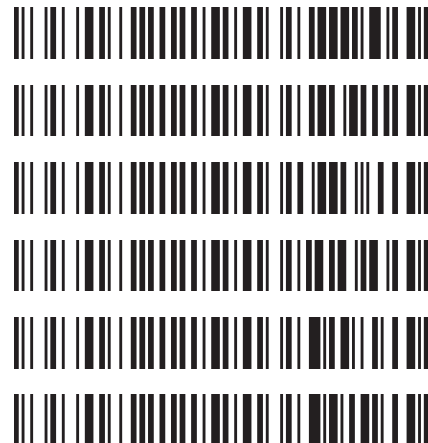


6/1/2021

Worklist: 5007

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2021-1038	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1039	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1079	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1146	3	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2021-1228	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1229	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ

REVIEWED
By Brittany Wylie at 11:49 am, Jun 02, 2021



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

Extraction Date 5/28/21
Plate lot#: 210412

Analyst: Anne Nord
Plate Expiration: 10/12/21

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: 20K20702 **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	1229-1			QC 1
b	cal 2	1228-1				cal 100 ng
c	cal 3	negative urine				cal 50 ng
d	cal 4	urine control				cal 25 ng
e	Cal 5	1038-1				cal 10ng
f	cal 6	1039-1				cal 5 ng
g	cal 7	1079-1				cal 3 ng
h	Internal control	1146-3				cal 1ng

C2021-____-__

Toxicology AM method 27/26 external prep information



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 8/26/20 Exp: 7/1/21 lot 82620 by AMN

Drug	lot	expiration
C-THC	FE01061702	3/1/2022
THC-OH	FE07221601	7/1/2021
THC	FE01041701	3/1/2022

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	
--	--	--	--

AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine

out of use

ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	1/12/2021
ppd 1/13/21 Exp 7/1/21 neg urine lot 10120	lot 11321	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	3/28/2021
ppd 3/29/21 Exp 7/1/21 neg urine lot 2121	lot 32921	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	5/27/2021
ppd 5/28/21 Exp 7/1/21 neg urine lot 5621	lot 52821	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

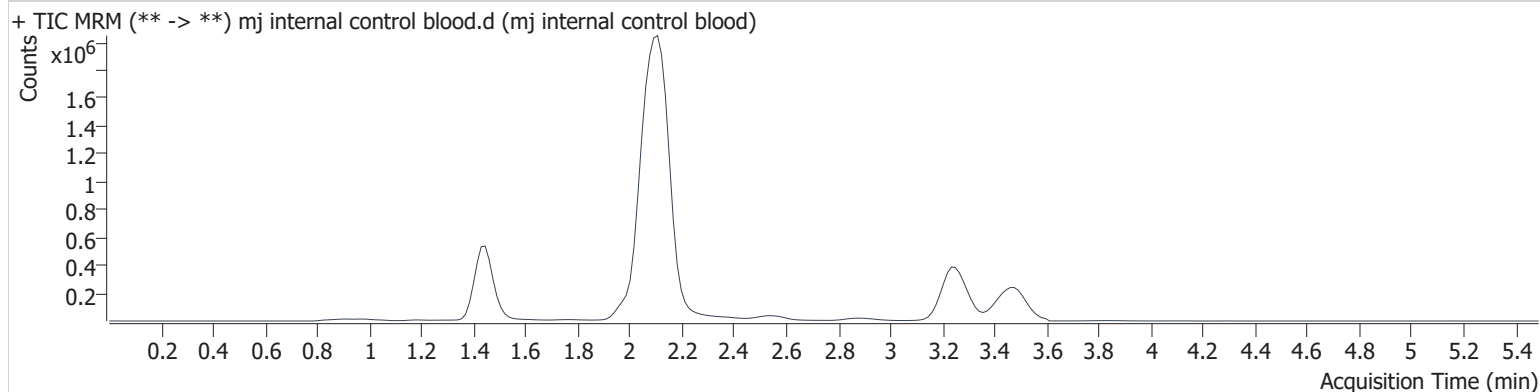
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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	5/28/2021 8:47:17 PM		

Sample Info.

Sample Chromatogram



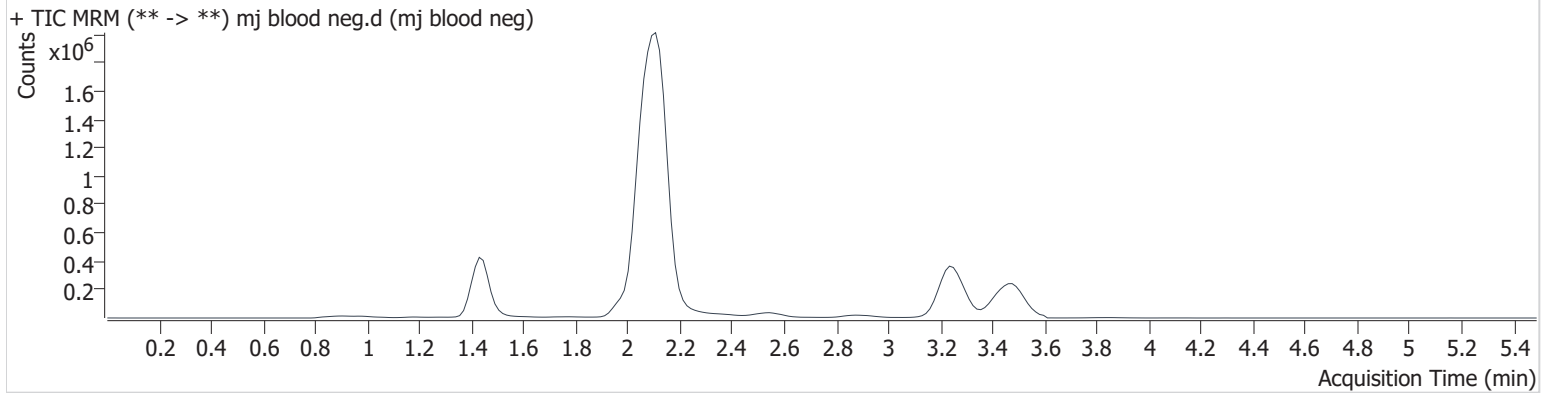
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	232383	∞	9.8	∞	1464247	5.024 ng/ml
THC-COOH	1.461	130860	269023.0	35.6	174.7	620835	14.962 ng/ml
THC	3.287	121743	7029.3	25.7	42958 62635 95557. 0	1076161	4.243 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 AM

Instrument	69679	Data File	mj blood neg.d
Type	Sample	Sample	mj blood neg
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	5/28/2021 8:54:00 PM		
Sample Info.			

Sample Chromatogram

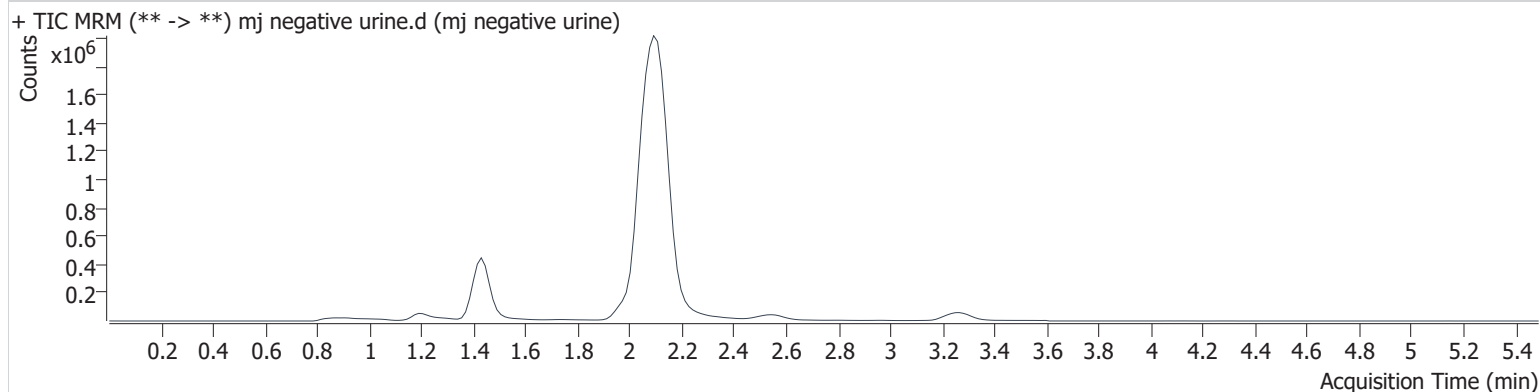


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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-C2	Comment	
Injection Volume	10		
Acq. Date-Time	5/28/2021 9:14:01 PM		
Sample Info.			

Sample Chromatogram



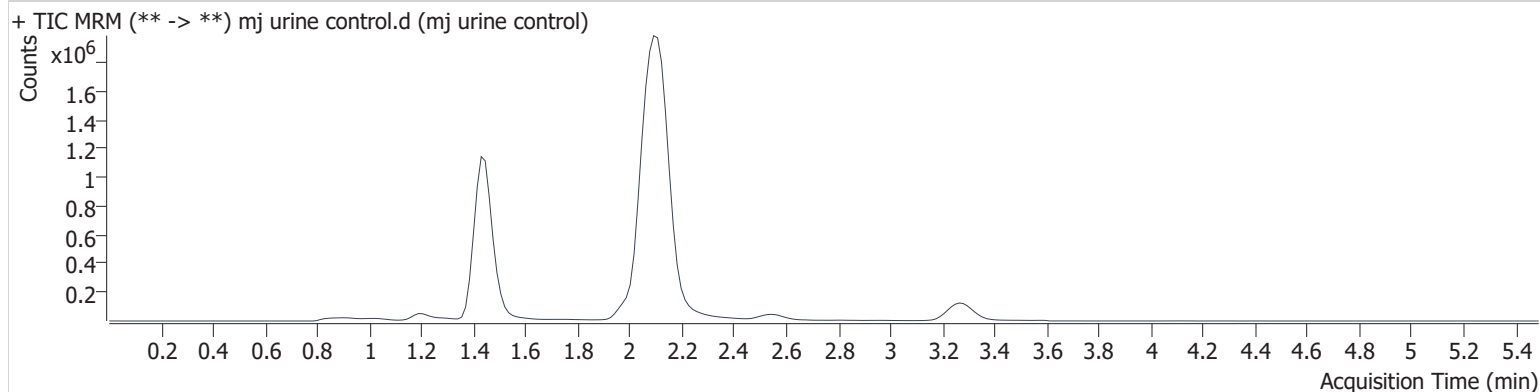
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 AM

Instrument	69679	Data File	mj urine control.d
Type	Sample	Sample	mj urine control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-D2	Comment	
Injection Volume	10		
Acq. Date-Time	5/28/2021 9:20:45 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	2372610	∞	12.4	∞	1625474	47.501 ng/ml
THC-COOH	1.461	311545	625.7	34.6	888.9	550374	38.393 ng/ml
THC	3.287	307224	∞	22.4	∞	484694	22.618 ng/ml

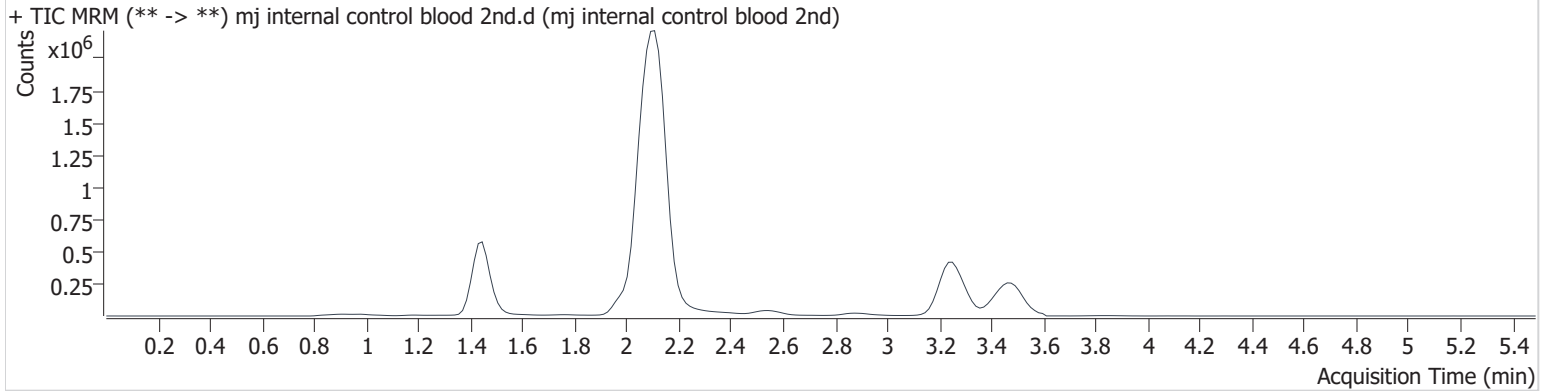
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 AM

Instrument	69679	Data File	mj internal control blood 2nd.d
Type	QC	Sample	mj internal control blood 2nd
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	5/28/2021 10:34:09 PM		

Sample Info.

Sample Chromatogram



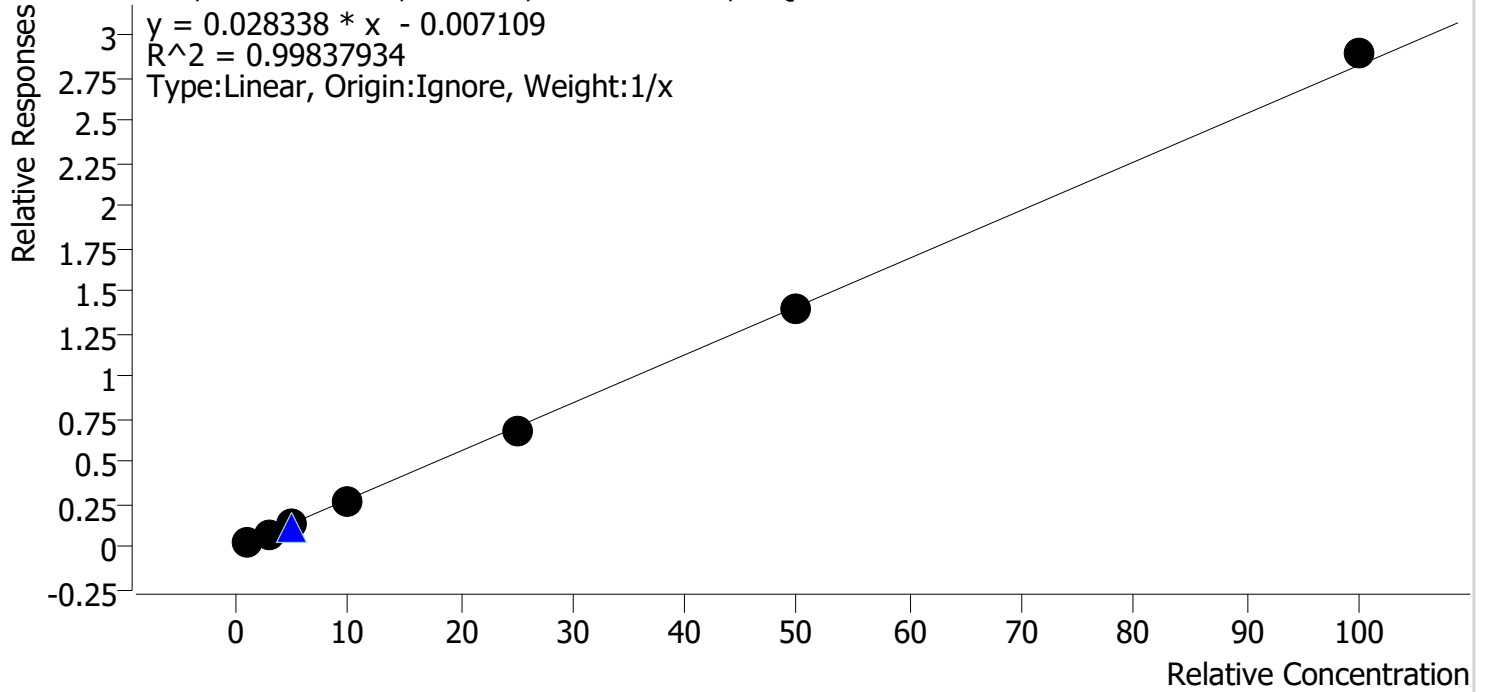
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	254070	∞	9.5	∞	1499314	5.375 ng/ml
THC-COOH	1.461	139641	375853.9	34.2	262.3	659750	15.020 ng/ml
THC	3.287	136214	17308.6	24.1	∞	1208220	4.229 ng/ml

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Last Cal. Update 5/29/2021 9:01 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

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



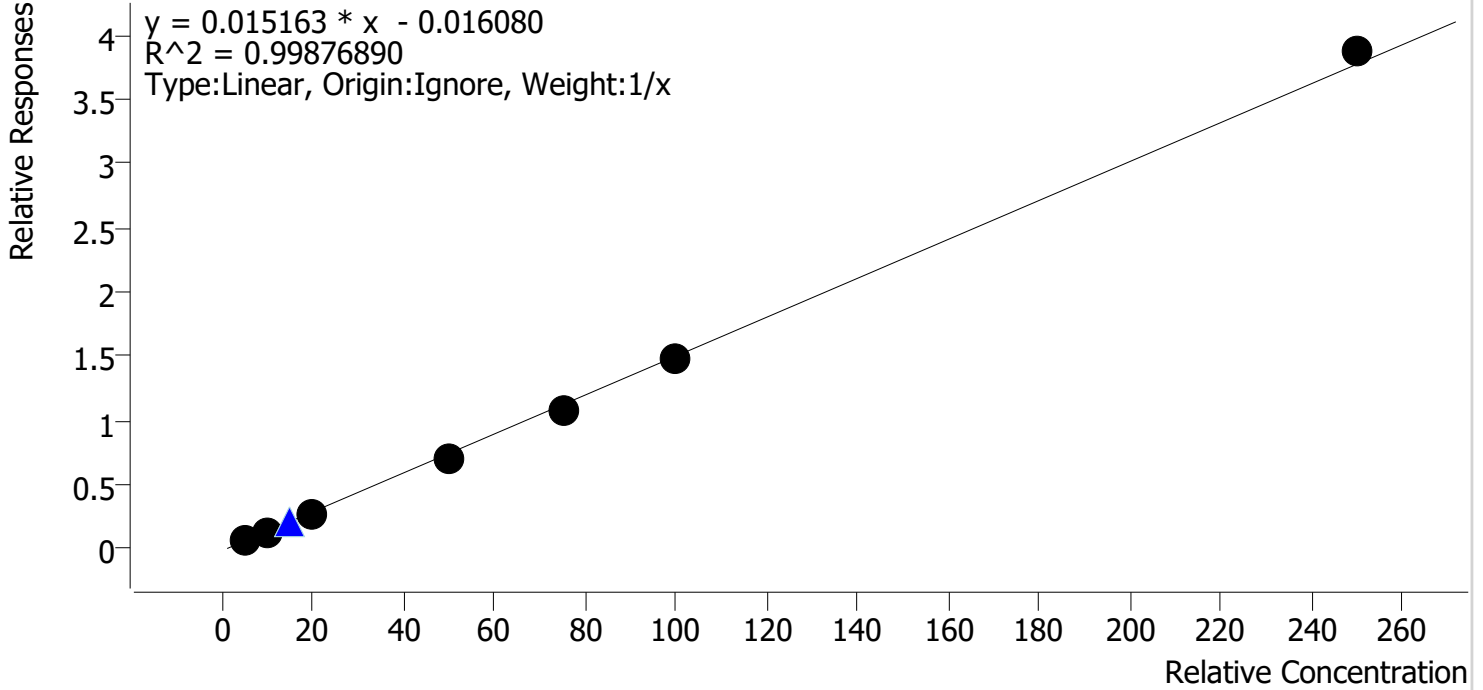
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	1.0	1.2	122.4
mj cal2	2	✓	3.0	2.9	96.5
mj cal 3	3	✓	5.0	4.6	91.8
mj cal 4	4	✓	10.0	9.2	91.7
mj cal 5	5	✓	25.0	23.8	95.4
mj cal 6	6	✓	50.0	49.9	99.7
mj cal 7	7	✓	100.0	102.4	102.4

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Last Cal. Update 5/29/2021 9:01 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	5.6	111.8
mj cal2	2	✓	10.0	9.9	98.9
mj cal 3	3	✓	20.0	19.2	96.0
mj cal 4	4	✓	50.0	47.5	95.1
mj cal 5	5	✓	75.0	72.8	97.0
mj cal 6	6	✓	100.0	98.6	98.6
mj cal 7	7	✓	250.0	256.4	102.6

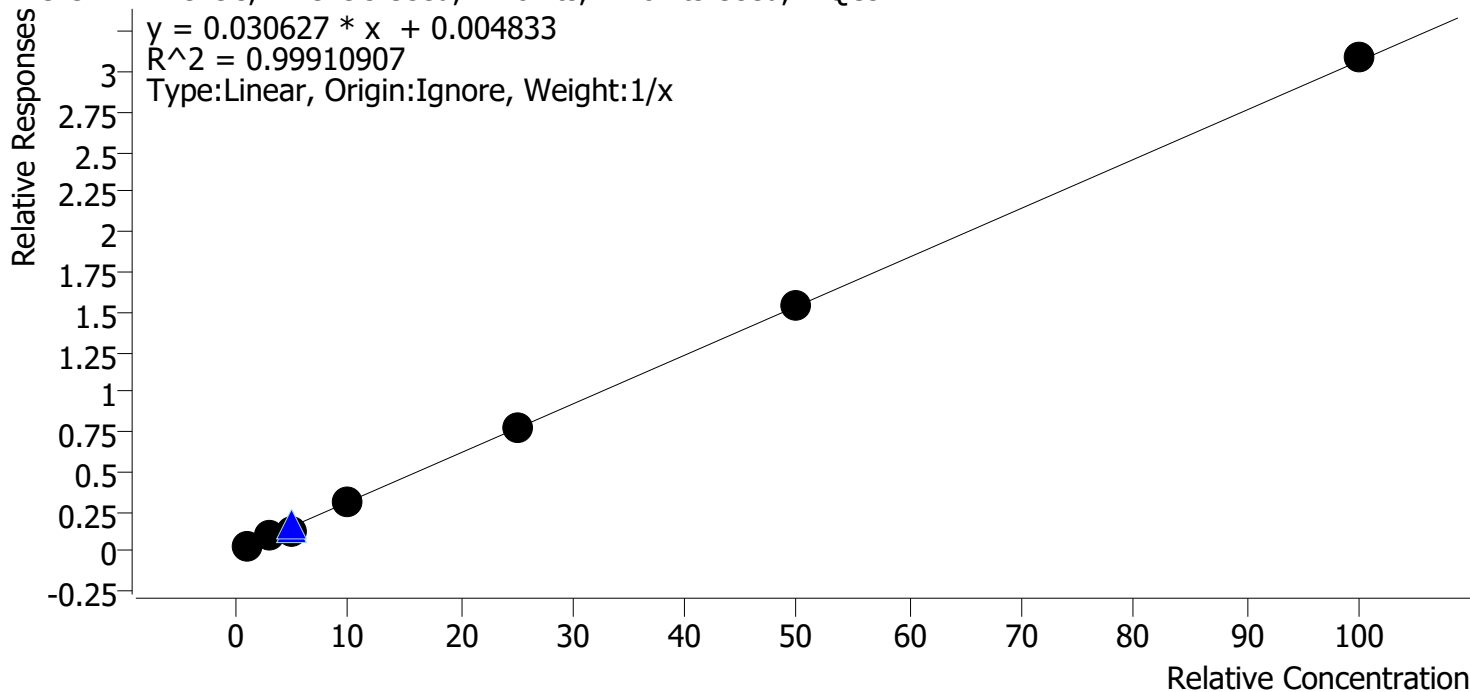
Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Last Cal. Update 5/29/2021 9:01 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	107.2
mj cal2	2	✓	3.0	3.3	108.3
mj cal 3	3	✓	5.0	4.2	84.8
mj cal 4	4	✓	10.0	9.9	99.3
mj cal 5	5	✓	25.0	25.0	100.0
mj cal 6	6	✓	50.0	49.8	99.6
mj cal 7	7	✓	100.0	100.7	100.7

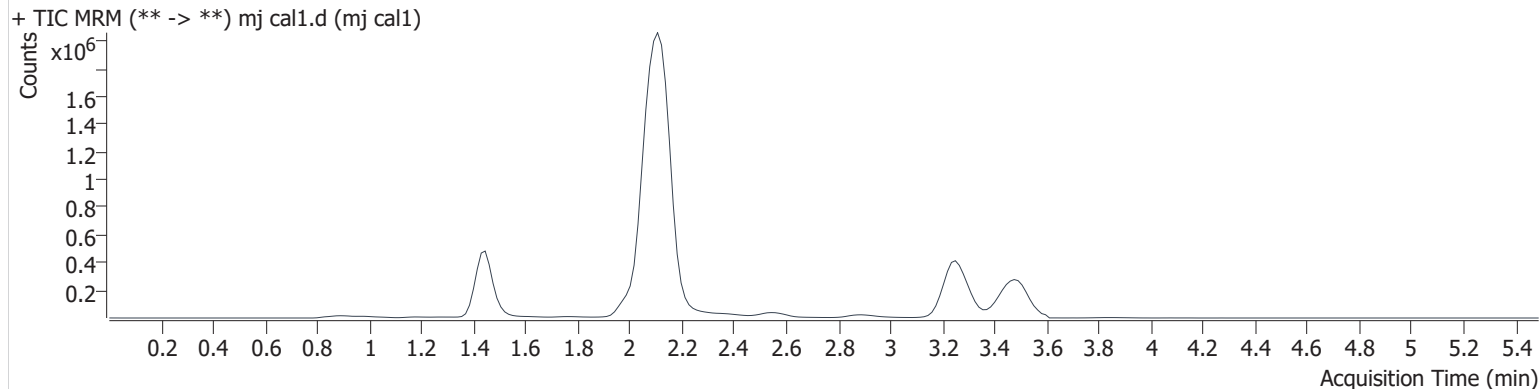
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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	5/28/2021 8:00:21 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.451	56248	∞	9.7	∞	1493281	1.072 ng/ml	Low
THC-COOH	1.476	40041	60.3	34.0	2590.6	582964	5.590 ng/ml	
THC	3.287	29172	559.3	25.0	397.4	1057516	1.224 ng/ml	



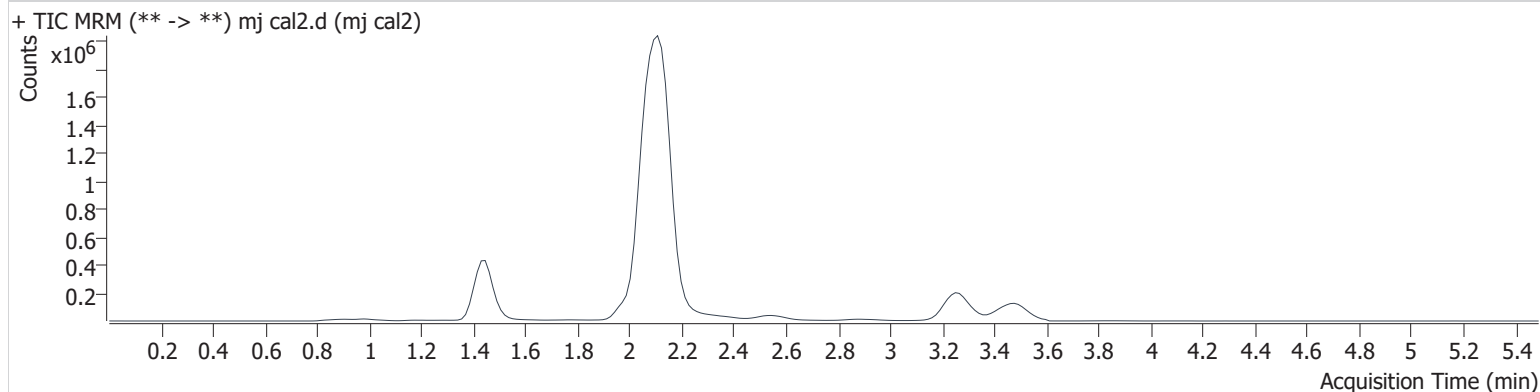
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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	5/28/2021 8:07:05 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	133690	∞	9.3	∞	1280877	3.250 ng/ml
THC-COOH	1.461	79152	27.2	34.4	99.1	591038	9.893 ng/ml
THC	3.287	53683	2559865927 129290.0	23.4	136.4	716036	2.896 ng/ml

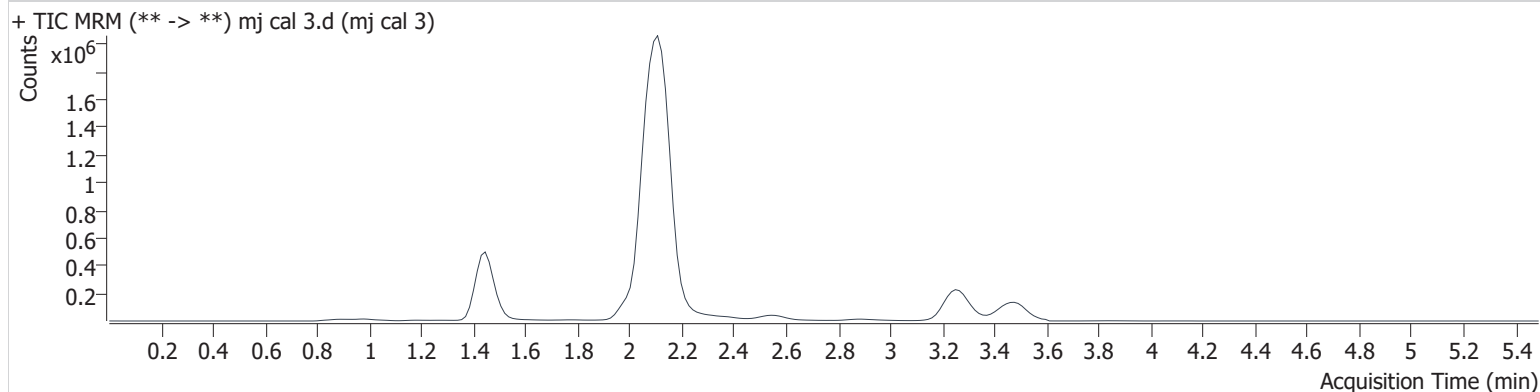
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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	5/28/2021 8:13:47 PM		

Sample Info.

Sample Chromatogram



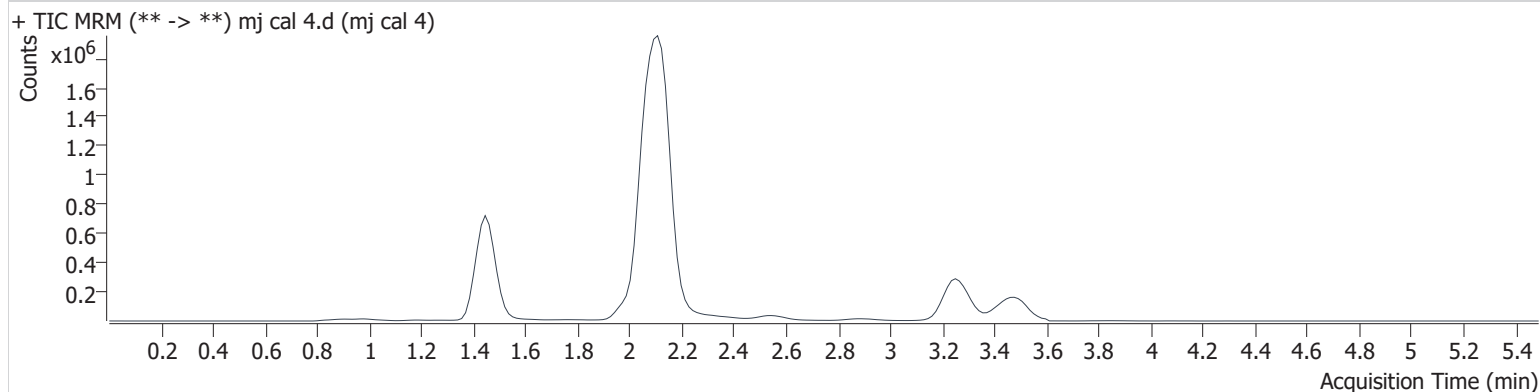
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	171031	∞	11.3	∞	1269543	4.241 ng/ml
THC-COOH	1.461	157257	187.9	35.1	∞	571571	19.206 ng/ml
THC	3.287	92750	1563009025 48820.0	25.3	164.2	754305	4.590 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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	5/28/2021 8:20:29 PM		
Sample Info.			

Sample Chromatogram



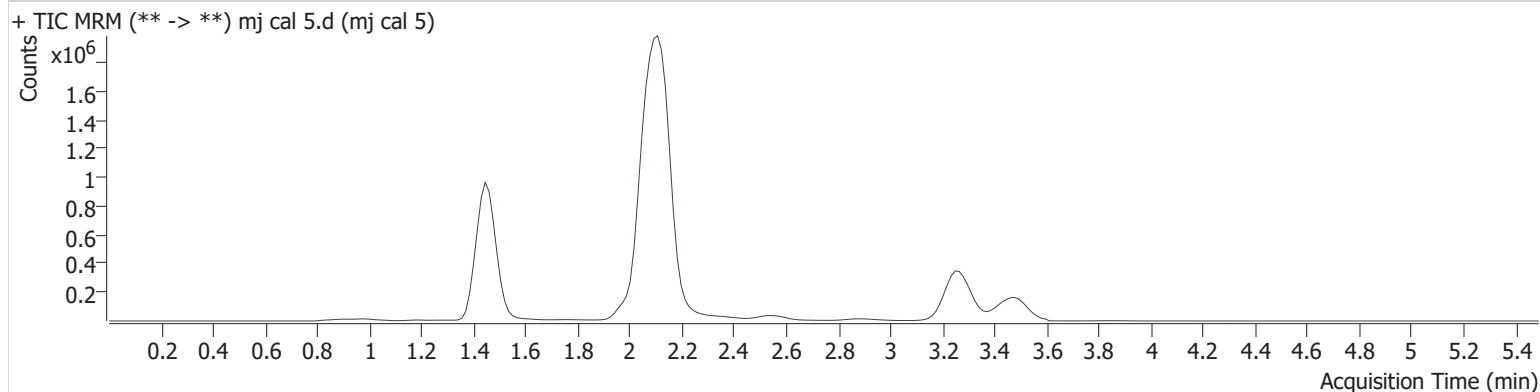
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	443001	∞	10.1	∞	1433263	9.934 ng/ml
THC-COOH	1.461	441782	2707.9	36.1	30283	627007	47.529 ng/ml
THC	3.287	215588	∞	24.3	915.6	852835	9.171 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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	5/28/2021 8:27:11 PM		
Sample Info.			

Sample Chromatogram



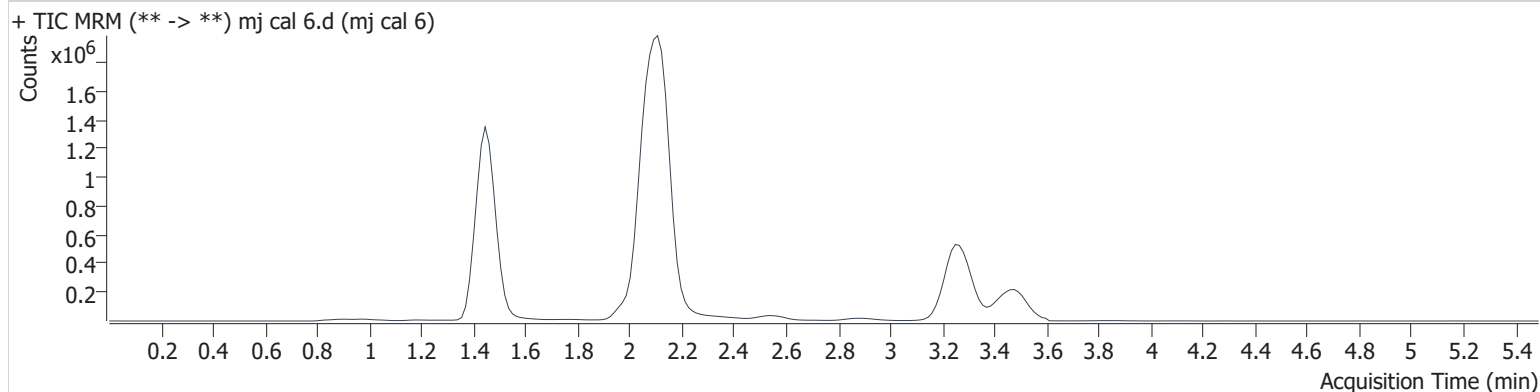
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	1062677	∞	10.7	∞	1379437	24.995 ng/ml
THC-COOH	1.461	668412	3932.4	36.2	3286.5	614863	72.756 ng/ml
THC	3.287	580762	∞	23.8	95281 89398 0879.0	868737	23.841 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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	5/28/2021 8:33:52 PM		
Sample Info.			

Sample Chromatogram



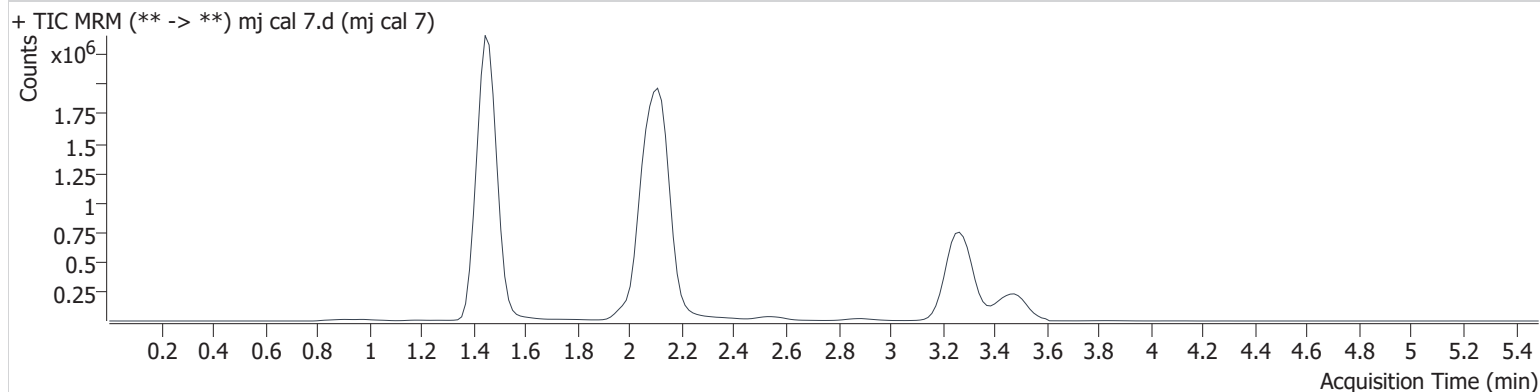
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	2219273	∞	11.5	∞	1450196	49.809 ng/ml
THC-COOH	1.461	899208	931222.3	36.7	601.3	608015	98.598 ng/ml
THC	3.287	1340948	∞	23.3	22247 84665 61468. 0	953665	49.869 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\052821\QuantResults\cann.batch.bin
Calibration Last Update 5/29/2021 9:01:22 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	5/28/2021 8:40:34 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.436	4293965	∞	11.9	∞	1390116	100.699 ng/ml
THC-COOH	1.461	2135422	2885978.5	37.1	16320 32.8	551495	256.428 ng/ml
THC	3.287	2692093	∞	23.7	∞	929924	102.408 ng/ml