REVIEWED
By Sarah Collins at 3:08 pm, Oct 12, 2021

10/7/2021

Worklist: 5277

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
M2021-3546	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2715	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2918	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2948	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2981	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2982	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2993	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3010	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3027	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3028	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3033	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3036	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3037	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3059	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: 10/1/2021 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: Lampire 20L20724 **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:** *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:
THC-OH not evaluated due to ratios being out.
Instrument stopped due to high pressure before this plate was ran. The issue was resolved 10/4/21 and the plate was run with the 10/4/2021 worklist. The plate was stored in the freezer until the clog was removed.

	1	2	3	4	5	6
A	IS + Cal. 1	Blood Negative	P2021-3010-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	M2021-3546-2	P2021-3027-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	P2021-2715-1	P2021-3028-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	P2021-2918-1	P2021-3033-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	P2021-2948-1	P2021-3036-1	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	P2021-2981-2	P2021-3037-1	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	P2021-2982-1	P2021-3059-1	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2021-2993-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 1

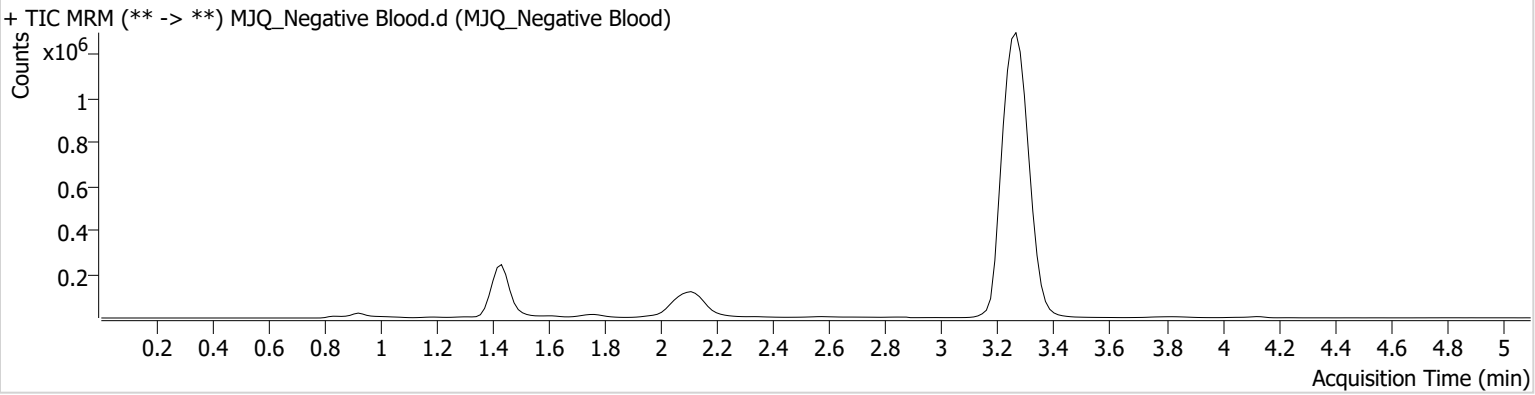
All wells to contain 100 µl of residual DMSO

AM #27 Cannabinoid Quant. Results

Batch results	D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update	10/7/2021 1:16:45 PM

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	10/5/2021 3:18:43 AM		
Sample Info.			

Sample Chromatogram

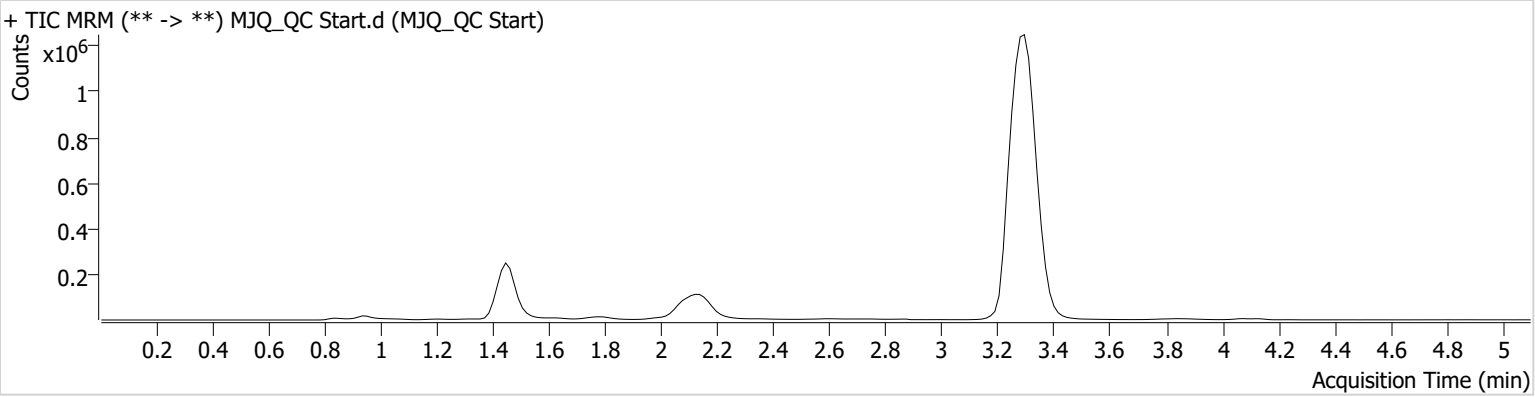


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 3:03:30 AM		
Sample Info.			

Sample Chromatogram



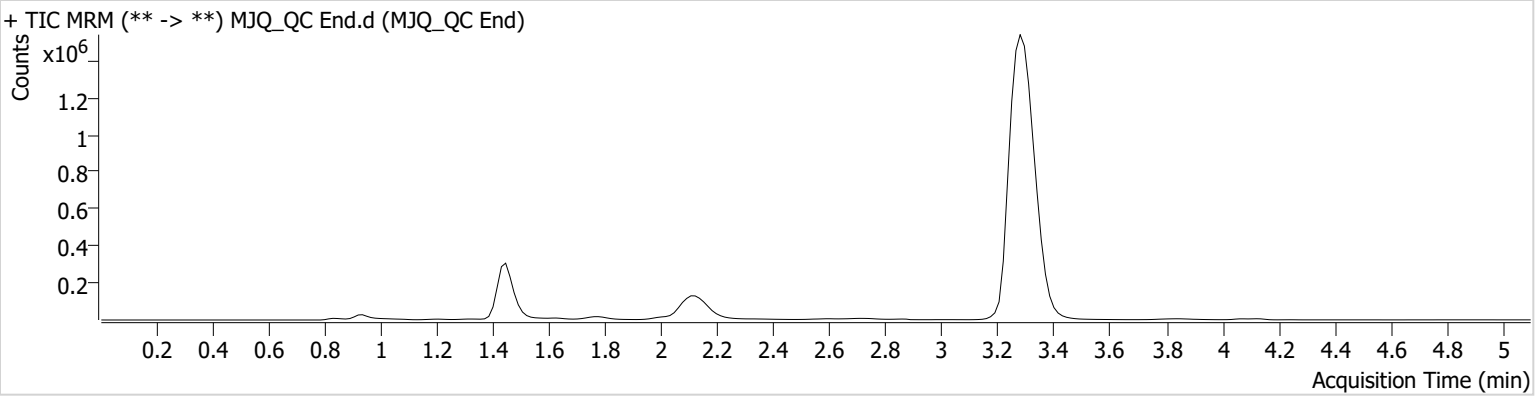
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	77214	∞	10.3	∞	798722	4.5320	ng/ml
THC-COOH	1.474	47212	78.18	65.0	∞	163074	14.4326	ng/ml
THC	3.300	354235	3760.57	27.3	∞	7838945	4.7431	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 7:06:55 AM		
Sample Info.			

Sample Chromatogram



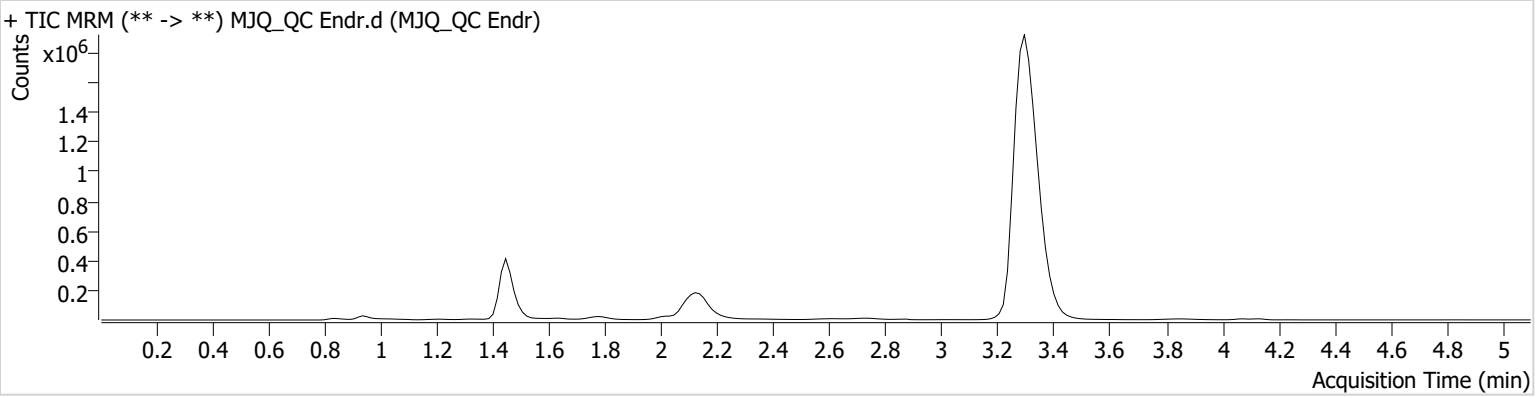
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	80692	∞	10.1	∞	874398	4.1713	ng/ml
THC-COOH	1.474	48095	∞	67.3	240.71	170390	14.0933	ng/ml
THC	3.300	440085	∞	27.3	∞	9150353	5.0349	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

Instrument	Falco (069901)	**Data File**	MJQ_QC Endr.d
Type	Sample	**Sample**	MJQ_QC Endr
Acq. Method	AM 27 THCQ.m	**Operator**	Amber Gerheart
Sample Position	P5-H1	**Comment**	
Injection Volume	10		
Acq. Date-Time	10/5/2021 11:25:26 AM		
Sample Info.			

Sample Chromatogram

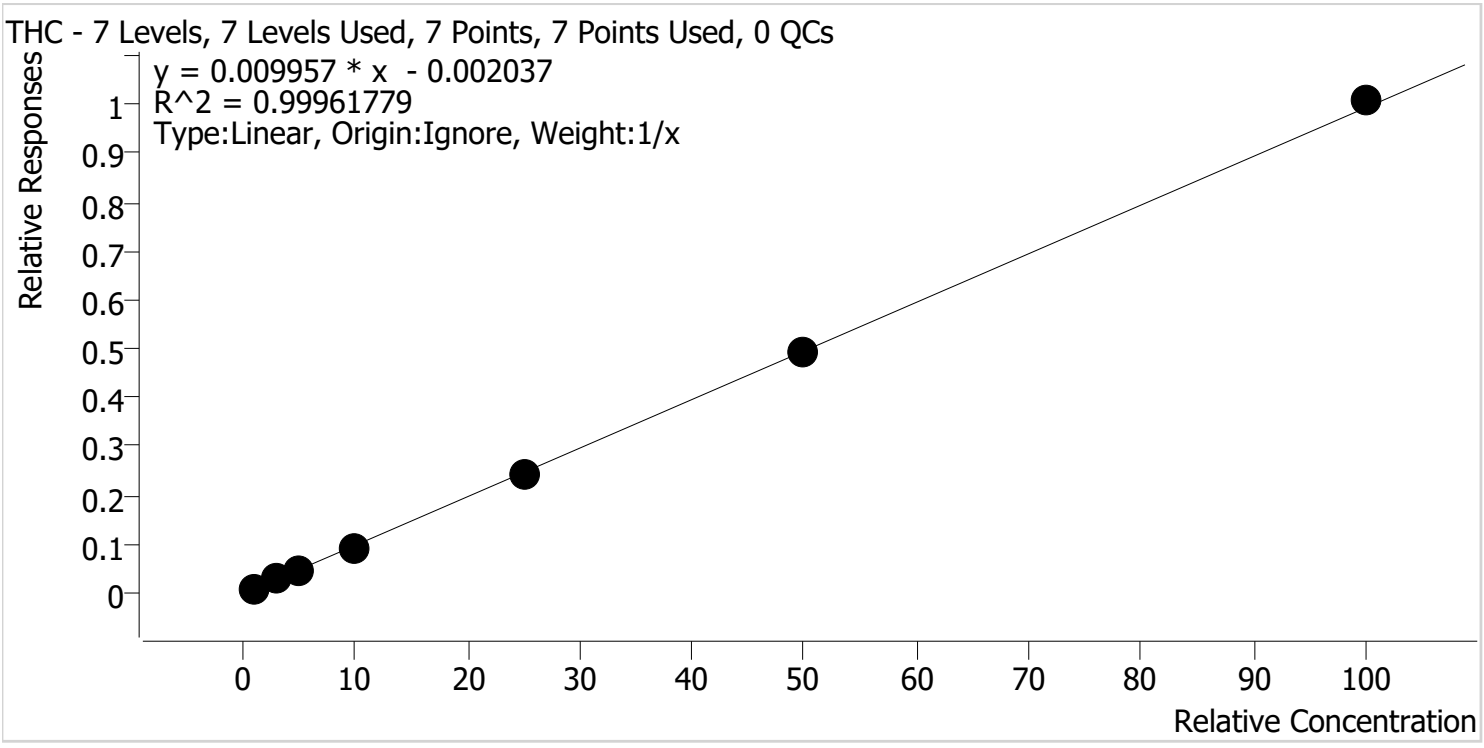


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	99172	∞	9.3	∞	1039626	4.4268	ng/ml
THC-COOH	1.474	53273	∞	65.3	878.08	193015	13.8005	ng/ml
THC	3.315	538035	∞	27.1	366.08	10800542	5.2077	ng/ml

Reinjected after samples were reinjected

AM #27 Cannabinoids Quant. Calibration Curve Report

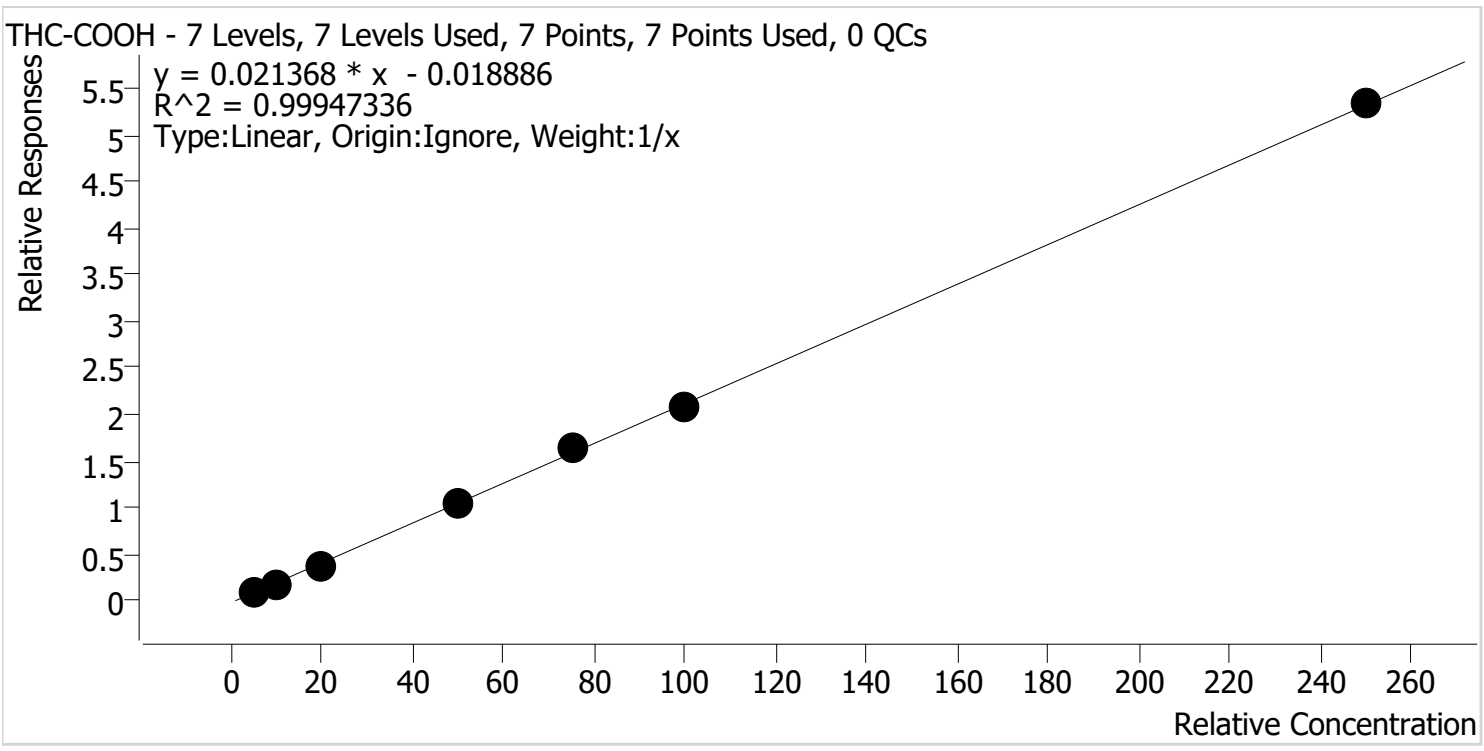
Batch results	D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update	10/7/2021 1:16 PM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	1.0	1.1	109.4
MJQ_Cal 2	2	✔	3.0	3.0	98.7
MJQ_Cal 3	3	✔	5.0	4.8	96.7
MJQ_Cal 4	4	✔	10.0	9.7	97.1
MJQ_Cal 5	5	✔	25.0	24.4	97.5
MJQ_Cal 6	6	✔	50.0	49.6	99.2
MJQ_Cal 7	7	✔	100.0	101.4	101.4

AM #27 Cannabinoids Quant. Calibration Curve Report

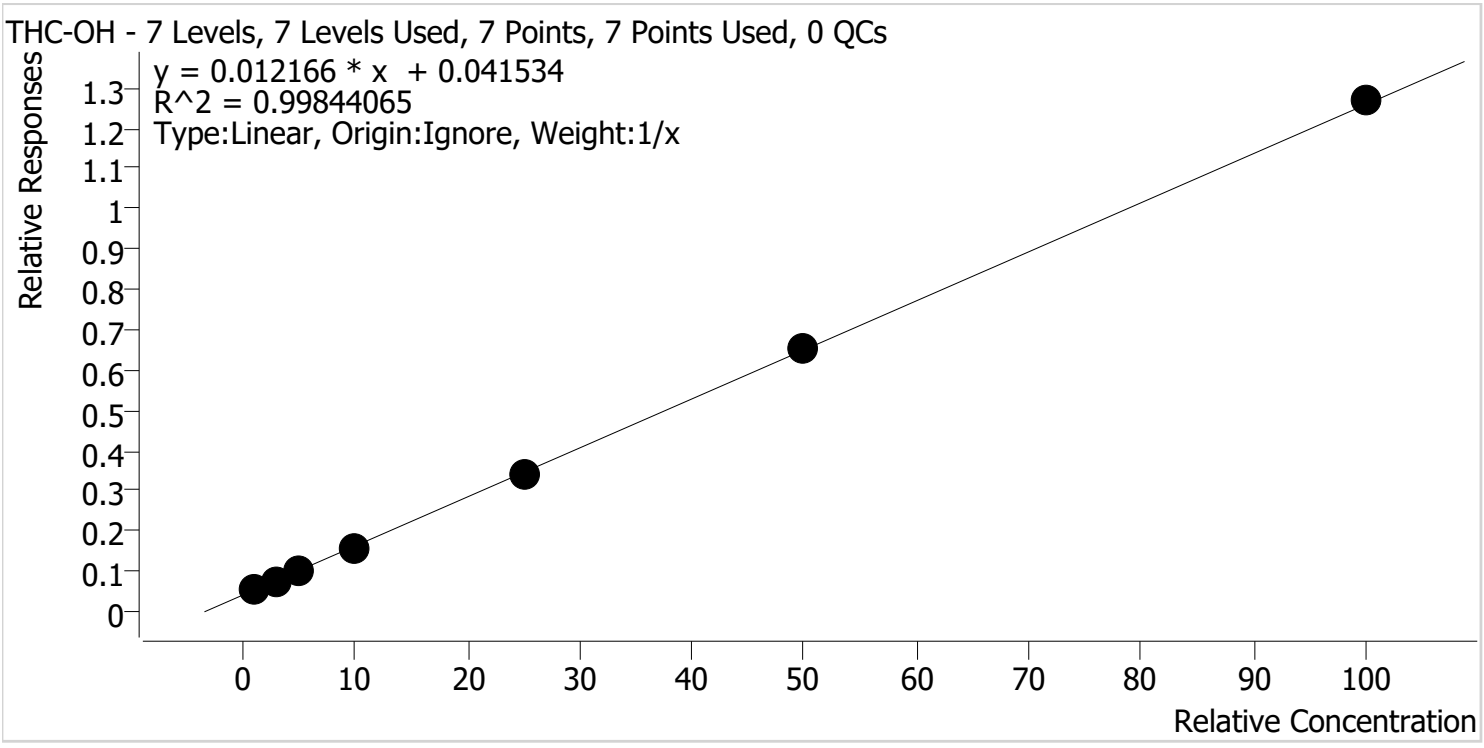
Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 10/7/2021 1:16 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	5.0	5.4	108.6
MJQ_Cal 2	2	✔	10.0	9.7	96.6
MJQ_Cal 3	3	✔	20.0	18.8	94.1
MJQ_Cal 4	4	✔	50.0	49.4	98.7
MJQ_Cal 5	5	✔	75.0	77.1	102.9
MJQ_Cal 6	6	✔	100.0	98.9	98.9
MJQ_Cal 7	7	✔	250.0	250.7	100.3

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update	10/7/2021 1:16 PM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	1.0	1.3	129.6
MJQ_Cal 2	2	✔	3.0	2.4	80.2
MJQ_Cal 3	3	✔	5.0	4.7	93.6
MJQ_Cal 4	4	✔	10.0	9.6	95.8
MJQ_Cal 5	5	✔	25.0	24.7	98.9
MJQ_Cal 6	6	✔	50.0	50.5	101.1
MJQ_Cal 7	7	✔	100.0	100.8	100.8

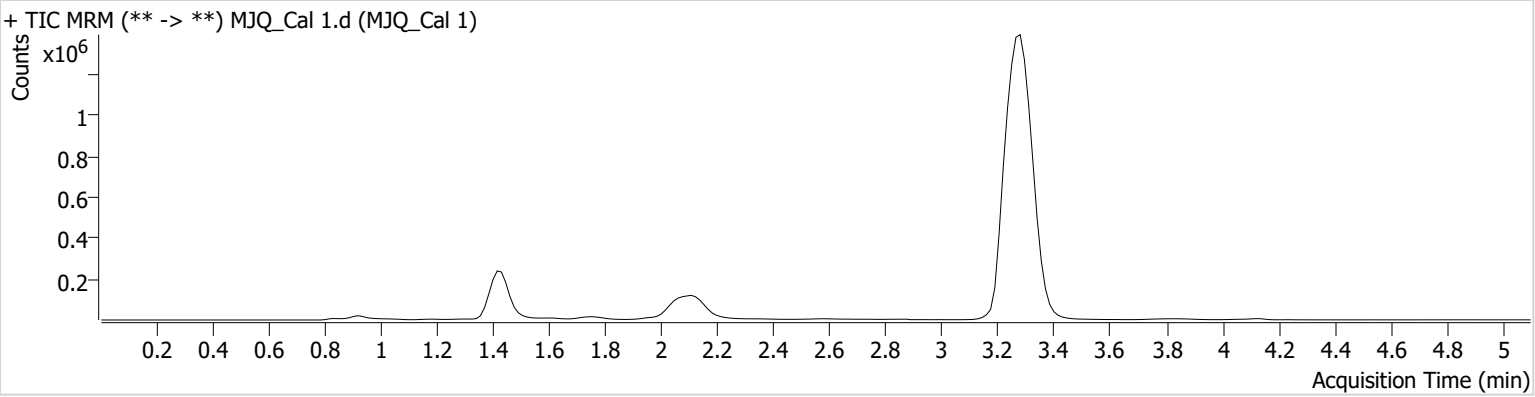
Compound not evaluated due to ratios being out.

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 2:02:35 AM		
Sample Info.			

Sample Chromatogram



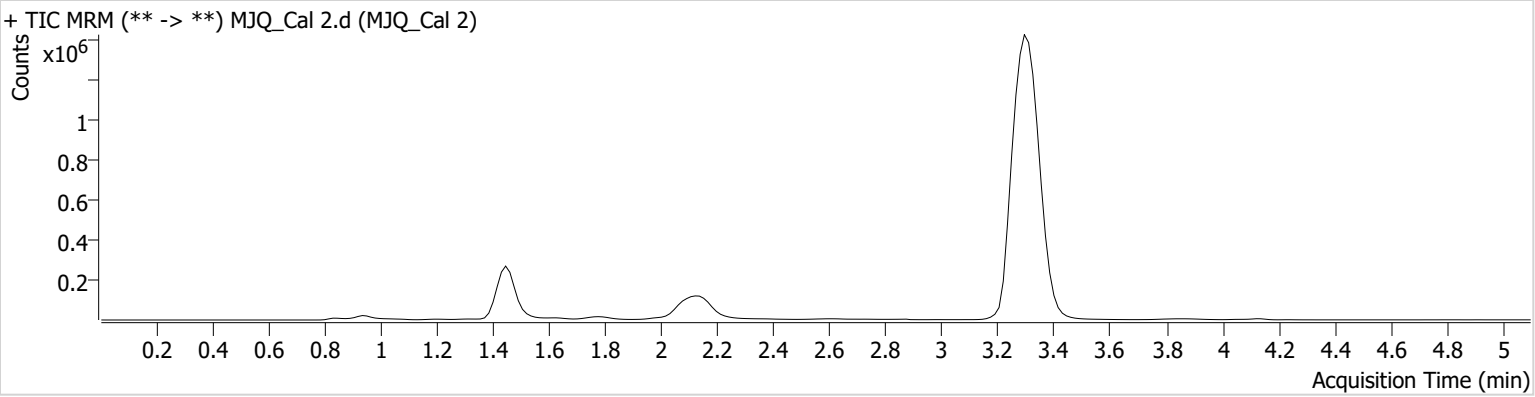
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.483	50563	∞	4.8 **Low**	7.20 **Low**	882339	1.2963	ng/ml **Low**
THC-COOH	1.444	17612	∞	59.2	157.05	181266	5.4310	ng/ml
THC	3.285	83872	∞	31.1	79.03	9465756	1.0945	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 2:10:21 AM		
Sample Info.			

Sample Chromatogram



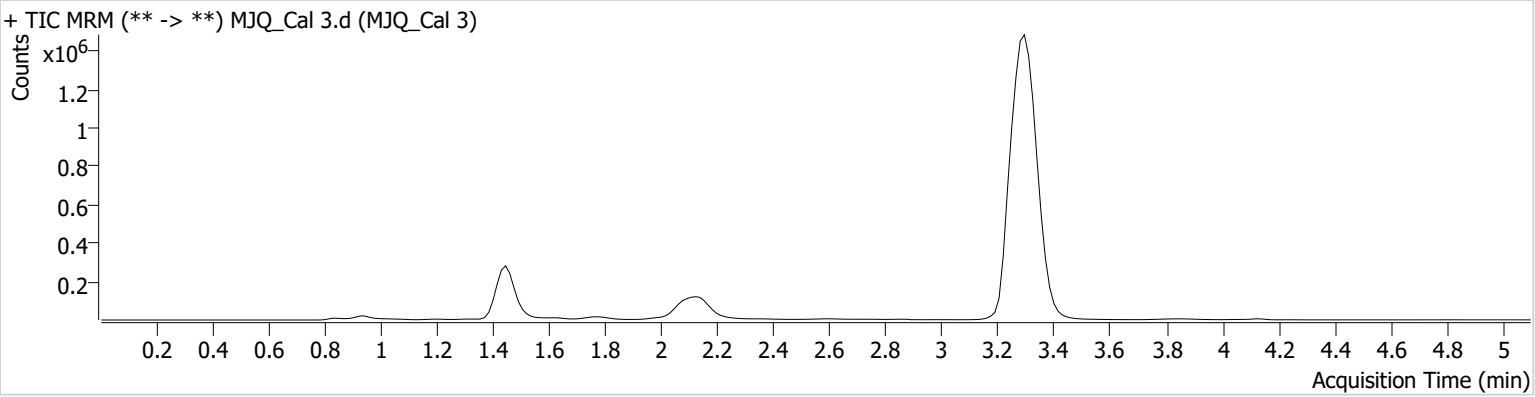
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.483	64089	∞	7.9 **Low**	75.22	905199	2.4055	ng/ml **Low**
THC-COOH	1.474	33791	∞	67.5	311.48	180254	9.6570	ng/ml
THC	3.315	254737	∞	28.5	∞	9284308	2.9602	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 2:17:57 AM		
Sample Info.			

Sample Chromatogram



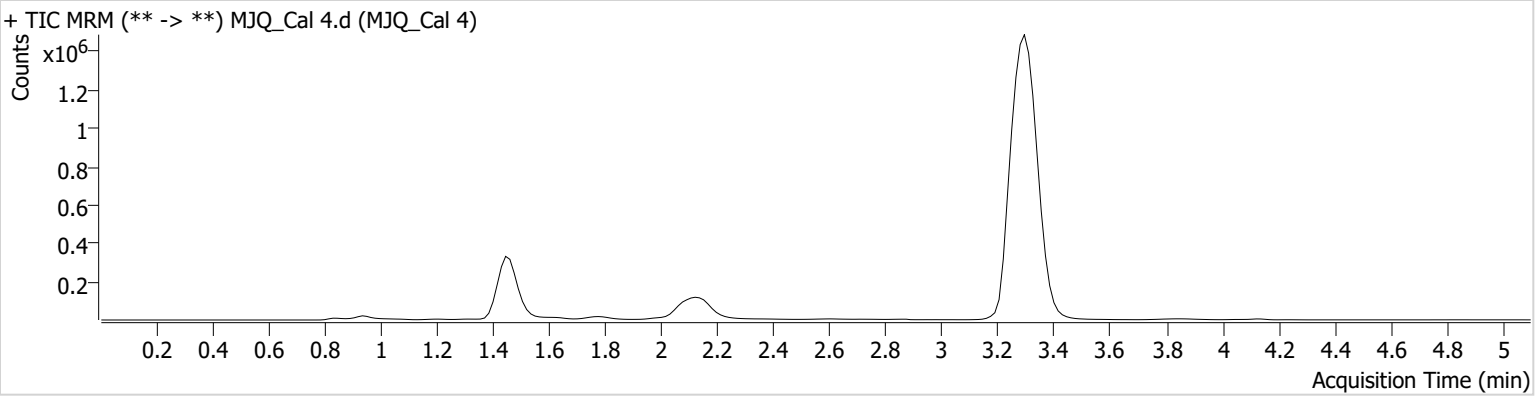
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	87036	∞	9.1	95.26	883678	4.6817	ng/ml
THC-COOH	1.474	68687	∞	66.0	1021.02	179242	18.8177	ng/ml
THC	3.315	429687	3092.03	26.0	690.08	9320106	4.8349	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 2:25:32 AM		
Sample Info.			

Sample Chromatogram



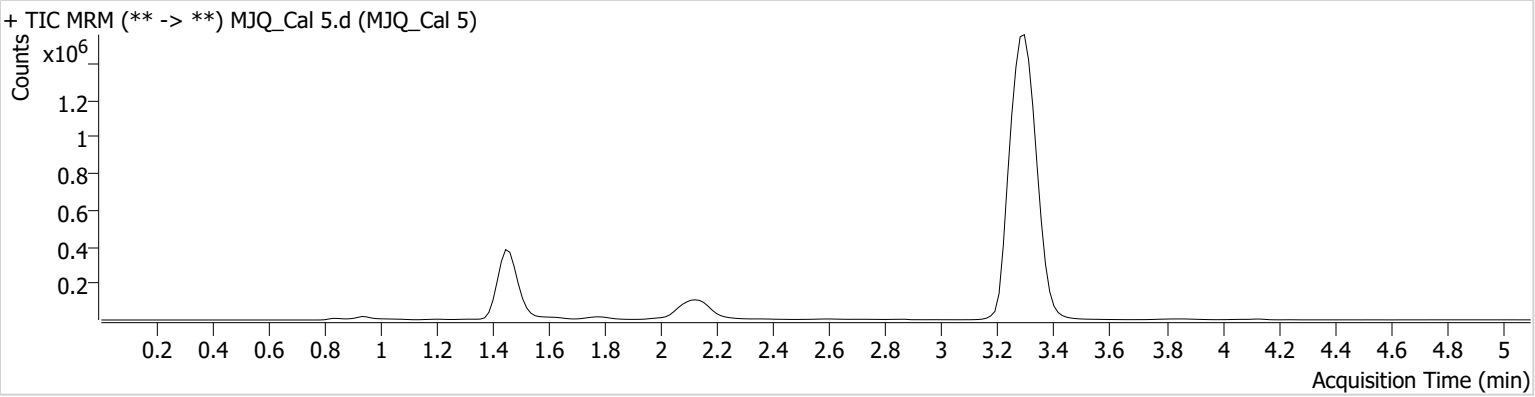
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	142451	∞	11.0	334.95	900977	9.5816	ng/ml
THC-COOH	1.474	190148	∞	66.8	1860.83	183583	49.3562	ng/ml
THC	3.315	839173	5186.24	26.2	∞	8869829	9.7066	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 2:33:07 AM		
Sample Info.			

Sample Chromatogram



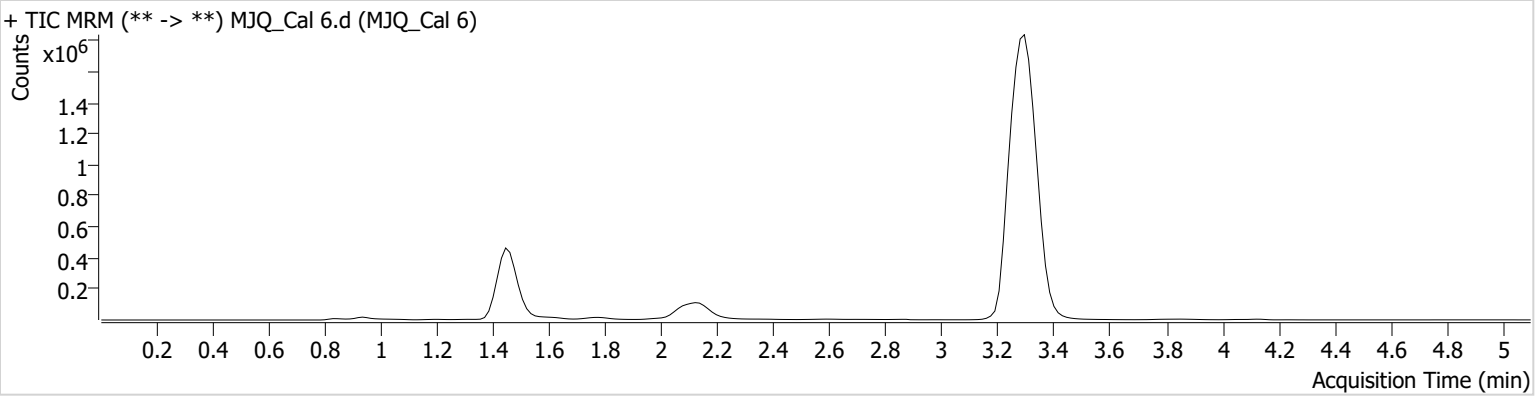
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	292159	∞	12.6 **High**	1161.97	853389	24.7255	ng/ml
THC-COOH	1.474	282710	1815.57	65.5	784.89	173489	77.1451	ng/ml
THC	3.300	1920663	∞	25.9	9025.54	7981756	24.3721	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 2:40:43 AM		
Sample Info.			

Sample Chromatogram



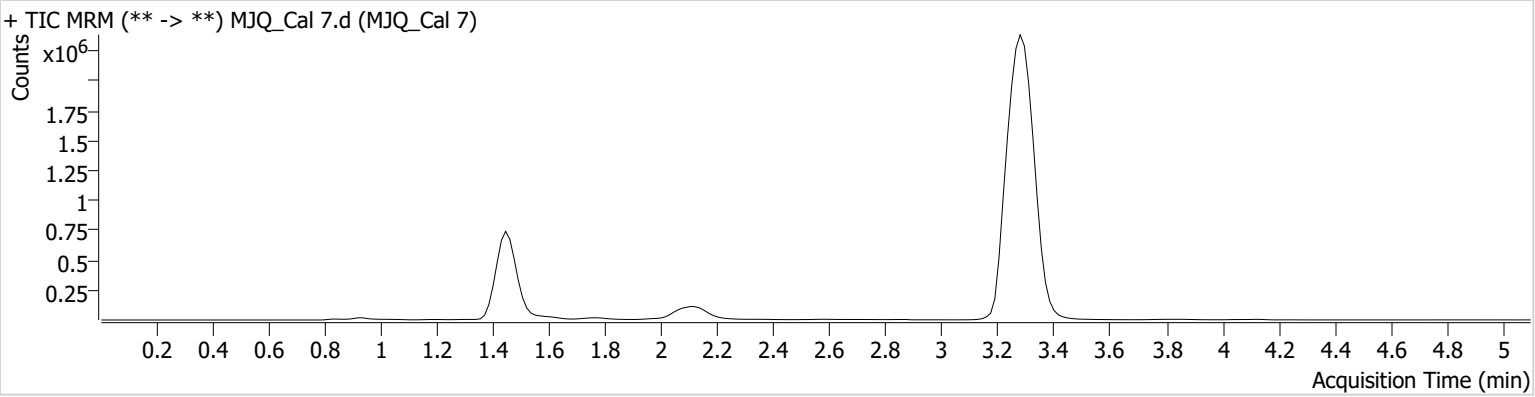
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	541429	∞	13.3 **High**	1550.13	824980	50.5298	ng/ml
THC-COOH	1.474	347628	760.34	72.0	2775.53	166055	98.8548	ng/ml
THC	3.300	3743532	∞	26.1	∞	7612212	49.5958	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100421 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 1:16:45 PM

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	10/5/2021 2:48:19 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.438	1000043	∞	13.9 **High**	∞	788896	100.7797	ng/ml
THC-COOH	1.459	832042	3459.66	69.5	∞	155845	250.7383	ng/ml
THC	3.300	7183187	∞	26.0	∞	7126568	101.4360	ng/ml