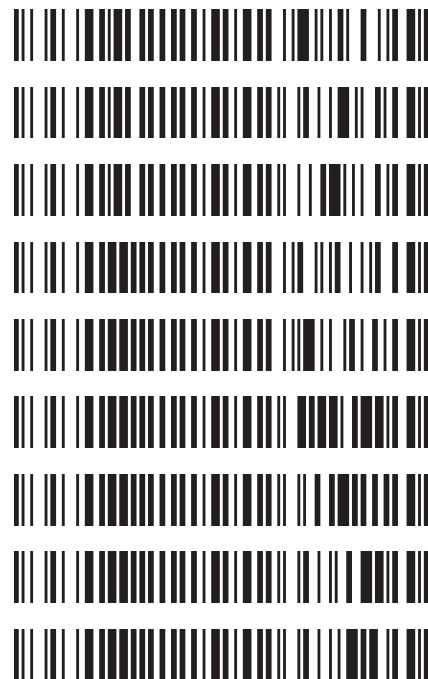


Worklist: 6033

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2022-2512	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-2522	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-2610	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1765	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1803	1	BLOOD	AM 27 Blood THC Quant by LC-QQQ
P2022-1921	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1972	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1990	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-1996	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: 07/18/2022

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 22B2015-1

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	cal 1ng	QC 2	P2022-1972-1			
b	cal 3 ng	Blood NEG	P2022-1990-1			
c	cal 5 ng	M2022-2512-1	P2022-1996-1			
d	cal 10ng	M2022-2522-2				
e	cal 25 ng	M2022-2610-2				
f	cal 50 ng	P2022-1765-2				
g	cal 100 ng	P2022-1803-1				
h	QC 1	P2022-1921-1				

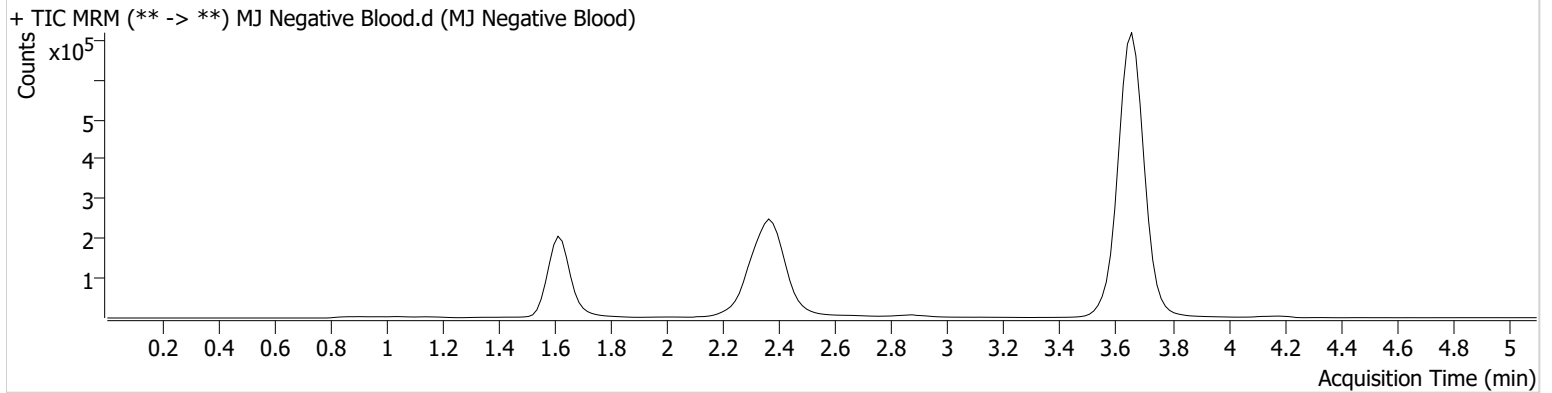
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-B2	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 6:47:11 PM		
Sample Info.			

Sample Chromatogram



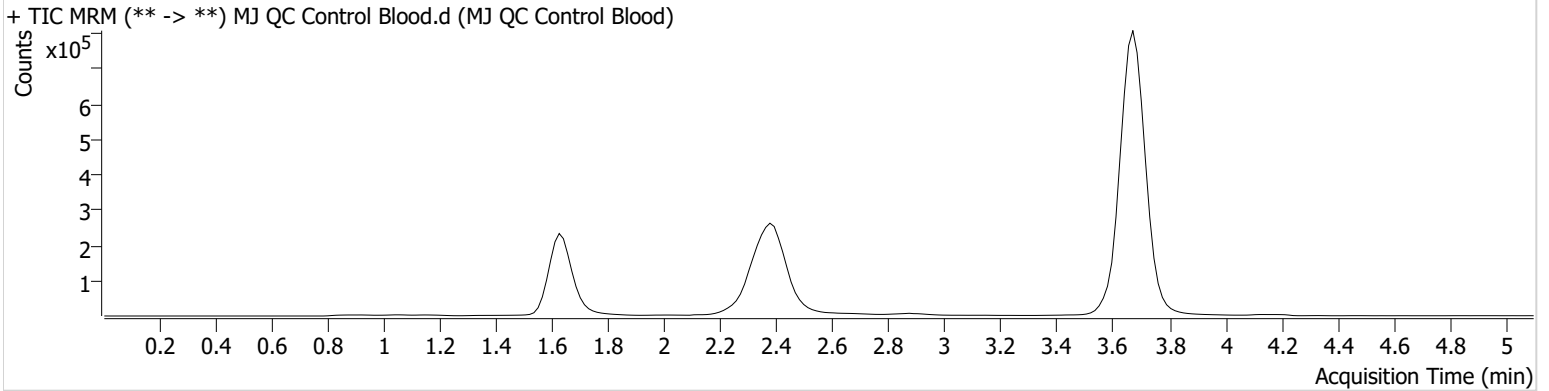
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-H1	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 6:31:55 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	58071	354.59	13.4	177.85	865816	4.8203 ng/ml
THC-COOH	1.670	97007	∞	50.7	184.99	266328	14.9365 ng/ml
THC	3.676	203352	3461.46	28.5	187.64	4918190	4.8481 ng/ml

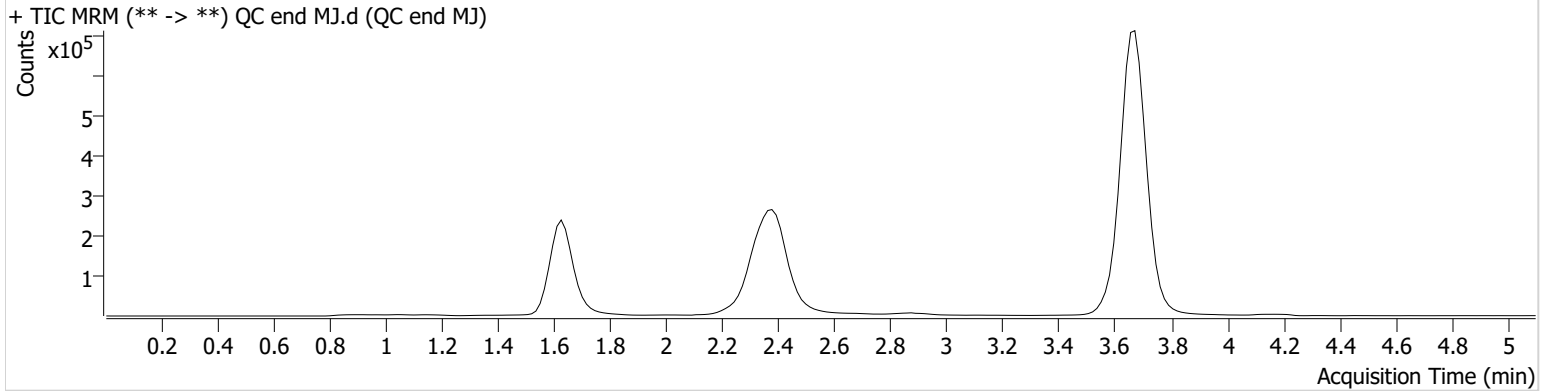
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-A2	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 9:19:30 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	60667	∞	13.1	112.00	900306	4.8419 ng/ml
THC-COOH	1.655	92104	130.27	55.6	370.81	272655	13.7858 ng/ml
THC	3.676	179719	602.82	28.1	98.28	4499521	4.6902 ng/ml

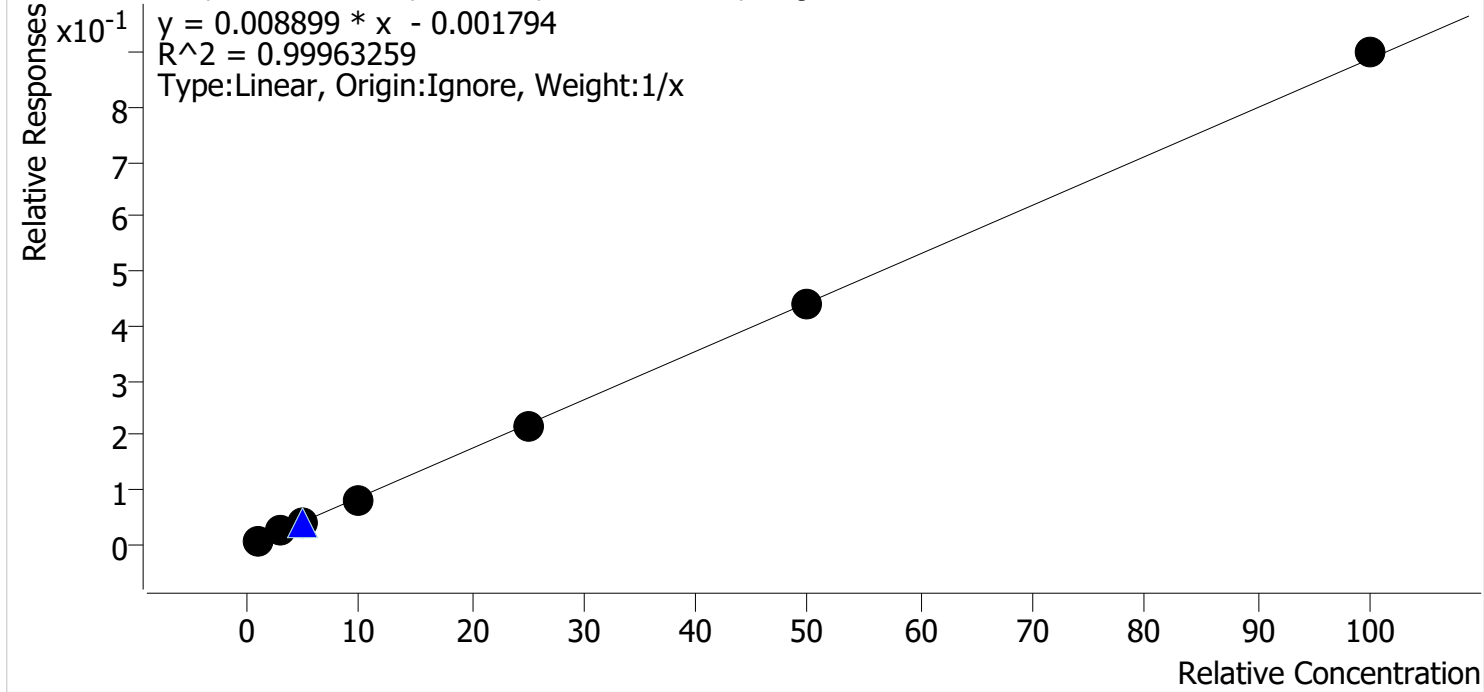
CS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/22/2022 8:33 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

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

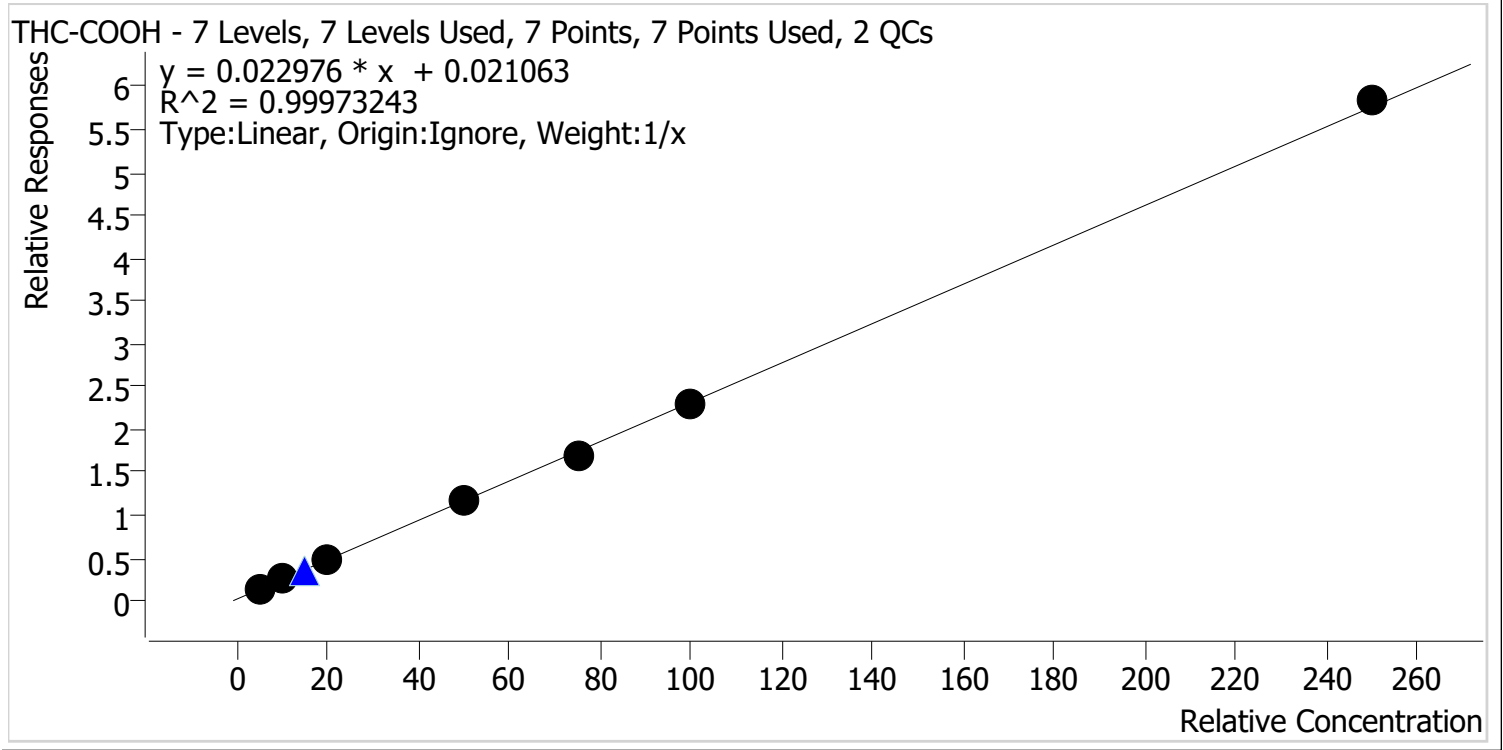


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	109.3
Cal 2 MJ	2	✓	3.0	2.9	97.9
Cal 3 MJ	3	✓	5.0	4.9	98.8
Cal 4 MJ	4	✓	10.0	9.5	95.4
Cal 5 MJ	5	✓	25.0	24.5	97.9
Cal 6 MJ	6	✓	50.0	49.7	99.5
Cal 7 MJ	7	✓	100.0	101.3	101.3



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/22/2022 8:33 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

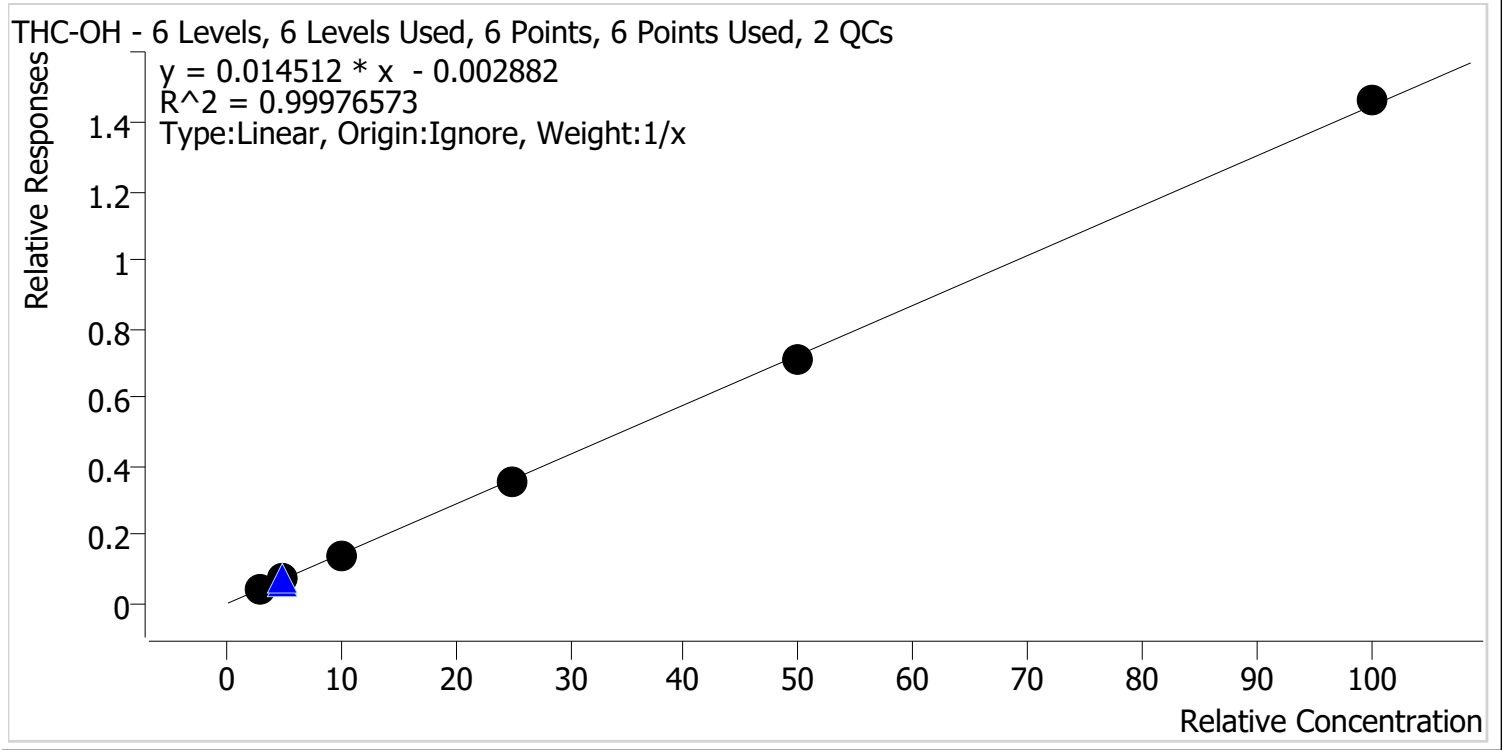


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.2	103.6
Cal 2 MJ	2	✓	10.0	9.9	99.5
Cal 3 MJ	3	✓	20.0	19.9	99.7
Cal 4 MJ	4	✓	50.0	49.7	99.5
Cal 5 MJ	5	✓	75.0	73.6	98.1
Cal 6 MJ	6	✓	100.0	98.4	98.4
Cal 7 MJ	7	✓	250.0	253.2	101.3



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/22/2022 8:33 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	3.1	103.3
Cal 3 MJ	3	✓	5.0	5.0	100.8
Cal 4 MJ	4	✓	10.0	9.7	97.1
Cal 5 MJ	5	✓	25.0	24.8	99.2
Cal 6 MJ	6	✓	50.0	49.3	98.7
Cal 7 MJ	7	✓	100.0	101.0	101.0

Cal 1 removed due to peak shape (it is also not within the curve range for the method).

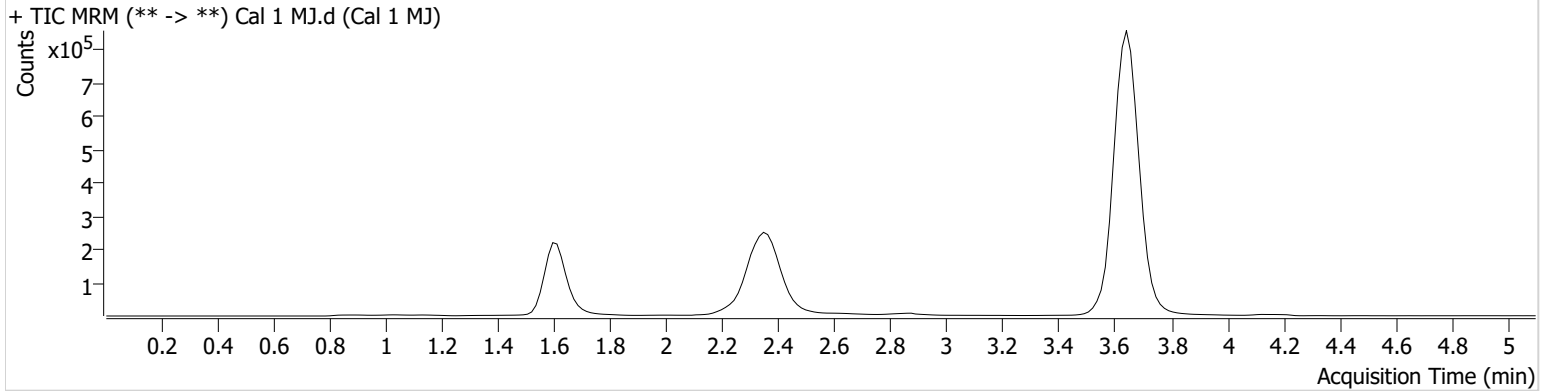
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-A1	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 5:30:57 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.640	38028	∞	45.8	396.96	271507	5.1794 ng/ml
THC	3.661	42849	409.65	32.7	46.83	5403620	1.0927 ng/ml

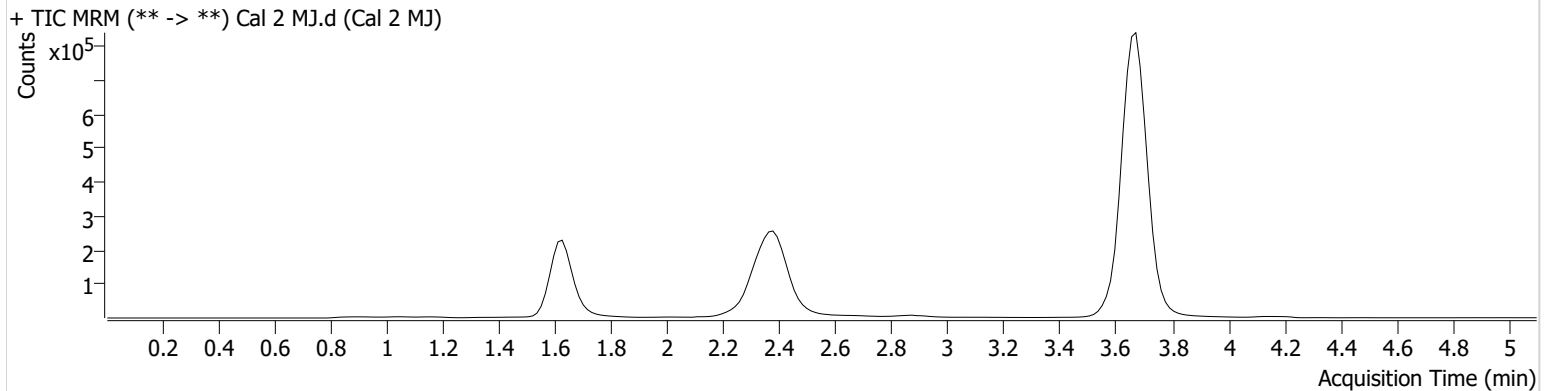
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-B1	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 5:38:43 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	37816	∞	14.4	46.31	898759	3.0979 ng/ml
THC-COOH	1.655	68941	1820.43	49.0	174.19	276219	9.9464 ng/ml
THC	3.676	129366	1188.31	30.7	∞	5316982	2.9358 ng/ml

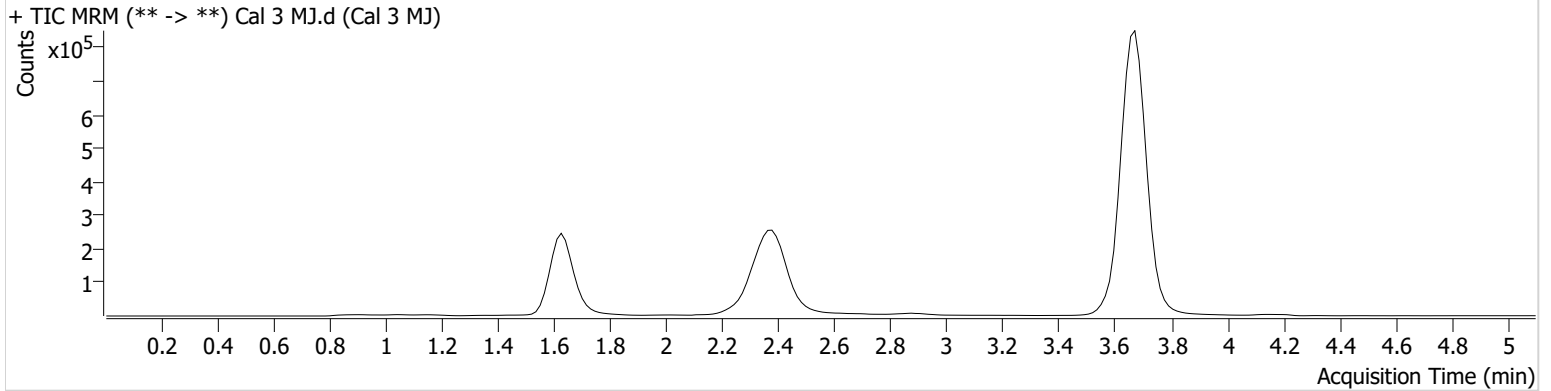
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-C1	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 5:46:19 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	62636	∞	13.3	1504.93	891525	5.0398 ng/ml
THC-COOH	1.655	130602	∞	52.7	220.31	272596	19.9358 ng/ml
THC	3.676	221416	591.68	28.2	350.05	5249297	4.9417 ng/ml

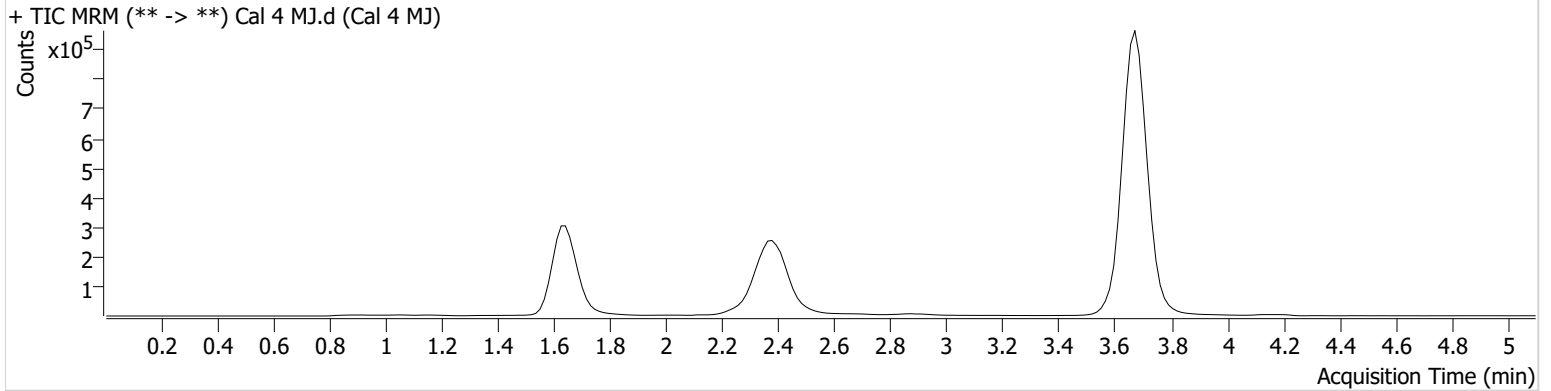
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-D1	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 5:53:55 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	125173	365.56	13.0	370.83	907127	9.7069 ng/ml
THC-COOH	1.670	317722	∞	56.4	∞	272964	49.7441 ng/ml
THC	3.676	456524	4489.00	28.2	449.82	5495718	9.5367 ng/ml

AM #27 Cannabinoid Quant. Results

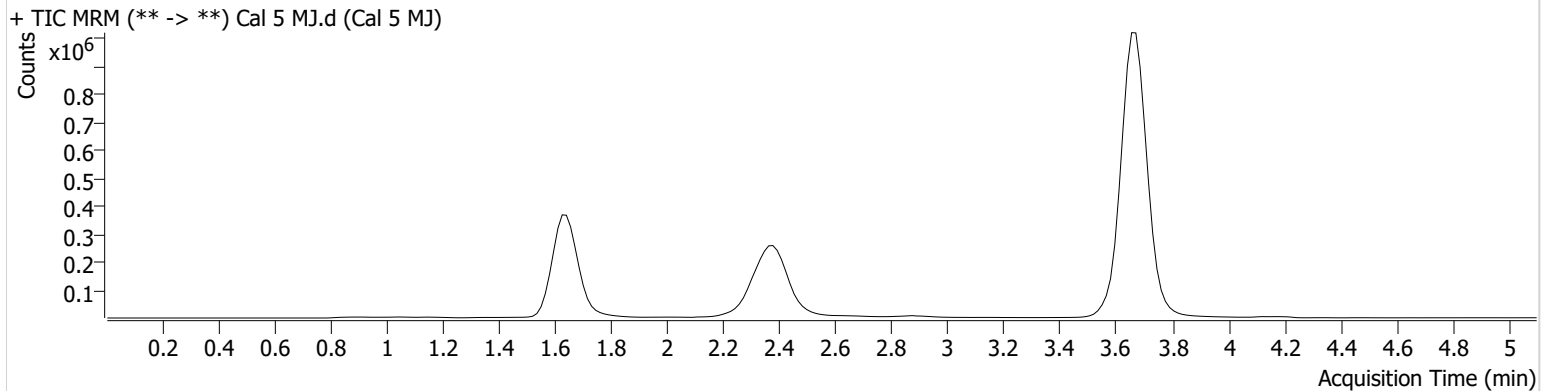


Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 AM

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-E1	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 6:01:31 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	319132	∞	13.2	904.88	894098	24.7936 ng/ml
THC-COOH	1.655	462978	∞	57.4	1824.40	270441	73.5939 ng/ml
THC	3.676	1143984	6222.54	27.2	∞	5295092	24.4804 ng/ml

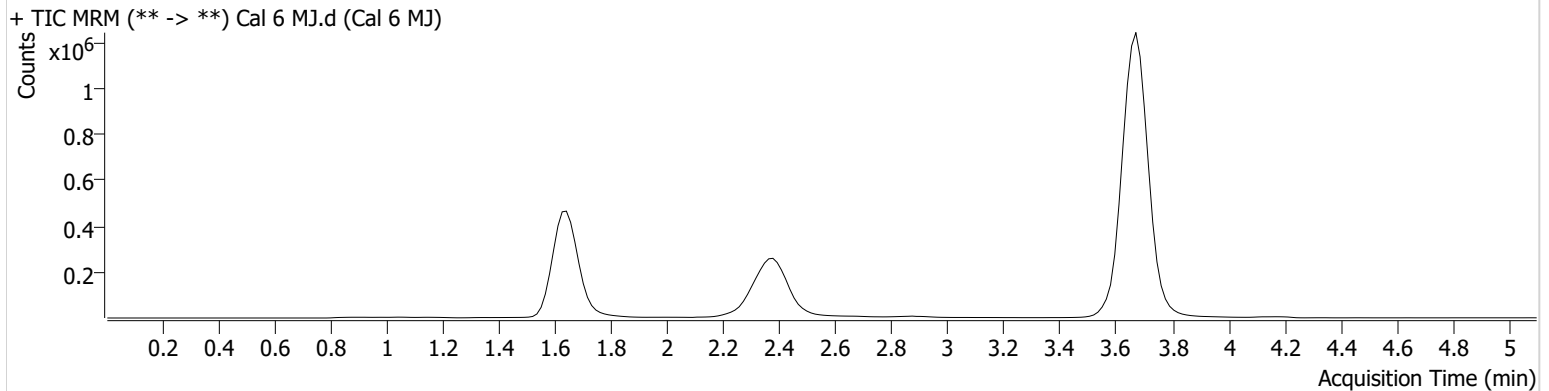
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-F1	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 6:09:07 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	640008	∞	13.5	2053.13	897537	49.3342 ng/ml
THC-COOH	1.655	605295	∞	57.0	∞	265368	98.3601 ng/ml
THC	3.676	2298731	3492.93	27.9	601.08	5215487	49.7323 ng/ml

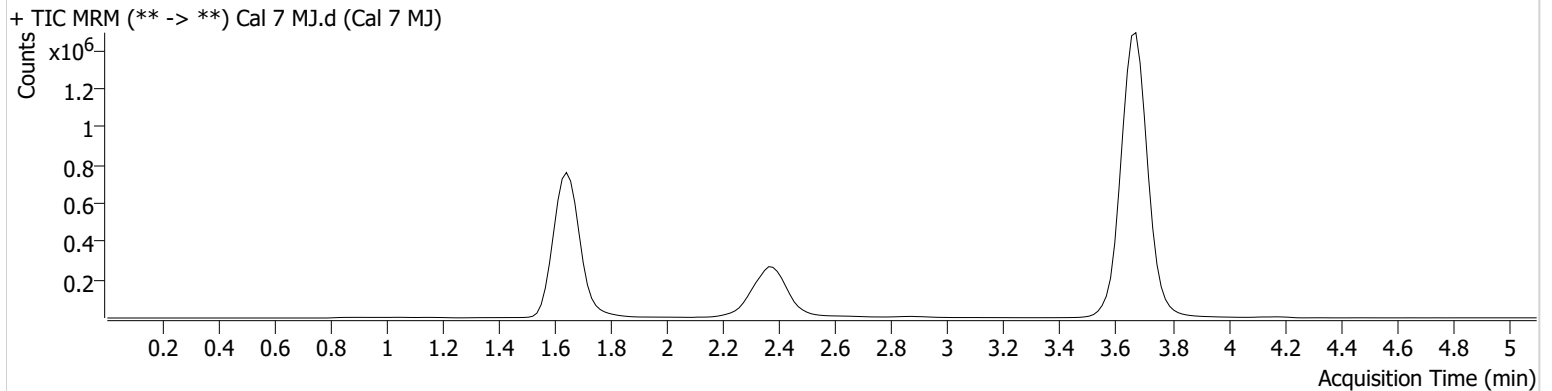
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2022\AM 27-28\071822 AM 27 28 CS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/22/2022 8:33:45 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-G1	Comment	
Injection Volume	10		
Acq. Date-Time	7/18/2022 6:16:43 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	1224644	∞	13.4	2235.03	836927	101.0275 ng/ml
THC-COOH	1.655	1386575	3708.50	57.5	2556.57	237449	253.2404 ng/ml
THC	3.676	4184829	82333.98	27.5	20446.4 1	4652645	101.2802 ng/ml