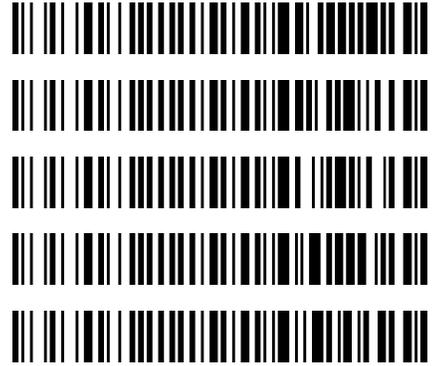


Worklist: 6290

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
C2023-0545	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-0550	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-0619	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-0637	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2023-0642	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 3-24-23
Plate lot#: 230113

Analyst: Anne Nord
Plate re-test: 7/13/23

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

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 22B52016-1 **Urine Blank:** **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: 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: *samples were extracted and injected on 3/24/23, there were interfering peaks in the THC and Carboxy-THC qualifiers. The acquisition parameters were adjusted to provide separation. The samples were reconstituted and re-injected 3/27/23, Those injections were evaluated.*

	1	2	3	4	5	6
a	cal 1	Internal control blood				
b	cal 2	negative blood				
c	cal 3	545-1				
d	cal 4	550-1				
e	Cal 5	619-2				
f	cal 6	637-1				
g	cal 7	642-1				
h	Internal control (blood)					

Plate position 3

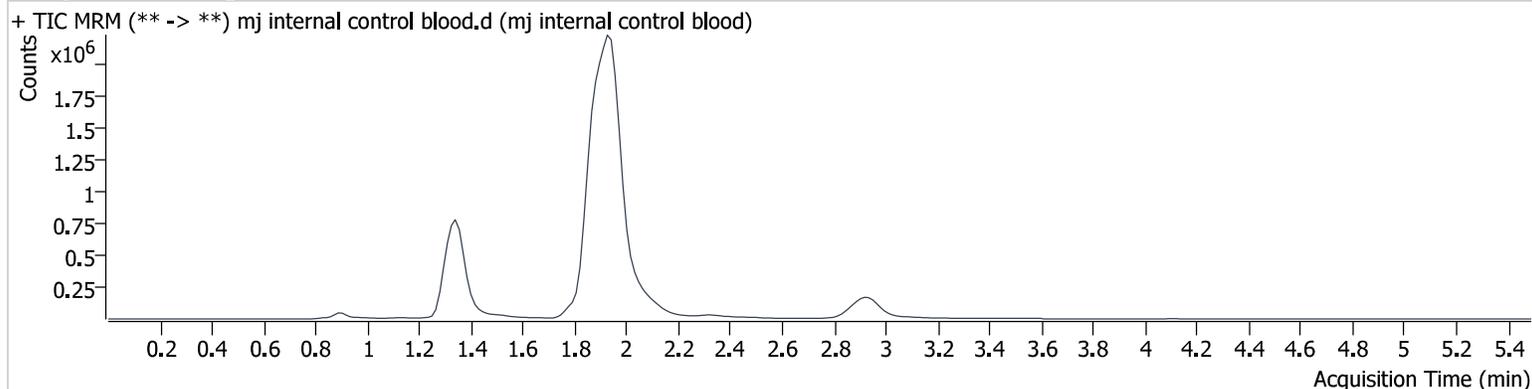
c2023-0 _____

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 AM

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	
Injection Volume	10		
Acq. Date-Time	3/27/2023 10:18:36 AM		
Sample Info.			

Sample Chromatogram



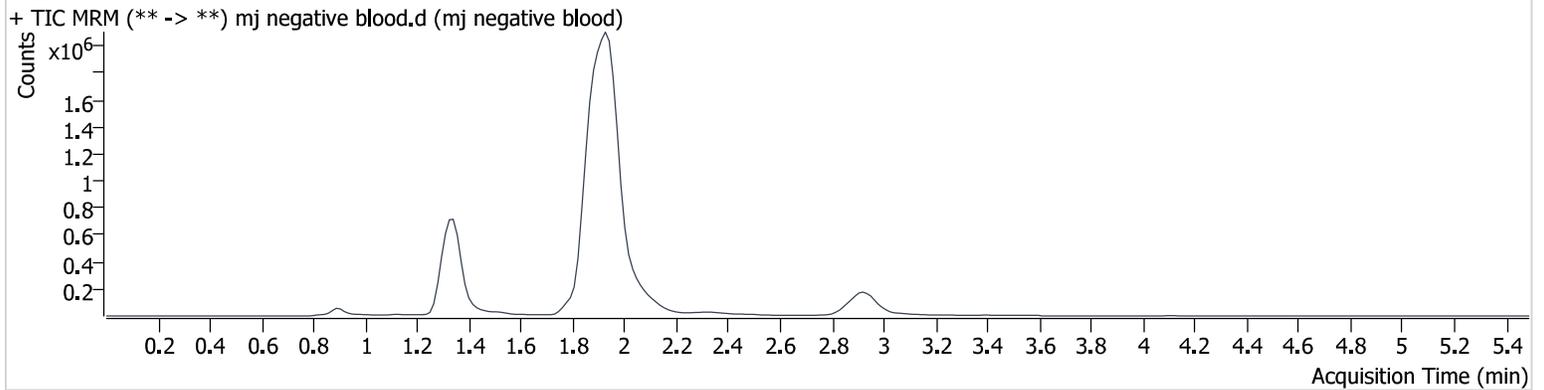
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	49439	∞	912.10	46915.5	2388350	4.370 ng/ml
THC-COOH	1.358	71007	1043.7	248.28	∞	945326	14.655 ng/ml
THC	2.941	147204	1200.8	24.20	213.3	1132349	4.833 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 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-B2	Comment	
Injection Volume	10		
Acq. Date-Time	3/27/2023 10:25:10 AM		
Sample Info.			

Sample Chromatogram

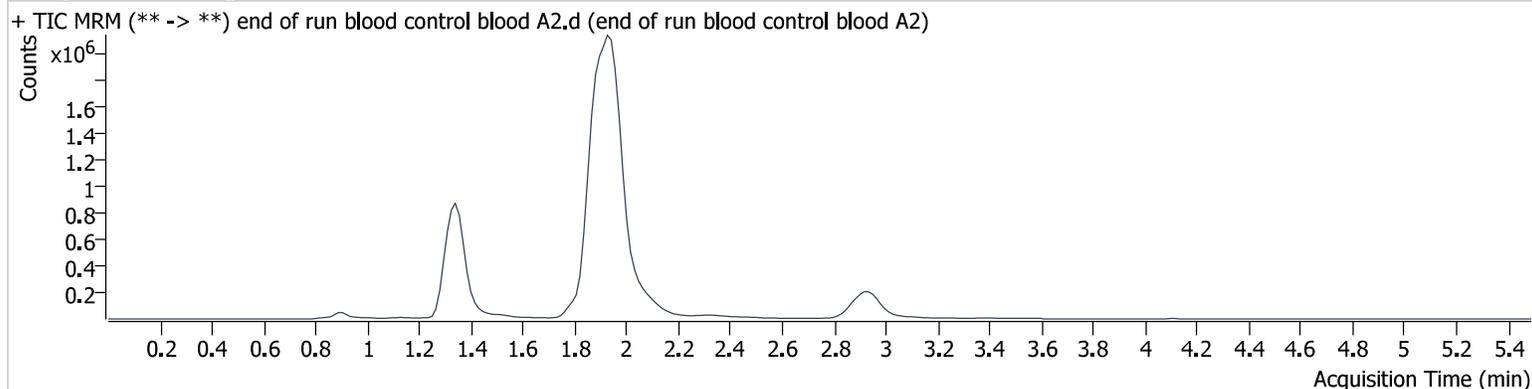


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 AM

Instrument	69679	Data File	end of run blood control blood A2.d
Type	Sample	Sample	end of run blood control blood A2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	3/27/2023 11:37:40 AM		
Sample Info.			

Sample Chromatogram



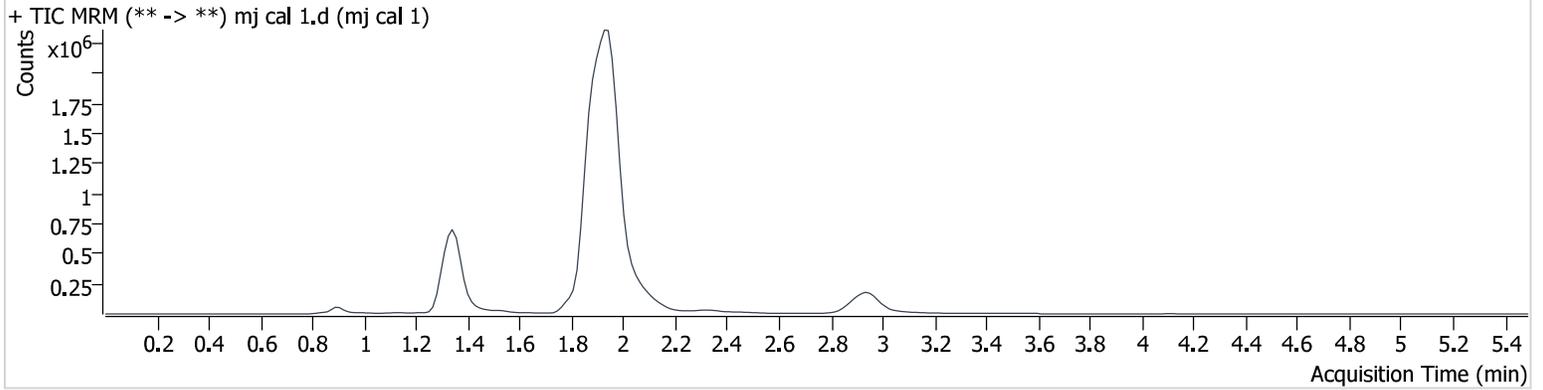
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	52640	422.9	917.01	∞	2605748	4.272 ng/ml
THC-COOH	1.358	80578	∞	242.31	602.1	1030951	15.196 ng/ml
THC	2.941	179167	987.5	24.03	149.3	1375007	4.843 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 AM

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**
Injection Volume 10
Acq. Date-Time 3/27/2023 9:32:26 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	11777	208.4	902.55	∞	2488208	1.234 ng/ml Low
THC-COOH	1.373	22293	27.4	257.23	∞	992905	5.303 ng/ml
THC	2.956	32478	496.9	26.64	56.4	1321980	1.251 ng/ml

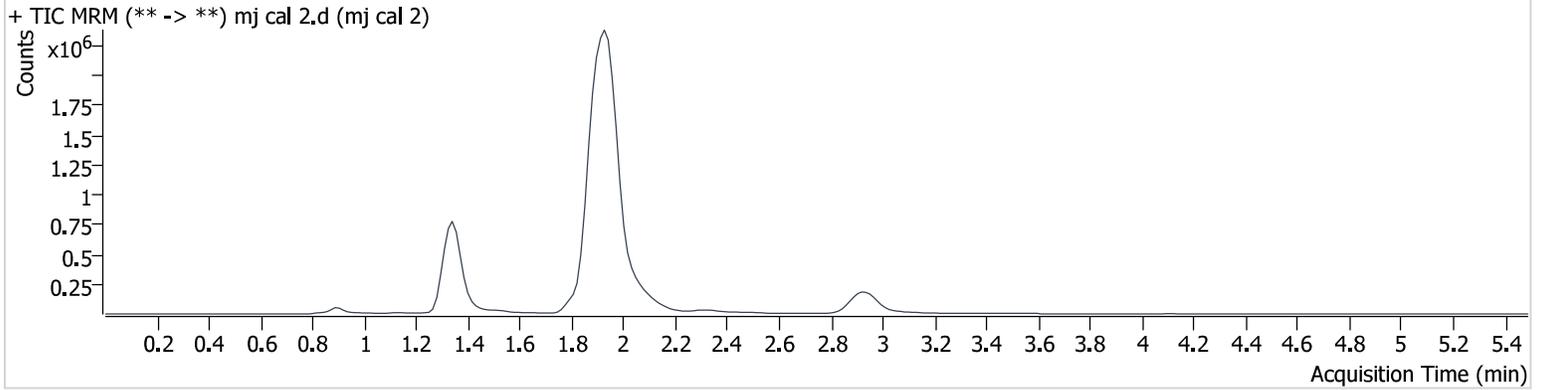
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 AM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-B1
Injection Volume 10
Acq. Date-Time 3/27/2023 9:39:10 AM
Sample Info.

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

Sample Chromatogram



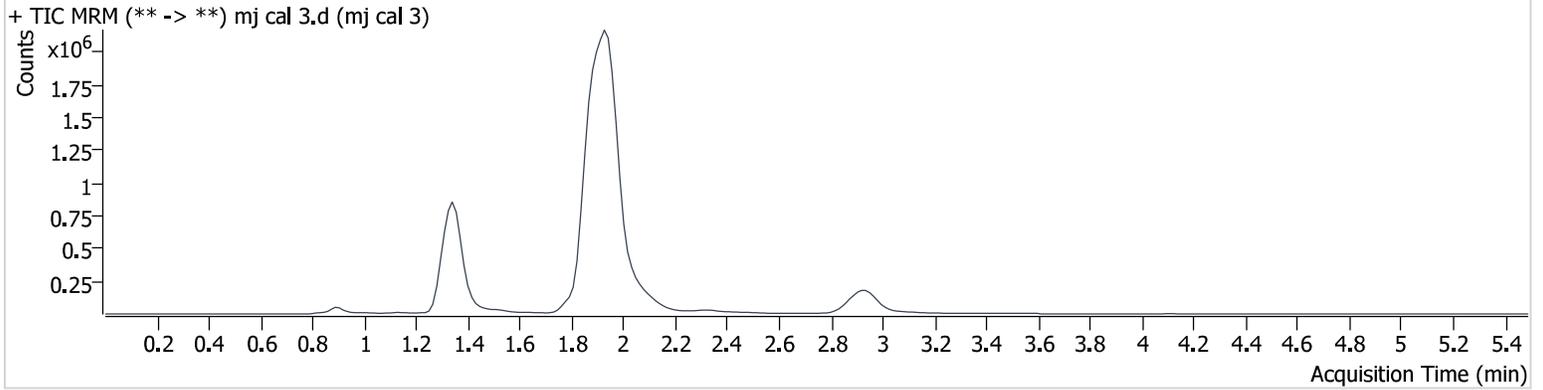
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.349	30614	125.3	1000.6	381.1	2403766	2.806 ng/ml	Low
THC-COOH	1.373	47184	∞	239.58	980.3	947289	10.161 ng/ml	
THC	2.956	89785	1059.5	24.94	58.2	1267615	2.822 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 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 3/27/2023 9:45:45 AM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.349	53204	324.8	937.92	1721.3	2451676	4.567 ng/ml
THC-COOH	1.358	97273	314.6	244.08	∞	974860	19.036 ng/ml
THC	2.956	149175	2697.8	25.61	385.5	1219032	4.573 ng/ml

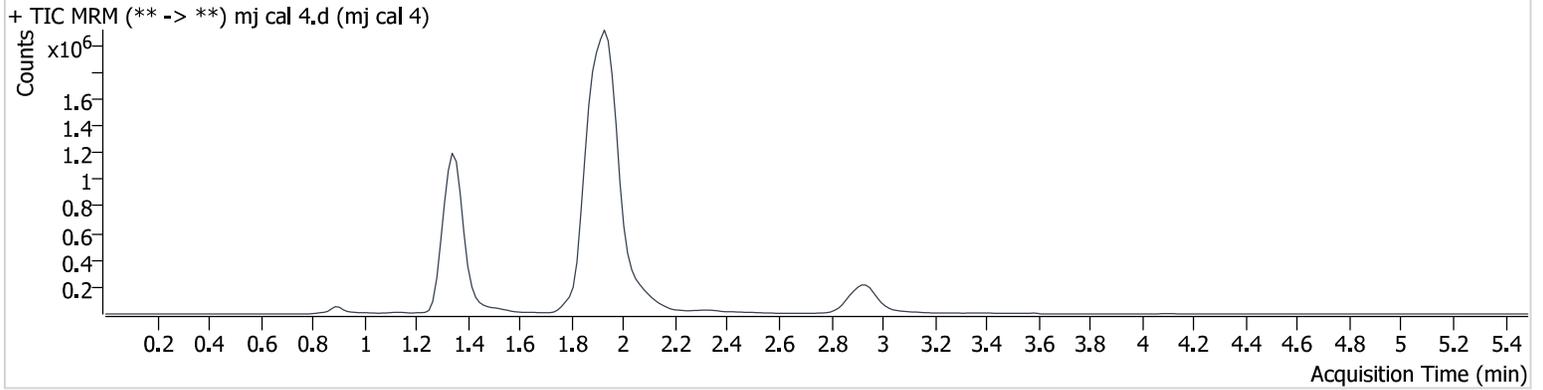
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 AM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-D1
Injection Volume 10
Acq. Date-Time 3/27/2023 9:52:19 AM
Sample Info.

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

Sample Chromatogram



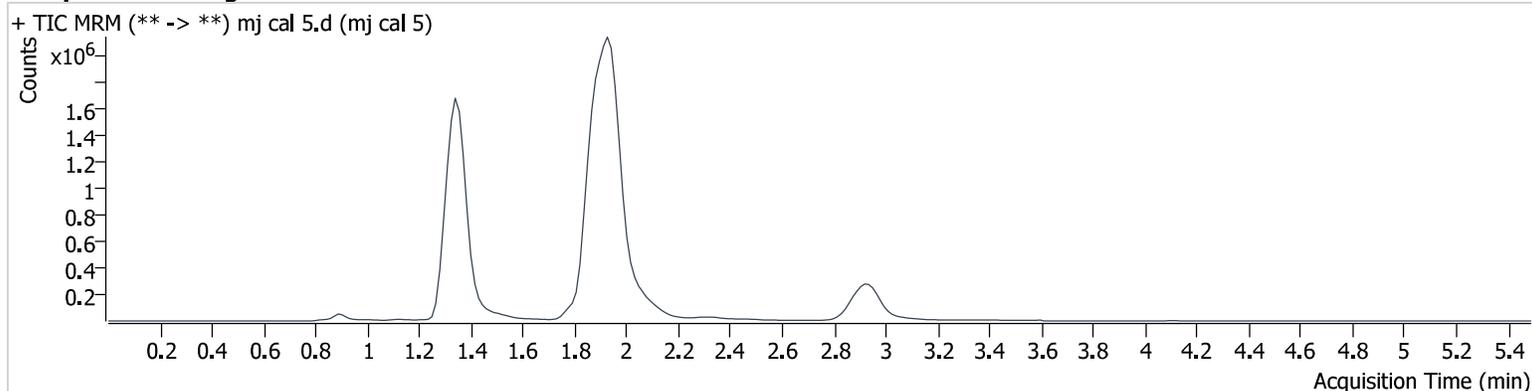
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.334	118002	263.2	881.73	∞	2546943	9.404 ng/ml
THC-COOH	1.358	264736	371.7	241.40	425.5	994436	48.593 ng/ml
THC	2.941	325693	41873.0	23.92	210.6	1275948	9.088 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 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	3/27/2023 9:58:54 AM		
Sample Info.			

Sample Chromatogram



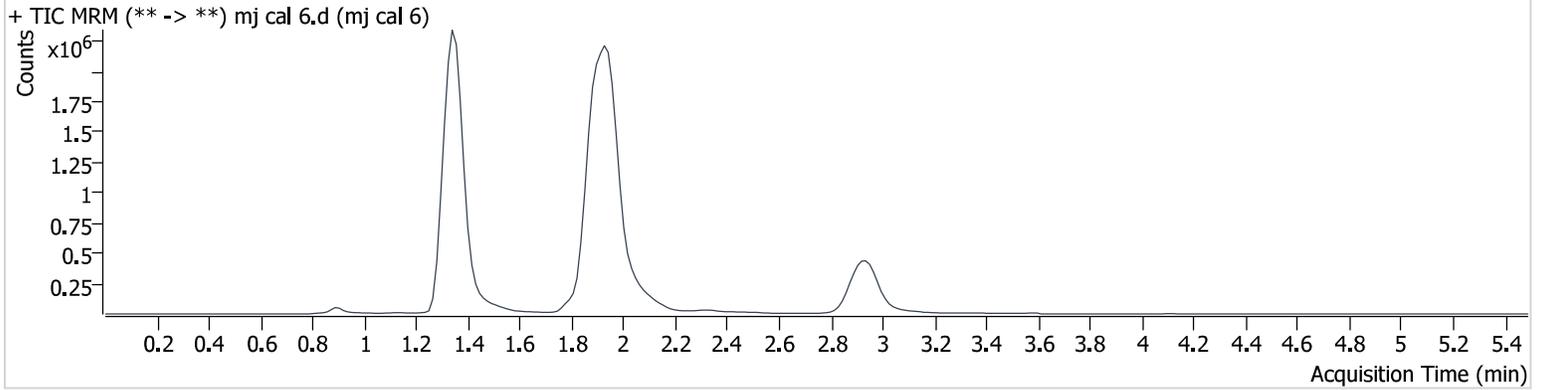
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.334	307720	6364.5	848.11	∞	2545164	24.051 ng/ml
THC-COOH	1.358	409691	6935.5	238.63	∞	993083	74.579 ng/ml
THC	2.941	823757	∞	23.42	817.3	1179657	24.140 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 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 3/27/2023 10:05:28 AM
Sample Info.

Sample Chromatogram



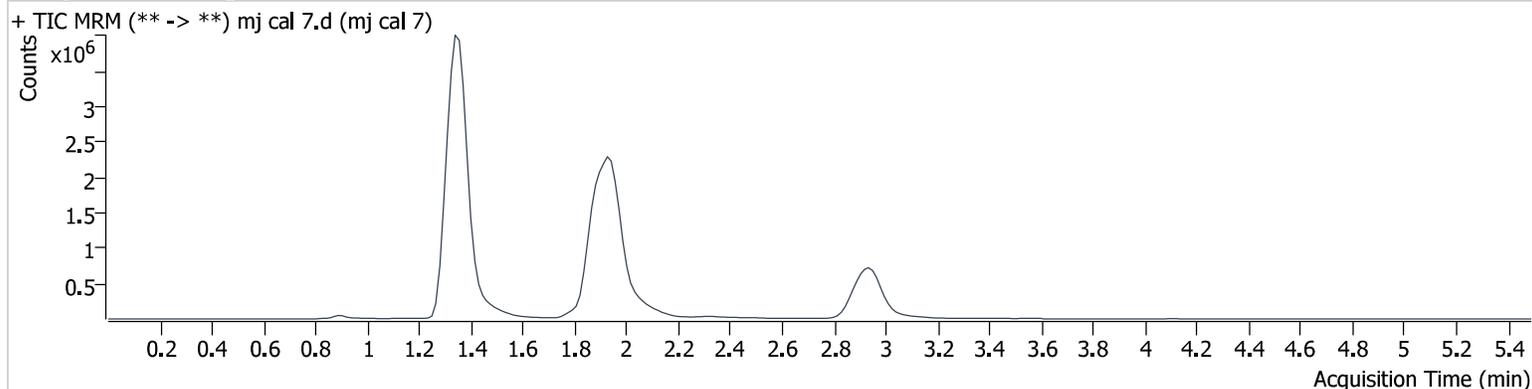
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.334	623672	1821.0	829.27	∞	2489793	49.502 ng/ml
THC-COOH	1.358	519117	956.2	248.11	∞	940566	99.331 ng/ml
THC	2.941	1779993	12994.1	23.77	753.3	1224034	49.821 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2023\am 27-28\032423r\QuantResults\cann.batch.bin
Calibration Last Update 3/27/2023 11:47:28 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	3/27/2023 10:12:02 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.334	1251512	∞	805.69	∞	2406760	102.435 ng/ml
THC-COOH	1.373	1194310	1161.2	246.35	20003.2	842714	252.998 ng/ml
THC	2.941	3642382	13365.4	23.75	3384.6	1214529	102.304 ng/ml