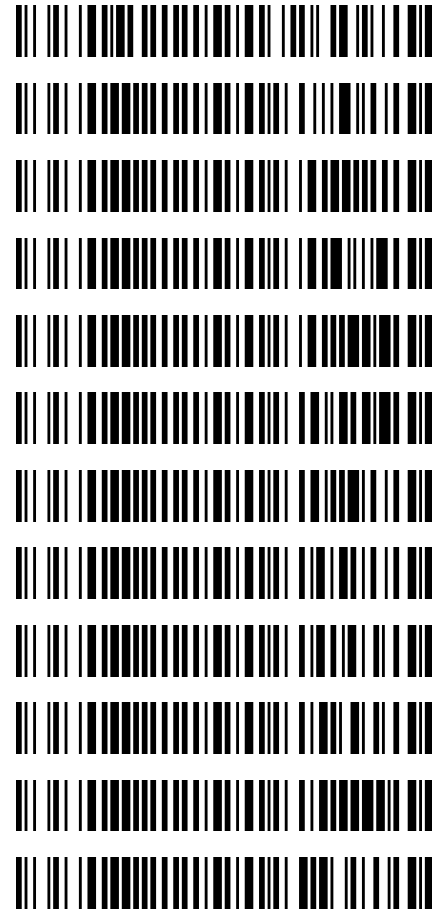


REVIEWED
By Sarah Collins at 3:14 pm, Aug 06, 2021

8/2/2021

Worklist: 5147

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-3036	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2340	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2451	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2457	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2458	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2459	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2471	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2472	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2473	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2478	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2479	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2528	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: 8/03/2021

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: 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: *Curve Range: THC 3-100 ng/mL*
THC-OH not evaluated

AA

During the injection of Cal 2 the needle clogged. The clog was fixed and Cal 2 was reinjected with no further issues. Cal 2 reinject data used.

AA

	1	2	3	4	5	6
A	IS + Cal. 1	Blood Negative	P2021-2472-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	M2021-3036-1	P2021-2473-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	P2021-2340-2	P2021-2478-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	P2021-2451-1	P2021-2479-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	P2021-2457-1	P2021-2528-1	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	P2021-2458-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	P2021-2459-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2021-2471-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

**Idaho State Police
Forensic Services
Toxicology Discipline**

Request for Departure from an Analytical Method

Date of Request

02/24/2021

Forensic Scientist

Anne Nord

Analytical Method

Toxicology AM #27: Quantitative Analysis of THC and Metabolites in Blood and Urine by LCMS-QQQ

Request

The method currently reads:

4.3.2.5 If any points are dropped from the approved quantitative range of the curve, the compound will be reported qualitatively. For calibrators and controls 10 ng and below, the accuracy must be within 30%, for calibrators and controls greater than 10 ng/mL the accuracy must be within 20%. If a control falls outside the accuracy range, at the analyst's discretion, the compound may be reported qualitatively.

I would like to add in the following exception:

If the 1ng/ml point is dropped for THC. If the 1 ng/ml point is dropped the quantitative range will be 3-50 ng/ml.

Discipline Leader Review

Departure approved

Comments: This deviation is approved and will remain in effect until it is changed in the actual method.

Departure Not Approved

Comments:

Celena Shrum

Toxicology Discipline Lead

Date: 02/24/2021

AG

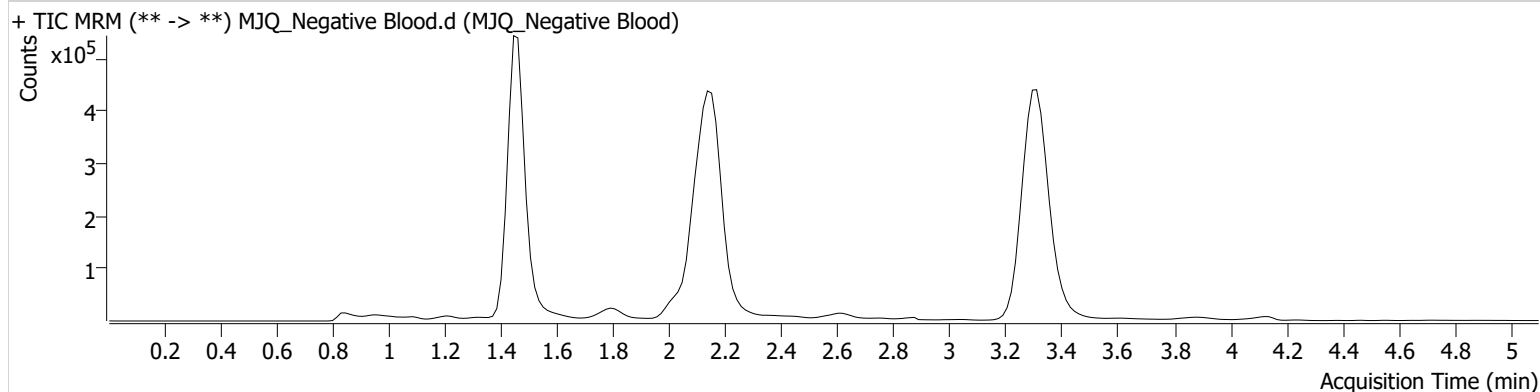


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/4/2021 8:23:51 AM		
Sample Info.			

Sample Chromatogram



AG

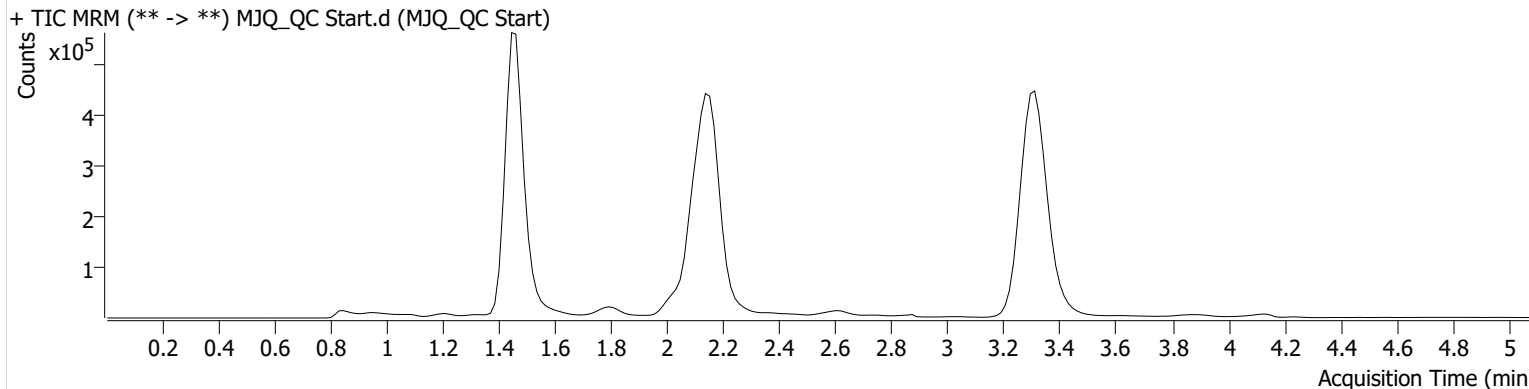


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/4/2021 8:08:39 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	191670	∞	7.6 Low	∞	1850696	5.2377 ng/ml
THC-COOH	1.489	121846	∞	52.1	∞	364639	15.6908 ng/ml
THC	3.315	134815	656.24	32.0	∞	2886233	5.0655 ng/ml

AG

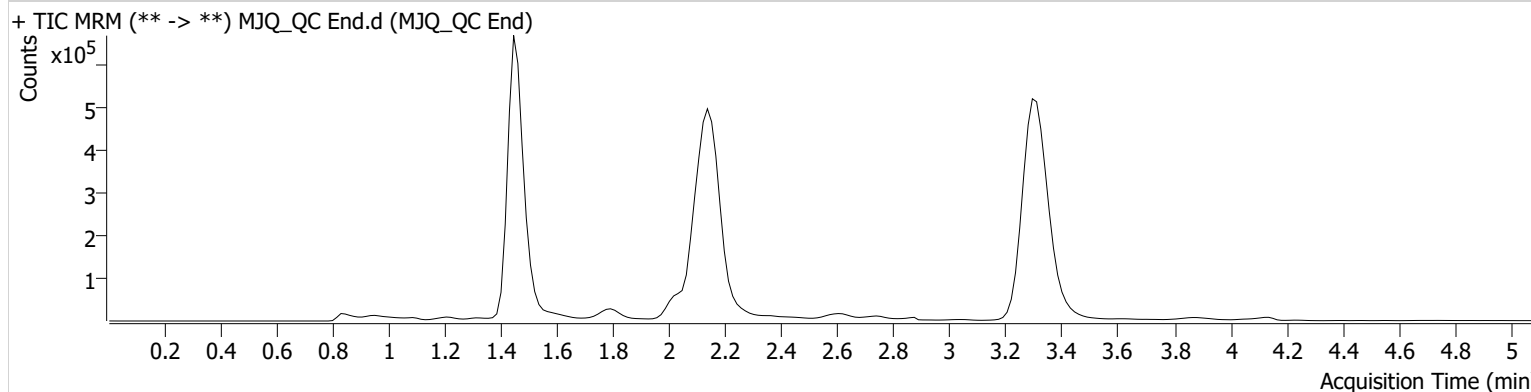


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/4/2021 11:41:45 AM		

Sample Chromatogram



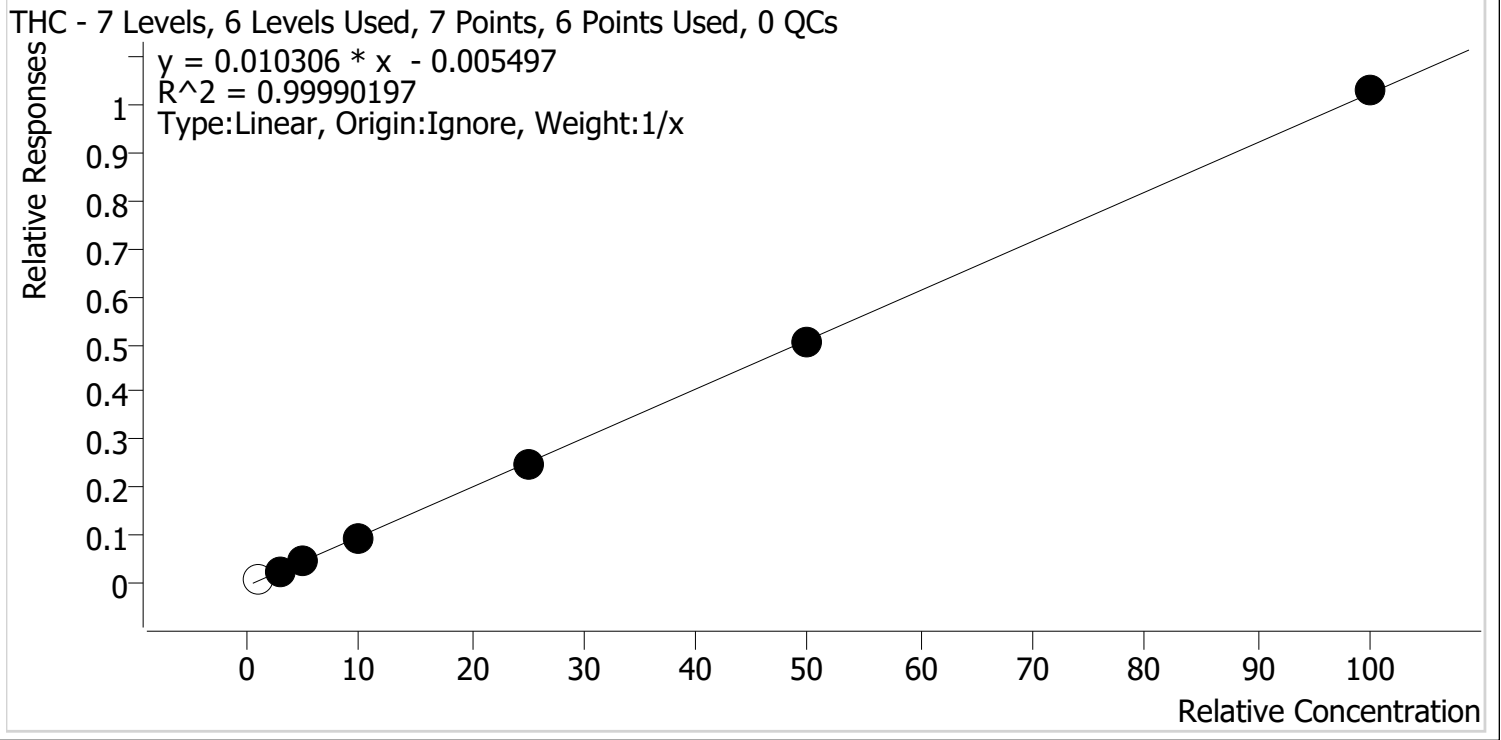
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	194150	∞	7.4 Low	∞	1919871	5.0679 ng/ml
THC-COOH	1.489	108690	∞	54.6	712.91	362554	14.1240 ng/ml
THC	3.315	151436	∞	32.1	243.09	3215490	5.1030 ng/ml

AA



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 8/5/2021 7:40 AM
Analyst Name ISP\lagerheart
Analyte THC **Internal Standard** **THC-D3**



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	x	1.0	1.5	146.2
MJQ_Cal 2r	2	✓	3.0	3.0	100.4
MJQ_Cal 3	3	✓	5.0	5.1	101.9
MJQ_Cal 4	4	✓	10.0	9.8	98.0
MJQ_Cal 5	5	✓	25.0	25.0	100.1
MJQ_Cal 6	6	✓	50.0	49.5	99.0
MJQ_Cal 7	7	✓	100.0	100.6	100.6

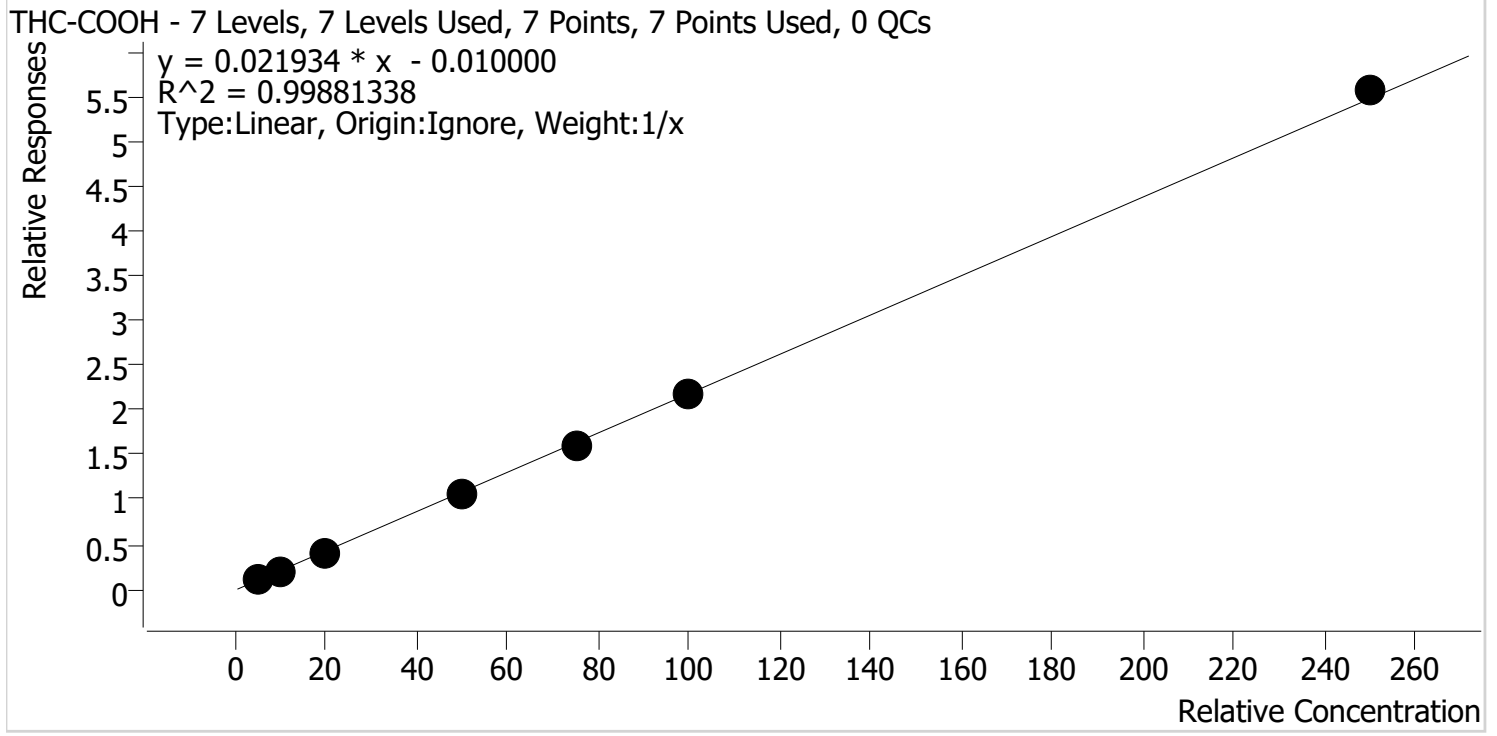
*Cal 1 dropped due to ratio being out

AA



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 8/5/2021 7:40 AM
Analyst Name ISP\lagerheart
Analyte THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	5.0	5.8	115.9
MJQ_Cal 2r	2	✓	10.0	9.7	96.5
MJQ_Cal 3	3	✓	20.0	18.1	90.6
MJQ_Cal 4	4	✓	50.0	48.6	97.2
MJQ_Cal 5	5	✓	75.0	73.3	97.8
MJQ_Cal 6	6	✓	100.0	100.4	100.4
MJQ_Cal 7	7	✓	250.0	254.1	101.6

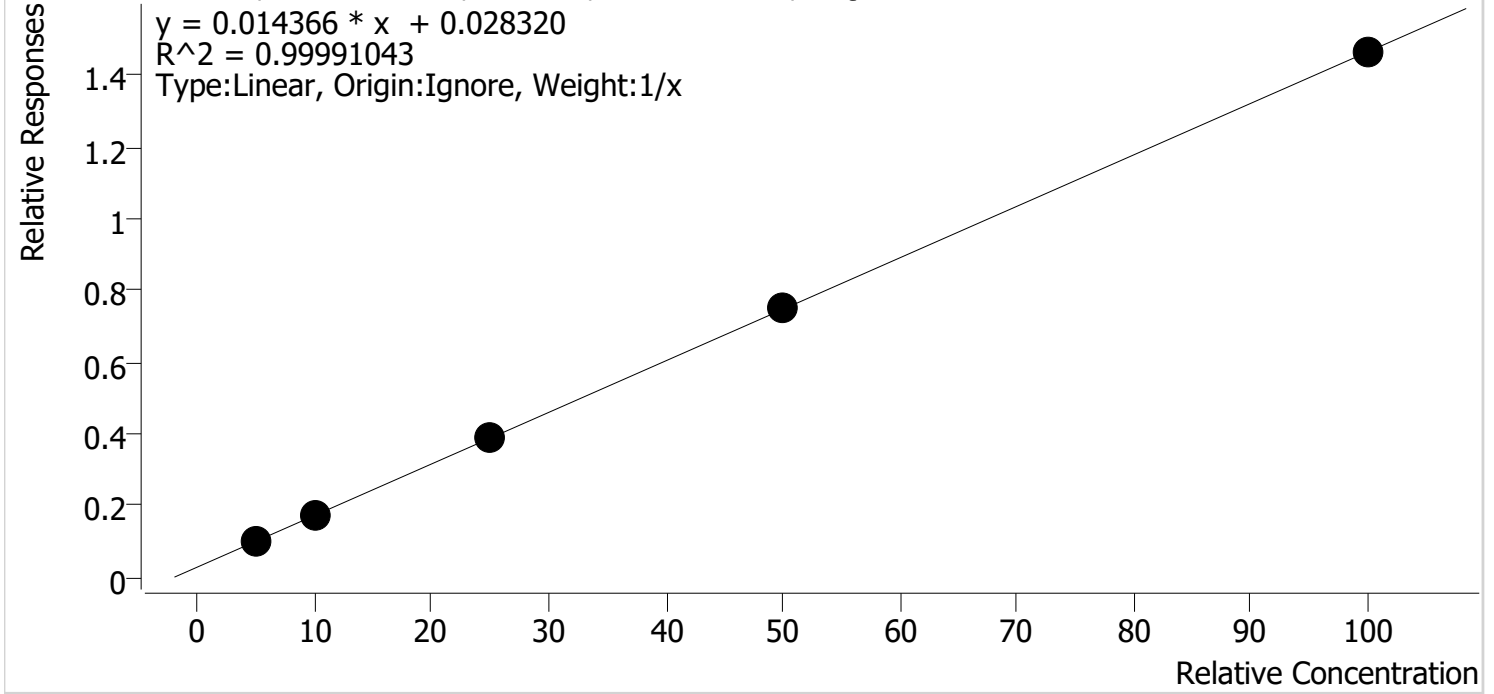
AA



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 8/5/2021 7:40 AM
Analyst Name ISP\lagerheart
Analyte THC-OH **Internal Standard** THC-OH-D3

THC-OH - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 3	3	✓	5.0	5.1	102.0
MJQ_Cal 4	4	✓	10.0	9.8	97.7
MJQ_Cal 5	5	✓	25.0	24.9	99.7
MJQ_Cal 6	6	✓	50.0	50.4	100.8
MJQ_Cal 7	7	✓	100.0	99.8	99.8

Compound not evaluated due to interfering peak in some calibrators and QCs.

AG

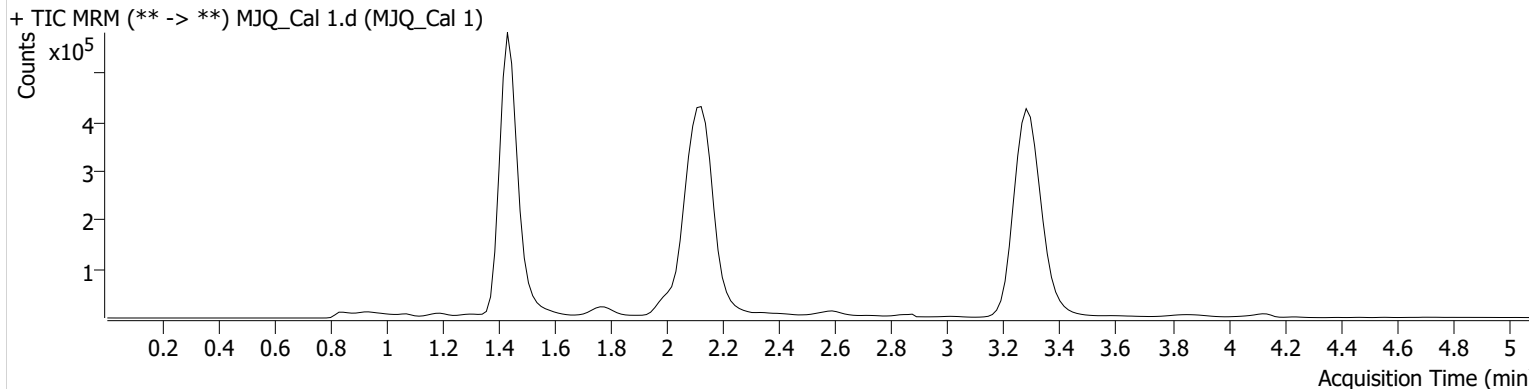


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/3/2021 11:37:41 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	46967	∞	48.5	84.81	401230	5.7929 ng/ml
THC	3.300	27800	47.46	34.6 High	∞	2904288	1.4621 ng/ml

AG

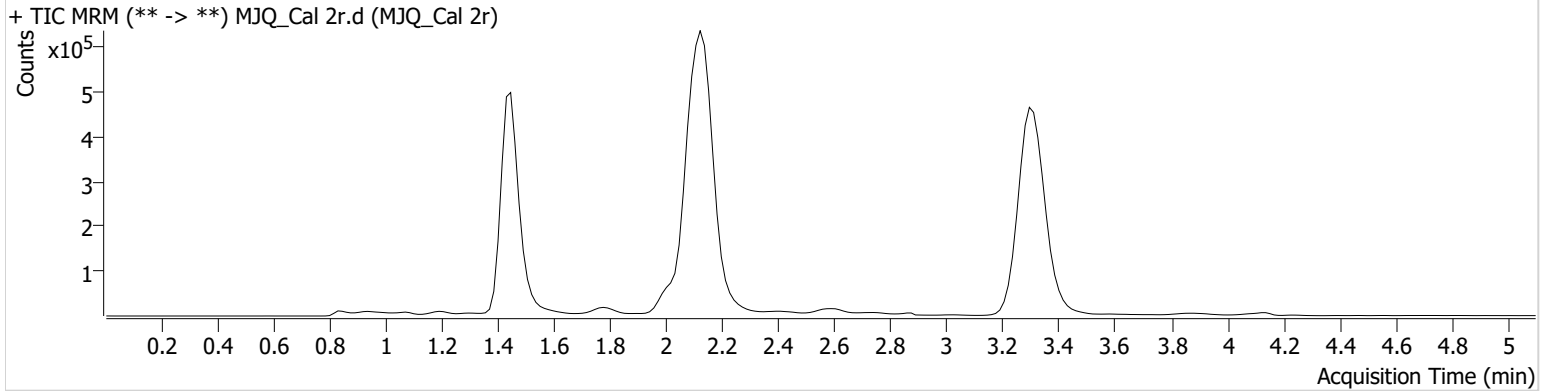


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 2r.d
Type	Cal	Sample	MJQ_Cal 2r
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P5-B1	Comment	
Injection Volume	10		
Acq. Date-Time	8/4/2021 3:14:12 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	70310	∞	51.6	254.60	348620	9.6510 ng/ml
THC	3.315	76817	478.24	32.2	116.08	3005802	3.0130 ng/ml

During the injection of Cal 2 the needle clogged. The clog was fixed and Cal 2 was reinjected with no further issues. Cal 2 reinject data used.

AG

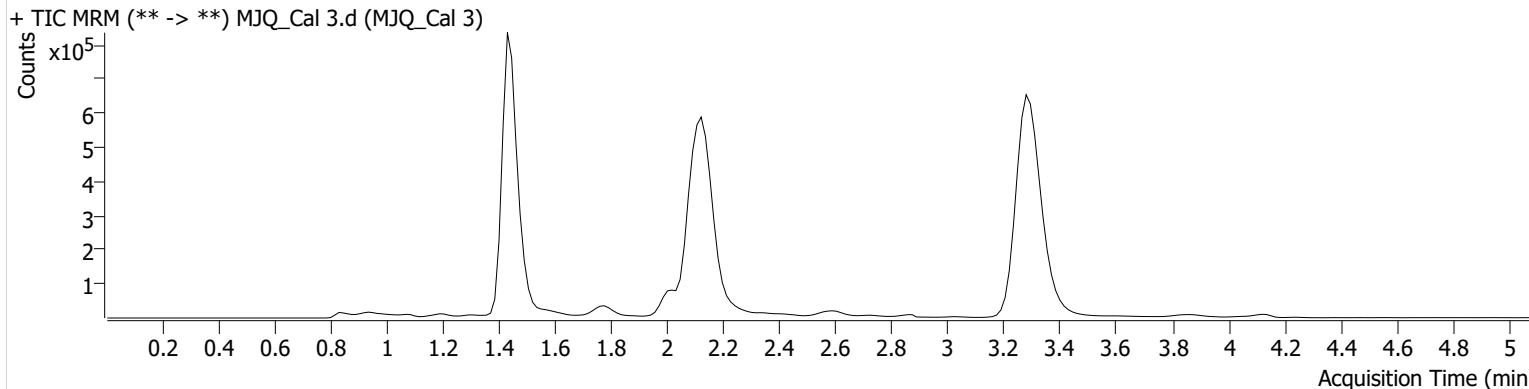


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/4/2021 7:22:55 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	230985	∞	7.7 Low	159.95	2273327	5.1013 ng/ml
THC-COOH	1.474	167366	∞	55.8	822.11	431914	18.1229 ng/ml
THC	3.300	184525	∞	31.7	∞	3924784	5.0952 ng/ml

AG

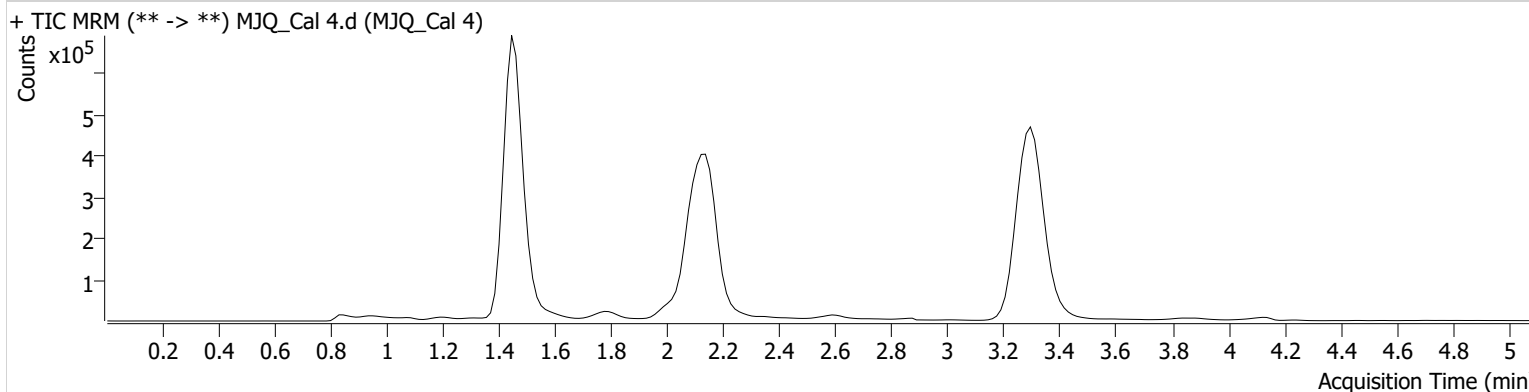


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/4/2021 7:30:41 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	324889	∞	9.1	∞	1925915	9.7709 ng/ml
THC-COOH	1.474	396942	∞	54.1	∞	375787	48.6148 ng/ml
THC	3.315	278913	∞	30.4	102.46	2921411	9.7968 ng/ml

AG

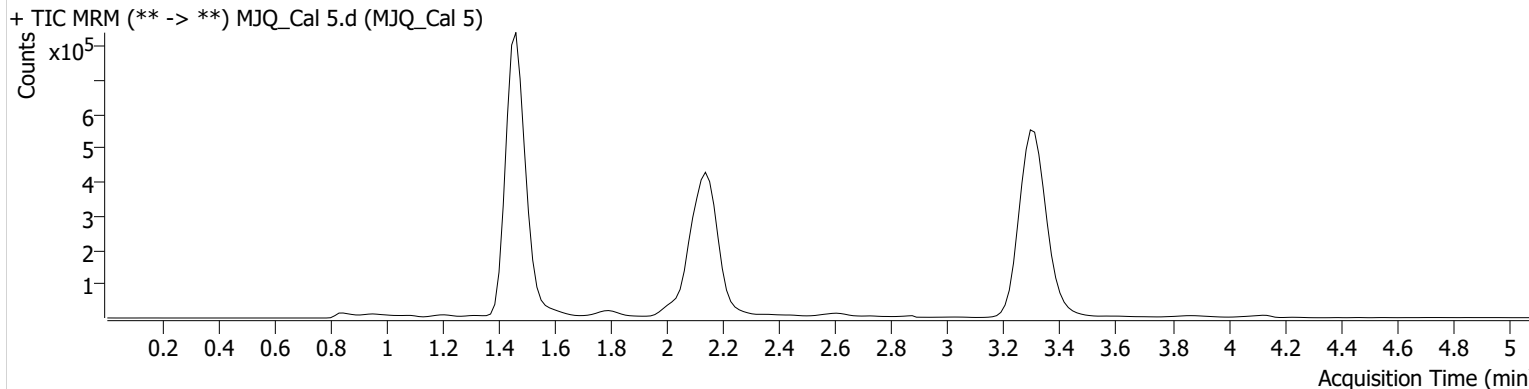


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/4/2021 7:38:17 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	731866	∞	10.2	401.20	1894721	24.9154 ng/ml
THC-COOH	1.489	593349	∞	55.4	∞	371273	73.3191 ng/ml
THC	3.315	729357	4139.88	25.9	678.42	2888942	25.0295 ng/ml

AG

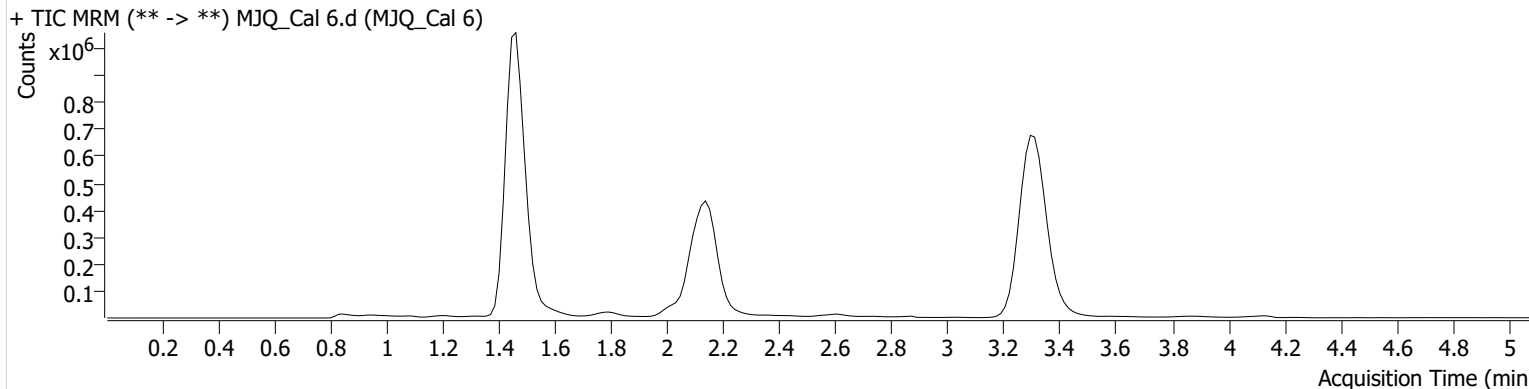


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/4/2021 7:45:52 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	1409925	∞	10.7	∞	1874240	50.3914 ng/ml
THC-COOH	1.489	794994	∞	55.7	∞	362721	100.3829 ng/ml
THC	3.315	1423006	∞	25.1	668.72	2819085	49.5106 ng/ml

AG

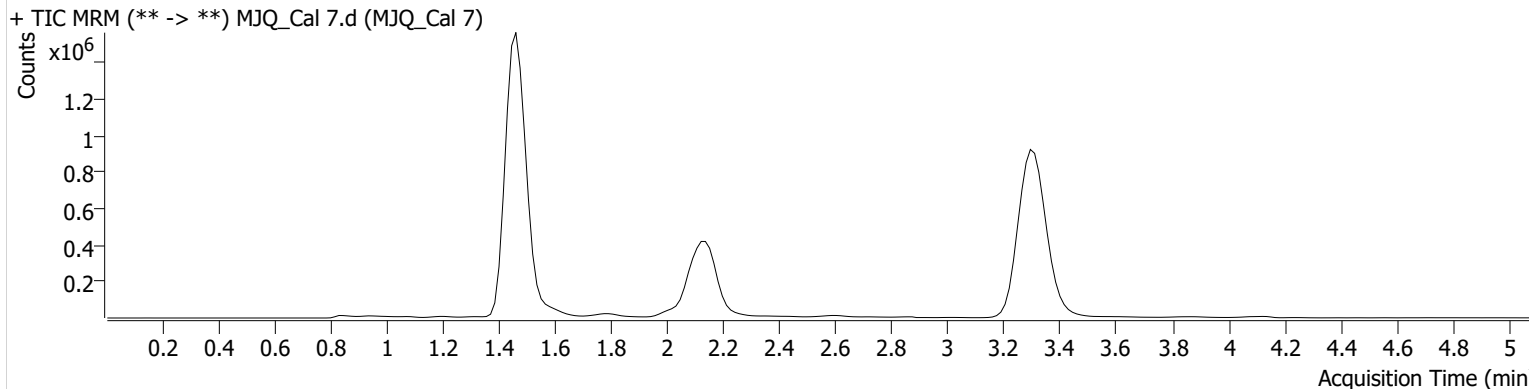


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080321 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/5/2021 7:40:57 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	8/4/2021 7:53:28 AM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	2534674	∞	11.1	2373.09	1733237	99.8210 ng/ml
THC-COOH	1.474	1814655	∞	56.3	∞	326161	254.1164 ng/ml
THC	3.315	2862884	∞	26.9	∞	2777188	100.5550 ng/ml