

**REVIEWED**

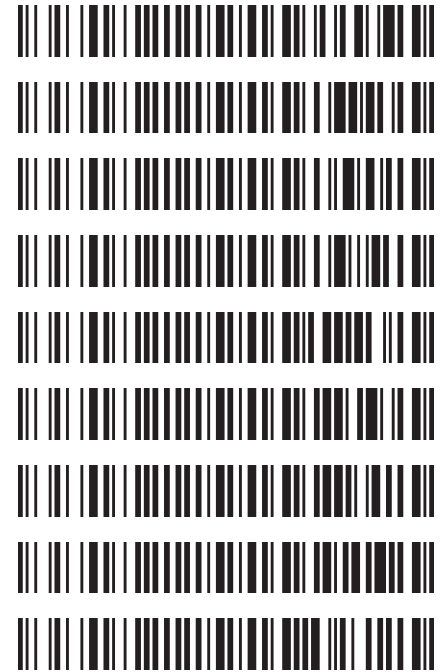
By Britany Wylie at 12:52 pm, May 02, 2019



4/30/2019

**Worklist: 3357**

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>
C2019-0669	1	150401	AM 27 Blood THC Quant by LC-QQQ
C2019-0693	1	150402	AM 27 Blood THC Quant by LC-QQQ
C2019-0694	1	150403	AM 27 Blood THC Quant by LC-QQQ
C2019-0695	1	150404	AM 27 Blood THC Quant by LC-QQQ
C2019-0712	1	150409	AM 27 Blood THC Quant by LC-QQQ
C2019-0720	1	150405	AM 27 Blood THC Quant by LC-QQQ
C2019-0721	1	150406	AM 27 Blood THC Quant by LC-QQQ
C2019-0722	1	150407	AM 27 Blood THC Quant by LC-QQQ
C2019-0768	1	150408	AM 27 Blood THC Quant by LC-QQQ





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

Extraction Date: 04/30/19  
Plate lot#: 0539904

Analyst: Anne Nord  
Plate Expiration: 09/10/19

**Mobile phase A:** 0.1% Formic Acid in LCMS Water  
MTBE  
**Mobile phase B:** 0.1% Formic acid in Acetonitrile  
LCMS Methanol  
Hexane  
**Blank Blood Lot:** 19A207P3  
**Column:** UCT Selectra DA 100 x 2.1mm 3um  
**LCMS-QQQ ID:** 69679

## 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: k52558g** in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- 4. Pipette **500µL 0.1% formic acid in water** 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: 66792*
- 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: 66819*
- 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: Data\2019\AM 27\043019 Batch Name: cann quant
- 2. Make any necessary integration changes, Curve weighting of Linear 1/x with  $r^2$  values  $\geq 0.98$  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:



Toxicology AM method 27 external prep information

working solution 1 ug/ml in meoh C-THC, THC-OH, THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 75 ul THC-OH in 9767.5 ul meOH

Ppd 3/13/19 Exp: 9/13/19 lot 91319 by AMN

Drug	lot	expiration
C-THC	FE01061702 cerillient	3/1/2022
THC-OH	318.1b18.1L1a	12/1/2021
THC	135.1b71.0L6	11/1/2021

AM 27 control 100 ul working solution lot (91319) in 9900 ul blood lot (19A207p3)

ppd 3/13/19 Exp 9/13/19 lot 31319 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-TH by AMN

# AM #27 Cannabinoids

**Batch results**

D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin

**Calibration Last Update**

5/2/2019 10:51:47 AM

**Instrument**

69679

**Type**

Sample

**Acq. Method**

AM 27 THC quant.m

**Sample Position**

P1-H1

**Injection Volume**

10

**Acq. Date-Time**

5/1/2019 1:26:22 PM

**Sample Info.****Data File**

QC.d

**Sample**

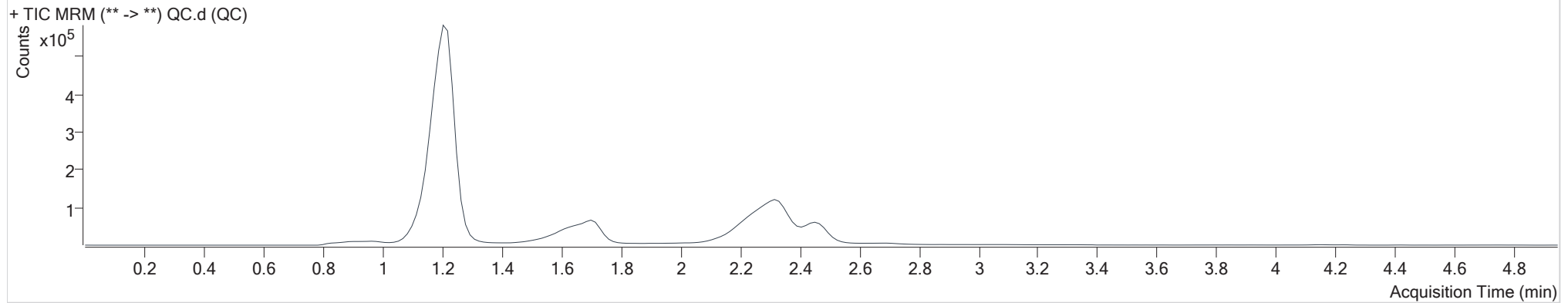
QC

**Operator**

Anne Nord

**Comment**

THC,THCOH 5ng CTHC 10

**Sample Chromatogram**

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	83400	144	40.3	164	908620	9.06 ng/ml <b>Low</b>
THC-OH	1.224	146892	295	9.2	81	1913584	5.31 ng/ml
THC	2.341	21058	185	24.2	10	671237	4.71 ng/ml

# AM #27 Cannabinoids



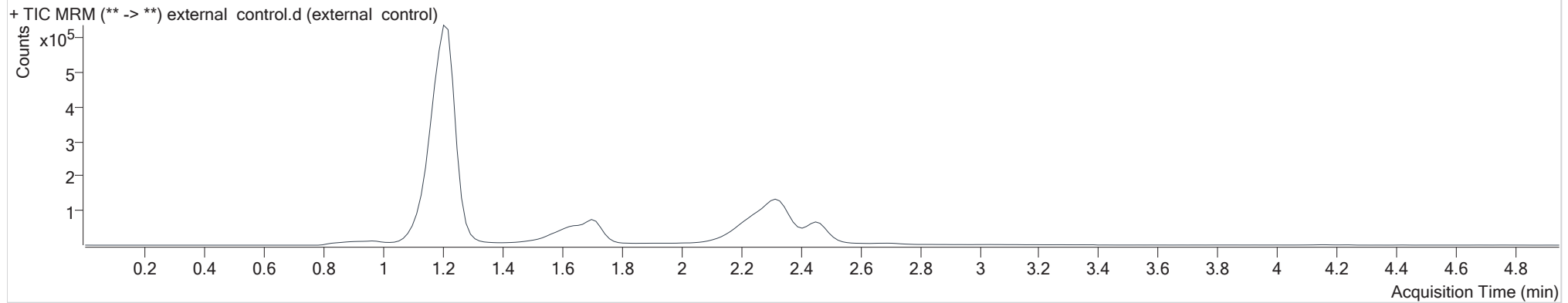
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
5/2/2019 10:51:47 AM

**Instrument** 69679  
**Type** Sample  
**Acq. Method** AM 27 THC quant.m  
**Sample Position** P1-B2  
**Injection Volume** 10  
**Acq. Date-Time** 5/1/2019 1:41:51 PM  
**Sample Info.**

**Data File** external control.d  
**Sample** external control  
**Operator** Anne Nord  
**Comment** THC, THCOH 7.5ng CTHC 15ng

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	129012	42	37.7	344	987755	12.70 ng/ml
THC-OH	1.224	204631	169	9.8	∞	2068065	6.78 ng/ml
THC	2.341	40148	325	23.2	23	690585	8.29 ng/ml

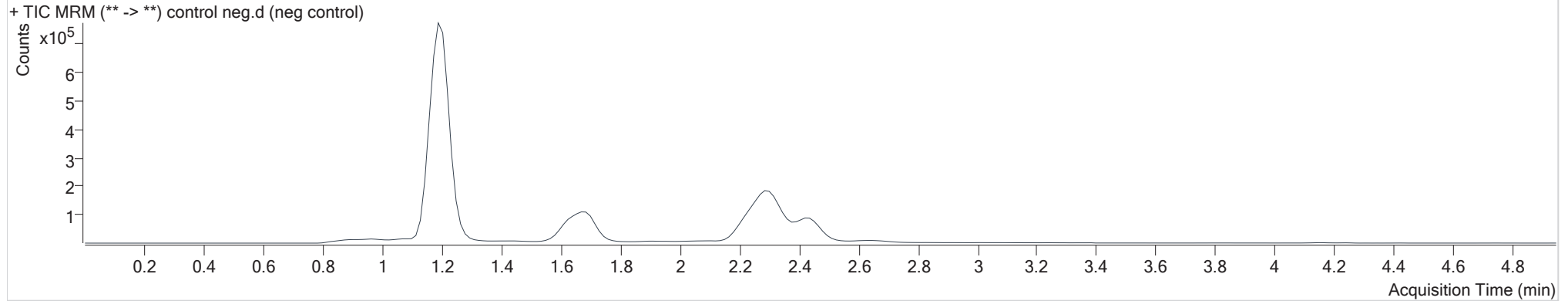
# AM #27 Cannabinoids



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 5/2/2019 10:51:47 AM

<b>Instrument</b>	69679	<b>Data File</b>	control neg.d
<b>Type</b>	Sample	<b>Sample</b>	neg control
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P1-A2	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/1/2019 2:42:30 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



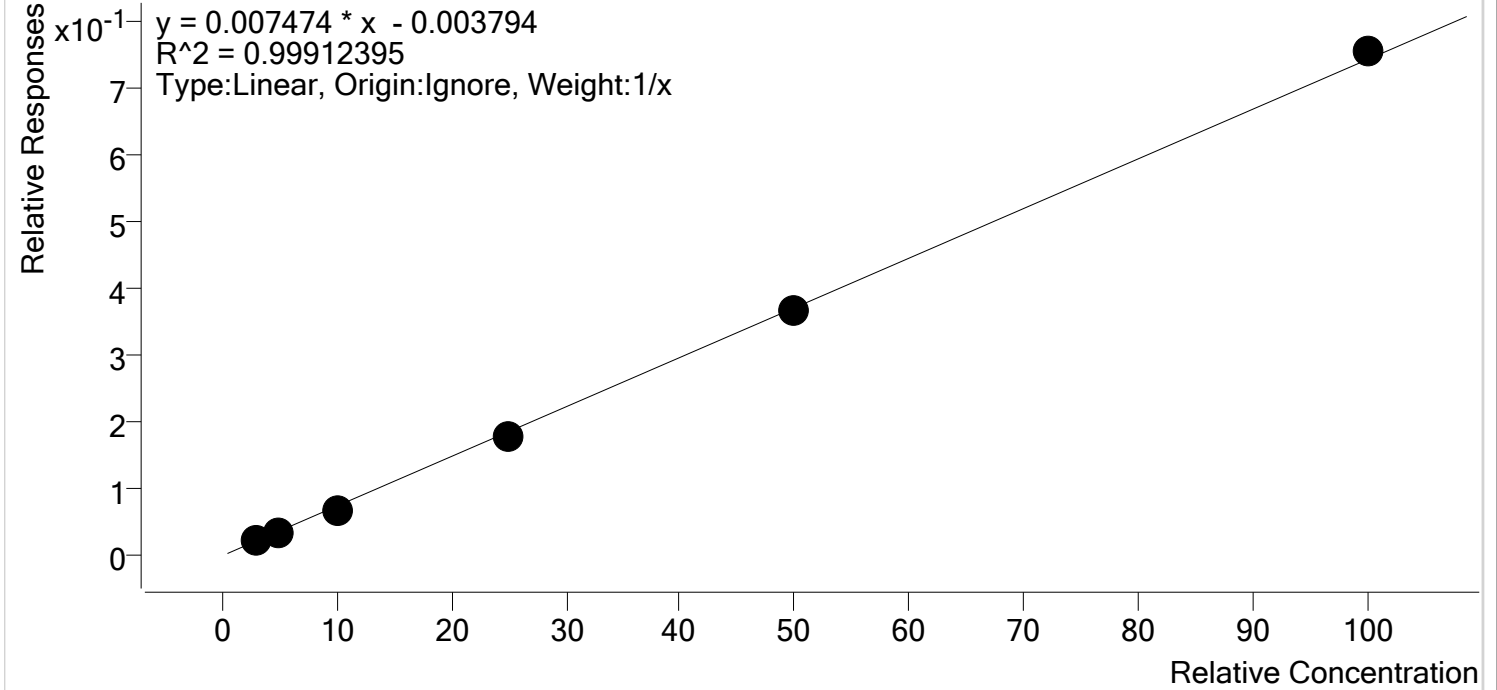
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.224	5772	0 <b>Low</b>	4.5 <b>Low</b>	3 <b>Low</b>	2446593	0.37 ng/ml <b>Low</b>

# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Last Cal. Update** 5/2/2019 10:51 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-d3

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



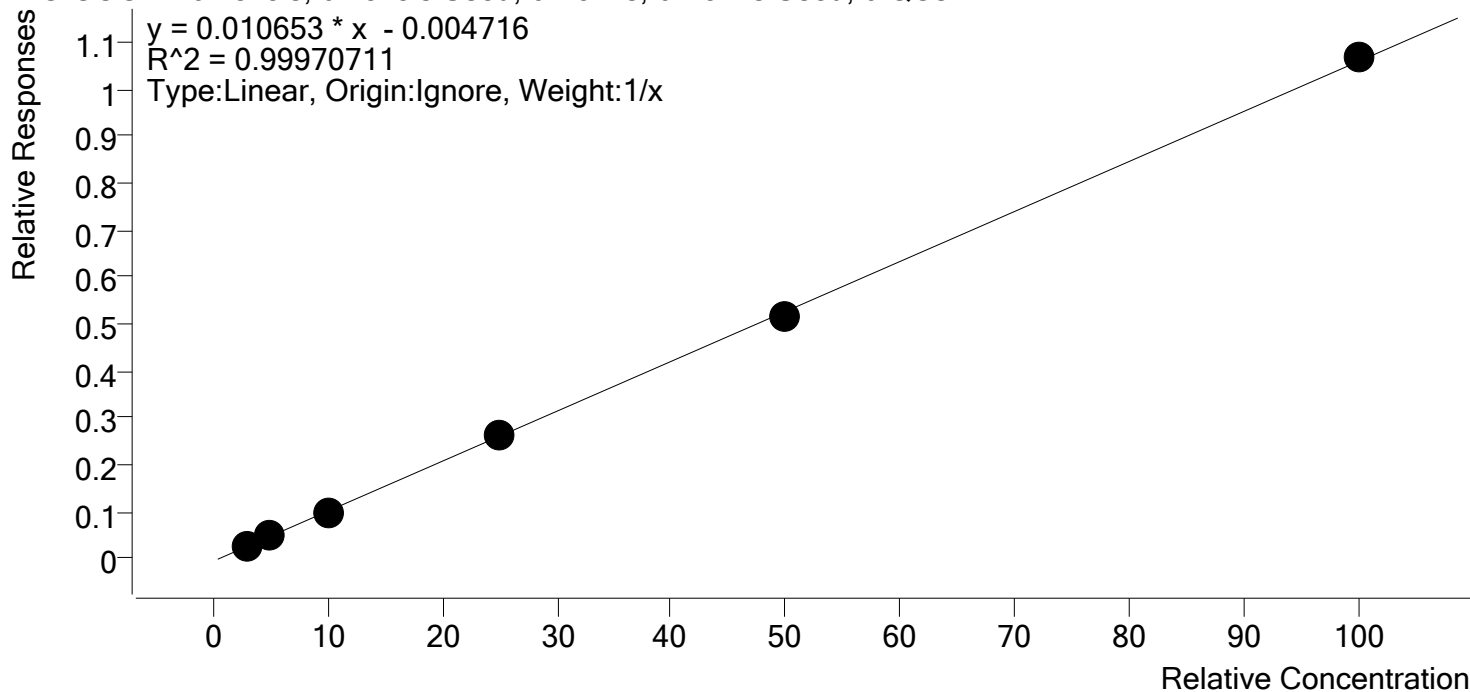
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✓	3.0	3.3	109.6
cal 3	3	✓	5.0	5.0	99.6
cal 4	4	✓	10.0	9.3	92.9
cal 5	5	✓	25.0	24.3	97.1
cal 6	6	✓	50.0	49.6	99.2
cal 7	7	✓	100.0	101.5	101.5

# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Last Cal. Update** 5/2/2019 10:51 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-d9

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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✓	3.0	3.1	104.9
cal 3	3	✓	5.0	4.9	98.1
cal 4	4	✓	10.0	9.6	96.4
cal 5	5	✓	25.0	25.3	101.3
cal 6	6	✓	50.0	49.3	98.6
cal 7	7	✓	100.0	100.7	100.7

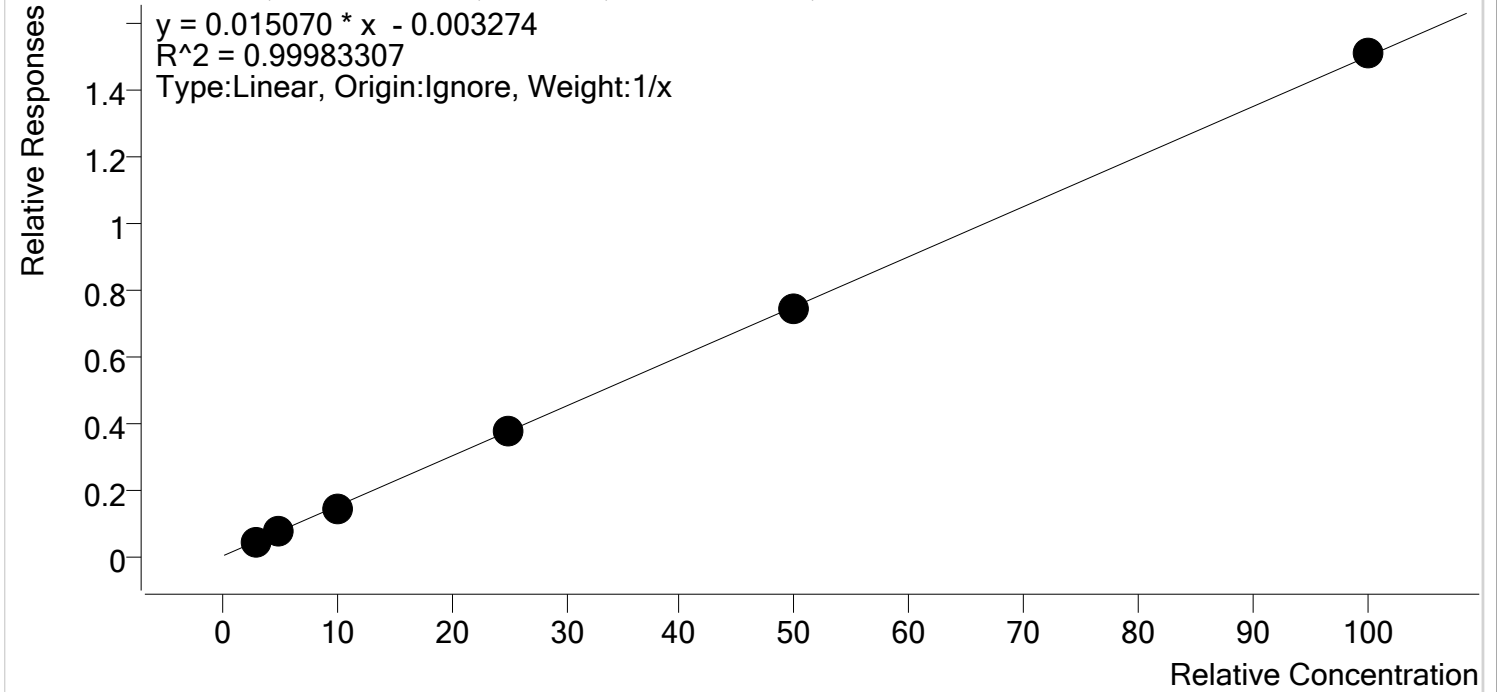


# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Last Cal. Update** 5/2/2019 10:51 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-d3

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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✓	3.0	2.9	98.2
cal 3	3	✓	5.0	5.2	104.6
cal 4	4	✓	10.0	9.8	97.7
cal 5	5	✓	25.0	25.0	99.9
cal 6	6	✓	50.0	49.6	99.1
cal 7	7	✓	100.0	100.5	100.5

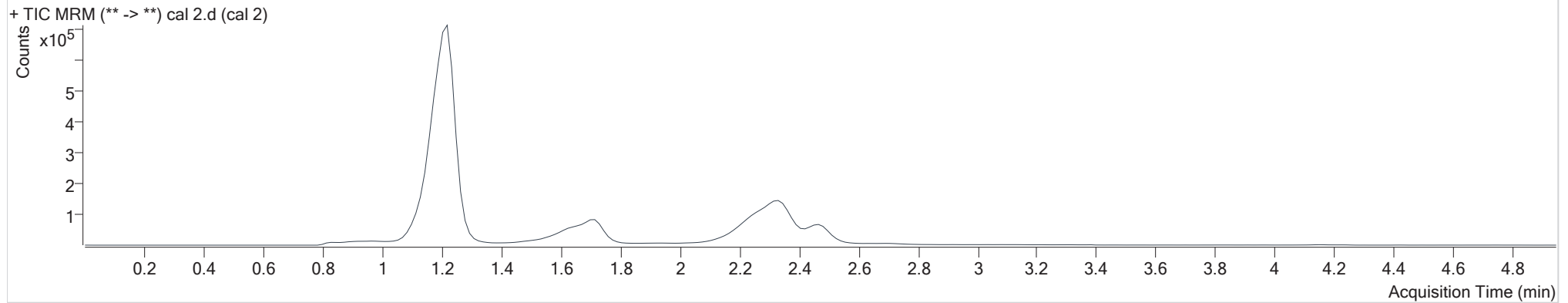
# AM #27 Cannabinoids



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 5/2/2019 10:51:47 AM

<b>Instrument</b>	69679	<b>Data File</b>	cal 2.d
<b>Type</b>	Cal	<b>Sample</b>	cal 2
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P1-B1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/1/2019 12:16:59 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.234	32444	44	33.9	∞	1125560	3.15 ng/ml <b>Low</b>
THC-OH	1.224	107929	∞	11.6	202	2625726	2.94 ng/ml <b>Low</b>
THC	2.356	17751	76	22.5	11	854458	3.29 ng/ml

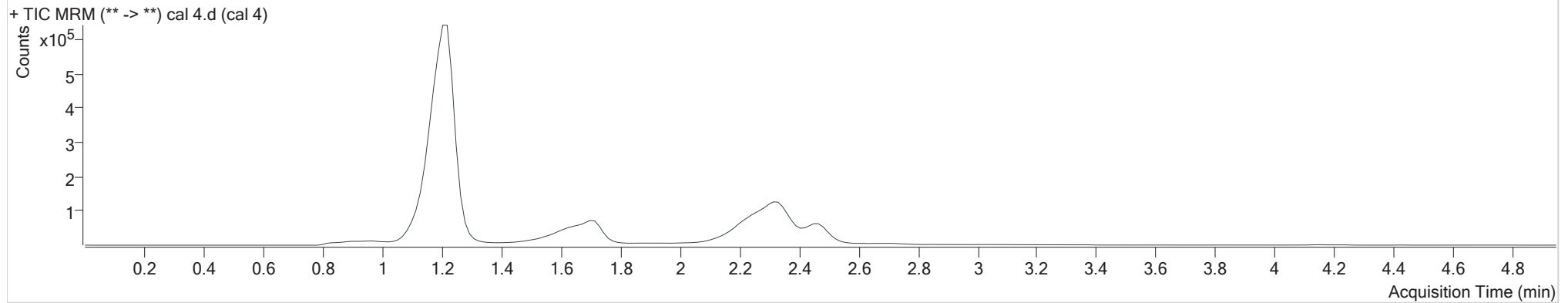
# AM #27 Cannabinoids



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 5/2/2019 10:51:47 AM

<b>Instrument</b>	69679	<b>Data File</b>	cal 4.d
<b>Type</b>	Cal	<b>Sample</b>	cal 4
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P1-D1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/1/2019 12:32:29 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	94525	∞	37.5	245	964792	9.64 ng/ml <b>Low</b>
THC-OH	1.224	315867	∞	10.1	863	2193515	9.77 ng/ml
THC	2.341	44906	588	24.5	44	683944	9.29 ng/ml

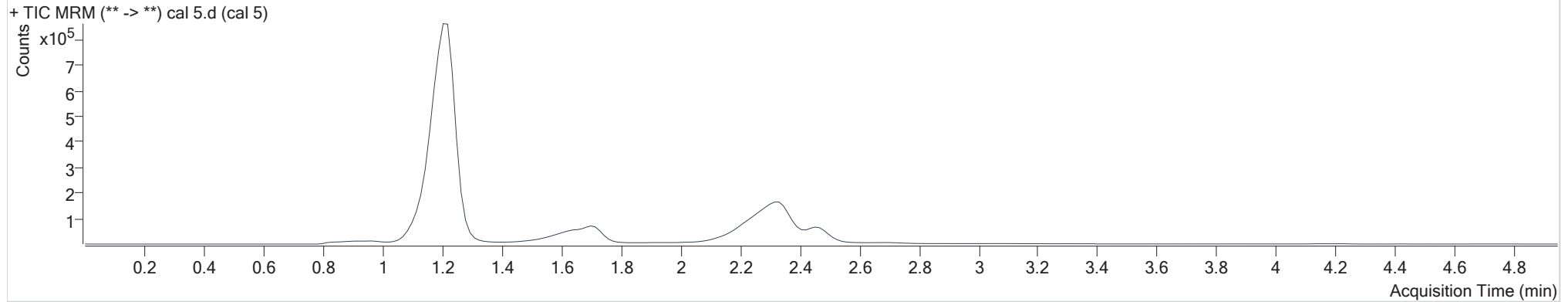
# AM #27 Cannabinoids



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 5/2/2019 10:51:47 AM

<b>Instrument</b>	69679	<b>Data File</b>	cal 5.d
<b>Type</b>	Cal	<b>Sample</b>	cal 5
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P1-E1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/1/2019 12:40:13 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	269228	∞	34.9	610	1015701	25.32 ng/ml
THC-OH	1.224	838228	∞	11.3	1544	2247590	24.97 ng/ml
THC	2.341	159411	578	24.6	349	896926	24.29 ng/ml

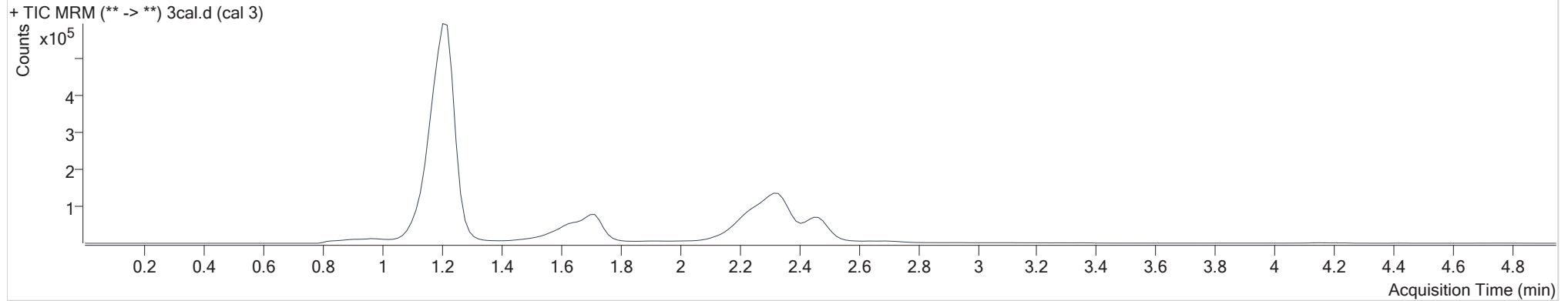
# AM #27 Cannabinoids



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 5/2/2019 10:51:47 AM

<b>Instrument</b>	69679	<b>Data File</b>	3cal.d
<b>Type</b>	Cal	<b>Sample</b>	cal 3
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P1-C1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/1/2019 12:47:57 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	45709	∞	33.2	∞	961540	4.90 ng/ml <b>Low</b>
THC-OH	1.224	166180	193	9.9	235	2199687	5.23 ng/ml
THC	2.341	25306	409	22.4	93	757238	4.98 ng/ml

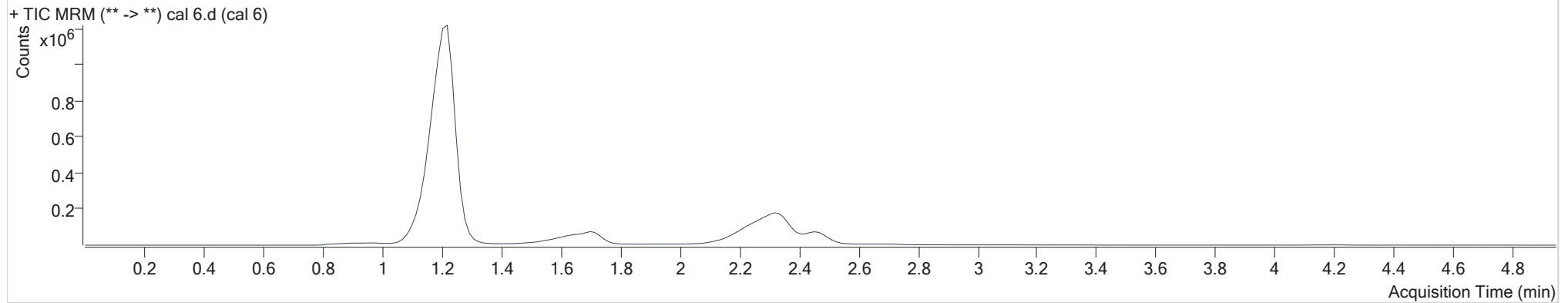
# AM #27 Cannabinoids



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 5/2/2019 10:51:47 AM

<b>Instrument</b>	69679	<b>Data File</b>	cal 6.d
<b>Type</b>	Cal	<b>Sample</b>	cal 6
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P1-F1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/1/2019 12:55:41 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	552386	∞	38.1	∞	1061655	49.28 ng/ml
THC-OH	1.224	1757882	∞	11.5	5511	2364377	49.55 ng/ml
THC	2.341	302850	6999	23.3	123	825034	49.62 ng/ml

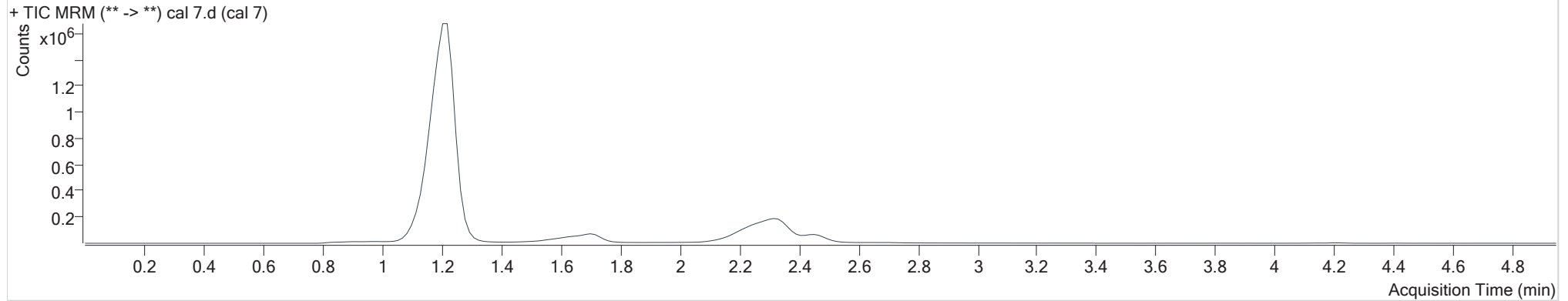
# AM #27 Cannabinoids



**Batch results** D:\MassHunter\Data\2019\AM 27\043019\QuantResults\cann quant.batch.bin  
**Calibration Last Update** 5/2/2019 10:51:47 AM

<b>Instrument</b>	69679	<b>Data File</b>	cal 7.d
<b>Type</b>	Cal	<b>Sample</b>	cal 7
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P1-G1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/1/2019 1:11:01 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.218	990954	∞	38.3	∞	927798	100.70 ng/ml
THC-OH	1.209	3308826	2703	11.7	∞	2188742	100.53 ng/ml
THC	2.341	542398	2019	24.0	158	718338	101.53 ng/ml