

REVIEWED

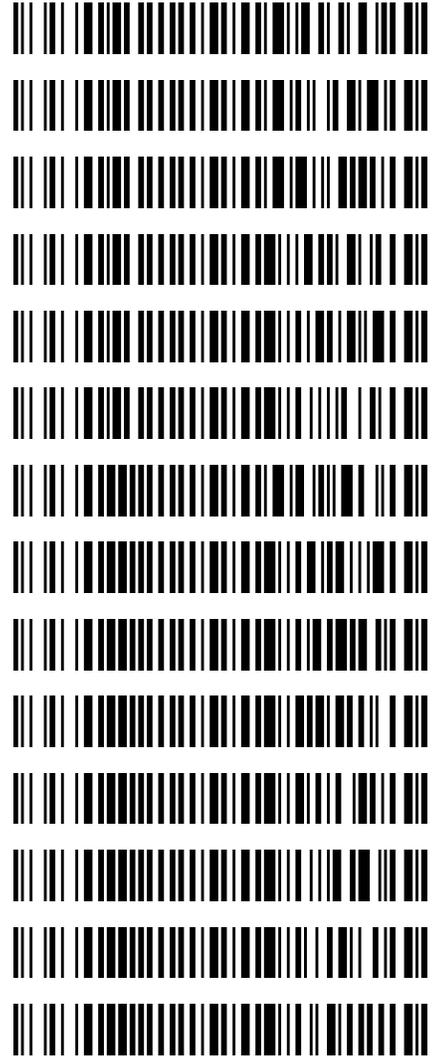
By Sarah Collins at 10:42 am, Oct 30, 2023

10/27/2023

CS

Worklist: 6546

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2023-3011	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4028	1 *	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4210	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4257	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4288	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4350	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-2841	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3094	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3114	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3123	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3132	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3147	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3194	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3219	1	BCK	AM 27 Blood THC Quant by LC-QQQ



*Inadvertently included in this run.

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

Extraction Date: 10/25/2023

Analyst: Celena Shrum

Plate lot#: 230627

Plate Retest Date: 12/27/2023

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 23E52981

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: #42**
- 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: THC curve limits 3-100 Cal 1 was dropped due to ratio and S/N. Due to the accuracy of the end QC being between 20 and 30%, the samples will be reported qualitatively between 3 and 5ng/mL.

	1	2	3	4	5	6
a				M2023-3011-1	P2023-3114-2	QC 1
b				M2023-4028-1	P2023-3123-2	cal 100 ng
c				M2023-4210-2	P2023-3132-1	cal 50 ng
d				M2023-4257-1	P2023-3147-1	cal 25 ng
e				M2023-4288-2	P2023-3194-1	cal 10ng
f				M2023-4350-2	P2023-3219-1	cal 5 ng
g				P2023-2841-1	NEG Blood	cal 3 ng
h				P2023-3094-1	QC 2	cal 1ng

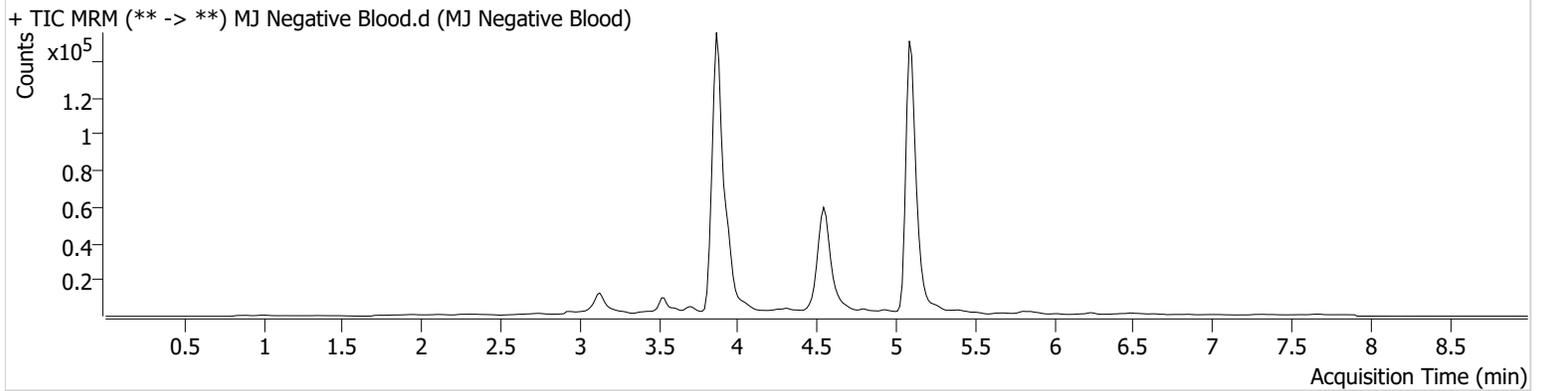


AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Calibration Last Update 10/27/2023 11:35:29 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	Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.
Injection Volume	10		
Acq. Date-Time	10/26/2023 2:54:07 PM		
Sample Info.			

Sample Chromatogram





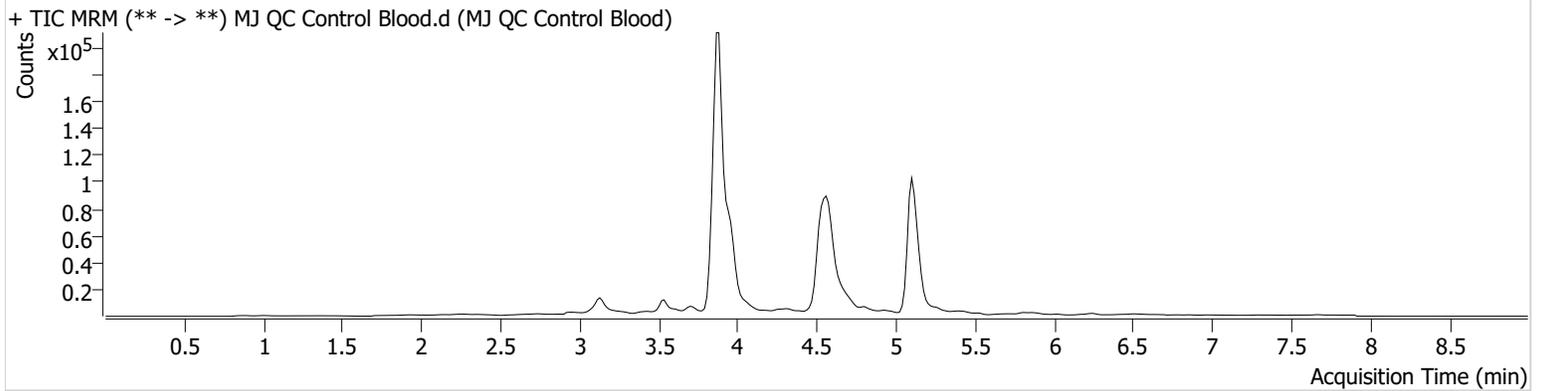
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Calibration Last Update 10/27/2023 11:35:29 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 10/26/2023 2:27:53 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.105	15569	56.91	32.4	∞	406398	4.2444 ng/ml
THC-COOH	3.969	16182	486.94	208.3	134.41	140503	15.0418 ng/ml
THC-OH	3.881	51781	∞	12.2	∞	862833	4.5598 ng/ml



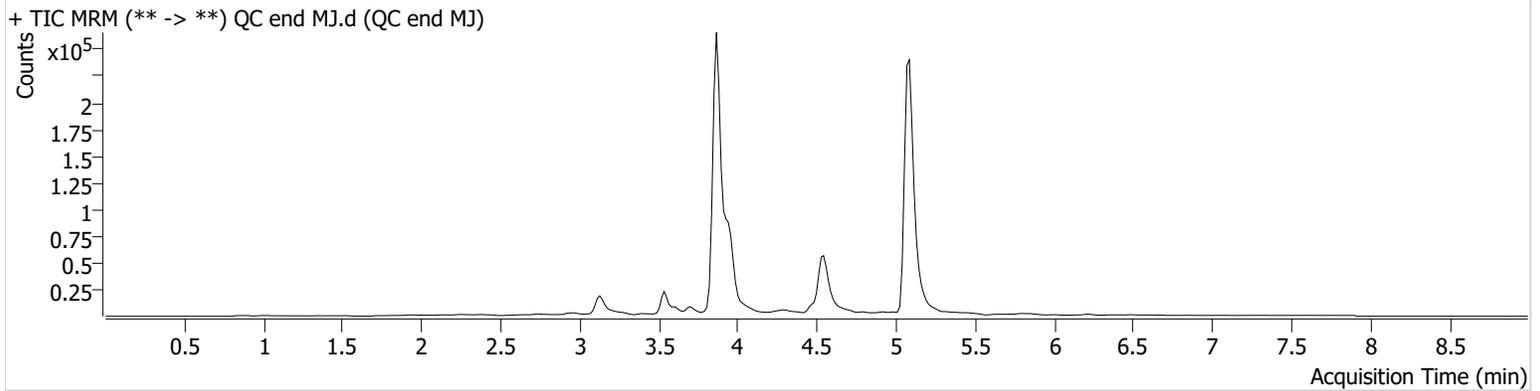
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Calibration Last Update 10/27/2023 11:35:29 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 10/26/2023 9:27:22 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.090	30144	166.82	32.9	∞	925221	3.6098 ng/ml
THC-COOH	3.954	17832	376.82	210.6	1158.68	156575	14.8806 ng/ml
THC-OH	3.865	57487	∞	12.8	∞	897207	4.8451 ng/ml

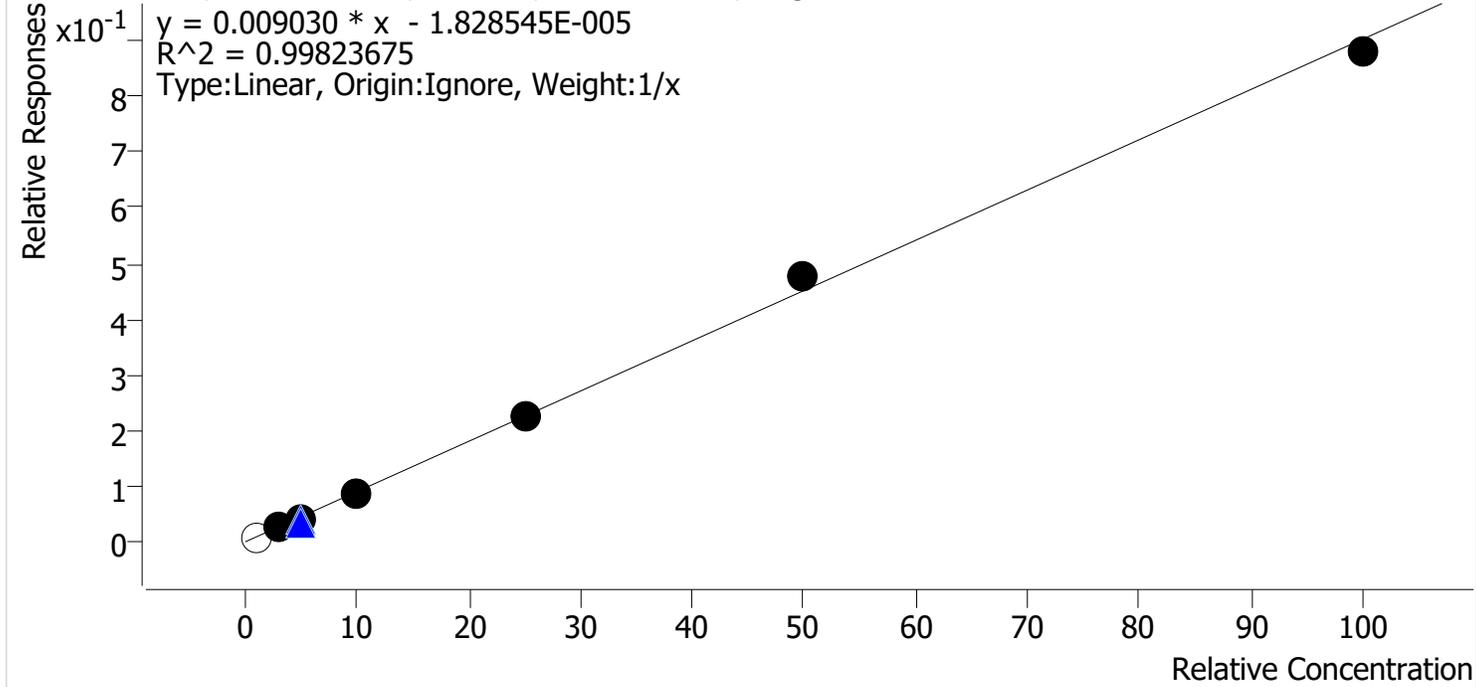
5



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Last Cal. Update 10/27/2023 11:35 AM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3

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

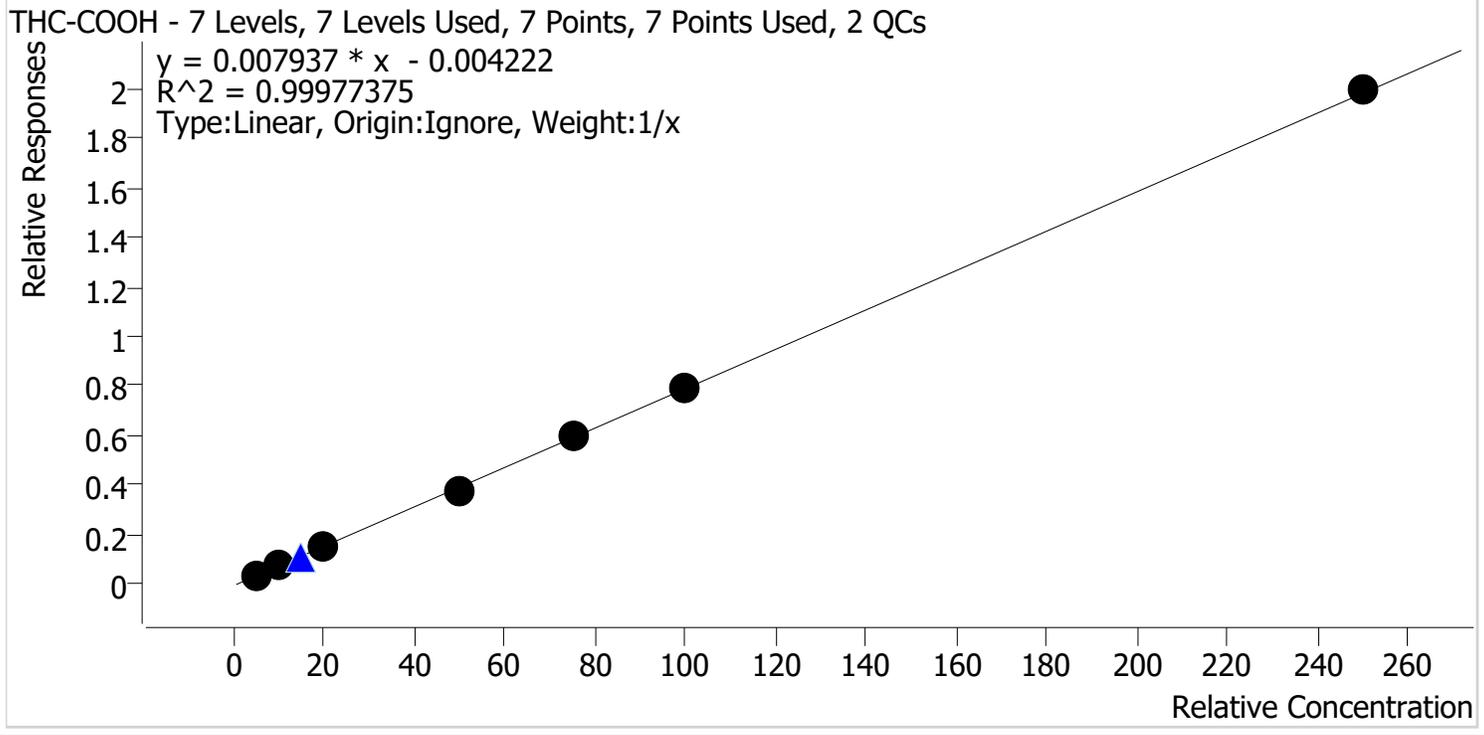


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	x	1.0	0.9	90.3
Cal 2 MJ	2	✓	3.0	3.1	103.0
Cal 3 MJ	3	✓	5.0	4.8	96.0
Cal 4 MJ	4	✓	10.0	9.8	97.7
Cal 5 MJ	5	✓	25.0	25.0	100.1
Cal 6 MJ	6	✓	50.0	52.9	105.8
Cal 7 MJ	7	✓	100.0	97.4	97.4



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Last Cal. Update 10/27/2023 11:35 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	104.0
Cal 2 MJ	2	✓	10.0	9.8	98.5
Cal 3 MJ	3	✓	20.0	20.0	100.1
Cal 4 MJ	4	✓	50.0	48.3	96.6
Cal 5 MJ	5	✓	75.0	75.6	100.8
Cal 6 MJ	6	✓	100.0	99.4	99.4
Cal 7 MJ	7	✓	250.0	251.6	100.7

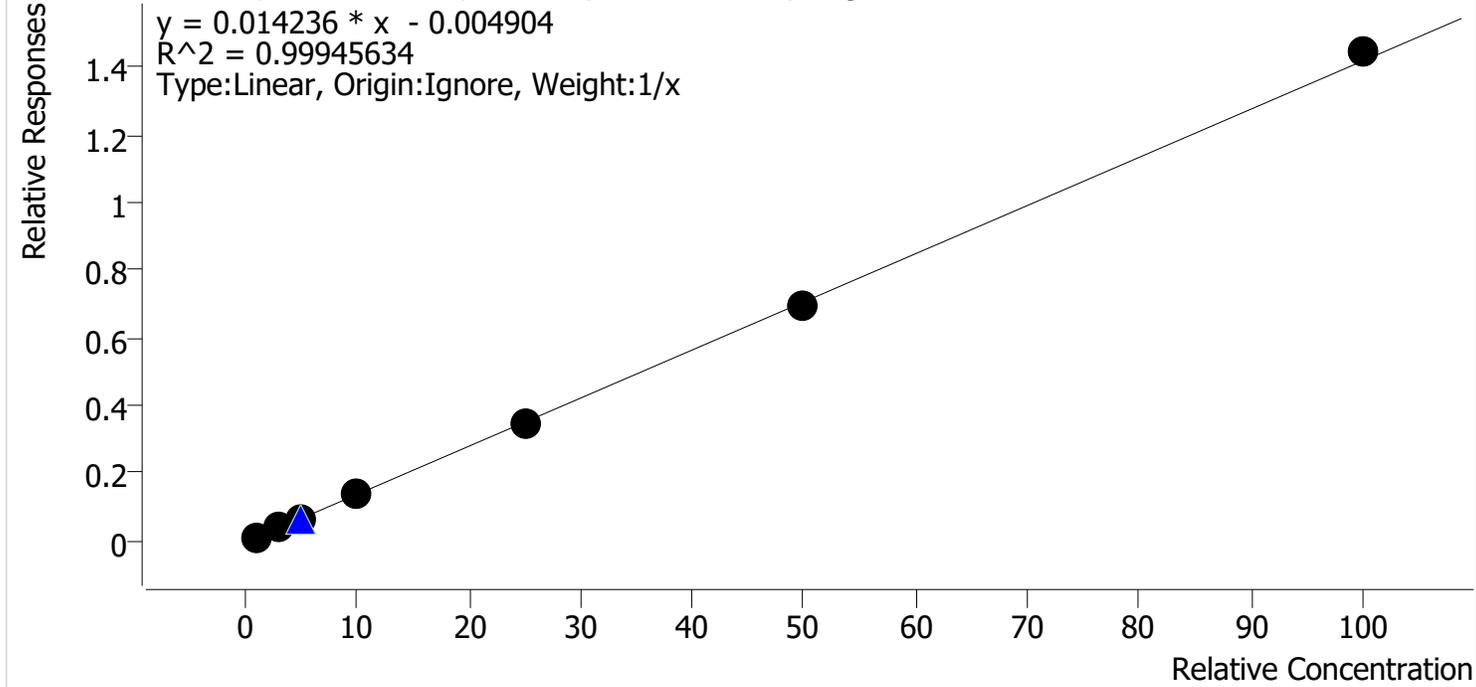
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Last Cal. Update 10/27/2023 11:35 AM
Analyst Name ISP\Datastor
Analyte THC-OH **Internal Standard** THC-OH-D3

THC-OH - 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	109.9
Cal 2 MJ	2	✓	3.0	3.0	98.9
Cal 3 MJ	3	✓	5.0	4.7	94.6
Cal 4 MJ	4	✓	10.0	9.9	98.8
Cal 5 MJ	5	✓	25.0	24.5	98.0
Cal 6 MJ	6	✓	50.0	49.0	98.0
Cal 7 MJ	7	✓	100.0	101.8	101.8



AM #27 Cannabinoids Quant. Results

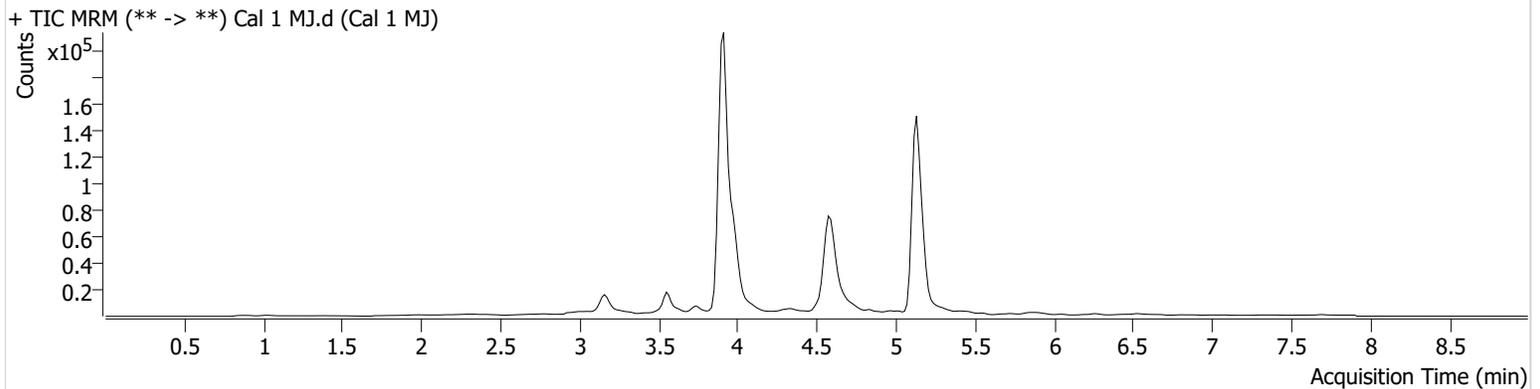
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Calibration Last Update 10/27/2023 11:35:29 AM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-H6
Injection Volume 10
Acq. Date-Time 10/26/2023 12:42:57 PM
Sample Info.

Data File Cal 1 MJ.d
Sample Cal 1 MJ
Operator Celena Shrum
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.135	4994	39.43	46.0 High	3.91 Low	614066	0.9025 ng/ml
THC-COOH	4.000	5581	∞	211.2	∞	150632	5.1998 ng/ml
THC-OH	3.911	9315	∞	13.7	36.79	867584	1.0986 ng/ml



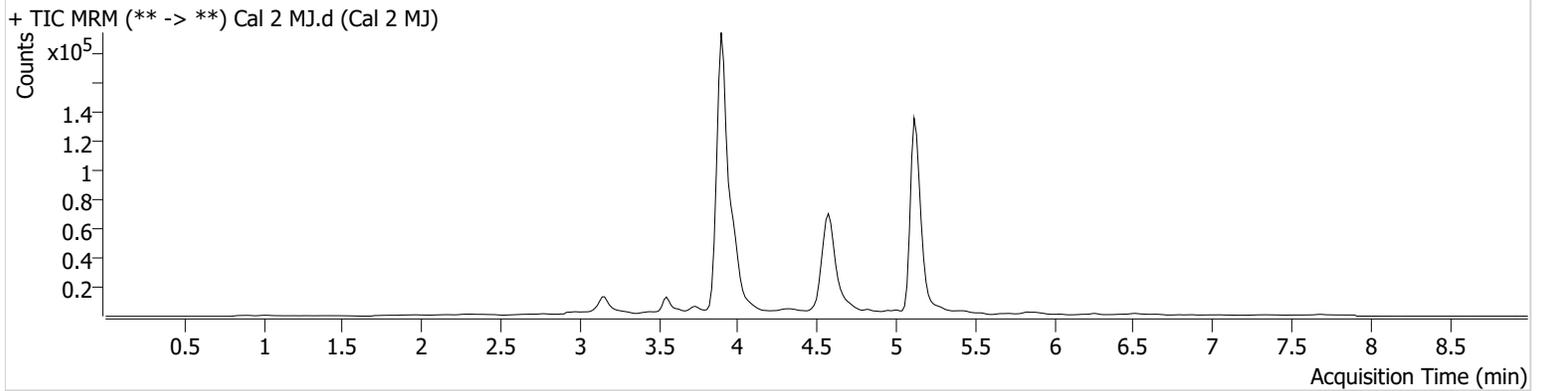
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Calibration Last Update 10/27/2023 11:35:29 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 10/26/2023 12:56:12 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.120	14916	∞	26.8	10.02	534892	3.0901 ng/ml
THC-COOH	3.985	10033	79.11	209.6	∞	135712	9.8464 ng/ml
THC-OH	3.911	29629	∞	14.9	∞	793565	2.9671 ng/ml



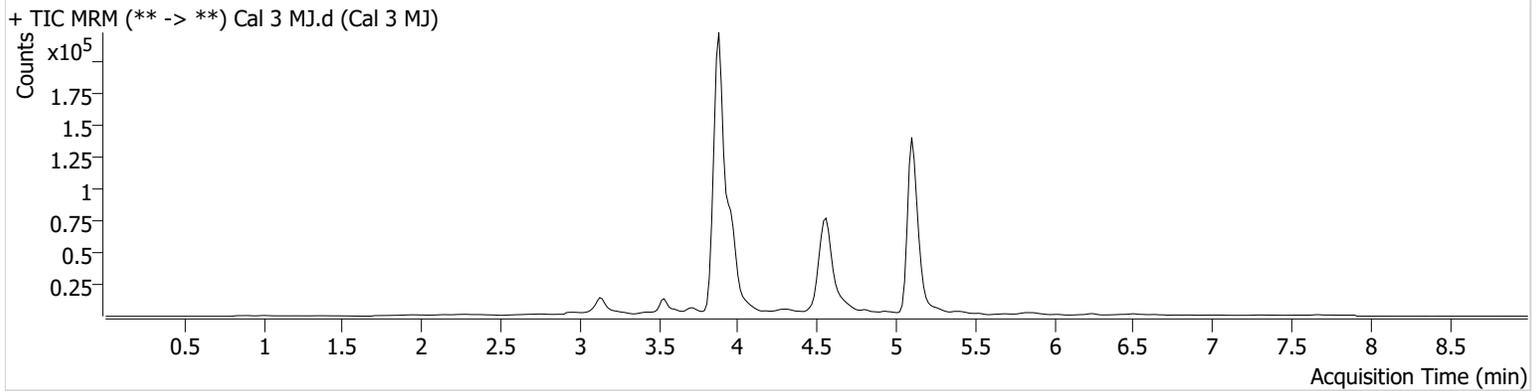
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Calibration Last Update 10/27/2023 11:35:29 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 10/26/2023 1:09:18 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.105	23888	∞	31.4	∞	551561	4.7980 ng/ml
THC-COOH	3.969	22453	∞	190.2	∞	145133	20.0228 ng/ml
THC-OH	3.881	57022	∞	12.8	∞	912807	4.7324 ng/ml



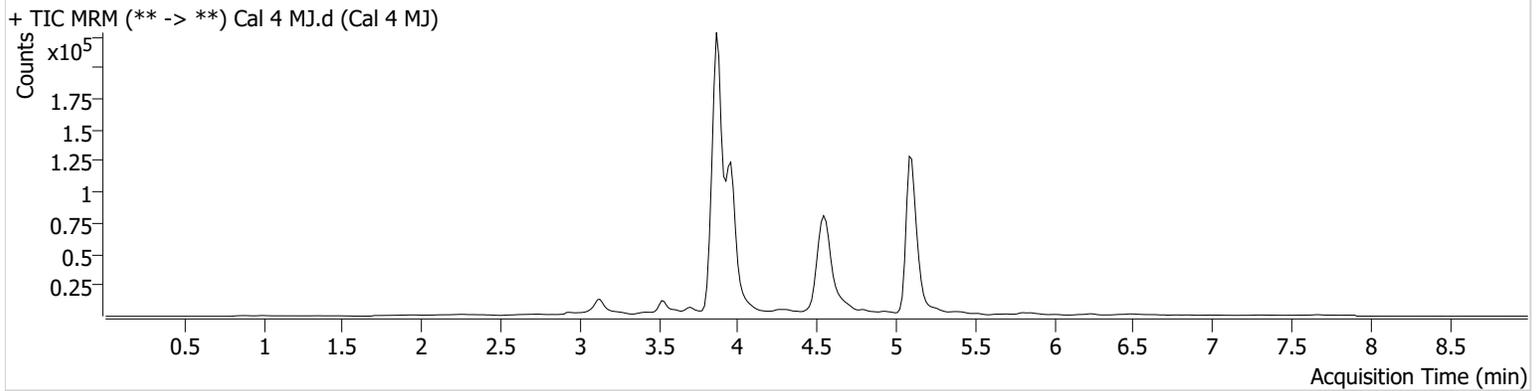
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Calibration Last Update 10/27/2023 11:35:29 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 10/26/2023 1:22:24 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.105	43306	224.54	28.8	∞	490981	9.7695 ng/ml
THC-COOH	3.954	52095	153.65	220.1	5151.46	137450	48.2827 ng/ml
THC-OH	3.881	115605	∞	13.9	∞	851759	9.8781 ng/ml



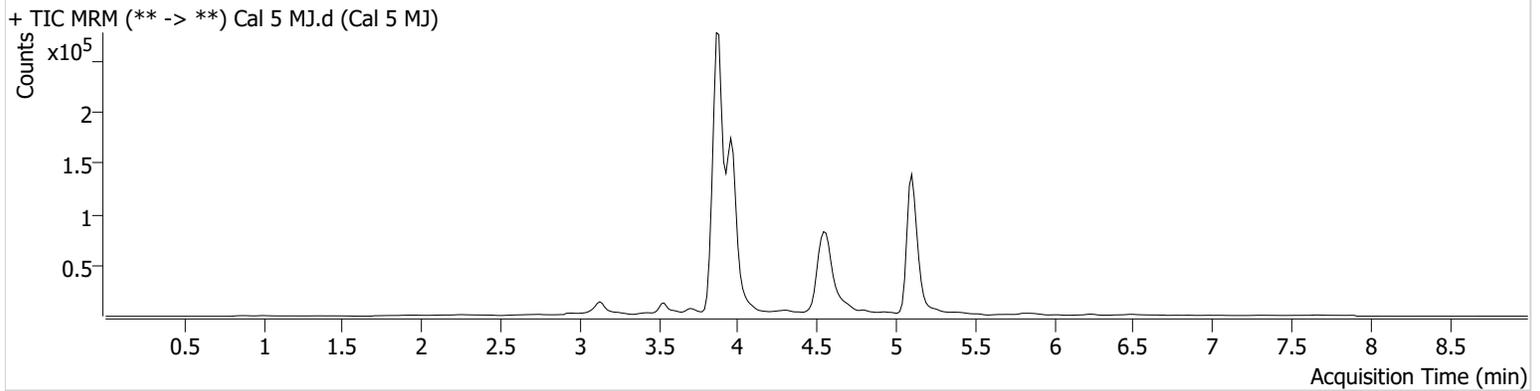
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Calibration Last Update 10/27/2023 11:35:29 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 10/26/2023 1:35:29 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.105	103470	802.31	25.3	∞	457698	25.0361 ng/ml
THC-COOH	3.954	84912	2049.95	207.6	∞	142559	75.5732 ng/ml
THC-OH	3.881	306096	85.63	13.2	4993.73	890299	24.4947 ng/ml



AM #27 Cannabinoids Quant. Results

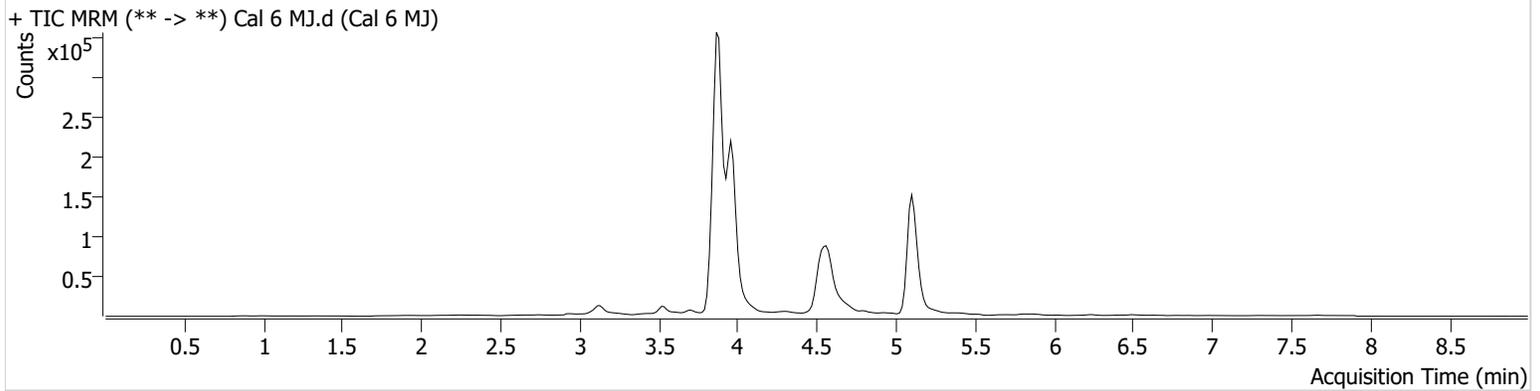
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Calibration Last Update 10/27/2023 11:35:29 AM

Instrument Falco (069901)
Type Cal
Acq. Method AM 27 Agilent Method.m
Sample Position P1-C6
Injection Volume 10
Acq. Date-Time 10/26/2023 1:48:35 PM
Sample Info.

Data File Cal 6 MJ.d
Sample Cal 6 MJ
Operator Celena Shrum
Comment

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.105	191868	∞	25.7	∞	401715	52.8928 ng/ml
THC-COOH	3.954	109872	∞	200.1	∞	139949	99.4424 ng/ml
THC-OH	3.881	623814	∞	13.2	∞	900655	48.9959 ng/ml



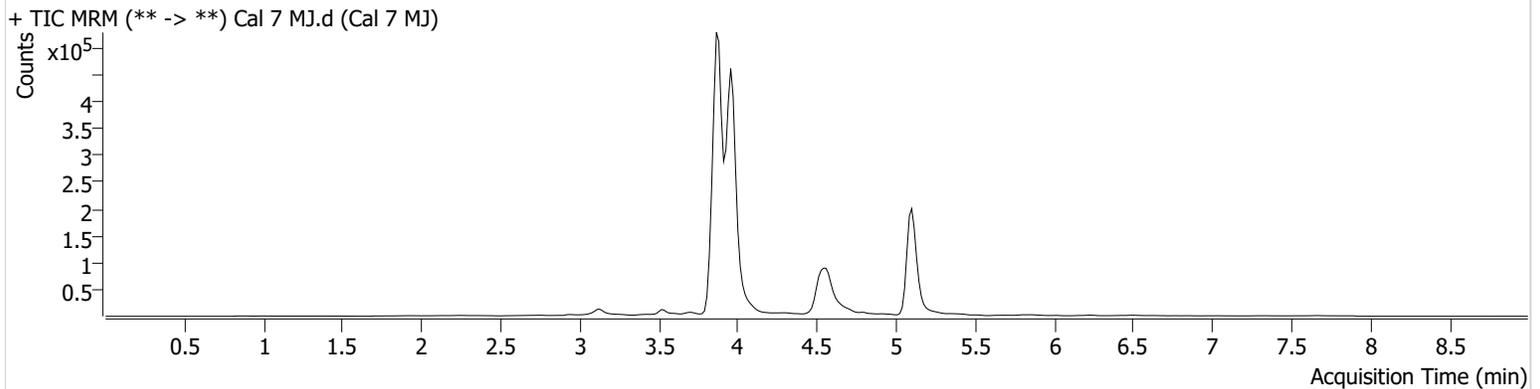
AM #27 Cannabinoids Quant. Results

Batch results D:\MassHunter\Data\2023\AM 27 28\102523 AM 27 28 TS CS\QuantResults\AM 27_CS.batch.bin
Calibration Last Update 10/27/2023 11:35:29 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 10/26/2023 2:01:41 PM
Sample Info.

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Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC	5.105	346150	3058.27	27.9	∞	393503	97.4136 ng/ml
THC-COOH	3.954	264100	8053.80	201.9	∞	132509	251.6327 ng/ml
THC-OH	3.881	1281076	∞	13.3	∞	886658	101.8332 ng/ml