REVIEWED
By Tamara Salazar at 9:21 am, Jul 20, 2021

7/12/2021

Worklist: 5102

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
M2021-2473	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-2828	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2082	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2086	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2087	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2088	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2120	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2134	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2135	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2136	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2185	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2189	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2199	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2204	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: 7/12/2021 Analyst: Amber Gerheart
Plate lot#: 210609 Plate Retest Date: 12/09/2021
Mobile phase A: 0.1% Formic Acid in LCMS Water **Mobile phase B:** 0.1% Formic acid in Acetonitrile
Blank Blood Lot: 20L20723 **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: *THC-OH not evaluated due to ratios being out of tolerances for some calibrators.*

	1	2	3	4	5	6
A	IS + Cal. 1	Blood Negative	P2021-2134-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	M2021-2473-1	P2021-2135-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2021-2828-3	P2021-2136-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	P2021-2082-1	P2021-2185-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	P2021-2086-1	P2021-2189-1	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	P2021-2087-1	P2021-2199-1	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	P2021-2088-1	P2021-2204-1	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2021-2120-2	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 1

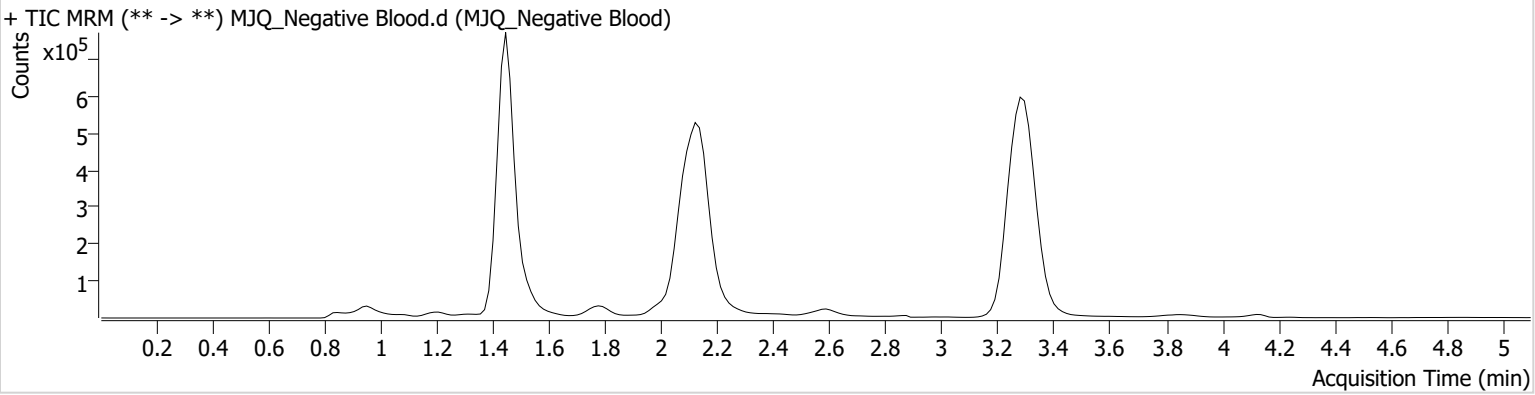
All wells to contain 100 µl of residual DMSO

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 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	P5-A2	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 3:18:17 PM		
Sample Info.			

Sample Chromatogram



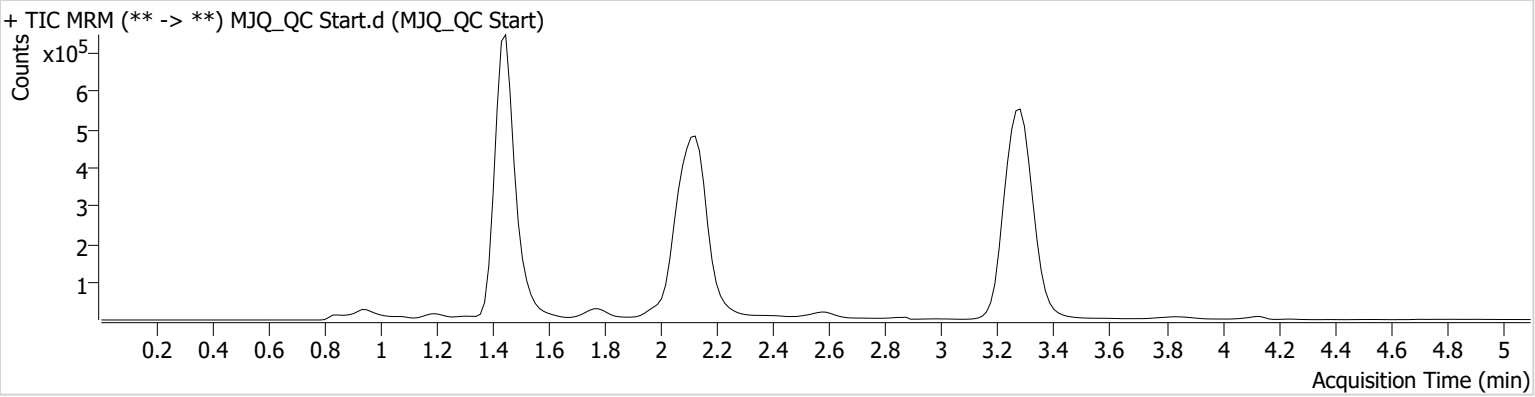
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.513 High	226556	∞	2.4 Low	105.00	2692823	0.4428	ng/ml Low

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 AM

Instrument	Falco (069901)	**Data File**	MJQ_QC Start.d
Type	Sample	**Sample**	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P5-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 3:03:04 PM		
Sample Info.			

Sample Chromatogram



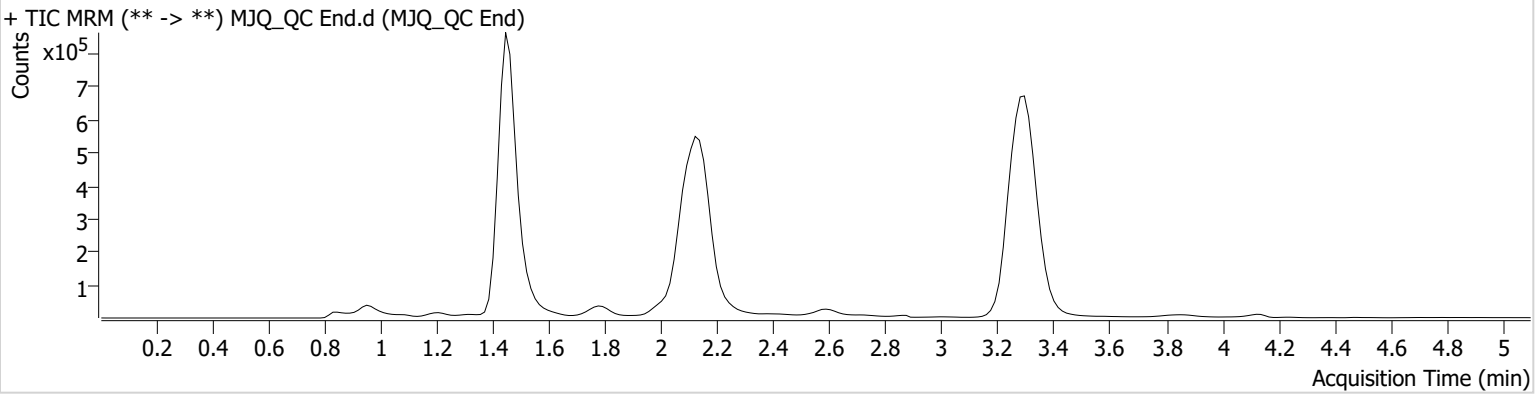
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	359827	∞	5.8 **Low**	212.67	2526210	4.7192	ng/ml
THC-COOH	1.474	193740	263.41	52.1	∞	503273	15.6342	ng/ml
THC	3.285	173911	∞	28.8	∞	3791964	4.7575	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 AM

Instrument	Falco (069901)	**Data File**	MJQ_QC End.d
Type	Sample	**Sample**	MJQ_QC End
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P5-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 7:06:25 PM		
Sample Info.			

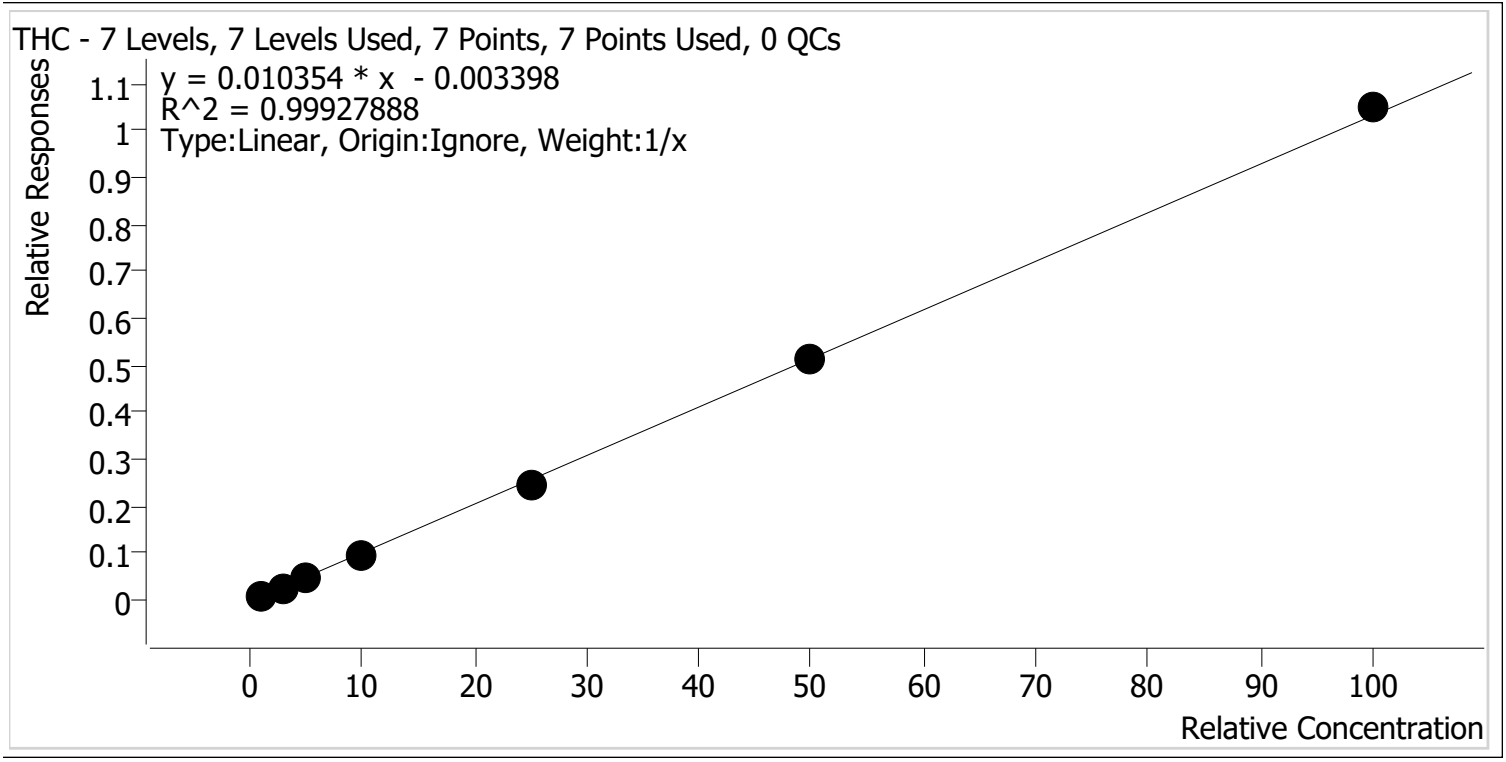
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	379108	∞	6.1	∞	2741697	4.4139	ng/ml
THC-COOH	1.489	199121	∞	53.3	938.74	550149	14.7048	ng/ml
THC	3.300	206061	∞	28.9	∞	4496417	4.7541	ng/ml

AM #27 Cannabinoids Quant. Calibration Curve Report

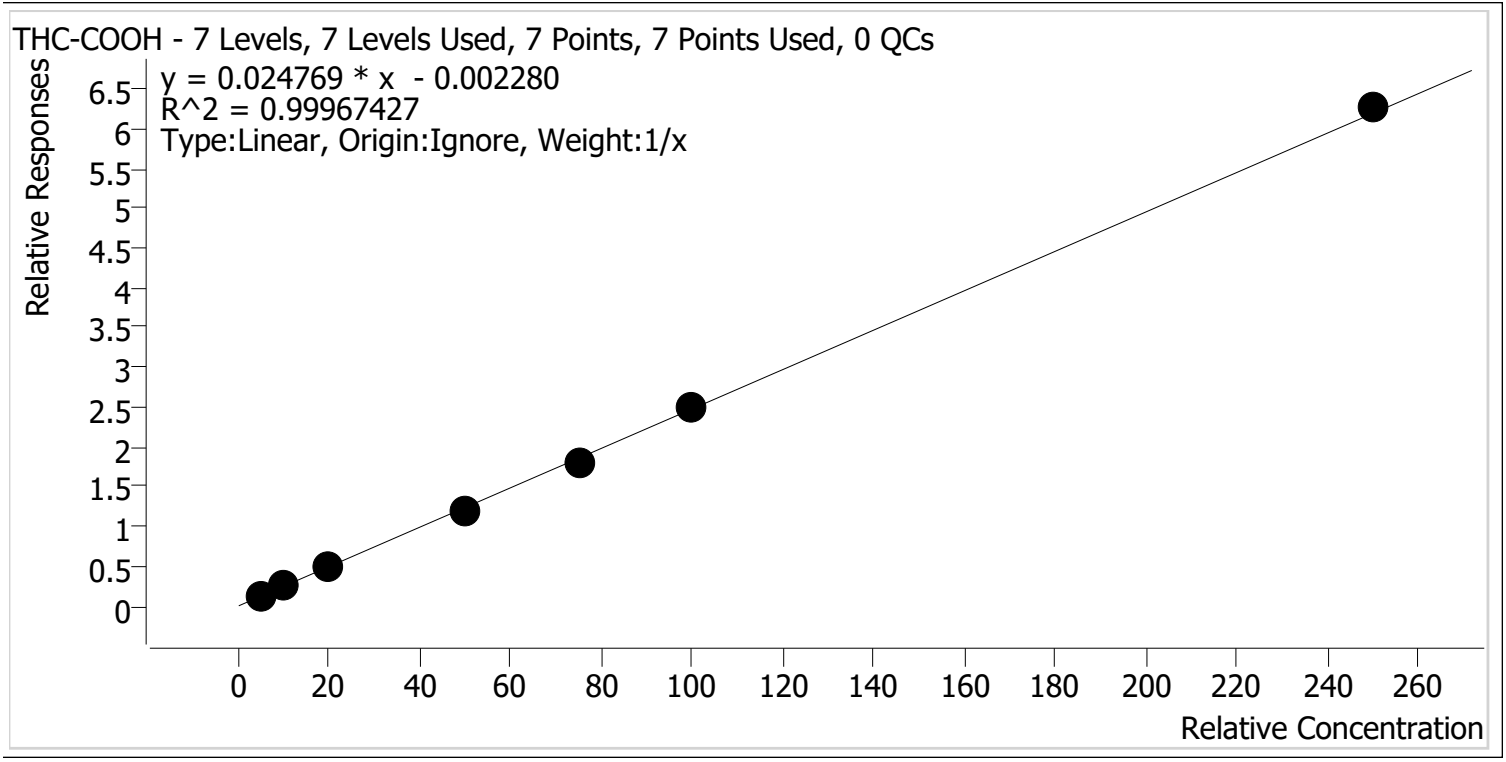
Batch results	C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Last Cal. Update	7/13/2021 10:53 AM
Analyst Name	ISP\agerheart
Analyte	THC
Internal Standard	THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	1.0	1.1	114.9
MJQ_Cal 2	2	✔	3.0	2.9	97.0
MJQ_Cal 3	3	✔	5.0	4.8	95.7
MJQ_Cal 4	4	✔	10.0	9.4	94.0
MJQ_Cal 5	5	✔	25.0	24.2	96.9
MJQ_Cal 6	6	✔	50.0	49.9	99.9
MJQ_Cal 7	7	✔	100.0	101.6	101.6

AM #27 Cannabinoids Quant. Calibration Curve Report

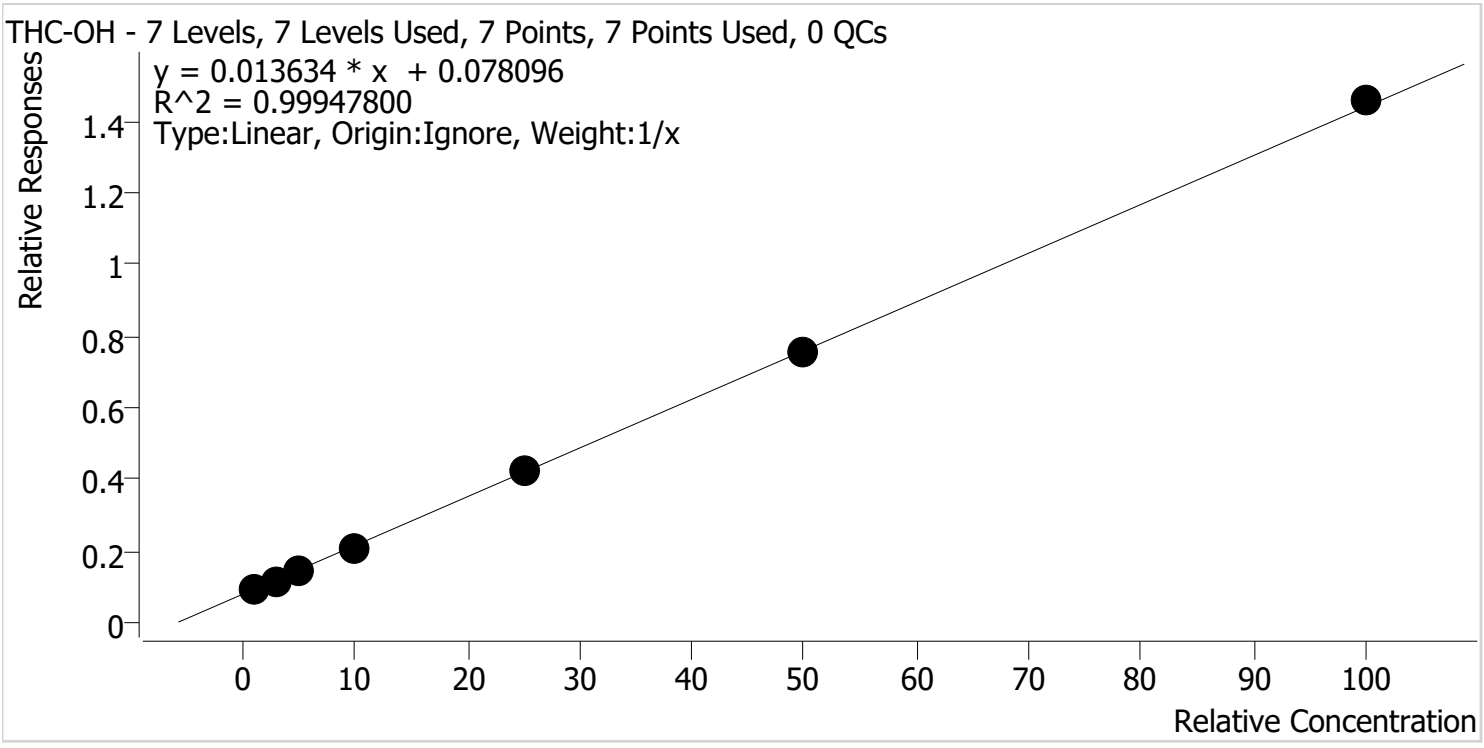
Batch results	C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Last Cal. Update	7/13/2021 10:53 AM
Analyst Name	ISP\agerheart
Analyte	THC-COOH
Internal Standard	THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	5.0	5.2	104.3
MJQ_Cal 2	2	✔	10.0	10.2	102.4
MJQ_Cal 3	3	✔	20.0	19.2	96.1
MJQ_Cal 4	4	✔	50.0	49.1	98.1
MJQ_Cal 5	5	✔	75.0	73.5	98.0
MJQ_Cal 6	6	✔	100.0	99.9	99.9
MJQ_Cal 7	7	✔	250.0	252.9	101.1

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Last Cal. Update	7/13/2021 10:53 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	1.1	112.6
MJQ_Cal 2	2	✔	3.0	2.9	96.2
MJQ_Cal 3	3	✔	5.0	4.8	96.5
MJQ_Cal 4	4	✔	10.0	9.4	94.1
MJQ_Cal 5	5	✔	25.0	25.1	100.6
MJQ_Cal 6	6	✔	50.0	49.4	98.7
MJQ_Cal 7	7	✔	100.0	101.3	101.3

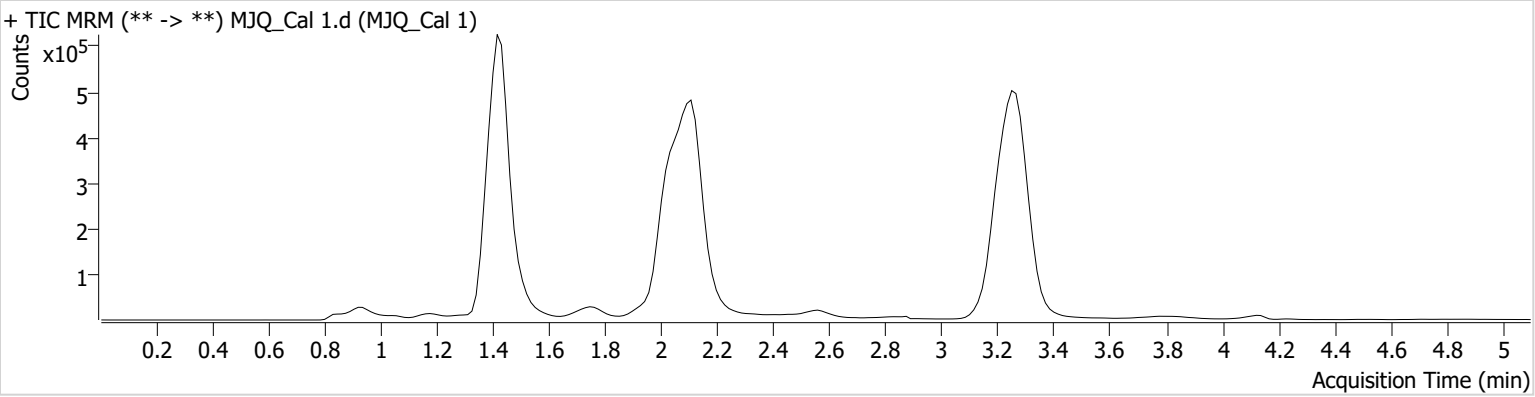
Compound not evaluated

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 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	P5-A1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 2:02:05 PM		
Sample Info.			

Sample Chromatogram



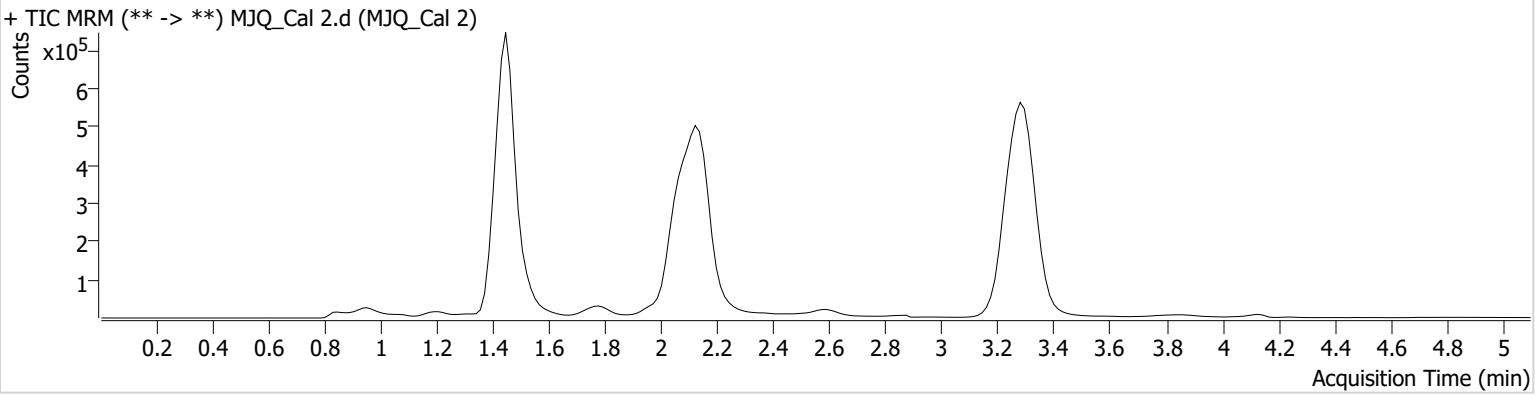
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.498 High	244288	∞	3.6 Low	27.32	2614035	1.1264	ng/ml Low
THC-COOH	1.459	66478	∞	49.1	328.25	524005	5.2140	ng/ml
THC	3.270	34096	∞	31.4	31.09	4010399	1.1492	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 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	P5-B1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 2:09:50 PM		
Sample Info.			

Sample Chromatogram



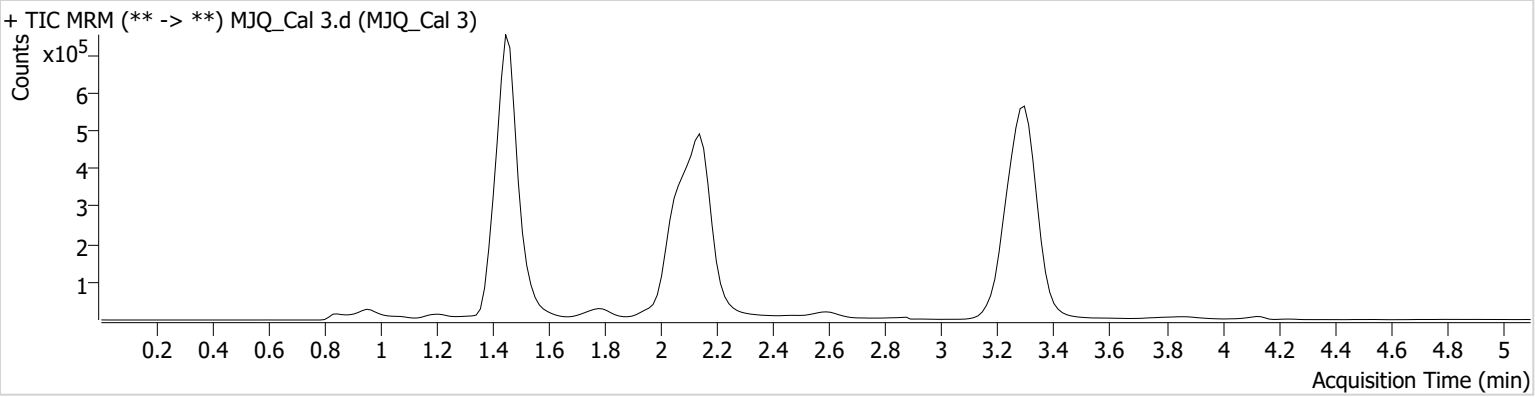
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.513 High	318838	∞	4.9 Low	∞	2714915	2.8857	ng/ml Low
THC-COOH	1.474	132651	∞	52.2	469.03	527595	10.2429	ng/ml
THC	3.300	109583	∞	32.9	∞	4100550	2.9091	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 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	P5-C1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 2:17:26 PM		
Sample Info.			

Sample Chromatogram



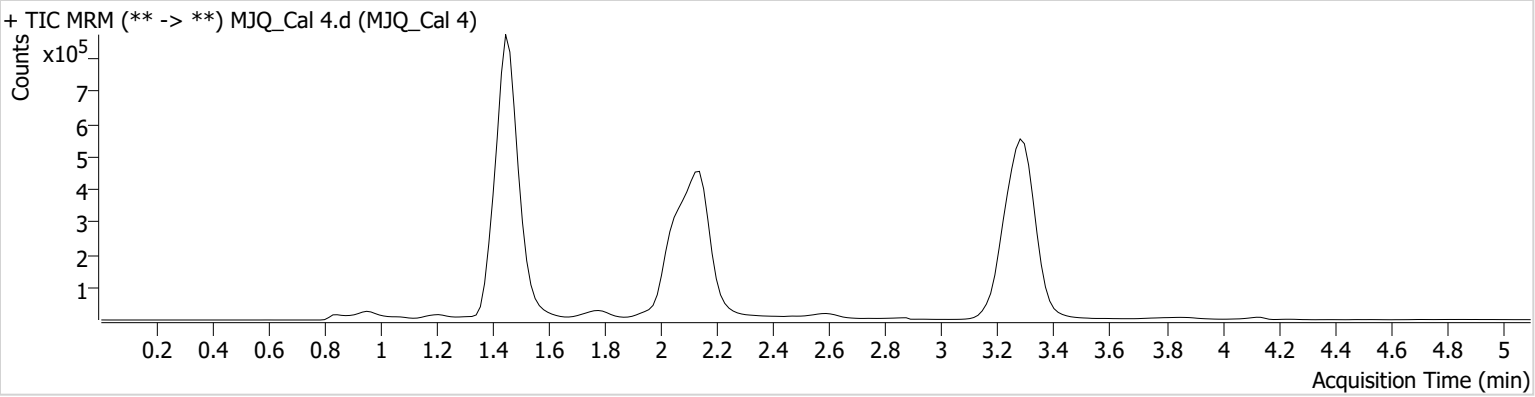
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	389047	∞	5.8 **Low**	∞	2703452	4.8271	ng/ml
THC-COOH	1.489	259777	298.39	54.2	428.15	548042	19.2294	ng/ml
THC	3.315	190721	∞	25.8	∞	4131398	4.7866	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 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	P5-D1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 2:25:02 PM		
Sample Info.			

Sample Chromatogram



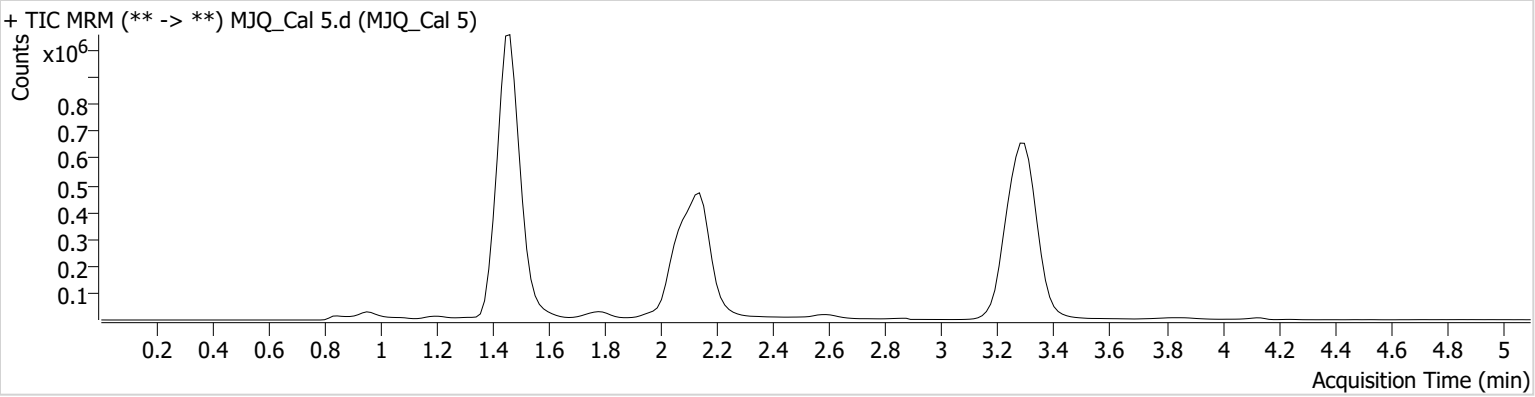
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	550083	∞	7.5	336.31	2665691	9.4075	ng/ml
THC-COOH	1.474	636307	∞	55.9	∞	524686	49.0546	ng/ml
THC	3.300	365665	∞	27.9	1092.97	3893426	9.3986	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 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	P5-E1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 2:32:37 PM		
Sample Info.			

Sample Chromatogram



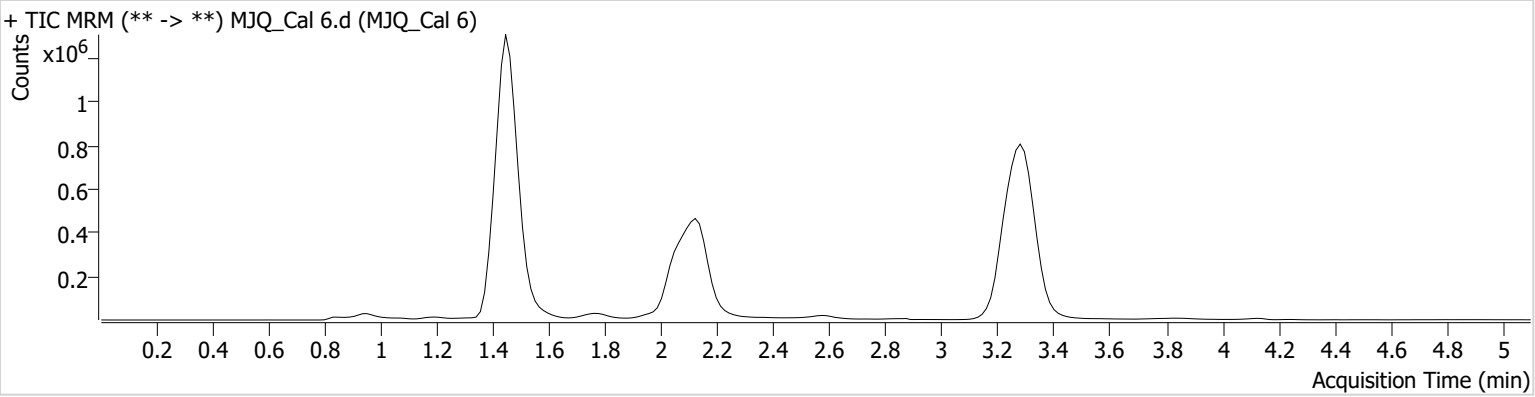
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	1092415	∞	9.3 **High**	1381.86	2594901	25.1497	ng/ml
THC-COOH	1.489	954307	669.77	56.4	2639.16	524909	73.4928	ng/ml
THC	3.300	966021	∞	25.6	∞	3902806	24.2330	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 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	P5-F1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 2:40:14 PM		
Sample Info.			

Sample Chromatogram



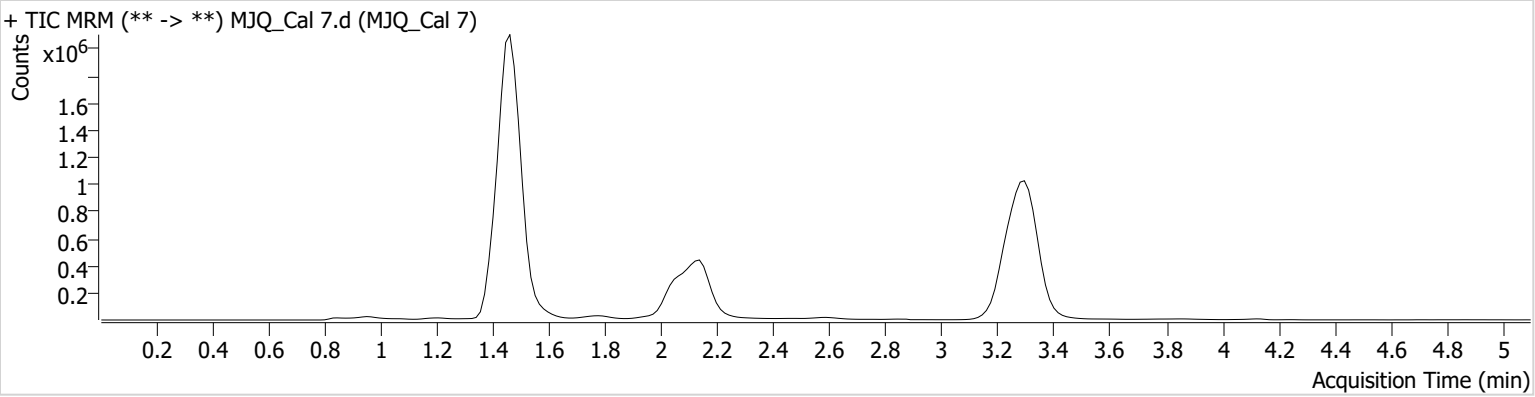
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	1888413	∞	10.5 **High**	729.91	2514602	49.3535	ng/ml
THC-COOH	1.474	1223419	∞	56.4	1636.61	494909	99.8955	ng/ml
THC	3.300	1977949	∞	25.4	∞	3850972	49.9327	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\071221 AM 27 28 P1 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 7/13/2021 10:53:46 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	P5-G1	**Comment**	
Injection Volume	10		
Acq. Date-Time	7/12/2021 2:47:50 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	3646526	∞	11.0 **High**	∞	2500131	101.2501	ng/ml
THC-COOH	1.474	2999025	∞	57.1	13802.37	479000	252.8708	ng/ml
THC	3.300	3810425	∞	25.8	∞	3634131	101.5908	ng/ml