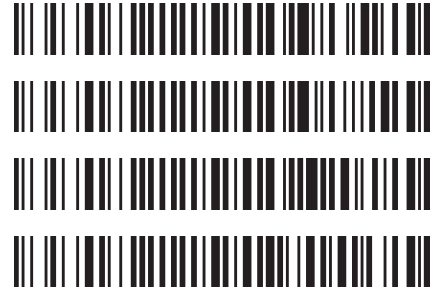


9/28/2020

REVIEWED
By Britany Wylie at 8:15 am, Sep 29, 2020

Worklist: 4542


<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2020-1837	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1838	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1851	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2020-1860	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 9/25/20
Plate lot#: 200723

Analyst: Anne Nord ^{10/20/20}
Plate Expiration: 01/23/2020 ¹ 

REVIEWED
10/20/2020 10:00 AM

Mobile phase A: 0.1% Formic Acid in LCMS Water MTBE
Mobile phase B: 0.1% Formic acid in Acetonitrile Hexane
LCMS Methanol

Blank Blood Lot: 20G20792 **Urine Blank:** 73020 **Column:** UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQ 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: 5 ng/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:

Blood only run.

GA

	1	2	3	4	5	6
a	cal 100 ng	neg blood				QC 1
b	cal 50 ng	1837-1				cal 100 ng
c	cal 25 ng	1838-1				cal 50 ng
d	cal 10ng	1851-1				cal 25 ng
e	cal 5 ng	1860-1				cal 10ng
f	cal 3 ng					cal 5 ng
g	cal 1ng					cal 3 ng
h	QC 1					cal 1ng

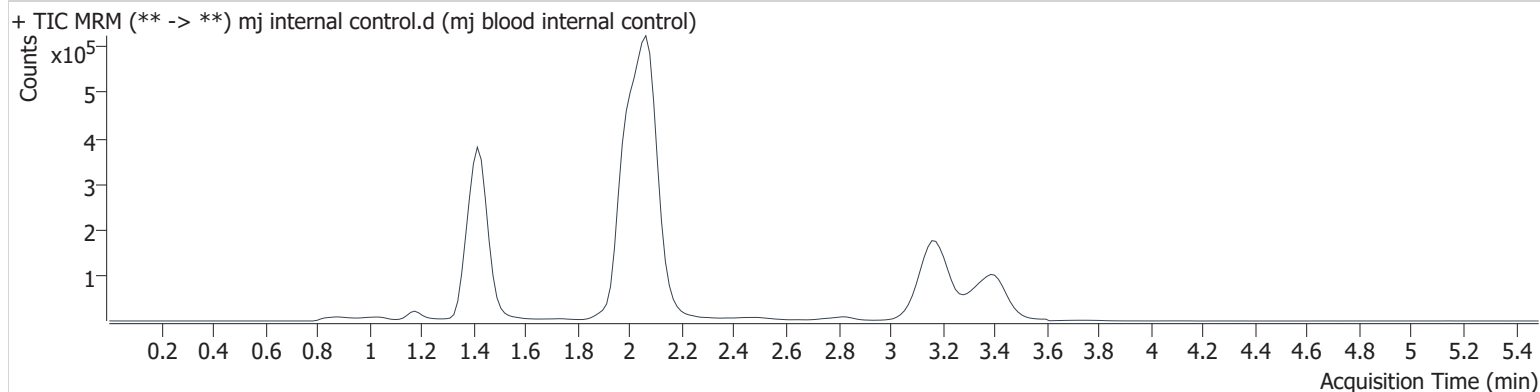
c2020-

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 AM

Instrument	69679	Data File	mj internal control.d
Type	QC	Sample	mj blood internal control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	9/25/2020 3:30:53 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	159783	∞	11.4	∞	1139988	4.494 ng/ml
THC-COOH	1.446	101504	292.6	34.9	402.9	499861	14.807 ng/ml
THC	3.227	60077	∞	27.3	146.7	564599	4.387 ng/ml

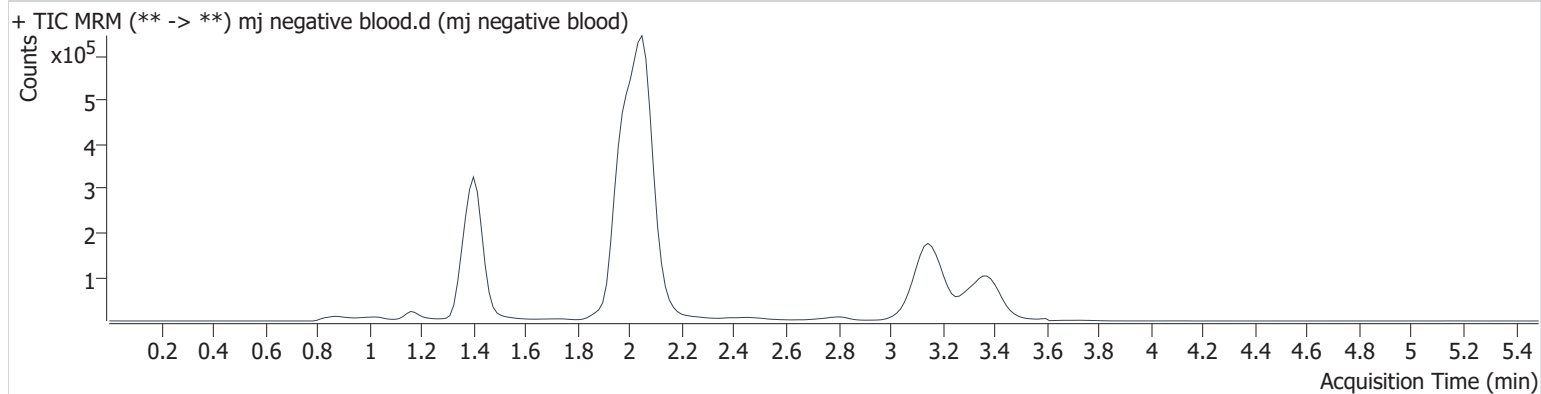
GA

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 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	9/25/2020 3:38:37 PM		
Sample Info.			

Sample Chromatogram

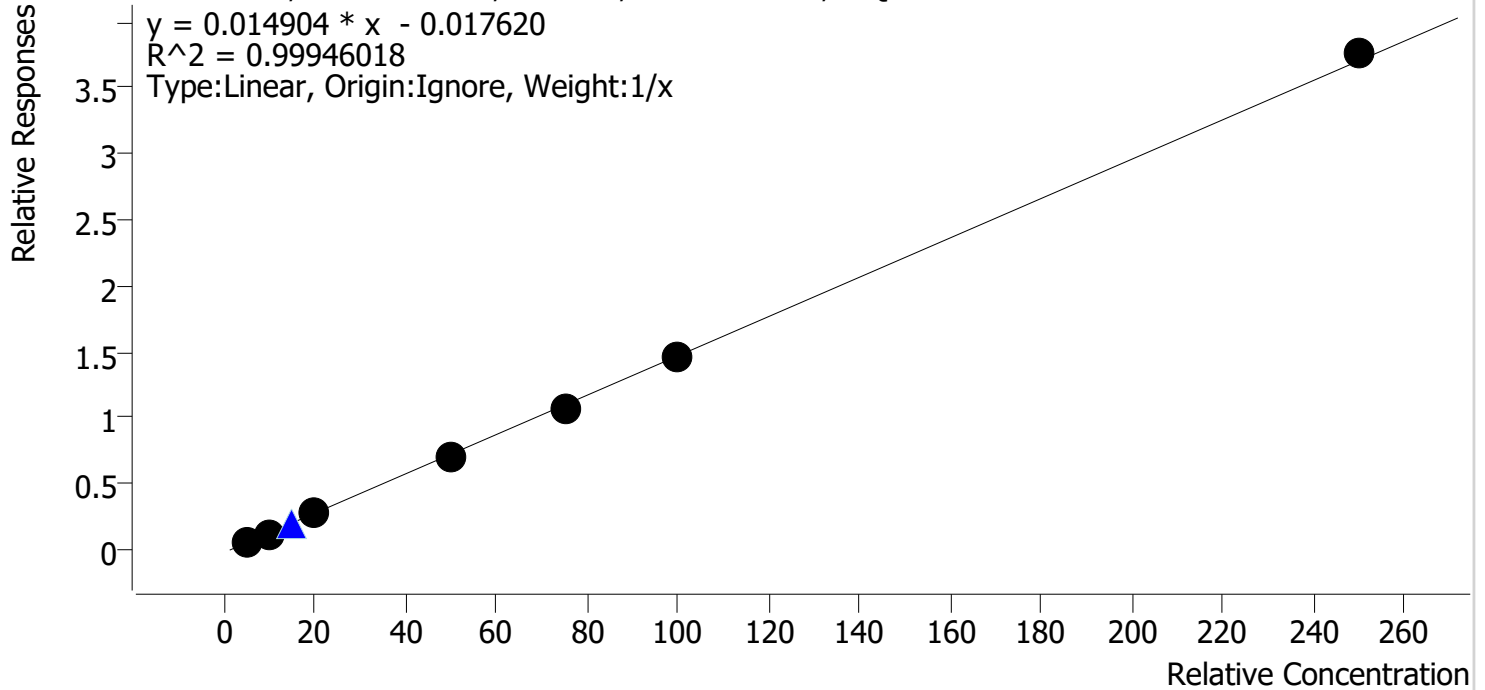


Compound Calibration Report



Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Last Cal. Update 9/26/2020 9:25 AM
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



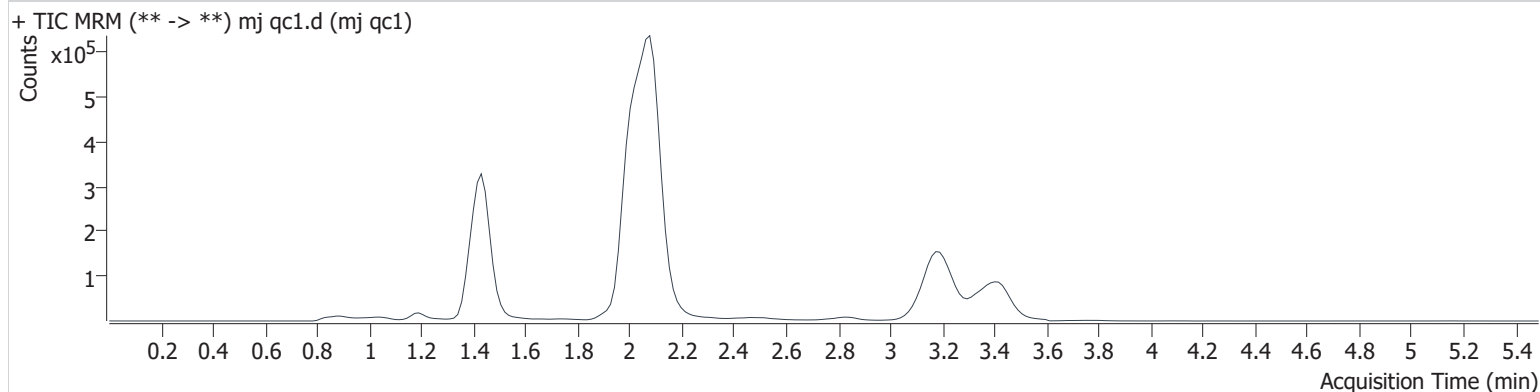
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.4	108.9
mj cal2	2	✓	10.0	9.8	97.9
mj cal 3	3	✓	20.0	19.5	97.3
mj cal 4	4	✓	50.0	48.3	96.7
mj cal 5	5	✓	75.0	73.2	97.6
mj cal 6	6	✓	100.0	100.1	100.1
mj cal 7	7	✓	250.0	253.6	101.4

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 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	9/25/2020 2:36:43 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.436	43752	∞	8.9	∞	1137648	1.035 ng/ml	Low
THC-COOH	1.461	31185	35785.6	33.9	43.6	490785	5.446 ng/ml	Low
THC	3.227	12846	∞	25.7	39.0	561476	1.200 ng/ml	Low

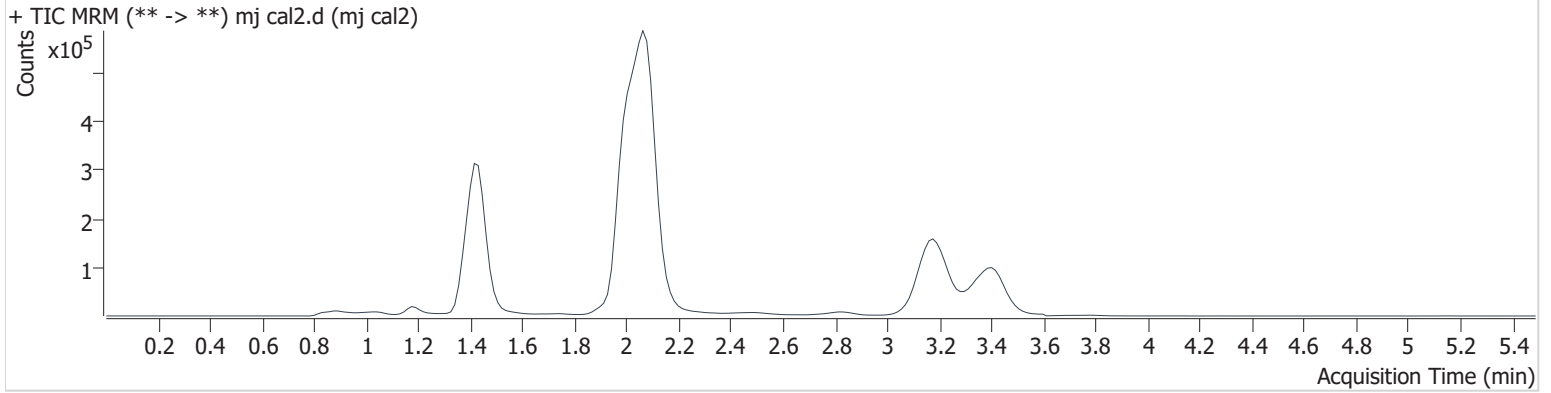
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 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	9/25/2020 2:44:27 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.421	103206	∞	10.2	∞	969742	3.347 ng/ml	
THC-COOH	1.446	59692	95707.3	33.7	49.6	465406	9.788 ng/ml	Low
THC	3.212	35814	6106.7	28.0	69.3	521085	2.949 ng/ml	Low

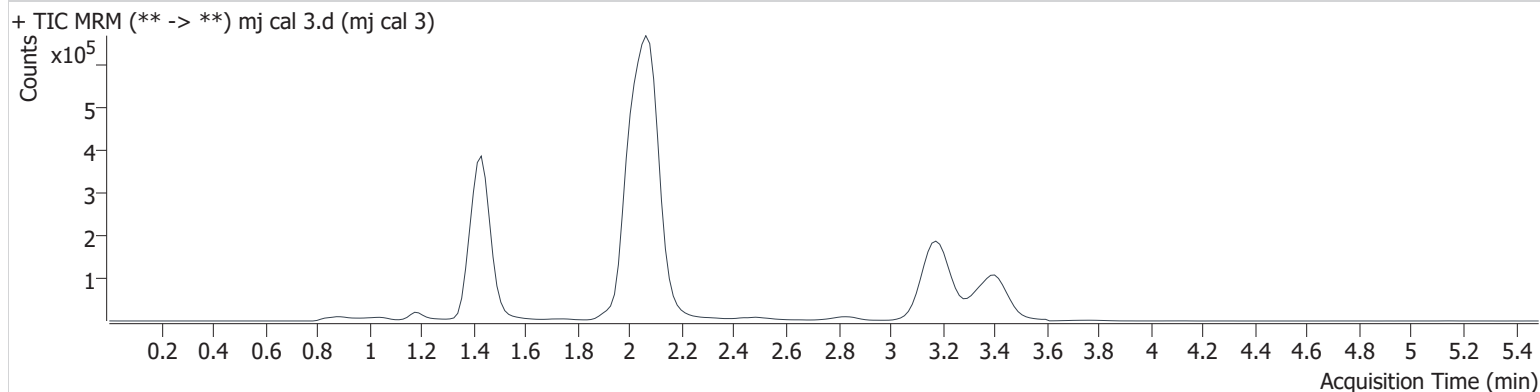
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 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	9/25/2020 2:52:12 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	153668	∞	12.4	∞	1089435	4.524 ng/ml
THC-COOH	1.461	132056	181844.7	32.4	222.9	484579	19.467 ng/ml
THC	3.212	68377	1483.4	26.3	322.5	591828	4.735 ng/ml

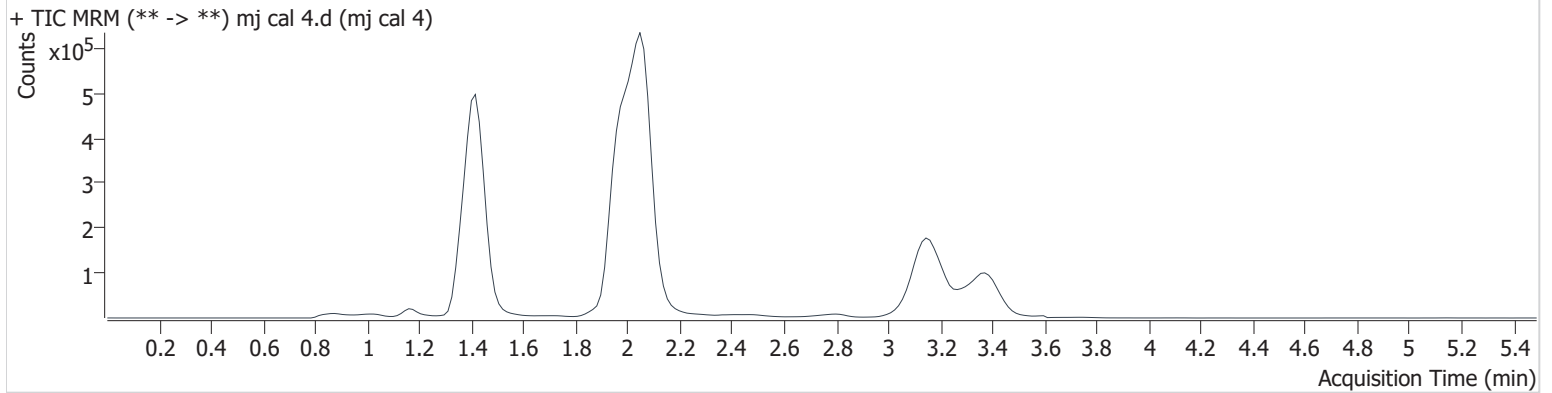
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 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	9/25/2020 2:59:56 PM		

Sample Info.

Sample Chromatogram



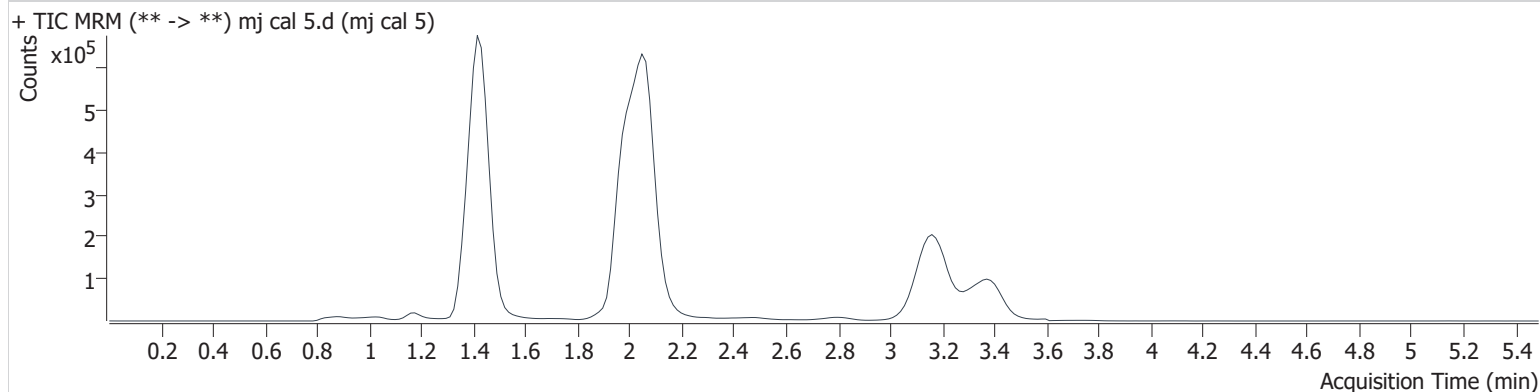
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	329350	∞	11.8	∞	1132788	9.616 ng/ml
THC-COOH	1.431	339298	543476.7	33.9	805.8	482809	48.335 ng/ml
THC	3.212	124904	∞	26.8	86104 27173 2901.9	551222	8.972 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 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	9/25/2020 3:07:40 PM		

Sample Chromatogram



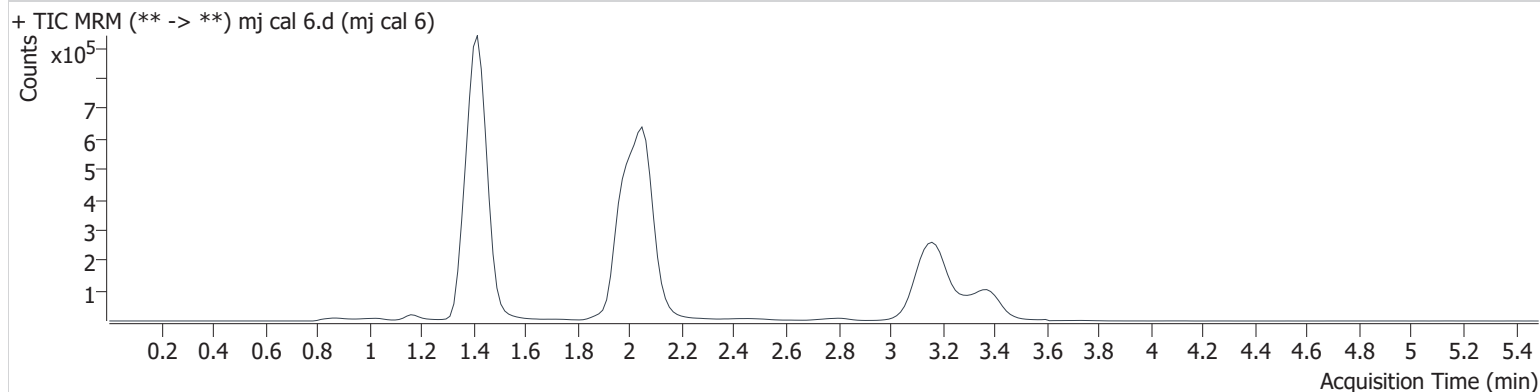
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	787744	∞	12.1	5357.2	1088766	24.336 ng/ml
THC-COOH	1.446	513483	907022.7	34.4	28847	478325	73.210 ng/ml
THC	3.197	347454	∞	23.8	∞	563441	23.855 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 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	9/25/2020 3:15:24 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	1653112	∞	12.4	∞	1122519	49.818 ng/ml
THC-COOH	1.431	687812	918.2	35.2	1214.2	466329	100.146 ng/ml
THC	3.197	738376	∞	24.3	∞	571708	49.603 ng/ml

GA

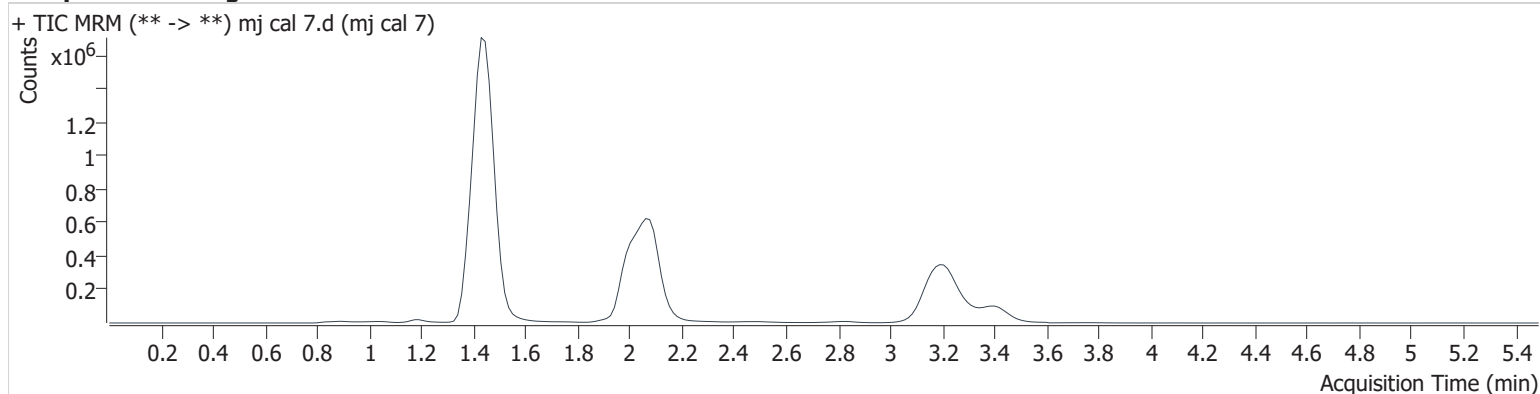
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 092520\QuantResults\cann.batch.bin
Calibration Last Update 9/26/2020 9:25:48 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	9/25/2020 3:23:09 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.421	3389005	∞	13.0	∞	1134595	101.324 ng/ml
THC-COOH	1.461	1678978	2764.0	35.4	1288.6	446284	253.608 ng/ml
THC	3.227	1493168	∞	24.3	∞	556559	102.687 ng/ml