

**REVIEWED**

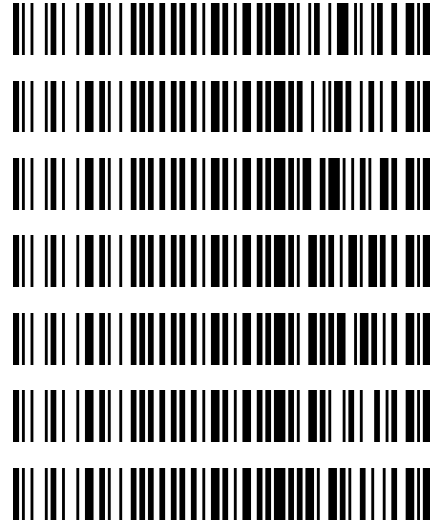
By Celena Shrum at 10:08 am, May 10, 2024



5/9/2024

**Worklist: 6806**

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
C2024-0775	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0800	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0829	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0831	3	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0840	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0843	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-0853	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: 05/08/24

Plate lot#: 231212

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: 24C52042

Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Anne Nord

Plate Retest Date: 6/12/24

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Urine Lot: blood only

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 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.** Using a calibrated pipette, add **1000µl blood and urine (if applicable) (calibrated pipette)** into the appropriate wells of analytical (standards) plate. **Pipette ID: I41142J**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Add **500µL of 0.1% formic acid in water to blood samples,** and **500µL of saturated phosphate buffer to urine samples** in the wells of the analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **700-800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate. Amount transferred: **800 µL**
- 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)**
- 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.
- 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. Did all QCs pass for each analyte? (if not, describe in comments section)
- 5. Enter QCs into control charting.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: *Calibrator 7 did not inject properly. The calibrator was injected the next day and that injection was evaluated.*

*THC-OH curve limits 3-100 dropped cal 1 due to accuracy.*

	1	2	3	4	5	6
a	cal 1	internal control blood	0853-1			
b	cal 2	negative blood				
c	cal 3	0775-1				
d	cal 4	0800-1				
e	cal 5	0829-1				
f	cal 6	0831-3				
g	cal 7	0840-1				
h	Internal control (blood)	0843-1				

Plate position 3

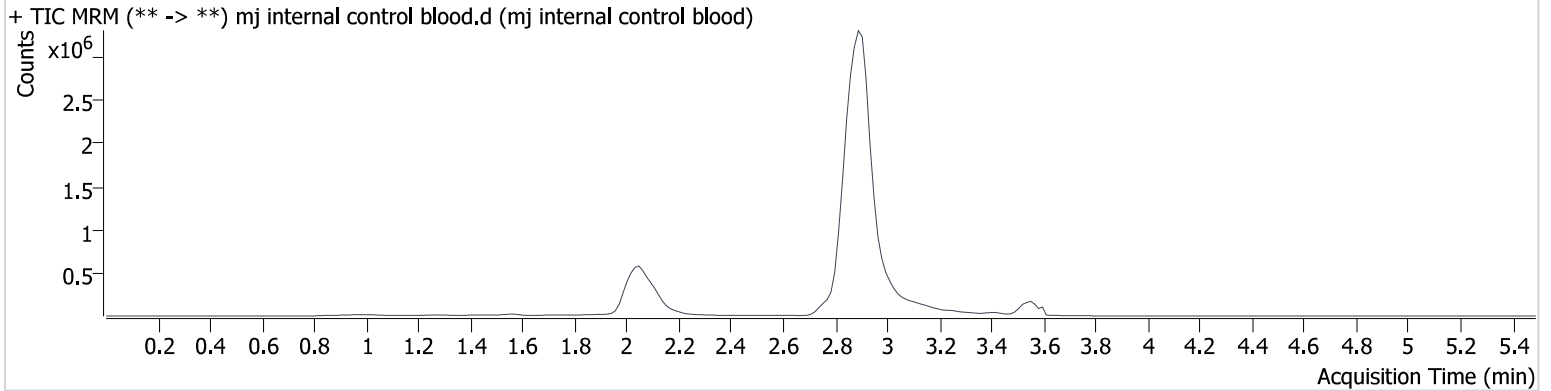
c2024-\_\_\_\_-\_\_

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679 **Data File** mj internal control blood.d  
**Type** QC **Sample** mj internal control blood  
**Acq. Method** thc quant 50 50.m **Operator** Anne Nord  
**Sample Position** P3-H1 **Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods  
**Injection Volume** 10  
**Acq. Date-Time** 5/9/2024 12:31:11 AM  
**Sample Info.**

## Sample Chromatogram



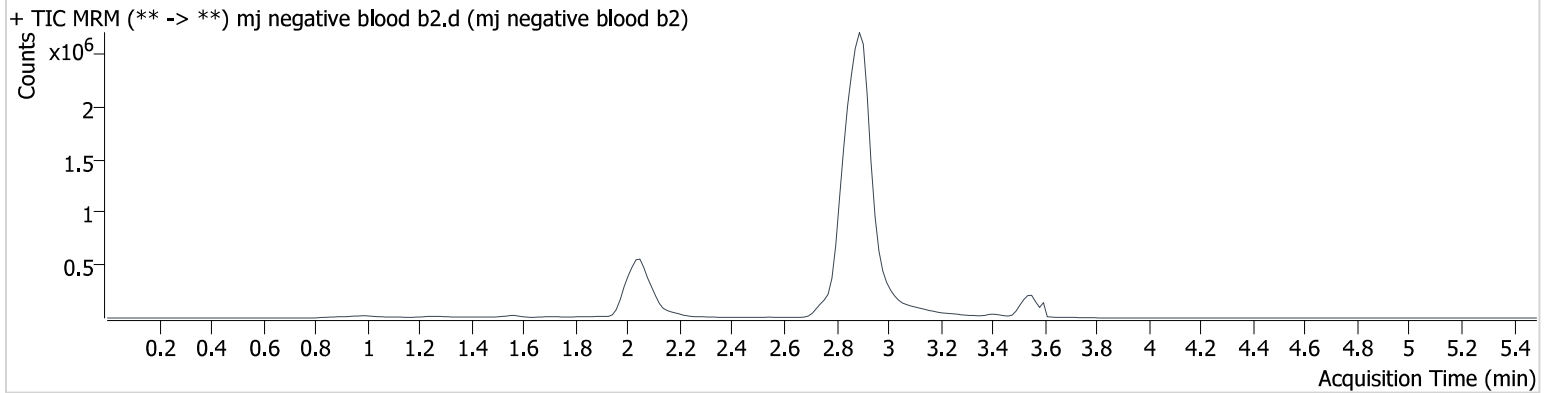
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	33514	127.7	860.55	∞	2220679	5.571 ng/ml
THC-COOH	2.107	62460	587.4	271.97	327.4	920202	16.225 ng/ml
THC	3.558	62981	∞	24.79	∞	424174	4.868 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

<b>Instrument</b>	69679	<b>Data File</b>	mj negative blood b2.d
<b>Type</b>	Sample	<b>Sample</b>	mj negative blood b2
<b>Acq. Method</b>	thc quant 50 50.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-B2	<b>Comment</b>	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/9/2024 12:37:45 AM		
<b>Sample Info.</b>			

## Sample Chromatogram

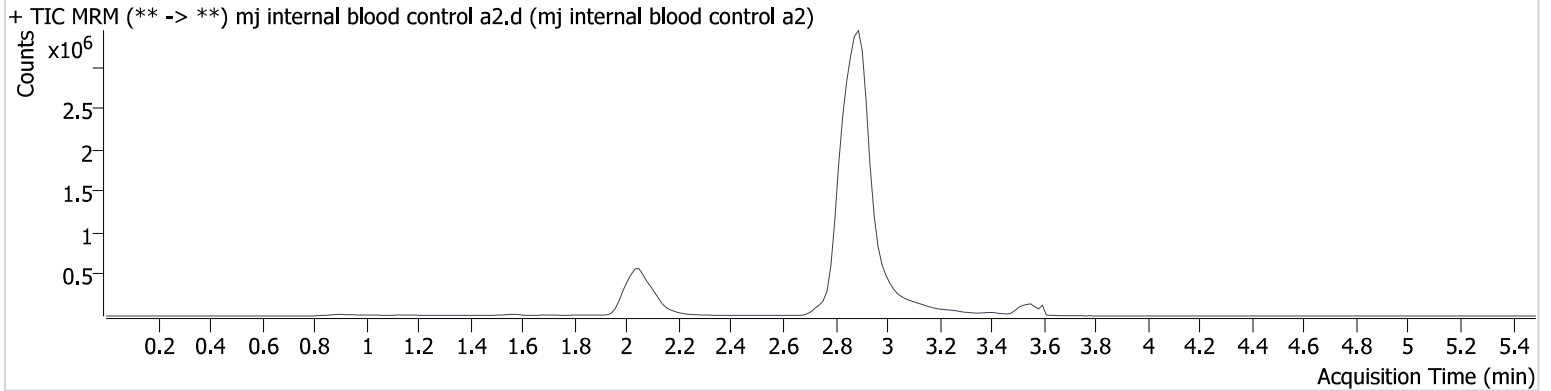


# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679 **Data File** mj internal blood control a2.d  
**Type** Sample **Sample** mj internal blood control a2  
**Acq. Method** thc quant 50 50.m **Operator** Anne Nord  
**Sample Position** P3-A2 **Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods  
**Injection Volume** 10  
**Acq. Date-Time** 5/9/2024 2:16:44 AM  
**Sample Info.**

## Sample Chromatogram

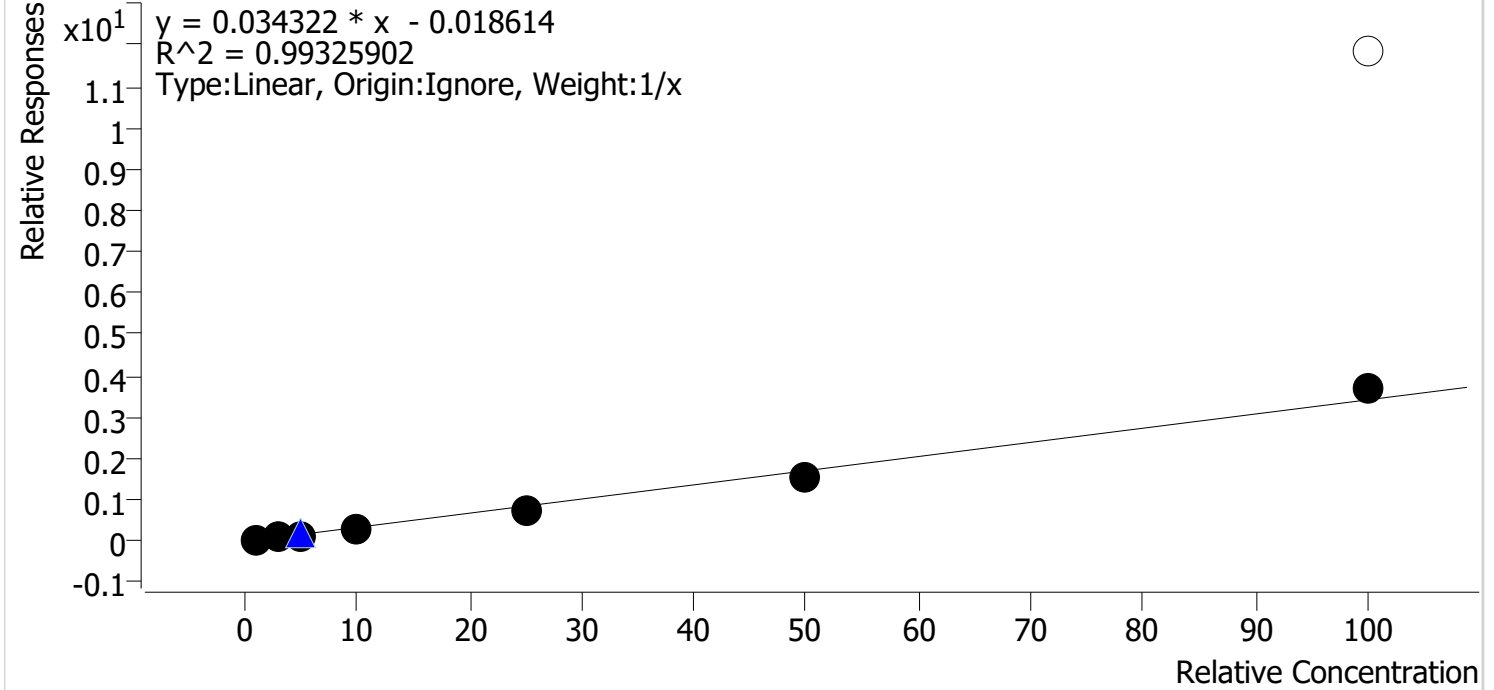


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.036	30874	∞	931.96	435.6	2336174	5.053 ng/ml
THC-COOH	2.107	60394	138197.0	283.72	783.8	1021977	14.457 ng/ml
THC	3.558	55310	∞	24.97	357.5	386439	4.712 ng/ml

# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Last Cal. Update** 5/9/2024 9:36 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-d3

THC - 7 Levels, 7 Levels Used, 8 Points, 7 Points Used, 1 QCs

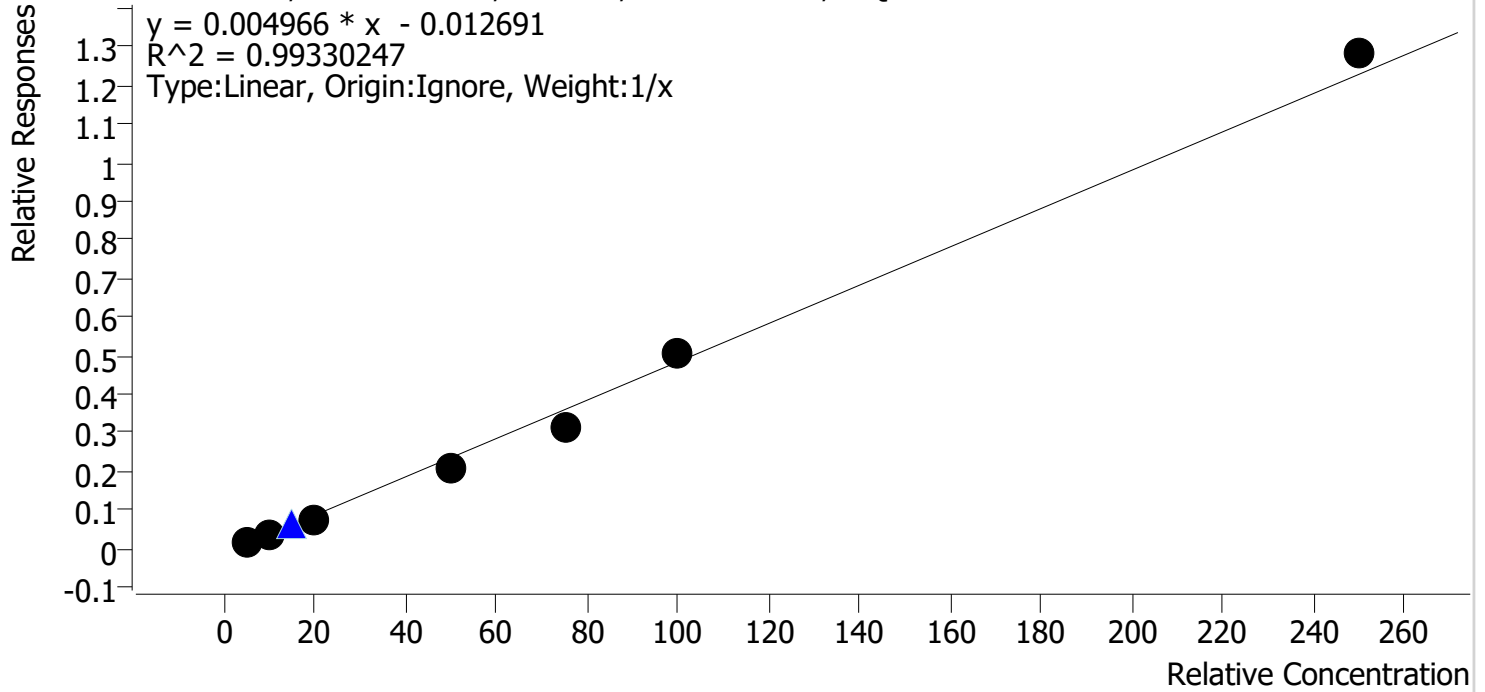


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.3	128.9
mj cal 2	2	✓	3.0	2.9	97.1
mj cal 3	3	✓	5.0	4.6	91.1
mj cal 4	4	✓	10.0	9.2	91.9
mj cal 5	5	✓	25.0	22.9	91.7
mj cal 6	6	✓	50.0	46.2	92.5
mj cal 7	7	x	100.0	345.7	345.7
mj cal 7r	7	✓	100.0	106.9	106.9

# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Last Cal. Update** 5/9/2024 9:36 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



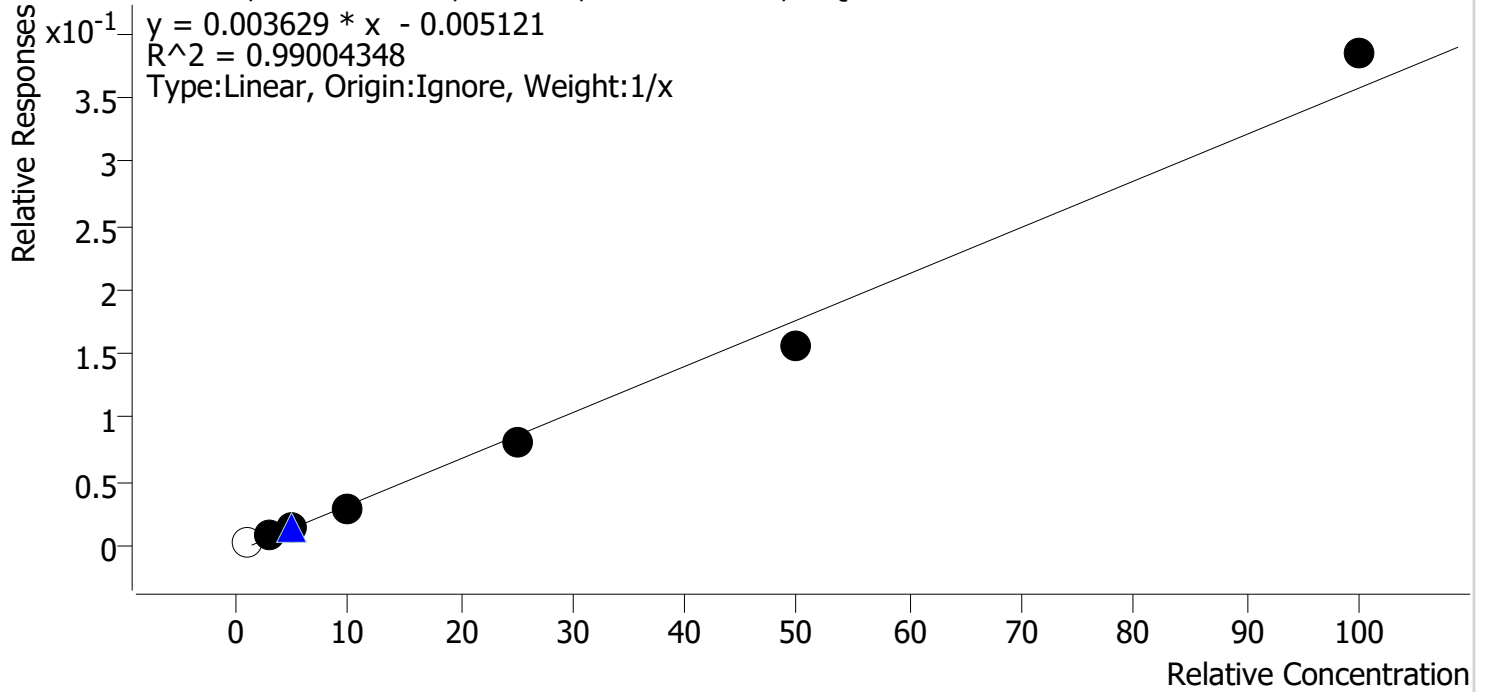
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	6.1	121.5
mj cal 2	2	✓	10.0	10.2	101.6
mj cal 3	3	✓	20.0	18.3	91.5
mj cal 4	4	✓	50.0	44.3	88.6
mj cal 5	5	✓	75.0	66.5	88.7
mj cal 6	6	✓	100.0	103.9	103.9
mj cal 7r	7	✓	250.0	260.8	104.3



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Last Cal. Update** 5/9/2024 9:36 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	2.2	215.9
mj cal 2	2	✓	3.0	3.5	116.3
mj cal 3	3	✓	5.0	5.1	102.7
mj cal 4	4	✓	10.0	9.1	91.3
mj cal 5	5	✓	25.0	23.3	93.3
mj cal 6	6	✓	50.0	44.5	89.0
mj cal 7r	7	✓	100.0	107.4	107.4

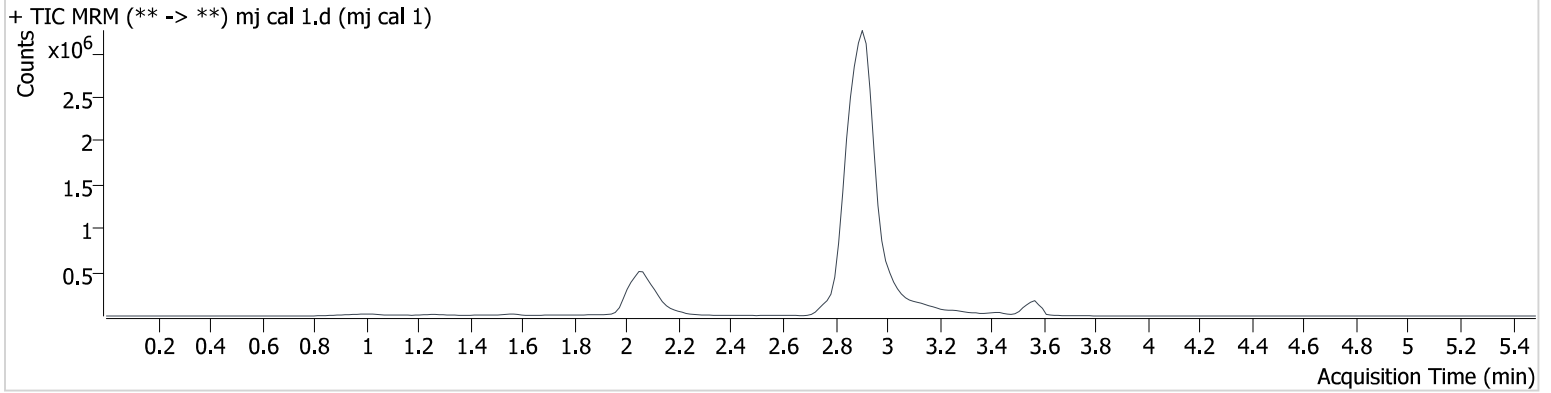
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** thc quant 50 50.m  
**Sample Position** P3-A1  
**Injection Volume** 10  
**Acq. Date-Time** 5/8/2024 11:44:55 PM  
**Sample Info.**

**Data File** mj cal 1.d  
**Sample** mj cal 1  
**Operator** Anne Nord  
**Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	2.066	5927	∞	728.73	∞	2183891	2.159 ng/ml	Low
THC-COOH	2.122	19808	426.3	288.17	133.1	1133877	6.074 ng/ml	
THC	3.573	11587	∞	27.43	25.3	452417	1.289 ng/ml	

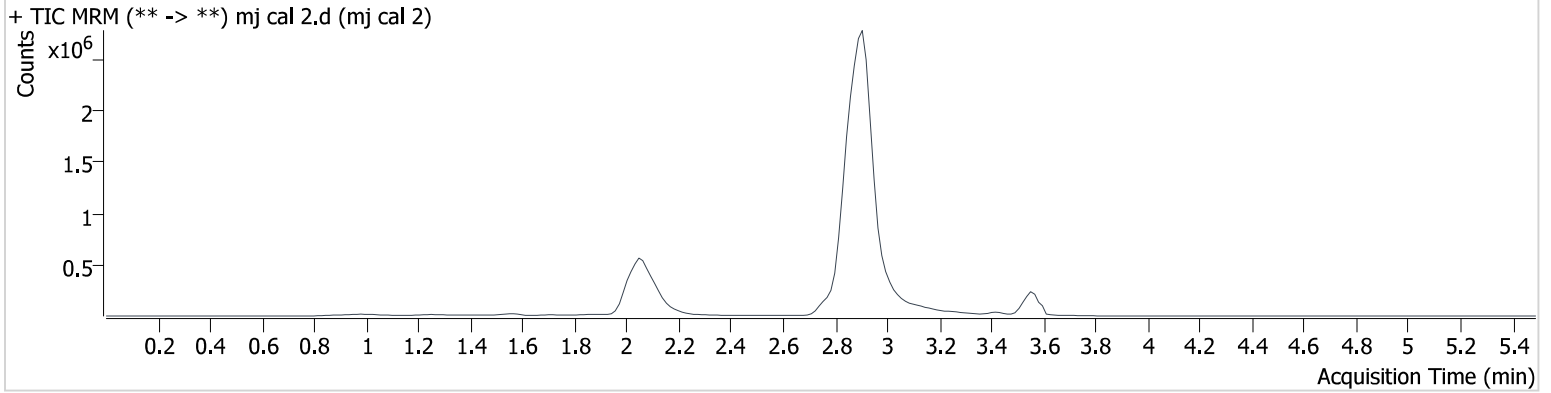
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** thc quant 50 50.m  
**Sample Position** P3-B1  
**Injection Volume** 10  
**Acq. Date-Time** 5/8/2024 11:51:39 PM  
**Sample Info.**

**Data File** mj cal 2.d  
**Sample** mj cal 2  
**Operator** Anne Nord  
**Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.051	18225	331.1	903.22	∞	2418610	3.488 ng/ml
THC-COOH	2.122	43472	210.5	276.70	1300.9	1151240	10.160 ng/ml
THC	3.558	46360	∞	24.51	∞	570050	2.912 ng/ml

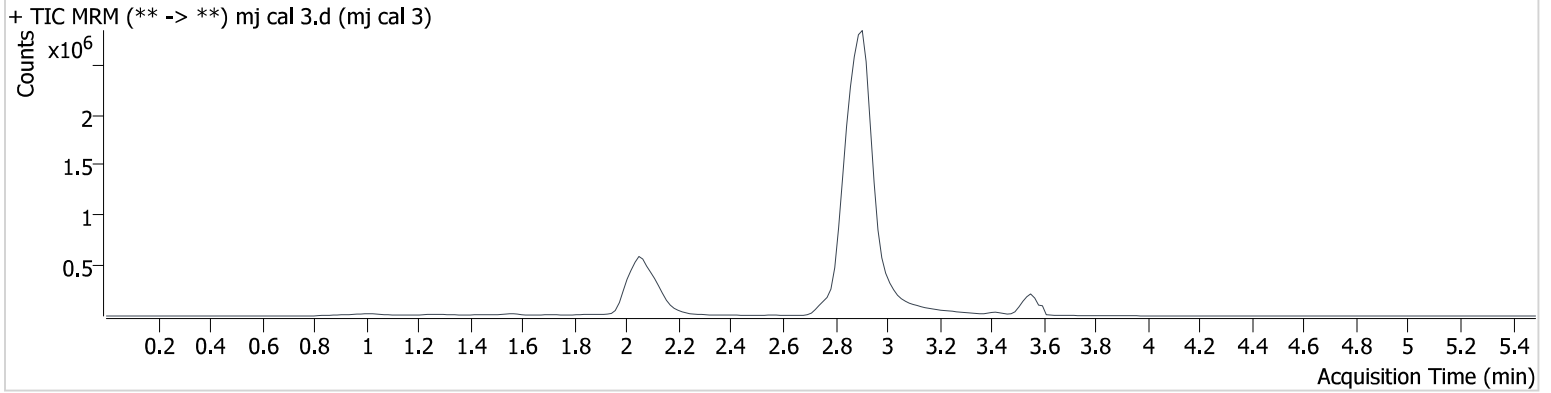
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** thc quant 50 50.m  
**Sample Position** P3-C1  
**Injection Volume** 10  
**Acq. Date-Time** 5/8/2024 11:58:13 PM  
**Sample Info.**

**Data File** mj cal 3.d  
**Sample** mj cal 3  
**Operator** Anne Nord  
**Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.051	32263	∞	858.22	4221.2	2388198	5.134 ng/ml
THC-COOH	2.122	83885	226.0	289.16	13030.7	1073623	18.290 ng/ml
THC	3.558	69960	∞	23.61	∞	507699	4.557 ng/ml

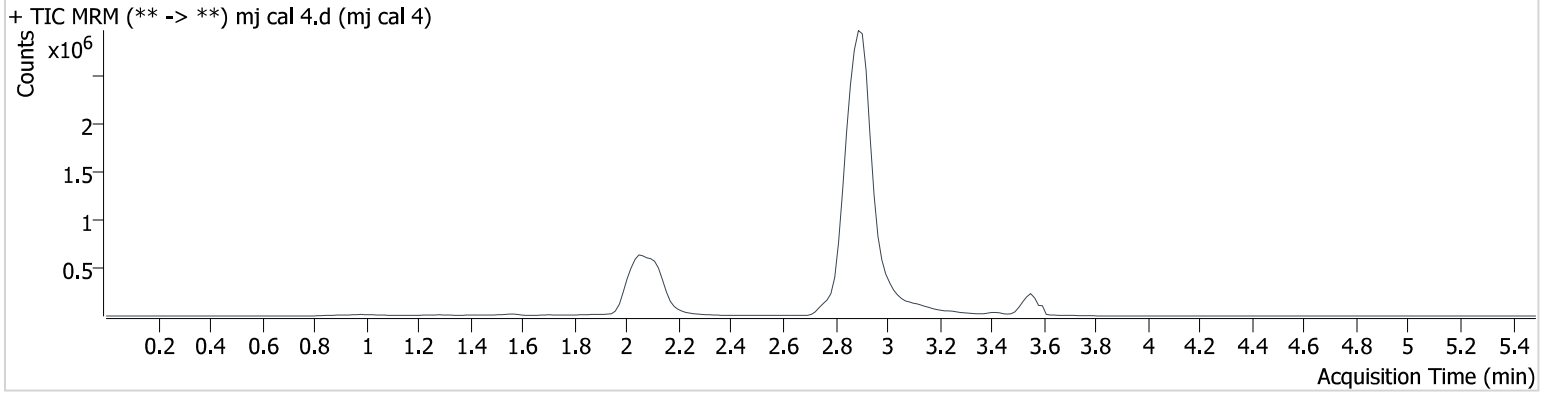
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** thc quant 50 50.m  
**Sample Position** P3-D1  
**Injection Volume** 10  
**Acq. Date-Time** 5/9/2024 12:04:49 AM  
**Sample Info.**

**Data File** mj cal 4.d  
**Sample** mj cal 4  
**Operator** Anne Nord  
**Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.051	58300	∞	963.07	∞	2081143	9.132 ng/ml
THC-COOH	2.122	215809	688163.7	273.26	816.1	1041596	44.281 ng/ml
THC	3.558	140842	∞	24.75	∞	474655	9.188 ng/ml

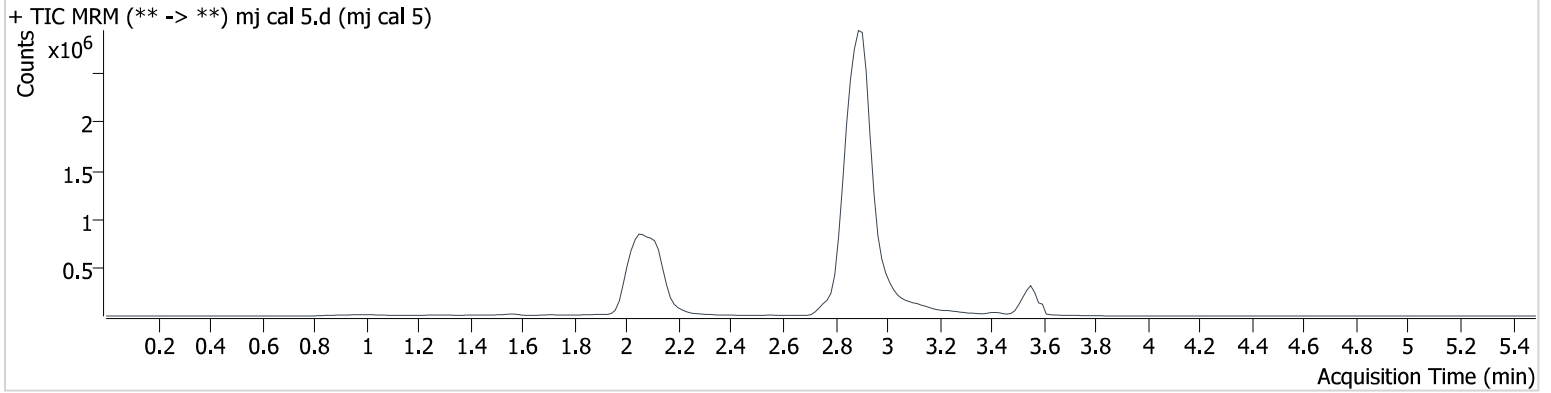
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** thc quant 50 50.m  
**Sample Position** P3-E1  
**Injection Volume** 10  
**Acq. Date-Time** 5/9/2024 12:11:25 AM  
**Sample Info.**

**Data File** mj cal 5.d  
**Sample** mj cal 5  
**Operator** Anne Nord  
**Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.051	169367	∞	875.70	∞	2129673	23.328 ng/ml
THC-COOH	2.107	328769	1752.3	279.88	149061 4.0	1035003	66.525 ng/ml
THC	3.558	364736	∞	27.80	∞	474772	22.925 ng/ml

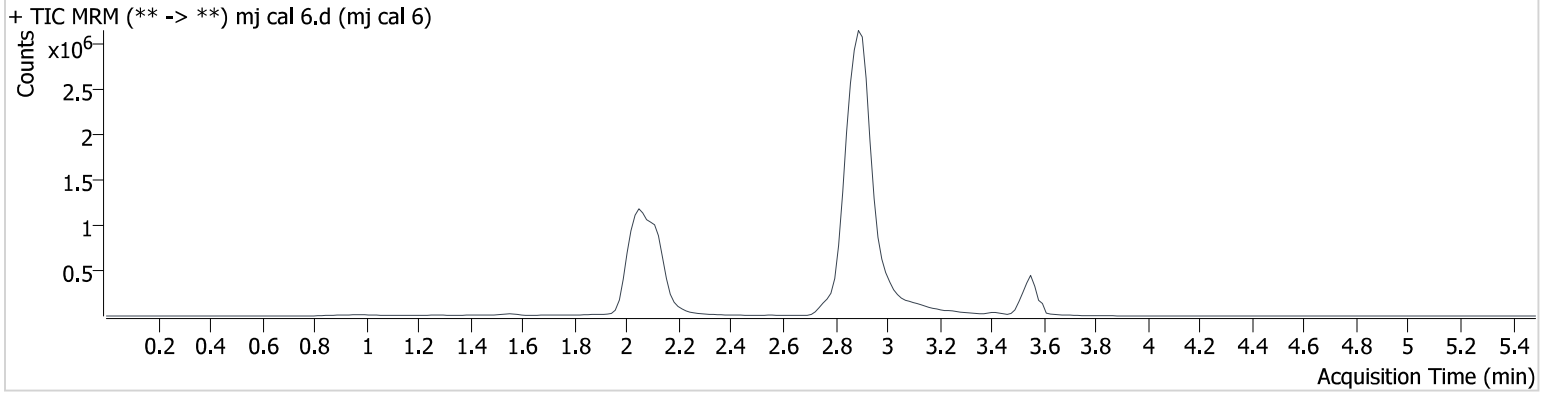
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** thc quant 50 50.m  
**Sample Position** P3-F1  
**Injection Volume** 10  
**Acq. Date-Time** 5/9/2024 12:18:01 AM  
**Sample Info.**

**Data File** mj cal 6.d  
**Sample** mj cal 6  
**Operator** Anne Nord  
**Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

## Sample Chromatogram



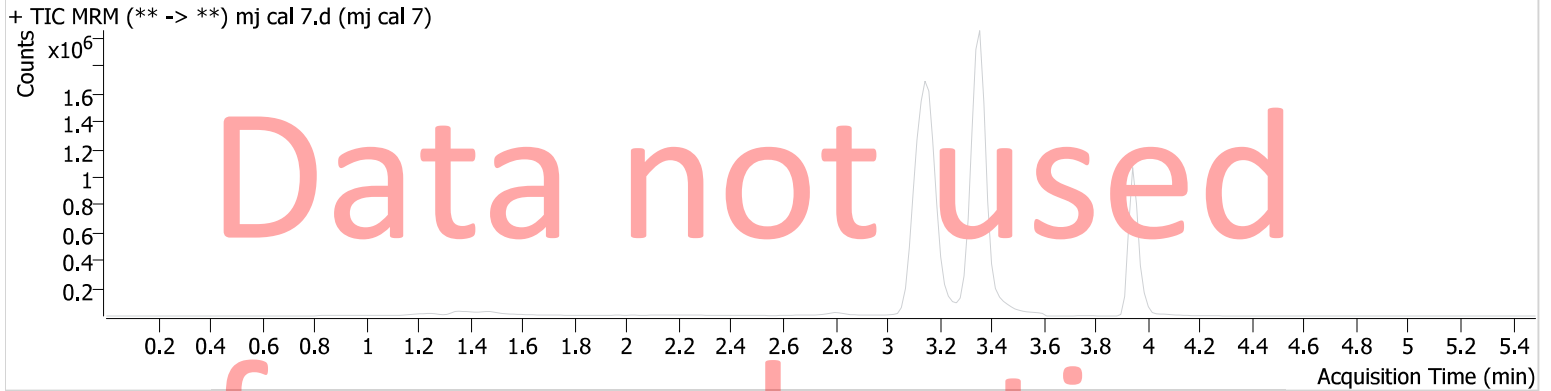
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.051	326673	21132.1	910.00	∞	2089496	44.498 ng/ml
THC-COOH	2.107	432957	1301.2	273.63	14750.1	860321	103.902 ng/ml
THC	3.558	734219	49928.5	25.75	∞	468150	46.237 ng/ml

# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 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>	thc quant 50 50.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-G1	<b>Comment</b>	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/9/2024 12:24:37 AM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	3.468	8740	∞	7.44 <b>Low</b>	∞	738	345.671 ng/ml

Sample did not inject properly little to no internal standard response. Sample was re-injected that injection was evaluated.



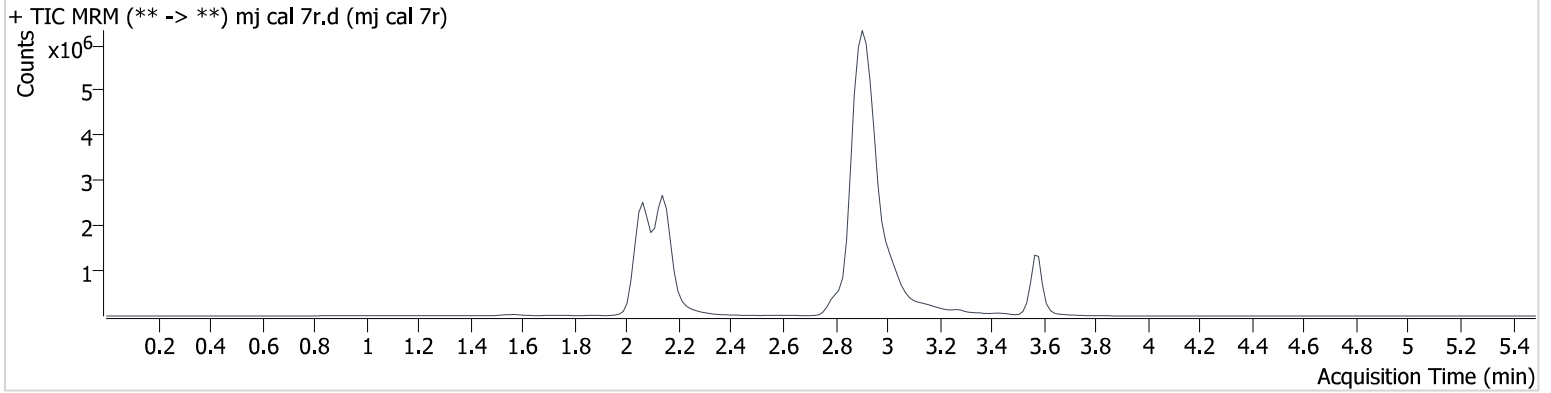
# AM #27 Cannabinoids

**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 9:36:21 AM

**Instrument** 69679  
**Type** Cal  
**Acq. Method** thc quant 50 50.m  
**Sample Position** P3-G1  
**Injection Volume** 10  
**Acq. Date-Time** 5/9/2024 8:50:43 AM  
**Sample Info.**

**Data File** mj cal 7r.d  
**Sample** mj cal 7r  
**Operator** Anne Nord  
**Comment** Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.066	930580	∞	808.41	∞	2419257	107.420 ng/ml
THC-COOH	2.137	1328788	3410.0	265.49	12647.2	1036336	260.768 ng/ml
THC	3.588	2550691	∞	26.87	∞	698792	106.892 ng/ml

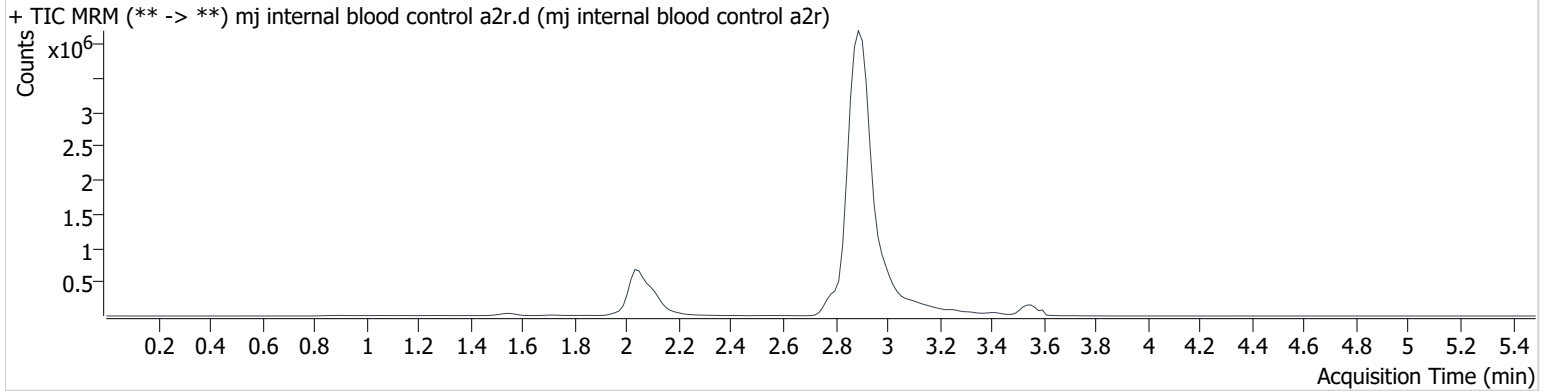
# AM #27 Cannabinoids



**Batch results** D:\MassHunter\Data\2024\am 27-28\050824r\QuantResults\am 27.batch.bin  
**Calibration Last Update** 5/9/2024 3:55:27 PM

<b>Instrument</b>	69679	<b>Data File</b>	mj internal blood control a2r.d
<b>Type</b>	Sample	<b>Sample</b>	mj internal blood control a2r
<b>Acq. Method</b>	thc quant 50 50.m	<b>Operator</b>	Anne Nord
<b>Sample Position</b>	P3-A2	<b>Comment</b>	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/9/2024 3:43:38 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	2.051	34636	∞	804.51	205.8	2275981	5.605 ng/ml
THC-COOH	2.107	66279	329.9	274.44	∞	990779	16.027 ng/ml
THC	3.558	63894	∞	25.31	313.1	422321	4.950 ng/ml