

AS

REVIEWED
By Sarah Collins at 8:06 am, Nov 04, 2022

Worklist: 6147

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2022-3940	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4086	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4087	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4205	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4211	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4332	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2022-4347	3	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3132	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3137	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3139	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3143	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3205	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3215	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3217	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3237	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3242	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3245	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3256	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-3283	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

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

Extraction Date: 10/31/2022

Plate lot#: 220802

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 22B52015-2 -1

LCMS-QQQ ID: 069901 ~~AG~~ 11/2/2022

Analyst: Amber Gerheart

Plate Retest Date: 02/02/2023

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: 16**
- 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. 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: Calibration Curve Range: THC-COOH 10-250 ng/mL
THC-COOH cal 1 dropped due to ratio being out

AG

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1 END	M2022-4347-3	P2022-3237-2	IS + Sample	IS + QC_1
B	IS + Cal. 2	Blood Negative	P2022-3132-1	P2022-3242-1	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2022-3940-1	P2022-3137-1	P2022-3245-1	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	M2022-4086-1	P2022-3139-1	P2022-3256-1	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	M2022-4087-1	P2022-3143-1	P2022-3283-1	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	M2022-4205-2	P2022-3205-1	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	M2022-4211-2	P2022-3215-1	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	M2022-4332-1	P2022-3217-1	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

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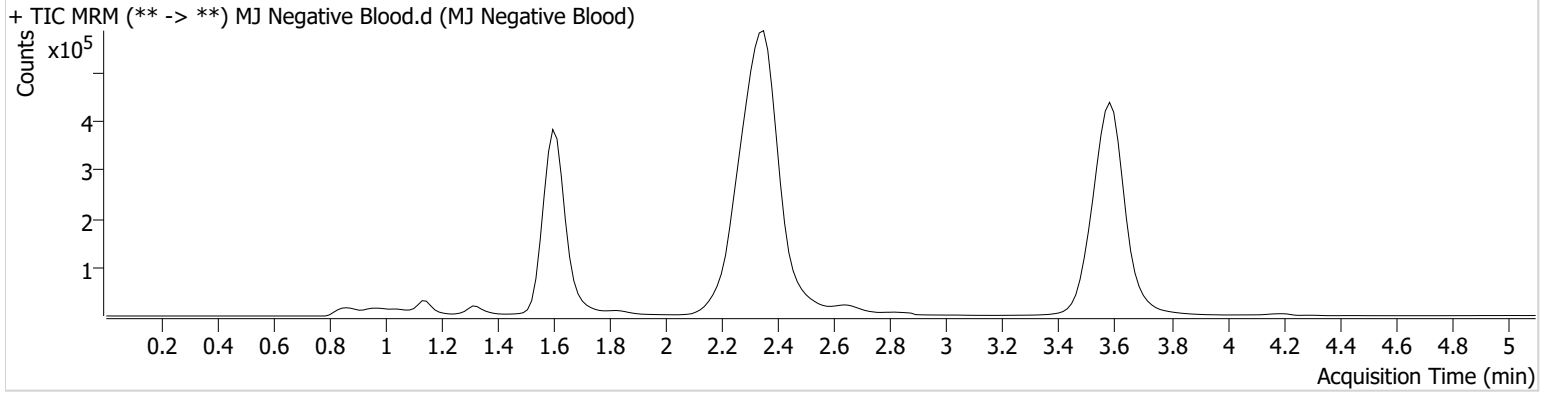


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-B2	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 8:23:16 PM		
Sample Info.			

Sample Chromatogram



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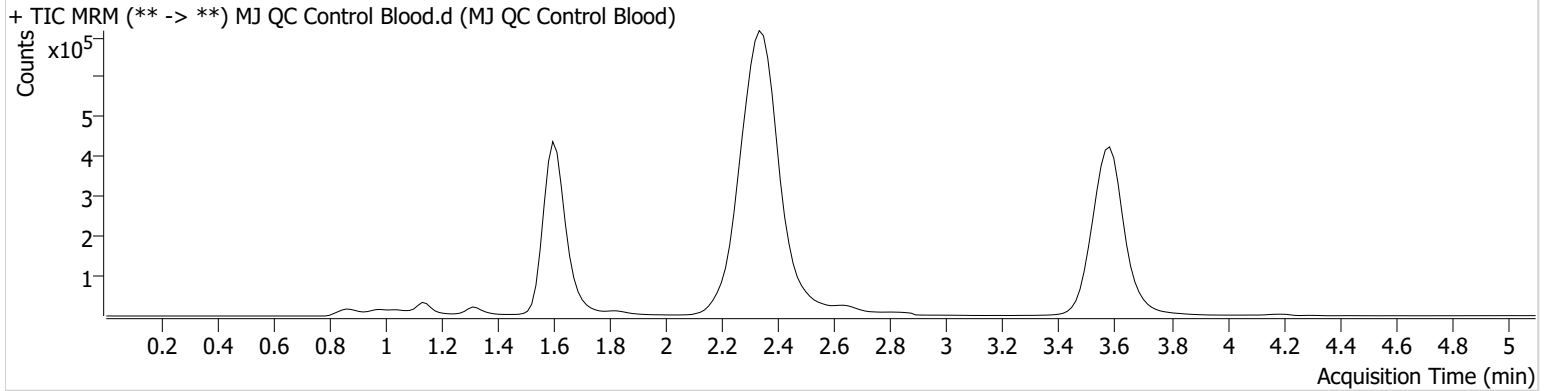
AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 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	Amber Gerheart
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 8:08:04 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	130401	654.78	13.1	436.51	1754338	4.6403 ng/ml
THC-COOH	1.640	141075	∞	46.8	∞	339114	15.0550 ng/ml
THC	3.601	147522	570.28	28.1	85.14	3228906	4.8864 ng/ml

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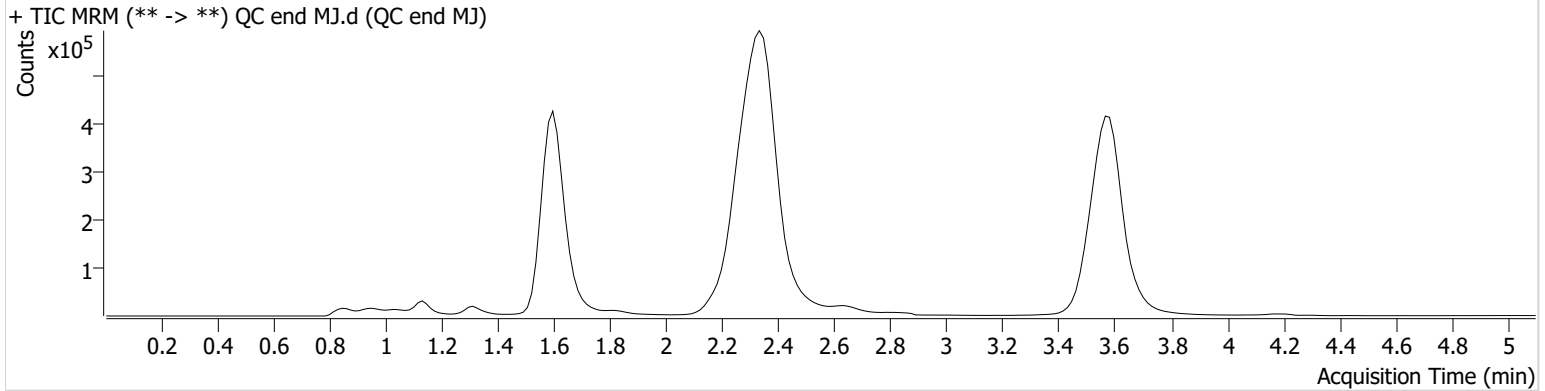


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-A2	Comment	
Injection Volume	10		
Acq. Date-Time	11/1/2022 1:27:51 AM		

Sample Chromatogram



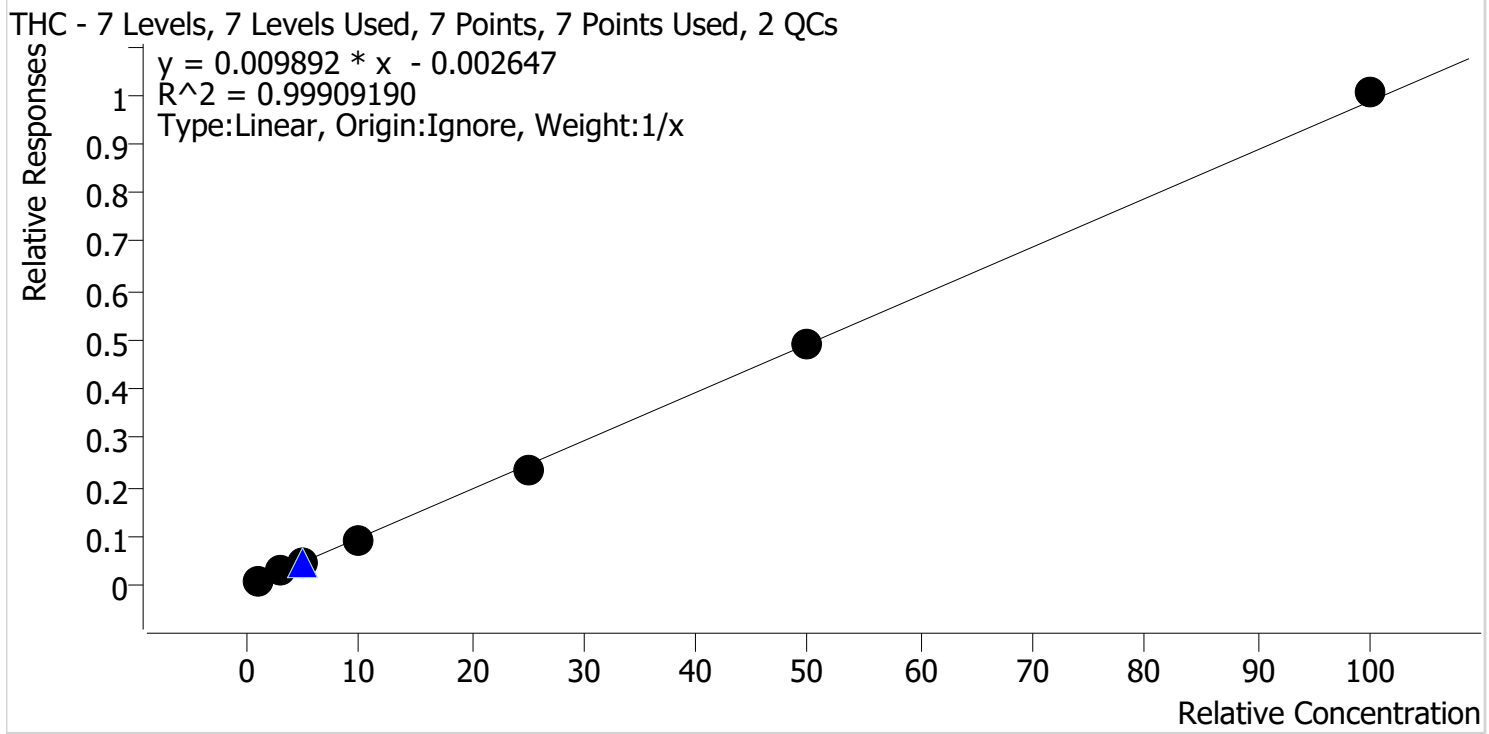
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	128948	875.32	13.7	302.55	1753688	4.5929 ng/ml
THC-COOH	1.640	130589	∞	51.0	∞	344137	13.5170 ng/ml
THC	3.586	147462	∞	27.2	28.41	3186168	4.9464 ng/ml

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 11/1/2022 9:29 AM
Analyst Name ISP\lagerheart
Analyte THC **Internal Standard** THC-D3



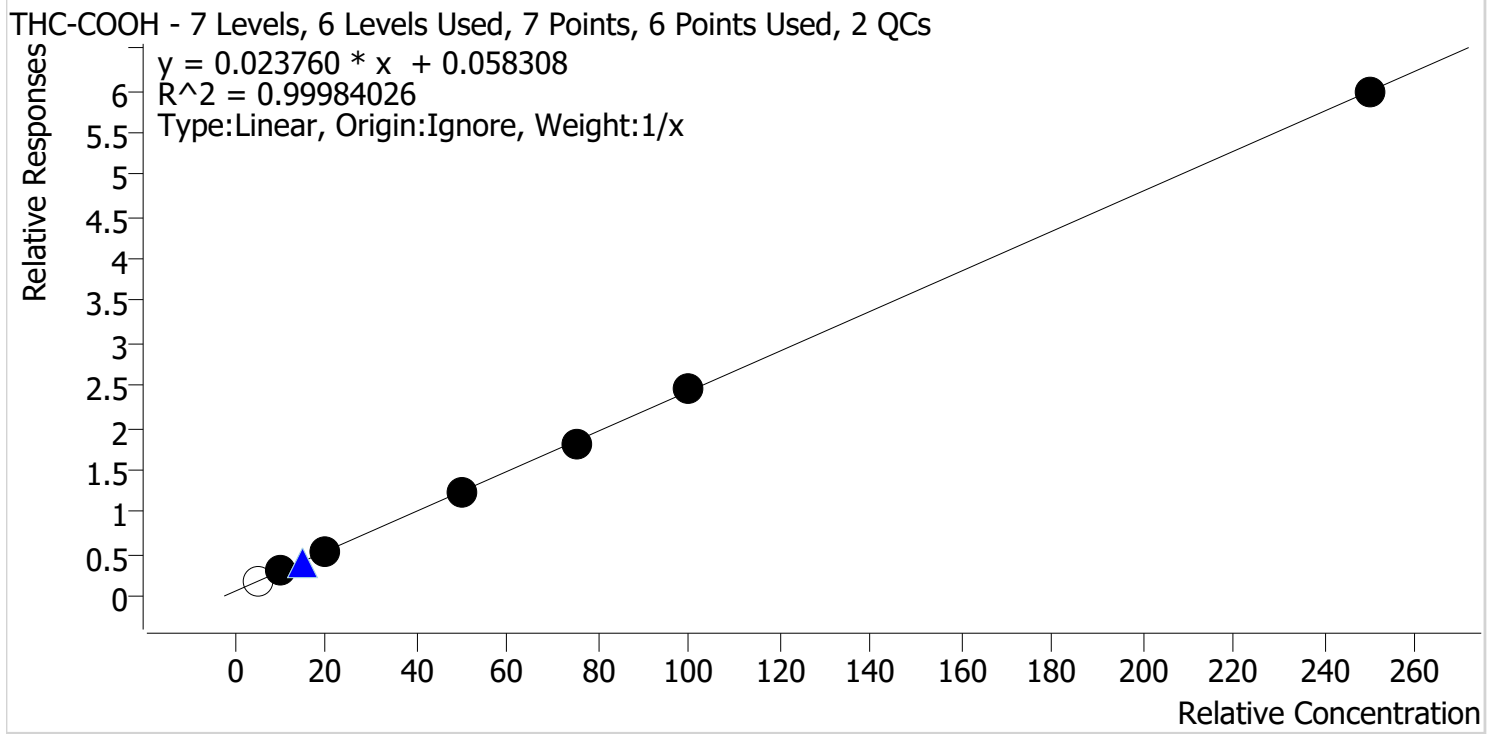
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.2	115.6
Cal 2 MJ	2	✓	3.0	3.0	98.4
Cal 3 MJ	3	✓	5.0	4.7	94.6
Cal 4 MJ	4	✓	10.0	9.3	93.5
Cal 5 MJ	5	✓	25.0	24.1	96.2
Cal 6 MJ	6	✓	50.0	50.0	99.9
Cal 7 MJ	7	✓	100.0	101.8	101.8

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 11/1/2022 9:29 AM
Analyst Name ISP\lagerheart
Analyte THC-COOH **Internal Standard** THC-COOH-D9



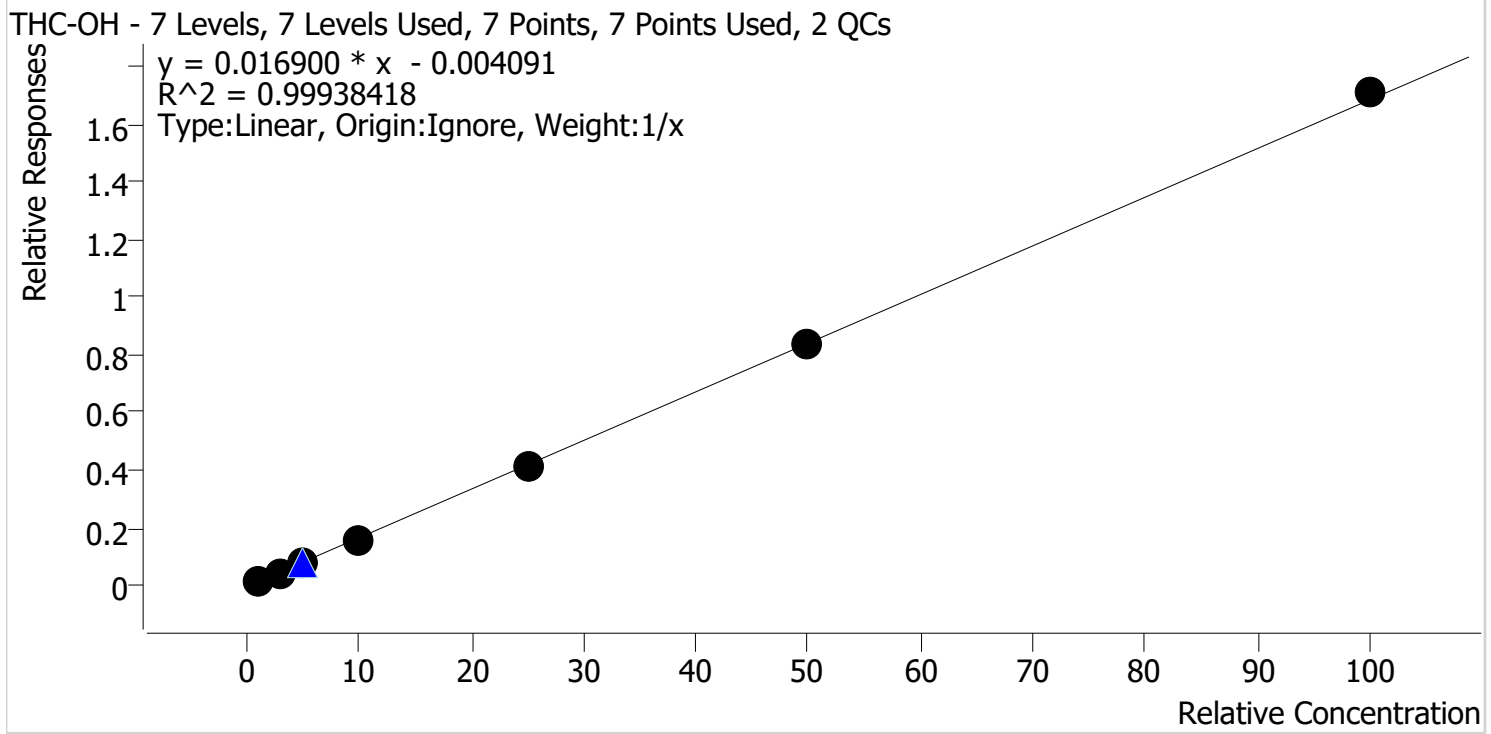
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	5.0	5.2	104.4
Cal 2 MJ	2	✓	10.0	10.1	101.4
Cal 3 MJ	3	✓	20.0	19.7	98.5
Cal 4 MJ	4	✓	50.0	49.7	99.4
Cal 5 MJ	5	✓	75.0	74.4	99.2
Cal 6 MJ	6	✓	100.0	101.9	101.9
Cal 7 MJ	7	✓	250.0	249.2	99.7

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 11/1/2022 9:29 AM
Analyst Name ISP\lagerheart
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.2	116.7
Cal 2 MJ	2	✓	3.0	2.8	93.7
Cal 3 MJ	3	✓	5.0	4.7	93.7
Cal 4 MJ	4	✓	10.0	9.6	96.4
Cal 5 MJ	5	✓	25.0	24.8	99.1
Cal 6 MJ	6	✓	50.0	49.5	98.9
Cal 7 MJ	7	✓	100.0	101.5	101.5

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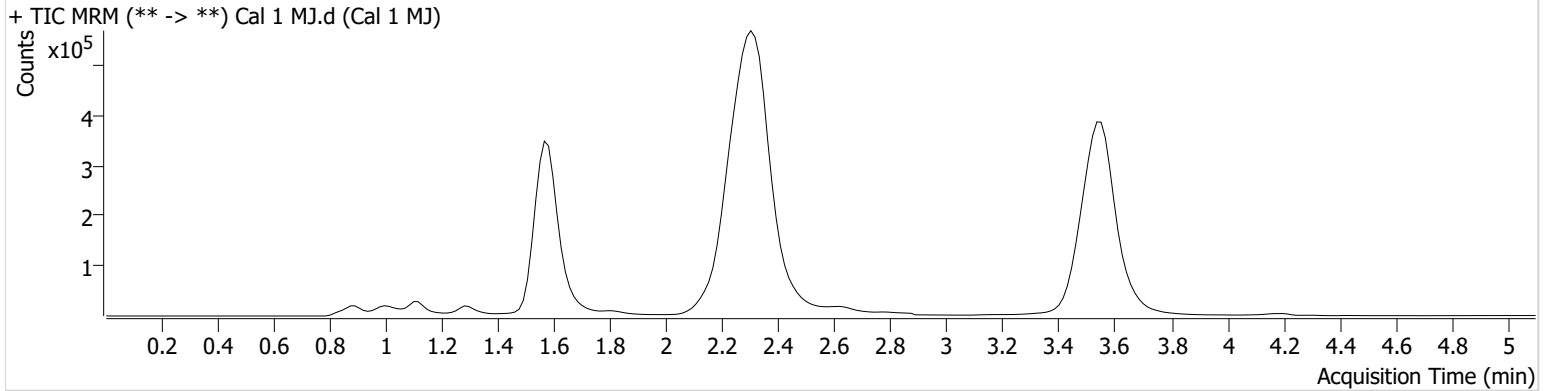
AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 7:07:01 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.573	25527	57.01	14.9	31.10	1632739	1.1672 ng/ml Low
THC-COOH	1.610	61756	∞	36.0 Low	∞	338790	5.2180 ng/ml
THC	3.555	29416	269.21	30.6	∞	3346490	1.1562 ng/ml

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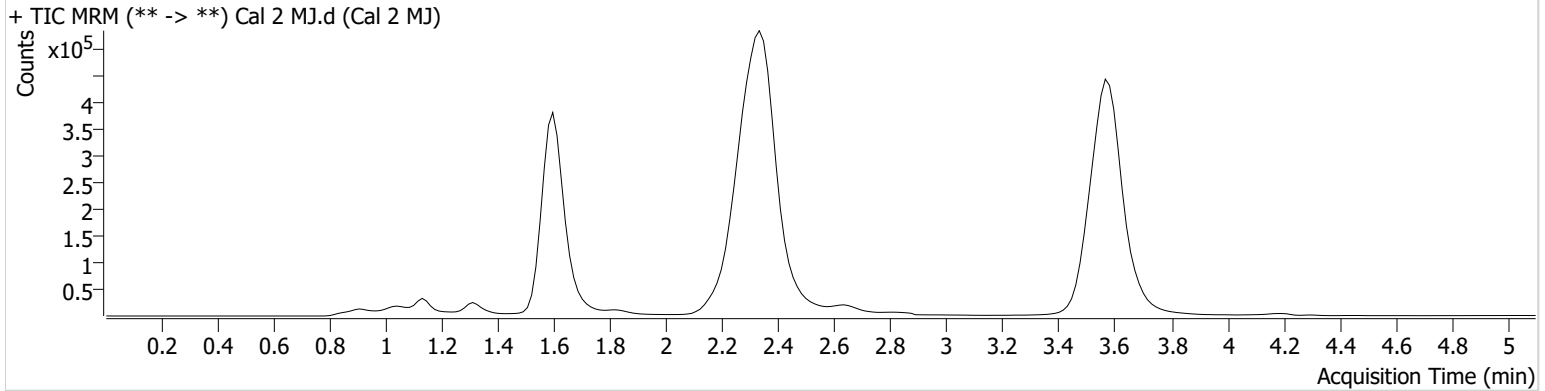


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-B1	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 7:14:48 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	69104	∞	14.5	162.05	1592250	2.8101 ng/ml Low
THC-COOH	1.640	94858	∞	44.5	274.26	317112	10.1357 ng/ml
THC	3.586	92758	211.13	27.7	51.68	3494229	2.9512 ng/ml

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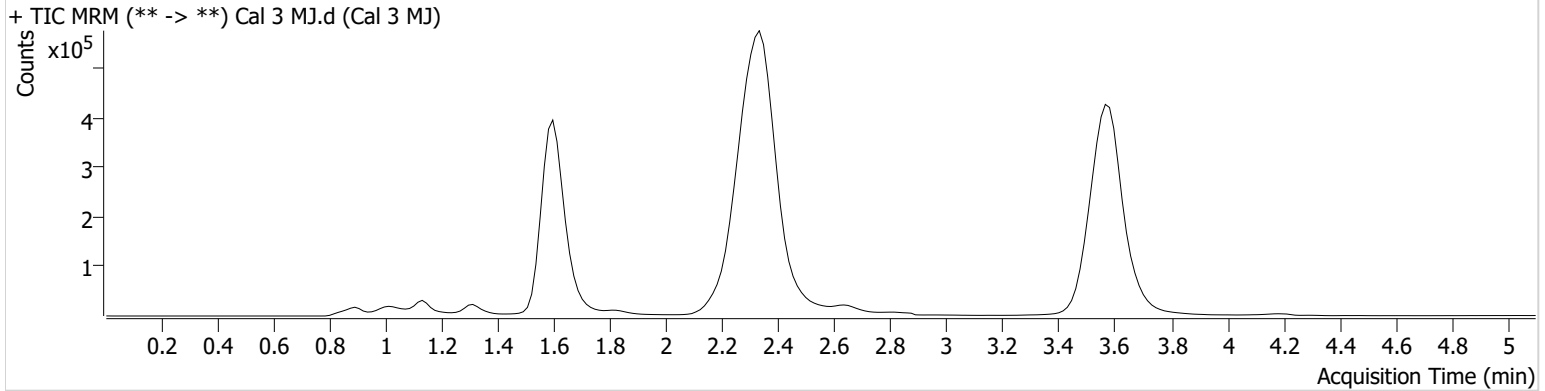
AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 7:22:24 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	118872	∞	13.8	188.91	1583427	4.6842 ng/ml
THC-COOH	1.625	168346	∞	48.3	∞	319883	19.6957 ng/ml
THC	3.586	149427	1019.48	28.0	279.87	3386033	4.7289 ng/ml

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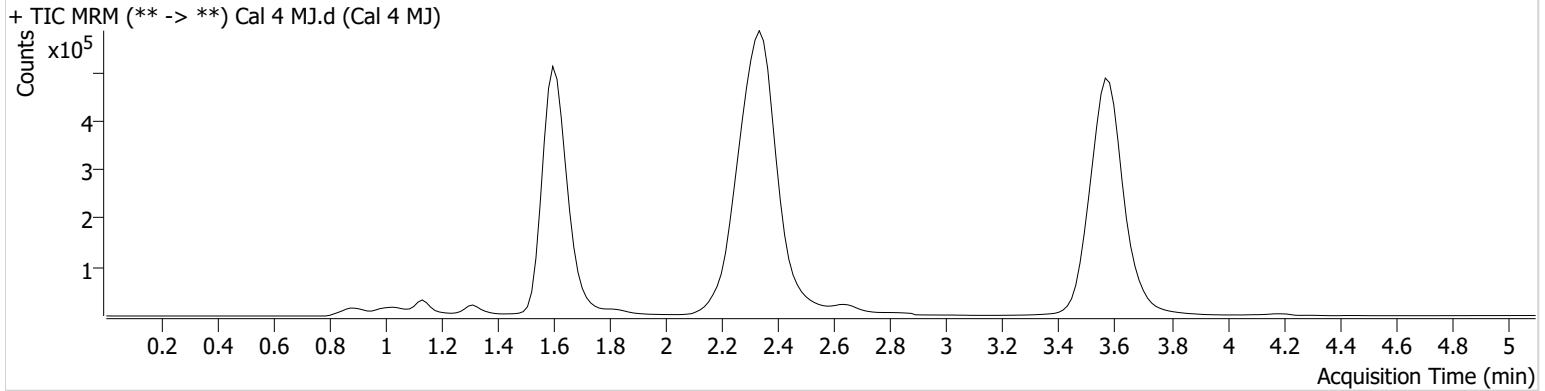


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-D1	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 7:30:01 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	280830	∞	13.3	508.65	1767359	9.6443 ng/ml
THC-COOH	1.625	417501	∞	56.2	∞	336771	49.7230 ng/ml
THC	3.586	330031	2207.60	28.2	230.84	3674630	9.3472 ng/ml

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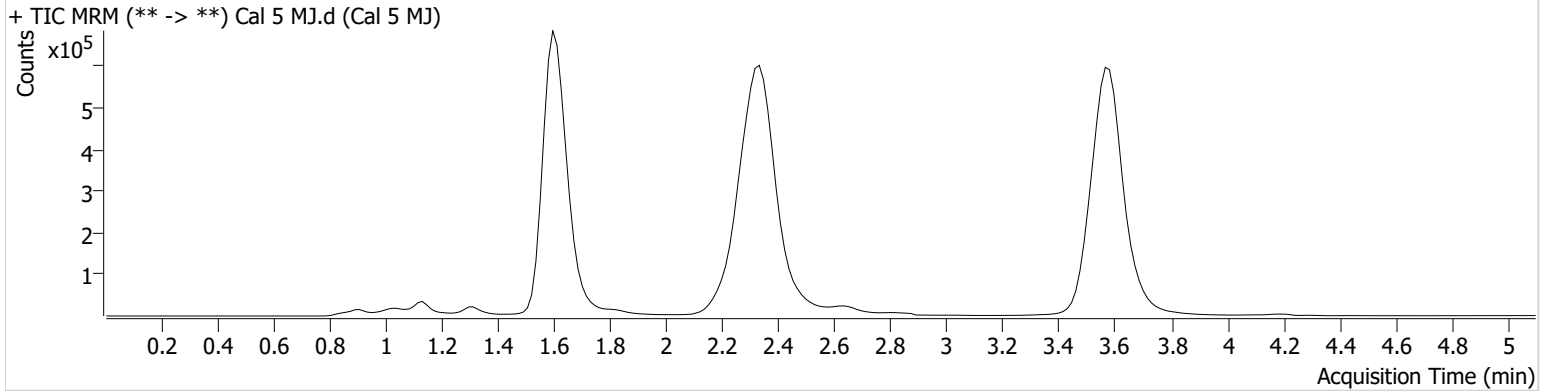


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 7:37:38 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	745572	∞	13.5	∞	1798016	24.7783 ng/ml
THC-COOH	1.625	620185	∞	57.3	5293.97	339782	74.3667 ng/ml
THC	3.586	880779	3877.09	26.9	∞	3742550	24.0593 ng/ml

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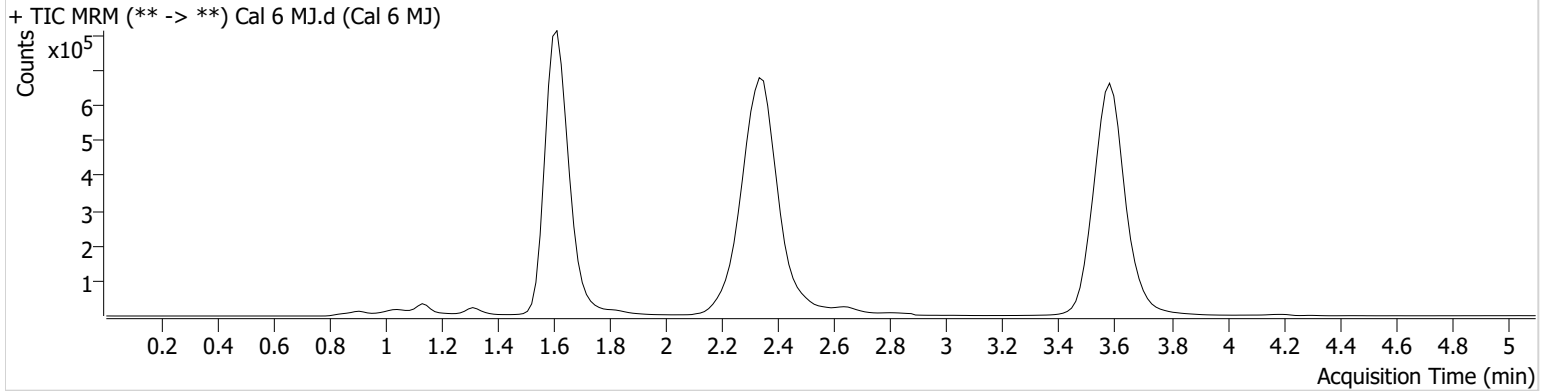
AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 7:45:14 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	1338332	∞	13.6	5898.41	1609156	49.4549 ng/ml
THC-COOH	1.640	768952	∞	56.4	∞	310174	101.8861 ng/ml
THC	3.586	1591166	∞	26.2	∞	3236998	49.9611 ng/ml

AG

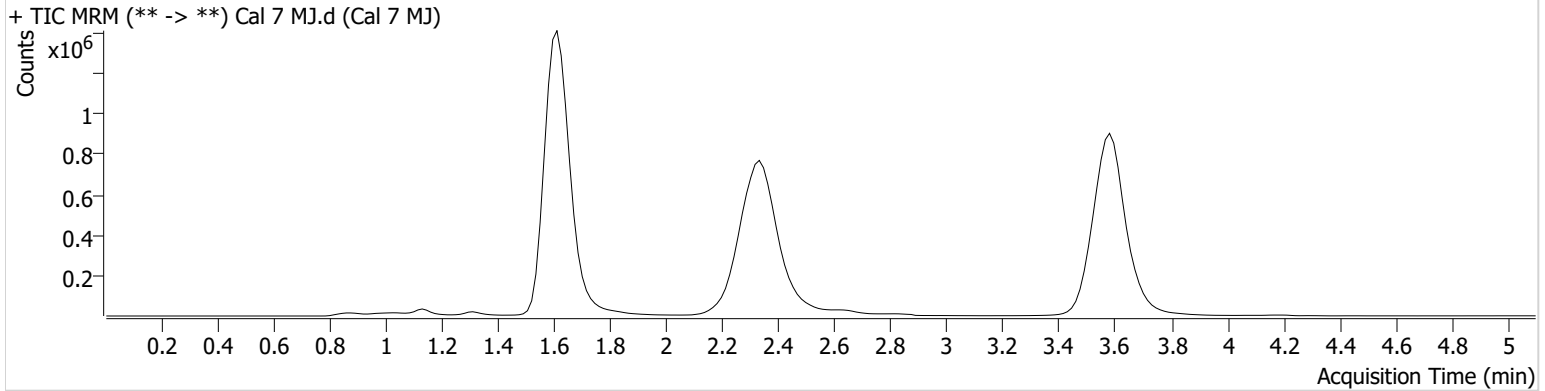


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\103122 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 11/1/2022 9:29:41 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-G1	Comment	
Injection Volume	10		
Acq. Date-Time	10/31/2022 7:52:49 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.603	2952187	14123.61	13.6	∞	1725819	101.4609 ng/ml
THC-COOH	1.625	1906000	∞	59.0	∞	318778	249.1928 ng/ml
THC	3.586	3240713	25388.59	27.0	15830.7 8	3226855	101.7960 ng/ml