REVIEWED
By Amber Gerheart at 2:39 pm, Apr 20, 2023

4/18/2023

Worklist: 6339

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
M2023-1024	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1145	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1226	3	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1317	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-1389	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0902	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0938	4	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0946	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0953	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0964	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0973	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0976	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-0982	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1023	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1025	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-1033	1	BCK	AM 27 Blood THC Quant by LC-QQQ

CS

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

Extraction Date: 04/14/2023
Analyst: Celena Shrum
Plate lot#: 220802
Plate Retest Date: 07/23/2023
Mobile phase A: 0.1% Formic Acid in LCMS Water
Mobile phase B: 0.1% Formic acid in Acetonitrile
Blank Blood Lot: Lampire 23A52593
Column: Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)
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. Urine hydrolysis (if applicable): add 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.
☒ 3. Using a calibrated pipette, add **1000µl blood or 1000µl hydrolyzed urine** into the appropriate wells of the analytical (standards) plate. **Pipette ID: ~~#27~~ 42** CS 4/20/23
☒ 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 5. Add **500µL of 0.1% formic acid in water to blood samples or 500µl of saturated phosphate buffer to urine samples** to the appropriate wells of the analytical plate.
☒ 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 7. Transfer **800µL of blood+acid mixture or urine+acid** to corresponding wells of SLE+ plate.
☒ 8. 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)*** Manifold ID: 067104
☒ 9. Wait 5 minutes.
☒ 10. Add **2.25mL MTBE. (*Add in 3 increments of 750uL)***
☒ 11. Wait 5 minutes.
☒ 12. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 13. Add **2.25mL Hexane. (*Add in 3 increments of 750uL)***
☒ 14. Wait 5 minutes.
☒ 15. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
☒ 16. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. *SPE Dry ID: 067103*
☒ 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. THC concentrations of 1-3ng/mL will be reported qualitatively.
☒ 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:

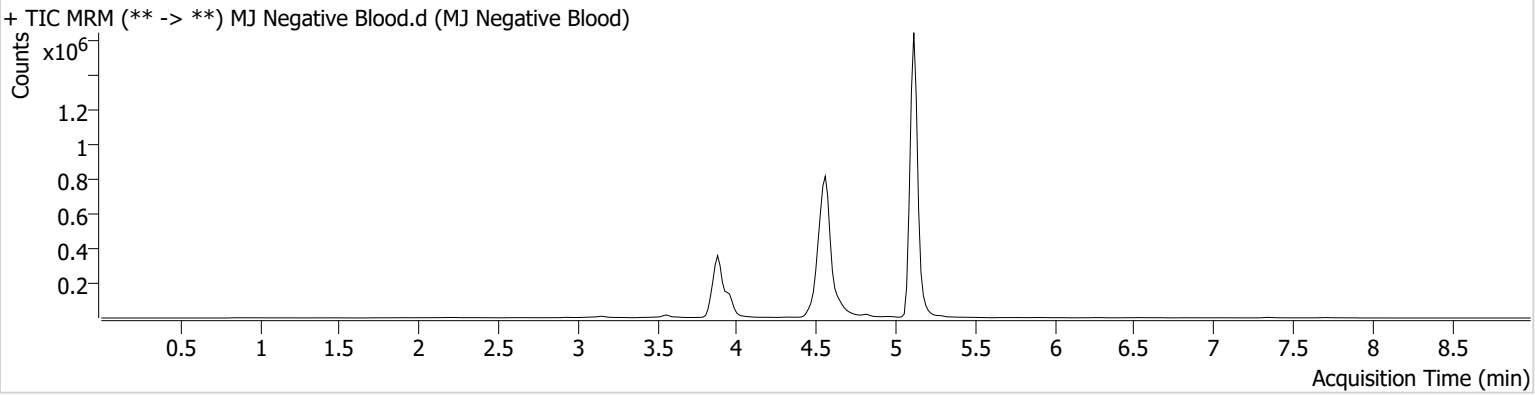
	1	2	3	4	5	6
a				P2023-1023-1	P2023-0902-1	QC 1
b				P2023-0982-2	M2023-1389-1	cal 100 ng
c				P2023-0976-1	M2023-1317-2	cal 50 ng
d				P2023-0973-2	M2023-1226-3	cal 25 ng
e				P2023-0964-1	M2023-1145-2	cal 10ng
f				P2023-0953-1	M2023-1024-1	cal 5 ng
g			P2023-1033-1	P2023-0946-1	NEG Blood	cal 3 ng
h			P2023-1025-1	P2023-0938-4	QC 2	cal 1ng

AM #27 Cannabinoid Quant. Results

Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update	4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	MJ Negative Blood.d
Type	Sample	**Sample**	MJ Negative Blood
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-G5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 6:21:35 PM		
Sample Info.			

Sample Chromatogram

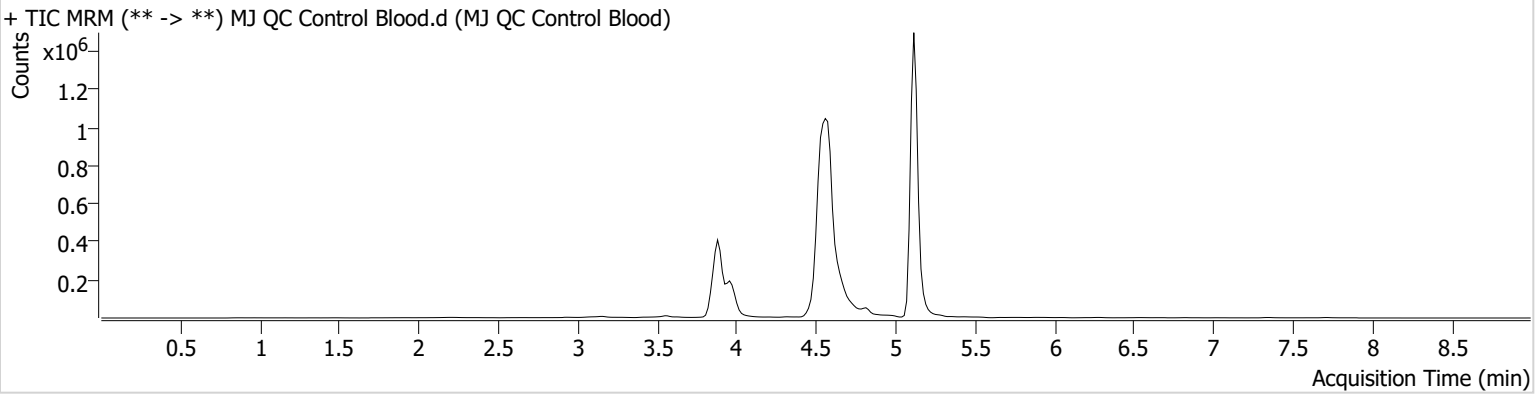


AM #27 Cannabinoid Quant. Results

Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update	4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	MJ QC Control Blood.d
Type	QC	**Sample**	MJ QC Control Blood
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-A6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 5:55:23 PM		
Sample Info.			

Sample Chromatogram



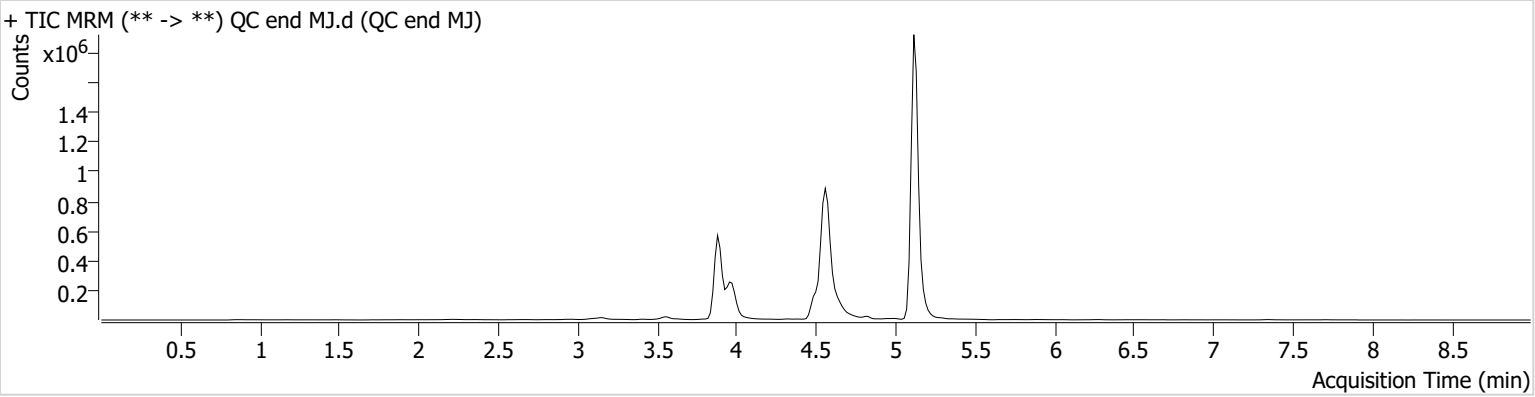
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	123343	420.61	11.6	∞	1555111	5.0683	ng/ml
THC-COOH	3.969	38033	756.42	240.7	985.69	456723	14.9370	ng/ml
THC	5.120	201031	∞	22.4	∞	4471428	5.0725	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	QC end MJ.d
Type	QC	**Sample**	QC end MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-H5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/15/2023 1:46:49 AM		
Sample Info.			

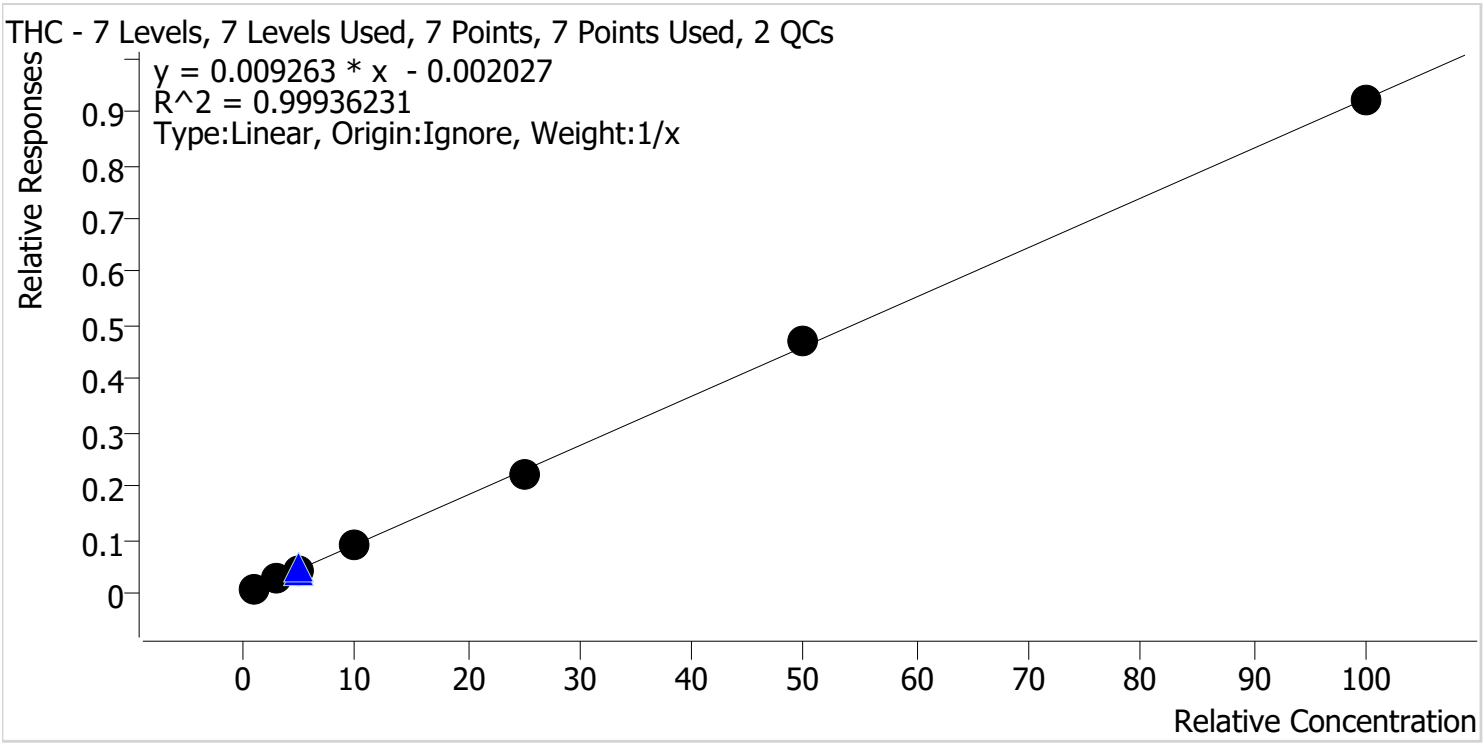
Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	128677	∞	13.2	1915.98	1845348	4.4464	ng/ml
THC-COOH	3.985	49070	1920.05	244.0	4772.74	582641	15.1026	ng/ml
THC	5.120	261804	∞	23.0	∞	5660601	5.2119	ng/ml

AM #27 Cannabinoids Quant. Calibration Curve Report

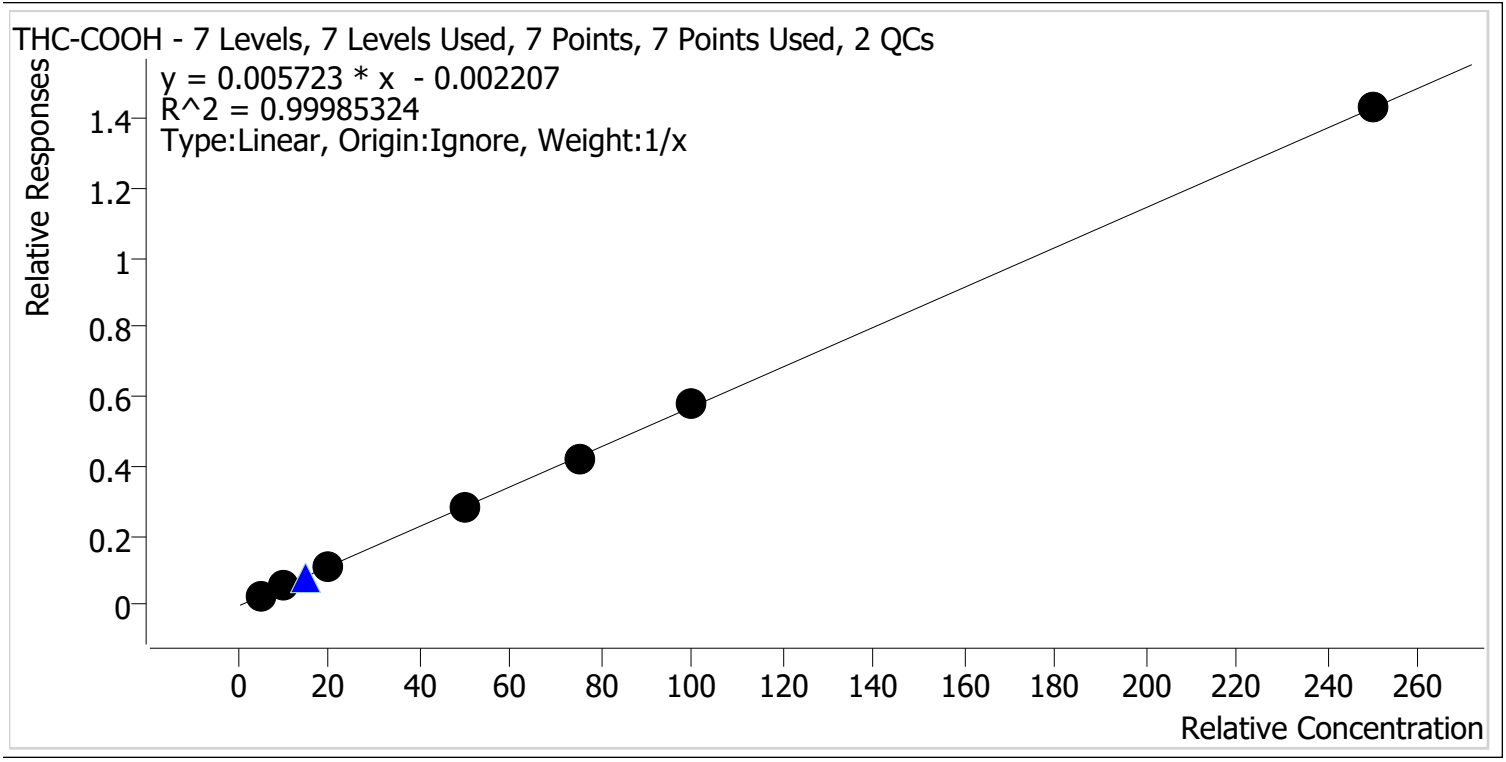
Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update	4/18/2023 10:23 AM
Analyst Name	ISP\datastor
Analyte	THC
Internal Standard	THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✔	1.0	1.1	110.6
Cal 2 MJ	2	✔	3.0	3.0	99.5
Cal 3 MJ	3	✔	5.0	4.6	91.2
Cal 4 MJ	4	✔	10.0	9.8	98.3
Cal 5 MJ	5	✔	25.0	24.5	97.9
Cal 6 MJ	6	✔	50.0	51.4	102.8
Cal 7 MJ	7	✔	100.0	99.6	99.6

AM #27 Cannabinoids Quant. Calibration Curve Report

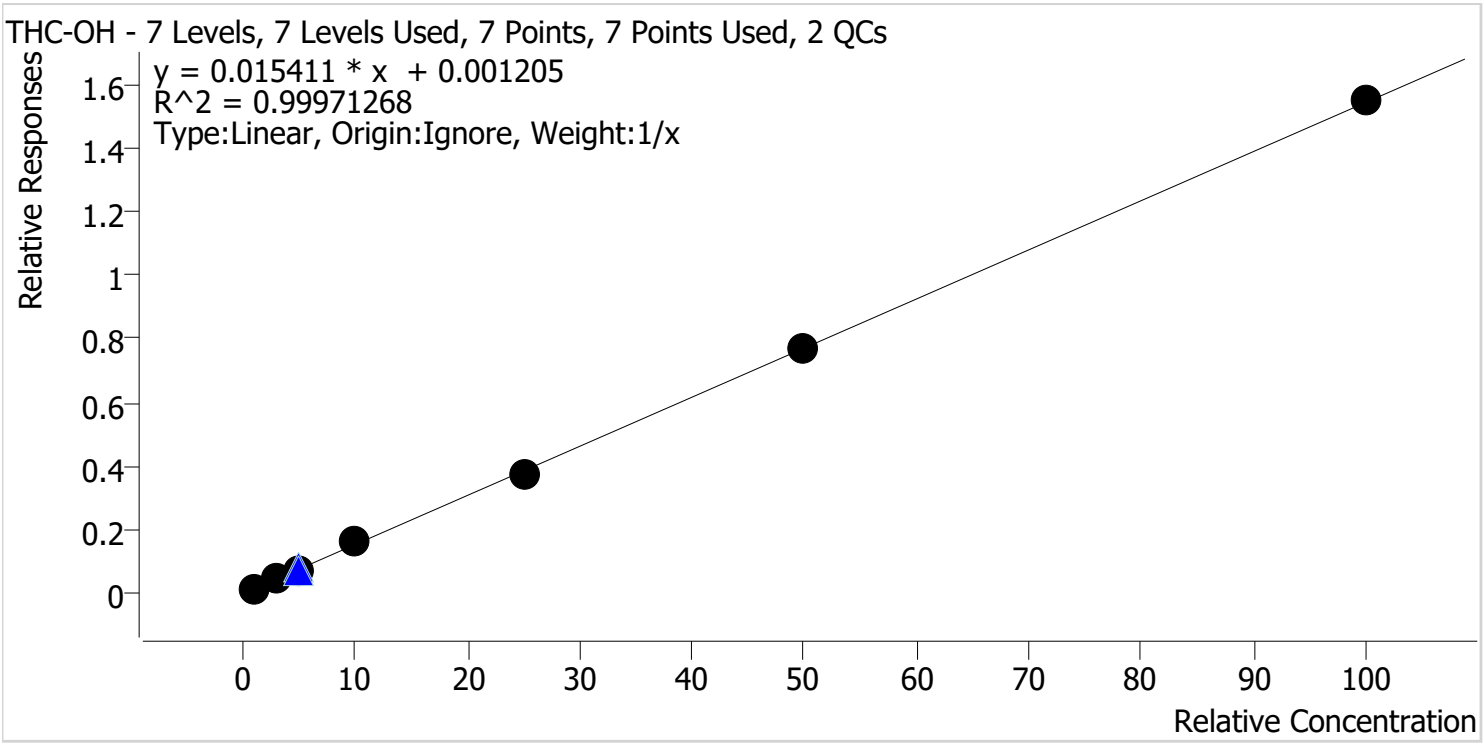
Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update	4/18/2023 10:23 AM
Analyst Name	ISP\datastor
Analyte	THC-COOH
Internal Standard	THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✔	5.0	5.2	104.1
Cal 2 MJ	2	✔	10.0	9.9	99.1
Cal 3 MJ	3	✔	20.0	19.4	97.2
Cal 4 MJ	4	✔	50.0	49.6	99.2
Cal 5 MJ	5	✔	75.0	74.2	99.0
Cal 6 MJ	6	✔	100.0	101.4	101.4
Cal 7 MJ	7	✔	250.0	250.2	100.1

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update	4/18/2023 10:23 AM
Analyst Name	ISP\datastor
Analyte	THC-OH
Internal Standard	THC-OH-D3



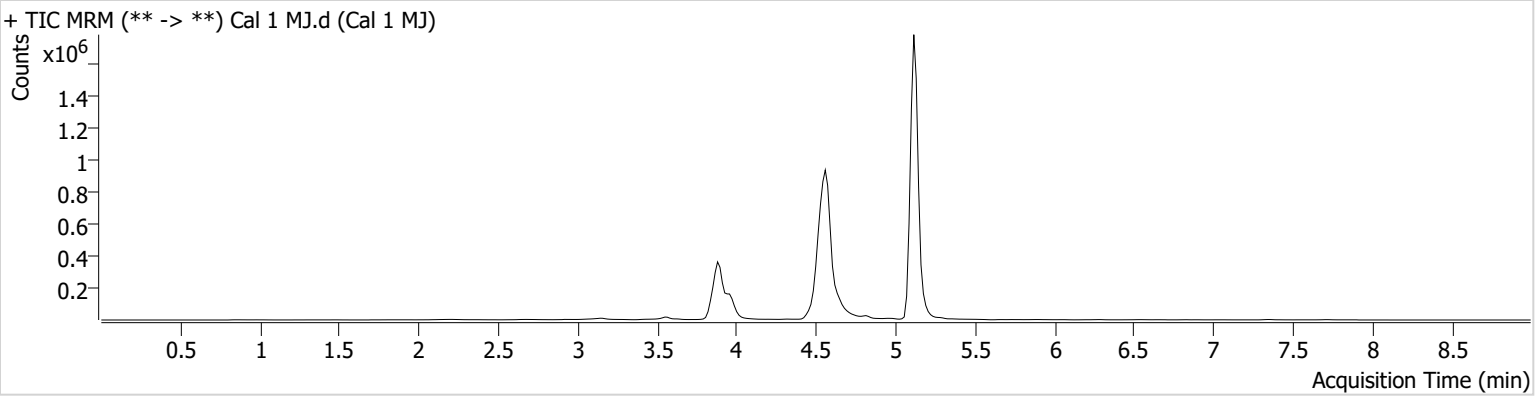
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✔	1.0	1.0	101.6
Cal 2 MJ	2	✔	3.0	2.9	96.6
Cal 3 MJ	3	✔	5.0	5.0	99.1
Cal 4 MJ	4	✔	10.0	10.5	105.1
Cal 5 MJ	5	✔	25.0	24.4	97.5
Cal 6 MJ	6	✔	50.0	49.9	99.8
Cal 7 MJ	7	✔	100.0	100.3	100.3

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	Cal 1 MJ.d
Type	Cal	**Sample**	Cal 1 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-H6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 4:10:25 PM		
Sample Info.			

Sample Chromatogram



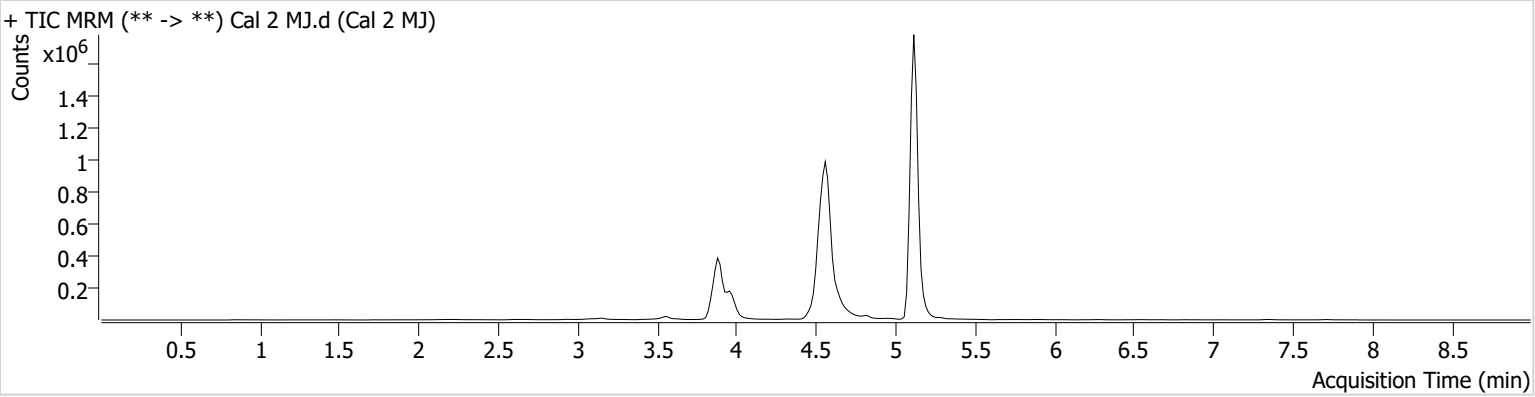
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	25291	∞	11.7	29.44	1499799	1.0160	ng/ml Low
THC-COOH	3.985	12983	203.87	247.2	∞	470849	5.2038	ng/ml
THC	5.120	48022	447.30	26.1	∞	5844509	1.1059	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update	4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	Cal 2 MJ.d
Type	Cal	**Sample**	Cal 2 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-G6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 4:23:41 PM		
Sample Info.			

Sample Chromatogram



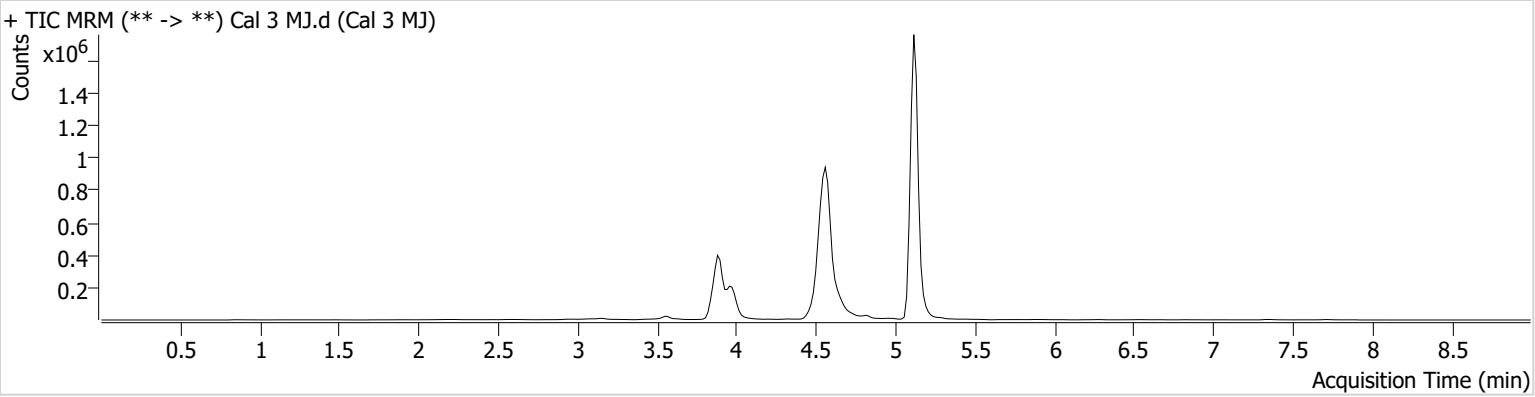
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	69995	163.98	12.9	958.03	1526685	2.8967	ng/ml Low
THC-COOH	3.985	25559	427.51	255.3	∞	469162	9.9054	ng/ml
THC	5.120	145816	2259.72	22.5	∞	5688865	2.9860	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update	4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	Cal 3 MJ.d
Type	Cal	**Sample**	Cal 3 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-F6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 4:36:47 PM		
Sample Info.			

Sample Chromatogram



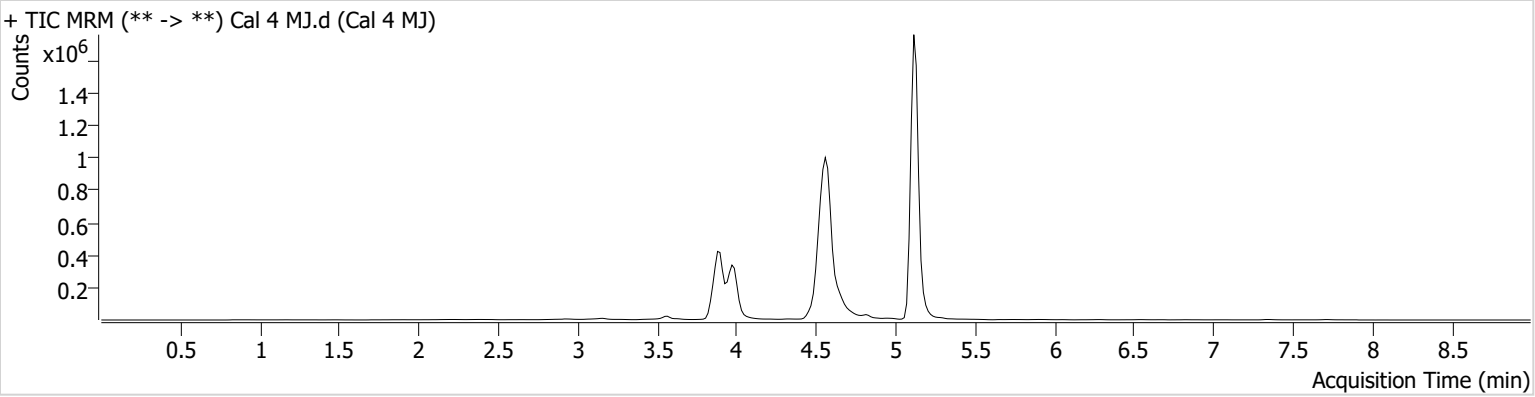
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	118710	250.33	12.6	168.22	1529926	4.9565	ng/ml
THC-COOH	3.985	50079	∞	246.5	973.30	459353	19.4363	ng/ml
THC	5.120	223236	1167.68	22.7	∞	5549799	4.5614	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update	4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	Cal 4 MJ.d
Type	Cal	**Sample**	Cal 4 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-E6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 4:49:53 PM		
Sample Info.			

Sample Chromatogram



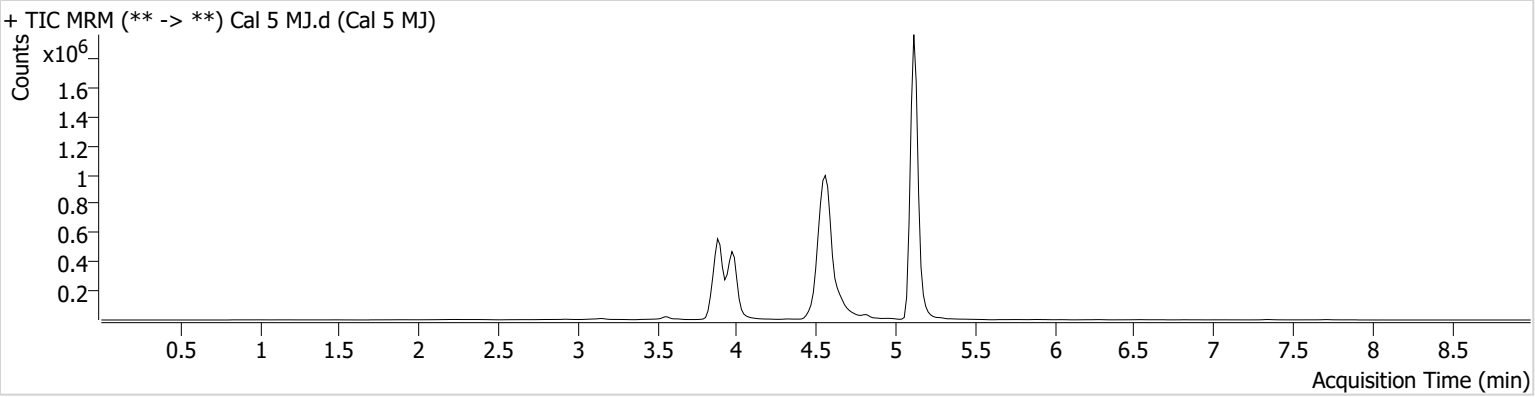
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	252585	∞	12.0	∞	1548217	10.5078	ng/ml
THC-COOH	3.985	130785	2786.88	241.8	∞	464321	49.6055	ng/ml
THC	5.120	468304	∞	21.5	∞	5262394	9.8261	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update	4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	Cal 5 MJ.d
Type	Cal	**Sample**	Cal 5 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-D6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 5:02:59 PM		
Sample Info.			

Sample Chromatogram



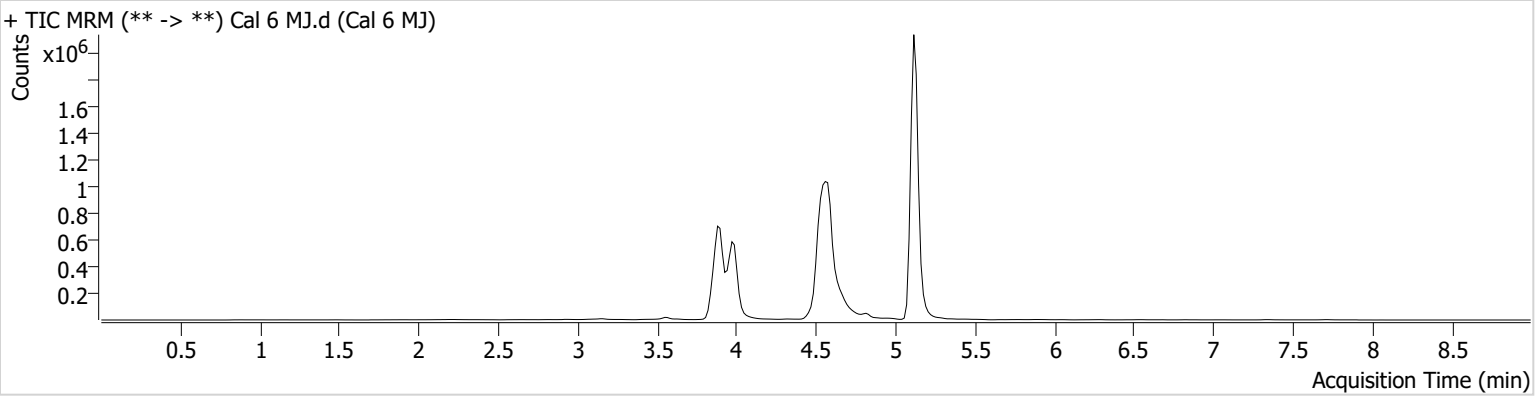
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	615527	∞	13.2	3970.33	1634138	24.3625	ng/ml
THC-COOH	3.985	198455	6397.59	236.0	∞	469628	74.2286	ng/ml
THC	5.120	1134937	28498.28	22.7	∞	5049778	24.4824	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	Cal 6 MJ.d
Type	Cal	**Sample**	Cal 6 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-C6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 5:16:05 PM		
Sample Info.			

Sample Chromatogram



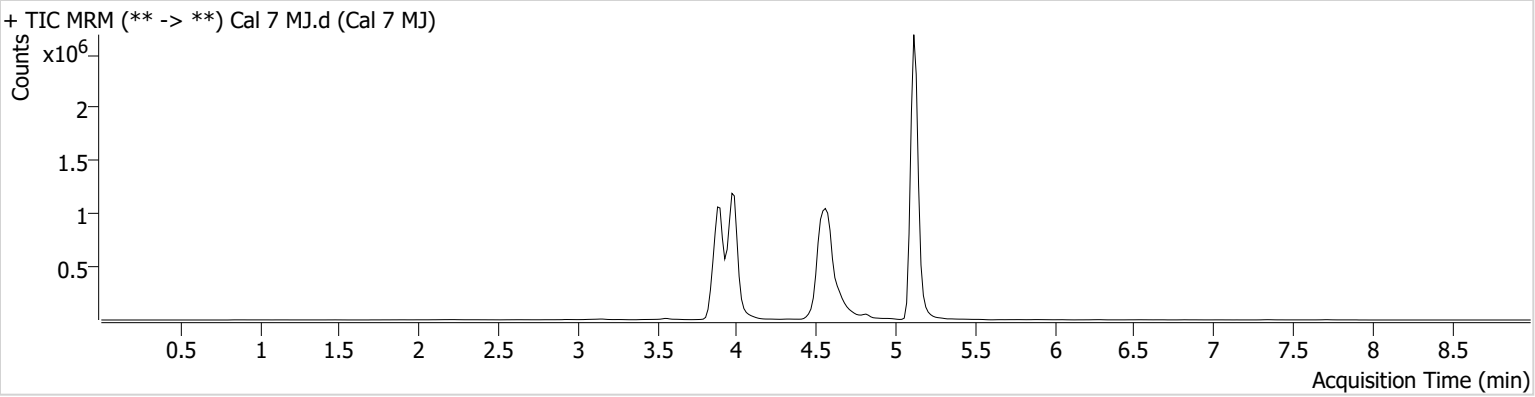
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	1246573	∞	13.2	5055.18	1617681	49.9230	ng/ml
THC-COOH	3.985	265126	16943.62	241.8	2044.89	458496	101.4311	ng/ml
THC	5.120	2101931	∞	22.5	∞	4431738	51.4223	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results	D:\MassHunter\Data\2023\AM 27 28\041423 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update	4/18/2023 10:23:56 AM

Instrument	Falco (069901)	**Data File**	Cal 7 MJ.d
Type	Cal	**Sample**	Cal 7 MJ
Acq. Method	AM 27 Agilent Method.m	**Operator**	Celena Shrum
Sample Position	P1-B6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2023 5:29:11 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	3.896	2594923	∞	13.0	8674.42	1676792	100.3373	ng/ml
THC-COOH	3.969	609446	∞	244.3	∞	426321	250.1893	ng/ml
THC	5.120	3854317	∞	23.1	∞	4186283	99.6160	ng/ml