









REVIEWED

By Anne Nord at 8:33 am, Mar 26, 2021

TS

3/24/2021

Worklist: 4844

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2021-0319	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
M2021-0395	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
M2021-0464	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-0129	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-0218	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-0380	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-0448	3	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2021-0577	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	

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

TS

Extraction Date: 3/19/2021

Analyst: Tamara Salazar – HOA Amber Gerheart

Plate lot#: 201206

Plate Expiration: 6/6/2021

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: 20L20724

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 069901

Blank Urine Lot: POC03132019

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: 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: 800 µL
- 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: *Only THC-COOH evaluated for urine samples*

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	1	2	3	4	5	6
A	IS + Cal. 1	Negative Blood	P2021-0218-1	IS + Sample	IS + Sample	IS + QC_1
B	IS + Cal. 2	Blood External Control	P2021-0380-1	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	Negative Urine	P2021-0448-3	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	Urine External Control	P2021-0577-1	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	M2021-0319-2	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 4
F	IS + Cal. 6	M2021-0395-2	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 3
G	IS + Cal. 7	M2021-0464-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 2
H	IS + QC_1	P2021-0129-1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 1

All wells to contain 100 µl of residual DMSO



Idaho State Police
Forensic Services

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**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		

Blood External Control Solution (Lot: 03052021)

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:	03/10/2021	
Prepared by:	Tamara Salazar	



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**AM #26 Screening of THC and Metabolites and AM #27
Confirmation of THC and Metabolites Urine 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: 03052021)

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

Approximately 20ng/mL each

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>
Negative Urine	Pocatello Lab	POC031319
Methanol External Control Solution	-	WS03052021
Prepared:	03/05/2021	
Prepared by:	Tamara Salazar/Amber Gerheart	

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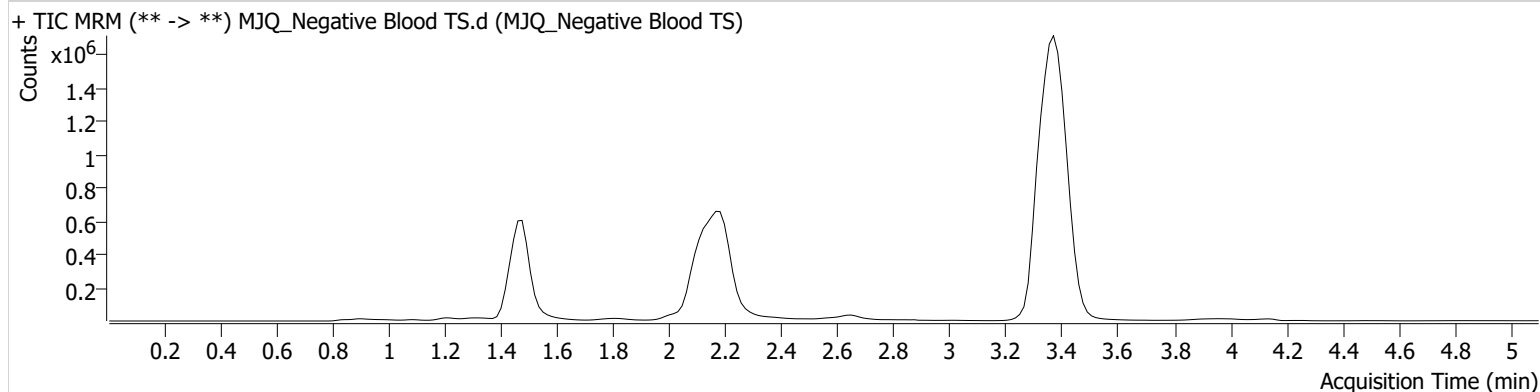


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_Negative Blood TS.d
Type	Sample	Sample	MJQ_Negative Blood TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A2	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 9:34:18 PM		
Sample Info.			

Sample Chromatogram



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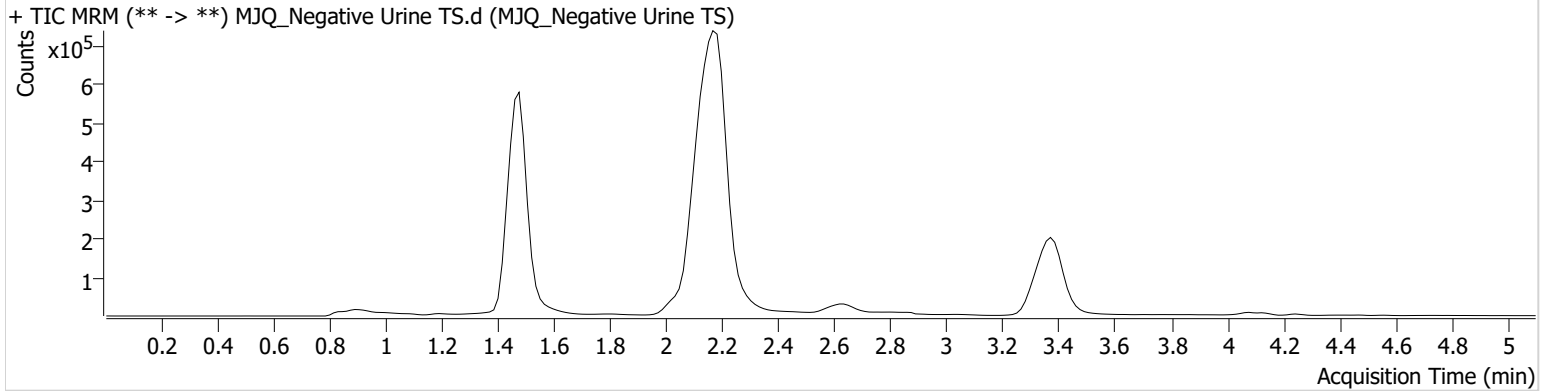


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_Negative Urine TS.d
Type	Sample	Sample	MJQ_Negative Urine TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C2	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 10:04:40 PM		
Sample Info.			

Sample Chromatogram



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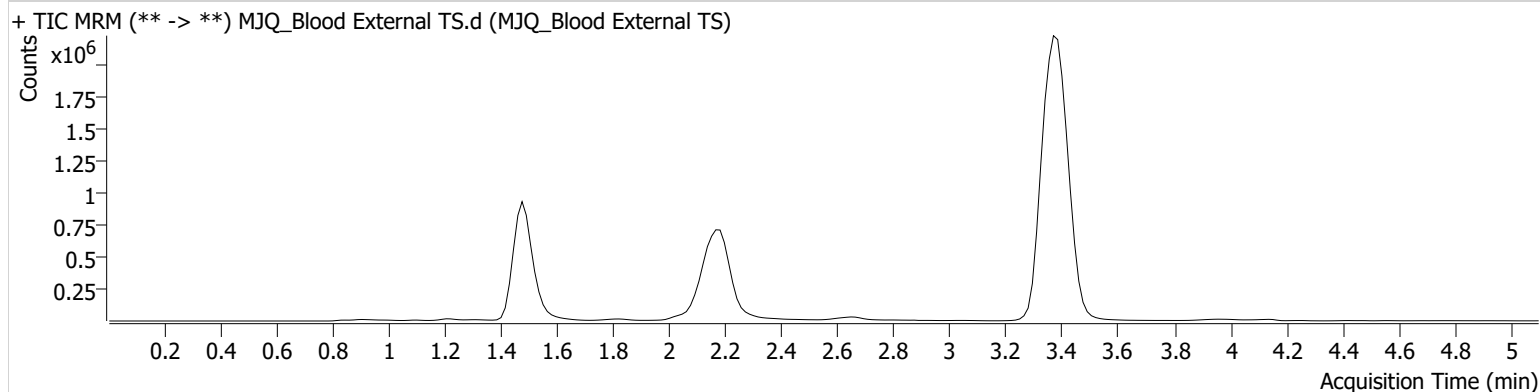


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_Blood External TS.d
Type	Sample	Sample	MJQ_Blood External TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-B2	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 9:49:29 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	695433	∞	10.9	∞	2431673	19.2781 ng/ml
THC-COOH	1.519	365070	466.30	54.5	∞	721078	19.3526 ng/ml
THC	3.390	1966473	∞	26.7	∞	12181897	17.0811 ng/ml

TS

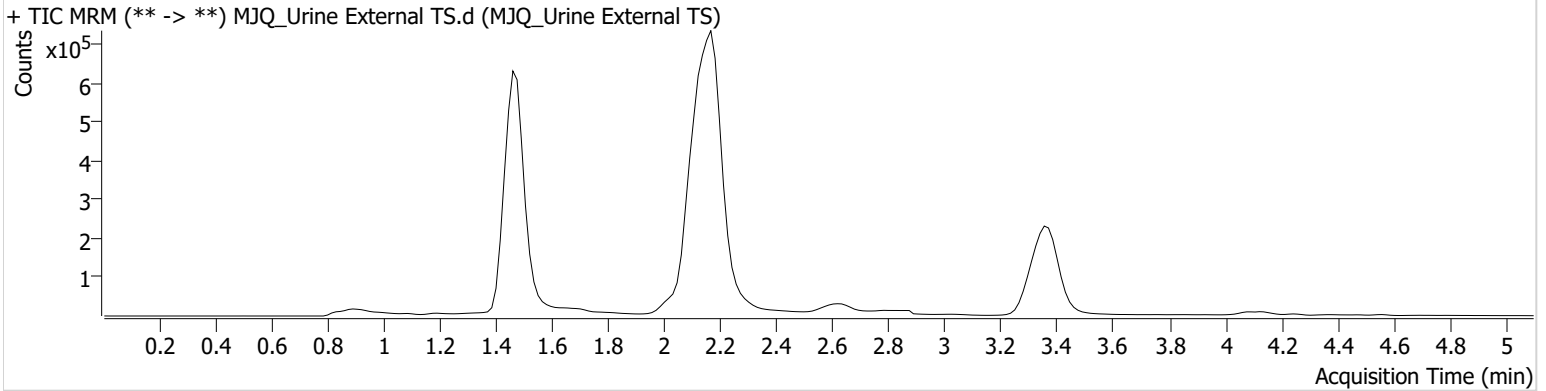


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_Urine External TS.d
Type	Sample	Sample	MJQ_Urine External TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-D2	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 10:19:53 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	248034	∞	11.2	∞	2113925	6.3482 ng/ml
THC-COOH	1.504	102202	∞	61.6	∞	557932	6.4823 ng/ml
THC	3.375	35282	69.95	44.8 High	72.13	1559817	2.6699 ng/ml

TS

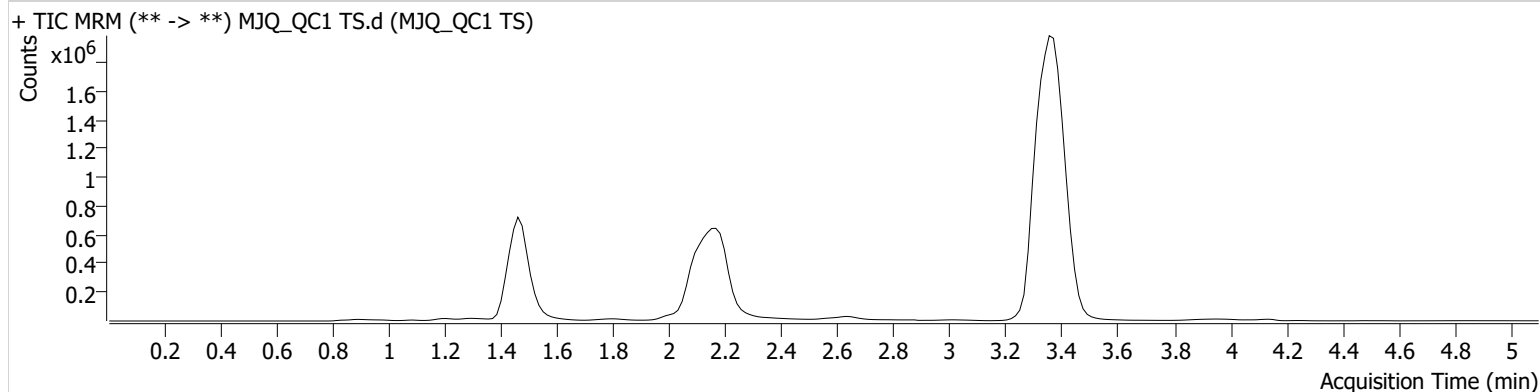


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_QC1 TS.d
Type	Sample	Sample	MJQ_QC1 TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 9:19:04 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.468	177670	∞	9.3	∞	2414837	2.9935 ng/ml Low
THC-COOH	1.504	269205	338.49	52.4	∞	724016	13.9965 ng/ml
THC	3.375	503520	∞	27.4	110.48	13926950	4.0751 ng/ml

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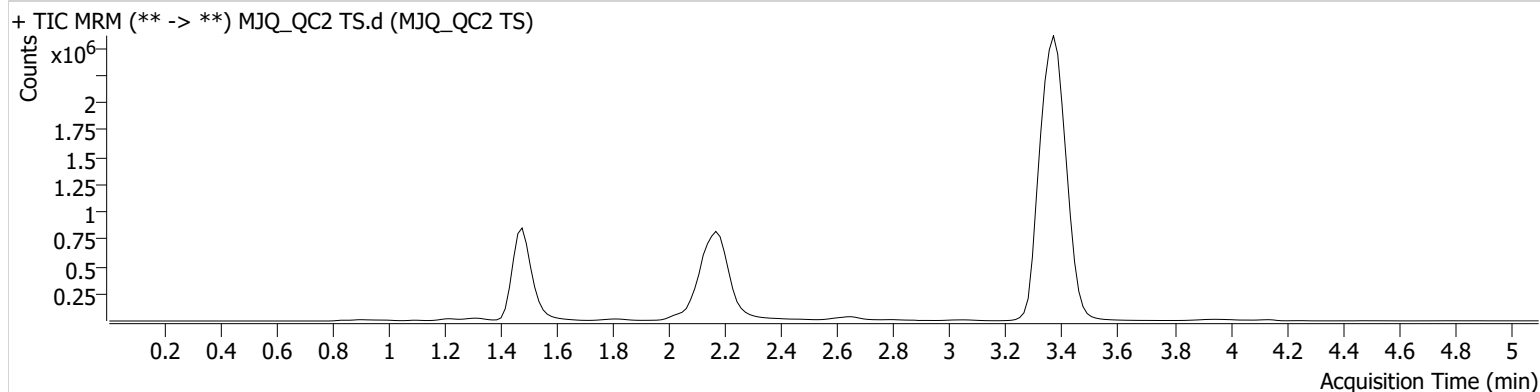
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument Instrument 1
Type Sample
Acq. Method AM 27 THCQ.m
Sample Position P1-H1
Injection Volume 10
Acq. Date-Time 3/20/2021 12:36:49 AM
Sample Info.

Data File MJQ_QC2 TS.d
Sample MJQ_QC2 TS
Operator Tamara Salazar
Comment Bracket QC

Sample Chromatogram



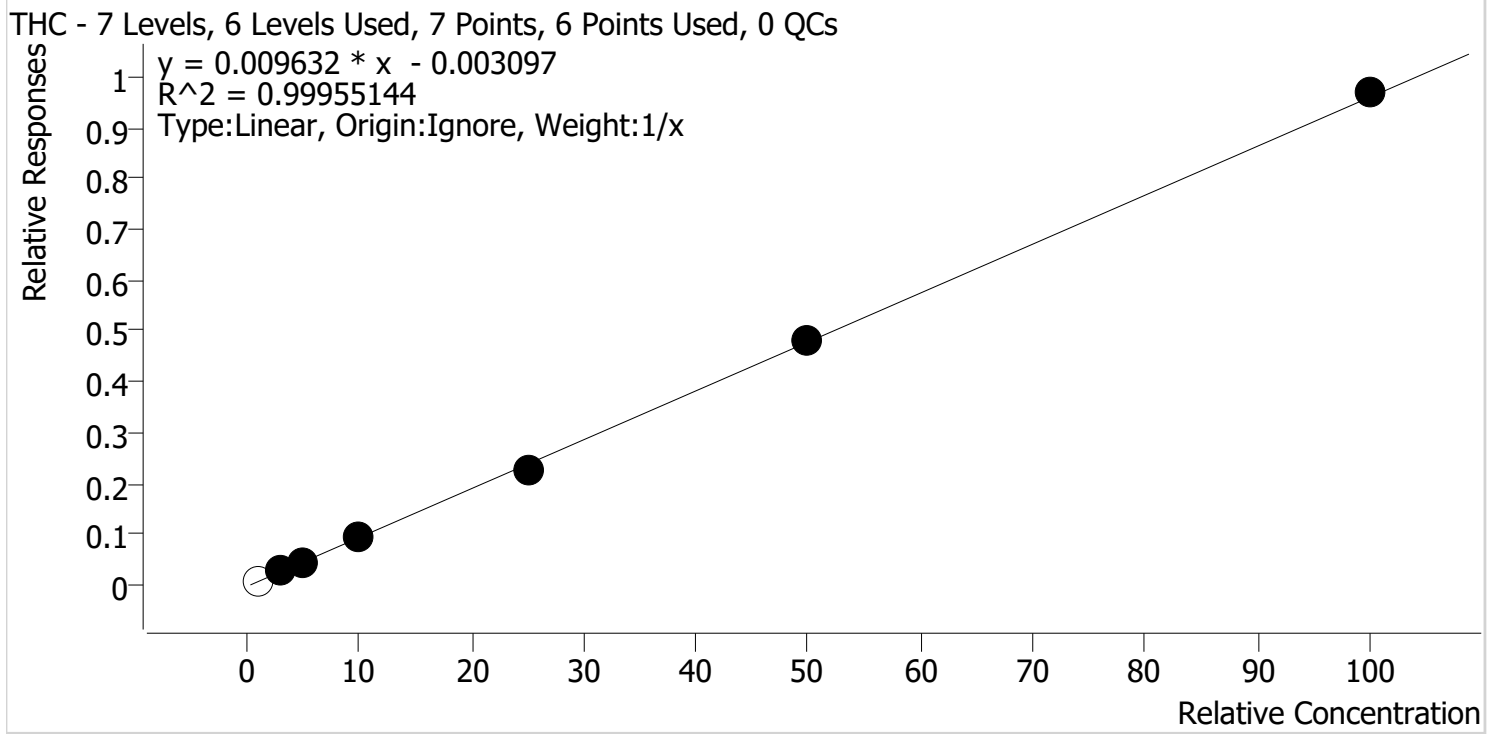
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.483	189068	∞	8.5	176.64	2628866	2.8666 ng/ml Low
THC-COOH	1.504	291189	∞	53.0	1668.38	783315	13.9933 ng/ml
THC	3.390	583472	2808.89	27.8	146.08	16491166	3.9948 ng/ml

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Last Cal. Update 3/24/2021 3:14 PM
Analyst Name ISP\Datastor
Analyte THC **Internal Standard** THC-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1 TS	1	x	1.0	1.2	118.9
MJQ_Cal 2 TS	2	✓	3.0	3.0	101.5
MJQ_Cal 3 TS	3	✓	5.0	5.1	102.0
MJQ_Cal 4 TS	4	✓	10.0	10.0	99.9
MJQ_Cal 5 TS	5	✓	25.0	23.9	95.4
MJQ_Cal 6 TS	6	✓	50.0	50.1	100.1
MJQ_Cal 7 TS	7	✓	100.0	100.9	100.9

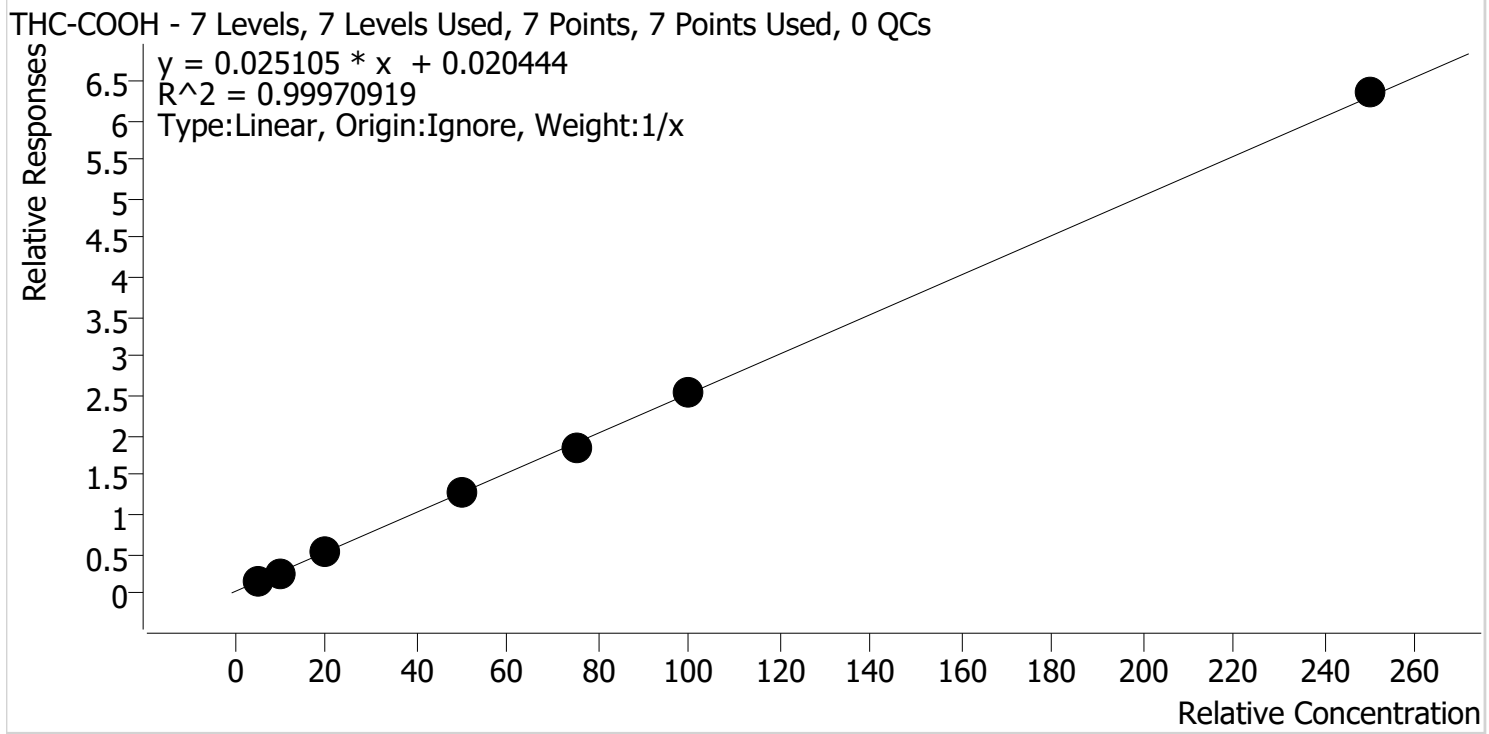
Not Evaluated

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Last Cal. Update 3/24/2021 3:14 PM
Analyst Name ISP\Datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 1 TS	1	✓	5.0	5.4	107.8
MJQ_Cal 2 TS	2	✓	10.0	9.7	96.5
MJQ_Cal 3 TS	3	✓	20.0	19.5	97.7
MJQ_Cal 4 TS	4	✓	50.0	49.4	98.8
MJQ_Cal 5 TS	5	✓	75.0	73.4	97.9
MJQ_Cal 6 TS	6	✓	100.0	100.5	100.5
MJQ_Cal 7 TS	7	✓	250.0	252.1	100.8

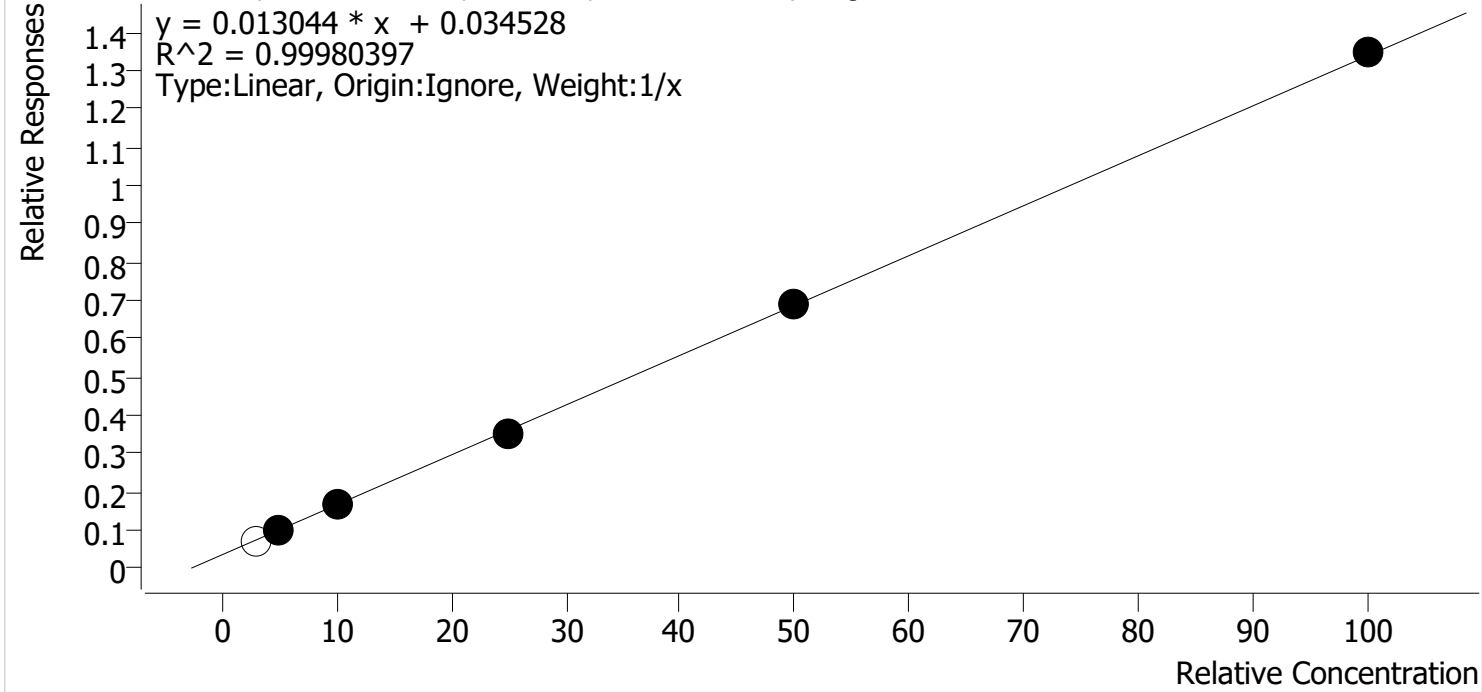
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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Last Cal. Update 3/24/2021 3:14 PM
Analyst Name ISP\Datastor
Analyte THC-OH **Internal Standard** THC-OH-D3

THC-OH - 6 Levels, 5 Levels Used, 6 Points, 5 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJQ_Cal 2 TS	2	x	3.0	2.9	95.2
MJQ_Cal 3 TS	3	✓	5.0	5.1	102.3
MJQ_Cal 4 TS	4	✓	10.0	10.0	99.8
MJQ_Cal 5 TS	5	✓	25.0	24.3	97.4
MJQ_Cal 6 TS	6	✓	50.0	50.0	99.9
MJQ_Cal 7 TS	7	✓	100.0	100.6	100.6

Not Evaluated

TS

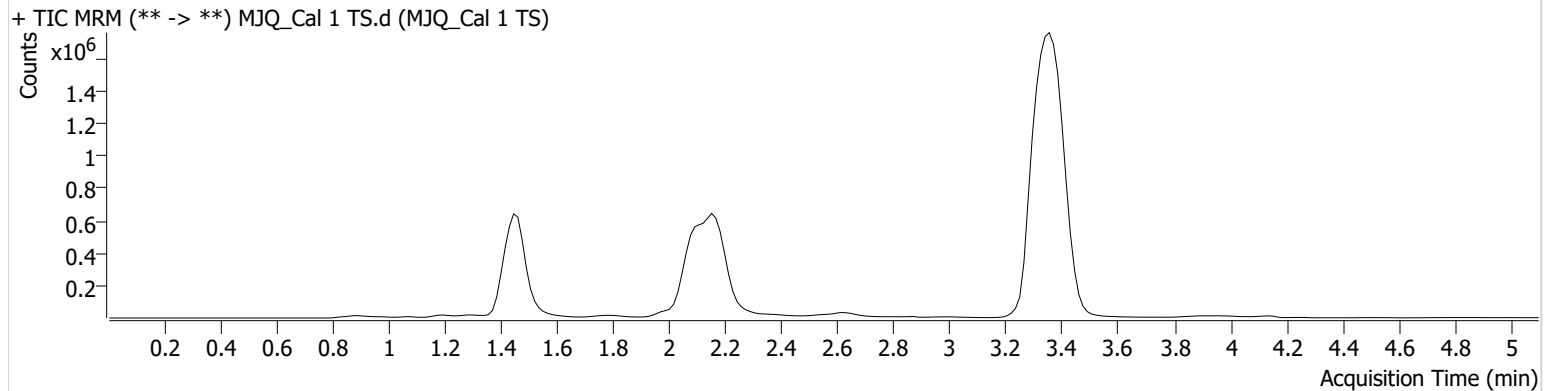


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_Cal 1 TS.d
Type	Cal	Sample	MJQ_Cal 1 TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 8:18:04 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.489	109839	∞	44.3	199.34	705472	5.3875 ng/ml
* THC	3.375	114469	290.15	33.8 High	86.30	13694997	1.1893 ng/ml

* Not Evaluated

TS



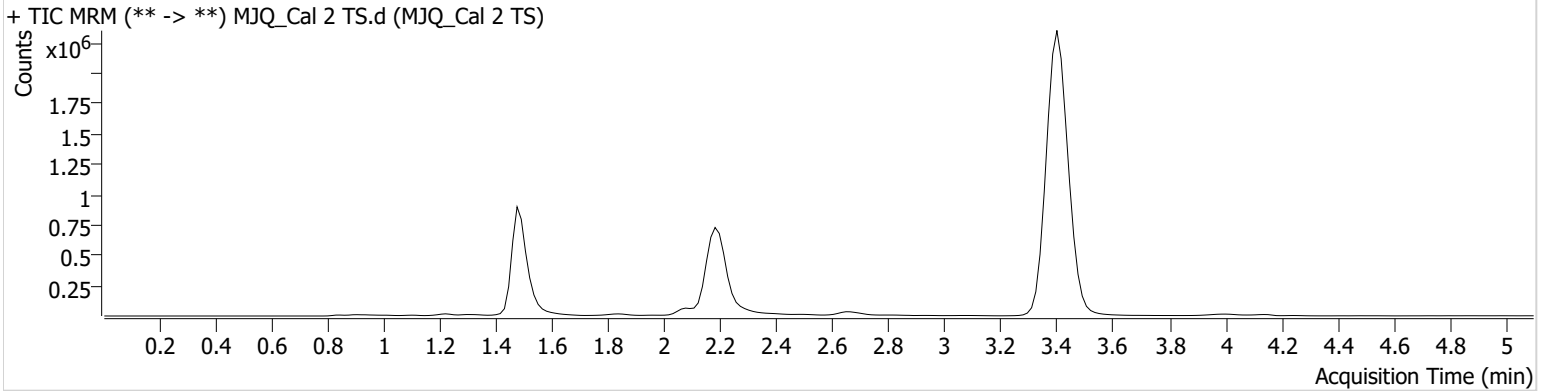
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument Instrument 1
Type Cal
Acq. Method AM 27 THCQ.m
Sample Position P1-B1
Injection Volume 10
Acq. Date-Time 3/19/2021 8:25:50 PM
Sample Info.

Data File MJQ_Cal 2 TS.d
Sample MJQ_Cal 2 TS
Operator Tamara Salazar
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.483	170231	19.81	6.4 Low	∞	2371483	2.8561 ng/ml Low
THC-COOH	1.519	170563	∞	51.2	∞	649088	9.6528 ng/ml
* THC	3.420	324859	753.59	32.2	∞	12382901	3.0452 ng/ml

* Not Evaluated

TS

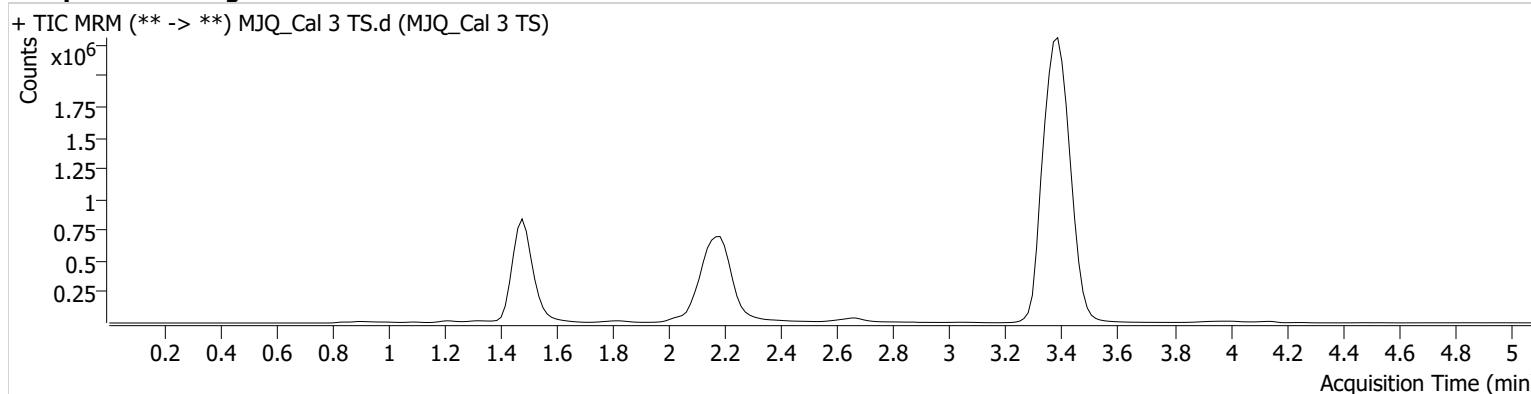


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_Cal 3 TS.d
Type	Cal	Sample	MJQ_Cal 3 TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 8:33:26 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.483	264112	∞	8.0 Low	∞	2607897	5.1170 ng/ml
THC-COOH	1.504	392382	417.42	53.9	∞	767965	19.5380 ng/ml
* THC	3.405	683912	3935.61	26.9	386.48	14851850	5.1024 ng/ml

* Not Evaluated

TS



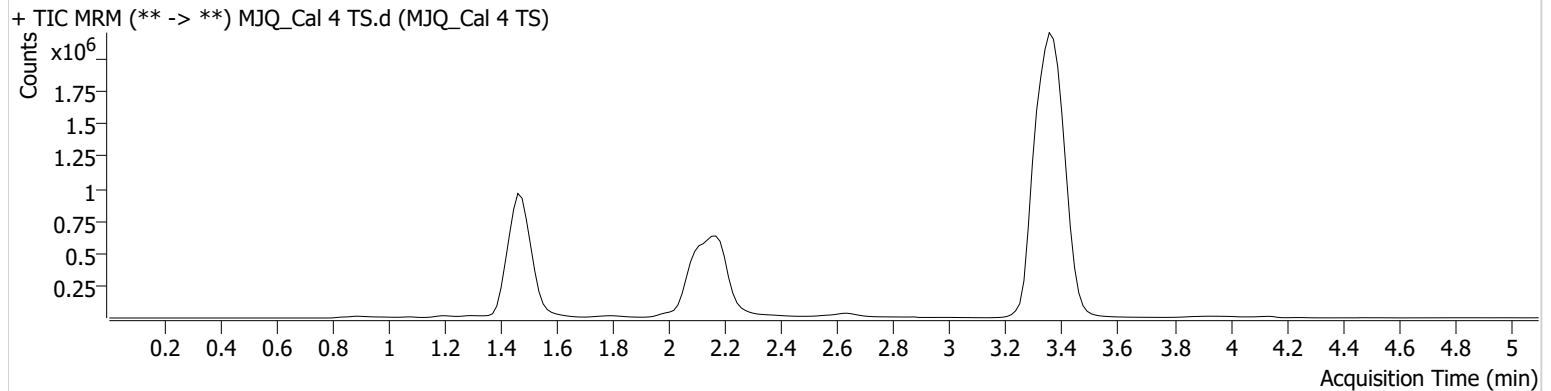
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument Instrument 1
Type Cal
Acq. Method AM 27 THCQ.m
Sample Position P1-D1
Injection Volume 10
Acq. Date-Time 3/19/2021 8:41:04 PM
Sample Info.

Data File MJQ_Cal 4 TS.d
Sample MJQ_Cal 4 TS
Operator Tamara Salazar
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.468	445792	∞	9.4	994.16	2706976	9.9782 ng/ml
THC-COOH	1.489	956546	1274.97	55.0	∞	758937	49.3905 ng/ml
* THC	3.375	1384863	∞	26.1	∞	14864720	9.9941 ng/ml

* Not Evaluated

TS



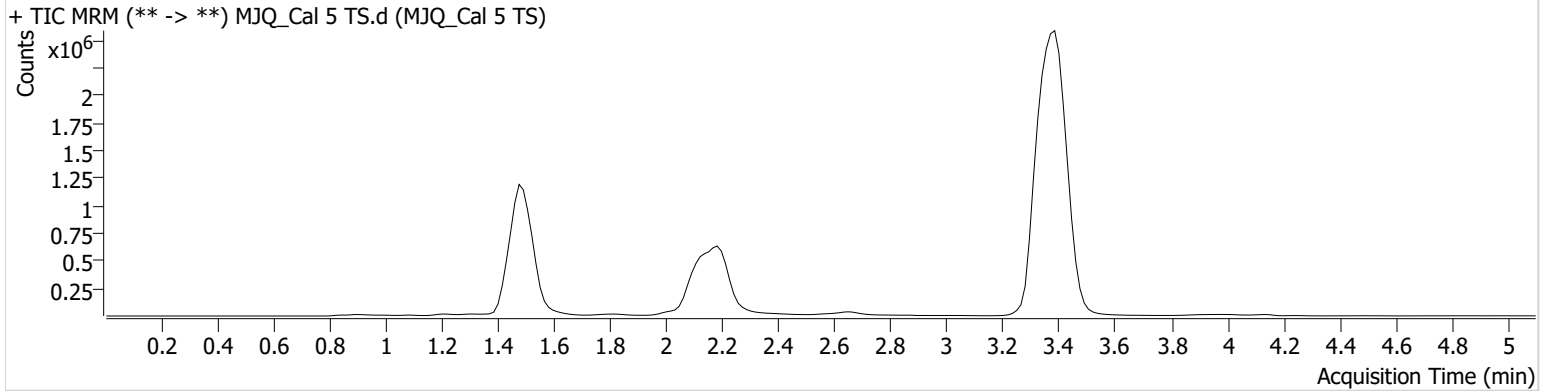
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_Cal 5 TS.d
Type	Cal	Sample	MJQ_Cal 5 TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 8:48:39 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.483	938080	∞	10.9	∞	2664850	24.3403 ng/ml
THC-COOH	1.504	1405517	∞	56.0	∞	753934	73.4445 ng/ml
* THC	3.390	3421895	∞	26.1	4133.44	15093979	23.8587 ng/ml

* Not Evaluated

TS



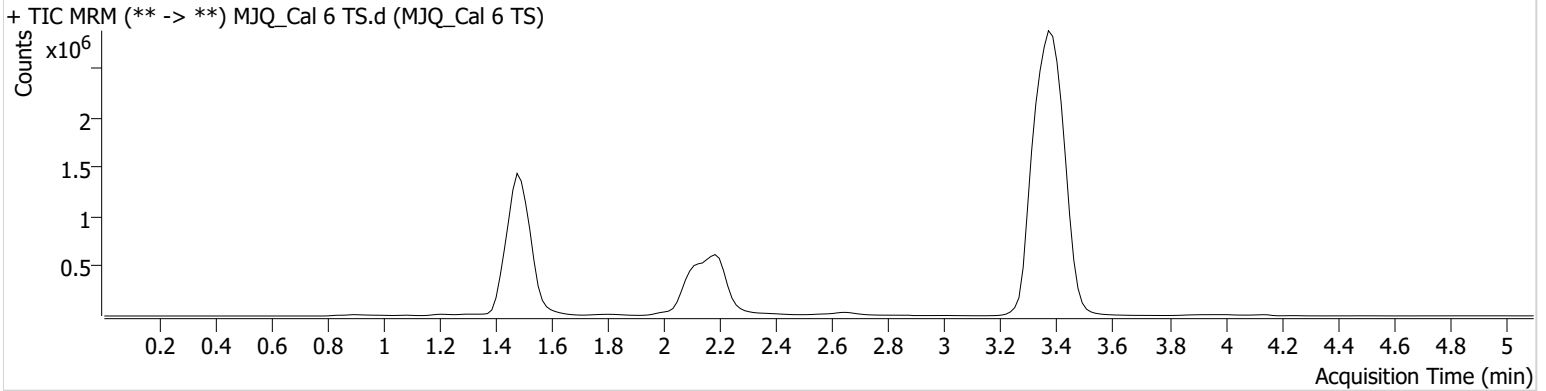
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument	Instrument 1	Data File	MJQ_Cal 6 TS.d
Type	Cal	Sample	MJQ_Cal 6 TS
Acq. Method	AM 27 THCQ.m	Operator	Tamara Salazar
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	3/19/2021 8:56:15 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.483	1785103	∞	11.4	∞	2601859	49.9513 ng/ml
THC-COOH	1.504	1839522	∞	55.1	∞	723353	100.4835 ng/ml
* THC	3.390	6742995	∞	26.1	7978.39	14072567	50.0690 ng/ml

* Not Evaluated

TS



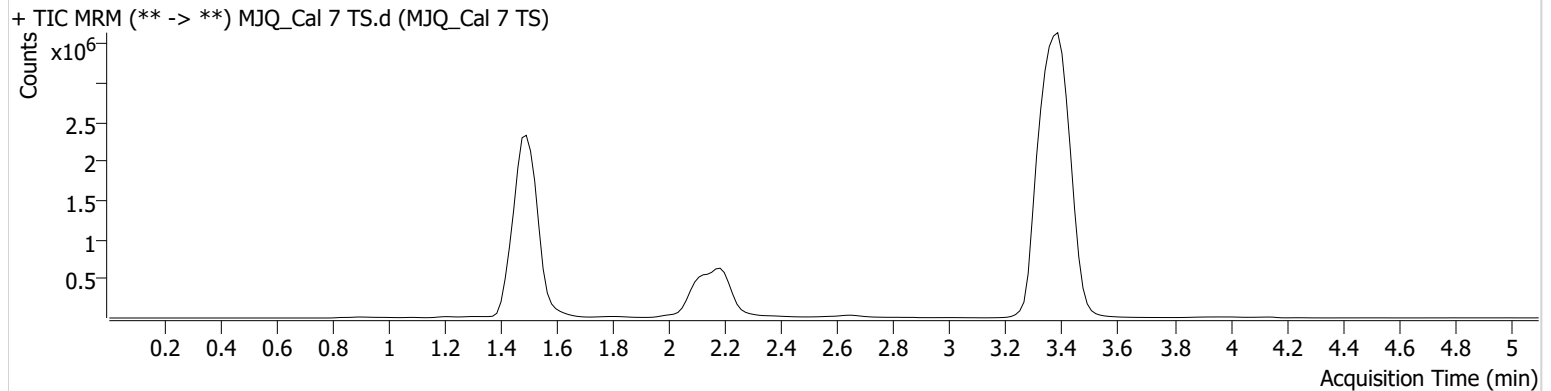
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2021\AM 27-28\P1 P2 Urine_031821_TS\QuantResults\AM 27 AG.batch.bin
Calibration Last Update 3/24/2021 3:14:08 PM

Instrument Instrument 1
Type Cal
Acq. Method AM 27 THCQ.m
Sample Position P1-G1
Injection Volume 10
Acq. Date-Time 3/19/2021 9:03:50 PM
Sample Info.

Data File MJQ_Cal 7 TS.d
Sample MJQ_Cal 7 TS
Operator Tamara Salazar
Comment

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
* THC-OH	1.483	3313877	∞	11.6	∞	2460342	100.6132 ng/ml
THC-COOH	1.504	4193440	∞	55.2	13270.0	660446	252.1032 ng/ml
* THC	3.390	12589559	∞	26.7	∞	12991676	100.9306 ng/ml

* Not Evaluated