2/25/2022

REVIEWED
By Sarah Collins at 1:30 pm, Mar 02, 2022

Worklist: 5629

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
M2022-0423	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0335	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-0336	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: 02/25/2022 Analyst: Amber Gerheart
Plate lot#: 211018 Plate Retest Date: 04/18/2022
Mobile phase A: 0.1% Formic Acid in LCMS Water **Mobile phase B:** 0.1% Formic acid in Acetonitrile
Blank Blood Lot: Lampire 22B52016-2 **Column**: UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 069901 **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. 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:

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	Blood Negative	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2022-0423-3	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	P2022-0335-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	P2022-0336-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1

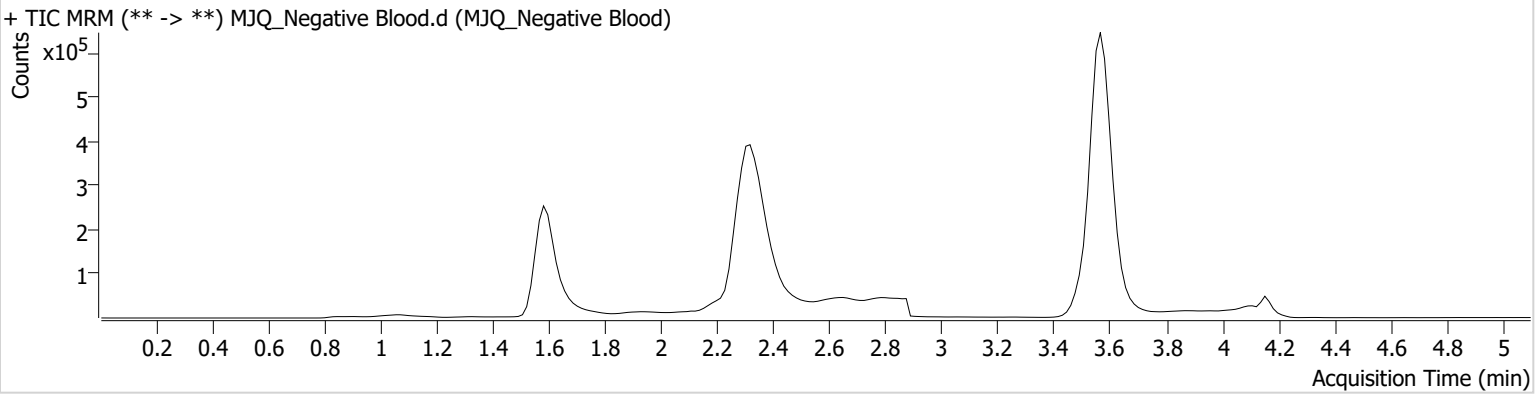
All wells to contain 100 µl of residual DMSO

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_Negative Blood.d
Type	Sample	**Sample**	MJQ_Negative Blood
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-B2	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 1:22:21 PM		
Sample Info.			

Sample Chromatogram

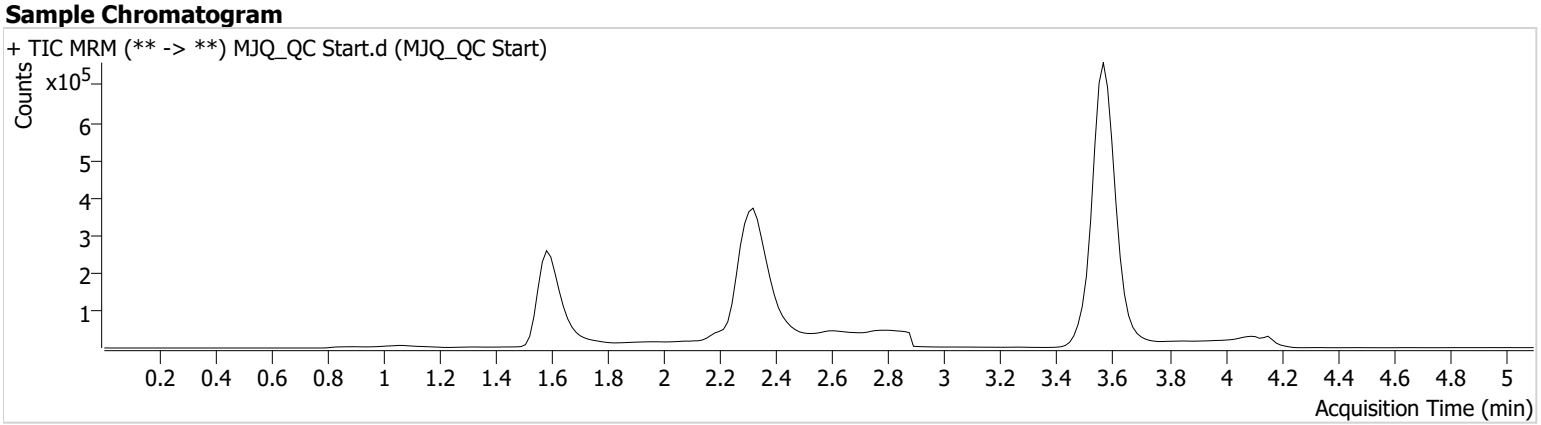


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_QC Start.d
Type	QC	**Sample**	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 1:07:08 PM		
Sample Info.			

Sample Chromatogram



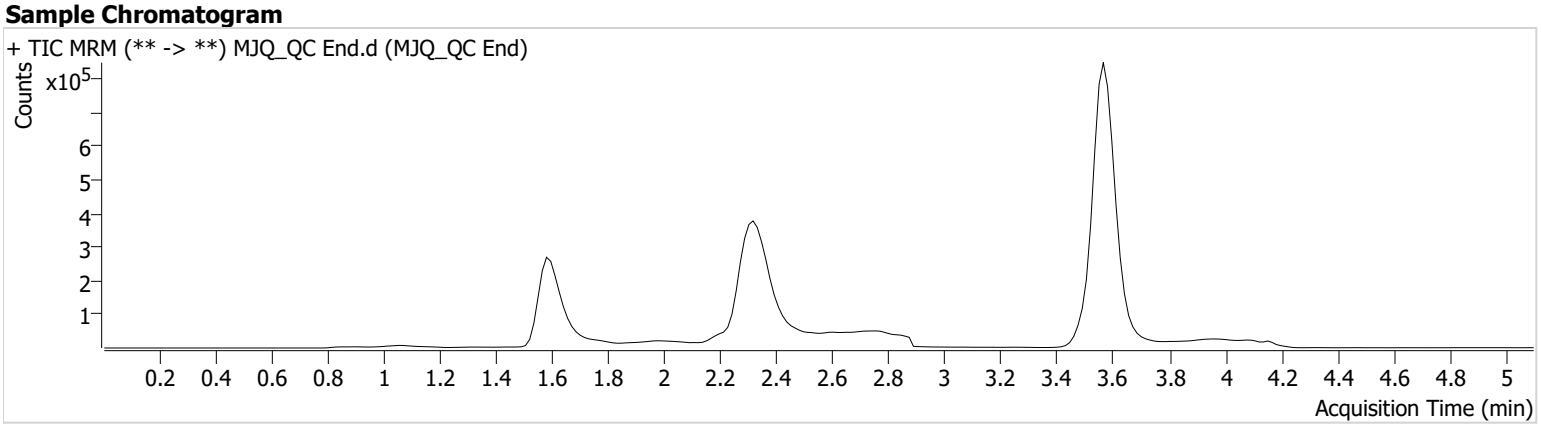
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.588	66284	∞	15.9	∞	838306	5.2987	ng/ml
THC-COOH	1.625	103919	174.94	66.4	1157.97	290239	14.3584	ng/ml
THC	3.570	172590	1160.13	28.6	∞	4249958	4.5976	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_QC End.d
Type	QC	**Sample**	MJQ_QC End
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 2:23:19 PM		
Sample Info.			

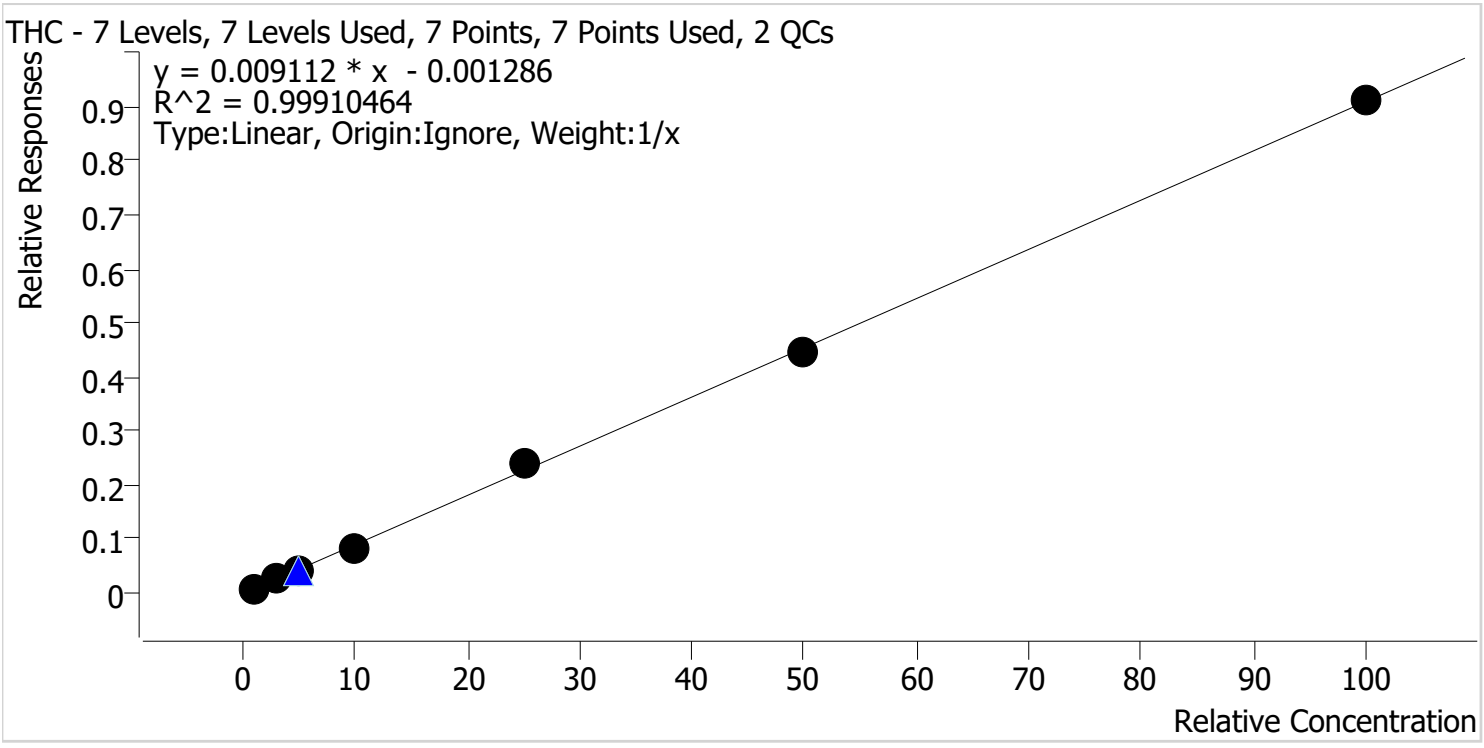
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.588	66781	∞	16.8	251.89	848146	5.2772	ng/ml
THC-COOH	1.625	108830	∞	65.9	527.33	311165	14.0372	ng/ml
THC	3.570	192796	1544.95	25.8	206.25	4430954	4.9160	ng/ml

AM #27 Cannabinoids Quant. Calibration Curve Report

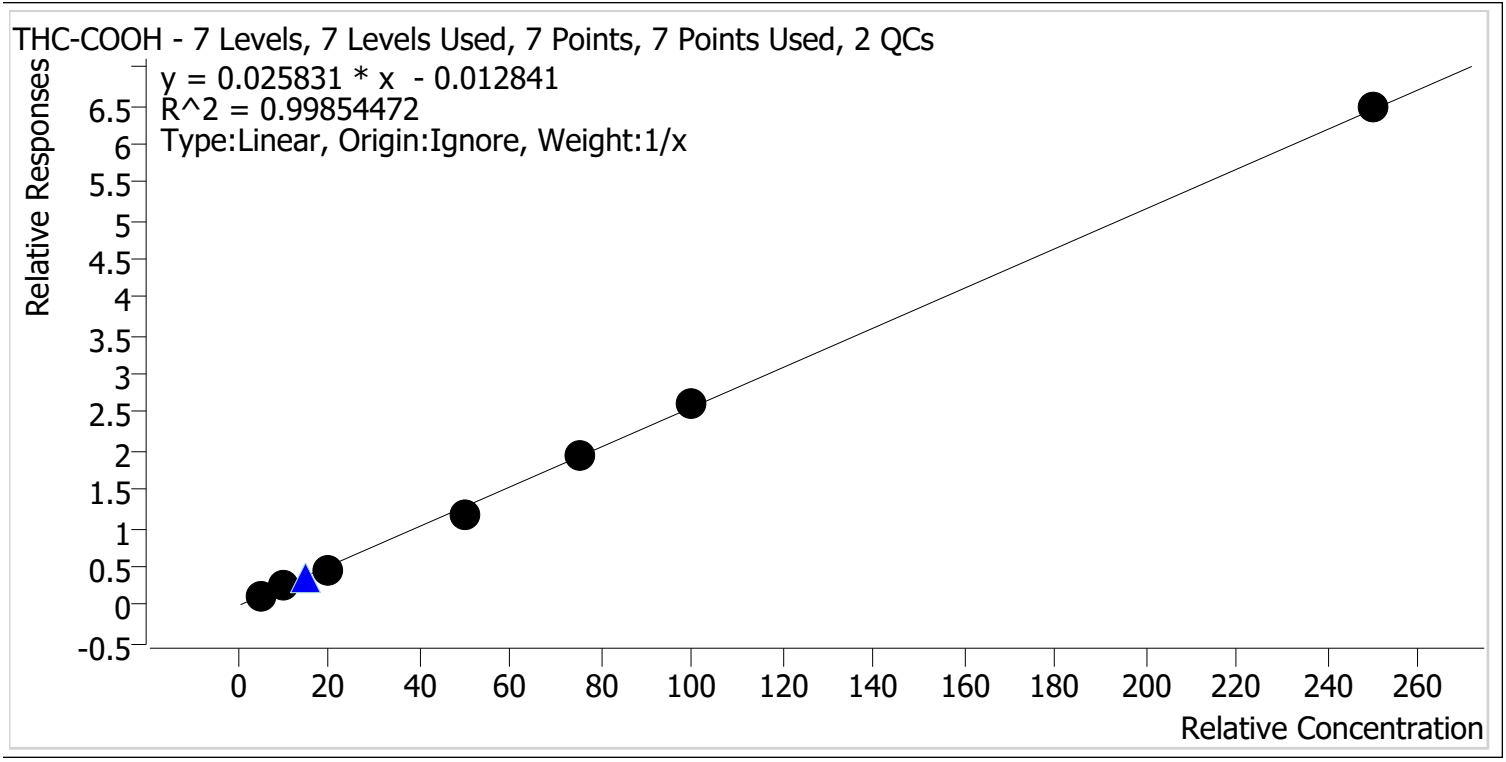
Batch results	C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Last Cal. Update	3/2/2022 7:54 AM
Analyst Name	ISP\agerheart
Analyte	THC
Internal Standard	THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	1.0	1.1	105.2
MJQ_Cal 2	2	✔	3.0	3.0	99.1
MJQ_Cal 3	3	✔	5.0	5.0	100.1
MJQ_Cal 4	4	✔	10.0	9.2	91.6
MJQ_Cal 5	5	✔	25.0	26.3	105.1
MJQ_Cal 6	6	✔	50.0	49.4	98.7
MJQ_Cal 7	7	✔	100.0	100.2	100.2

AM #27 Cannabinoids Quant. Calibration Curve Report

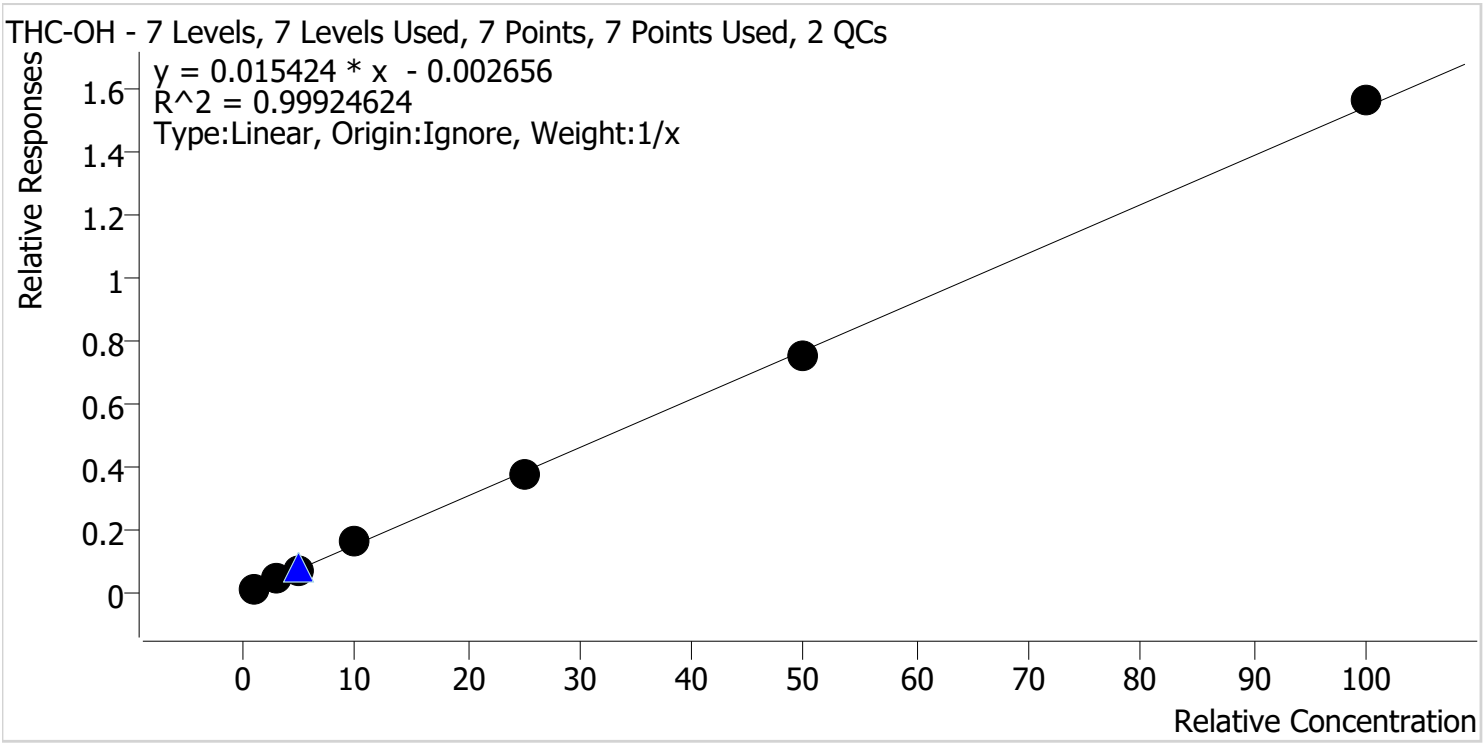
Batch results	C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Last Cal. Update	3/2/2022 7:54 AM
Analyst Name	ISP\agerheart
Analyte	THC-COOH
Internal Standard	THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	5.0	5.0	100.3
MJQ_Cal 2	2	✔	10.0	11.1	110.6
MJQ_Cal 3	3	✔	20.0	18.5	92.6
MJQ_Cal 4	4	✔	50.0	46.3	92.5
MJQ_Cal 5	5	✔	75.0	76.2	101.7
MJQ_Cal 6	6	✔	100.0	101.9	101.9
MJQ_Cal 7	7	✔	250.0	251.0	100.4

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Last Cal. Update	3/2/2022 7:54 AM
Analyst Name	ISP\agerheart
Analyte	THC-OH
Internal Standard	THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	1.0	1.0	98.6
MJQ_Cal 2	2	✔	3.0	3.0	100.0
MJQ_Cal 3	3	✔	5.0	4.9	97.7
MJQ_Cal 4	4	✔	10.0	10.7	107.4
MJQ_Cal 5	5	✔	25.0	24.4	97.5
MJQ_Cal 6	6	✔	50.0	48.7	97.4
MJQ_Cal 7	7	✔	100.0	101.3	101.3

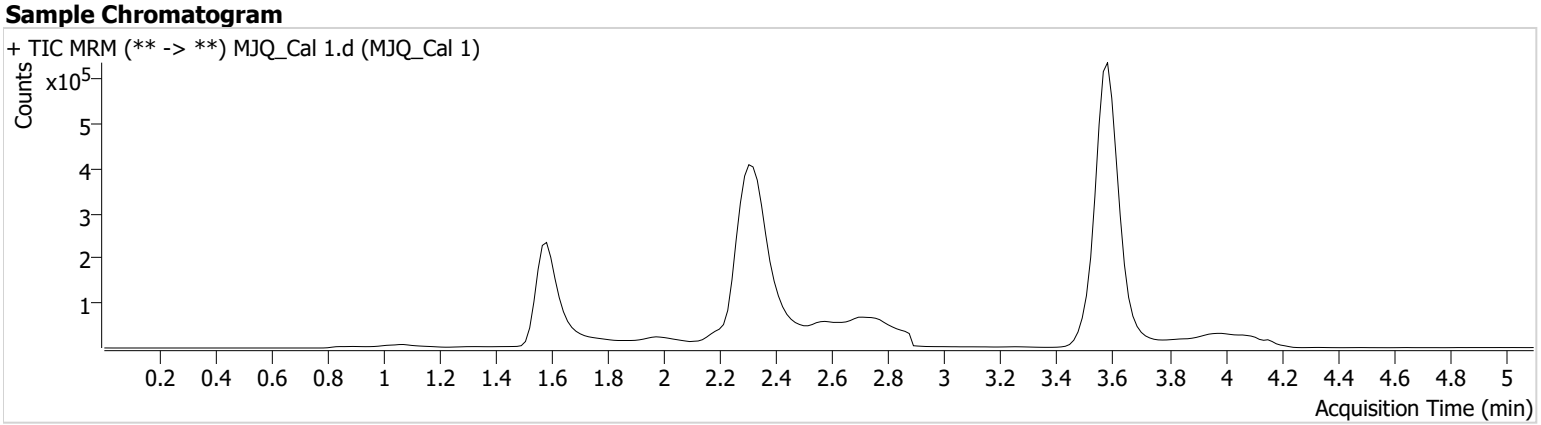
Generated at 8:39 AM on 3/2/2022

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_Cal 1.d
Type	Cal	**Sample**	MJQ_Cal 1
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 12:06:06 PM		
Sample Info.			

Sample Chromatogram



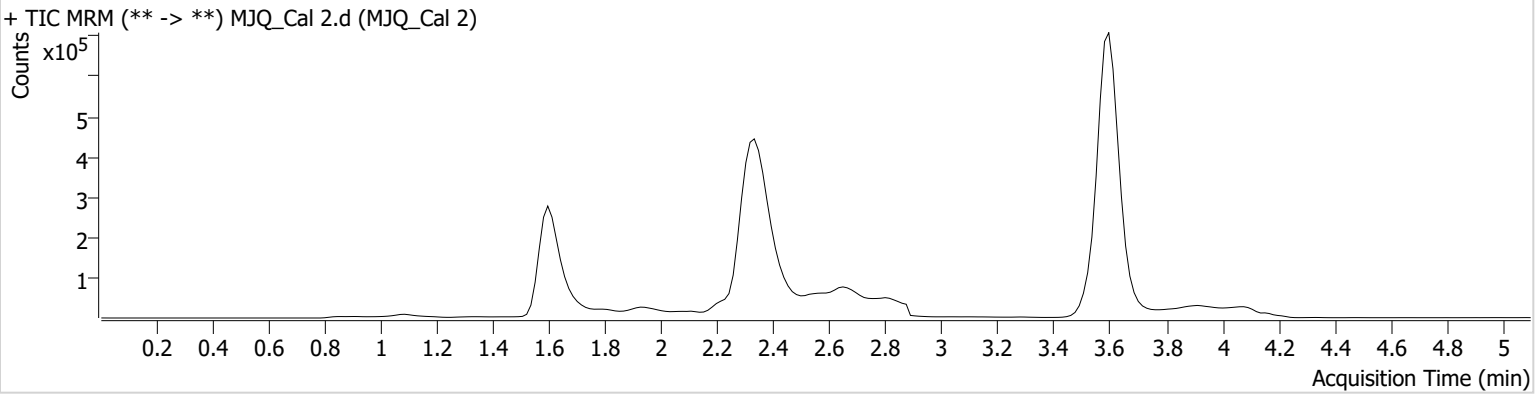
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.588	10916	14.54	16.7	10.27	869187	0.9865	ng/ml Low
THC-COOH	1.625	35616	∞	48.2	416.25	305095	5.0164	ng/ml
THC	3.586	29417	151.97	30.5	61.70	3544363	1.0519	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_Cal 2.d
Type	Cal	**Sample**	MJQ_Cal 2
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 12:13:52 PM		
Sample Info.			

Sample Chromatogram



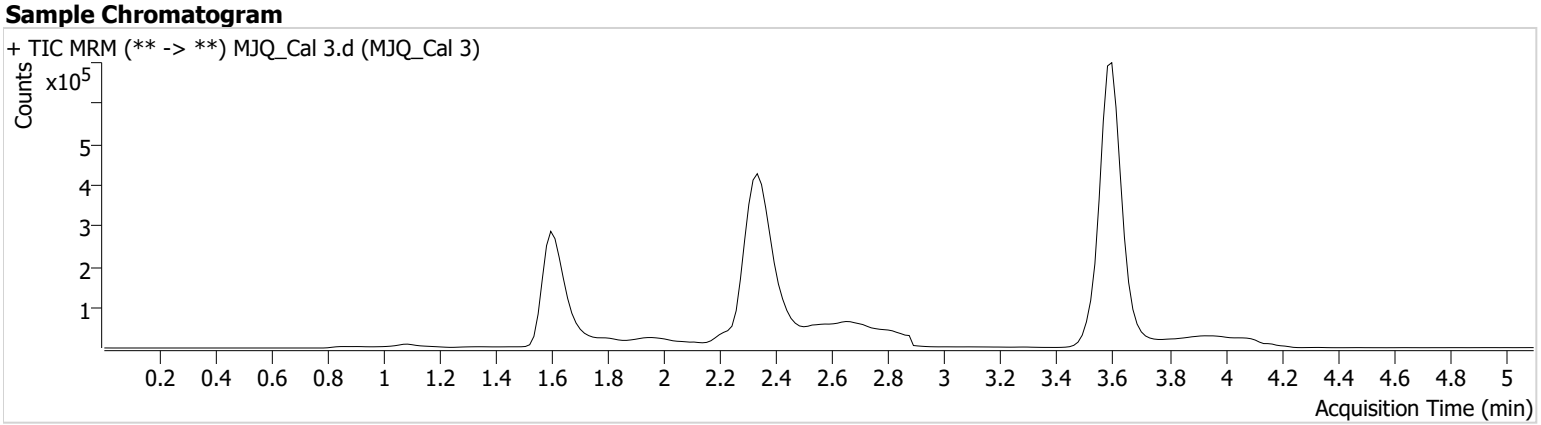
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.603	38629	88.64	14.9	151.67	885326	3.0012	ng/ml
THC-COOH	1.640	82968	509.76	56.1	∞	304075	11.0602	ng/ml
THC	3.601	94029	411.88	29.2	∞	3643950	2.9728	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_Cal 3.d
Type	Cal	**Sample**	MJQ_Cal 3
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 12:21:27 PM		
Sample Info.			

Sample Chromatogram



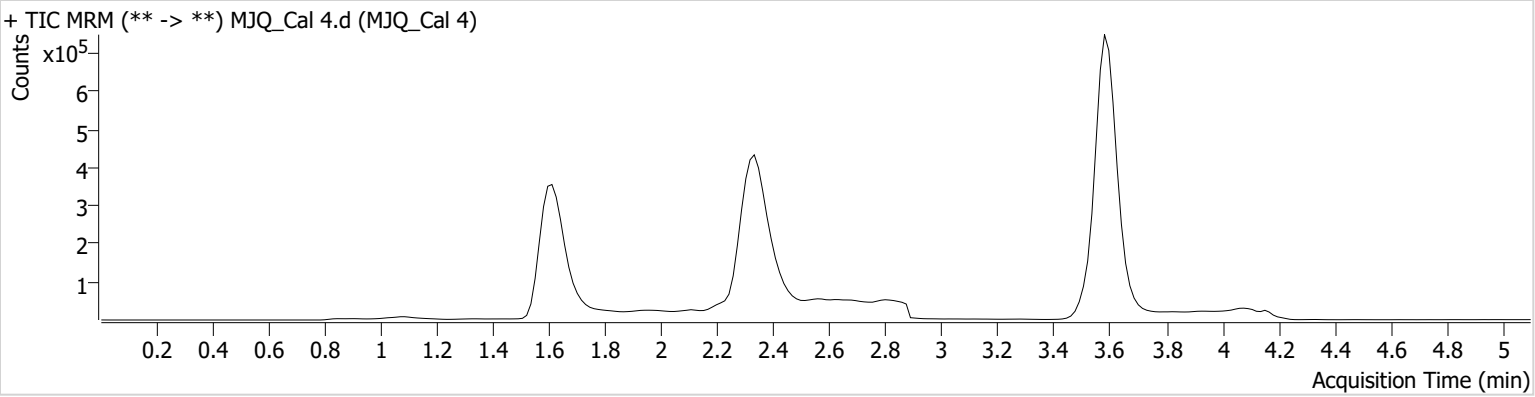
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.603	61131	∞	14.6	127.68	841002	4.8850	ng/ml
THC-COOH	1.640	138441	∞	63.3	∞	297496	18.5127	ng/ml
THC	3.601	154491	163.07	28.3	∞	3485941	5.0046	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_Cal 4.d
Type	Cal	**Sample**	MJQ_Cal 4
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 12:29:03 PM		
Sample Info.			

Sample Chromatogram



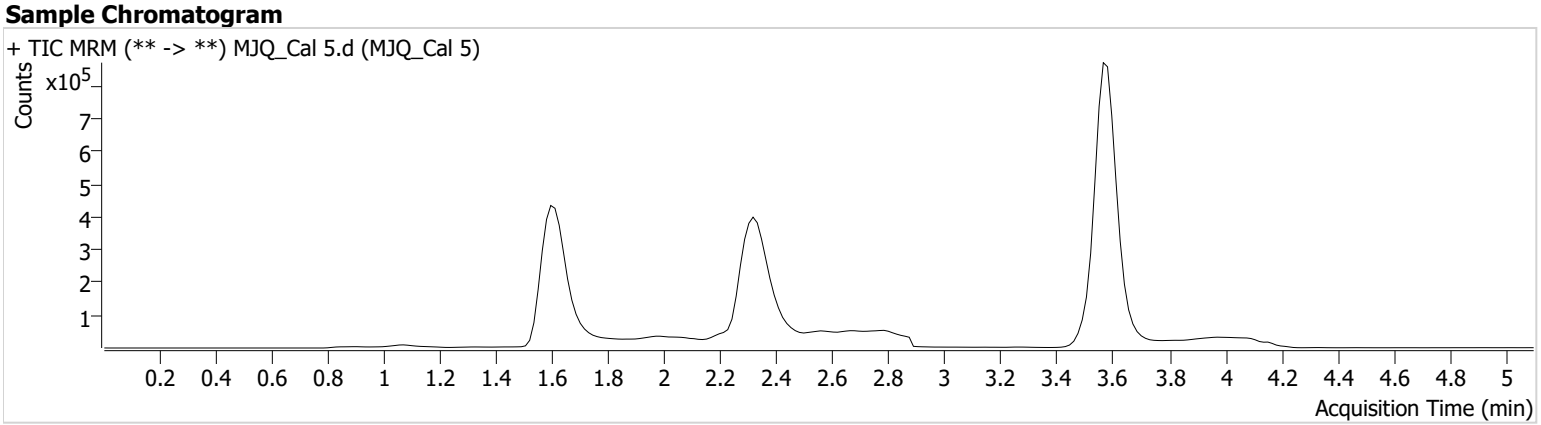
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.603	148790	824.76	15.1	391.33	912804	10.7407	ng/ml
THC-COOH	1.640	367638	∞	57.4	2089.33	310974	46.2649	ng/ml
THC	3.601	321613	2570.80	27.6	334.14	3913018	9.1607	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_Cal 5.d
Type	Cal	**Sample**	MJQ_Cal 5
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 12:36:40 PM		
Sample Info.			

Sample Chromatogram



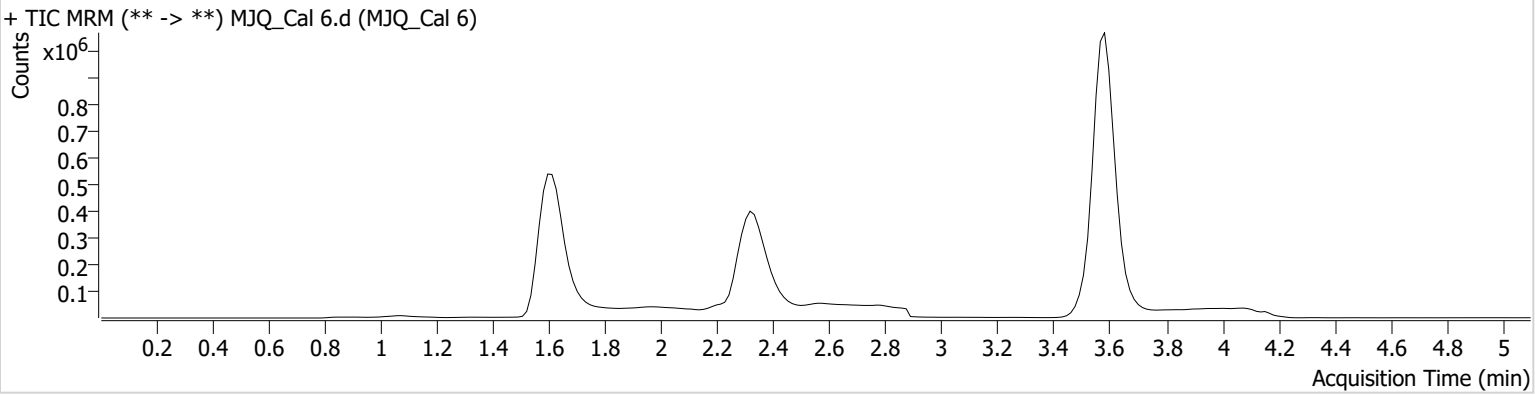
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.588	343446	∞	15.5	∞	920063	24.3745	ng/ml
THC-COOH	1.625	572066	1020.94	53.6	213.73	292363	76.2480	ng/ml
THC	3.586	867615	7600.31	24.6	167.67	3641895	26.2847	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_Cal 6.d
Type	Cal	**Sample**	MJQ_Cal 6
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 12:44:17 PM		
Sample Info.			

Sample Chromatogram



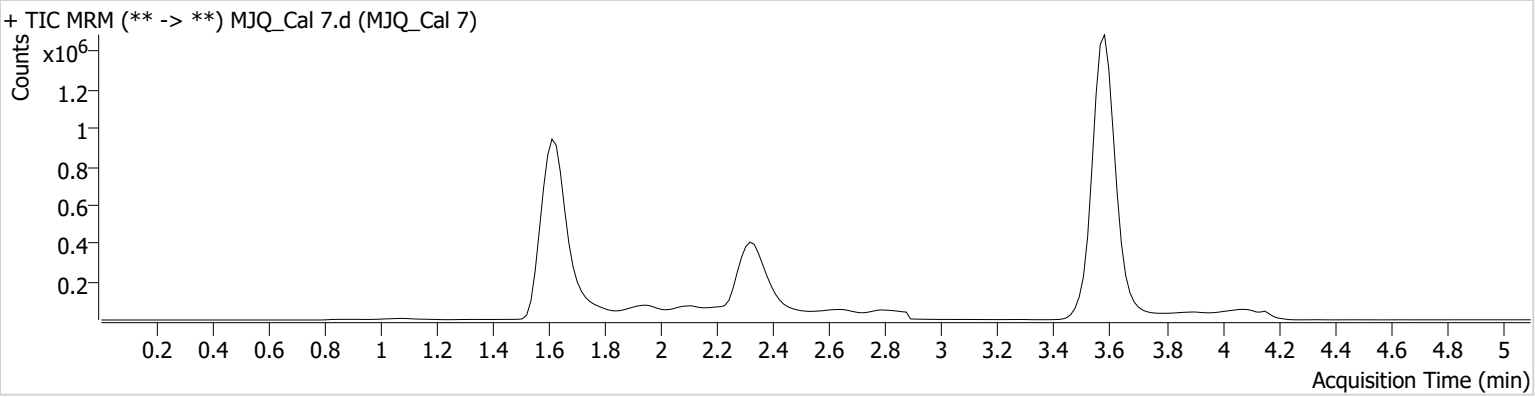
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.588	681030	4220.75	14.1	2696.03	909963	48.6963	ng/ml
THC-COOH	1.625	743793	∞	54.0	∞	283909	101.9202	ng/ml
THC	3.586	1746613	16599.45	24.8	∞	3894868	49.3529	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\022522 AM 27 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 3/2/2022 7:54:18 AM

Instrument	Falco (069901)	**Data File**	MJQ_Cal 7.d
Type	Cal	**Sample**	MJQ_Cal 7
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P1-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	2/25/2022 12:51:52 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.588	1342879	∞	14.1	7042.04	860823	101.3159	ng/ml
THC-COOH	1.625	1769483	3302.10	55.1	9205.75	273487	250.9774	ng/ml
THC	3.586	3636426	28746.30	25.5	6472.25	3989368	100.1724	ng/ml