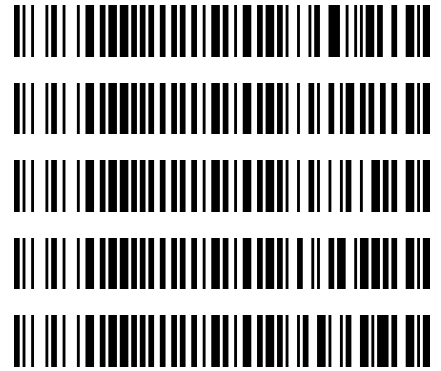


Worklist: 4594

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
P2020-2951	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-2969	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-2972	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-3003	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-3011	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: 11/05/20
 Plate lot#: IDP-108-2-200723

Analyst: Sarah Pickle
 Plate Expiration: 01/23/21

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 445283-4

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: 3382167**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 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)
- 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.
- 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.
- 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: 5ng/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: *THC-OH 5-100 (reported qualitatively)*

A new column was installed 11/05/20 and the samples were injected but due to poor peak shape, the original column was reinstalled and the samples were reinjected 11/06/20. The reinject data was used for analysis.

\$

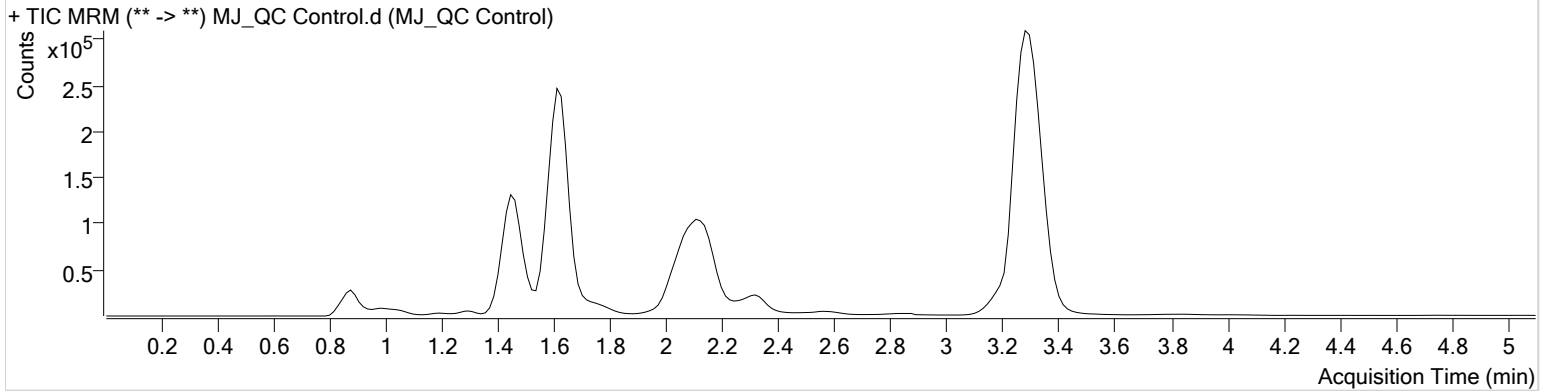


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument	Falco	Data File	MJ_QC Control.d
Type	Sample	Sample	MJ_QC Control
Acq. Method	AM 27 THCQ.m	Operator	Sarah Pickle
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	11/6/2020 4:01:08 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	77375	∞	8.8	∞	411524	5.4994 ng/ml
THC-COOH	1.474	52814	∞	56.7	∞	105850	14.2274 ng/ml
THC	3.300	99535	545.96	28.2	88.86	2092234	4.6243 ng/ml

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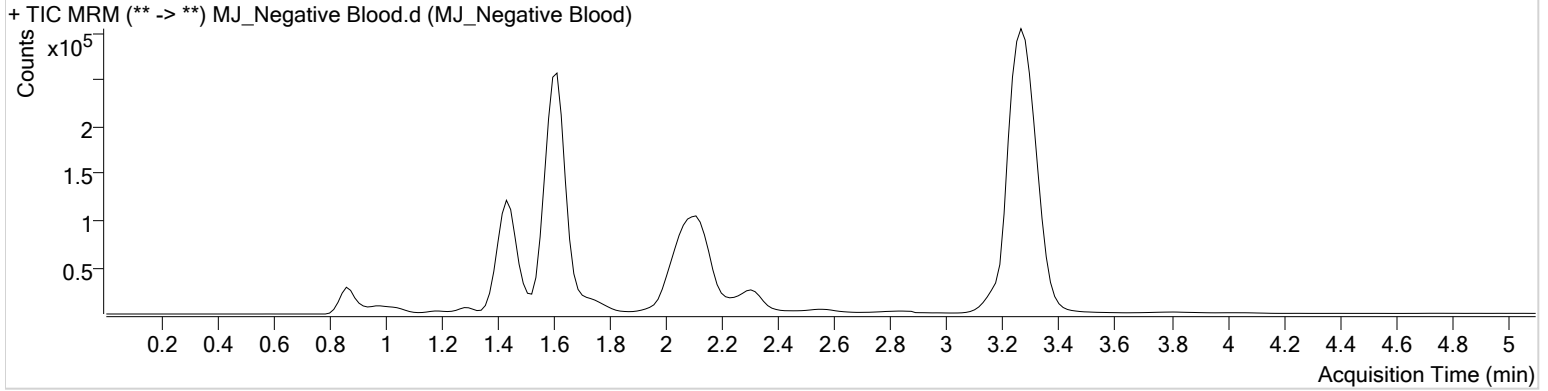


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument	Falco	Data File	MJ_Negative Blood.d
Type	Sample	Sample	MJ_Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Sarah Pickle
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	11/6/2020 4:16:22 PM		
Sample Info.			

Sample Chromatogram



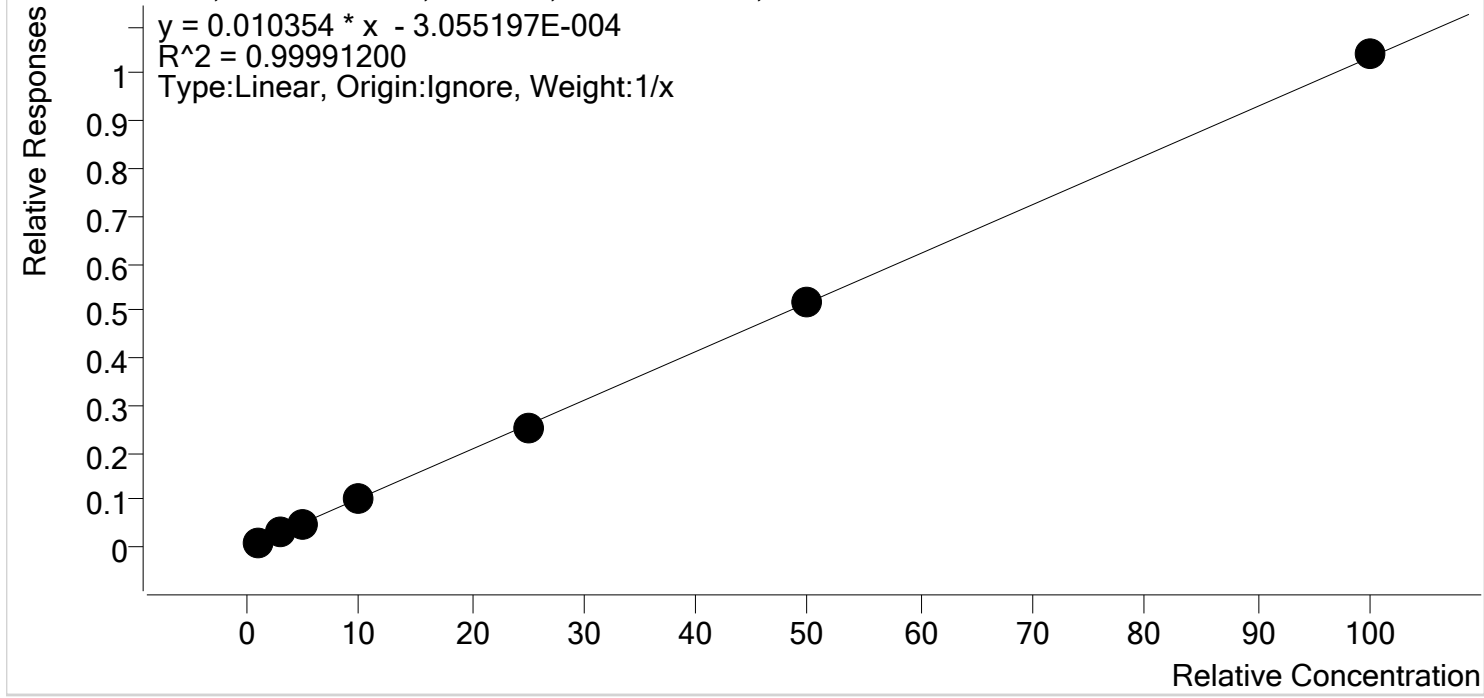
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Last Cal. Update 11/9/2020 11:27 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

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

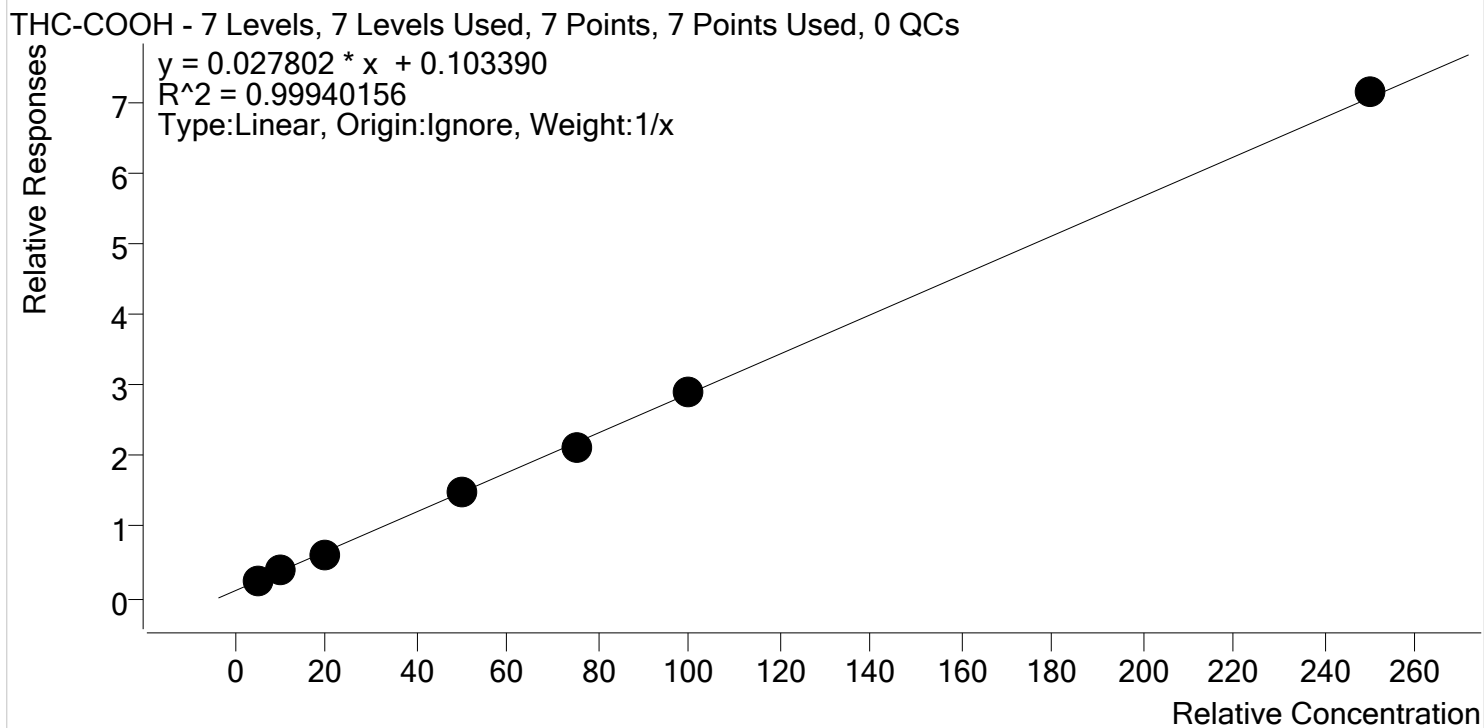


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	✓	1.0	1.0	102.6
MJ_Cal 2	2	✓	3.0	3.0	100.3
MJ_Cal 3	3	✓	5.0	5.0	100.3
MJ_Cal 4	4	✓	10.0	9.8	97.7
MJ_Cal 5	5	✓	25.0	24.7	98.8
MJ_Cal 6	6	✓	50.0	49.9	99.7
MJ_Cal 7	7	✓	100.0	100.6	100.6



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Last Cal. Update 11/9/2020 11:27 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

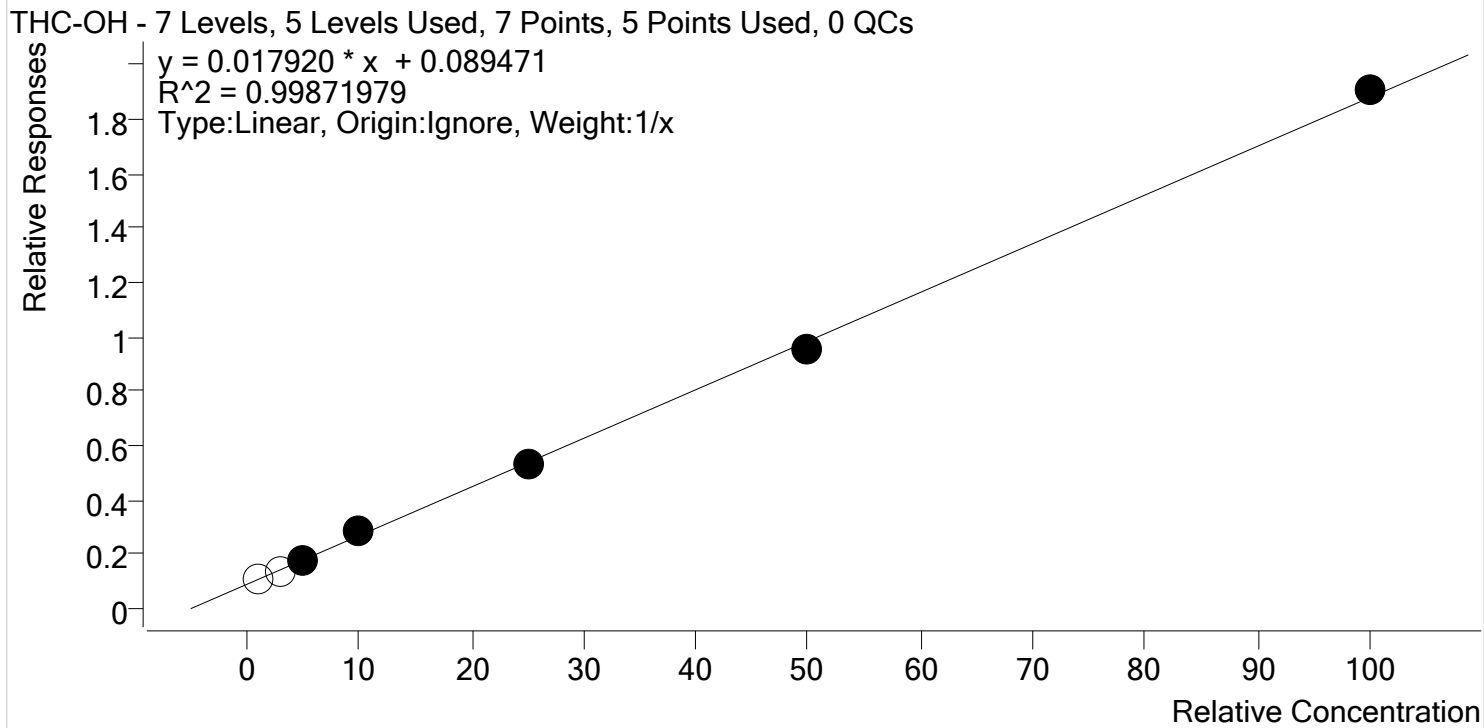


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	✓	5.0	5.2	104.5
MJ_Cal 2	2	✓	10.0	10.3	103.4
MJ_Cal 3	3	✓	20.0	19.0	94.8
MJ_Cal 4	4	✓	50.0	49.4	98.7
MJ_Cal 5	5	✓	75.0	72.2	96.3
MJ_Cal 6	6	✓	100.0	101.2	101.2
MJ_Cal 7	7	✓	250.0	252.7	101.1



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Last Cal. Update 11/9/2020 11:27 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	1.2	123.0
MJ_Cal 2	2	x	3.0	2.5	84.4
MJ_Cal 3	3	✓	5.0	4.8	95.7
MJ_Cal 4	4	✓	10.0	10.8	108.2
MJ_Cal 5	5	✓	25.0	24.4	97.6
MJ_Cal 6	6	✓	50.0	48.5	97.1
MJ_Cal 7	7	✓	100.0	101.5	101.5

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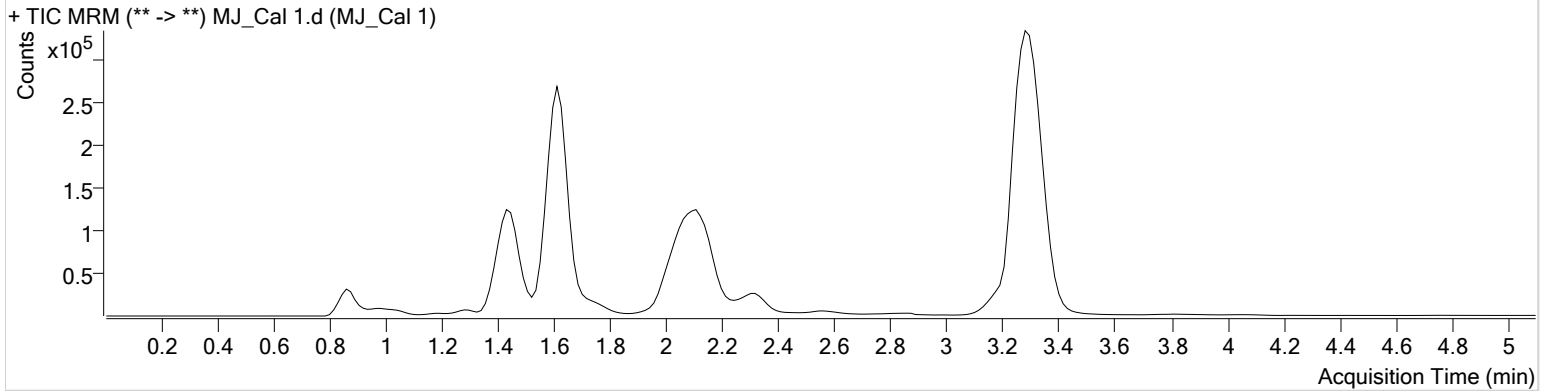


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument	Falco	Data File	MJ_Cal 1.d
Type	Cal	Sample	MJ_Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Sarah Pickle
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	11/6/2020 3:00:08 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	55215	∞	4.5 Low	11.95	495164	1.2298 ng/ml Low
THC-COOH	1.459	29383	∞	53.1	∞	118138	5.2270 ng/ml
THC	3.300	25448	188.55	34.5	70.69	2466082	1.0262 ng/ml Low

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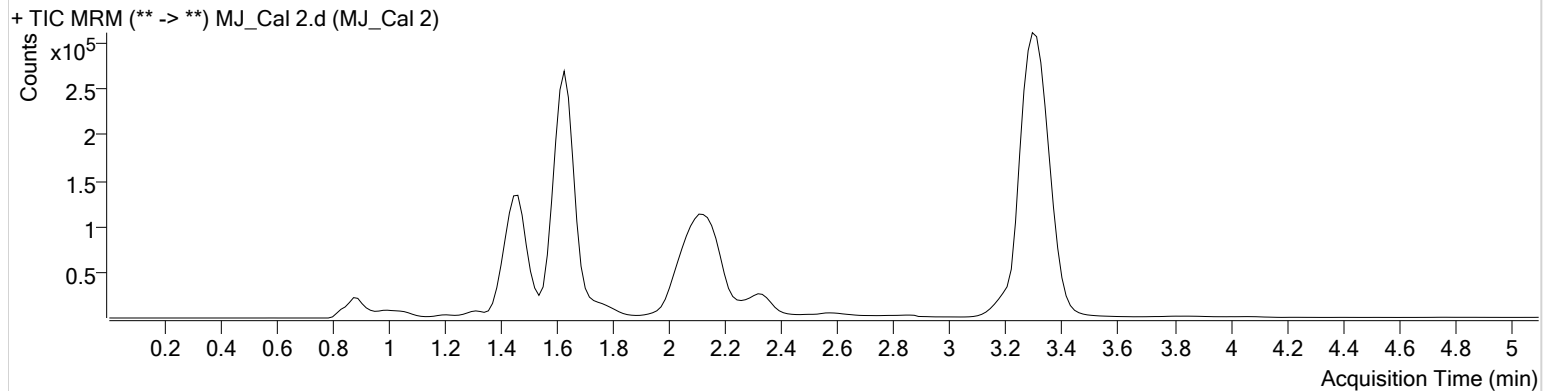


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument Falco **Data File** MJ_Cal 2.d
Type Cal **Sample** MJ_Cal 2
Acq. Method AM 27 THCQ.m **Operator** Sarah Pickle
Sample Position P3-B1 **Comment**
Injection Volume 10
Acq. Date-Time 11/6/2020 3:07:55 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.498	68147	∞	6.9 Low	19.93	505289	2.5333 ng/ml Low
THC-COOH	1.474	45596	∞	50.5	∞	116662	10.3389 ng/ml
THC	3.315	69082	563.19	28.1	53.61	2239928	3.0083 ng/ml

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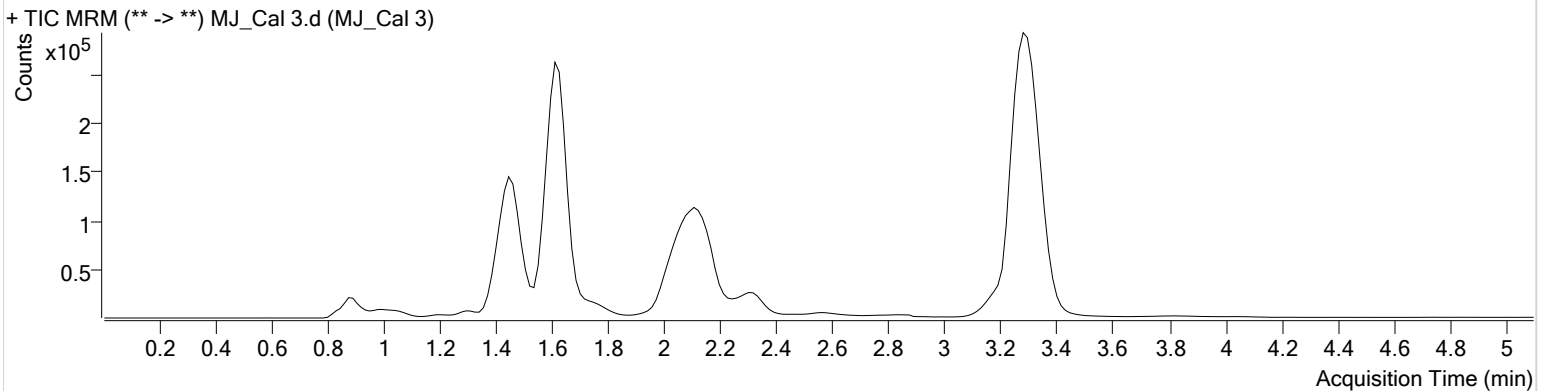


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument Falco **Data File** MJ_Cal 3.d
Type Cal **Sample** MJ_Cal 3
Acq. Method AM 27 THCQ.m **Operator** Sarah Pickle
Sample Position P3-C1 **Comment**
Injection Volume 10
Acq. Date-Time 11/6/2020 3:15:32 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	85923	∞	8.7	164.60	490450	4.7835 ng/ml
THC-COOH	1.474	73183	∞	57.3	∞	116070	18.9594 ng/ml
THC	3.300	104951	557.27	31.4	47.82	2032498	5.0168 ng/ml

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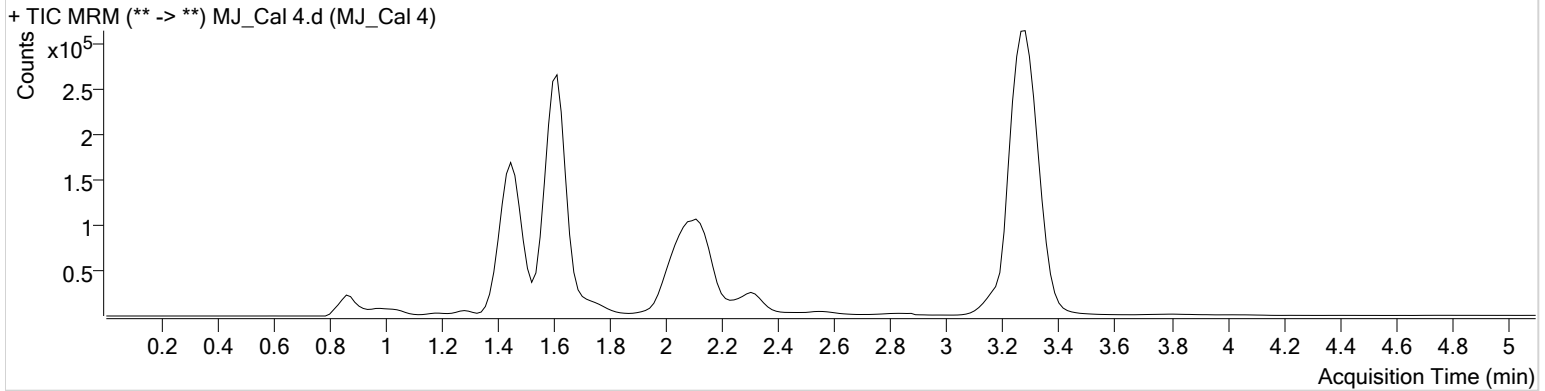


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument	Falco	Data File	MJ_Cal 4.d
Type	Cal	Sample	MJ_Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Sarah Pickle
Sample Position	P3-D1	Comment	
Injection Volume	10		
Acq. Date-Time	11/6/2020 3:23:10 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	118166	∞	9.8	∞	416936	10.8228 ng/ml
THC-COOH	1.459	157303	∞	59.9	∞	106582	49.3660 ng/ml
THC	3.285	206270	909.21	27.5	370.96	2046260	9.7655 ng/ml

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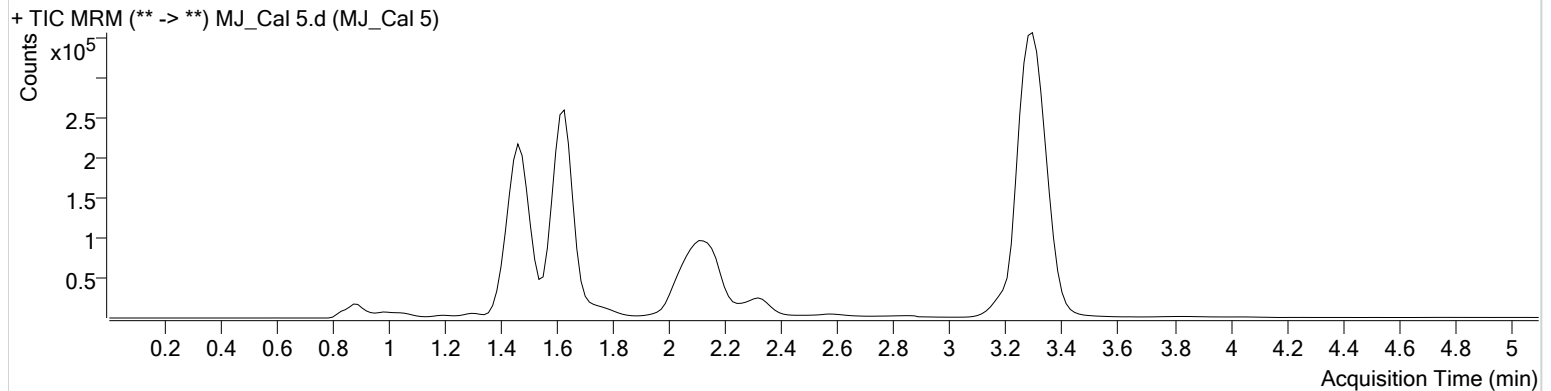


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument Falco **Data File** MJ_Cal 5.d
Type Cal **Sample** MJ_Cal 5
Acq. Method AM 27 THCQ.m **Operator** Sarah Pickle
Sample Position P3-E1 **Comment**
Injection Volume 10
Acq. Date-Time 11/6/2020 3:30:44 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	239358	∞	11.1	159.67	454529	24.3938 ng/ml
THC-COOH	1.489	219219	∞	60.7	7153.71	103850	72.2069 ng/ml
THC	3.300	506088	2755.05	27.4	∞	1981890	24.6928 ng/ml

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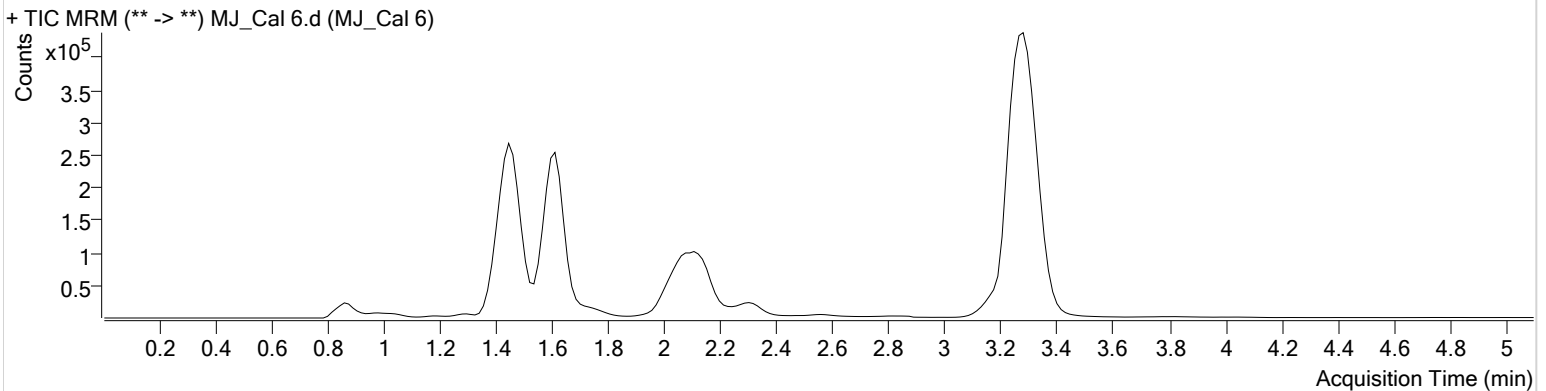


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument Falco **Data File** MJ_Cal 6.d
Type Cal **Sample** MJ_Cal 6
Acq. Method AM 27 THCQ.m **Operator** Sarah Pickle
Sample Position P3-F1 **Comment**
Injection Volume 10
Acq. Date-Time 11/6/2020 3:38:21 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	414685	∞	12.2	∞	432388	48.5262 ng/ml
THC-COOH	1.474	296805	∞	57.3	∞	101776	101.1738 ng/ml
THC	3.285	1021869	8755.35	26.9	2372.65	1980934	49.8525 ng/ml

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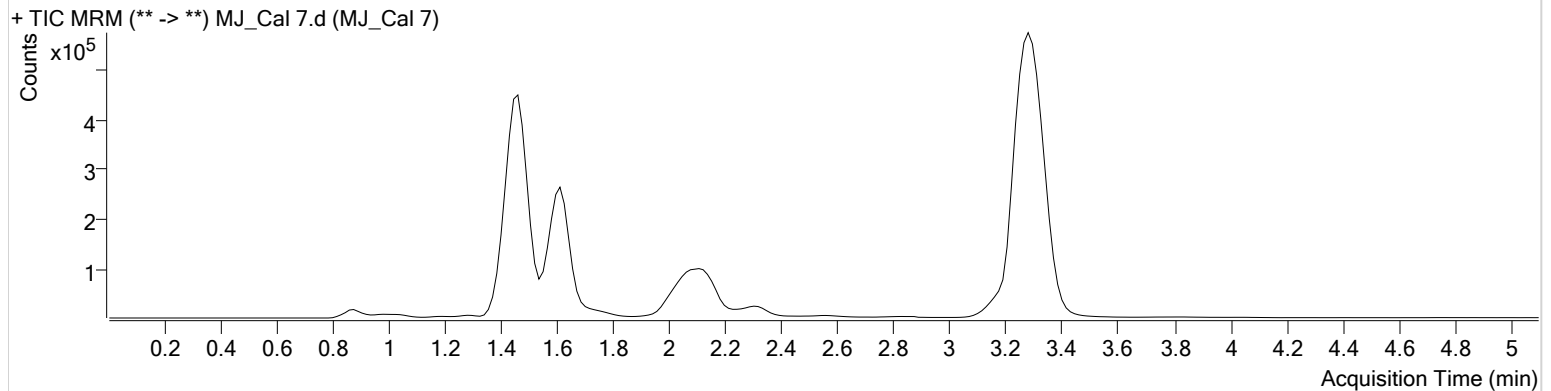


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\110620 AM 27 reinjects SP\QuantResults\AM 27 reinjects.batch.bin
Calibration Last Update 11/9/2020 11:27:03 AM

Instrument Falco **Data File** MJ_Cal 7.d
Type Cal **Sample** MJ_Cal 7
Acq. Method AM 27 THCQ.m **Operator** Sarah Pickle
Sample Position P3-G1 **Comment**
Injection Volume 10
Acq. Date-Time 11/6/2020 3:45:58 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	761263	∞	12.6	2044.66	399011	101.4737 ng/ml
THC-COOH	1.474	659550	∞	61.4	∞	92505	252.7279 ng/ml
THC	3.285	1977805	14106.04	26.9	1090.72	1898690	100.6379 ng/ml