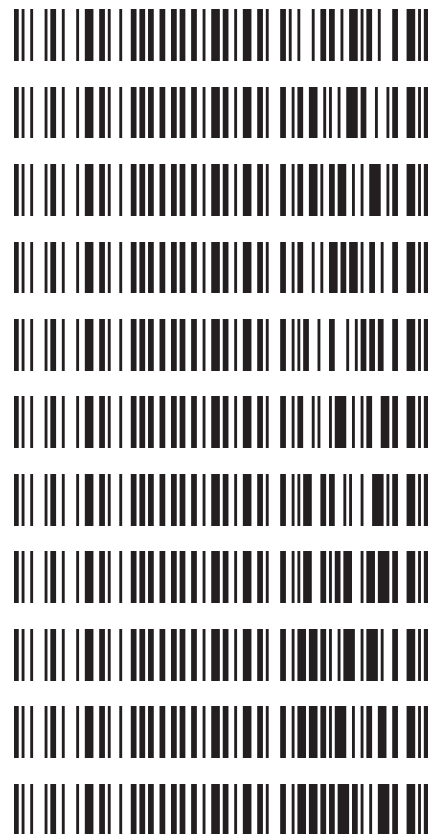


4/14/2022

**Worklist: 5781**

REVIEWED  
By Brittany Wolfe at 11:22 am, Apr 18, 2022

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2022-0478	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0636	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0686	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0706	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0730	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0767	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0792	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0793	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0800	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0801	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0804	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 4/13/22  
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:** 22B52020 **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.

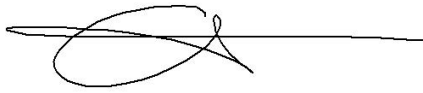
### Analytic:

- 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  $\geq 0.98$  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	Internal control	793-1			
b	cal 2	negative blood	800-1			
c	cal 3	478-1	801-1			
d	cal 4	686-1	804-1			
e	Cal 5	706-1	636-1			
f	cal 6	730-1				
g	cal 7	767-2				
h	Internal control (blood)	792-1				

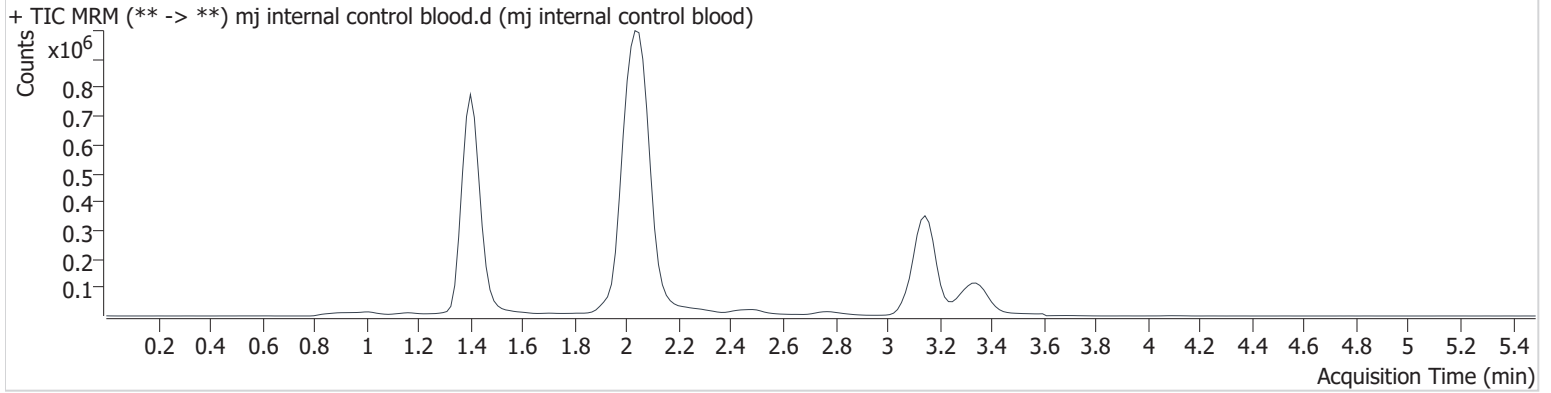
c2022-0\_\_\_\_-\_\_

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj internal control blood.d
<b>Type</b>	QC	<b>Sample</b>	mj internal control blood
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-H1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 11:24:25 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



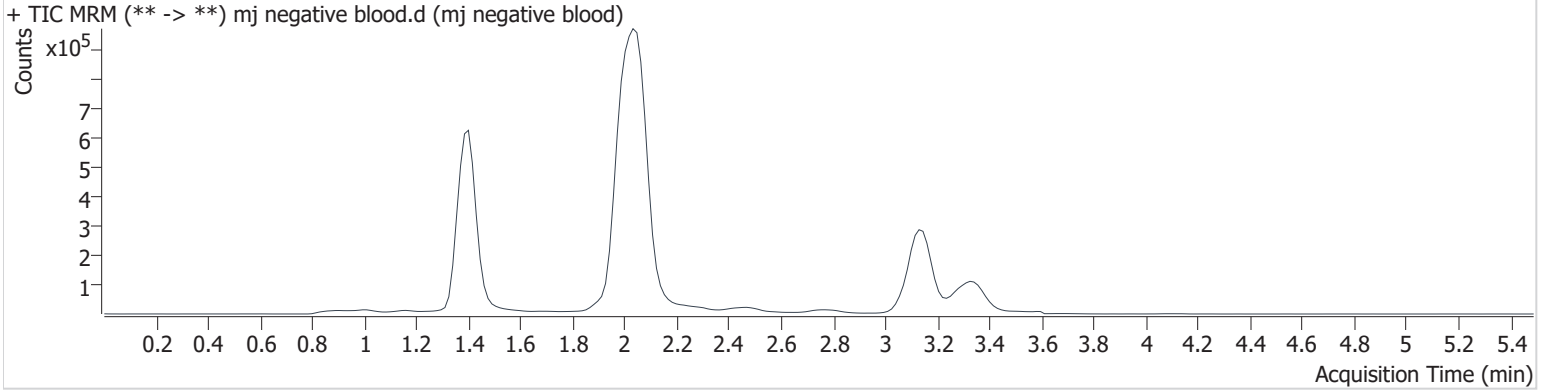
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	321697	∞	12.3	11829.0	2339382	5.295 ng/ml
THC-COOH	1.431	138315	1423.7	38.1	∞	605034	15.966 ng/ml
THC	3.167	171857	∞	25.4	∞	1468791	4.541 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj negative blood.d
<b>Type</b>	Sample	<b>Sample</b>	mj negative blood
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-B2	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 11:31:09 AM		
<b>Sample Info.</b>			

## Sample Chromatogram

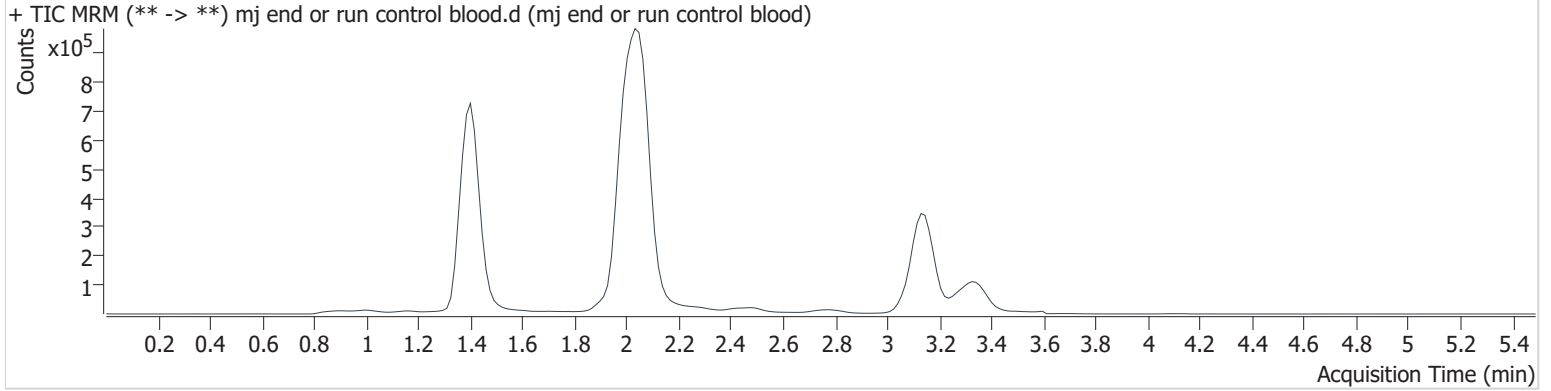


# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

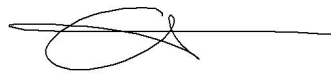
<b>Instrument</b>	69679	<b>Data File</b>	mj end or run control blood.d
<b>Type</b>	Sample	<b>Sample</b>	mj end or run control blood
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-A2	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 2:04:52 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



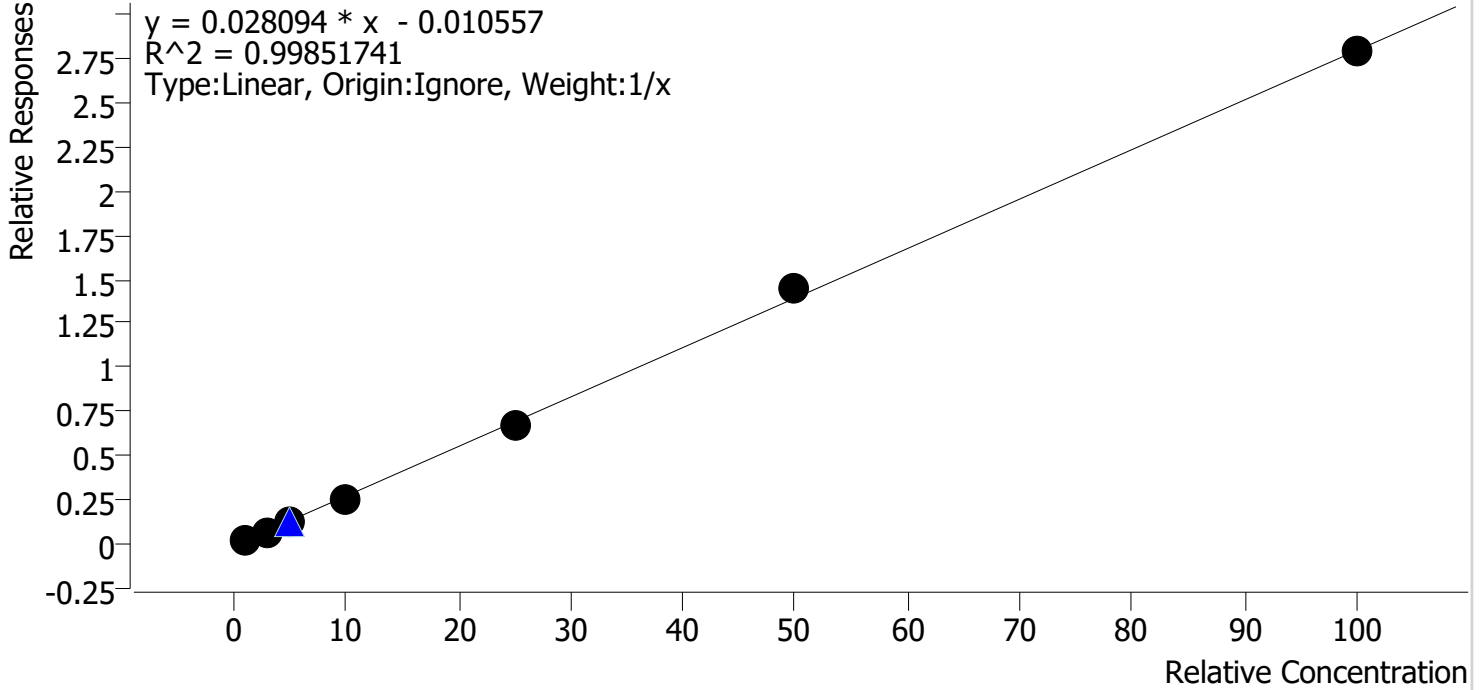
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	306935	∞	13.8	∞	2285763	5.179 ng/ml
THC-COOH	1.431	139782	∞	38.0	657.6	639907	15.312 ng/ml
THC	3.152	167865	∞	27.3	∞	1477306	4.420 ng/ml

# Compound Calibration Report



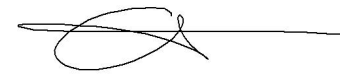
**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Last Cal. Update** 4/13/2022 2:48 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-d3

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



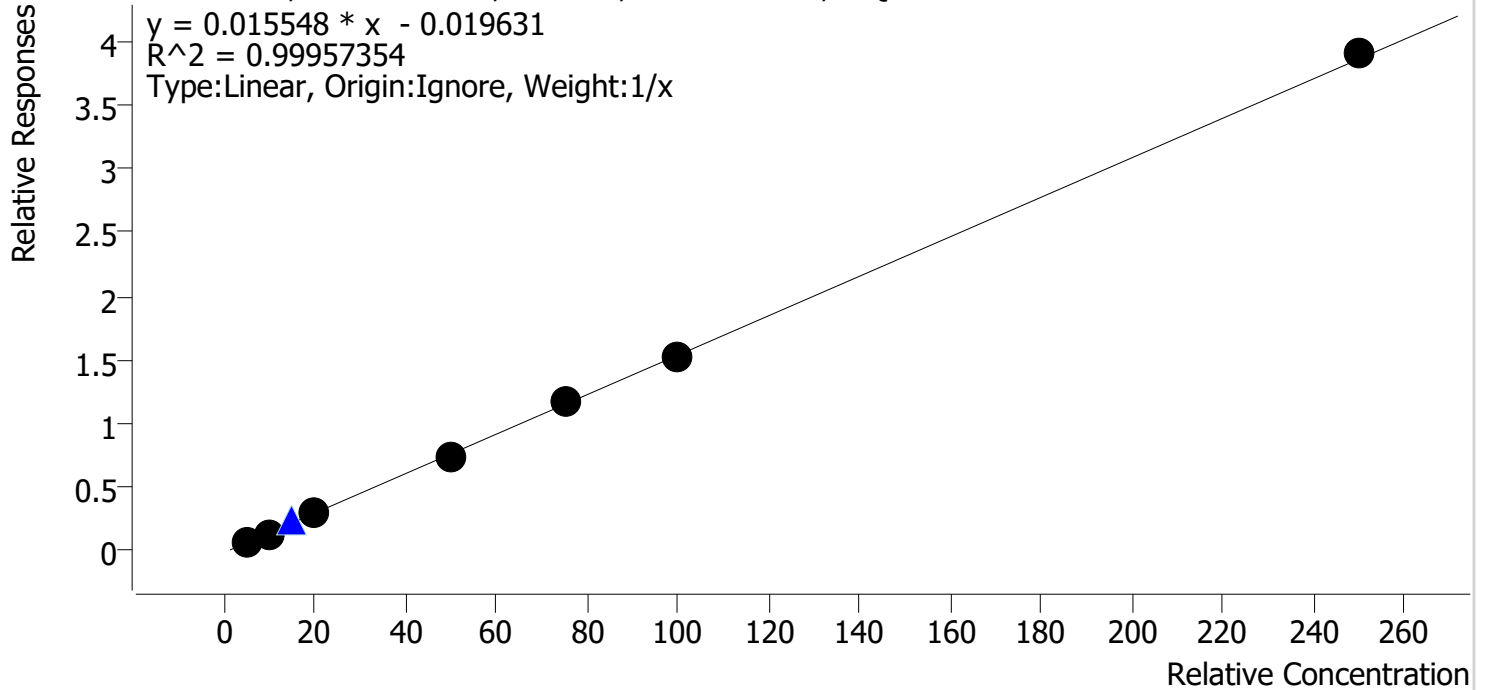
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	117.5
mj cal 2	2	✓	3.0	2.9	96.0
mj cal 3	3	✓	5.0	4.6	92.6
mj cal 4	4	✓	10.0	9.4	94.1
mj cal 5	5	✓	25.0	23.9	95.7
mj cal 6	6	✓	50.0	52.2	104.5
mj cal 7	7	✓	100.0	99.7	99.7

# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Last Cal. Update** 4/13/2022 2:48 PM  
**Analyst Name** ISP\datastor  
**Analyte** THC-COOH **Internal Standard** THC-COOH-d9

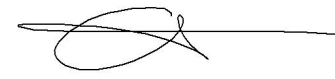
THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.4	108.8
mj cal 2	2	✓	10.0	9.7	97.1
mj cal 3	3	✓	20.0	19.3	96.5
mj cal 4	4	✓	50.0	48.5	97.1
mj cal 5	5	✓	75.0	75.6	100.8
mj cal 6	6	✓	100.0	98.7	98.7
mj cal 7	7	✓	250.0	252.7	101.1



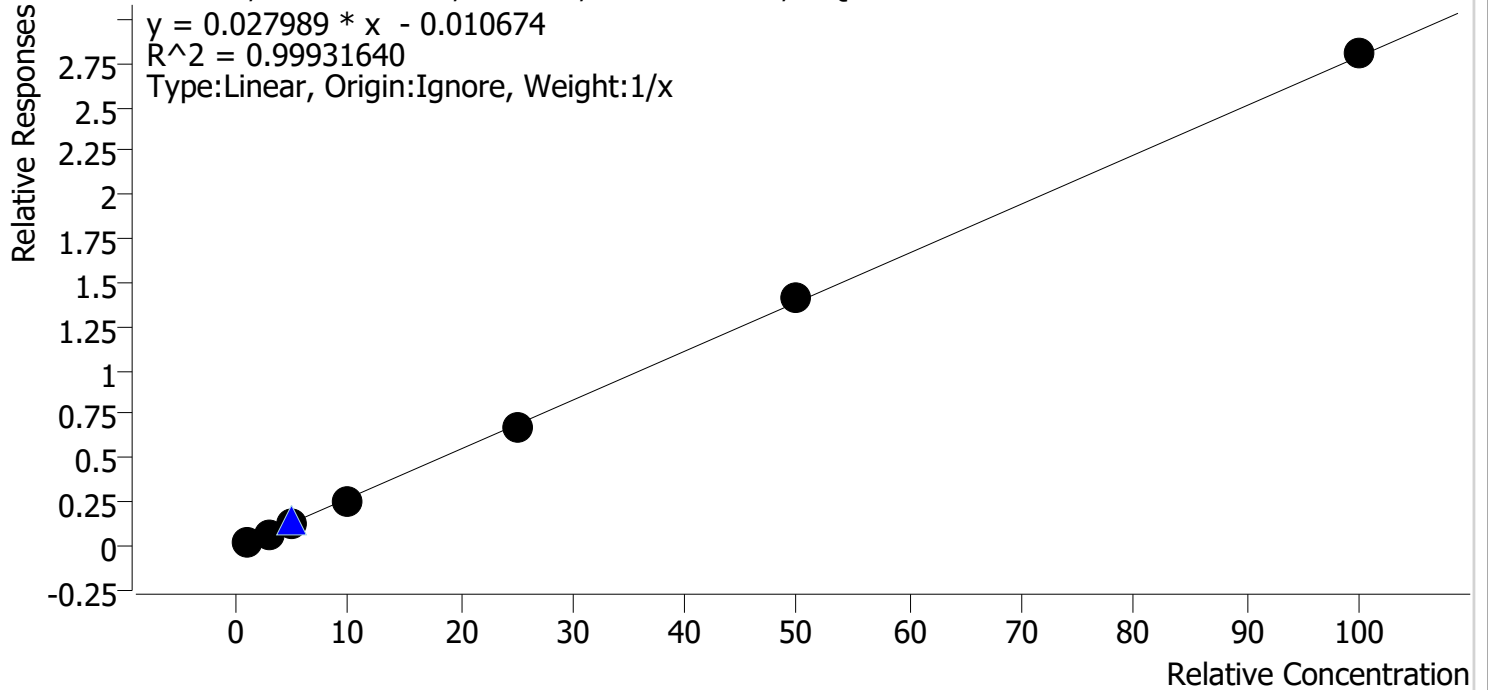
# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Last Cal. Update** 4/13/2022 2:48 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



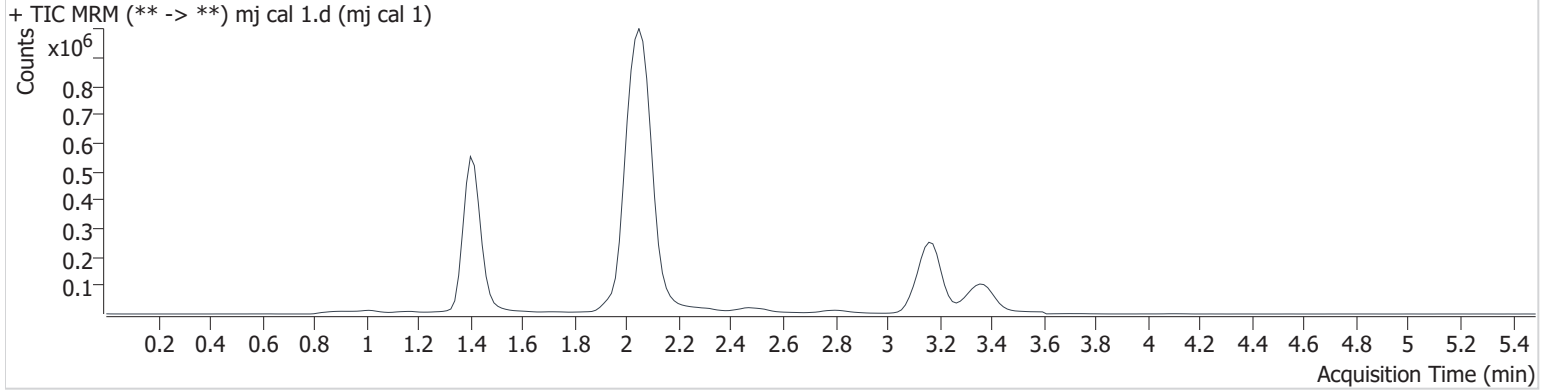
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	116.1
mj cal 2	2	✓	3.0	2.9	97.1
mj cal 3	3	✓	5.0	4.6	91.2
mj cal 4	4	✓	10.0	9.6	95.6
mj cal 5	5	✓	25.0	24.5	97.9
mj cal 6	6	✓	50.0	50.8	101.5
mj cal 7	7	✓	100.0	100.6	100.6

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 1.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 1
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-A1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 10:30:50 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.406	40896	∞	14.4	∞	1873047	1.161 ng/ml	<b>Low</b>
THC-COOH	1.431	33430	99.8	41.4	∞	514937	5.438 ng/ml	
THC	3.197	24937	∞	28.5	930.8	1111051	1.175 ng/ml	

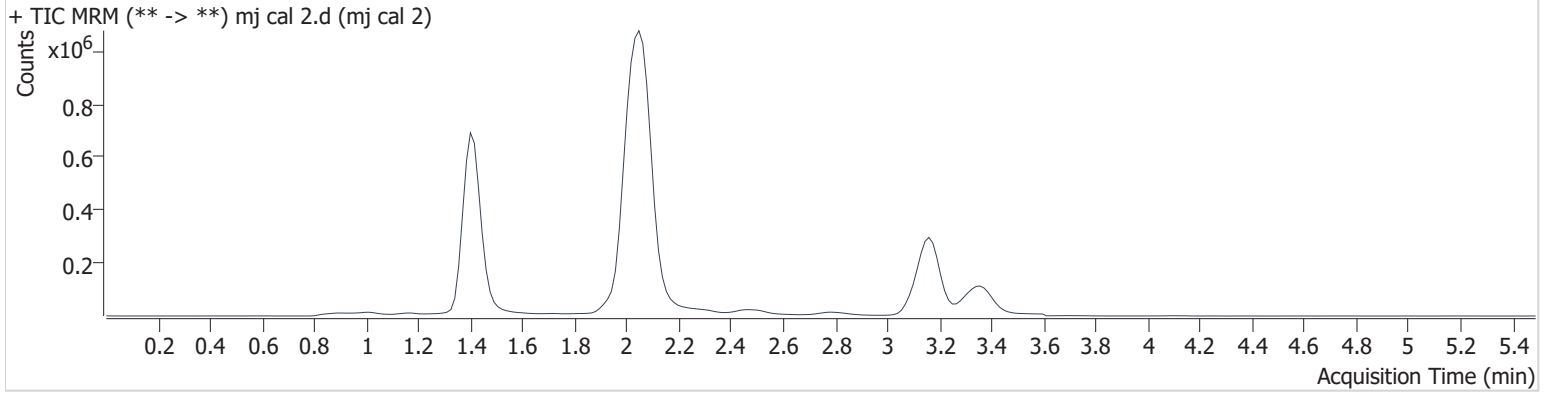
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 2.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 2
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-B1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 10:37:34 AM		

**Sample Info.**

## Sample Chromatogram



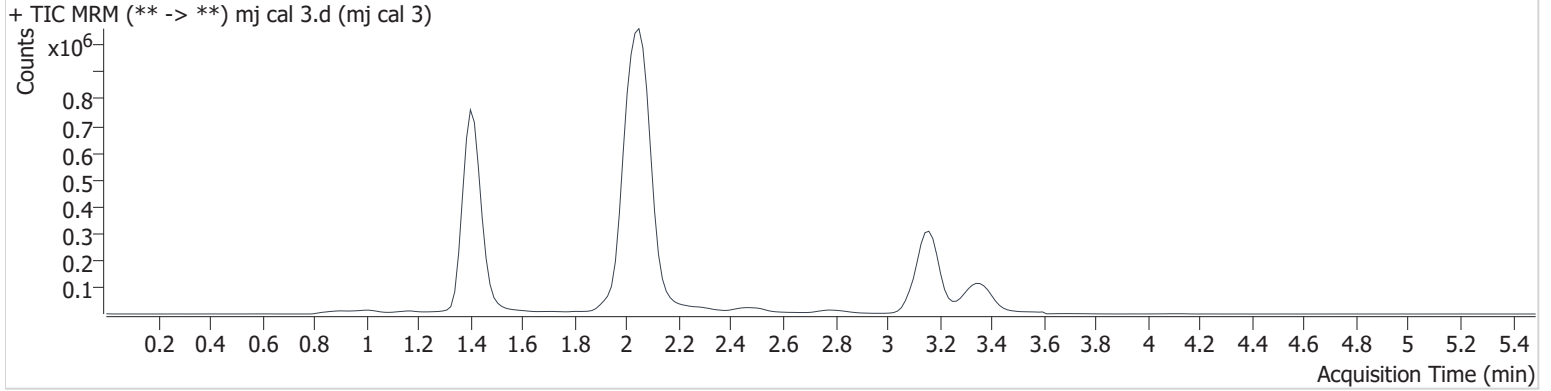
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.406	158433	531.2	13.9	470.9	2235136	2.914 ng/ml	<b>Low</b>
THC-COOH	1.431	79284	∞	38.3	171.0	603794	9.708 ng/ml	
THC	3.182	88730	∞	27.3	717.7	1261689	2.879 ng/ml	

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 3.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 3
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-C1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 10:44:17 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



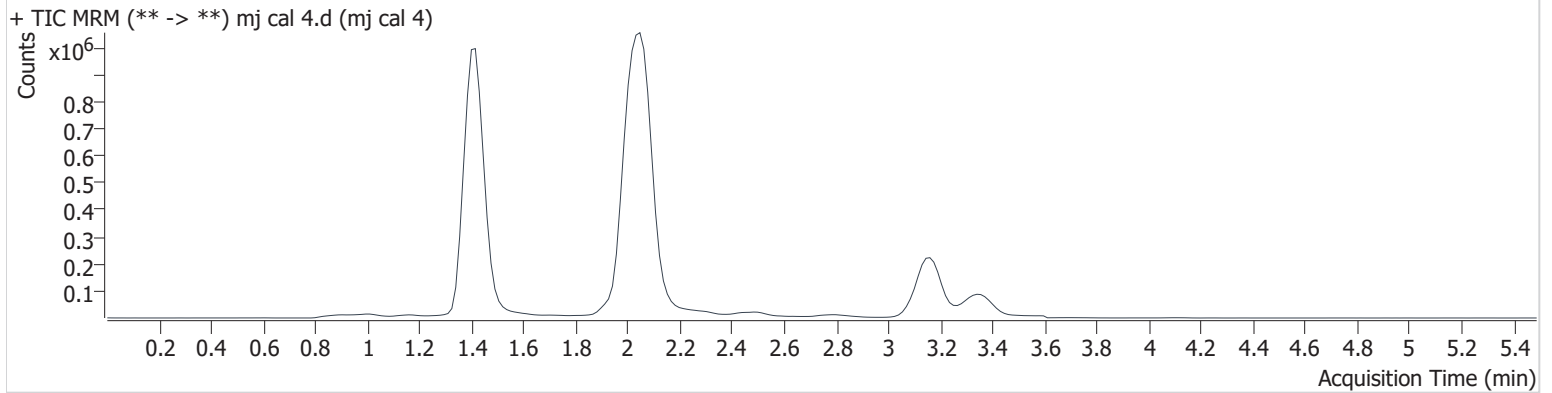
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	263759	∞	13.6	∞	2255583	4.559 ng/ml
THC-COOH	1.431	171742	∞	38.7	∞	612496	19.297 ng/ml
THC	3.182	150443	∞	25.5	∞	1259449	4.628 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 4.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 4
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-D1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 10:50:59 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



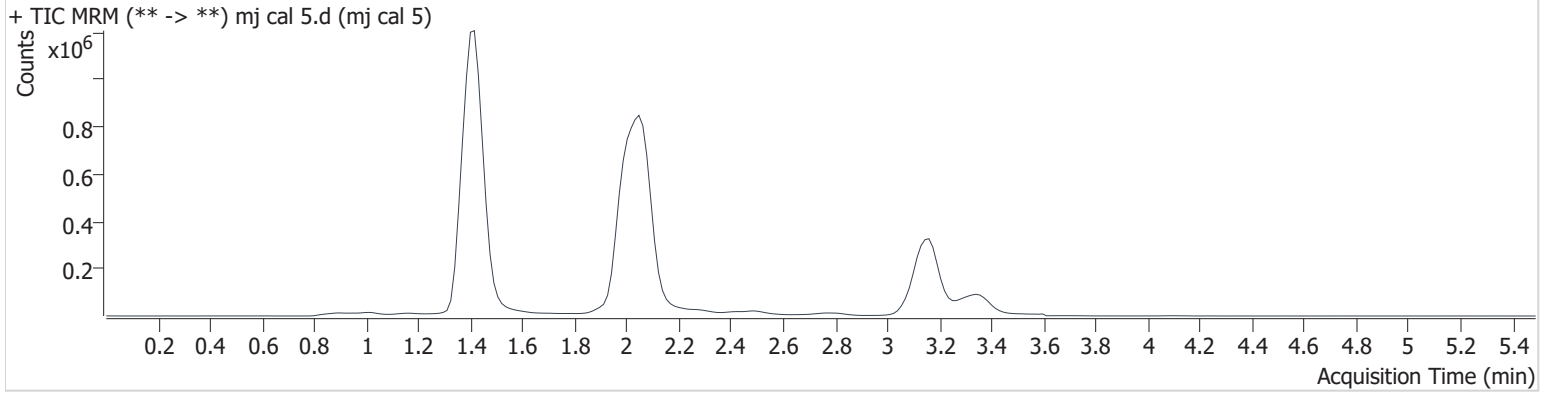
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	640215	∞	12.2	1899.3	2493047	9.557 ng/ml
THC-COOH	1.431	474000	3109.3	39.7	3415.9	644746	48.547 ng/ml
THC	3.182	222604	∞	25.1	∞	877363	9.407 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 5.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 5
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-E1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 10:57:42 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



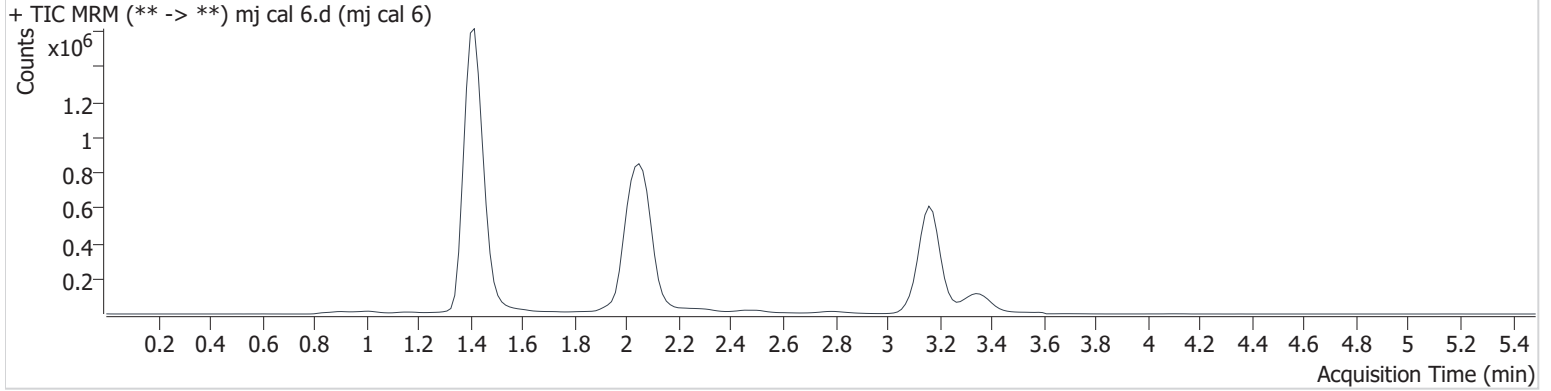
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	1615535	∞	12.1	∞	2396233	24.470 ng/ml
THC-COOH	1.431	644624	∞	39.4	∞	557759	75.596 ng/ml
THC	3.167	663812	∞	24.5	∞	1003204	23.929 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 6.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 6
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-F1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 11:04:24 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



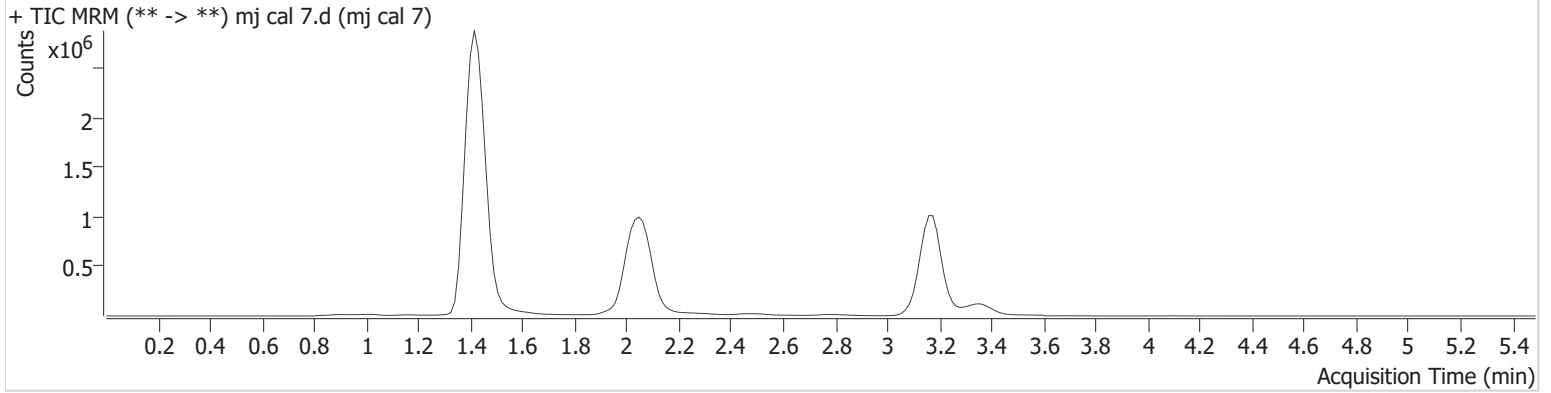
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	3093082	∞	12.2	1208.1	2194013	50.751 ng/ml
THC-COOH	1.431	712063	∞	39.6	4632.3	470021	98.700 ng/ml
THC	3.182	1628182	∞	24.3	∞	1117415	52.242 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\041322\QuantResults\cann.batch.bin  
**Calibration Last Update** 4/13/2022 2:48:30 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj cal 7.d
<b>Type</b>	Cal	<b>Sample</b>	mj cal 7
<b>Acq. Method</b>	AM 27 THC quant.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-G1	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	4/13/2022 11:11:06 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	5951943	∞	12.6	∞	2122189	100.588 ng/ml
THC-COOH	1.446	2028008	19579.5	38.9	24034.5	518729	252.714 ng/ml
THC	3.182	3511109	∞	24.6	∞	1257774	99.741 ng/ml