REVIEWED
By Celena Shrum at 7:24 am, Nov 03, 2023

11/2/2023

Worklist: 6552

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
C2023-2265	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-2284	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2023-2301	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2023-2316	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-2337	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-2344	1	BLOOD	AM 27 Blood THC Quant by LC-QQQ
C2023-2381	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-2414	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-2429	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-2430	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-2432	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ

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

Extraction Date 11/1/23 Analyst: Anne Nord
Plate lot#: 230627 Plate re-test: 12/27/2023

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: 23J52629 **Urine Blank:** 8423 **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 (calibrated pipette) blood or 1000µL hydrolyzed urine Pipette ID: I41142J** 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. SN > 10
☒ 4. Case sample response for THC 1ng/ml LOD 3ng/ml LOQ, OH-THC 3ng/mL LOD and LOQ, Carboxy-THC: 5 ng/mL (qualitative only). 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. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:
The samples were injected on 11/1/23, there was an interfering peak that was co-eluting in THC-COOH and THC-OH. The acquisition method was adjusted and the batch was reinjected on 11/1/23, the blood QC failed to inject so the batch could not be evaluated. 11/2/23 I reconstituted all of the samples and the batch was re-injected. That injection batch was evaluated.

	1	2	3	4	5	6
a	cal 1	Internal control urine	2429-2			
b	cal 2	negative blood	2430-1			
c	cal 3	2265-1	negative urine			
d	cal 4	2316-1	2284-1			
e	cal 5	2337-1	2301-1			
f	cal 6	2344-1	2432-1			
g	cal 7	2381-1				
h	Internal control (blood)	2414-1				

Plate position 3

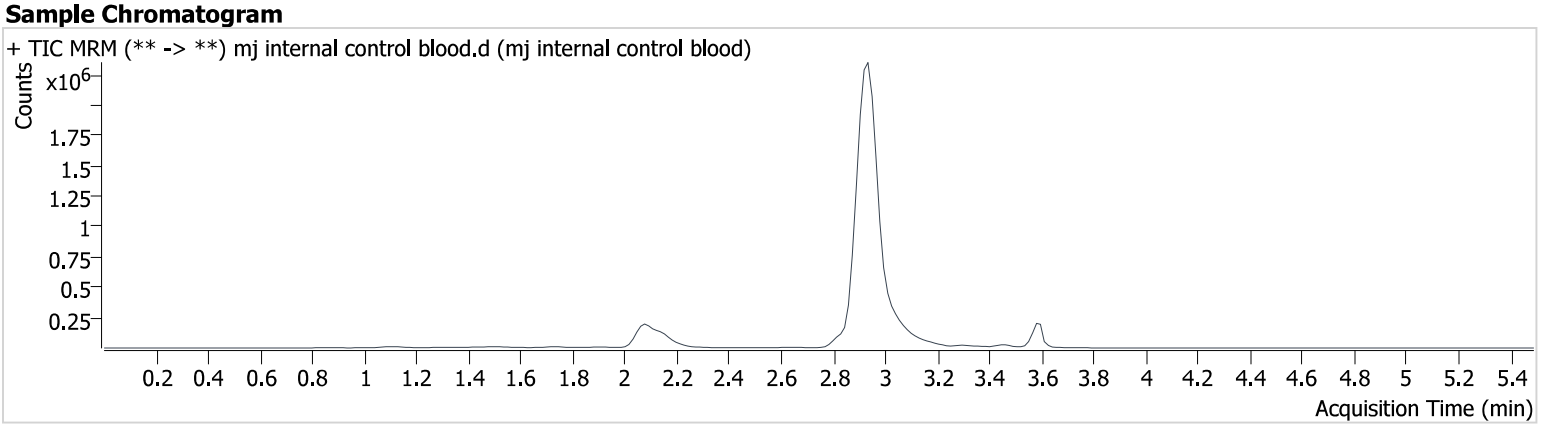
c2023-____-_

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj internal control blood.d
Type	QC	**Sample**	mj internal control blood
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 10:10:08 AM		
Sample Info.			

Sample Chromatogram



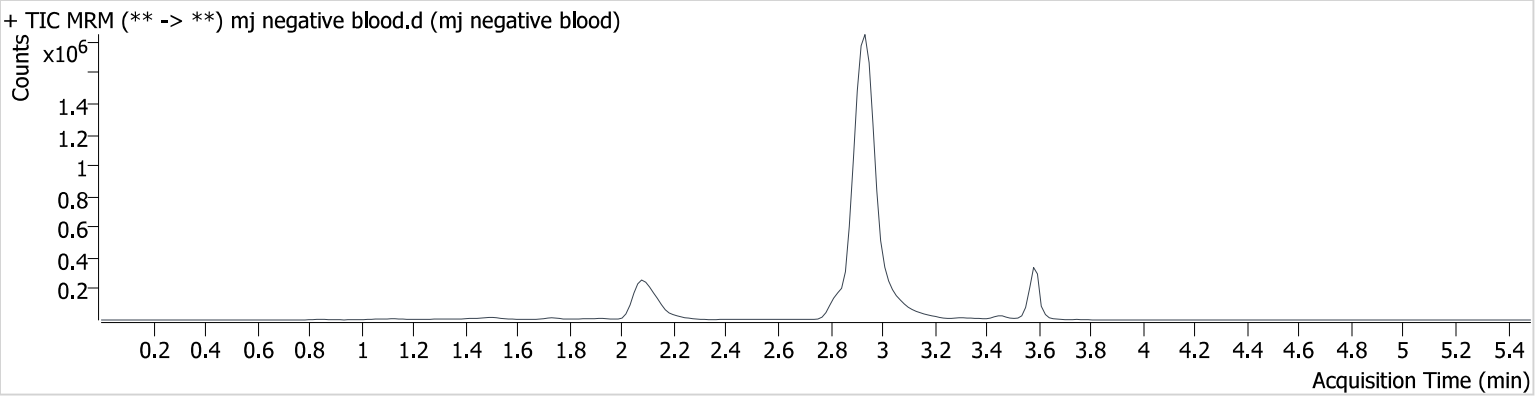
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.081	9445	566.0	903.97	∞	702623	4.264 ng/ml
THC-COOH	2.152	23306	18712.8	268.87	96.4	317198	13.929 ng/ml
THC	3.588	33146	4067.5	24.51	∞	273229	4.043 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj negative blood.d
Type	Sample	**Sample**	mj negative blood
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-B2	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 10:16:42 AM		
Sample Info.			

Sample Chromatogram

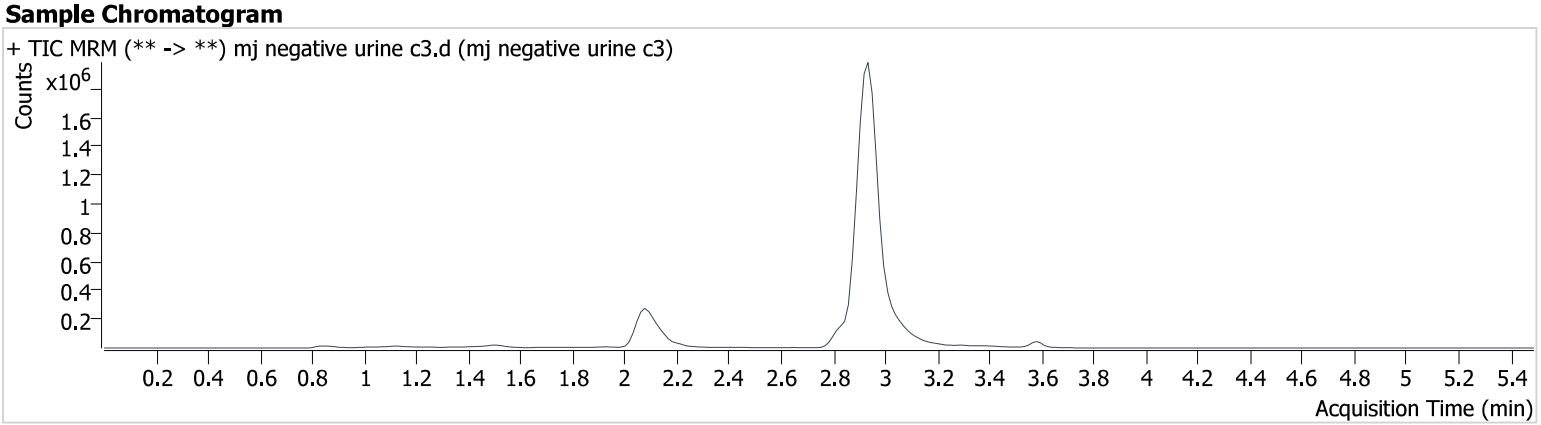


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj negative urine c3.d
Type	Sample	**Sample**	mj negative urine c3
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-C3	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 12:08:48 PM		
Sample Info.			

Sample Chromatogram

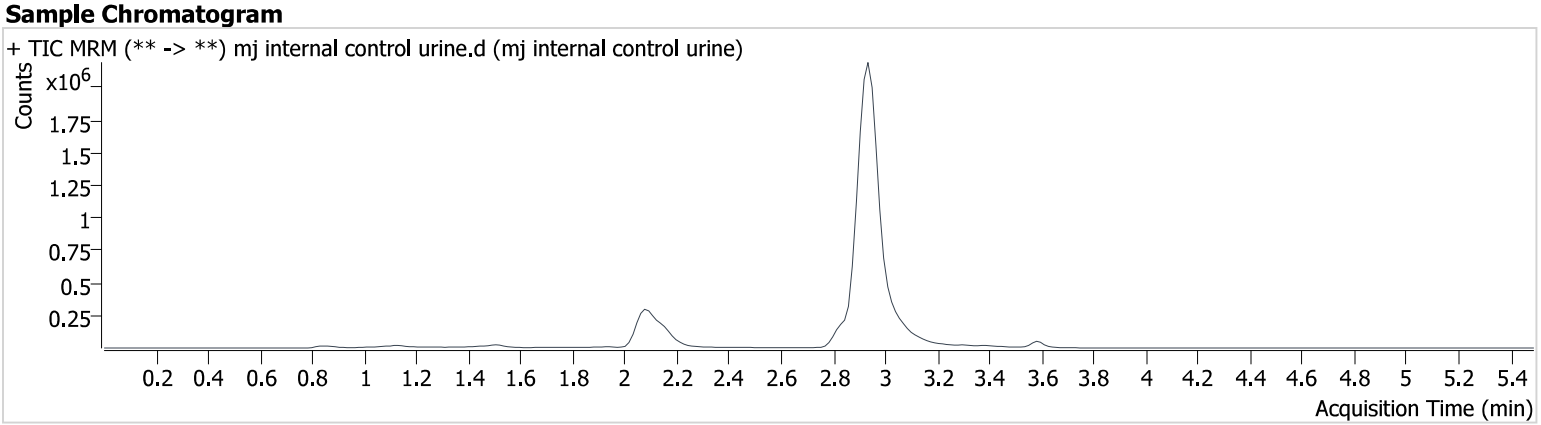


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj internal control urine.d
Type	Sample	**Sample**	mj internal control urine
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-A2	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 1:21:26 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.081	16382	∞	787.27	470.9	1139812	4.540 ng/ml
THC-COOH	2.152	31306	239.8	278.35	149.4	425598	13.943 ng/ml
THC	3.588	16420	∞	28.99	∞	138355	3.964 ng/ml

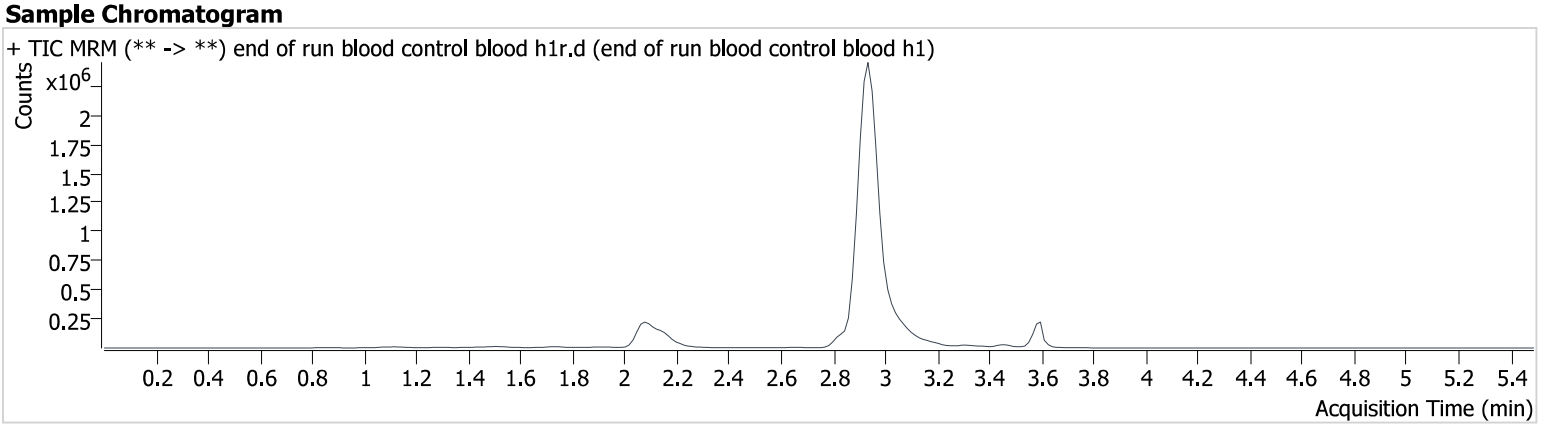
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	end of run blood control blood h1r.d
Type	Sample	**Sample**	end of run blood control blood h1
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2023 1:28:02 PM		
Sample Info.			

Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

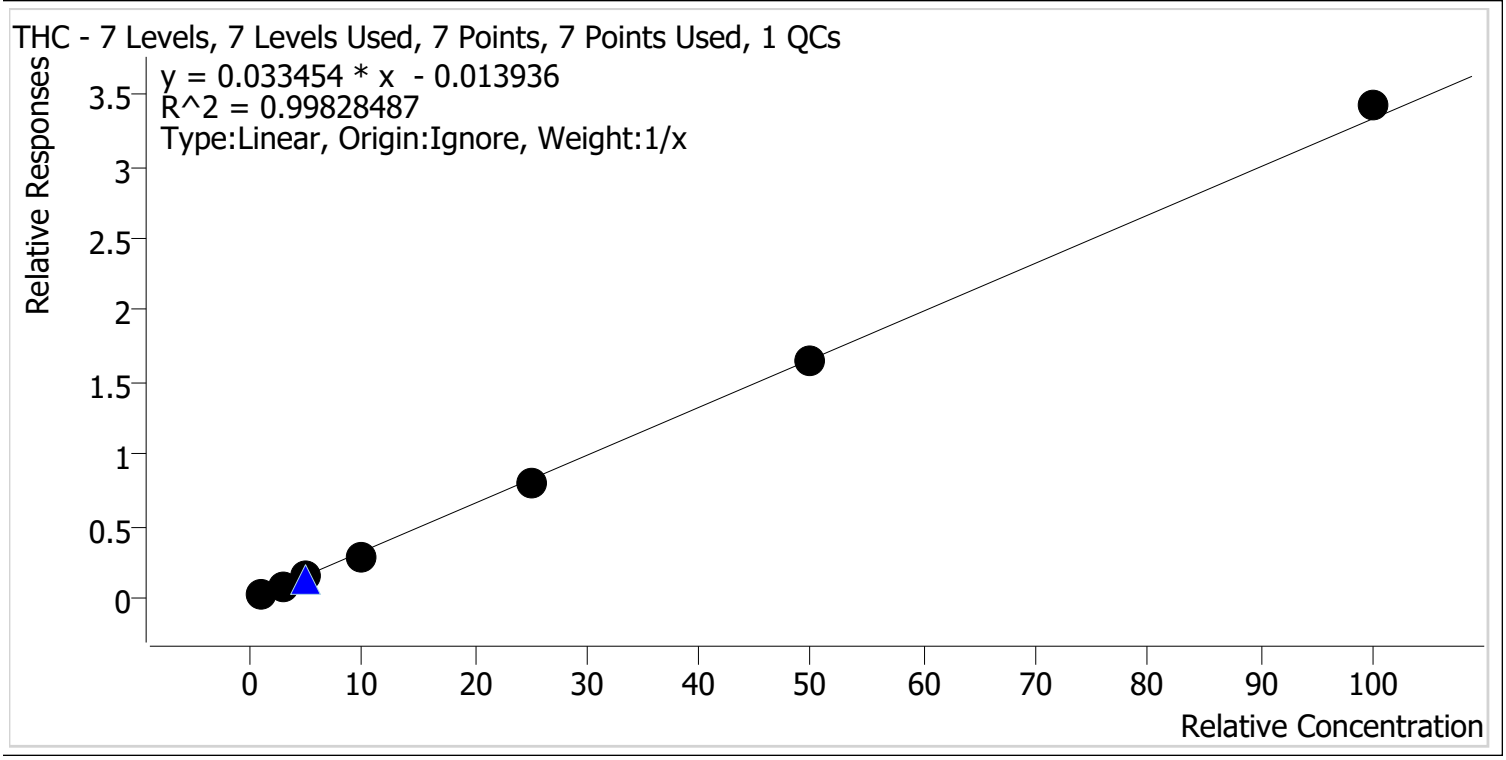
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.081	10858	239.8	763.97	∞	795106	4.328 ng/ml
THC-COOH	2.152	25890	216.6	267.63	187.6	352454	13.926 ng/ml
THC	3.603	36355	∞	24.27	∞	299291	4.048 ng/ml

Compound Calibration Report

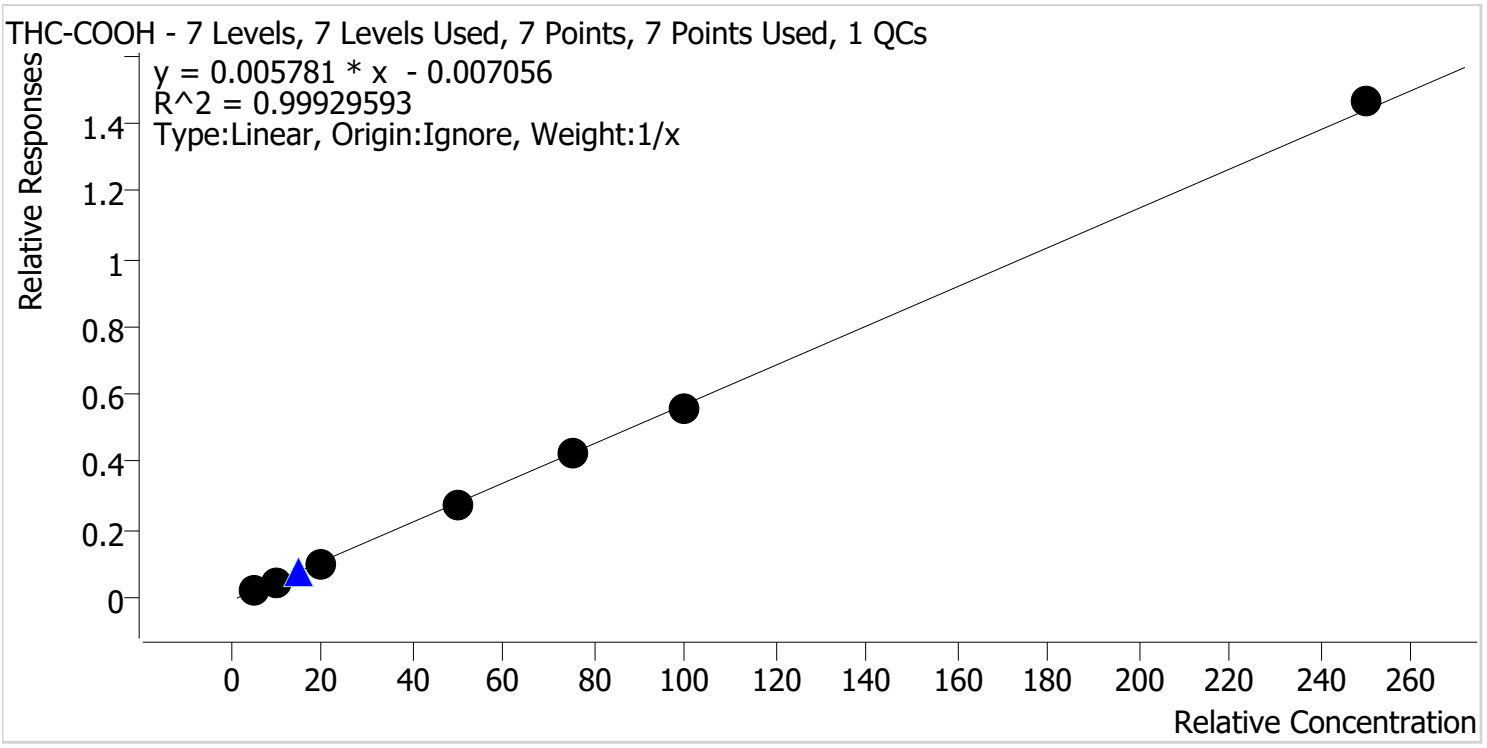
Batch results	D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Last Cal. Update	11/2/2023 1:34 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-d3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.2	123.8
mj cal 2	2	✔	3.0	2.8	93.5
mj cal 3	3	✔	5.0	4.7	93.5
mj cal 4	4	✔	10.0	9.2	91.9
mj cal 5	5	✔	25.0	23.9	95.5
mj cal 6	6	✔	50.0	49.5	99.0
mj cal 7	7	✔	100.0	102.7	102.7

Compound Calibration Report

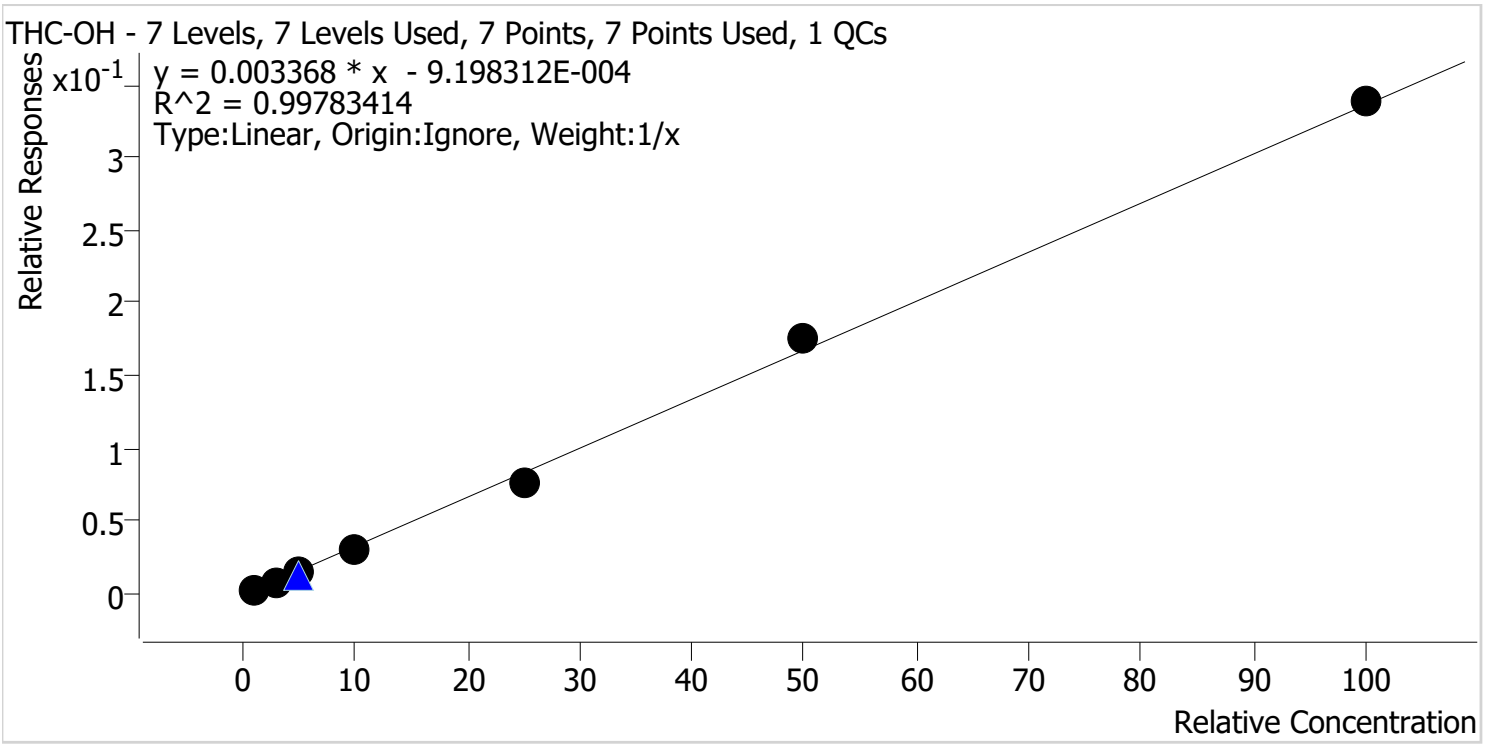
Batch results	D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Last Cal. Update	11/2/2023 1:34 PM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-d9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	5.0	5.5	110.0
mj cal 2	2	✔	10.0	9.8	98.3
mj cal 3	3	✔	20.0	18.9	94.6
mj cal 4	4	✔	50.0	49.0	98.0
mj cal 5	5	✔	75.0	75.0	100.0
mj cal 6	6	✔	100.0	97.4	97.4
mj cal 7	7	✔	250.0	254.3	101.7

Compound Calibration Report

Batch results	D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Last Cal. Update	11/2/2023 1:34 PM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-d3



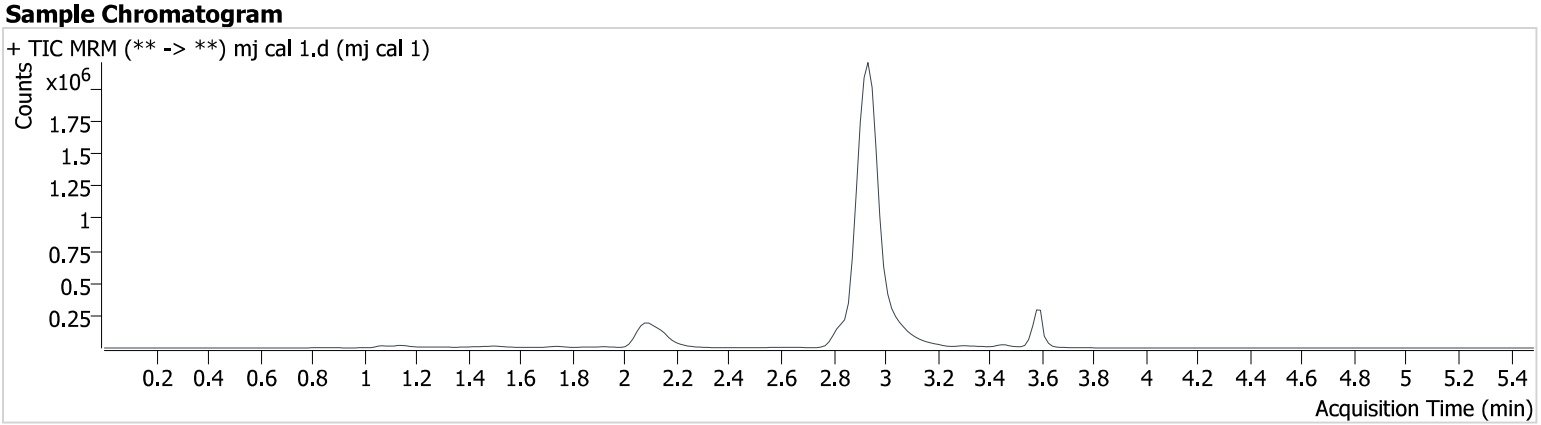
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✔	1.0	1.1	114.7
mj cal 2	2	✔	3.0	2.9	96.7
mj cal 3	3	✔	5.0	4.9	98.9
mj cal 4	4	✔	10.0	9.3	93.4
mj cal 5	5	✔	25.0	22.8	91.3
mj cal 6	6	✔	50.0	52.2	104.4
mj cal 7	7	✔	100.0	100.7	100.7

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj cal 1.d
Type	Cal	**Sample**	mj cal 1
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-A1	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 9:17:20 AM		
Sample Info.			

Sample Chromatogram



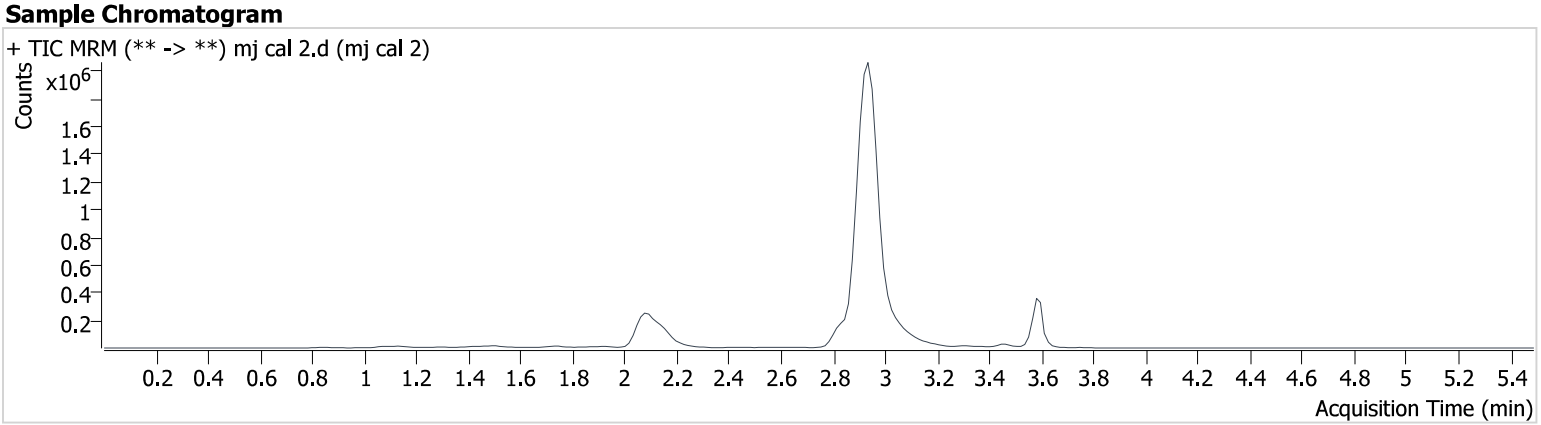
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.081	2393	55.0	701.82	245.2	812723	1.147 ng/ml	Low
THC-COOH	2.152	9984	90.3	264.73	48717.5	403464	5.500 ng/ml	
THC	3.588	13005	5791.0	28.18	∞	473315	1.238 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj cal 2.d
Type	Cal	**Sample**	mj cal 2
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-B1	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 9:24:03 AM		
Sample Info.			

Sample Chromatogram



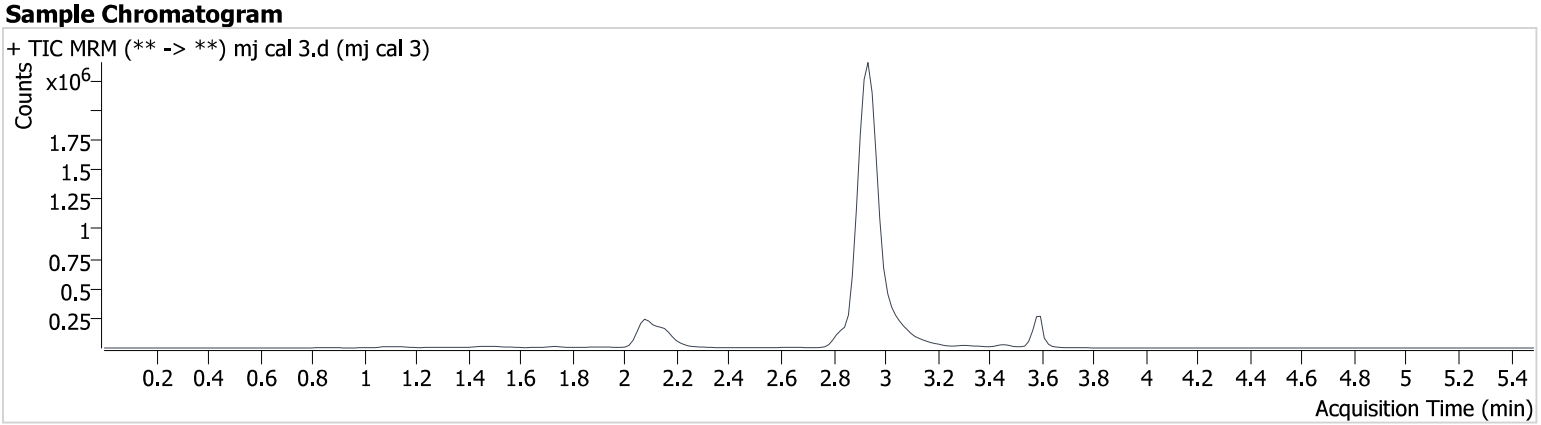
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.081	8686	∞	837.77	379.4	981259	2.901 ng/ml	Low
THC-COOH	2.152	21809	328.9	263.32	77863.9	438252	9.828 ng/ml	
THC	3.588	43867	2861.2	26.65	345.3	548681	2.806 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj cal 3.d
Type	Cal	**Sample**	mj cal 3
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-C1	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 9:30:40 AM		
Sample Info.			

Sample Chromatogram



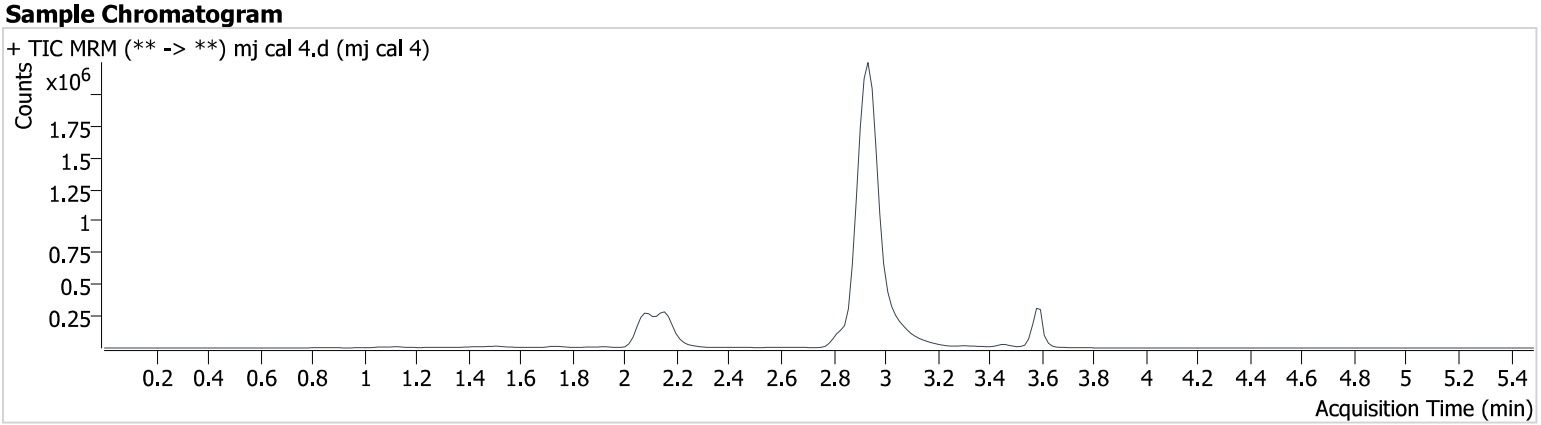
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.081	13260	∞	724.48	∞	842713	4.945 ng/ml
THC-COOH	2.152	38493	123.8	265.90	232.7	376365	18.911 ng/ml
THC	3.603	52721	2348.4	25.05	930.2	369867	4.677 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj cal 4.d
Type	Cal	**Sample**	mj cal 4
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-D1	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 9:37:16 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.081	27255	∞	881.74	∞	892743	9.337 ng/ml
THC-COOH	2.152	106208	1216.7	265.85	1094.3	384547	48.992 ng/ml
THC	3.588	119138	1623.4	25.41	∞	406046	9.187 ng/ml

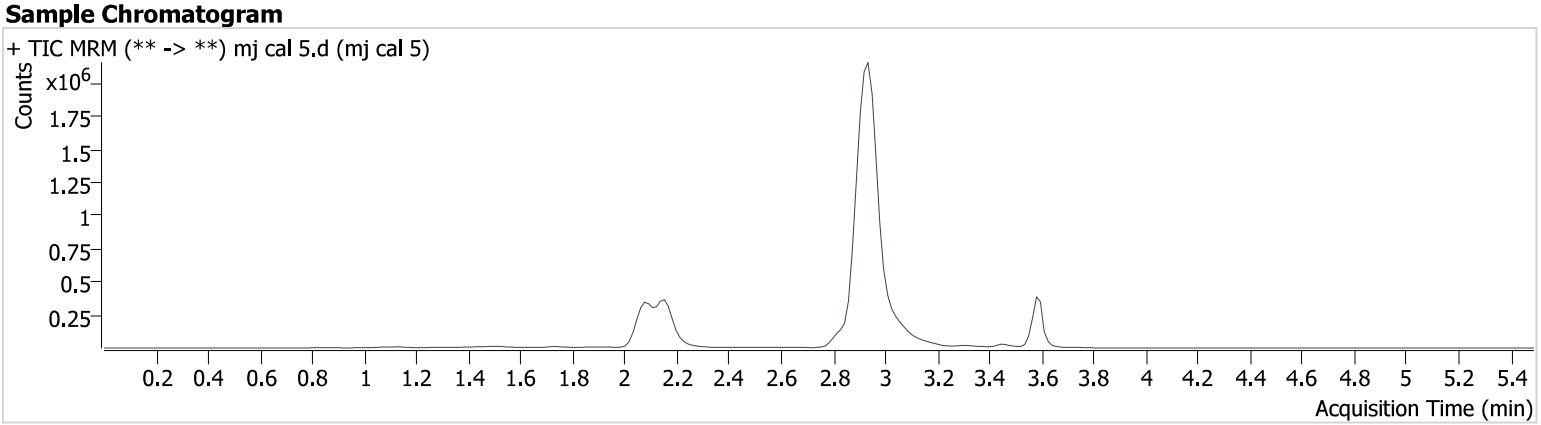
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj cal 5.d
Type	Cal	**Sample**	mj cal 5
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2023 9:43:51 AM		
Sample Info.			

Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.081	63515	∞	934.87	∞	836212	22.823 ng/ml
THC-COOH	2.152	154722	186620.7	258.64	1344.0	362747	74.996 ng/ml
THC	3.588	301223	∞	23.35	2959.7	383652	23.886 ng/ml

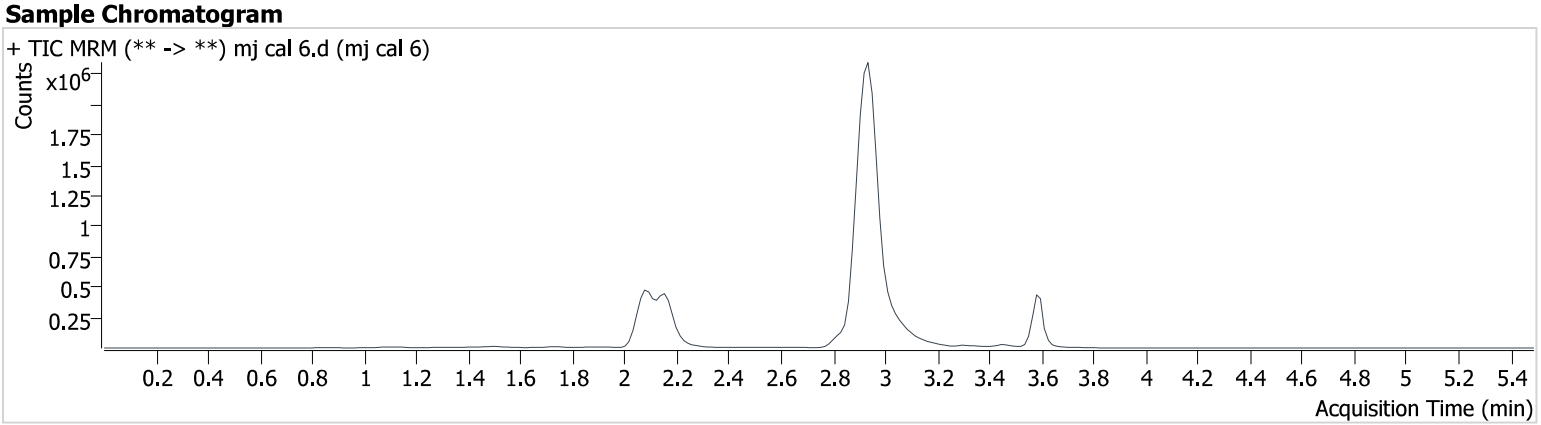
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj cal 6.d
Type	Cal	**Sample**	mj cal 6
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	11/2/2023 9:57:00 AM		
Sample Info.			

Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

Sample Chromatogram



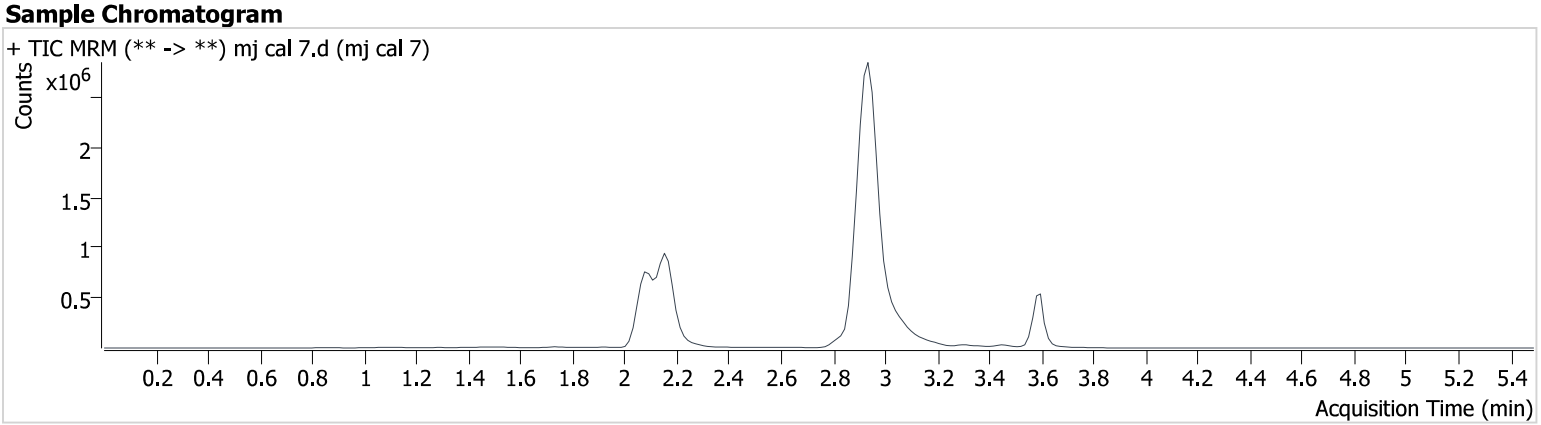
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.081	139421	∞	838.02	∞	797389	52.183 ng/ml
THC-COOH	2.152	195088	15391.3	260.90	438.7	350662	97.450 ng/ml
THC	3.588	531469	∞	24.11	∞	323668	49.499 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\110123r\QuantResults\am27.batch.bin
Calibration Last Update 11/2/2023 1:34:49 PM

Instrument	69679	**Data File**	mj cal 7.d
Type	Cal	**Sample**	mj cal 7
Acq. Method	thc quant 50 50.m	**Operator**	Anne Nord
Sample Position	P3-G1	**Comment**	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
Injection Volume	10		
Acq. Date-Time	11/2/2023 10:03:34 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.081	269250	9169.1	870.02	∞	796255	100.664 ng/ml
THC-COOH	2.152	473010	526153.5	261.52	82303.2	323251	254.323 ng/ml
THC	3.603	902698	32883.1	23.98	∞	263791	102.706 ng/ml