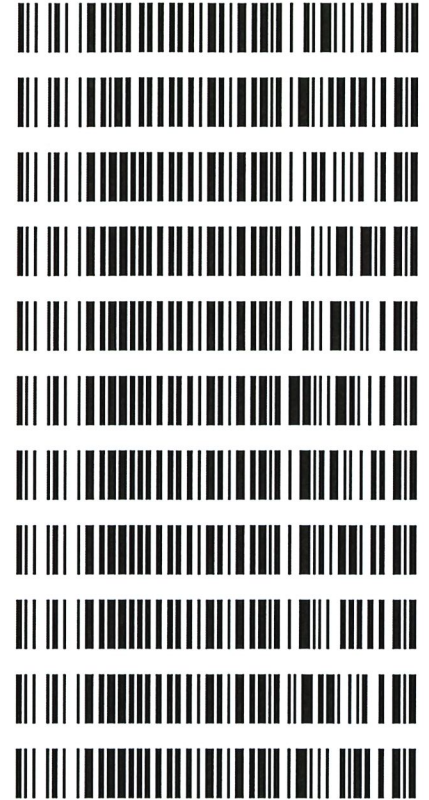


REVIEWED
By Sarah Pickle at 2:33 pm, Jun 08, 2020

TS 6/4/2020

Worklist: 4286

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2020-1905	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2020-2008	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1523	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1560	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1586	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1604	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1623	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1624	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1627	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1634	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2020-1657	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: 06/05/2020
Plate lot#: IDP-108-200303

Analyst: Tamara Salazar
Plate Expiration: 09/03/2020

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
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.
- 3. Create worklist:

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Pipette **1000µL blood (calibrated pipette) Pipette ID: 42** in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 067105*
- 4. Pipette **500µL 0.1% formic acid in water for blood samples** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **800µL 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 85-100 PSI- Selector to the right) Manifold ID: 067104
- 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: 067103
- 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, 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: *Curves limited: THC-OH 5-100 (reported qualitatively)*

Calibrator 2 was rerejected due to possible interfering peak 6/8/2020

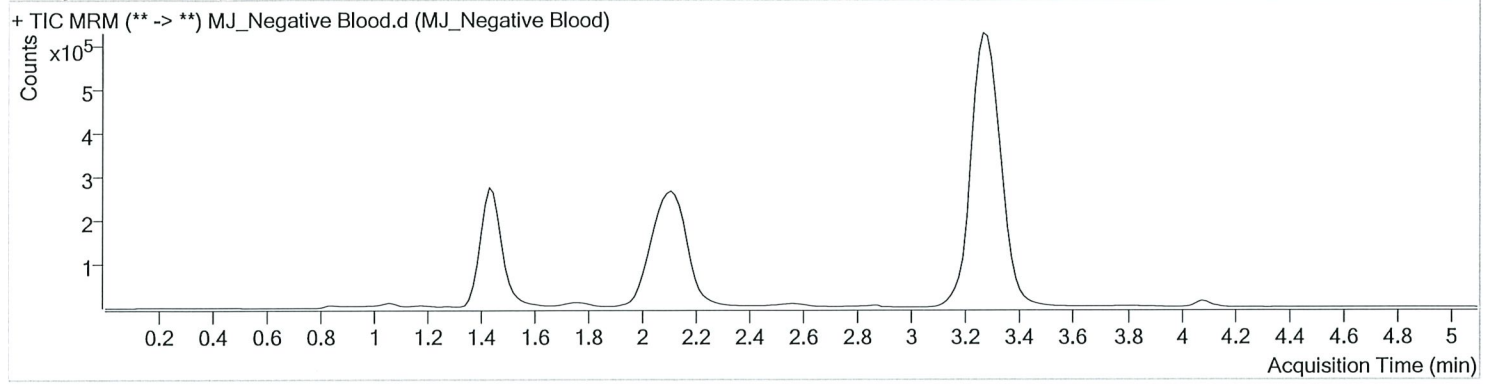


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument	Falco	Data File	MJ_Negative Blood.d
Type	Sample	Sample	MJ_Negative Blood
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-H5	Comment	
Injection Volume	10		
Acq. Date-Time	6/5/2020 1:00:08 PM		
Sample Info.			

Sample Chromatogram



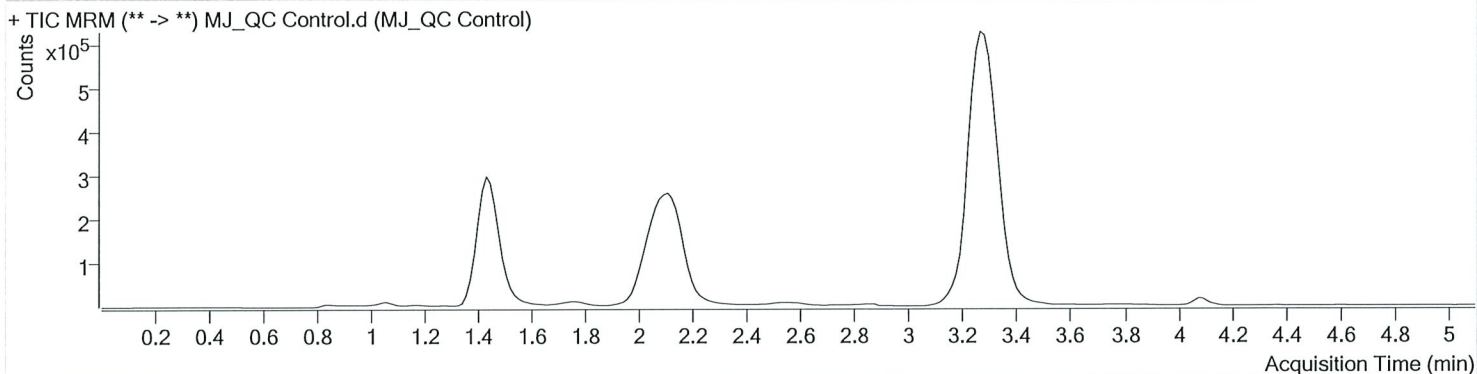


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument	Falco	Data File	MJ_QC Control.d
Type	Sample	Sample	MJ_QC Control
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-A6	Comment	
Injection Volume	10		
Acq. Date-Time	6/5/2020 12:44:56 PM		
Sample Info.			

Sample Chromatogram

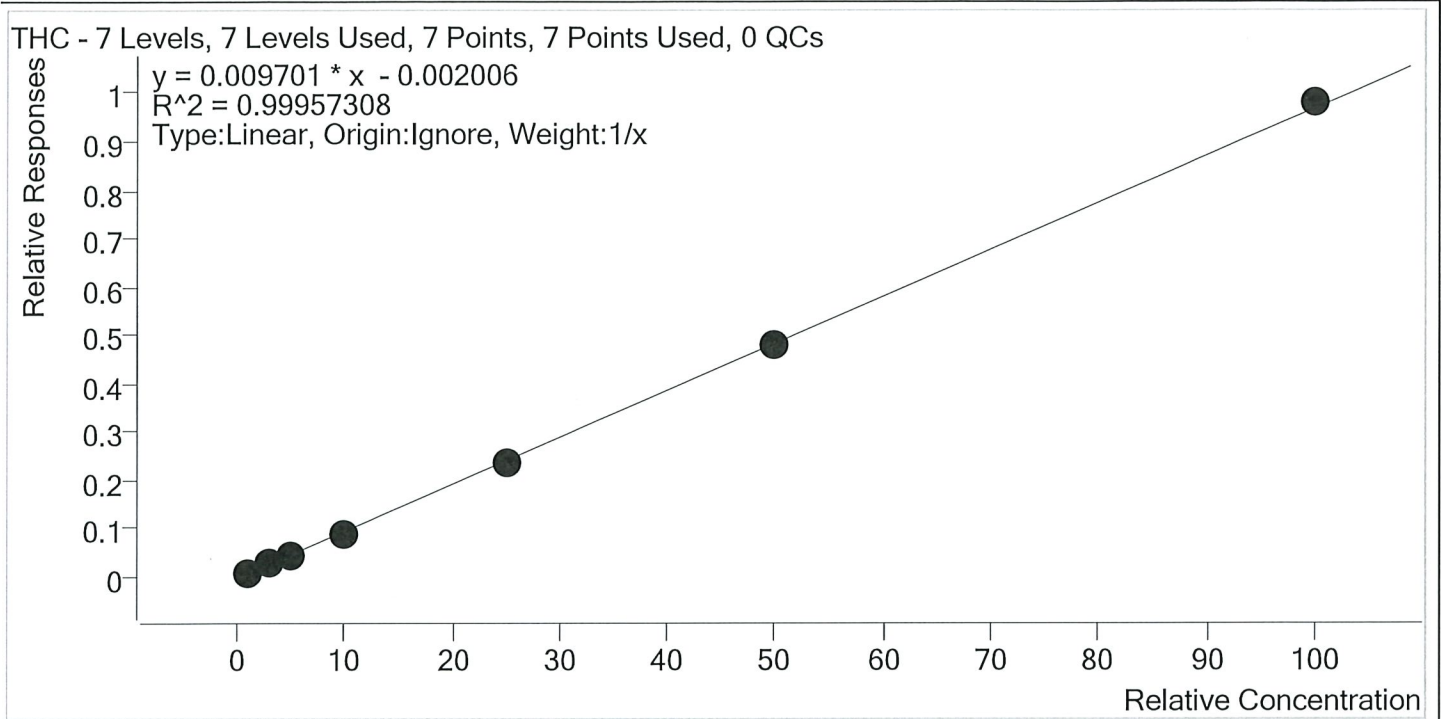


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	133467	∞	9.6	168.35	1061423	4.2875 ng/ml
THC-COOH	1.474	113595	∞	55.0	∞	324863	15.7352 ng/ml
THC	3.285	183321	1029.84	27.8	∞	4584231	4.3290 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wklst 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Last Cal. Update 6/8/2020 9:37 AM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3



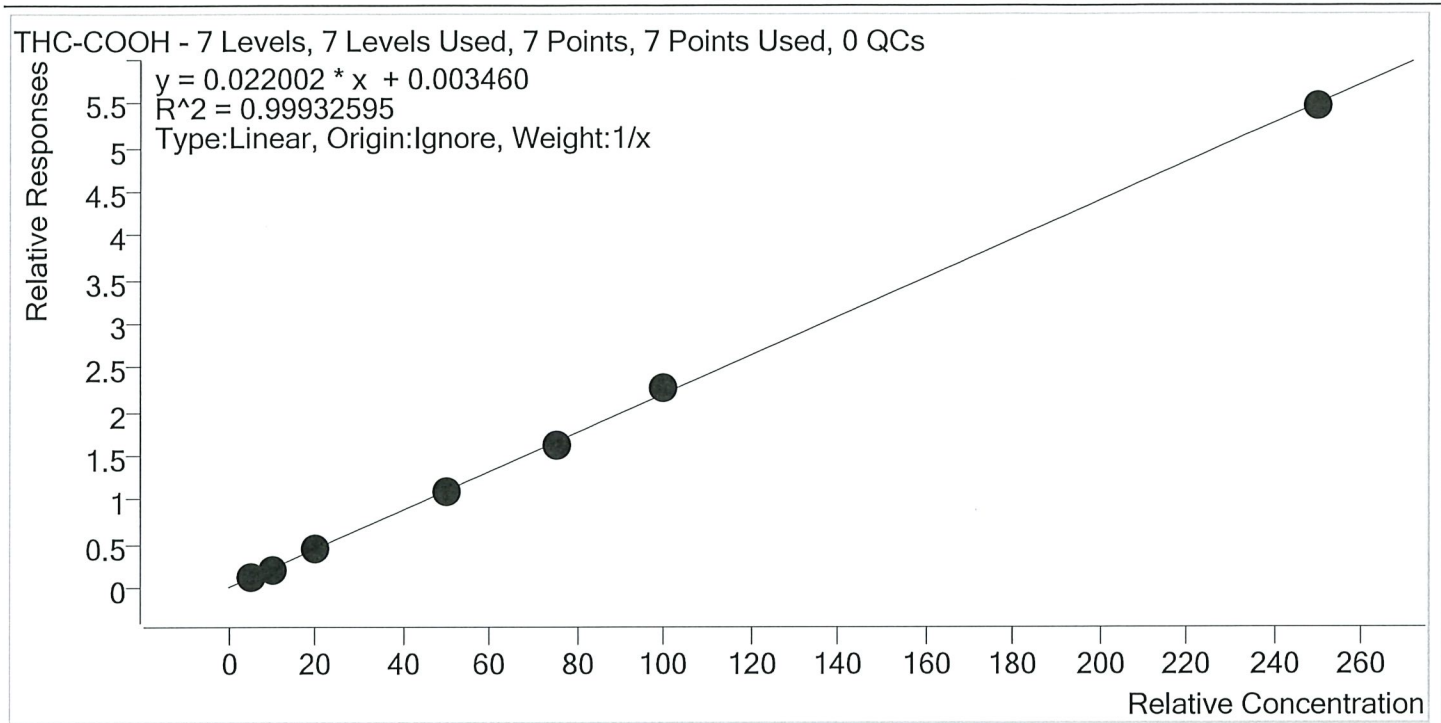
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.1	111.5
MJ Cal 2 r	2	✓	3.0	2.9	96.8
MJ Cal 3	3	✓	5.0	4.9	97.8
MJ Cal 4	4	✓	10.0	9.5	95.1
MJ Cal 5	5	✓	25.0	24.4	97.8
MJ Cal 6	6	✓	50.0	49.9	99.9
MJ Cal 7	7	✓	100.0	101.2	101.2

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk\lst 4286 4287 TS\QuantResults\AM 27
 TS.batch.bin
Last Cal. Update 6/8/2020 9:37 AM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

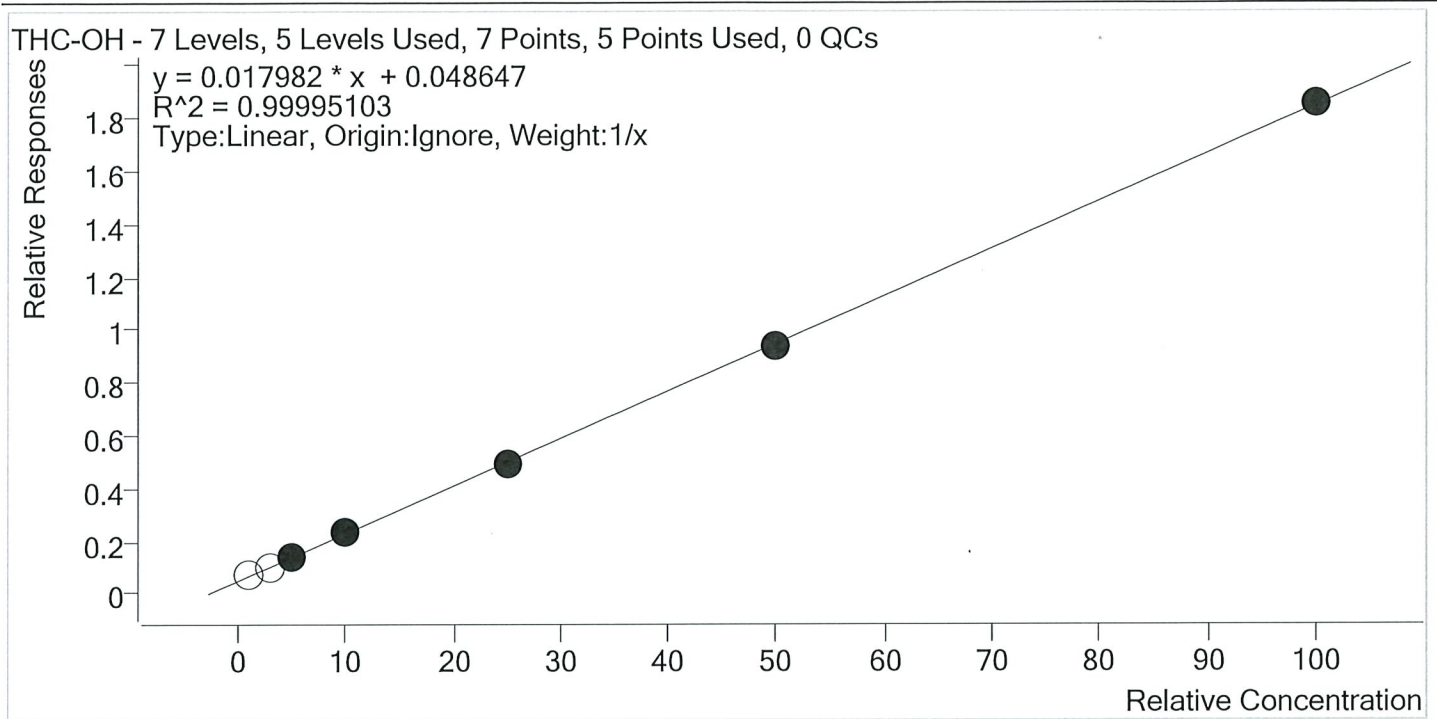


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	5.0	5.4	108.2
MJ Cal 2 r	2	✓	10.0	9.4	93.7
MJ Cal 3	3	✓	20.0	19.4	97.1
MJ Cal 4	4	✓	50.0	49.8	99.6
MJ Cal 5	5	✓	75.0	73.7	98.3
MJ Cal 6	6	✓	100.0	103.7	103.7
MJ Cal 7	7	✓	250.0	248.5	99.4



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Last Cal. Update 6/8/2020 9:37 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	1.5	148.9
MJ Cal 2 r	2	×	3.0	2.9	97.8
MJ Cal 3	3	✓	5.0	5.0	99.5
MJ Cal 4	4	✓	10.0	10.1	101.2
MJ Cal 5	5	✓	25.0	24.9	99.8
MJ Cal 6	6	✓	50.0	49.6	99.2
MJ Cal 7	7	✓	100.0	100.4	100.4

* Compound reported qualitatively. 6/8/2020 - TS

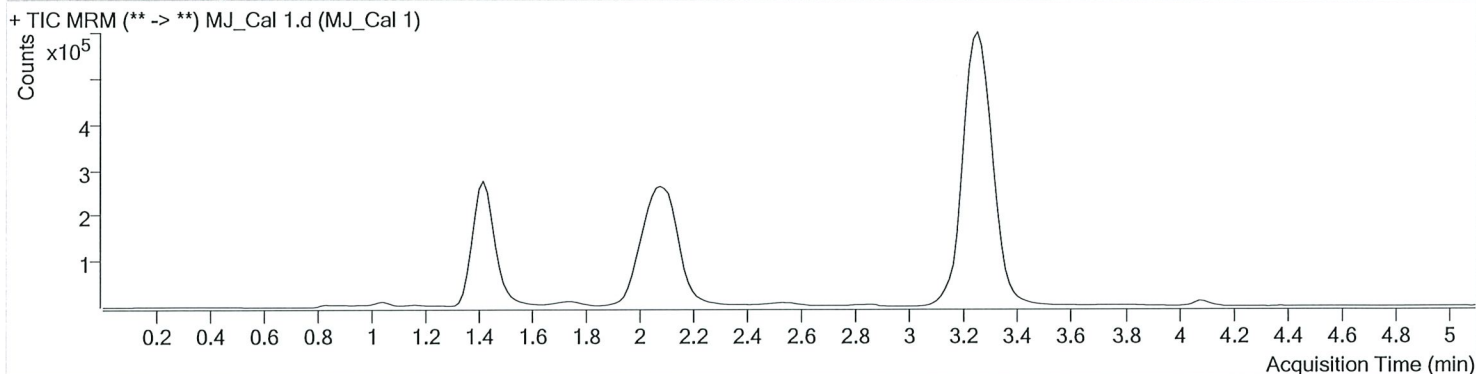


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument	Falco	Data File	MJ_Cal 1.d
Type	Cal	Sample	MJ_Cal 1
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-H6	Comment	
Injection Volume	10		
Acq. Date-Time	6/5/2020 11:44:05 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	83077	∞	5.3 Low	14.10	1101584	1.4887 ng/ml Low
THC-COOH	1.459	40779	∞	50.2	225.89	333033	5.4079 ng/ml Low
THC	3.254	40398	281.50	30.6	103.38	4583293	1.1154 ng/ml Low

TS

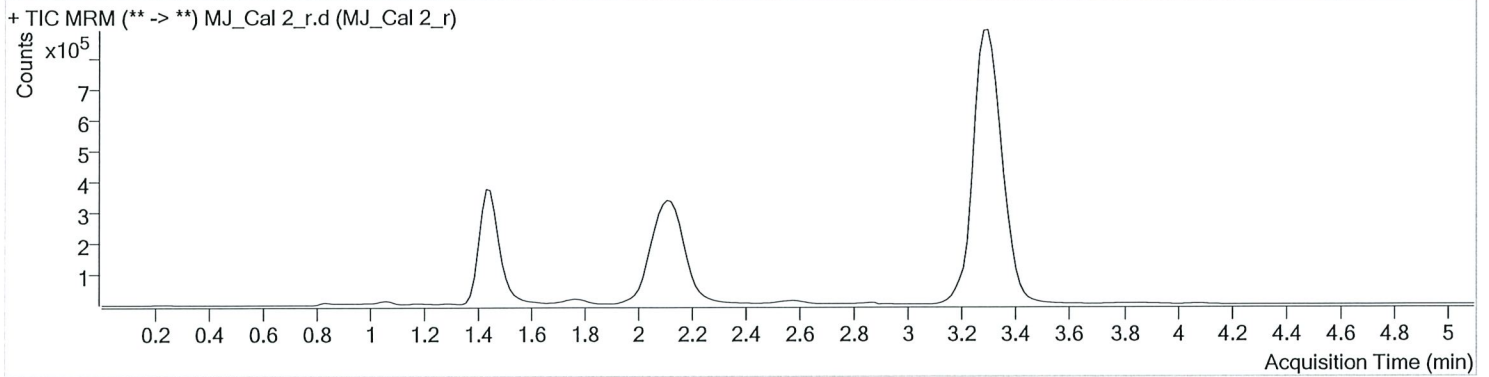


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument	Falco	Data File	MJ_Cal 2_r.d
Type	Cal	Sample	MJ_Cal 2_r
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-G6	Comment	
Injection Volume	10		
Acq. Date-Time	6/5/2020 4:02:18 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	133667	∞	8.0 Low	∞	1317998	2.9346 ng/ml Low
THC-COOH	1.474	81304	∞	59.3	340.63	387856	9.3701 ng/ml Low
THC	3.300	169782	472.74	28.9	201.51	6489533	2.9037 ng/ml Low

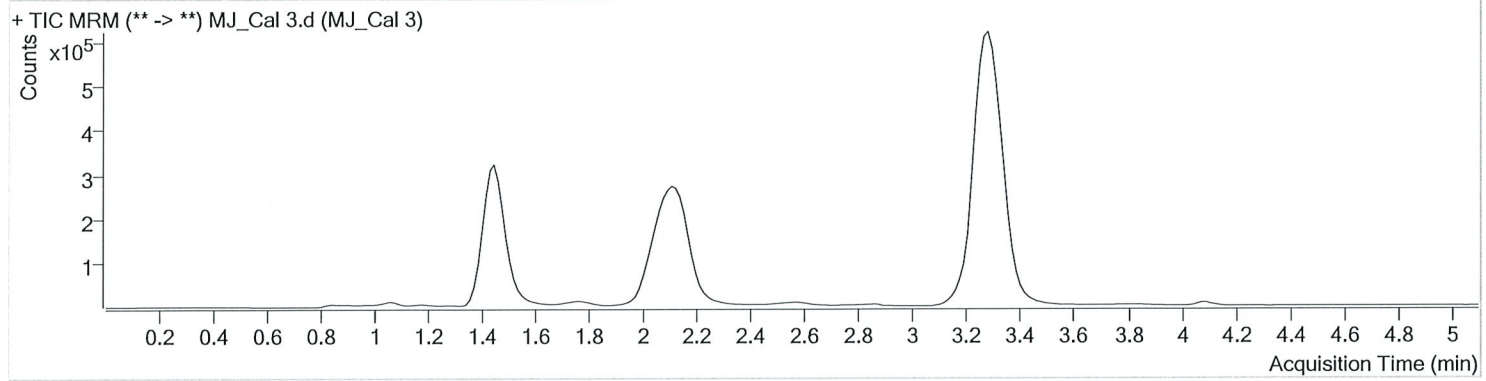


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument	Falco	Data File	MJ_Cal 3.d
Type	Cal	Sample	MJ_Cal 3
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-F6	Comment	
Injection Volume	10		
Acq. Date-Time	6/5/2020 11:59:24 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	155088	∞	10.0	172.37	1123099	4.9740 ng/ml
THC-COOH	1.474	144437	∞	58.2	∞	335400	19.4153 ng/ml
THC	3.285	204129	1253.98	27.0	120.17	4494454	4.8885 ng/ml

TS

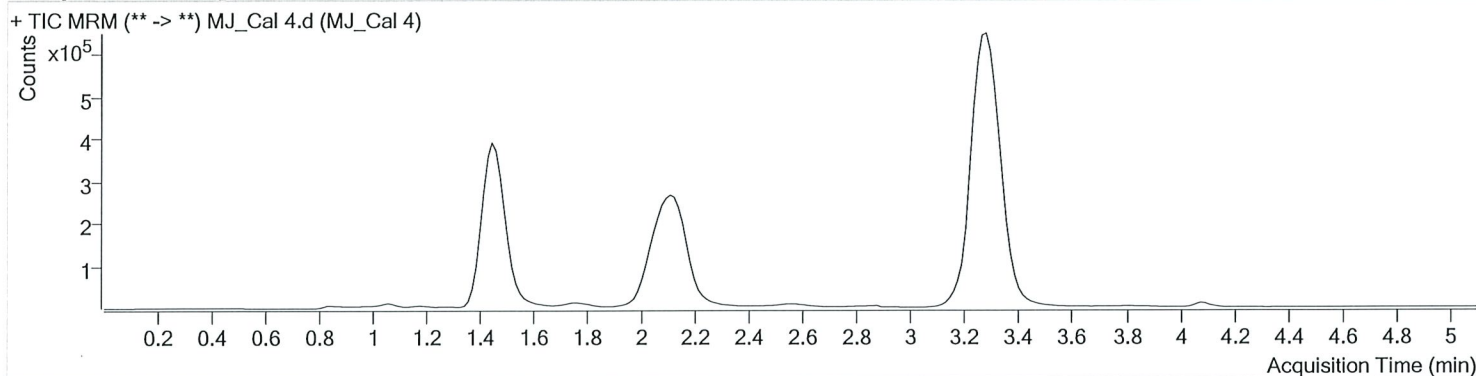


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wkst 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument	Falco	Data File	MJ_Cal 4.d
Type	Cal	Sample	MJ_Cal 4
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-E6	Comment	
Injection Volume	10		
Acq. Date-Time	6/5/2020 12:06:59 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	252553	∞	10.6	677.13	1095369	10.1167 ng/ml
THC-COOH	1.474	358576	∞	60.4	2767.13	326201	49.8031 ng/ml
THC	3.285	400903	1129.99	27.5	423.34	4444103	9.5058 ng/ml

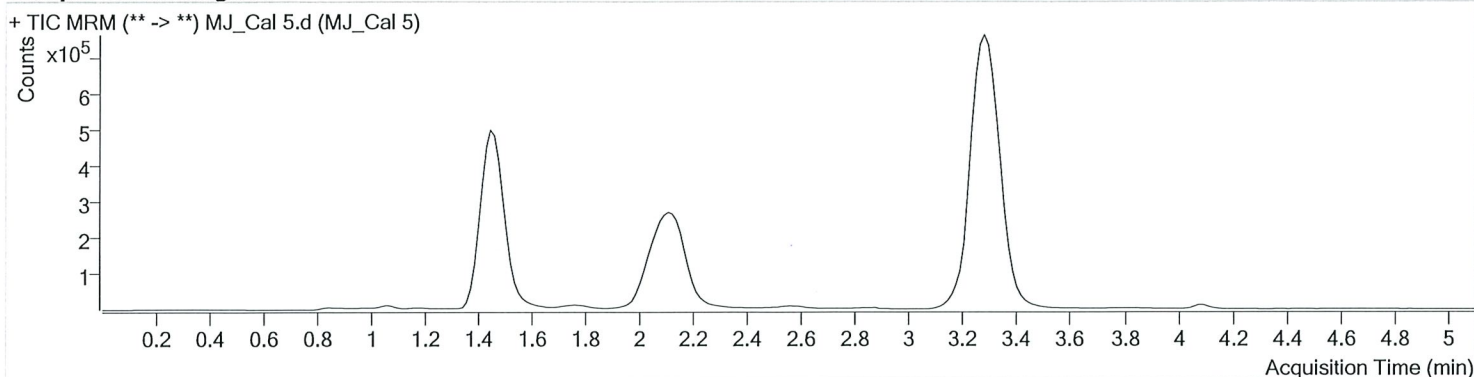


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument	Falco	Data File	MJ_Cal 5.d
Type	Cal	Sample	MJ_Cal 5
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-D6	Comment	
Injection Volume	10		
Acq. Date-Time	6/5/2020 12:14:33 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	554023	∞	12.2	977.13	1114243	24.9458 ng/ml
THC-COOH	1.474	540239	∞	63.2	∞	332317	73.7290 ng/ml
THC	3.300	1059827	7231.64	25.5	661.35	4507796	24.4422 ng/ml

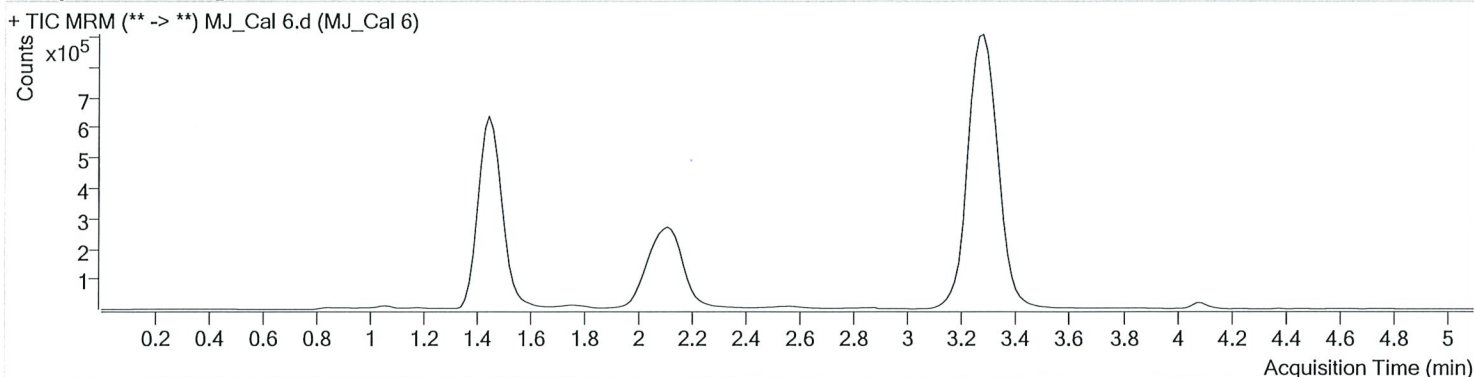


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument	Falco	Data File	MJ_Cal 6.d
Type	Cal	Sample	MJ_Cal 6
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-C6	Comment	
Injection Volume	10		
Acq. Date-Time	6/5/2020 12:22:09 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	1022520	∞	13.0	1372.46	1087026	49.6061 ng/ml
THC-COOH	1.474	739884	∞	57.3	∞	323660	103.7400 ng/ml
THC	3.285	2120869	3455.28	25.7	1634.55	4396649	49.9314 ng/ml

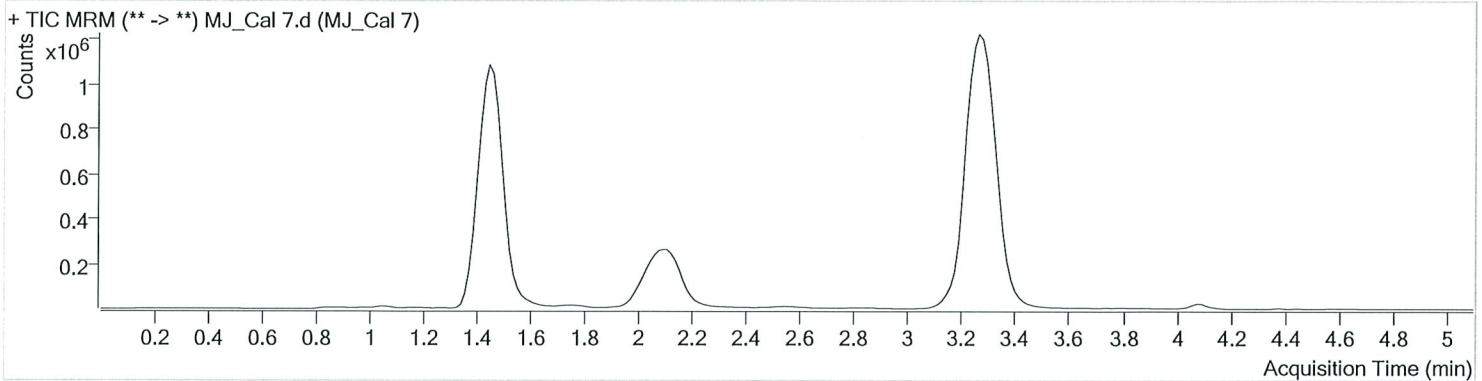


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\060520 AM 27 28 wk1st 4286 4287 TS\QuantResults\AM 27 TS.batch.bin
Calibration Last Update 6/8/2020 9:37:32 AM

Instrument Falco Data File MJ_Cal 7.d
Type Cal Sample MJ_Cal 7
Acq. Method AM 27 THC quant.m Operator Tamara Salazar
Sample Position P3-B6 Comment
Injection Volume 10
Acq. Date-Time 6/5/2020 12:29:45 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	1989883	∞	13.5	1828.30	1073716	100.3575 ng/ml
THC-COOH	1.474	1687922	∞	60.8	∞	308476	248.5347 ng/ml
THC	3.285	4290222	50868.11	26.0	∞	4378357	101.2130 ng/ml