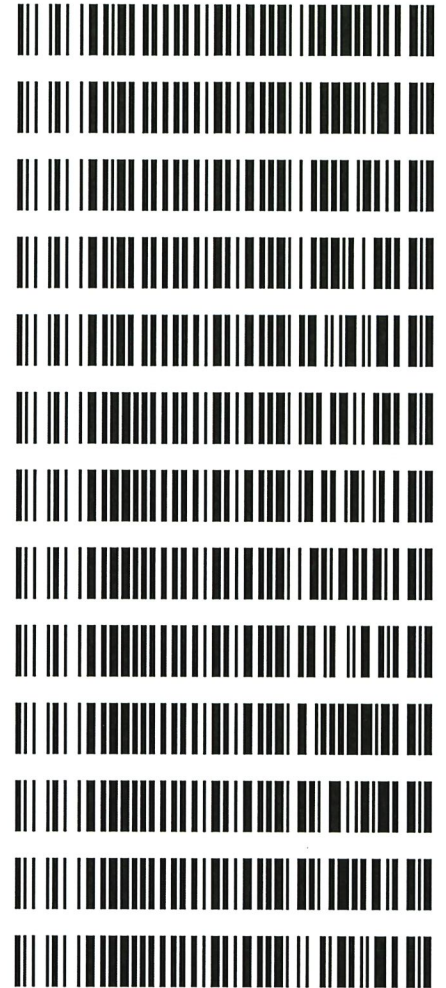


15

Worklist: 3896

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2019-3507	3	BCK	AM 27 Blood THC Quant by LC-QQQ
M2019-4718	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2019-4835	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2019-5191	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2019-5282	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2019-3530	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2019-3532	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2019-3534	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2019-3592	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2019-3631	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2019-3644	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2019-3645	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2019-3677	1	BCK	AM 27 Blood THC Quant by LC-QQQ



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

15

Extraction Date: 12/19/19
Plate lot#: IDP-108-190716

Analyst: Tamara Salazar
Plate Expiration: 01/16/2020

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 445283-3
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.
- 3. Create worklist:

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Pipette **1000µL blood/urine (calibrated pipette) Pipette ID: 16** 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, 500µl saturated phosphate buffer for urine 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.
Worklist path: D:\MassHunter\Data\2019\AM 27\121919 THCQ wklst 3896 TS
Batch Name: *THCQ TS*
- 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 3-100*

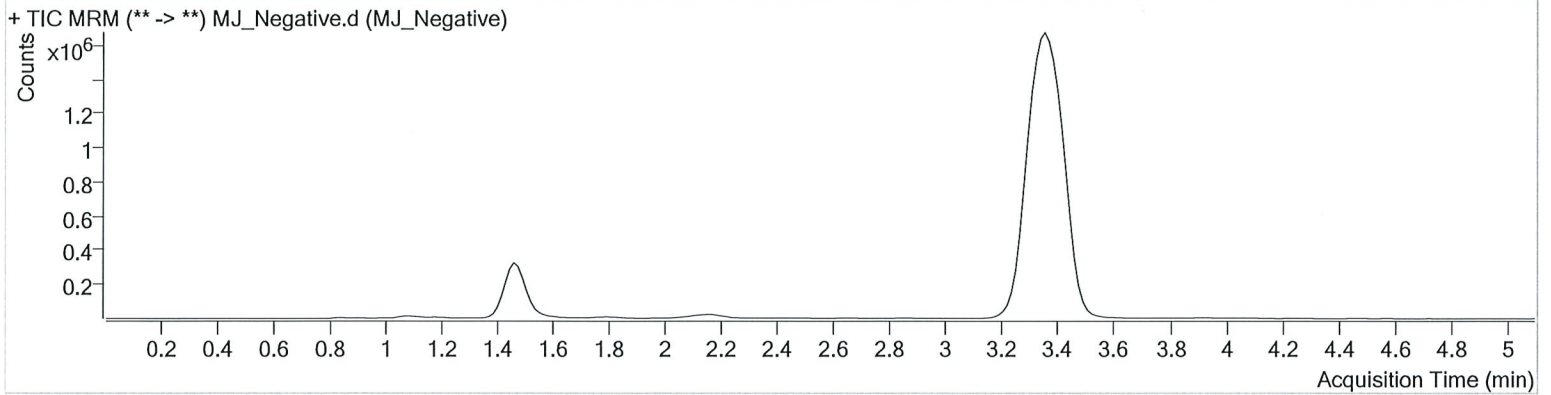
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklst 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 AM

Instrument	Falco	Data File	MJ_Negative.d
Type	Sample	Sample	MJ_Negative
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 12:29:08 PM		
Sample Info.			

Sample Chromatogram



AM #27 Cannabinoid Quant. Results

TS

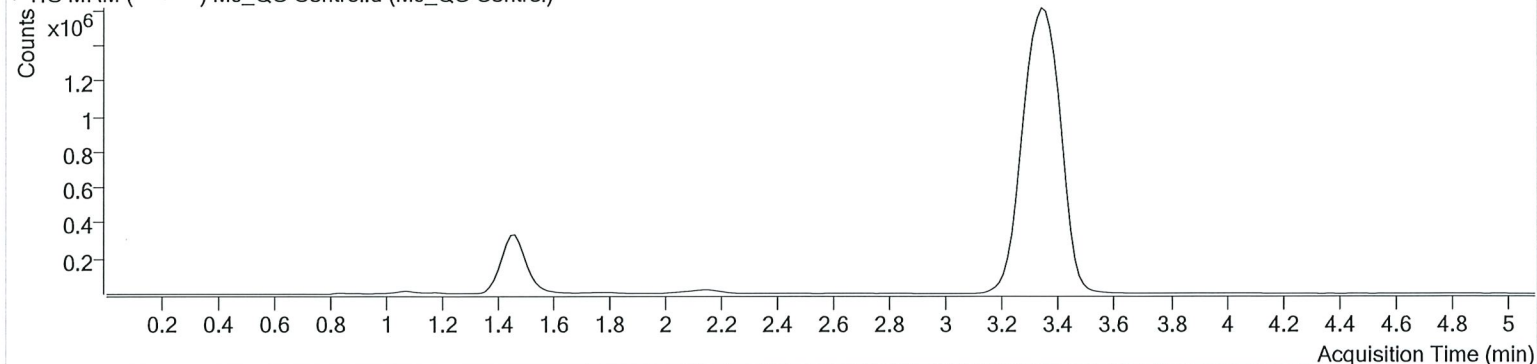


Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklst 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 AM

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-H1	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 12:13:57 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJ_QC Control.d (MJ_QC Control)

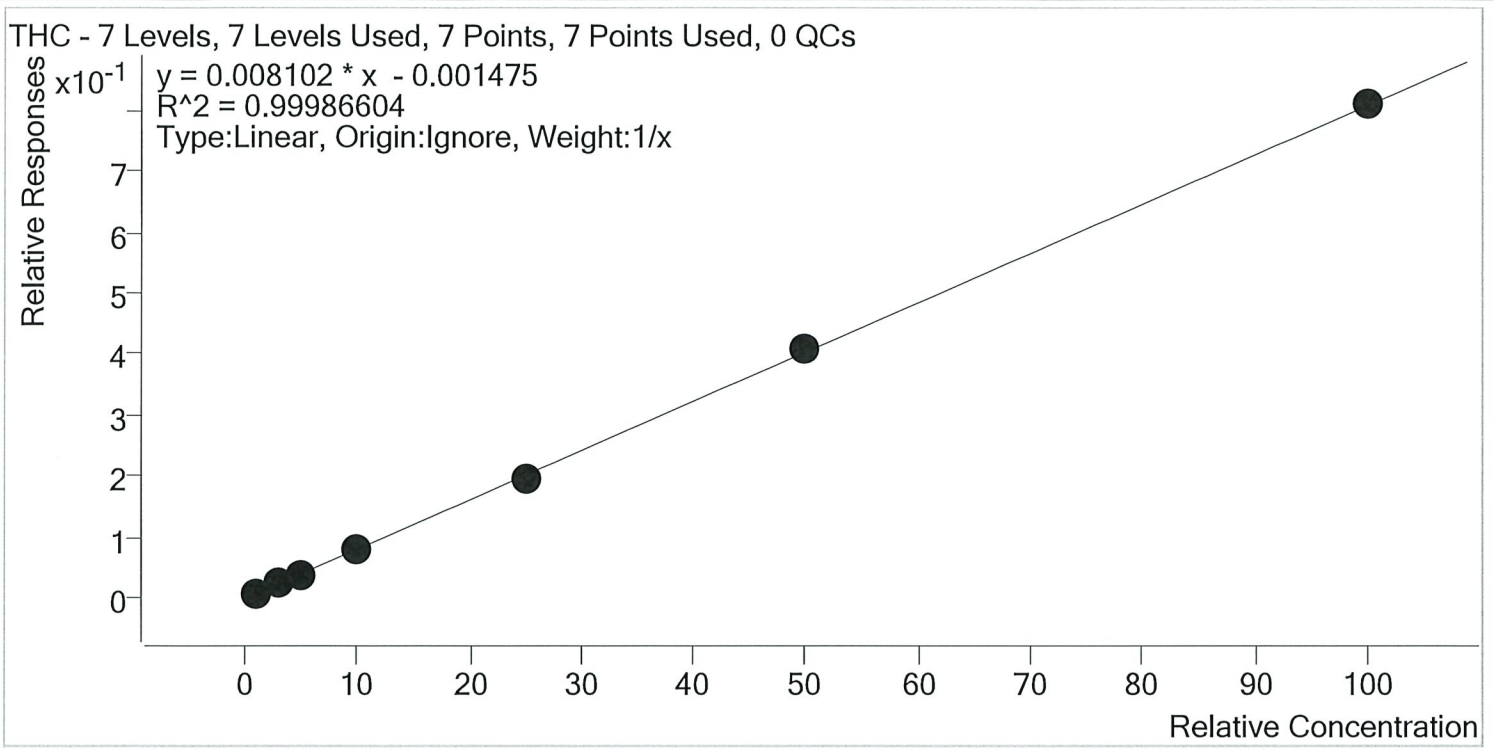


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	126422	∞	10.1	273.50	1319100	5.0611 ng/ml
THC-COOH	1.489	136041	1490.25	58.8	812.86	396868	13.4940 ng/ml
THC	3.360	489934	1800.60	26.2	232.67	14548920	4.3386 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wk1st 3896 TS\QuantResults\THCQ TS.batch.bin
Last Cal. Update 12/23/2019 8:26 AM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3

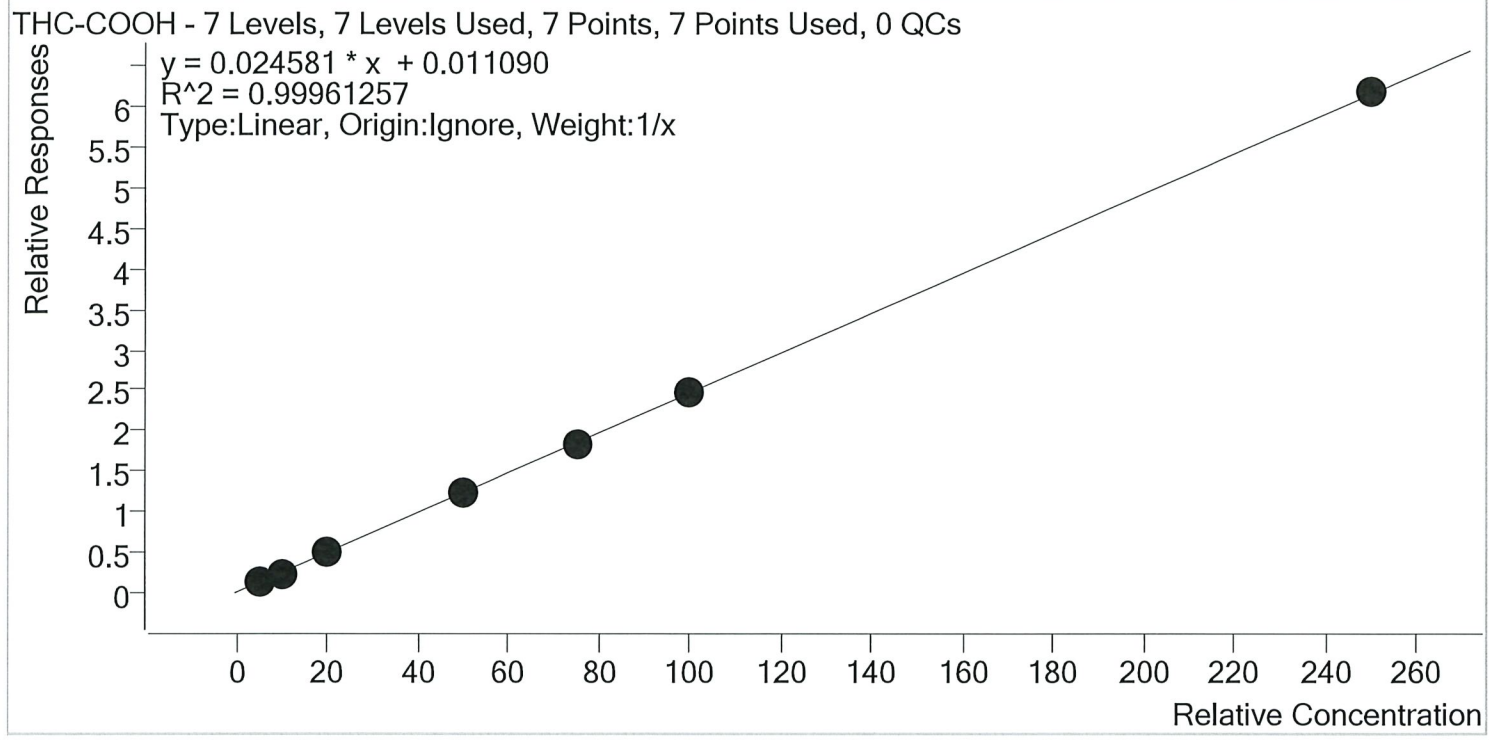


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.1	107.2
MJ Cal 2	2	✓	3.0	2.9	97.4
MJ Cal 3	3	✓	5.0	4.8	96.6
MJ Cal 4	4	✓	10.0	9.9	99.2
MJ Cal 5	5	✓	25.0	24.6	98.5
MJ Cal 6	6	✓	50.0	50.3	100.7
MJ Cal 7	7	✓	100.0	100.3	100.3



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wk1st 3896 TS\QuantResults\THCQ TS.batch.bin
Last Cal. Update 12/23/2019 8:26 AM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



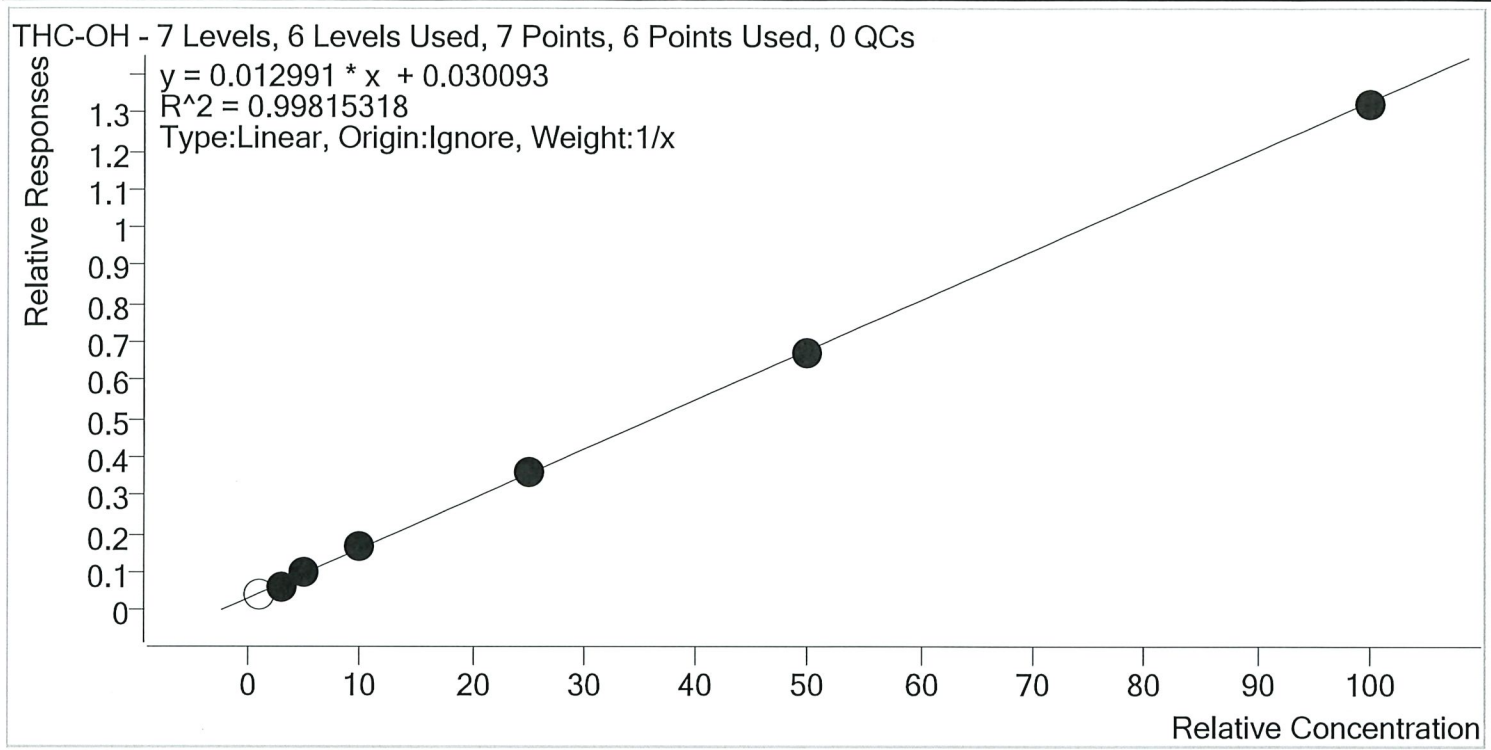
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	5.0	5.4	108.6
MJ Cal 2	2	✓	10.0	9.1	91.1
MJ Cal 3	3	✓	20.0	20.4	101.8
MJ Cal 4	4	✓	50.0	49.3	98.6
MJ Cal 5	5	✓	75.0	74.1	98.8
MJ Cal 6	6	✓	100.0	100.6	100.6
MJ Cal 7	7	✓	250.0	251.1	100.4

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklist 3896 TS\QuantResults\THCQ TS.batch.bin
Last Cal. Update 12/23/2019 8:26 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	0.9	90.4
MJ Cal 2	2	✓	3.0	2.4	80.6
MJ Cal 3	3	✓	5.0	5.6	112.1
MJ Cal 4	4	✓	10.0	10.7	106.7
MJ Cal 5	5	✓	25.0	25.7	102.7
MJ Cal 6	6	✓	50.0	49.3	98.7
MJ Cal 7	7	✓	100.0	99.3	99.3

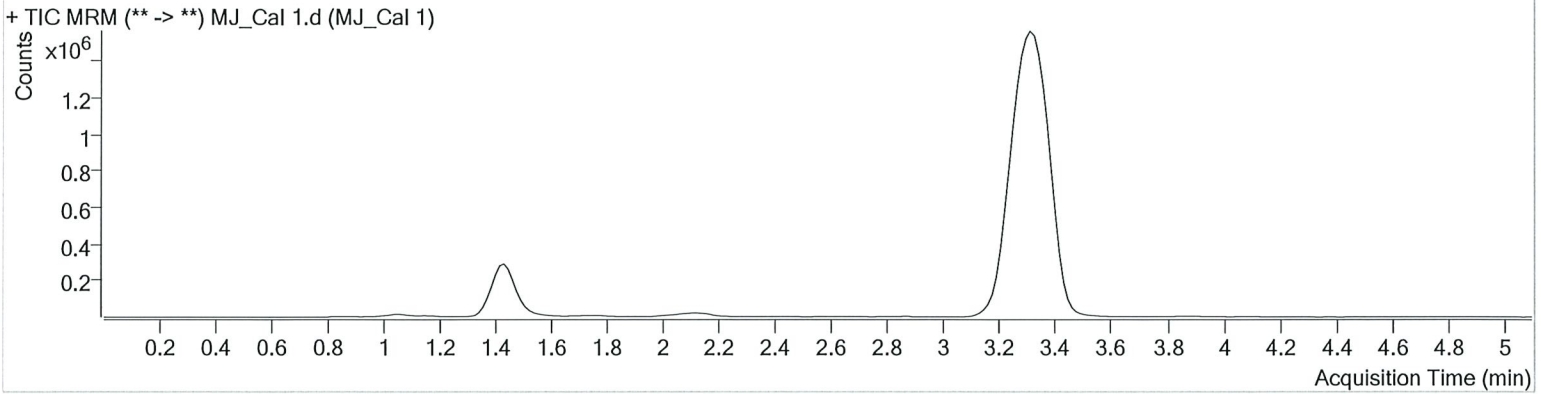
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklt 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 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-A1	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 11:13:10 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	52906	∞	5.5 Low	13.27	1264642	0.9039 ng/ml Low
THC-COOH	1.474	55388	∞	47.8	370.47	383183	5.4292 ng/ml Low
THC	3.330	106838	347.73	29.4	∞	14810585	1.0725 ng/ml Low

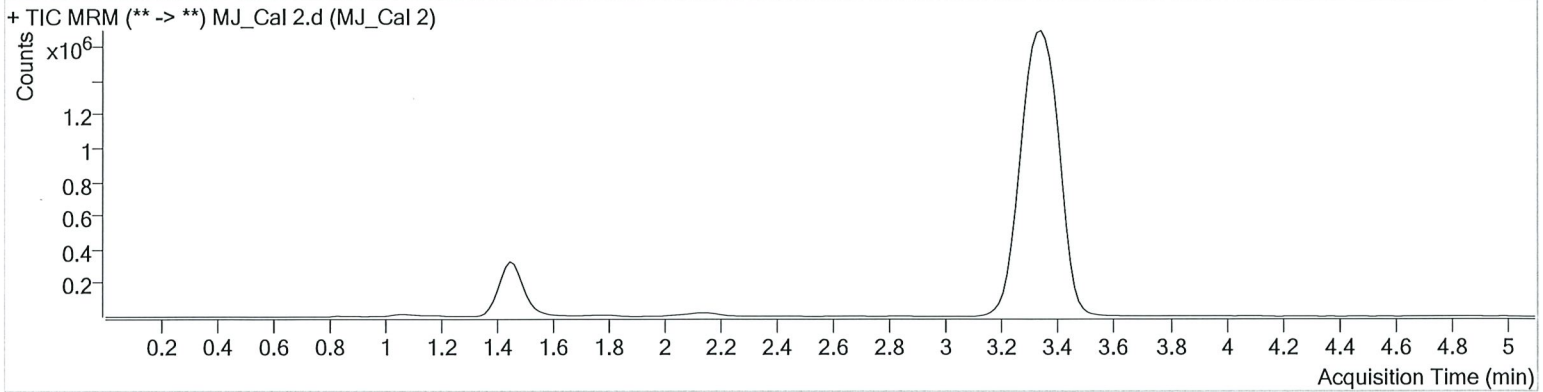
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklt 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 AM

Instrument	Falco	Data File	MJ_Cal 2.d
Type	Cal	Sample	MJ_Cal 2
Acq. Method	AM 27 THC quant.m	Operator	Tamara Salazar
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 11:20:55 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	82282	∞	9.3	100.71	1338015	2.4173 ng/ml Low
THC-COOH	1.489	94531	∞	58.3	617.89	402151	9.1116 ng/ml Low
THC	3.360	348098	1447.80	27.5	1584.47	15683368	2.9217 ng/ml Low

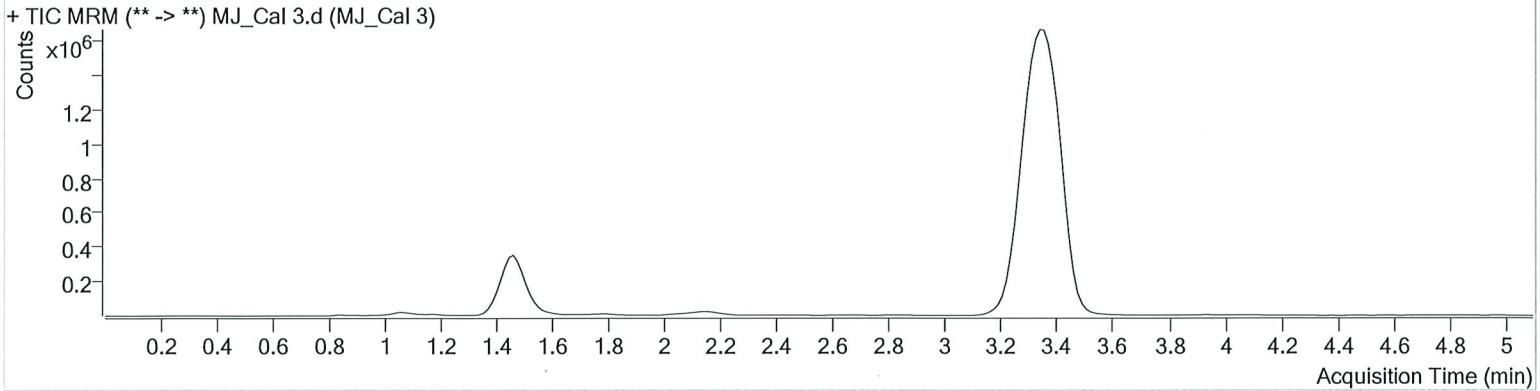
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklst 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 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-C1	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 11:28:29 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	139458	∞	9.6	257.16	1355146	5.6054 ng/ml
THC-COOH	1.504	204629	∞	56.4	1021.76	399940	20.3635 ng/ml
THC	3.360	574690	∞	27.3	∞	15254630	4.8322 ng/ml

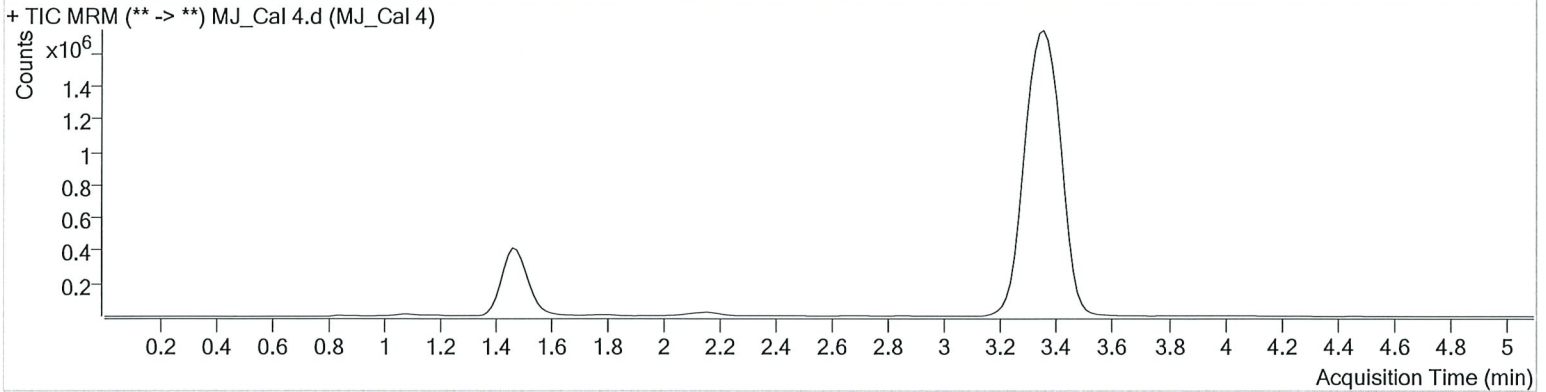
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wk1st 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 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-D1	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 11:36:03 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	213146	∞	10.6	214.02	1263913	10.6652 ng/ml
THC-COOH	1.504	460287	720.21	59.8	1369.05	376248	49.3170 ng/ml
THC	3.375	1128066	1545.62	26.8	555.39	14292592	9.9241 ng/ml

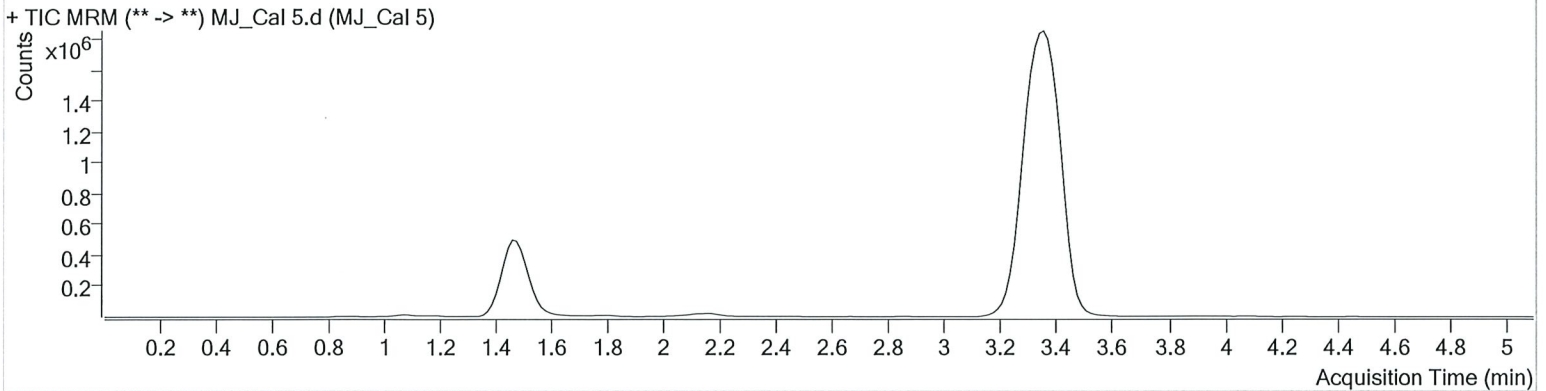
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklt 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 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-E1	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 11:43:38 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	453484	∞	12.2	∞	1247278	25.6715 ng/ml
THC-COOH	1.504	676116	∞	59.9	∞	368817	74.1264 ng/ml
THC	3.360	2716737	6480.73	26.2	∞	13716928	24.6286 ng/ml

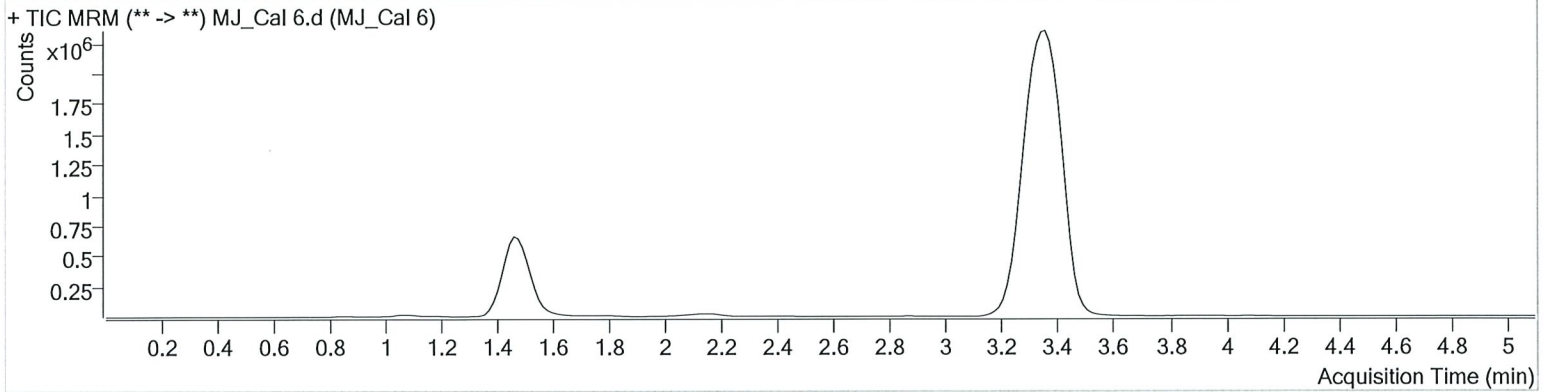
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklt 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 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-F1	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 11:51:12 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	893956	∞	13.6	2173.48	1332262	49.3370	ng/ml
THC-COOH	1.504	957304	∞	59.5	3884.17	385448	100.5863	ng/ml
THC	3.360	6017696	6661.18	26.5	1464.70	14808336	50.3412	ng/ml

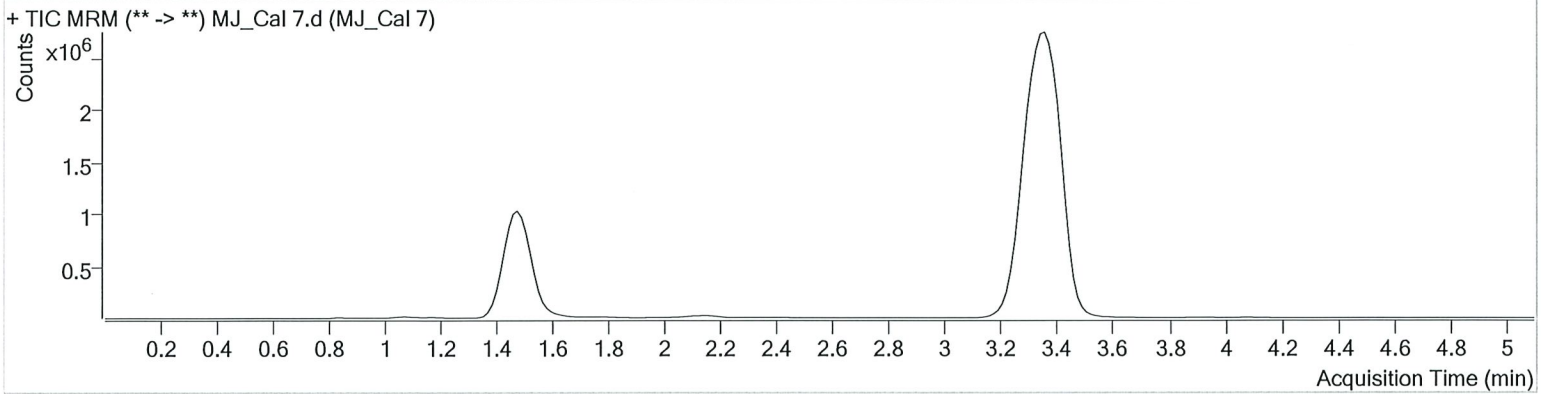
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2019\AM 27\121919 THCQ wklt 3896 TS\QuantResults\THCQ TS.batch.bin
Calibration Last Update 12/23/2019 8:26:42 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-G1	Comment	
Injection Volume	10		
Acq. Date-Time	12/19/2019 11:58:47 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	1558020	∞	13.7	4549.52	1180231	99.3036 ng/ml
THC-COOH	1.489	2053668	∞	60.3	∞	332171	251.0659 ng/ml
THC	3.360	10225274	9082.27	26.9	∞	12608882	100.2798 ng/ml