

Worklist: 5522

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
P2021-3893	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-3945	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4078	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4124	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4201	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4202	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4209	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4222	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4246	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4248	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4249	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2021-4250	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-0007	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2022-0013	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

TS

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

Extraction Date: 01/18/2022

Analyst: Tamara Salazar

Plate lot#: IDP-108-3-211018

Plate Re-test Date: 04-18-22

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 20L20725

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 069901

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. 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: 750uL
- 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 possible interfering peak.

TS

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	IS + Sample	P2022-0013-1	P2021-4202-1	IS + QC_1
B	IS + Cal. 2	IS + Sample	IS + Sample	P2022-0007-1	P2021-4201-1	IS + Cal. 7
C	IS + Cal. 3	IS + Sample	IS + Sample	P2021-4250-1	P2021-4124-1	IS + Cal. 6
D	IS + Cal. 4	IS + Sample	IS + Sample	P2021-4249-1	P2021-4078-1	IS + Cal. 5
E	IS + Cal. 5	IS + Sample	IS + Sample	P2021-4248-1	P2021-3945-1	IS + Cal. 4
F	IS + Cal. 6	IS + Sample	IS + Sample	P2021-4246-1	P2021-3893-1	IS + Cal. 3
G	IS + Cal. 7	IS + Sample	IS + Sample	P2021-4222-1	Neg Blood	IS + Cal. 2
H	IS + QC_1	IS + Sample	IS + Sample	P2021-4209-1	IS + QC_1	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

TS

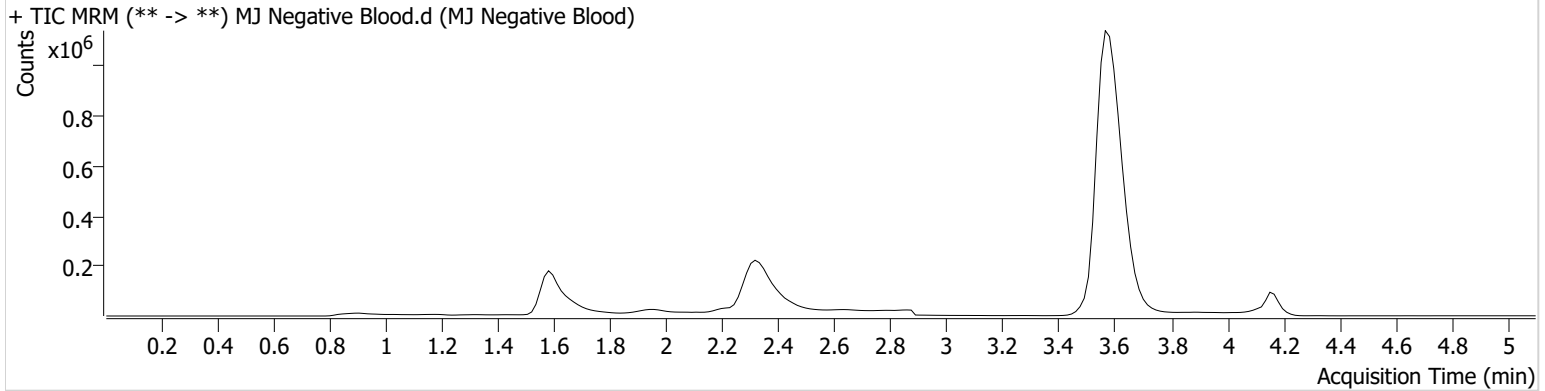


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G5	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 1:56:44 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663 High	123974	∞	2.9 Low	60.09	666275	0.4908 ng/ml Low

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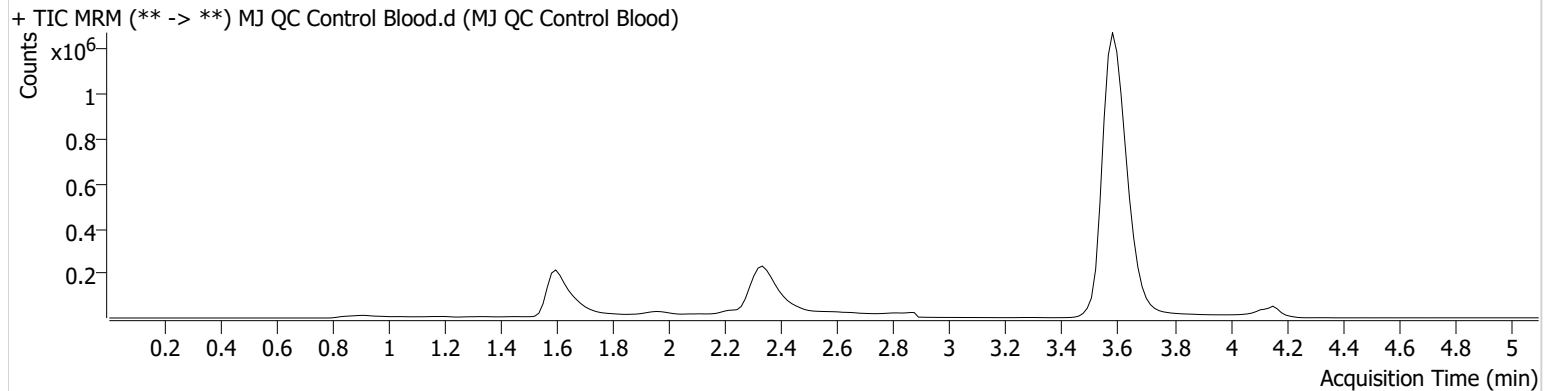


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 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	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 1:41:30 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.663 High	179552	∞	6.3 Low	74.93	666165	5.4238 ng/ml
THC-COOH	1.625	64939	∞	61.0	223.59	195657	15.8055 ng/ml
THC	3.601	302870	∞	25.9	799.05	7296095	4.6425 ng/ml

*Compound not evaluated.

TS

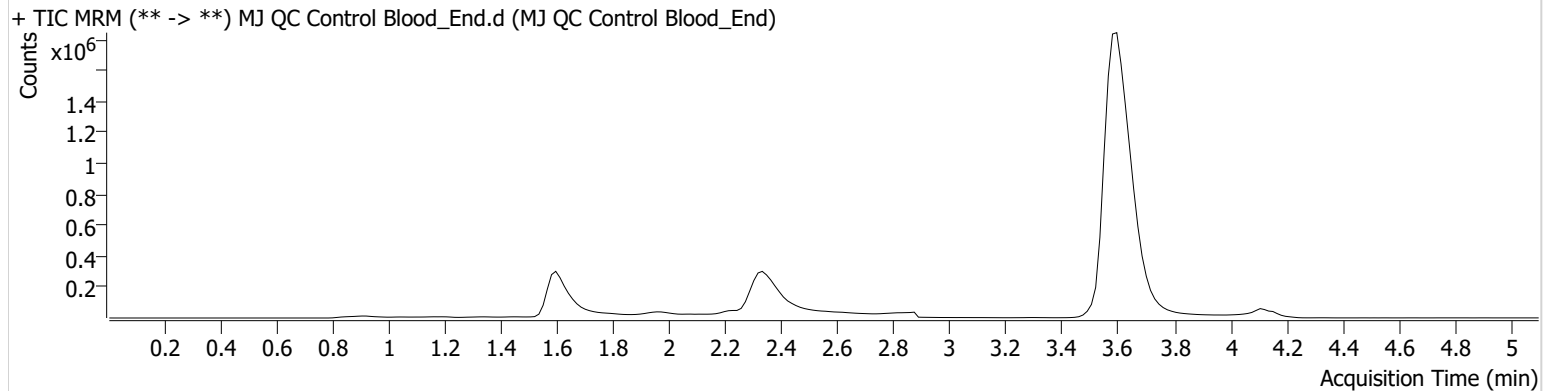


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ QC Control Blood_End.d
Type	QC	Sample	MJ QC Control Blood_End
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 5:45:15 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.663 High	211875	∞	6.7 Low	146.86	893503	3.5086 ng/ml
THC-COOH	1.625	77404	∞	72.9	776.25	254077	14.6070 ng/ml
THC	3.601	531558	2286.30	25.9	∞	11667087	5.0762 ng/ml

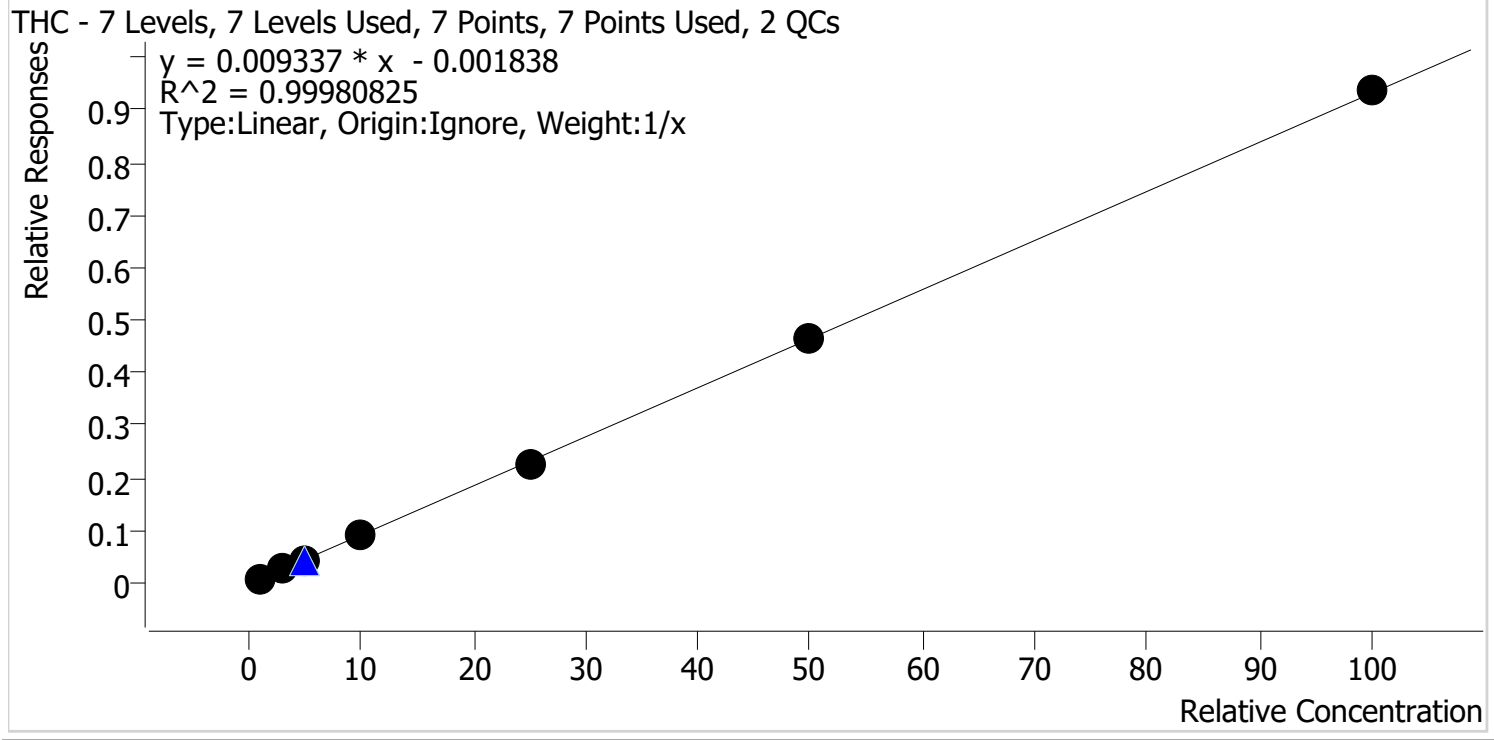
*Compound not evaluated.

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 1/24/2022 8:45 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



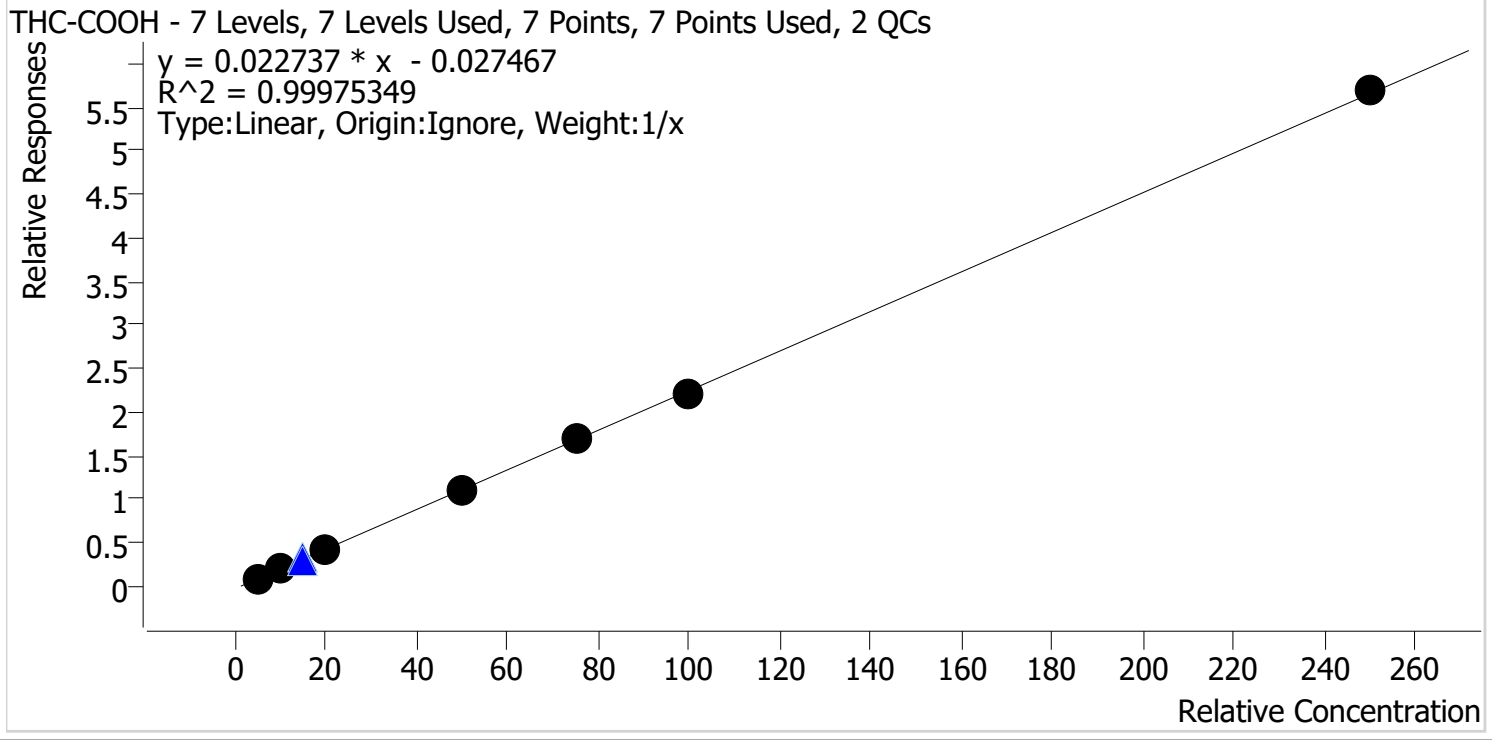
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.1	108.7
MJ Cal 2	2	✓	3.0	2.9	95.7
MJ Cal 3	3	✓	5.0	4.8	95.8
MJ Cal 4	4	✓	10.0	10.1	100.8
MJ Cal 5	5	✓	25.0	24.6	98.4
MJ Cal 6	6	✓	50.0	50.1	100.2
MJ Cal 7	7	✓	100.0	100.5	100.5

TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 1/24/2022 8:45 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	5.0	5.0	99.6
MJ Cal 2	2	✓	10.0	10.3	103.3
MJ Cal 3	3	✓	20.0	19.5	97.5
MJ Cal 4	4	✓	50.0	50.0	100.0
MJ Cal 5	5	✓	75.0	75.9	101.3
MJ Cal 6	6	✓	100.0	97.7	97.7
MJ Cal 7	7	✓	250.0	251.5	100.6

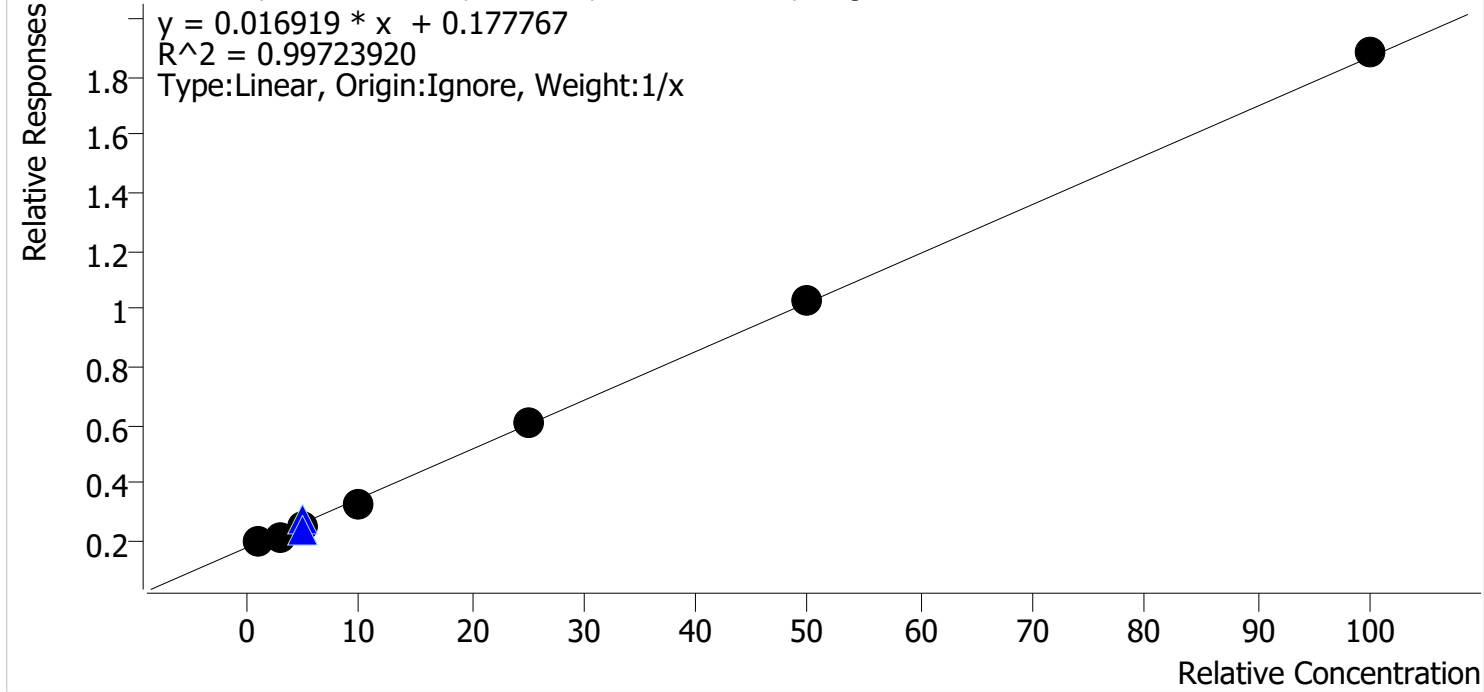
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
 Last Cal. Update 1/24/2022 8:45 AM
 Analyst Name ISP\datastor
 Analyte THC-OH Internal Standard THC-OH-D3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.4	139.2
MJ Cal 2	2	✓	3.0	2.3	77.3
MJ Cal 3	3	✓	5.0	4.4	89.0
MJ Cal 4	4	✓	10.0	9.1	91.5
MJ Cal 5	5	✓	25.0	25.3	101.2
MJ Cal 6	6	✓	50.0	50.5	101.0
MJ Cal 7	7	✓	100.0	100.9	100.9

*Compound not evaluated.

TS

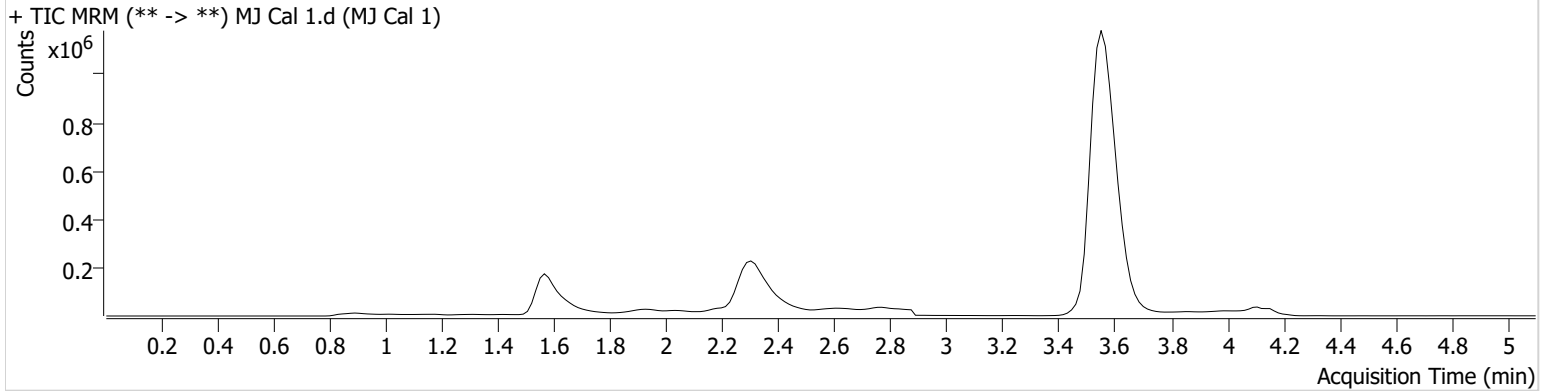


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ Cal 1.d
Type	Cal	Sample	MJ Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 12:48:07 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648 High	128404	∞	3.0 Low	12.25	637839	1.3916 ng/ml Low
THC-COOH	1.610	15654	∞	73.0	∞	182620	4.9780 ng/ml Low
THC	3.555	62876	292.75	28.6	238.10	7567644	1.0866 ng/ml

TS

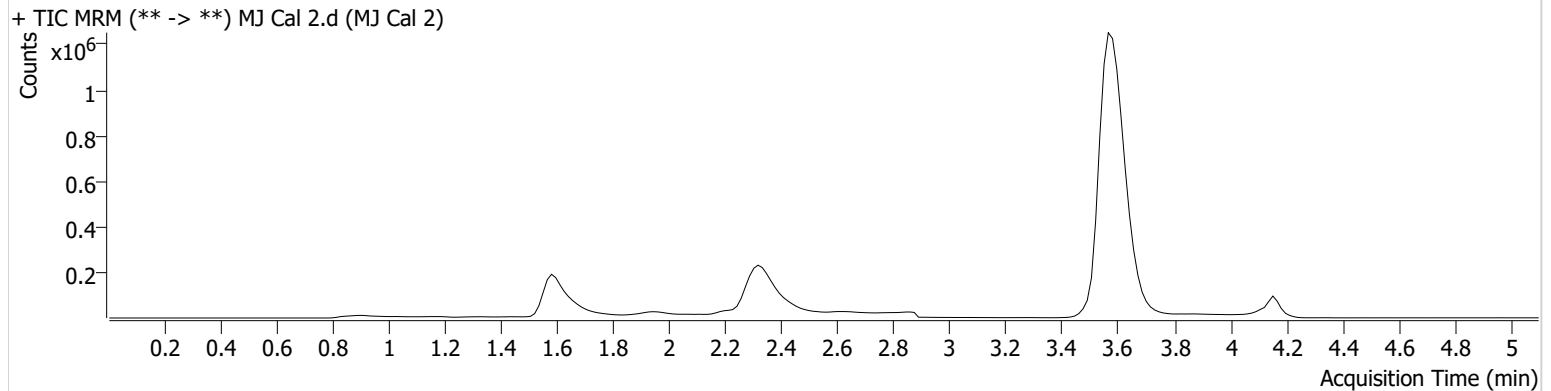


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ Cal 2.d
Type	Cal	Sample	MJ Cal 2
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 12:55:54 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663 High	143875	∞	4.7 Low	18.06	662940	2.3204 ng/ml Low
THC-COOH	1.625	41309	∞	59.9	322.86	199106	10.3331 ng/ml
THC	3.586	197307	782.79	25.8	93.28	7902380	2.8708 ng/ml

TS

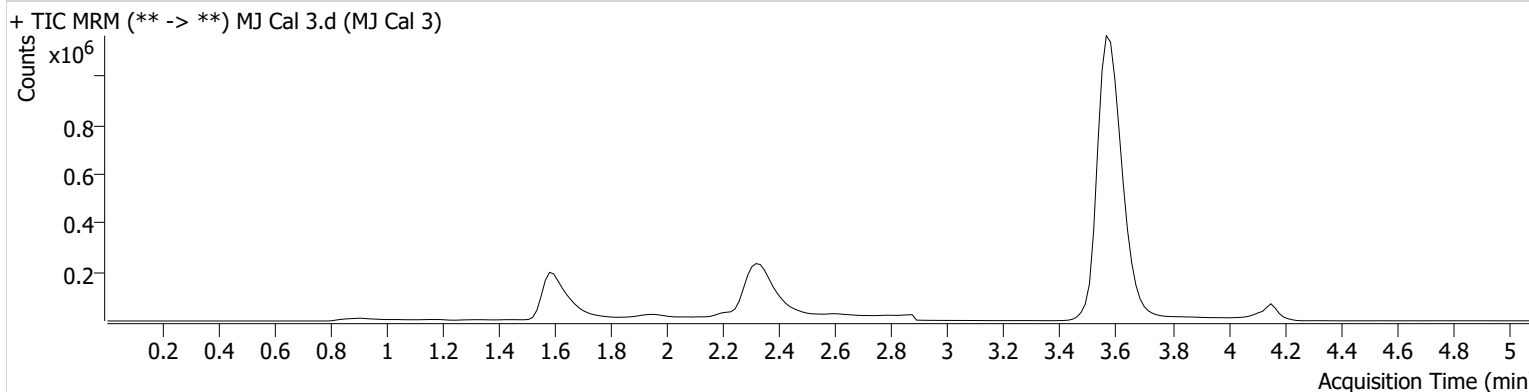


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ Cal 3.d
Type	Cal	Sample	MJ Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 1:03:31 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663 High	161459	∞	6.4 Low	∞	638135	4.4478 ng/ml
THC-COOH	1.625	79055	∞	71.2	∞	190069	19.5013 ng/ml
THC	3.586	288344	1503.88	26.1	263.05	6724199	4.7893 ng/ml

TS

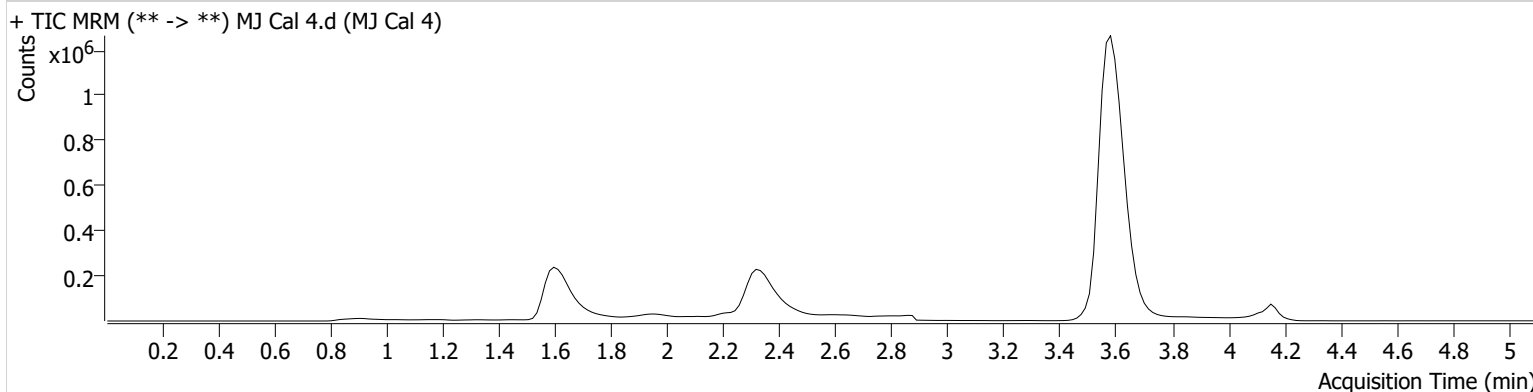


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ Cal 4.d
Type	Cal	Sample	MJ Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 1:11:07 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.663 High	216472	∞	8.5	∞	650979	9.1477 ng/ml
THC-COOH	1.625	210265	∞	62.3	1015.94	189524	50.0028 ng/ml
THC	3.586	656071	4574.38	24.5	138.70	7108517	10.0812 ng/ml

TS



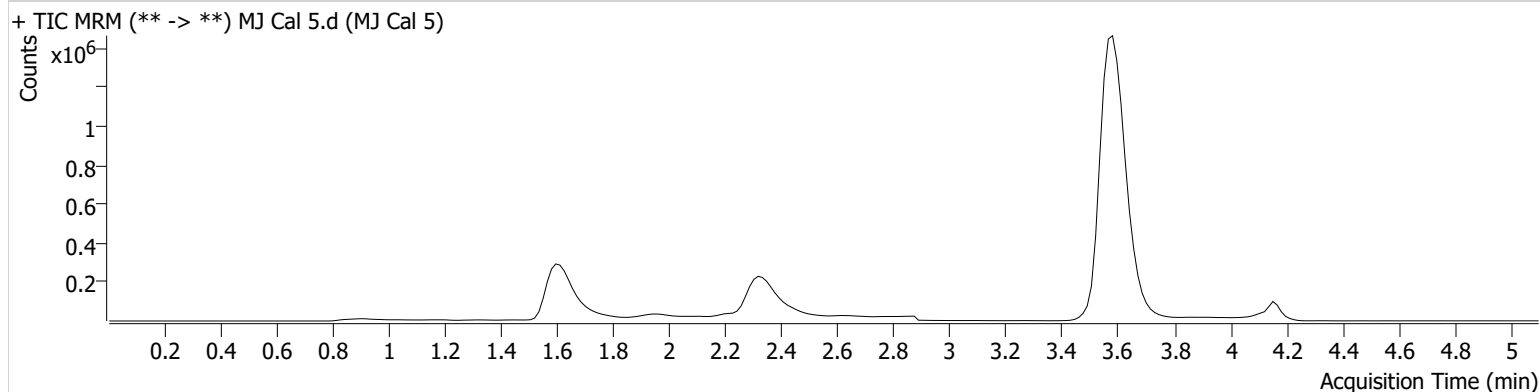
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ Cal 5.d
Type	Cal	Sample	MJ Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 1:18:43 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	394117	∞	10.3 High	∞	650647	25.2956 ng/ml
THC-COOH	1.625	317373	249.17	63.2	1495.23	186782	75.9395 ng/ml
THC	3.586	1678142	13534.46	25.2	1754.30	7365766	24.5967 ng/ml

TS

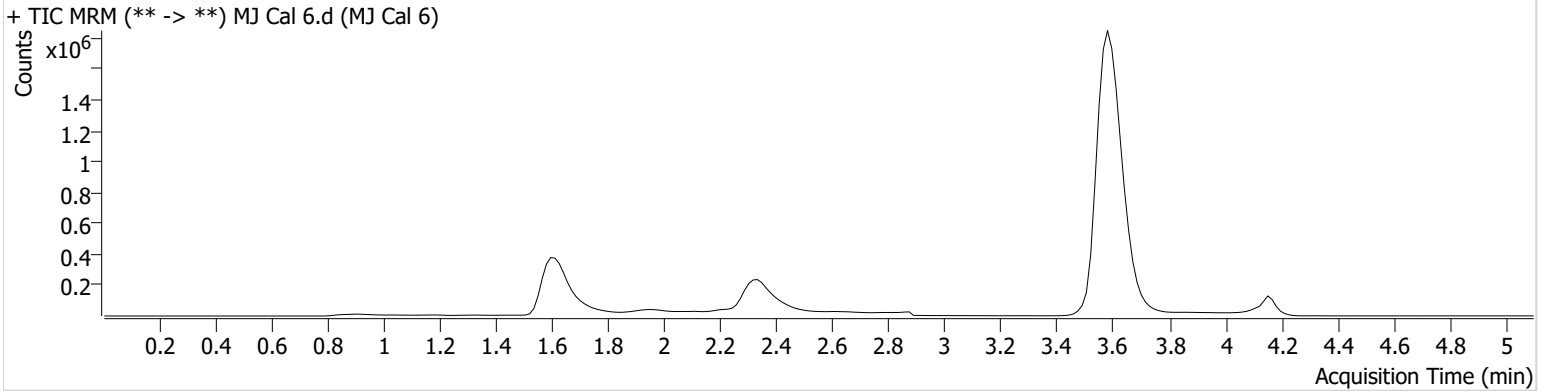


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ Cal 6.d
Type	Cal	Sample	MJ Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 1:26:19 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	684742	∞	12.2 High	∞	663629	50.4800 ng/ml
THC-COOH	1.625	429147	814.76	62.2	∞	195519	97.7434 ng/ml
THC	3.586	3399280	54624.19	25.6	∞	7297905	50.0813 ng/ml

TS

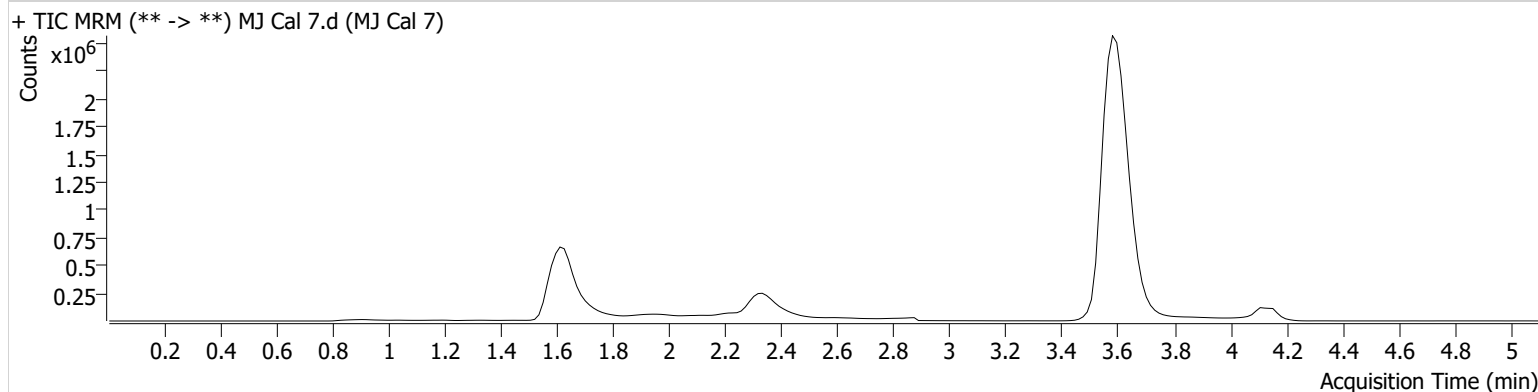
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\011822 AM 25 AM 27 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 1/24/2022 8:45:43 AM

Instrument	Falco (069901)	Data File	MJ Cal 7.d
Type	Cal	Sample	MJ Cal 7
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-B6	Comment	
Injection Volume	10		
Acq. Date-Time	1/18/2022 1:33:54 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.588	1263743	∞	13.4 High	∞	670374	100.9169 ng/ml
THC-COOH	1.625	1038355	911.61	62.3	∞	182459	251.5020 ng/ml
THC	3.586	7107096	37257.92	25.7	5734.51	7588906	100.4941 ng/ml