

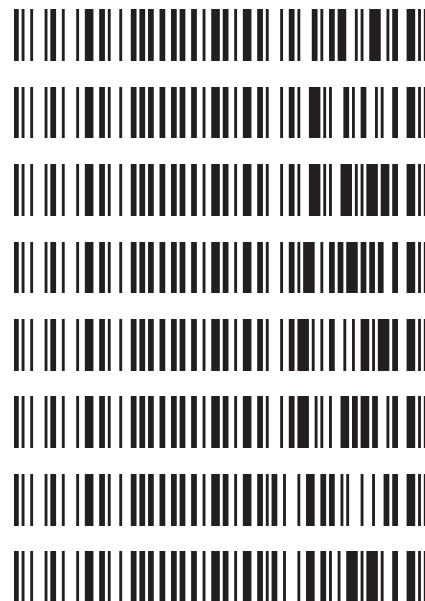
7/29/2021

Worklist: 5134

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2021-1585	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1619	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1622	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1651	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1661	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1671	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1700	3	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-1701	1	BCK	AM 27 Blood THC Quant by LC-QQQ

REVIEWED

By Britany Wylie at 12:21 pm, Jul 29, 2021





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

Extraction Date 7/28/21
Plate lot#: 210412

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

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:**

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.

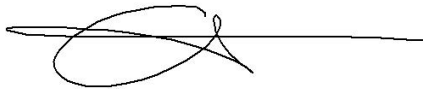
Analytic:

- 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: *blood only run.*



	1	2	3	4	5	6
a	cal 1	neg blood	1651-1			QC 1
b	cal 2	1585-1				cal 100 ng
c	cal 3	1619-1				cal 50 ng
d	cal 4	1622-1				cal 25 ng
e	Cal 5	1661-1				cal 10ng
f	cal 6	1671-1				cal 5 ng
g	cal 7	1700-3				cal 3 ng
h	Internal control	1701-1				cal 1ng

C2021-____-__

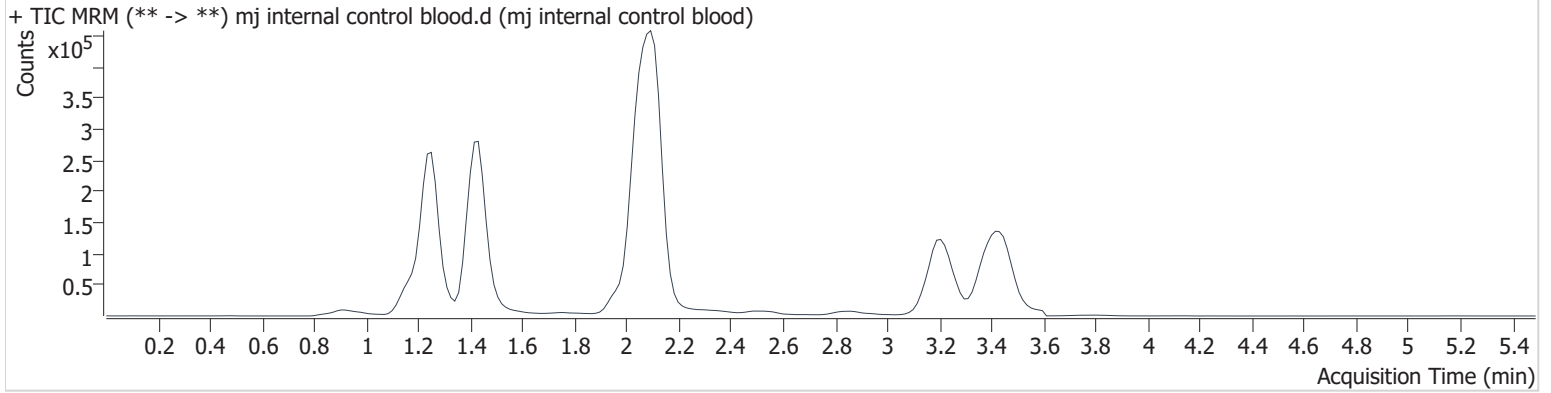
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 6:34:03 PM		

Sample Info.

Sample Chromatogram



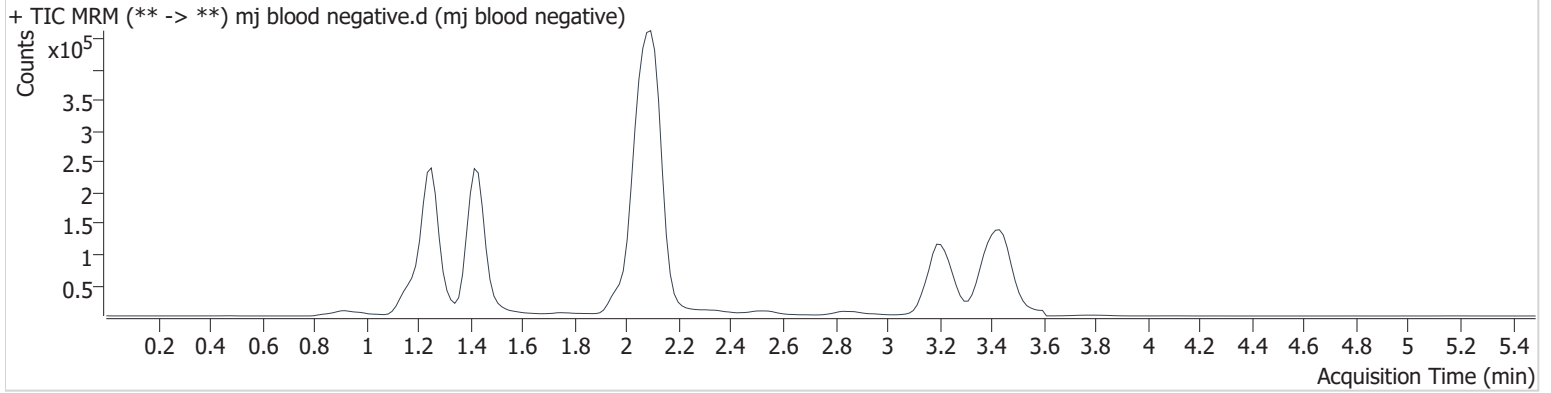
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.424	13648	∞	793.9	208.1	916290	4.820 ng/ml
THC-COOH	1.446	54199	81.1	35.2	278.2	281374	14.709 ng/ml
THC	3.227	42344	∞	24.0	191.6	422713	4.214 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 6:40:45 PM		
Sample Info.			

Sample Chromatogram

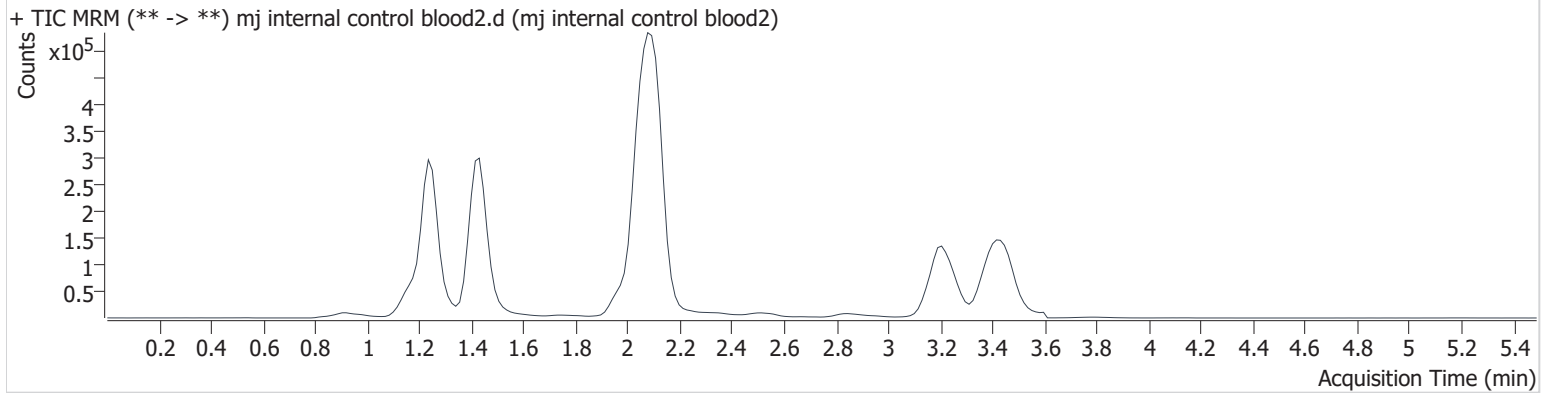


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 8:34:13 PM		
Sample Info.			

Sample Chromatogram

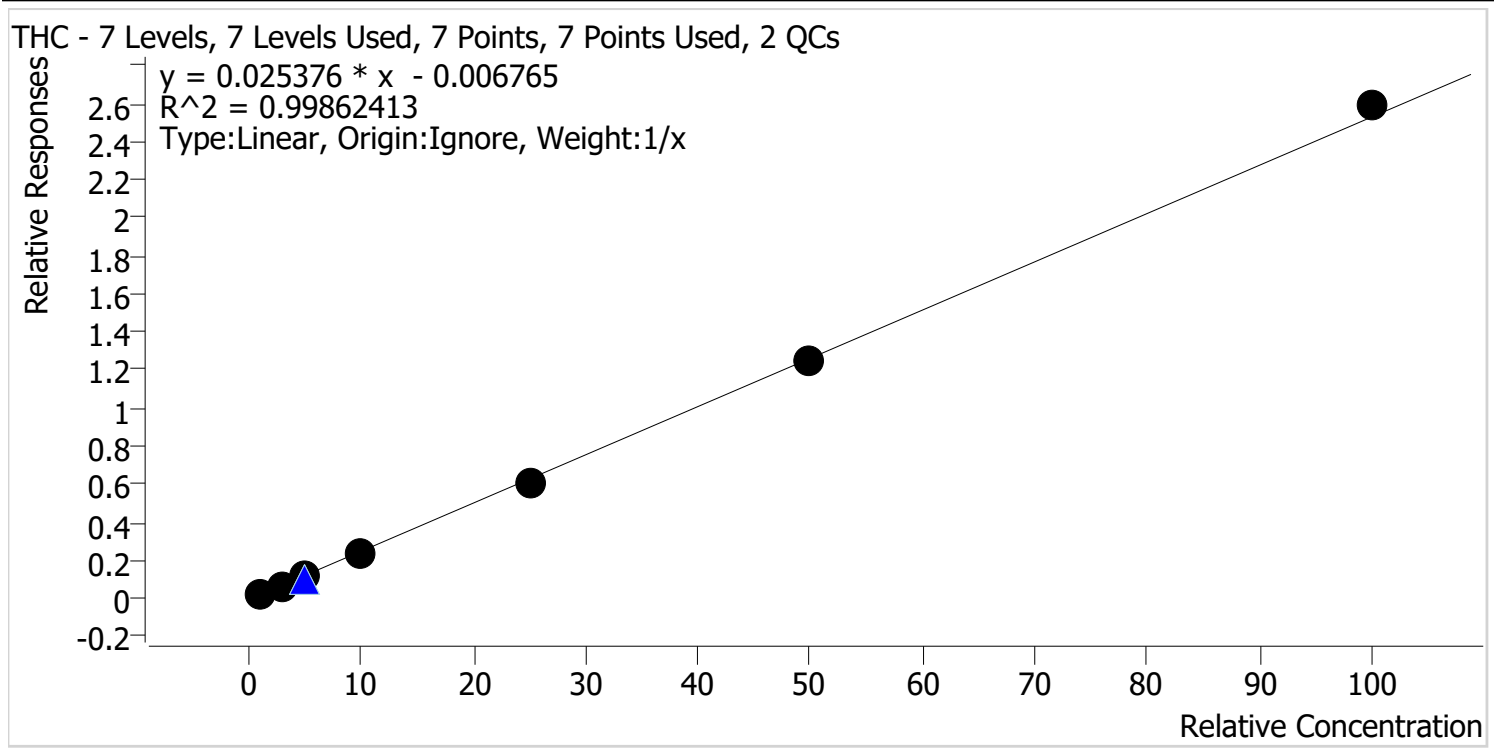


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.424	12872	∞	834.8	∞	922195	4.519 ng/ml
THC-COOH	1.446	52564	406.9	37.9	165.4	285257	14.117 ng/ml
THC	3.242	46541	∞	26.1	∞	467923	4.186 ng/ml

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Last Cal. Update 7/29/2021 8:37 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3



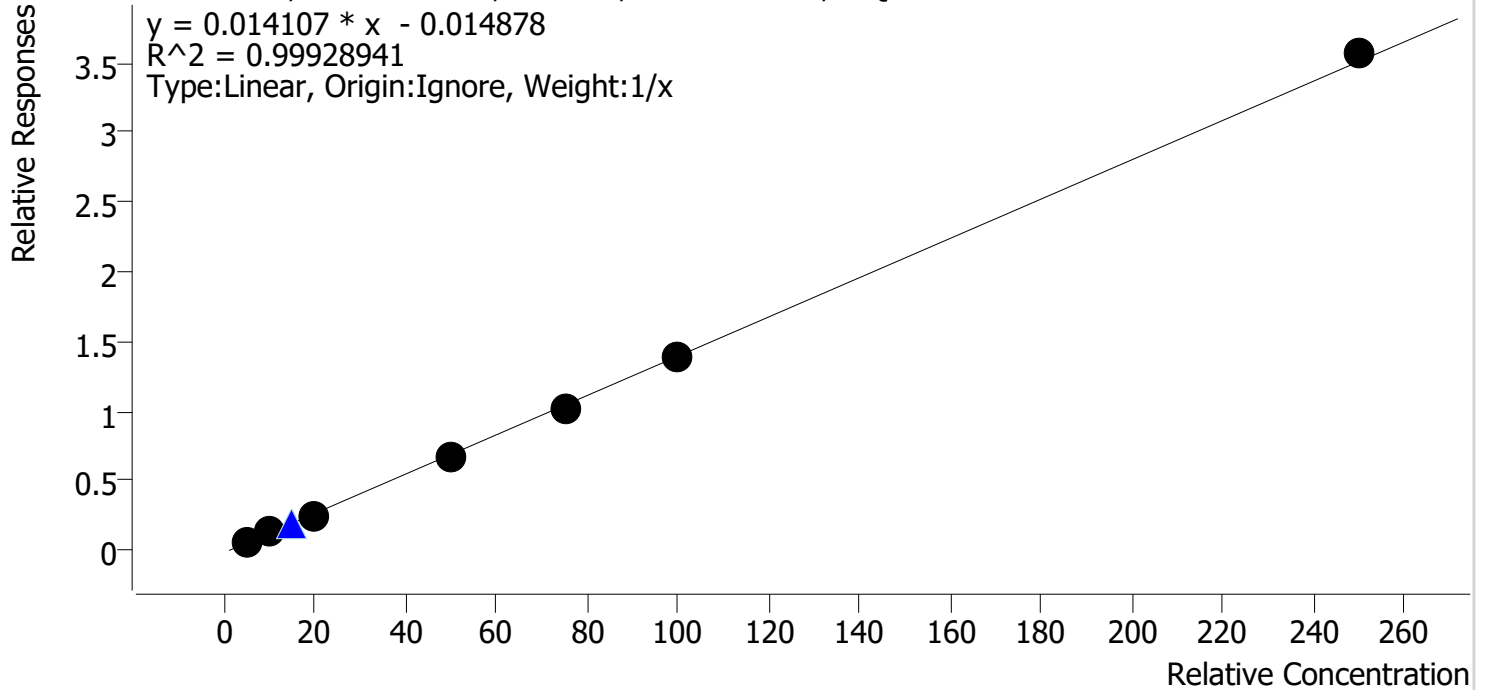
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	1.0	1.2	121.3
mj cal2	2	✓	3.0	2.9	95.0
mj cal 3	3	✓	5.0	4.7	93.8
mj cal 4	4	✓	10.0	9.2	91.9
mj cal 5	5	✓	25.0	24.1	96.4
mj cal 6	6	✓	50.0	49.6	99.3
mj cal 7	7	✓	100.0	102.3	102.3

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Last Cal. Update 7/29/2021 8:37 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



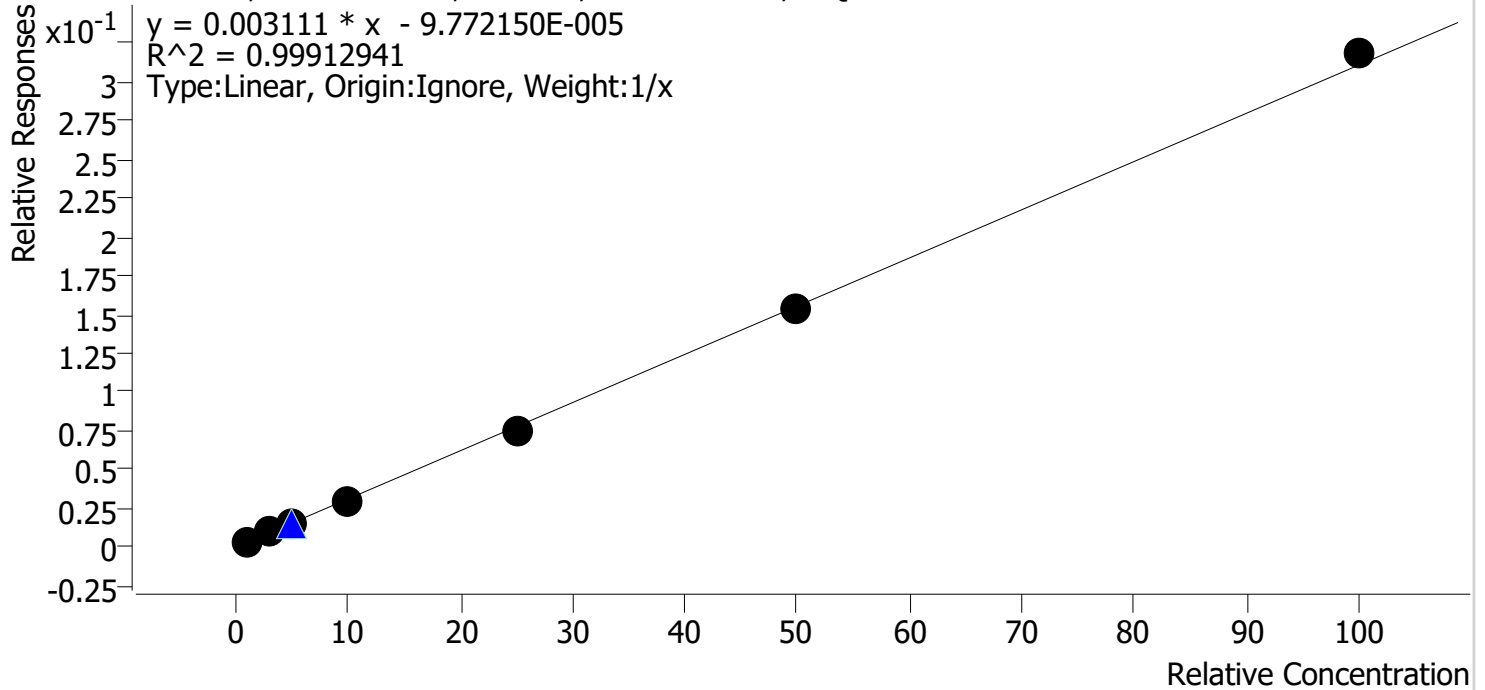
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal1	1	✓	5.0	5.5	110.3
mj cal2	2	✓	10.0	10.0	99.6
mj cal 3	3	✓	20.0	18.6	92.9
mj cal 4	4	✓	50.0	49.2	98.3
mj cal 5	5	✓	75.0	73.2	97.6
mj cal 6	6	✓	100.0	99.6	99.6
mj cal 7	7	✓	250.0	254.0	101.6

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Last Cal. Update 7/29/2021 8:37 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	112.4
mj cal2	2	✓	3.0	2.9	97.8
mj cal 3	3	✓	5.0	4.9	97.0
mj cal 4	4	✓	10.0	9.6	95.9
mj cal 5	5	✓	25.0	24.1	96.3
mj cal 6	6	✓	50.0	49.1	98.1
mj cal 7	7	✓	100.0	102.3	102.3

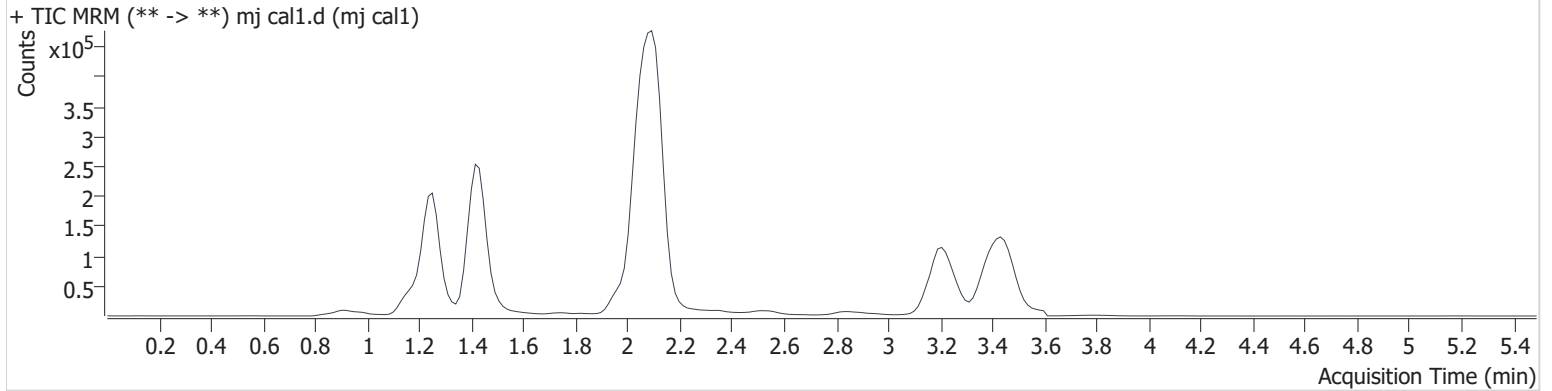
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 5:47:05 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	3223	∞	1002.8	∞	947778	1.124 ng/ml	Low
THC-COOH	1.461	17159	36.4	33.6	54.2	272562	5.517 ng/ml	
THC	3.242	9924	∞	25.0	68.8	413123	1.213 ng/ml	

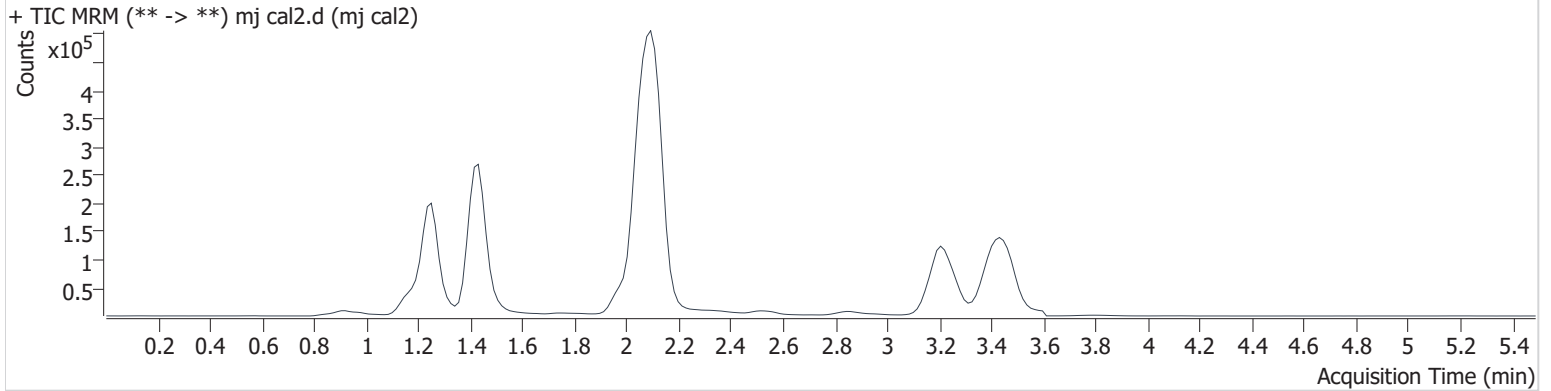
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 5:53:50 PM		

Sample Info.

Sample Chromatogram



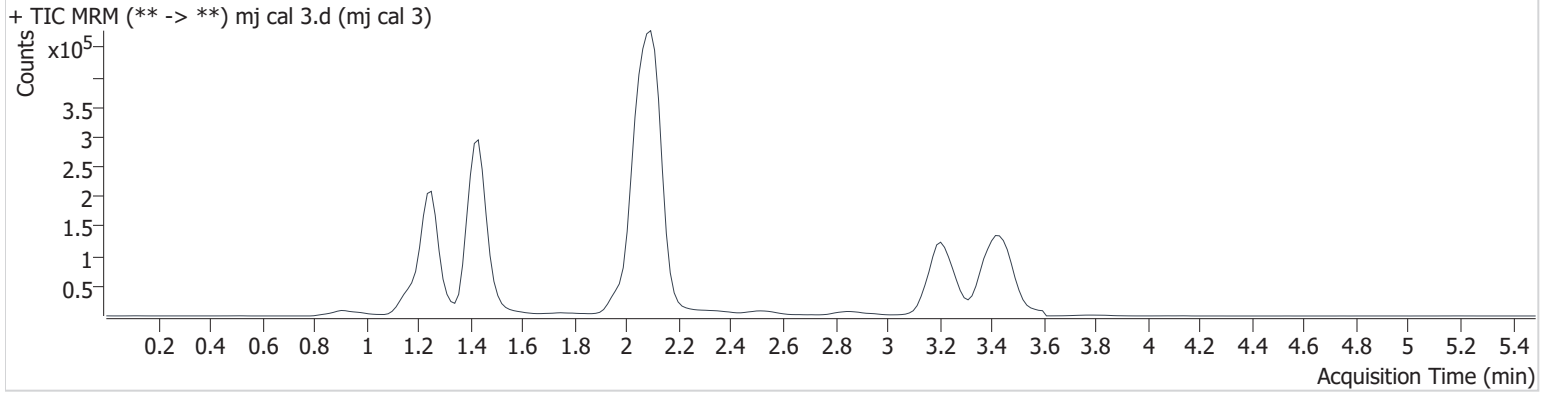
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.424	8187	∞	786.3	154.4	906499	2.935 ng/ml	Low
THC-COOH	1.461	33225	101.5	35.1	11599.0	264486	9.960 ng/ml	
THC	3.242	27239	∞	27.7	∞	415462	2.850 ng/ml	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 6:00:32 PM		
Sample Info.			

Sample Chromatogram



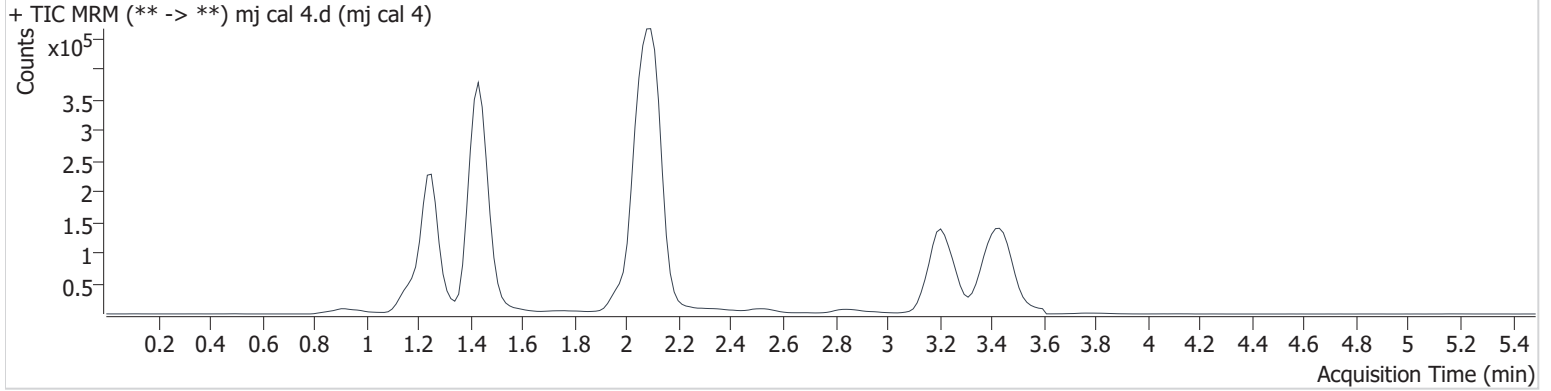
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.424	14085	2522.4	827.8	1435.3	939657	4.850 ng/ml
THC-COOH	1.461	69777	141.1	36.5	15751.4	282165	18.585 ng/ml
THC	3.242	47469	∞	22.5	203.9	422840	4.691 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 6:07:14 PM		
Sample Info.			

Sample Chromatogram



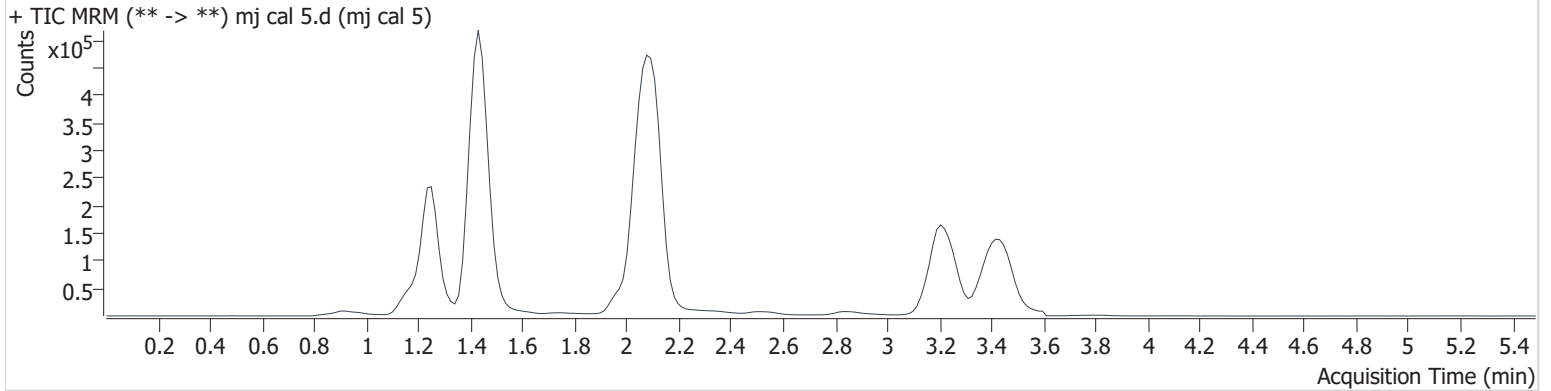
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.424	26766	∞	951.0	∞	900227	9.590 ng/ml
THC-COOH	1.446	186467	1855.2	34.7	285.6	274738	49.168 ng/ml
THC	3.242	97891	∞	23.5	∞	432411	9.188 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 6:13:57 PM		
Sample Info.			

Sample Chromatogram



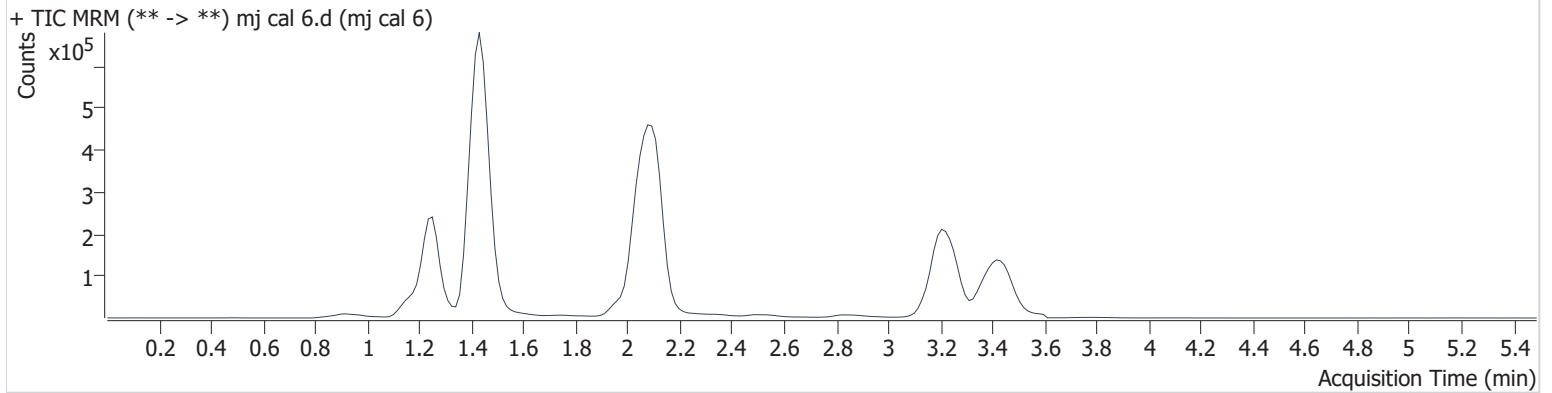
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.424	68855	∞	891.4	∞	920320	24.083 ng/ml
THC-COOH	1.446	279379	628.2	35.8	785.5	274439	73.220 ng/ml
THC	3.242	254645	∞	23.8	∞	421165	24.093 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 6:20:39 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.424	138572	∞	873.0	∞	908375	49.073 ng/ml
THC-COOH	1.446	365686	3367.8	35.9	477.7	263125	99.575 ng/ml
THC	3.242	515720	∞	23.3	∞	411630	49.639 ng/ml

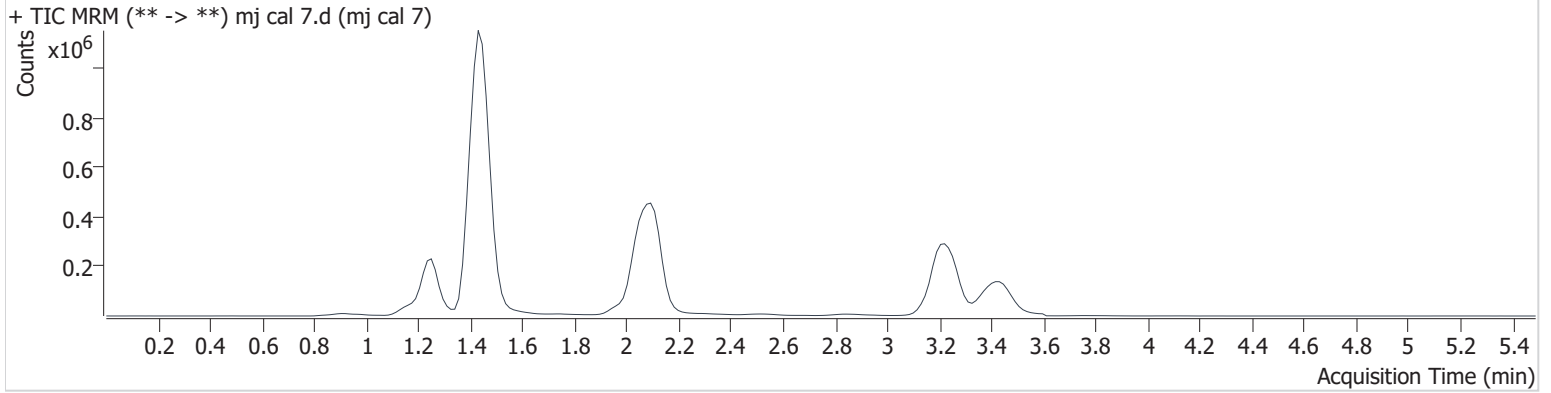
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\072821\QuantResults\cann.batch.bin
Calibration Last Update 7/29/2021 8:37:06 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	7/28/2021 6:27:21 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.424	266699	∞	871.3	12962.0	838008	102.344 ng/ml
THC-COOH	1.461	871596	1411.4	36.6	3155.9	244292	253.975 ng/ml
THC	3.242	964762	∞	23.4	∞	372517	102.326 ng/ml