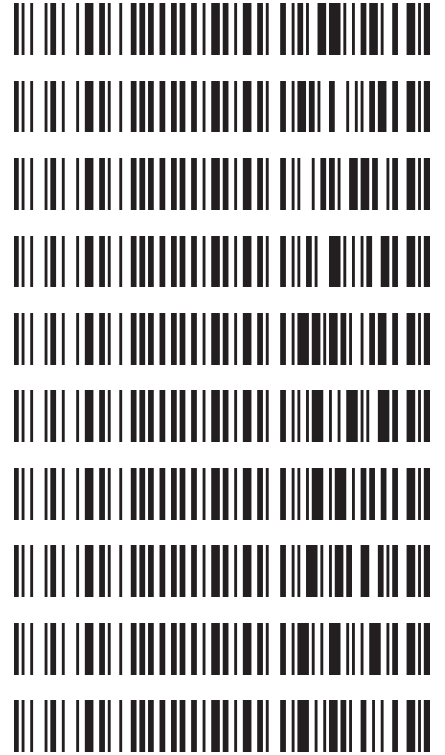


5/10/2022

**Worklist: 5862****REVIEWED***By Britany Wylie at 7:44 am, May 12, 2022*

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
C2022-0808	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0852	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0888	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0906	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0942	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0967	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0967	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0974	2	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-0984	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2022-1001	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 5/5/22  
Plate lot#: 220309

Analyst: Anne Nord  
Plate re-test: 9/9/2022

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

**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.

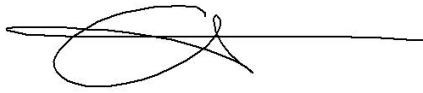
## Analytic:

- 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, THC-OH curve range 3-100*



	1	2	3	4	5	6
a	cal 1	Internal control blood end of run	967-2			
b	cal 2	negative blood	974-2			
c	cal 3	808-1	984-1			
d	cal 4	852-1	1001-1			
e	Cal 5	888-2				
f	cal 6	906-1				
g	cal 7	942-1				
h	Internal control (blood)	967-1				

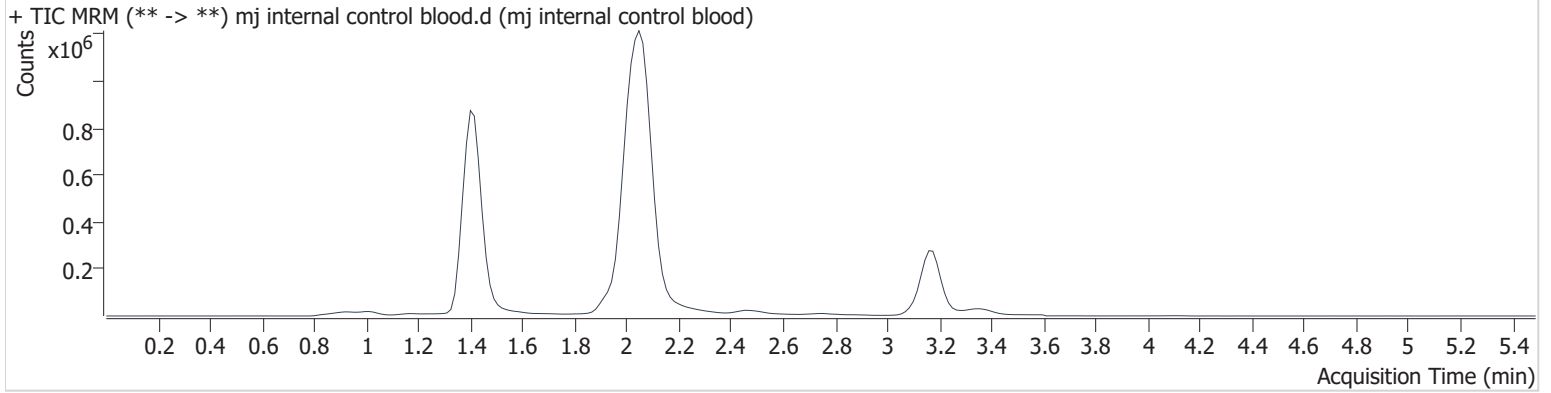
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 1:56:25 PM		

**Sample Info.**

## Sample Chromatogram



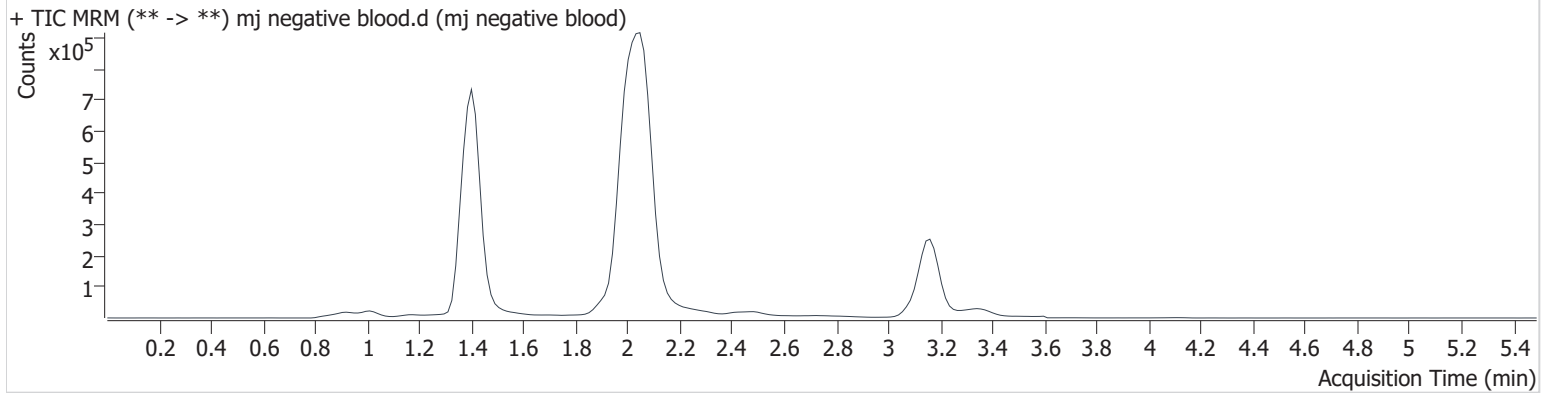
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	316420	$\infty$	12.1	519.7	2797205	4.835 ng/ml
THC-COOH	1.431	155903	$\infty$	38.6	657.7	787025	14.396 ng/ml
THC	3.182	148887	4986.4	25.2	141.8	1327536	4.713 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 2:03:07 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



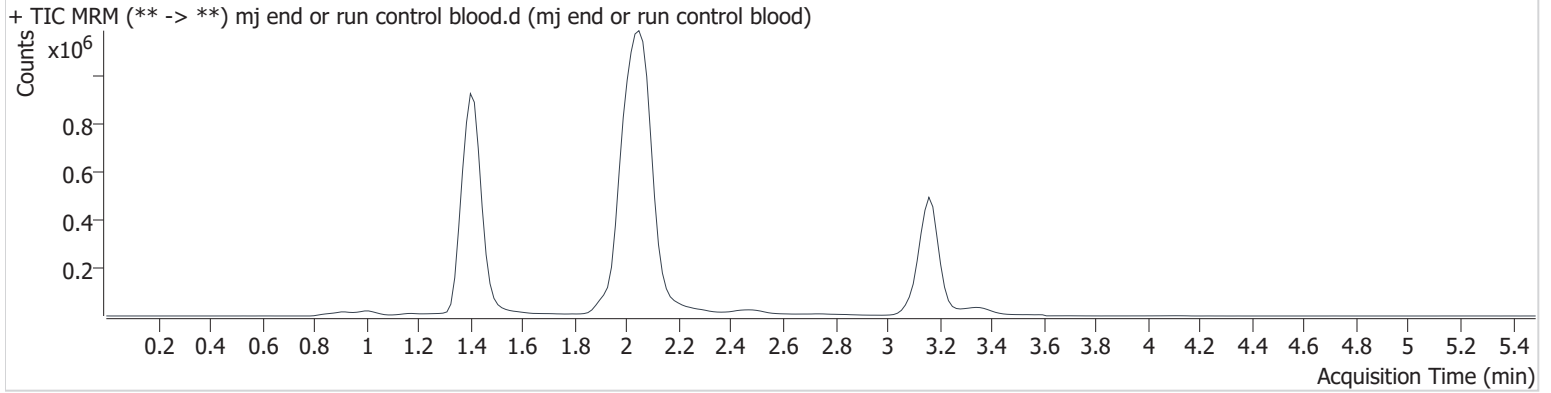
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 4:23:22 PM		

**Sample Info.**

## Sample Chromatogram



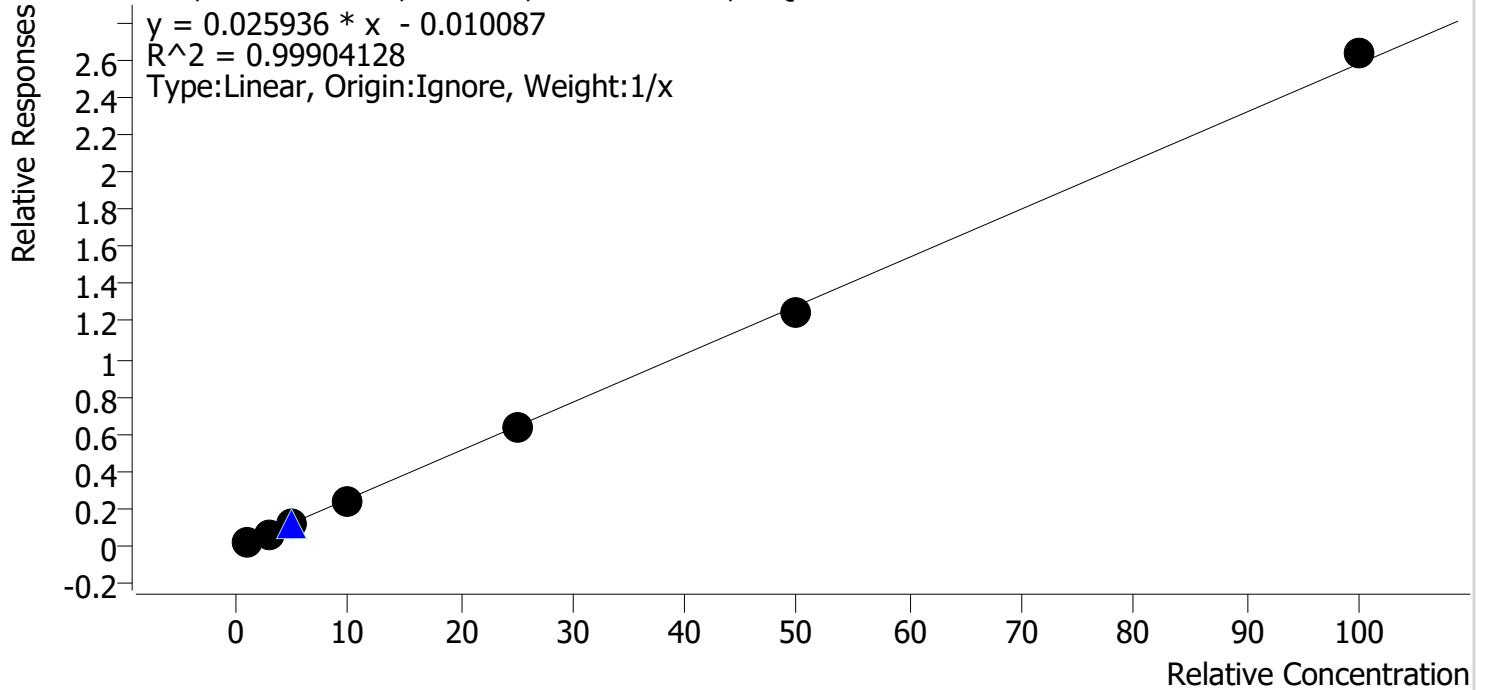
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	345280	∞	11.9	601.4	3062812	4.821 ng/ml
THC-COOH	1.431	176146	∞	37.4	∞	890406	14.378 ng/ml
THC	3.182	259950	∞	25.7	1120.9	2309210	4.729 ng/ml

# Compound Calibration Report



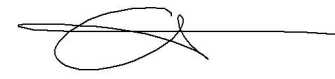
**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Last Cal. Update** 5/10/2022 10:36 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-d3

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



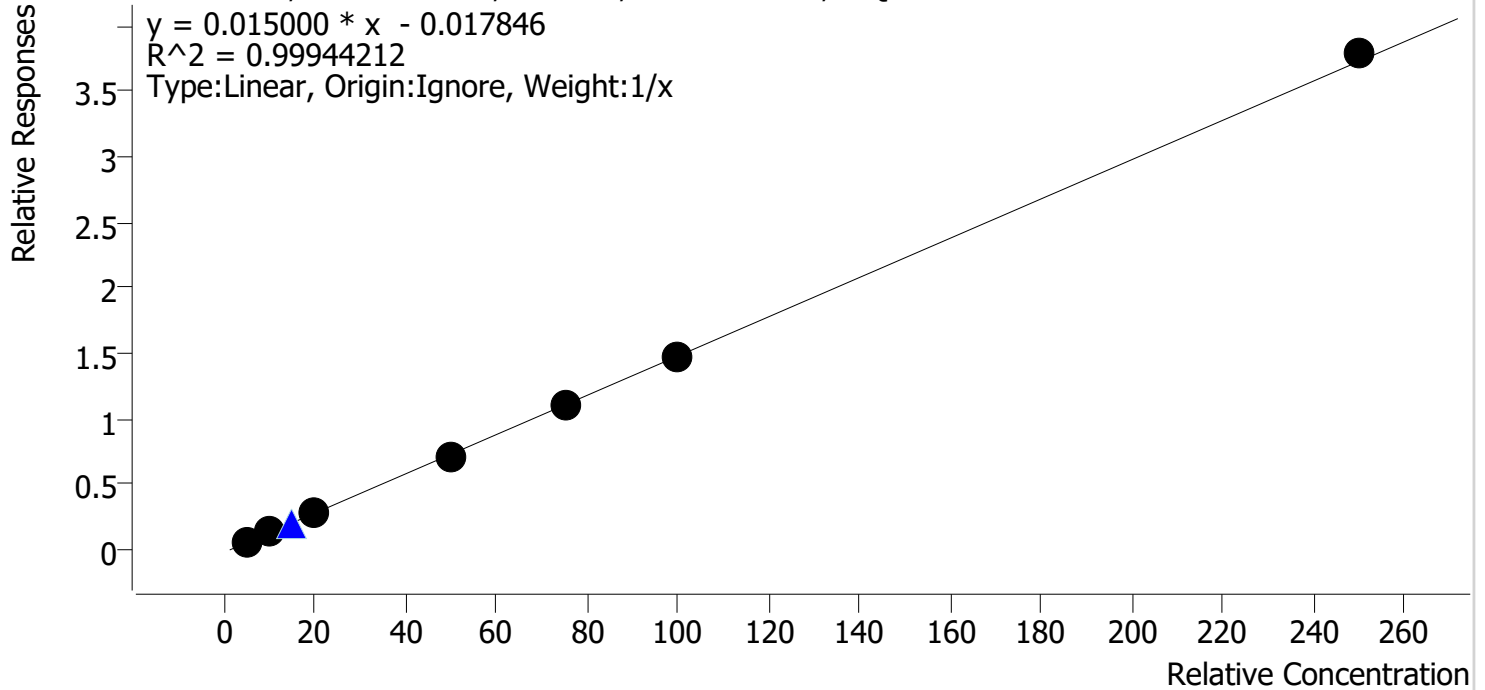
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	116.9
mj cal 2	2	✓	3.0	2.9	95.5
mj cal 3	3	✓	5.0	4.7	94.6
mj cal 4	4	✓	10.0	9.4	93.6
mj cal 5	5	✓	25.0	24.9	99.6
mj cal 6	6	✓	50.0	48.9	97.8
mj cal 7	7	✓	100.0	102.1	102.1

# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Last Cal. Update** 5/10/2022 10:36 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, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.4	107.8
mj cal 2	2	✓	10.0	10.0	99.9
mj cal 3	3	✓	20.0	19.4	96.8
mj cal 4	4	✓	50.0	47.9	95.8
mj cal 5	5	✓	75.0	74.1	98.8
mj cal 6	6	✓	100.0	99.4	99.4
mj cal 7	7	✓	250.0	253.9	101.6

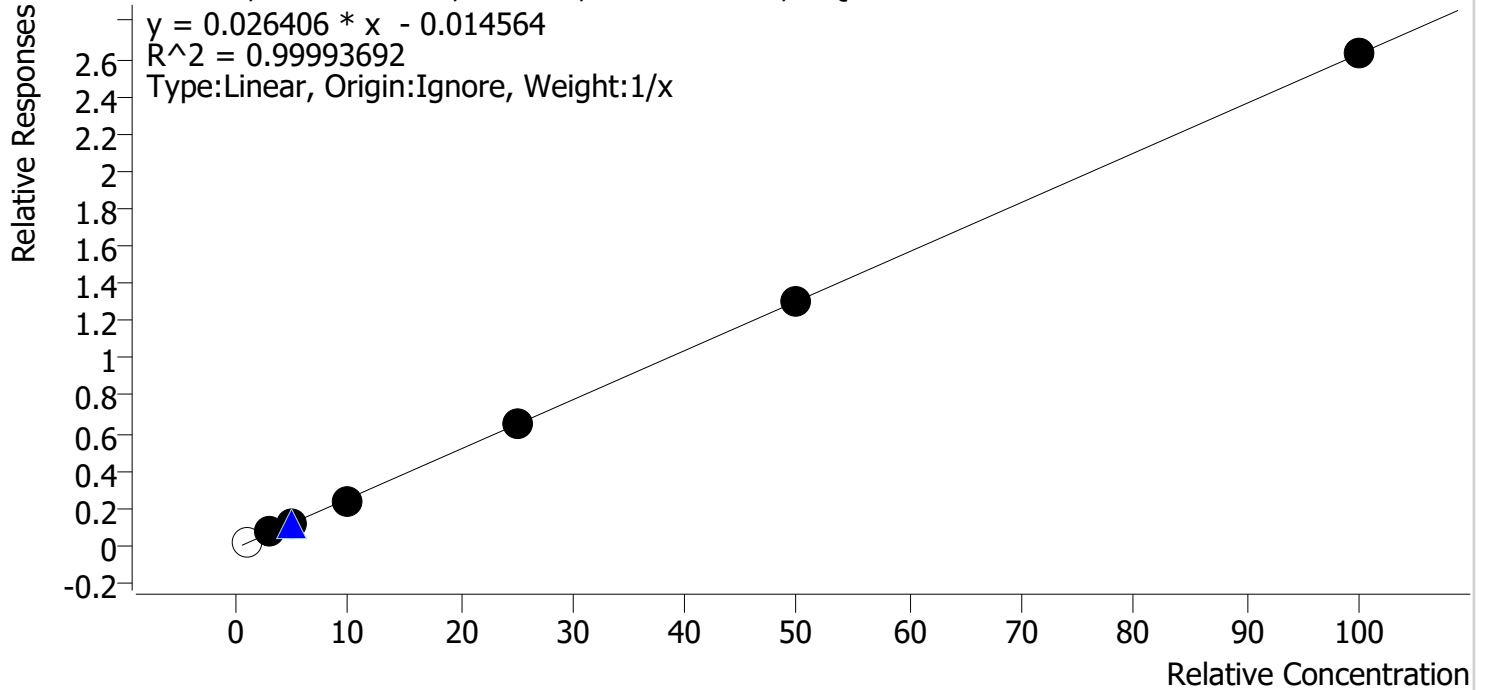


# Compound Calibration Report



**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Last Cal. Update** 5/10/2022 10:36 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-d3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	x	1.0	1.3	127.8
mj cal 2	2	✓	3.0	3.0	101.5
mj cal 3	3	✓	5.0	5.0	101.0
mj cal 4	4	✓	10.0	9.7	97.5
mj cal 5	5	✓	25.0	24.9	99.6
mj cal 6	6	✓	50.0	50.2	100.4
mj cal 7	7	✓	100.0	100.1	100.1

dropped cal 1 due to ratio being out of range.

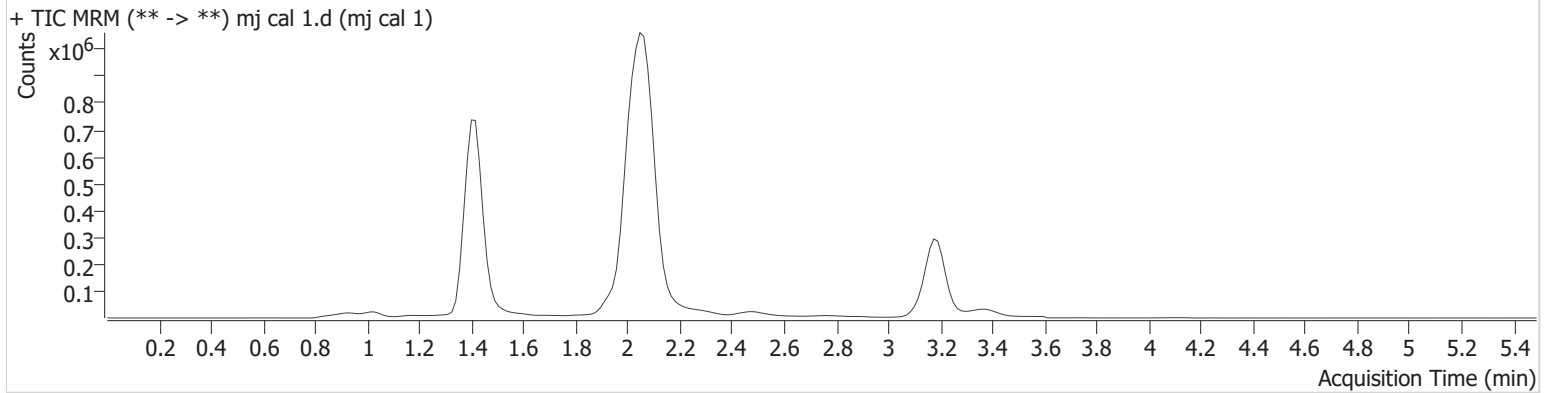
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 1:09:27 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	52567	204.6	15.2 <b>High</b>	1346.9	2738925	1.278 ng/ml <b>Low</b>
THC-COOH	1.446	43456	∞	36.2	∞	689694	5.390 ng/ml
THC	3.197	31737	103.8	31.3	81.4	1569299	1.169 ng/ml

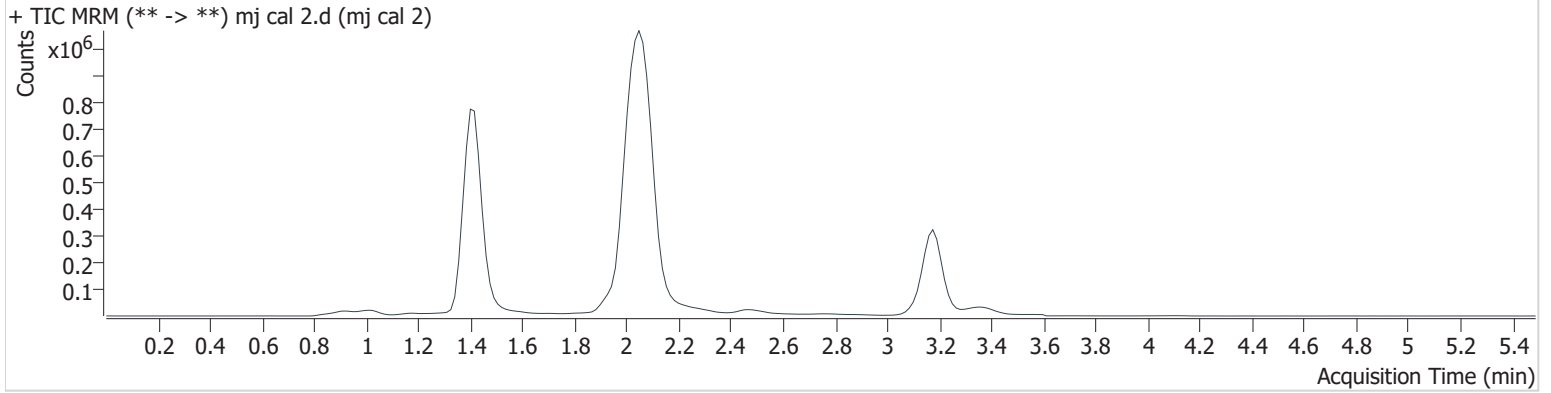
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 1:16:11 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	175604	4317.5	12.7	∞	2666813	3.045 ng/ml
THC-COOH	1.446	91838	378.4	37.2	∞	696041	9.986 ng/ml
THC	3.182	101416	807.8	26.7	297.0	1580132	2.864 ng/ml

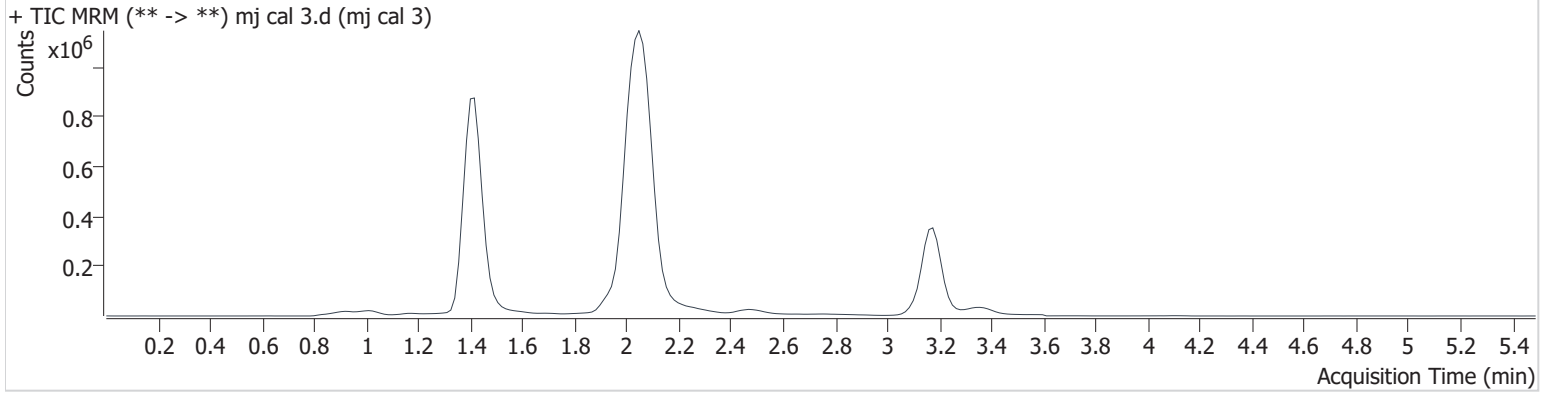
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 1:22:53 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	323738	∞	11.4	∞	2725393	5.050 ng/ml
THC-COOH	1.446	196064	∞	37.3	∞	719080	19.367 ng/ml
THC	3.182	186140	665.6	25.9	174.9	1653022	4.731 ng/ml

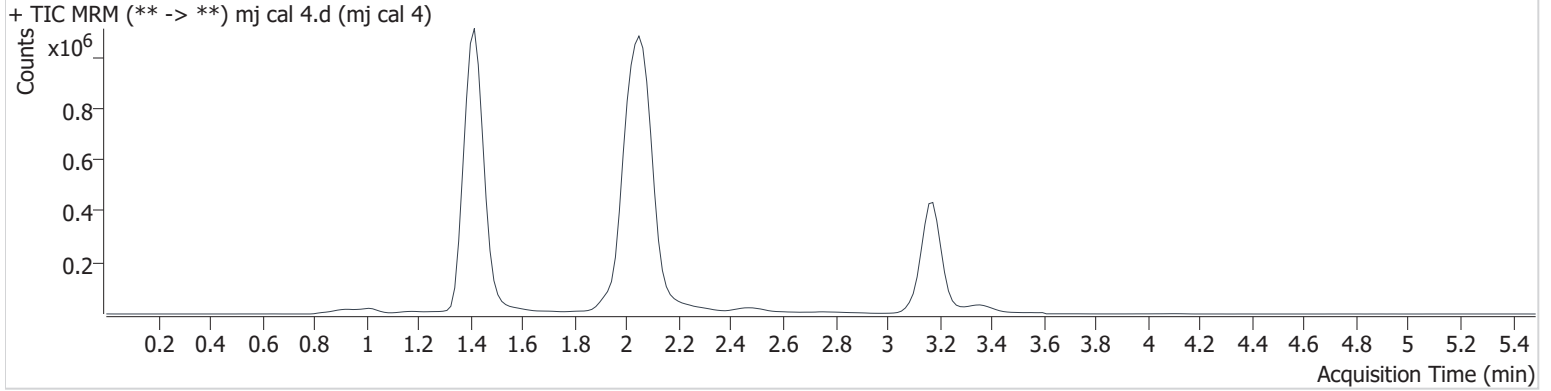
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 1:29:35 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	681371	∞	11.5	∞	2806693	9.745 ng/ml
THC-COOH	1.431	511742	∞	38.3	2495.8	730429	47.898 ng/ml
THC	3.182	424087	3653.5	24.4	1133.2	1823342	9.357 ng/ml

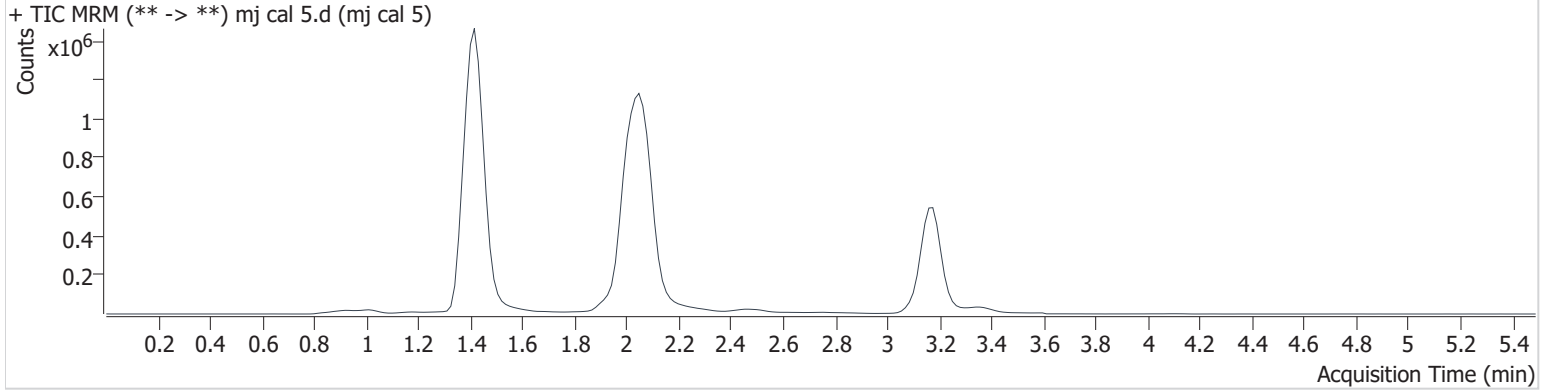
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 1:36:18 PM		

**Sample Info.**

## Sample Chromatogram



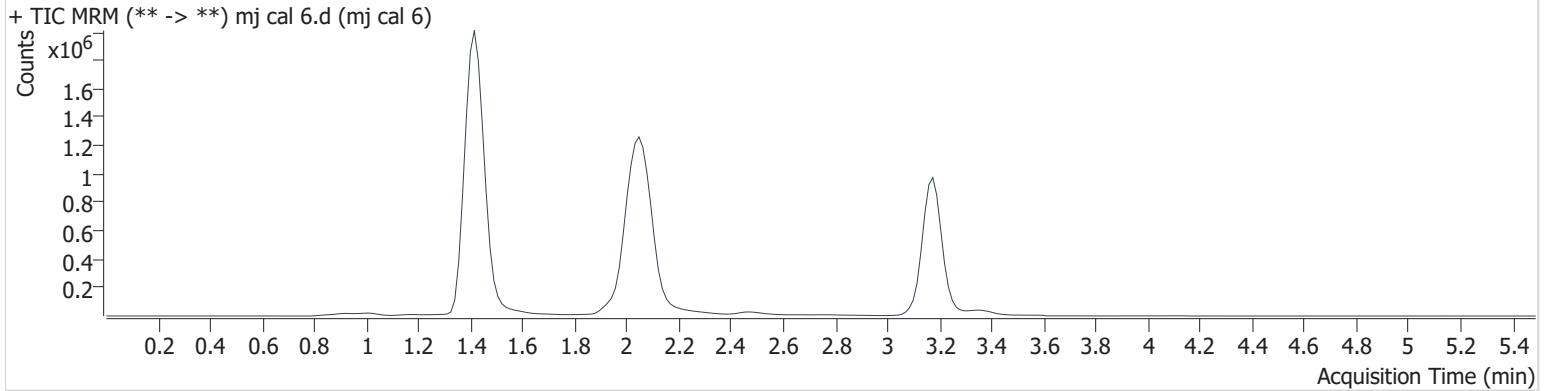
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	1775704	∞	12.0	∞	2762399	24.895 ng/ml
THC-COOH	1.431	786492	∞	37.8	∞	719340	74.081 ng/ml
THC	3.182	1087937	28452.3	24.2	7241.3	1710785	24.908 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 1:43:00 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	3459876	∞	12.3	∞	2639179	50.198 ng/ml
THC-COOH	1.431	1056146	∞	37.8	1987.2	717276	99.355 ng/ml
THC	3.182	2495615	33251.3	24.6	1637.0	1983768	48.894 ng/ml

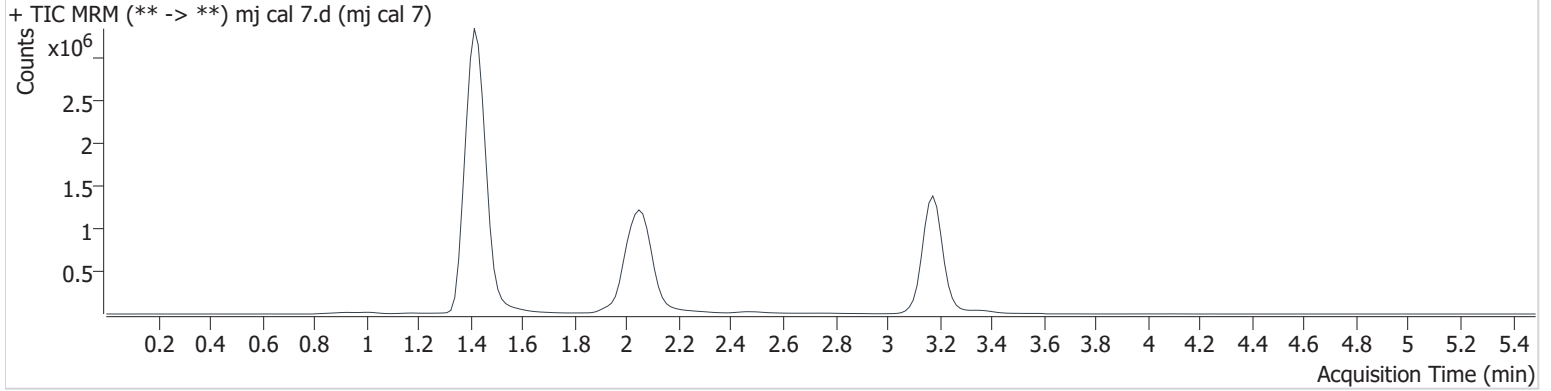
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2022\am 27-28\050522\QuantResults\cann.batch.bin  
**Calibration Last Update** 5/10/2022 10:36:23 AM

<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>	5/5/2022 1:49:42 PM		

**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.406	6730083	∞	12.4	∞	2561097	100.067 ng/ml
THC-COOH	1.446	2520308	18433.5	37.9	21004.7	664830	253.922 ng/ml
THC	3.182	4671590	35167.8	24.7	12520.1	1771280	102.078 ng/ml