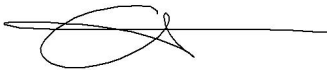


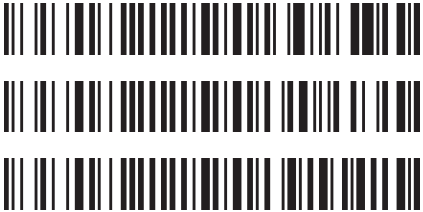
REVIEWED
By Sarah Collins at 9:00 am, Dec 30, 2021



12/29/2021

Worklist: 5485

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2021-2664	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2720		BCK	AM 27 Blood THC Quant by LC-QQQ
C2021-2745		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 12/28/21
Plate lot#: 211018

Analyst: Anne Nord
Plate re-test: 4/18/22

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:**
LCMS-QQQ ID: 69679

Column: UCT Selectra DA 100 x 2.1mm 3um

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.

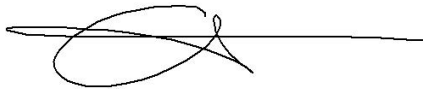
Analytic:

- 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: THC-OH not evaluated in this run, ratios indicate an interferant.



	1	2	3	4	5	6
A	cal 1	Internal control				
B	cal 2	negative blood				
C	cal 3	c2021-2664-2 *				
D	cal 4	c2021-2720-1				
E	Cal 5	c2021-2745-1				
F	cal 6	c2021-2664-2				
G	cal 7					
H	Internal control					

C2021- ____ - _

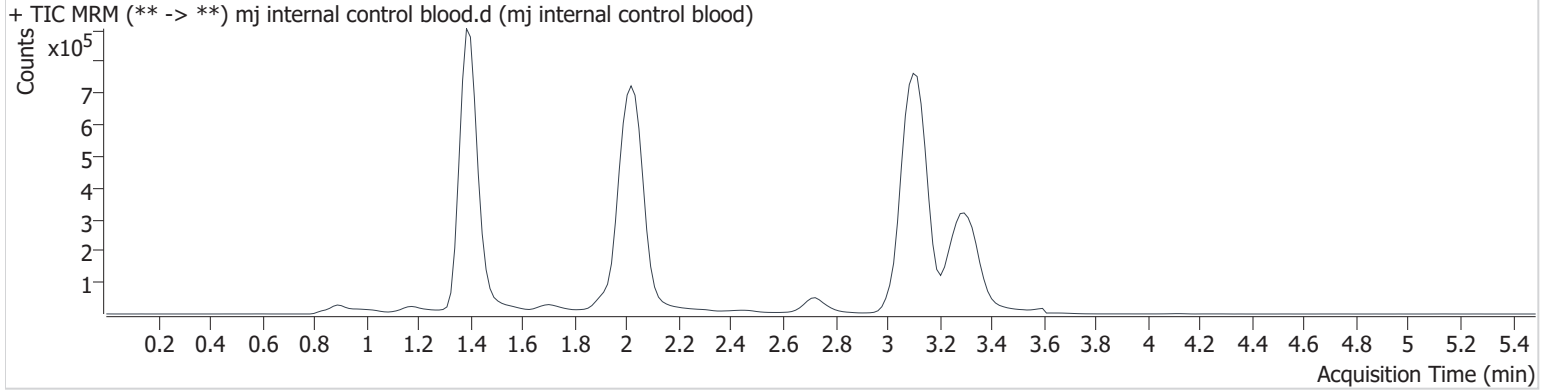
* well clogged, this well was not used, another aliquot was taken and extracted in another well.

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

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	12/28/2021 12:40:26 PM		
Sample Info.			

Sample Chromatogram



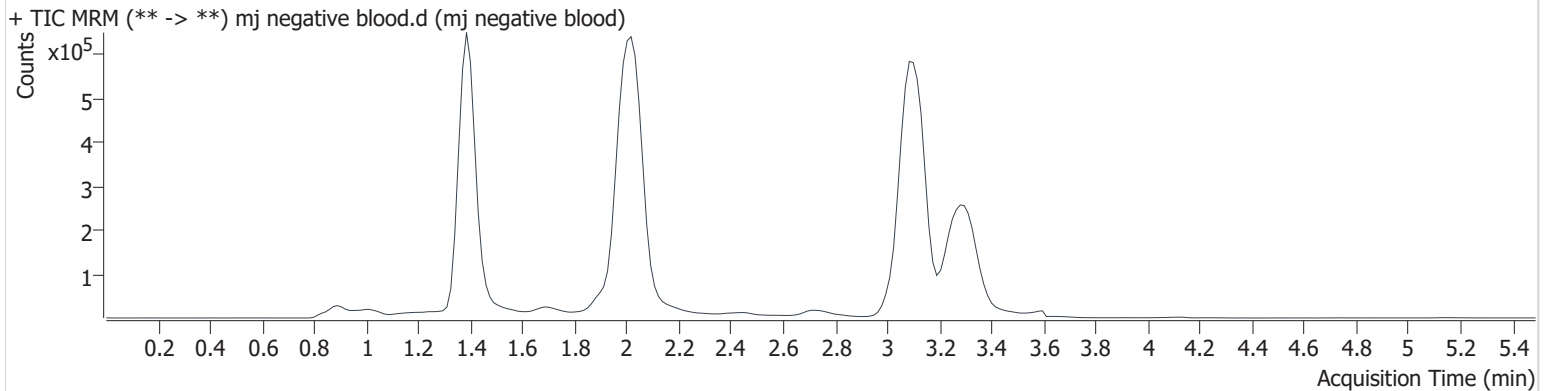
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	41232	346.4	1068.0	31.0	2487748	5.005 ng/ml
THC-COOH	1.418	157391	37.6	36.3	90.1	785166	14.869 ng/ml
THC	3.131	426771	1933.1	23.8	220.3	3678185	4.700 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM


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	12/28/2021 12:47:10 PM		
Sample Info.			

Sample Chromatogram

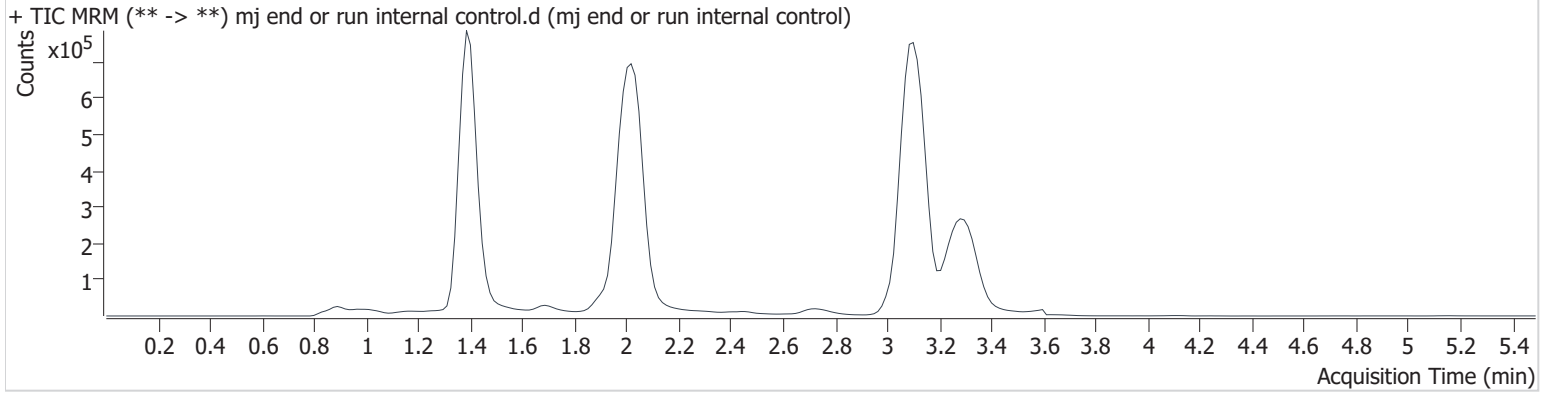


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

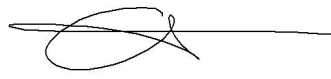
Instrument	69679	Data File	mj end or run internal control.d
Type	Sample	Sample	mj end or run internal control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord  12/29/21
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	12/28/2021 1:33:59 PM		
Sample Info.			

Sample Chromatogram



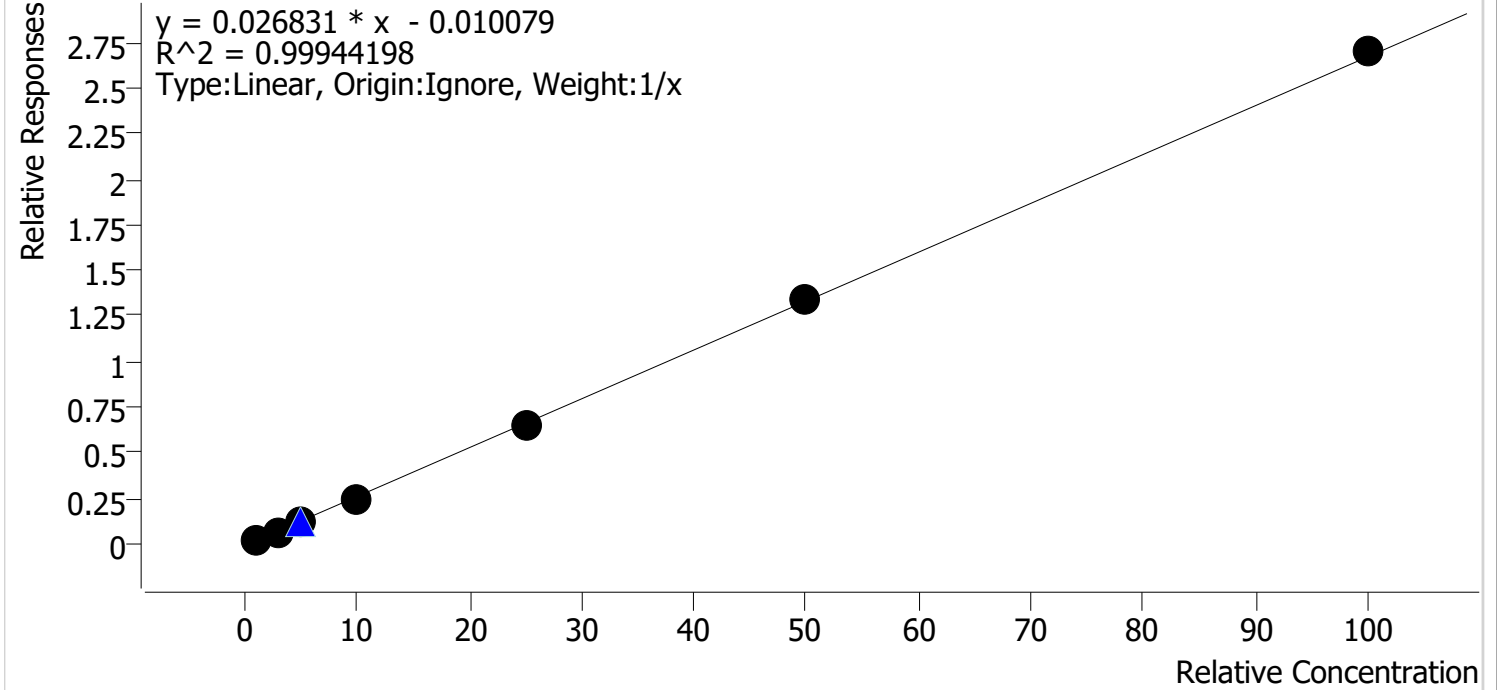
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	37501	86.1	940.7	127.0	2140859	5.293 ng/ml
THC-COOH	1.416	122024	27.1	43.0	51.2	784771	11.812 ng/ml
THC	3.119	419963	1335.7	26.0	201.2	3697015	4.609 ng/ml

Compound Calibration Report



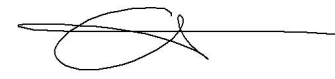
Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Last Cal. Update 12/28/2021 2:17 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



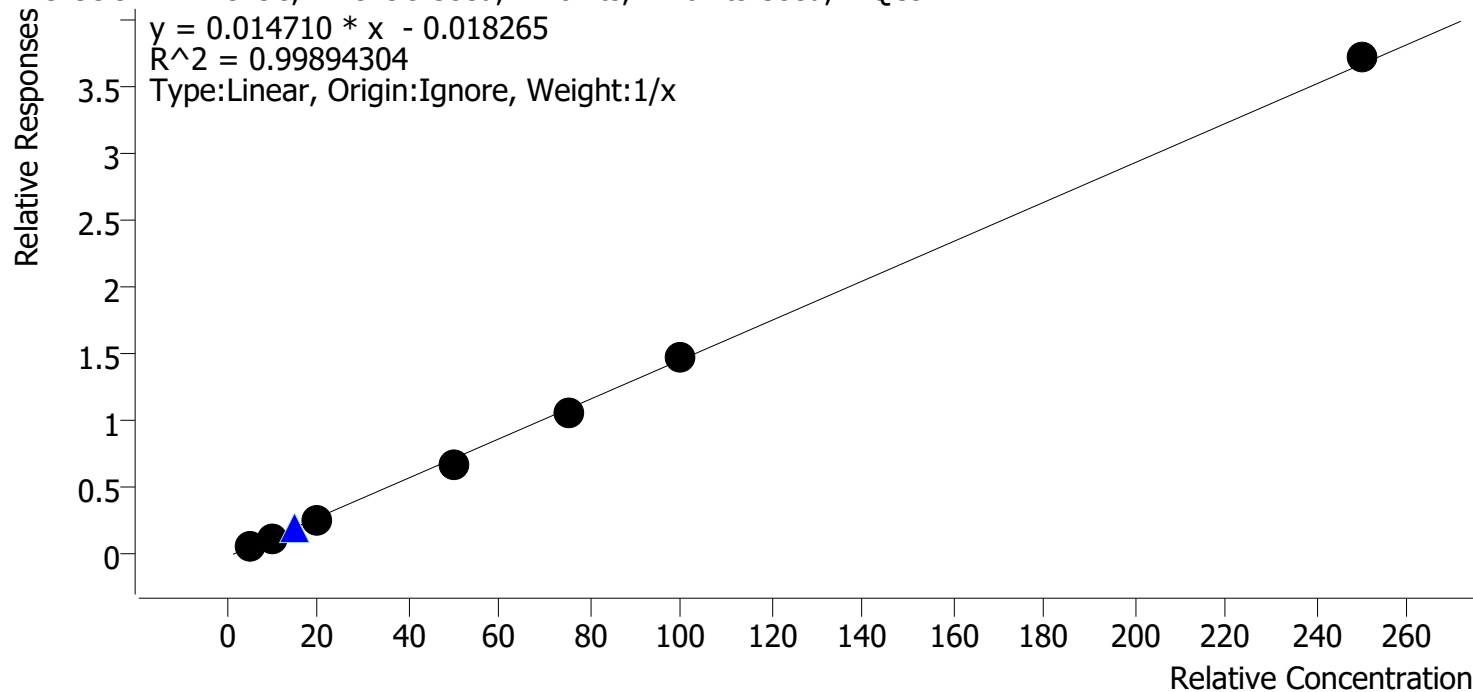
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	115.7
mj cal2	2	✓	3.0	2.9	96.4
mj cal 3	3	✓	5.0	4.6	92.5
mj cal 4	4	✓	10.0	9.6	95.8
mj cal 5	5	✓	25.0	24.6	98.3
mj cal 6	6	✓	50.0	50.1	100.3
mj cal 7	7	✓	100.0	101.0	101.0

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Last Cal. Update 12/28/2021 2:17 PM
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



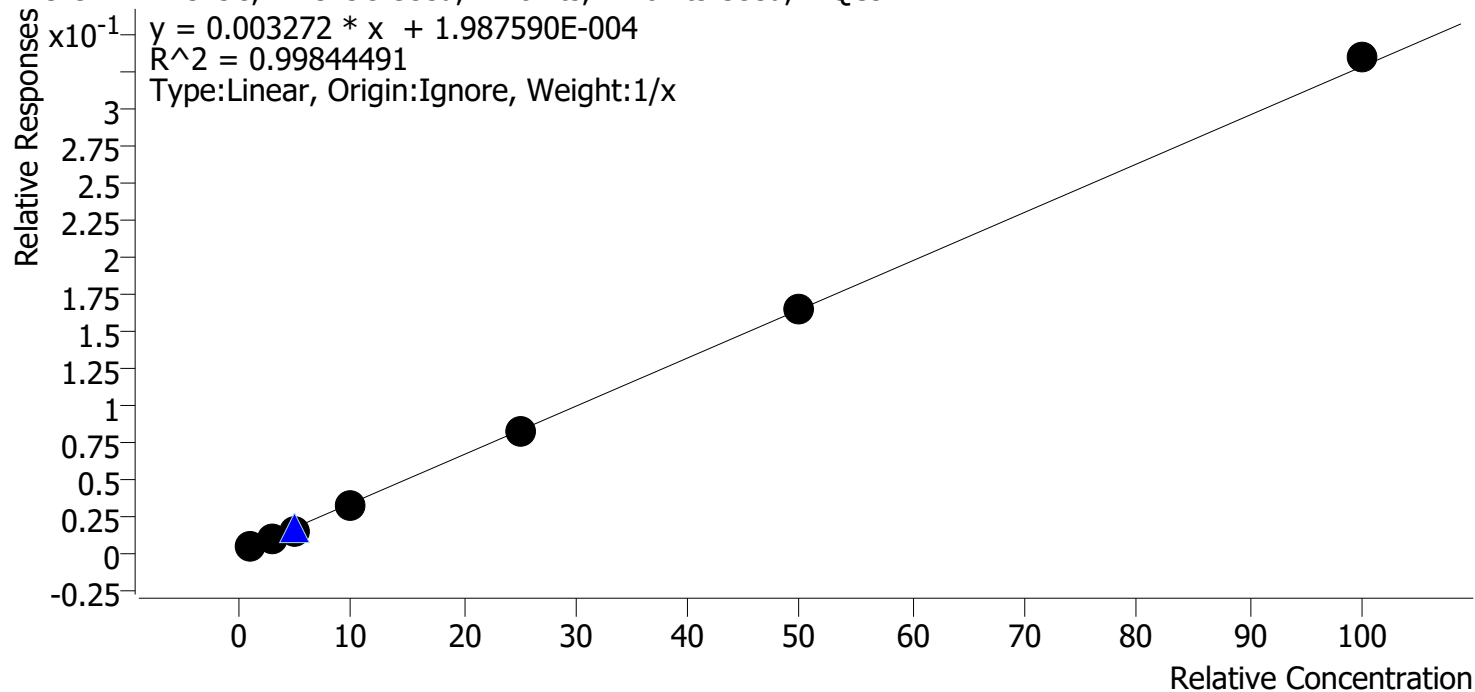
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.6	112.2
mj cal2	2	✓	10.0	9.8	98.3
mj cal 3	3	✓	20.0	18.8	94.1
mj cal 4	4	✓	50.0	47.1	94.1
mj cal 5	5	✓	75.0	73.7	98.3
mj cal 6	6	✓	100.0	101.6	101.6
mj cal 7	7	✓	250.0	253.3	101.3

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Last Cal. Update 12/28/2021 2:17 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.3	126.3
mj cal2	2	✓	3.0	2.8	93.2
mj cal 3	3	✓	5.0	4.5	89.1
mj cal 4	4	✓	10.0	9.2	91.7
mj cal 5	5	✓	25.0	24.5	97.9
mj cal 6	6	✓	50.0	50.0	100.0
mj cal 7	7	✓	100.0	101.8	101.8

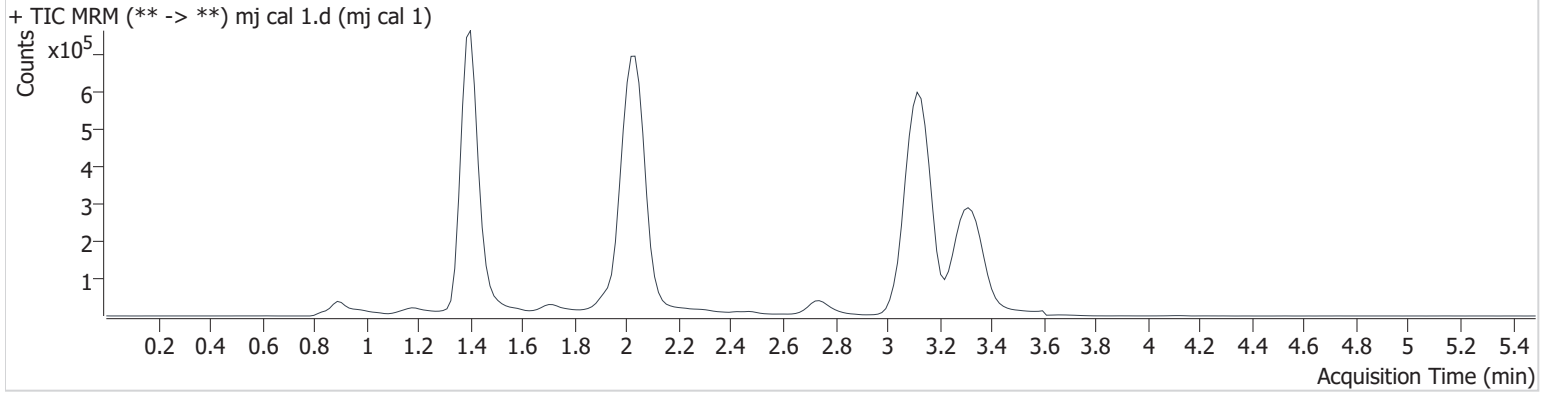
not evaluated due to ratios decreasing as calibrators increased, indicating an interferant.

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

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 12/28/2021 11:46:51 AM
Sample Info.

Sample Chromatogram



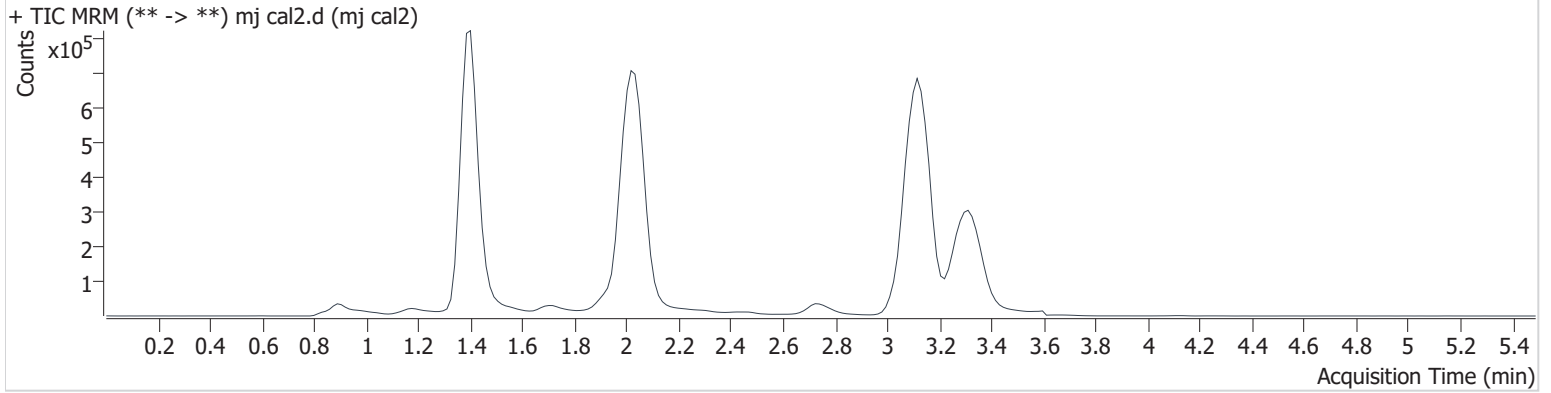
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.424	10600	26.0	1485.4 High	11.1	2446928	1.263 ng/ml Low
THC-COOH	1.426	46151	14.1	31.8	19.8	718222	5.610 ng/ml
THC	3.149	63422	185.9	23.9	71.0	3026933	1.157 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

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	12/28/2021 11:53:35 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	23154	107.2	1208.4 High	18.3	2477213	2.796 ng/ml Low
THC-COOH	1.423	91262	32.3	38.7	146.3	722780	9.825 ng/ml
THC	3.143	224199	746.8	24.7	135.0	3319619	2.893 ng/ml

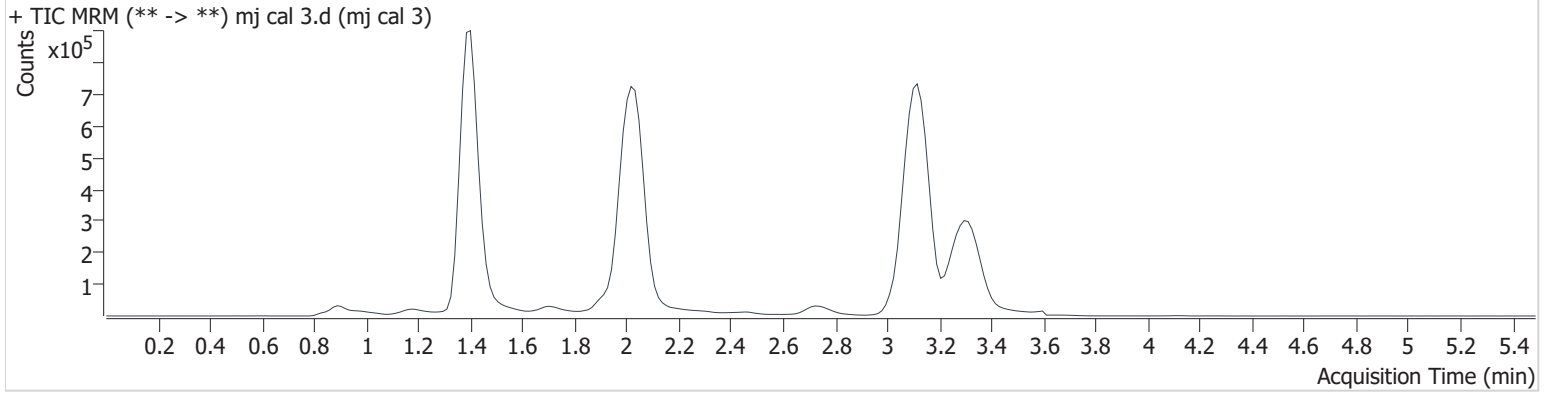
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

Instrument 69679
Type Cal
Acq. Method AM 27 THC quant.m
Sample Position P3-C1
Injection Volume 10
Acq. Date-Time 12/28/2021 12:00:17 PM
Sample Info.

Data File mj cal 3.d
Sample mj cal 3
Operator Anne Nord
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	37362	70.3	1121.5	32.5	2529712	4.453 ng/ml
THC-COOH	1.422	194848	74.3	38.7	134.7	753174	18.828 ng/ml
THC	3.136	399536	1105.0	23.7	157.8	3502895	4.627 ng/ml

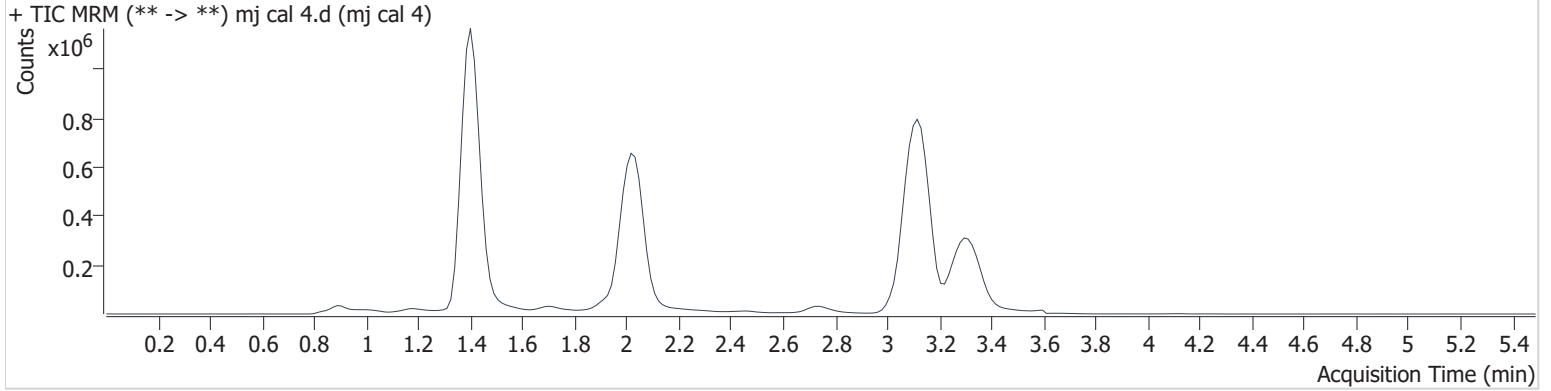
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

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	12/28/2021 12:06:59 PM		

Sample Info.

Sample Chromatogram



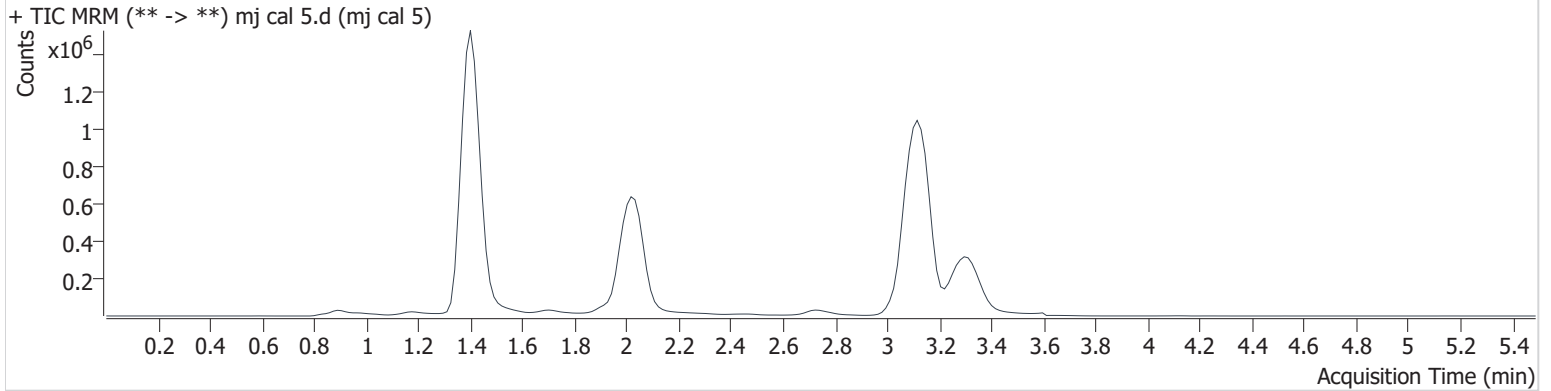
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	75857	267.9	1002.8	66.1	2511731	9.169 ng/ml
THC-COOH	1.421	515805	101.5	37.1	569.8	765188	47.066 ng/ml
THC	3.137	837534	∞	23.0	939.7	3393123	9.575 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

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	12/28/2021 12:13:42 PM		
Sample Info.			

Sample Chromatogram



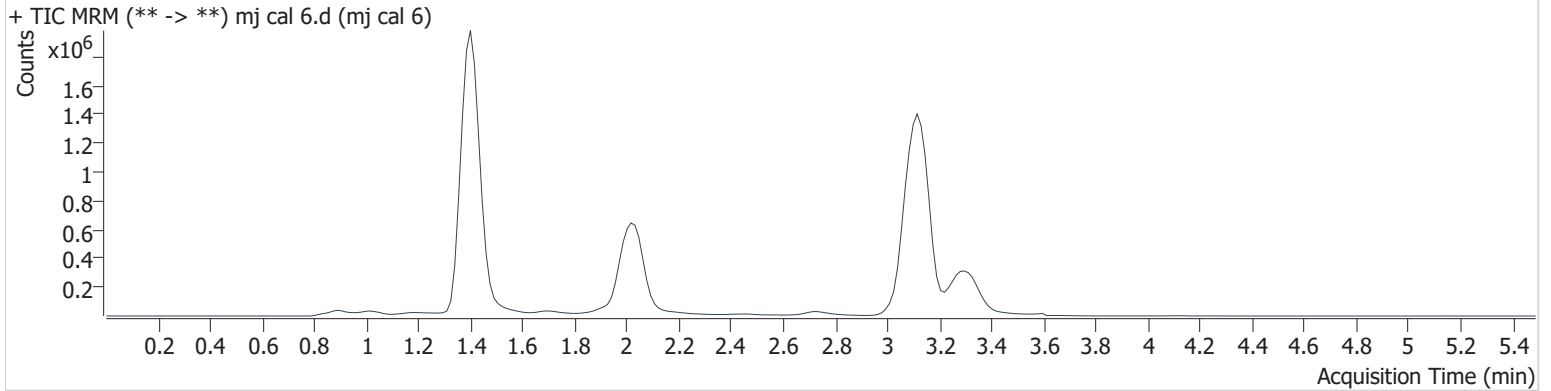
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	196329	740.6	911.9	172.7	2446153	24.469 ng/ml
THC-COOH	1.421	773528	215.6	37.3	961.8	725230	73.748 ng/ml
THC	3.135	2204588	14324.7	23.8	1478.1	3394780	24.579 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

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 12/28/2021 12:20:24 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	378363	469.3	876.0	307.2	2309771	50.003 ng/ml
THC-COOH	1.421	988709	134.2	37.3	730.5	669493	101.634 ng/ml
THC	3.128	4087198	12326.4	24.5	898.4	3060768	50.145 ng/ml

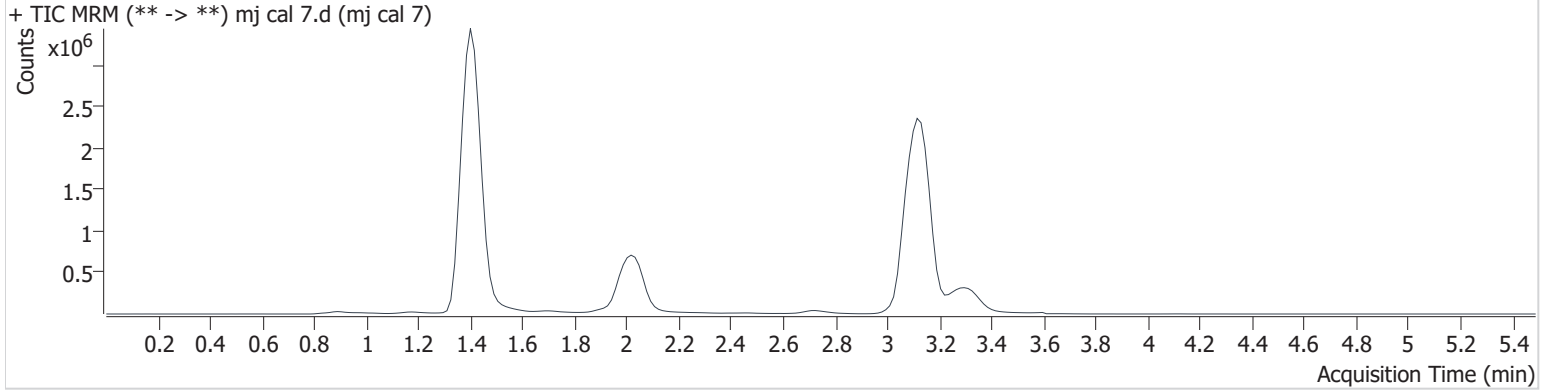
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\122821\QuantResults\cann.batch.bin
Calibration Last Update 12/28/2021 2:17:11 PM

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	12/28/2021 12:27:06 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.394	773832	1709.3	852.1	478.2	2320758	101.846 ng/ml
THC-COOH	1.420	2399800	549.5	37.9	1991.3	647249	253.289 ng/ml
THC	3.131	9065855	11073.6	24.6	8985.7	3357105	101.025 ng/ml