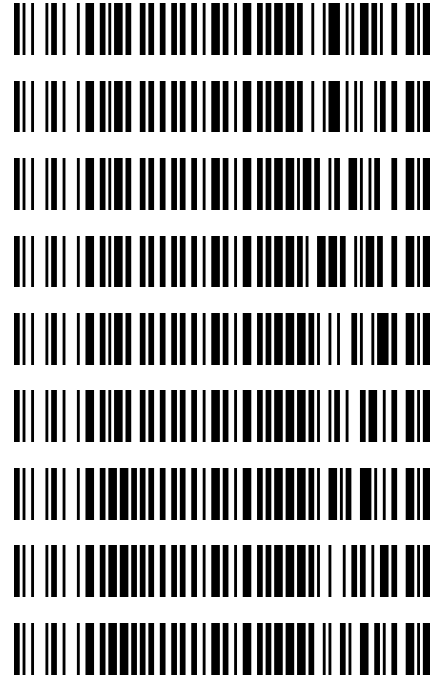


Worklist: 4136

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2020-0944	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-0945	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-0981	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-1029	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-1049	10	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-1085	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-0910	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-0914	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-0920	1	BCK	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 03/30/20
 Plate lot#: IDP-108-2-200303

Analyst: Sarah Pickle
 Plate Expiration: 09/03/20

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: Hemostat 445283-3
LCMS-QQQ ID: 069901

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. Pipette **1000 µL blood (calibrated pipette)** in wells of analytical (standards) plate. **Pipette ID: 3382167**
- 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\2020\AM 27-28\033020 AM 27 SP Batch Name: AM 27
- 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r² 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: *Curves limited: THC 3-100, COOH 10-250, THC-OH 5-100 (reported qualitatively)*

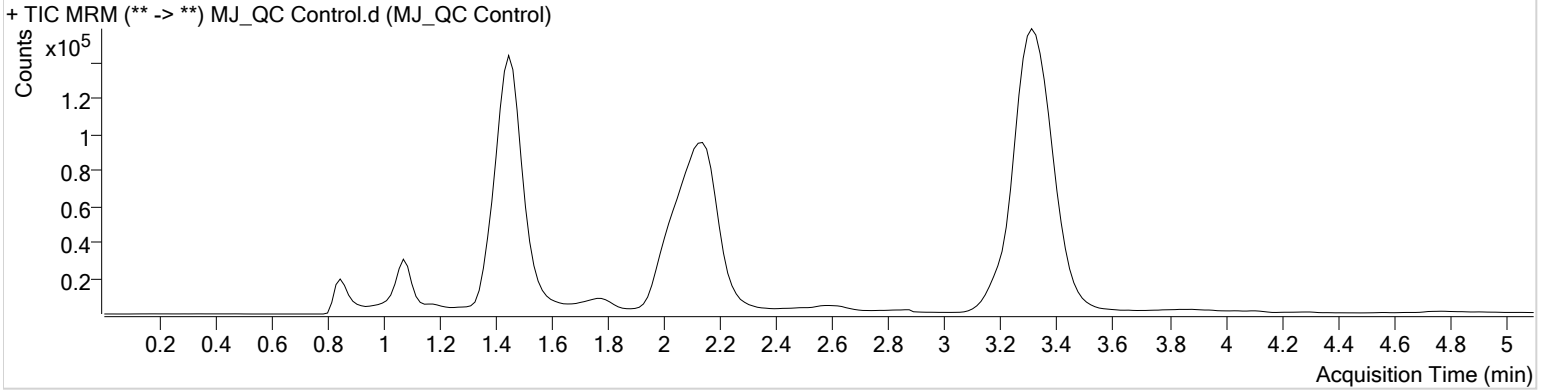


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument Falco **Data File** MJ_QC Control.d
Type Sample **Sample** MJ_QC Control
Acq. Method AM 27 THC quant.m **Operator** Sarah Pickle
Sample Position P3-A6 **Comment**
Injection Volume 10
Acq. Date-Time 3/30/2020 4:19:22 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	103652	∞	9.4	∞	583914	4.4341 ng/ml
THC-COOH	1.474	70973	∞	47.0	∞	176069	16.6060 ng/ml
THC	3.330	58515	400.02	28.2	44.93	1494370	4.5732 ng/ml

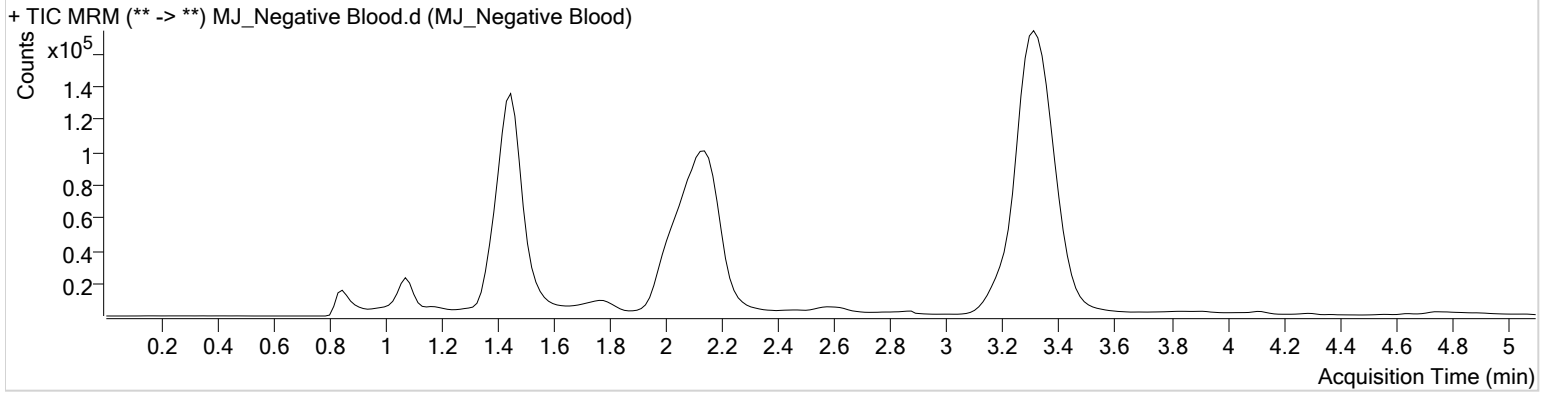
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument	Falco	Data File	MJ_Negative Blood.d
Type	Sample	Sample	MJ_Negative Blood
Acq. Method	AM 27 THC quant.m	Operator	Sarah Pickle
Sample Position	P3-H5	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2020 4:34:34 PM		
Sample Info.			

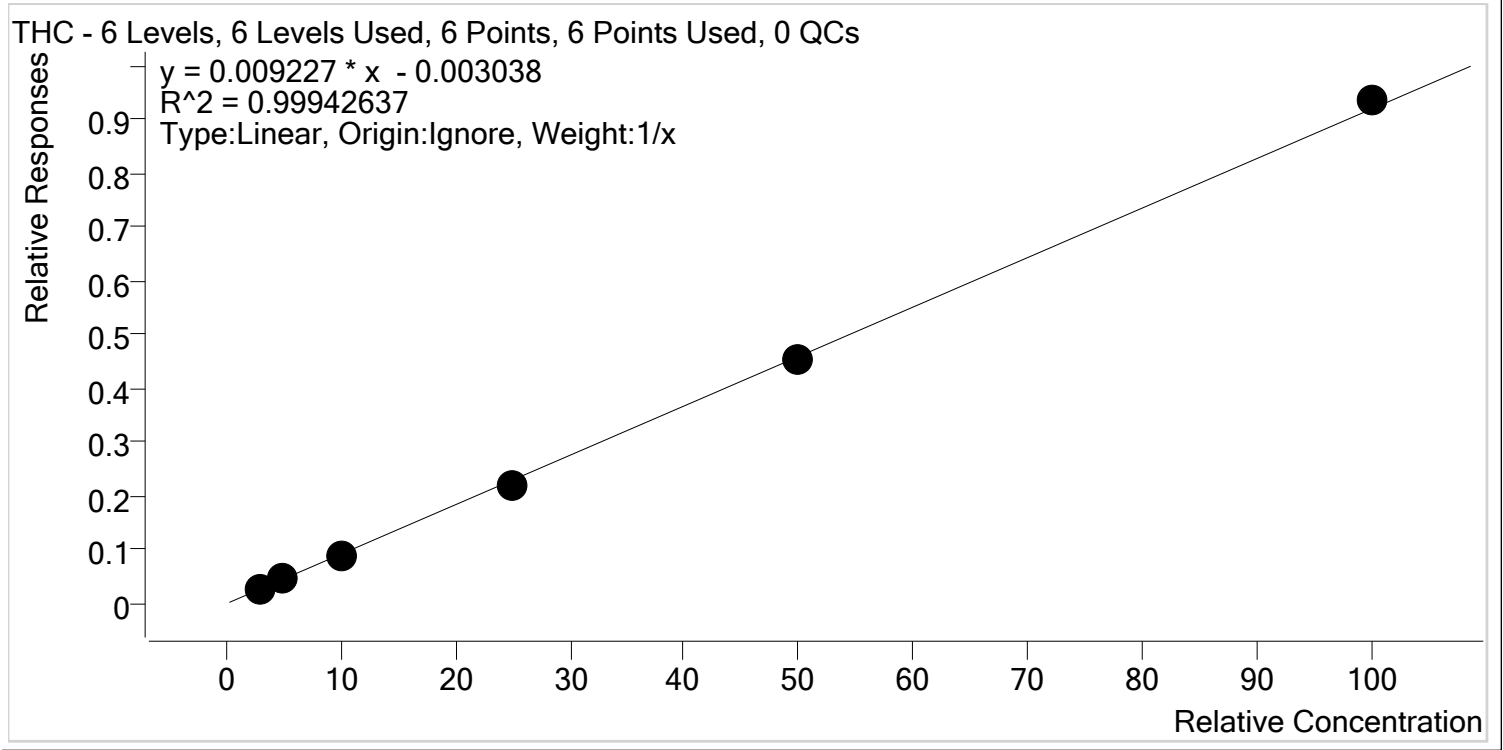
Sample Chromatogram





AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Last Cal. Update 4/1/2020 10:30 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



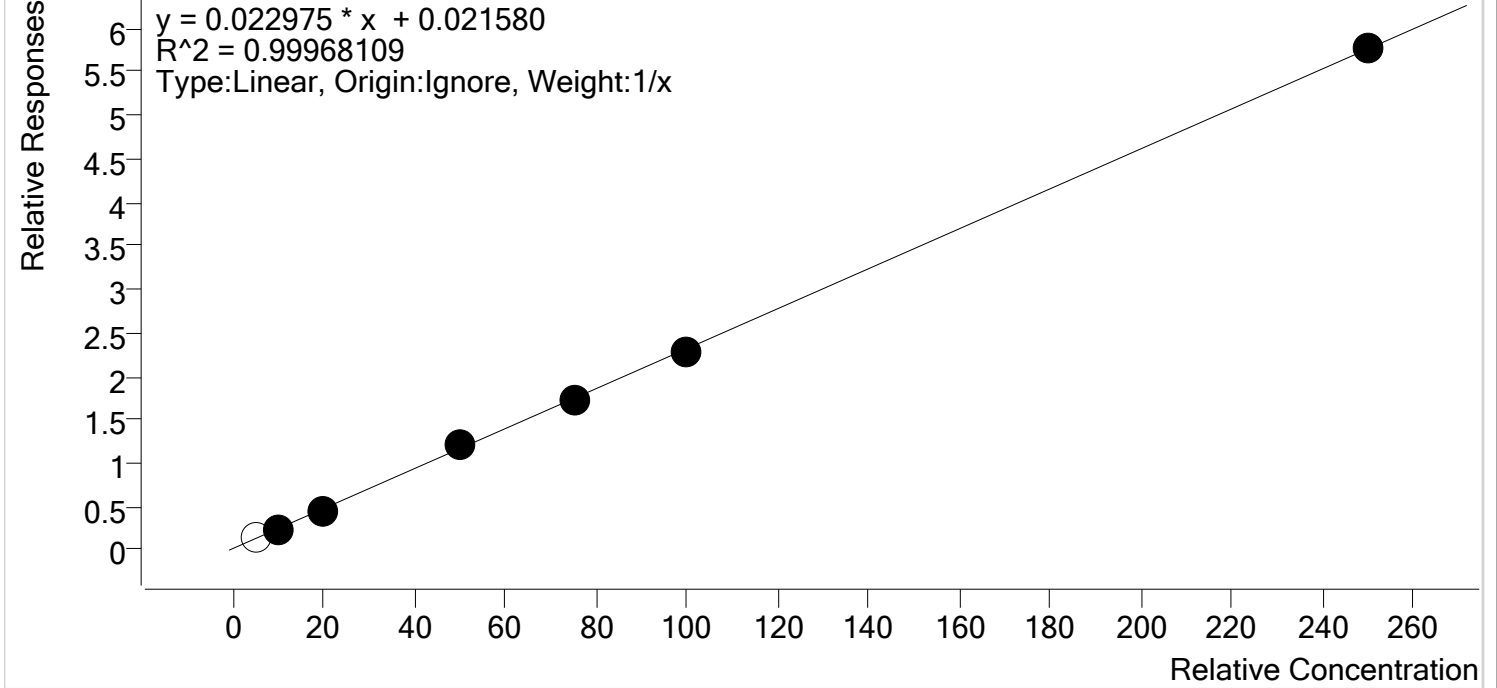
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 2	2	✓	3.0	3.1	102.9
MJ_Cal 3	3	✓	5.0	5.1	102.4
MJ_Cal 4	4	✓	10.0	9.8	98.3
MJ_Cal 5	5	✓	25.0	24.1	96.3
MJ_Cal 6	6	✓	50.0	49.3	98.5
MJ_Cal 7	7	✓	100.0	101.6	101.6



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Last Cal. Update 4/1/2020 10:30 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

THC-COOH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs



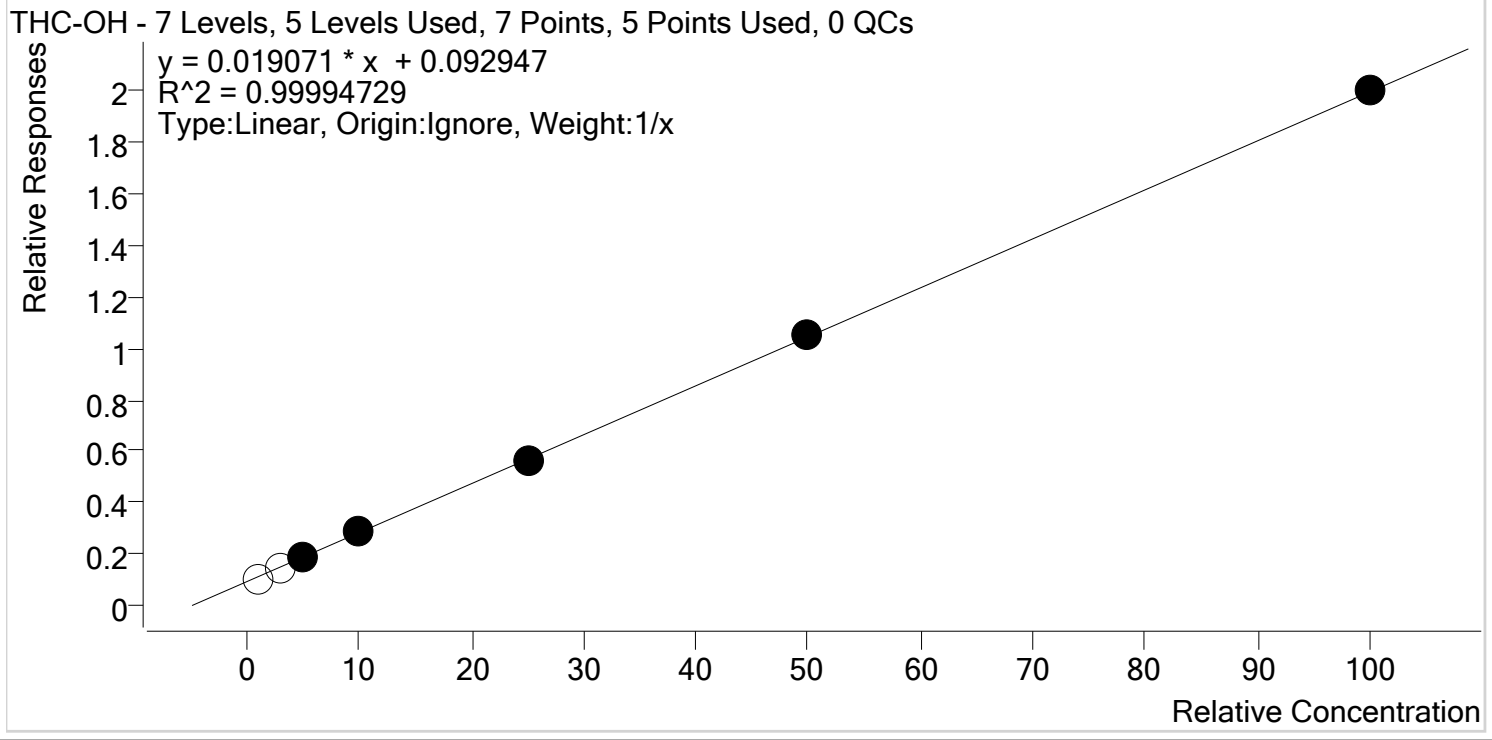
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	x	5.0	5.8	116.3
MJ_Cal 2	2	✓	10.0	10.1	101.3
MJ_Cal 3	3	✓	20.0	19.3	96.5
MJ_Cal 4	4	✓	50.0	51.8	103.6
MJ_Cal 5	5	✓	75.0	74.7	99.6
MJ_Cal 6	6	✓	100.0	99.0	99.0
MJ_Cal 7	7	✓	250.0	250.1	100.0

§



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Last Cal. Update 4/1/2020 10:30 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	x	1.0	0.6	59.9
MJ_Cal 2	2	x	3.0	3.0	99.2
MJ_Cal 3	3	✓	5.0	5.0	100.5
MJ_Cal 4	4	✓	10.0	10.1	100.7
MJ_Cal 5	5	✓	25.0	24.6	98.5
MJ_Cal 6	6	✓	50.0	50.0	100.1
MJ_Cal 7	7	✓	100.0	100.2	100.2

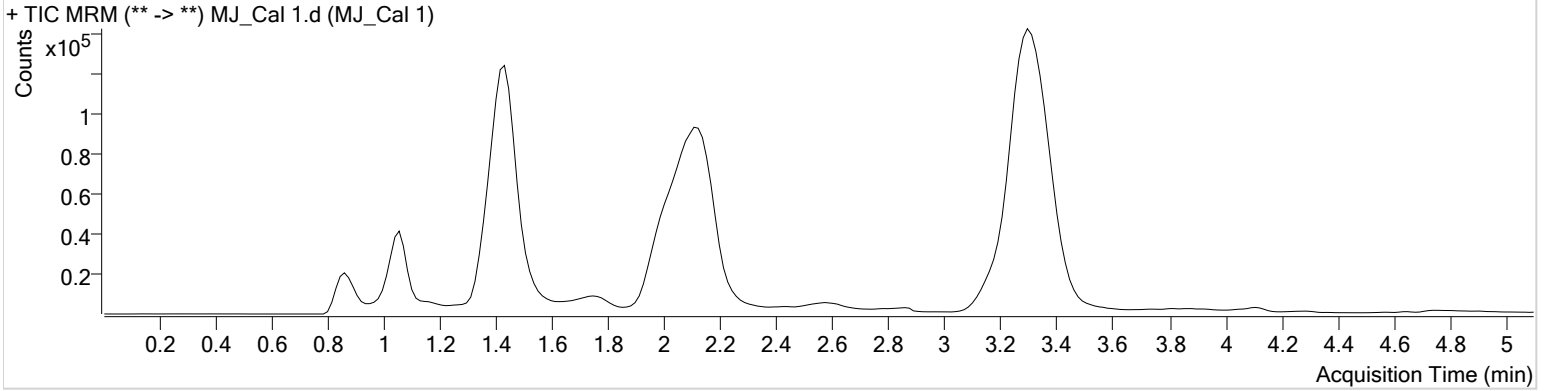


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument Falco **Data File** MJ_Cal 1.d
Type Cal **Sample** MJ_Cal 1
Acq. Method AM 27 THC quant.m **Operator** Sarah Pickle
Sample Position P3-H6 **Comment**
Injection Volume 10
Acq. Date-Time 3/30/2020 3:18:31 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	61812	∞	4.0 Low	7.03 Low	592250	0.5989 ng/ml Low
THC-COOH	1.459	25556	55.69	40.9 Low	102.69	164679	5.8153 ng/ml Low

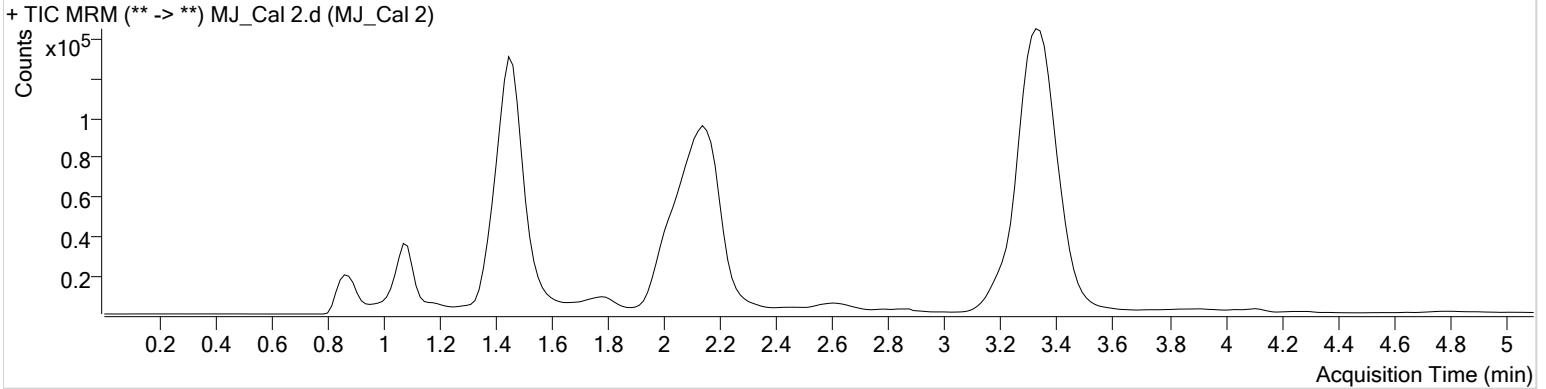


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument Falco Data File MJ_Cal 2.d
Type Cal Sample MJ_Cal 2
Acq. Method AM 27 THC quant.m Operator Sarah Pickle
Sample Position P3-G6 Comment
Injection Volume 10
Acq. Date-Time 3/30/2020 3:26:16 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.498	86320	∞	7.2 Low	44.70	576638	2.9756 ng/ml Low
THC-COOH	1.489	42334	362.66	48.1	321.08	166526	10.1258 ng/ml
THC	3.345	35998	228.55	30.7	37.39	1414030	3.0884 ng/ml

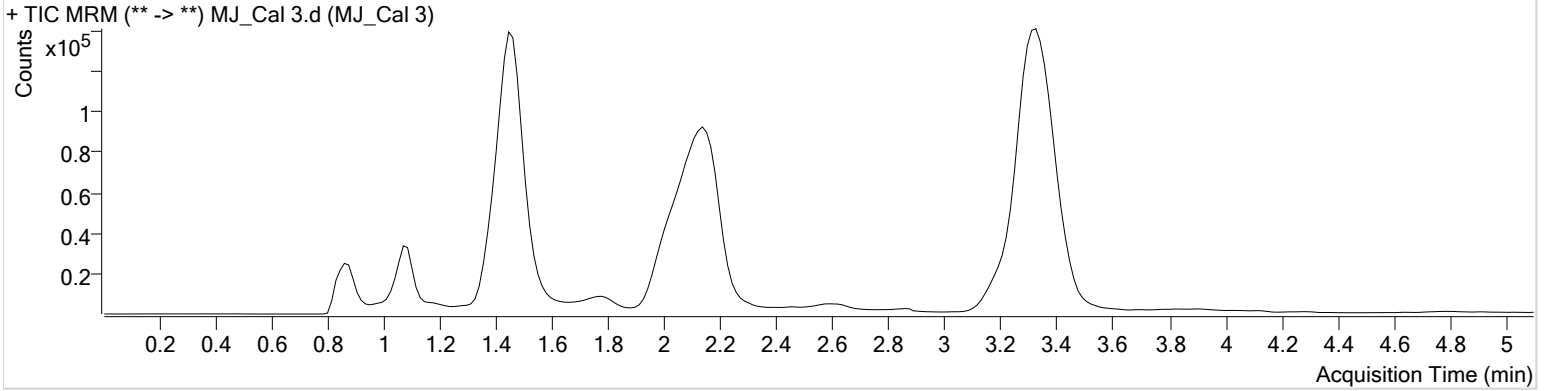


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument Falco **Data File** MJ_Cal 3.d
Type Cal **Sample** MJ_Cal 3
Acq. Method AM 27 THC quant.m **Operator** Sarah Pickle
Sample Position P3-F6 **Comment**
Injection Volume 10
Acq. Date-Time 3/30/2020 3:33:50 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	105718	∞	9.3	∞	560117	5.0230 ng/ml
THC-COOH	1.489	79834	∞	50.8	345.97	171646	19.3050 ng/ml
THC	3.330	59133	275.85	27.0	39.65	1338244	5.1183 ng/ml

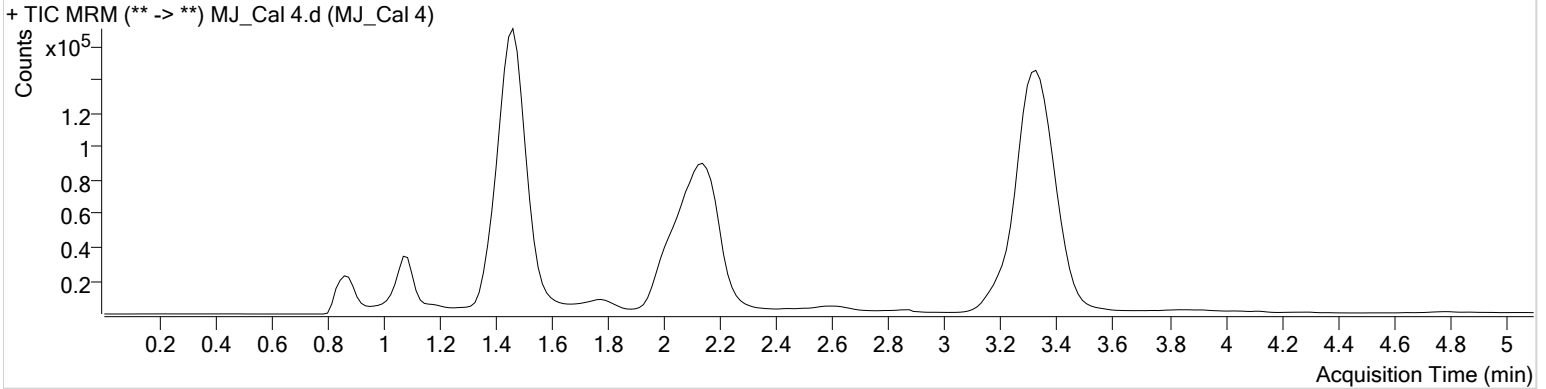


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument Falco **Data File** MJ_Cal 4.d
Type Cal **Sample** MJ_Cal 4
Acq. Method AM 27 THC quant.m **Operator** Sarah Pickle
Sample Position P3-E6 **Comment**
Injection Volume 10
Acq. Date-Time 3/30/2020 3:41:25 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	155823	∞	10.3	207.25	546825	10.0681 ng/ml
THC-COOH	1.489	194134	186.04	52.8	700.74	160198	51.8073 ng/ml
THC	3.330	113239	519.32	28.5	∞	1292328	9.8261 ng/ml

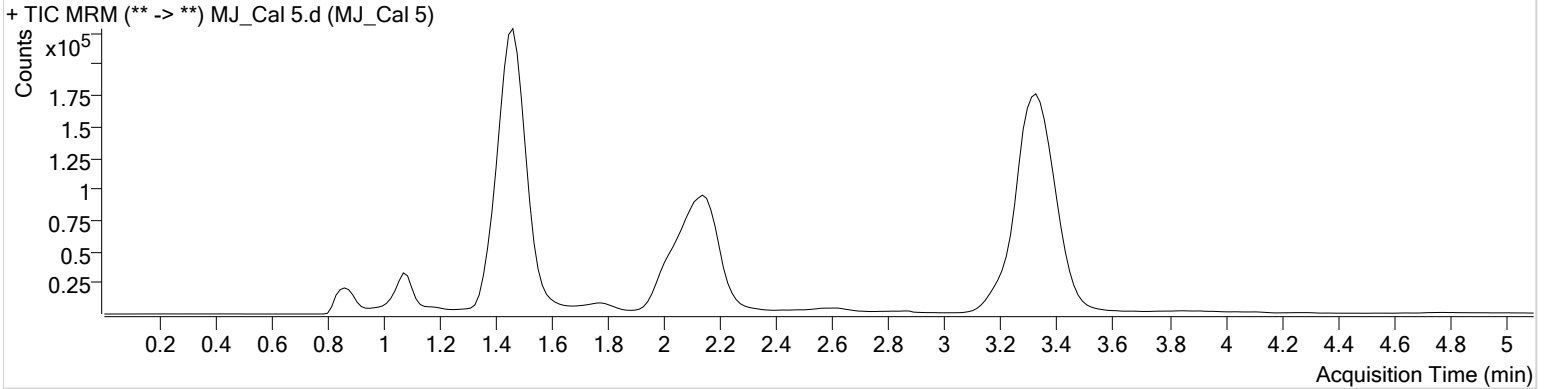


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument Falco **Data File** MJ_Cal 5.d
Type Cal **Sample** MJ_Cal 5
Acq. Method AM 27 THC quant.m **Operator** Sarah Pickle
Sample Position P3-D6 **Comment**
Injection Volume 10
Acq. Date-Time 3/30/2020 3:49:00 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	326107	∞	12.0	398.16	579460	24.6353 ng/ml
THC-COOH	1.489	288581	1016.77	57.3	2210.46	166076	74.6932 ng/ml
THC	3.330	303231	1546.19	27.6	∞	1384385	24.0689 ng/ml

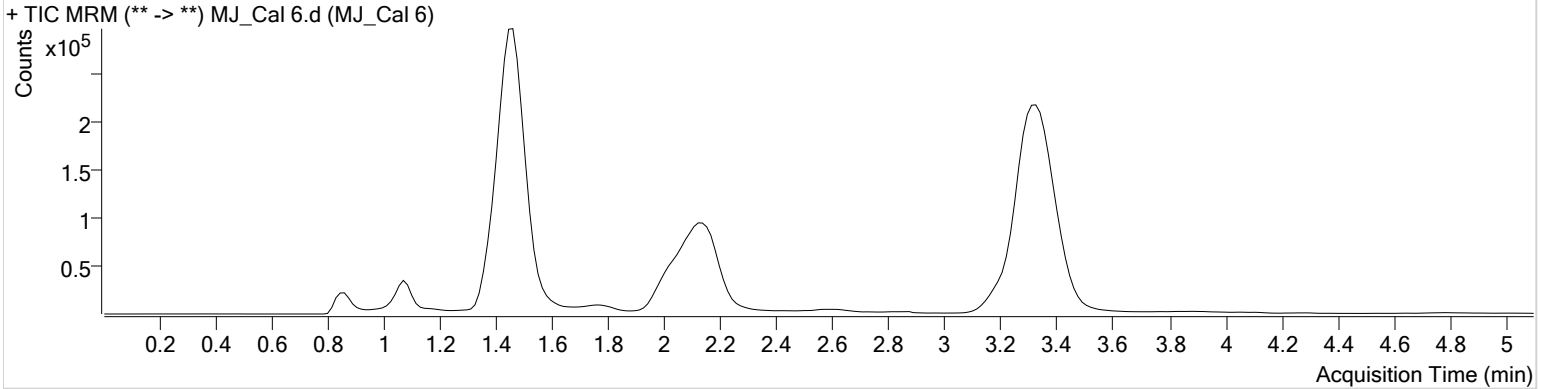


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument Falco **Data File** MJ_Cal 6.d
Type Cal **Sample** MJ_Cal 6
Acq. Method AM 27 THC quant.m **Operator** Sarah Pickle
Sample Position P3-C6 **Comment**
Injection Volume 10
Acq. Date-Time 3/30/2020 3:56:35 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	601587	∞	12.8	443.72	574373	50.0451 ng/ml
THC-COOH	1.474	386032	∞	57.4	1703.34	168172	98.9728 ng/ml
THC	3.330	629258	1491.14	25.0	701.43	1394032	49.2522 ng/ml

AM #27 Cannabinoid Quant. Results

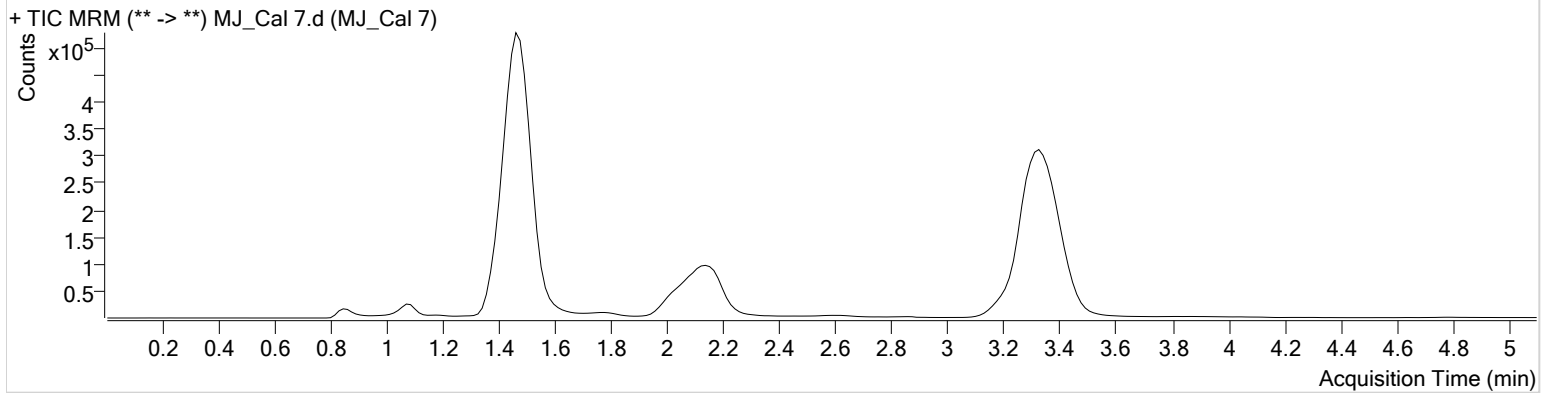


Batch results D:\MassHunter\Data\2020\AM 27-28\033020 AM 27 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/1/2020 10:30:52 AM

Instrument	Falco	Data File	MJ_Cal 7.d
Type	Cal	Sample	MJ_Cal 7
Acq. Method	AM 27 THC quant.m	Operator	Sarah Pickle
Sample Position	P3-B6	Comment	
Injection Volume	10		
Acq. Date-Time	3/30/2020 4:04:10 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	1142008	∞	12.9	672.98	569735	100.2286 ng/ml
THC-COOH	1.489	937596	1334.45	58.6	2926.99	162566	250.0958 ng/ml
THC	3.330	1353014	4540.99	26.0	408.24	1447359	101.6462 ng/ml