

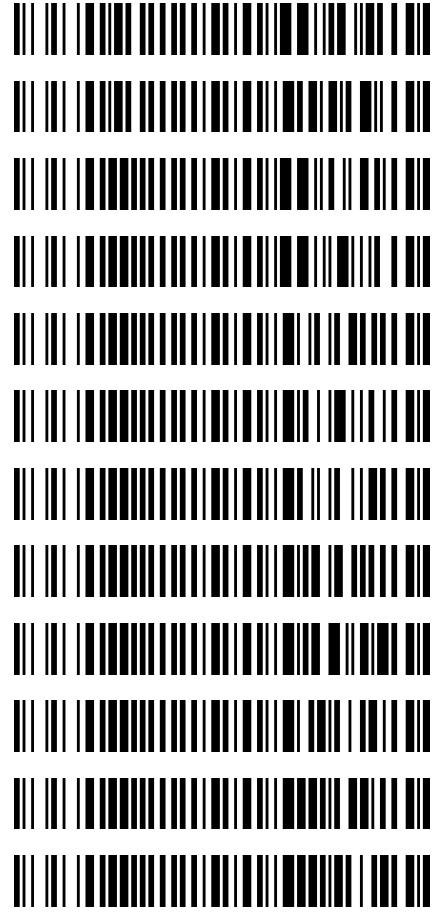
REVIEWED
By Sarah Collins at 9:46 am, May 05, 2023

TS

5/4/2023

Worklist: 6357

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-1331	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1418	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0934	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0951	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1022	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1046	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1086	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1088	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1092	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1099	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1133	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1134	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: 05/02/2023

Plate lot#: 230113

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 23A52593

Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Tamara Salazar

Plate Retest Date: 07/13/2023

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Urine Lot: [Click here to enter text.](#)

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: 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 and urine (if applicable) (calibrated pipette)** into the appropriate wells of 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, and 500µL of saturated phosphate buffer to urine samples** in the wells of the analytical plate.
- 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 7. Transfer **700-800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
Amount transferred: 750µL
- 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)
- 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.
- 17. 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. 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:

Internal standard blank ran out midway through the run. The blank was remade and any sample with a following a blank with low internal standard response was reinjected. More reconstitution solvent was added to the samples as the original solvent had evaporated overnight.

THC-OH -- 3-100 -- calibrator 1 dropped due to S/N

Analytical Plate Map

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	P2023-1046-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	IS + Sample	P2023-1022-1	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	P2023-1134-1	P2023-0951-1	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	P2023-1133-1	P2023-0934-1	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	P2023-1099-1	M2023-1418-1	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	P2023-1092-1	M2023-1331-1	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2023-1088-3	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2023-1086-1	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

SLE Plate Map

TS

	1	2	3	4	5	6
A				P2023-1046-1	P2023-1046-1*	IS + QC_1
B				P2023-1086-1	P2023-1022-1	IS + Cal. 7
C				P2023-1134-1	P2023-0951-1	IS + Cal. 6
D				P2023-1133-1	P2023-0934-1	IS + Cal. 5
E				P2023-1099-1	M2023-1418-1	IS + Cal. 4
F				P2023-1092-1	M2023-1331-1	IS + Cal. 3
G				P2023-1088-3	Neg Blood	IS + Cal. 2
H				P2023-1086-1*	IS + QC_1	IS + Cal. 1

*Sample moved during step 7 of the extraction process due to a clot

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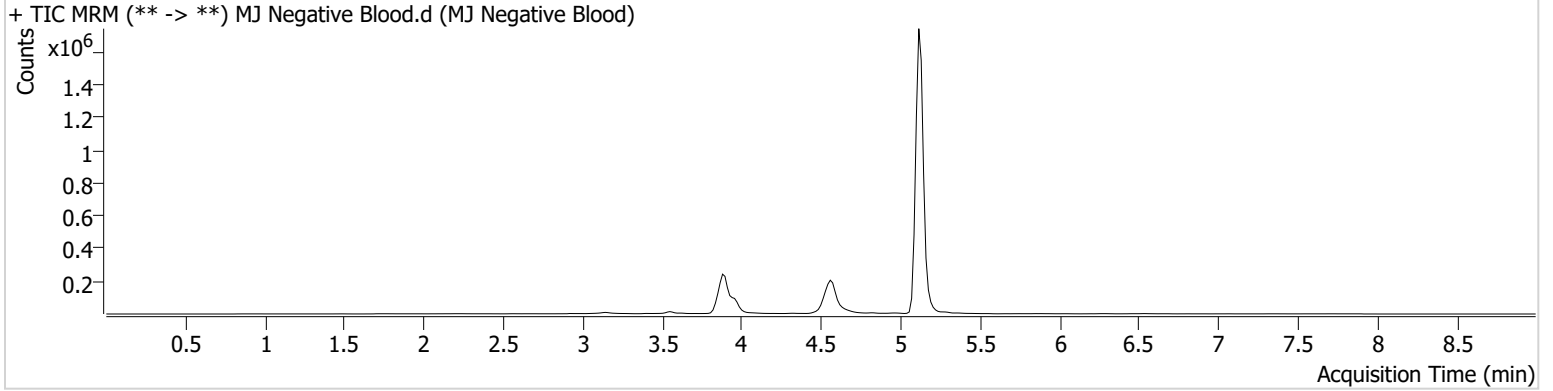


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-G5	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 4:54:57 PM		
Sample Info.			

Sample Chromatogram



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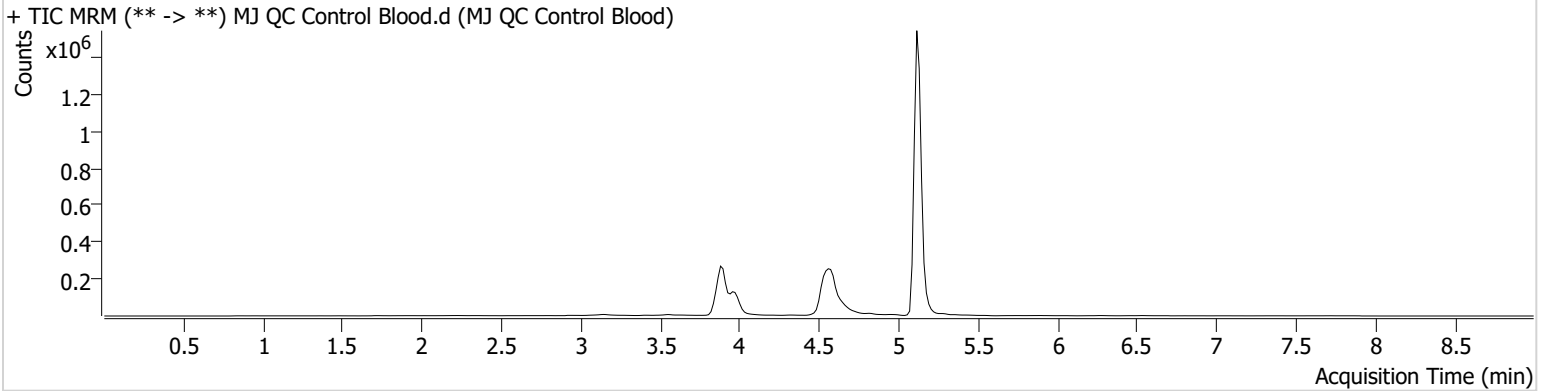


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	MJ QC Control Blood.d
Type	QC	Sample	MJ QC Control Blood
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 4:28:45 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	80989	∞	14.1	∞	1012652	4.7568 ng/ml
THC-COOH	3.985	31014	414.79	226.0	477.78	285294	15.0961 ng/ml
THC	5.120	191556	∞	26.9	∞	4489158	5.0482 ng/ml

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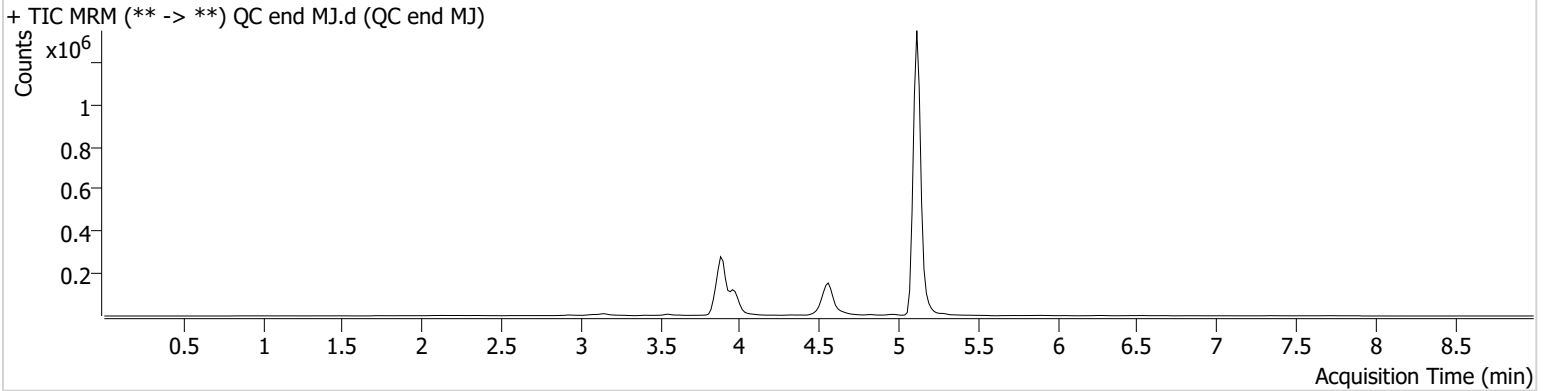


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-H5	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 10:35:30 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	84217	2905.91	15.2	∞	1050967	4.7656 ng/ml
THC-COOH	3.985	27479	644.32	230.2	521.08	265364	14.4053 ng/ml
THC	5.120	161812	∞	26.6	∞	4178650	4.6090 ng/ml

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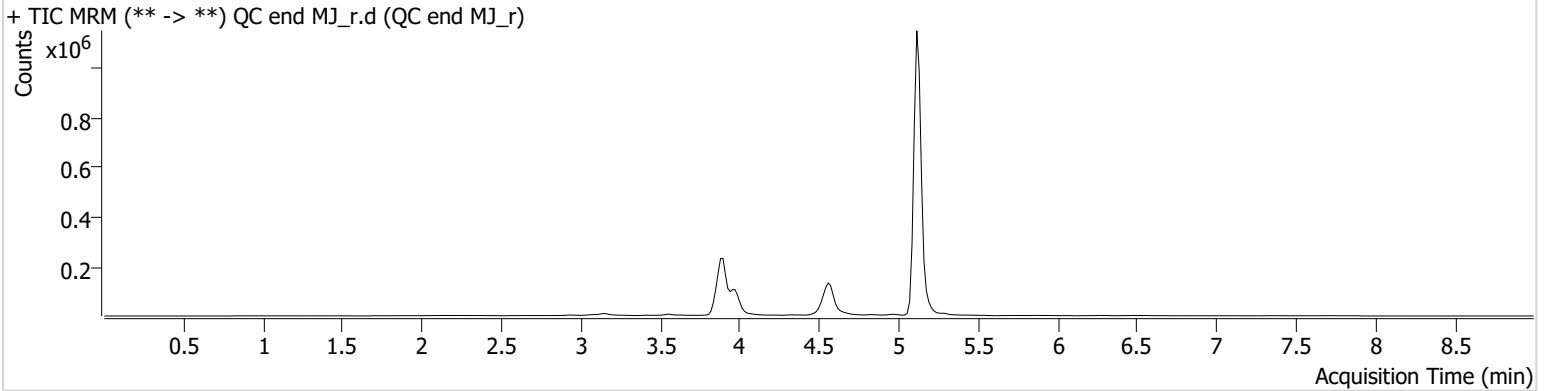
AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument Falco (069901) **Data File** QC end MJ_r.d
Type QC **Sample** QC end MJ_r
Acq. Method AM 27 Agilent Method.m **Operator** Tamara Salazar
Sample Position P1-H5 **Comment**
Injection Volume
Acq. Date-Time 3/20/2005 1:30:47 PM
Sample Info.

QC ran after reinjected samples.

Sample Chromatogram



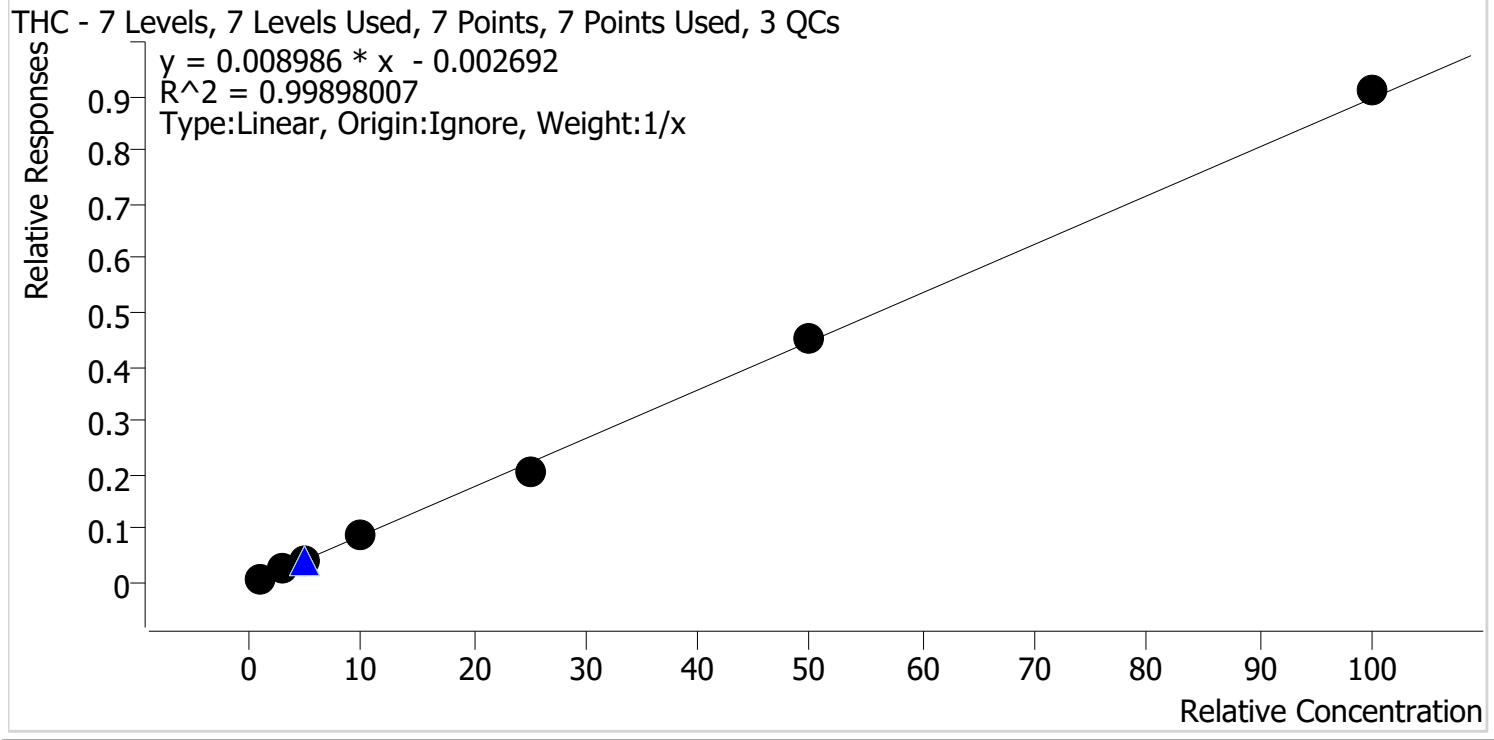
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	70876	136.55	14.2	48.12	904429	4.6654 ng/ml
THC-COOH	3.985	25409	133.46	235.9	∞	234828	15.0285 ng/ml
THC	5.120	139986	1607.25	26.7	∞	3456312	4.8068 ng/ml

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 5/4/2023 8:29 AM
Analyst Name ISP\tsalazar
Analyte THC **Internal Standard** THC-D3



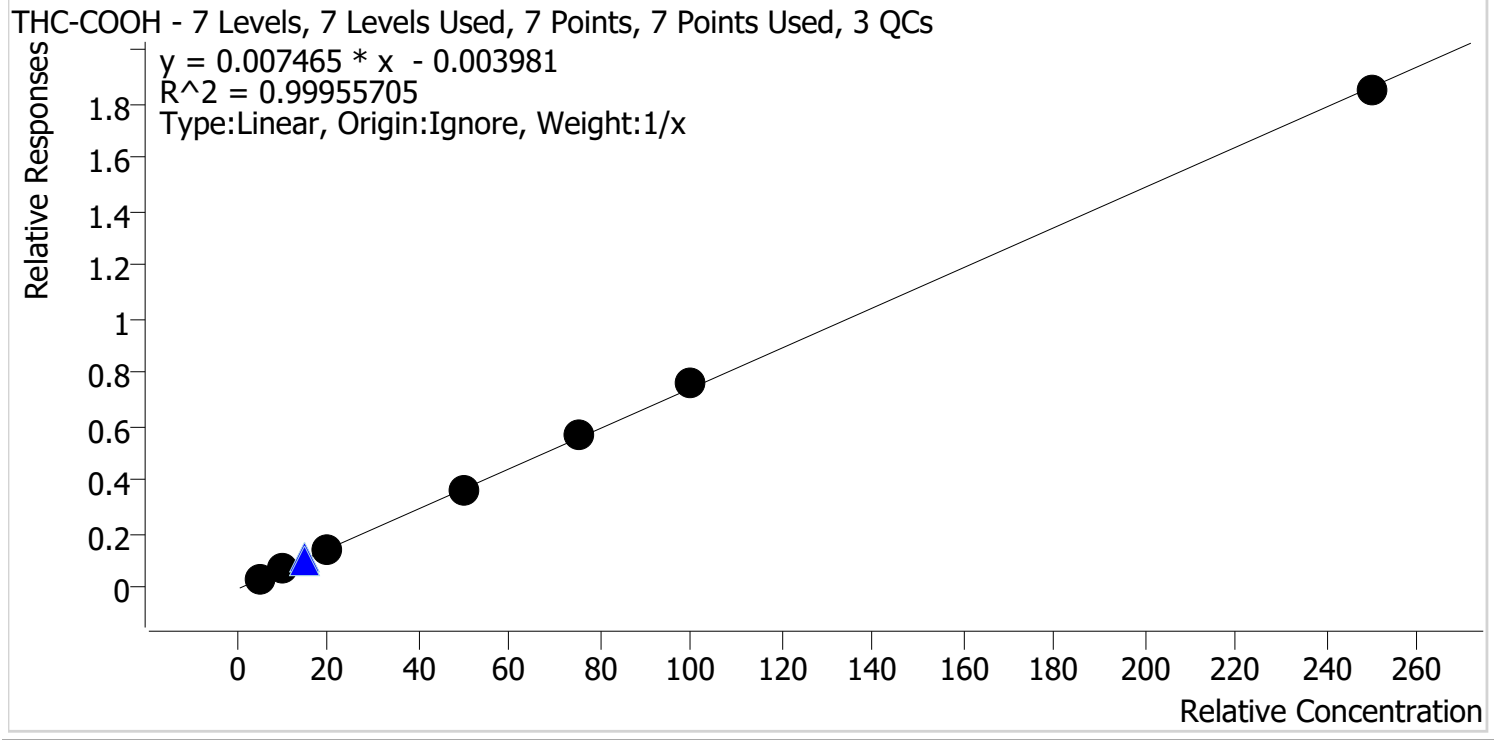
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	113.2
Cal 2 MJ	2	✓	3.0	3.0	100.0
Cal 3 MJ	3	✓	5.0	4.6	92.7
Cal 4 MJ	4	✓	10.0	9.8	97.9
Cal 5 MJ	5	✓	25.0	23.5	93.9
Cal 6 MJ	6	✓	50.0	50.4	100.7
Cal 7 MJ	7	✓	100.0	101.6	101.6

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 5/4/2023 8:29 AM
Analyst Name ISP\tsalazar
Analyte THC-COOH **Internal Standard** THC-COOH-D9



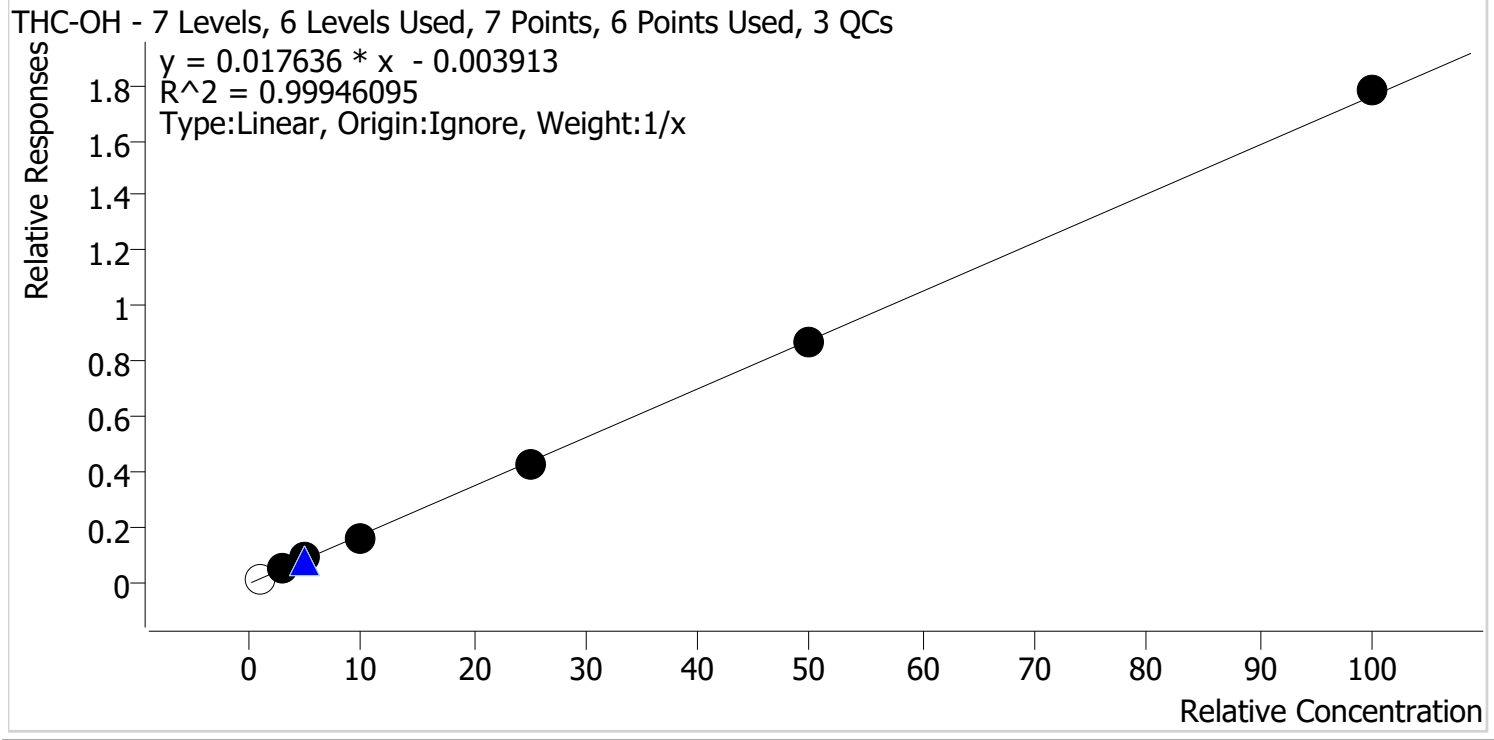
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.2	104.0
Cal 2 MJ	2	✓	10.0	10.0	99.5
Cal 3 MJ	3	✓	20.0	19.0	94.9
Cal 4 MJ	4	✓	50.0	49.0	98.1
Cal 5 MJ	5	✓	75.0	76.6	102.1
Cal 6 MJ	6	✓	100.0	102.1	102.1
Cal 7 MJ	7	✓	250.0	248.1	99.3

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 5/4/2023 8:29 AM
Analyst Name ISP\tsalazar
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	1.0	1.0	102.3
Cal 2 MJ	2	✓	3.0	3.1	104.9
Cal 3 MJ	3	✓	5.0	5.1	102.9
Cal 4 MJ	4	✓	10.0	9.4	93.9
Cal 5 MJ	5	✓	25.0	24.4	97.7
Cal 6 MJ	6	✓	50.0	49.8	99.5
Cal 7 MJ	7	✓	100.0	101.1	101.1

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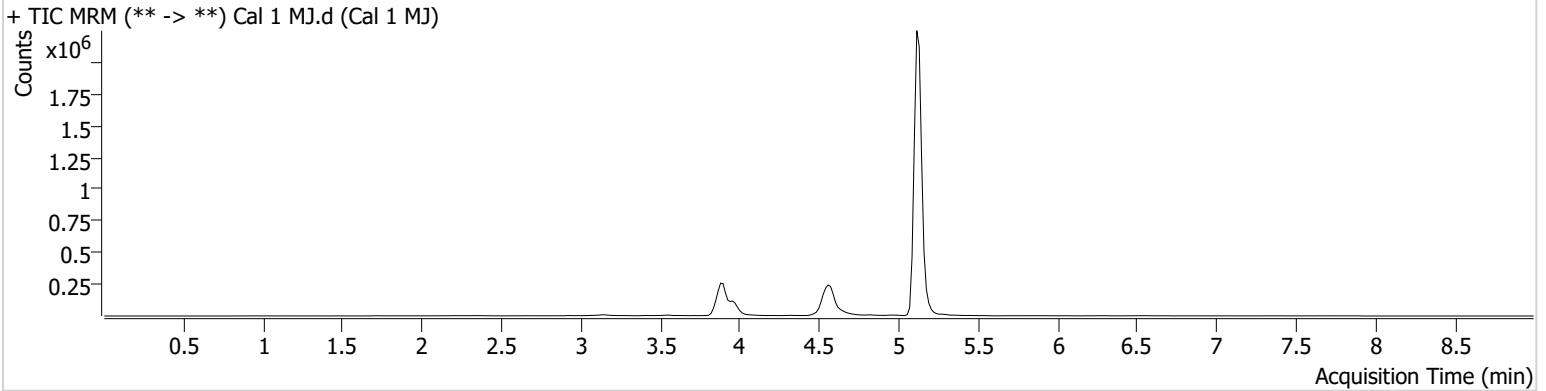


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 2:42:30 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	14890	1.72 Low	16.4	12.42	1054269	1.0227 ng/ml Low
THC-COOH	3.985	11022	151.56	236.4	1365.66	316435	5.1996 ng/ml
THC	5.135	55208	2038.21	29.4	∞	7383460	1.1316 ng/ml

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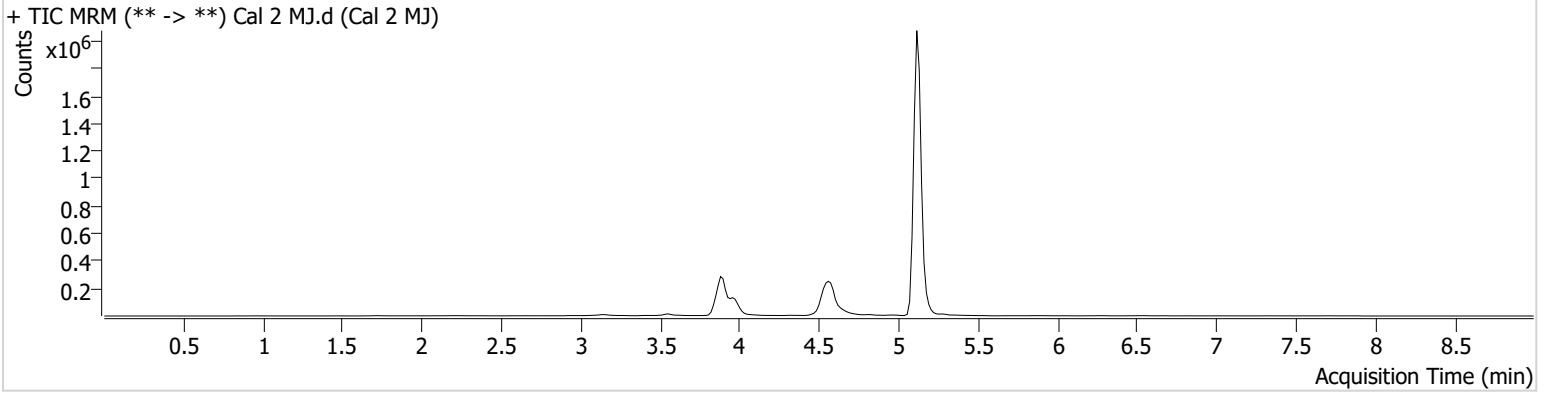


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-G6	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 2:55:46 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	57842	∞	13.7	146.72	1121416	3.1466 ng/ml
THC-COOH	3.985	22843	∞	226.9	∞	324807	9.9548 ng/ml
THC	5.120	158437	111.77	26.3	∞	6528236	3.0004 ng/ml

TS

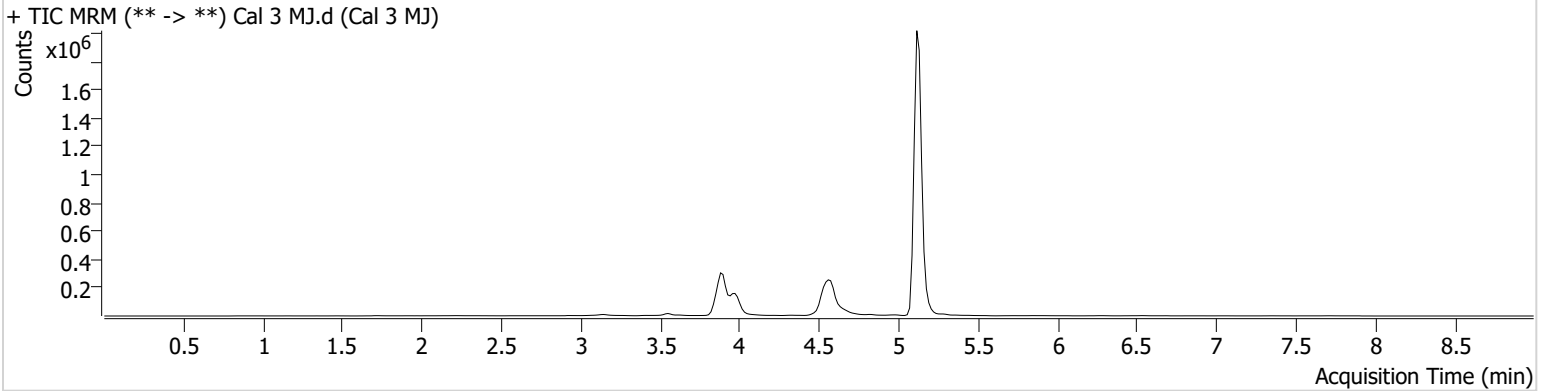


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 3:08:51 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	101599	∞	13.6	245.80	1170335	5.1443 ng/ml
THC-COOH	3.985	44963	463.78	236.0	∞	326429	18.9855 ng/ml
THC	5.135	248453	3108.12	27.0	∞	6378617	4.6343 ng/ml

TS

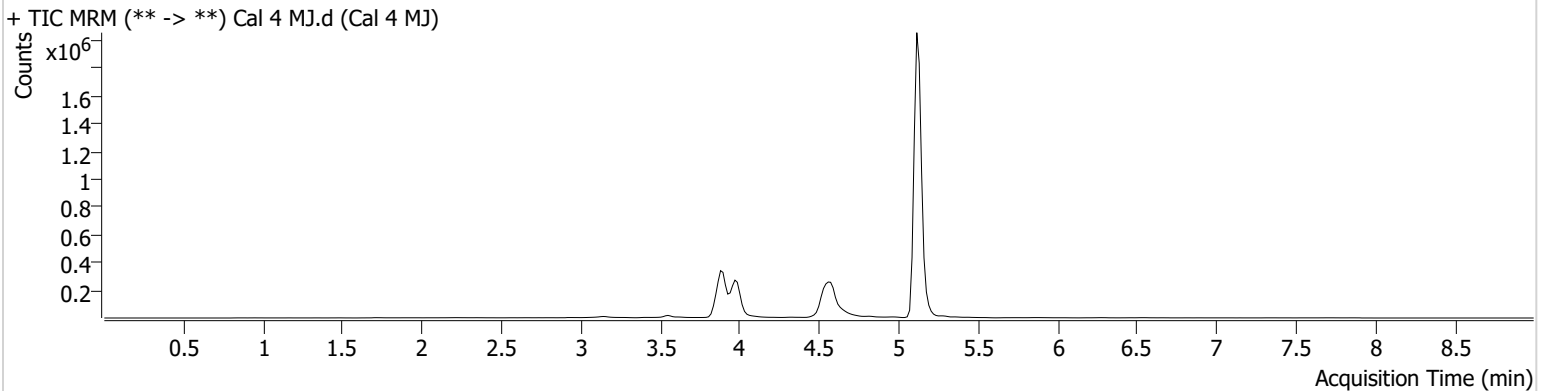


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 3:21:57 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	193641	∞	15.0	694.50	1197477	9.3910 ng/ml
THC-COOH	3.985	116550	2084.58	228.9	∞	321977	49.0252 ng/ml
THC	5.120	508840	∞	26.0	∞	5968439	9.7873 ng/ml

TS

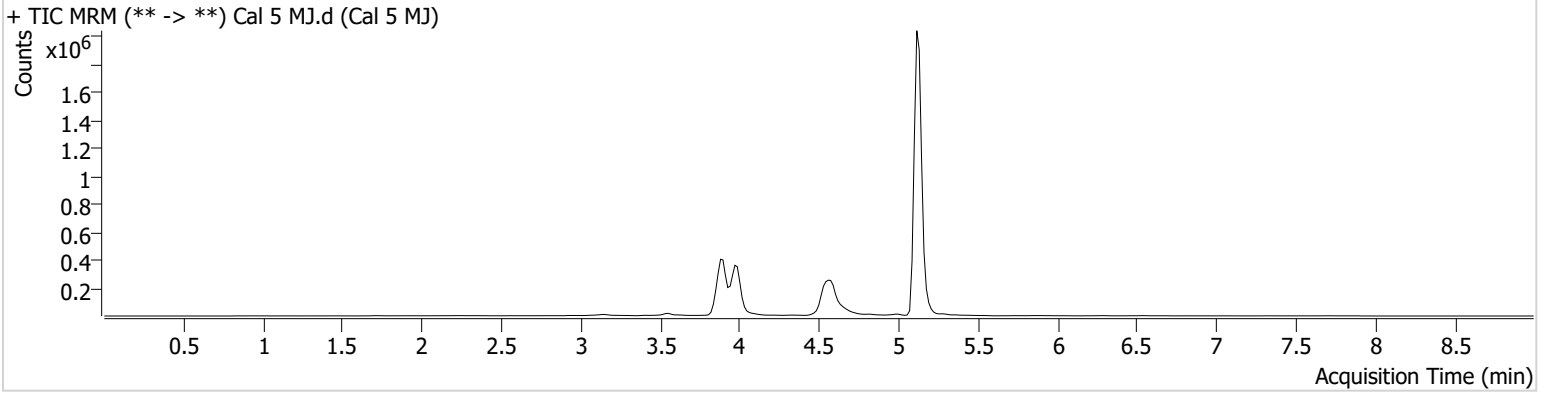


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 3:35:05 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	482849	995.03	15.2	∞	1131649	24.4155 ng/ml
THC-COOH	3.985	171278	4133.12	217.6	∞	301768	76.5680 ng/ml
THC	5.135	1105141	8521.93	26.7	∞	5304707	23.4842 ng/ml

TS

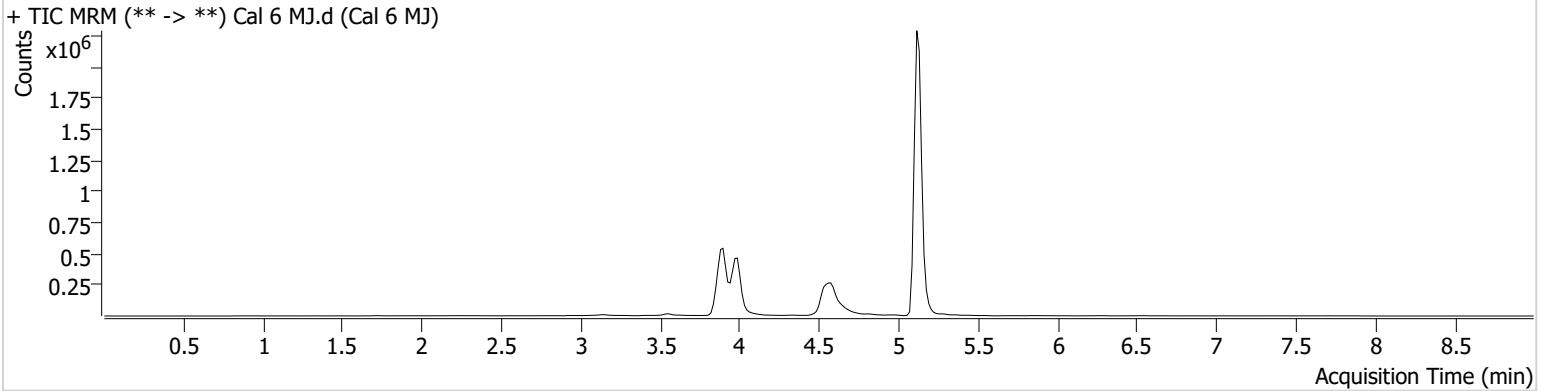


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 3:49:15 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	978043	∞	15.3	5987.22	1119633	49.7536 ng/ml
THC-COOH	3.985	230945	∞	223.5	3719.91	304482	102.1418 ng/ml
THC	5.135	2123755	∞	24.9	∞	4720776	50.3646 ng/ml

TS



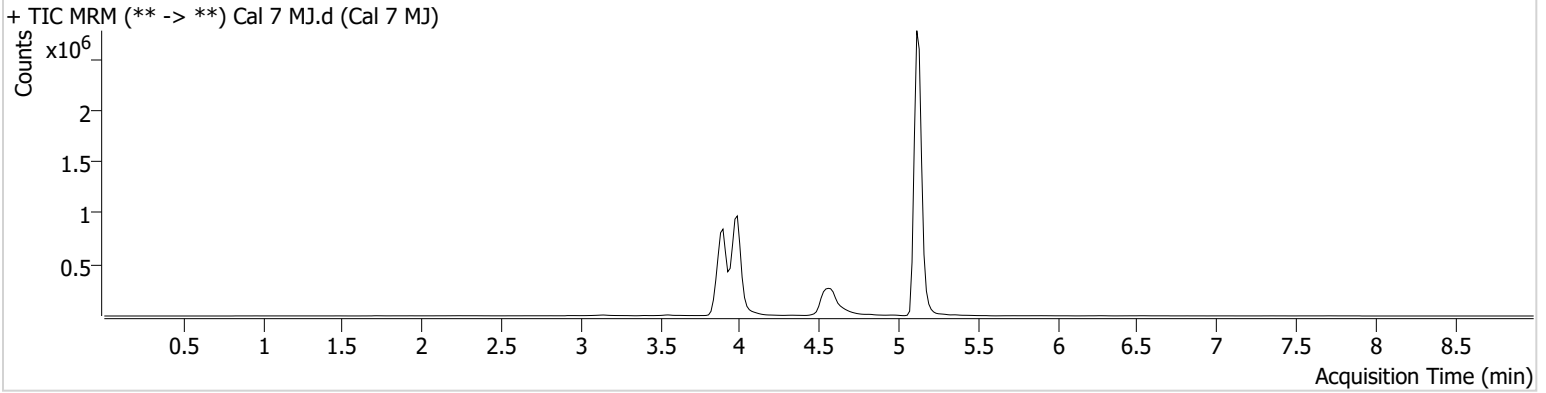
AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\050223 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 5/4/2023 8:29:26 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Tamara Salazar
Sample Position	P1-B6	Comment	
Injection Volume	10		
Acq. Date-Time	5/2/2023 4:02:30 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	2029541	∞	15.0	∞	1140229	101.1489 ng/ml
THC-COOH	3.985	529695	5754.57	221.6	∞	286597	248.1252 ng/ml
THC	5.135	3885246	∞	25.2	∞	4268366	101.5975 ng/ml