

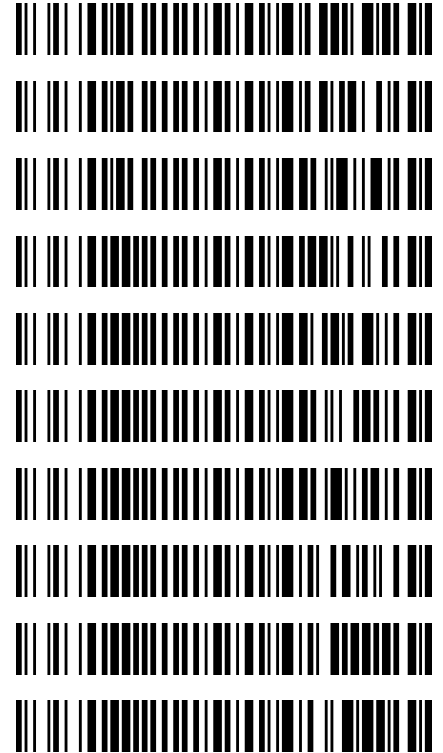
REVIEWED

By Celena Shrum at 7:30 am, Apr 11, 2023

4/5/2023

Worklist: 6318

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-0918	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-0920	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1069	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0716	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0725	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0765	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0768	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0787	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0789	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0811	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: 4/6/2023

Plate lot#: 220802

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 23A52593

LCMS-QQQ ID: 069901

Analyst: Amber Gerheart

Plate Retest Date: 07/23/2023

Mobile phase B: 0.1% Formic acid in Acetonitrile

Column: UCT Selectra DA 100 x 2.1mm 3um

Blank Urine Lot: N/A

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: 42**
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **500µL 0.1% formic acid in water blood sample, 500 µL saturated phosphate buffer in urine** in wells of 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 ≥ 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)
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:

2021

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1 END	P2023-0768-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	Blood Negative	P2023-0787-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2023-0918-1	P2023-0789-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	M2023-0920-1	P2023-0811-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	M2023-1069-2	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	P2023-0716-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	P2023-0725-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2023-0765-1	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

2021

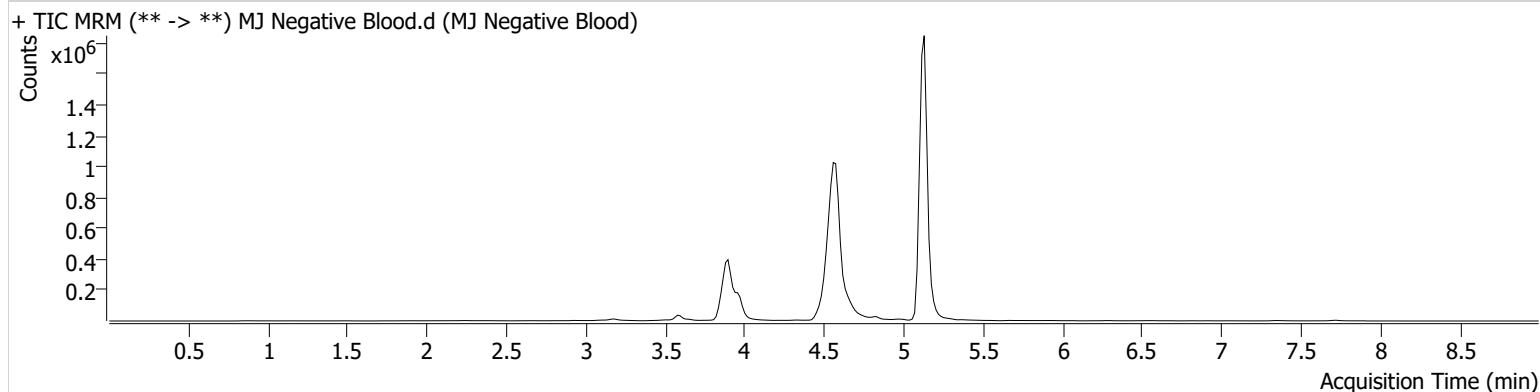


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-B2	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 2:48:32 PM		
Sample Info.			

Sample Chromatogram



201

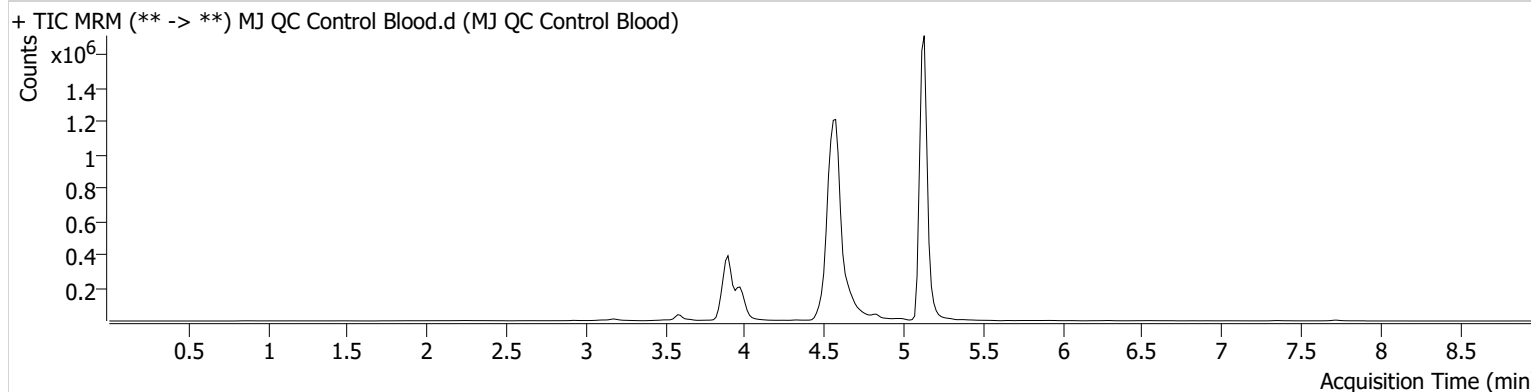


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

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	Amber Gerheart
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 2:22:19 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	100294	∞	13.5	289.49	1503839	4.4221 ng/ml
THC-COOH	3.985	40536	206.54	241.1	175.31	488463	14.4976 ng/ml
THC	5.135	219748	3621.55	23.9	140.78	5392171	4.6784 ng/ml

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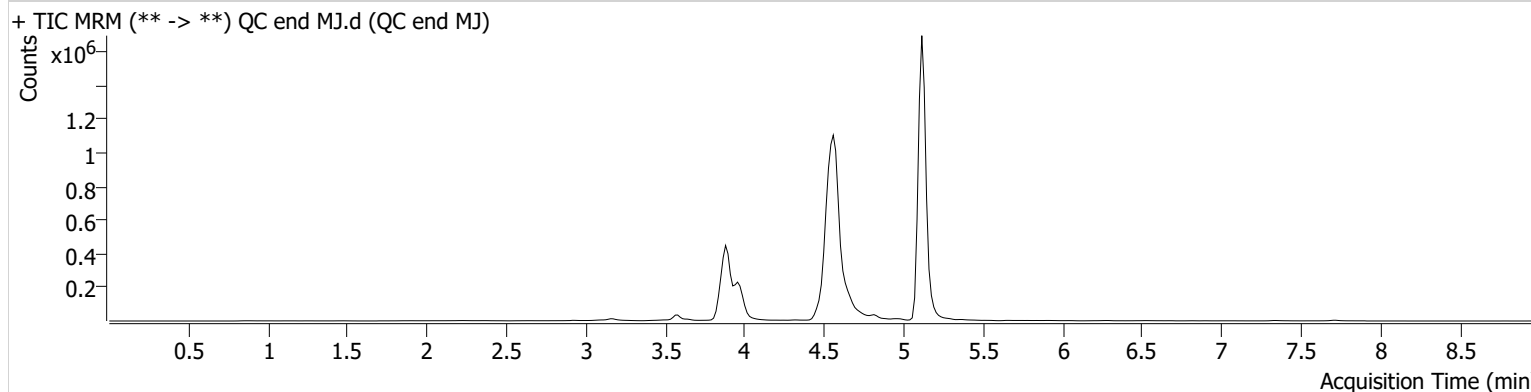


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-A2	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 7:36:39 PM		

Sample Chromatogram



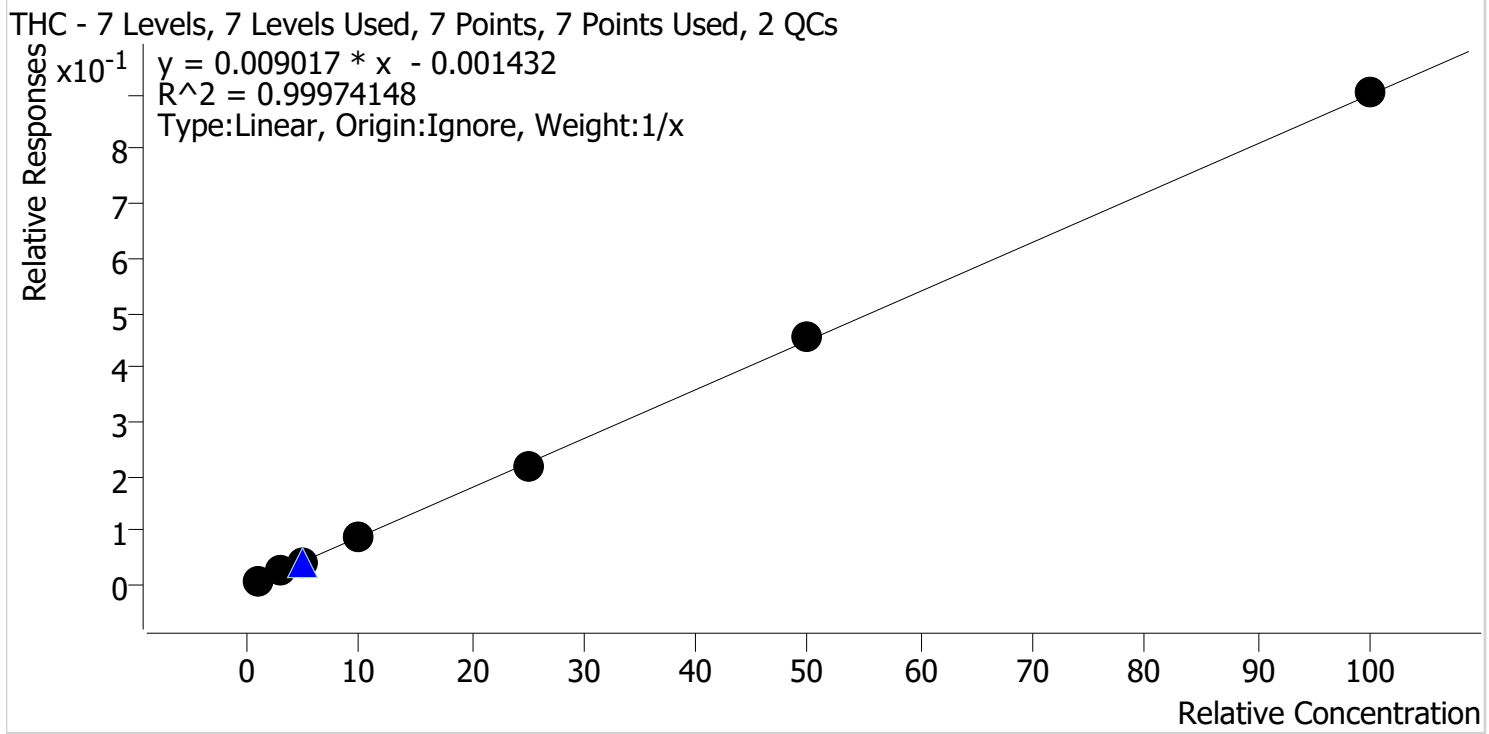
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	130879	∞	11.8	44.60	1730306	5.0111 ng/ml
THC-COOH	3.969	44101	433.79	255.1	321.10	553055	13.9452 ng/ml
THC	5.120	229621	4355.59	23.1	∞	5323164	4.9427 ng/ml

2021



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Last Cal. Update 4/10/2023 2:19 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



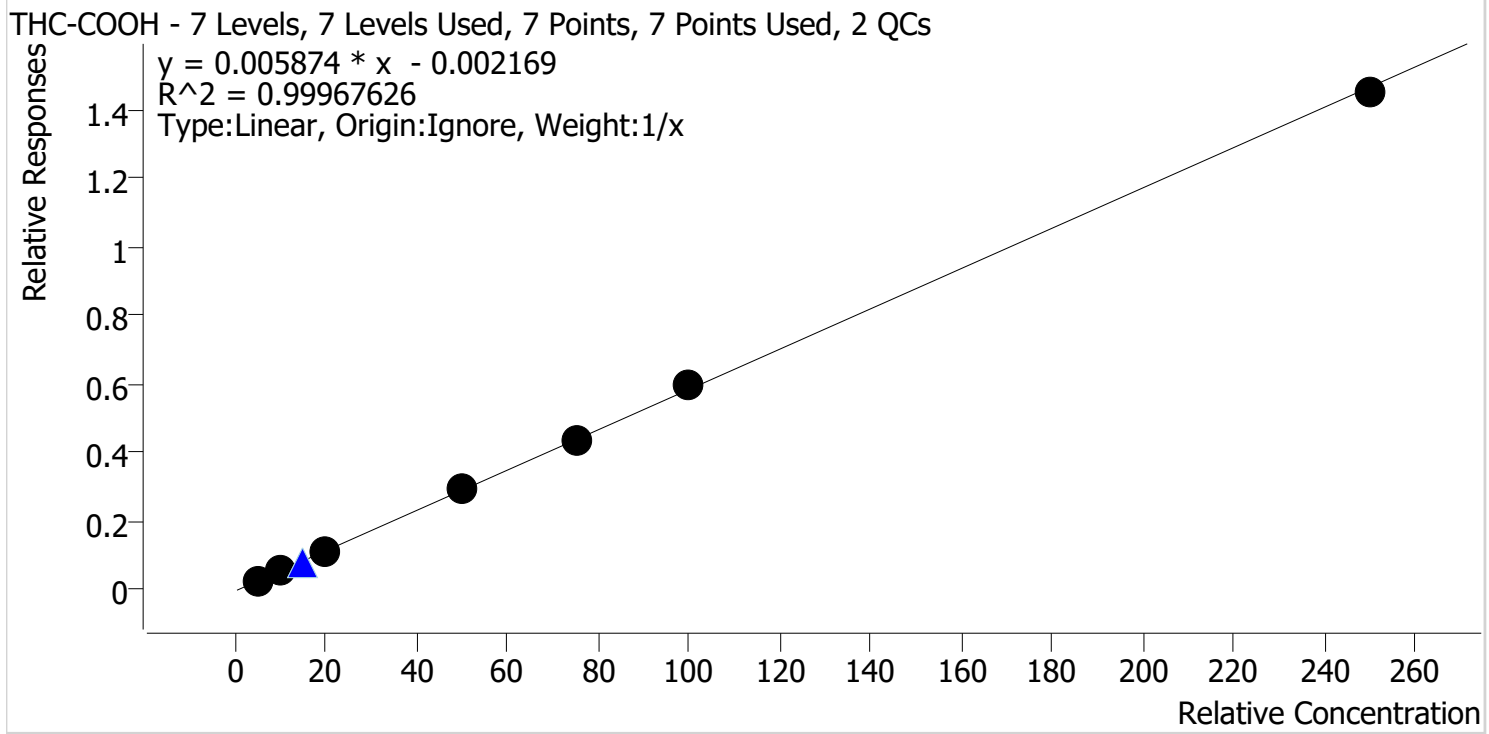
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	109.4
Cal 2 MJ	2	✓	3.0	2.9	96.9
Cal 3 MJ	3	✓	5.0	4.9	97.2
Cal 4 MJ	4	✓	10.0	9.7	97.1
Cal 5 MJ	5	✓	25.0	24.5	97.9
Cal 6 MJ	6	✓	50.0	50.5	101.1
Cal 7 MJ	7	✓	100.0	100.4	100.4

2021



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\lam 27.batch.bin
Last Cal. Update 4/10/2023 2:19 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.1	101.9
Cal 2 MJ	2	✓	10.0	9.8	98.2
Cal 3 MJ	3	✓	20.0	19.4	97.0
Cal 4 MJ	4	✓	50.0	50.4	100.8
Cal 5 MJ	5	✓	75.0	75.6	100.8
Cal 6 MJ	6	✓	100.0	102.5	102.5
Cal 7 MJ	7	✓	250.0	247.2	98.9

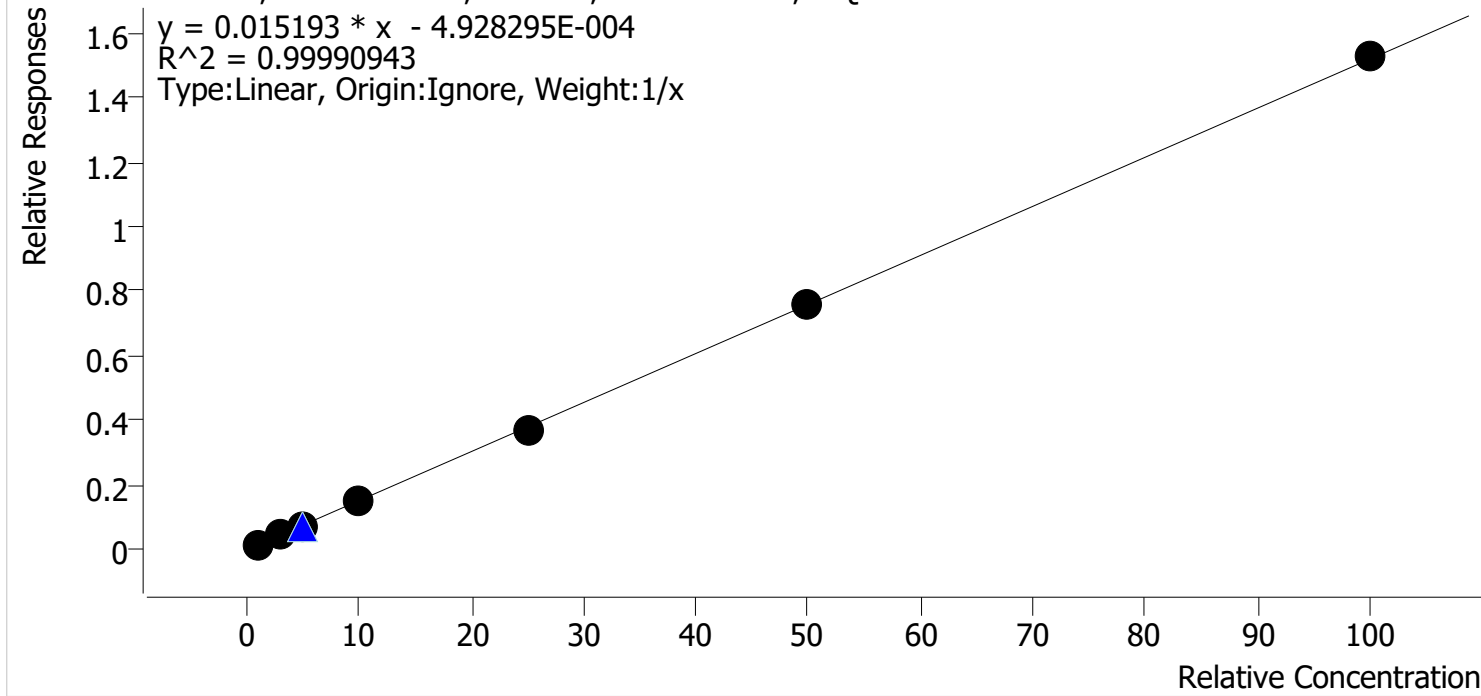
2021



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Last Cal. Update 4/10/2023 2:19 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, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.0	100.5
Cal 2 MJ	2	✓	3.0	3.0	100.4
Cal 3 MJ	3	✓	5.0	5.0	100.1
Cal 4 MJ	4	✓	10.0	10.1	100.8
Cal 5 MJ	5	✓	25.0	24.5	97.8
Cal 6 MJ	6	✓	50.0	50.0	99.9
Cal 7 MJ	7	✓	100.0	100.5	100.5

2021

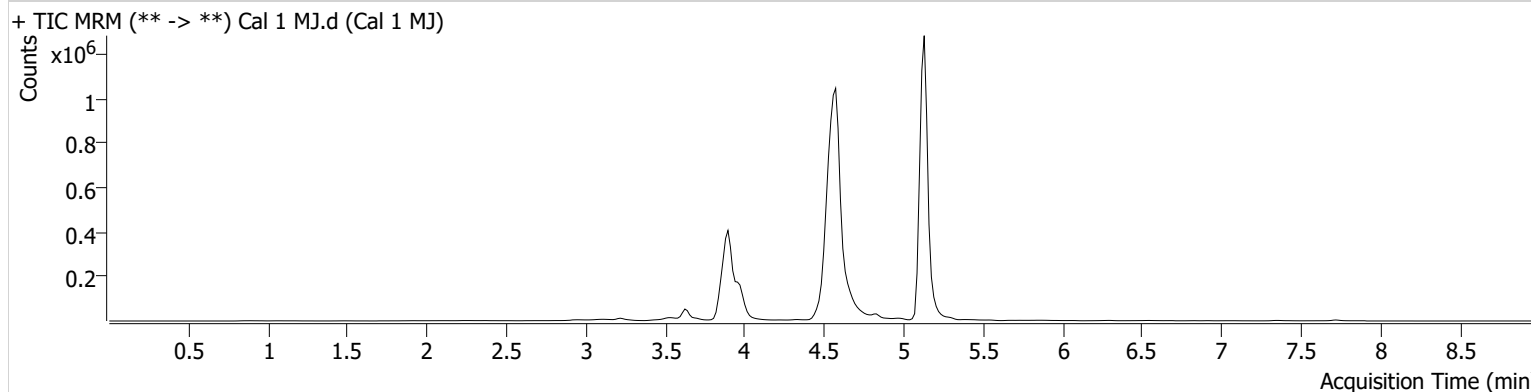


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 12:37:28 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	25589	∞	12.9	15.00	1731672	1.0051 ng/ml Low
THC-COOH	3.985	13673	97.24	240.9	223.06	492821	5.0926 ng/ml
THC	5.135	36400	∞	27.0	∞	4318301	1.0937 ng/ml

201

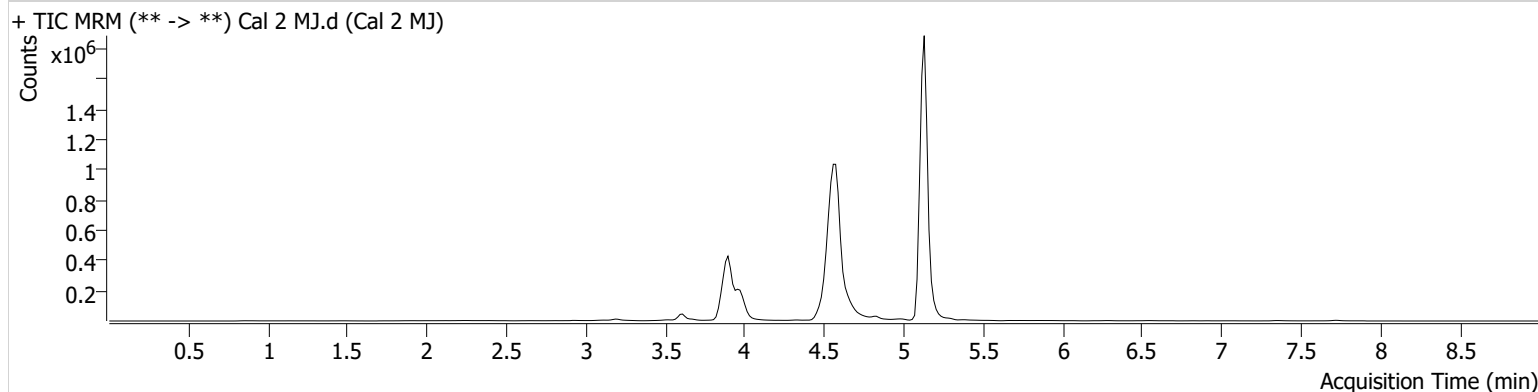


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-B1	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 12:50:42 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	78185	749.97	12.0	523.53	1727836	3.0108 ng/ml
THC-COOH	3.985	30323	233.04	251.1	362.22	546318	9.8189 ng/ml
THC	5.135	148904	∞	23.9	∞	6008693	2.9071 ng/ml

2021

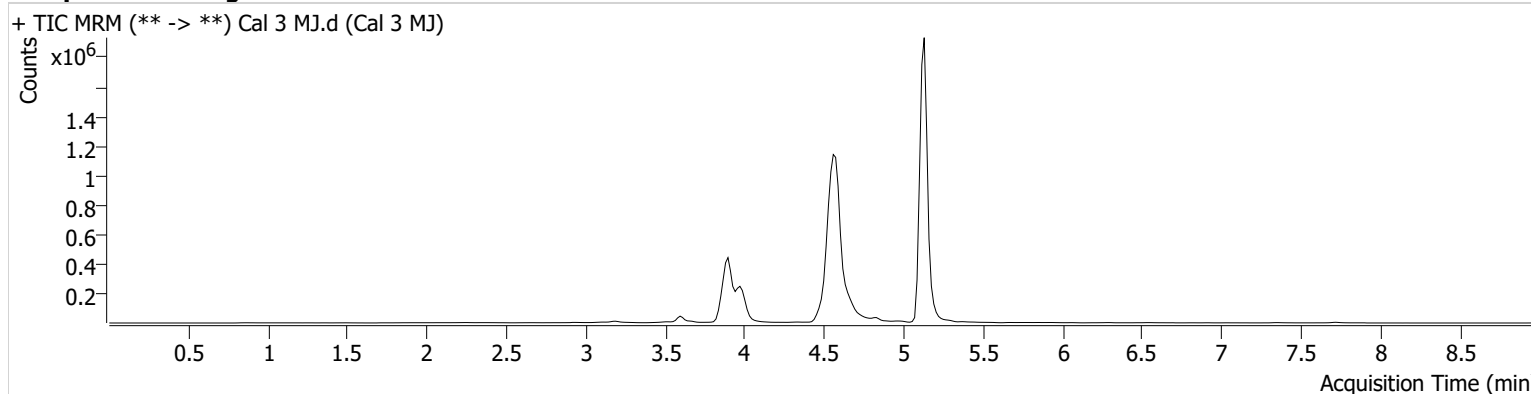


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 1:03:47 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	129331	∞	11.8	161.63	1712190	5.0042 ng/ml
THC-COOH	3.985	61589	194.45	249.9	542.32	551138	19.3943 ng/ml
THC	5.135	257426	∞	23.1	∞	6071259	4.8611 ng/ml

201

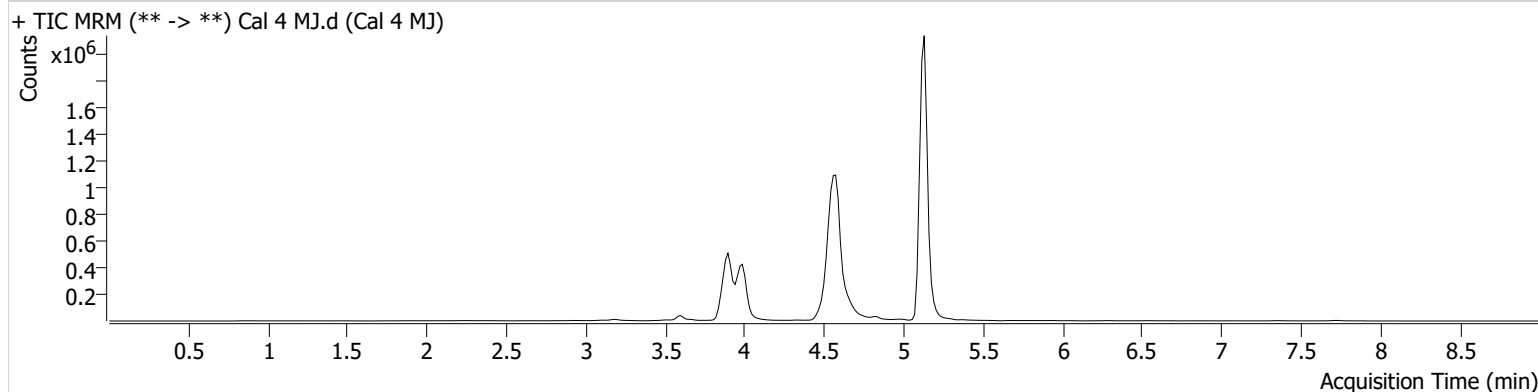


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-D1	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 1:16:53 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	281217	∞	11.9	∞	1841781	10.0824 ng/ml
THC-COOH	3.985	172176	481.96	241.1	1958.64	585607	50.4246 ng/ml
THC	5.135	562050	∞	23.2	348.29	6522866	9.7147 ng/ml

2021

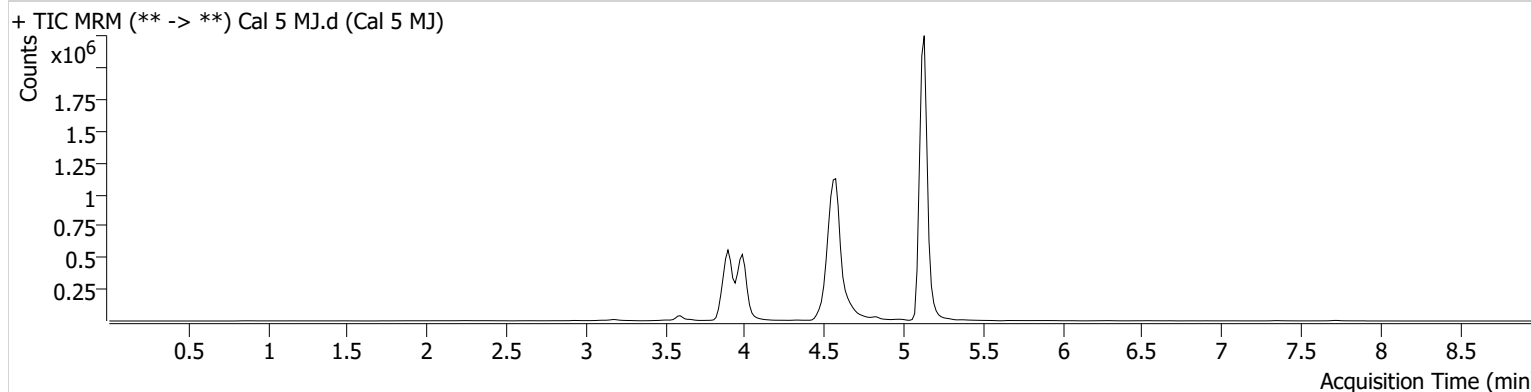


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 1:29:59 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	613194	∞	13.4	∞	1652514	24.4563 ng/ml
THC-COOH	3.985	230425	1177.66	242.0	8504.06	521686	75.5669 ng/ml
THC	5.135	1309530	∞	22.8	∞	5975029	24.4646 ng/ml

2021

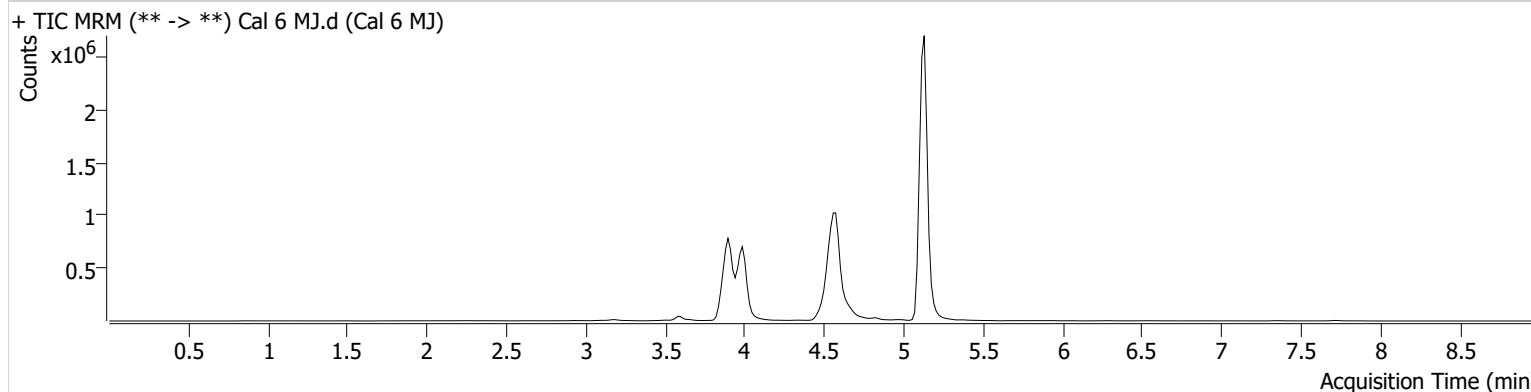


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 1:43:03 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	1348727	∞	13.6	2029.09	1778209	49.9557 ng/ml
THC-COOH	3.985	322250	1614.02	245.2	10910.2	537167	102.5027 ng/ml
THC	5.135	2714160	∞	22.7	∞	5974210	50.5424 ng/ml

201

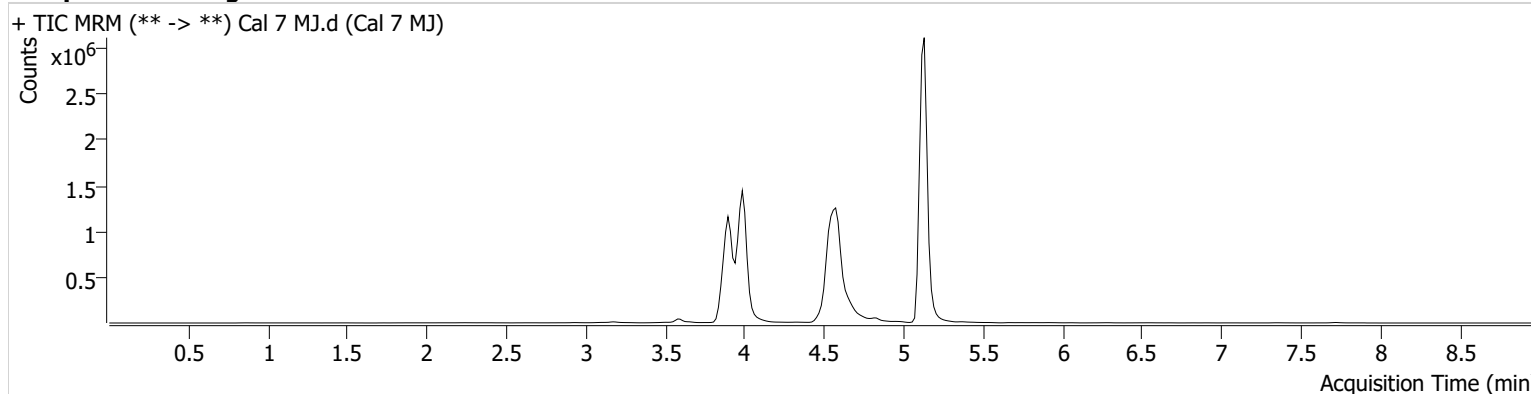


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2023\AM 27 28\040623 AM 27 28 AG\QuantResults\am 27.batch.bin
Calibration Last Update 4/10/2023 2:19:28 PM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 Agilent Method.m	Operator	Amber Gerheart
Sample Position	P1-G1	Comment	
Injection Volume	10		
Acq. Date-Time	4/6/2023 1:56:09 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	3.896	2698031	8764.03	13.5	∞	1767854	100.4854 ng/ml
THC-COOH	3.985	723796	1906.49	242.8	∞	499231	247.2000 ng/ml
THC	5.135	4546731	∞	22.9	∞	5029403	100.4163 ng/ml