










Worklist: 6478

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>	
C2023-1735	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2023-1739	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-1766	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-1767	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-1874	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2023-1890	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2023-1904	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 8/23/23
Plate lot#: 230627

Analyst: Anne Nord
Plate re-test: 12/27/2023

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 23C57106 **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: 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. 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:

End of the run blood control did not inject. Sample was reinjected the same day.

	1	2	3	4	5	6
a	cal 1	Internal control urine	1735-1			
b	cal 2	negative blood	1890-1			
c	cal 3	1739-1				
d	cal 4	1766-1				
e	cal 5	1767-1				
f	cal 6	1874-1				
g	cal 7	1904-1				
h	Internal control (blood)	negative urine				

Plate position 3

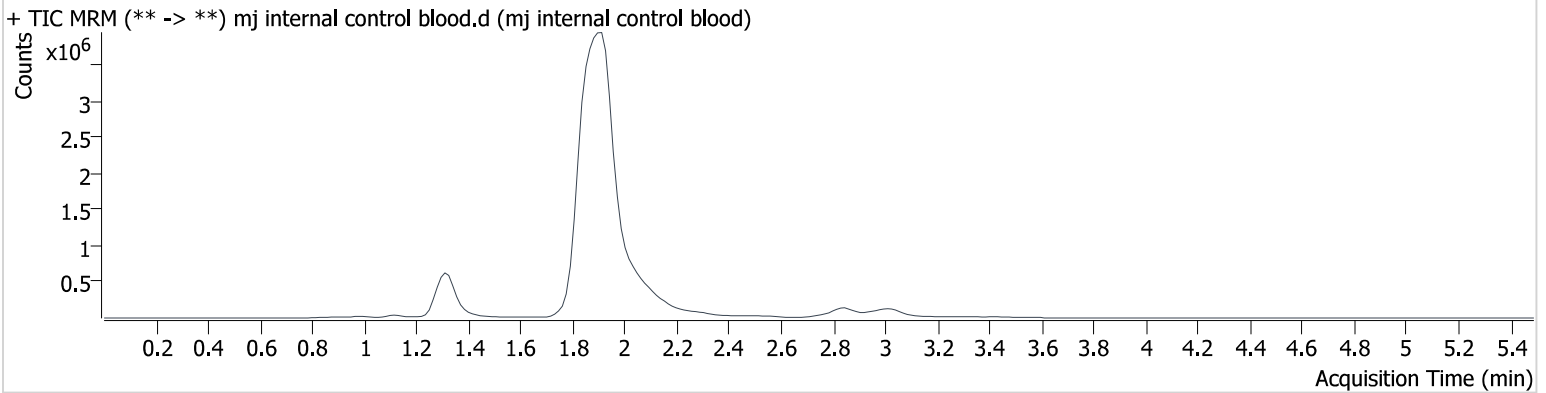
c2023-____-__

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument	69679	Data File	mj internal control blood.d
Type	QC	Sample	mj internal control blood
Acq. Method	AM 27 THC quant.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	8/23/2023 1:07:01 PM		
Sample Info.			

Sample Chromatogram



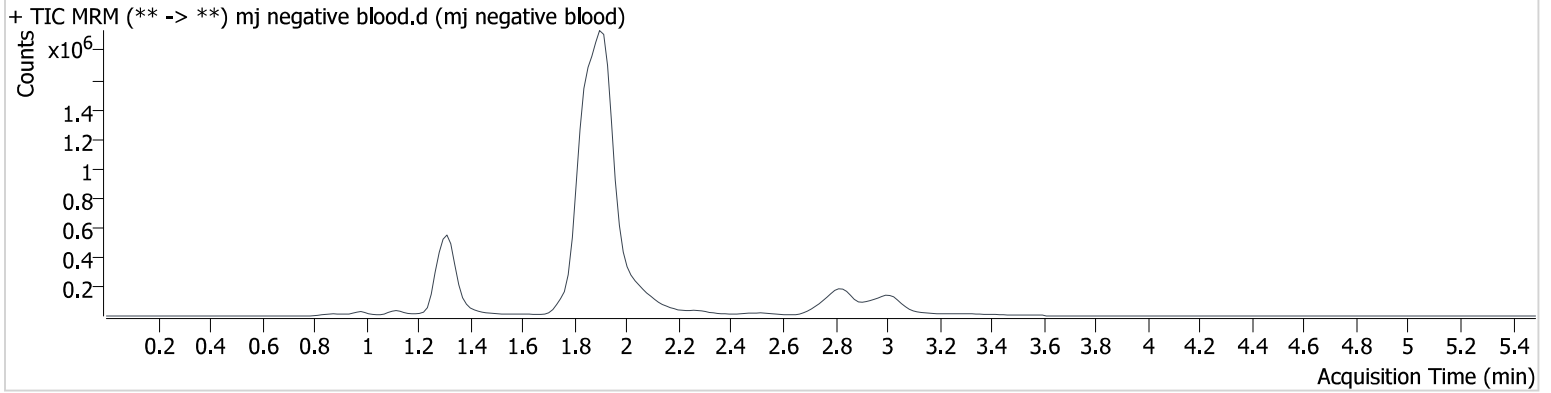
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	27405	∞	839.92	∞	1962940	4.647 ng/ml
THC-COOH	1.327	31131	∞	275.77	∞	473369	14.033 ng/ml
THC	2.854	16074	444.3	317.91	∞	460787	4.900 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

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-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	8/23/2023 1:13:35 PM		
Sample Info.			

Sample Chromatogram

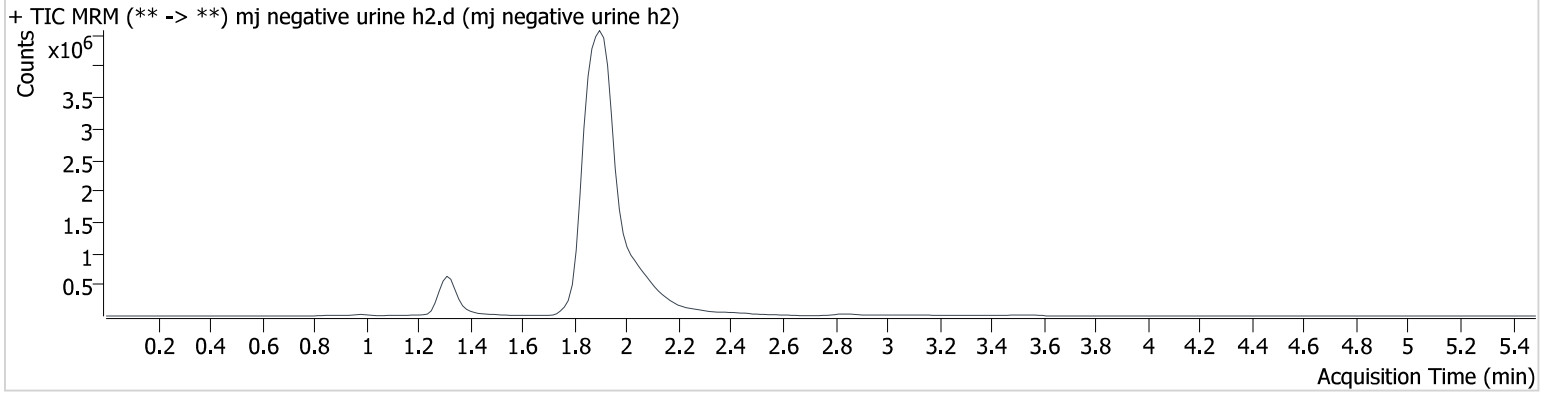


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument	69679	Data File	mj negative urine h2.d
Type	Sample	Sample	mj negative urine h2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H2	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	8/23/2023 2:26:10 PM		
Sample Info.			

Sample Chromatogram

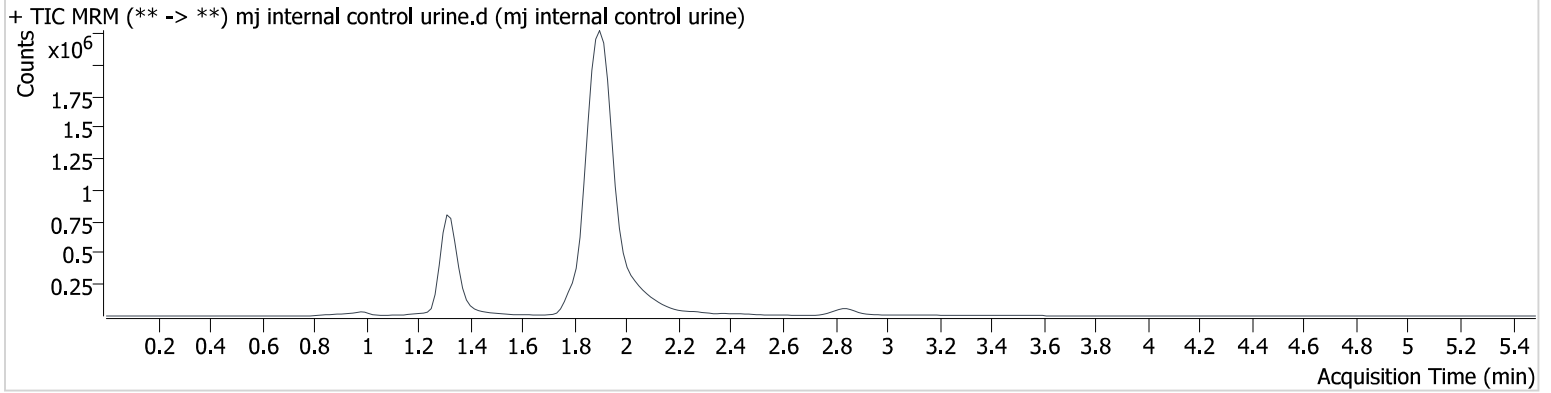


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument	69679	Data File	mj internal control urine.d
Type	Sample	Sample	mj internal control urine
Acq. Method	AM 27 THC quant.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	8/23/2023 2:59:08 PM		
Sample Info.			

Sample Chromatogram



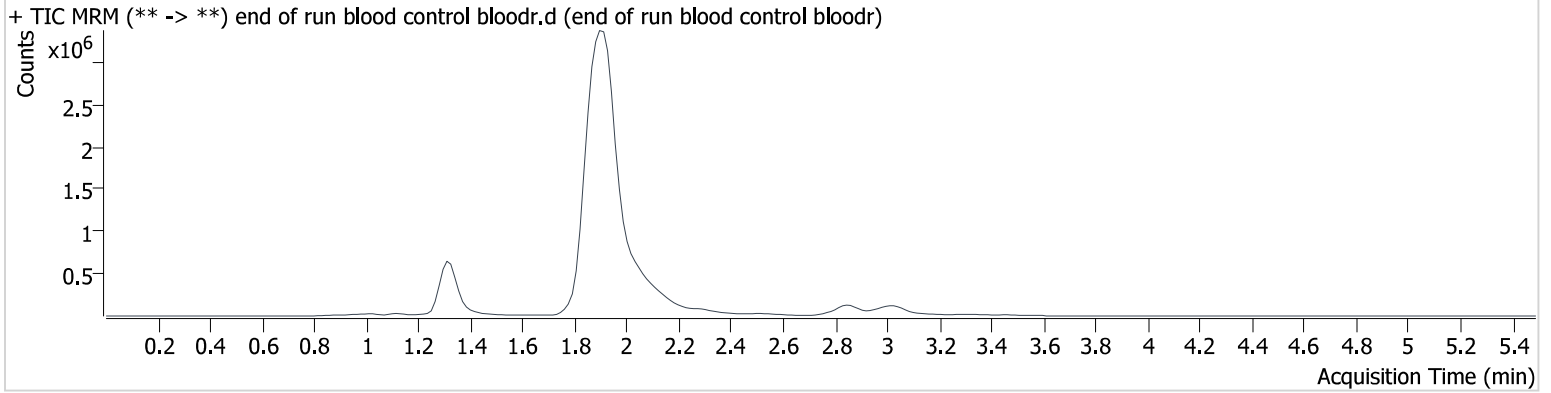
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	31026	709.7	804.95	∞	2375850	4.367 ng/ml
THC-COOH	1.342	42424	352.4	286.73	∞	629576	14.356 ng/ml
THC	2.839	8147	94.2	444.31	323.8	300080	3.841 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument	69679	Data File	end of run blood control bloodr.d
Type	Sample	Sample	end of run blood control bloodr
Acq. Method	AM 27 THC quant.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	8/23/2023 4:37:24 PM		
Sample Info.			

Sample Chromatogram



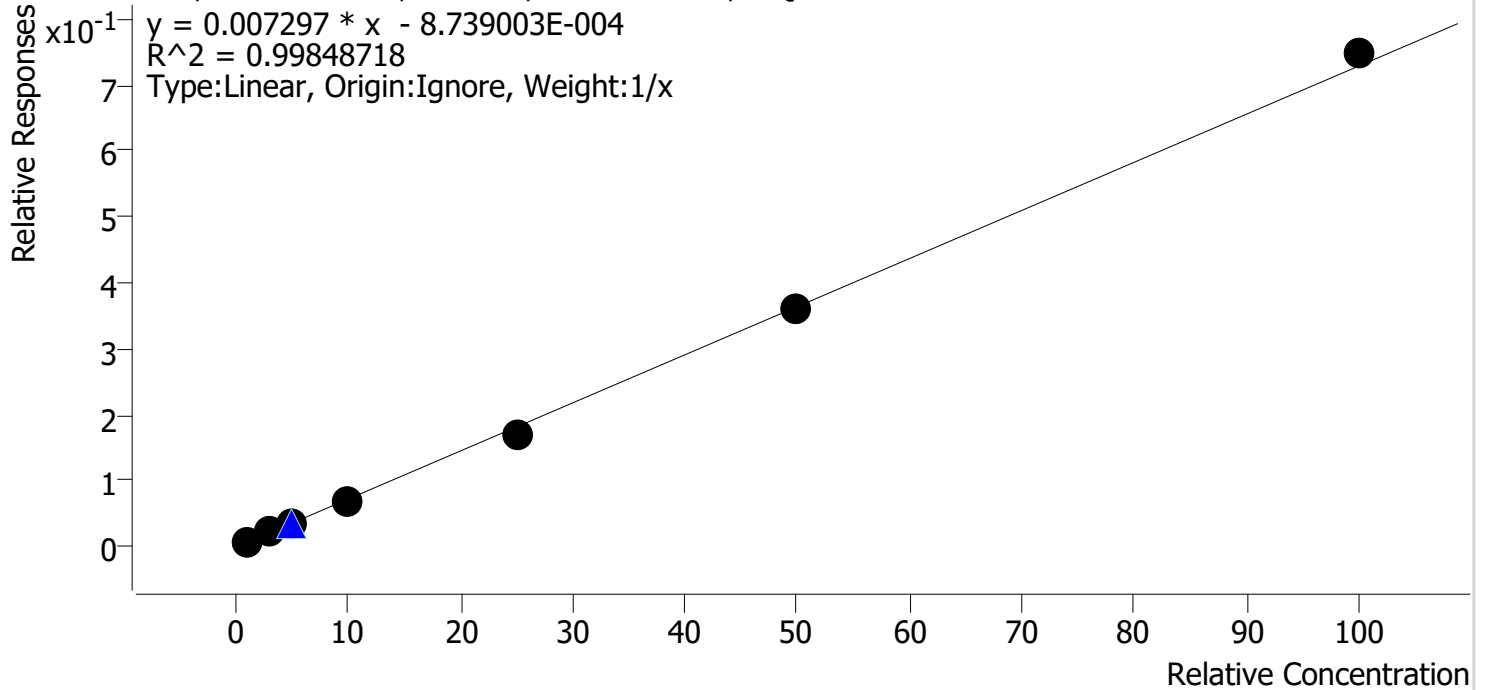
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	28426	∞	869.23	∞	2020621	4.680 ng/ml
THC-COOH	1.342	22998	∞	293.63	∞	437615	11.400 ng/ml
THC	2.869	14410	157.8	325.77	25290.9	427188	4.743 ng/ml

Compound Calibration Report



Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Last Cal. Update 8/23/2023 4:48 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.1	114.8
mj cal 2	2	✓	3.0	3.0	100.4
mj cal 3	3	✓	5.0	4.8	96.5
mj cal 4	4	✓	10.0	9.2	92.5
mj cal 5	5	✓	25.0	23.4	93.7
mj cal 6	6	✓	50.0	49.8	99.6
mj cal 7	7	✓	100.0	102.5	102.5

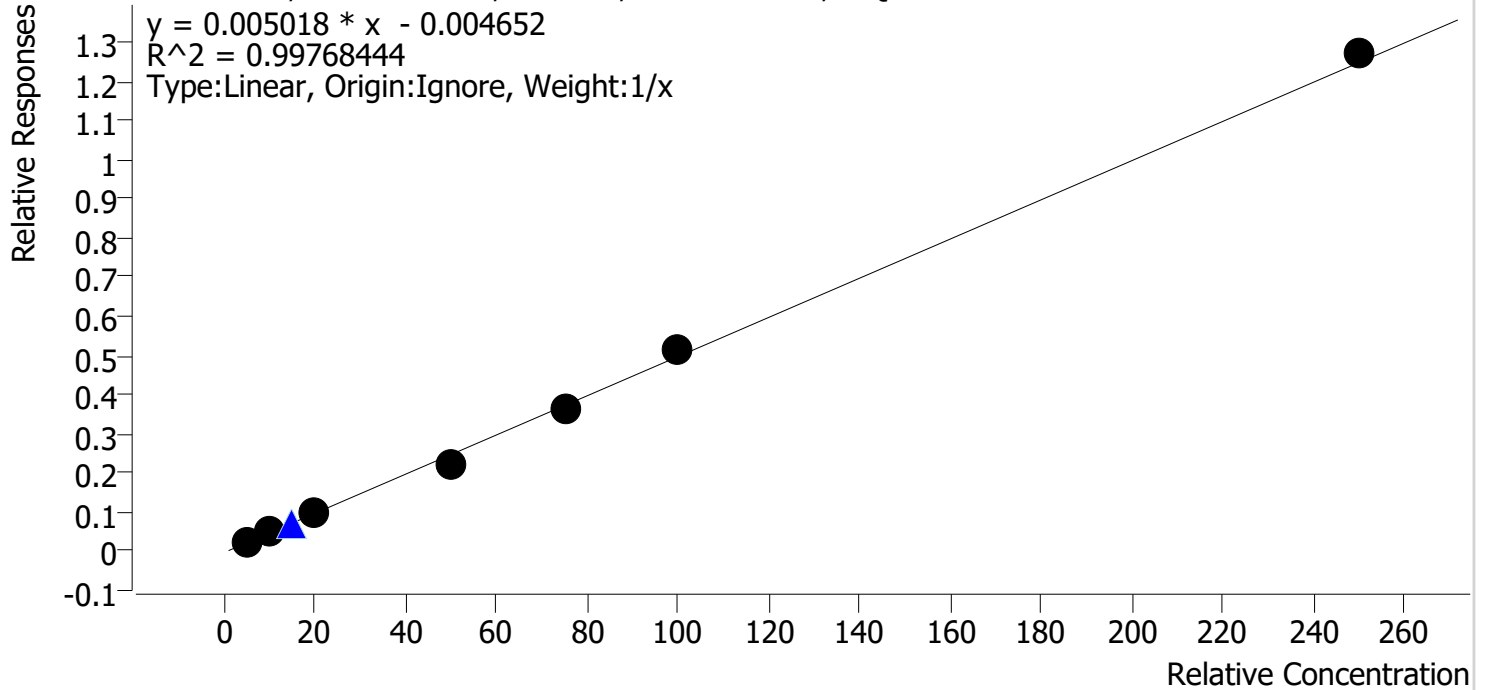
Compound Calibration Report



Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Last Cal. Update 8/23/2023 4:48 PM
Analyst Name ISP\datastor
Analyte THC-COOH

Internal Standard THC-COOH-d9

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



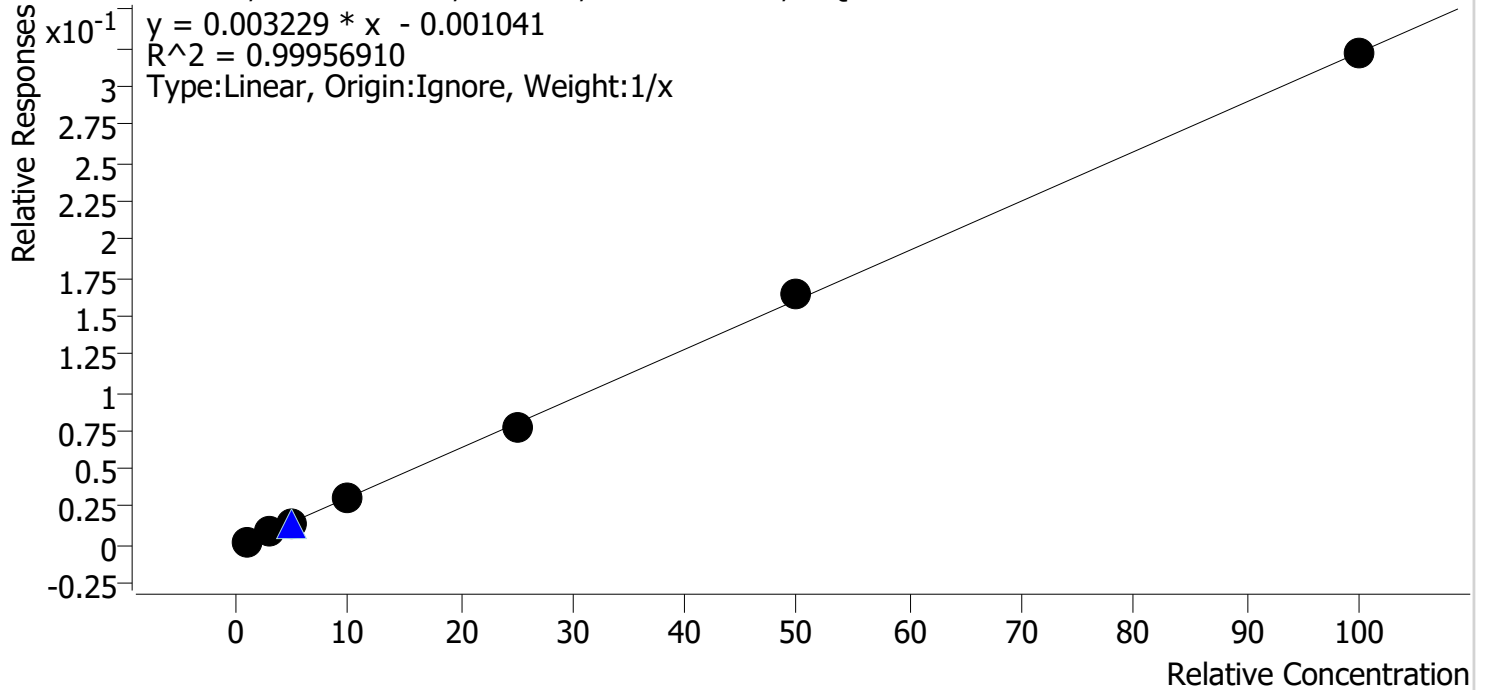
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.5	109.4
mj cal 2	2	✓	10.0	10.2	101.8
mj cal 3	3	✓	20.0	19.6	98.0
mj cal 4	4	✓	50.0	44.5	89.0
mj cal 5	5	✓	75.0	72.8	97.0
mj cal 6	6	✓	100.0	102.9	102.9
mj cal 7	7	✓	250.0	254.6	101.8

Compound Calibration Report



Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Last Cal. Update 8/23/2023 4:48 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



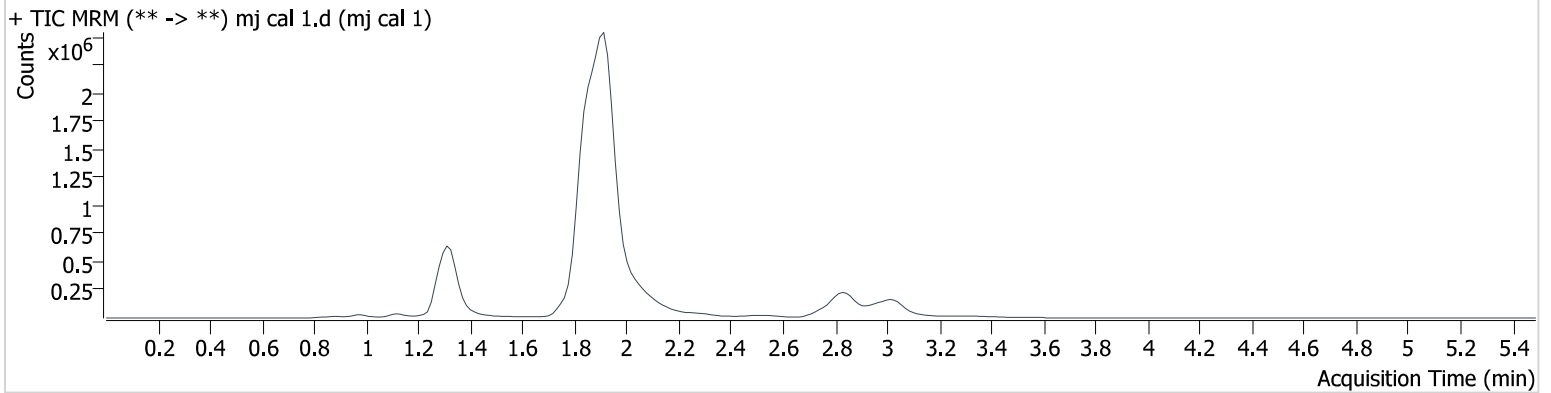
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.1	106.5
mj cal 2	2	✓	3.0	3.0	101.1
mj cal 3	3	✓	5.0	4.8	96.3
mj cal 4	4	✓	10.0	9.6	95.9
mj cal 5	5	✓	25.0	24.5	97.9
mj cal 6	6	✓	50.0	51.3	102.5
mj cal 7	7	✓	100.0	99.8	99.8

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument	69679	Data File	mj cal 1.d
Type	Cal	Sample	mj cal 1
Acq. Method	AM 27 THC quant.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	8/23/2023 12:20:51 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.319	5705	∞	1009.9 6	∞	2380165	1.065 ng/ml	Low
THC-COOH	1.342	13750	149.4	287.27	∞	602853	5.472 ng/ml	
THC	2.854	6624	53.7	343.34	∞	882912	1.148 ng/ml	

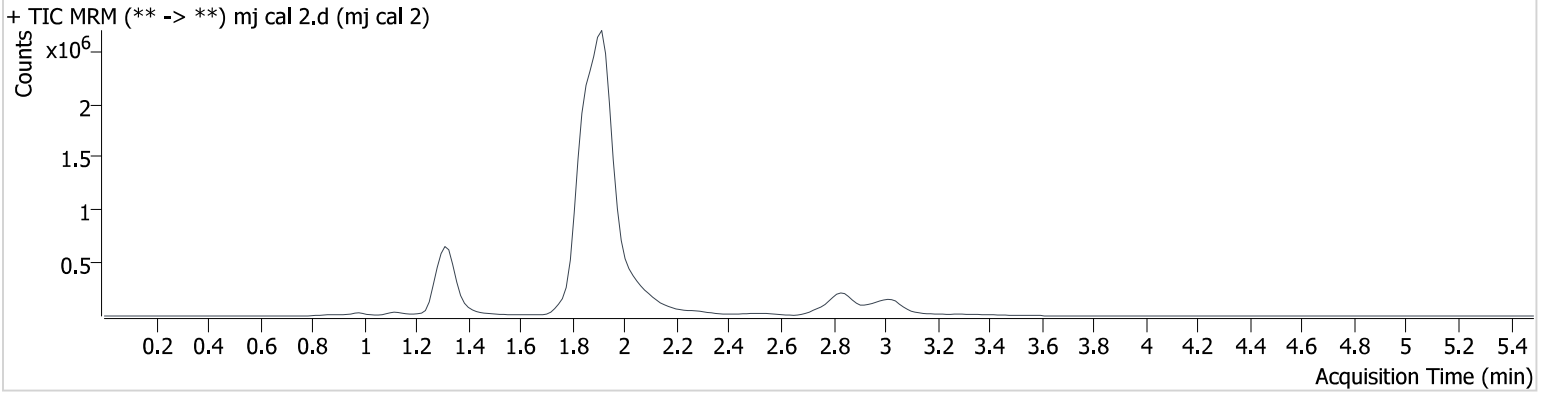
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-B1
Injection Volume 10
Acq. Date-Time 8/23/2023 12:27:35 PM
Sample Info.

Data File mj cal 2.d
Sample mj cal 2
Operator Anne Nord
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

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	20019	∞	830.31	∞	2287699	3.033 ng/ml
THC-COOH	1.342	26039	437.0	276.03	∞	560691	10.182 ng/ml
THC	2.854	16839	357.0	337.43	514.2	797758	3.012 ng/ml

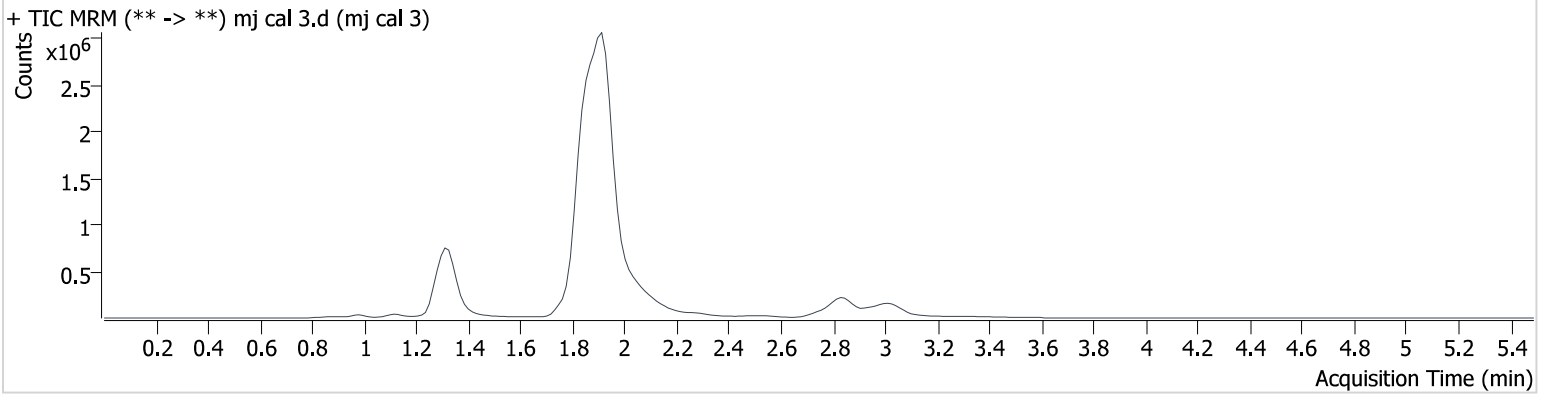
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-C1
Injection Volume 10
Acq. Date-Time 8/23/2023 12:34:09 PM
Sample Info.

Data File mj cal 3.d
Sample mj cal 3
Operator Anne Nord
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

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	35021	720.4	849.63	∞	2414431	4.815 ng/ml
THC-COOH	1.342	56463	1759.1	286.66	∞	602817	19.593 ng/ml
THC	2.854	26838	204.1	380.56	∞	781822	4.824 ng/ml

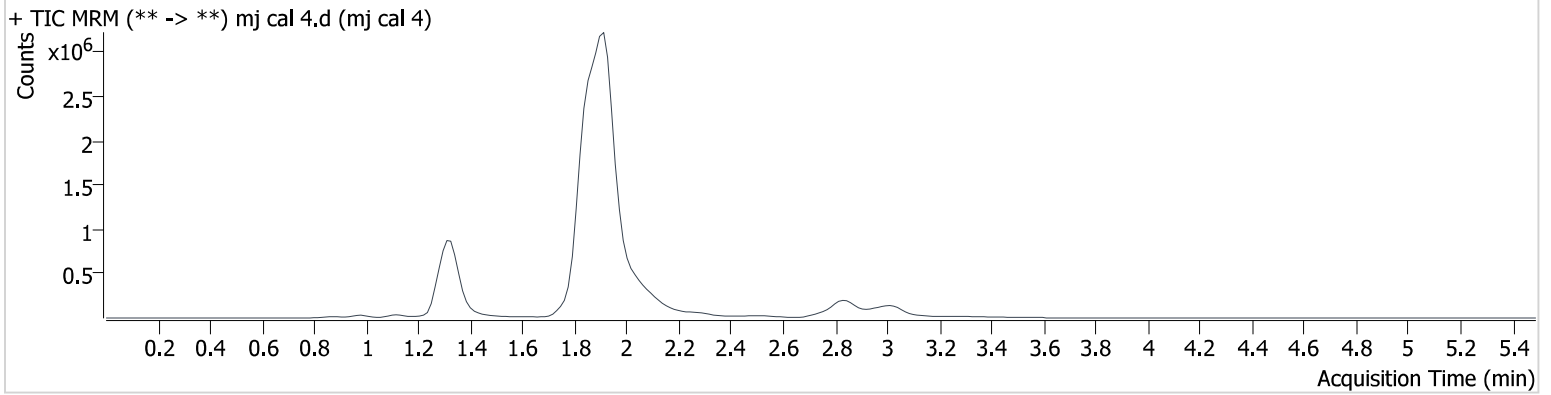
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-D1
Injection Volume 10
Acq. Date-Time 8/23/2023 12:40:43 PM
Sample Info.

Data File mj cal 4.d
Sample mj cal 4
Operator Anne Nord
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

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	67906	13318.6	865.71	∞	2268662	9.594 ng/ml
THC-COOH	1.342	132141	∞	288.97	∞	604348	44.500 ng/ml
THC	2.869	43614	545.3	386.30	3103.5	654631	9.250 ng/ml

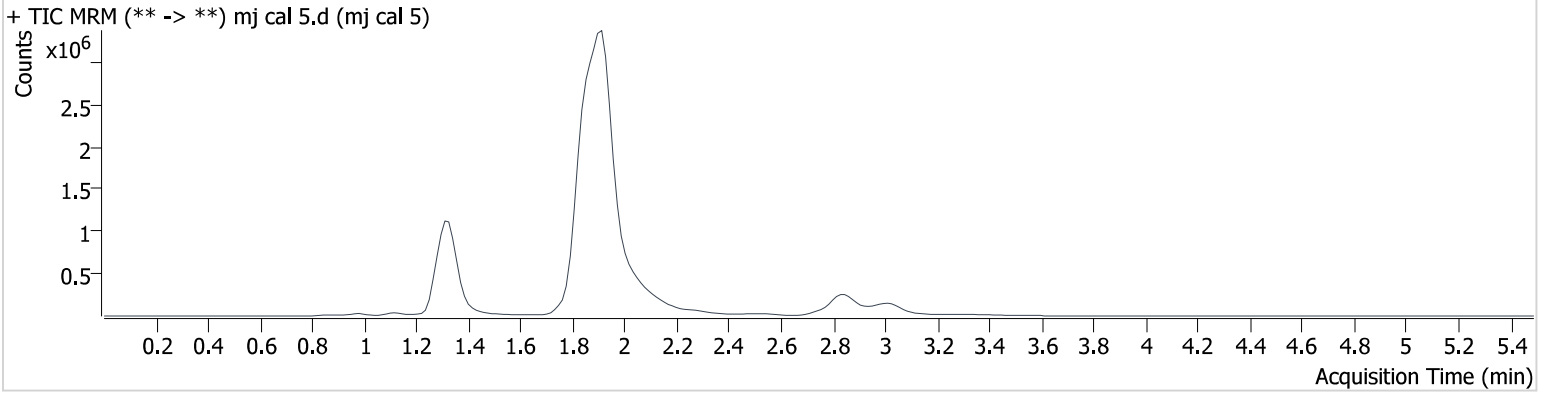
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-E1
Injection Volume 10
Acq. Date-Time 8/23/2023 12:47:19 PM
Sample Info.

Data File mj cal 5.d
Sample mj cal 5
Operator Anne Nord
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

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	171002	∞	856.91	915749 895775 99.8	2193019	24.475 ng/ml
THC-COOH	1.342	191641	1952.8	287.82	∞	531670	72.759 ng/ml
THC	2.854	113356	∞	409.64	4722.2	666790	23.417 ng/ml

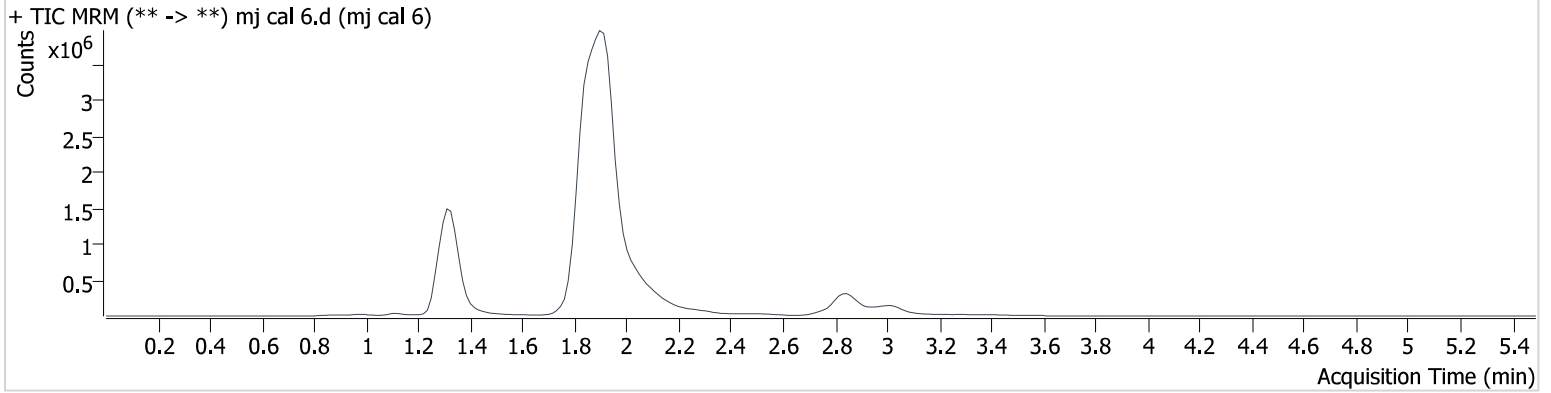
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-F1
Injection Volume 10
Acq. Date-Time 8/23/2023 12:53:53 PM
Sample Info.

Data File mj cal 6.d
Sample mj cal 6
Operator Anne Nord
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

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	343480	∞	839.96	∞	2088668	51.259 ng/ml
THC-COOH	1.342	258431	∞	281.71	∞	504855	102.938 ng/ml
THC	2.854	210664	∞	407.23	∞	581117	49.799 ng/ml

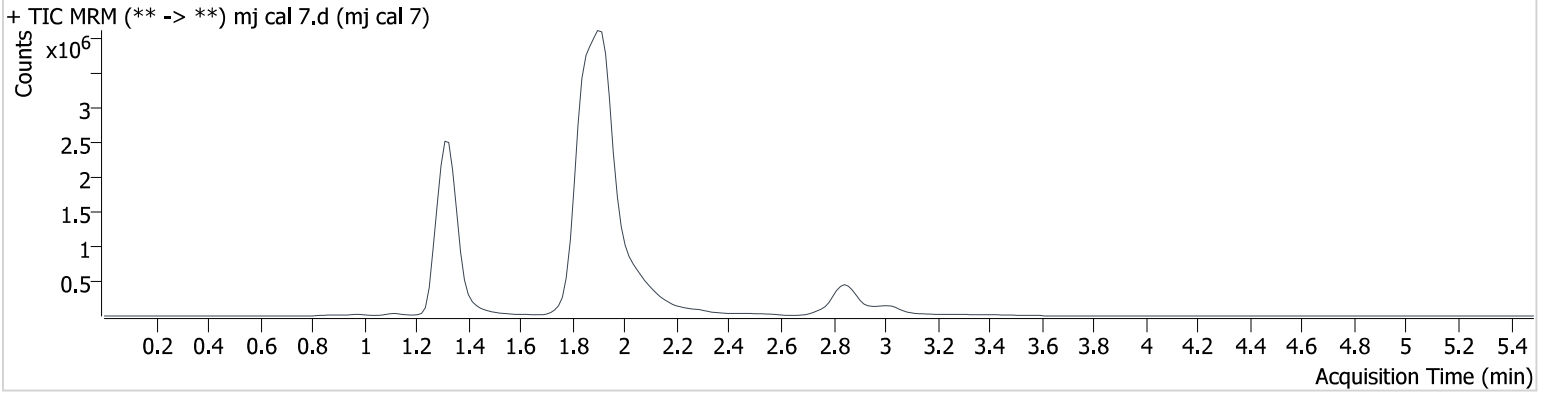
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\082323\QuantResults\cann.batch.bin
Calibration Last Update 8/23/2023 4:48:29 PM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-G1
Injection Volume 10
Acq. Date-Time 8/23/2023 1:00:27 PM
Sample Info.

Data File mj cal 7.d
Sample mj cal 7
Operator Anne Nord
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

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.319	665712	12036.2	876.92	∞	2073649	99.760 ng/ml
THC-COOH	1.327	602989	∞	283.88	∞	473784	254.556 ng/ml
THC	2.854	419313	∞	395.91	∞	561004	102.549 ng/ml