

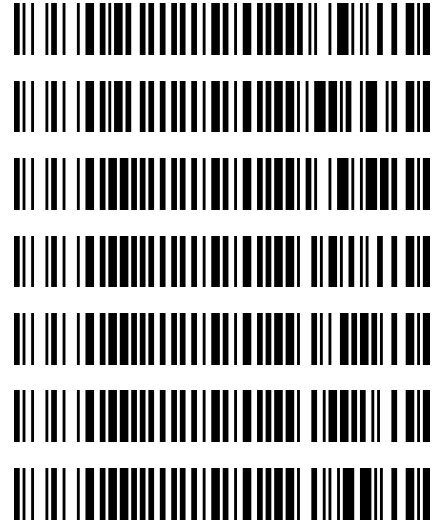
REVIEWED

By Celena Shrum at 3:00 pm, Apr 15, 2020

§ 4/14/2020

Worklist: 4162

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2020-1204	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-1270	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-0983	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-0993	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-0999	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1007	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1010	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: 04/14/20
 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\041420 AM 27 28 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)*

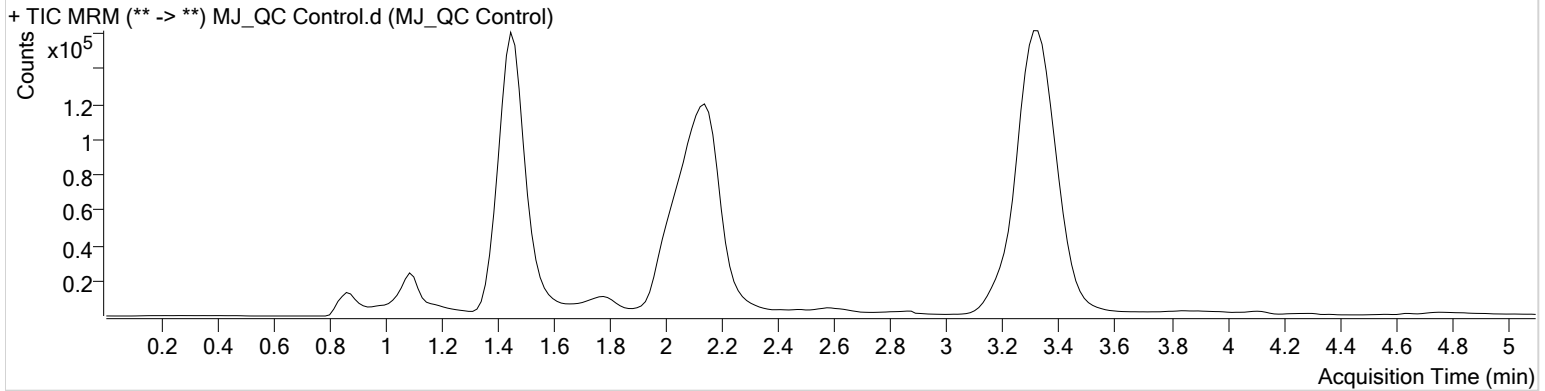
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-H1	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 12:24:10 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	120002	83.38	8.9	∞	626228	4.2372 ng/ml
THC-COOH	1.489	74955	105.57	47.7	283.77	183253	16.0729 ng/ml
THC	3.345	58906	172.70	32.6	108.18	1542120	4.5494 ng/ml

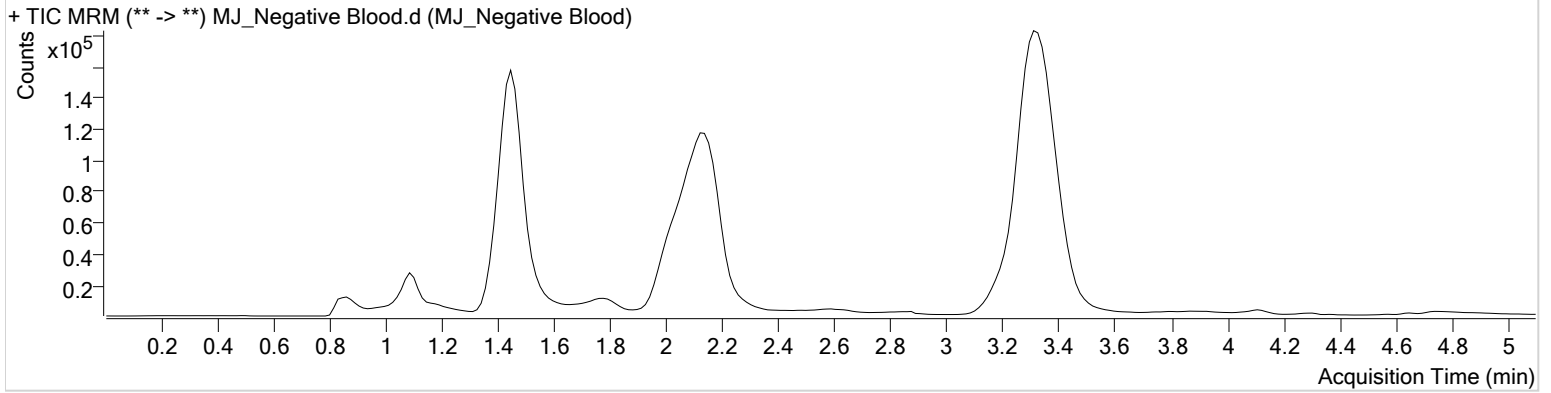
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-A2	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 12:39:22 PM		
Sample Info.			

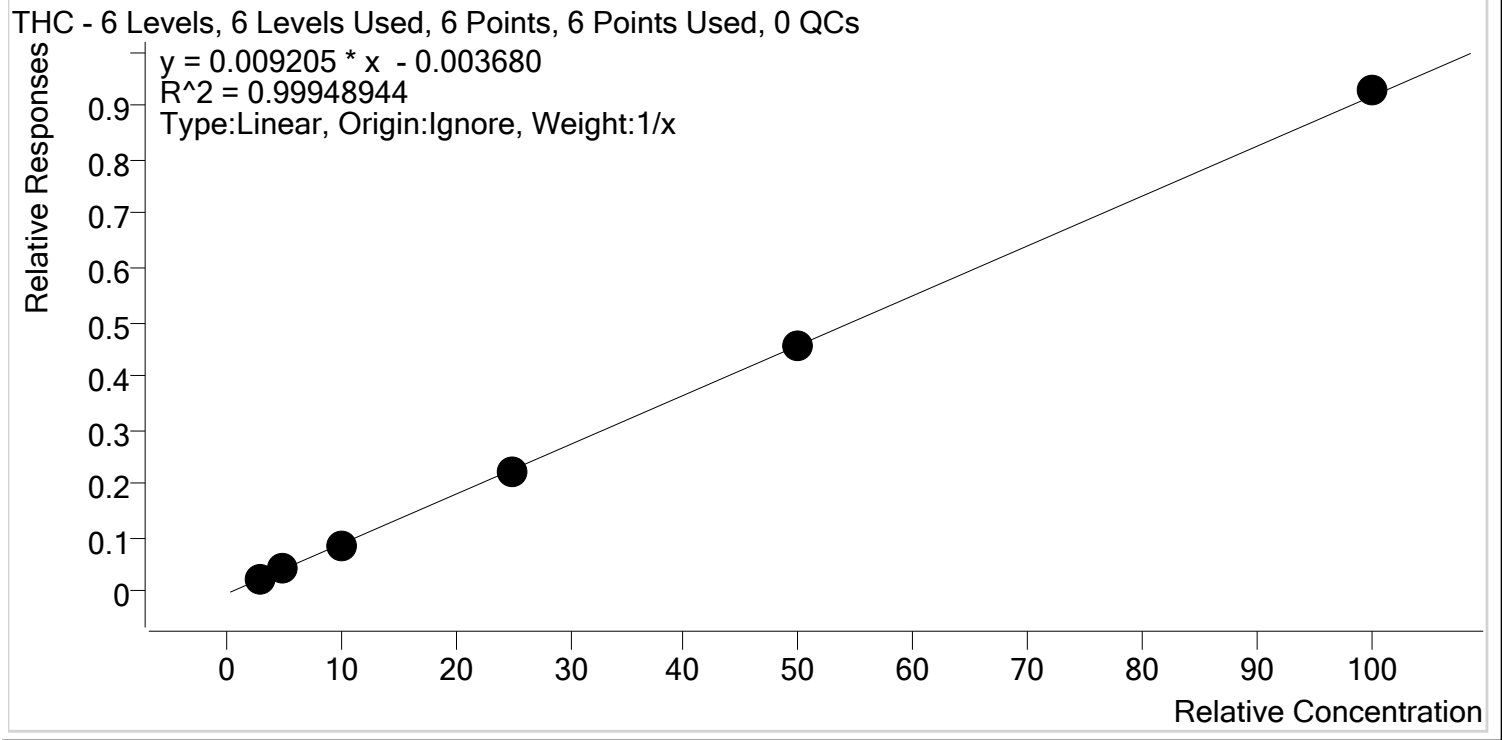
Sample Chromatogram





AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Last Cal. Update 4/14/2020 2:52 PM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3

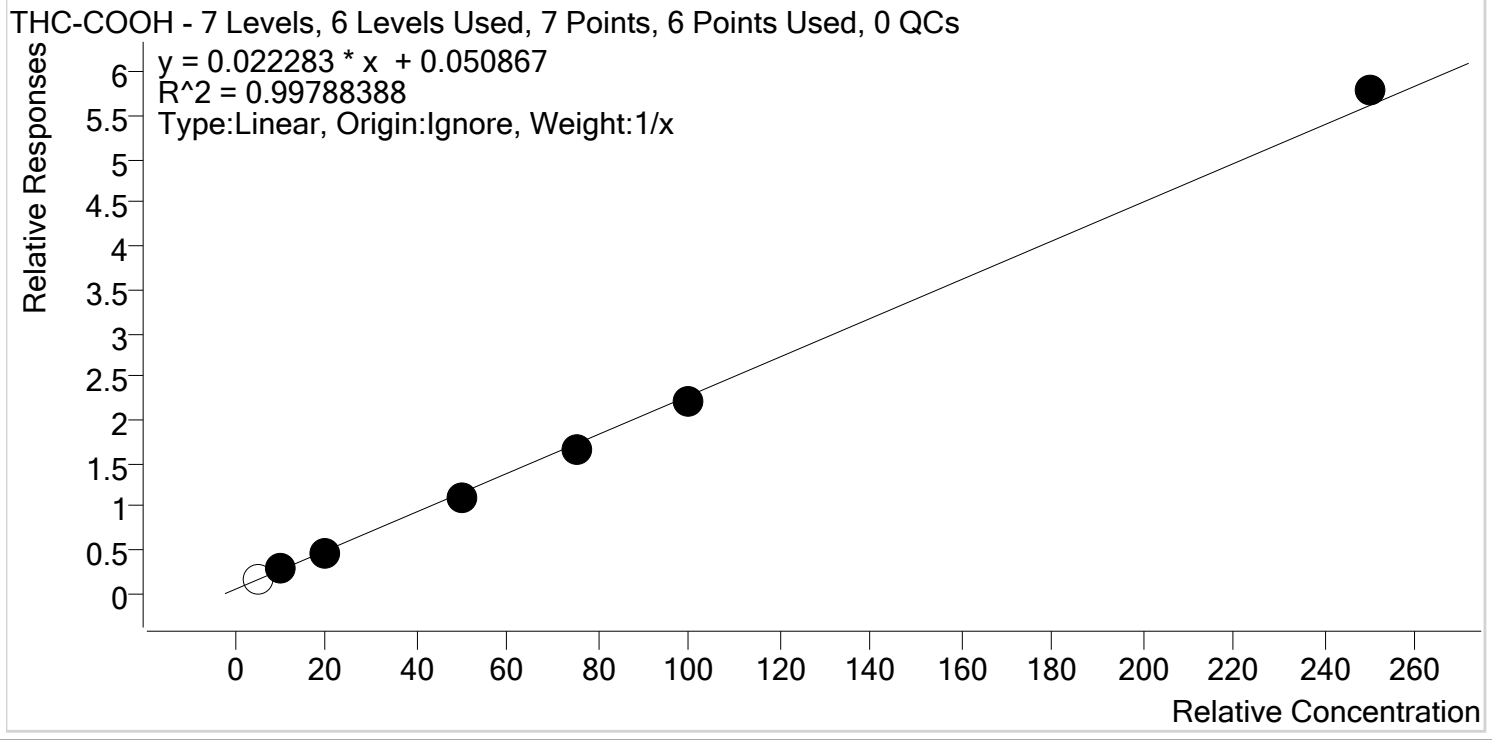


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 2	2	✓	3.0	3.1	103.6
MJ_Cal 3	3	✓	5.0	5.2	103.9
MJ_Cal 4	4	✓	10.0	9.4	94.4
MJ_Cal 5	5	✓	25.0	24.4	97.5
MJ_Cal 6	6	✓	50.0	49.8	99.5
MJ_Cal 7	7	✓	100.0	101.1	101.1



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Last Cal. Update 4/14/2020 2:52 PM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

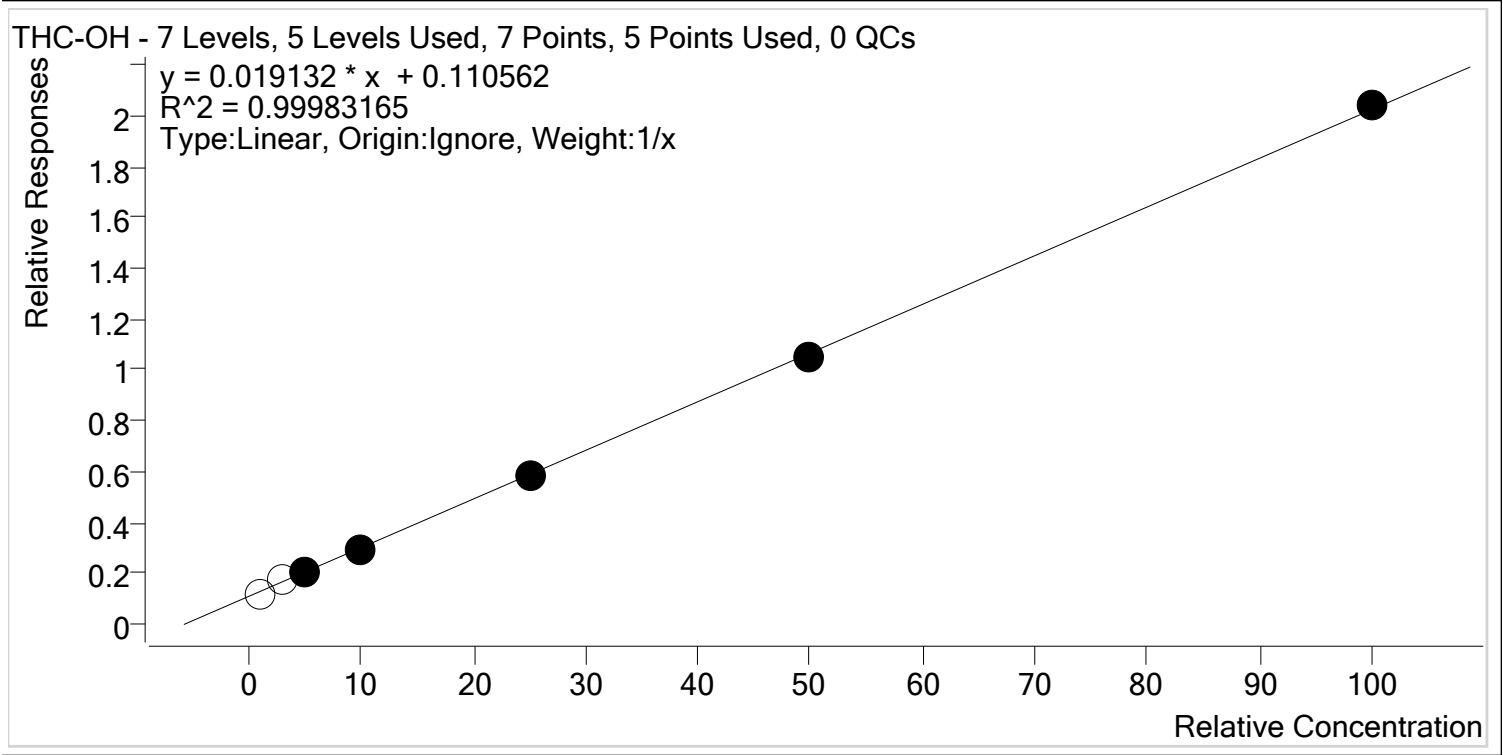


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1	1	x	5.0	5.5	109.1
MJ_Cal 2	2	✓	10.0	11.1	111.2
MJ_Cal 3	3	✓	20.0	19.5	97.4
MJ_Cal 4	4	✓	50.0	47.4	94.9
MJ_Cal 5	5	✓	75.0	72.4	96.5
MJ_Cal 6	6	✓	100.0	97.0	97.0
MJ_Cal 7	7	✓	250.0	257.6	103.0



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Last Cal. Update 4/14/2020 2:52 PM
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	0.5	54.2
MJ_Cal 2	2	x	3.0	3.8	125.6
MJ_Cal 3	3	✓	5.0	5.1	101.9
MJ_Cal 4	4	✓	10.0	9.9	98.9
MJ_Cal 5	5	✓	25.0	25.0	99.9
MJ_Cal 6	6	✓	50.0	49.3	98.5
MJ_Cal 7	7	✓	100.0	100.8	100.8

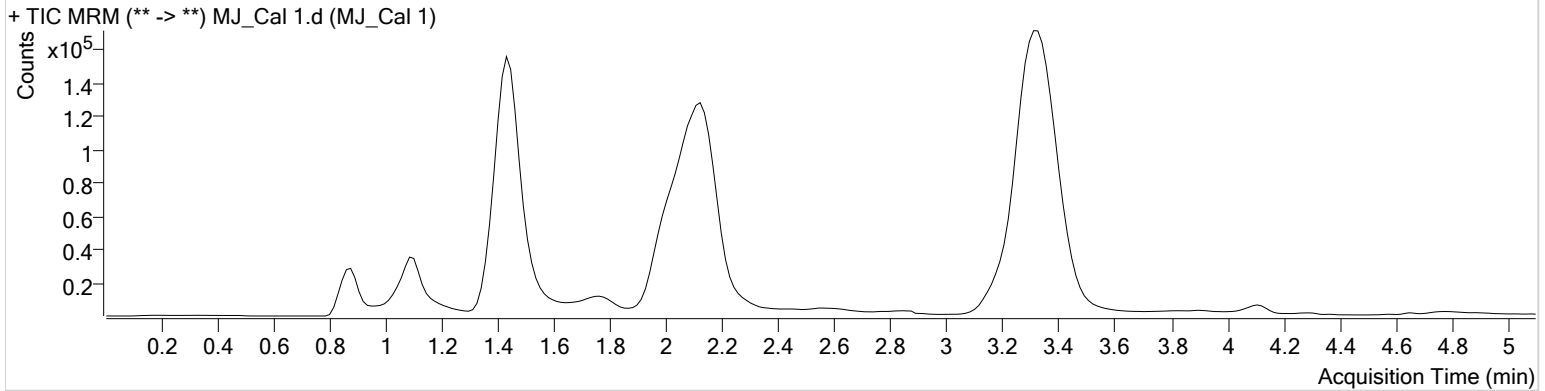
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-A1	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 11:23:22 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.498	85182	∞	4.8 Low	11.01	704405	0.5418 ng/ml Low
THC-COOH	1.474	35423	37.24	35.2 Low	126.98	205499	5.4527 ng/ml Low

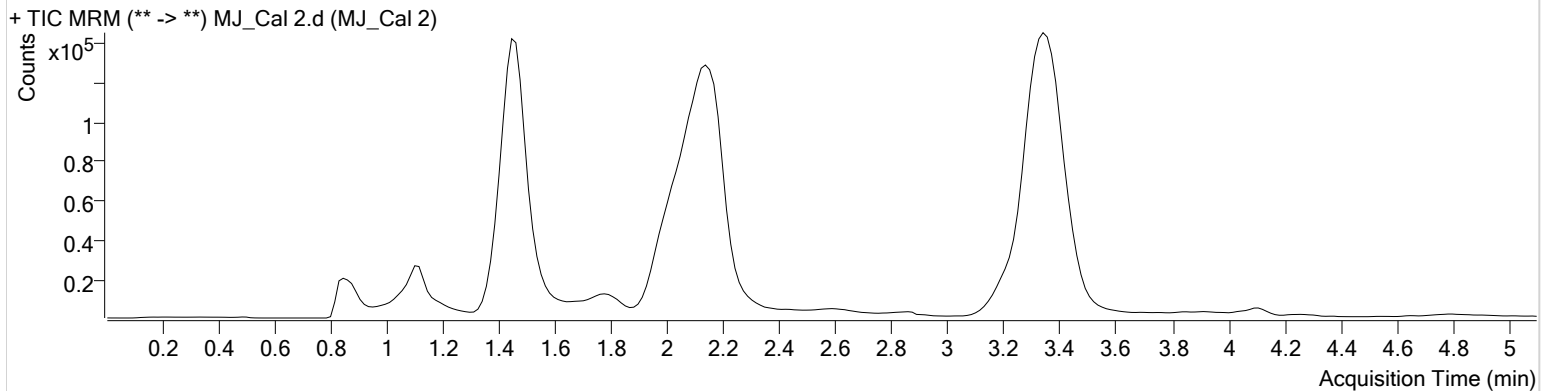
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-B1	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 11:31:07 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.513	107978	∞	6.3 Low	21.75	591119	3.7689 ng/ml
THC-COOH	1.489	52370	256.76	43.9	157.11	175331	11.1214 ng/ml
THC	3.360	36728	96.11	33.1	80.73	1473651	3.1074 ng/ml

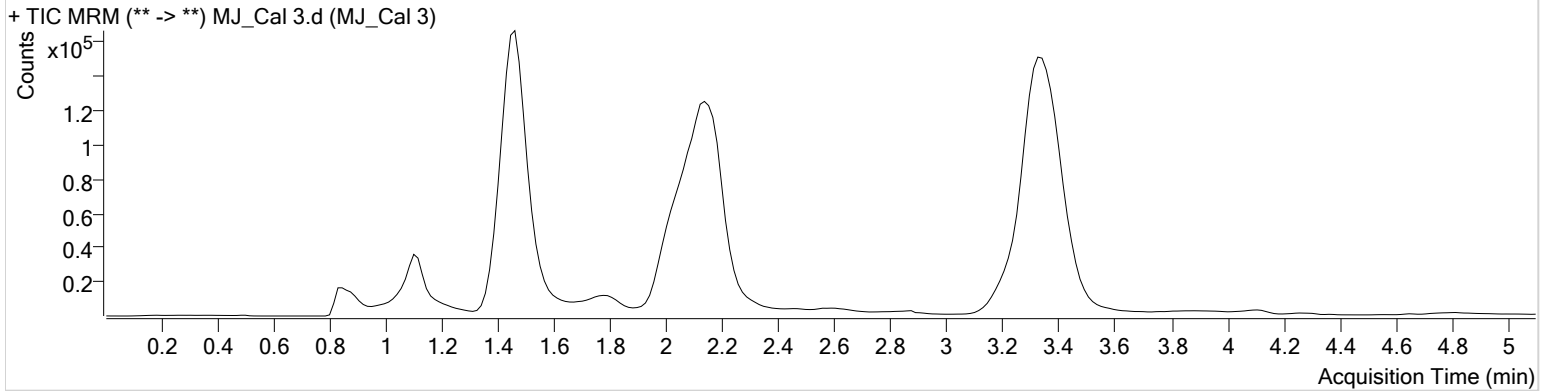
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-C1	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 11:38:42 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	133070	∞	9.0	∞	639503	5.0974 ng/ml
THC-COOH	1.489	96916	∞	47.8	∞	199852	19.4795 ng/ml
THC	3.345	64251	328.81	30.4	41.04	1455817	5.1943 ng/ml

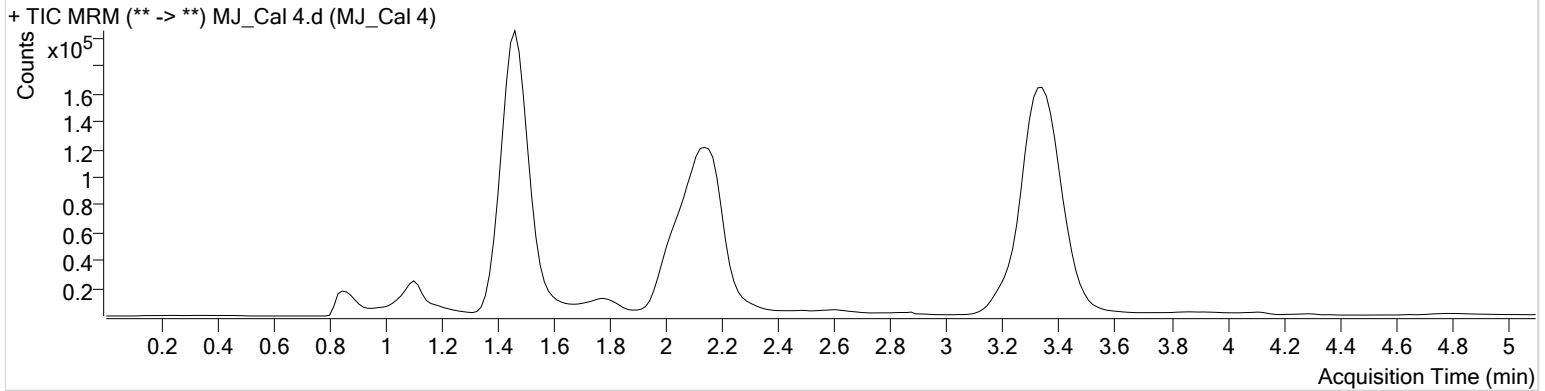
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-D1	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 11:46:16 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	193391	231.19	9.4	492.23	645253	9.8869 ng/ml
THC-COOH	1.489	213710	∞	53.7	711.25	192938	47.4250 ng/ml
THC	3.345	128236	310.35	27.1	92.29	1540850	9.4409 ng/ml

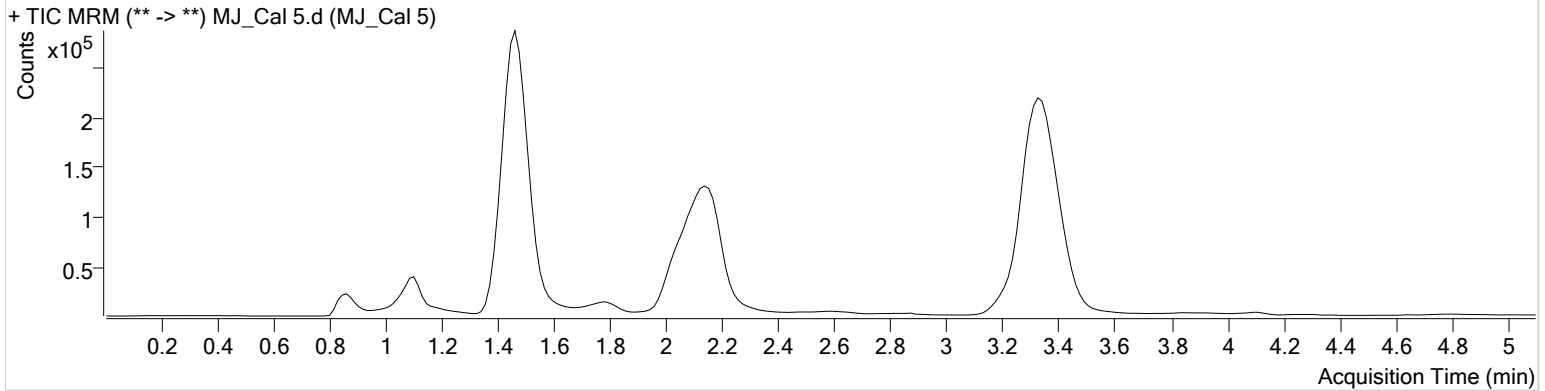
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-E1	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 11:53:51 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	392517	∞	10.9	565.48	667205	24.9712 ng/ml
THC-COOH	1.489	338608	270.52	54.9	1064.50	203582	72.3578 ng/ml
THC	3.345	358340	1965.00	27.1	752.24	1624056	24.3698 ng/ml

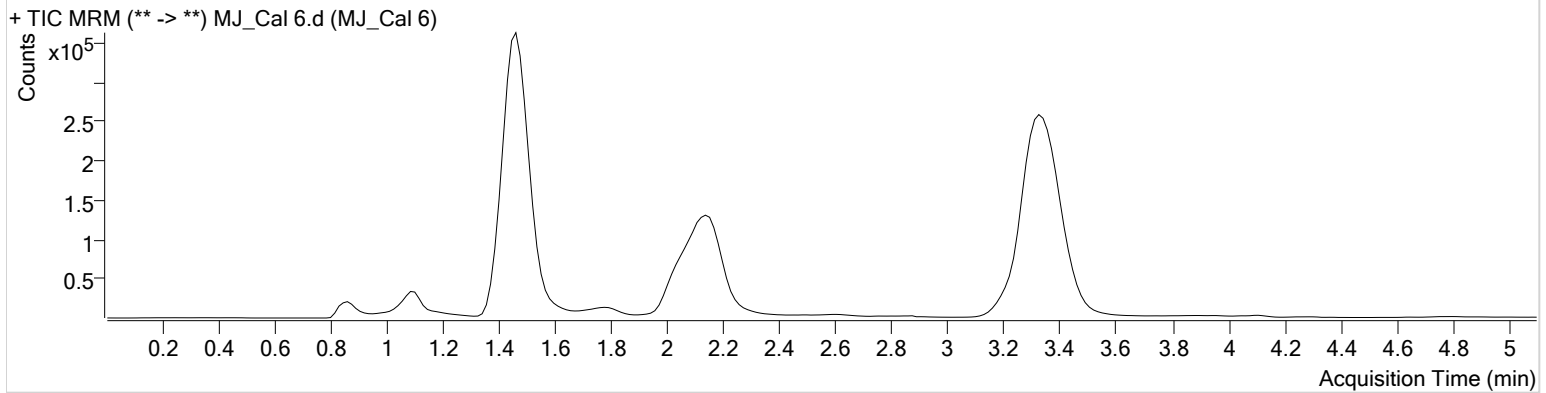
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-F1	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 12:01:25 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	704614	537.39	12.2	664.67	669240	49.2535 ng/ml
THC-COOH	1.489	441144	597.08	56.0	∞	199350	97.0247 ng/ml
THC	3.345	741873	1610.57	25.1	572.34	1632804	49.7590 ng/ml

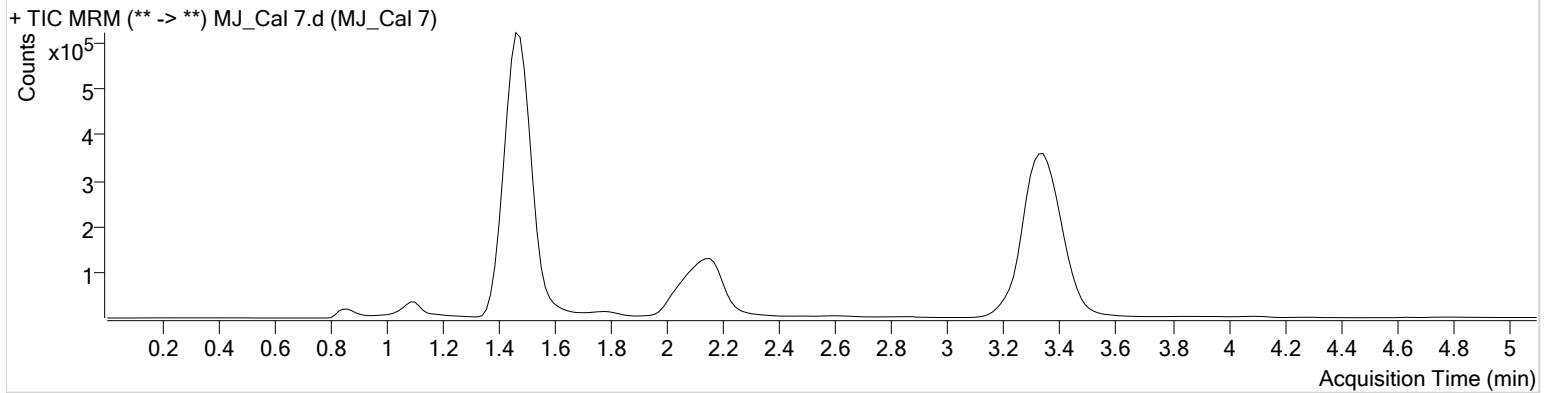
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\041420 AM 27 28 SP\QuantResults\AM 27.batch.bin
Calibration Last Update 4/14/2020 2:52:55 PM

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-G1	Comment	
Injection Volume	10		
Acq. Date-Time	4/14/2020 12:08:59 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	1319427	∞	12.1	∞	647142	100.7911 ng/ml
THC-COOH	1.489	1035969	∞	58.4	3091.25	178896	257.5915 ng/ml
THC	3.345	1526576	3866.21	24.9	926.51	1646407	101.1287 ng/ml