











8/28/2021

Worklist: 5202

REVIEWED

By Brittany Wylie at 11:48 am, Aug 31, 2021

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2021-1739	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-1797	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-1804	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-1816	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-1868	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-1884	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-1889	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-1906	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 8/27/21
Plate lot#: 210609

Analyst: Anne Nord
Plate Expiration: 12-9-21

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

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 21D52496 **Urine Blank:** 5621 **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.

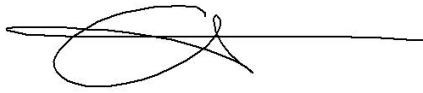
Analytic:

- 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 1ng/ml, 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

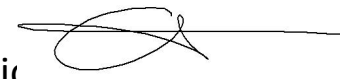
COMMENTS:



	1	2	3	4	5	6
a	cal 1	neg blood	1739-2			QC 1
b	cal 2	1804-1	1797-1			cal 100 ng
c	cal 3	1868-1	1816-1			cal 50 ng
d	cal 4	1884-1				cal 25 ng
e	Cal 5	1889-1				cal 10ng
f	cal 6	1906-1				cal 5 ng
g	cal 7	negative urine				cal 3 ng
h	Internal control	urine postive control				cal 1ng

C2021-____-__

Toxicology AM method 27/26 external prep informati



working solution 15 ug/ml in meoh C-THC, THC-OH, 7.5 ug/ml THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 150 ul THC-OH in 9692.5 ul meOH
 Ppd 8/26/21 Exp: 8/26/22 lot 82621 by AMN

Drug	lot	expiration
C-THC	FE04151901	6/1/2024
THC-OH	FE06152002	6/1/2025
THC	FE04222001	5/1/2025

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

		Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	
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AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine

out of use

ppd 8/26/21 Exp 8/26/22 neg urine lot 5621	lot u82621	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

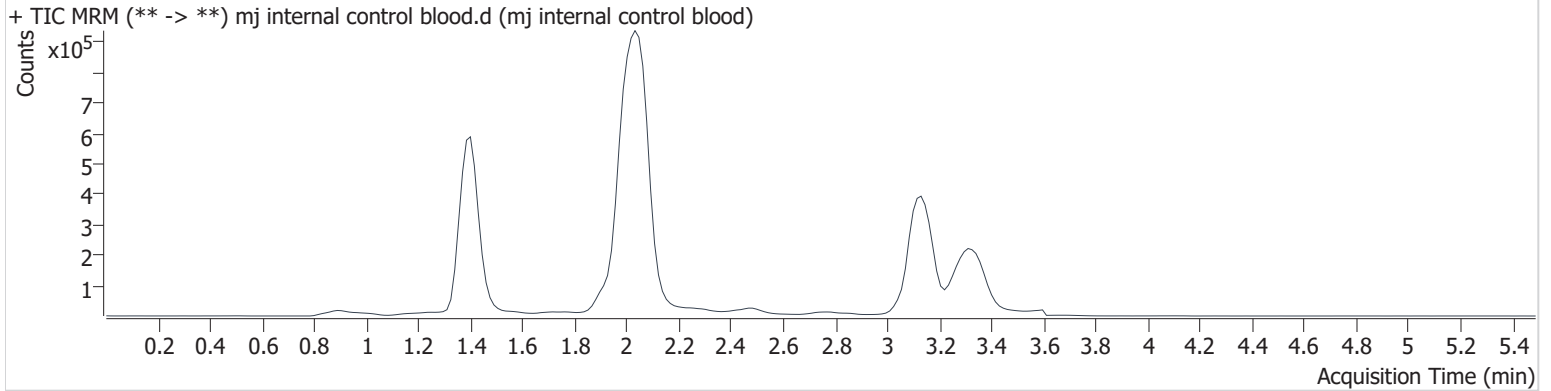
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 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	8/27/2021 3:15:00 PM		

Sample Info.

Sample Chromatogram



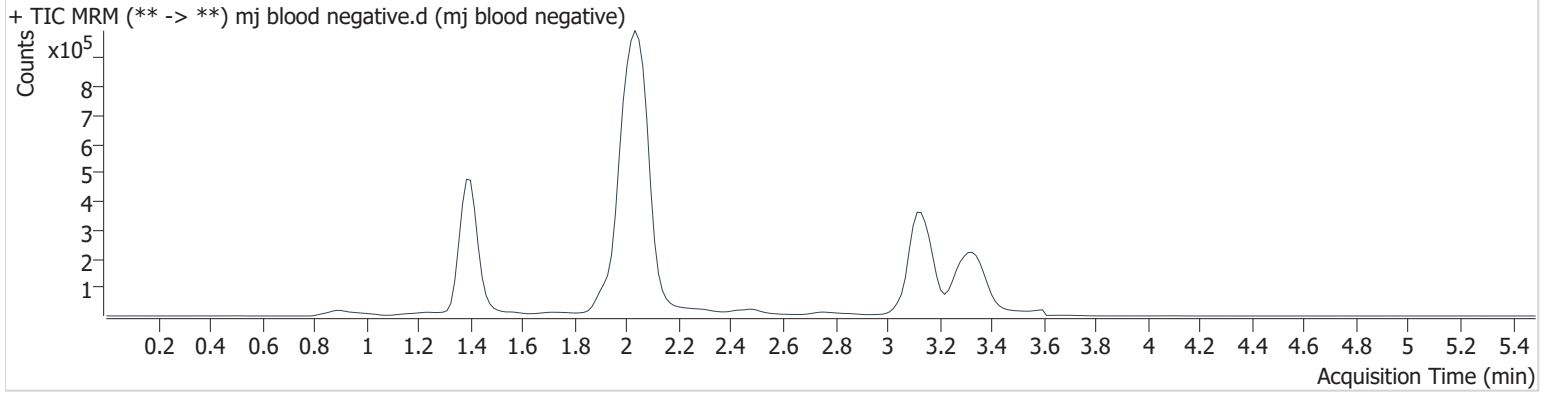
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	28784	∞	865.0	195.3	1948688	4.599 ng/ml
THC-COOH	1.431	99848	220.0	34.8	301.5	480767	14.893 ng/ml
THC	3.122	195541	∞	26.1	∞	1773952	4.571 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 AM

Instrument	69679	Data File	mj blood negative.d
Type	Sample	Sample	mj blood negative
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2021 3:21:42 PM		
Sample Info.			

Sample Chromatogram

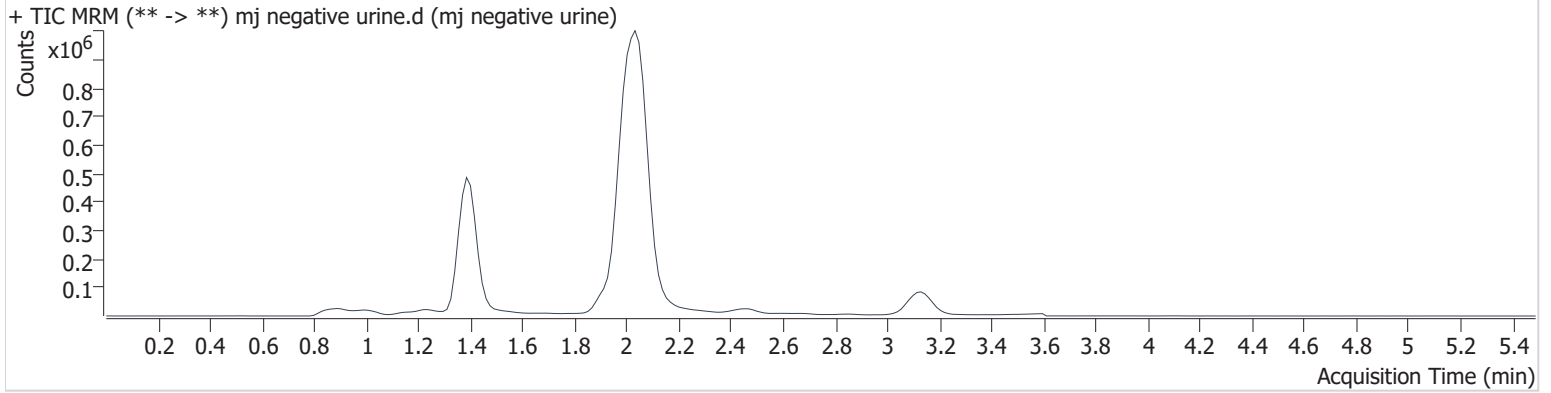


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 AM

Instrument	69679	Data File	mj negative urine.d
Type	Sample	Sample	mj negative urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-G2	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2021 4:48:21 PM		
Sample Info.			

Sample Chromatogram



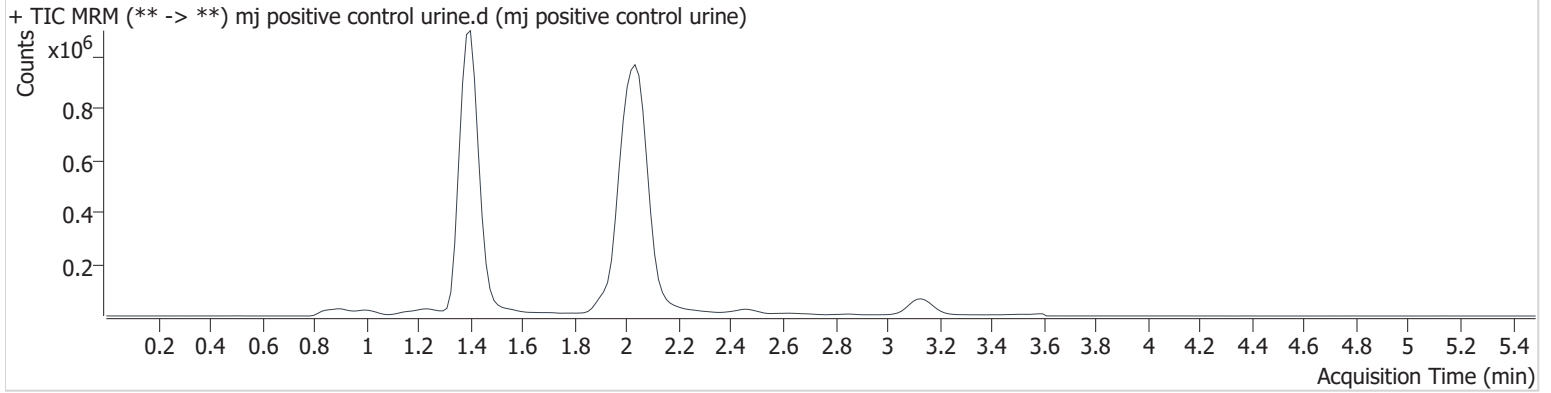
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 AM

Instrument	69679	Data File	mj positive control urine.d
Type	Sample	Sample	mj positive control urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H2	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2021 4:55:04 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	291234	7862.9	812.9	∞	1901917	45.858 ng/ml
THC-COOH	1.416	273767	153.0	34.7	11196 3.9	423575	46.286 ng/ml
THC	3.122	41075	∞	25.8	30.1	365301	4.654 ng/ml

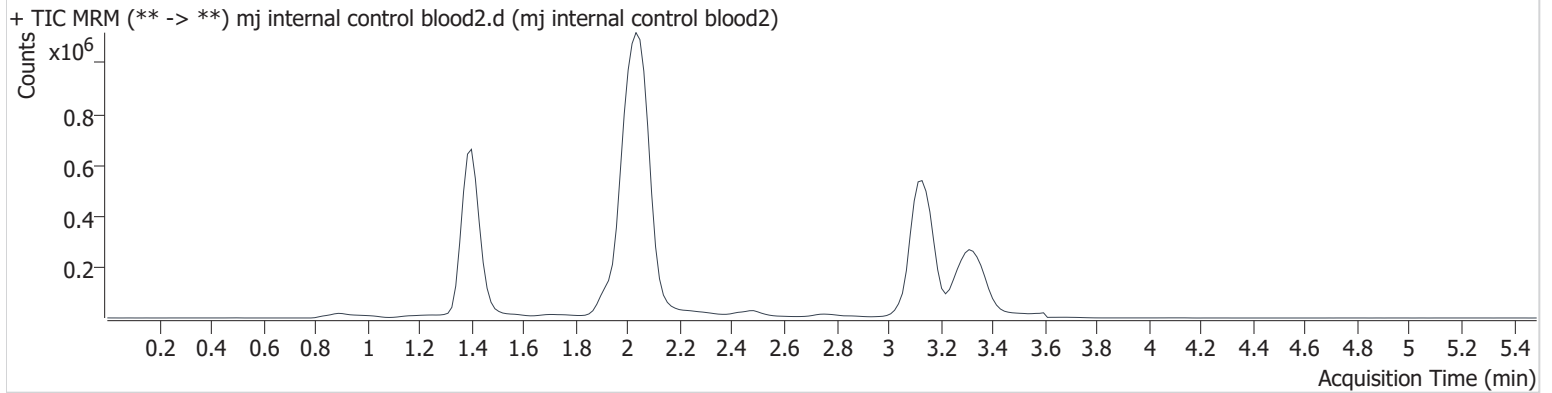
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 AM

Instrument	69679	Data File	mj internal control blood2.d
Type	QC	Sample	mj internal control blood2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2021 5:55:01 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	31862	∞	880.3	375.1	2055306	4.818 ng/ml
THC-COOH	1.431	109517	251.7	34.5	136.3	542280	14.483 ng/ml
THC	3.122	284188	∞	24.1	∞	2436454	4.812 ng/ml

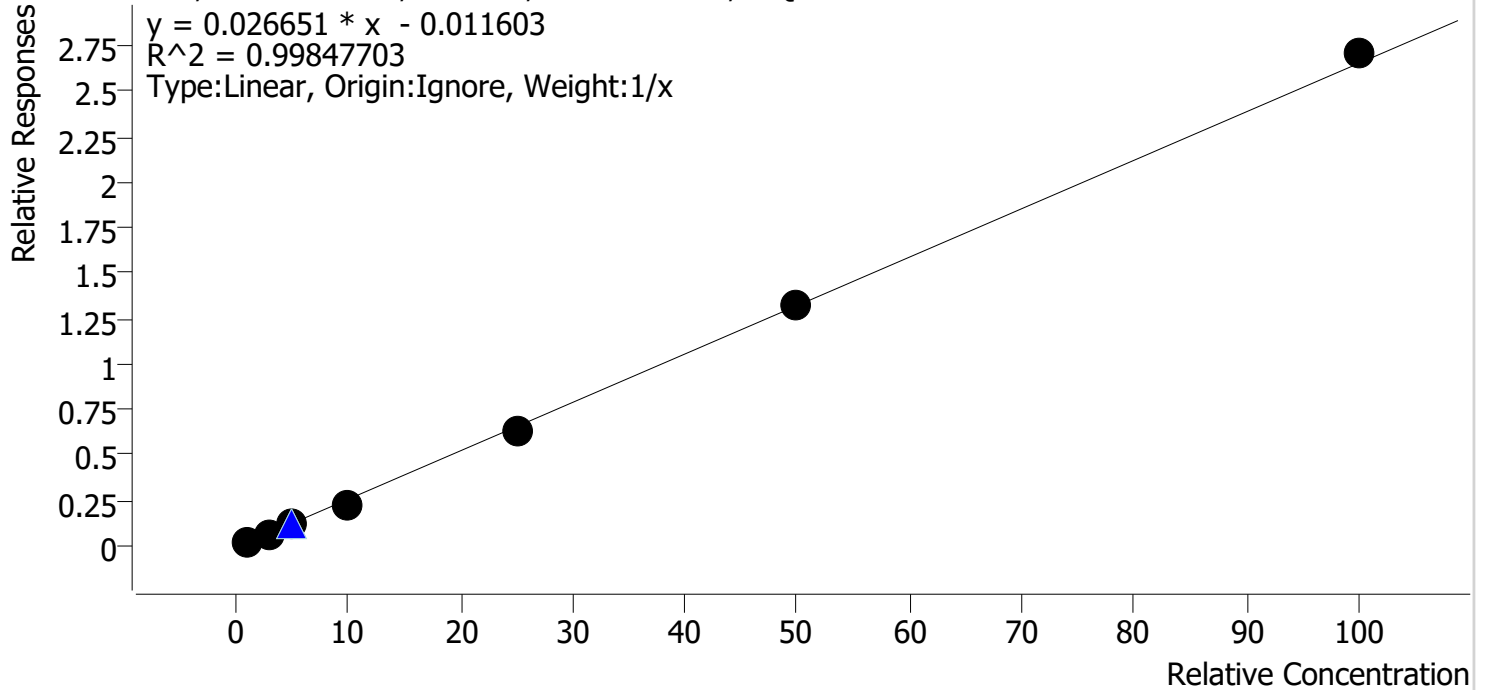
Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Last Cal. Update 8/28/2021 9:26 AM
Analyst Name ISP\datastor
Analyte THC

Internal Standard THC-d3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs



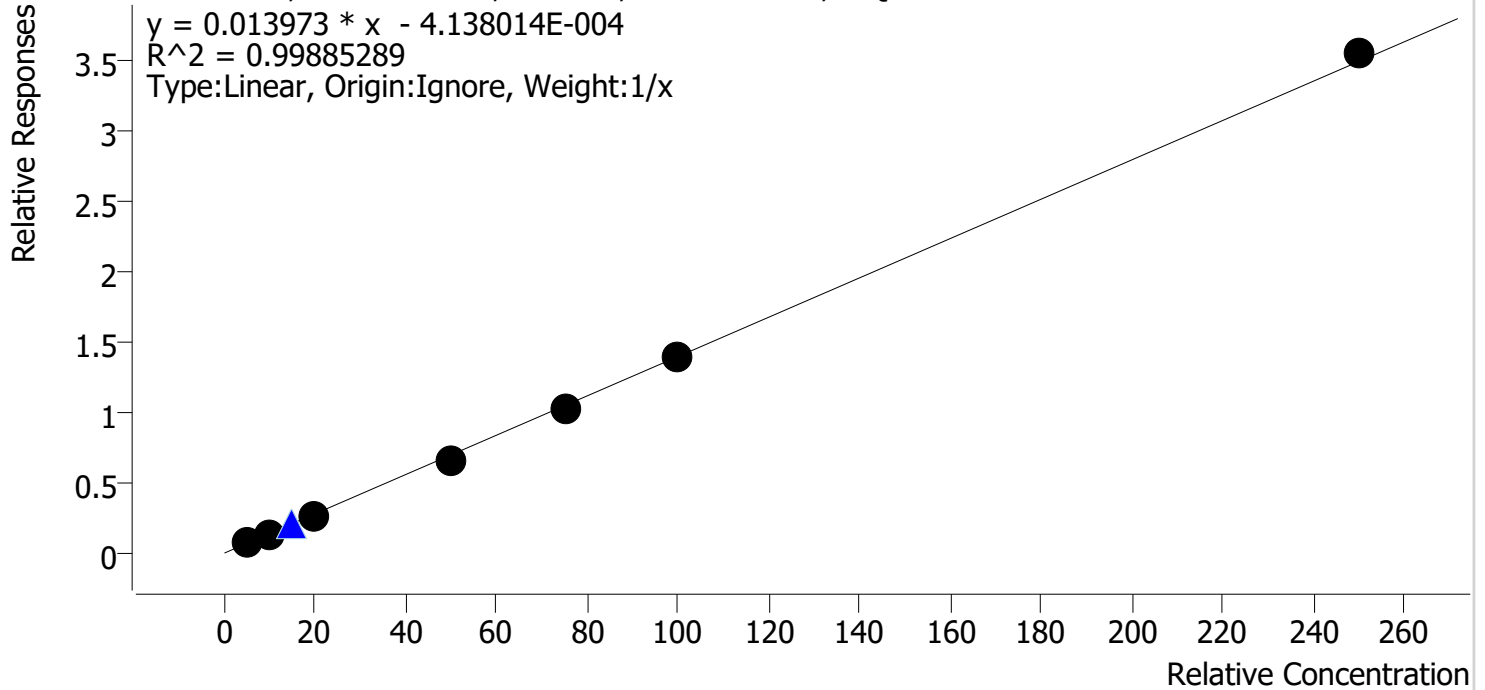
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	1.0	1.2	124.0
mj cal2	2	✓	3.0	2.8	94.5
mj cal 3	3	✓	5.0	4.6	91.8
mj cal 4	4	✓	10.0	9.1	91.0
mj cal 5	5	✓	25.0	24.1	96.5
mj cal 6	6	✓	50.0	50.1	100.3
mj cal 7	7	✓	100.0	102.0	102.0

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Last Cal. Update 8/28/2021 9:26 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, 2 QCs

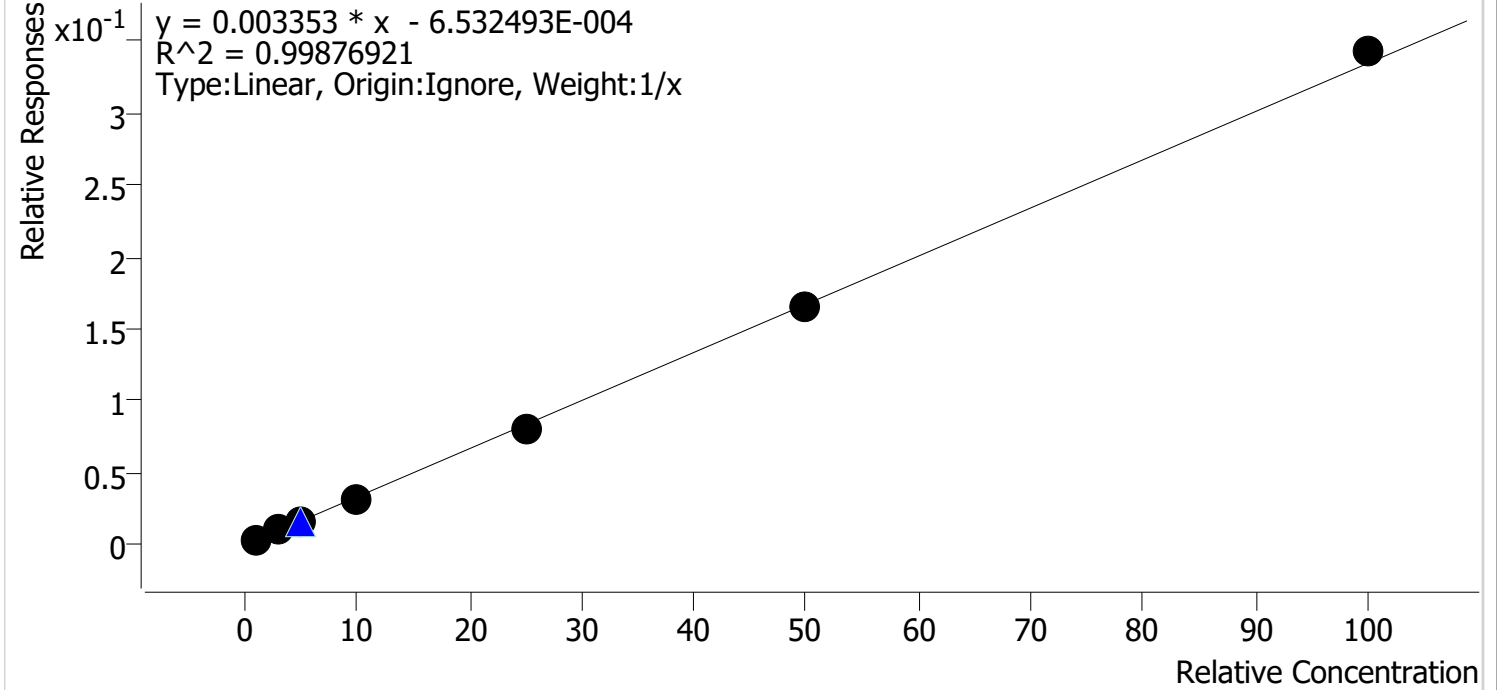


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	5.0	5.8	116.2
mj cal2	2	✓	10.0	9.5	94.9
mj cal 3	3	✓	20.0	18.5	92.7
mj cal 4	4	✓	50.0	47.7	95.4
mj cal 5	5	✓	75.0	74.0	98.7
mj cal 6	6	✓	100.0	100.5	100.5
mj cal 7	7	✓	250.0	253.9	101.6

Compound Calibration Report

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Last Cal. Update 8/28/2021 9:26 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	1.0	1.1	111.0
mj cal2	2	✓	3.0	3.1	103.0
mj cal 3	3	✓	5.0	4.9	97.4
mj cal 4	4	✓	10.0	9.2	92.0
mj cal 5	5	✓	25.0	23.8	95.2
mj cal 6	6	✓	50.0	49.5	99.1
mj cal 7	7	✓	100.0	102.4	102.4

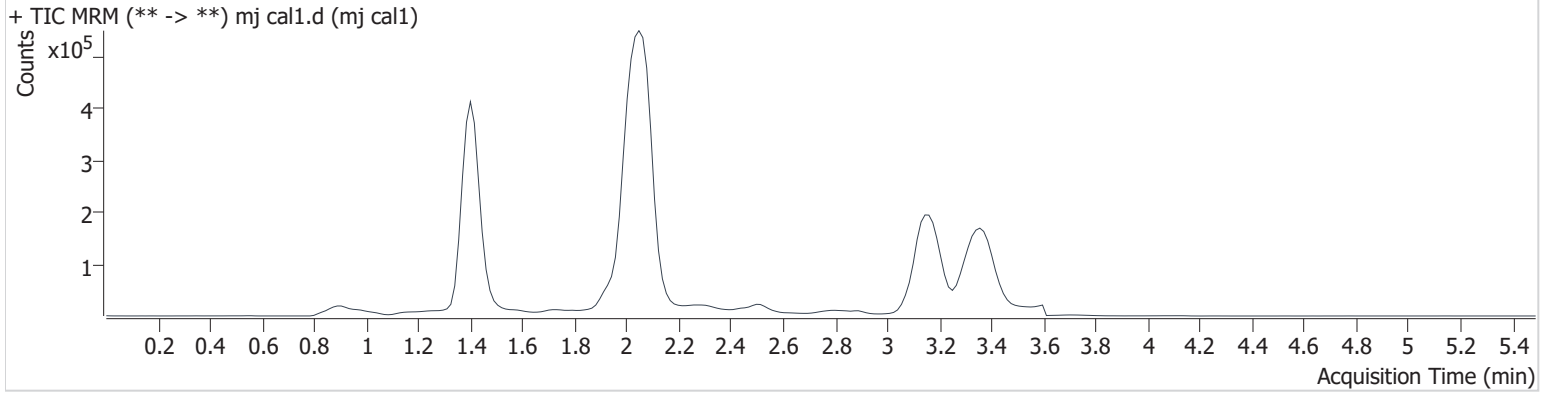
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 AM

Instrument	69679	Data File	mj cal1.d
Type	Cal	Sample	mj cal1
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	8/27/2021 2:28:05 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.409	4952	∞	922.2	∞	1613484	1.110 ng/ml	Low
THC-COOH	1.431	20987	687.9	31.5	31.2	259857	5.810 ng/ml	
THC	3.182	19174	91.2	28.9	23.7	893860	1.240 ng/ml	

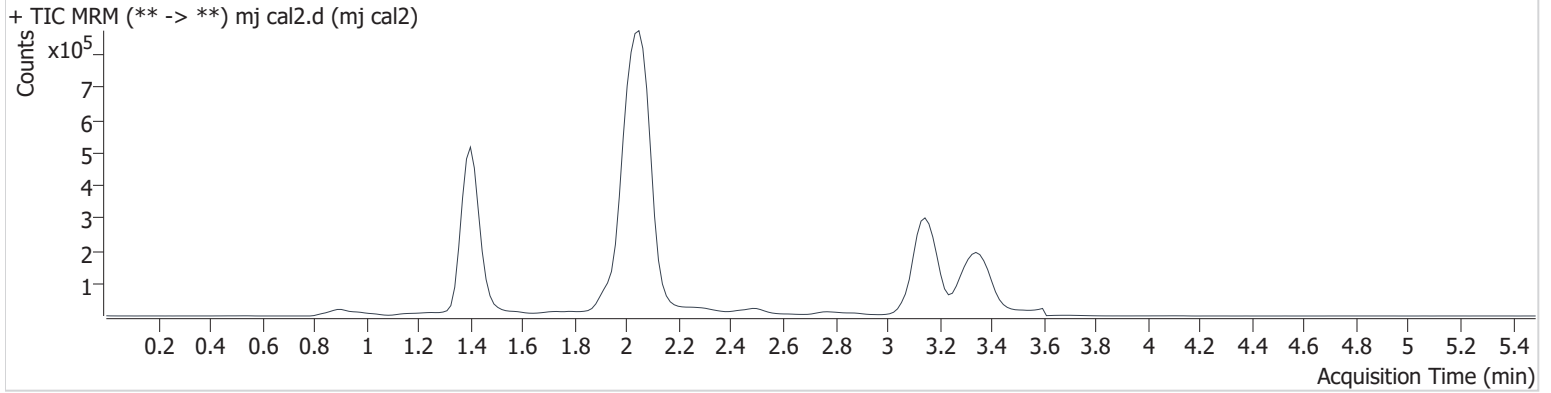
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 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	8/27/2021 2:34:49 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	17649	∞	816.3	159.3	1817945	3.090 ng/ml
THC-COOH	1.431	53018	563.9	36.1	84.4	401166	9.488 ng/ml
THC	3.167	86181	∞	24.9	∞	1348103	2.834 ng/ml

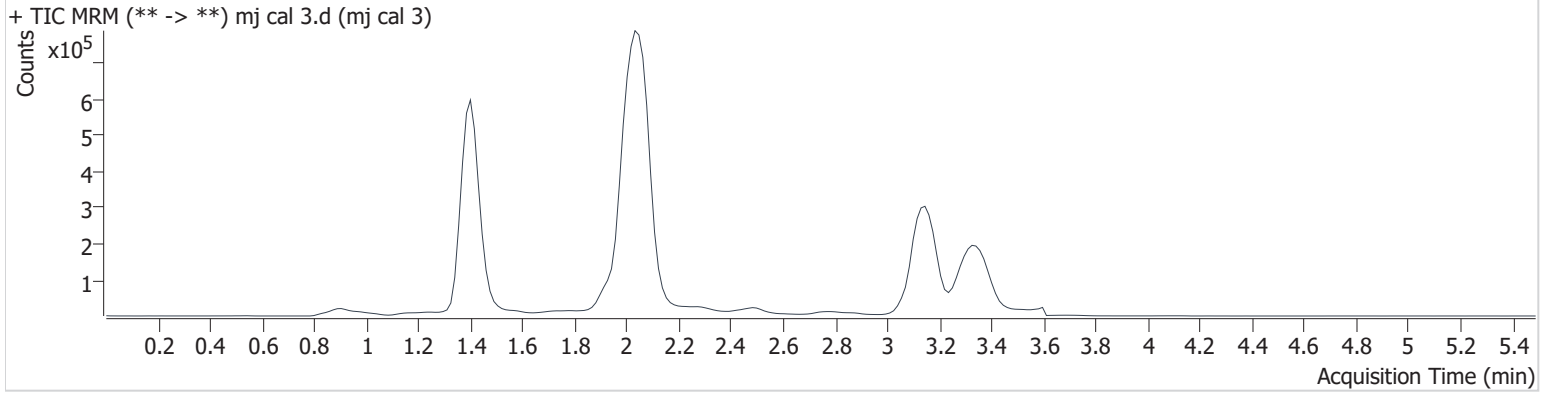
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 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	8/27/2021 2:41:31 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	30025	∞	842.3	1626.3	1915504	4.869 ng/ml
THC-COOH	1.431	108970	51.5	34.8	44420. 7	421111	18.549 ng/ml
THC	3.167	147359	∞	26.5	∞	1331484	4.588 ng/ml

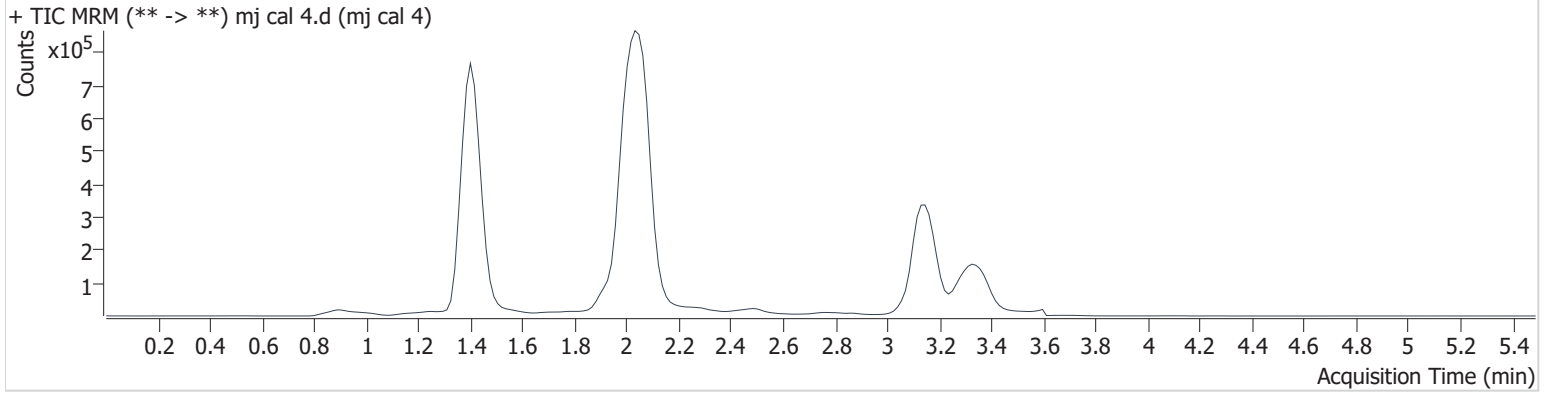
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 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	8/27/2021 2:48:13 PM		

Sample Info.

Sample Chromatogram



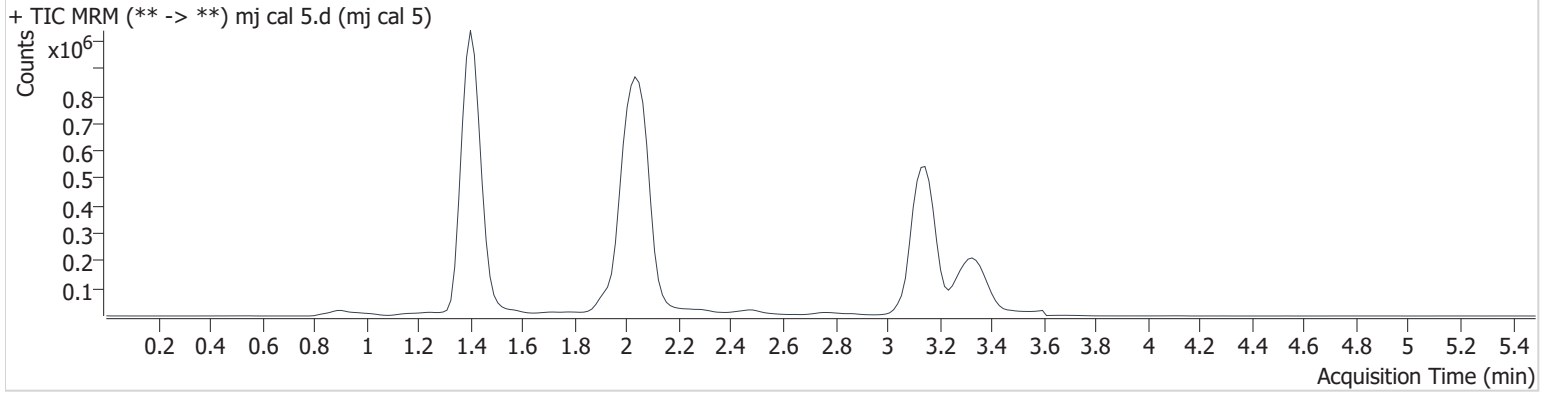
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	60537	∞	1001.1	∞	2005157	9.198 ng/ml
THC-COOH	1.431	313287	373.7	35.5	159.1	470119	47.723 ng/ml
THC	3.152	312240	∞	25.1	∞	1351905	9.101 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 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	8/27/2021 2:54:55 PM		
Sample Info.			

Sample Chromatogram



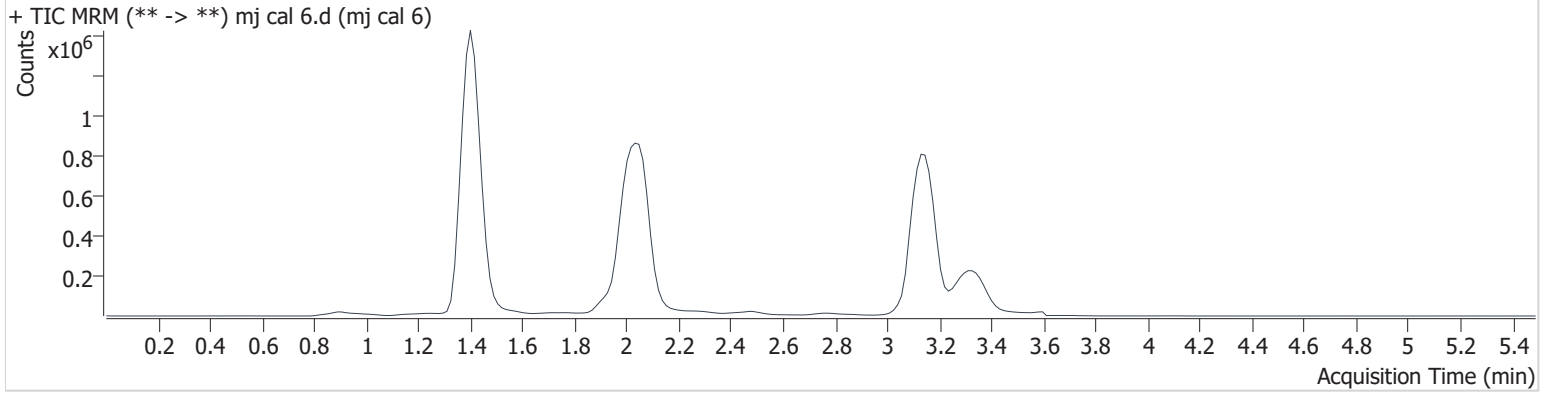
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	157623	∞	874.7	5603.4	1992151	23.789 ng/ml
THC-COOH	1.431	486261	305.8	36.8	8261.2	470458	74.002 ng/ml
THC	3.152	1020554	∞	23.7	∞	1616931	24.118 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 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	8/27/2021 3:01:37 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	328088	∞	845.7	3625.5	1982894	49.535 ng/ml
THC-COOH	1.431	653951	1542.1	36.4	249.0	465896	100.486 ng/ml
THC	3.122	2232291	∞	24.0	∞	1684932	50.146 ng/ml

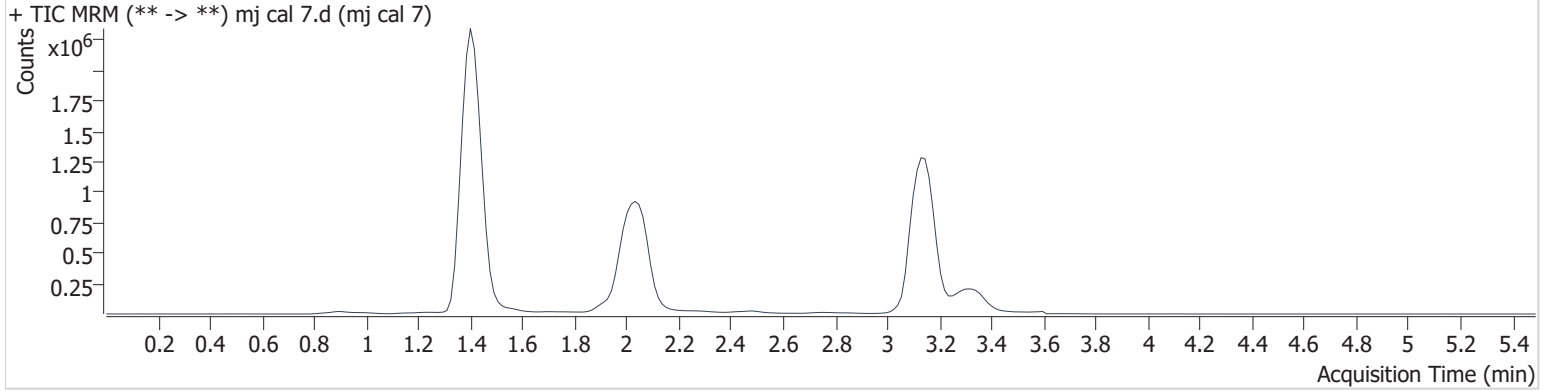
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\082721\QuantResults\cann.batch.bin
Calibration Last Update 8/28/2021 9:26:02 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	8/27/2021 3:08:18 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	638987	∞	830.4	4394.3	1864196	102.409 ng/ml
THC-COOH	1.431	1512365	1335.5	37.8	7448.0	426281	253.942 ng/ml
THC	3.122	4431332	∞	24.6	2432.1	1637547	101.972 ng/ml