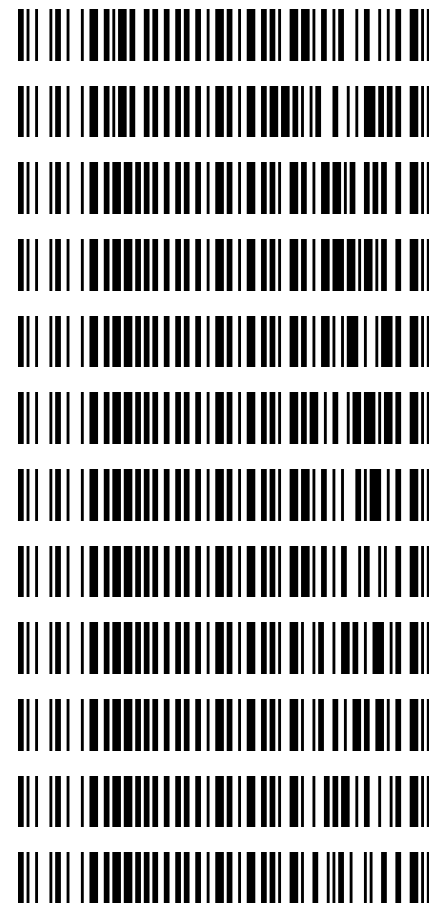


**Worklist: 3629**

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>
M2019-2843	2	161527	AM 27 Blood THC Quant by LC-QQQ
M2019-2859	1	161528	AM 27 Blood THC Quant by LC-QQQ
P2019-2193	1	161529	AM 27 Blood THC Quant by LC-QQQ
P2019-2196	1	161530	AM 27 Blood THC Quant by LC-QQQ
P2019-2198	1	161531	AM 27 Blood THC Quant by LC-QQQ
P2019-2211	1	161532	AM 27 Blood THC Quant by LC-QQQ
P2019-2238	1	161533	AM 27 Blood THC Quant by LC-QQQ
P2019-2240	1	161534	AM 27 Blood THC Quant by LC-QQQ
P2019-2264	1	161535	AM 27 Blood THC Quant by LC-QQQ
P2019-2266	1	161536	AM 27 Blood THC Quant by LC-QQQ
P2019-2275	1	161537	AM 27 Blood THC Quant by LC-QQQ
P2019-2318	1	161538	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 8/23/19  
 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\082319 THCQ SP Batch Name: THCQ
- 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r<sup>2</sup> 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 10-100*

# AM #27 Cannabinoids Quant. Results



## Batch results

D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin

## Calibration Last Update

8/28/2019 1:06:31 PM

## Instrument

Falco

## Data File

Negative.d

## Type

Sample

## Sample

Negative

## Acq. Method

AM 27 THC quant.m

## Sample Position

P3-A2

## Comment

## Injection Volume

10

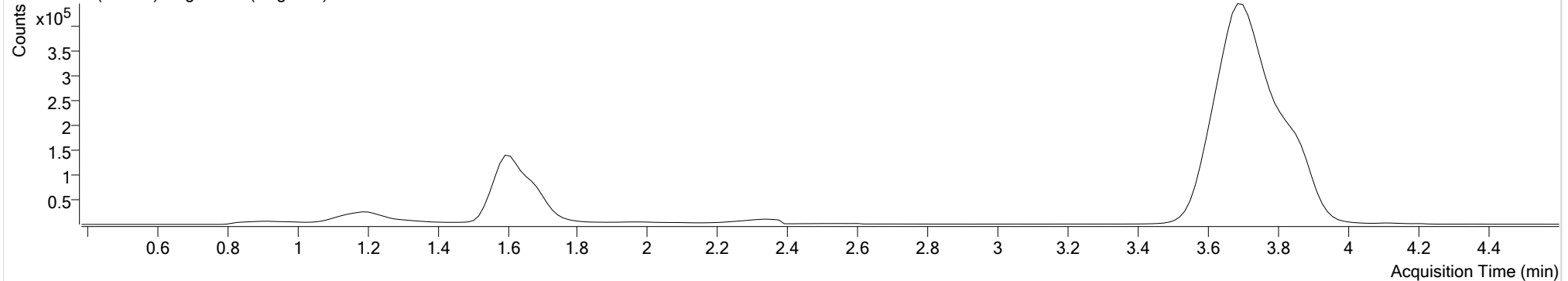
## Acq. Date-Time

8/23/2019 11:58:54 AM

## Sample Info.

## Sample Chromatogram

+ TIC MRM (\*\* -> \*\*) Negative.d (Negative)



# AM #27 Cannabinoids Quant. Results

**Batch results**

D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin

**Calibration Last Update**

8/28/2019 1:06:31 PM

**Instrument**

Falco

**Data File**

QC Control.d

**Type**

Sample

**Sample**

QC Control

**Acq. Method**

AM 27 THC quant.m

**Sample Position**

P3-H1

**Comment****Injection Volume**

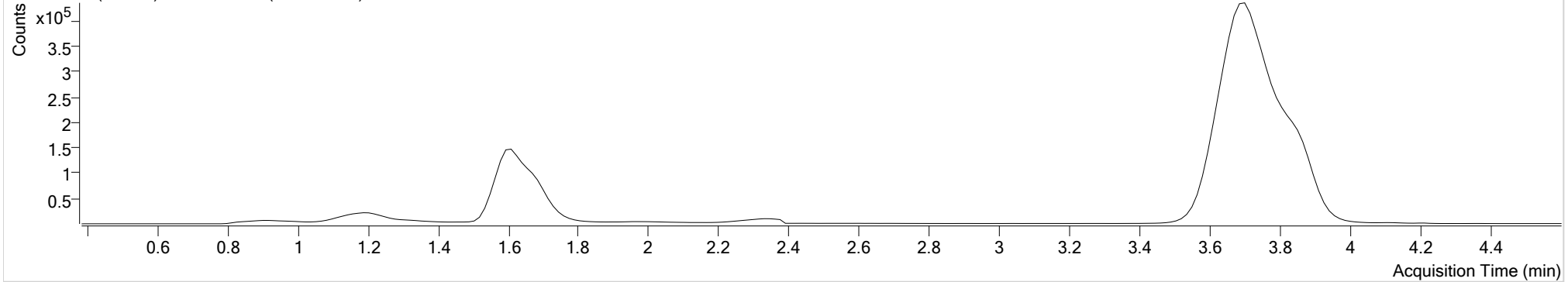
10

**Acq. Date-Time**

8/23/2019 11:43:41 AM

**Sample Info.****Sample Chromatogram**

+ TIC MRM (\*\* -&gt; \*\*) QC Control.d (QC Control)



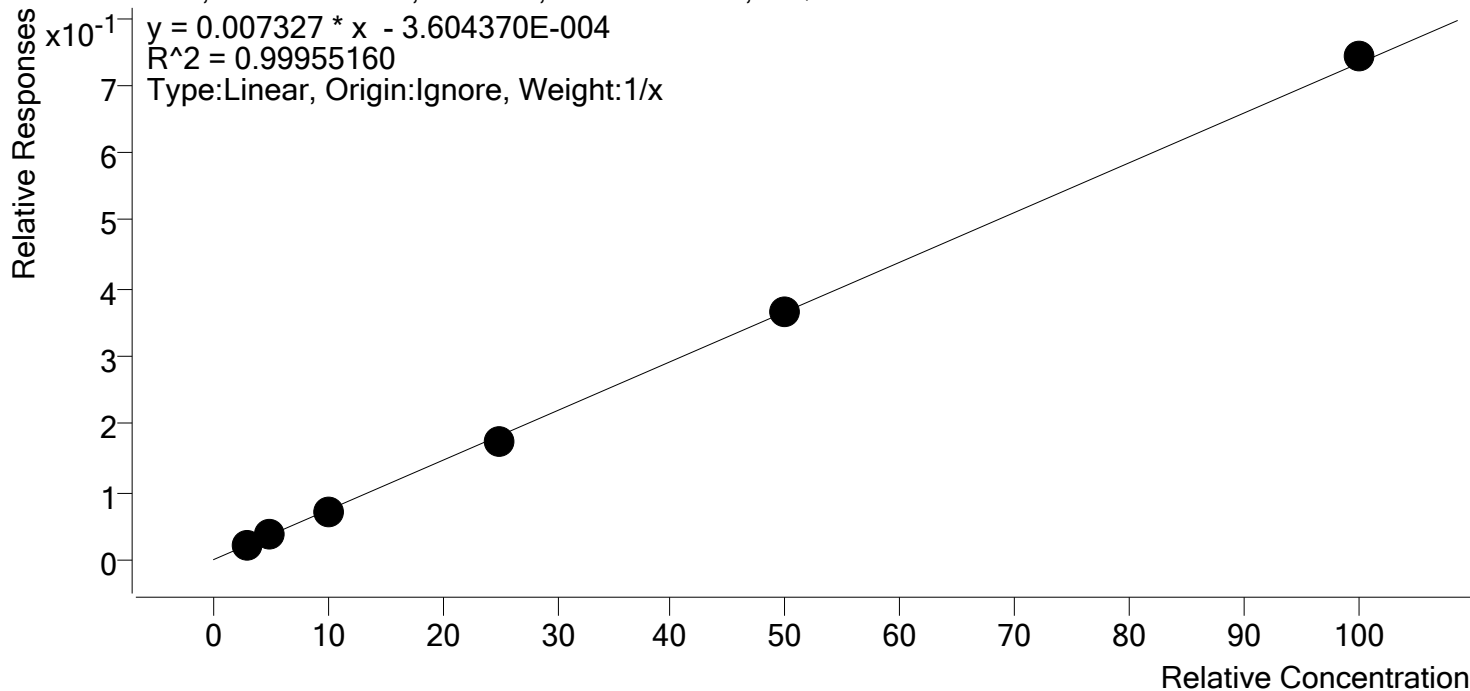
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.702	184858	619.35	29.3	∞	5294673	4.8141 ng/ml
THC-COOH	1.655	60762	301.83	49.0	203.68	253846	10.0036 ng/ml
THC-OH	1.618	69340	∞	10.5	85.00	767097	4.7956 ng/ml



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin  
**Last Cal. Update** 8/28/2019 1:06 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-D3

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

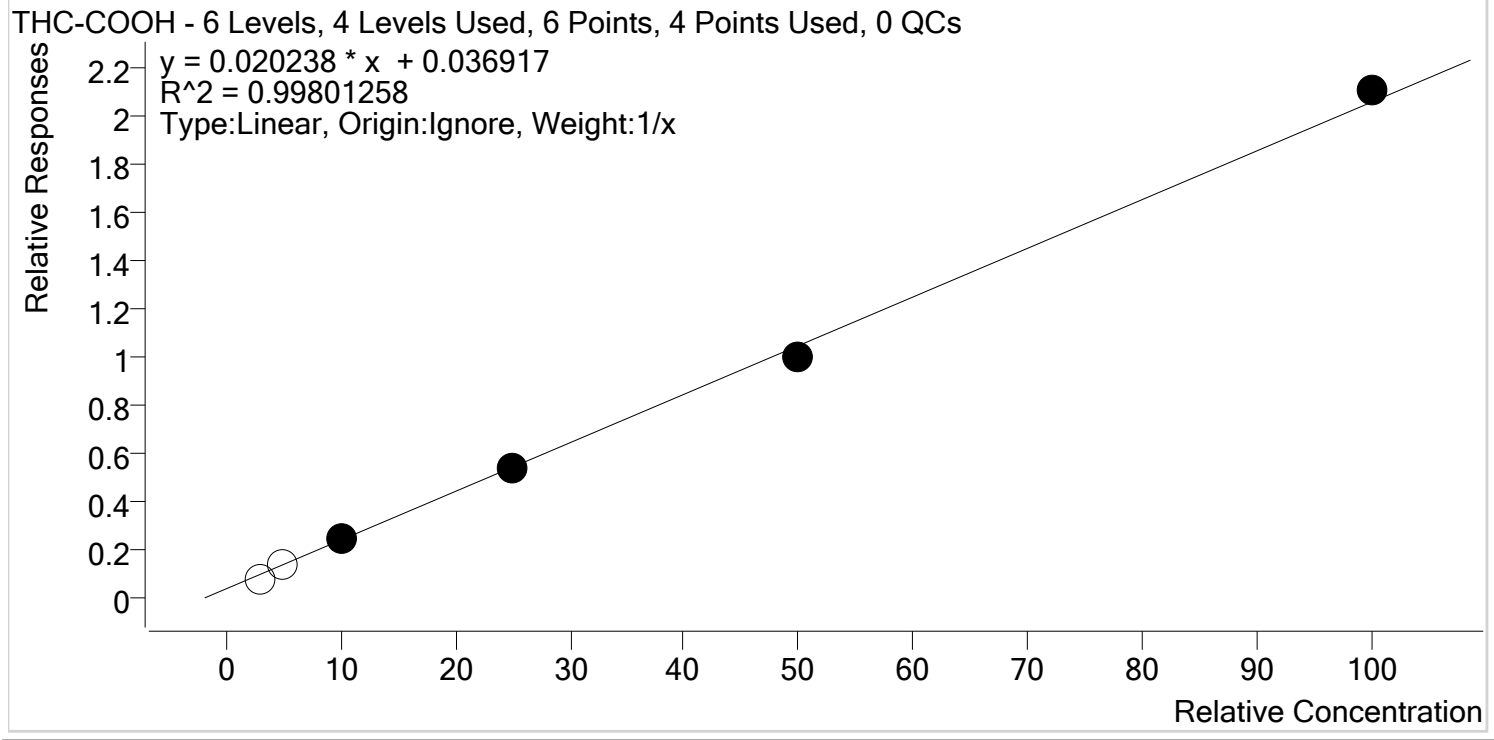


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-3ng	1	✓	3.0	3.1	102.8
Cal 2- 5ng	2	✓	5.0	5.1	102.6
Cal 3 -10ng	3	✓	10.0	9.8	97.6
Cal 4-25ng	4	✓	25.0	24.1	96.4
Cal 5-50ng	5	✓	50.0	49.6	99.2
Cal 6-100ng	6	✓	100.0	101.3	101.3



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin  
**Last Cal. Update** 8/28/2019 1:06 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-D9

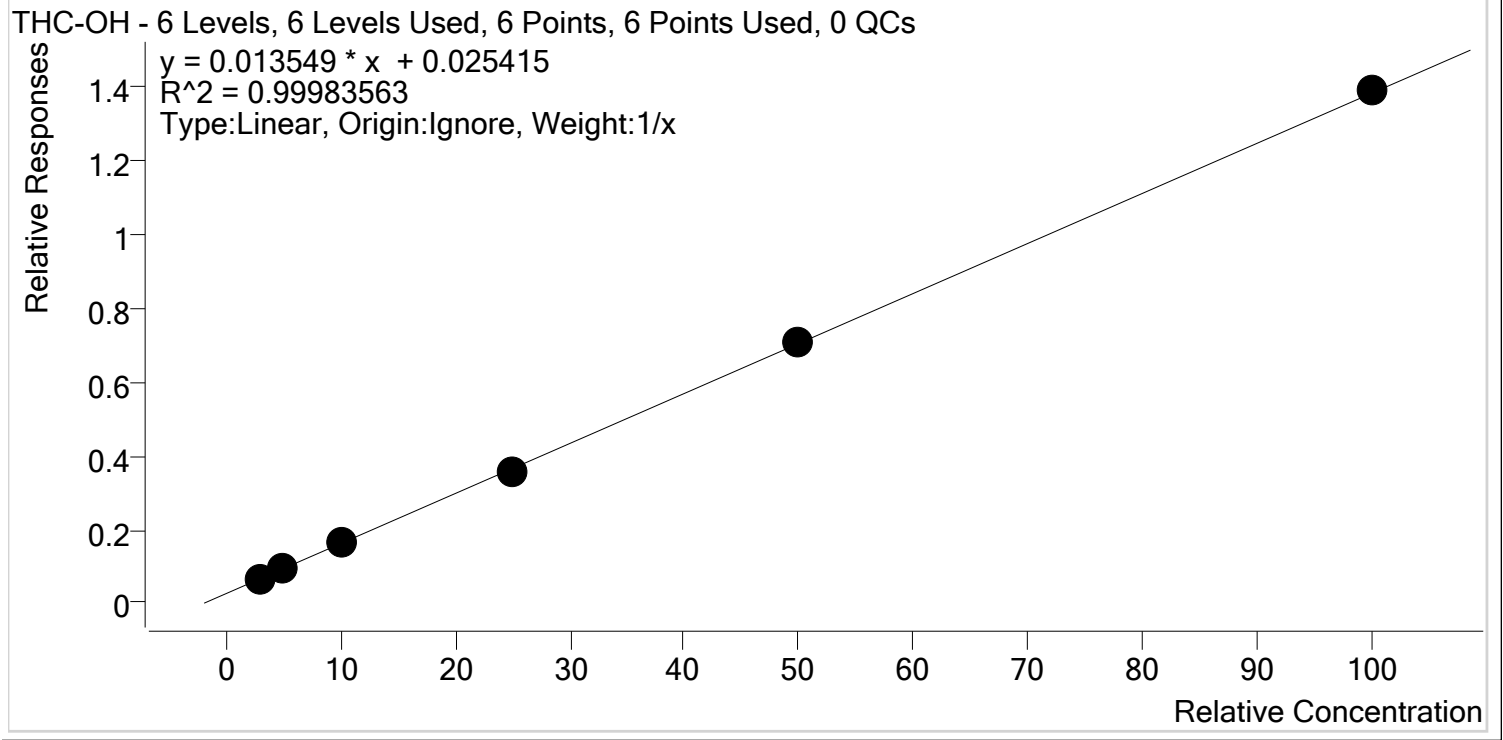


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-3ng	1	x	3.0	2.1	70.8
Cal 2- 5ng	2	x	5.0	5.3	105.2
Cal 3 -10ng	3	✓	10.0	10.4	104.1
Cal 4-25ng	4	✓	25.0	24.5	98.2
Cal 5-50ng	5	✓	50.0	47.7	95.5
Cal 6-100ng	6	✓	100.0	102.3	102.3



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin  
**Last Cal. Update** 8/28/2019 1:06 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	3.0	100.5
Cal 2- 5ng	2	✓	5.0	5.1	101.3
Cal 3 -10ng	3	✓	10.0	10.0	100.3
Cal 4-25ng	4	✓	25.0	24.3	97.2
Cal 5-50ng	5	✓	50.0	50.1	100.2
Cal 6-100ng	6	✓	100.0	100.5	100.5

# AM #27 Cannabinoids Quant. Results

**Batch results**

D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin

**Calibration Last Update**

8/28/2019 1:06:31 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-B1

**Comment****Injection Volume**

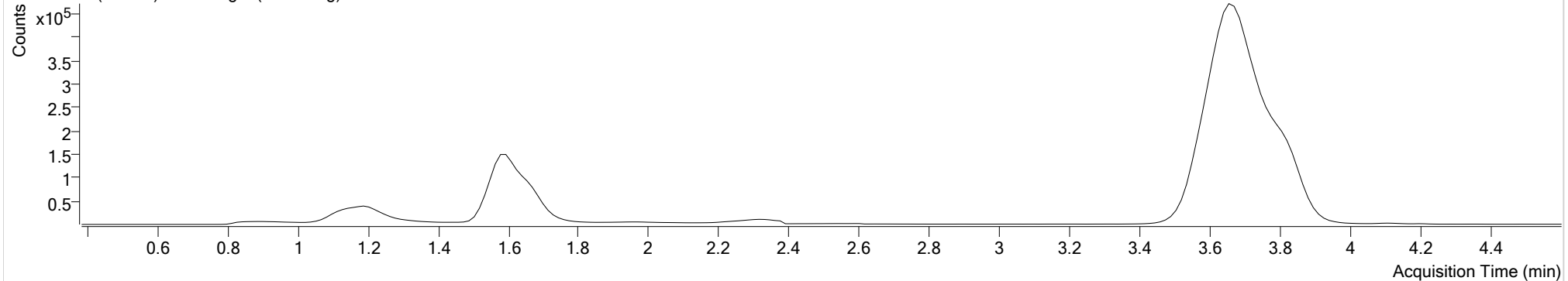
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**Acq. Date-Time**

8/23/2019 10:58:15 AM

**Sample Info.****Sample Chromatogram**

+ TIC MRM (\*\* -&gt; \*\*) Cal 1-3ng.d (Cal 1-3ng)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.672	128376	∞	30.0	∞	5770870	3.0852 ng/ml
THC-COOH	1.625	20942	64.63	49.5	53.36	262171	2.1229 ng/ml
THC-OH	1.603	53189	∞	10.3	17.28	802529	3.0157 ng/ml



# AM #27 Cannabinoids Quant. Results

**Batch results**

D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin

**Calibration Last Update**

8/28/2019 1:06:31 PM

**Instrument**

Falco

**Data File**

Cal 2- 5ng.d

**Type**

Cal

**Sample**

Cal 2- 5ng

**Acq. Method**

AM 27 THC quant.m

**Sample Position**

P3-C1

**Comment****Injection Volume**

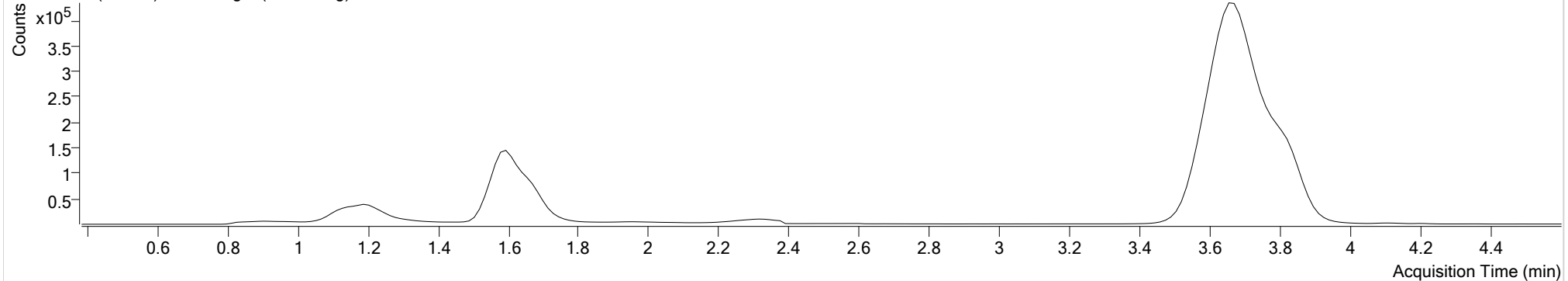
10

**Acq. Date-Time**

8/23/2019 11:05:49 AM

**Sample Info.****Sample Chromatogram**

+ TIC MRM (\*\* -&gt; \*\*) Cal 2- 5ng.d (Cal 2- 5ng)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.672	193313	∞	28.3	∞	5193770	5.1289 ng/ml
THC-COOH	1.625	38224	∞	40.1 <b>Low</b>	152.10	266558	5.2616 ng/ml
THC-OH	1.603	70269	∞	11.5	126.49	747303	5.0640 ng/ml

# AM #27 Cannabinoids Quant. Results

**Batch results**

D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin

**Calibration Last Update**

8/28/2019 1:06:31 PM

**Instrument**

Falco

**Data File**

Cal 3 -10ng.d

**Type**

Cal

**Sample**

Cal 3 -10ng

**Acq. Method**

AM 27 THC quant.m

**Sample Position**

P3-D1

**Comment****Injection Volume**

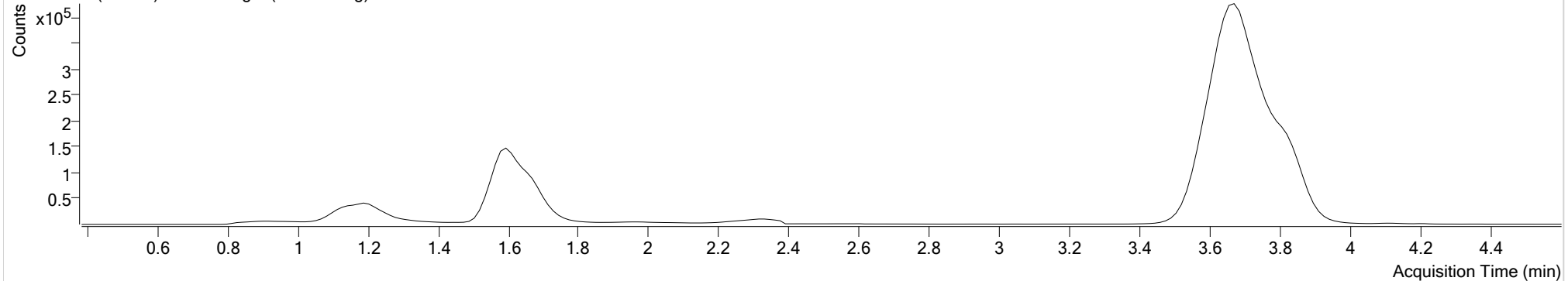
10

**Acq. Date-Time**

8/23/2019 11:13:24 AM

**Sample Info.****Sample Chromatogram**

+ TIC MRM (\*\* -&gt; \*\*) Cal 3 -10ng.d (Cal 3 -10ng)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.672	351740	642.91	28.0	321.93	4941547	9.7636 ng/ml
THC-COOH	1.640	58830	360.26	49.4	156.66	237707	10.4051 ng/ml
THC-OH	1.603	116044	206.30	11.5	111.72	719303	10.0309 ng/ml

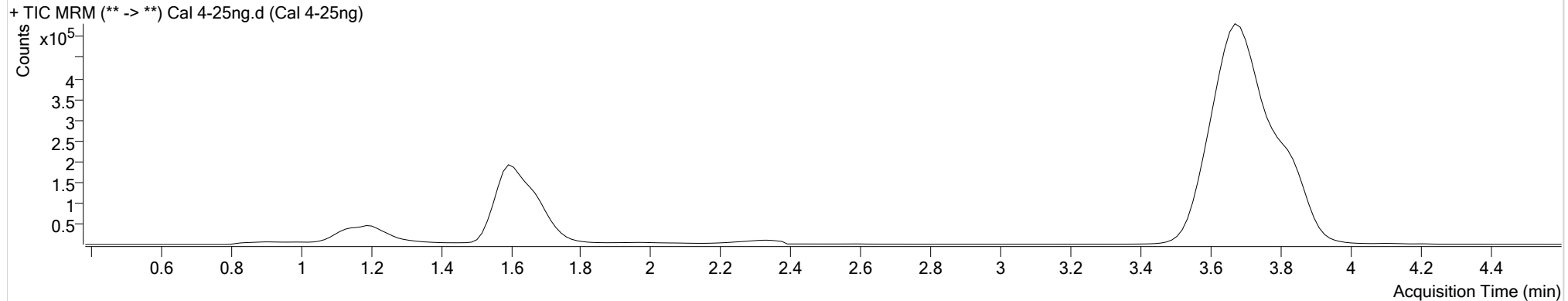
# AM #27 Cannabinoids Quant. Results



**Batch results** D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin  
**Calibration Last Update** 8/28/2019 1:06:31 PM

<b>Instrument</b>	Falco	<b>Data File</b>	Cal 4-25ng.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 4-25ng
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Comment</b>	
<b>Sample Position</b>	P3-E1		
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	8/23/2019 11:20:58 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.687	966244	4125.13	27.5	1847.53	5480975	24.1087 ng/ml
THC-COOH	1.640	135027	458.25	55.7	3480.96	253086	24.5387 ng/ml
THC-OH	1.603	276353	837.77	12.7	∞	779256	24.2978 ng/ml

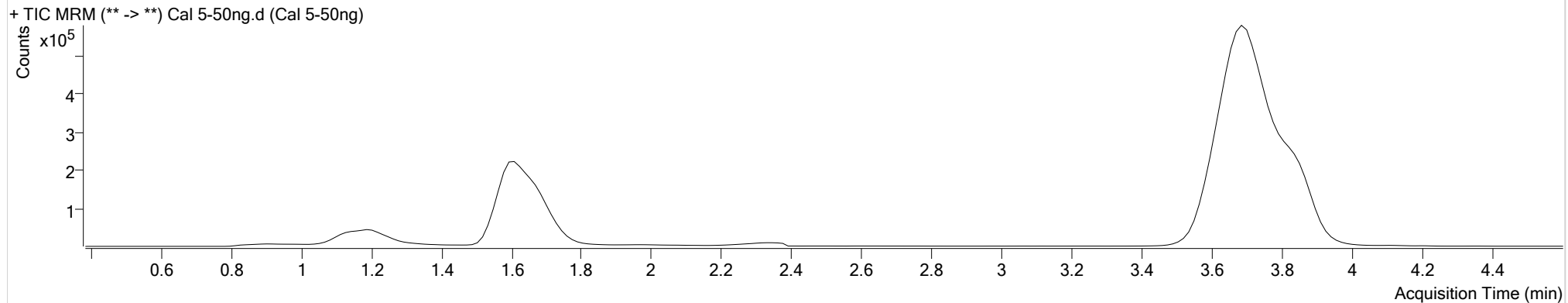
# AM #27 Cannabinoids Quant. Results



**Batch results** D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin  
**Calibration Last Update** 8/28/2019 1:06:31 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-F1 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 8/23/2019 11:28:32 AM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.702	1790063	7351.28	27.6	711.40	4930456	49.5987 ng/ml
THC-COOH	1.640	245542	∞	57.7	938.46	244802	47.7381 ng/ml
THC-OH	1.603	497335	∞	13.0	295.54	706141	50.1043 ng/ml

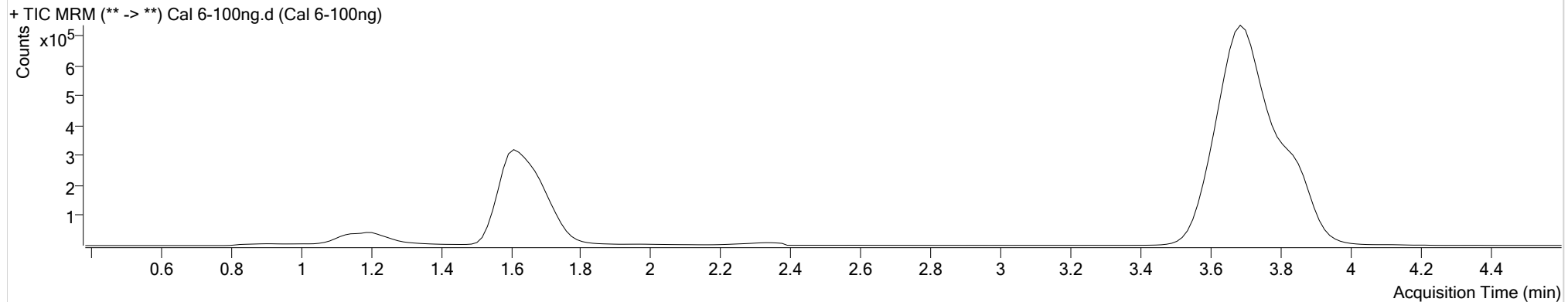
# AM #27 Cannabinoids Quant. Results



**Batch results** D:\MassHunter\Data\2019\AM 27\082319 THCQ SP\QuantResults\THCQ.batch.bin  
**Calibration Last Update** 8/28/2019 1:06:31 PM

<b>Instrument</b>	Falco	<b>Data File</b>	Cal 6-100ng.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 6-100ng
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Comment</b>	
<b>Sample Position</b>	P3-G1		
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	8/23/2019 11:36:06 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.687	3449991	11672.43	27.6	2676.63	4649572	101.3150 ng/ml
THC-COOH	1.655	465167	762.31	60.5	1325.71	220710	102.3181 ng/ml
THC-OH	1.603	957580	∞	12.9	471.03	690414	100.4873 ng/ml