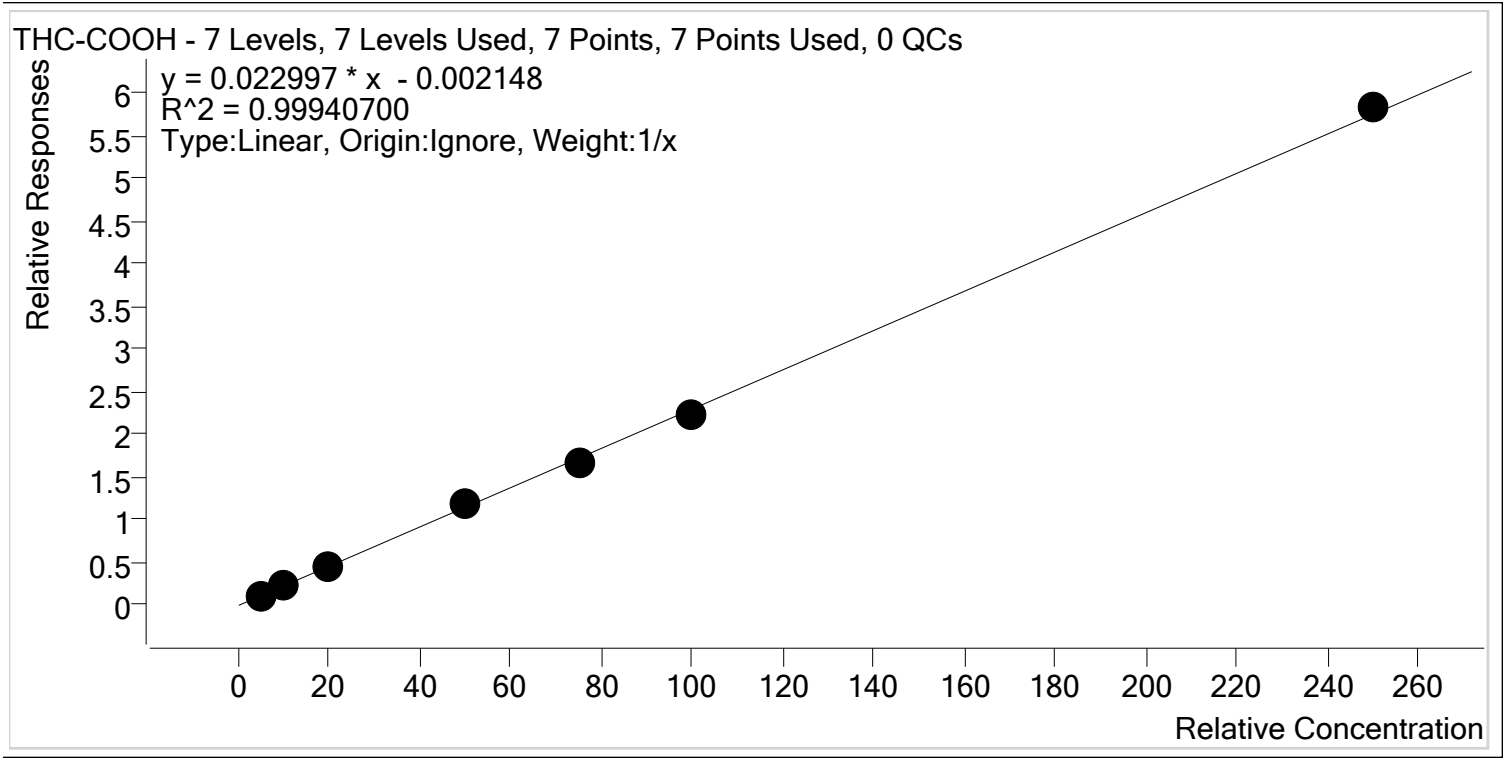
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Last Cal. Update** | 10/21/2019 8:15 AM |
| **Analyst Name** | ISP\datastor |
| **Analyte** | THC |
| **Internal Standard** | THC-D3 |




| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|---|---|---|---|---|---|
| MJ_Cal 1 | 1 | ✔ | 1.0 | 1.1 | 112.0 |
| MJ_Cal 2 | 2 | ✔ | 3.0 | 2.9 | 97.2 |
| MJ_Cal 3 | 3 | ✔ | 5.0 | 4.5 | 90.9 |
| MJ_Cal 4 | 4 | ✔ | 10.0 | 10.1 | 100.7 |
| MJ_Cal 5 | 5 | ✔ | 25.0 | 24.6 | 98.6 |
| MJ_Cal 6 | 6 | ✔ | 50.0 | 49.9 | 99.9 |
| MJ_Cal 7R2 | 7 | ✔ | 100.0 | 100.8 | 100.8 |

# AM #27 Cannabinoids Quant. Calibration Curve Report

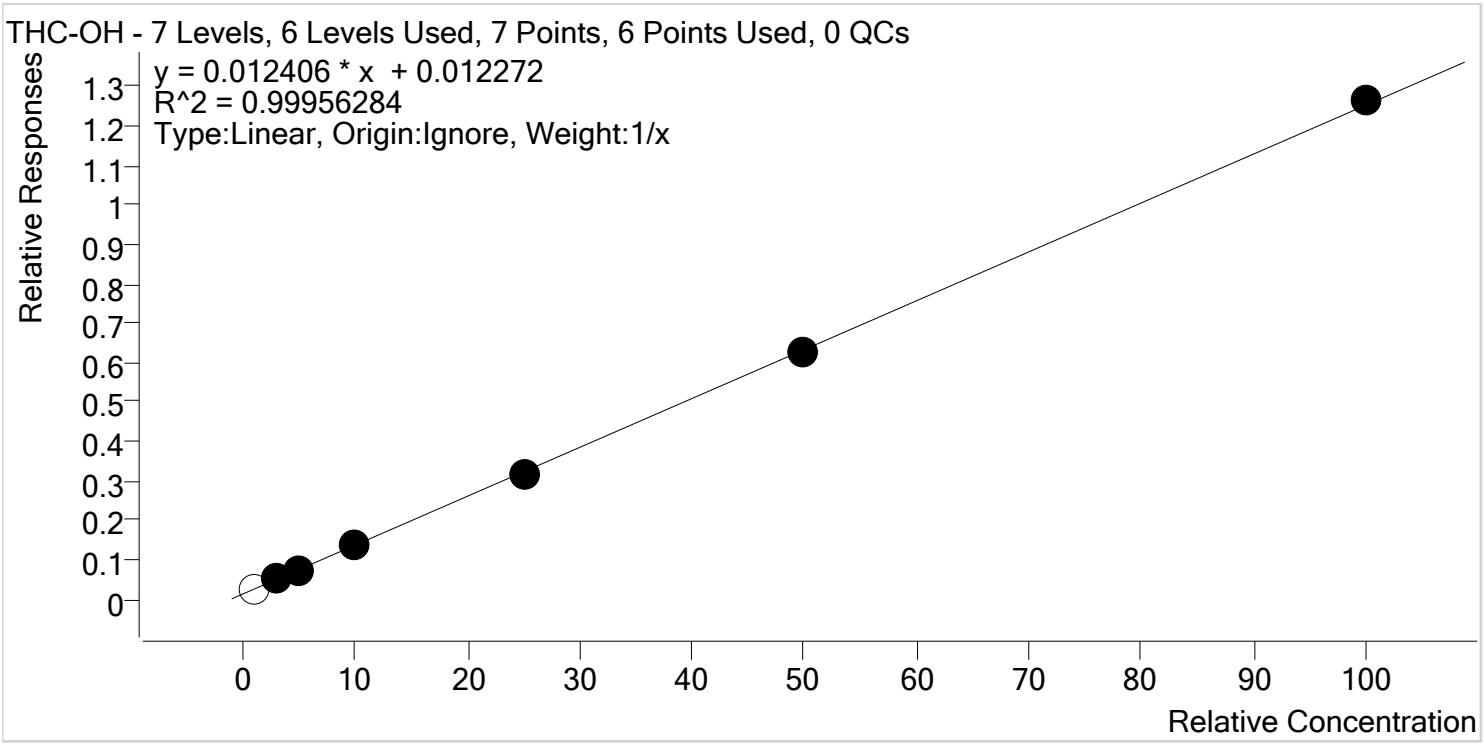
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Last Cal. Update** | 10/21/2019 8:15 AM |
| **Analyst Name** | ISP\datastor |
| **Analyte** | THC-COOH |
| **Internal Standard** | THC-COOH-D9 |




| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|---|---|---|---|---|---|
| MJ_Cal 1 | 1 | ✔ | 5.0 | 5.0 | 100.1 |
| MJ_Cal 2 | 2 | ✔ | 10.0 | 10.4 | 103.7 |
| MJ_Cal 3 | 3 | ✔ | 20.0 | 19.5 | 97.4 |
| MJ_Cal 4 | 4 | ✔ | 50.0 | 51.3 | 102.6 |
| MJ_Cal 5 | 5 | ✔ | 75.0 | 73.4 | 97.9 |
| MJ_Cal 6 | 6 | ✔ | 100.0 | 97.0 | 97.0 |
| MJ_Cal 7R2 | 7 | ✔ | 250.0 | 253.4 | 101.4 |

# AM #27 Cannabinoids Quant. Calibration Curve Report

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Last Cal. Update** | 10/21/2019 8:15 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 | ✗ | 1.0 | 1.1 | 109.0 |
| MJ_Cal 2 | 2 | ✔ | 3.0 | 3.1 | 103.0 |
| MJ_Cal 3 | 3 | ✔ | 5.0 | 4.8 | 95.1 |
| MJ_Cal 4 | 4 | ✔ | 10.0 | 10.4 | 104.4 |
| MJ_Cal 5 | 5 | ✔ | 25.0 | 24.5 | 97.9 |
| MJ_Cal 6 | 6 | ✔ | 50.0 | 49.3 | 98.7 |
| MJ_Cal 7R2 | 7 | ✔ | 100.0 | 100.9 | 100.9 |

# AM #27 Cannabinoids Quant. Results

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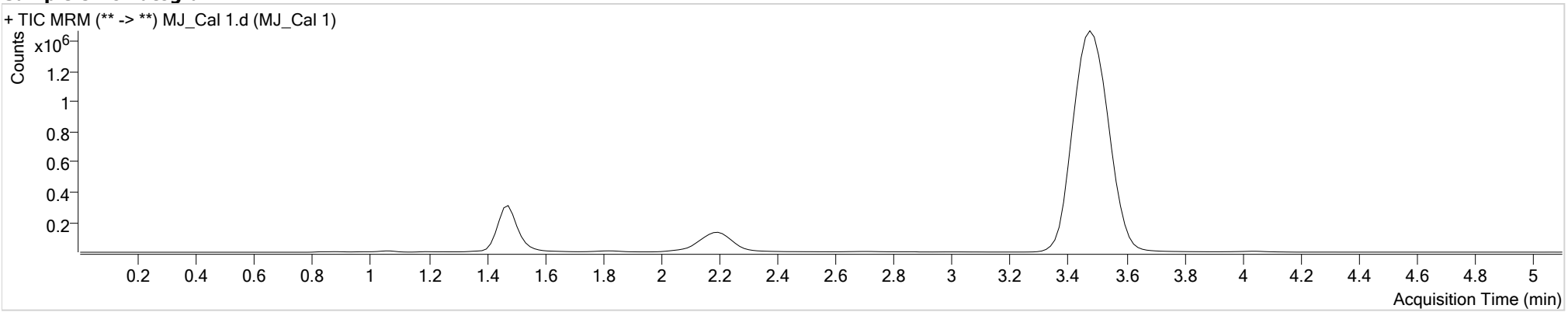
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Calibration Last Update** | 10/21/2019 8:15:22 AM |

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_Cal 1.d |
| **Type** | Cal | **Sample** | MJ_Cal 1 |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-B6 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 11:34:33 AM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC | 3.495 | 115288 | 954.91 | 31.0 | 134.08 | 12445996 | 1.1196 ng/ml |
| THC-COOH | 1.519 | 31896 | ∞ | 65.4 | 193.52 | 282493 | 5.0031 ng/ml |
| THC-OH | 1.543 **High** | 29743 | ∞ | 7.6 **Low** | 10.40 | 1152942 | 1.0902 ng/ml |

# AM #27 Cannabinoids Quant. Results

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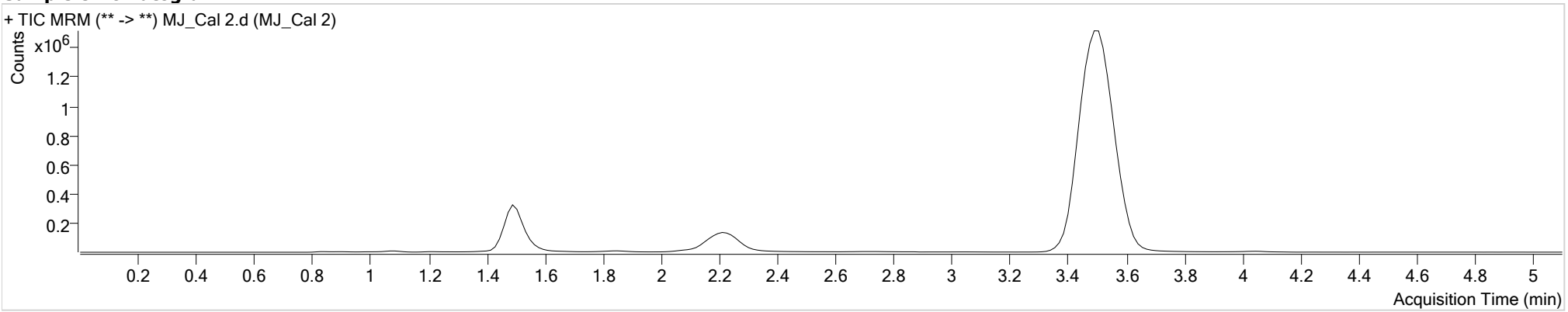
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Calibration Last Update** | 10/21/2019 8:15:22 AM |

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_Cal 2.d |
| **Type** | Cal | **Sample** | MJ_Cal 2 |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-C6 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 11:42:17 AM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC | 3.510 | 294555 | 1209.54 | 29.1 | 2169.03 | 12332139 | 2.9166 ng/ml |
| THC-COOH | 1.534 | 65278 | ∞ | 58.8 | 1357.50 | 276218 | 10.3697 ng/ml |
| THC-OH | 1.498 | 57116 | ∞ | 10.3 | 108.86 | 1128239 | 3.0913 ng/ml |

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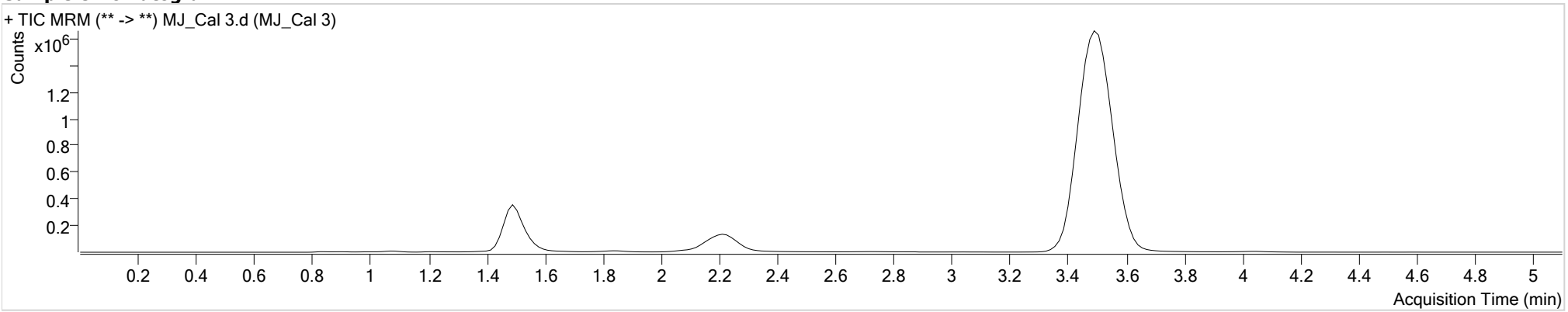
# AM #27 Cannabinoids Quant. Results

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Calibration Last Update** | 10/21/2019 8:15:22 AM |

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_Cal 3.d |
| **Type** | Cal | **Sample** | MJ_Cal 3 |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-D6 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 11:49:52 AM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC | 3.510 | 488635 | 616.88 | 28.0 | 430.91 | 13155841 | 4.5457 ng/ml |
| THC-COOH | 1.534 | 128871 | ∞ | 61.3 | 807.33 | 289131 | 19.4749 ng/ml |
| THC-OH | 1.498 | 84758 | ∞ | 12.1 | ∞ | 1189153 | 4.7559 ng/ml |

# AM #27 Cannabinoids Quant. Results

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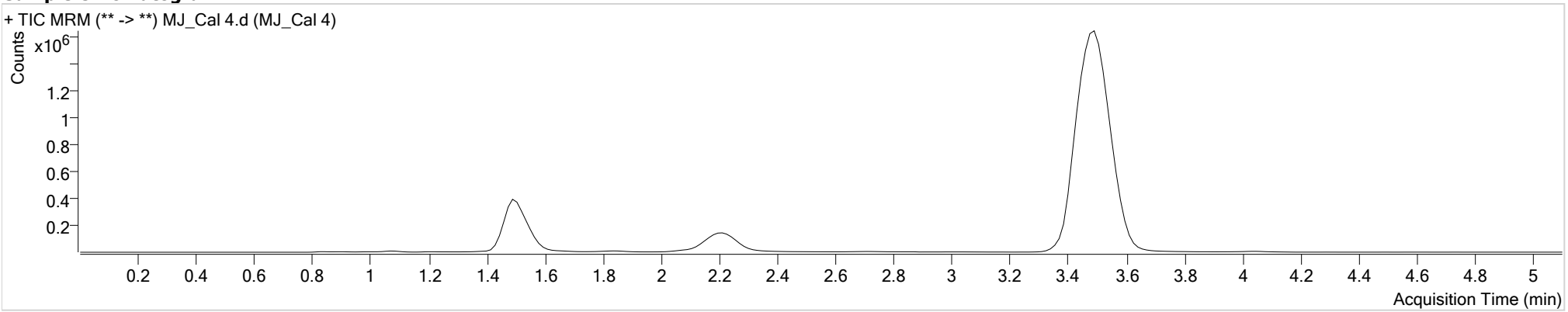
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Calibration Last Update** | 10/21/2019 8:15:22 AM |

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_Cal 4.d |
| **Type** | Cal | **Sample** | MJ_Cal 4 |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-E6 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 11:57:27 AM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**



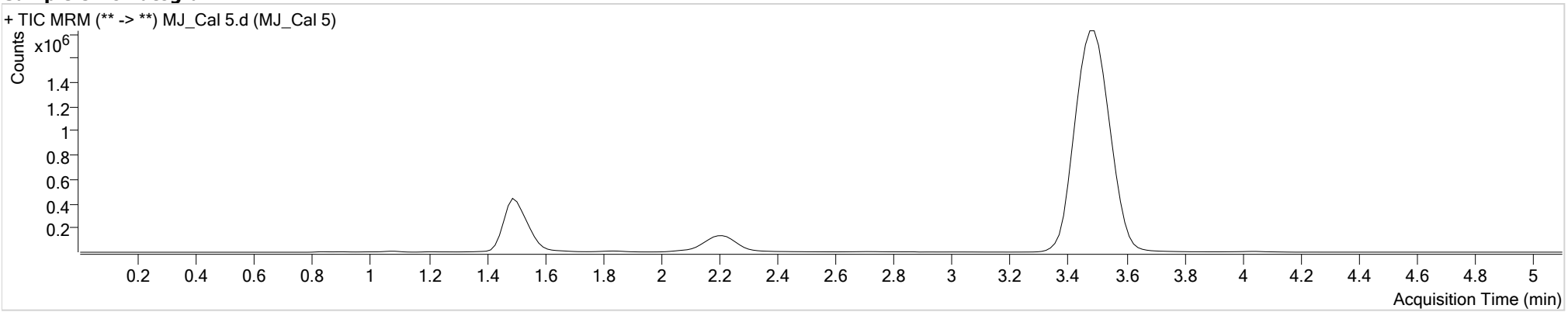

| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC | 3.510 | 1003536 | ∞ | 26.9 | ∞ | 12223076 | 10.0710 ng/ml |
| THC-COOH | 1.534 | 329792 | 3713.92 | 62.6 | 1732.67 | 280082 | 51.2948 ng/ml |
| THC-OH | 1.498 | 163602 | ∞ | 12.9 | 289.39 | 1154097 | 10.4369 ng/ml |

# AM #27 Cannabinoids Quant. Results

| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Calibration Last Update** | 10/21/2019 8:15:22 AM |

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_Cal 5.d |
| **Type** | Cal | **Sample** | MJ_Cal 5 |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-F6 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 12:05:02 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC | 3.510 | 2444887 | ∞ | 26.8 | ∞ | 12184093 | 24.6413 ng/ml |
| THC-COOH | 1.534 | 429902 | ∞ | 61.9 | 2713.45 | 254993 | 73.4044 ng/ml |
| THC-OH | 1.498 | 343580 | 367.97 | 13.7 | 534.78 | 1087623 | 24.4733 ng/ml |

# AM #27 Cannabinoids Quant. Results

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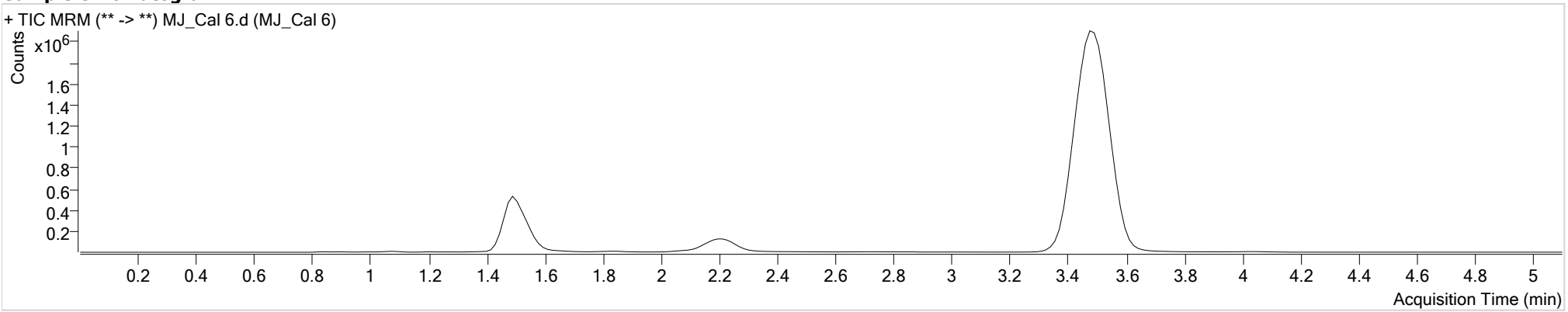
| | |
|---|---|
| **Batch results** | D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin |
| **Calibration Last Update** | 10/21/2019 8:15:22 AM |

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_Cal 6.d |
| **Type** | Cal | **Sample** | MJ_Cal 6 |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-G6 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 12:12:37 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC | 3.495 | 4715535 | 39480.25 | 27.1 | 4786.79 | 11602927 | 49.9263 ng/ml |
| THC-COOH | 1.534 | 532377 | ∞ | 61.3 | 5911.24 | 238797 | 97.0366 ng/ml |
| THC-OH | 1.498 | 640740 | ∞ | 13.7 | 1559.40 | 1026288 | 49.3336 ng/ml |

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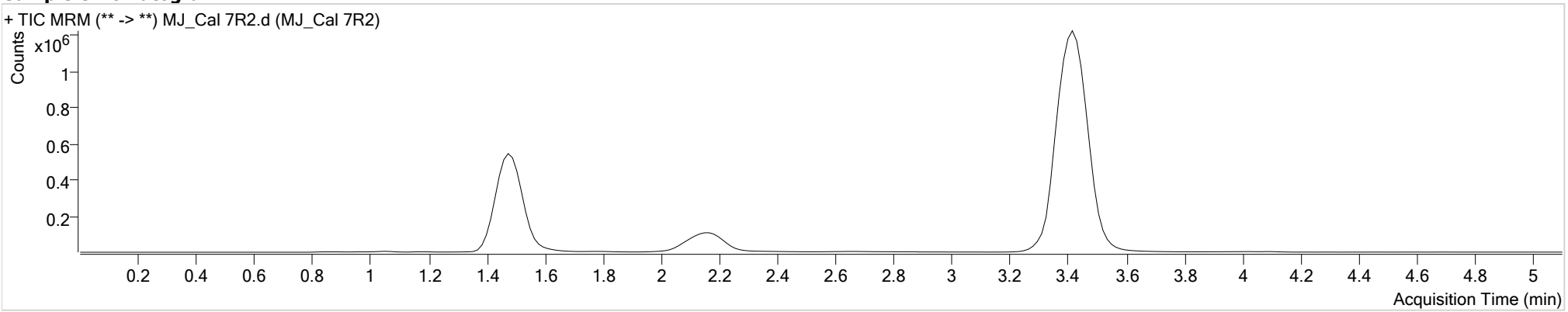
# AM #27 Cannabinoids Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 27\101819 THCQ reinjects SP\QuantResults\THCQ SP.batch.bin
**Calibration Last Update** 10/21/2019 8:15:22 AM

| | | | |
|---|---|---|---|
| **Instrument** | Falco | **Data File** | MJ_Cal 7R2.d |
| **Type** | Cal | **Sample** | MJ_Cal 7R2 |
| **Acq. Method** | AM 27 THC quant.m | | |
| **Sample Position** | P3-H6 | **Comment** | |
| **Injection Volume** | 10 | | |
| **Acq. Date-Time** | 10/18/2019 3:42:53 PM | | |
| **Sample Info.** | | | |

**Sample Chromatogram**




| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---|---|---|---|---|---|---|---|
| THC | 3.420 | 3705865 | 8083.70 | 26.8 | 1602.28 | 4518209 | 100.7794 ng/ml |
| THC-COOH | 1.489 | 1032459 | 3770.18 | 60.8 | 10354.54 | 177225 | 253.4164 ng/ml |
| THC-OH | 1.453 | 745705 | ∞ | 14.0 | 2223.67 | 589865 | 100.9090 ng/ml |