REVIEWED
By Celena Shrum at 9:22 am, Jun 13, 2019

6/12/2019

Worklist: 3471

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2019-0985	1	154412	AM 27 Blood THC Quant by LC-QQQ
C2019-1003	2	154413	AM 27 Blood THC Quant by LC-QQQ
C2019-1057	1	154414	AM 27 Blood THC Quant by LC-QQQ
C2019-1079	1	154415	AM 27 Blood THC Quant by LC-QQQ

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

Extraction Date: 06/11/19 Analyst: Anne Nord
Plate lot#: 0539904 Plate Expiration: 09/10/19

Mobile phase A: 0.1% Formic Acid in LCMS Water **Mobile phase B:** 0.1% Formic acid in Acetonitrile
MTBE 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\061119 Batch Name: cann quant
☒ 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:

Acquisition Method Report

Agilent Technologies

Acquisition Method Info

Method Name AM 27 THC quant.m

Method Path D:\MassHunter\methods\AM 27 THC quant.m

Method Description UCT Column

Device List

Binary Pump

Name: Binary Pump **Module: G7120A**

Flow	0.600 mL/min
Use Solvent Types	Yes
Stroke Mode	Synchronized
Low Pressure Limit	0.00 bar
High Pressure Limit	1000.00 bar
Max. Flow Ramp Up	100.000 mL/min²
Max. Flow Ramp Down	100.000 mL/min²
Expected Mixer	No check
Stroke A	
Automatic Stroke Calculation A	Yes
Stop Time	
Stoptime Mode	Time set
Stoptime	7.00 min
Post Time	
Posttime Mode	Off

Solvent Composition

	Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
1	A	100.0 % Water V.03		100.0 % Water V.03	0.1 Formic Acid	Ch. 2	Yes	40.00 %
2	B	100.0 % Acetonitrile V.03		100.0 % Acetonitrile V.03	0.1 Formic Acid	Ch. 2	Yes	60.00 %

Timetable

	Time	A	B	Flow
1	3.50 min	40.00 %	60.00 %	--- mL/min
2	3.51 min	5.00 %	95.00 %	--- mL/min
3	4.50 min	5.00 %	95.00 %	--- mL/min
4	4.51 min	45.00 %	55.00 %	--- mL/min

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 ulTHC-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

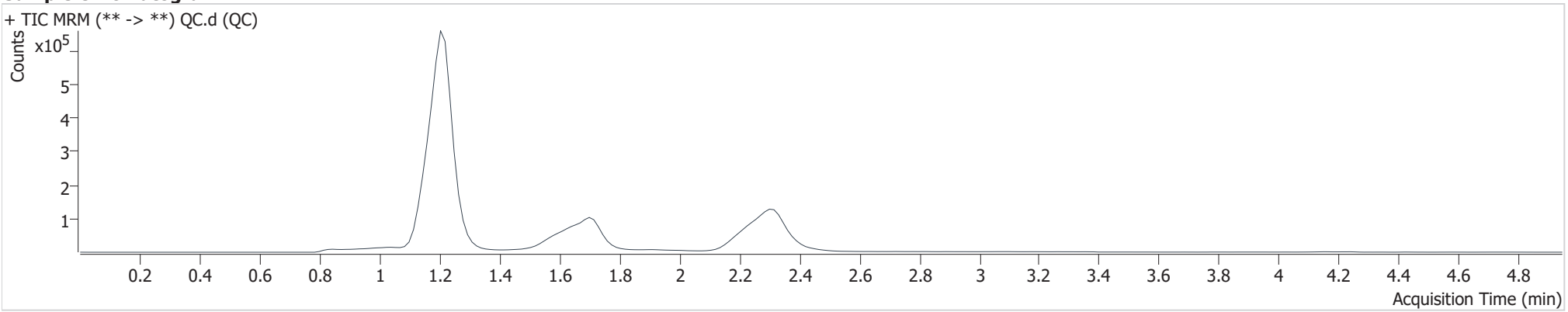
AM 27 control 100 ul working solution lot (91319) in 9900 ul blood lot (19A207p3)
ppd 3/13/19 Exp 9/13/19 lot 31319 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-TH by AMN

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	QC.d
Type	QC	**Sample**	QC
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 4:14:13 PM		
Sample Info.			

Sample Chromatogram



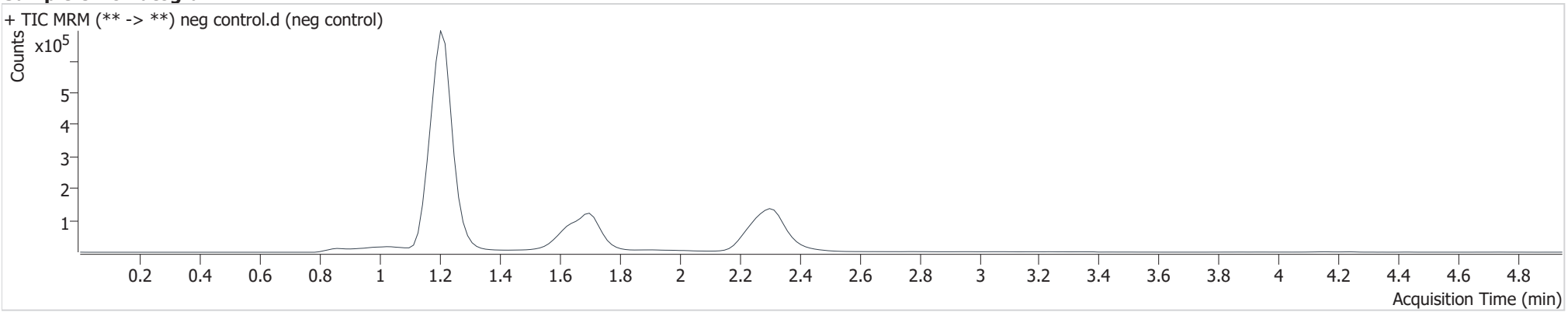
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.209	140574	∞	8.3	∞	2199810	4.553 ng/ml
THC-COOH	1.220	102360	128.1	35.1	1338.0	1092557	8.987 ng/ml Low
THC	2.326	36970	351.5	24.5	219.1	1141139	4.933 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	neg control.d
Type	Sample	**Sample**	neg control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 4:21:58 PM		
Sample Info.			

Sample Chromatogram



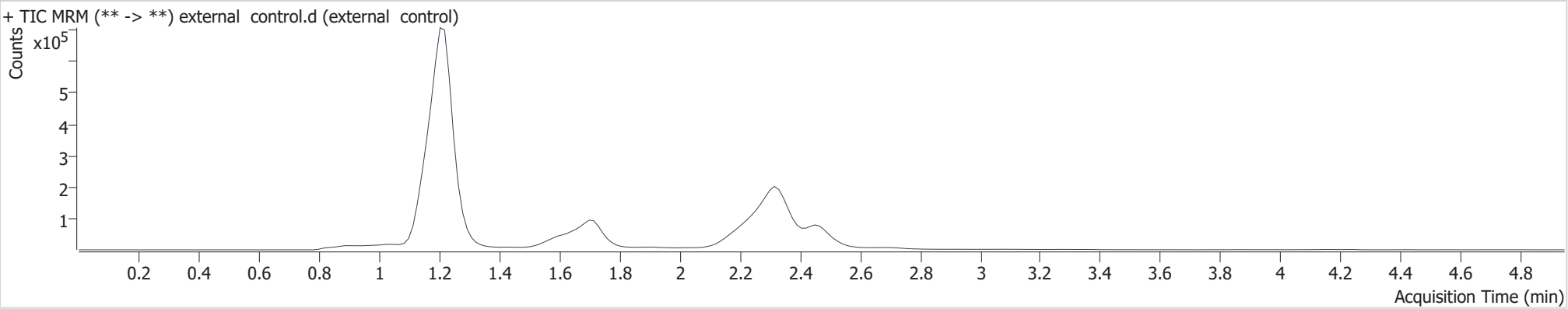
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.209	27689	∞			2414957	0.910 ng/ml Low

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	external control.d
Type	Sample	**Sample**	external control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B2	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 4:29:42 PM		
Sample Info.			

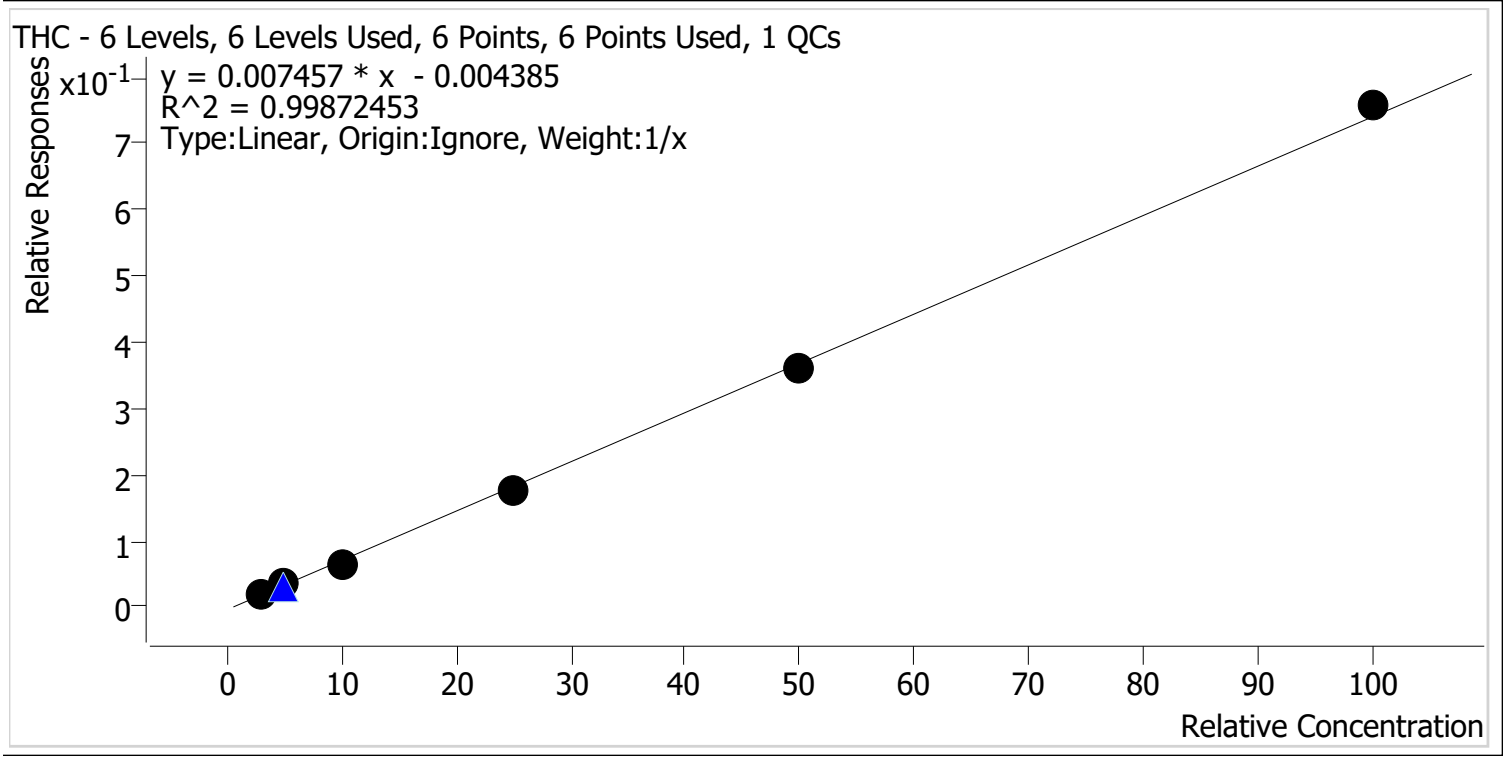
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.224	244036	∞	8.8	∞	2361134	7.294 ng/ml
THC-COOH	1.220	143620	516.5	35.4	283.7	1166812	11.771 ng/ml
THC	2.341	54416	3095.7	23.4	274.6	1077785	7.359 ng/ml

Compound Calibration Report

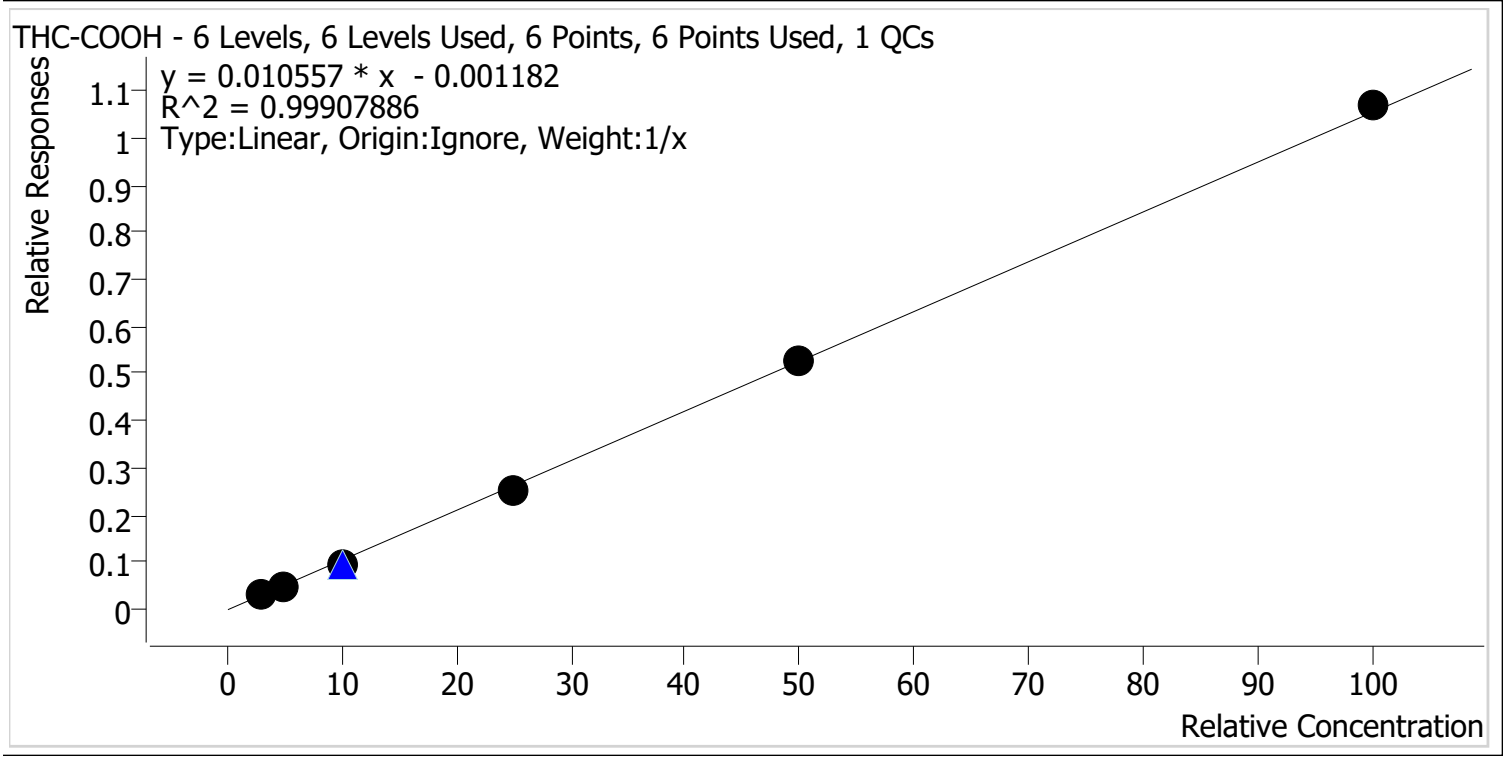
Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Last Cal. Update	6/12/2019 10:22 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✔	3.0	3.3	109.3
cal 3	3	✔	5.0	5.1	101.2
cal 4	4	✔	10.0	9.3	93.3
cal 5	5	✔	25.0	23.9	95.7
cal 6	6	✔	50.0	49.2	98.4
cal 7	7	✔	100.0	102.2	102.2

Compound Calibration Report

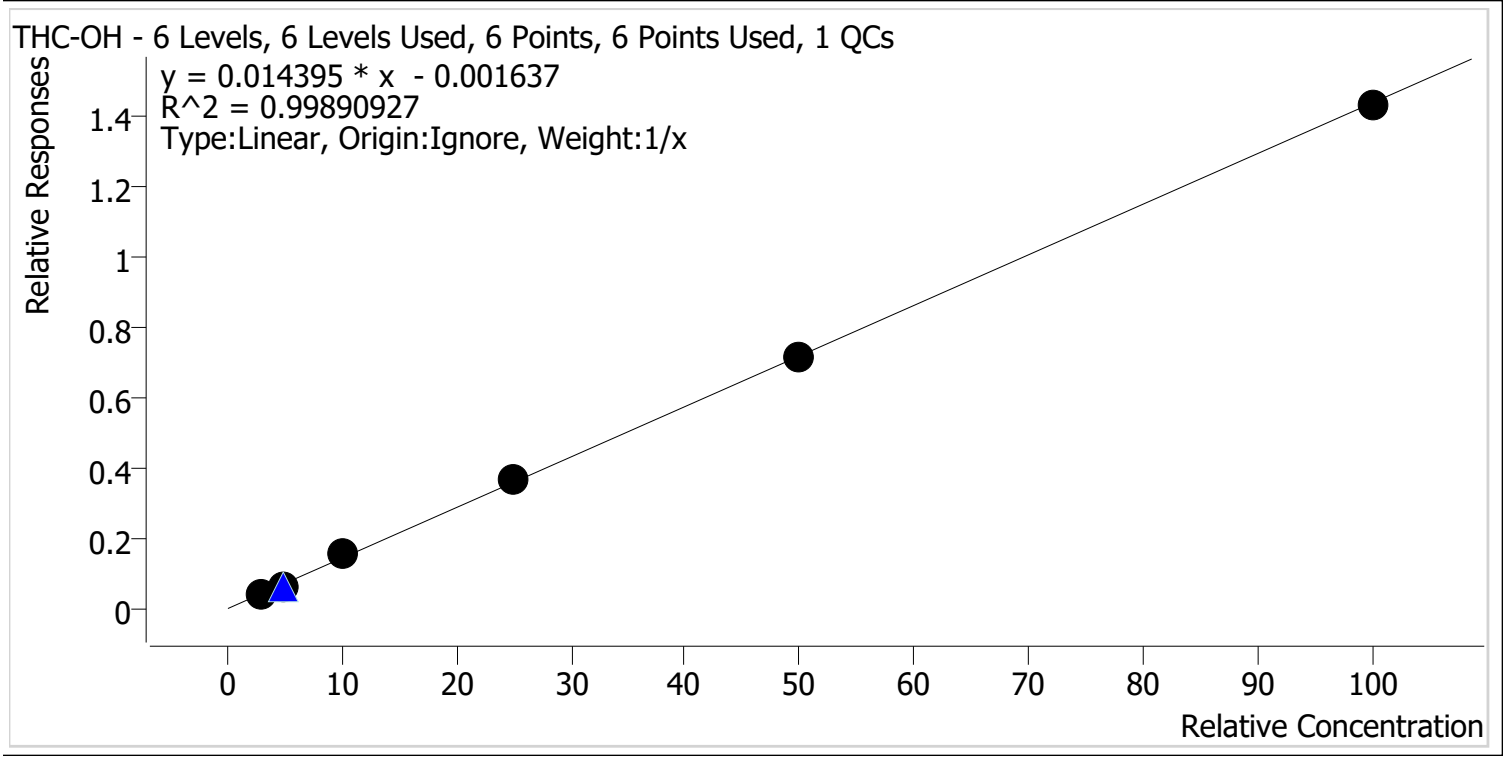
Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Last Cal. Update	6/12/2019 10:22 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✔	3.0	3.3	110.7
cal 3	3	✔	5.0	4.9	98.3
cal 4	4	✔	10.0	9.3	93.3
cal 5	5	✔	25.0	24.1	96.3
cal 6	6	✔	50.0	50.1	100.1
cal 7	7	✔	100.0	101.3	101.3

Compound Calibration Report

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Last Cal. Update	6/12/2019 10:22 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



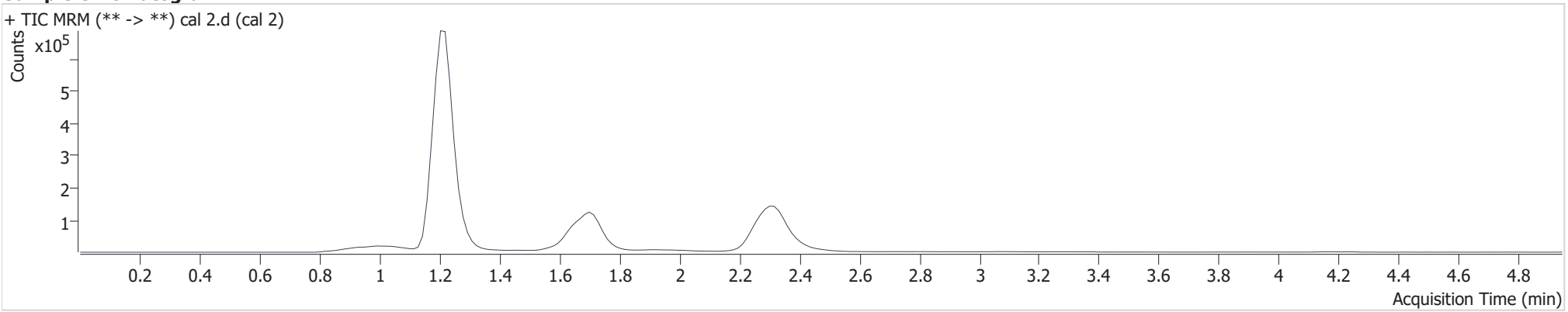
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
cal 2	2	✔	3.0	3.0	101.2
cal 3	3	✔	5.0	4.5	89.0
cal 4	4	✔	10.0	10.9	109.2
cal 5	5	✔	25.0	25.4	101.5
cal 6	6	✔	50.0	49.8	99.6
cal 7	7	✔	100.0	99.4	99.4

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	cal 2.d
Type	Cal	**Sample**	cal 2
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 3:20:13 PM		
Sample Info.			

Sample Chromatogram



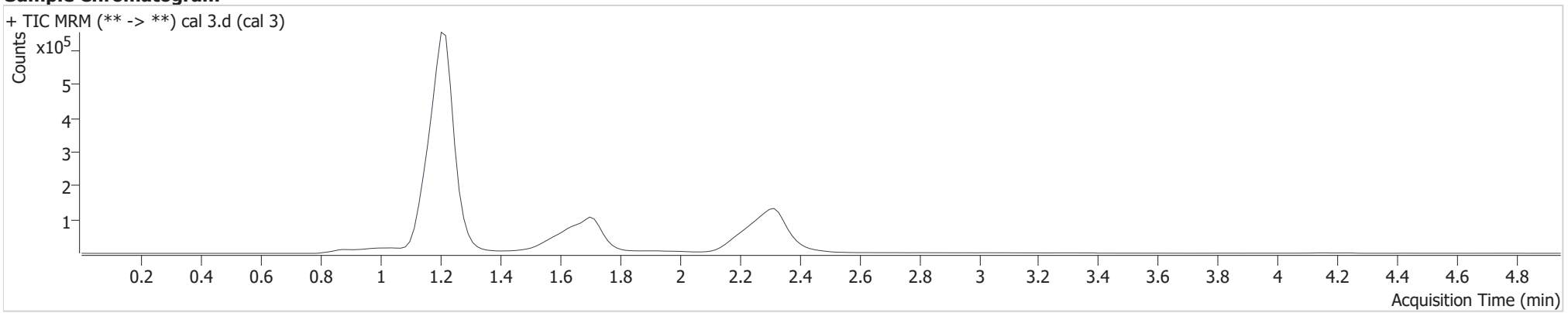
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.224	90717	∞	12.2	∞	2155886	3.037 ng/ml
THC-COOH	1.220	35565	59.4	30.7	117.8	1050189	3.320 ng/ml Low
THC	2.326	21516	1413.4	26.6	51.4	1072500	3.278 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	cal 3.d
Type	Cal	**Sample**	cal 3
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 3:27:57 PM		
Sample Info.			

Sample Chromatogram



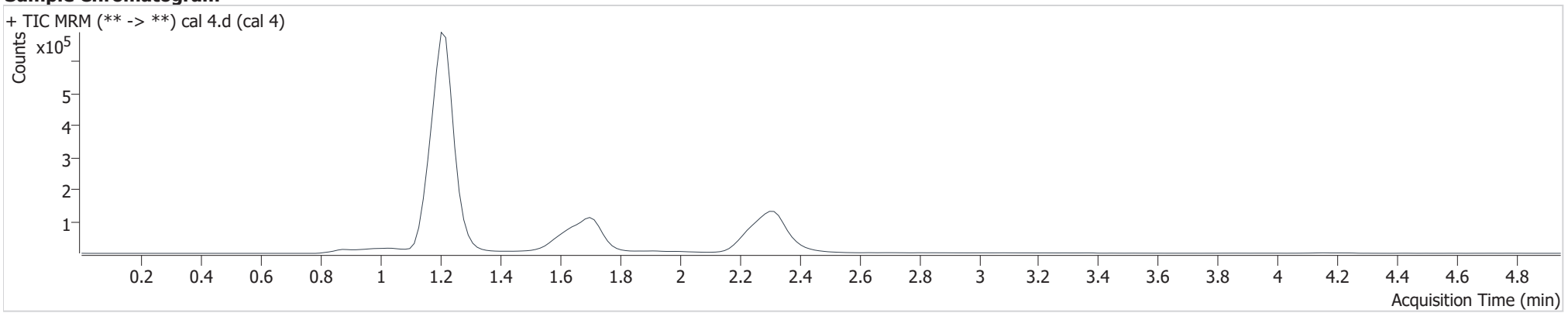
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.224	148383	∞	9.5	∞	2375946	4.452 ng/ml
THC-COOH	1.220	57433	179.6	34.8	681.4	1132761	4.915 ng/ml Low
THC	2.326	39834	405.6	23.7	45.0	1195105	5.058 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	cal 4.d
Type	Cal	**Sample**	cal 4
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 3:35:41 PM		
Sample Info.			

Sample Chromatogram



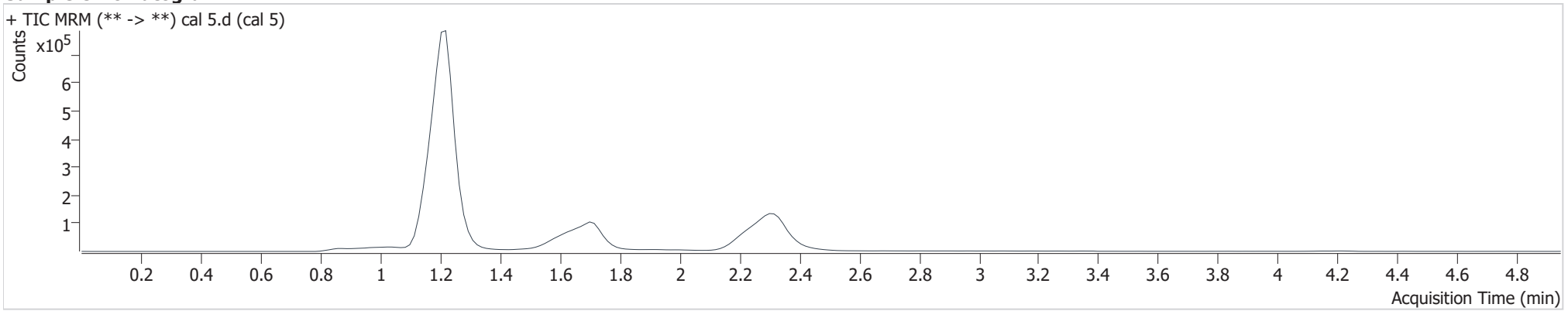
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.224	328760	∞	9.2	∞	2113672	10.919 ng/ml
THC-COOH	1.220	101055	782.9	36.6	152.9	1038623	9.328 ng/ml Low
THC	2.326	68612	635.0	25.0	168.2	1052623	9.329 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	cal 5.d
Type	Cal	**Sample**	cal 5
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 3:43:25 PM		
Sample Info.			

Sample Chromatogram



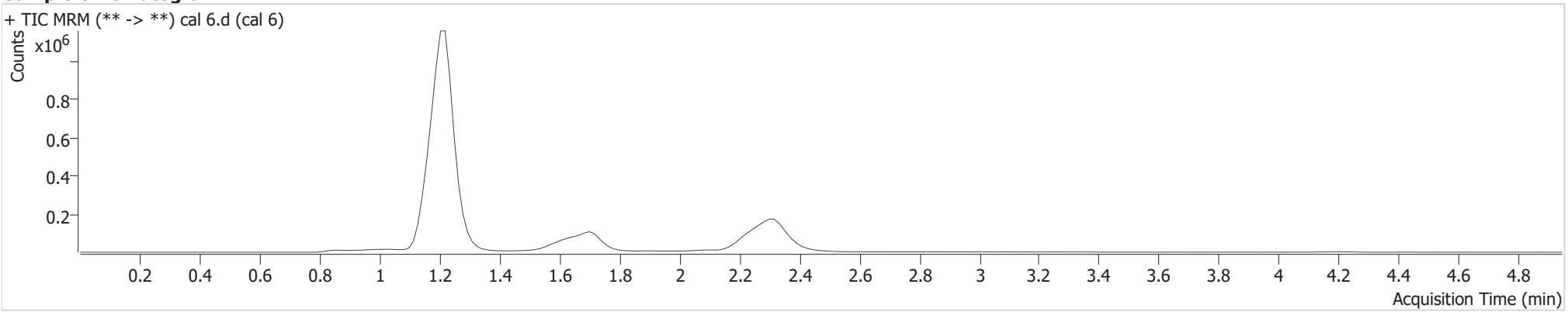
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.224	729365	∞	10.4	∞	2005664	25.376 ng/ml
THC-COOH	1.220	249890	429.7	36.7	953.5	987414	24.085 ng/ml
THC	2.326	174653	10603.4	26.4	374.4	1004124	23.913 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	cal 6.d
Type	Cal	**Sample**	cal 6
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 3:51:09 PM		
Sample Info.			

Sample Chromatogram



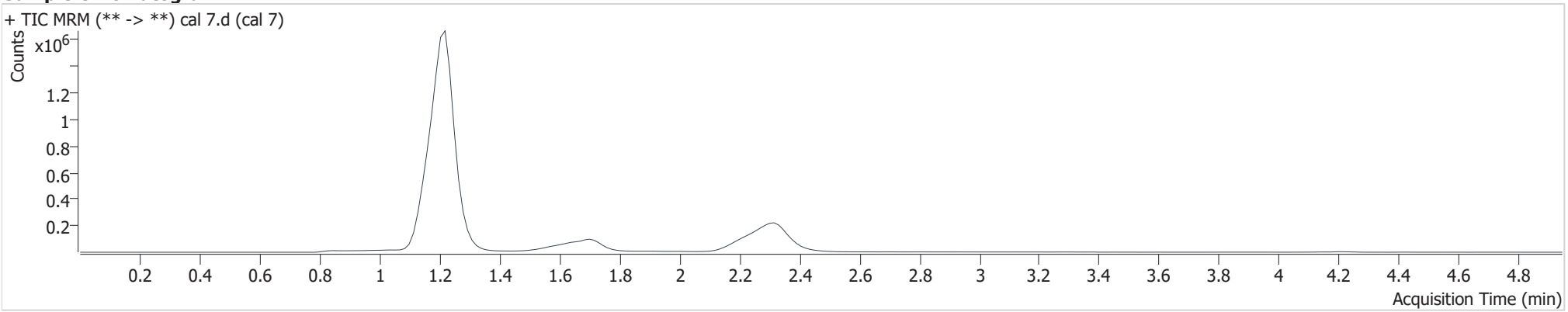
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.224	1539927	∞	11.3	∞	2152003	49.823 ng/ml
THC-COOH	1.220	555483	1373.4	37.7	3778.2	1053281	50.069 ng/ml
THC	2.326	389565	2057.7	24.2	532.2	1074754	49.197 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\AM 27\061119\QuantResults\cann quant.batch.bin
Calibration Last Update	6/12/2019 10:22:38 AM

Instrument	69679	**Data File**	cal 7.d
Type	Cal	**Sample**	cal 7
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/11/2019 3:58:53 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.224	3072826	∞	11.5	∞	2150118	99.393 ng/ml
THC-COOH	1.220	1078033	2049.5	38.1	2250.6	1009341	101.284 ng/ml
THC	2.326	832355	∞	24.2	3019.1	1098251	102.224 ng/ml