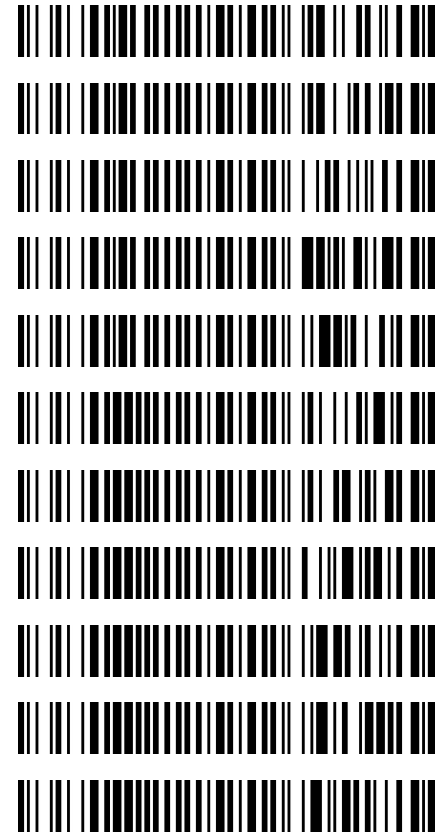


REVIEWED
By Sarah Collins at 9:48 am, Aug 11, 2022

8/8/2022

Worklist: 6055

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
M2022-2776	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-2777	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-2995	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3002	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2022-3139	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2217	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2223	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2324	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2371	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2372	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2022-2406	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: 8/8/2022

Plate lot#: 220309

Mobile phase A: 0.1% Formic Acid in LCMS Water

Blank Blood Lot: Lampire 22B52015-1

LCMS-QQQ ID: 069901

Analyst: Amber Gerheart

Plate Retest Date: 09/09/2022

Mobile phase B: 0.1% Formic acid in Acetonitrile

Column: UCT Selectra DA 100 x 2.1mm 3um

Blank Urine Lot: N/A

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: 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: 800 µL
- 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: Calibration Curve Range: THC-OH 3-100 ng/mL

	1	2	3	4	5	6
A	IS + Cal. 1	IS + QC_1	P2022-2223-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	Blood Negative	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	M2022-2776-1	P2022-2324-1	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	M2022-2777-1	P2022-2371-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	M2022-2995-1	P2022-2372-1	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	M2022-3002-2	P2022-2406-1	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	M2022-3139-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2022-2217-1	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

AG

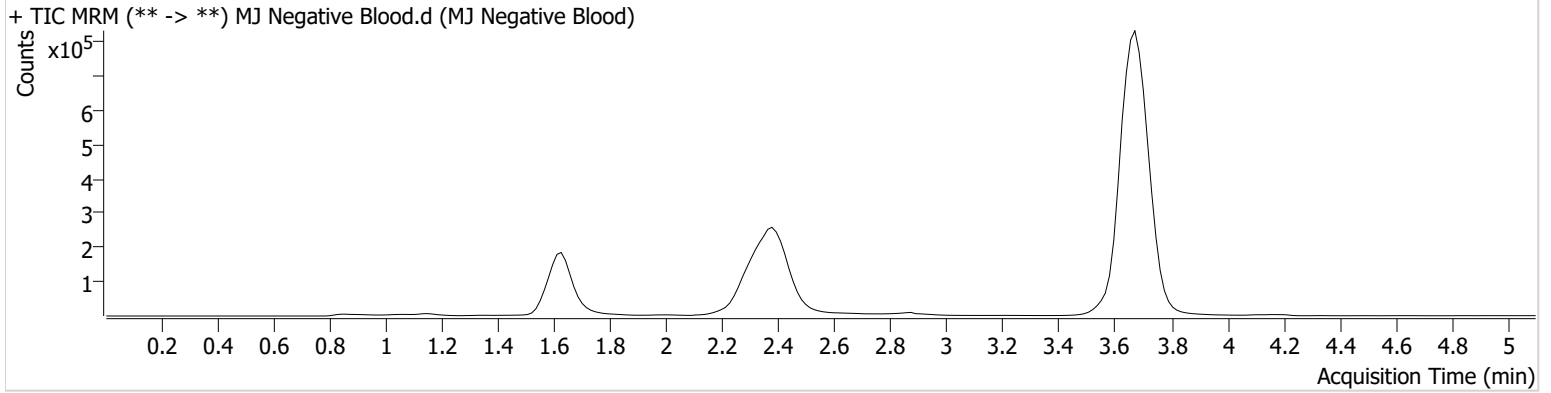


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	MJ Negative Blood.d
Type	Sample	Sample	MJ Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-B2	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 6:04:49 PM		
Sample Info.			

Sample Chromatogram



AG



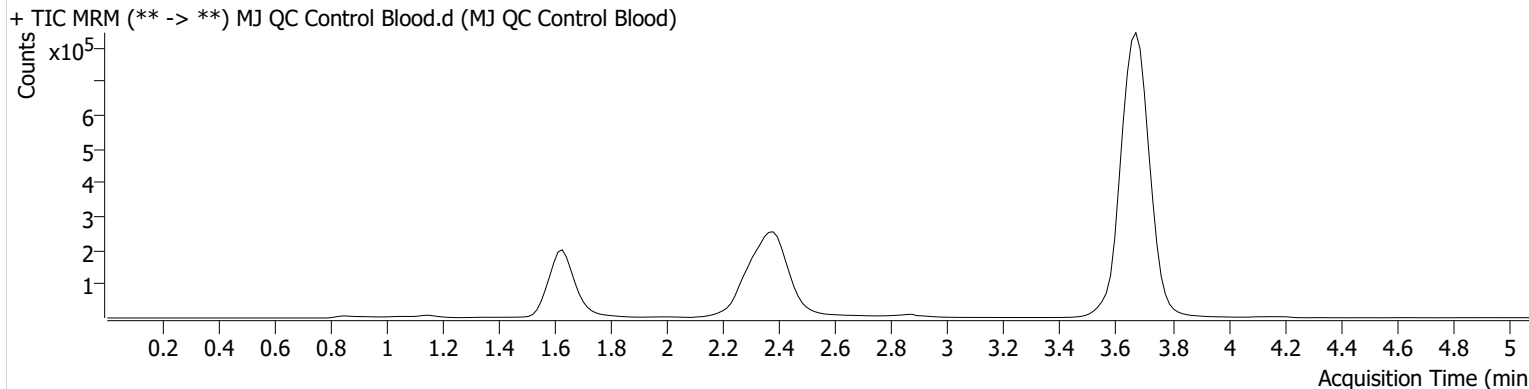
AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	MJ QC Control Blood.d
Type	QC	Sample	MJ QC Control Blood
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 5:49:36 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	67138	230.09	12.4	222.24	861560	4.5494 ng/ml
THC-COOH	1.655	81629	242.70	54.2	630.63	235523	14.9655 ng/ml
THC	3.676	239507	∞	28.5	∞	5800076	4.8857 ng/ml

AG

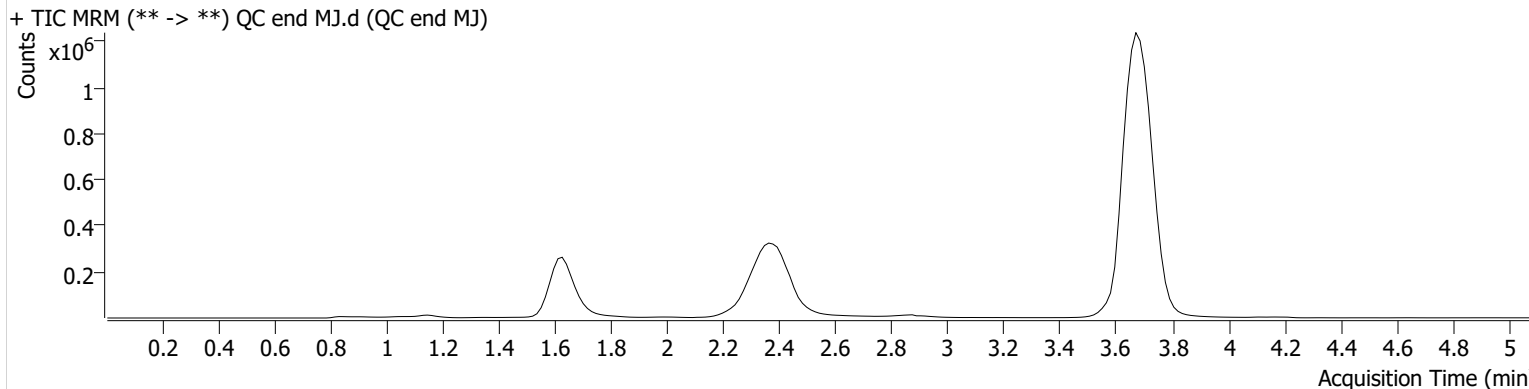


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	QC end MJ.d
Type	QC	Sample	QC end MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 9:07:28 PM		

Sample Chromatogram



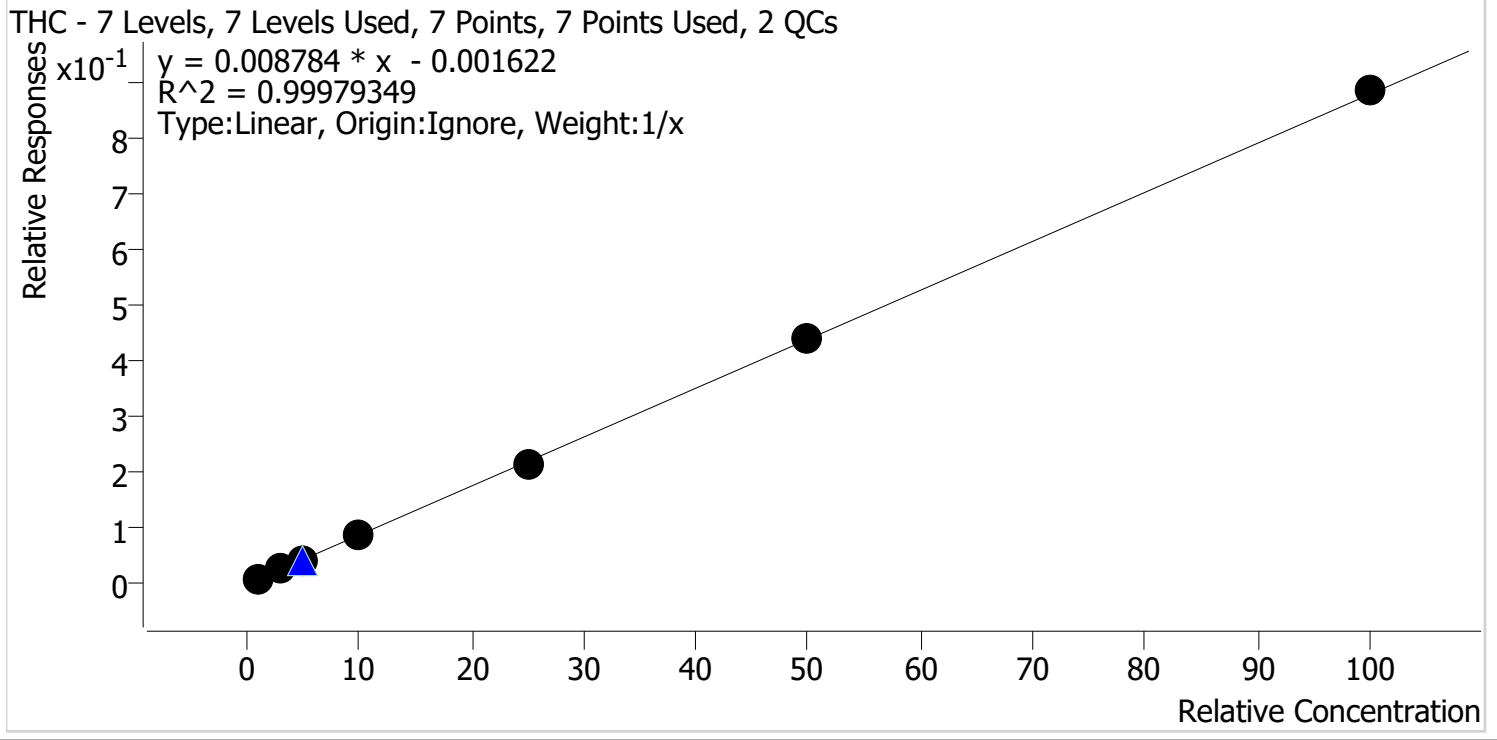
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	88354	∞	11.6	160.33	1100210	4.7060 ng/ml
THC-COOH	1.655	98011	955.74	58.3	∞	291998	14.4881 ng/ml
THC	3.691	356147	1077.89	28.9	806.97	8580031	4.9102 ng/ml

AA



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 8/10/2022 3:43 PM
Analyst Name ISP\lagerheart
Analyte THC **Internal Standard** THC-D3



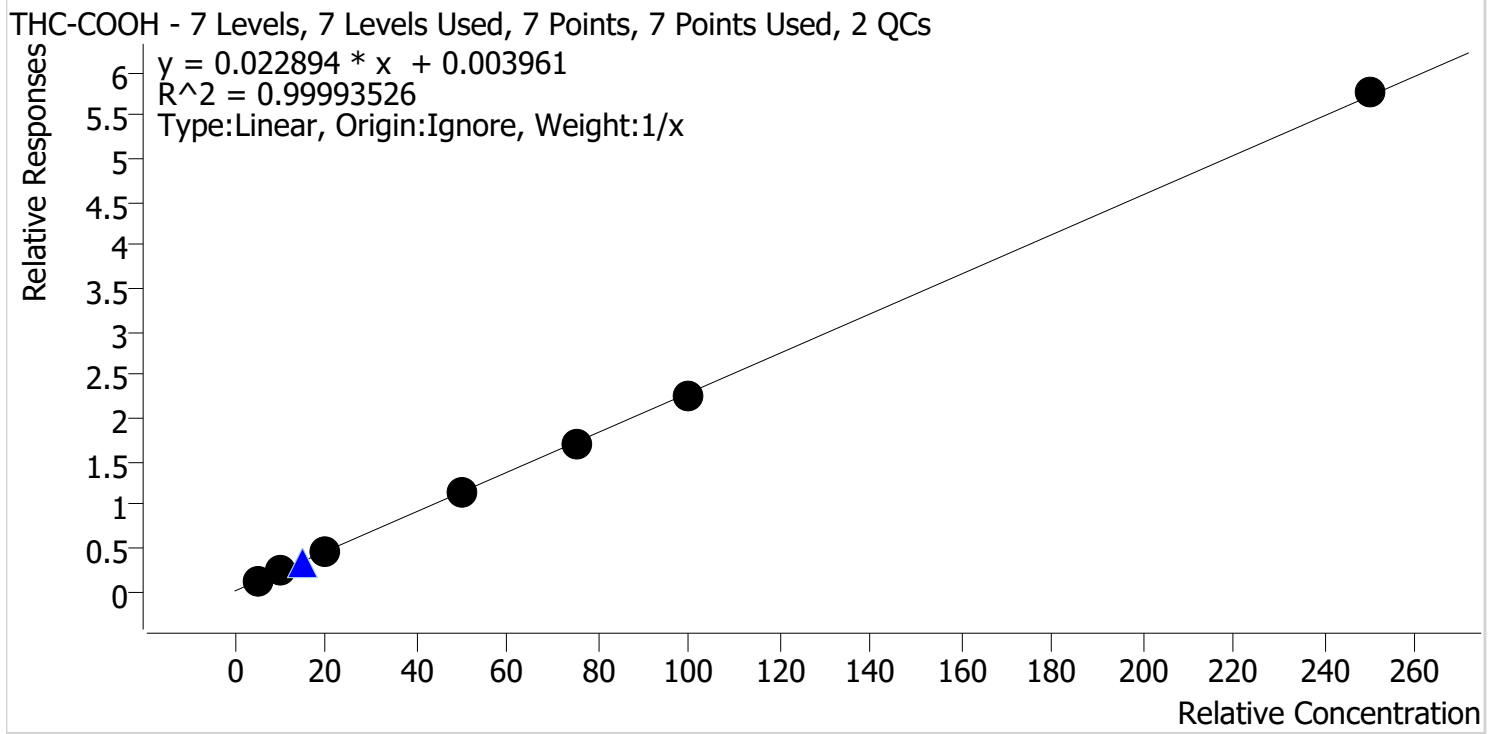
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	1.0	1.1	108.8
Cal 2 MJ	2	✓	3.0	2.9	97.9
Cal 3 MJ	3	✓	5.0	4.8	96.9
Cal 4 MJ	4	✓	10.0	9.7	97.0
Cal 5 MJ	5	✓	25.0	24.6	98.5
Cal 6 MJ	6	✓	50.0	50.1	100.2
Cal 7 MJ	7	✓	100.0	100.7	100.7

AA



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 8/10/2022 3:43 PM
Analyst Name ISP\lagerheart
Analyte THC-COOH **Internal Standard** THC-COOH-D9



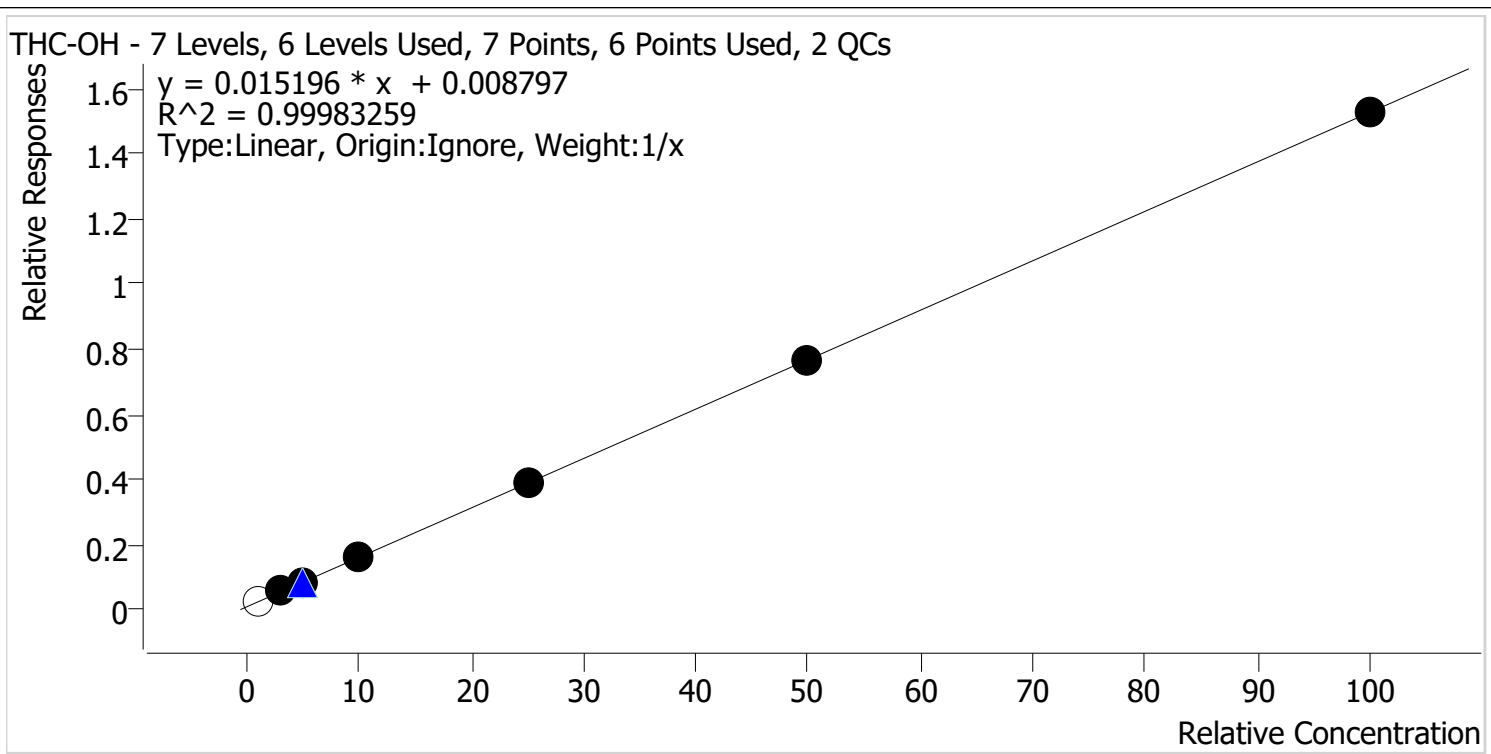
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	✓	5.0	5.1	101.2
Cal 2 MJ	2	✓	10.0	10.1	100.7
Cal 3 MJ	3	✓	20.0	19.9	99.6
Cal 4 MJ	4	✓	50.0	49.7	99.3
Cal 5 MJ	5	✓	75.0	74.5	99.3
Cal 6 MJ	6	✓	100.0	99.1	99.1
Cal 7 MJ	7	✓	250.0	251.6	100.7

AA



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results C:\Users\lagerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Last Cal. Update 8/10/2022 3:43 PM
Analyst Name ISP\lagerheart
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	1.0	1.2	123.1
Cal 2 MJ	2	✓	3.0	3.1	104.4
Cal 3 MJ	3	✓	5.0	4.8	95.5
Cal 4 MJ	4	✓	10.0	9.9	98.7
Cal 5 MJ	5	✓	25.0	25.4	101.6
Cal 6 MJ	6	✓	50.0	49.9	99.8
Cal 7 MJ	7	✓	100.0	99.9	99.9

Cal 1 dropped due to ratio being out

AG



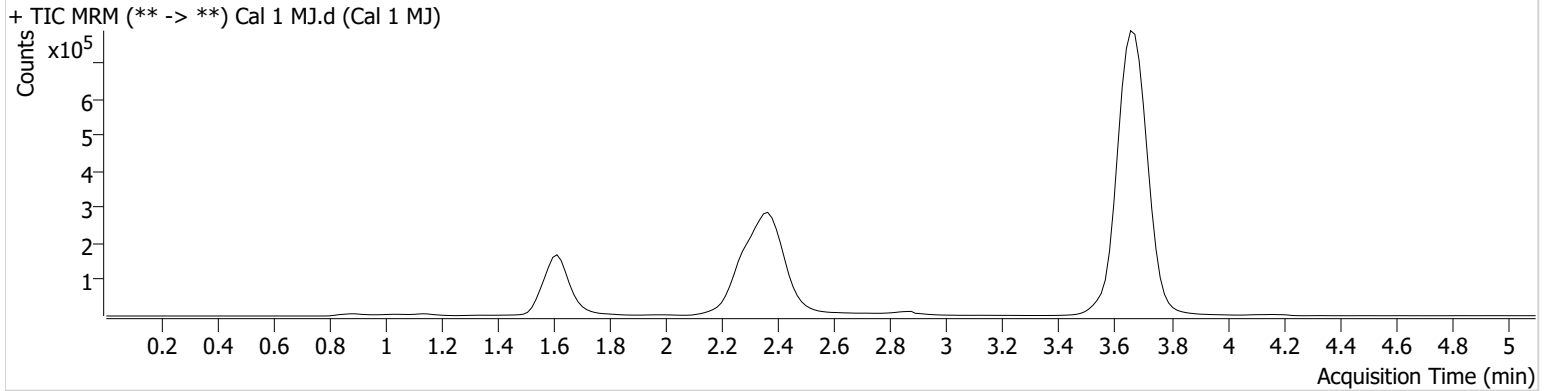
AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	Cal 1 MJ.d
Type	Cal	Sample	Cal 1 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 4:48:37 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.678	22326	∞	8.1 Low	9.34 Low	811595	1.2314 ng/ml Low
THC-COOH	1.655	27427	40.60	46.8	158.87	229011	5.0582 ng/ml
THC	3.676	46819	92.31	32.2	∞	5899114	1.0882 ng/ml

AG

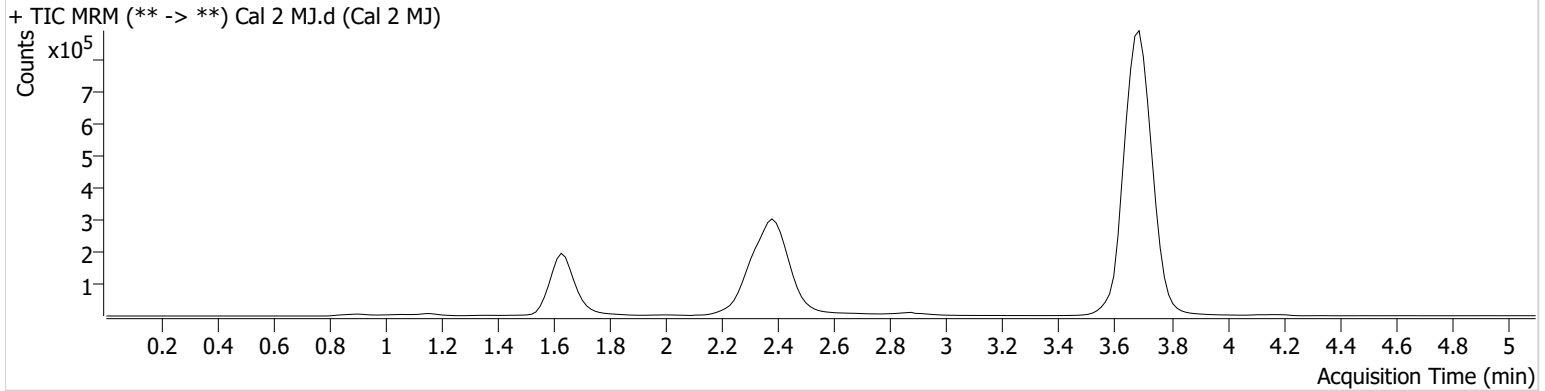


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	Cal 2 MJ.d
Type	Cal	Sample	Cal 2 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-B1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 4:56:22 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.648	46941	61.39	11.9	39.97	832221	3.1330 ng/ml
THC-COOH	1.670	54870	∞	53.9	300.52	233881	10.0743 ng/ml
THC	3.691	147395	2033.90	28.4	91.67	6094029	2.9382 ng/ml

AG

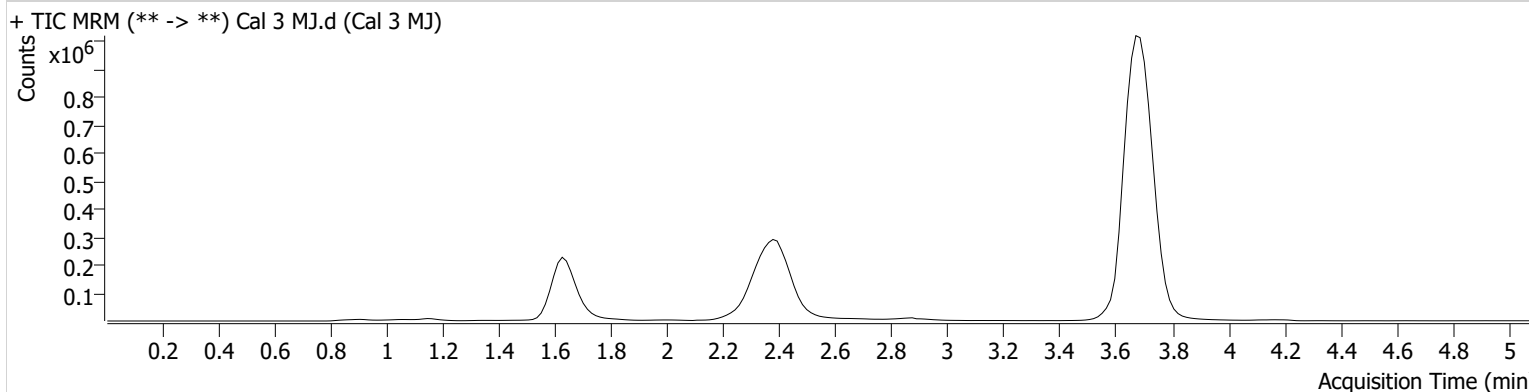


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	Cal 3 MJ.d
Type	Cal	Sample	Cal 3 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 5:03:58 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	72748	74.68	12.5	427.22	893823	4.7773 ng/ml
THC-COOH	1.670	113344	776.12	53.3	697.22	246339	19.9243 ng/ml
THC	3.691	286666	1281.56	28.8	∞	7004429	4.8439 ng/ml

AG



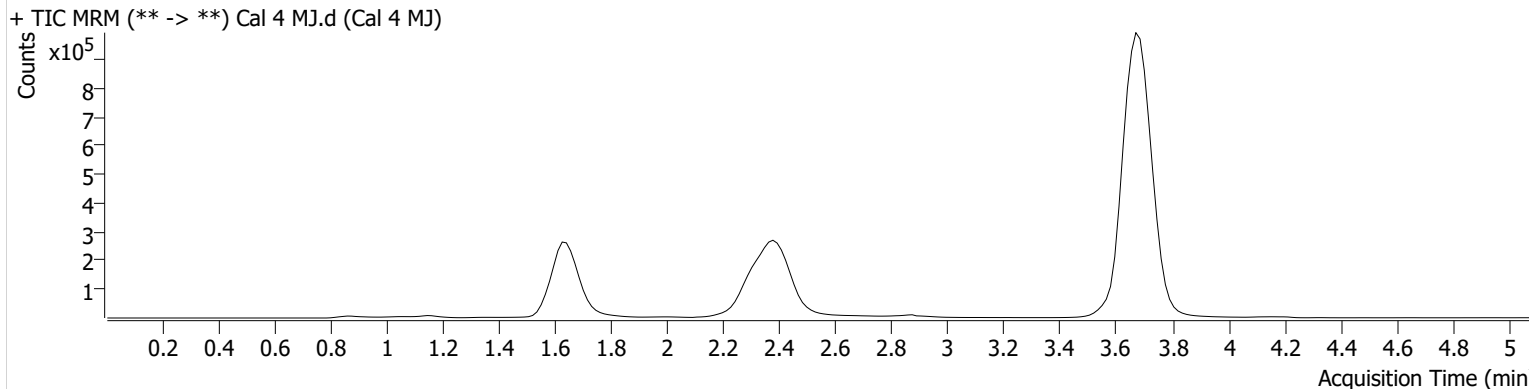
AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	Cal 4 MJ.d
Type	Cal	Sample	Cal 4 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-D1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 5:11:34 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	143445	577.57	12.4	281.93	903138	9.8735 ng/ml
THC-COOH	1.670	282748	1802.96	55.5	860.80	247783	49.6695 ng/ml
THC	3.691	547611	4014.75	27.8	637.93	6552961	9.6982 ng/ml

AG



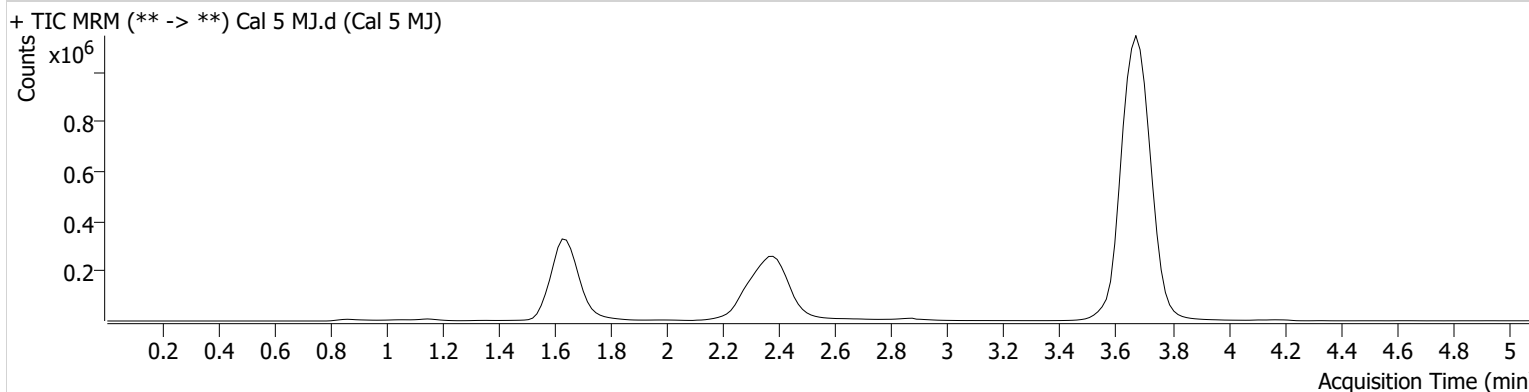
AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	Cal 5 MJ.d
Type	Cal	Sample	Cal 5 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 5:19:10 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	354042	∞	12.8	599.97	897199	25.3898 ng/ml
THC-COOH	1.655	414013	2973.90	56.5	715.79	242141	74.5092 ng/ml
THC	3.676	1428532	5516.87	27.9	1242.74	6654445	24.6239 ng/ml

AG

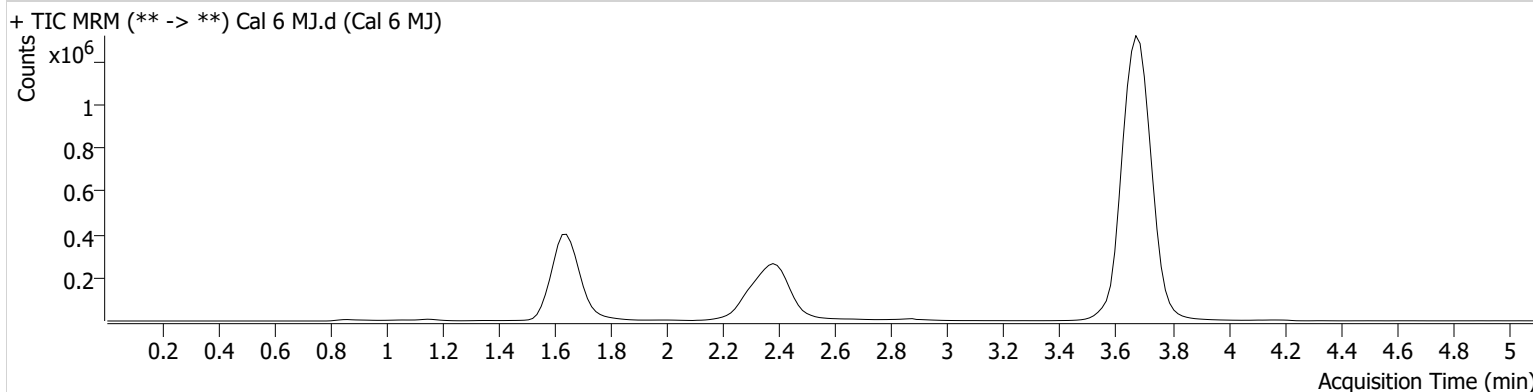


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	Cal 6 MJ.d
Type	Cal	Sample	Cal 6 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 5:26:46 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	669023	∞	13.3	28598.0	872172	49.9016 ng/ml
THC-COOH	1.670	532924	1481.77	57.1	1377.57	234399	99.1343 ng/ml
THC	3.691	2733600	6095.20	27.7	1362.80	6237259	50.0788 ng/ml

AG

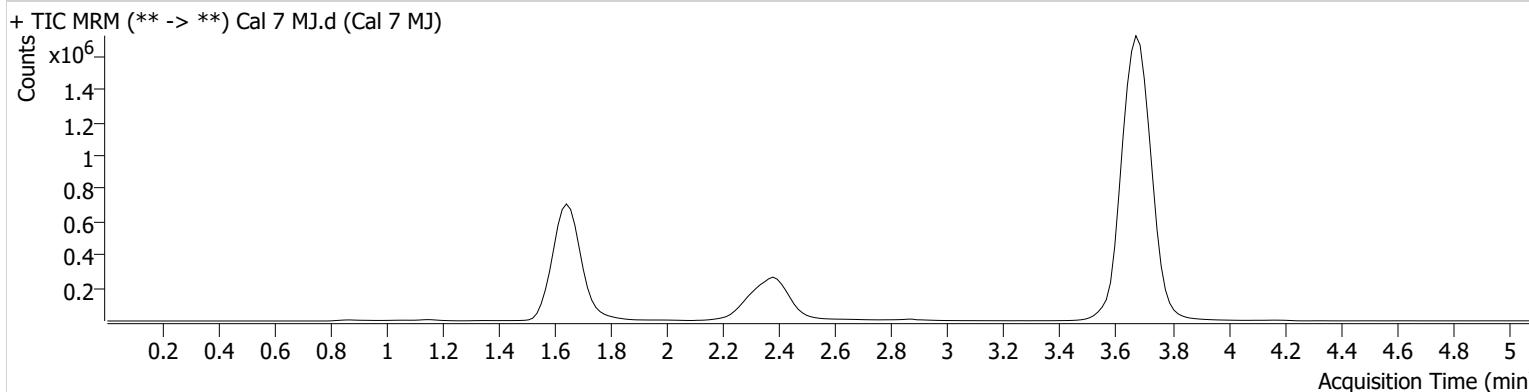


AM #27 Cannabinoid Quant. Results

Batch results C:\Users\agerheart\Desktop\080822 AM 27 28 AG\QuantResults\AM 27.batch.bin
Calibration Last Update 8/10/2022 3:43:29 PM

Instrument	Falco (069901)	Data File	Cal 7 MJ.d
Type	Cal	Sample	Cal 7 MJ
Acq. Method	AM 27 THCQ.m	Operator	Amber Gerheart
Sample Position	P1-G1	Comment	
Injection Volume	10		
Acq. Date-Time	8/8/2022 5:34:22 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.633	1340414	∞	13.1	2782.37	877690	99.9249 ng/ml
THC-COOH	1.670	1296498	∞	56.8	7878.41	224896	251.6301 ng/ml
THC	3.691	5323543	20088.63	28.1	∞	6027710	100.7289 ng/ml