

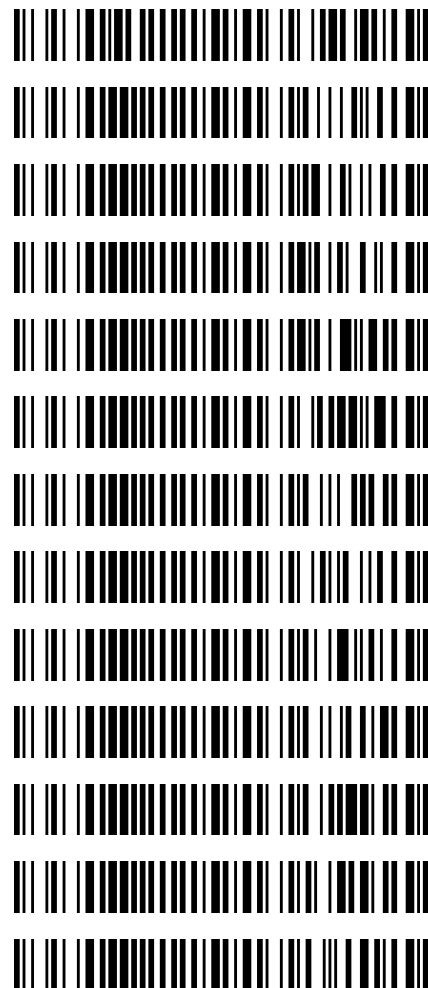
REVIEWED
By Sarah Collins at 8:50 am, Jul 21, 2021

TS

7/16/2021

Worklist: 5110

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-2895	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-1991	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2033	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2168	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2170	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2227	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2228	3	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2231	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2254	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2260	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2269	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2270	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-2286	1	BCK	AM 27 Blood THC Quant by LC-QQQ



TS

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

Extraction Date: 07/15/2021

Analyst: Tamara Salazar

Plate lot#: IDP-108-2-21609

Plate Re-test Date: 12-09-21

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 20L20723

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, 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: 800uL
- 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: *THC-OH not evaluated due to a possible interfering compound.*

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	1	2	3	4	5	6
A	IS + Cal. 1				P2021-2228-3	IS + QC_1
B	IS + Cal. 2			P2021-2033-1	P2021-2227-1	IS + Cal. 7
C	IS + Cal. 3			P2021-2286-1	P2021-2170-1	IS + Cal. 6
D	IS + Cal. 4			P2021-2270-1	P2021-2168-1	IS + Cal. 5
E	IS + Cal. 5			P2021-2269-1	P2021-2033-1 (Moved to B4 due to blood clot)	IS + Cal. 4
F	IS + Cal. 6			P2021-2260-1	P2021-1991-2	IS + Cal. 3
G	IS + Cal. 7			P2021-2254-1	M2021-2895-3	IS + Cal. 2
H	IS + QC_1			P2021-2231-1	Neg Blood	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

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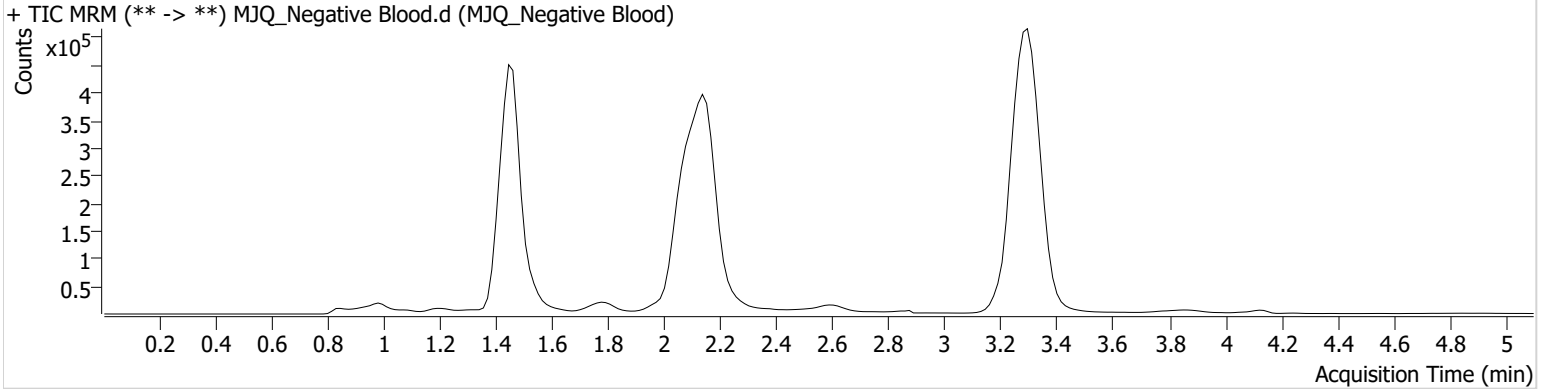


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument	Falco (069901)	Data File	MJQ_Negative Blood.d
Type	Sample	Sample	MJQ_Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H5	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 2:52:57 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.528 High	163035	∞	2.1 Low	15.39	1815334	0.6141 ng/ml Low

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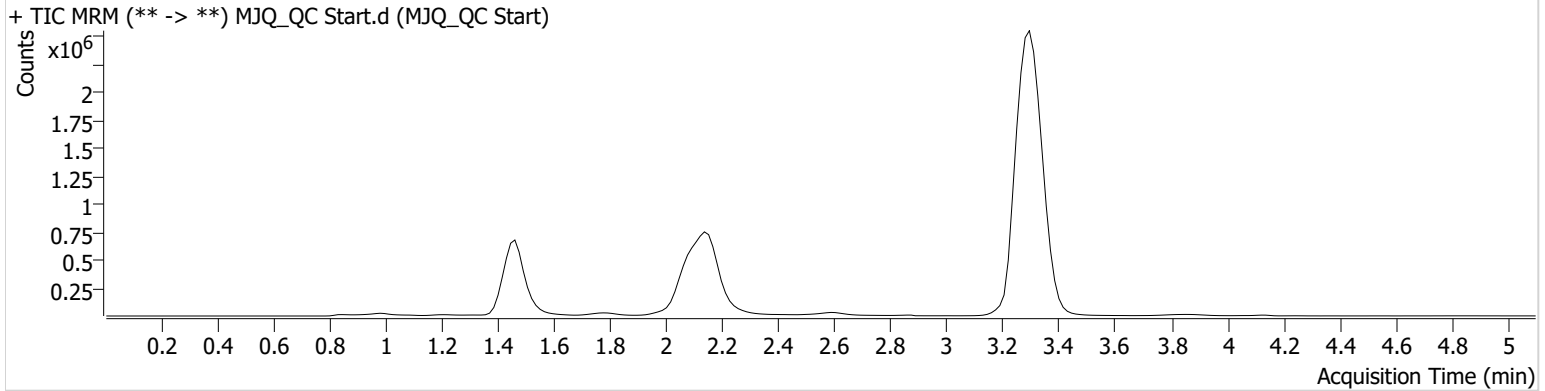


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument	Falco (069901)	Data File	MJQ_QC Start.d
Type	Sample	Sample	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 2:37:45 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	308187	∞	6.2 Low	180.98	2173676	4.3665 ng/ml
THC-COOH	1.489	246574	∞	52.6	605.17	664079	15.1581 ng/ml
THC	3.315	768866	∞	26.2	725.49	16028769	4.7829 ng/ml

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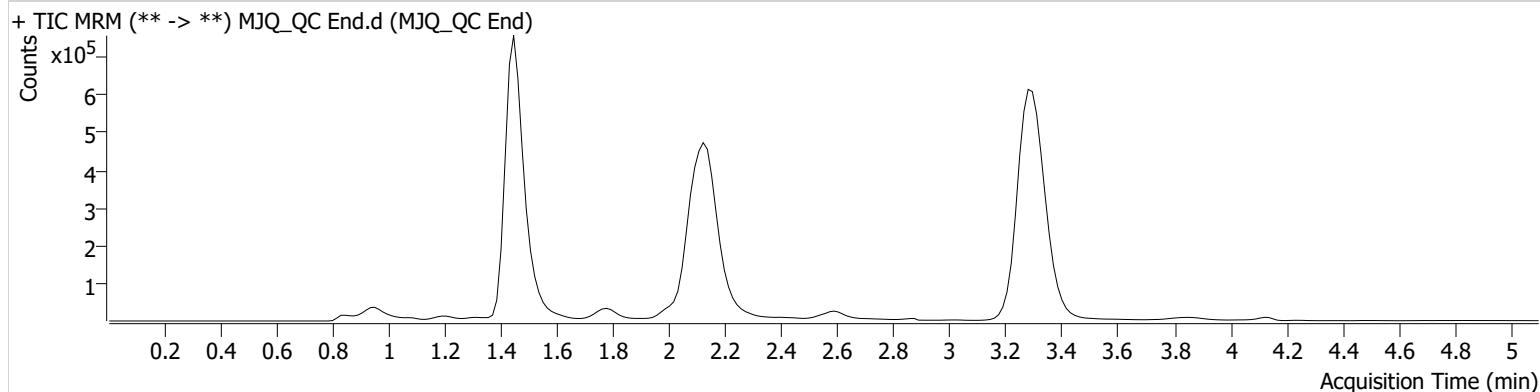


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:56:00 AM

Instrument	Falco (069901)	Data File	MJQ_QC End.d
Type	Sample	Sample	MJQ_QC End
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 7:05:31 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.498	353247	∞	6.2 Low	∞	2383693	4.8294 ng/ml
THC-COOH	1.474	159779	∞	58.6	885.67	487830	13.3910 ng/ml
THC	3.315	183963	∞	30.0	94.71	4034446	4.5589 ng/ml

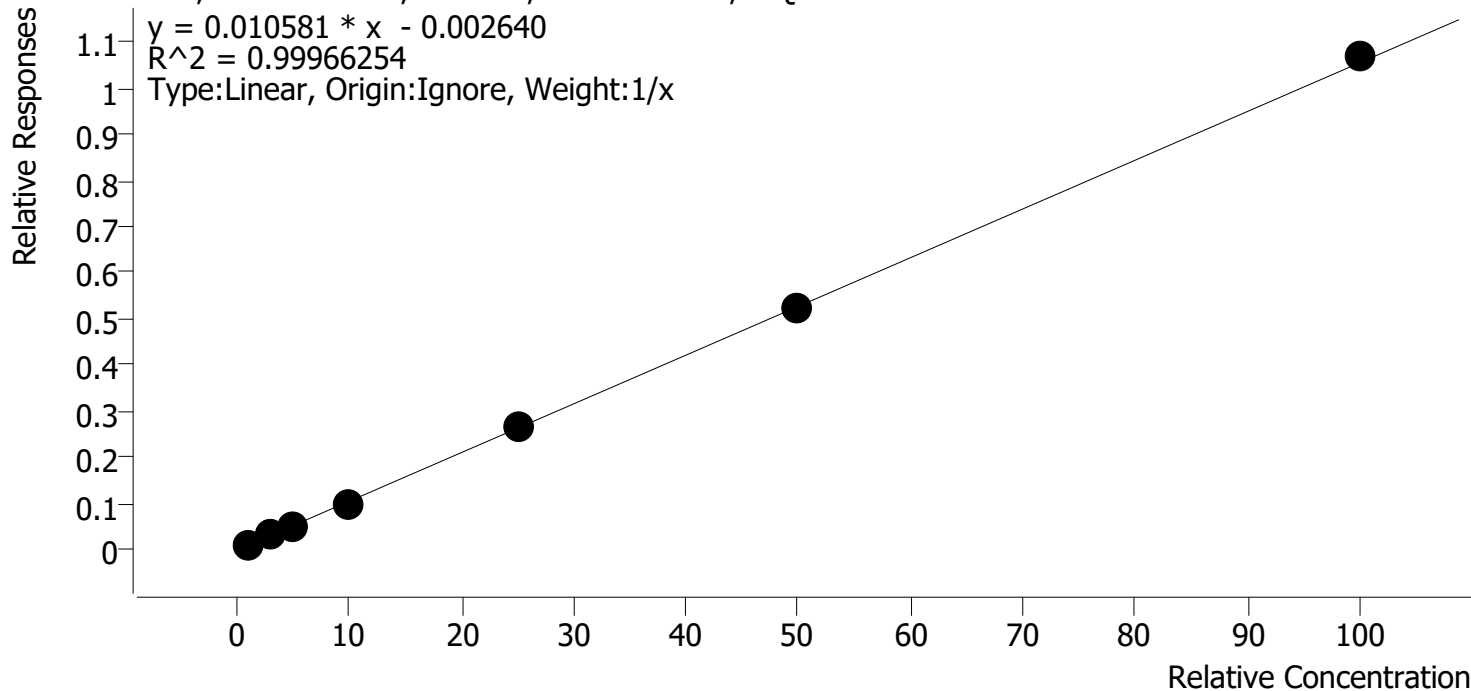
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/20/2021 9:33 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



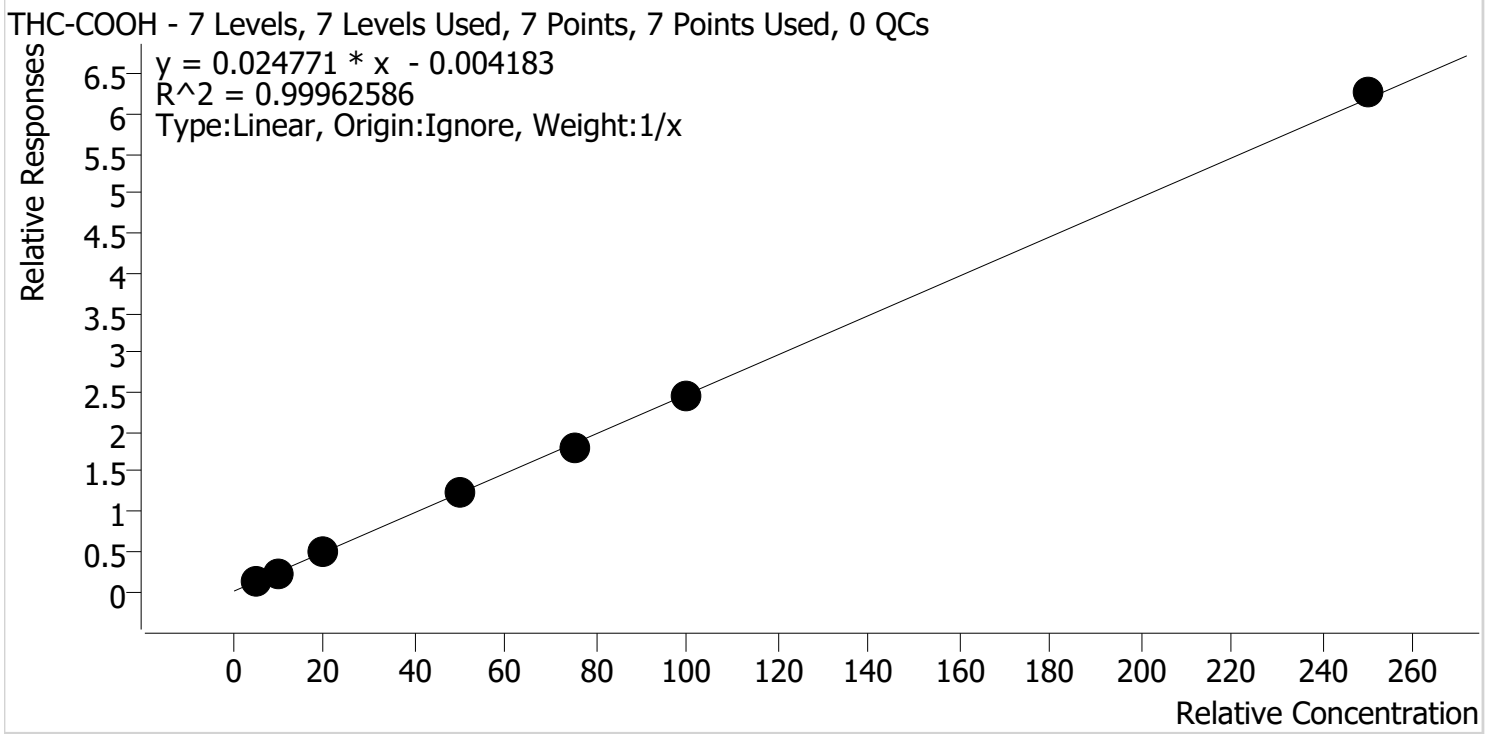
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	109.7
MJQ_Cal 2	2	✓	3.0	2.9	97.5
MJQ_Cal 3	3	✓	5.0	4.9	97.2
MJQ_Cal 4	4	✓	10.0	9.6	95.9
MJQ_Cal 5	5	✓	25.0	25.0	99.8
MJQ_Cal 6	6	✓	50.0	49.4	98.7
MJQ_Cal 7	7	✓	100.0	101.2	101.2



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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 7/20/2021 9:33 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	5.0	5.4	108.4
MJQ_Cal 2	2	✓	10.0	9.6	96.3
MJQ_Cal 3	3	✓	20.0	19.4	96.9
MJQ_Cal 4	4	✓	50.0	50.1	100.2
MJQ_Cal 5	5	✓	75.0	73.7	98.3
MJQ_Cal 6	6	✓	100.0	98.7	98.7
MJQ_Cal 7	7	✓	250.0	253.1	101.2

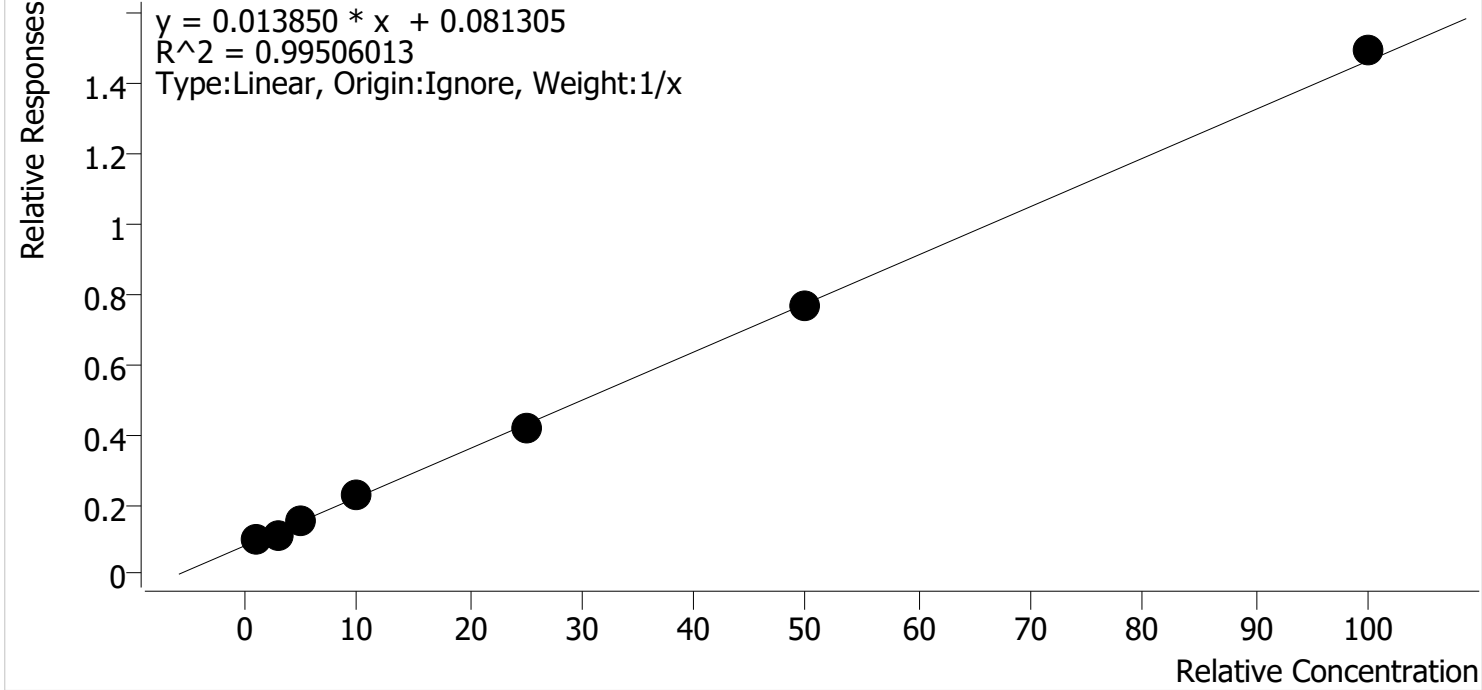


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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
 Last Cal. Update 7/20/2021 9:33 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, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.5	145.8
MJQ_Cal 2	2	✓	3.0	1.8	58.3
MJQ_Cal 3	3	✓	5.0	4.8	96.1
MJQ_Cal 4	4	✓	10.0	10.2	102.0
MJQ_Cal 5	5	✓	25.0	24.2	96.7
MJQ_Cal 6	6	✓	50.0	49.5	99.0
MJQ_Cal 7	7	✓	100.0	102.1	102.1

Compound not evaluated due to interfering peak.

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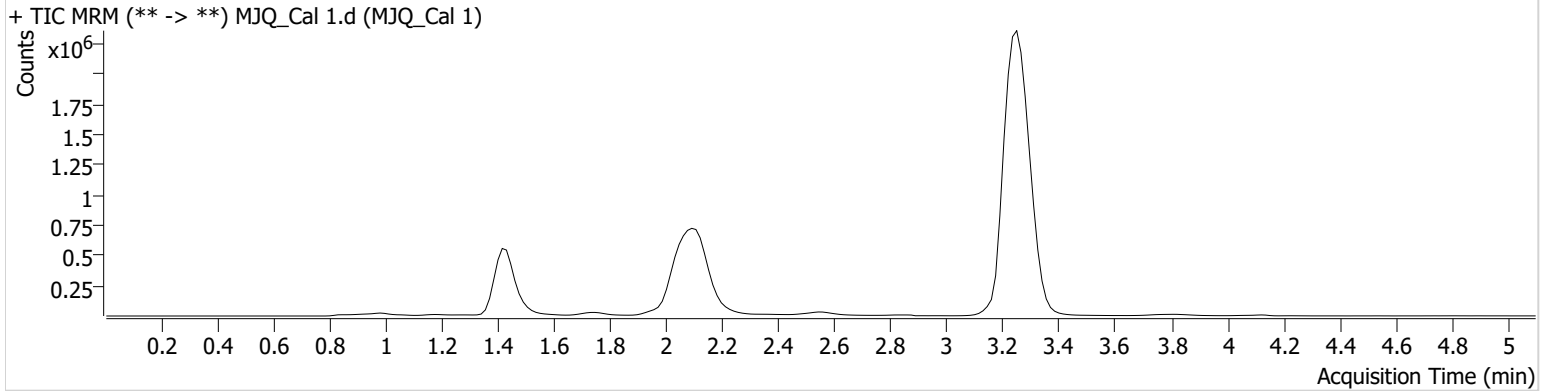


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 1.d
Type	Cal	Sample	MJQ_Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 1:36:47 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	204733	∞	3.6 Low	20.53	2017031	1.4582 ng/ml Low
THC-COOH	1.459	70695	∞	45.7	∞	543459	5.4202 ng/ml
THC	3.254	136053	∞	30.0	127.42	15169769	1.0971 ng/ml

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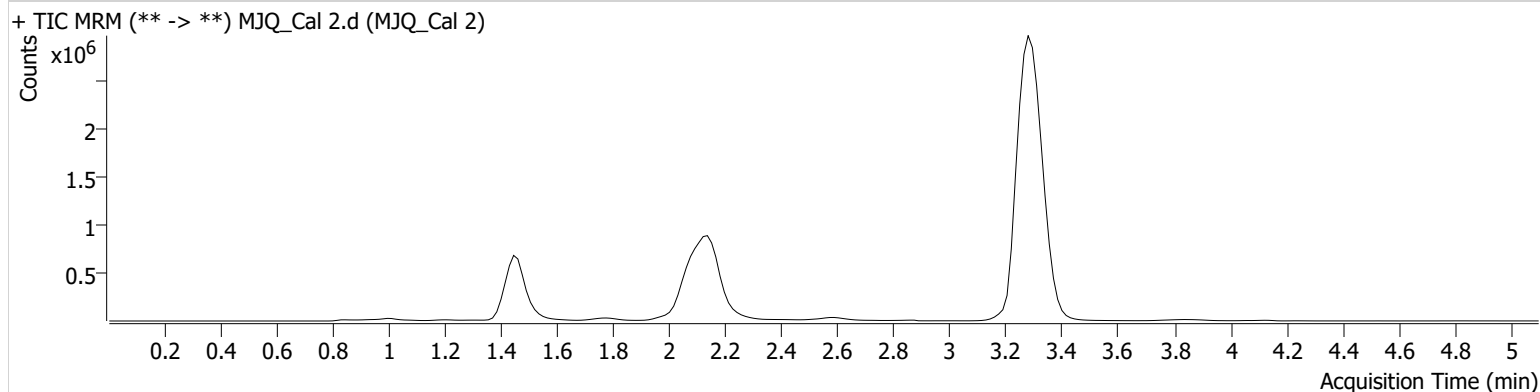
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 2.d
Type	Cal	Sample	MJQ_Cal 2
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-G6	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 1:44:33 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.513 High	237765	91.54	6.1 Low	∞	2252692	1.7503 ng/ml Low
THC-COOH	1.489	159460	∞	54.0	∞	680201	9.6326 ng/ml
THC	3.300	519509	∞	25.3	782.50	18354838	2.9244 ng/ml

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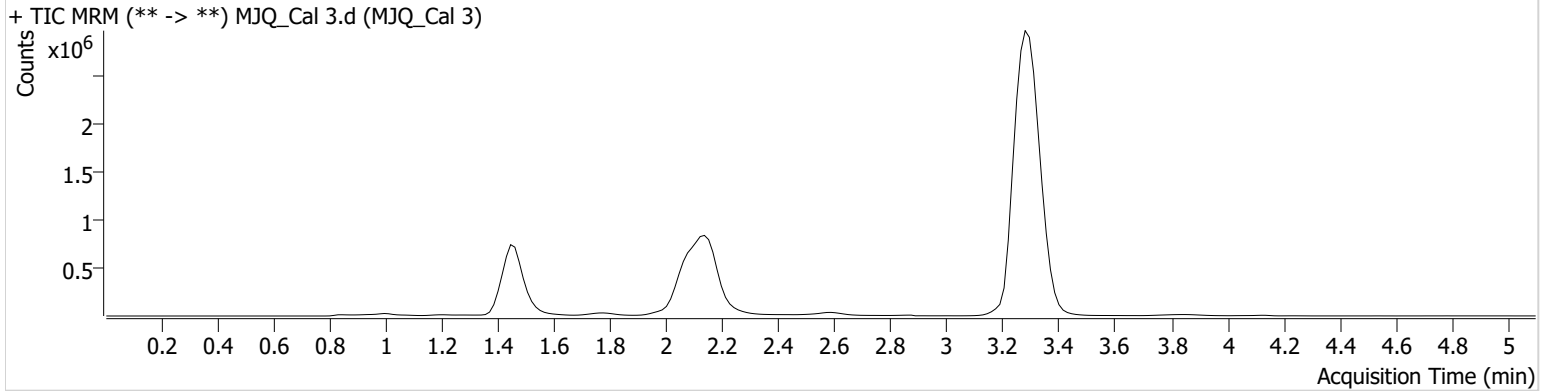
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 3.d
Type	Cal	Sample	MJQ_Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 1:52:09 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	343258	∞	6.5	∞	2321969	4.8032 ng/ml
THC-COOH	1.489	331379	1515.79	55.3	∞	696390	19.3787 ng/ml
THC	3.300	893000	9076.52	26.1	∞	18305250	4.8600 ng/ml

TS

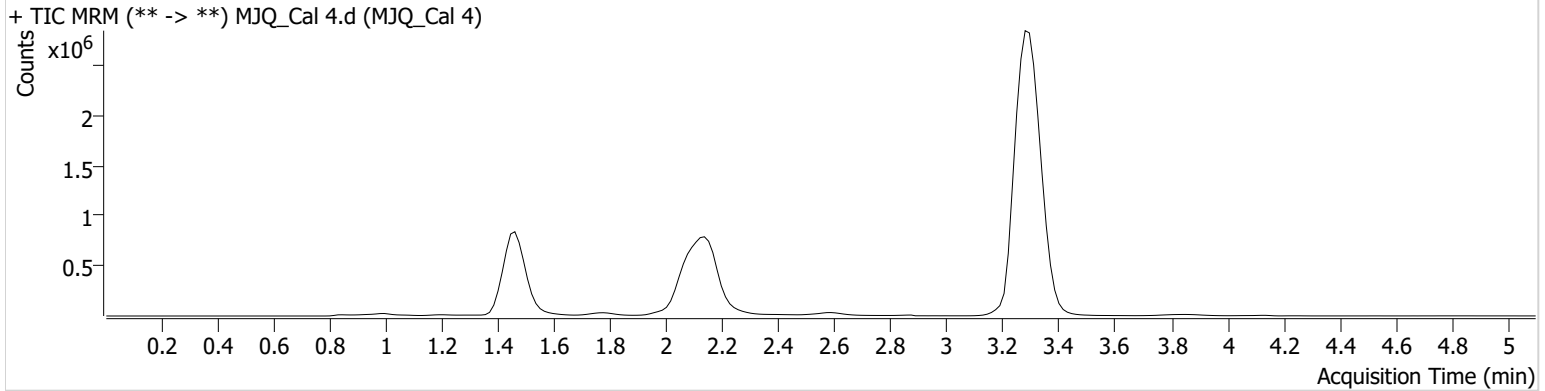


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 4.d
Type	Cal	Sample	MJQ_Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 1:59:44 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	476065	∞	7.4	203.80	2139171	10.1978 ng/ml
THC-COOH	1.489	797977	1502.55	53.6	∞	645414	50.0806 ng/ml
THC	3.300	1621630	∞	25.2	∞	16416531	9.5851 ng/ml

TS

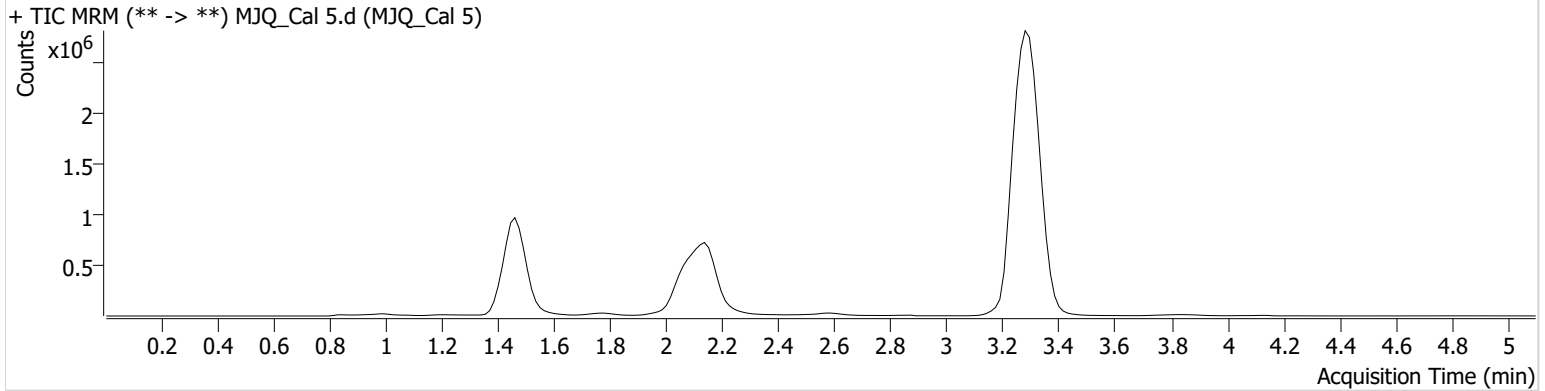


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 5.d
Type	Cal	Sample	MJQ_Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 2:07:19 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	819175	∞	9.7 High	∞	1968954	24.1688 ng/ml
THC-COOH	1.489	1083019	∞	55.6	∞	594589	73.6997 ng/ml
THC	3.300	3733359	∞	25.0	∞	14280850	24.9565 ng/ml

TS

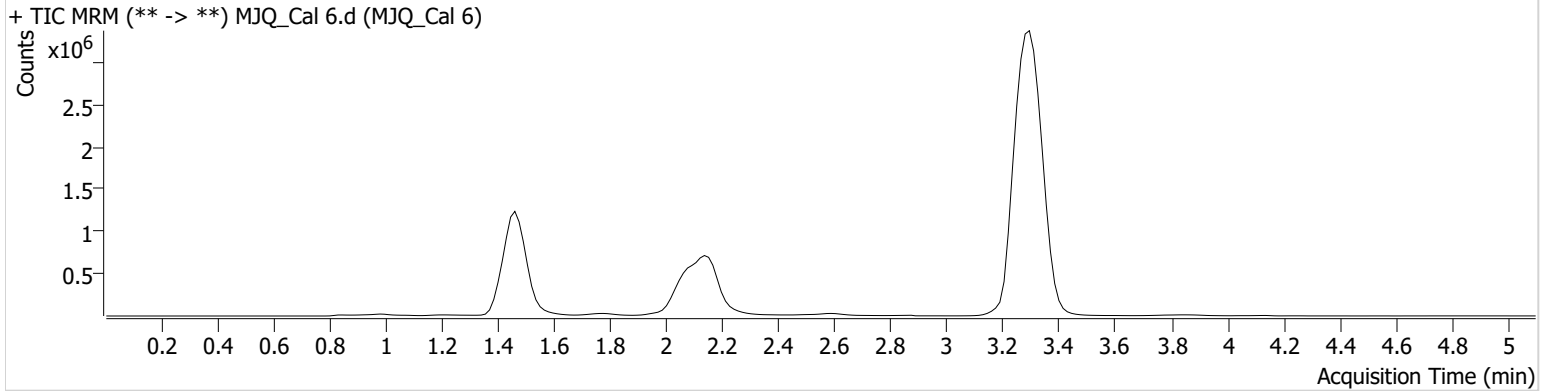


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 6.d
Type	Cal	Sample	MJQ_Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	7/15/2021 2:14:56 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	1583112	234.91	10.7 High	865.49	2064578	49.4935 ng/ml
THC-COOH	1.489	1475688	∞	56.4	∞	604470	98.7220 ng/ml
THC	3.300	7409661	∞	26.3	4663.56	14260709	49.3552 ng/ml

TS

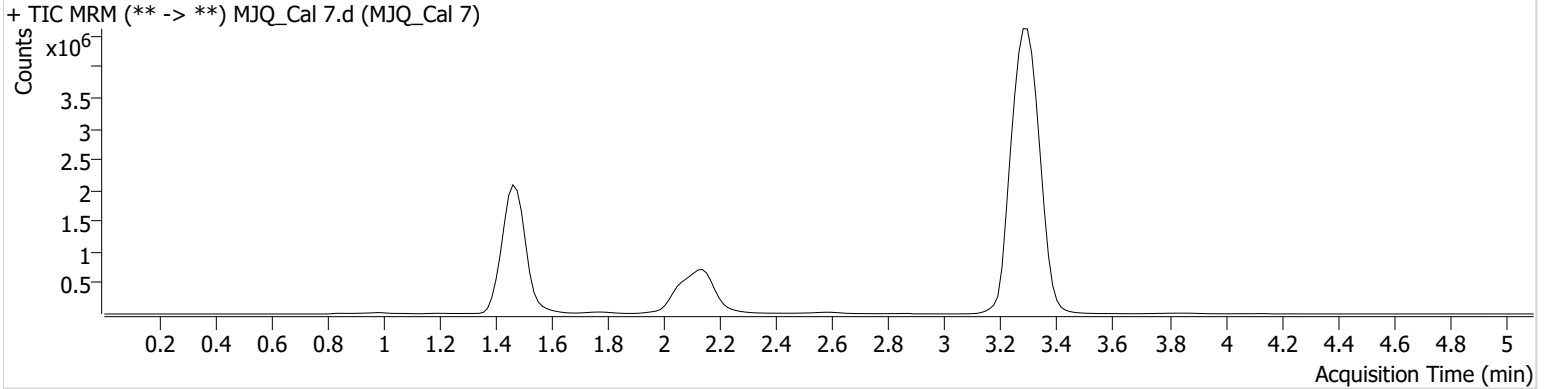


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\071521 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 7/20/2021 9:33:47 AM

Instrument Falco (069901) **Data File** MJQ_Cal 7.d
Type Cal **Sample** MJQ_Cal 7
Acq. Method AM 27 THCQ.m **Operator** Tamara Salazar
Sample Position P1-B6 **Comment**
Injection Volume 10
Acq. Date-Time 7/15/2021 2:22:31 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	2925908	∞	11.2 High	∞	1956090	102.1281 ng/ml
THC-COOH	1.474	3479535	∞	56.6	27572.6 3	555428	253.0662 ng/ml
THC	3.300	15076285	∞	26.0	∞	14111319	101.2216 ng/ml