

REVIEWED

By Anne Nord at 3:18 pm, Dec 01, 2020

SJ

12/1/2020

Worklist: 4627

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
M2020-3163	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2020-4493	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2020-4497	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
M2020-4593	6	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3315	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3334	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3336	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3338	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3368	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3401	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3402	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3403	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3404	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3406	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3407	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3408	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

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

Extraction Date: 11/25/2020

Analyst: Sophia Jackson

Plate lot#: IDP-108-2-200723

Plate Expiration: 01/23/2021

Mobile phase A: 0.1% Formic Acid in LCMS Water

Mobile phase B: 0.1% Formic acid in Acetonitrile

Blank Blood Lot: Lampire 20L20725

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.
- 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:

Curves limited: THC 3-100

Sample P2020-3338-1 reinjected due to poor ISTD response. Reinject data analyzed.

SJ

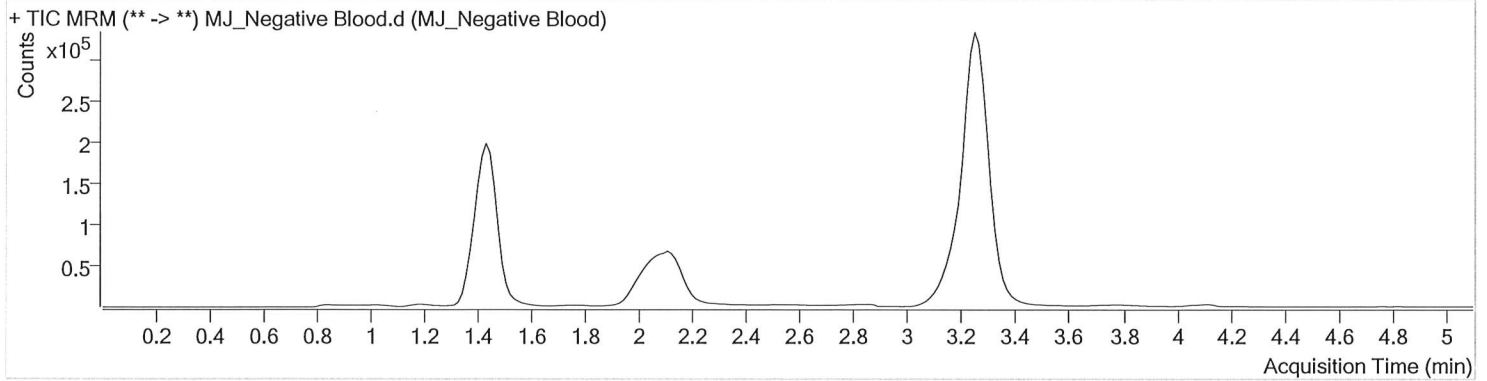


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument	Instrument 1	Data File	MJ_Negative Blood.d
Type	Sample	Sample	MJ_Negative Blood
Acq. Method	AM 27 THCQ.m	Operator	Sophia Jackson
Sample Position	P1-A2	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2020 3:26:37 PM		
Sample Info.			

Sample Chromatogram



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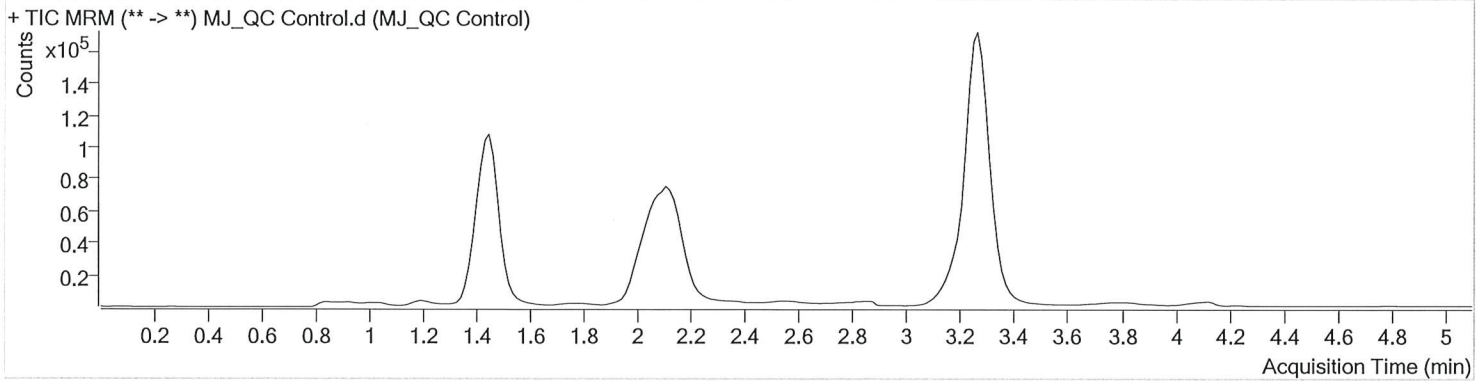


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument	Instrument 1	Data File	MJ_QC Control.d
Type	Sample	Sample	MJ_QC Control
Acq. Method	AM 27 THCQ.m	Operator	Sophia Jackson
Sample Position	P1-H1	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2020 3:11:26 PM		

Sample Chromatogram

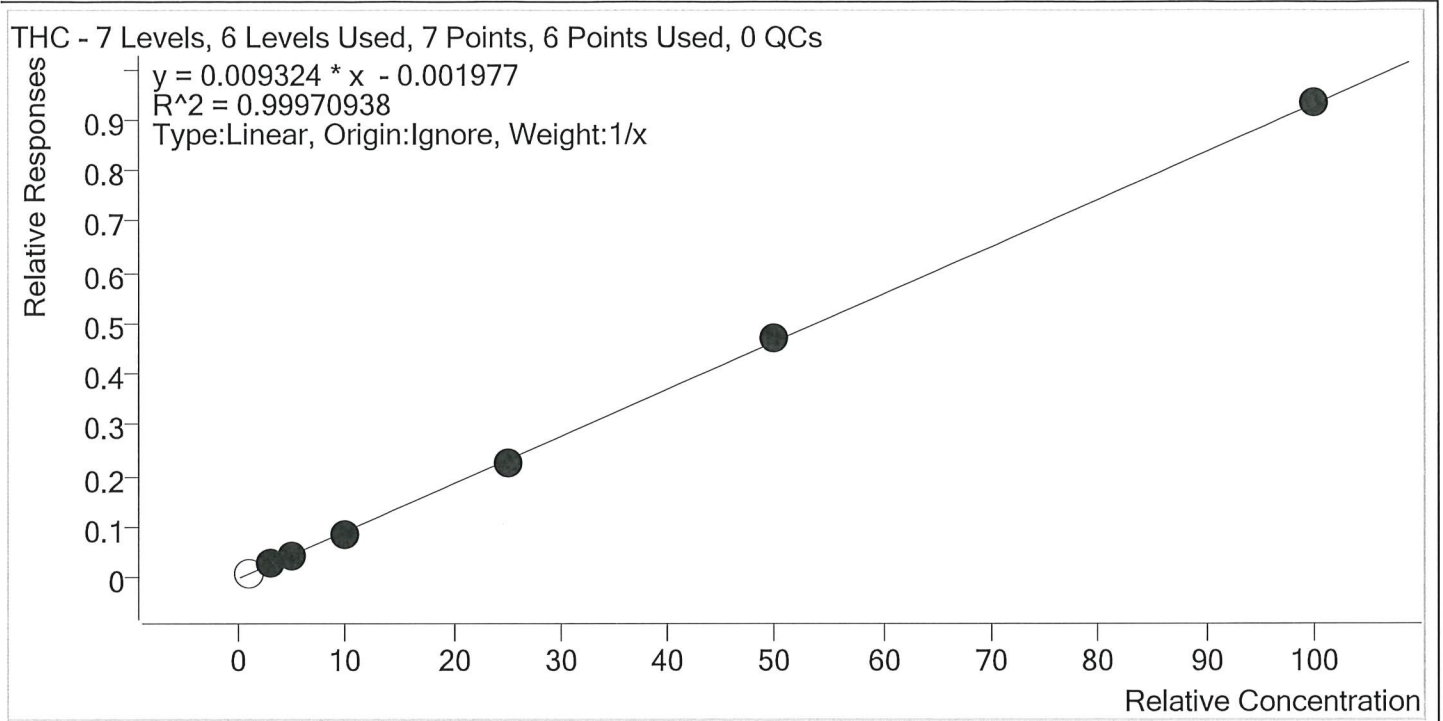


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	27285	∞	13.6	20.88	366545	4.7710 ng/ml
THC-COOH	1.474	54533	∞	60.5	351.55	134567	15.3621 ng/ml
THC	3.270	47533	656.50	30.5	∞	1110918	4.8010 ng/ml



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Last Cal. Update 12/1/2020 3:04 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-D3



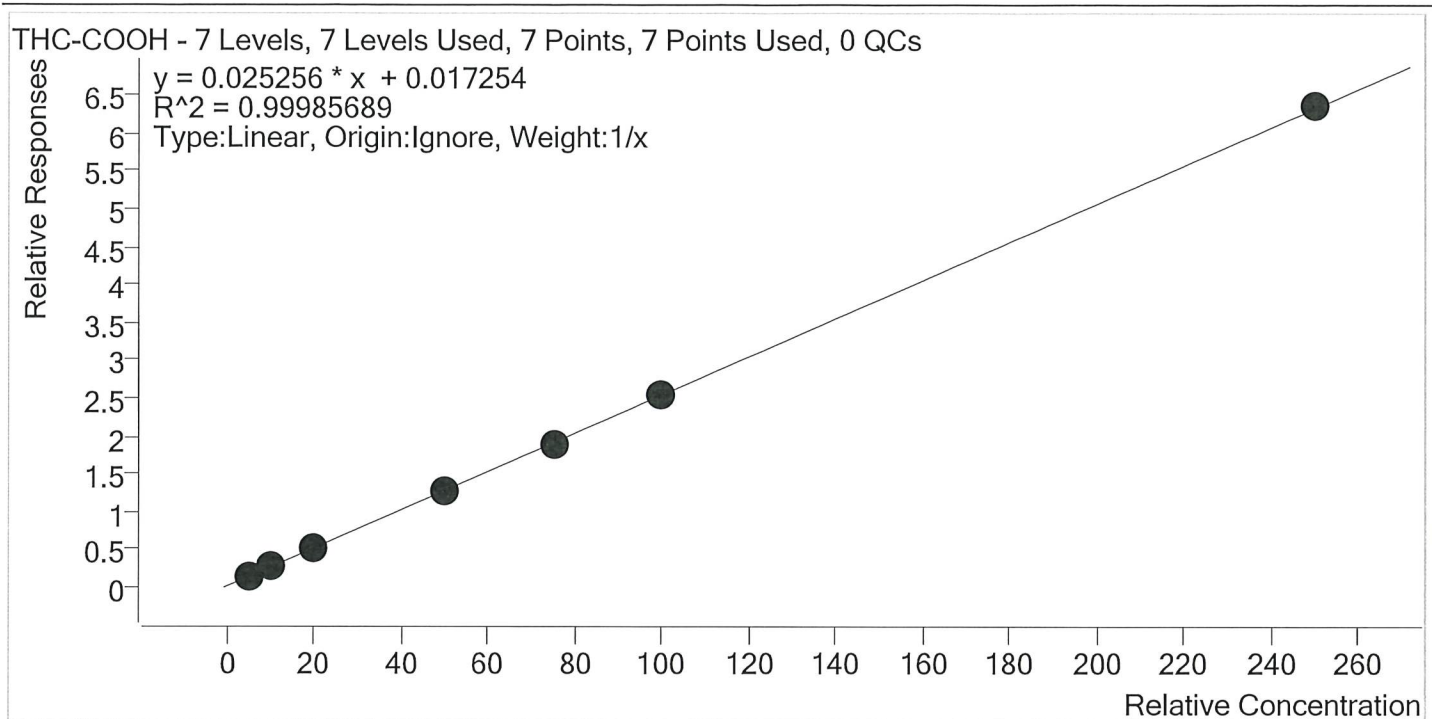
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	×	1.0	1.2	119.2
MJ Cal 2	2	✓	3.0	3.1	102.0
MJ Cal 3	3	✓	5.0	5.1	102.9
MJ Cal 4	4	✓	10.0	9.6	96.4
MJ Cal 5	5	✓	25.0	24.3	97.4
MJ Cal 6	6	✓	50.0	50.5	101.0
MJ Cal 7	7	✓	100.0	100.3	100.3

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Last Cal. Update 12/1/2020 3:04 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-D9



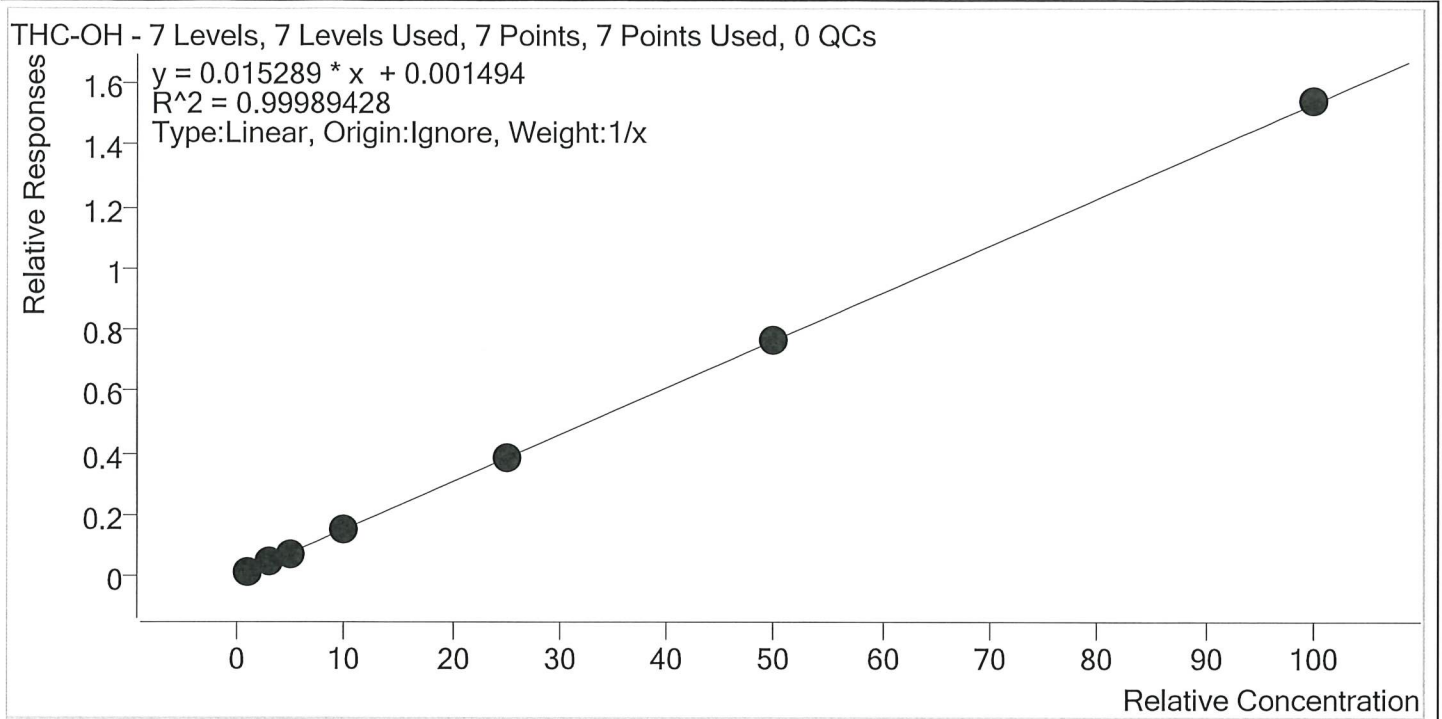
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	5.0	5.2	104.9
MJ Cal 2	2	✓	10.0	9.7	96.5
MJ Cal 3	3	✓	20.0	20.1	100.5
MJ Cal 4	4	✓	50.0	48.9	97.8
MJ Cal 5	5	✓	75.0	74.8	99.8
MJ Cal 6	6	✓	100.0	100.0	100.0
MJ Cal 7	7	✓	250.0	251.3	100.5

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AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Last Cal. Update 12/1/2020 3:04 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-D3



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
MJ Cal 1	1	✓	1.0	1.1	105.9
MJ Cal 2	2	✓	3.0	2.9	96.9
MJ Cal 3	3	✓	5.0	5.0	99.6
MJ Cal 4	4	✓	10.0	9.7	97.5
MJ Cal 5	5	✓	25.0	25.0	100.1
MJ Cal 6	6	✓	50.0	49.7	99.5
MJ Cal 7	7	✓	100.0	100.6	100.6

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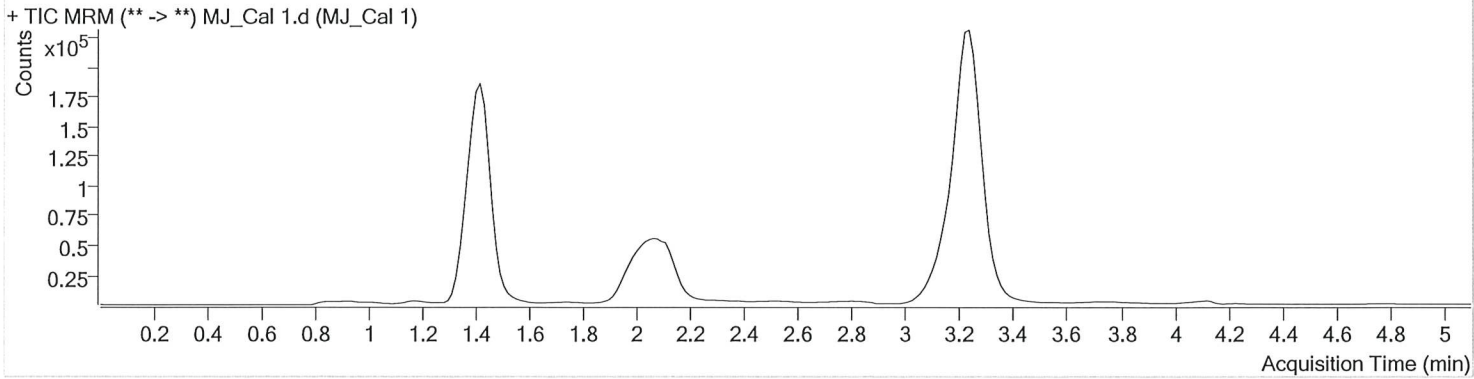
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument	Instrument 1	Data File	MJ_Cal 1.d
Type	Cal	Sample	MJ_Cal 1
Acq. Method	AM 27 THCQ.m	Operator	Sophia Jackson
Sample Position	P1-A1	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2020 2:10:34 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.423	14202	∞	11.8	17.84	802850	1.0593 ng/ml Low
THC-COOH	1.444	38447	∞	51.1	153.96	256726	5.2464 ng/ml
THC	3.239	16628	72.16	38.2 High	87.06	1819612	1.1921 ng/ml Low

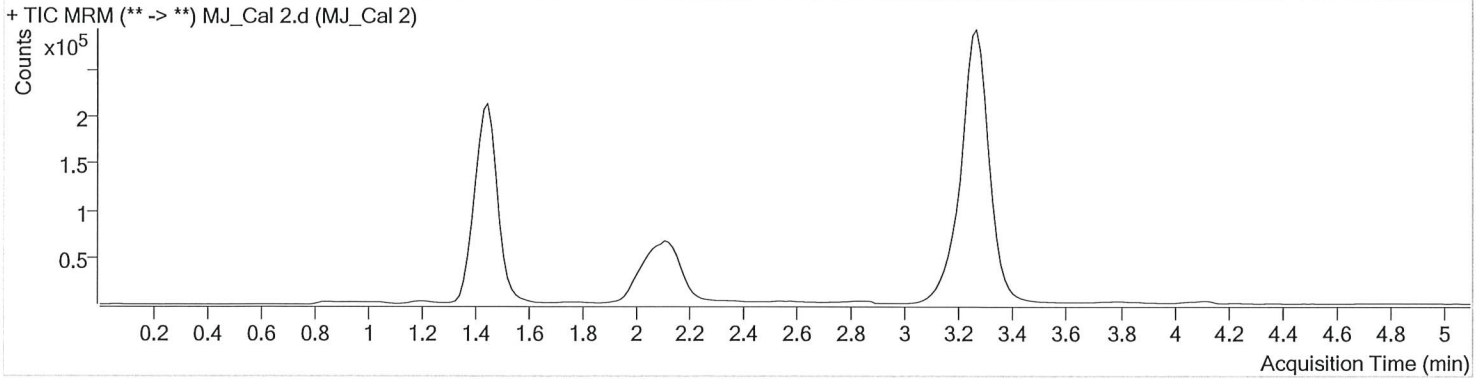
AM #27 Cannabinoid Quant. Results



Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument Instrument 1 Data File MJ_Cal 2.d
Type Cal Sample MJ_Cal 2
Acq. Method AM 27 THCQ.m Operator Sophia Jackson
Sample Position P1-B1 Comment
Injection Volume 10
Acq. Date-Time 11/25/2020 2:18:19 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	37111	∞	13.6	36.22	807688	2.9075 ng/ml Low
THC-COOH	1.474	73359	∞	61.0	500.48	281041	9.6520 ng/ml
THC	3.270	55265	480.43	30.7	50.10	2081469	3.0597 ng/ml

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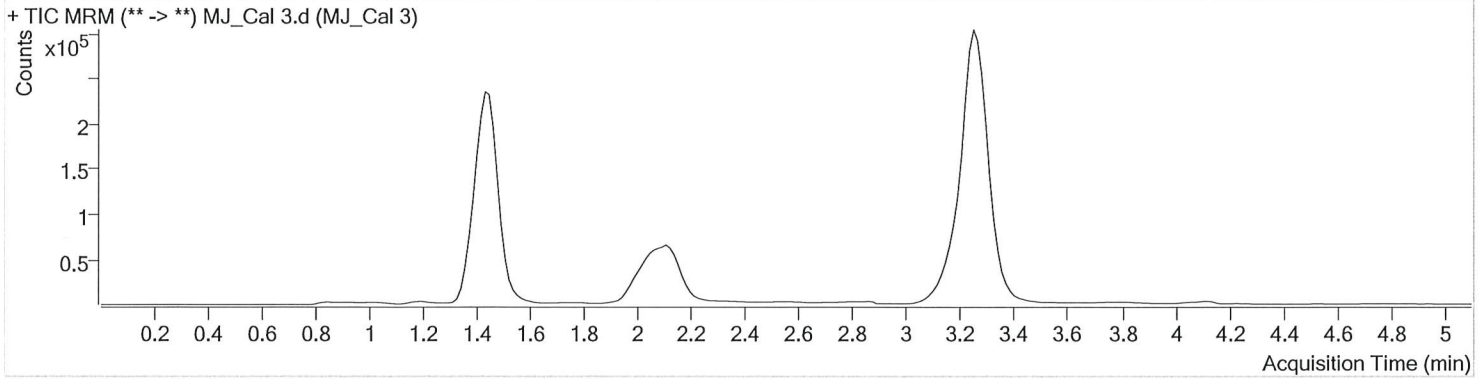
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument	Instrument 1	Data File	MJ_Cal 3.d
Type	Cal	Sample	MJ_Cal 3
Acq. Method	AM 27 THCQ.m	Operator	Sophia Jackson
Sample Position	P1-C1	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2020 2:25:55 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	64102	∞	13.1	166.37	825915	4.9786 ng/ml
THC-COOH	1.459	144533	825.98	63.0	622.68	275481	20.0901 ng/ml
THC	3.254	98350	466.53	29.6	100.62	2138113	5.1454 ng/ml

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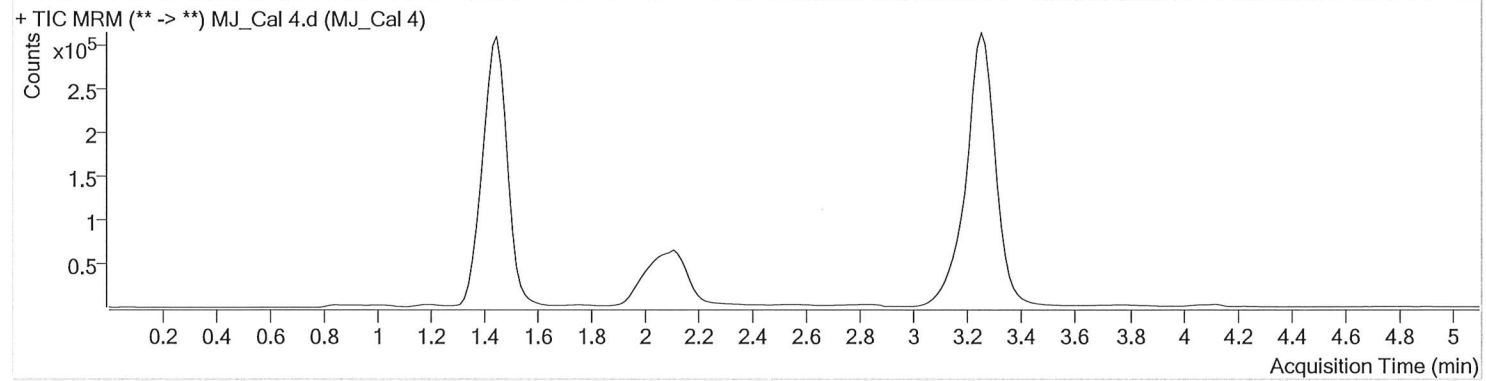


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument	Instrument 1	Data File	MJ_Cal 4.d
Type	Cal	Sample	MJ_Cal 4
Acq. Method	AM 27 THCQ.m	Operator	Sophia Jackson
Sample Position	P1-D1	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2020 2:33:31 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	127414	∞	13.5	232.12	846476	9.7473 ng/ml
THC-COOH	1.459	359595	2517.63	65.1	2348.77	287109	48.9069 ng/ml
THC	3.254	188505	1976.56	28.7	181.40	2143918	9.6421 ng/ml

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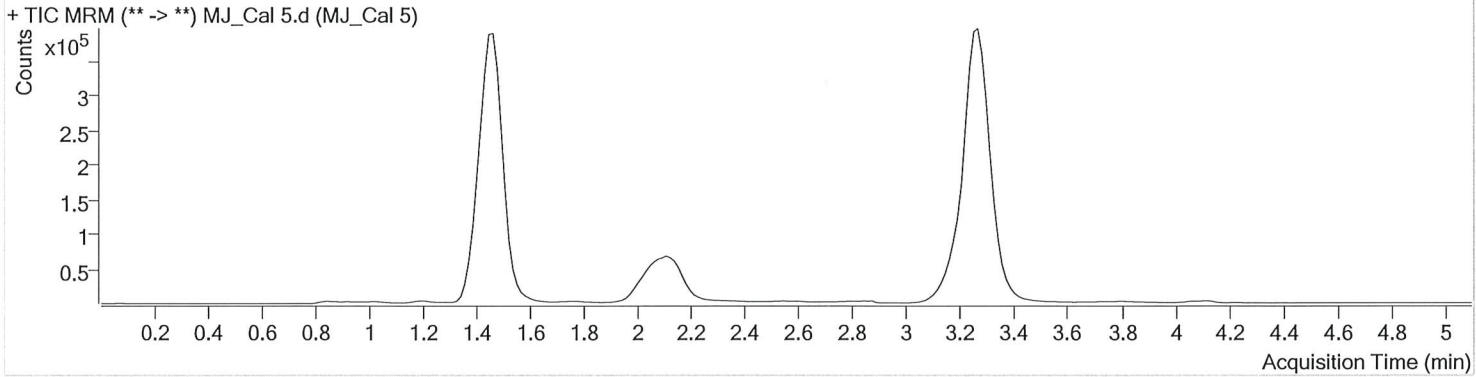


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument	Instrument 1	Data File	MJ_Cal 5.d
Type	Cal	Sample	MJ_Cal 5
Acq. Method	AM 27 THCQ.m	Operator	Sophia Jackson
Sample Position	P1-E1	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2020 2:41:05 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	306640	∞	13.1	1123.49	798323	25.0250 ng/ml
THC-COOH	1.474	521192	3323.69	65.4	8862.03	273306	74.8220 ng/ml
THC	3.270	503814	5582.99	29.2	∞	2239023	24.3452 ng/ml



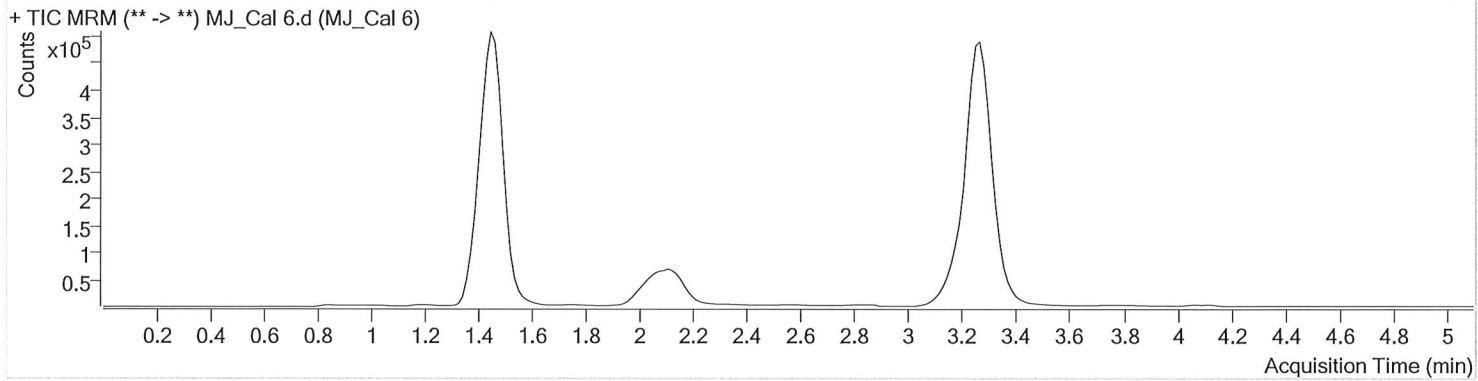
AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument	Instrument 1	Data File	MJ_Cal 6.d
Type	Cal	Sample	MJ_Cal 6
Acq. Method	AM 27 THCQ.m	Operator	Sophia Jackson
Sample Position	P1-F1	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2020 2:48:41 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.438	621396	∞	13.4	1486.33	815735	49.7259 ng/ml
THC-COOH	1.474	704410	4524.39	64.9	∞	276952	100.0215 ng/ml
THC	3.270	1056053	6719.85	28.7	∞	2252596	50.4931 ng/ml

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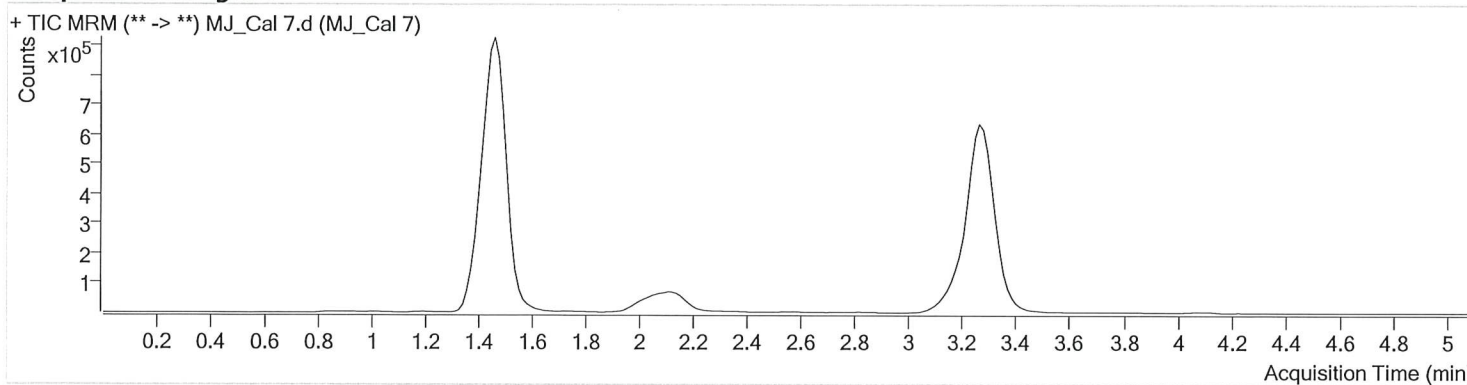


AM #27 Cannabinoid Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\112520 AM 27 28 SJ SP\QuantResults\AM 27 SJ.batch.bin
Calibration Last Update 12/1/2020 3:04:59 PM

Instrument	Instrument 1	Data File	MJ_Cal 7.d
Type	Cal	Sample	MJ_Cal 7
Acq. Method	AM 27 THCQ.m	Operator	Sophia Jackson
Sample Position	P1-G1	Comment	
Injection Volume	10		
Acq. Date-Time	11/25/2020 2:56:15 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.453	1295614	∞	13.2	1870.58	841898	100.5565 ng/ml
THC-COOH	1.474	1724843	5527.39	66.0	16660.9	271064	251.2612 ng/ml
THC	3.270	2079824	12104.66	28.1	1982.50	2228352	100.3145 ng/ml