

REVIEWED

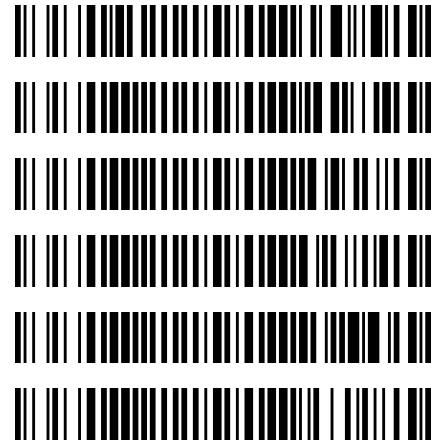
By Tamara Salazar at 1:54 pm, Jul 05, 2019

7/1/2019

TS

Worklist: 3520

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>
M2019-2555	1	156236	AM 27 Blood THC Quant by LC-QQQ
P2019-1758	1	156237	AM 27 Blood THC Quant by LC-QQQ
P2019-1873	1	156238	AM 27 Blood THC Quant by LC-QQQ
P2019-1903	1	156239	AM 27 Blood THC Quant by LC-QQQ
P2019-1924	1	156240	AM 27 Blood THC Quant by LC-QQQ
P2019-1927	1	156241	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 7/2/19
 Plate lot#: 0539904

Analyst: Sarah Pickle
 Plate Expiration: 9/10/19

Mobile phase A: 0.1% Formic Acid in LCMS Water
 MTBE
Blank Blood Lot: 445283-2
LCMS-QQQ ID: 069901

Mobile phase B: 0.1% Formic acid in Acetonitrile
 LCMS Methanol
 Hexane
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: #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\070219 THCQ MDQ 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 Range limited: THC-COOH 5-100*



Idaho State Police Forensic Services

AM #27 Quantitative Analysis of THC and Metabolites in Blood by LCMS-QQQ

Methanol External Control Solution (Lot: WS041619)

10 ul of 1mg/mL THC, 100 ul of 100 ug/mL THC-OH, C-THC in 9790 ul MeOH
Approximate concentration 1ug/mL.

Component	Source	Source Lot Number	Expiration Date
Methanol (LCMS)	Fisher	184782	
THC	Cerilliant	FE09101501	11/30/2020
C-THC	Cerilliant	FE07171501	09/30/2020
THC-OH	Cerilliant	FE01121503	01/31/2020
Prepared:	04/16/2019		
Prepared By:	Tamara Salazar		
Expires:	01/31/2020		

Blood External Control Solution (Lot: 041619)

100 ul of methanol external control solution was added to 9900 ul of blood.
Approximately 10ng/mL of each compound.

Component	Source	Source Lot Number
Negative Blood	Hemostat	445283-1
Methanol External Control Solution	-	WS041619
Prepared:	04/16/19	
Prepared by:	Tamara Salazar	
Expires:	01/31/2020	

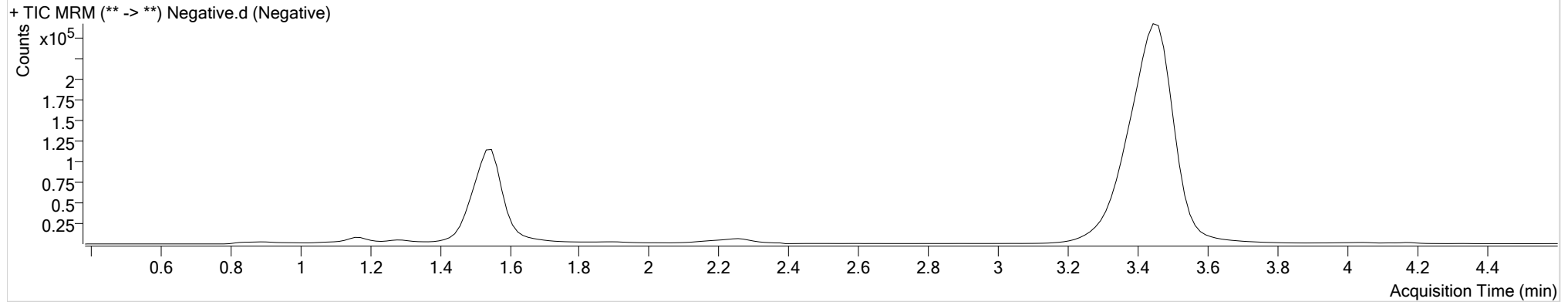
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument Type	Falco	Data File	Negative.d
Acq. Method	Sample	Sample	Negative
Acq. Method	AM 27 THC quant.m		
Sample Position	P3-H5	Comment	
Injection Volume	10		
Acq. Date-Time	7/2/2019 11:52:48 AM		
Sample Info.			

Sample Chromatogram



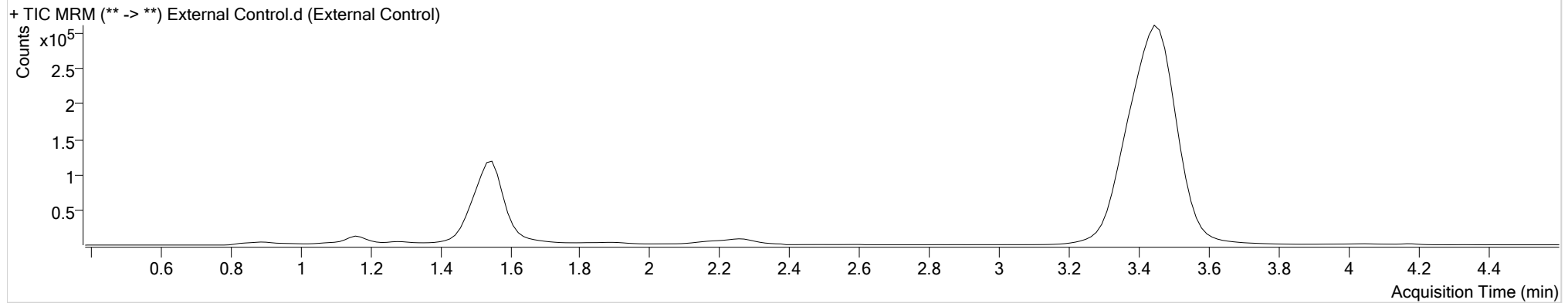
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument Falco **Data File** External Control.d
Type Sample **Sample** External Control
Acq. Method AM 27 THC quant.m
Sample Position P3-G5 **Comment**
Injection Volume 10
Acq. Date-Time 7/2/2019 12:08:00 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.461	170514	670.87	25.5	163.76	2861528	7.1491 ng/ml
THC-COOH	1.580	32885	159.77	43.0	191.34	235530	8.2469 ng/ml
THC-OH	1.558	55256	∞	11.3	142.84	424579	8.0774 ng/ml

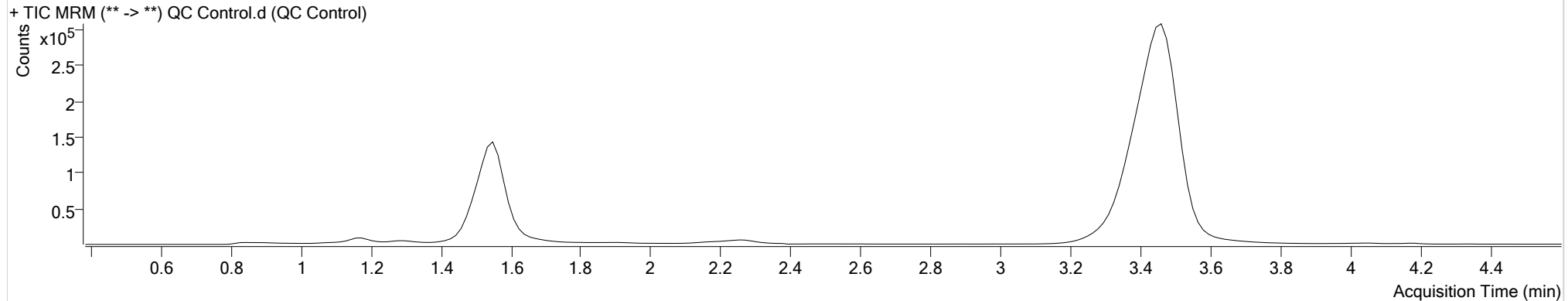
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument	Falco	Data File	QC Control.d
Type	Sample	Sample	QC Control
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-A6		
Injection Volume	10		
Acq. Date-Time	7/2/2019 11:37:37 AM		
Sample Info.			

Sample Chromatogram



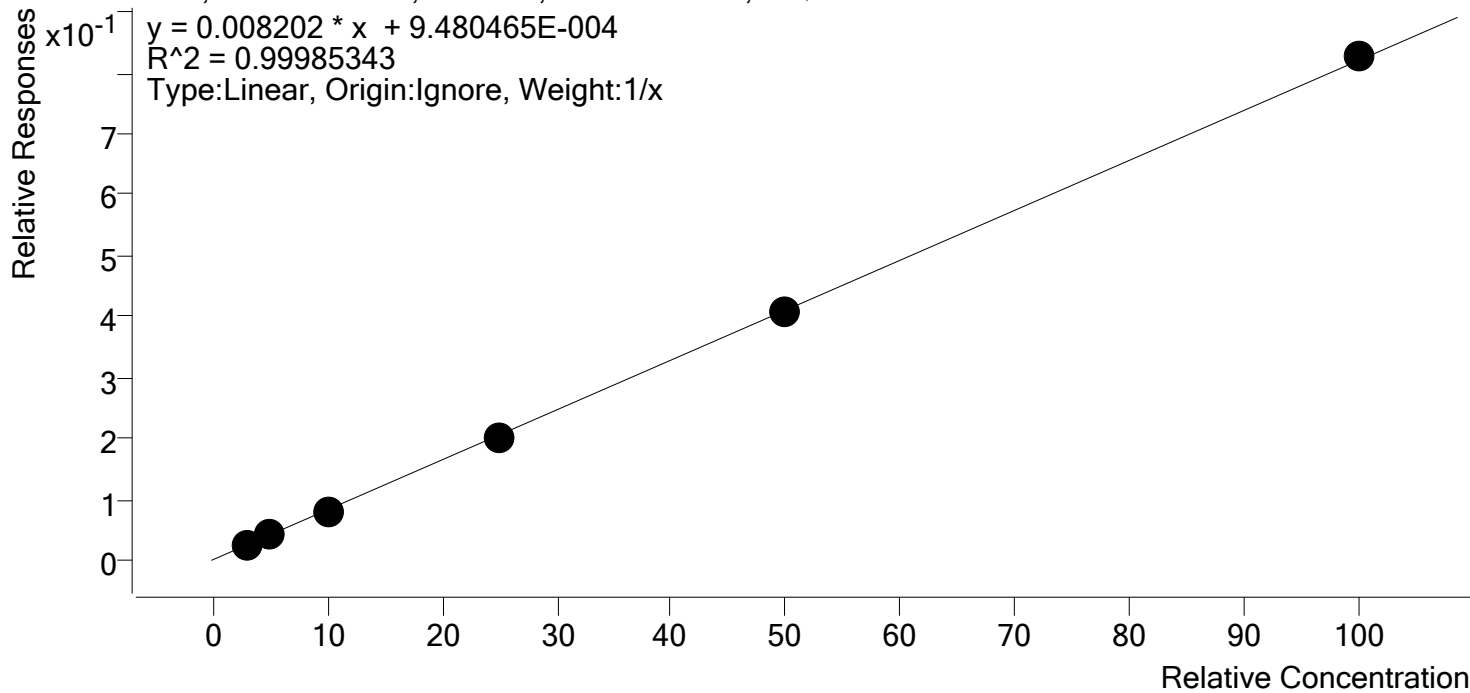
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.476	109303	338.77	28.1	228.91	2680096	4.8565 ng/ml
THC-COOH	1.580	39390	138.19	50.1	567.89	246193	9.7143 ng/ml
THC-OH	1.558	46767	∞	12.1	136.64	522881	5.2238 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Last Cal. Update 7/2/2019 3:05 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

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

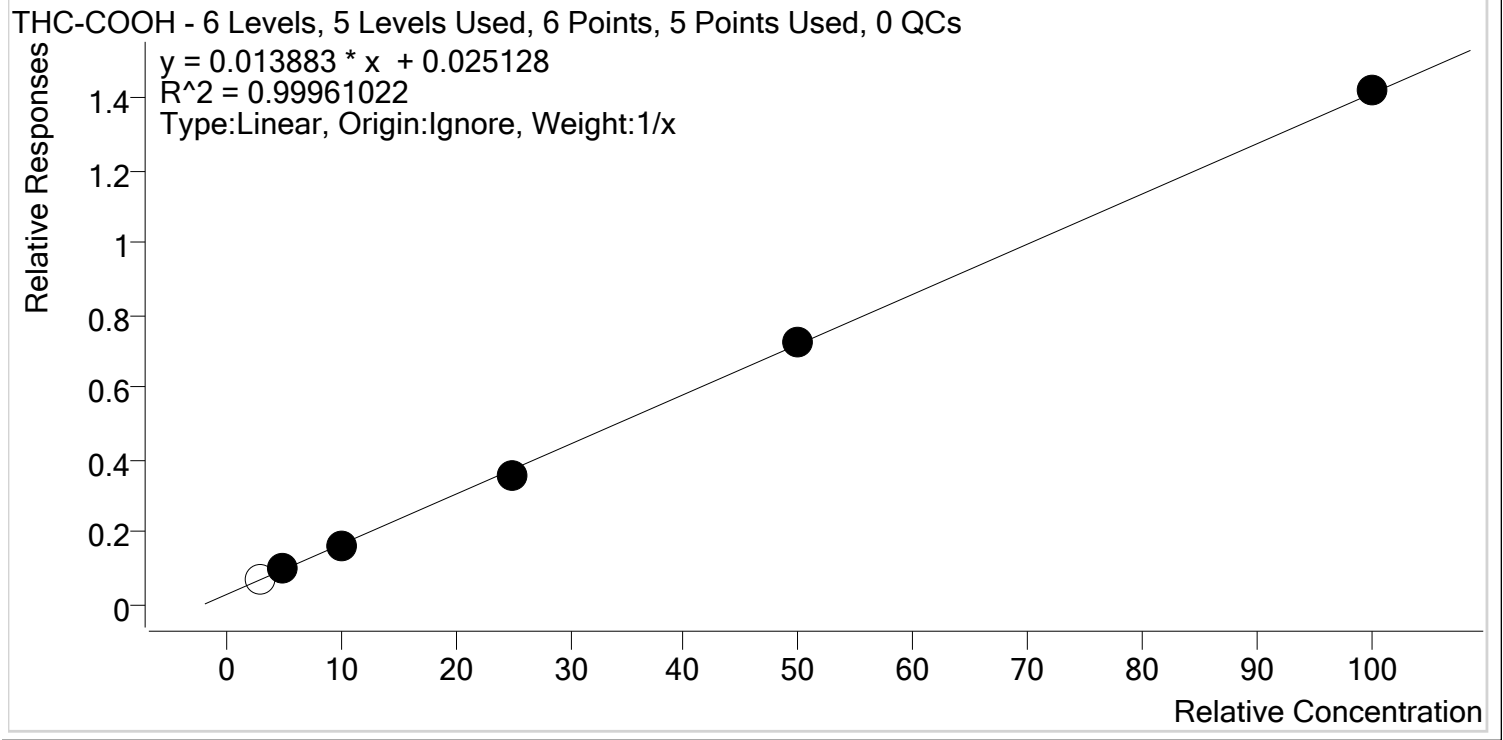


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-3ng	1	✓	3.0	3.0	101.0
Cal 2- 5ng	2	✓	5.0	5.1	102.2
Cal 3 -10ng	3	✓	10.0	9.9	98.5
Cal 4-25ng	4	✓	25.0	24.5	98.0
Cal 5-50ng	5	✓	50.0	49.8	99.5
Cal 6-100ng	6	✓	100.0	100.7	100.7



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Last Cal. Update 7/2/2019 3:05 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

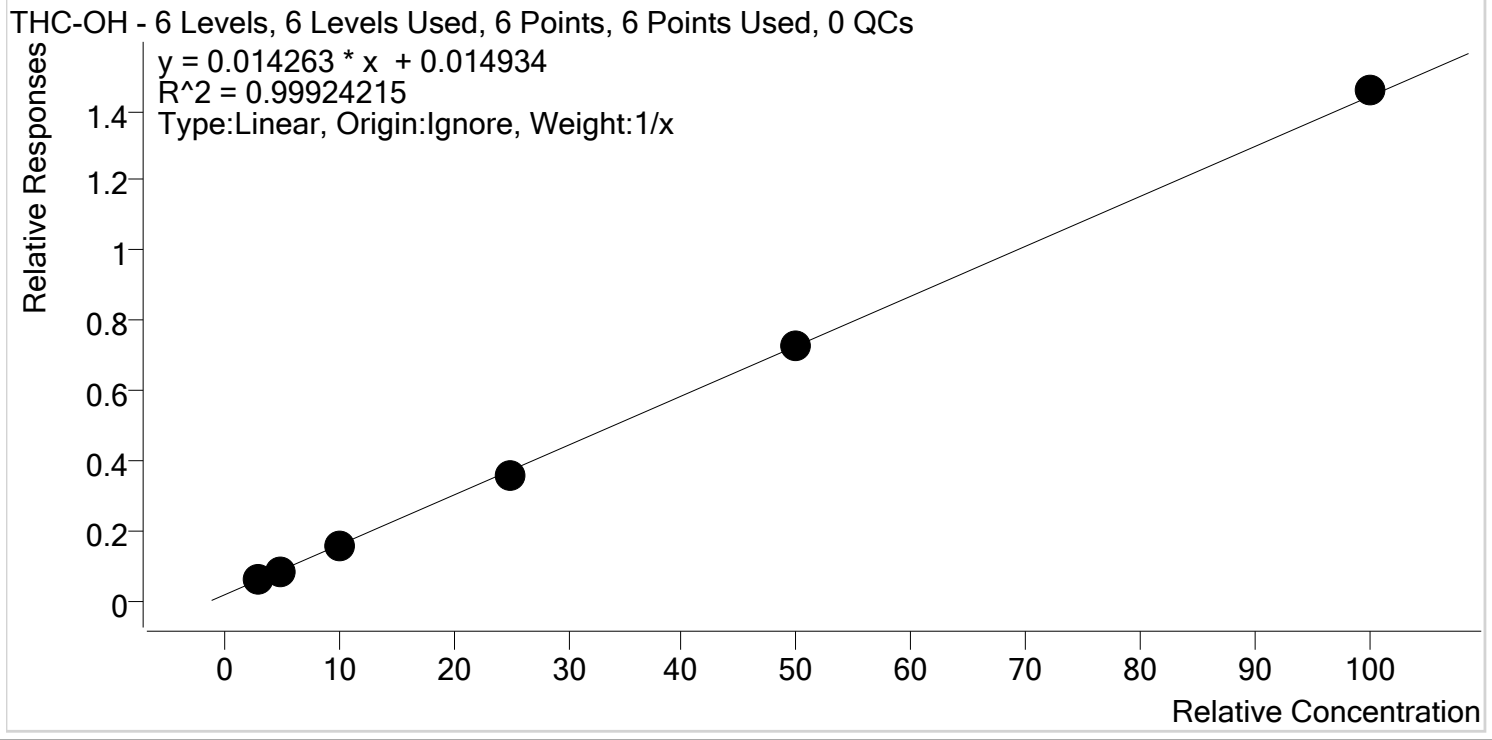


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-3ng	1	x	3.0	3.2	107.5
Cal 2- 5ng	2	✓	5.0	5.2	104.0
Cal 3 -10ng	3	✓	10.0	9.9	98.5
Cal 4-25ng	4	✓	25.0	24.1	96.4
Cal 5-50ng	5	✓	50.0	50.3	100.6
Cal 6-100ng	6	✓	100.0	100.6	100.6



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Last Cal. Update 7/2/2019 3:05 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-3ng	1	✓	3.0	3.3	110.4
Cal 2- 5ng	2	✓	5.0	4.8	96.3
Cal 3 -10ng	3	✓	10.0	9.6	95.7
Cal 4-25ng	4	✓	25.0	24.0	96.2
Cal 5-50ng	5	✓	50.0	50.1	100.2
Cal 6-100ng	6	✓	100.0	101.1	101.1

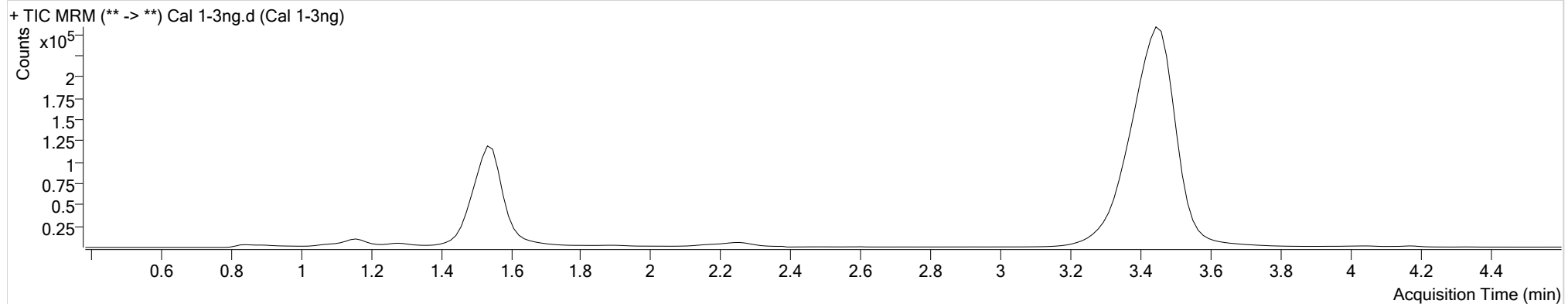
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument Falco **Data File** Cal 1-3ng.d
Type Cal **Sample** Cal 1-3ng
Acq. Method AM 27 THC quant.m
Sample Position P3-G6 **Comment**
Injection Volume 10
Acq. Date-Time 7/2/2019 10:52:08 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.461	58516	141.77	28.5	71.69	2267893	3.0301 ng/ml
THC-COOH	1.565	15522	∞	40.5 Low	99.89	222013	3.2261 ng/ml
THC-OH	1.558	28561	∞	11.1	24.50	459337	3.3124 ng/ml

AM #27 Cannabinoids Quant. Results

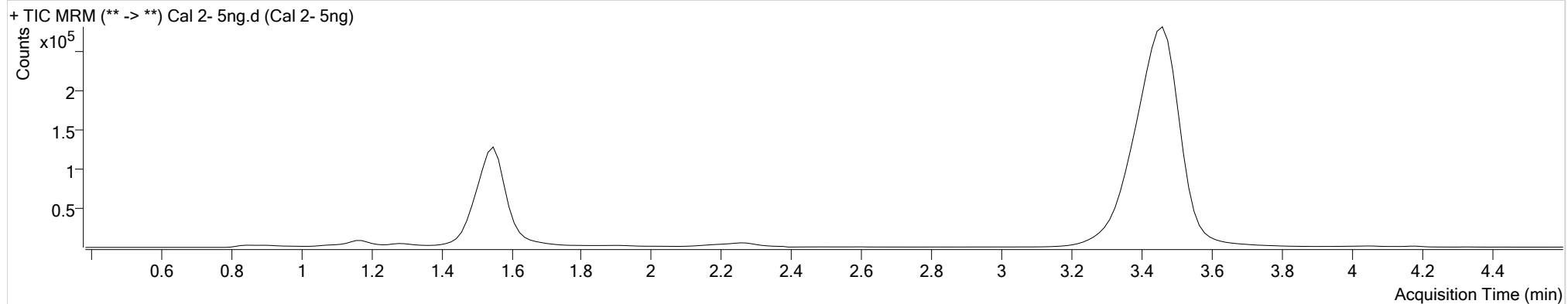


Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument Falco
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-F6
Injection Volume 10
Acq. Date-Time 7/2/2019 10:59:43 AM
Sample Info.

Data File Cal 2- 5ng.d
Sample Cal 2- 5ng
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.476	103430	918.56	26.3	118.58	2413989	5.1080 ng/ml
THC-COOH	1.580	22451	240.55	45.7	234.64	230763	5.1979 ng/ml
THC-OH	1.558	39856	∞	13.3	46.52	476496	4.8174 ng/ml

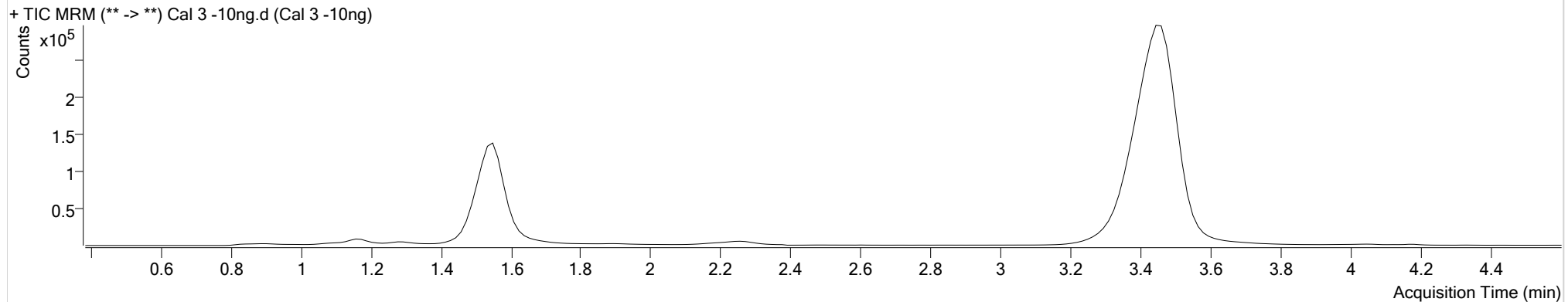
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument	Falco	Data File	Cal 3 -10ng.d
Type	Cal	Sample	Cal 3 -10ng
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-E6		
Injection Volume	10		
Acq. Date-Time	7/2/2019 11:07:18 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.461	191259	2199.20	27.5	1120.52	2339028	9.8532 ng/ml
THC-COOH	1.580	36227	∞	51.2	284.15	223784	9.8503 ng/ml
THC-OH	1.558	69984	∞	13.3	259.15	462181	9.5693 ng/ml

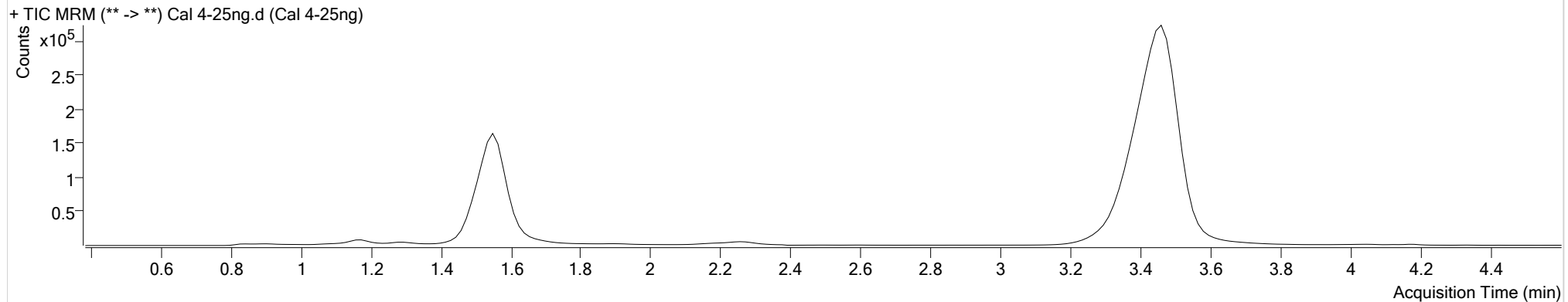
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument Falco **Data File** Cal 4-25ng.d
Type Cal **Sample** Cal 4-25ng
Acq. Method AM 27 THC quant.m
Sample Position P3-D6 **Comment**
Injection Volume 10
Acq. Date-Time 7/2/2019 11:14:52 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.476	467639	1530.57	27.3	393.32	2315291	24.5085 ng/ml
THC-COOH	1.580	80625	∞	55.8	645.71	224179	24.0950 ng/ml
THC-OH	1.558	164149	∞	14.1	478.91	458630	24.0463 ng/ml

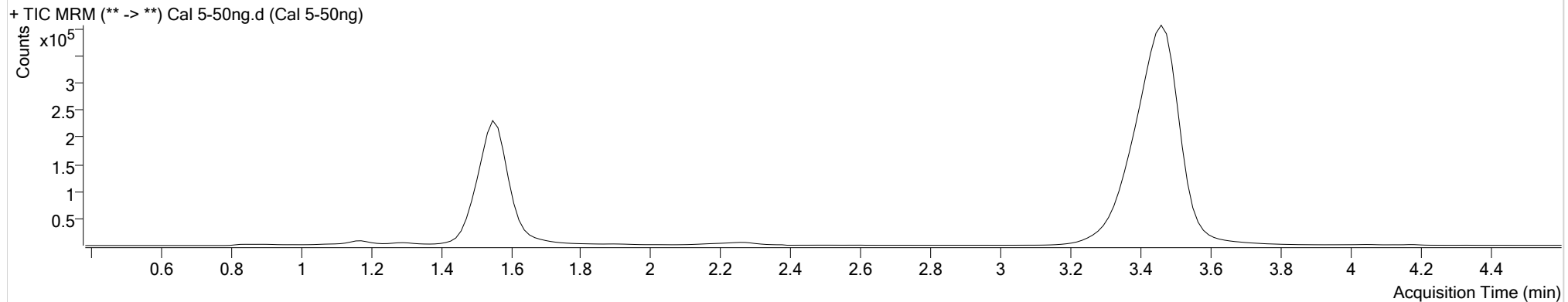
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument Falco **Data File** Cal 5-50ng.d
Type Cal **Sample** Cal 5-50ng
Acq. Method AM 27 THC quant.m
Sample Position P3-C6 **Comment**
Injection Volume 10
Acq. Date-Time 7/2/2019 11:22:27 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.476	998375	1938.09	26.9	∞	2439798	49.7723 ng/ml
THC-COOH	1.580	166568	∞	56.3	2044.62	230232	50.3016 ng/ml
THC-OH	1.558	362030	1006.15	14.0	977.62	496198	50.1061 ng/ml

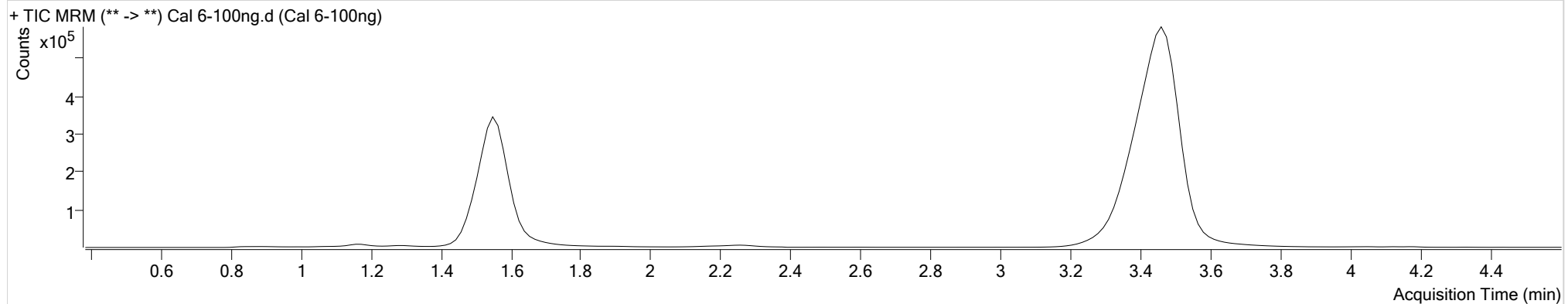
AM #27 Cannabinoids Quant. Results



Batch results D:\MassHunter\Data\2019\AM 28\070219 THCQ MDQ SP\QuantResults\THCQ.batch.bin
Calibration Last Update 7/2/2019 3:05:08 PM

Instrument	Falco	Data File	Cal 6-100ng.d
Type	Cal	Sample	Cal 6-100ng
Acq. Method	AM 27 THC quant.m	Comment	
Sample Position	P3-B6		
Injection Volume	10		
Acq. Date-Time	7/2/2019 11:30:02 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.461	2131533	5478.27	27.2	8299.80	2576906	100.7280 ng/ml
THC-COOH	1.580	327450	∞	57.7	3829.05	230410	100.5552 ng/ml
THC-OH	1.558	734348	1405.83	14.1	953.87	503794	101.1485 ng/ml