REVIEWED
By Britany Wylie at 2:12 pm, Sep 10, 2019

9/9/2019

Worklist: 3667

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2019-1385	1	162873	AM 27 Blood THC Quant by LC-QQQ
C2019-1589	1	162864	AM 27 Blood THC Quant by LC-QQQ
C2019-1628	1	162865	AM 27 Blood THC Quant by LC-QQQ
C2019-1655	1	162866	AM 27 Blood THC Quant by LC-QQQ
C2019-1658	2	162867	AM 27 Blood THC Quant by LC-QQQ
C2019-1691	1	162868	AM 27 Blood THC Quant by LC-QQQ
C2019-1712	1	162869	AM 27 Blood THC Quant by LC-QQQ
C2019-1714	1	162870	AM 27 Blood THC Quant by LC-QQQ
C2019-1722	1	162871	AM 27 Blood THC Quant by LC-QQQ
M2019-3587	3	162872	AM 27 Blood THC Quant by LC-QQQ

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

Extraction Date: 09-06-19 Analyst: Anne Nord
Plate lot#: 190716 Plate Expiration: 1-16-2020

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: 445283-2 **Urine Blank:** 8919 **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.

Analytic:

☒ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
☒ 2. Urine hydrolysis: add 1.5 ml urine to blank plate, add 250 ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
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 or urine 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.
☒ 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 blood), 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 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

Urine control 1 300 ul working solution to 9700 ul neg urine (031319)
ppd 3/13/19 Exp 9/13/19 urine1 Concentration 22.5 ng/ml THC, THC-OH and 45 ng/ml C-THC by AMN

Urine control 2 600 ul working solution to 9400 ul neg urine (031319)
ppd 3/13/19 Exp 9/13/19 urine1 Concentration 45 ng/ml THC, THC-OH and 90 ng/ml C-THC by AMN

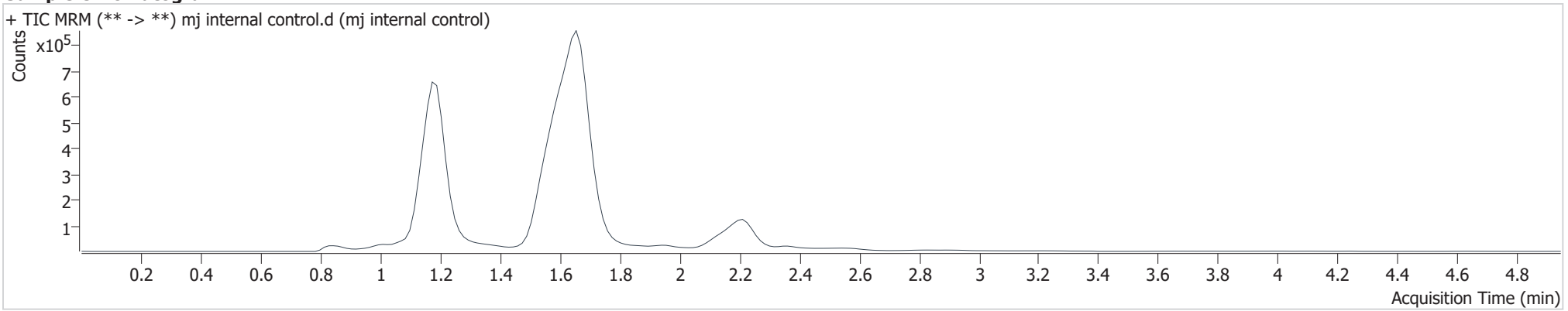
Urine control 3 1200 ul working solution to 8800 ul neg urine (031319)
ppd 3/13/19 Exp 9/13/19 urine1 Concentration 90 ng/ml THC, THC-OH and 180 ng/ml C-THC by AMN
ppd 9/6/19 diluted 1:2 with negative urine 031319 Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC By AMN
used 9/6/19

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj internal control.d
Type	QC	**Sample**	mj internal control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 2:10:06 PM		
Sample Info.			

Sample Chromatogram



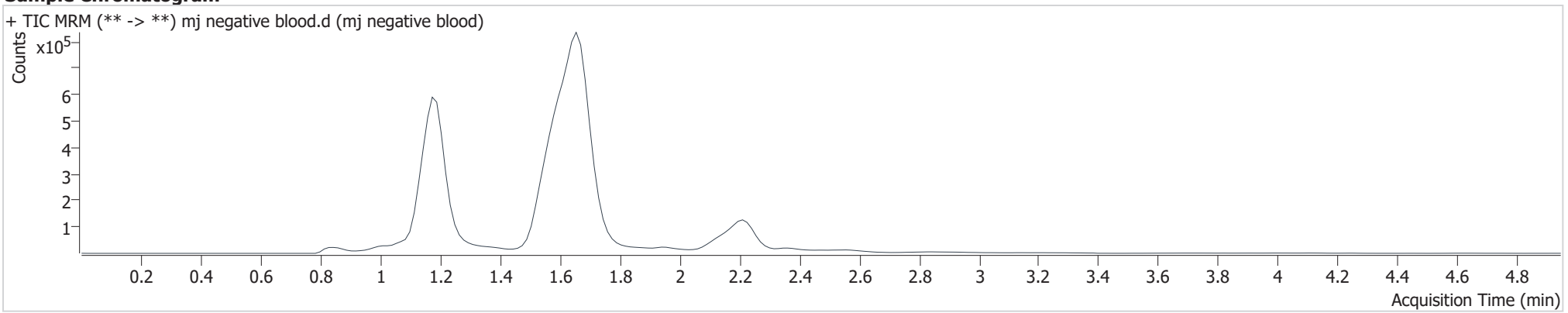
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.196	18633	∞	1077.8	∞	2310590	4.550 ng/ml
THC-COOH	1.190	122355	452.34	36.6	728.41	743074	14.426 ng/ml
THC	2.221	25617	329.73	24.9	130.01	830694	4.196 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj negative blood.d
Type	Sample	**Sample**	mj negative blood
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 2:17:48 PM		
Sample Info.			

Sample Chromatogram

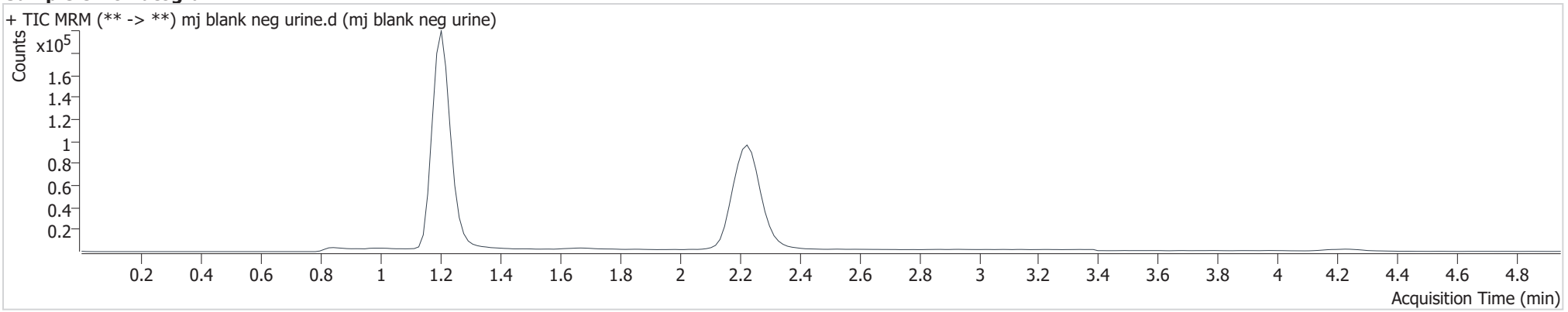


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj blank neg urine.d
Type	Sample	**Sample**	mj blank neg urine
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	Vial 2	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 4:13:02 PM		
Sample Info.			

Sample Chromatogram

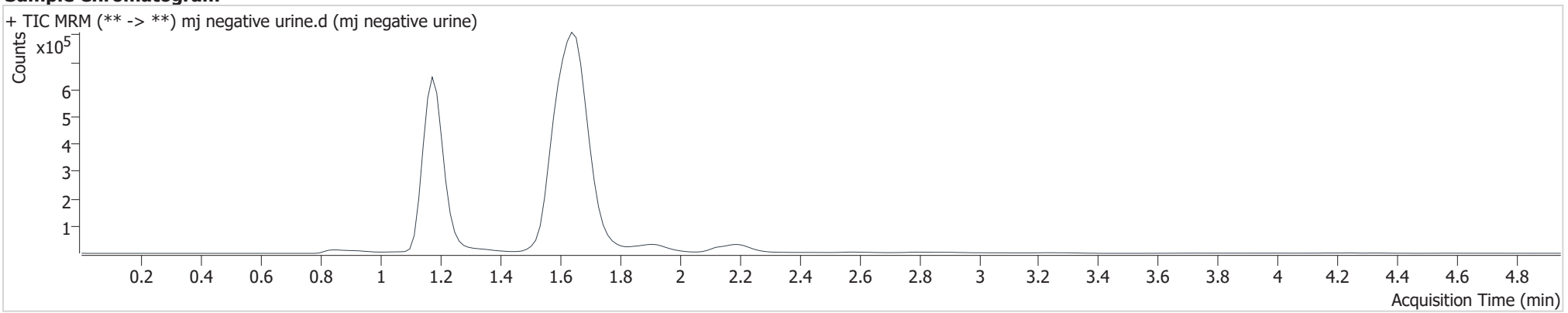


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj negative urine.d
Type	Sample	**Sample**	mj negative urine
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A3	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 4:20:40 PM		
Sample Info.			

Sample Chromatogram

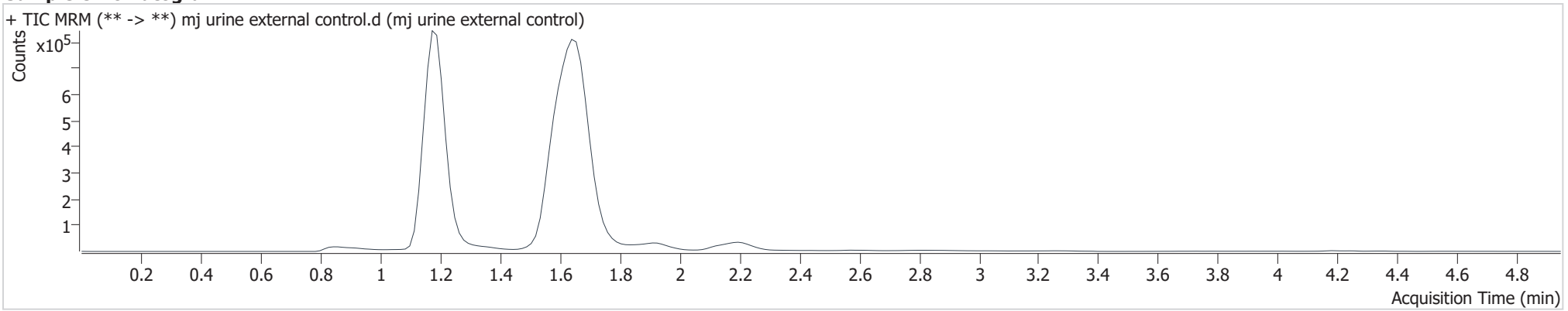


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj urine external control.d
Type	Sample	**Sample**	mj urine external control
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B3	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 4:28:25 PM		
Sample Info.			

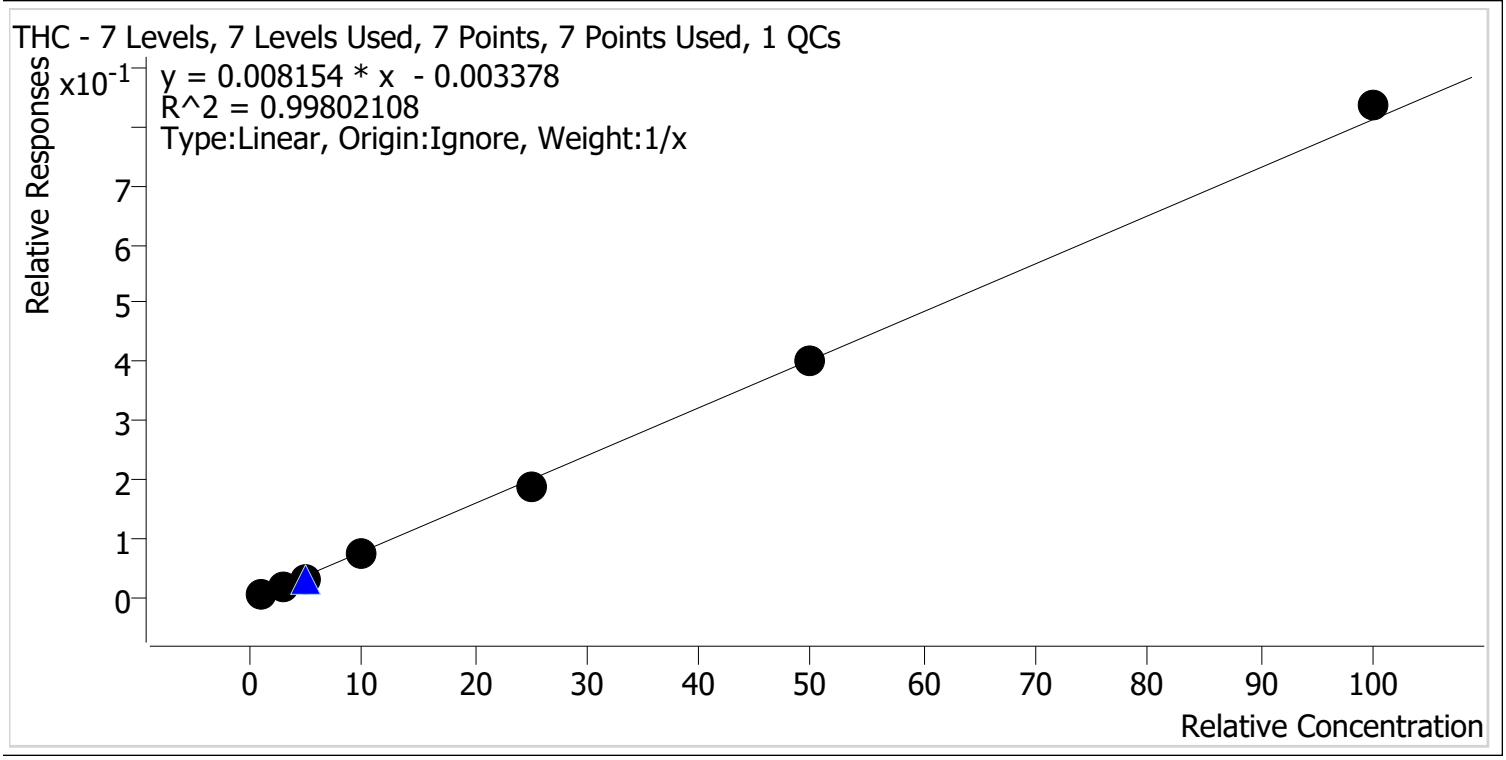
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.181	55769	24922	980.6	∞	2406900	14.126 ng/ml
THC-COOH	1.190	251849	402.65	35.4	952.57	625533	35.232 ng/ml
THC	2.221	34607	380.96	24.0	52.467	239858	18.109 ng/ml

Compound Calibration Report

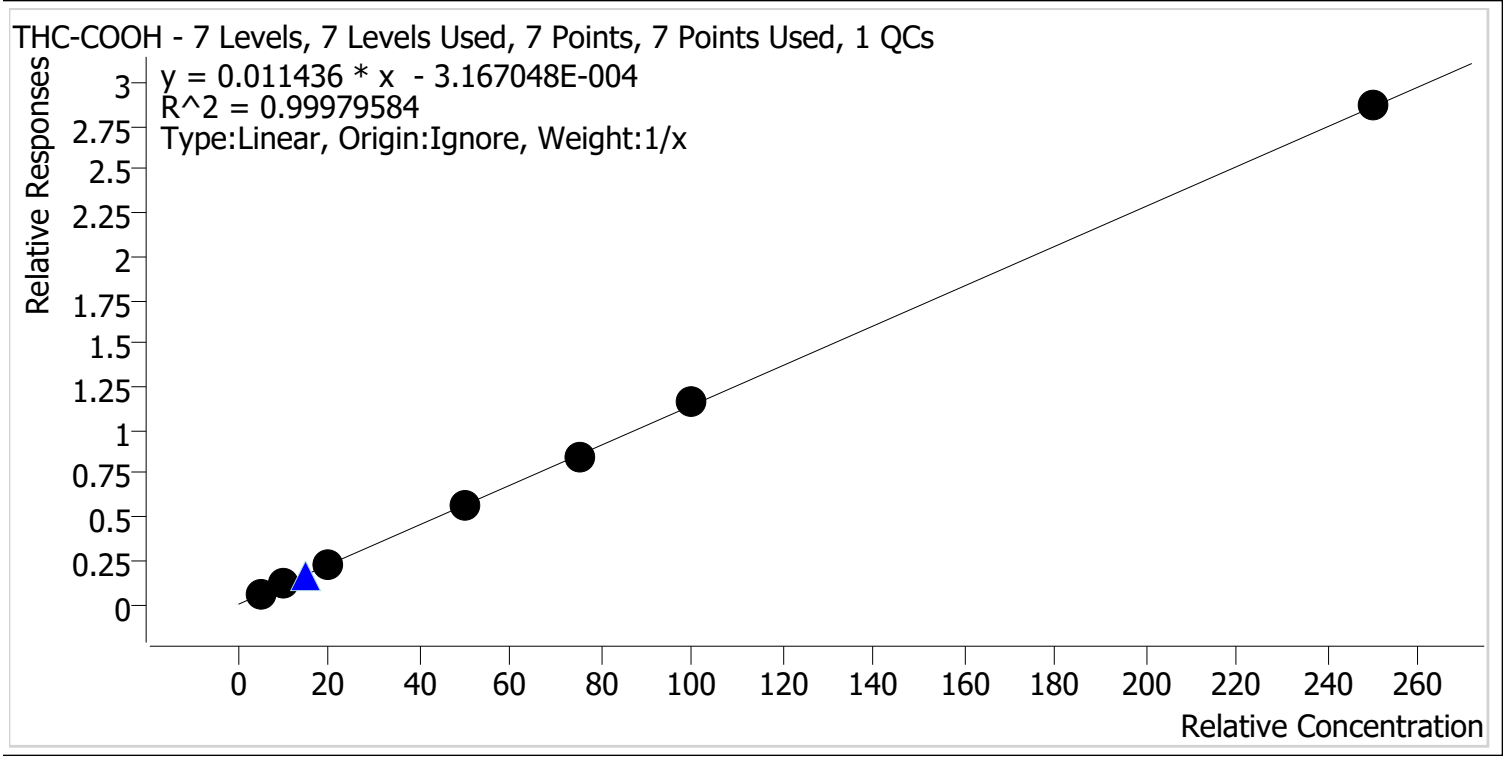
Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Last Cal. Update	9/7/2019 8:50 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✔	1.0	1.3	125.6
mj cal2	2	✔	3.0	2.8	93.6
mj cal 3	3	✔	5.0	4.6	91.7
mj cal 4	4	✔	10.0	9.2	92.4
mj cal 5	5	✔	25.0	23.7	94.7
mj cal 6	6	✔	50.0	49.5	99.1
mj cal 7	7	✔	100.0	102.9	102.9

Compound Calibration Report

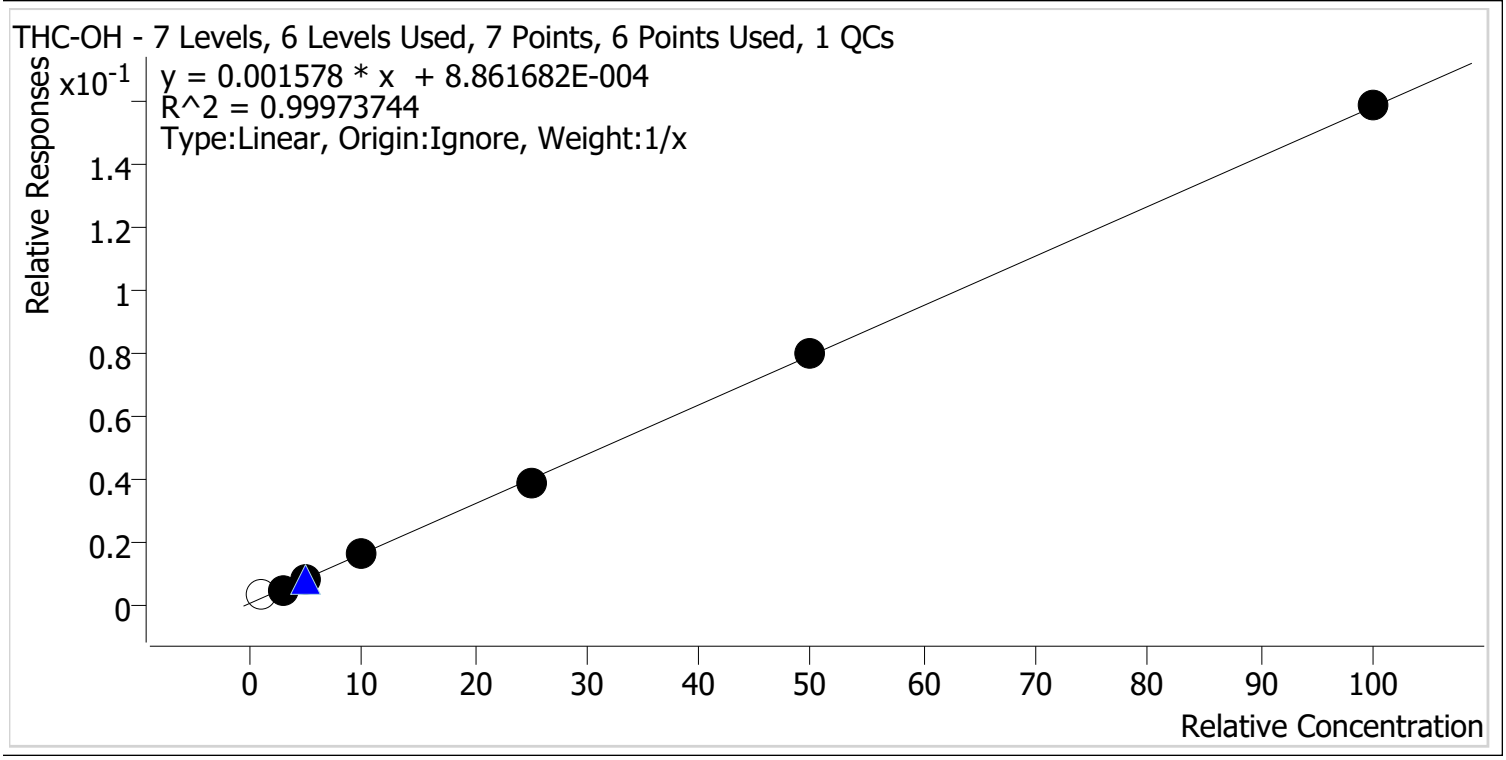
Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Last Cal. Update	9/7/2019 8:50 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✔	5.0	5.3	106.8
mj cal2	2	✔	10.0	9.7	97.3
mj cal 3	3	✔	20.0	19.3	96.4
mj cal 4	4	✔	50.0	49.3	98.6
mj cal 5	5	✔	75.0	74.8	99.7
mj cal 6	6	✔	100.0	101.0	101.0
mj cal 7	7	✔	250.0	250.6	100.2

Compound Calibration Report

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Last Cal. Update	9/7/2019 8:50 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



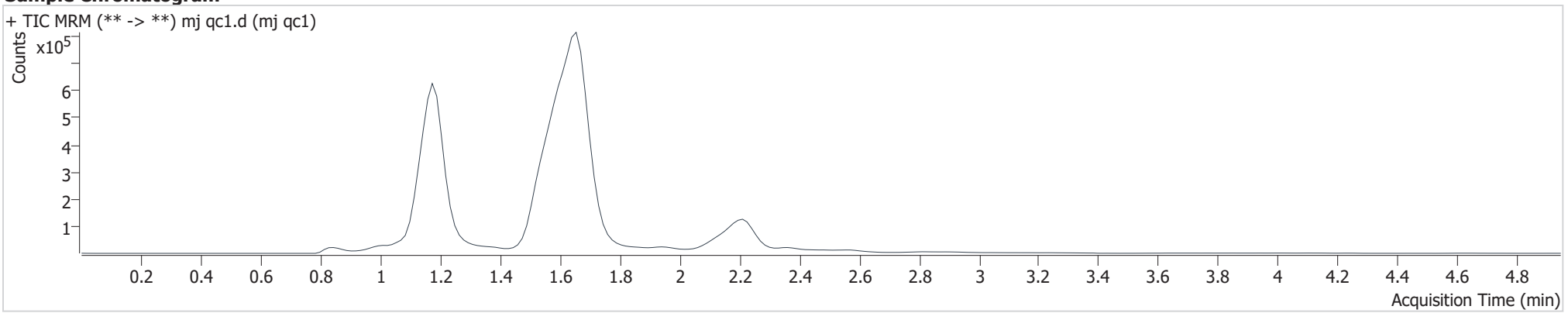
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✗	1.0	1.9	194.2
mj cal2	2	✔	3.0	2.9	97.8
mj cal 3	3	✔	5.0	5.0	100.6
mj cal 4	4	✔	10.0	10.4	103.9
mj cal 5	5	✔	25.0	24.3	97.2
mj cal 6	6	✔	50.0	50.1	100.2
mj cal 7	7	✔	100.0	100.3	100.3

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj qc1.d
Type	Cal	**Sample**	mj qc1
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 1:16:00 PM		
Sample Info.			

Sample Chromatogram



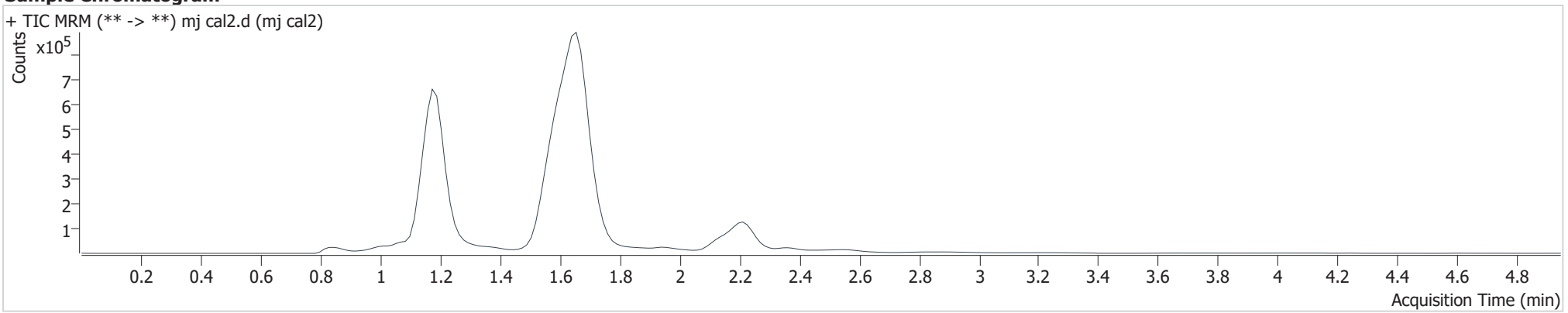
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.196	9765	165.75	1494.0 **High**	∞	2472686	1.942 ng/ml **Low**
THC-COOH	1.190	47524	48.868	32.8	187.09	782399	5.339 ng/ml **Low**
THC	2.236	6211	64.318	30.3	∞	904964	1.256 ng/ml **Low**

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj cal2.d
Type	Cal	**Sample**	mj cal2
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 1:23:44 PM		
Sample Info.			

Sample Chromatogram



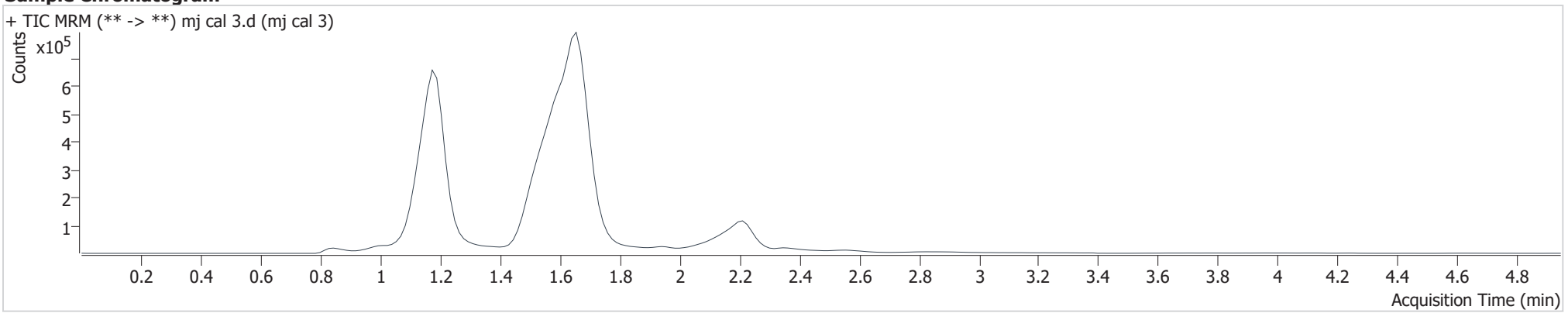
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.181	12851	∞	1031.0	∞	2329285	2.935 ng/ml Low
THC-COOH	1.190	82984	206.2	37.0	589.51	747569	9.734 ng/ml Low
THC	2.221	15816	247.82	24.7	20.121	810371	2.808 ng/ml Low

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj cal 3.d
Type	Cal	**Sample**	mj cal 3
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 1:31:28 PM		
Sample Info.			

Sample Chromatogram



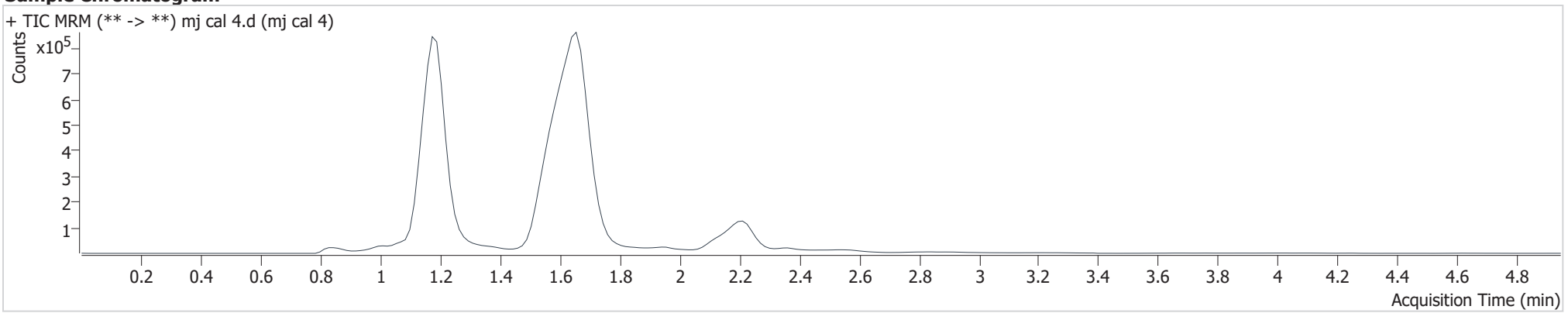
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.181	21024	∞	977.7	∞	2383421	5.030 ng/ml
THC-COOH	1.190	166497	617.62	38.2	551.01	756561	19.271 ng/ml
THC	2.221	28519	985.11	30.5	∞	838469	4.586 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj cal 4.d
Type	Cal	**Sample**	mj cal 4
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 1:39:12 PM		
Sample Info.			

Sample Chromatogram



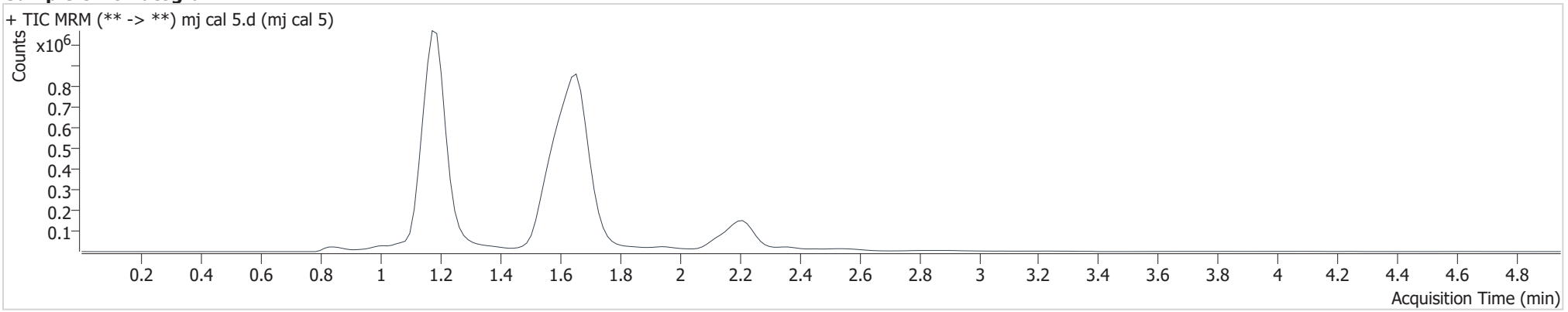
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.181	39219	∞	1113.2	∞	2269570	10.392 ng/ml
THC-COOH	1.190	405113	3480.3	38.2	2099.6	718955	49.298 ng/ml
THC	2.221	57561	1013.5	22.6	102.88	799862	9.240 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj cal 5.d
Type	Cal	**Sample**	mj cal 5
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 1:46:56 PM		
Sample Info.			

Sample Chromatogram



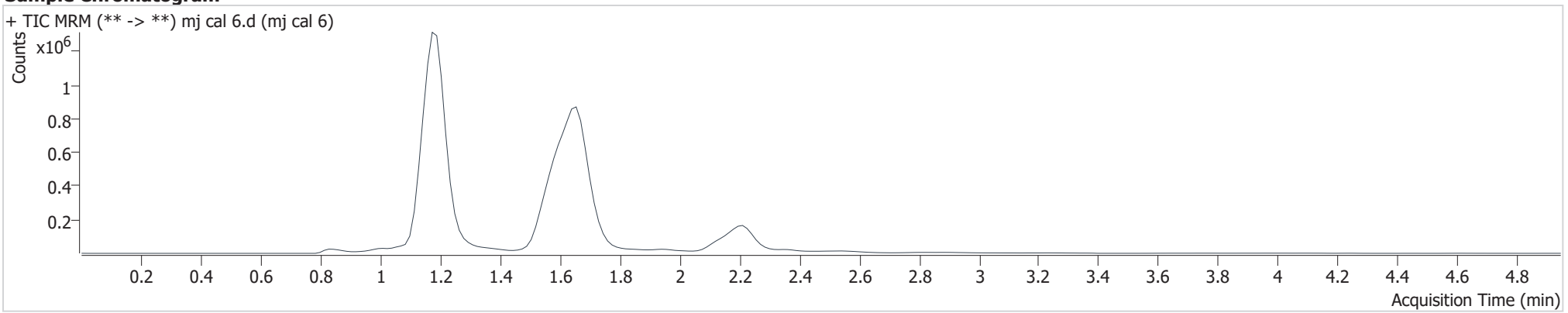
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.181	90179	∞	1125.1	∞	2299085	24.302 ng/ml
THC-COOH	1.190	616913	1.0392E+06	38.1	18898	721819	74.760 ng/ml
THC	2.221	160600	2085	24.8	1415.5	846551	23.680 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj cal 6.d
Type	Cal	**Sample**	mj cal 6
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 1:54:40 PM		
Sample Info.			

Sample Chromatogram



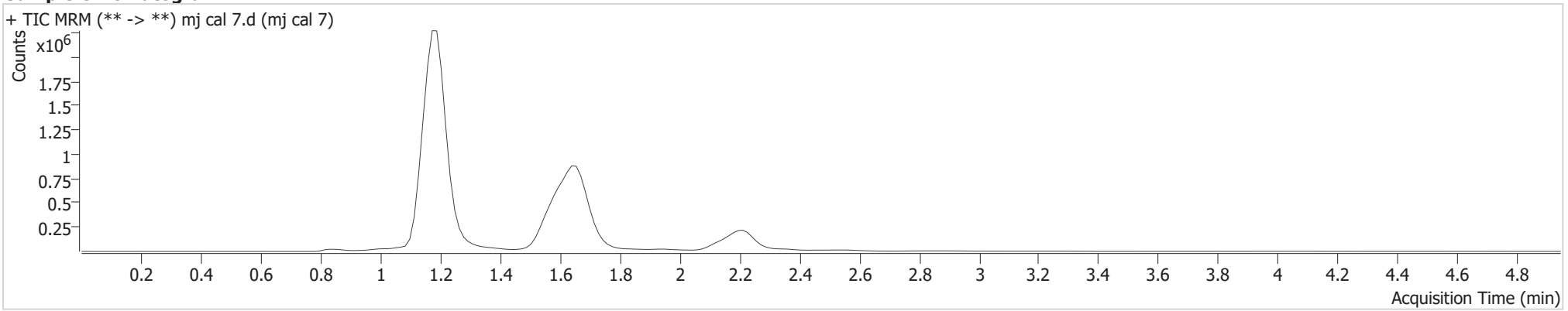
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.181	178807	∞	1037.4	∞	2238099	50.082 ng/ml
THC-COOH	1.190	789010	7621.1	38.7	3818.4	683151	101.018 ng/ml
THC	2.221	308975	2828.1	25.0	527.5	771391	49.537 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2019\am28-27 090619\QuantResults\cann quant.batch.bin
Calibration Last Update	9/7/2019 8:50:00 AM

Instrument	69679	**Data File**	mj cal 7.d
Type	Cal	**Sample**	mj cal 7
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	9/6/2019 2:02:24 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.181	354286	∞	948.2	∞	2227517	100.259 ng/ml
THC-COOH	1.190	1979354	3826.3	39.2	2025.2	690775	250.580 ng/ml
THC	2.221	638788	9373	25.1	∞	764459	102.893 ng/ml