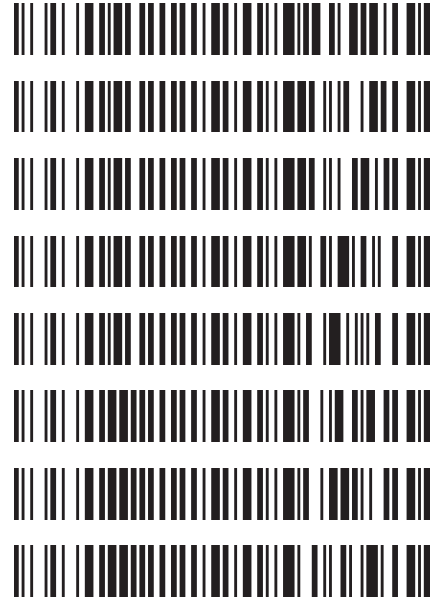


**Worklist: 6374**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-1560	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1662	3	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1663	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1678	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1781	4	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1259	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1260	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1289	1	BLOOD	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: 05/12/2023

Analyst: Celena Shrum

Plate lot#: 220802

Plate Retest Date: 07/23/2023

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

**Mobile phase B:** 0.1% Formic acid in Acetonitrile

**Blank Blood Lot:** Lampire 23A52593

**Column:** Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

**LCMS-QQQ ID:** 069901

### 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 (if applicable): add 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.
- 3. Using a calibrated pipette, add **1000µl blood or 1000µl hydrolyzed urine** into the appropriate wells of the analytical (standards) plate. **Pipette ID: #42**
- 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 5. Add **500µL of 0.1% formic acid in water to blood samples or 500µl of saturated phosphate buffer to urine samples** to the appropriate wells of the analytical plate.
- 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 7. Transfer **800µL of blood+acid mixture or urine+acid** to corresponding wells of SLE+ plate.
- 8. 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: 067104
- 9. Wait 5 minutes.
- 10. Add **2.25mL MTBE. (Add in 3 increments of 750uL)**
- 11. Wait 5 minutes.
- 12. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 13. Add **2.25mL Hexane. (Add in 3 increments of 750uL)**
- 14. Wait 5 minutes.
- 15. Apply positive pressure for approx. 15 seconds. **(10-15 PSI- Selector to the left).**
- 16. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. **SPE Dry ID: 067103**
- 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 and OH-THC 3ng/mL (quantitative), Carboxy-THC: 5ng/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. THC concentrations of 1-3ng/mL will be reported qualitatively.
- 5. Did all QCs pass for each analyte? (if not, 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:

	1	2	3	4	5	6
a					P2023-1259-1	QC 1
b					M2023-1781-4	cal 100 ng
c					M2023-1678-1	cal 50 ng
d					M2023-1663-2	cal 25 ng
e					M2023-1662-3	cal 10ng
f					M2023-1560-2	cal 5 ng
g				P2023-1289-1	NEG Blood	cal 3 ng
h				P2023-1260-1	QC 2	cal 1ng

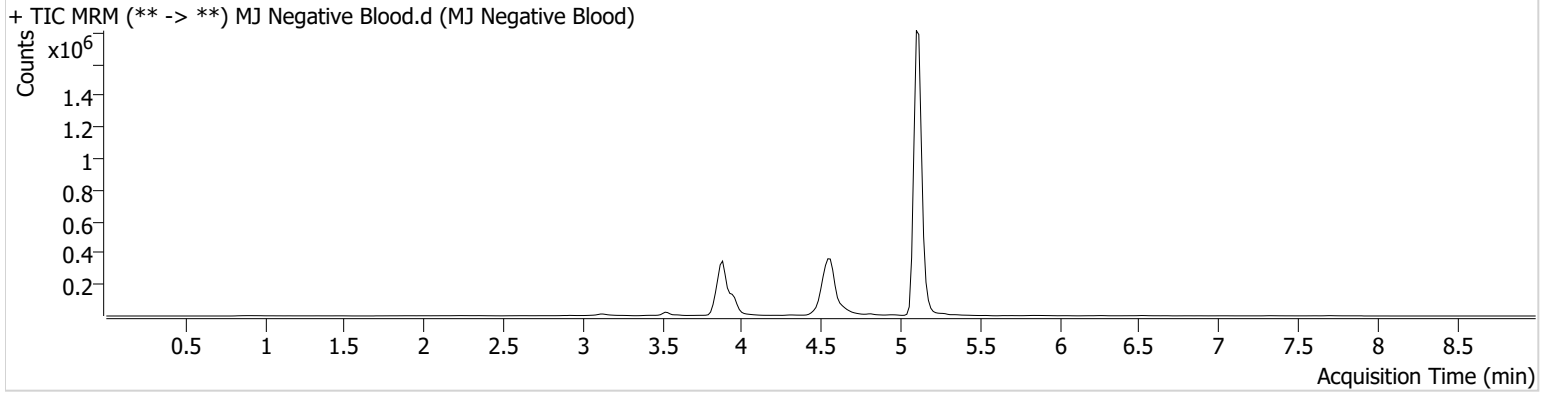


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ Negative Blood.d
<b>Type</b>	Sample	<b>Sample</b>	MJ Negative Blood
<b>Acq. Method</b>	AM 27 Agilent Method.m	<b>Operator</b>	Celena Shrum
<b>Sample Position</b>	P1-G5	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/12/2023 7:44:42 PM		
<b>Sample Info.</b>			

## Sample Chromatogram





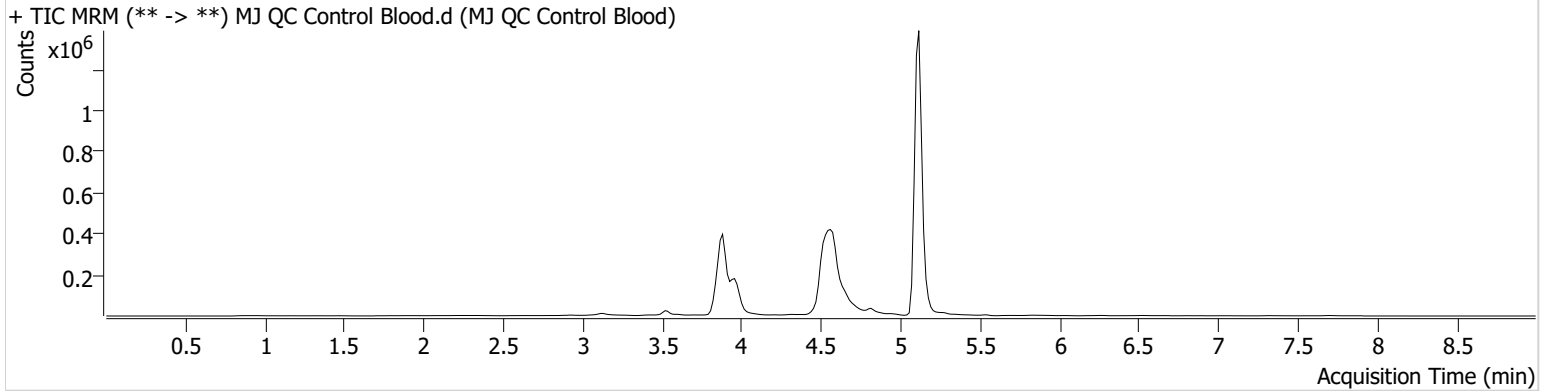
# AM #27 Cannabinoid Quant. Results



**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	MJ QC Control Blood.d
<b>Type</b>	QC	<b>Sample</b>	MJ QC Control Blood
<b>Acq. Method</b>	AM 27 Agilent Method.m	<b>Operator</b>	Celena Shrum
<b>Sample Position</b>	P1-A6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/12/2023 7:18:30 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	107304	260.96	14.0	39.13	1498989	4.6252 ng/ml
THC-COOH	3.969	42653	∞	225.3	579.91	411864	13.8988 ng/ml
THC	5.120	185628	2794.80	26.3	∞	4217979	5.0803 ng/ml

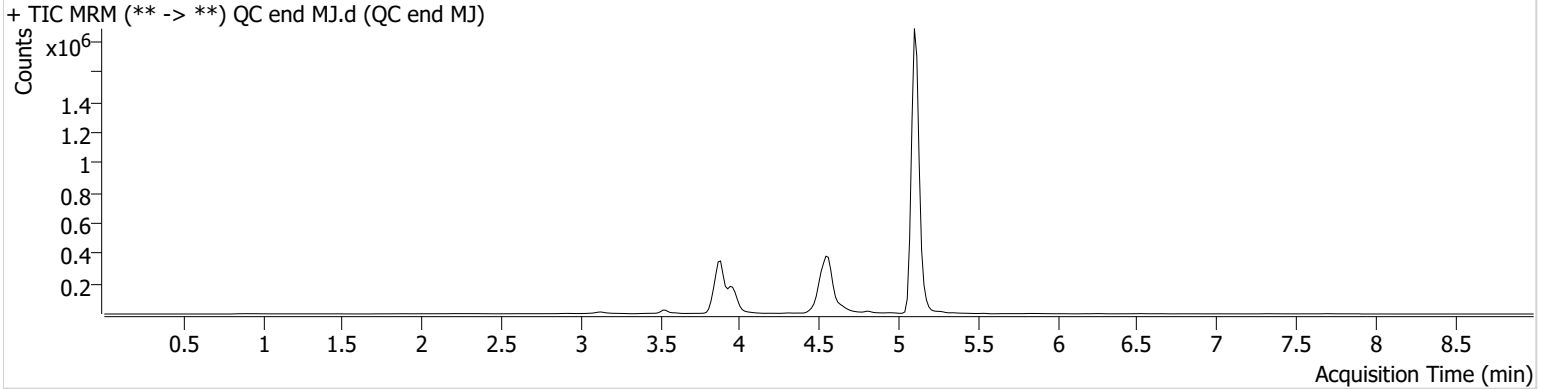


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

**Instrument** Falco (069901) **Data File** QC end MJ.d  
**Type** QC **Sample** QC end MJ  
**Acq. Method** AM 27 Agilent Method.m **Operator** Celena Shrum  
**Sample Position** P1-H5 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 5/12/2023 11:40:28 PM  
**Sample Info.**

## Sample Chromatogram

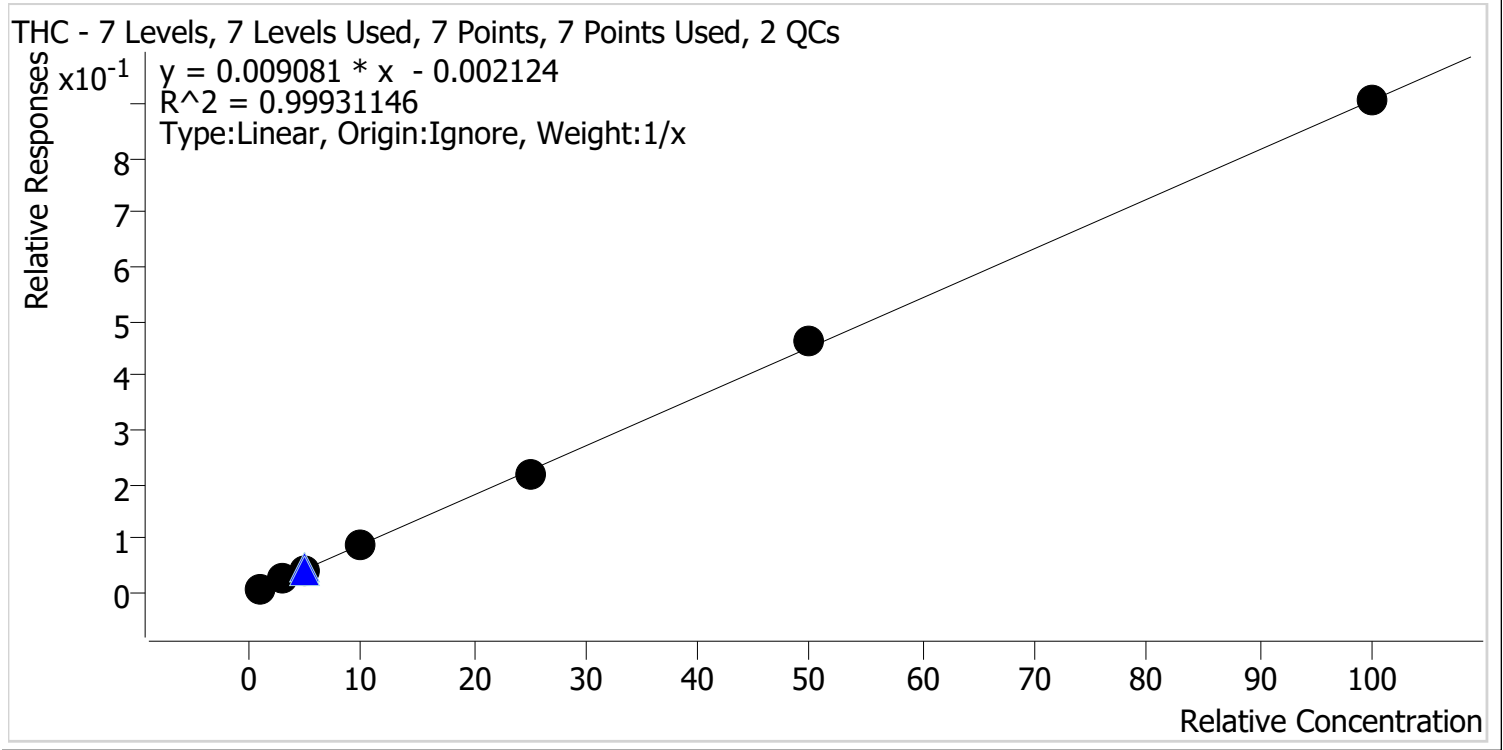


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	99607	∞	13.9	∞	1373346	4.6839 ng/ml
THC-COOH	3.969	44785	747.18	225.5	∞	410784	14.5965 ng/ml
THC	5.105	239961	∞	25.6	∞	5985471	4.6488 ng/ml



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 5/15/2023 9:01 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC **Internal Standard** THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	105.8
Cal 2 MJ	2	✓	3.0	3.0	99.8
Cal 3 MJ	3	✓	5.0	5.0	99.5
Cal 4 MJ	4	✓	10.0	9.6	96.4
Cal 5 MJ	5	✓	25.0	23.9	95.5
Cal 6 MJ	6	✓	50.0	51.5	103.0
Cal 7 MJ	7	✓	100.0	99.9	99.9

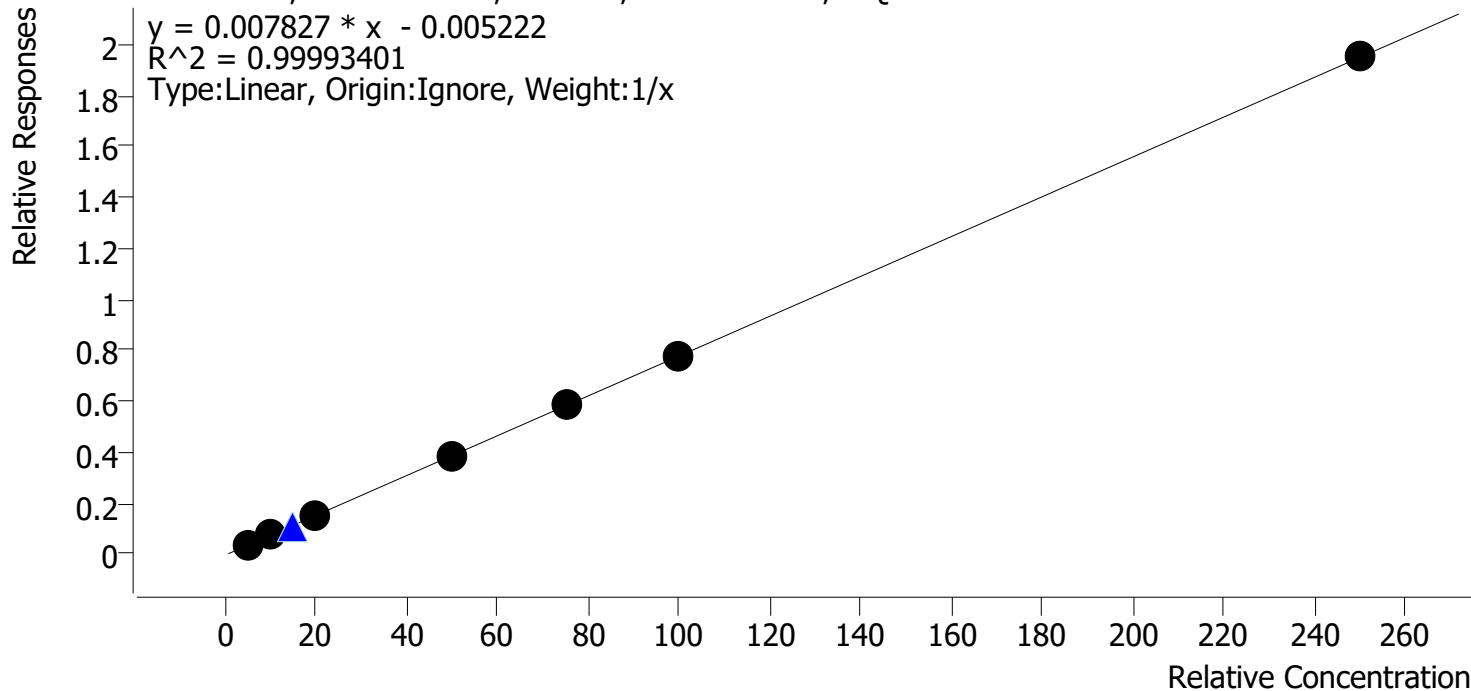
CS



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 5/15/2023 9:01 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

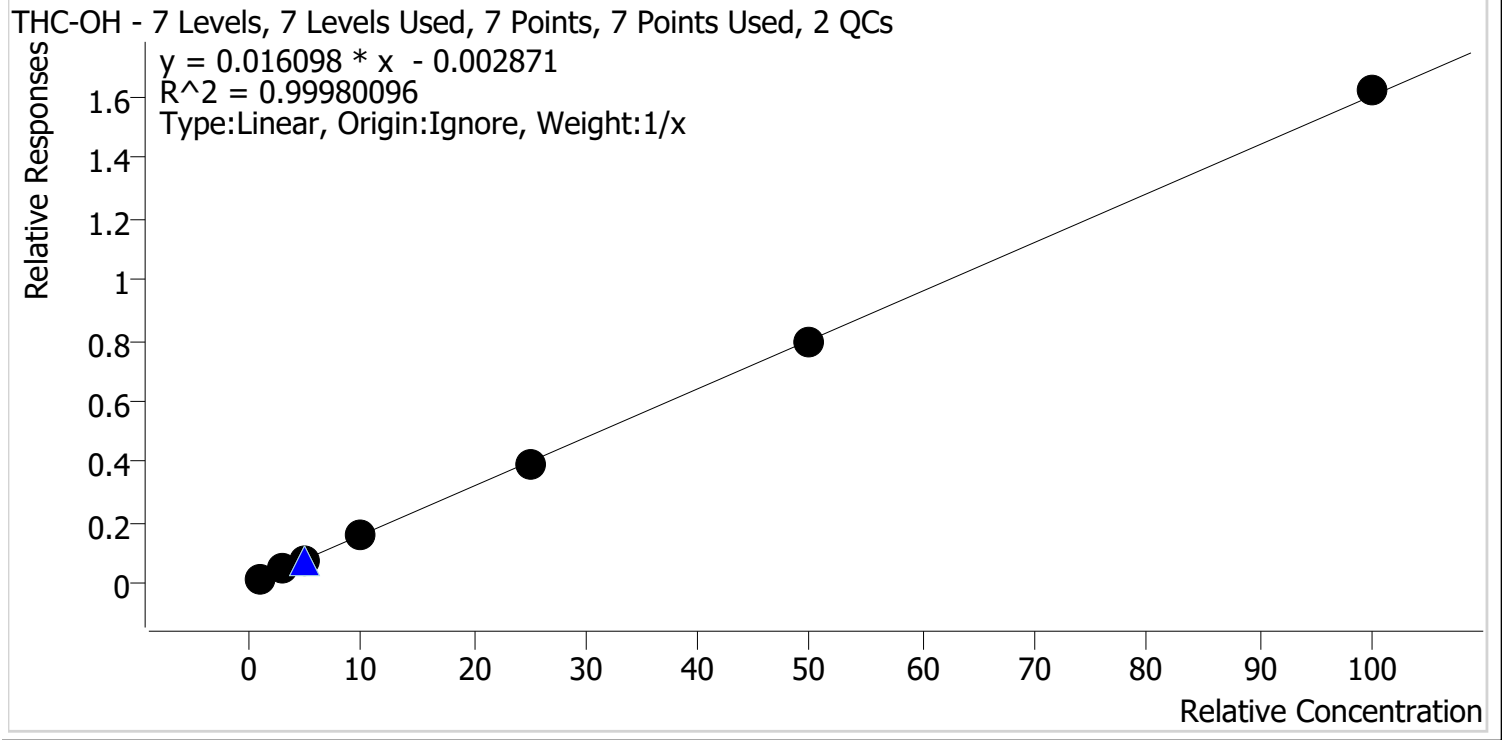


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.2	104.2
Cal 2 MJ	2	✓	10.0	9.6	96.2
Cal 3 MJ	3	✓	20.0	19.9	99.5
Cal 4 MJ	4	✓	50.0	50.1	100.1
Cal 5 MJ	5	✓	75.0	74.8	99.7
Cal 6 MJ	6	✓	100.0	100.1	100.1
Cal 7 MJ	7	✓	250.0	250.3	100.1



# AM #27 Cannabinoids Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Last Cal. Update** 5/15/2023 9:01 AM  
**Analyst Name** ISP\datastor  
**Analyte** THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	105.2
Cal 2 MJ	2	✓	3.0	3.0	99.3
Cal 3 MJ	3	✓	5.0	5.0	99.6
Cal 4 MJ	4	✓	10.0	9.7	96.7
Cal 5 MJ	5	✓	25.0	24.8	99.2
Cal 6 MJ	6	✓	50.0	49.5	98.9
Cal 7 MJ	7	✓	100.0	101.1	101.1

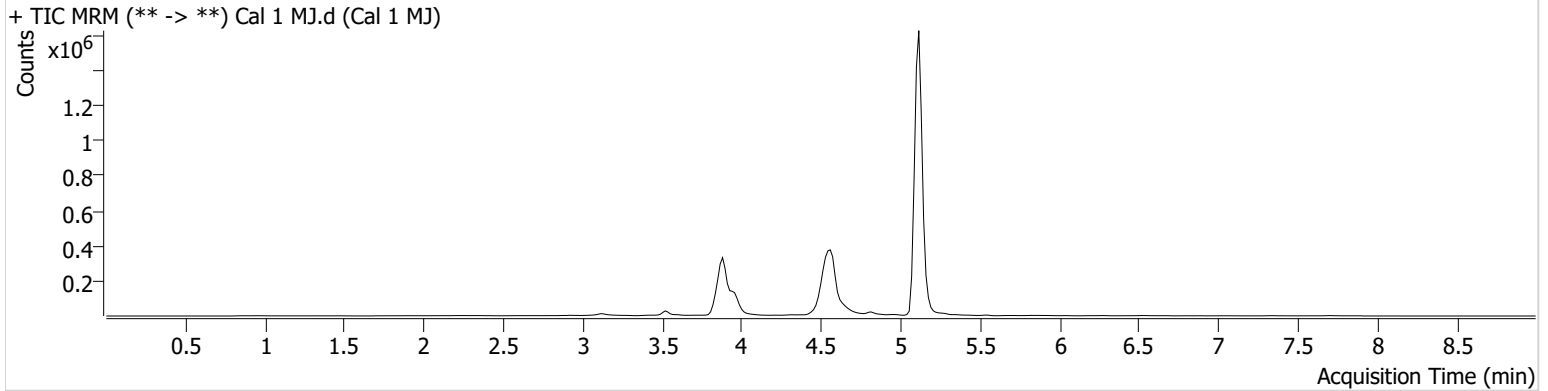


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	Cal 1 MJ.d
<b>Type</b>	Cal	<b>Sample</b>	Cal 1 MJ
<b>Acq. Method</b>	AM 27 Agilent Method.m	<b>Operator</b>	Celena Shrum
<b>Sample Position</b>	P1-H6	<b>Comment</b>	
<b>Injection Volume</b>	10		
<b>Acq. Date-Time</b>	5/12/2023 5:33:33 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	19074	36.46	13.3	10.24	1355543	1.0525 ng/ml <b>Low</b>
THC-COOH	3.969	13590	∞	232.0	∞	382035	5.2123 ng/ml
THC	5.120	40329	∞	31.1	∞	5386622	1.0584 ng/ml

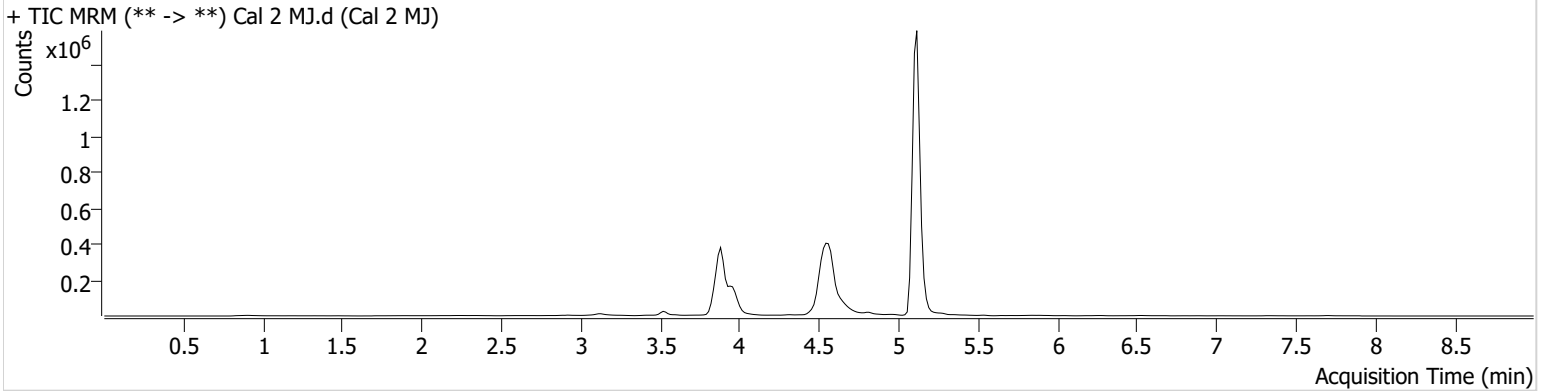


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

**Instrument** Falco (069901) **Data File** Cal 2 MJ.d  
**Type** Cal **Sample** Cal 2 MJ  
**Acq. Method** AM 27 Agilent Method.m **Operator** Celena Shrum  
**Sample Position** P1-G6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 5/12/2023 5:46:48 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	67220	∞	13.8	79.88	1491121	2.9788 ng/ml <b>Low</b>
THC-COOH	3.969	28447	217.63	228.8	∞	406114	9.6168 ng/ml
THC	5.120	127353	1887.28	25.2	∞	5080880	2.9942 ng/ml

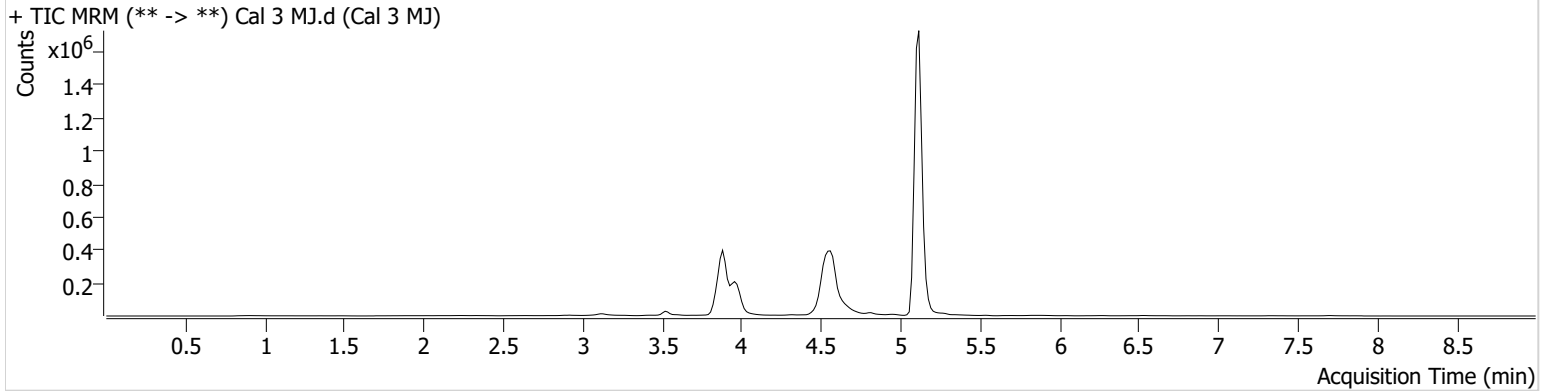


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

**Instrument** Falco (069901) **Data File** Cal 3 MJ.d  
**Type** Cal **Sample** Cal 3 MJ  
**Acq. Method** AM 27 Agilent Method.m **Operator** Celena Shrum  
**Sample Position** P1-F6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 5/12/2023 5:59:54 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	115809	∞	14.2	∞	1498381	4.9797 ng/ml
THC-COOH	3.969	63233	1229.31	217.9	∞	419958	19.9049 ng/ml
THC	5.120	235203	∞	23.9	∞	5465429	4.9731 ng/ml



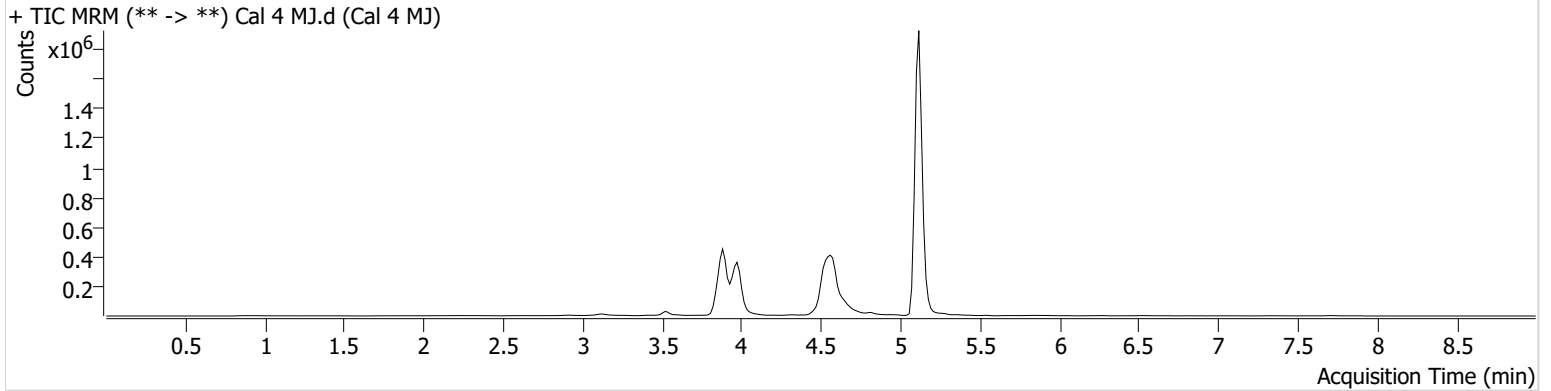


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

**Instrument** Falco (069901) **Data File** Cal 4 MJ.d  
**Type** Cal **Sample** Cal 4 MJ  
**Acq. Method** AM 27 Agilent Method.m **Operator** Celena Shrum  
**Sample Position** P1-E6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 5/12/2023 6:12:59 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	238334	∞	14.4	∞	1559833	9.6701 ng/ml
THC-COOH	3.969	160306	1941.07	216.1	1753.74	414563	50.0725 ng/ml
THC	5.120	469189	∞	25.4	∞	5490735	9.6441 ng/ml

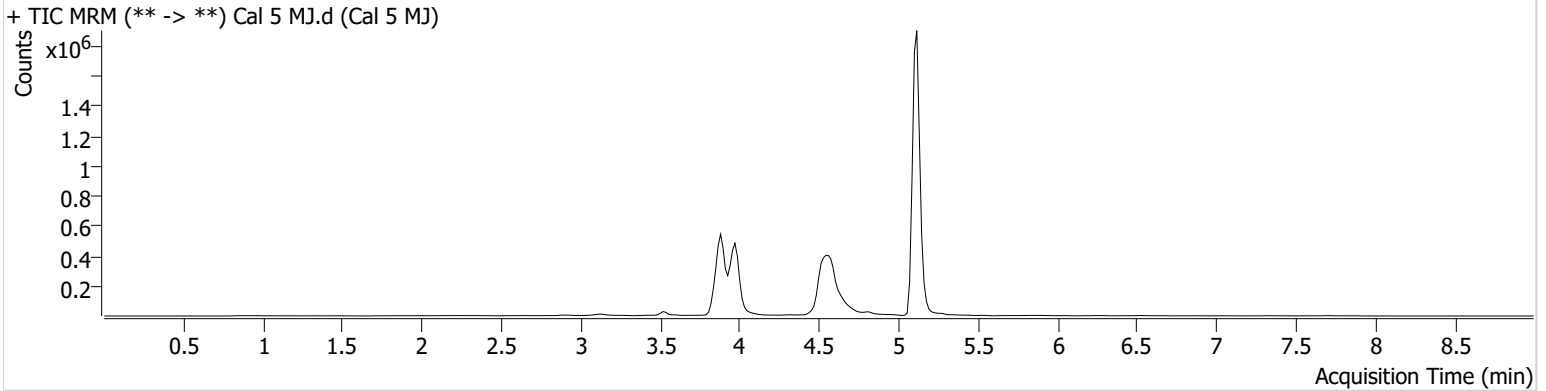


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

**Instrument** Falco (069901) **Data File** Cal 5 MJ.d  
**Type** Cal **Sample** Cal 5 MJ  
**Acq. Method** AM 27 Agilent Method.m **Operator** Celena Shrum  
**Sample Position** P1-D6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 5/12/2023 6:26:05 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	612931	∞	14.3	737.07	1546590	24.7977 ng/ml
THC-COOH	3.969	234239	∞	214.5	∞	403893	74.7655 ng/ml
THC	5.120	1041497	20517.88	24.4	∞	4852863	23.8682 ng/ml

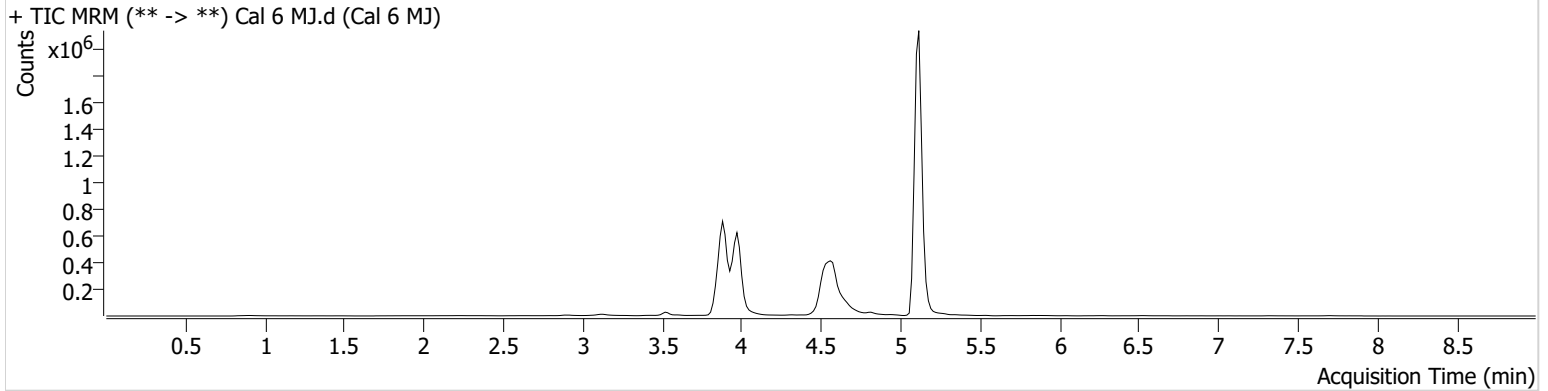


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

**Instrument** Falco (069901) **Data File** Cal 6 MJ.d  
**Type** Cal **Sample** Cal 6 MJ  
**Acq. Method** AM 27 Agilent Method.m **Operator** Celena Shrum  
**Sample Position** P1-C6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 5/12/2023 6:39:10 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	1226103	1808.67	14.3	∞	1545760	49.4532 ng/ml
THC-COOH	3.969	305010	6191.04	220.7	∞	391934	100.0970 ng/ml
THC	5.120	2064269	∞	26.4	∞	4432316	51.5223 ng/ml

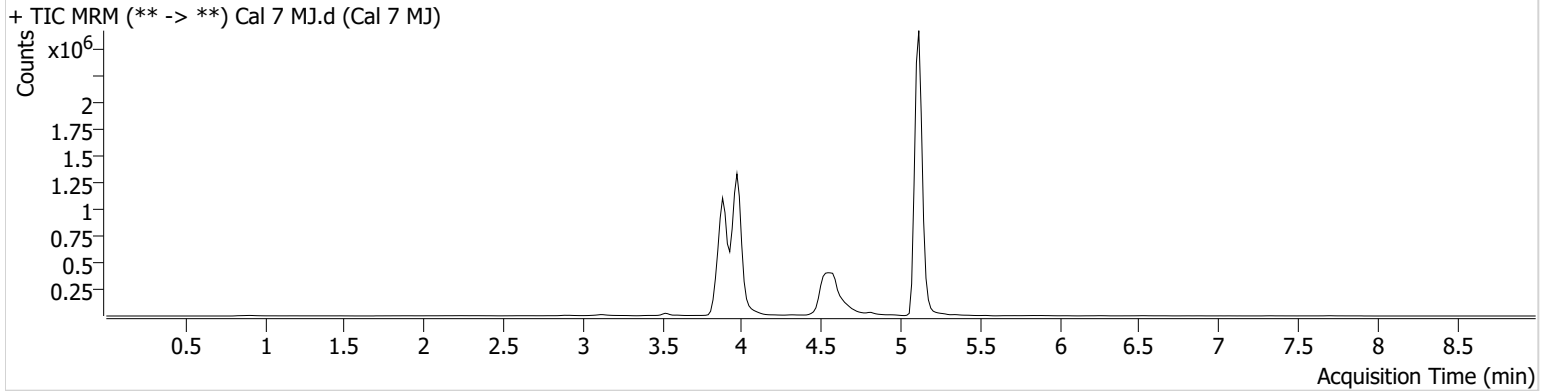


# AM #27 Cannabinoid Quant. Results

**Batch results** D:\MassHunter\Data\2023\AM 27 28\051223 AM 27 28 CS\QuantResults\AM 27.batch.bin  
**Calibration Last Update** 5/15/2023 9:01:05 AM

**Instrument** Falco (069901) **Data File** Cal 7 MJ.d  
**Type** Cal **Sample** Cal 7 MJ  
**Acq. Method** AM 27 Agilent Method.m **Operator** Celena Shrum  
**Sample Position** P1-B6 **Comment**  
**Injection Volume** 10  
**Acq. Date-Time** 5/12/2023 6:52:15 PM  
**Sample Info.**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.881	2644580	∞	14.3	∞	1628358	101.0681 ng/ml
THC-COOH	3.969	758118	11232.23	213.4	∞	387968	250.3310 ng/ml
THC	5.120	3737595	20090.29	27.0	∞	4128153	99.9397 ng/ml