

REVIEWED

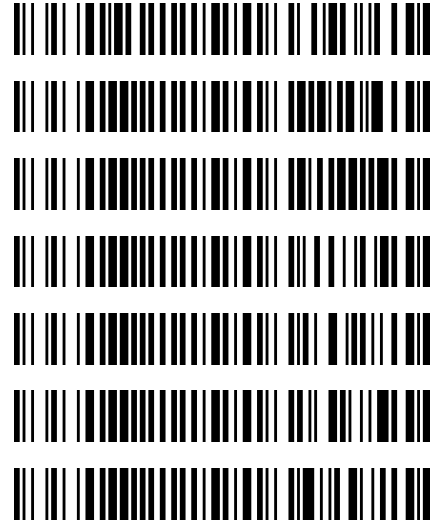
By Celena Shrum at 12:45 pm, Apr 30, 2021

SC

4/27/2021

Worklist: 4926

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>
M2021-1522	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
P2021-0703	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
P2021-0712	3	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
P2021-0732	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
P2021-1023	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
P2021-1090	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
P2021-1135	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ



8C

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

Extraction Date: 04/28/21

Analyst: Sarah Collins

Plate lot#: IDP-108-2-210412

Plate Expiration: 10/12/2021

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

Blank Urine Lot: POCO31319

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 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 range limited: THC-OH 3-100

All samples in this batch were urine, results reported qualitatively.

8C

	1	2	3	4	5	6
A	IS + Cal. 1				p2021-1023-1	IS + QC_1
B	IS + Cal. 2				p2021-0732-1	IS + Cal. 7
C	IS + Cal. 3				p2021-0712-3	IS + Cal. 6
D	IS + Cal. 4				p2021-0703-1	IS + Cal. 5
E	IS + Cal. 5				m2021-1522-2	IS + Cal. 4
F	IS + Cal. 6				external urine	IS + Cal. 3
G	IS + Cal. 7			p2021-1135-1	negative urine	IS + Cal. 2
H	IS + QC_1			p2021-1090-1	negative blood	IS + Cal. 1

All wells to contain 100 µl of residual DMSO

80



Idaho State Police Forensic Services

AM #26 Screening of THC and Metabolites and AM #27 Confirmation of THC and Metabolites Blood External Control Prep Sheet

Methanol External Control Solution (Lot: WS03052021)

10 µL of 1mg/mL THC, 100 µL of 100 µg/mL THC-OH, C-THC in 9790 µL MeOH

Approximate concentration 1ug/mL.

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>	<i>Expiration Date</i>
Methanol (LCMS)	Fisher	200921	
THC	Cerilliant	FE01041701	03/31/2022
C-THC	Cerilliant	FE08011801	08/31/2023
THC-OH	Cerilliant	FE07221601	07/31/2021
Prepared:	03/05/2021		
Prepared By:	Tamara Salazar/Amber Gerheart		

Urine External Control Solution (Lot: 04232021)

200 ul of methanol external control solution was added to 9800 ul of blood.

Approximately 20ng/mL each

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>
Negative Blood	Lampire	20L20724
Methanol External Control Solution	-	WS03052021
Prepared:	04/23/2021	
Prepared by:	Sarah Collins	

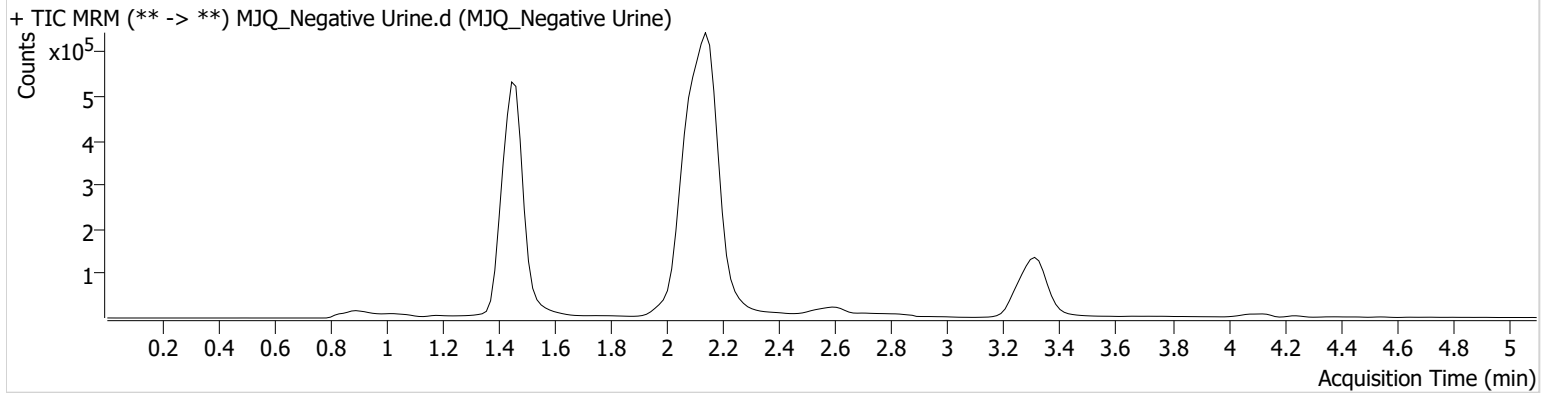
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	Data File	MJQ_Negative Urine.d
Type	Sample	Sample	MJQ_Negative Urine
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-G5	Comment	
Injection Volume	10		
Acq. Date-Time	4/28/2021 12:30:19 PM		
Sample Info.			

Sample Chromatogram



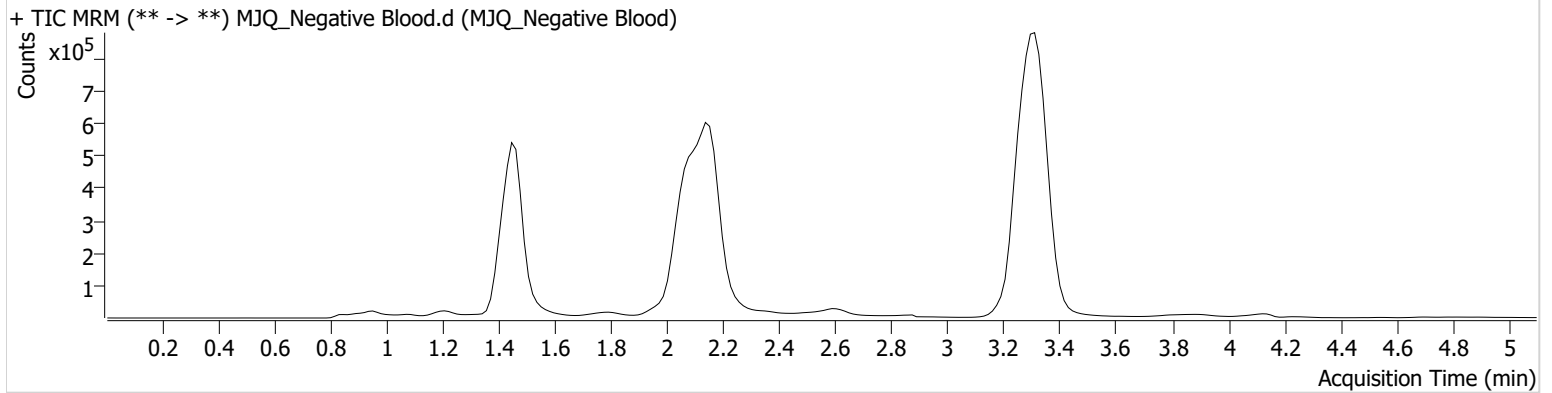
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument Type	Instrument 1 Sample	Data File	MJQ_Negative Blood.d
Acq. Method	AM 27 THCQ.m	Sample	MJQ_Negative Blood
Sample Position	P1-H5	Operator	Sarah Collins
Injection Volume	10	Comment	
Acq. Date-Time	4/28/2021 11:59:52 AM		
Sample Info.			

Sample Chromatogram



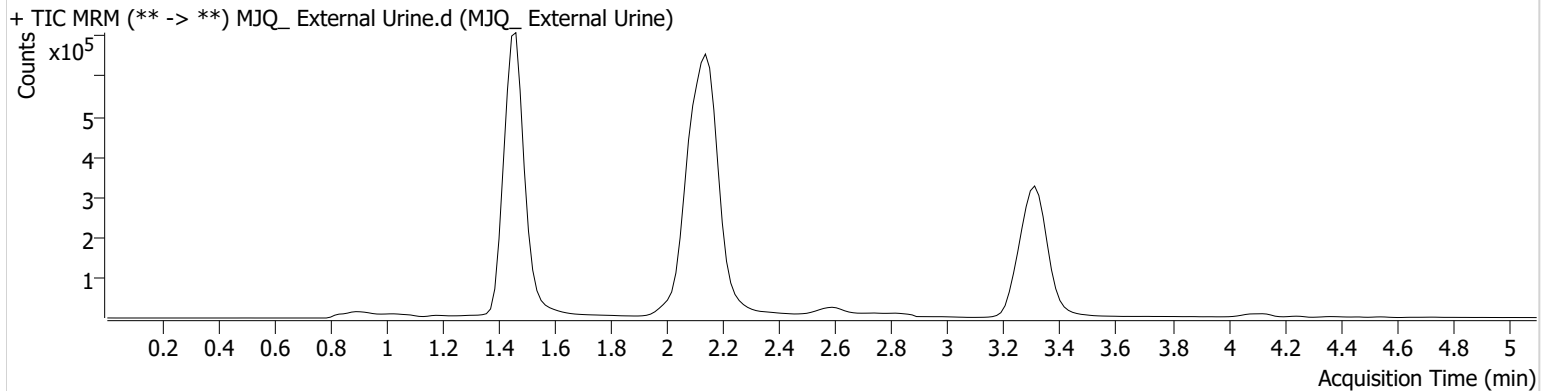
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	Data File	MJQ_ External Urine.d
Type	Sample	Sample	MJQ_ External Urine
Acq. Method	AM 27 THCQ.m	Operator	Sarah Collins
Sample Position	P1-F5	Comment	
Injection Volume	10		
Acq. Date-Time	4/28/2021 12:45:33 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	381451	∞	11.4	274.01	2335180	9.7464 ng/ml
THC-COOH	1.489	179754	∞	56.1	∞	577617	11.8954 ng/ml
THC	3.330	204830	330.33	28.0	∞	2047595	10.0928 ng/ml

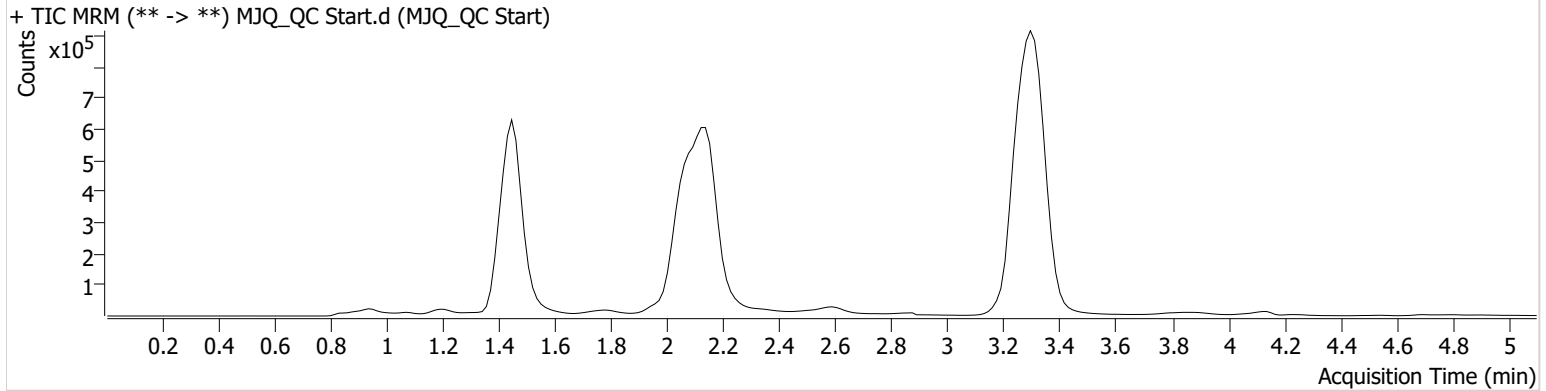
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	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	4/28/2021 12:15:07 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	193575	∞	9.5	∞	2209257	4.4514 ng/ml
THC-COOH	1.474	226964	∞	53.4	1843.23	627362	13.9683 ng/ml
THC	3.315	279010	648.09	28.3	51.81	6609697	4.3674 ng/ml

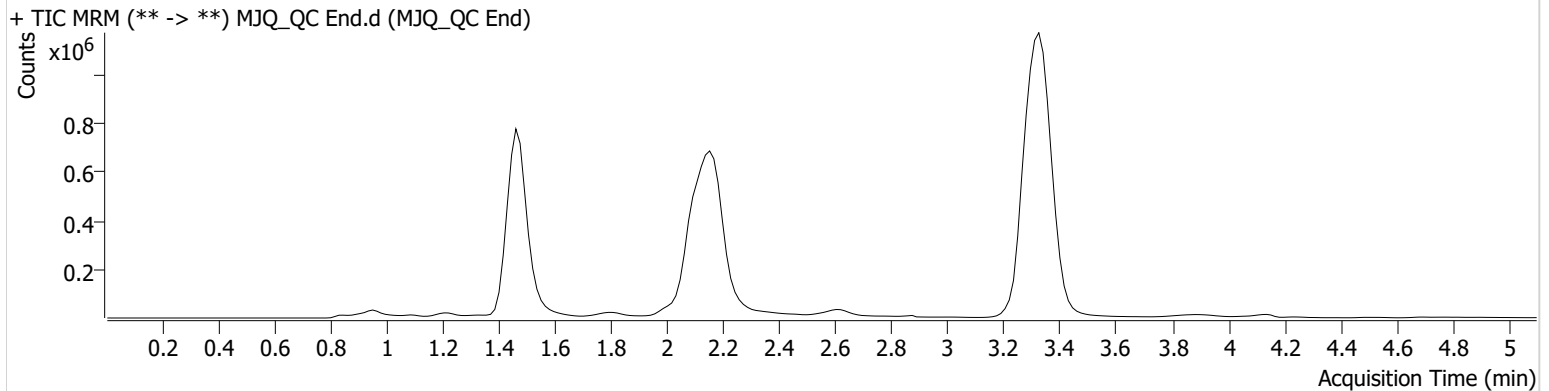
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	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	4/28/2021 2:47:25 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	227393	∞	9.2	∞	2539122	4.5868 ng/ml
THC-COOH	1.504	251984	∞	55.7	∞	688137	14.1490 ng/ml
THC	3.345	343749	∞	26.5	∞	7714314	4.5999 ng/ml

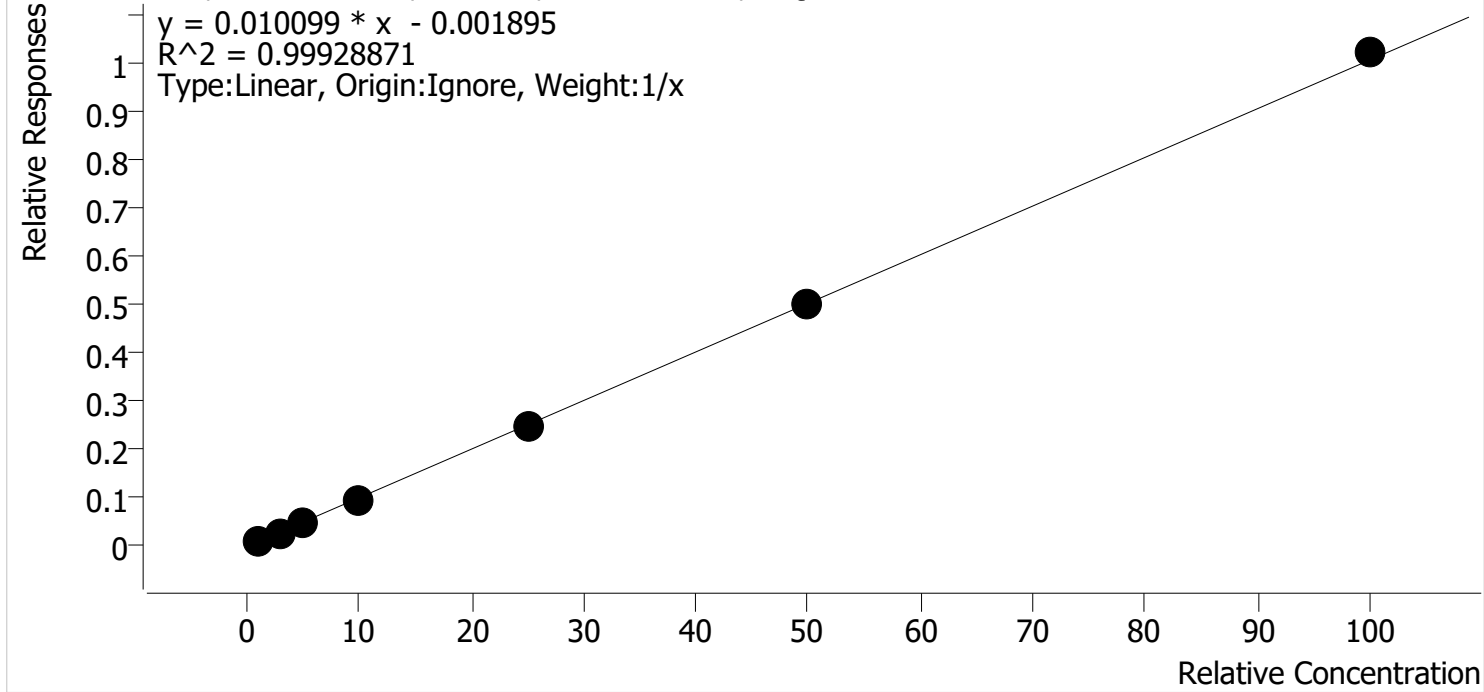


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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 4/28/2021 3:58 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3

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



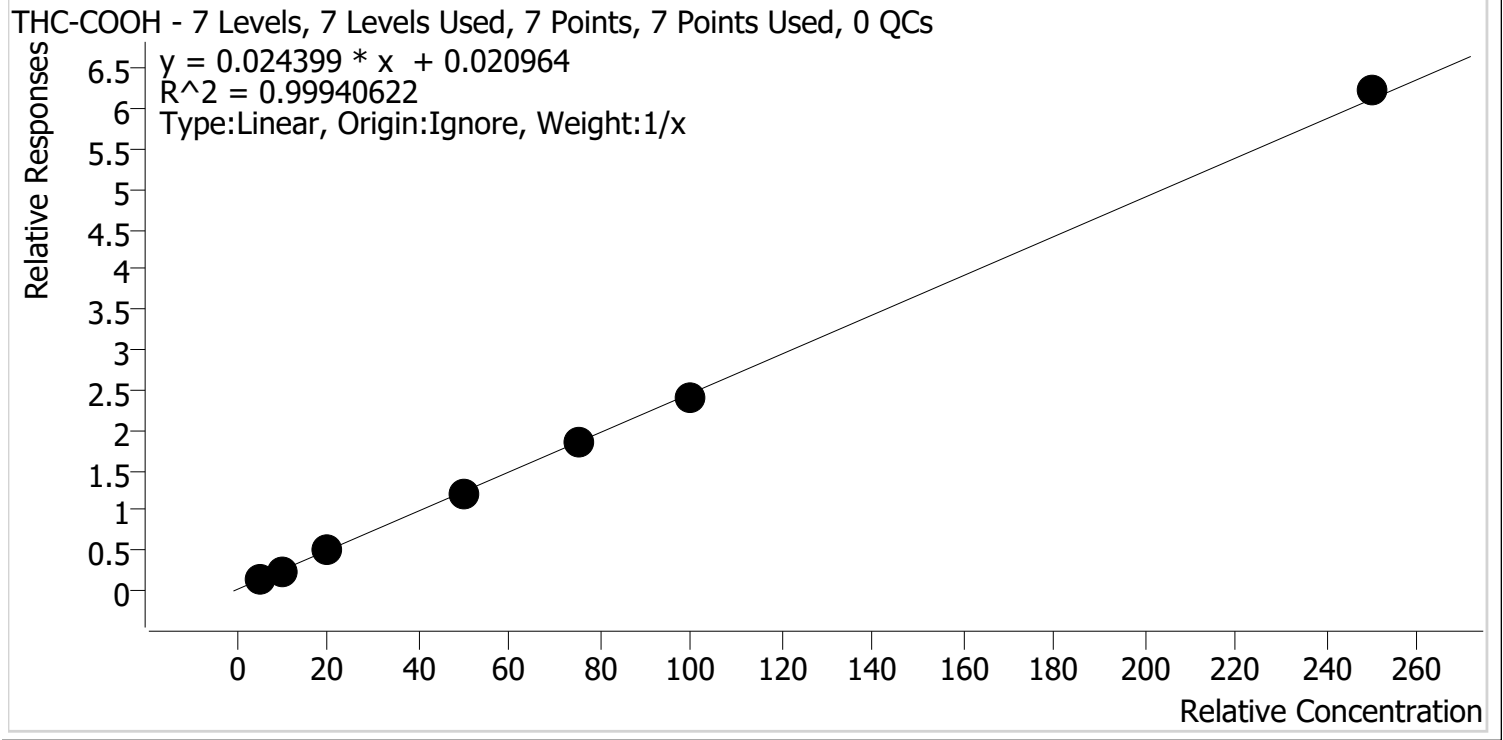
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	✓	1.0	1.2	117.3
MJQ_Cal 2	2	✓	3.0	2.9	95.1
MJQ_Cal 3	3	✓	5.0	4.7	93.6
MJQ_Cal 4	4	✓	10.0	9.5	94.9
MJQ_Cal 5	5	✓	25.0	24.5	97.9
MJQ_Cal 6	6	✓	50.0	49.8	99.7
MJQ_Cal 7	7	✓	100.0	101.5	101.5

SC



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 4/28/2021 3:58 PM
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	108.5
MJQ_Cal 2	2	✓	10.0	9.8	97.8
MJQ_Cal 3	3	✓	20.0	19.7	98.4
MJQ_Cal 4	4	✓	50.0	48.0	96.1
MJQ_Cal 5	5	✓	75.0	74.5	99.3
MJQ_Cal 6	6	✓	100.0	98.3	98.3
MJQ_Cal 7	7	✓	250.0	254.3	101.7

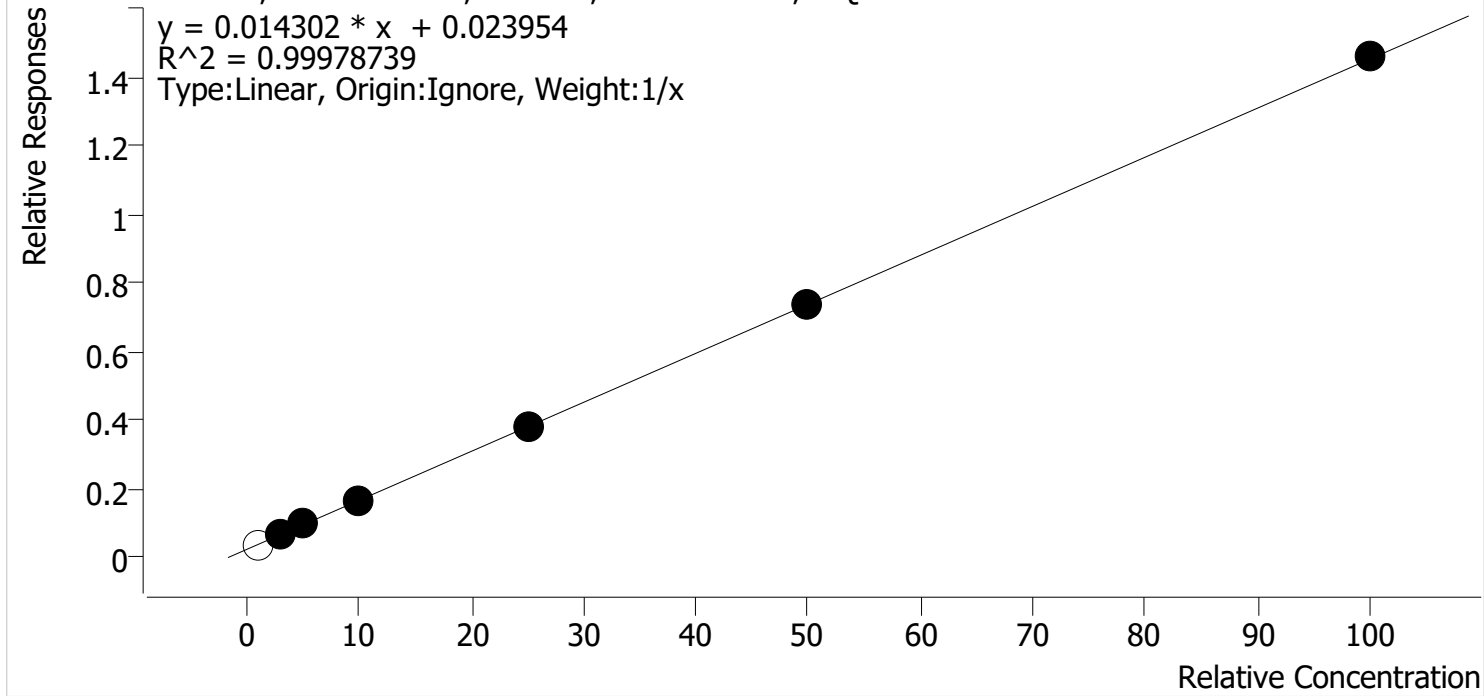


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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Last Cal. Update 4/28/2021 3:58 PM
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



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1	1	x	1.0	0.9	95.0
MJQ_Cal 2	2	✓	3.0	3.1	104.0
MJQ_Cal 3	3	✓	5.0	5.0	100.8
MJQ_Cal 4	4	✓	10.0	9.5	95.4
MJQ_Cal 5	5	✓	25.0	24.8	99.4
MJQ_Cal 6	6	✓	50.0	50.0	100.0
MJQ_Cal 7	7	✓	100.0	100.5	100.5

Calibrator 1 dropped due to not meeting ratio requirement

AM #27 Cannabinoid Quant. Results

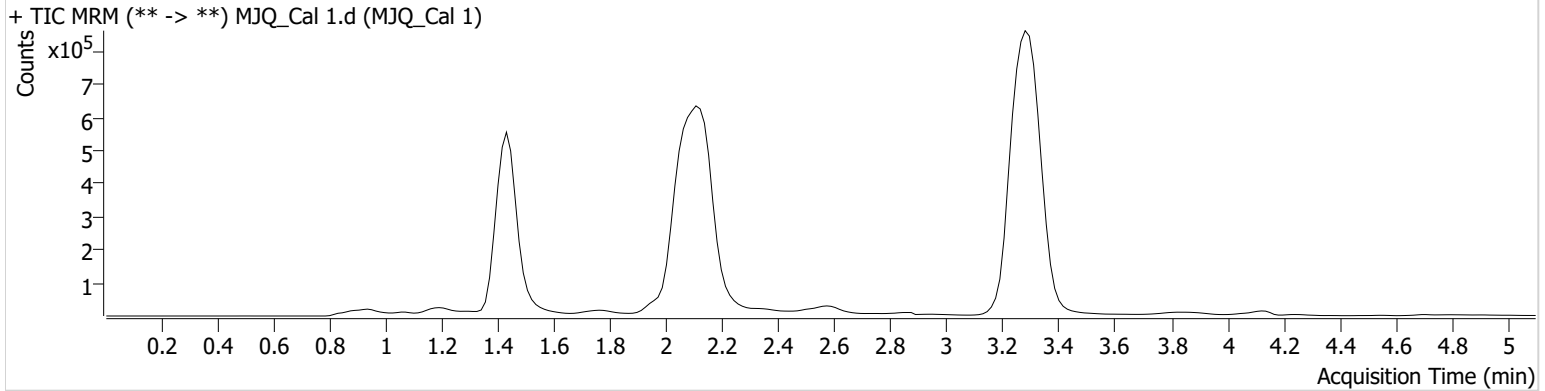
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Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	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	4/28/2021 10:58:57 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	78045	∞	6.0 Low	27.25	2079031	0.9498 ng/ml Low
THC-COOH	1.459	87451	∞	43.5	∞	570574	5.4225 ng/ml
THC	3.300	63538	270.35	32.2	∞	6385213	1.1729 ng/ml

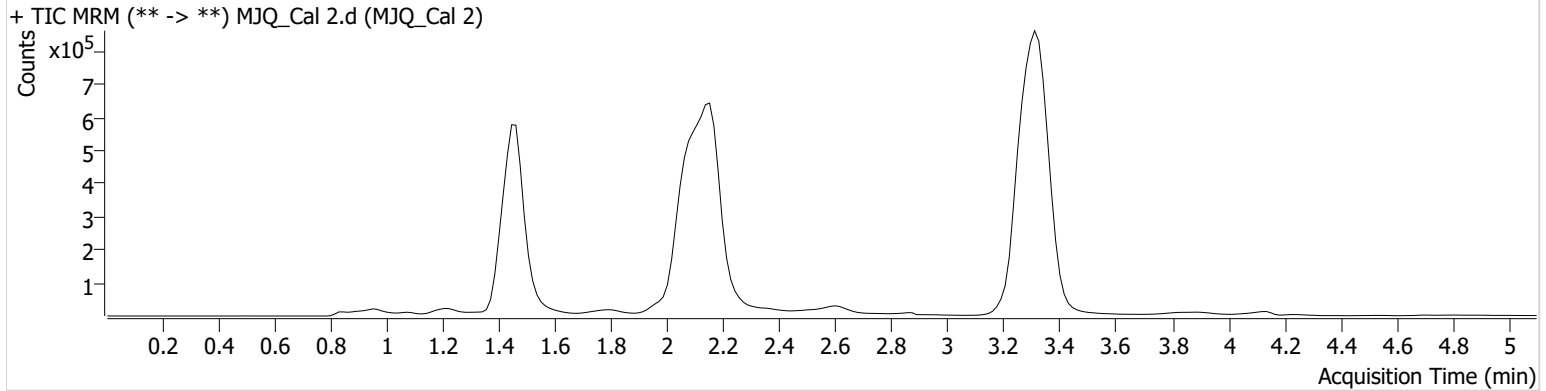
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	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	4/28/2021 11:06:43 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	148368	∞	8.3	45.90	2163088	3.1209 ng/ml
THC-COOH	1.489	155696	∞	50.4	566.53	599936	9.7774 ng/ml
THC	3.330	169855	601.29	31.2	96.02	6306919	2.8543 ng/ml

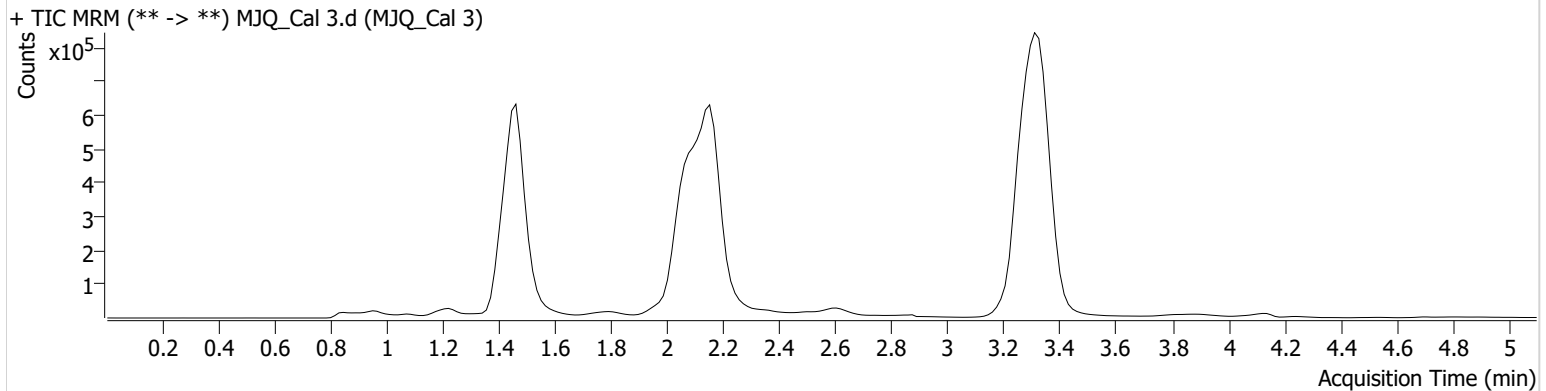
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	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	4/28/2021 11:14:18 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	213843	∞	8.8	102.75	2226491	5.0405 ng/ml
THC-COOH	1.489	290973	359.61	53.3	796.46	580859	19.6719 ng/ml
THC	3.330	278120	881.36	26.9	∞	6130668	4.6796 ng/ml

AM #27 Cannabinoid Quant. Results

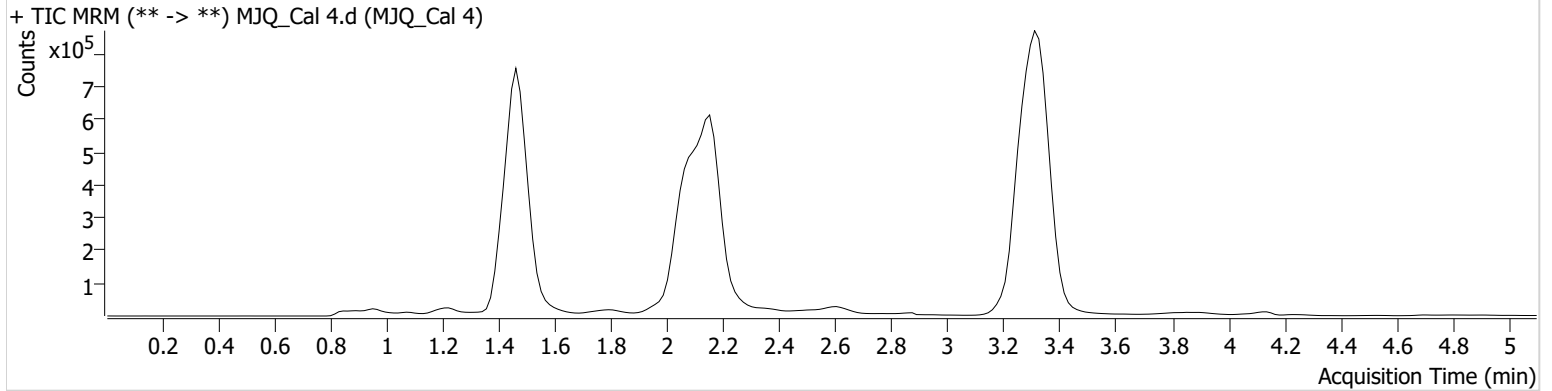


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Instrument Instrument 1
Type Cal
Acq. Method AM 27 THCQ.m
Sample Position P1-E6
Injection Volume 10
Acq. Date-Time 4/28/2021 11:21:53 AM
Sample Info.

Data File MJQ_Cal 4.d
Sample MJQ_Cal 4
Operator Sarah Collins
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	348935	188.68	10.0	∞	2176358	9.5352 ng/ml
THC-COOH	1.489	691123	∞	56.0	1220.00	579209	48.0454 ng/ml
THC	3.330	565502	∞	25.1	182.21	6017014	9.4937 ng/ml

AM #27 Cannabinoid Quant. Results

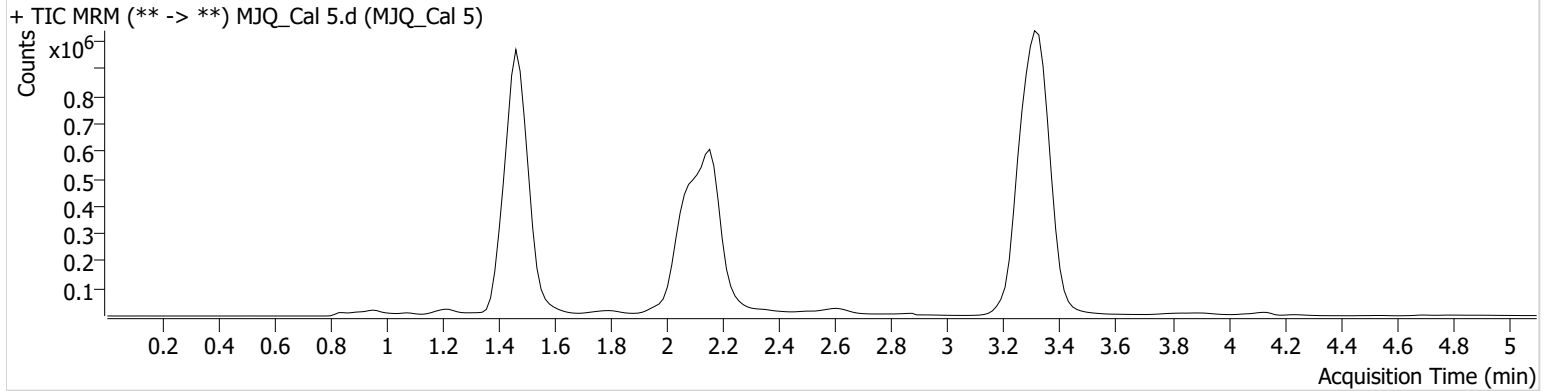
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Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	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	4/28/2021 11:29:28 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	838574	∞	11.1	2474.27	2211118	24.8421 ng/ml
THC-COOH	1.489	1066576	∞	57.4	3099.88	580177	74.4871 ng/ml
THC	3.330	1502810	1697.98	24.7	∞	6128776	24.4674 ng/ml

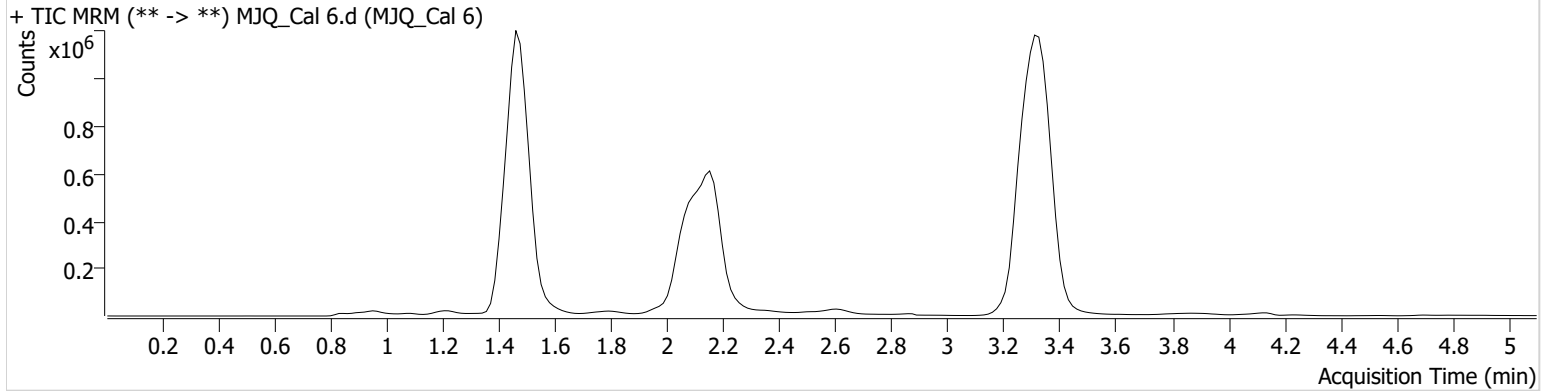
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	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	4/28/2021 11:37:04 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	1602345	∞	11.5	2154.98	2169008	49.9773 ng/ml
THC-COOH	1.489	1372168	∞	56.6	∞	567248	98.2845 ng/ml
THC	3.330	2848421	34675.77	25.2	3178.24	5681467	49.8305 ng/ml

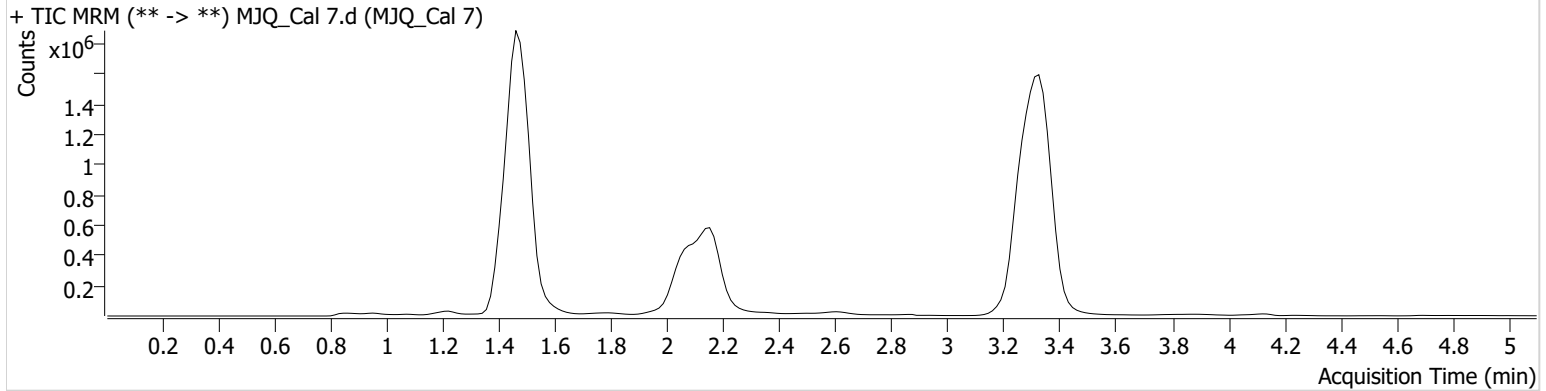
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2021\AM 27-28\042821 AM 27 28 SC\QuantResults\AM 27.batch.bin
Calibration Last Update 4/28/2021 3:58:57 PM

Instrument	Instrument 1	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	4/28/2021 11:44:40 AM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	3080829	∞	11.7	∞	2108555	100.4840 ng/ml
THC-COOH	1.489	3026036	890.95	58.4	5109.40	486042	254.3112 ng/ml
THC	3.330	5773002	∞	25.3	∞	5642172	101.5015 ng/ml