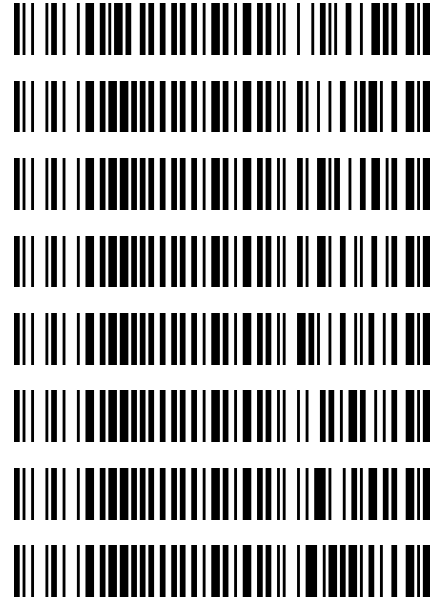


Worklist: 6068

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-2996	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2025	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2096	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2097	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2171	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2203	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2385	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2409	1	BCK	AM 27 Blood THC Quant by LC-QQQ



TS

AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 08/18/2022
Plate lot#: 220309
Mobile phase A: 0.1% Formic Acid in LCMS Water
Blank Blood Lot: Lampire 22B52015-1
Column: UCT Selectra DA 100 x 2.1mm 3um

Analyst: Tamara Salazar
Plate Retest Date: 09/09/2022
Mobile phase B: 0.1% Formic acid in Acetonitrile
Blank Urine Lot: N/A
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. TS
08/23/202
- 2. ~~Urine hydrolysis: add 1.5mL urine to blank plate, add 250ul 1N KOH. Shake and incubate at 40 degrees for 15 minutes.~~
- 3. Using a calibrated pipette, add **1000ul 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. TS
08/23/202
- 5. Add **500ul of 0.1% formic acid in water to blood samples, and 500ul 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 **700-800ul of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate. Amount transferred: 750ul
- 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 **100ul 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. Case samples with calculated concentrations for THC at 1ng/mL or greater and OH-THC at 3ng/mL or greater may be reported quantitatively (blood only). Calculated concentrations for carboxy-THC of 5ng/mL may be reported qualitatively. 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, 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: THC-OH 3-100

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	P2022-2203-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	IS + Sample	P2022-2171-1	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	IS + Sample	P2022-2097-1	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	IS + Sample	P2022-2096-1	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	IS + Sample	P2022-2025-1	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	IS + Sample	M2022-2996-1	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2022-2409-1	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2022-2385-1	IS + QC_1	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	P2022-2203-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	IS + Sample	P2022-2171-1*	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	IS + Sample	P2022-2097-1*	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	IS + Sample	P2022-2096-1	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	P2022-2171-1	P2022-2025-1	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	P2022-2097-1	M2022-2996-1	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2022-2409-1	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2022-2385-1	IS + QC_1	IS + Cal. 1

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

TS

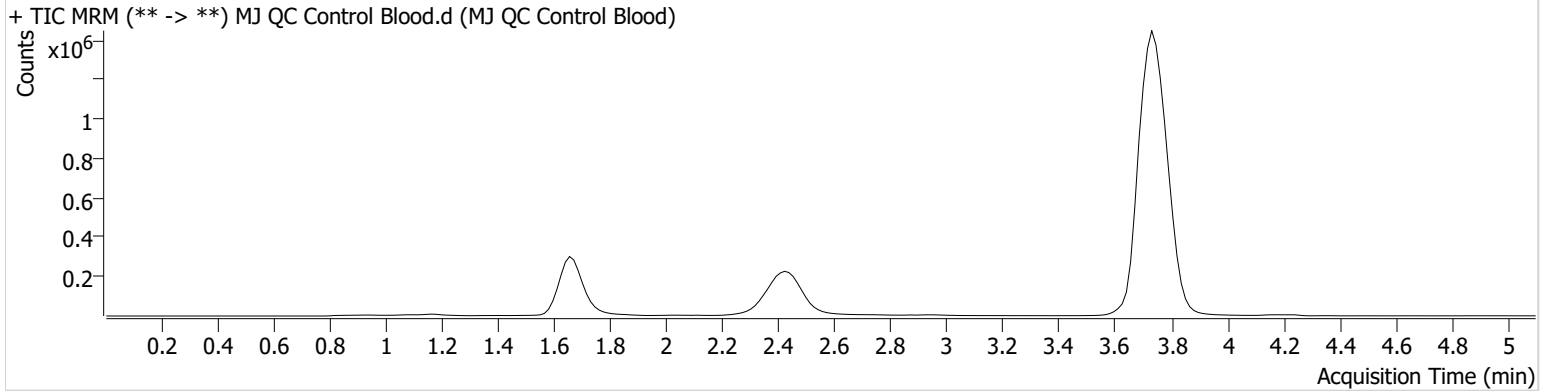


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	MJ QC Control Blood.d
Type	QC	Sample	MJ QC Control Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 5:47:21 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663	111201	156.99	10.0	259.64	1184724	4.8101 ng/ml
THC-COOH	1.700	106444	∞	55.8	255.30	318545	14.6042 ng/ml
THC	3.751	393901	2799.23	29.5	∞	9803273	4.6798 ng/ml

TS

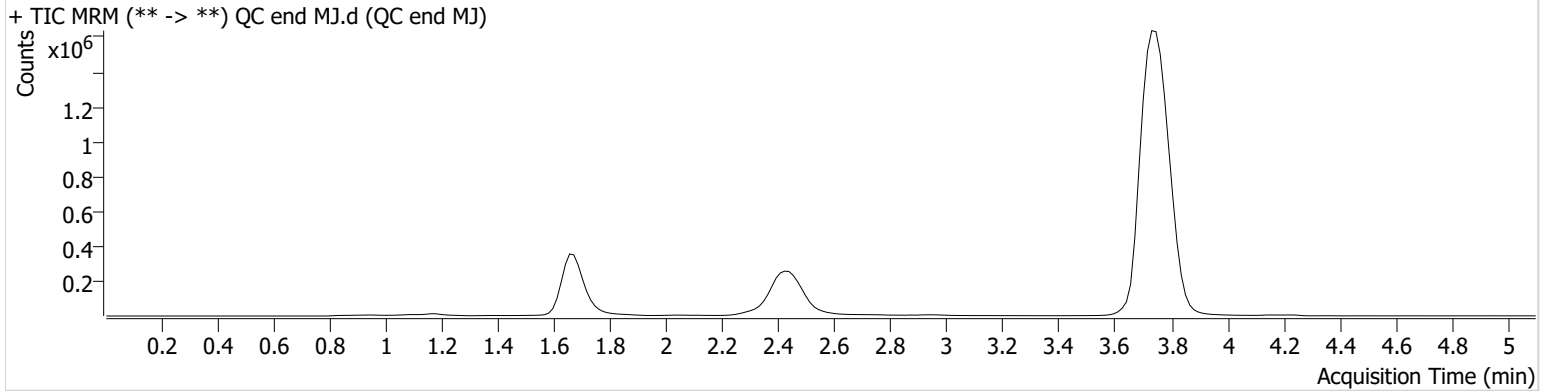


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 8:19:30 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663	126097	∞	10.2	231.84	1328819	4.8802 ng/ml
THC-COOH	1.700	119223	∞	55.2	496.15	342794	15.1920 ng/ml
THC	3.751	464431	3675.53	29.1	∞	11169157	4.8364 ng/ml

TS

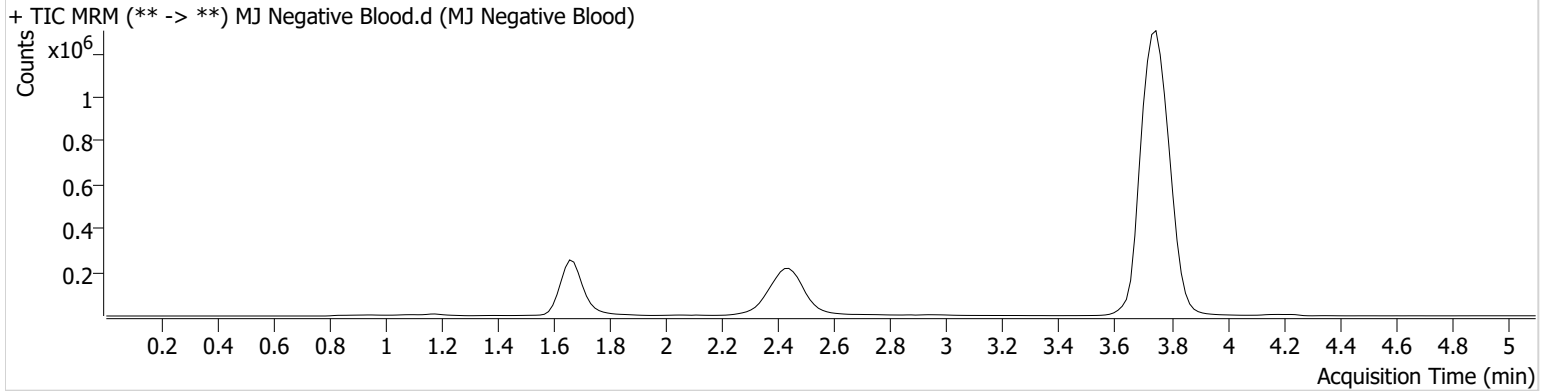


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G5	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 6:02:33 PM		

Sample Chromatogram



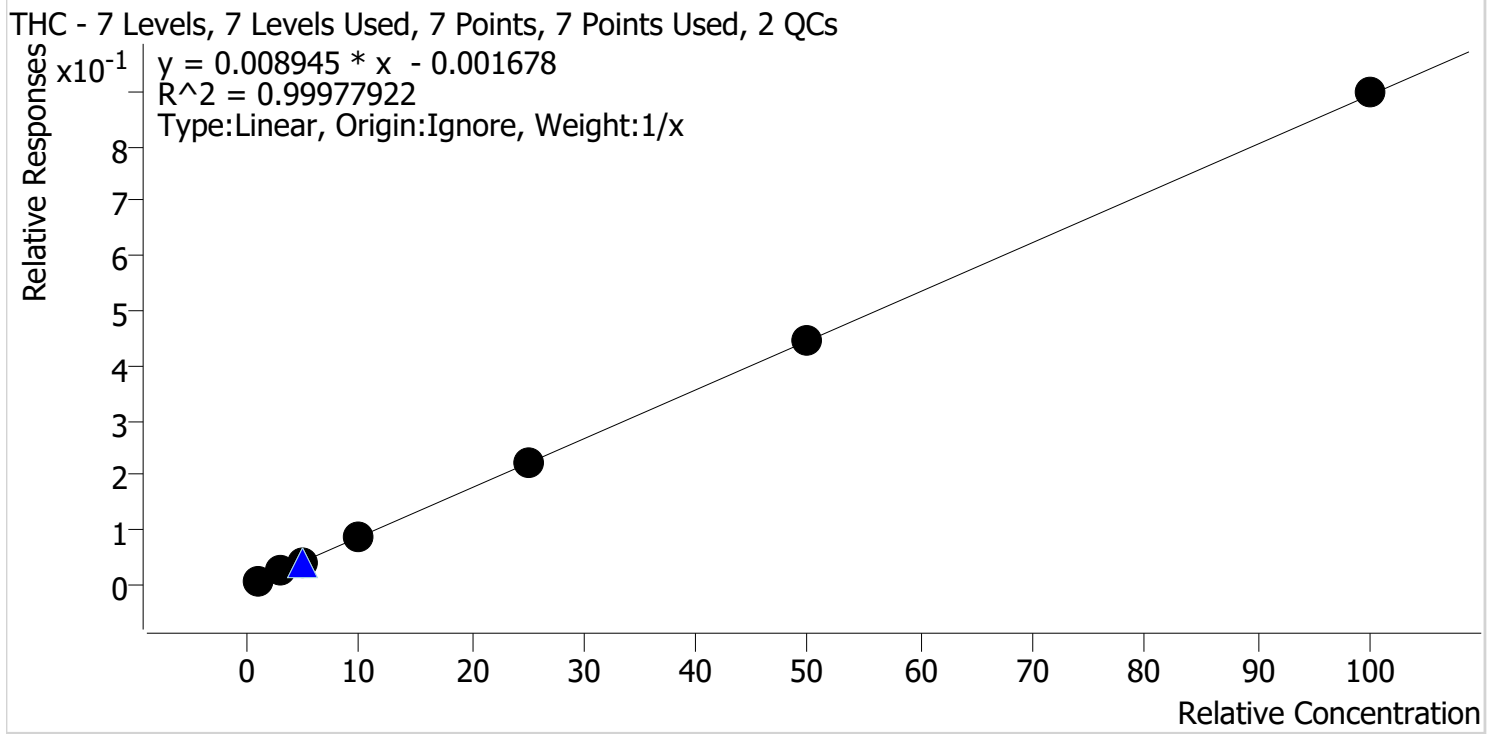
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.753 High	30345	∞	3.0 Low	7.01 Low	1146227	0.2335 ng/ml Low

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 8/19/2022 7:35 AM
Analyst Name ISP\tsalazar
Analyte THC **Internal Standard** THC-D3



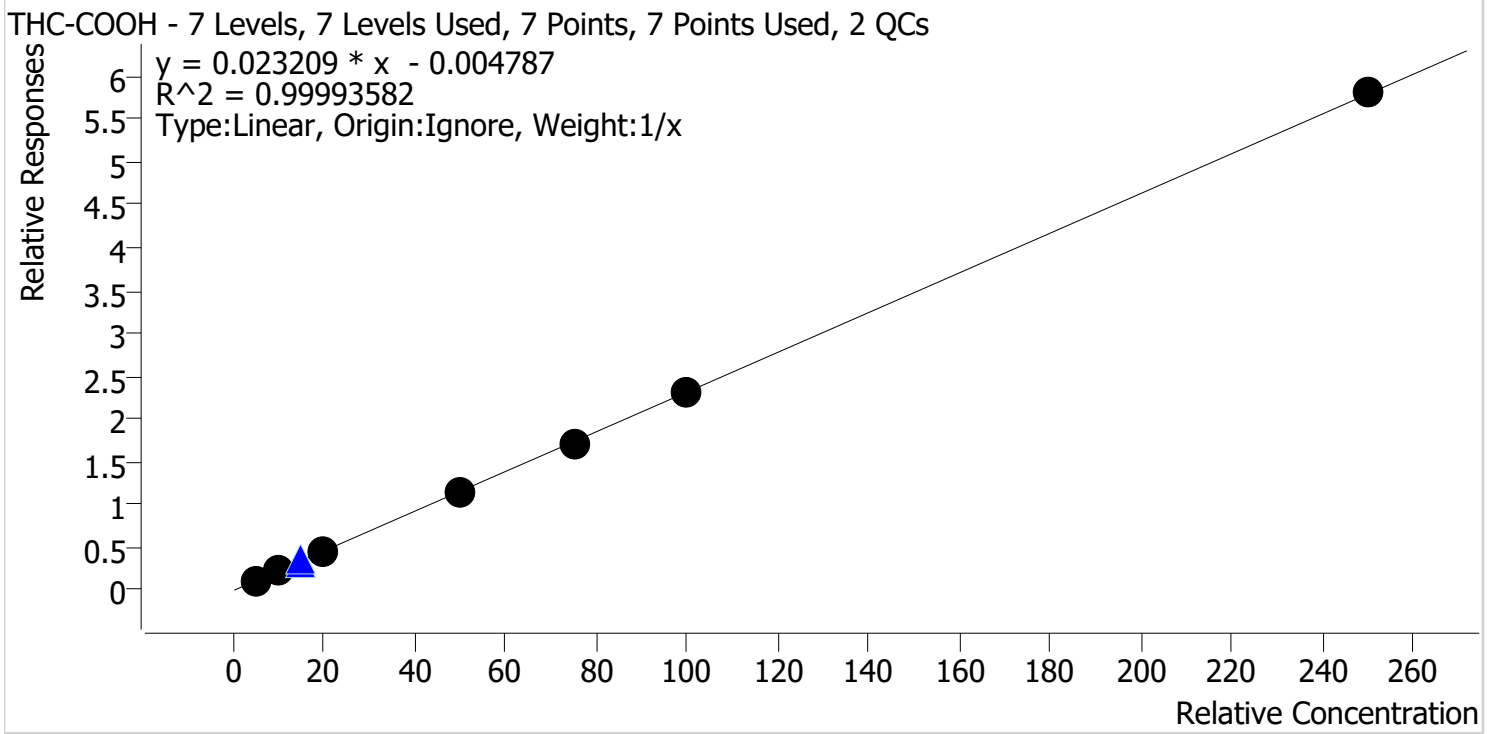
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	110.4
Cal 2 MJ	2	✓	3.0	2.9	96.2
Cal 3 MJ	3	✓	5.0	4.8	96.3
Cal 4 MJ	4	✓	10.0	9.7	97.0
Cal 5 MJ	5	✓	25.0	24.8	99.1
Cal 6 MJ	6	✓	50.0	50.2	100.4
Cal 7 MJ	7	✓	100.0	100.5	100.5

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 8/19/2022 7:35 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	5.0	99.5
Cal 2 MJ	2	✓	10.0	10.3	102.8
Cal 3 MJ	3	✓	20.0	19.7	98.5
Cal 4 MJ	4	✓	50.0	49.8	99.6
Cal 5 MJ	5	✓	75.0	74.2	98.9
Cal 6 MJ	6	✓	100.0	100.3	100.3
Cal 7 MJ	7	✓	250.0	250.7	100.3

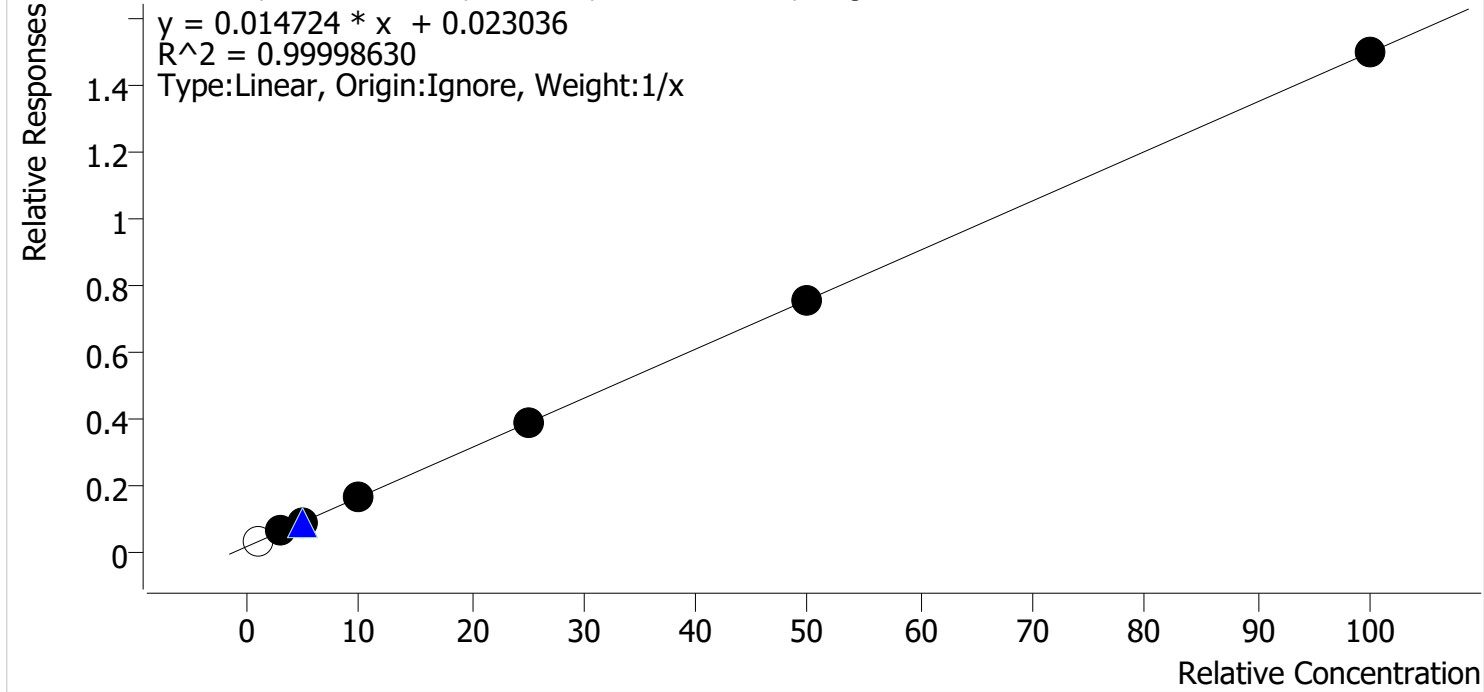
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 8/19/2022 7:35 AM
Analyst Name ISP\tsalazar
Analyte THC-OH **Internal Standard** THC-OH-D3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	1.0	1.2	118.5
Cal 2 MJ	2	✓	3.0	3.0	100.5
Cal 3 MJ	3	✓	5.0	5.0	99.6
Cal 4 MJ	4	✓	10.0	10.0	100.2
Cal 5 MJ	5	✓	25.0	24.8	99.4
Cal 6 MJ	6	✓	50.0	50.2	100.4
Cal 7 MJ	7	✓	100.0	100.0	100.0

Calibrator 1 dropped due to ratio.

TS

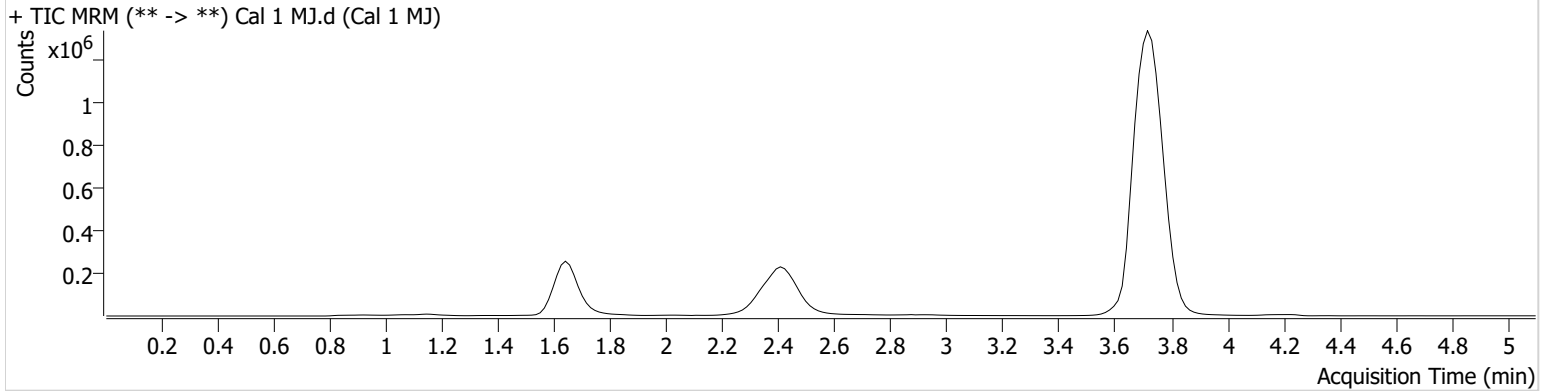


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 4:46:22 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.723	46896	∞	6.5 Low	15.47	1158451	1.1848 ng/ml Low
THC-COOH	1.685	34929	∞	55.6	214.96	315537	4.9760 ng/ml Low
THC	3.736	80222	550.56	34.9	52.26	9785493	1.1041 ng/ml

TS

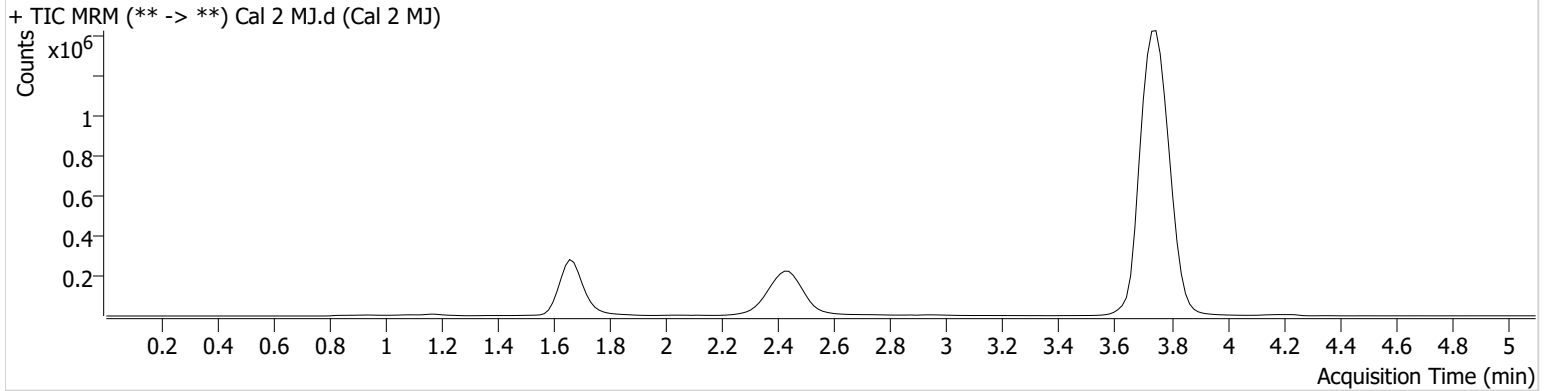


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 4:54:08 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678	78239	∞	9.4	78.82	1160415	3.0145 ng/ml
THC-COOH	1.700	73425	181.29	53.7	∞	313971	10.2826 ng/ml
THC	3.751	242514	∞	31.4	∞	10042191	2.8875 ng/ml

TS

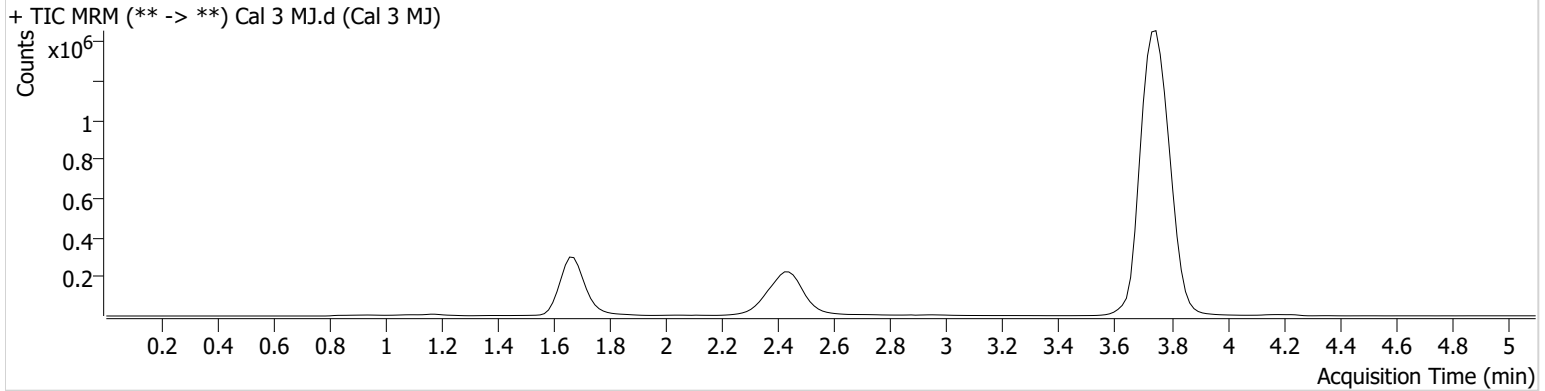


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 5:01:44 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678	115892	∞	10.0	311.03	1202451	4.9811 ng/ml
THC-COOH	1.700	144067	363.09	54.2	∞	318431	19.7002 ng/ml
THC	3.751	420169	2533.29	29.4	∞	10148848	4.8162 ng/ml

TS

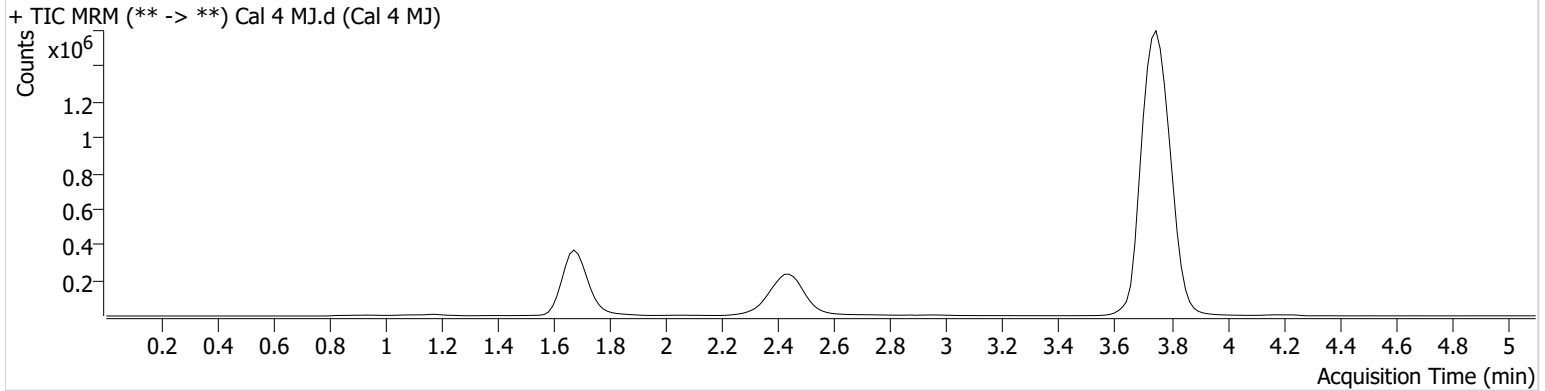


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 5:09:20 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678	201594	∞	11.1	252.26	1181940	10.0191 ng/ml
THC-COOH	1.700	360007	∞	56.6	3962.49	312800	49.7963 ng/ml
THC	3.751	888427	12509.06	28.1	∞	10445315	9.6967 ng/ml

TS

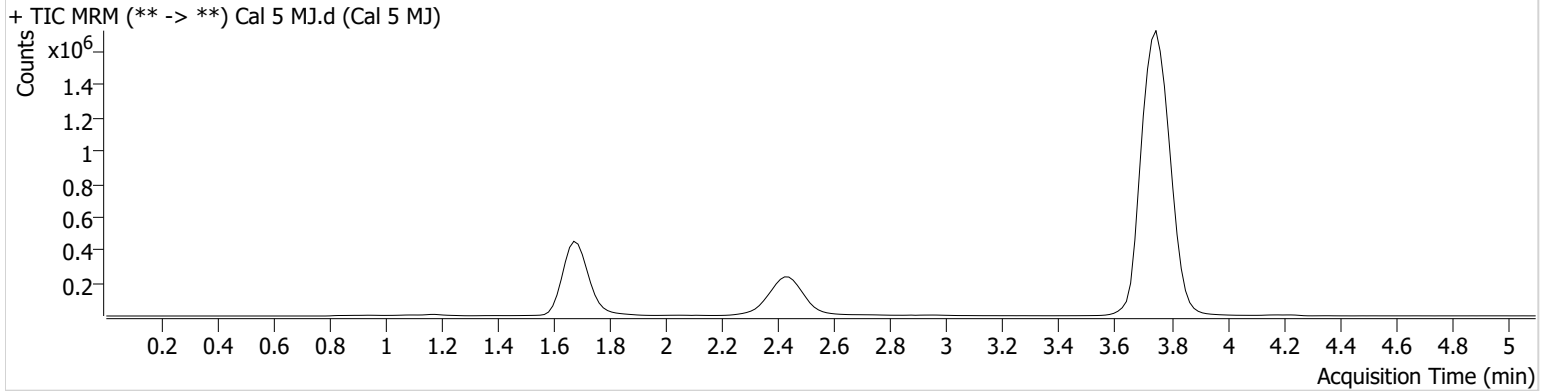


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 5:16:56 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663	448846	∞	12.4	777.35	1154382	24.8420 ng/ml
THC-COOH	1.700	523574	∞	56.3	4056.57	304841	74.2103 ng/ml
THC	3.751	2134189	∞	28.2	∞	9703747	24.7761 ng/ml

TS

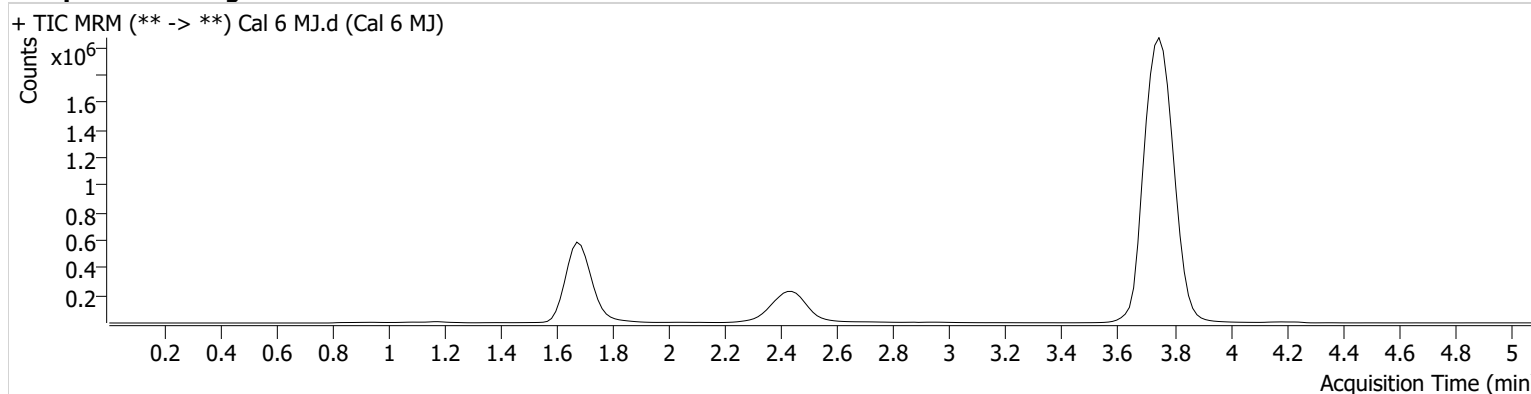


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 5:24:31 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663	902874	∞	12.8	1849.79	1184731	50.1926 ng/ml
THC-COOH	1.700	713001	26378.62	56.2	∞	306826	100.3328 ng/ml
THC	3.751	4356826	40997.46	28.1	7920.50	9734988	50.2225 ng/ml

TS

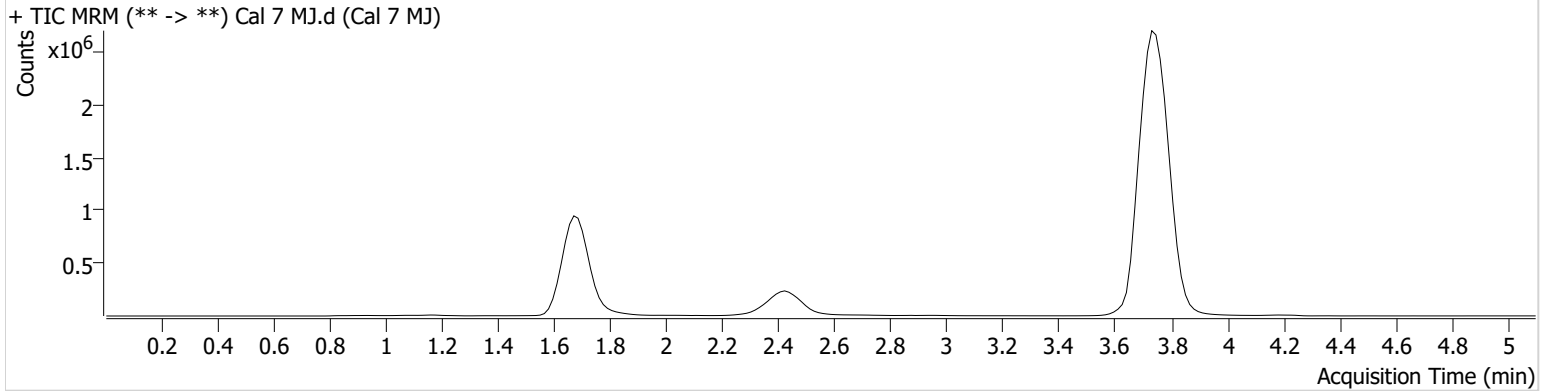


AM #27 Cannabinoid Quant. Results

Batch results G:\TOX\Pocatello\Falco\2022\AM 27-28\081822 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/19/2022 7:35:26 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-B6	Comment	
Injection Volume	10		
Acq. Date-Time	8/18/2022 5:32:08 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663	1703877	∞	13.0	2549.02	1139906	99.9508 ng/ml
THC-COOH	1.700	1654300	1397.69	56.5	∞	284554	250.7018 ng/ml
THC	3.751	8357271	∞	27.9	12852.8 1	9314544	100.4969 ng/ml