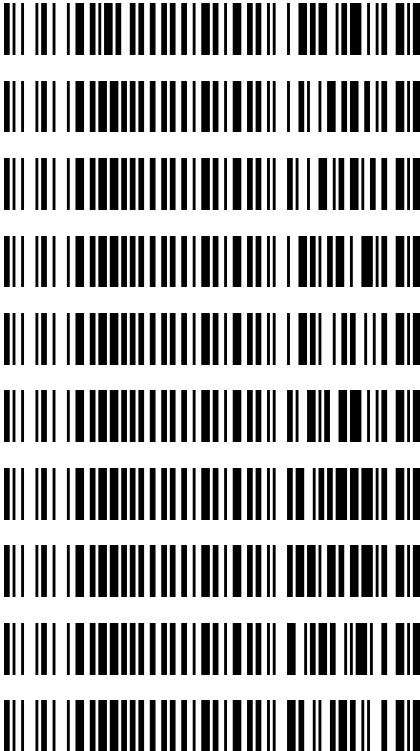




Worklist: 6058

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-2728	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2000	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2024	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2080	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2082	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2098	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2120	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2121	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2162	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2188	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: 08/05/2022

Analyst: Celena Shrum

Plate lot#: 220309

Plate Retest Date: 09/09/2022

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 22B52015-1

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** of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
- 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: THC-OH curve range: 3-100.

	1	2	3	4	5	6
a					P2022-2098-1	QC 1
b					P2022-2082-1	cal 100 ng
c					P2022-2080-1	cal 50 ng
d					P2022-2024-1	cal 25 ng
e				P2022-2188-1	P2022-2000-1	cal 10ng
f				P2022-2162-1	M2022-2728-3	cal 5 ng
g				P2022-2121-1	NEG Blood	cal 3 ng
h				P2022-2120-1	QC 2	cal 1ng

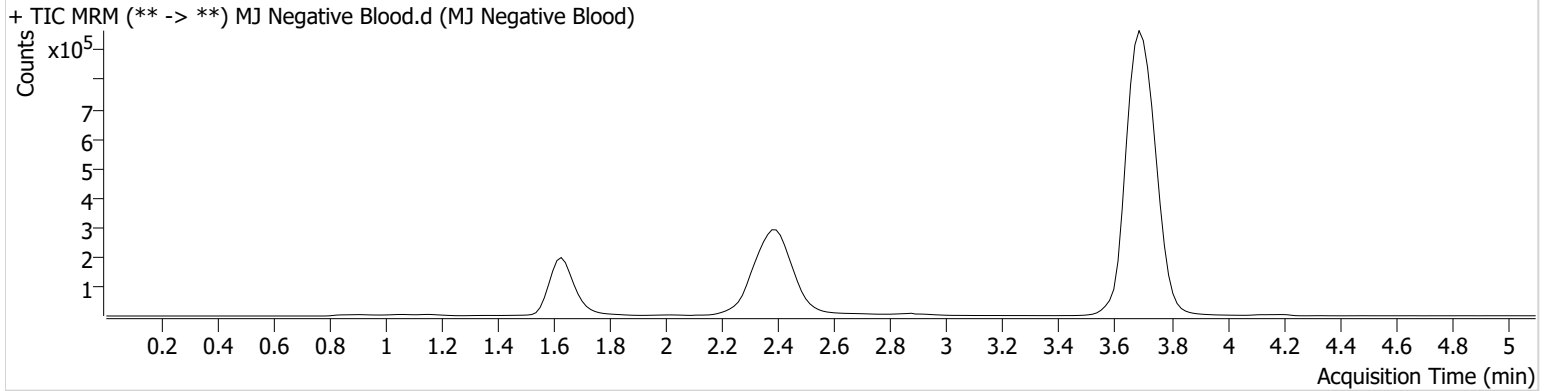
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-G5	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 2:21:31 PM		
Sample Info.			

Sample Chromatogram



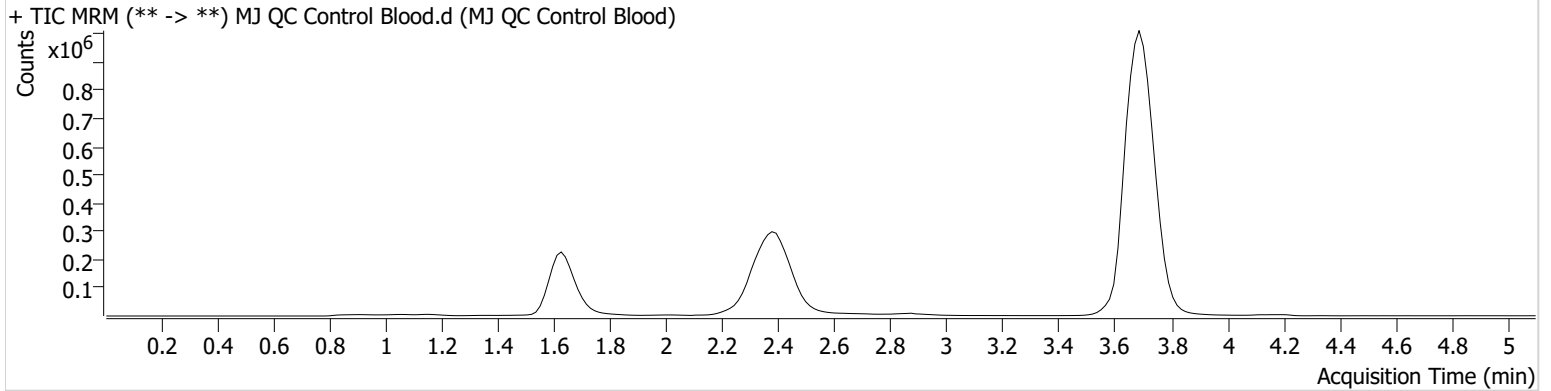
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 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	Celena Shrum
Sample Position	P5-A6	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 2:06:17 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	79204	198.80	11.3	∞	943844	5.0565 ng/ml
THC-COOH	1.655	94507	335.30	51.7	∞	263611	14.6164 ng/ml
THC	3.691	284510	∞	29.0	150.38	7060673	4.8254 ng/ml

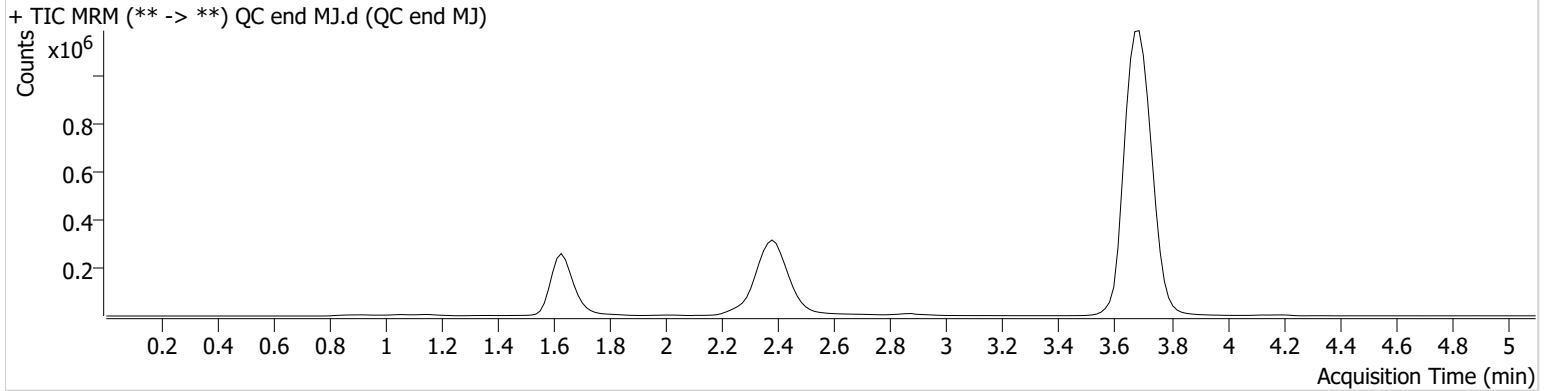
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-H5	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 5:09:05 PM		

Sample Chromatogram

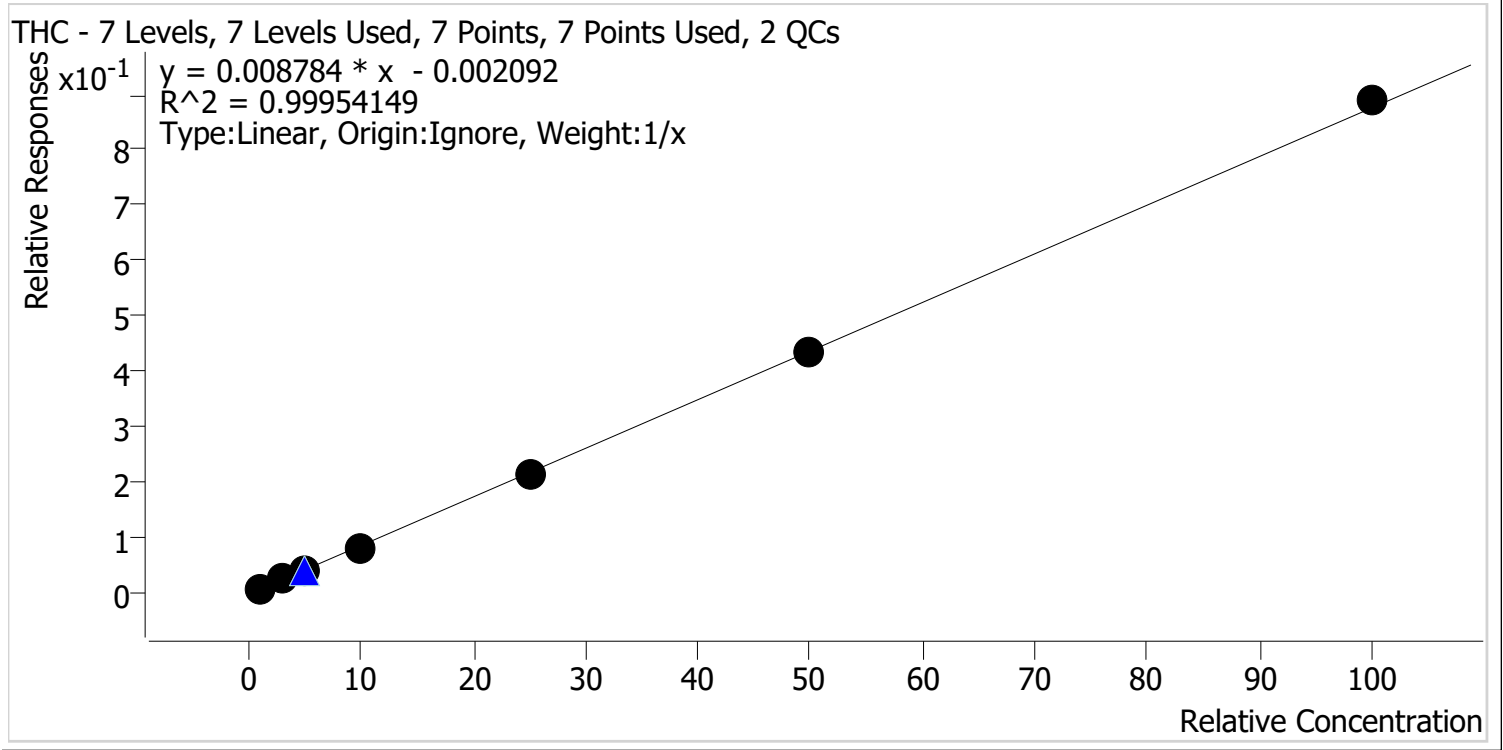


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	79548	∞	11.5	∞	953355	5.0258 ng/ml
THC-COOH	1.655	94377	231.46	54.1	∞	267644	14.3652 ng/ml
THC	3.691	310929	2390.63	28.8	163.85	7791446	4.7812 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 8/10/2022 10:19 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

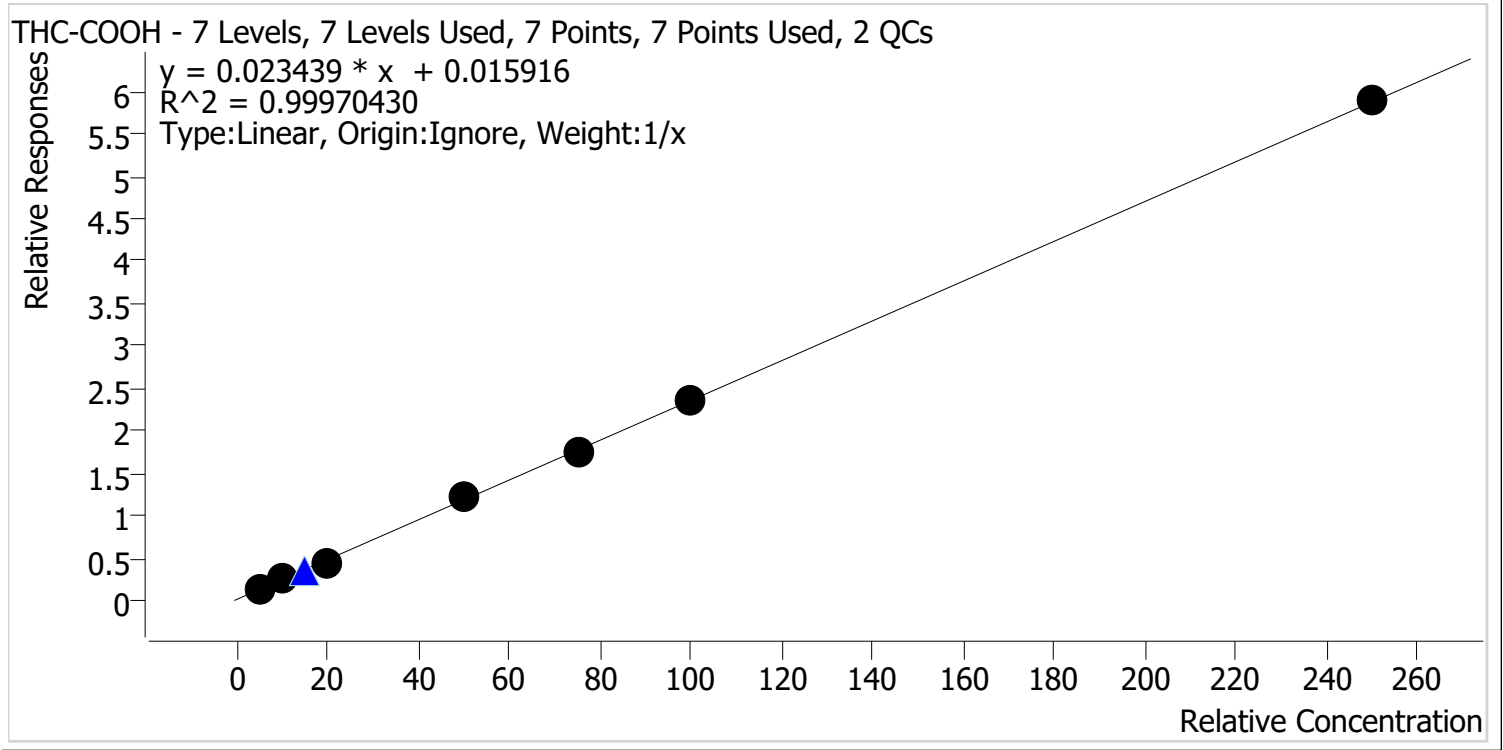


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	111.6
Cal 2 MJ	2	✓	3.0	2.9	97.9
Cal 3 MJ	3	✓	5.0	4.8	96.2
Cal 4 MJ	4	✓	10.0	9.5	95.3
Cal 5 MJ	5	✓	25.0	24.6	98.5
Cal 6 MJ	6	✓	50.0	49.5	99.1
Cal 7 MJ	7	✓	100.0	101.5	101.5



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 8/10/2022 10:19 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



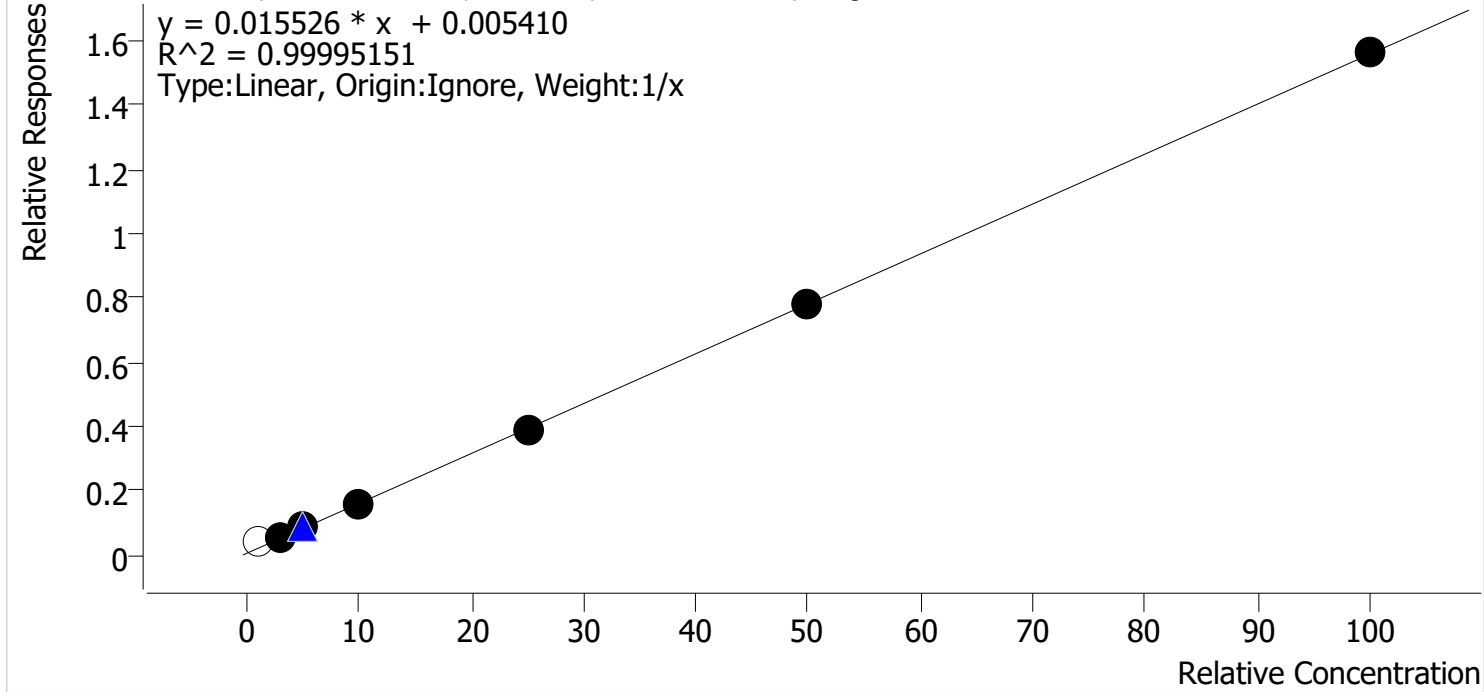
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.2	104.9
Cal 2 MJ	2	✓	10.0	10.0	100.4
Cal 3 MJ	3	✓	20.0	18.8	93.8
Cal 4 MJ	4	✓	50.0	50.6	101.3
Cal 5 MJ	5	✓	75.0	74.0	98.7
Cal 6 MJ	6	✓	100.0	100.6	100.6
Cal 7 MJ	7	✓	250.0	250.7	100.3



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 8/10/2022 10:19 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 2 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	1.0	2.5	251.9
Cal 2 MJ	2	✓	3.0	2.9	98.1
Cal 3 MJ	3	✓	5.0	5.1	102.6
Cal 4 MJ	4	✓	10.0	10.0	99.8
Cal 5 MJ	5	✓	25.0	24.9	99.6
Cal 6 MJ	6	✓	50.0	49.8	99.5
Cal 7 MJ	7	✓	100.0	100.3	100.3

Calibrator 1 dropped due to poor peak shape/response.

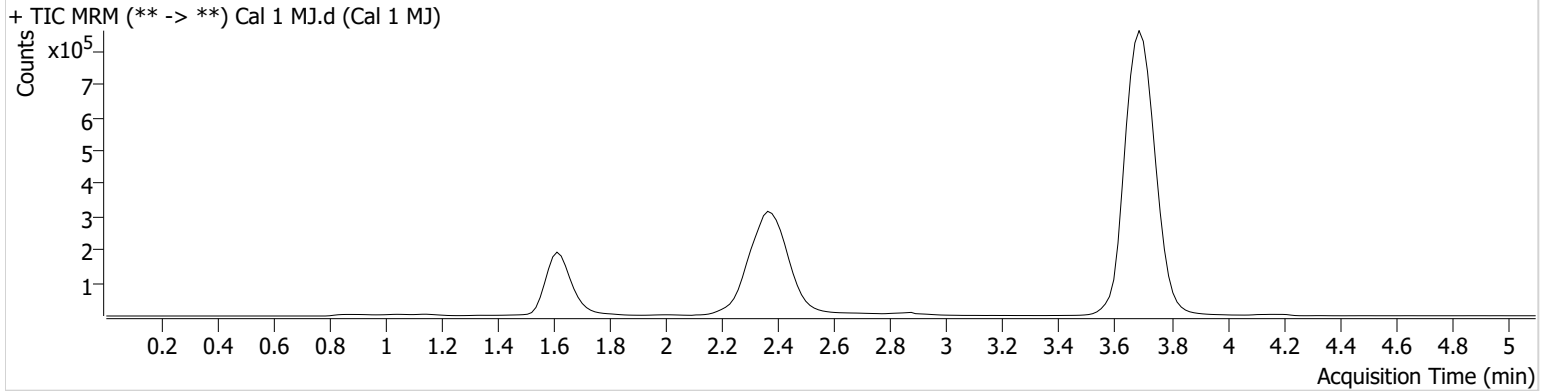
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-H6	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 1:05:13 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.693	40290	∞	6.1 Low	16.05	905033	2.5189 ng/ml Low
THC-COOH	1.655	34361	∞	42.3	187.80	247483	5.2444 ng/ml
THC	3.706	50206	115.37	32.0	28.27	6509571	1.1162 ng/ml

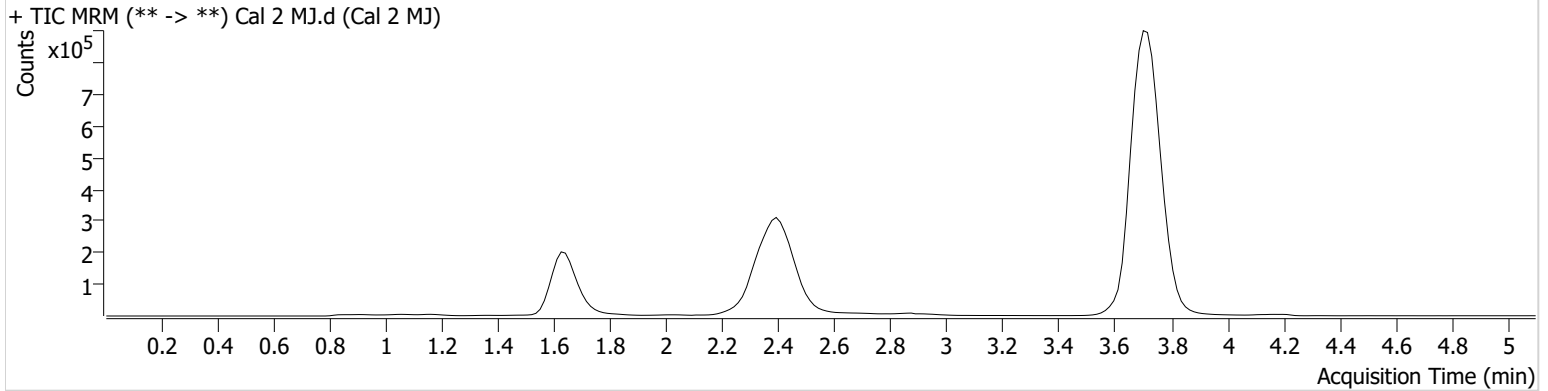
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-G6	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 1:12:59 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	46474	42.08	12.1	42.39	909011	2.9445 ng/ml Low
THC-COOH	1.670	62465	∞	48.0	866.29	248562	10.0426 ng/ml
THC	3.721	155754	473.54	29.7	242.32	6569646	2.9371 ng/ml

AM #27 Cannabinoid Quant. Results

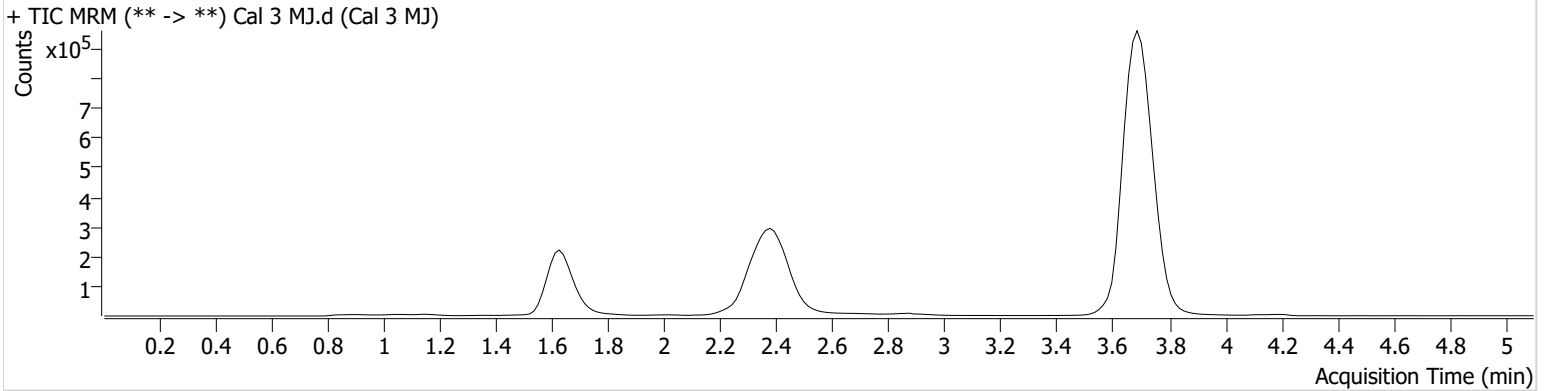


Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-F6	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 1:20:37 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	78123	∞	12.8	∞	918398	5.1304 ng/ml
THC-COOH	1.655	117477	201.75	52.2	∞	257737	18.7673 ng/ml
THC	3.691	274920	1380.58	28.4	151.99	6848266	4.8083 ng/ml

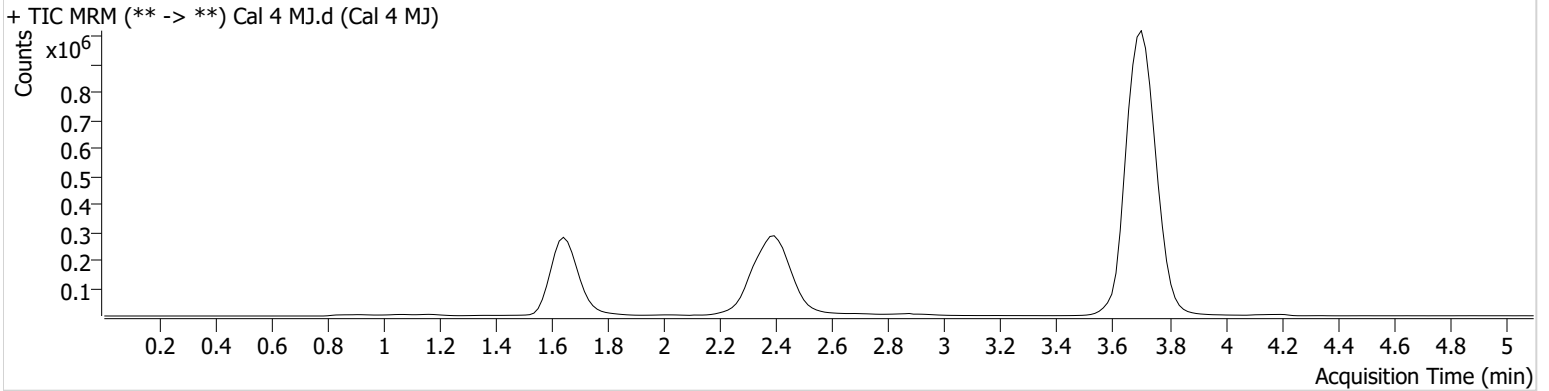
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-E6	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 1:28:12 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	153024	∞	12.0	145.85	953971	9.9832 ng/ml
THC-COOH	1.670	307031	∞	53.6	1502.52	255204	50.6490 ng/ml
THC	3.706	567112	∞	27.6	238.69	6945123	9.5340 ng/ml

AM #27 Cannabinoid Quant. Results

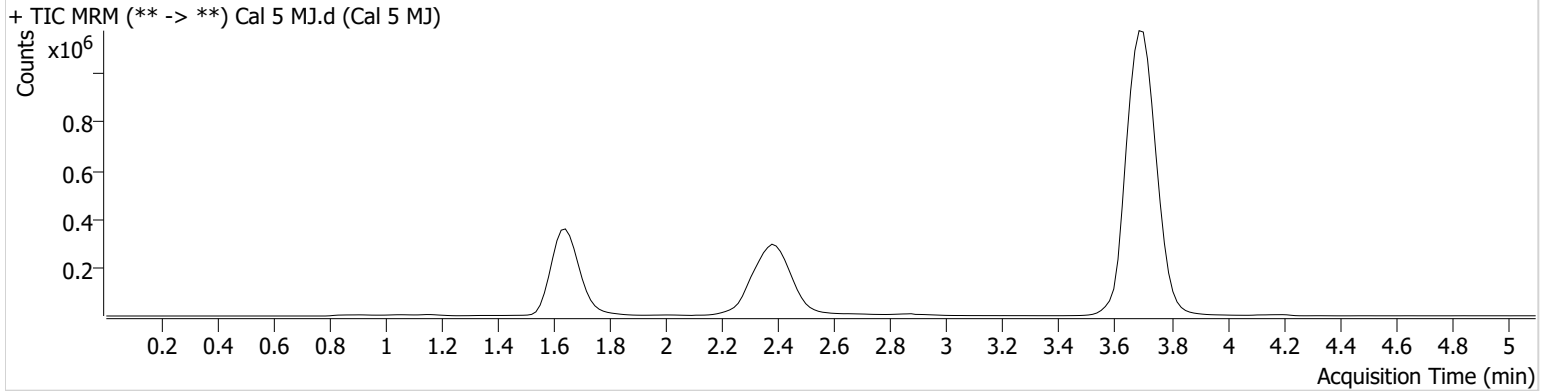


Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-D6	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 1:35:50 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	386763	∞	12.8	∞	986417	24.9057 ng/ml
THC-COOH	1.670	465229	∞	54.8	805.08	265690	74.0264 ng/ml
THC	3.706	1491473	1569.58	27.4	2920.08	6965466	24.6144 ng/ml

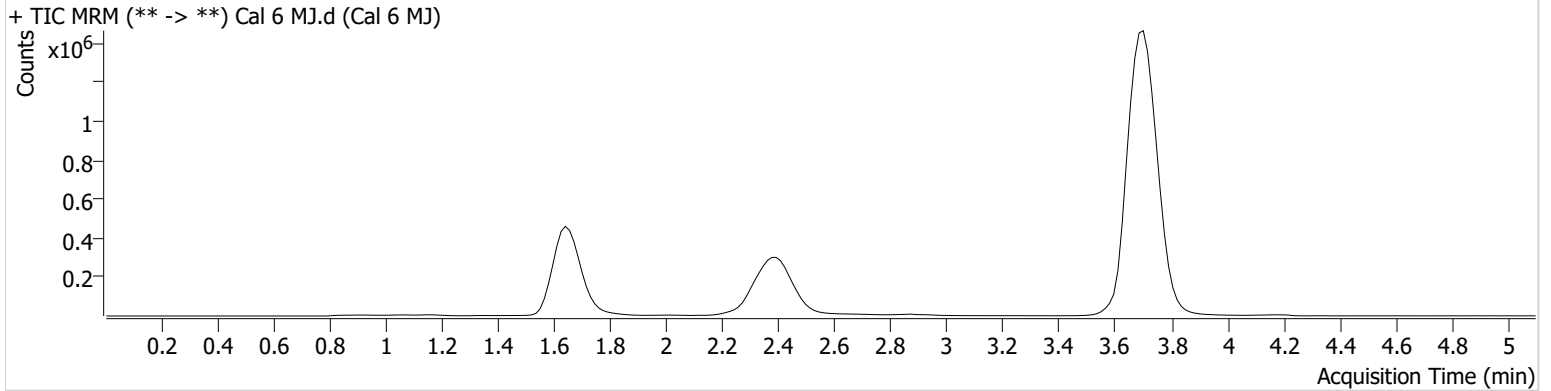
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-C6	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 1:43:26 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	750027	∞	13.2	2563.09	964269	49.7503 ng/ml
THC-COOH	1.670	612972	∞	54.9	∞	258296	100.5681 ng/ml
THC	3.706	3067041	7021.95	27.9	5771.46	7084164	49.5251 ng/ml

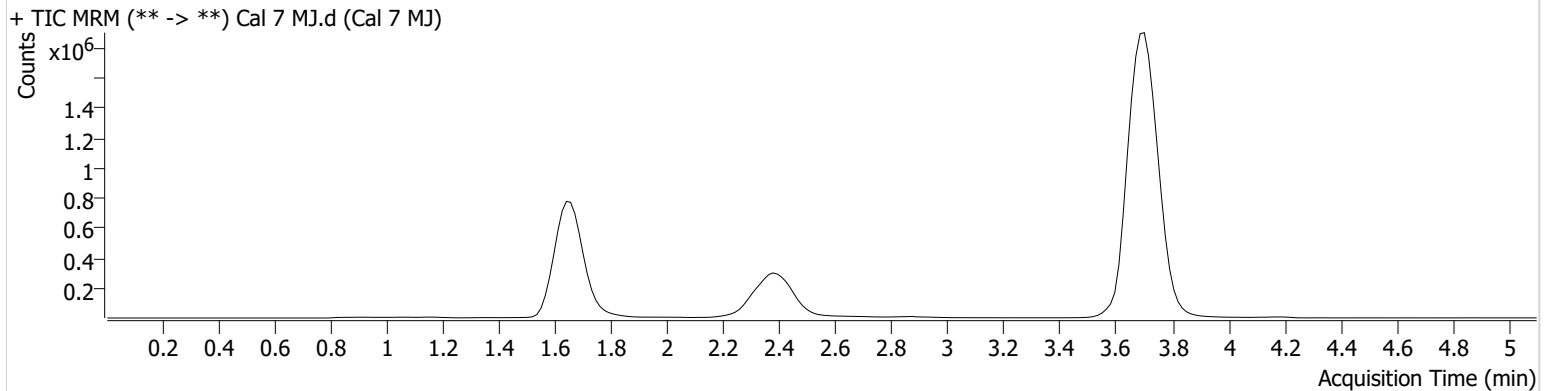
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\080522 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 10:19:55 AM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Celena Shrum
Sample Position	P5-B6	Comment	
Injection Volume	10		
Acq. Date-Time	8/5/2022 1:51:02 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	1493186	∞	13.2	5187.17	955688	100.2858 ng/ml
THC-COOH	1.670	1465240	∞	56.1	7383.79	248677	250.7023 ng/ml
THC	3.706	6053473	20004.71	27.8	∞	6807860	101.4649 ng/ml