




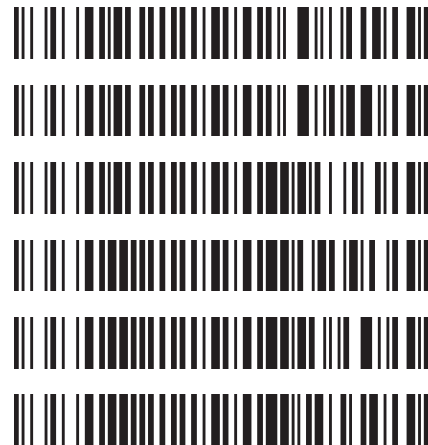


Worklist: 6090

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2022-3014	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2022-2589	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2022-2597	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2022-2646	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2022-2786	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	

Worklist: 6094

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-3175	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3195	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3617	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2680	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2748	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2749	1	BCK	AM 27 Blood THC Quant by LC-QQQ



SC CS

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

Extraction Date: 09/08/2022
Plate lot#: IDP-108-3-220309
Mobile phase A: 0.1% Formic Acid in LCMS Water
Blank Blood Lot: Lampire 22B52015-1
Column: UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 069901

Analyst: Sarah Collins
Retest Date: 09/09/2022
Mobile phase B: 0.1% Formic acid in Acetonitrile
Blank Urine Lot: POC021022

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:** 3382167
- 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 uL
- 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 sample response for THC 1ng/mL and OH-THC 3ng/mL (quantitative), Carboxy-THC: 5ng/mL (qualitative only) will be reported. 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-OH 3-100

SC

Celena Shrum had samples in this batch. Sarah Collins acted as the primary analyst and performed steps 3-16. I, Celena Shrum, approved of all steps utilized in this method. CS

Request for Departure from an Analytical Method or Quality Standard

Deviation Number (assigned by QM): TOX-22-02

Date of Request:
03/02/2022

Requestor/Discipline:
Celena Shrum/Toxicology

Analytical Method/Quality Standard, Revision #:
Toxicology AM #25, AM #26, and AM #27, Revision 13

Temporary or Permanent Deviation:
Permanent

Scope of Deviation (record specific information, e.g. affected programs, evidence types, expected end date; etc):

Deviation will remain in place until the change is made in the next method revision.

Deviation Request (Describe detailed instructions of the changes being made; include reference to specific section number(s) in the method manual):

Toxicology AM #25 3.3.1.1 Internal standards are prepared by the ToxBox plate manufacturer and contained on the 96 well plate. If the run contains urine samples, a positive external urine control must also be run.

Toxicology AM #26 3.3.2 A negative control will be run with each extraction. If the run contains urine samples, a negative urine control and external positive urine control must also be included.

Toxicology AM #27 3.3.2 A negative control will be run with each extraction. If the run contains urine samples, a negative urine control and positive external urine control will also be included in the run.

The deviation is to include the option of using an internal urine control in lieu of an external urine control.

SC CS

Technical Justification for Analytical Method Deviations:

Internal controls serve the same purpose as external controls but also helps to avoid the possible issues that can occur with using external controls (incorrect spiking, incorrect preparation, evaporation of compounds, etc.). If these errors occur, runs need to be repeated and this wastes time, sample, and supplies.

Technical Review

Departure approved
Comments:

Departure Not Approved
Comments:



Approver: Rachel Cutler
Title: Lab Manager

Date: 3/2/22

Quality Review

Quality Approver: Jason Crowe
Title: Quality Manager
Date: 3/2/2022



CS SC

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1 urine			p2022-2786-1	IS + QC_1 blood
B	IS + Cal. 2				p2022-2646-1	IS + Cal. 7
C	IS + Cal. 3			p2022-2680-1 CS	p2022-2597-1	IS + Cal. 6
D	IS + Cal. 4			p2022-2749-1 CS	p2022-2589-1	IS + Cal. 5
E	IS + Cal. 5			p2022-2748-1 CS	m2022-3014-2	IS + Cal. 4
F	IS + Cal. 6			m2022-3617-2 CS	negative urine	IS + Cal. 3
G	IS + Cal. 7			m2022-3195-1 CS	negative blood	IS + Cal. 2
H	IS + QC_1 blood			m2022-3175-1 CS	IS + QC_1 urine	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

SC CS

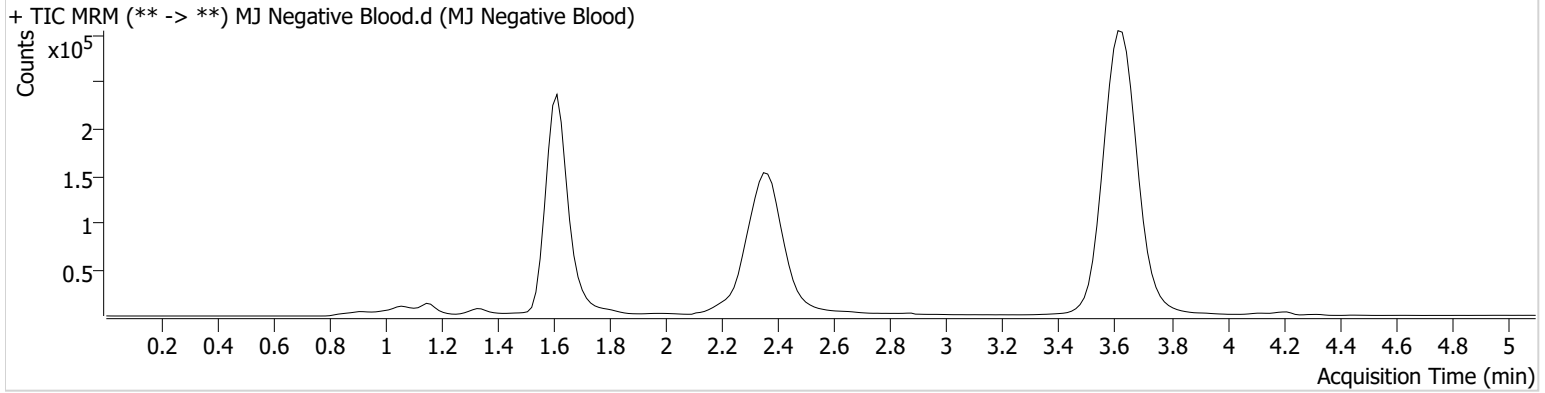


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-G5	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 11:12:22 PM		
Sample Info.			

Sample Chromatogram



SC CS

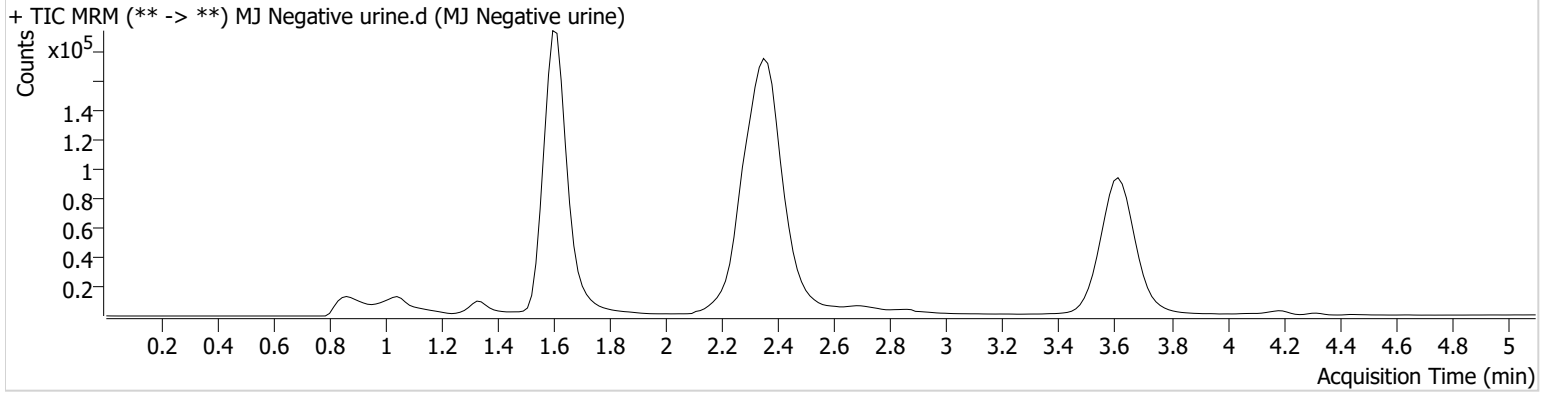


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	MJ Negative urine.d
Type	Sample	Sample	MJ Negative urine
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-F5	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 11:27:36 PM		
Sample Info.			

Sample Chromatogram



SC CS

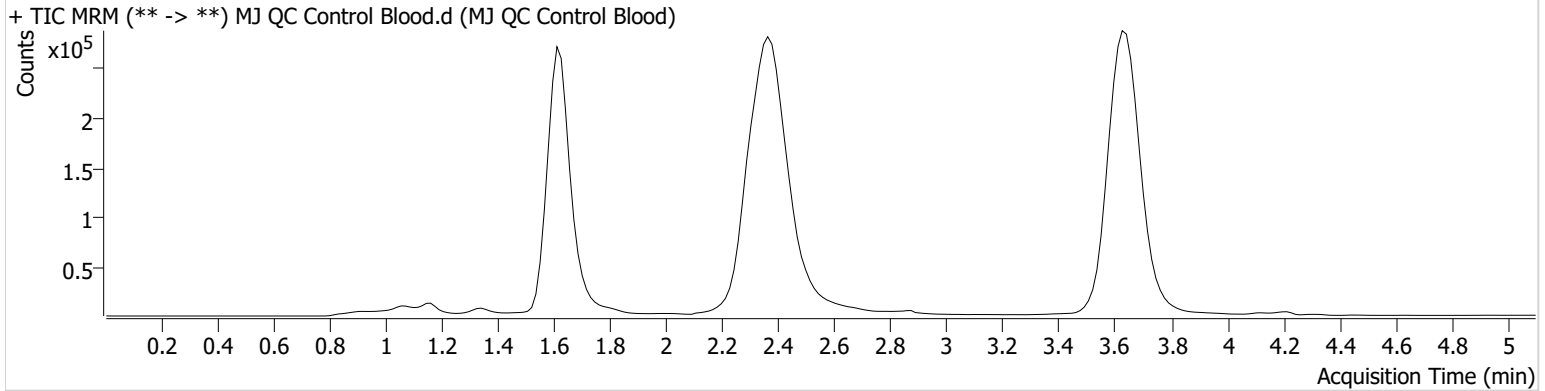


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 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	Sarah Collins
Sample Position	P5-A6	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 10:57:09 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	85309	∞	12.0	223.56	1123259	4.3741 ng/ml
THC-COOH	1.655	78395	∞	50.2	761.77	212741	15.5906 ng/ml
THC	3.646	90082	159.05	32.6	305.48	2220407	4.8662 ng/ml

SC CS

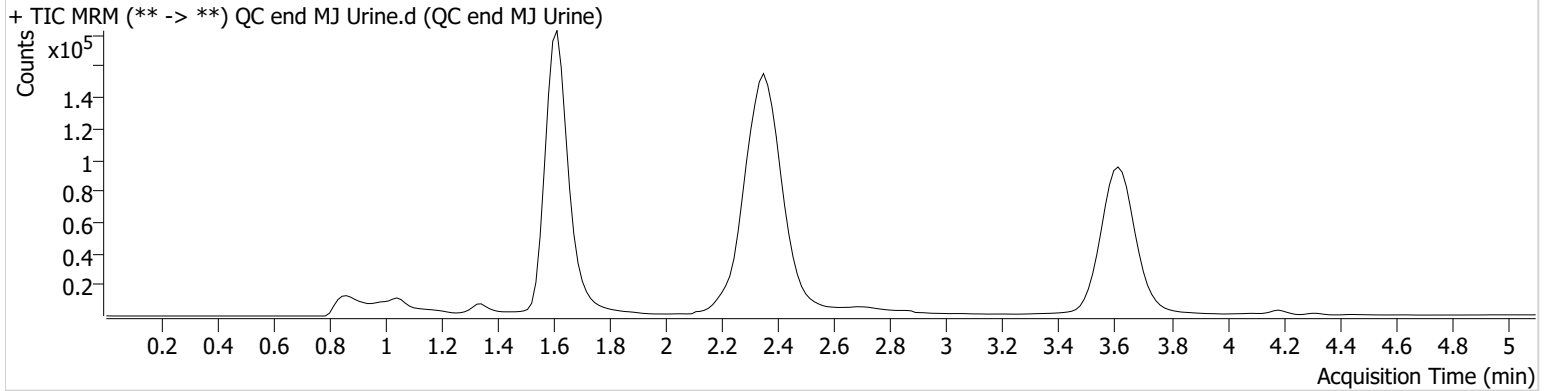


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	QC end MJ Urine.d
Type	QC	Sample	QC end MJ Urine
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-H5	Comment	
Injection Volume	10		
Acq. Date-Time	9/9/2022 2:30:10 AM		
Sample Info.			

Sample Chromatogram



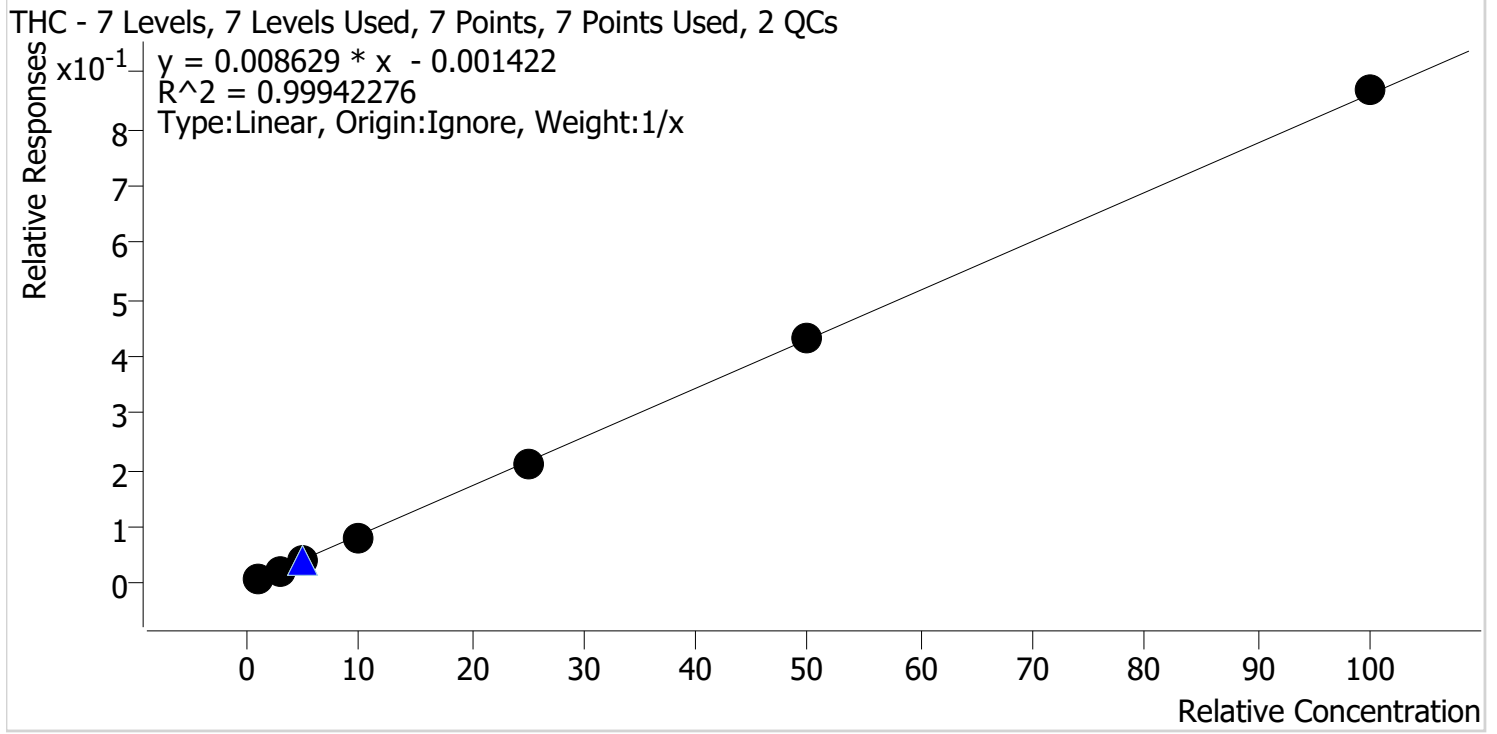
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	52916	∞	13.6	192.05	799407	3.7037 ng/ml
THC-COOH	1.640	44240	100.35	50.3	398.96	121790	15.3475 ng/ml
THC	3.631	31510	204.67	30.5	13.42	777122	4.8635 ng/ml

SC CS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Last Cal. Update 9/9/2022 8:51 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



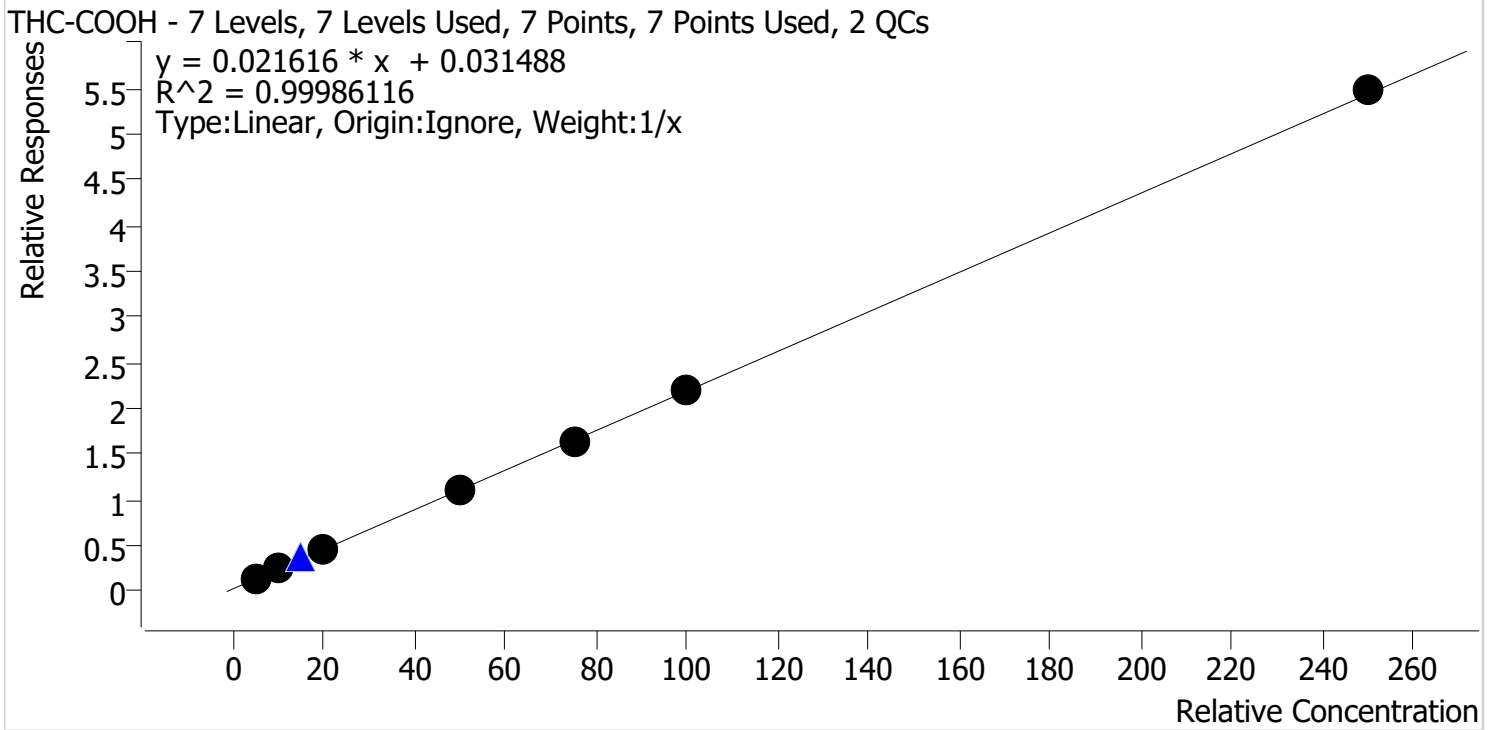
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.2	115.5
Cal 2 MJ	2	✓	3.0	2.9	96.2
Cal 3 MJ	3	✓	5.0	4.7	93.6
Cal 4 MJ	4	✓	10.0	9.5	95.0
Cal 5 MJ	5	✓	25.0	24.5	97.9
Cal 6 MJ	6	✓	50.0	50.5	101.0
Cal 7 MJ	7	✓	100.0	100.8	100.8

SC CS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Last Cal. Update 9/9/2022 8:51 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



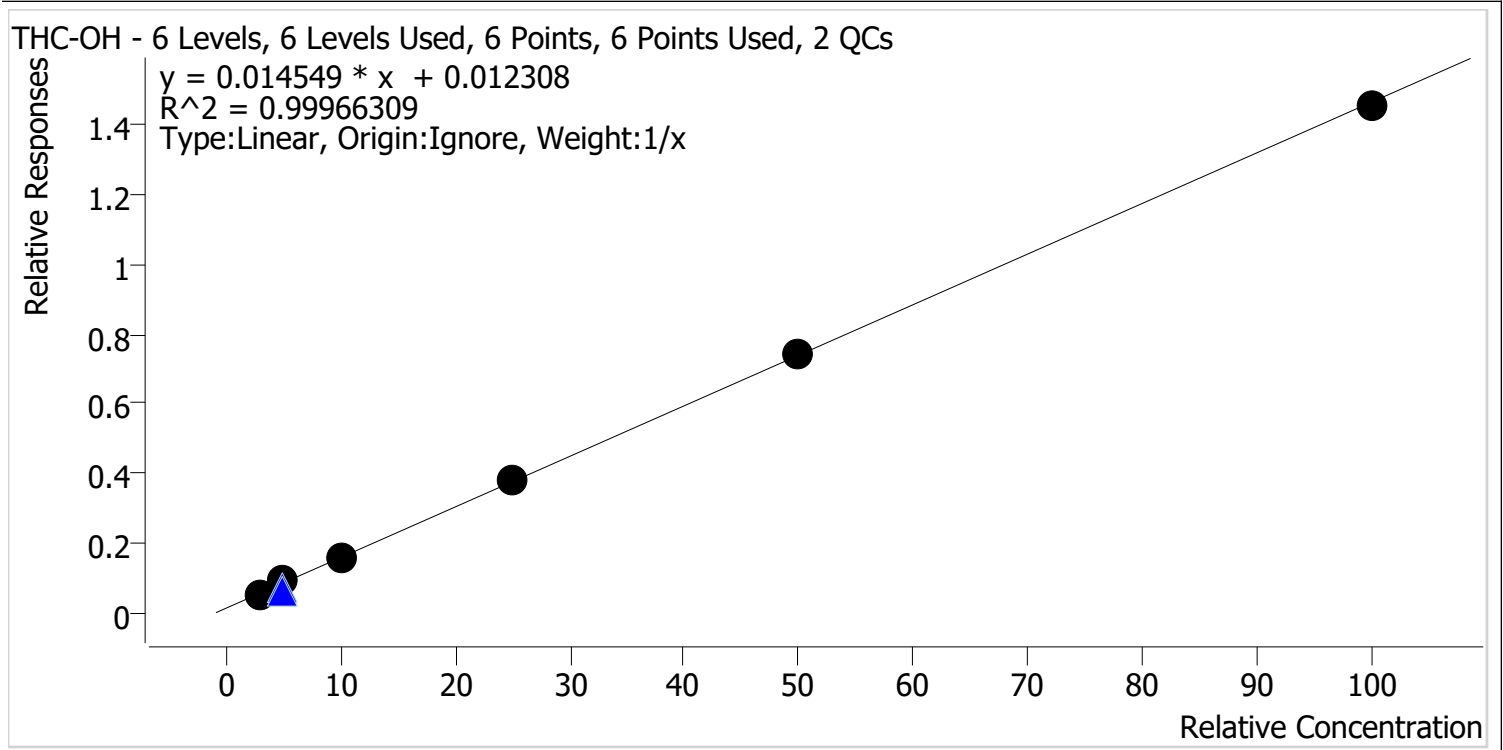
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.2	104.4
Cal 2 MJ	2	✓	10.0	9.9	98.5
Cal 3 MJ	3	✓	20.0	19.8	98.8
Cal 4 MJ	4	✓	50.0	49.6	99.3
Cal 5 MJ	5	✓	75.0	73.8	98.3
Cal 6 MJ	6	✓	100.0	99.8	99.8
Cal 7 MJ	7	✓	250.0	251.9	100.8

SC CS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Last Cal. Update 9/9/2022 8:51 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 2 MJ	2	✓	3.0	2.8	92.1
Cal 3 MJ	3	✓	5.0	5.2	104.1
Cal 4 MJ	4	✓	10.0	10.3	102.8
Cal 5 MJ	5	✓	25.0	25.3	101.1
Cal 6 MJ	6	✓	50.0	50.3	100.7
Cal 7 MJ	7	✓	100.0	99.1	99.1

Calibrator 1 dropped due to poor peak shape/response.

SC CS

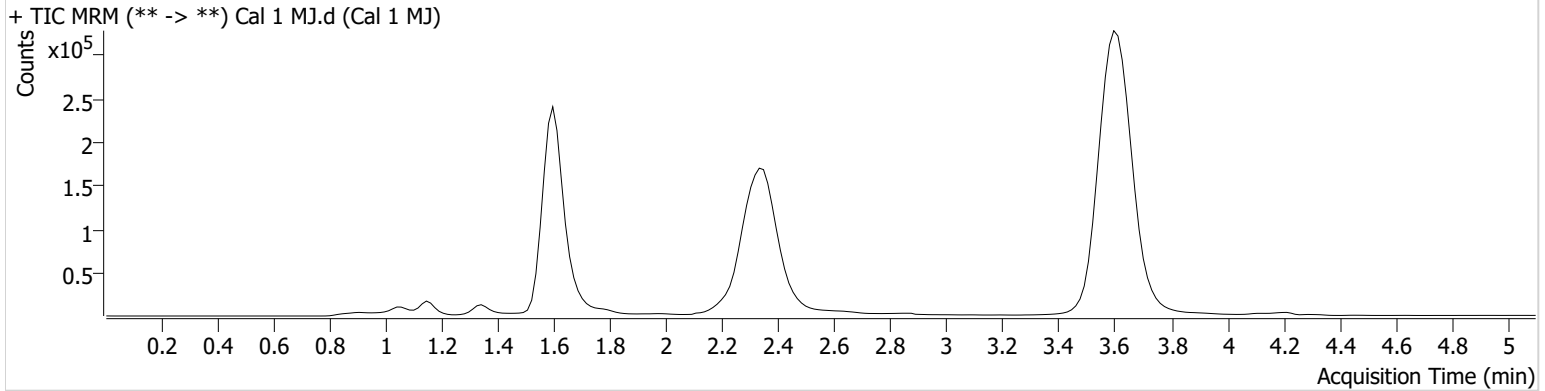


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-H6	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 9:56:06 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.625	28977	85.21	44.7	160.42	200707	5.2223 ng/ml
THC	3.616	23086	112.49	31.9	∞	2702614	1.1547 ng/ml

SC CS

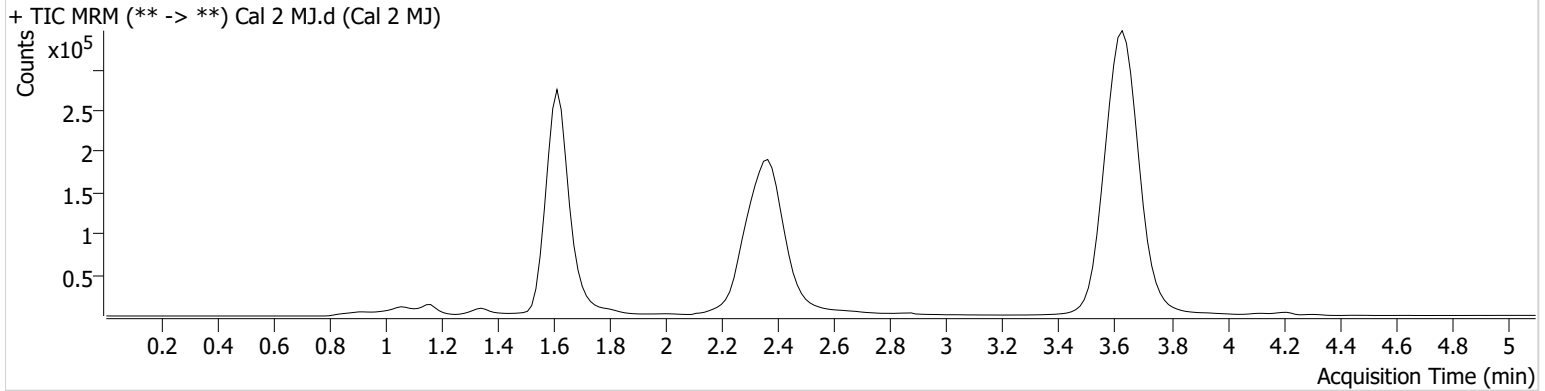


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-G6	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 10:03:52 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	63161	∞	11.4	82.38	1202428	2.7644 ng/ml Low
THC-COOH	1.655	54638	80.57	50.0	∞	223525	9.8514 ng/ml
THC	3.646	67243	1319.19	31.1	356.63	2863733	2.8859 ng/ml

SC CS

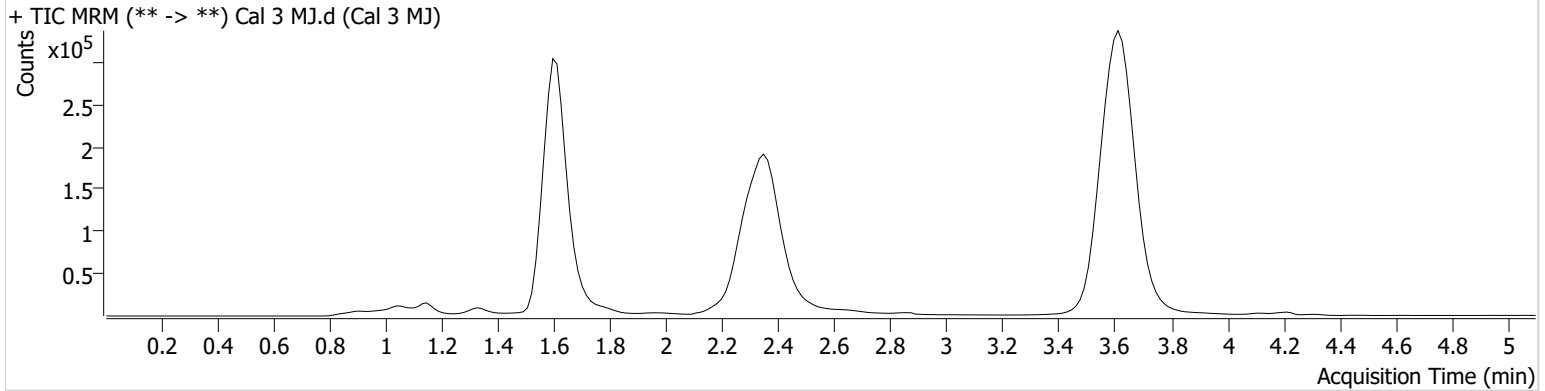


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-F6	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 10:11:31 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	113913	∞	10.6	248.85	1293887	5.2052 ng/ml
THC-COOH	1.640	108160	∞	52.8	252.53	235787	19.7642 ng/ml
THC	3.631	107020	1311.29	29.4	48.04	2747986	4.6779 ng/ml

SC CS

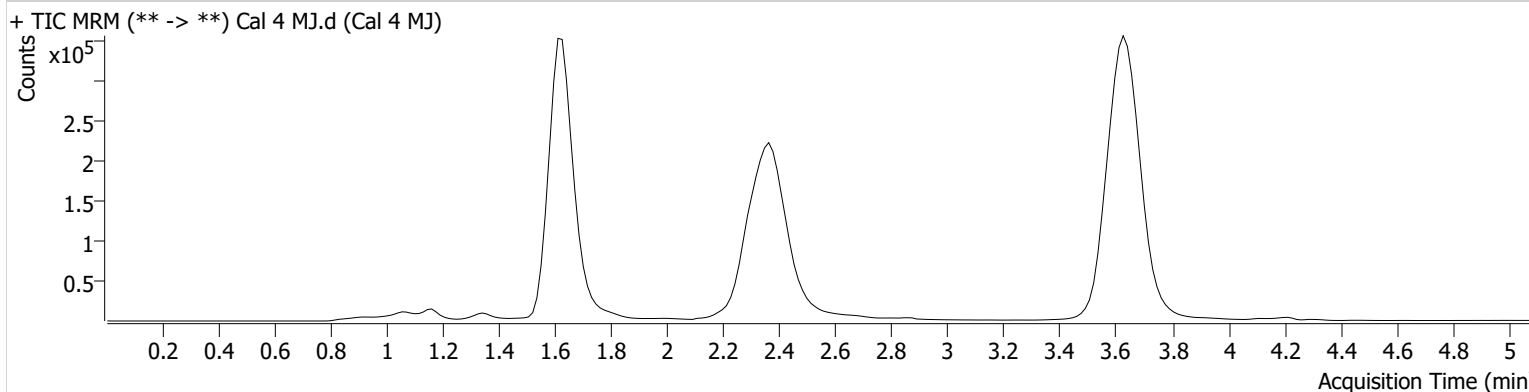


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-E6	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 10:19:06 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	206514	∞	11.9	573.54	1275237	10.2846 ng/ml
THC-COOH	1.655	255586	∞	57.4	2197.05	231386	49.6427 ng/ml
THC	3.646	217300	∞	28.3	∞	2696426	9.5036 ng/ml

SC CS

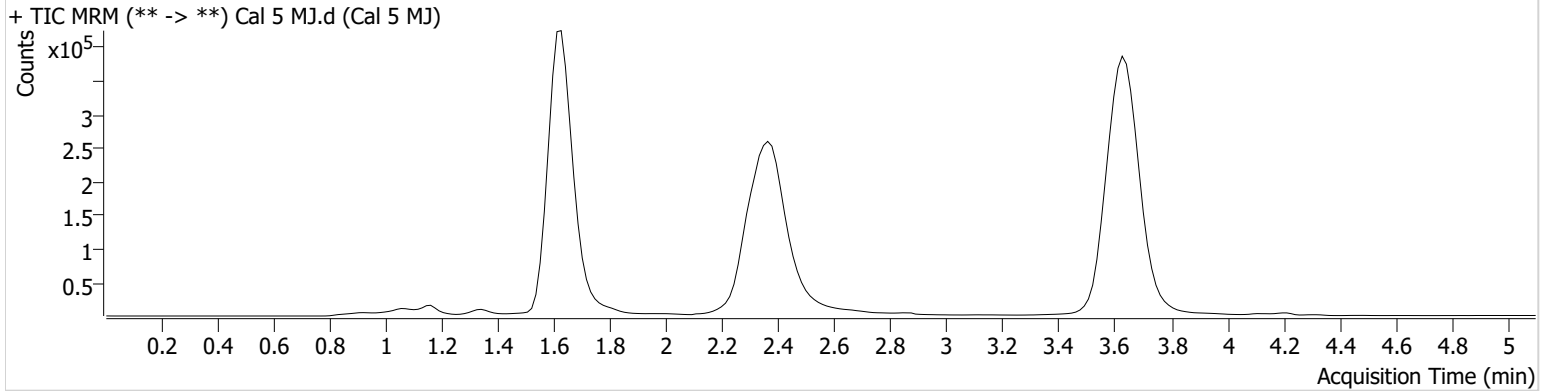


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-D6	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 10:26:42 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	471242	305.19	12.4	∞	1240069	25.2731 ng/ml
THC-COOH	1.655	368697	248.35	58.7	1999.53	226785	73.7530 ng/ml
THC	3.646	525992	933.12	27.7	827.02	2506072	24.4873 ng/ml

SC CS

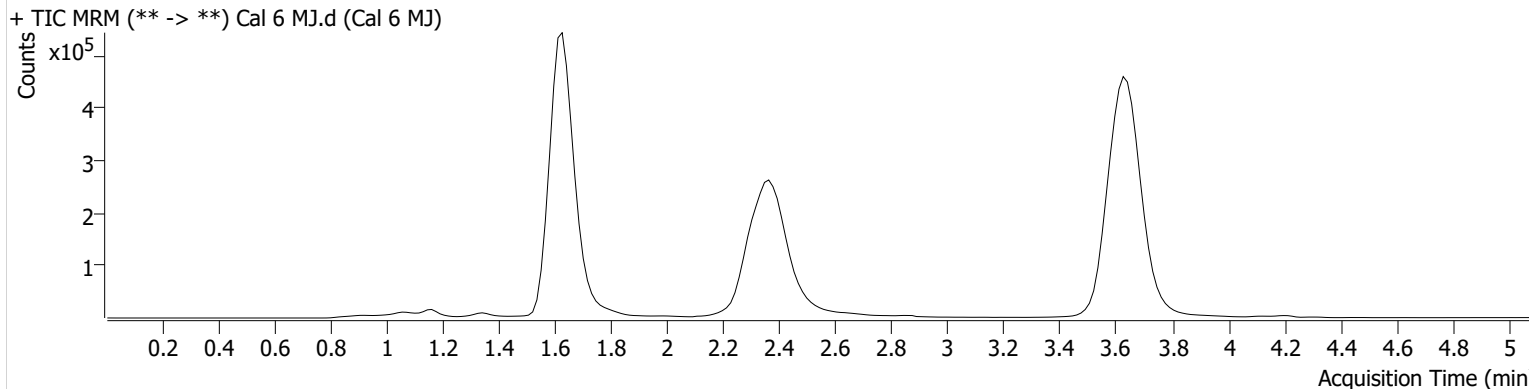


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-C6	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 10:34:18 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	916734	∞	12.8	2694.37	1231022	50.3384 ng/ml
THC-COOH	1.655	487145	∞	58.8	3714.76	222525	99.8171 ng/ml
THC	3.646	1072034	5088.67	27.0	∞	2468064	50.5004 ng/ml

SC CS

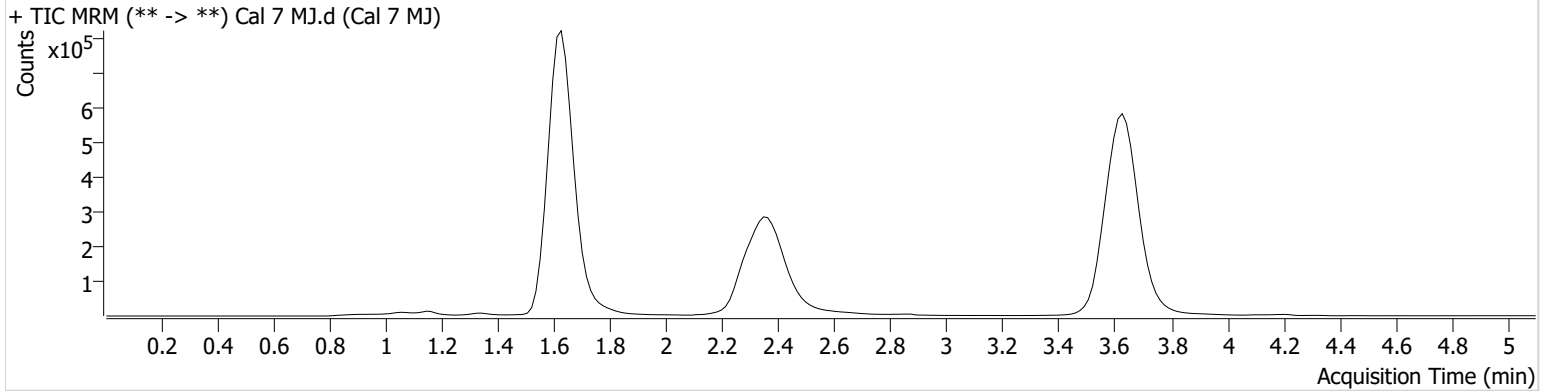


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2022\AM 27-28\090822 AM 27 28 CS SC\QuantResults\AM 27 SC CS.batch.bin
Calibration Last Update 9/9/2022 8:51:58 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P5-B6	Comment	
Injection Volume	10		
Acq. Date-Time	9/8/2022 10:41:54 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.618	1651647	∞	13.4	2406.32	1135438	99.1342 ng/ml
THC-COOH	1.640	1149896	3385.05	58.8	2733.21	209922	251.9493 ng/ml
THC	3.631	2007045	5092.01	27.2	∞	2311387	100.7902 ng/ml