REVIEWED
By Tamara Salazar at 7:38 pm, Apr 19, 2021

Worklist: 4911

LAB_CASE	ITEM	ITEM_TYPE	DESCRIPTION
M2021-0429	4	BCK	AM 27 Blood THC Quant by LC-QQQ

M2021-0777-1, M2021-0921-1, P2021-0673-1, P2021-0762-1, P2021-0779-1, and P2021-0782-1 were also included in this run.

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

Extraction Date: <u>04/14/2021</u>
Plate lot#: IDP-108-2-201206
Mobile phase A: 0.1% Formic Acid in LCMS Water
Blank Blood Lot: Lampire 20L20724
LCMS-QQQ ID: 069901

Analyst: <u>Celena Shrum</u>
Plate Expiration: 06/06/2021
Mobile phase B: 0.1% Formic acid in Acetonitrile
Column: UCT Selectra DA 100 x 2.1mm 3um

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** of analytical plate.
☒ 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
☒ 6. Transfer **800µL of blood+acid or urine+acid** mixture to corresponding wells of SLE+ plate.
☒ 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: Curve Limitations: THC 3-100, c-THC 5-250, THC-OH 3-100.

A possible manufacturing error with some of the analytical plates in this lot was discovered and as such this run was done to ensure that the error did not affect the plate(s) that had been used for Worklist 4863. The results of the samples from this plate were consistent with the previous run and the concentrations were within the expected uncertainty ranges. Since both runs were valid, the lower value of the runs will be reported. With further investigation, it was determined that the possible manufacturing error had no impact. Note: M2021-0429-4 was run with Worklist 4820 (not Worklist 4863).

Idaho State Police
Forensic Services
Toxicology Discipline

Request for Departure from an Analytical Method

Date of Request
02/24/2021

Forensic Scientist
Anne Nord

Analytical Method
Toxicology AM #27: Quantitative Analysis of THC and Metabolites in Blood and Urine by LCMS-QQQ

Request

The method currently reads:
4.3.2.5 If any points are dropped from the approved quantitative range of the curve, the compound will be reported qualitatively. For calibrators and controls 10 ng and below, the accuracy must be within 30%, for calibrators and controls greater than 10 ng/mL the accuracy must be within 20%. If a control falls outside the accuracy range, at the analyst's discretion, the compound may be reported qualitatively.

I would like to add in the following exception:

If the 1ng/ml point is dropped for THC, the quantitative range will be 3-50 ng/ml.

Discipline Leader Review

☒ Departure approved
  Comments: This deviation is approved and will remain in effect until it is changed in the actual method.

☐ Departure Not Approved
  Comments:

Celena Shrum
Toxicology Discipline Lead
Date: 02/24/2021

	1	2	3	4	5	6
a	cal 1ng	Blood NC				
b	cal 3 ng	M2021-0429-4				
c	cal 5 ng	M2021-0777-1				
d	cal 10ng	M2021-0921-1				
e	cal 25 ng	P2021-0673-1				
f	cal 50 ng	P2021-0762-1				
g	cal 100 ng	P2021-0779-1				
h	QC 1	P2021-0782-1				

Samples were moved to columns 5 and 6 on the SPE plate (A1 became A5, A2 became A6, etc.).

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update 4/19/2021 8:38:16 AM

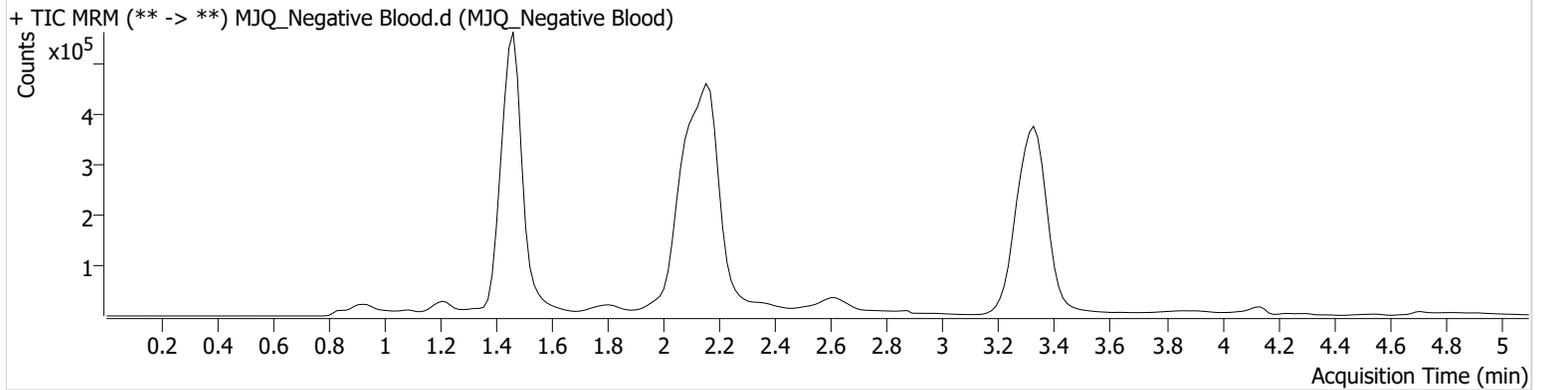|			
Instrument	Instrument 1	**Data File**	MJQ_Negative Blood.d
Type	Sample	**Sample**	MJQ_Negative Blood
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-A6	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 1:16:56 PM		
Sample Info.			

Sample Chromatogram

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update   4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_QC Start.d
Type	Sample	**Sample**	MJQ_QC Start
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-H5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 1:32:09 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJQ_QC Start.d (MJQ_QC Start)

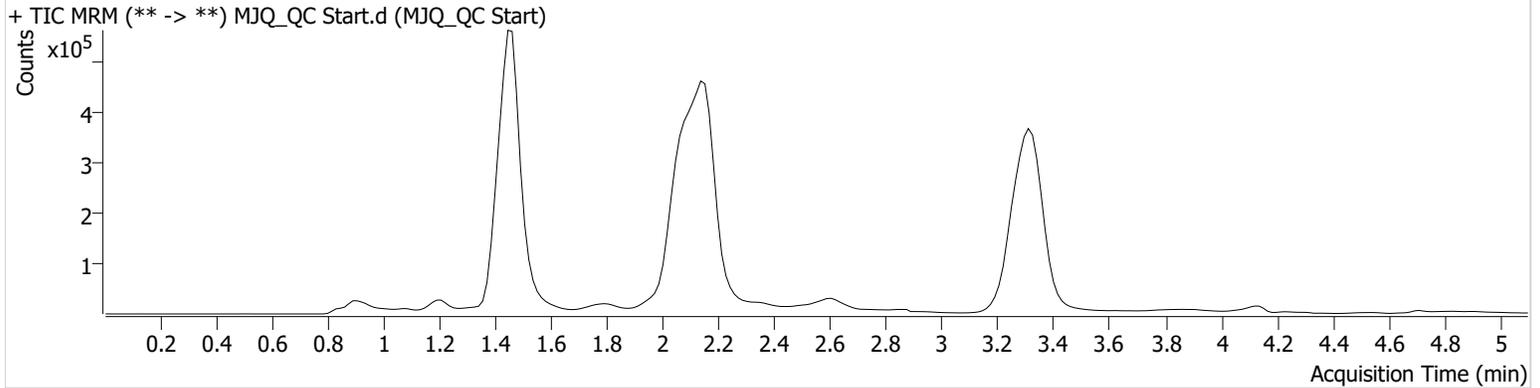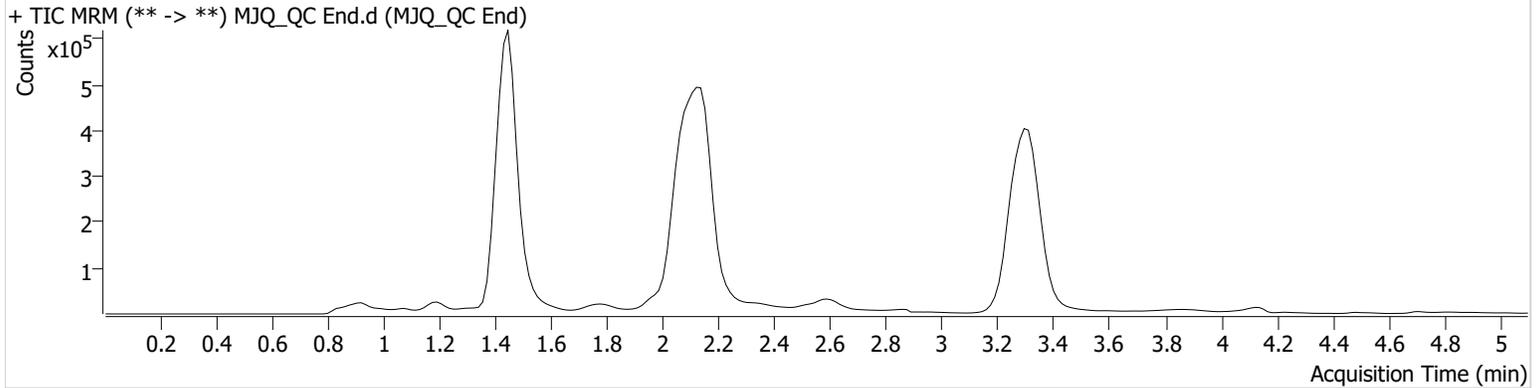| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	171165	∞	8.7	∞	2202796	3.9472	ng/ml
THC-COOH	1.489	169372	∞	54.4	625.52	456151	14.4573	ng/ml
THC	3.330	100363	∞	34.2	∞	2660507	4.2734	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update   4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_QC End.d
Type	Sample	**Sample**	MJQ_QC End
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-H5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 3:34:57 PM		
Sample Info.			

Sample Chromatogram

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.453	178967	∞	8.8	∞	2268005	4.0380	ng/ml
THC-COOH	1.474	179126	1052.22	53.6	498.59	492041	14.1709	ng/ml
THC	3.315	107803	∞	35.4	175.34	2967237	4.1262	ng/ml

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin

Last Cal. Update   4/19/2021 8:38 AM

Analyst Name   ISP\Datastor

Analyte   THC   **Internal Standard**   THC-D3
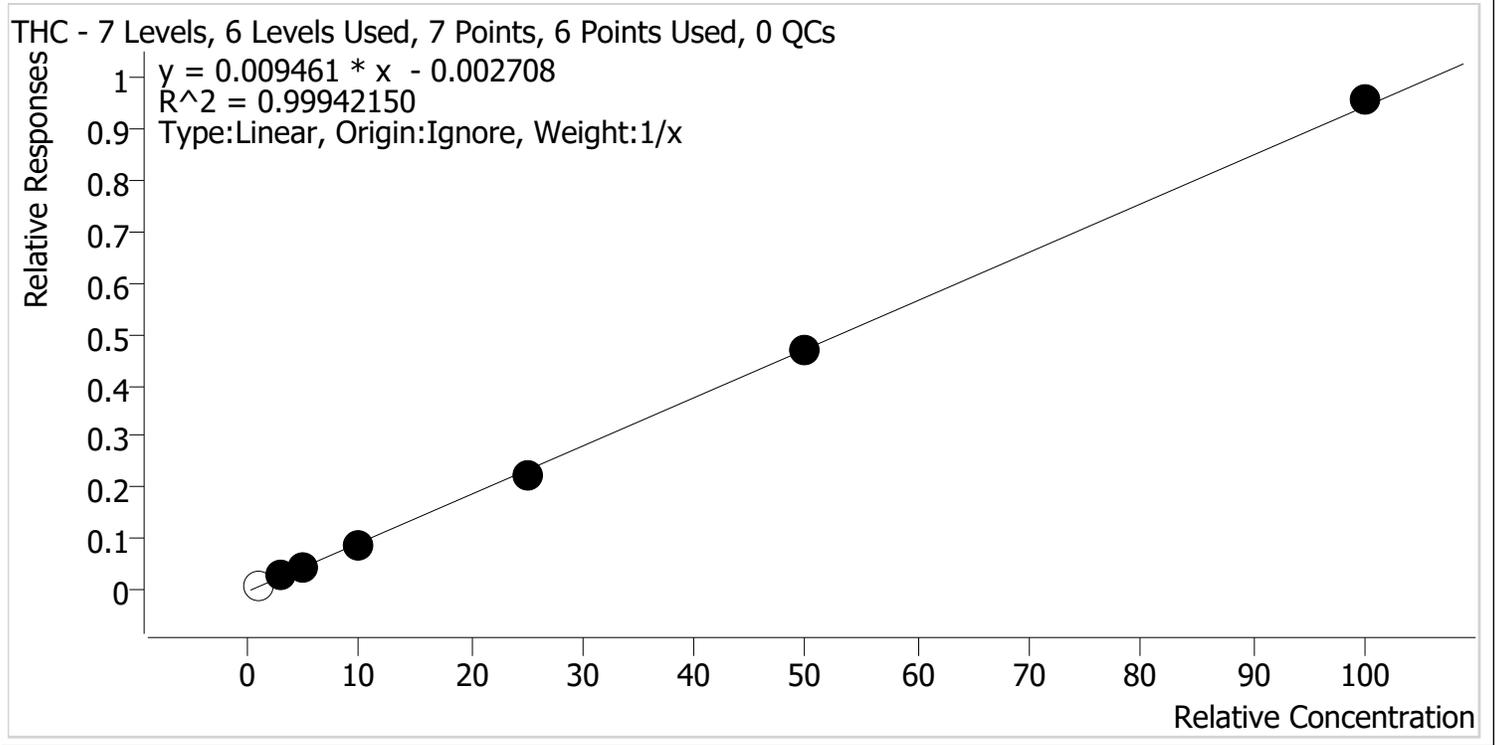
THC - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs

$y = 0.009461 * x - 0.002708$
$R^2 = 0.99942150$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✗	1.0	1.3	128.5
MJQ_Cal 2	2	✔	3.0	3.1	103.9
MJQ_Cal 3	3	✔	5.0	5.1	102.1
MJQ_Cal 4	4	✔	10.0	9.8	97.5
MJQ_Cal 5	5	✔	25.0	23.9	95.5
MJQ_Cal 6	6	✔	50.0	49.8	99.6
MJQ_Cal 7	7	✔	100.0	101.4	101.4

Calibrator 1 dropped due to poor secondary peak shape/response and ratio.

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Last Cal. Update   4/19/2021 8:38 AM
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

$y = 0.025350 * x + 0.004810$
$R^2 = 0.99960700$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✔	5.0	5.3	106.1
MJQ_Cal 2	2	✔	10.0	9.7	97.5
MJQ_Cal 3	3	✔	20.0	19.7	98.7
MJQ_Cal 4	4	✔	50.0	47.7	95.5
MJQ_Cal 5	5	✔	75.0	75.6	100.8
MJQ_Cal 6	6	✔	100.0	101.2	101.2
MJQ_Cal 7	7	✔	250.0	250.6	100.3

AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Last Cal. Update   4/19/2021 8:38 AM
Analyst Name   ISP\Datastor
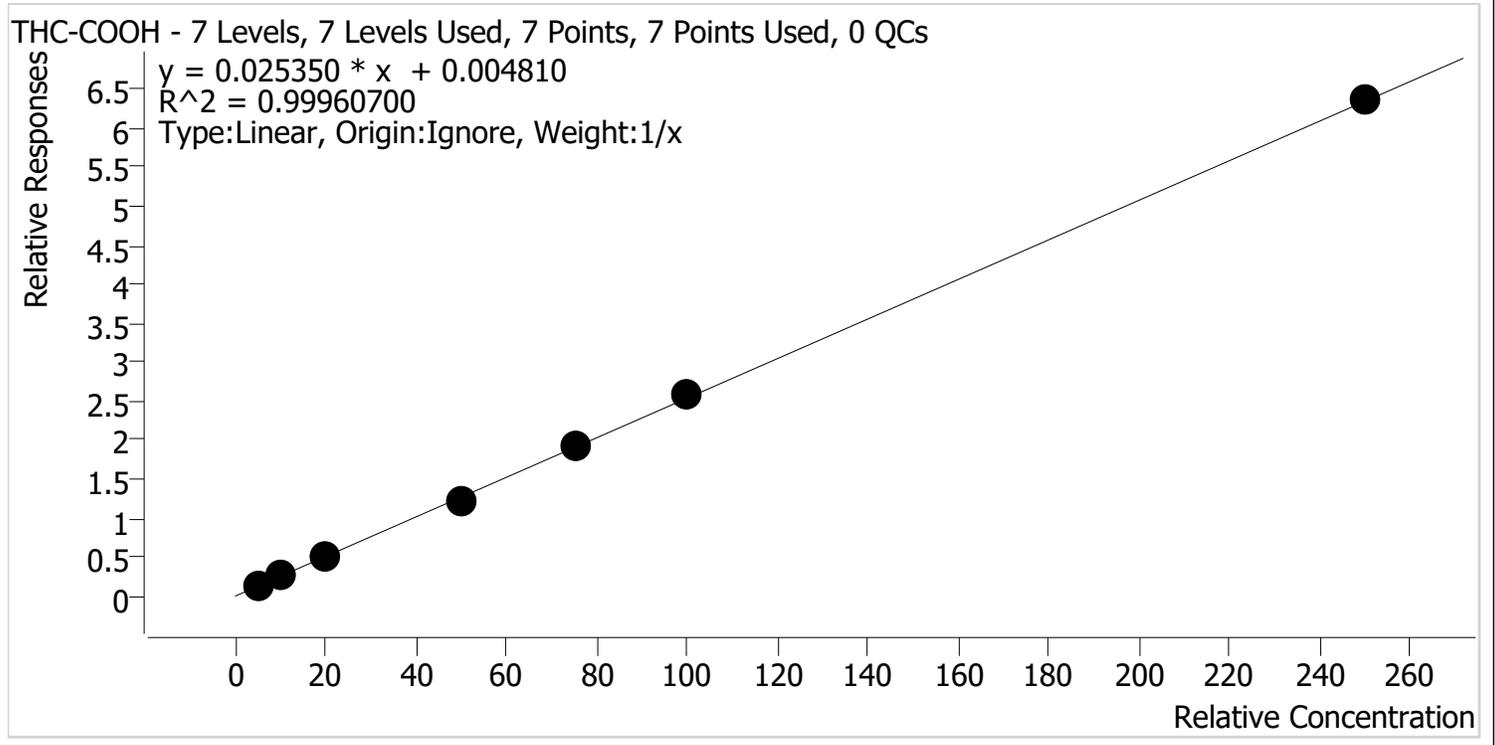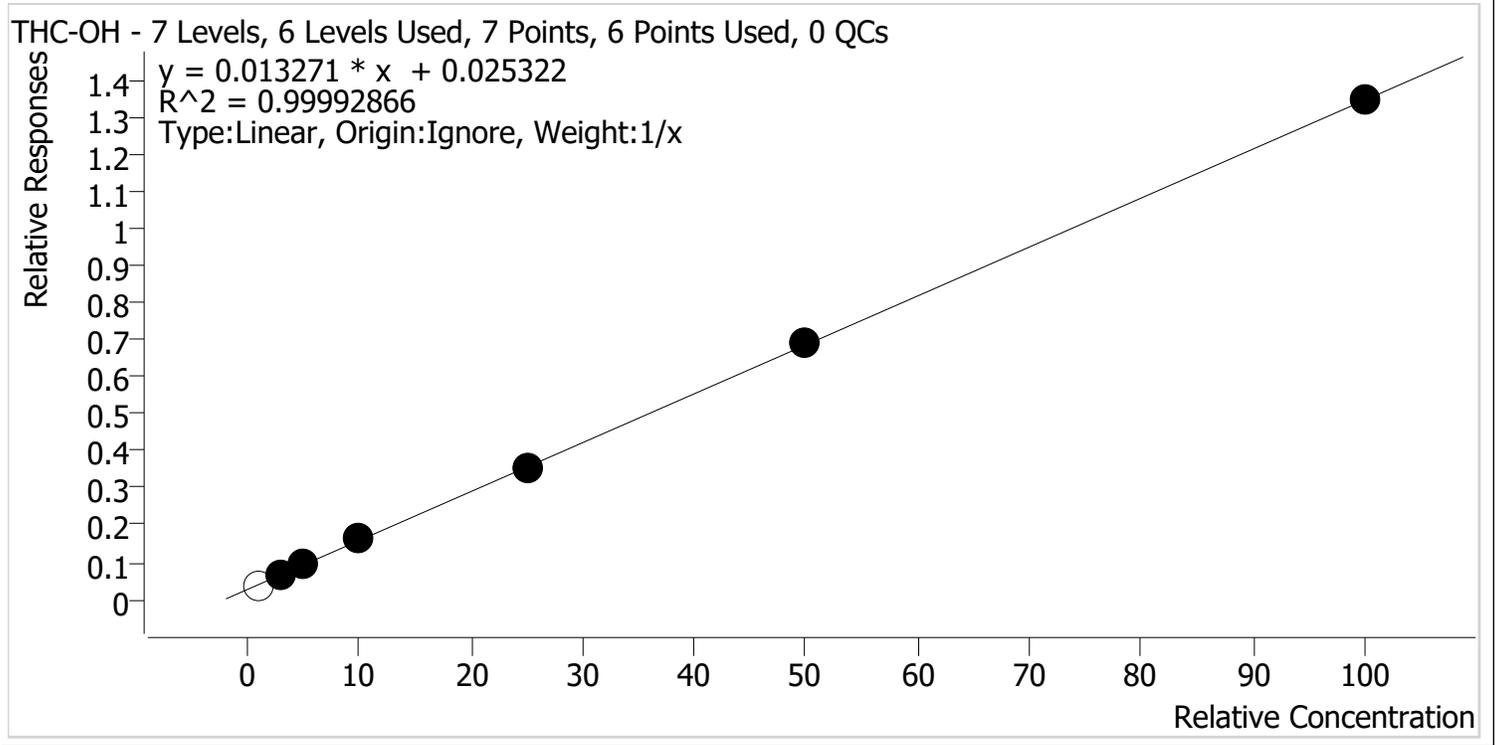**Analyte**   THC-OH   **Internal Standard**   THC-OH-D3

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs

$y = 0.013271 * x + 0.025322$
$R^2 = 0.99992866$
Type:Linear, Origin:Ignore, Weight:1/x

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✗	1.0	0.9	86.3
MJQ_Cal 2	2	✔	3.0	2.9	97.0
MJQ_Cal 3	3	✔	5.0	5.1	102.3
MJQ_Cal 4	4	✔	10.0	10.2	101.5
MJQ_Cal 5	5	✔	25.0	24.8	99.0
MJQ_Cal 6	6	✔	50.0	50.1	100.1
MJQ_Cal 7	7	✔	100.0	100.0	100.0

Calibrator 1 dropped due to not meeting ratio requirement.

AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update 4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_Cal 1.d
Type	Cal	**Sample**	MJQ_Cal 1
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-A5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 12:15:58 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJQ_Cal 1.d (MJQ_Cal 1)
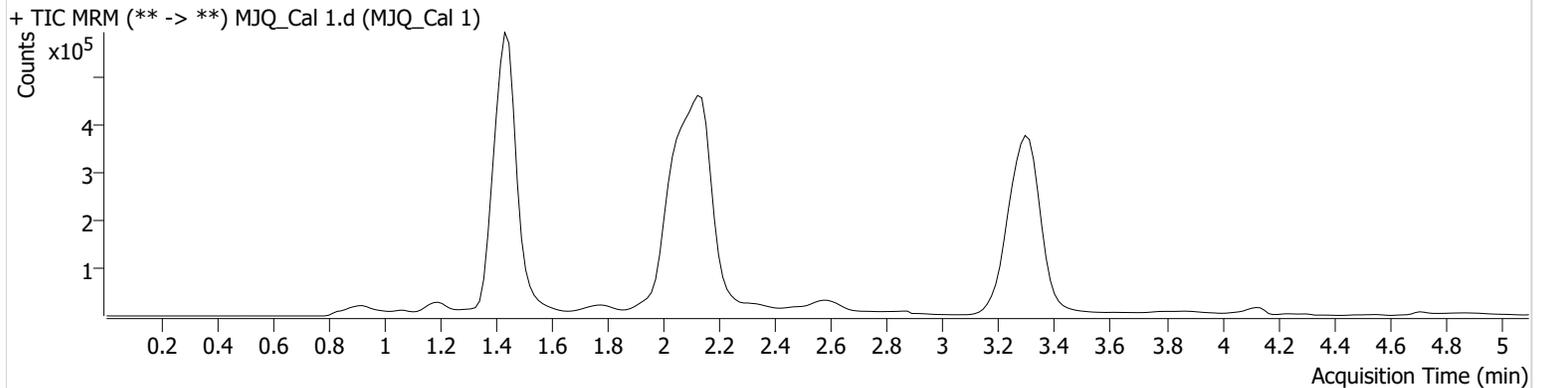
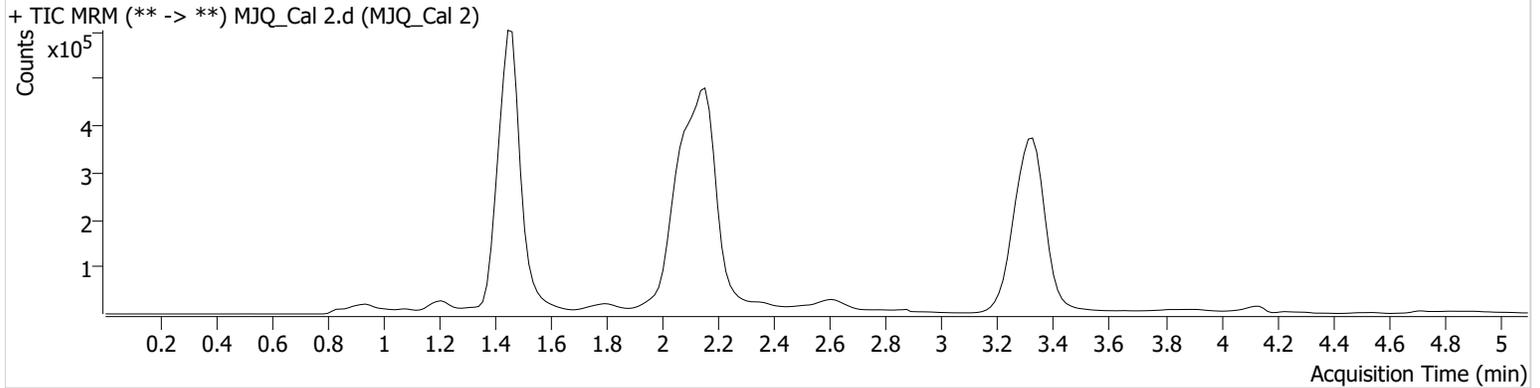| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.513 **High**	93737	∞	5.4 **Low**	24.42	2549172	0.8627	ng/ml **Low**
THC-COOH	1.474	73028	∞	48.7	118.03	524503	5.3026	ng/ml
THC	3.315	28565	∞	38.4 **High**	∞	3022496	1.2851	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update   4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_Cal 2.d
Type	Cal	**Sample**	MJQ_Cal 2
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-B5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 12:23:44 PM		
Sample Info.			

Sample Chromatogram

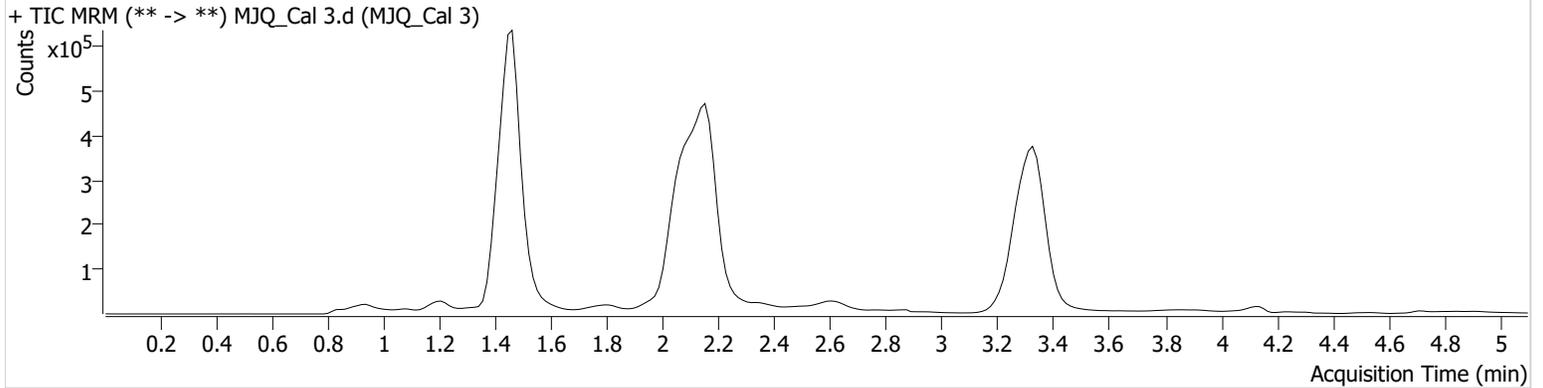| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	154701	∞	8.0	∞	2419479	2.9100	ng/ml **Low**
THC-COOH	1.489	128130	∞	52.2	723.08	508732	9.7455	ng/ml
THC	3.330	75573	∞	33.4	31.68	2820655	3.1180	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update   4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_Cal 3.d
Type	Cal	**Sample**	MJQ_Cal 3
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-C5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 12:31:20 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJQ_Cal 3.d (MJQ_Cal 3)

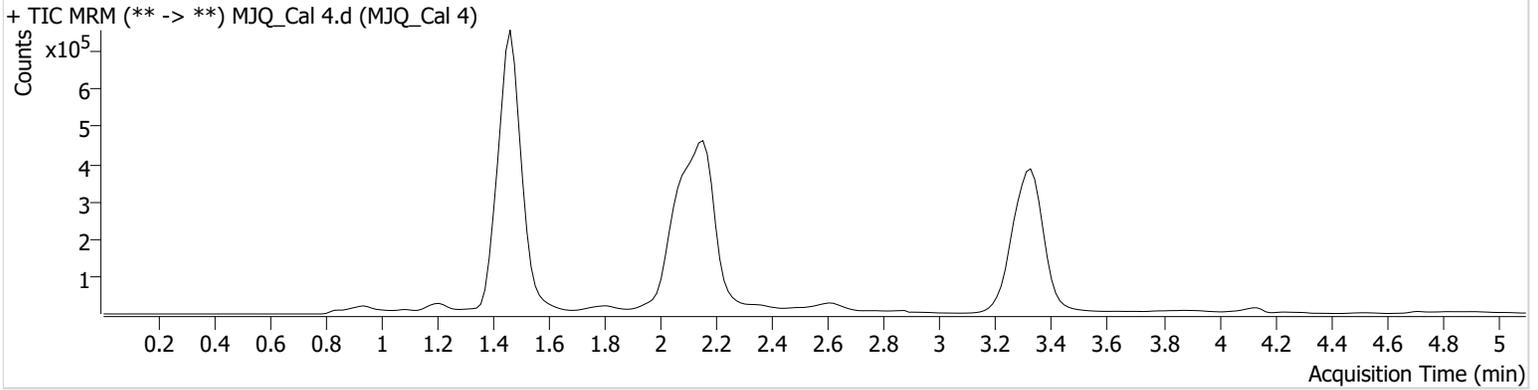| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	224290	∞	8.7	∞	2406033	5.1163	ng/ml
THC-COOH	1.489	254281	∞	55.2	4725.29	503227	19.7430	ng/ml
THC	3.345	127480	∞	35.9	∞	2797015	5.1035	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results　　　D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update　4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_Cal 4.d
Type	Cal	**Sample**	MJQ_Cal 4
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-D5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 12:38:56 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJQ_Cal 4.d (MJQ_Cal 4)

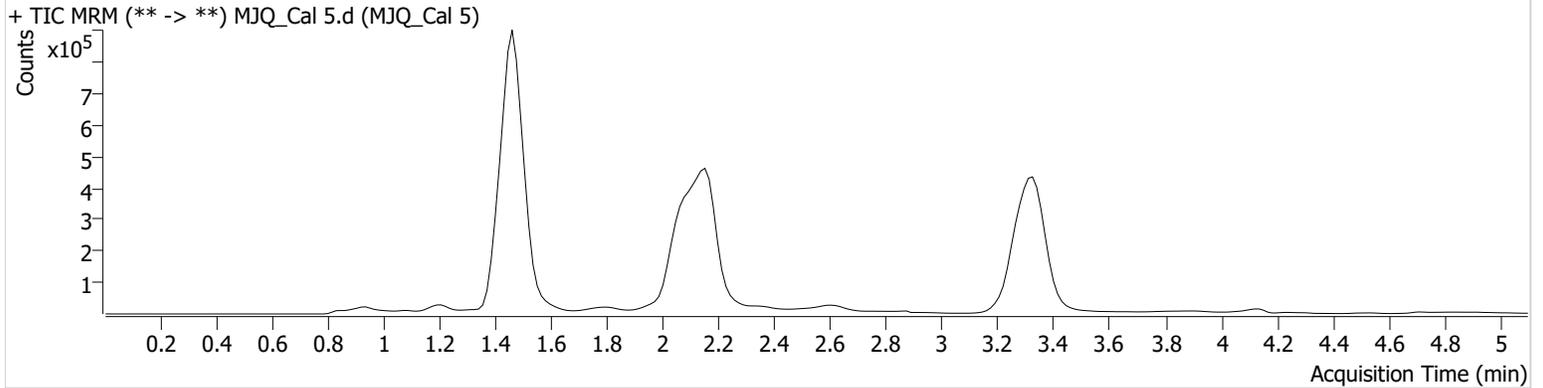| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	378470	∞	9.8	∞	2364899	10.1513	ng/ml
THC-COOH	1.489	600363	∞	57.6	∞	494144	47.7370	ng/ml
THC	3.345	244364	∞	30.1	∞	2728098	9.7536	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update   4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_Cal 5.d
Type	Cal	**Sample**	MJQ_Cal 5
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-E5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 12:46:32 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJQ_Cal 5.d (MJQ_Cal 5)

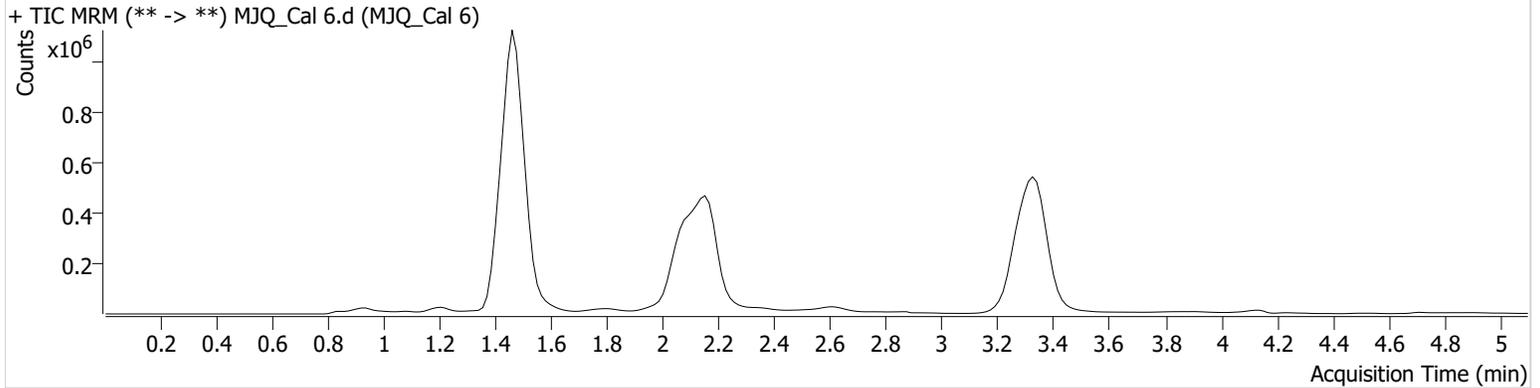| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	811623	∞	10.7	278.79	2293503	24.7581	ng/ml
THC-COOH	1.489	890257	∞	56.7	∞	463167	75.6323	ng/ml
THC	3.330	605327	∞	27.3	121.21	2711517	23.8819	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update   4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_Cal 6.d
Type	Cal	**Sample**	MJQ_Cal 6
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-F5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 12:54:08 PM		
Sample Info.			

Sample Chromatogram

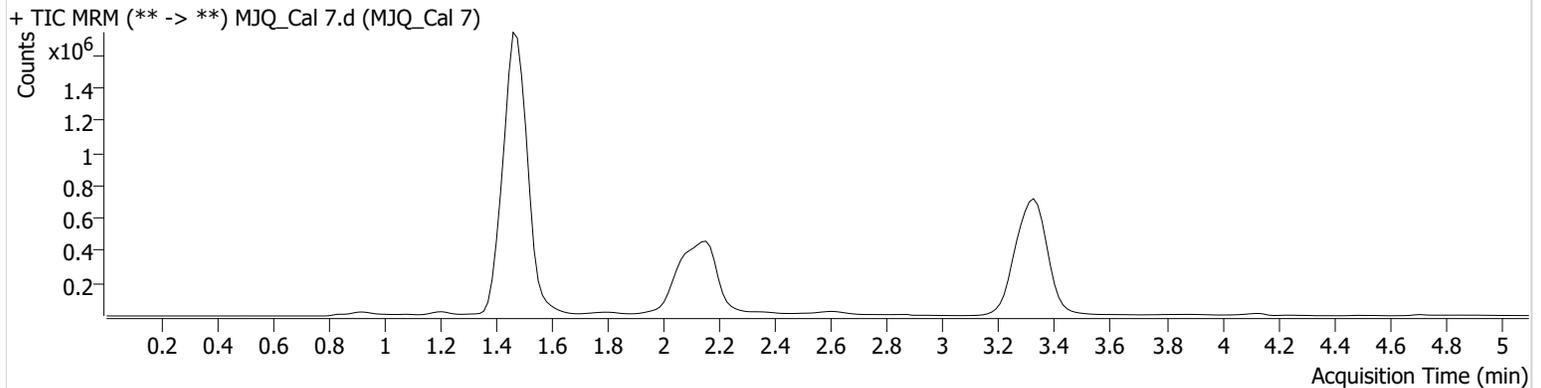| Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	1565013	∞	11.1	∞	2269098	50.0640	ng/ml
THC-COOH	1.489	1166439	∞	58.1	12117.50	453787	101.2077	ng/ml
THC	3.345	1284586	∞	25.7	∞	2742760	49.7890	ng/ml

AM #27 Cannabinoid Quant. Results

Batch results   D:\MassHunter\Data\2021\AM 27-28\041421 AM 27 CS\QuantResults\AM 27 THCQ.batch.bin
Calibration Last Update   4/19/2021 8:38:16 AM

Instrument	Instrument 1	**Data File**	MJQ_Cal 7.d
Type	Cal	**Sample**	MJQ_Cal 7
Acq. Method	AM 27 THCQ.m	**Operator**	Celena Shrum
Sample Position	P1-G5	**Comment**	
Injection Volume	10		
Acq. Date-Time	4/14/2021 1:01:43 PM		
Sample Info.			

Sample Chromatogram

+ TIC MRM (** -> **) MJQ_Cal 7.d (MJQ_Cal 7)

Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.468	2870788	∞	11.5	∞	2122738	100.0003	ng/ml
THC-COOH	1.489	2643653	∞	58.5	∞	415774	250.6318	ng/ml
THC	3.330	2511517	∞	25.3	∞	2626504	101.3540	ng/ml