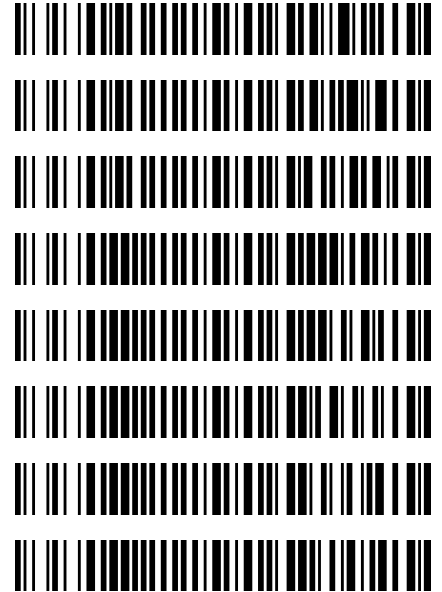


Worklist: 3660

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>
M2019-3274	2	162759	AM 27 Blood THC Quant by LC-QQQ
M2019-3277	1	162760	AM 27 Blood THC Quant by LC-QQQ
M2019-3530	2	162761	AM 27 Blood THC Quant by LC-QQQ
P2019-2441	1	162762	AM 27 Blood THC Quant by LC-QQQ
P2019-2444	1	162763	AM 27 Blood THC Quant by LC-QQQ
P2019-2464	1	162764	AM 27 Blood THC Quant by LC-QQQ
P2019-2473	1	162765	AM 27 Blood THC Quant by LC-QQQ
P2019-2478	1	162766	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 9/6/19

Analyst: Sarah Pickle

Plate lot#: Lot # 190716 Item # IDP-108

Plate Expiration: 1/16/20

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 445283-2

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: #3**
- 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\2019\AM 27\090619 MDS SP Batch Name: THCQ
- 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: *Curve Ranges: THC: 3-100, THC-COOH 10-250, THC-OH 3-100*

Samples were originally extracted 9/6/19. Due to internal standard retention time shifts, the samples were reconstituted and reinjected 9/9/19.

AM #27 Cannabinoids Quant. Results



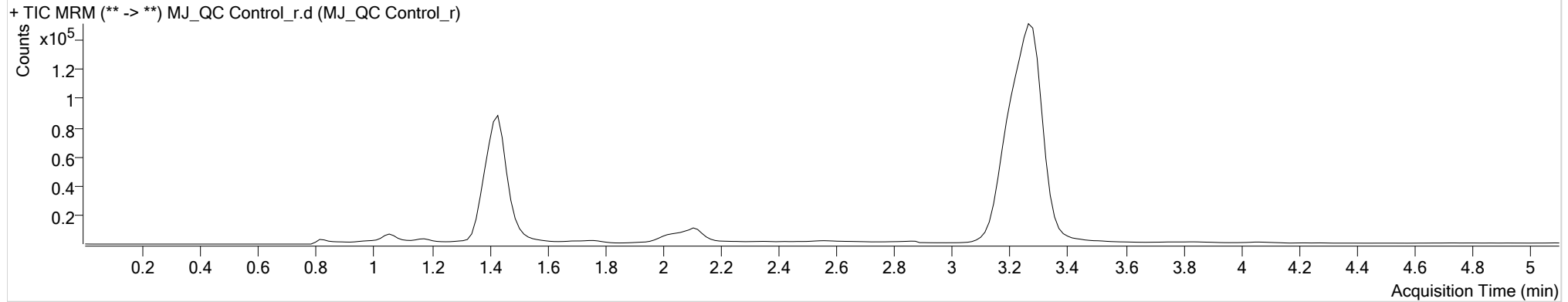
Batch results
Calibration Last Update

D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
9/9/2019 7:22:20 PM

Instrument Falco
Type Sample
Acq. Method AM 27 THC quant.m
Sample Position P3-H1
Injection Volume 10
Acq. Date-Time 9/9/2019 10:47:52 AM
Sample Info.

Data File MJ_QC Control_r.d
Sample MJ_QC Control_r
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.285	42586	165.67	28.5	15.88	1180076	4.5446 ng/ml
THC-COOH	1.459	33860	∞	47.8	407.70	86524	15.4254 ng/ml
THC-OH	1.438	28410	∞	9.5	25.26	313172	5.0622 ng/ml

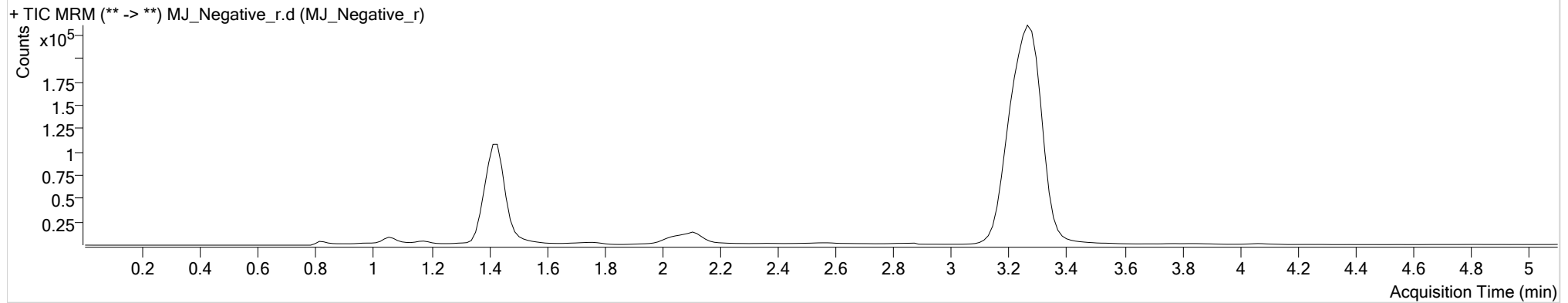
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Calibration Last Update 9/9/2019 7:22:20 PM

Instrument	Falco	Data File	MJ_Negative_r.d
Type	Sample	Sample	MJ_Negative_r
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-A2		
Injection Volume	10		
Acq. Date-Time	9/9/2019 11:03:04 AM		
Sample Info.			

Sample Chromatogram



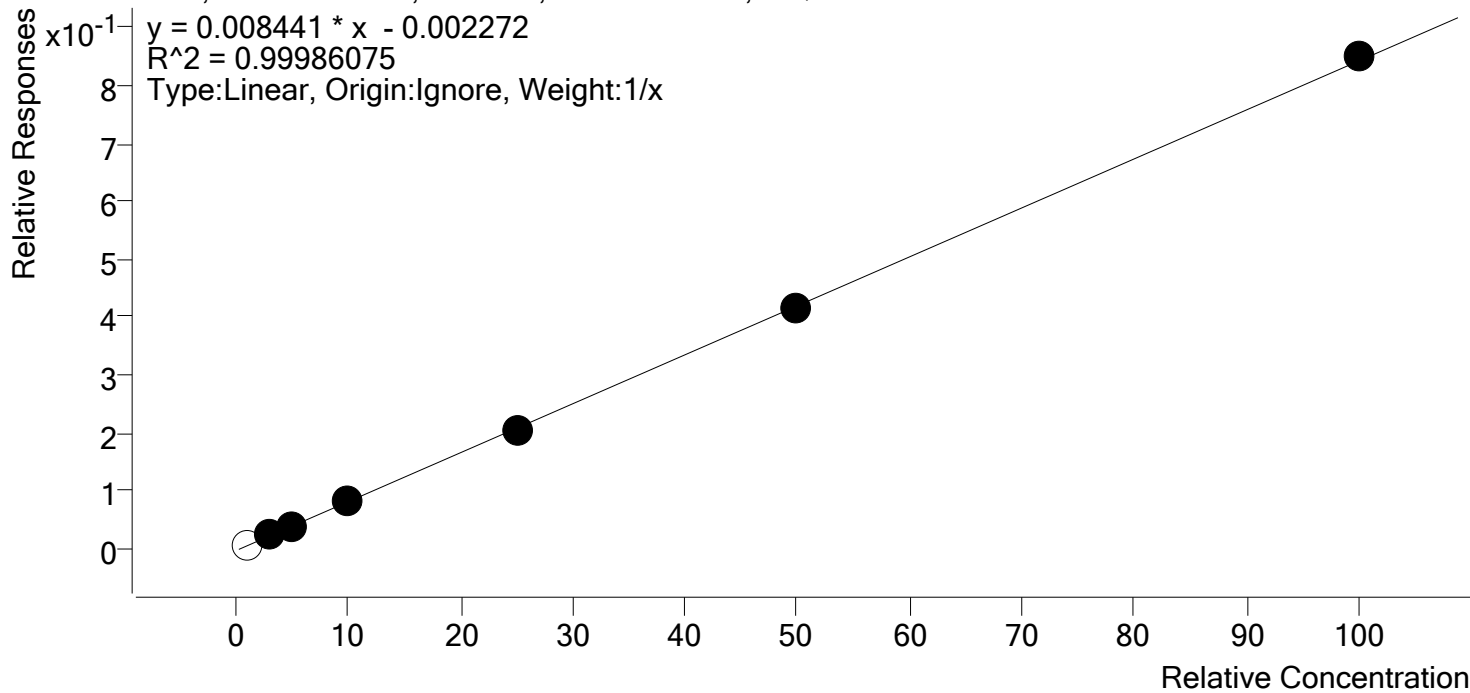
SP



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Last Cal. Update 9/9/2019 7:22 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

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

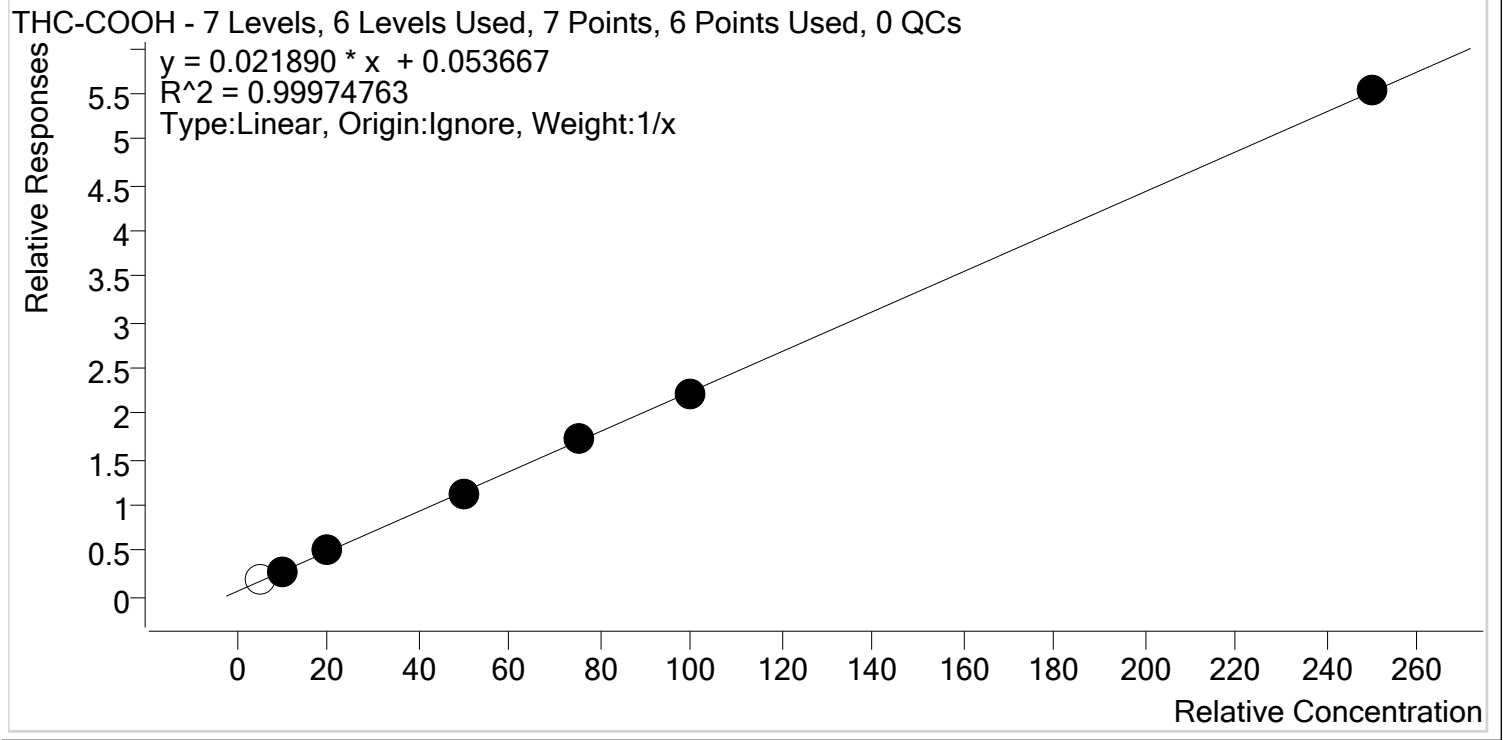


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1_r	1	x	1.0	1.2	122.6
MJ_Cal 2_r	2	✓	3.0	3.1	102.7
MJ_Cal 3_r	3	✓	5.0	5.0	99.6
MJ_Cal 4_r	4	✓	10.0	9.9	99.1
MJ_Cal 5_r	5	✓	25.0	24.7	98.7
MJ_Cal 6_r	6	✓	50.0	49.5	99.0
MJ_Cal 7_r	7	✓	100.0	100.9	100.9



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Last Cal. Update 9/9/2019 7:22 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



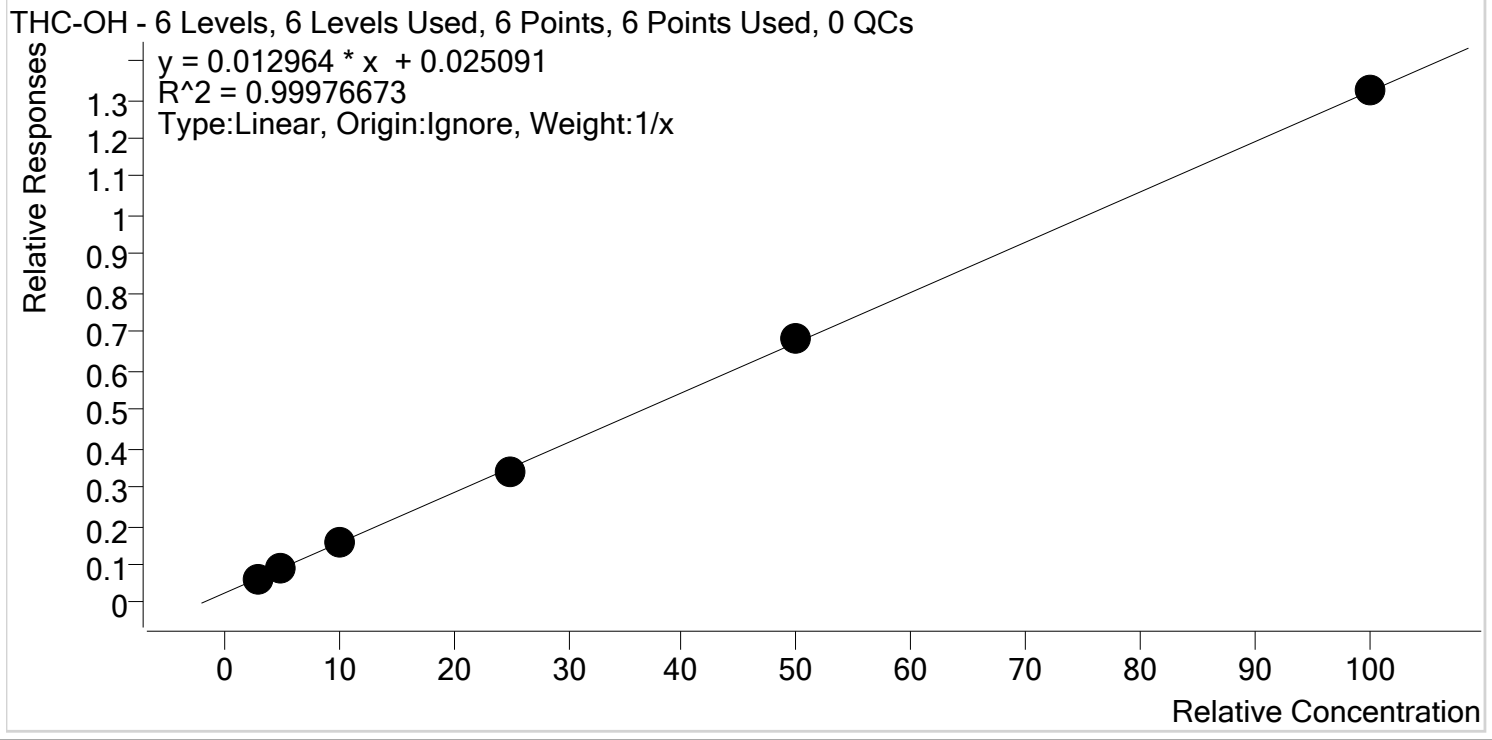
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 1_r	1	x	5.0	6.3	126.0
MJ_Cal 2_r	2	✓	10.0	9.8	98.2
MJ_Cal 3_r	3	✓	20.0	20.7	103.6
MJ_Cal 4_r	4	✓	50.0	48.8	97.5
MJ_Cal 5_r	5	✓	75.0	76.0	101.3
MJ_Cal 6_r	6	✓	100.0	99.1	99.1
MJ_Cal 7_r	7	✓	250.0	250.6	100.2

5



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Last Cal. Update 9/9/2019 7:22 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ_Cal 2_r	2	✓	3.0	2.9	98.0
MJ_Cal 3_r	3	✓	5.0	5.2	103.2
MJ_Cal 4_r	4	✓	10.0	10.1	100.8
MJ_Cal 5_r	5	✓	25.0	24.3	97.0
MJ_Cal 6_r	6	✓	50.0	50.4	100.9
MJ_Cal 7_r	7	✓	100.0	100.1	100.1

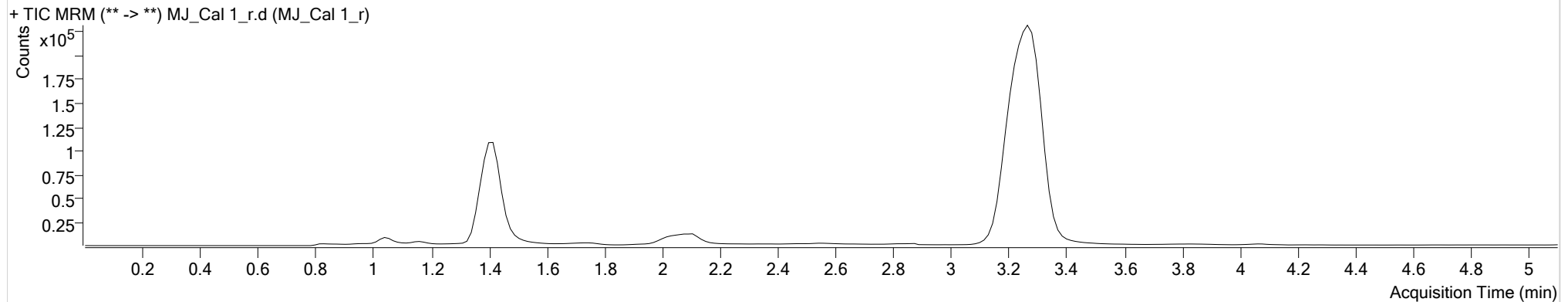
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Calibration Last Update 9/9/2019 7:22:20 PM

Instrument	Falco	Data File	MJ_Cal 1_r.d
Type	Cal	Sample	MJ_Cal 1_r
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-A1		
Injection Volume	10		
Acq. Date-Time	9/9/2019 9:54:39 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.285	15034	67.80	28.8	18.54	1862531	1.2255 ng/ml
THC-COOH	1.444	21434	∞	34.7 Low	83.62	111891	6.2992 ng/ml

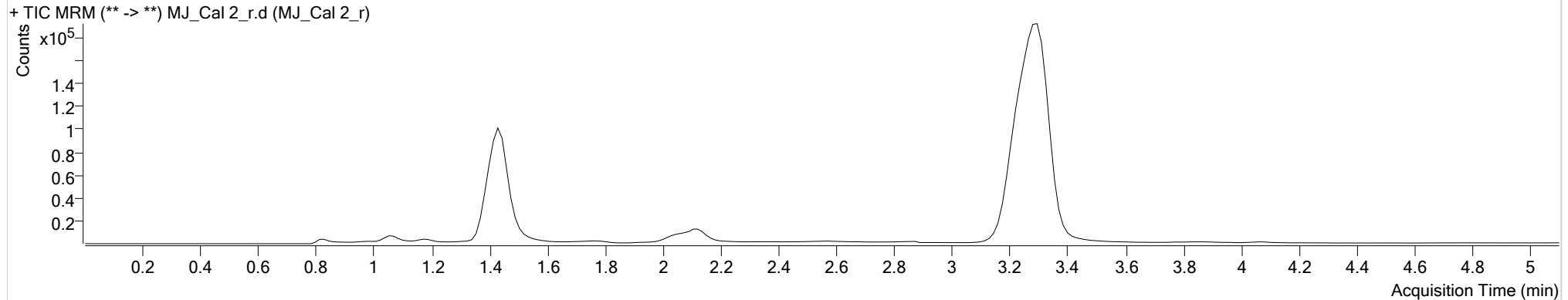
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Calibration Last Update 9/9/2019 7:22:20 PM

Instrument Falco **Data File** MJ_Cal 2_r.d
Type Cal **Sample** MJ_Cal 2_r
Acq. Method AM 27 THC quant.m
Sample Position P3-B1 **Comment**
Injection Volume 10
Acq. Date-Time 9/9/2019 10:02:24 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.315	35358	126.57	29.7	27.26	1489283	3.0819 ng/ml
THC-COOH	1.459	26252	∞	47.2	232.70	97740	9.8182 ng/ml
THC-OH	1.453	22875	∞	9.7	20.72	361941	2.9395 ng/ml

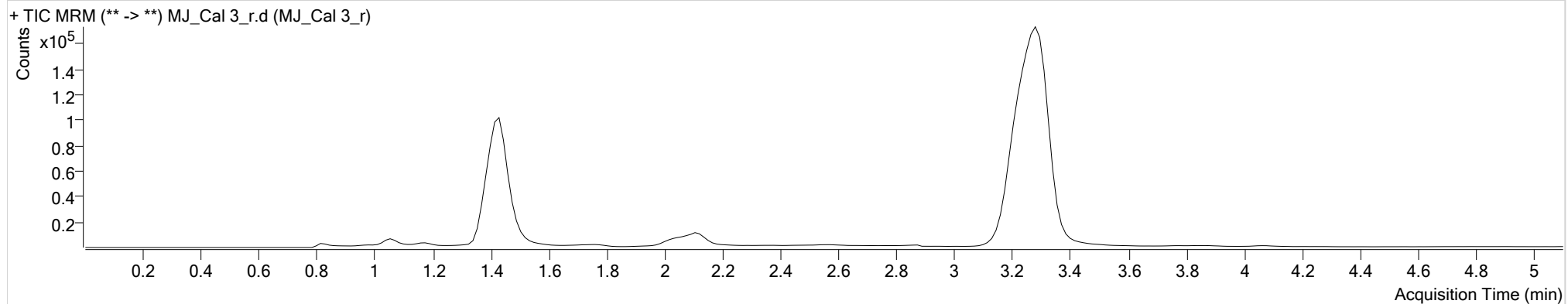
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Calibration Last Update 9/9/2019 7:22:20 PM

Instrument Falco **Data File** MJ_Cal 3_r.d
Type Cal **Sample** MJ_Cal 3_r
Acq. Method AM 27 THC quant.m
Sample Position P3-C1 **Comment**
Injection Volume 10
Acq. Date-Time 9/9/2019 10:10:00 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.300	52284	390.86	31.5	62.10	1314993	4.9797 ng/ml
THC-COOH	1.459	45782	135.24	52.1	377.77	90246	20.7229 ng/ml
THC-OH	1.438	32149	∞	10.4	29.72	349478	5.1605 ng/ml

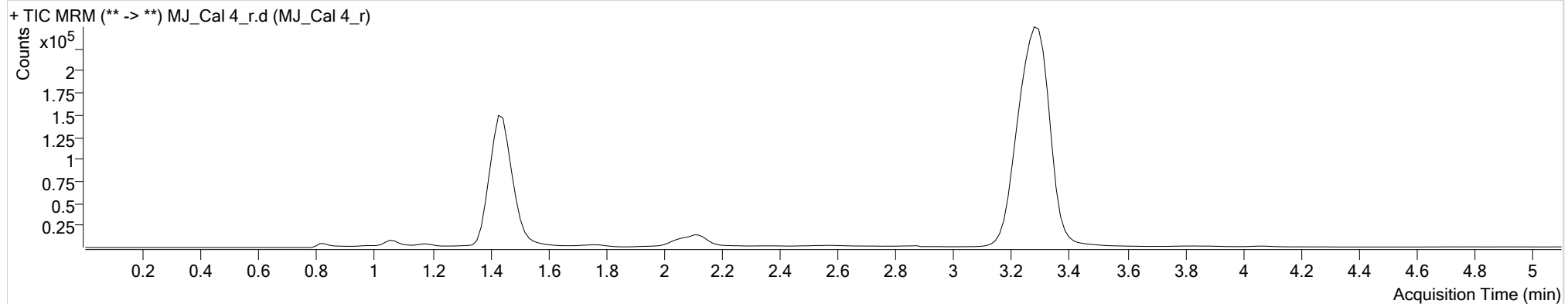
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Calibration Last Update 9/9/2019 7:22:20 PM

Instrument Falco **Data File** MJ_Cal 4_r.d
Type Cal **Sample** MJ_Cal 4_r
Acq. Method AM 27 THC quant.m
Sample Position P3-D1 **Comment**
Injection Volume 10
Acq. Date-Time 9/9/2019 10:17:34 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.300	140226	520.26	28.8	80.20	1722818	9.9122 ng/ml
THC-COOH	1.474	122303	311.48	58.2	2268.11	109069	48.7738 ng/ml
THC-OH	1.438	65653	∞	11.2	202.92	421596	10.0766 ng/ml

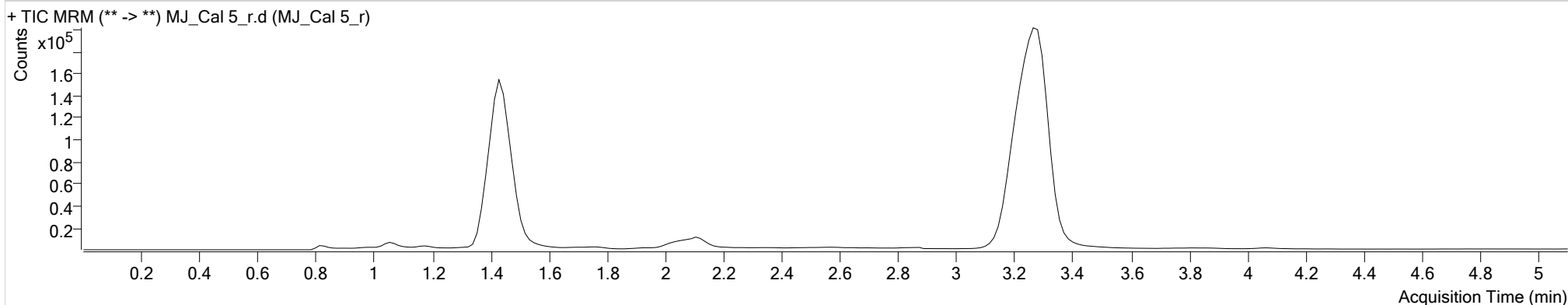
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Calibration Last Update 9/9/2019 7:22:20 PM

Instrument Falco **Data File** MJ_Cal 5_r.d
Type Cal **Sample** MJ_Cal 5_r
Acq. Method AM 27 THC quant.m
Sample Position P3-E1 **Comment**
Injection Volume 10
Acq. Date-Time 9/9/2019 10:25:09 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.285	260032	487.16	28.2	175.18	1262065	24.6791 ng/ml
THC-COOH	1.459	149266	752.10	58.7	578.24	86943	75.9769 ng/ml
THC-OH	1.438	120871	∞	12.5	425.70	355958	24.2574 ng/ml

AM #27 Cannabinoids Quant. Results

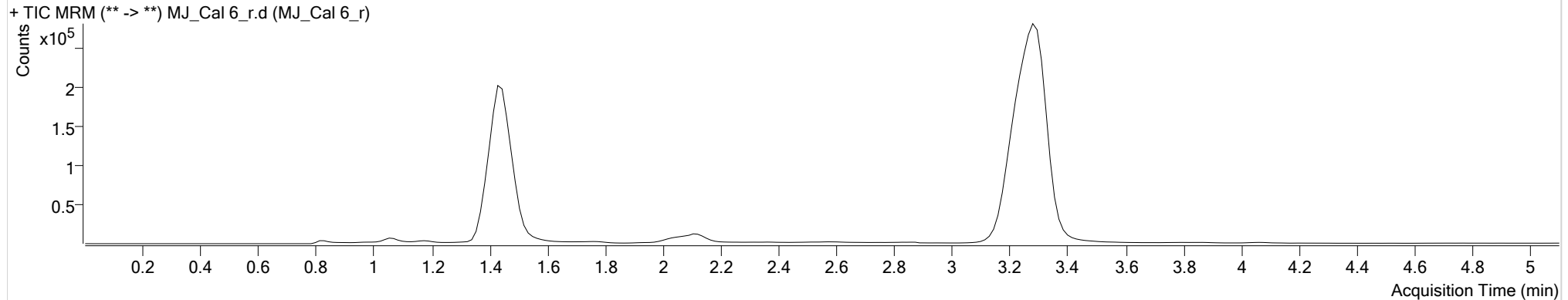


Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Calibration Last Update 9/9/2019 7:22:20 PM

Instrument Falco
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-F1
Injection Volume 10
Acq. Date-Time 9/9/2019 10:32:43 AM
Sample Info.

Data File MJ_Cal 6_r.d
Sample MJ_Cal 6_r
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.300	604492	5971.39	27.6	229.55	1454990	49.4903 ng/ml
THC-COOH	1.474	211977	∞	59.3	2353.87	95340	99.1173 ng/ml
THC-OH	1.438	258305	∞	12.9	241.89	380403	50.4425 ng/ml

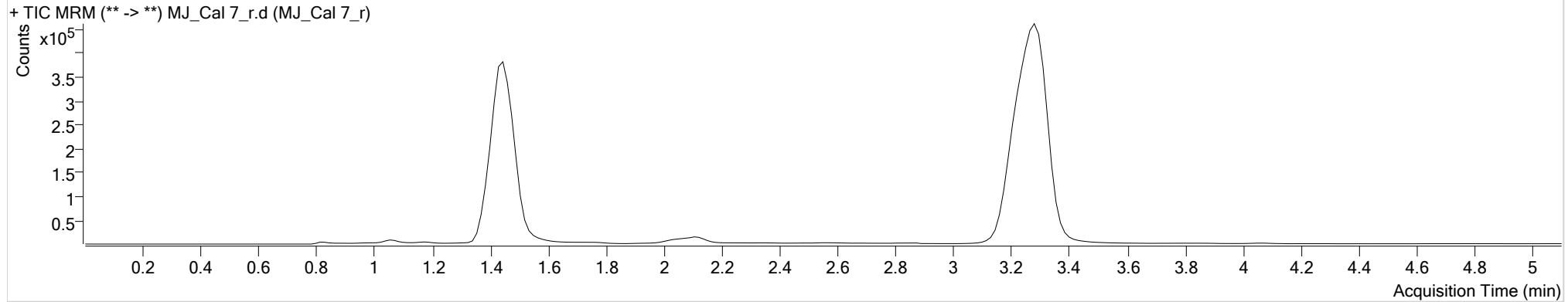
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 25\090619 MDS SP\QuantResults\THCQ reinjects.batch.bin
Calibration Last Update 9/9/2019 7:22:20 PM

Instrument Falco **Data File** MJ_Cal 7_r.d
Type Cal **Sample** MJ_Cal 7_r
Acq. Method AM 27 THC quant.m
Sample Position P3-G1 **Comment**
Injection Volume 10
Acq. Date-Time 9/9/2019 10:40:17 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.285	1498857	1621.92	27.3	5771.32	1765372	100.8569 ng/ml
THC-COOH	1.459	584694	8246.79	60.2	2818.45	105556	250.5910 ng/ml
THC-OH	1.438	550502	∞	13.2	1034.12	416071	100.1234 ng/ml