

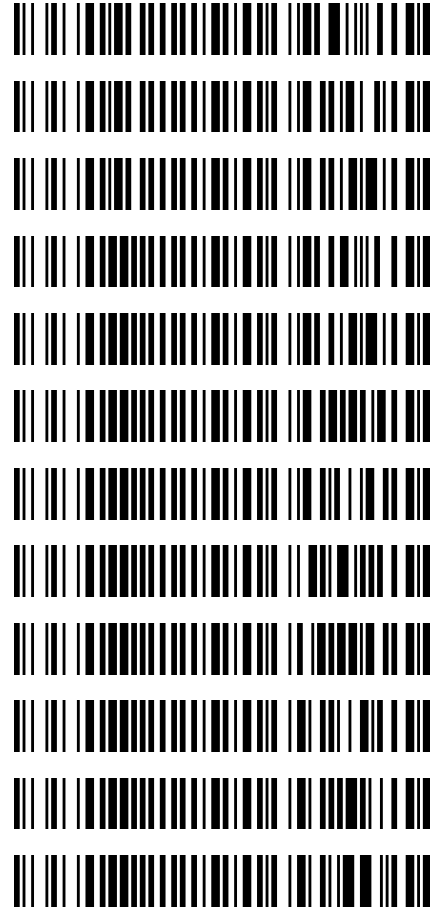
REVIEWED

By Tamara Salazar at 8:45 am, Oct 14, 2021

10/7/2021

Worklist: 5279

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-4178	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-4198	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-4202	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3235	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3236	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3244	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3246	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3280	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3319	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3341	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3345	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3347	1	BCK	AM 27 Blood THC Quant by LC-QQQ



SC

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

Extraction Date: 10/06/21

Analyst: Sarah Collins

Plate lot#: IDP-108-2-210609

Retest Date: 12/09/2021

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 20L20724

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. 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:** 3382167
- 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 uL
- 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: Did not evaluate THC-OH due to interfering peak.

SC

	1	2	3	4	5	6
A	IS + Cal. 1			p2021-3244-1	p2021-3246-1	IS + QC_1
B	IS + Cal. 2				p2021-3244-1 *	IS + Cal. 7
C	IS + Cal. 3				p2021-3236-1	IS + Cal. 6
D	IS + Cal. 4			p2021-3347-1	p2021-3235-1	IS + Cal. 5
E	IS + Cal. 5			p2021-3345-2	m2021-4202-2	IS + Cal. 4
F	IS + Cal. 6			p2021-3341-1	m2021-4198-2	IS + Cal. 3
G	IS + Cal. 7			p2021-3319-1	m2021-4178-2	IS + Cal. 2
H	IS + QC_1			p2021-3280-1	negative blood	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

*Sample moved during analytical step 6 due to blood clot

SC



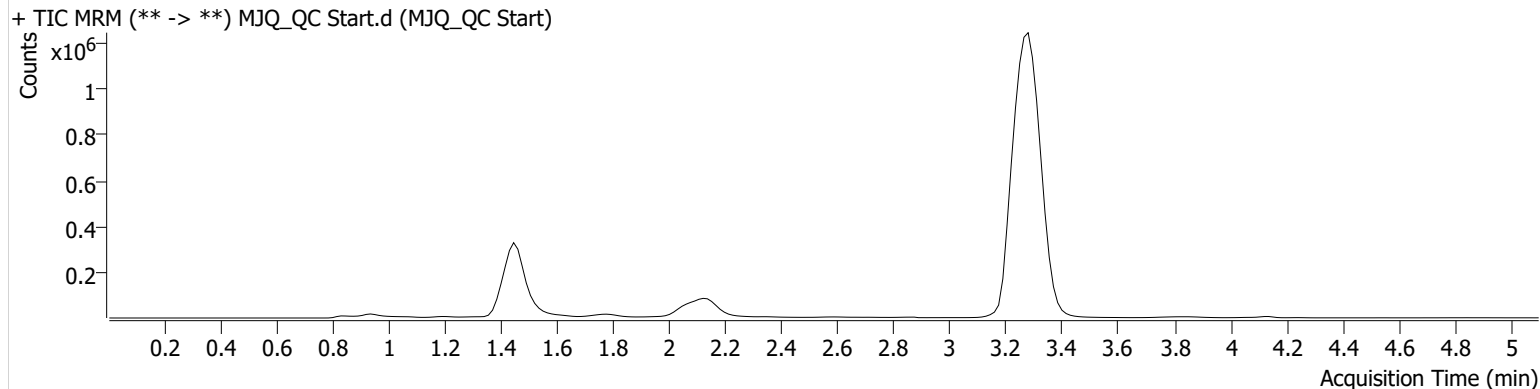
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_QC Start.d
Type	Sample	Sample	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 6:22:05 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	105480	∞	59.5	∞	271257	15.4655 ng/ml
THC-OH	1.468	156206	∞	8.1	∞	1177948	4.9648 ng/ml
THC	3.300	360697	1792.17	27.2	∞	8263189	4.5841 ng/ml

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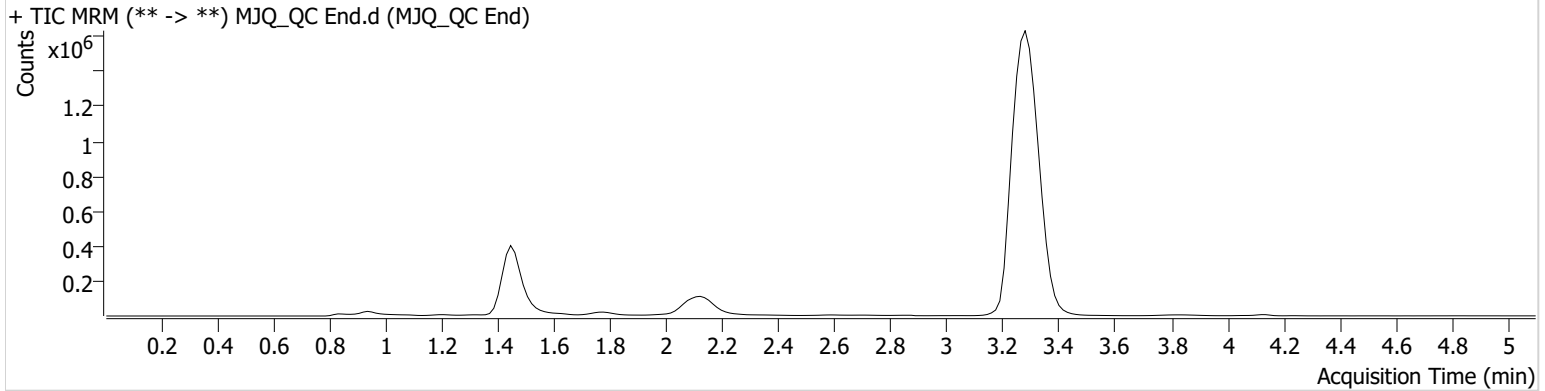


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_QC End.d
Type	Sample	Sample	MJQ_QC End
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-A6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 9:55:06 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	104390	∞	65.8	∞	293869	14.1534 ng/ml
THC-OH	1.498	173931	∞	8.2	∞	1321447	4.8836 ng/ml
THC	3.300	472816	2602.92	27.5	∞	10315284	4.8035 ng/ml

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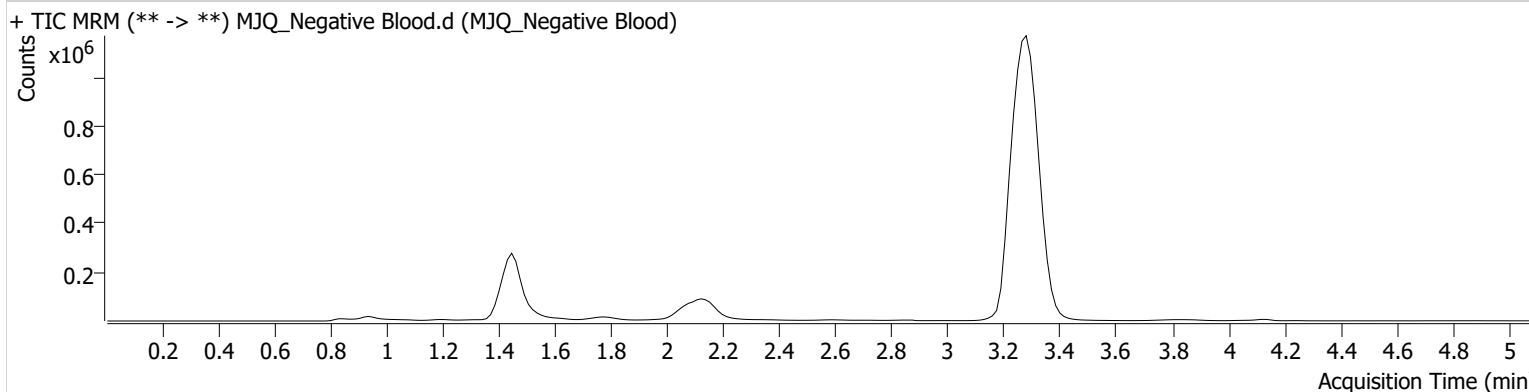


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_Negative Blood.d
Type	Sample	Sample	MJQ_Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-H5	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 6:37:18 PM		

Sample Chromatogram



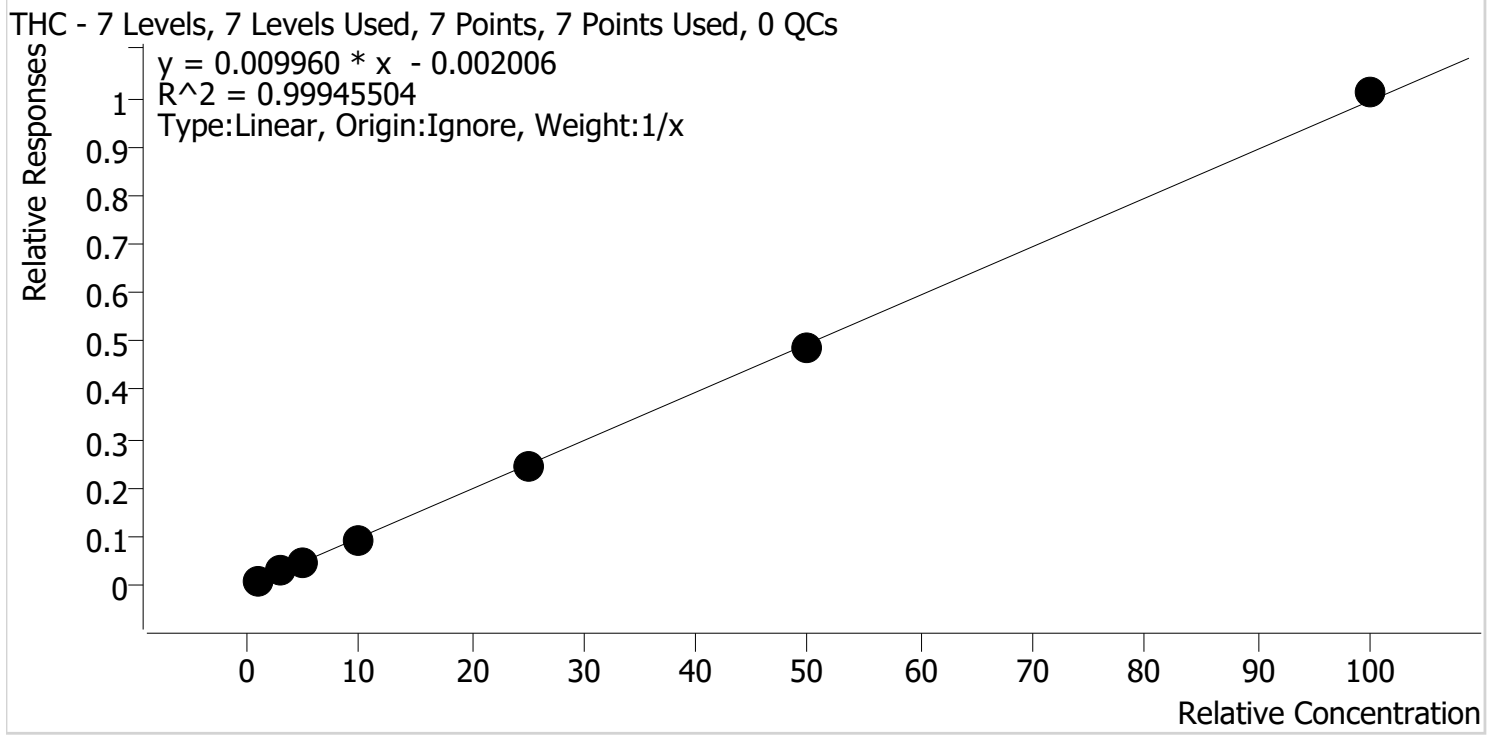
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.513	89450	∞	3.3 Low	18.63	1103878	0.7213 ng/ml Low

SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 10/7/2021 7:24 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



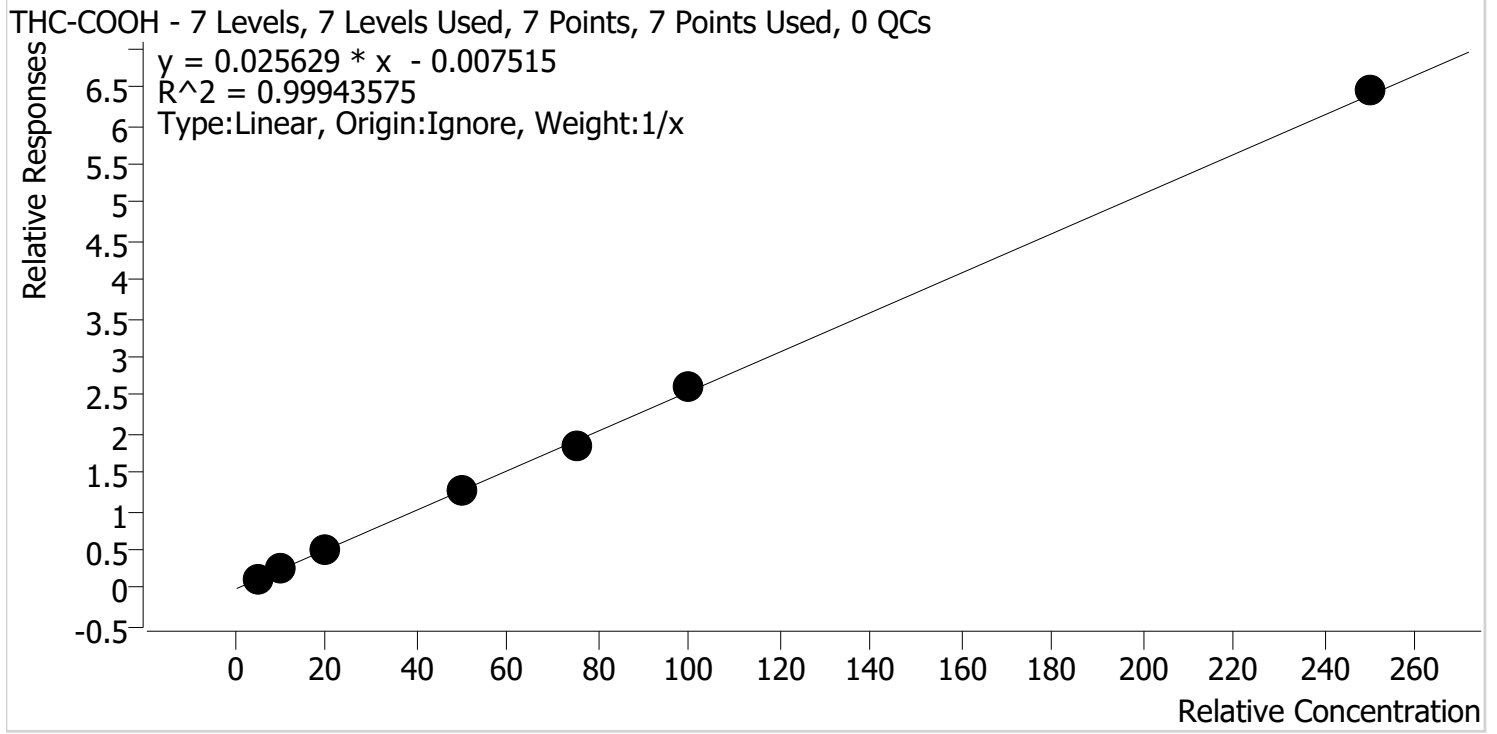
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.1	112.7
MJQ_Cal 2	2	✓	3.0	2.9	97.0
MJQ_Cal 3	3	✓	5.0	4.8	95.6
MJQ_Cal 4	4	✓	10.0	9.6	96.1
MJQ_Cal 5	5	✓	25.0	24.6	98.3
MJQ_Cal 6	6	✓	50.0	49.3	98.6
MJQ_Cal 7	7	✓	100.0	101.7	101.7

SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 10/7/2021 7:24 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



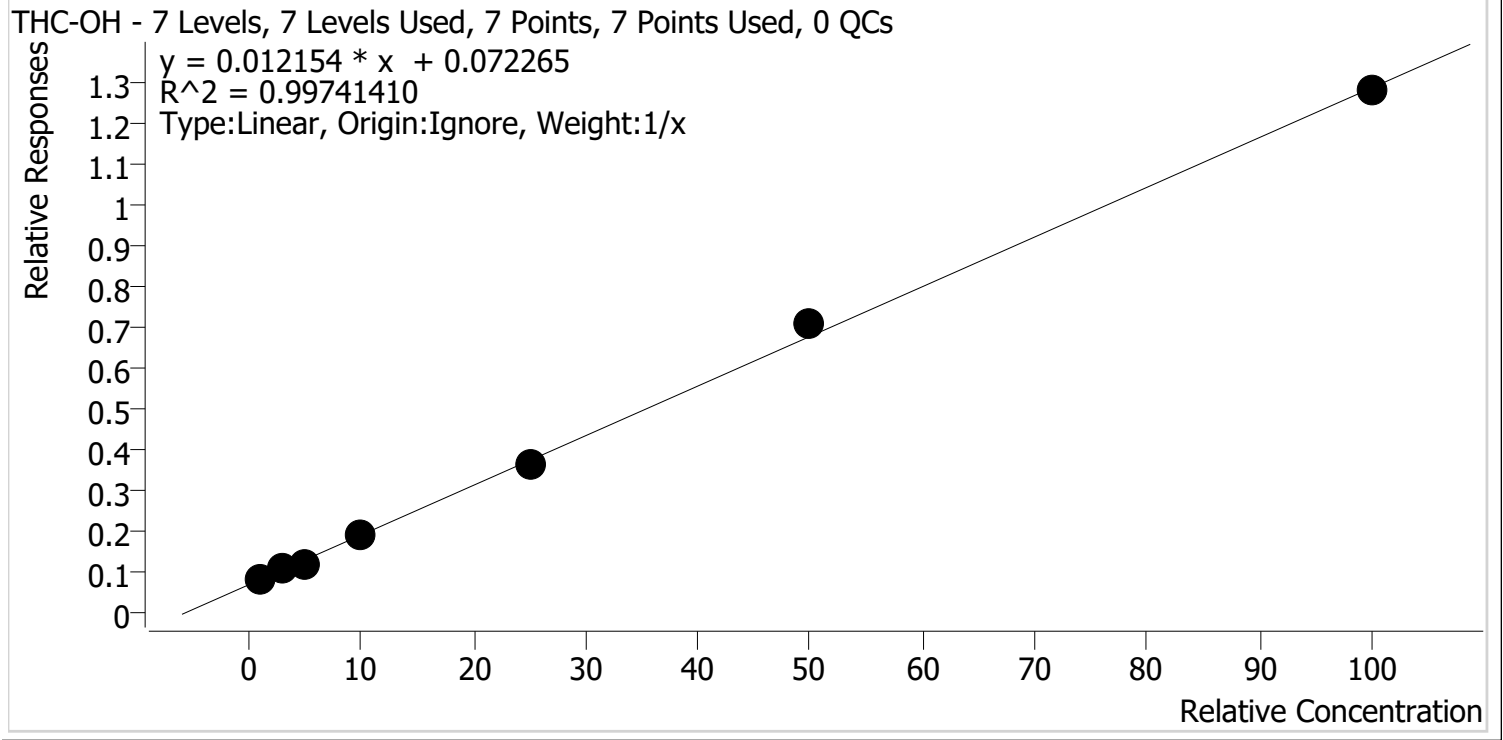
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	5.0	5.3	105.7
MJQ_Cal 2	2	✓	10.0	9.8	97.6
MJQ_Cal 3	3	✓	20.0	20.0	99.8
MJQ_Cal 4	4	✓	50.0	49.3	98.6
MJQ_Cal 5	5	✓	75.0	71.8	95.7
MJQ_Cal 6	6	✓	100.0	101.7	101.7
MJQ_Cal 7	7	✓	250.0	252.3	100.9

SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 10/7/2021 7:24 AM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.2	121.7
MJQ_Cal 2	2	✓	3.0	3.0	99.4
MJQ_Cal 3	3	✓	5.0	4.1	81.1
MJQ_Cal 4	4	✓	10.0	9.7	97.4
MJQ_Cal 5	5	✓	25.0	23.9	95.6
MJQ_Cal 6	6	✓	50.0	52.7	105.4
MJQ_Cal 7	7	✓	100.0	99.4	99.4

Compound not evaluated in this batch.

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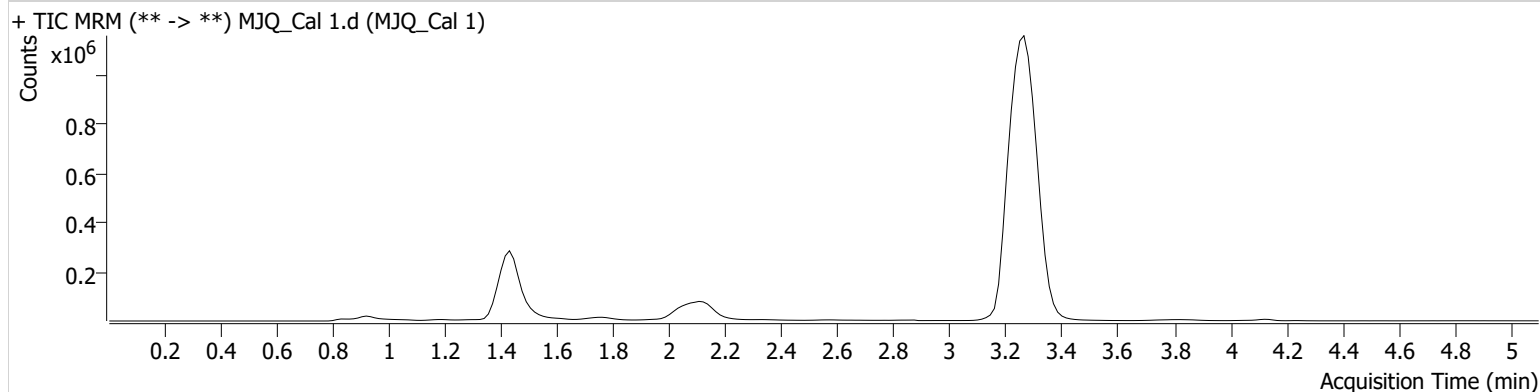


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 1.d
Type	Cal	Sample	MJQ_Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-H6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 5:21:10 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	32262	∞	58.0	∞	252149	5.2855 ng/ml
THC-OH	1.498	98909	∞	4.2 Low	18.16	1136197	1.2167 ng/ml Low
THC	3.285	74845	447.49	31.4	∞	8115320	1.1274 ng/ml

SC

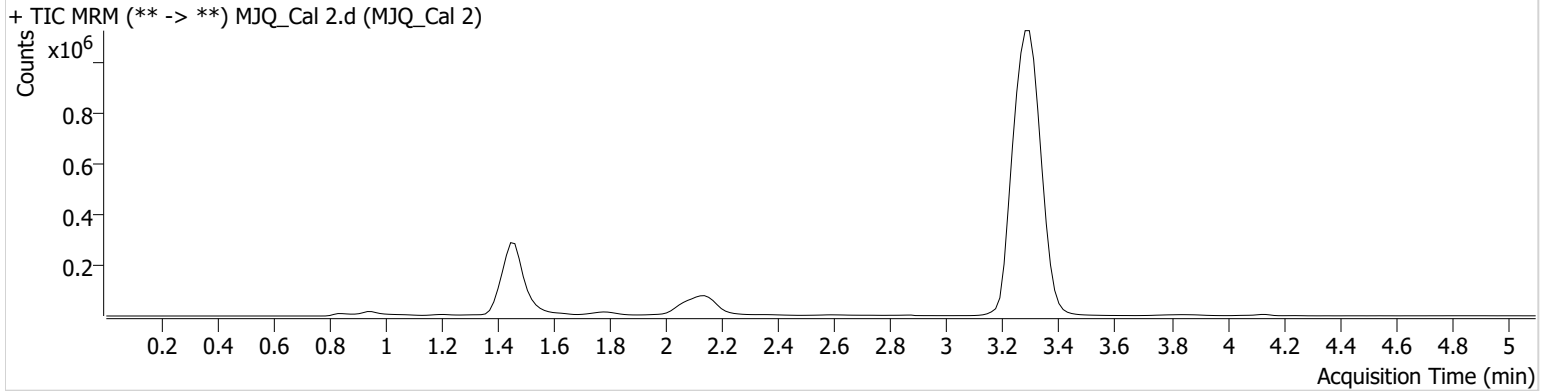


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 2.d
Type	Cal	Sample	MJQ_Cal 2
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-G6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 5:28:56 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	61251	∞	61.4	460.46	252371	9.7629 ng/ml
THC-OH	1.528 High	120104	∞	6.9 Low	∞	1106740	2.9830 ng/ml Low
THC	3.300	207764	∞	28.2	170.54	7699002	2.9108 ng/ml

SC

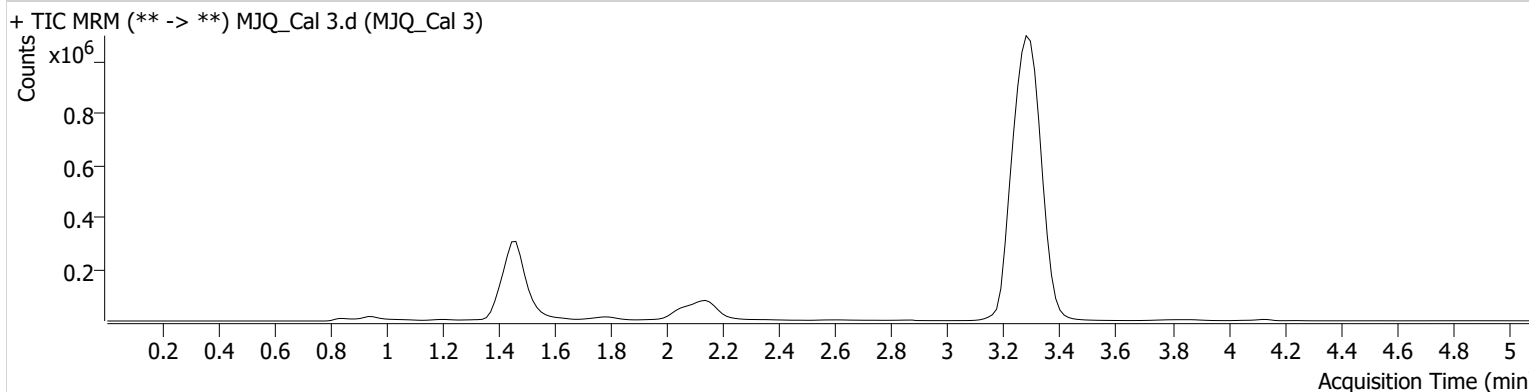


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 3.d
Type	Cal	Sample	MJQ_Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-F6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 5:36:31 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	127501	249.89	64.0	∞	252897	19.9645 ng/ml
THC-OH	1.468	140448	∞	8.8	∞	1155443	4.0553 ng/ml
THC	3.300	343120	∞	27.3	∞	7527533	4.7780 ng/ml

SC

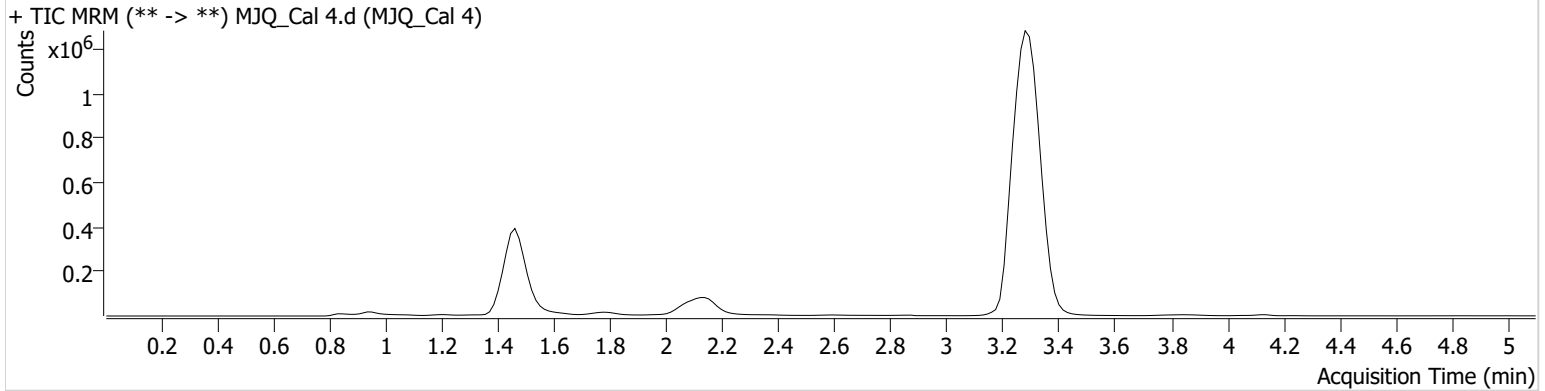


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 4.d
Type	Cal	Sample	MJQ_Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-E6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 5:44:06 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	320915	∞	67.4	∞	255582	49.2849 ng/ml
THC-OH	1.468	212950	∞	9.4	285.75	1117110	9.7382 ng/ml
THC	3.300	742264	6221.20	26.5	∞	7922099	9.6087 ng/ml

SC

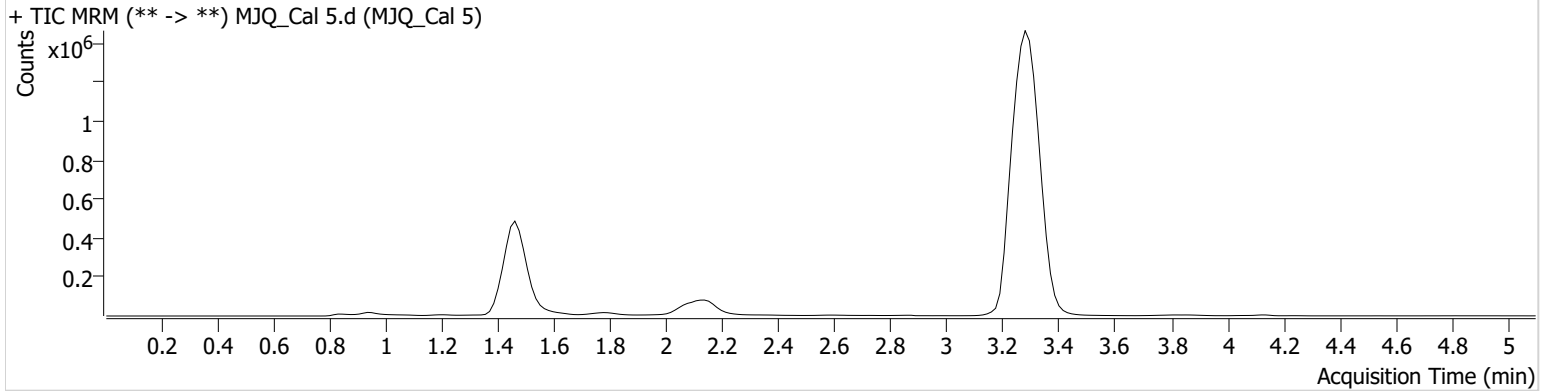


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 5.d
Type	Cal	Sample	MJQ_Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-D6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 5:51:42 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	469419	1092.37	70.5	2444.56	256208	71.7809 ng/ml
THC-OH	1.453	406897	∞	11.7 High	∞	1121461	23.9062 ng/ml
THC	3.300	1890480	∞	26.1	2188.96	7789253	24.5695 ng/ml

SC

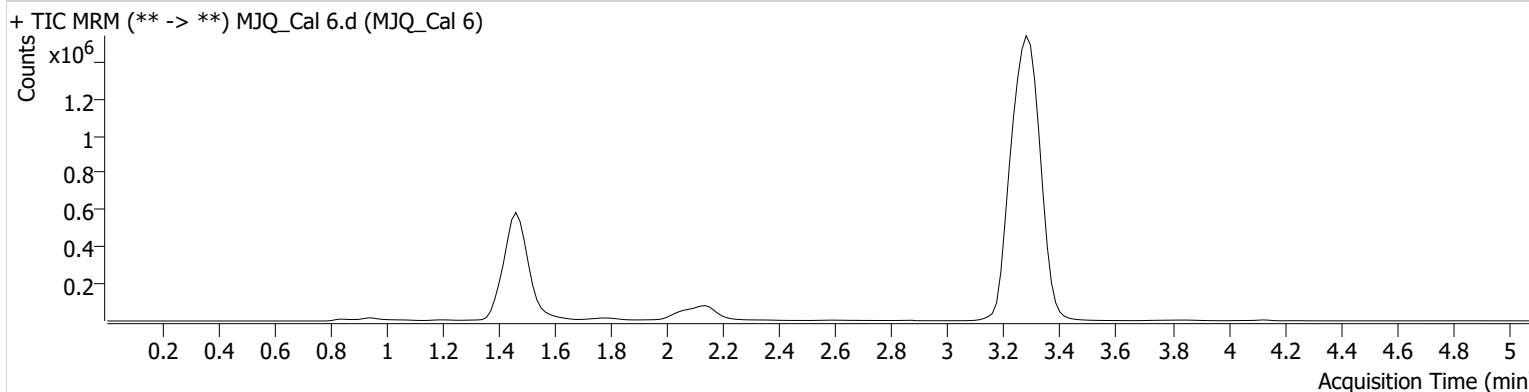


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 6.d
Type	Cal	Sample	MJQ_Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-C6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 5:59:17 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	654872	∞	64.8	2802.14	252087	101.6537 ng/ml
THC-OH	1.453	805560	∞	12.1 High	1920.84	1130346	52.6895 ng/ml
THC	3.300	3423499	∞	26.4	∞	7000160	49.3043 ng/ml

SC

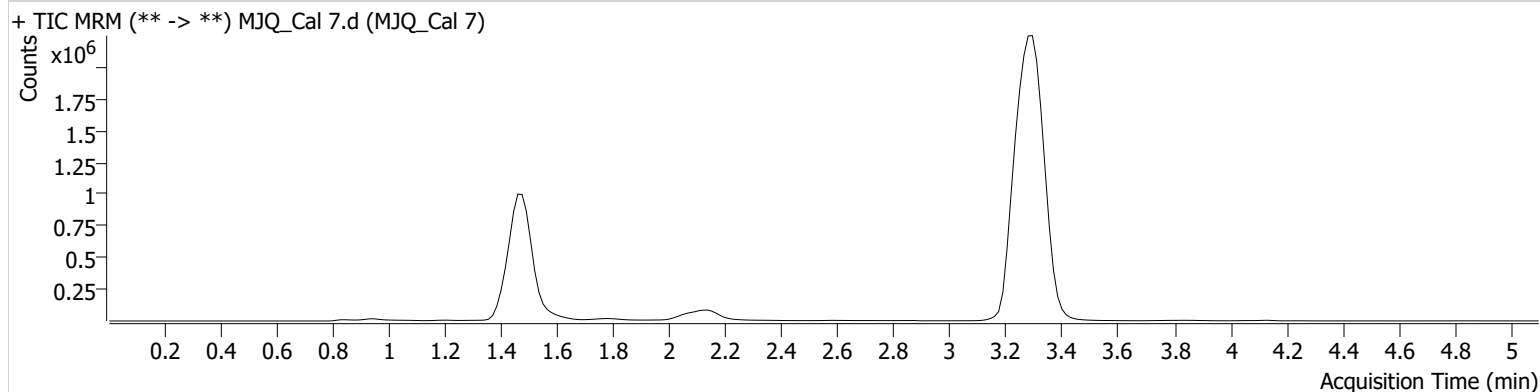


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100621 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 10/7/2021 7:24:53 AM

Instrument	Falco (069901)	Data File	MJQ_Cal 7.d
Type	Cal	Sample	MJQ_Cal 7
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-B6	Comment	
Injection Volume	10		
Acq. Date-Time	10/6/2021 6:06:53 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	1589946	2028.50	66.0	13458.79	246200	252.2677 ng/ml
THC-OH	1.453	1382349	∞	13.6 High	3385.14	1079509	99.4112 ng/ml
THC	3.300	7329217	∞	26.5	6302.14	7249971	101.7014 ng/ml