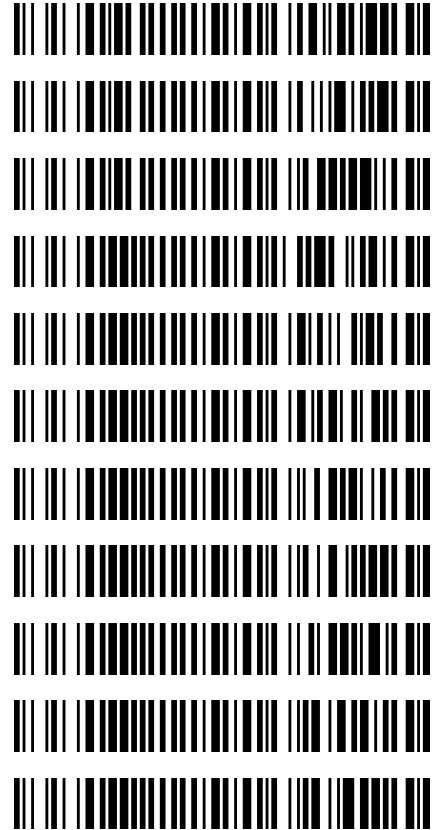


Worklist: 5274

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-4018	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-4137	4	BCK	AM 27 Blood THC Quant by LC-QQQ
M2021-4187	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3008	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3067	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3088	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3101	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3136	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3145	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3186	1	BCK	AM 27 Blood THC Quant by LC-QQQ
P2021-3190	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: 10/07/2021

Analyst: Tamara Salazar

Plate lot#: IDP-108-2-210609

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 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. 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.*

TS

	1	2	3	4	5	6
A	IS + Cal. 1				P2021-3101-1	IS + QC_1
B	IS + Cal. 2				P2021-3088-1	IS + Cal. 7
C	IS + Cal. 3				P2021-3067-1	IS + Cal. 6
D	IS + Cal. 4				P2021-3008-1	IS + Cal. 5
E	IS + Cal. 5			P2021-3190-1	M2021-4187-2	IS + Cal. 4
F	IS + Cal. 6			P2021-3186-1	M2021-4137-4	IS + Cal. 3
G	IS + Cal. 7			P2021-3145-1	M2021-4018-1	IS + Cal. 2
H	IS + QC_1			P2021-3136-1	Neg	IS + Cal. 1

All wells to contain 100 μ l of residual DMSO

TS

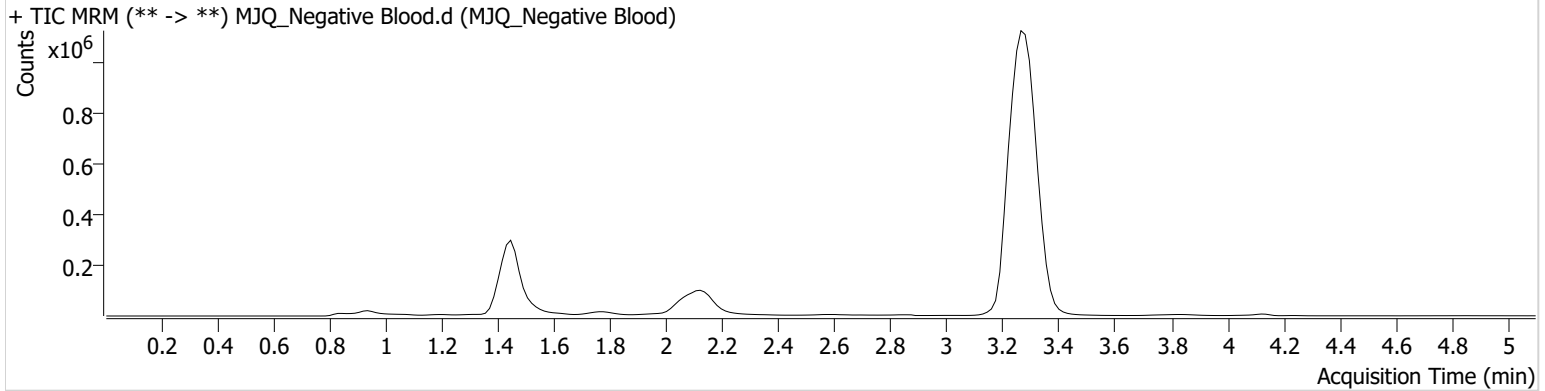


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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	10/7/2021 1:14:06 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.513	97710	∞	3.3 Low	17.39	1163999	0.2877 ng/ml Low

TS

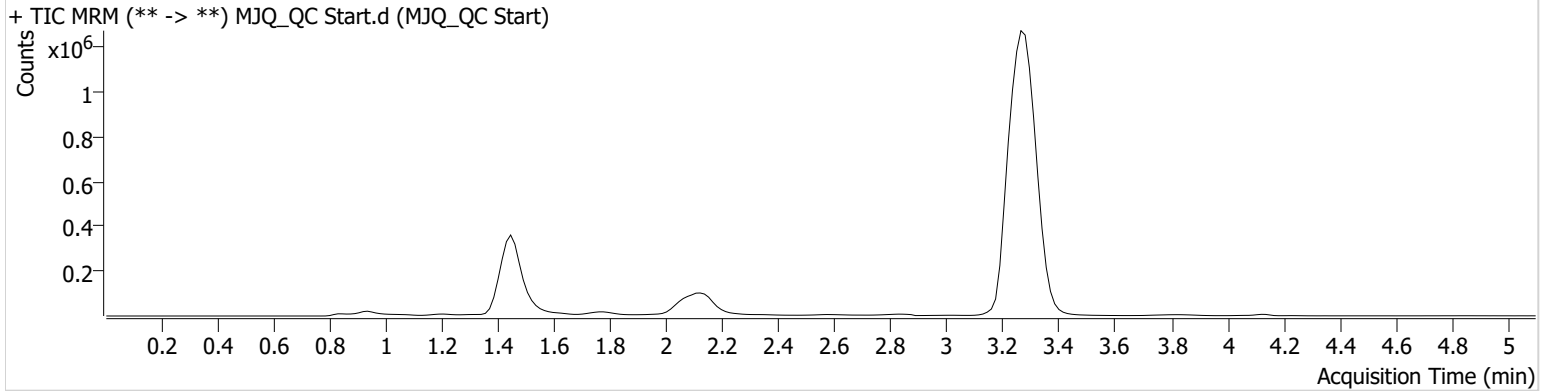


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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	10/7/2021 12:58:53 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	108552	∞	62.1	∞	276082	15.7670 ng/ml
THC-OH	1.468	172539	∞	7.9	∞	1231694	4.6877 ng/ml
THC	3.285	377132	3211.81	27.1	1221.36	8310110	4.7632 ng/ml

TS

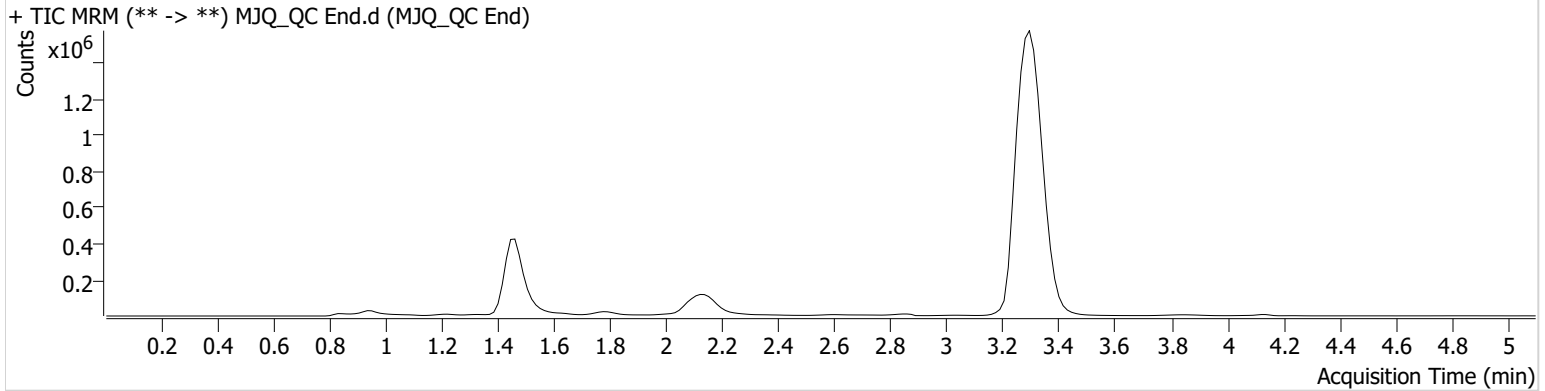


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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	10/7/2021 4:16:45 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	109572	101.69	63.7	347.31	289075	15.2327 ng/ml
THC-OH	1.513	188934	∞	7.6	∞	1349062	4.6850 ng/ml
THC	3.315	469015	3092.56	26.9	∞	9869825	4.9787 ng/ml

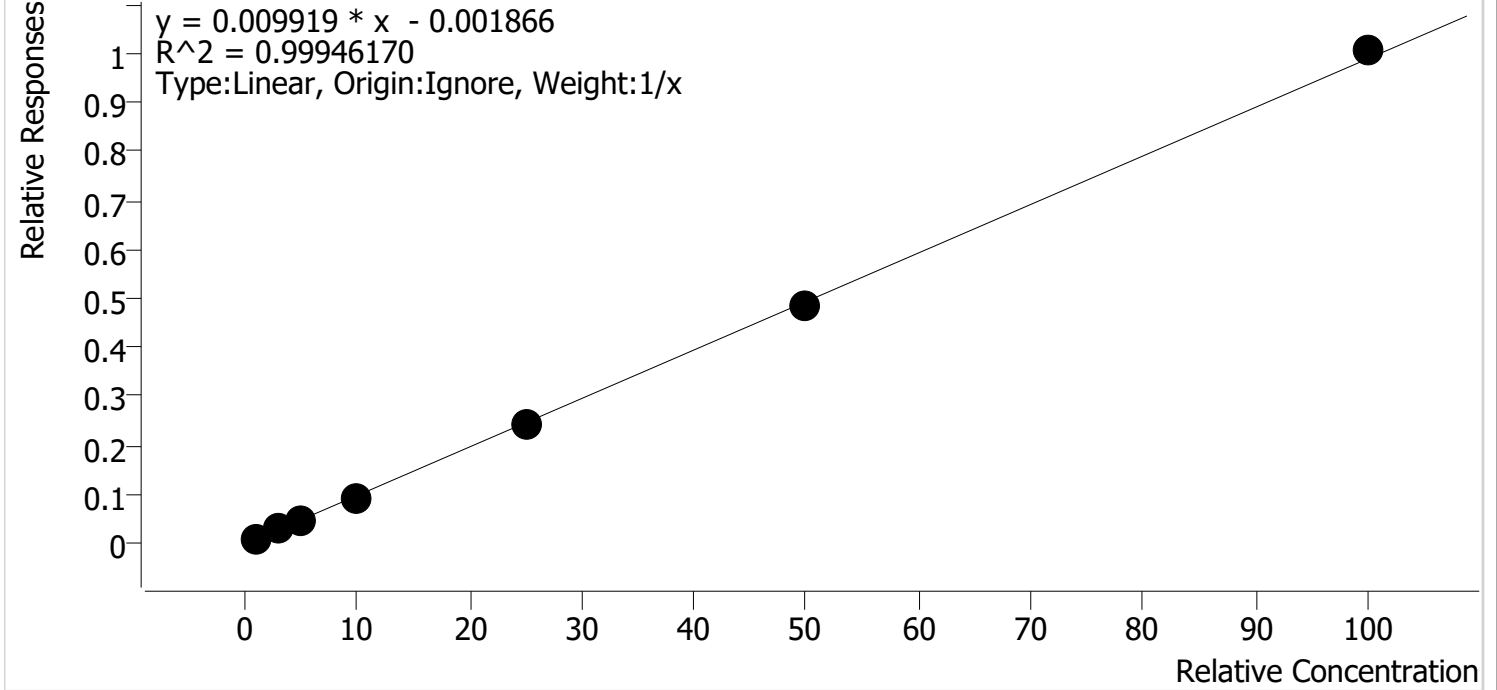


TS

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 10/12/2021 2:23 PM
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	111.1
MJQ_Cal 2	2	✓	3.0	3.0	98.9
MJQ_Cal 3	3	✓	5.0	4.8	96.1
MJQ_Cal 4	4	✓	10.0	9.5	94.8
MJQ_Cal 5	5	✓	25.0	24.7	98.9
MJQ_Cal 6	6	✓	50.0	49.3	98.5
MJQ_Cal 7	7	✓	100.0	101.7	101.7

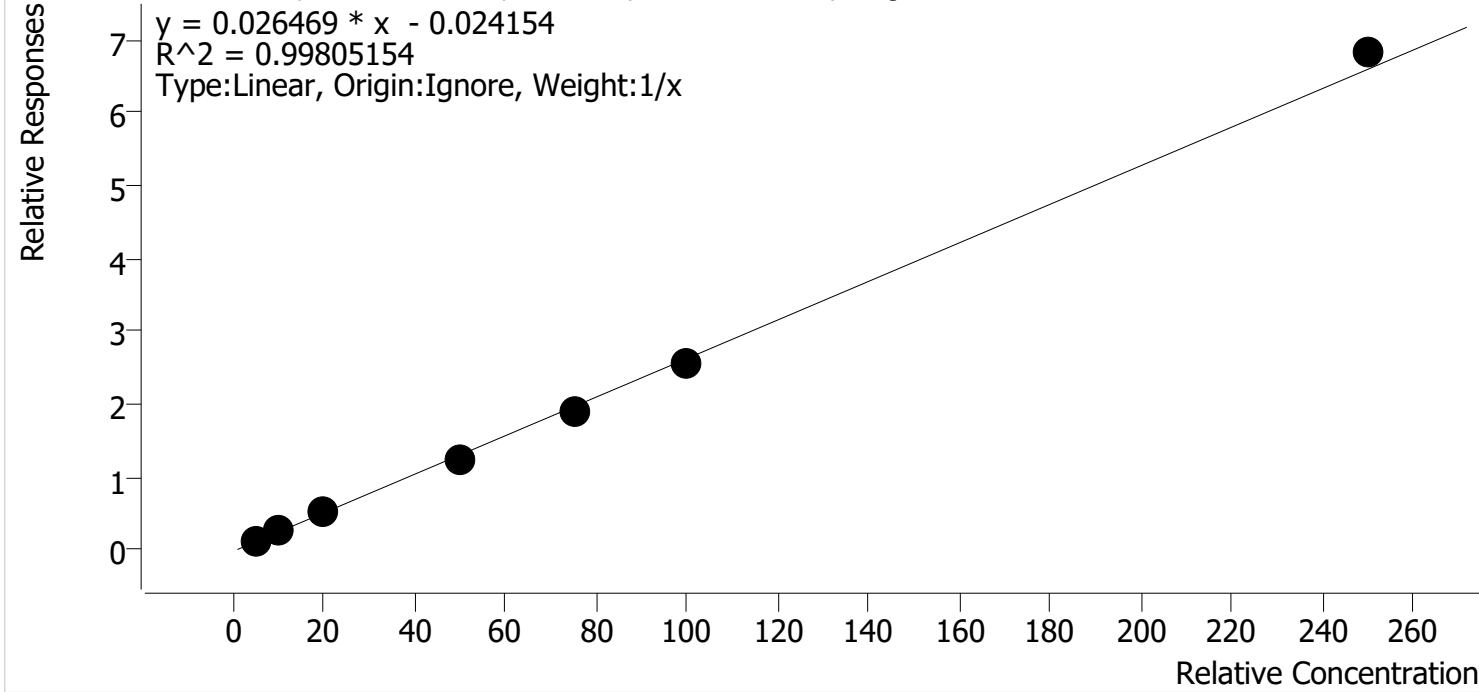
TS



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 10/12/2021 2:23 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9

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



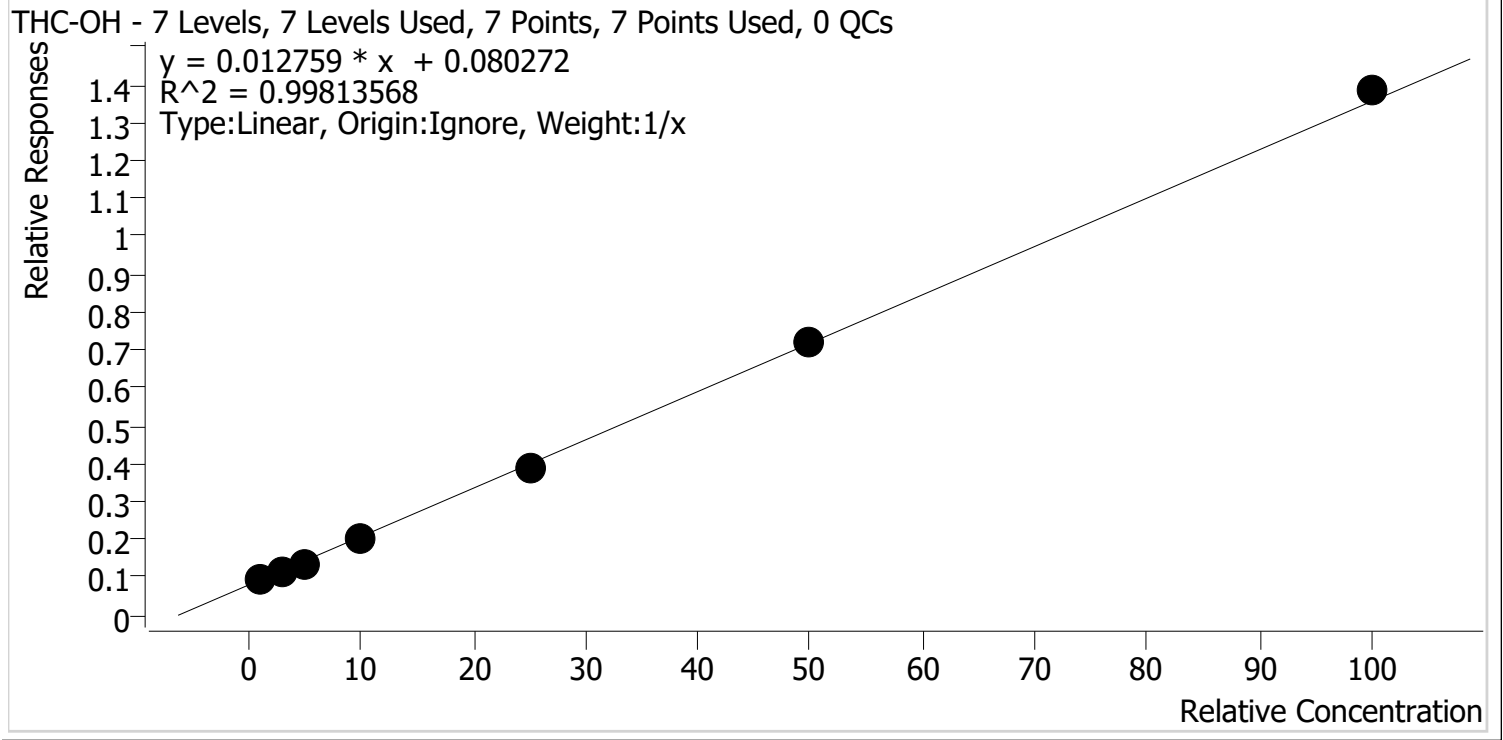
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	5.0	5.5	109.5
MJQ_Cal 2	2	✓	10.0	10.2	101.7
MJQ_Cal 3	3	✓	20.0	19.7	98.6
MJQ_Cal 4	4	✓	50.0	46.8	93.7
MJQ_Cal 5	5	✓	75.0	71.7	95.6
MJQ_Cal 6	6	✓	100.0	97.5	97.5
MJQ_Cal 7	7	✓	250.0	258.6	103.4



TS

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Last Cal. Update 10/12/2021 2:23 PM
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	1.3	127.9
MJQ_Cal 2	2	✓	3.0	2.8	94.5
MJQ_Cal 3	3	✓	5.0	4.3	85.0
MJQ_Cal 4	4	✓	10.0	9.4	93.7
MJQ_Cal 5	5	✓	25.0	24.3	97.0
MJQ_Cal 6	6	✓	50.0	49.8	99.6
MJQ_Cal 7	7	✓	100.0	102.2	102.2

TS

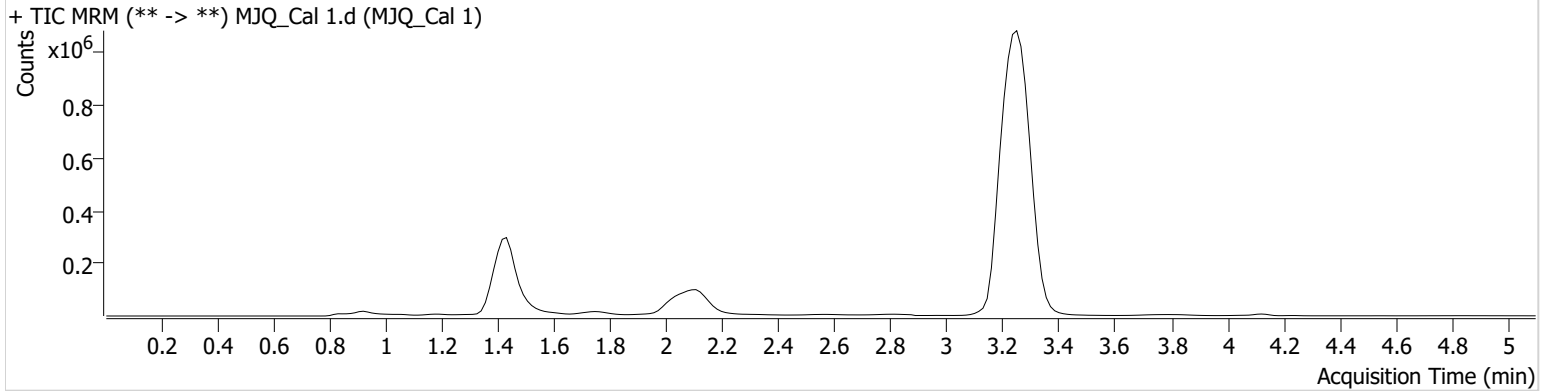


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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	10/7/2021 11:57:57 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.459	32504	∞	62.0	149.00	269153	5.4749 ng/ml
THC-OH	1.498	116098	∞	4.6 Low	24.12	1201986	1.2788 ng/ml Low
THC	3.270	71842	∞	32.2	∞	7843425	1.1115 ng/ml

TS

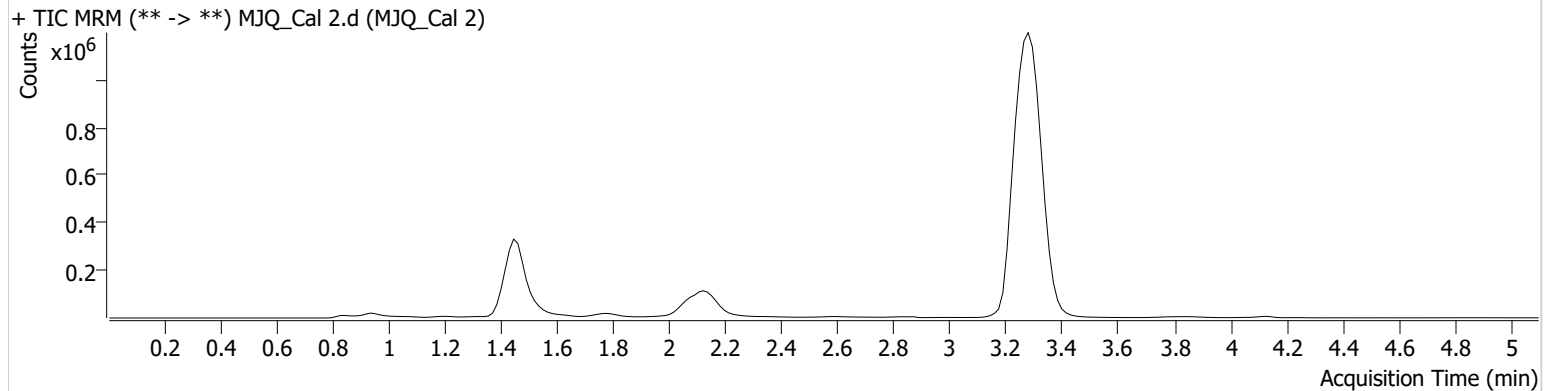


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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 10/7/2021 12:05:42 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	65227	∞	60.1	1170.94	266218	10.1691 ng/ml
THC-OH	1.513	136705	∞	6.1 Low	∞	1173995	2.8350 ng/ml Low
THC	3.300	218667	4698.25	28.3	195.80	7933155	2.9669 ng/ml

TS



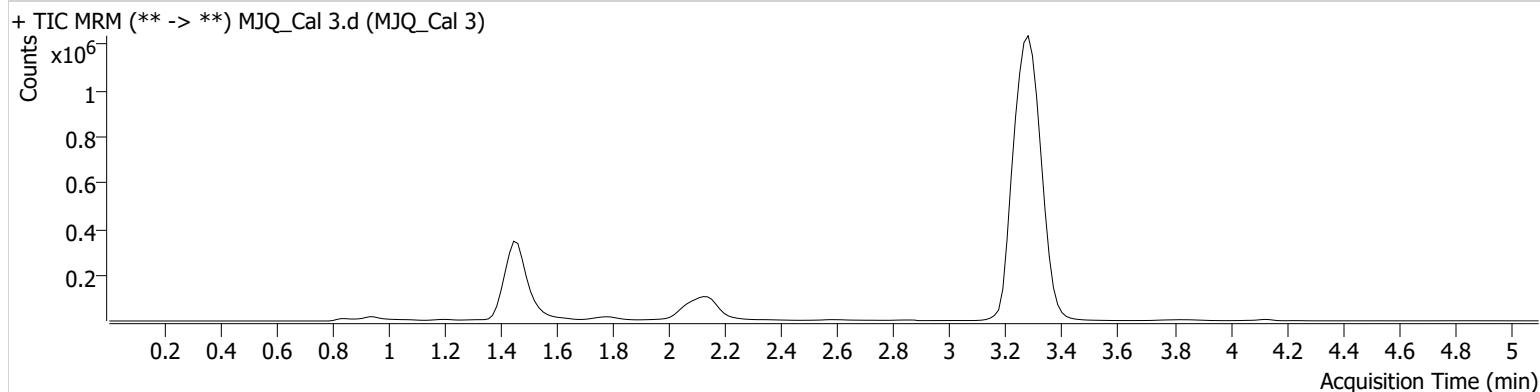
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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	10/7/2021 12:13:17 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	133735	367.12	63.6	388.76	268653	19.7192 ng/ml
THC-OH	1.513	159203	∞	7.4 Low	∞	1183442	4.2521 ng/ml
THC	3.300	371164	1723.62	26.9	∞	8104831	4.8049 ng/ml

TS



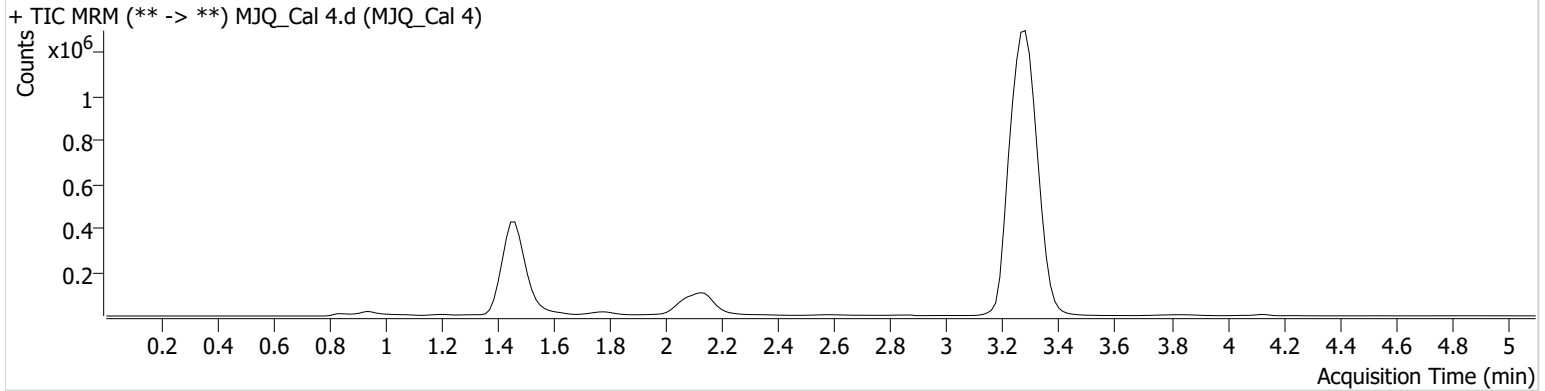
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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	10/7/2021 12:20:53 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.474	334197	∞	71.4	∞	274904	46.8408 ng/ml
THC-OH	1.468	243142	∞	9.2	91.94	1216446	9.3743 ng/ml
THC	3.300	743247	∞	26.5	∞	8064756	9.4790 ng/ml

TS



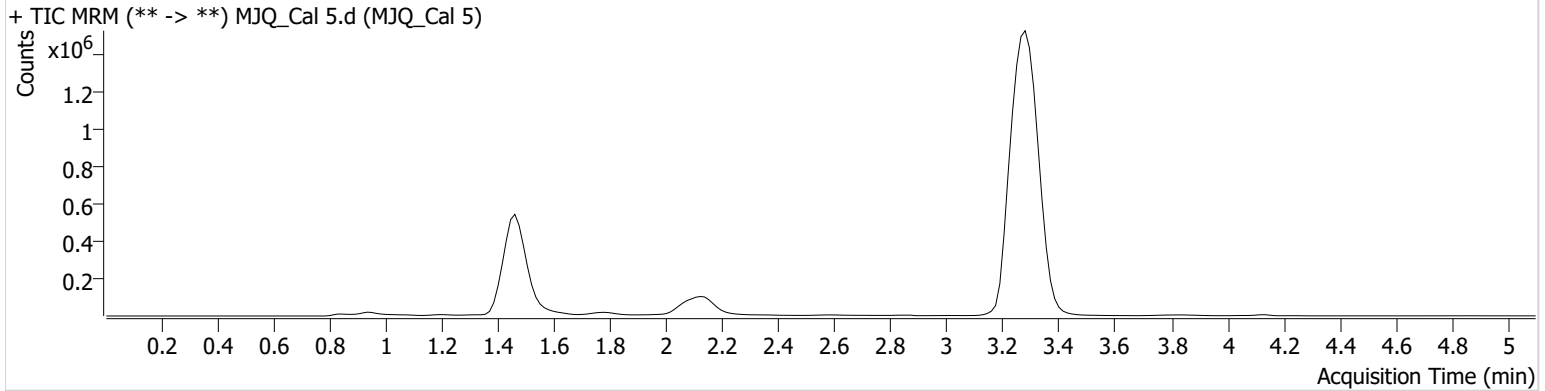
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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	10/7/2021 12:28:29 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	513285	5105.41	66.5	26486.1	274053	71.6716 ng/ml
THC-OH	1.453 Low	469642	∞	11.8 High	695.91	1205113	24.2523 ng/ml
THC	3.300	1996389	∞	26.0	∞	8202578	24.7246 ng/ml

TS

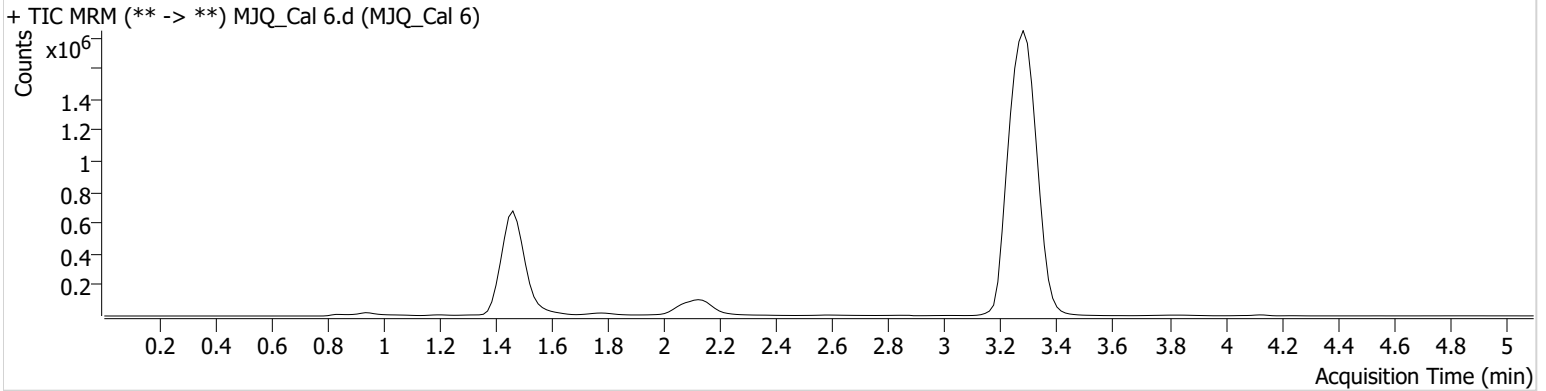


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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 10/7/2021 12:36:04 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	684813	∞	66.8	4506.02	267759	97.5367 ng/ml
THC-OH	1.453 Low	860733	∞	13.1 High	591.57	1202258	49.8205 ng/ml
THC	3.300	3903398	∞	26.7	∞	8019460	49.2580 ng/ml

TS



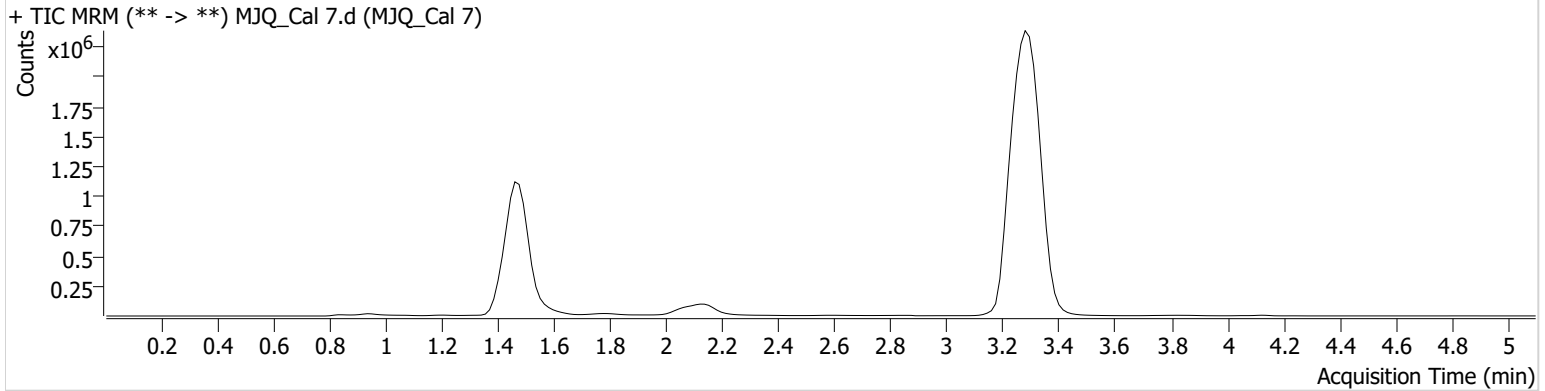
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\100721 AM 27 28 TS\QuantResults\AM 27.batch.bin
Calibration Last Update 10/12/2021 2:23:25 PM

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	10/7/2021 12:43:40 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	1798203	∞	63.3	∞	263648	258.5877 ng/ml
THC-OH	1.453 Low	1583287	∞	13.5 High	393.89	1143933	102.1870 ng/ml
THC	3.300	7841681	∞	26.1	∞	7791151	101.6552 ng/ml