REVIEWED
By Britany Wylie at 10:10 am, Jun 15, 2020

6/12/2020

Worklist: 4301

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2020-0959	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0982	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-0994	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-0995	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2020-1038	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1039	4	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1047	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1076	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1084	1	BCK	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: 6/10/2020 Analyst: Anne Nord
Plate lot#: 200303 Plate Expiration: 09-03-2020

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: 20A52255 **Urine Blank:** 6920 **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.
☒ 4. Pipette **500µL 0.1% formic acid in water blood sample**, **500 ul saturated phosphate buffer in urine** 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? (if not is it describe in comments section)
☒ 6 Enter QCs into control charting.
☒ 7 Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *THC curve 3-100*
THC-OH not evaluated for urine samples.

Toxicology AM method 27/26 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 2/13/20 Exp: 8/13/20 lot 21320 by AMN

Drug	lot	expiration
C-THC	FE07171501	9/1/2020
THC-OH	FE07721601	7/1/2021
THC	FE001041701	3/1/2022

AM 27/26 blood control 100 ul working solution lot (91319) in 9900 ul blood lot (20A52255)

ppd 02/13/20 Exp 08/13/20 lot b81320 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-THC by AMN

AM 27/26 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (6920) out of use

ppd 4/17/20 Exp 9120 lot u101720 Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC by BAW 6/8/2020

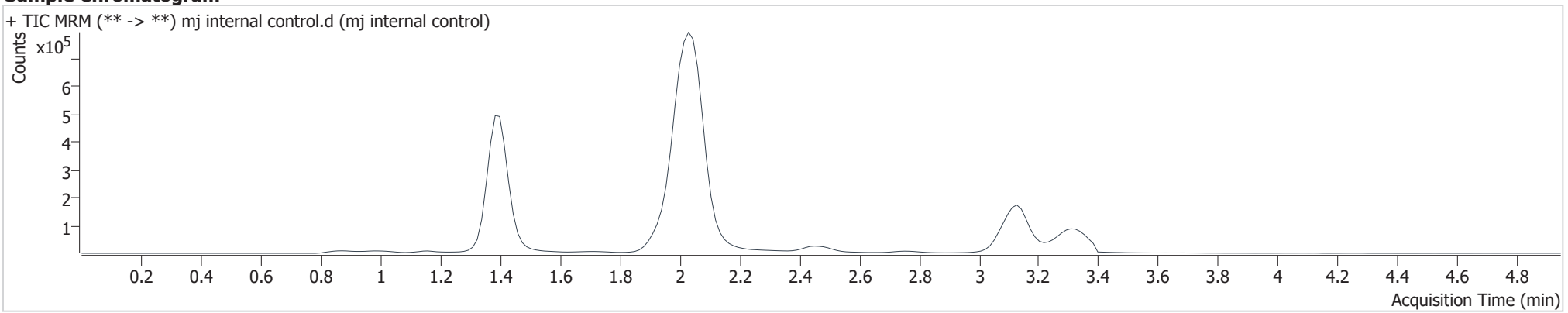
ppd 6/9/20 exp 8/13/20 lot 6920 Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC by amn

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 12:38:09 PM		
Sample Info.			

Sample Chromatogram



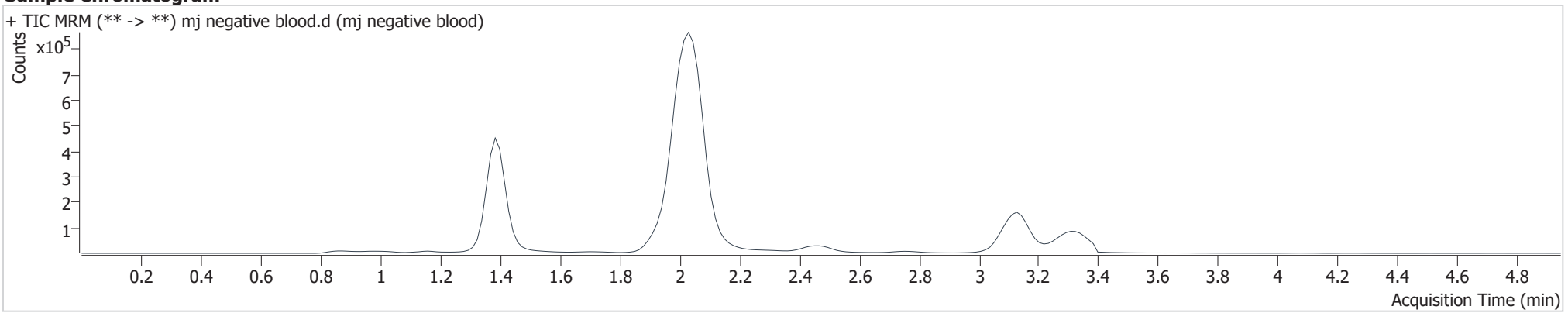
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	113407	∞	9.7	∞	1186669	4.985 ng/ml
THC-COOH	1.415	133206	665.1	37.9	1684.1	773839	15.311 ng/ml
THC	3.153	30160	∞	23.4	∞	809359	4.518 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 12:45:51 PM		
Sample Info.			

Sample Chromatogram

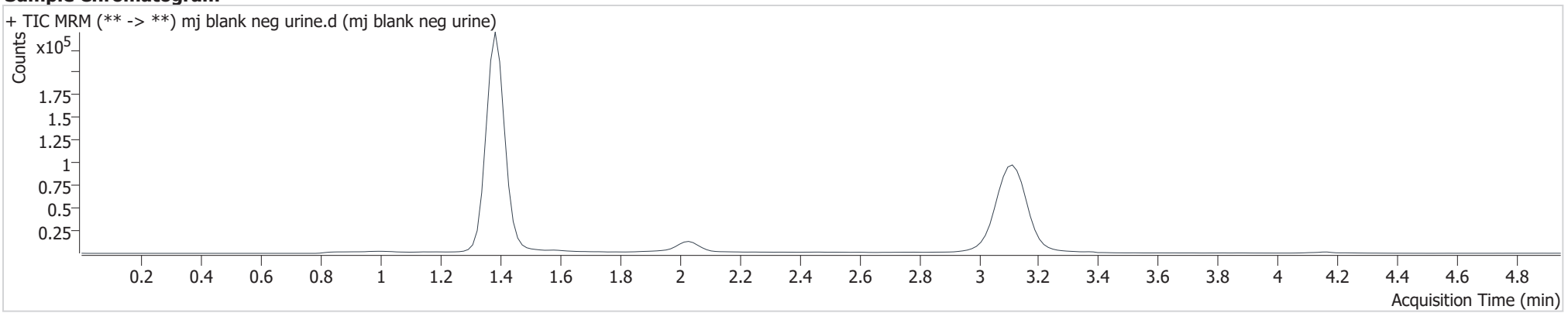


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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 4	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/10/2020 2:41:15 PM		
Sample Info.			

Sample Chromatogram

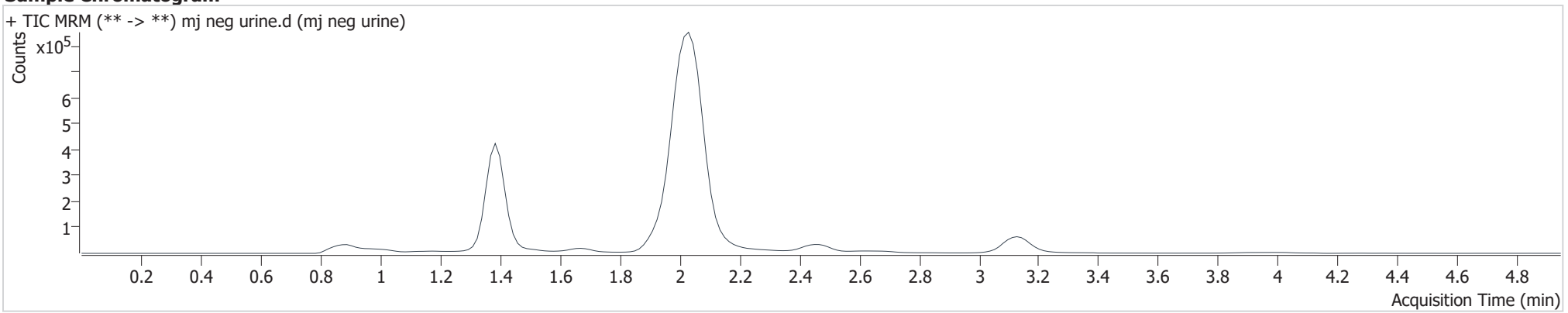


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 AM

Instrument	69679	**Data File**	mj neg urine.d
Type	Sample	**Sample**	mj neg urine
Acq. Method	AM 27 THC quant.m	**Operator**	Anne Nord
Sample Position	P3-A3	**Comment**	
Injection Volume	10		
Acq. Date-Time	6/10/2020 2:48:53 PM		
Sample Info.			

Sample Chromatogram

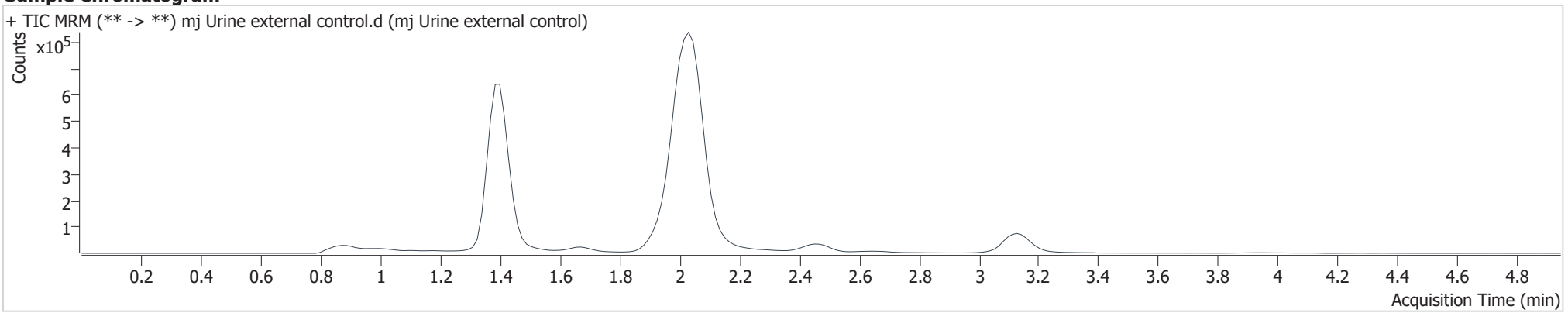


AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 2:56:38 PM		
Sample Info.			

Sample Chromatogram

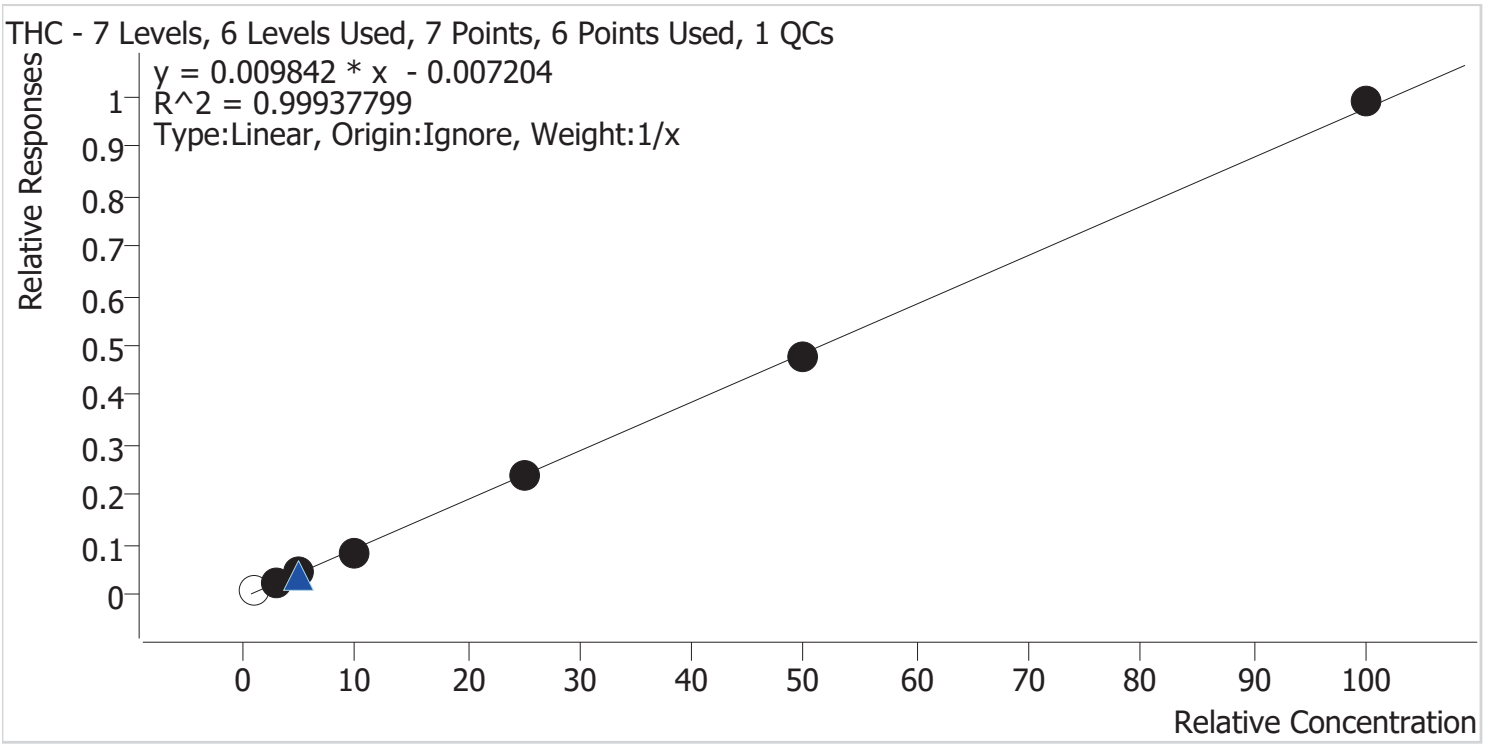


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	398815	∞	56.8 **High**	∞	1265314	16.874 ng/ml
THC-COOH	1.415	272270	320.5	37.2	654.1	563156	42.026 ng/ml
THC	3.153	57209	∞	24.5	∞	430973	14.220 ng/ml

Urine evaluated for THC and THC-COOH only.

Compound Calibration Report

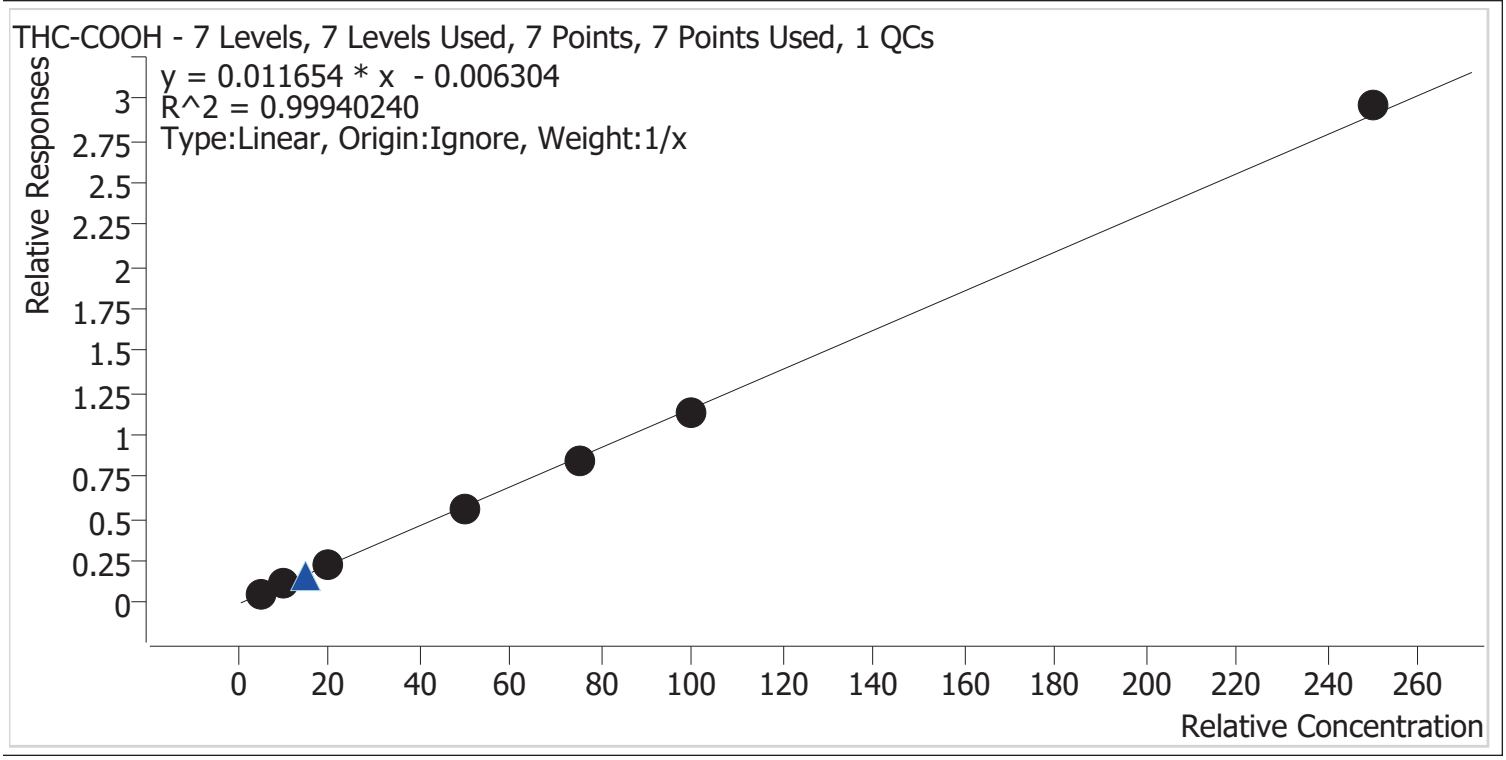
Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Last Cal. Update	6/11/2020 8:34 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	×	1.0	1.6	160.0
mj cal2	2	✓	3.0	3.2	105.1
mj cal 3	3	✓	5.0	5.1	102.4
mj cal 4	4	✓	10.0	9.3	93.5
mj cal 5	5	✓	25.0	24.8	99.4
mj cal 6	6	✓	50.0	49.1	98.3
mj cal 7	7	✓	100.0	101.4	101.4

Compound Calibration Report

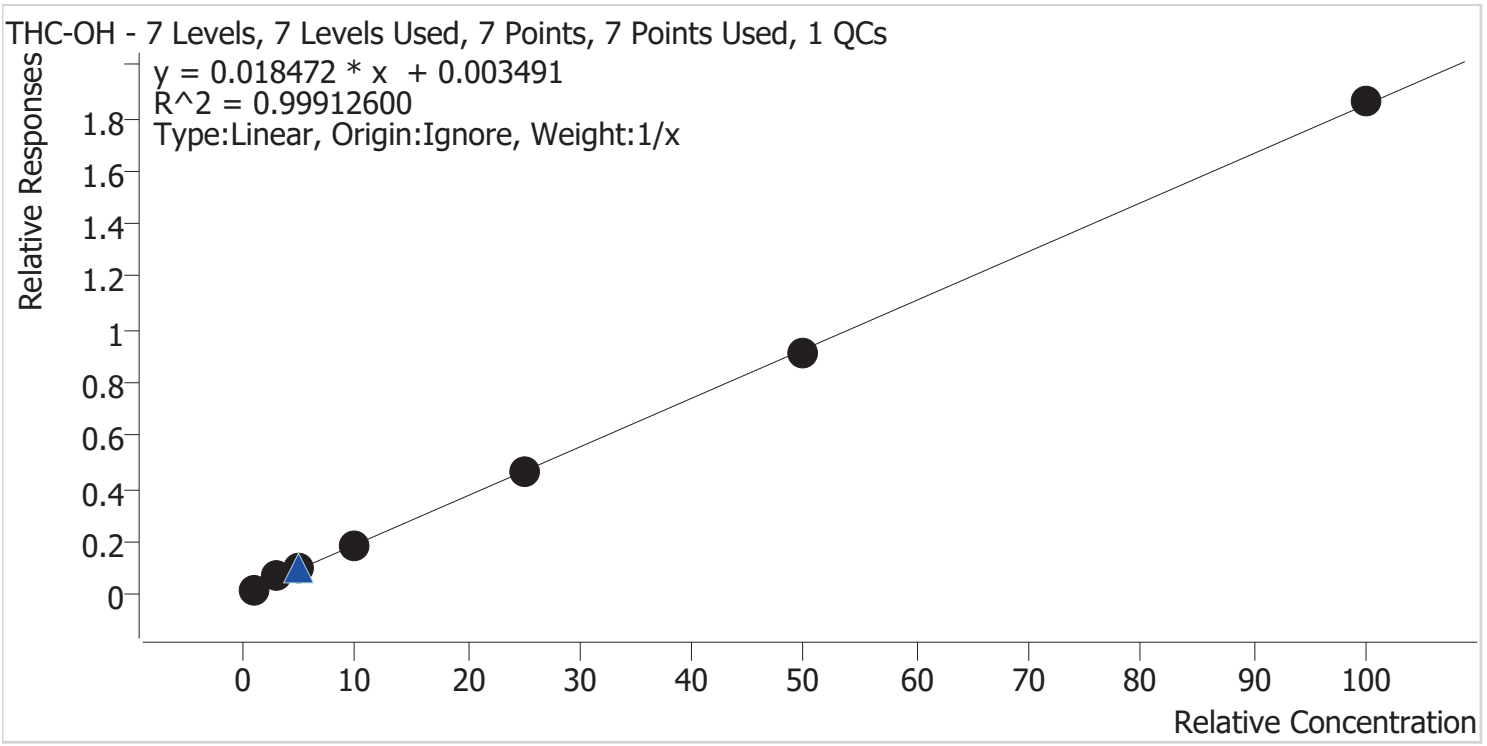
Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Last Cal. Update	6/11/2020 8:34 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.2	104.8
mj cal2	2	✓	10.0	10.0	99.7
mj cal 3	3	✓	20.0	20.2	100.9
mj cal 4	4	✓	50.0	48.6	97.2
mj cal 5	5	✓	75.0	72.9	97.2
mj cal 6	6	✓	100.0	98.4	98.4
mj cal 7	7	✓	250.0	254.8	101.9

Compound Calibration Report

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Last Cal. Update	6/11/2020 8:34 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



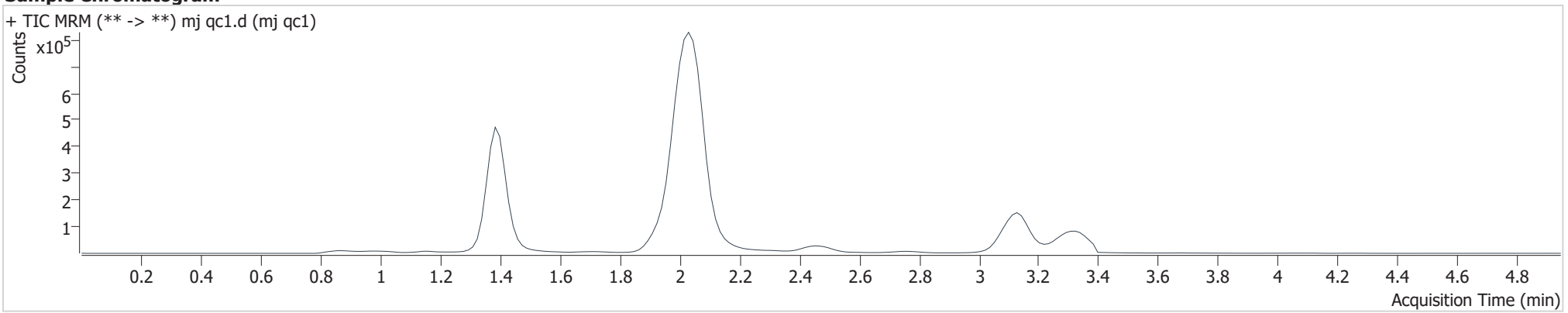
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	0.8	81.5
mj cal2	2	✓	3.0	3.4	113.7
mj cal 3	3	✓	5.0	5.4	107.2
mj cal 4	4	✓	10.0	9.9	99.3
mj cal 5	5	✓	25.0	25.0	99.8
mj cal 6	6	✓	50.0	48.9	97.8
mj cal 7	7	✓	100.0	100.6	100.6

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 11:44:01 AM		
Sample Info.			

Sample Chromatogram



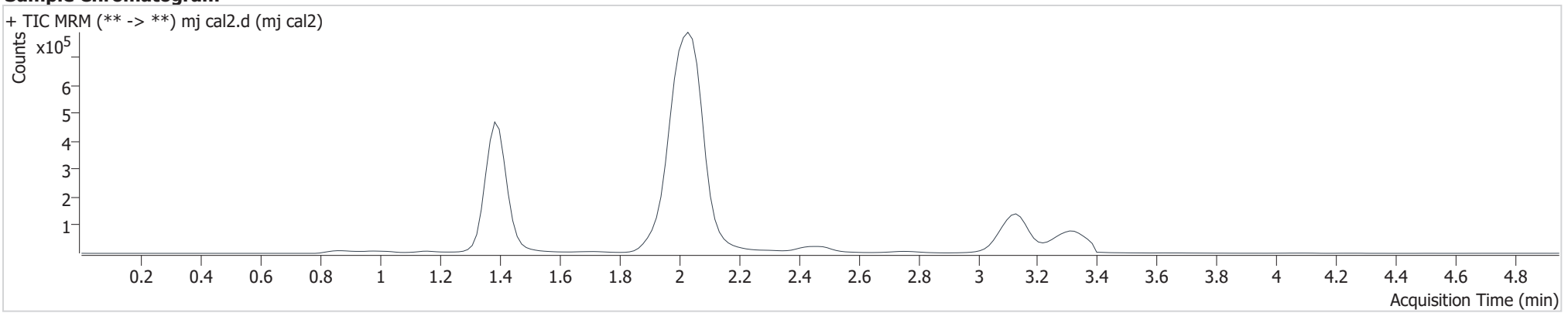
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	24030	∞	11.1	∞	1295639	0.815 ng/ml Low
THC-COOH	1.415	42605	159.8	35.1	236.3	778026	5.240 ng/ml Low
THC	3.153	6358	256.0	28.9	110.1	744521	1.600 ng/ml Low

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 11:51:46 AM		
Sample Info.			

Sample Chromatogram



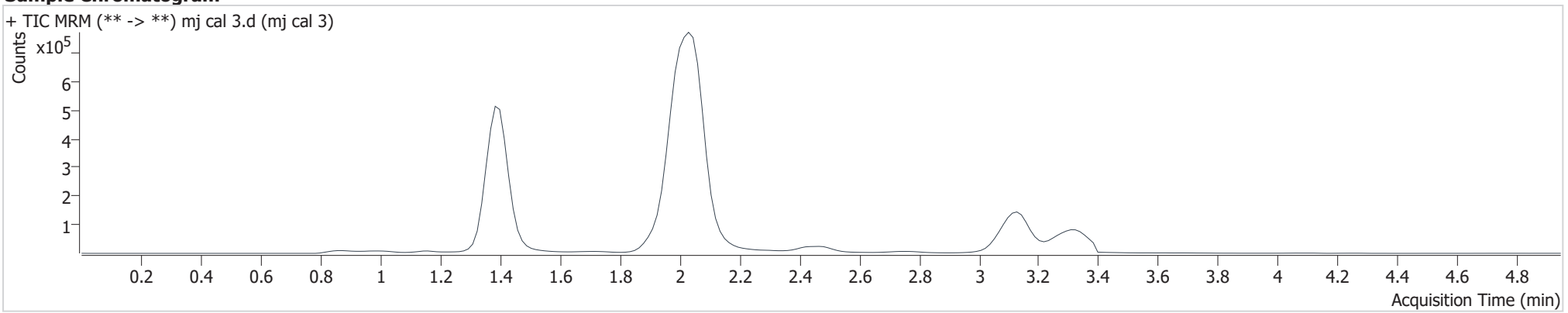
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	84162	∞	8.4	∞	1265104	3.412 ng/ml
THC-COOH	1.415	85309	501.1	36.9	375.3	776675	9.966 ng/ml Low
THC	3.153	16789	∞	25.4	69.7	704413	3.154 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 11:59:30 AM		
Sample Info.			

Sample Chromatogram



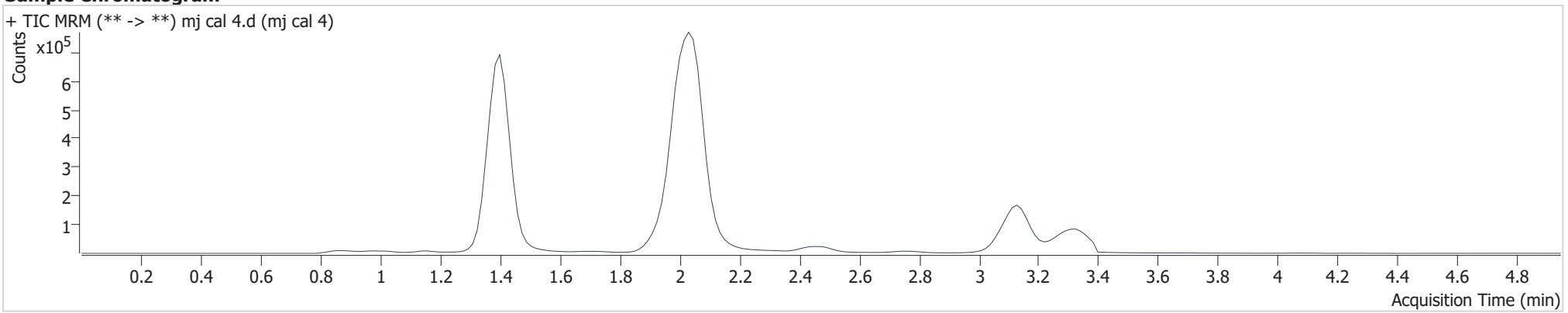
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	129249	∞	9.9	∞	1261272	5.359 ng/ml
THC-COOH	1.415	180802	226466.2	37.0	565.0	789667	20.187 ng/ml
THC	3.153	31206	∞	27.4	22082445 295712.0	722937	5.118 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 12:07:14 PM		
Sample Info.			

Sample Chromatogram



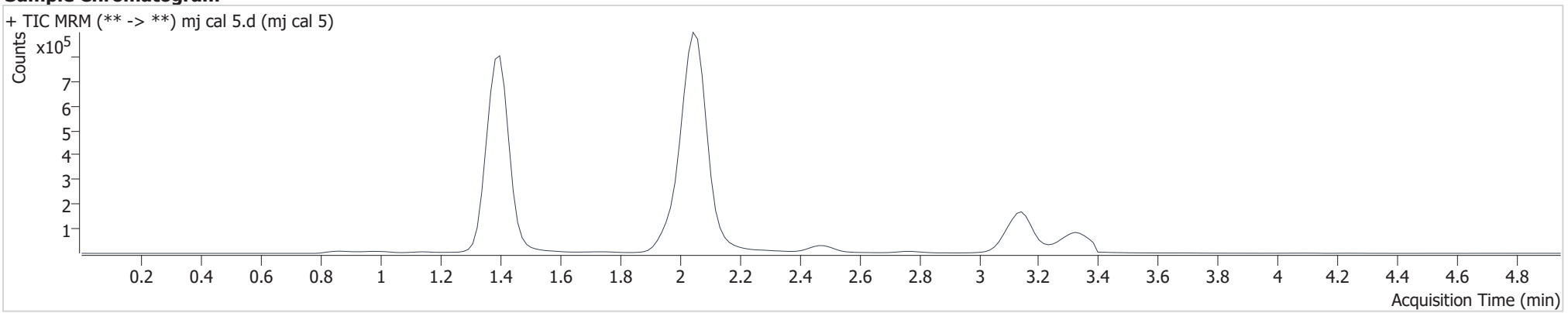
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	235140	∞	10.1	∞	1257780	9.932 ng/ml
THC-COOH	1.415	445147	9956.5	38.6	1765.8	795089	48.581 ng/ml
THC	3.153	65649	∞	26.4	∞	774319	9.347 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 12:14:58 PM		
Sample Info.			

Sample Chromatogram



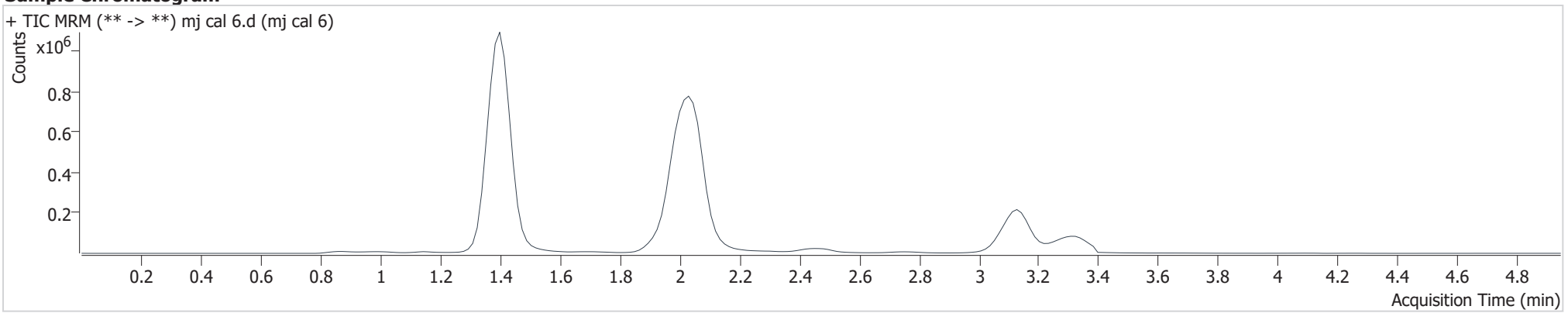
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	537889	∞	11.0	∞	1158262	24.952 ng/ml
THC-COOH	1.400	584958	1215.4	38.9	2940.7	693969	72.868 ng/ml
THC	3.153	150718	∞	25.1	20470718 283739.9	635216	24.841 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 12:22:42 PM		
Sample Info.			

Sample Chromatogram



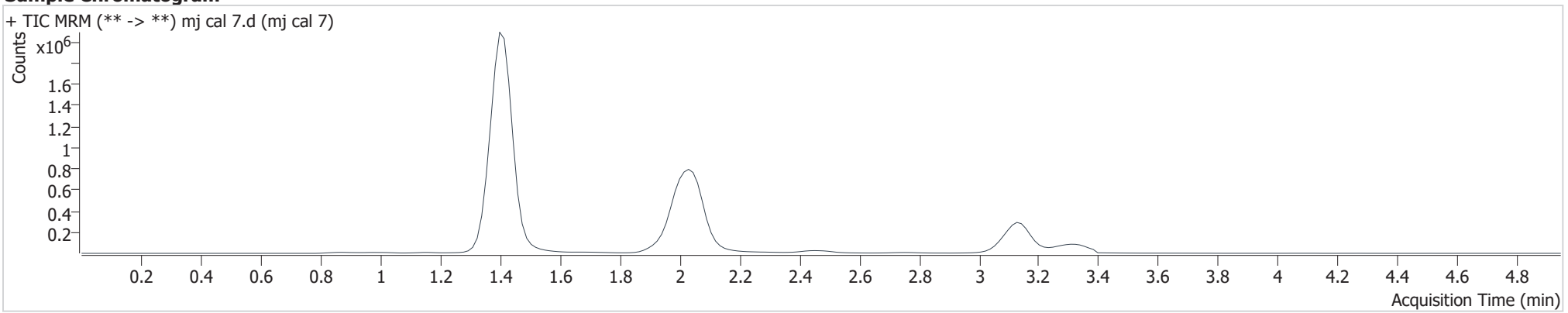
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	1123282	∞	11.6	∞	1238414	48.915 ng/ml
THC-COOH	1.415	880879	1325.8	39.3	4631.2	772478	98.388 ng/ml
THC	3.153	355600	∞	25.1	∞	746273	49.148 ng/ml

AM #27 Cannabinoids

Batch results	D:\MassHunter\Data\2020 Data\am 27-28 6-10-20\QuantResults\mj.batch.bin
Calibration Last Update	6/11/2020 8:34:00 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	6/10/2020 12:30:27 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	2278680	∞	11.7	∞	1223760	100.615 ng/ml
THC-COOH	1.415	2128815	14260.5	39.7	4084.8	718505	254.770 ng/ml
THC	3.153	735859	∞	26.1	∞	742785	101.393 ng/ml