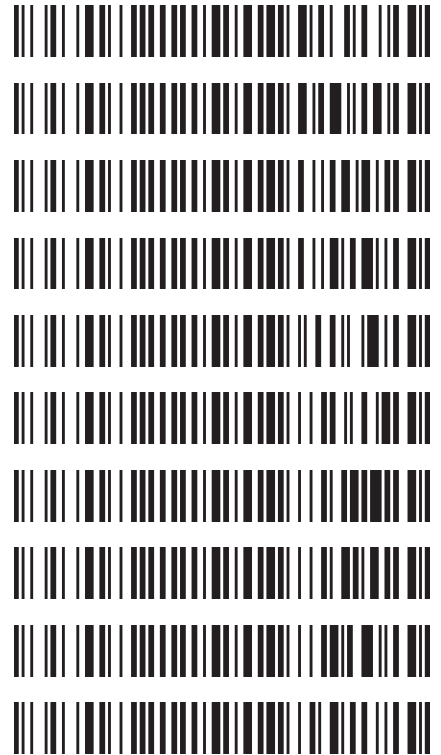


REVIEWED
By Briany Wylie at 2:53 pm, Oct 22, 2020

Worklist: 4570

| <u>LAB CASE</u> | <u>ITEM</u> | <u>ITEM TYPE</u> | <u>DESCRIPTION</u> |
|-----------------|-------------|------------------|-----------------------------------|
| C2020-1912 | 1 | UCK | AM 30 Urine THC Screen by LC-QTOF |
| C2020-1916 | 2 | UCK | AM 30 Urine THC Screen by LC-QTOF |
| C2020-1918 | 1 | UCK | AM 30 Urine THC Screen by LC-QTOF |
| C2020-1919 | 1 | BCK | AM 30 Blood THC Screen by LC-QTOF |
| C2020-1925 | 1 | UCK | AM 30 Urine THC Screen by LC-QTOF |
| C2020-1934 | 1 | BCK | AM 30 Blood THC Screen by LC-QTOF |
| C2020-1935 | 1 | BCK | AM 30 Blood THC Screen by LC-QTOF |
| C2020-1936 | 1 | BCK | AM 30 Blood THC Screen by LC-QTOF |
| C2020-1937 | 1 | UCK | AM 30 Urine THC Screen by LC-QTOF |
| C2020-1948 | 2 | BCK | AM 30 Blood THC Screen by LC-QTOF |



AM# 30: Screening of THC and Metabolites in Blood and Urine by LC-QTOF

Extraction Date: 10/6/20

Analyst: Anne Nord

Plate lot#: 200723

Plate Expiration: 1-23-2021

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.1% Formic acid in Methanol

Blank Blood Lot: 20G20792

Column: Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

LCMS-QQ ID: 70044

Blank Urine Lot: 10120

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 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.
Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: K52558G**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **500µL 0.1% formic acid in water blood sample, 500 µL 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, r^2 values ≥ 0.98 for each analyte
- 3. Evaluate samples: RT within +/- 2% or 0.1 min of administrative control, Mass Accuracy of 0 (+/- 10), and/or Mass Abundance Score of 40 or greater.
- 4. Did all QCs pass for each analyte? (if not, describe in comments section)
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

10/21/20
✎

COMMENTS: Evaluated ~~C-THC~~ and THC 3-100 curve range. THC-OH not evaluated responses variable on calibrators and internal standards. Evaluated C-THC 5-250 curve range.

GA

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|------------|----------------|--------|---|---|------------|
| a | cal 100 ng | neg blood | 1912 | | | QC 1 |
| b | cal 50 ng | 1919 | 1916-2 | | | cal 100 ng |
| c | cal 25 ng | 1934 | 1918 | | | cal 50 ng |
| d | cal 10ng | 1935 | 1925 | | | cal 25 ng |
| e | cal 5 ng | 1936 | 1937 | | | cal 10ng |
| f | cal 3 ng | 1948-2 | | | | cal 5 ng |
| g | cal 1ng | neg urine | | | | cal 3 ng |
| h | QC 1 | external urine | | | | cal 1ng |

c2020-

Toxicology AM method 27/26 external prep information



working solution 15 ug/ml in meoh C-THC, THC-OH, 7.5 ug/ml 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, 15 ng/ml C-THC, THC-OH | |
|--|--|--|--|

AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine

out of use

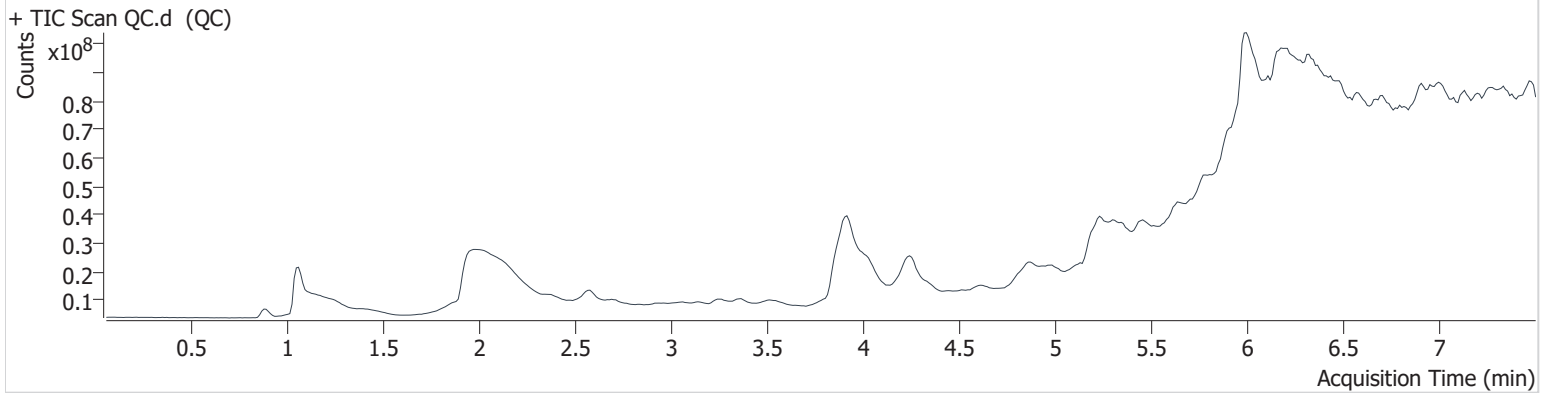
| | | | | |
|---|------------|---|--------|-----------|
| ppd 8/26/20 Exp 7/1/21 neg urine lot 73020 | lot u82620 | Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH | by amn | 10/4/2020 |
| ppd 10/5/20 Exp 7/1/21 neg urine lot 10120 | lot 10520 | Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH | by amn | |
| | | | | |
| | | | | |

AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

Instrument 69679 **Data File** QC.d
Type QC **Sample** QC
Acq. Method THC Screen 1122.m **Operator** Anne Nord
Sample Position P2-H1 **Comment**
Injection Volume 10
Acq. Date-Time 10/6/2020 12:53:03 PM
Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|--------|---------------|----------------------|------------|---------------|
| THC-COOH | 6.651 | 390238 | 1.23 | 52.7 | 2153772 | 25.0803 ng/ml |
| THC | 7.157 | 32546 | 1.60 | 8.1 | 1283349 | 3.8734 ng/ml |

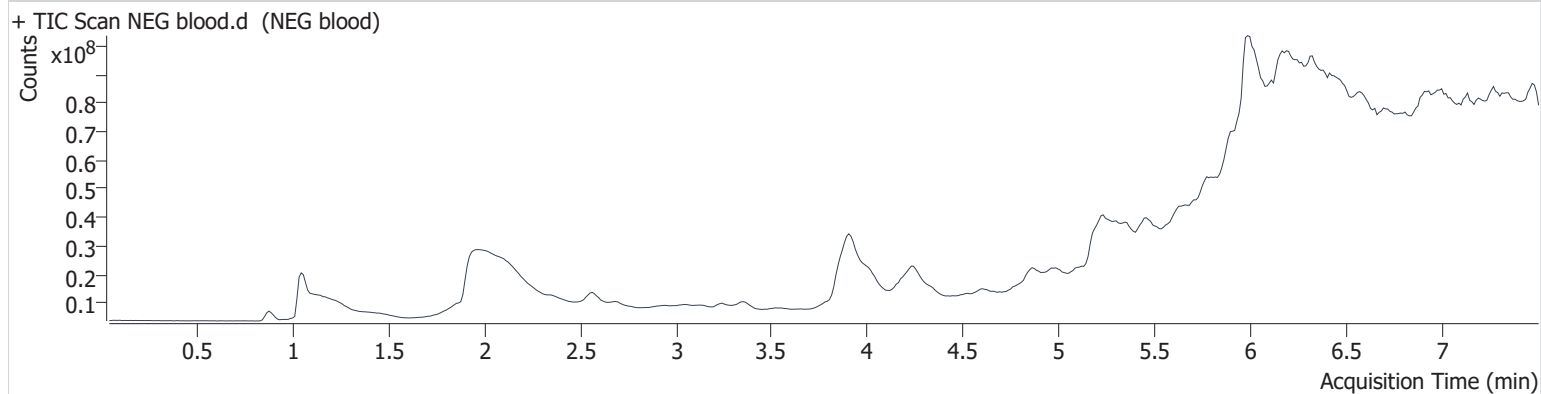
GA

AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

| | | | |
|-------------------------|----------------------|------------------|-------------|
| Instrument | 69679 | Data File | NEG blood.d |
| Type | Sample | Sample | NEG blood |
| Acq. Method | THC Screen 1122.m | Operator | Anne Nord |
| Sample Position | P2-A2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 10/6/2020 1:02:40 PM | | |
| Sample Info. | | | |

Sample Chromatogram



OA

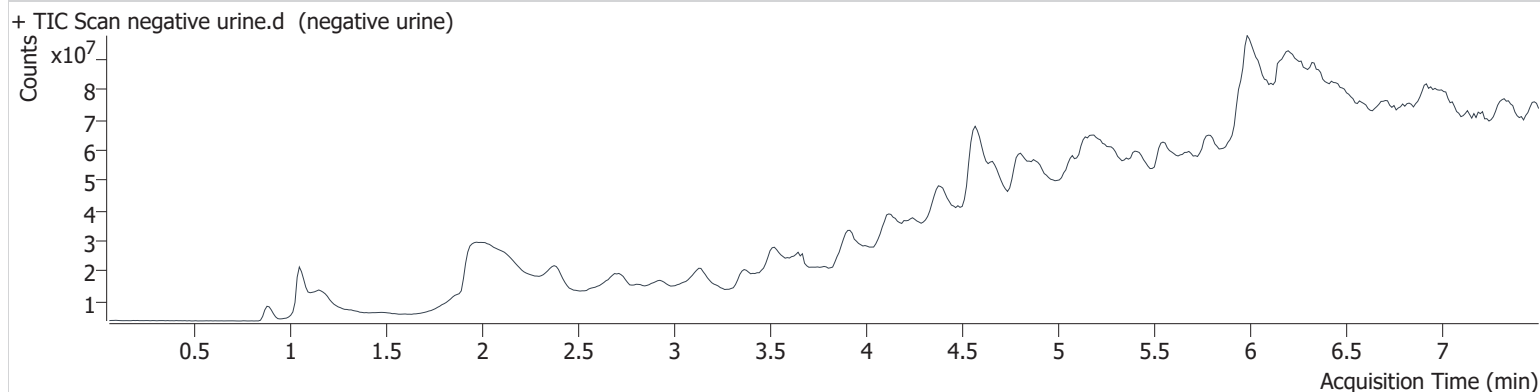
AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

| | | | |
|-------------------------|----------------------|------------------|------------------|
| Instrument | 69679 | Data File | negative urine.d |
| Type | Sample | Sample | negative urine |
| Acq. Method | THC Screen 1122.m | Operator | Anne Nord |
| Sample Position | P2-G2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 10/6/2020 2:00:11 PM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|------|-------|-------|-------------------|----------------------|------------|--------------|
| THC | 7.156 | 5707 | -14.84 Low | 0.0 | 826854 | 1.3586 ng/ml |

GA

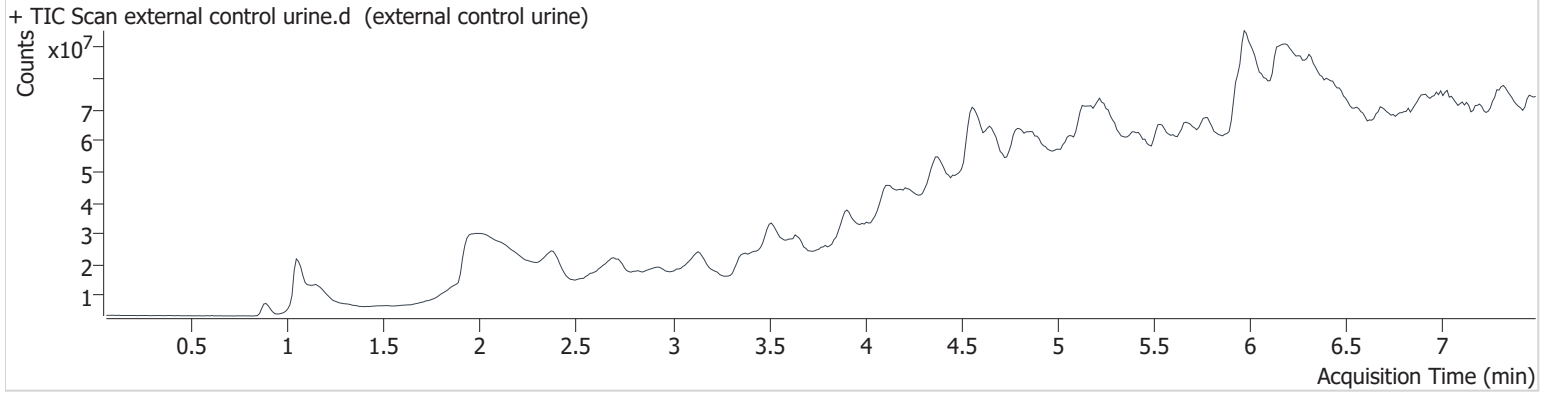
AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

| | | | |
|-------------------------|----------------------|------------------|--------------------------|
| Instrument | 69679 | Data File | external control urine.d |
| Type | Sample | Sample | external control urine |
| Acq. Method | THC Screen 1122.m | Operator | Anne Nord |
| Sample Position | P2-H2 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 10/6/2020 2:09:48 PM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|--------|---------------|----------------------|------------|---------------|
| THC-COOH | 6.648 | 363096 | 2.93 | 76.4 | 1335628 | 38.8914 ng/ml |
| THC | 7.154 | 50761 | 3.13 | 33.1 | 496588 | 14.3453 ng/ml |

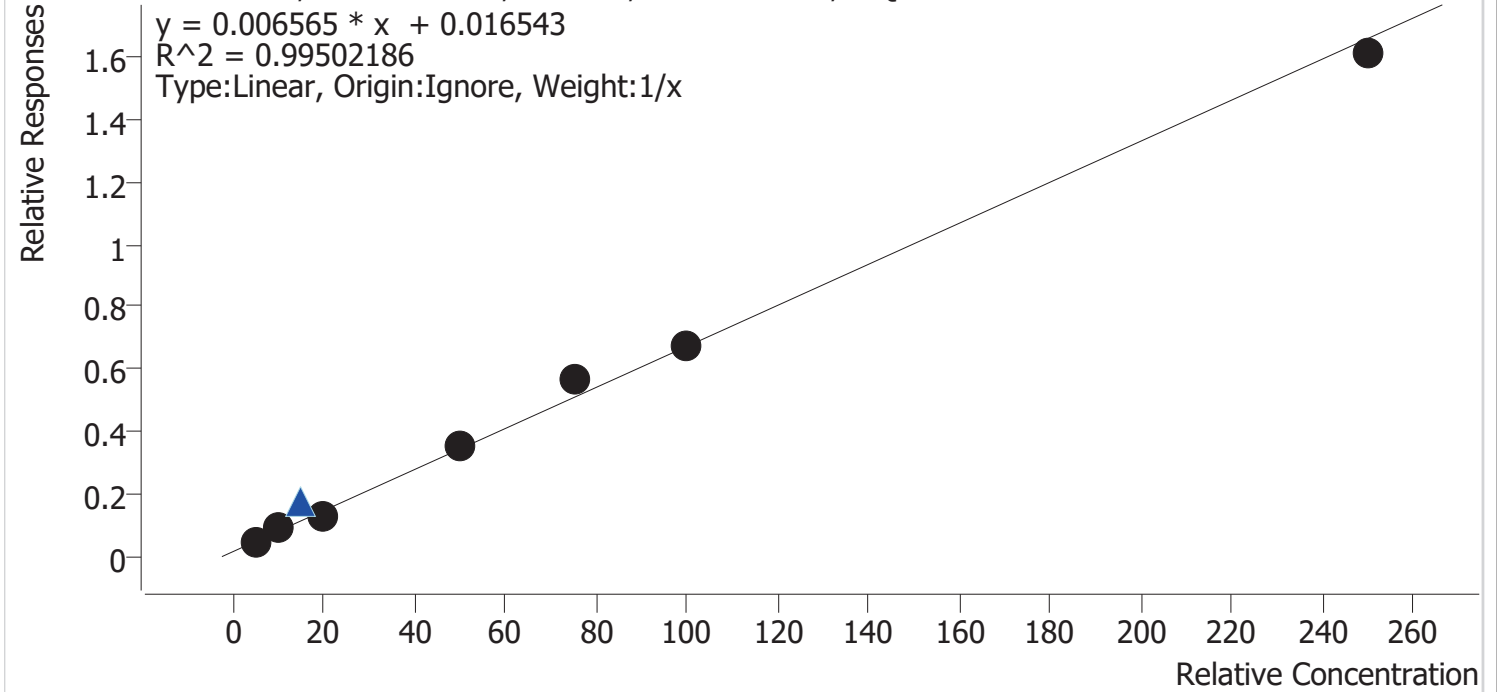
Compound Calibration Report



Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Last Cal. Update 10/16/2020 4:24 PM
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



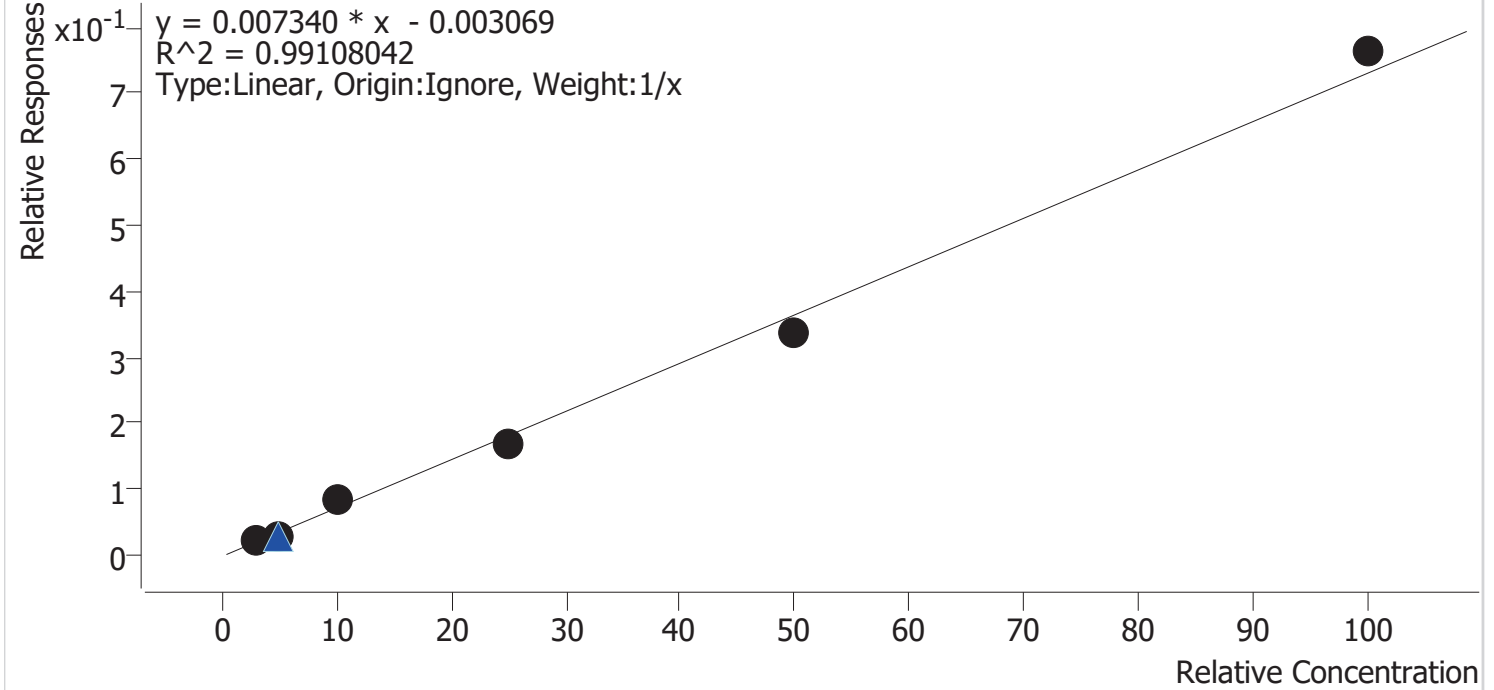
| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|--------|-------|---------|------------------------|---------------------|----------|
| CAL 1 | 1 | ✓ | 5.0 | 4.8 | 95.1 |
| CAL 2 | 2 | ✓ | 10.0 | 11.0 | 109.7 |
| CAL 3 | 3 | ✓ | 20.0 | 16.7 | 83.4 |
| CAL 4 | 4 | ✓ | 50.0 | 51.8 | 103.6 |
| CAL 5 | 5 | ✓ | 75.0 | 83.6 | 111.5 |
| CAL 6 | 6 | ✓ | 100.0 | 99.6 | 99.6 |
| CAL 7 | 7 | ✓ | 250.0 | 242.6 | 97.0 |

Compound Calibration Report



Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Last Cal. Update 10/16/2020 4:24 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

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



| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|--------|-------|---------|------------------------|---------------------|----------|
| CAL 2 | 2 | ✓ | 3.0 | 3.5 | 116.2 |
| CAL 3 | 3 | ✓ | 5.0 | 3.8 | 75.7 |
| CAL 4 | 4 | ✓ | 10.0 | 11.7 | 117.4 |
| CAL 5 | 5 | ✓ | 25.0 | 23.6 | 94.4 |
| CAL 6 | 6 | ✓ | 50.0 | 46.0 | 92.0 |
| CAL 7 | 7 | ✓ | 100.0 | 104.4 | 104.4 |

GA

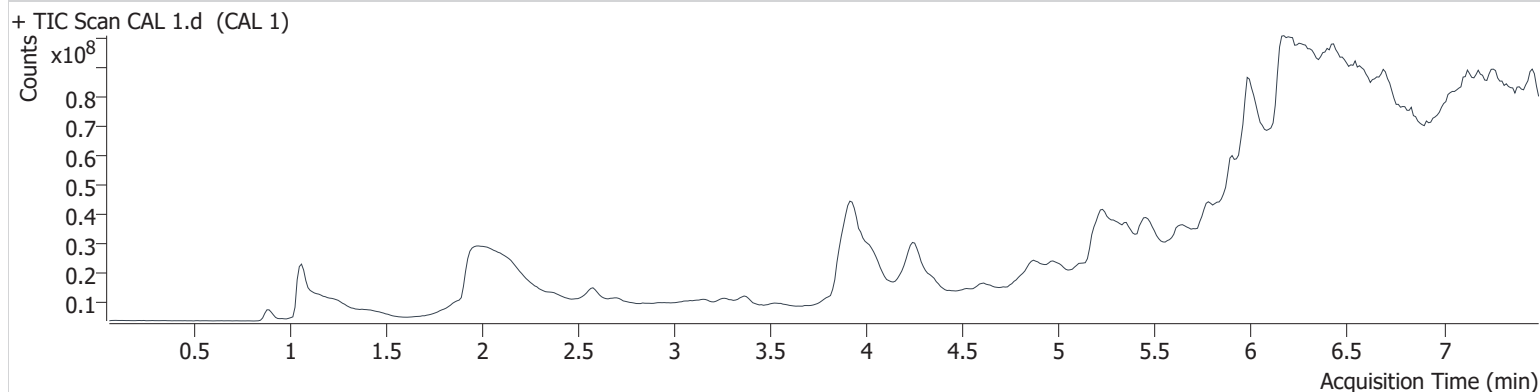
AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

| | | | |
|-------------------------|-----------------------|------------------|-----------|
| Instrument | 69679 | Data File | CAL 1.d |
| Type | Cal | Sample | CAL 1 |
| Acq. Method | THC Screen 1122.m | Operator | Anne Nord |
| Sample Position | P2-A1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 10/6/2020 11:45:48 AM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|--------|---------------|----------------------|------------|--------------|
| THC-COOH | 6.661 | 105262 | -3.49 | 67.0 | 2203339 | 4.7574 ng/ml |

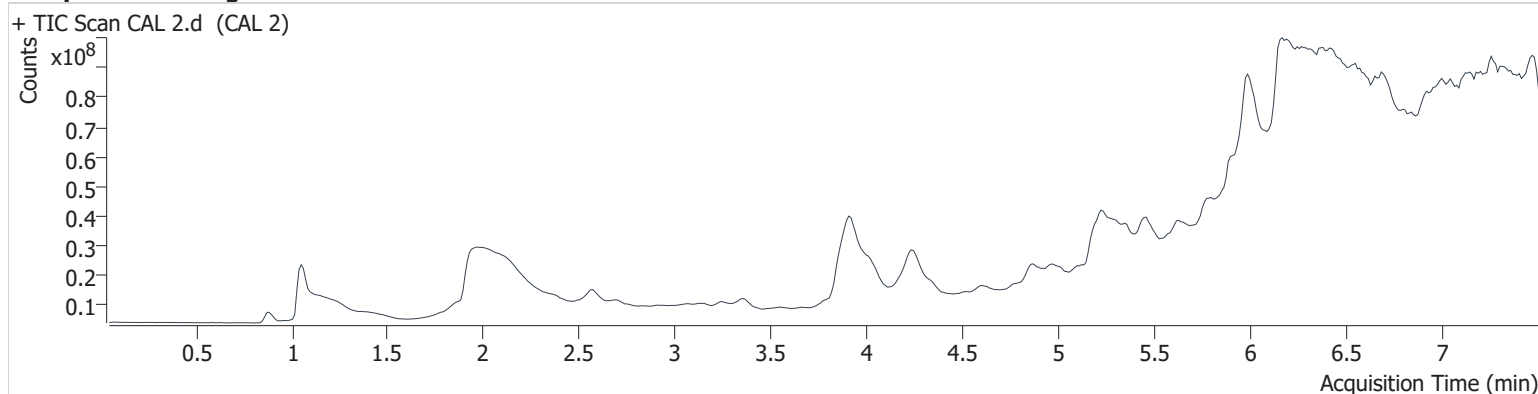
GA

AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

Instrument 69679 **Data File** CAL 2.d
Type Cal **Sample** CAL 2
Acq. Method THC Screen 1122.m **Operator** Anne Nord
Sample Position P2-B1 **Comment**
Injection Volume 10
Acq. Date-Time 10/6/2020 11:55:32 AM
Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|--------|---------------|----------------------|------------|---------------|
| THC-COOH | 6.646 | 194230 | -0.43 | 73.3 | 2192906 | 10.9721 ng/ml |
| THC | 7.152 | 31931 | -1.73 | 51.5 | 1418086 | 3.4861 ng/ml |

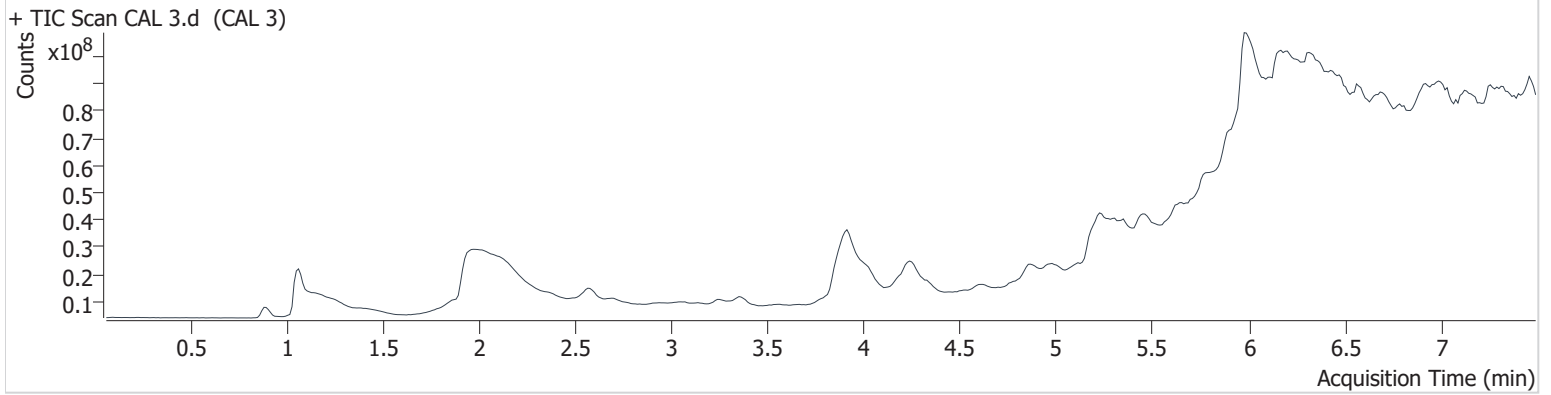
GA

AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

Instrument 69679 **Data File** CAL 3.d
Type Cal **Sample** CAL 3
Acq. Method THC Screen 1122.m **Operator** Anne Nord
Sample Position P2-C1 **Comment**
Injection Volume 10
Acq. Date-Time 10/6/2020 12:05:08 PM
Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|--------|---------------|----------------------|------------|---------------|
| THC-COOH | 6.649 | 297434 | 1.03 | 76.5 | 2359613 | 16.6814 ng/ml |
| THC | 7.155 | 34028 | -0.85 | 0.0 | 1377692 | 3.7834 ng/ml |

GA

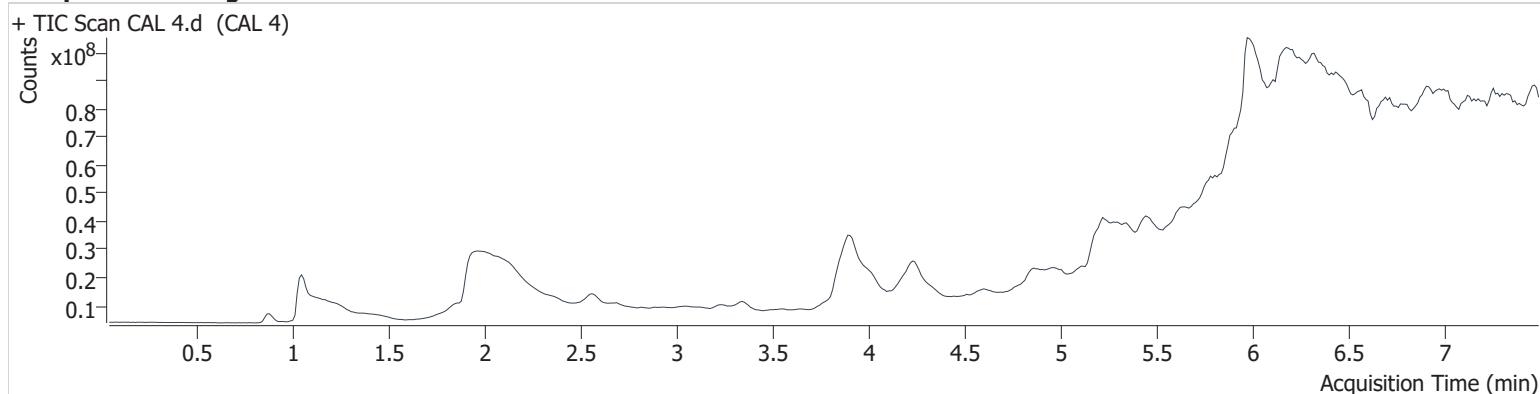
AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

| | | | |
|-------------------------|-----------------------|------------------|-----------|
| Instrument | 69679 | Data File | CAL 4.d |
| Type | Cal | Sample | CAL 4 |
| Acq. Method | THC Screen 1122.m | Operator | Anne Nord |
| Sample Position | P2-D1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 10/6/2020 12:14:42 PM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|--------|---------------|----------------------|------------|---------------|
| THC-COOH | 6.647 | 843148 | 2.32 | 86.8 | 2363834 | 51.8139 ng/ml |
| THC | 7.176 | 123731 | 2.14 | 43.5 | 1489156 | 11.7388 ng/ml |

GA

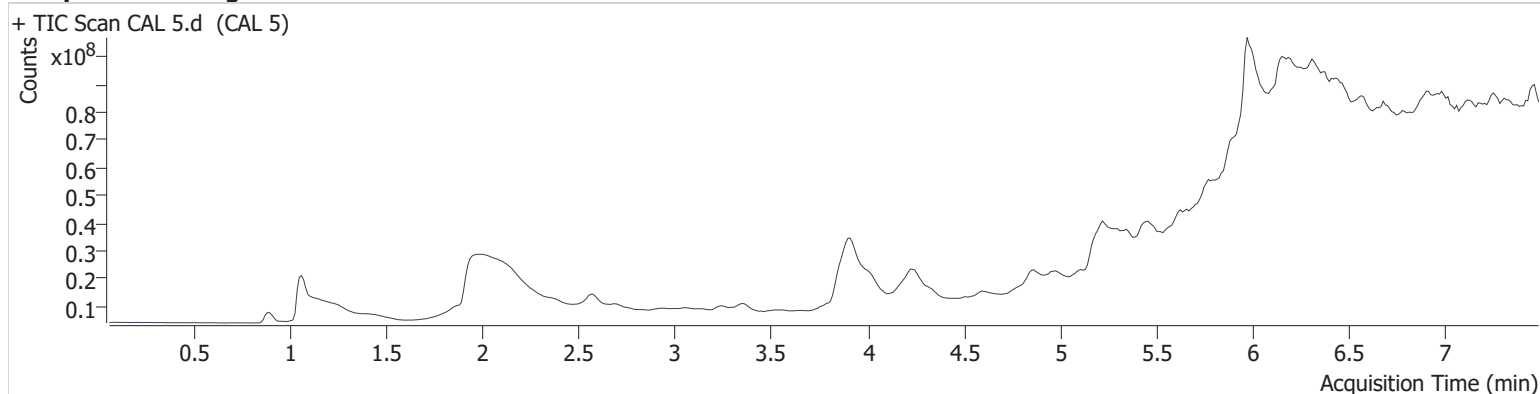
AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

| | | | |
|-------------------------|-----------------------|------------------|-----------|
| Instrument | 69679 | Data File | CAL 5.d |
| Type | Cal | Sample | CAL 5 |
| Acq. Method | THC Screen 1122.m | Operator | Anne Nord |
| Sample Position | P2-E1 | Comment | |
| Injection Volume | 10 | | |
| Acq. Date-Time | 10/6/2020 12:24:18 PM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|---------|---------------|----------------------|------------|---------------|
| THC-COOH | 6.649 | 1289889 | 1.66 | 93.9 | 2281106 | 83.6173 ng/ml |
| THC | 7.154 | 239393 | 3.21 | 58.2 | 1407519 | 23.5915 ng/ml |

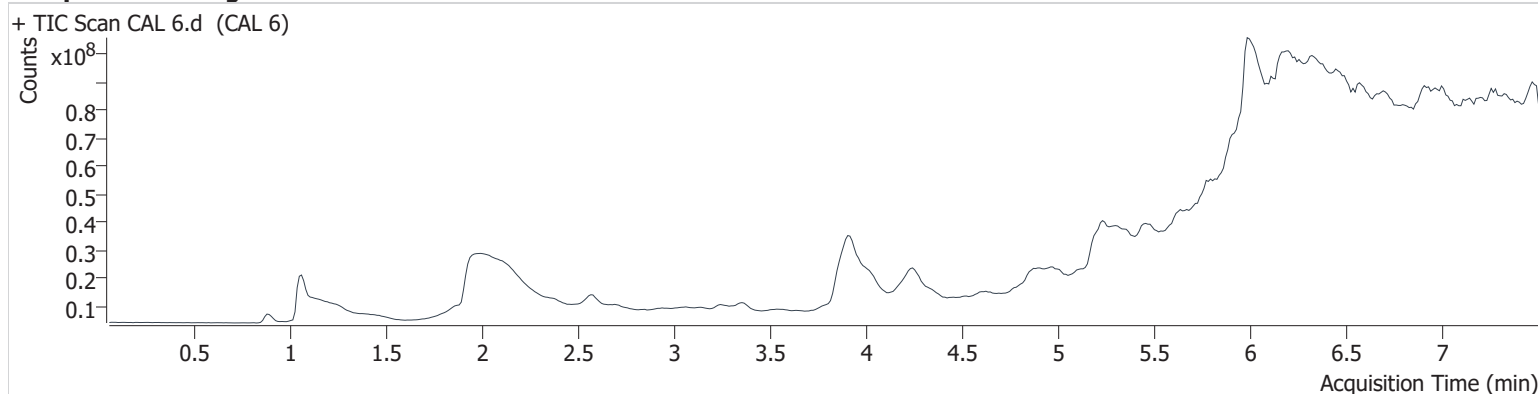
OA

AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

Instrument 69679 **Data File** CAL 6.d
Type Cal **Sample** CAL 6
Acq. Method THC Screen 1122.m **Operator** Anne Nord
Sample Position P2-F1 **Comment**
Injection Volume 10
Acq. Date-Time 10/6/2020 12:33:53 PM
Sample Info.

Sample Chromatogram



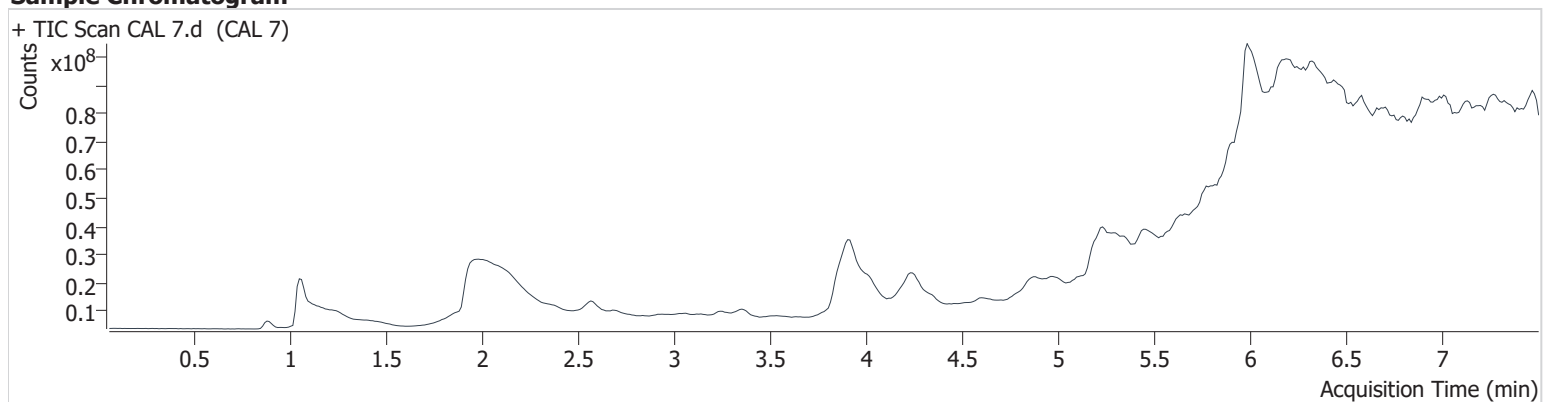
| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|---------|---------------|----------------------|------------|---------------|
| THC-COOH | 6.650 | 1632371 | 3.80 | 97.5 | 2435618 | 99.5725 ng/ml |
| THC | 7.156 | 465402 | 2.96 | 79.1 | 1391893 | 45.9750 ng/ml |

AM #30 Cannabinoids

Batch results D:\MassHunter\Data\2020\am 30\100620\QuantResults\cann.batch.bin
Calibration Last Update 10/16/2020 4:24:52 PM

Instrument 69679 **Data File** CAL 7.d
Type Cal **Sample** CAL 7
Acq. Method THC Screen 1122.m **Operator** Anne Nord
Sample Position P2-G1 **Comment**
Injection Volume 10
Acq. Date-Time 10/6/2020 12:43:29 PM
Sample Info.

Sample Chromatogram



| Name | RT | Resp. | Mass Accuracy | Mass Abundance Score | ISTD Resp. | Final Conc. |
|----------|-------|---------|---------------|----------------------|------------|----------------|
| THC-COOH | 6.653 | 3385569 | 3.86 | 99.4 | 2104085 | 242.5853 ng/ml |
| THC | 7.159 | 1053597 | 3.20 | 95.3 | 1380204 | 104.4253 ng/ml |