









REVIEWED

By Celena Shrum at 2:29 pm, Jan 22, 2020

1/15/2020

BW

Worklist: 3944

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2019-2453	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2456	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2457	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2019-2466	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-0012	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0030	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-0041	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0077	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: 1/15/20
Plate lot#: 190716

Analyst: Britany Wylie
Plate Expiration: 1-16-2020

Mobile phase A: 0.1% Formic Acid in LCMS Water
MTBE LCMS Methanol

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 19H52275 **Urine Blank:** 11420
LCMS-QQQ ID: 69679

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. Urine hydrolysis: add 1.5 ml urine to blank plate, add 250 ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
Pipette **1000µL blood (calibrated pipette) Pipette ID: k52558g** in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- 4. Pipette **500µL 0.1% formic acid in water blood sample, 500 ul saturated phosphate buffer in urine** in wells 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) Manifold ID: 66792*
- 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. *SPE Dry ID: 66819*
- 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 blood), Carboxy-THC: 10ng/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 is it describe in comments section)
- 6. Enter QCs into control charting.
- 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

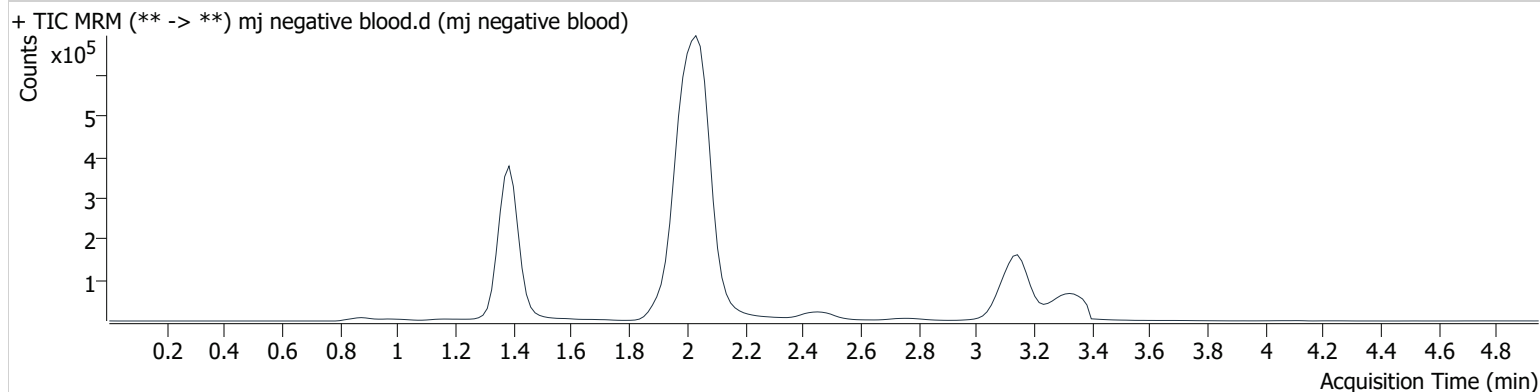
COMMENTS:

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj negative blood.d
Type	Sample	Sample	mj negative blood
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 3:26:14 PM		
Sample Info.			

Sample Chromatogram

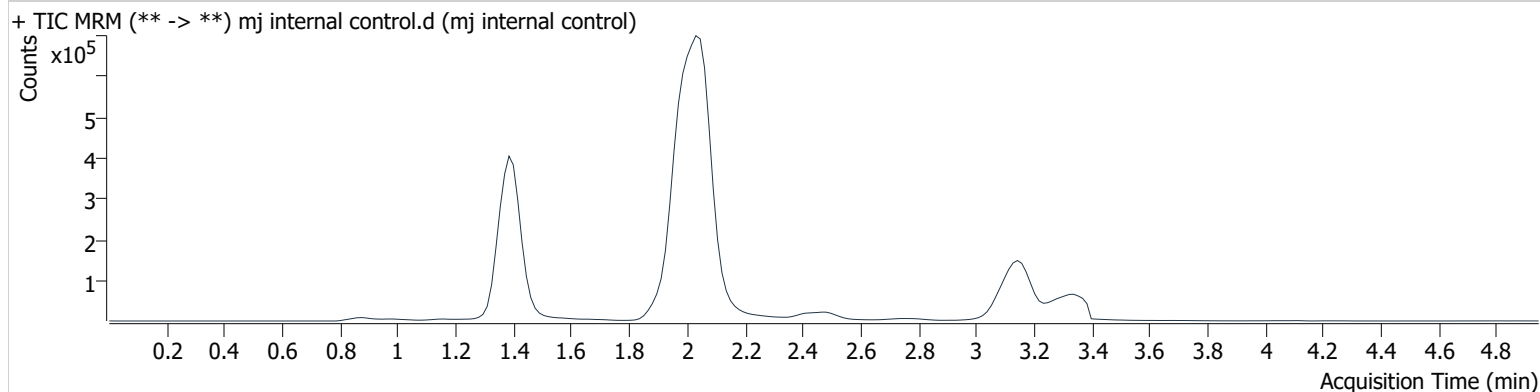


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj internal control.d
Type	QC	Sample	mj internal control
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 3:18:28 PM		

Sample Chromatogram



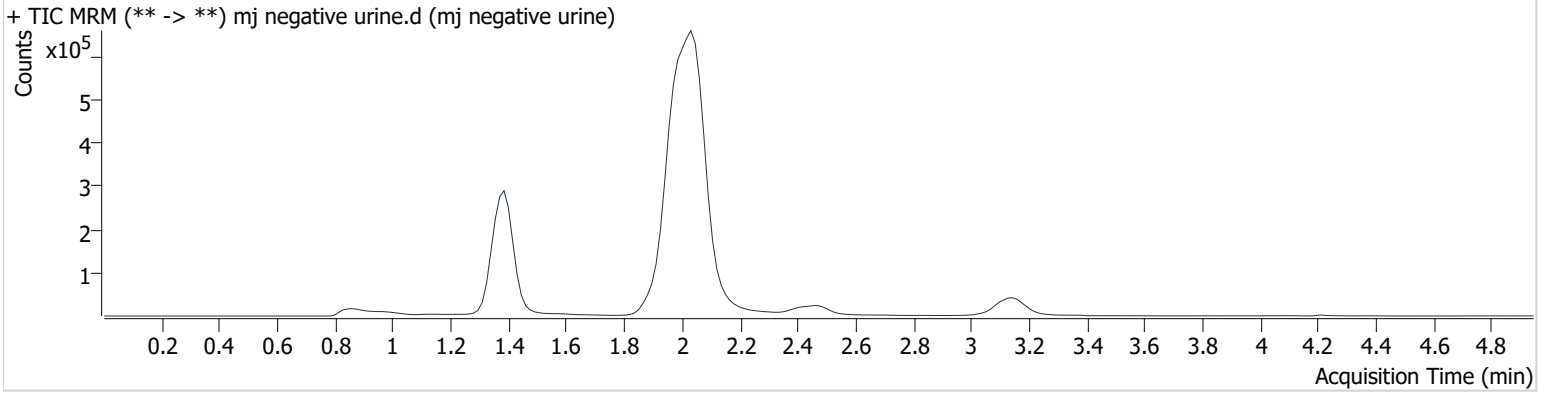
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	81670	∞	12.6	∞	1244736	3.699 ng/ml
THC-COOH	1.415	96350	639.9	44.4	728.6	536777	15.313 ng/ml
THC	3.168	29022	∞	26.2	103.8	878444	4.485 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj negative urine.d
Type	Sample	Sample	mj negative urine
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-H2	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 5:13:49 PM		
Sample Info.			

Sample Chromatogram

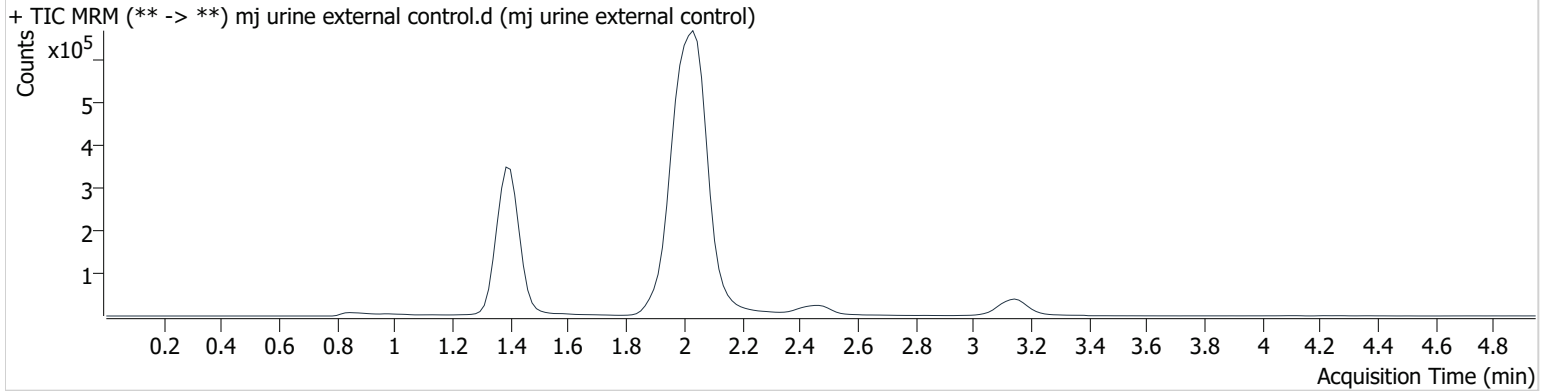


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj urine external control.d
Type	Sample	Sample	mj urine external control
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A3	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 5:29:12 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-COOH	1.415	169566	876.0	45.3	1198.0	374387	36.276 ng/ml

Toxicology AM method 27 external urine preparation information

Stock solution 8 ul (100 ug/ml) C-THC in 9.992 mls urine
Ppd 11/8/19 Exp: 3/1/20 lot 3120 by AMN

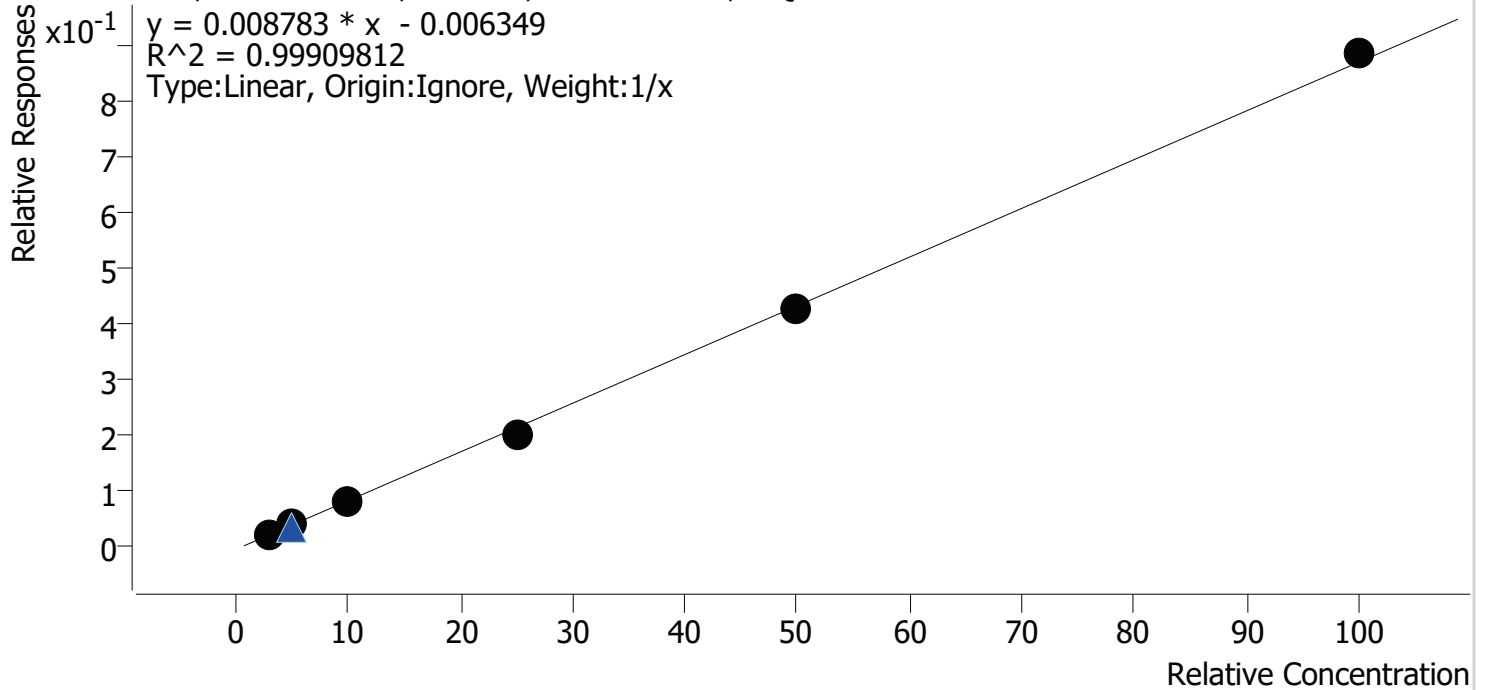
Drug	lot	expiration	lot
C-THC	FE03121501	3/1/2020	3120

concentration ~ 80 ng/ml Carboxy THC

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Last Cal. Update 1/15/2020 6:14 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

THC - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 2 QCs

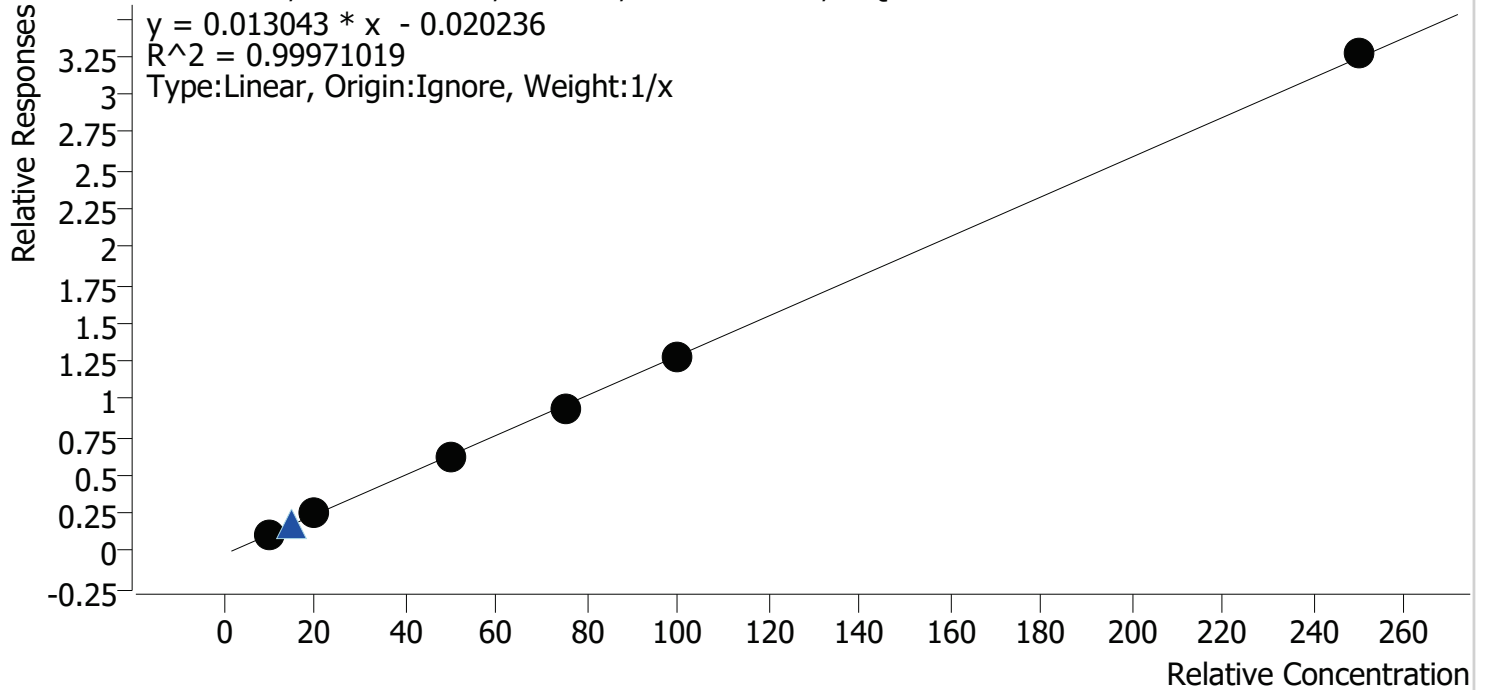


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	✓	3.0	3.2	107.6
mj cal 3	3	✓	5.0	5.0	99.1
mj cal 4	4	✓	10.0	9.8	97.6
mj cal 5	5	✓	25.0	23.6	94.6
mj cal 6	6	✓	50.0	49.7	99.4
mj cal 7	7	✓	100.0	101.7	101.7

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\lam 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Last Cal. Update 1/15/2020 6:14 PM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-d9

THC-COOH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 2 QCs

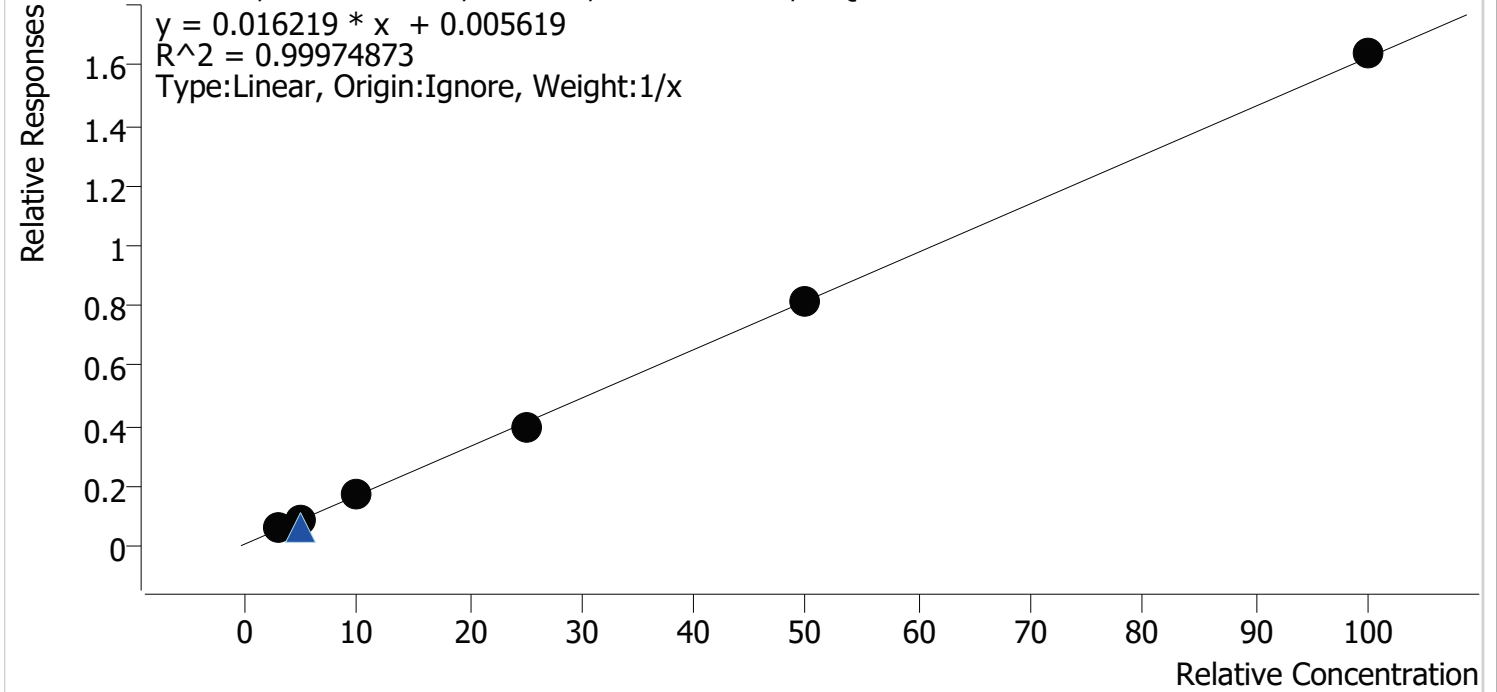


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	✓	10.0	10.0	99.8
mj cal 3	3	✓	20.0	20.7	103.6
mj cal 4	4	✓	50.0	49.1	98.1
mj cal 5	5	✓	75.0	73.4	97.9
mj cal 6	6	✓	100.0	99.8	99.8
mj cal 7	7	✓	250.0	252.1	100.8

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\lam 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Last Cal. Update 1/15/2020 6:14 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

THC-OH - 6 Levels, 6 Levels Used, 6 Points, 6 Points Used, 2 QCs



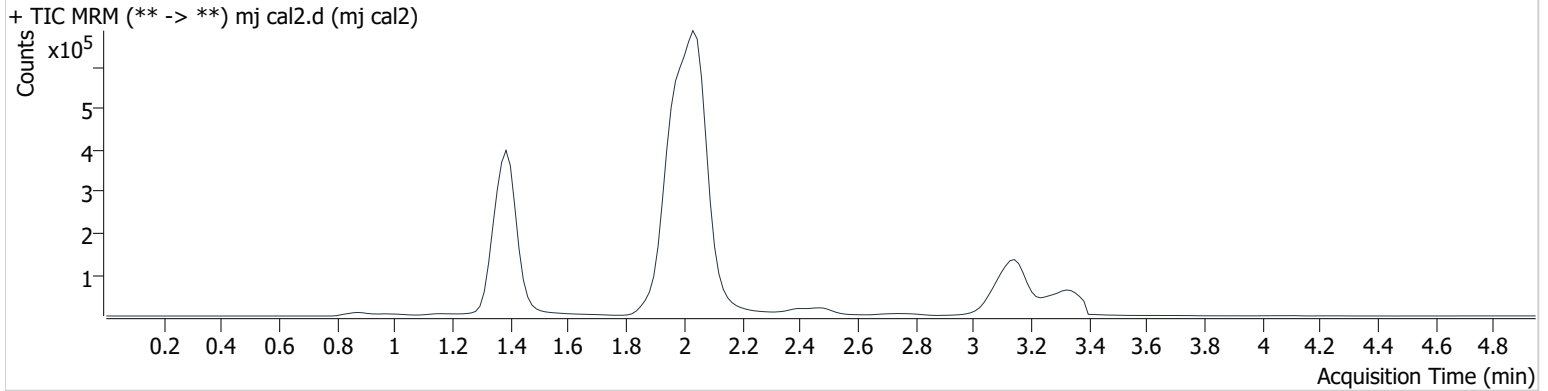
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal2	2	✓	3.0	3.1	103.3
mj cal 3	3	✓	5.0	5.0	100.1
mj cal 4	4	✓	10.0	9.9	99.0
mj cal 5	5	✓	25.0	24.2	96.8
mj cal 6	6	✓	50.0	50.0	100.0
mj cal 7	7	✓	100.0	100.8	100.8

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal2
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 2:32:06 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	74336	∞	9.1	∞	1330707	3.098 ng/ml
THC-COOH	1.415	65205	134.7	46.2	331.5	592901	9.983 ng/ml Low
THC	3.153	18739	∞	27.3	408.7	852244	3.227 ng/ml

AM #27 Cannabinoids

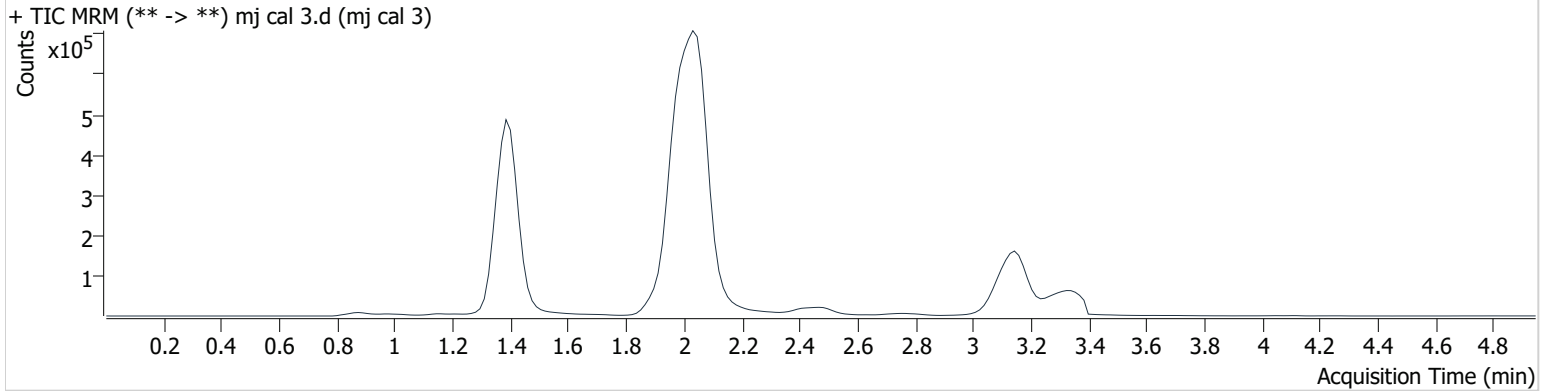
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Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj cal 3.d
Type	Cal	Sample	mj cal 3
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-C1	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 2:39:51 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	122744	∞	10.5	∞	1413859	5.006 ng/ml
THC-COOH	1.415	151220	376.4	45.2	254.8	605068	20.713 ng/ml
THC	3.153	35833	∞	24.9	153.2	963512	4.957 ng/ml

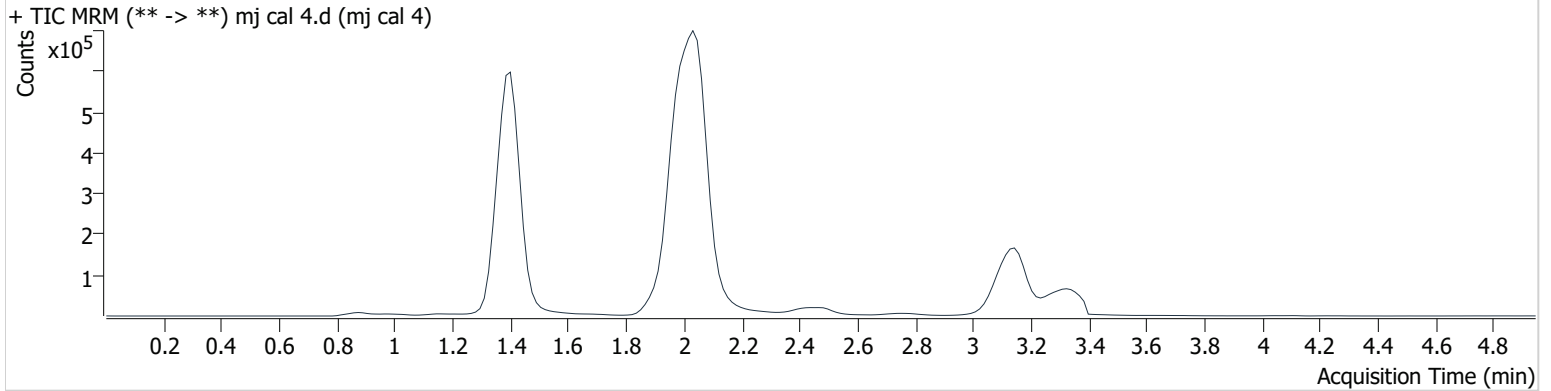
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj cal 4.d
Type	Cal	Sample	mj cal 4
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-D1	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 2:47:35 PM		

Sample Chromatogram



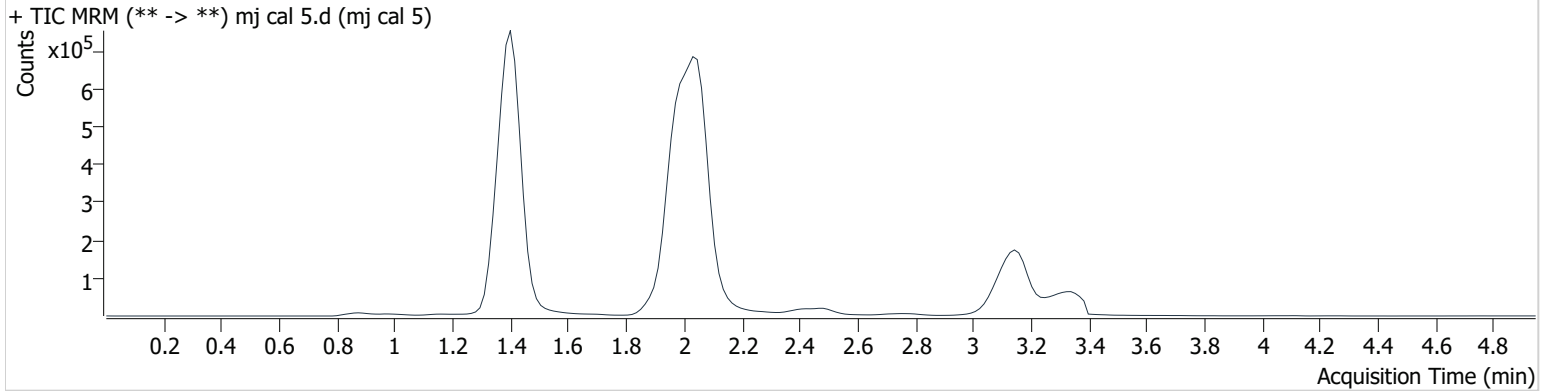
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	221381	∞	10.7	∞	1331965	9.901 ng/ml
THC-COOH	1.415	361249	771.2	46.2	851.7	583067	49.053 ng/ml
THC	3.168	75487	∞	25.7	1947.3	950869	9.762 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-E1	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 2:55:19 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	531664	∞	11.3	∞	1334937	24.209 ng/ml
THC-COOH	1.415	543900	13895.1	46.5	4633.4	580126	73.433 ng/ml
THC	3.168	183603	∞	25.5	∞	911933	23.647 ng/ml

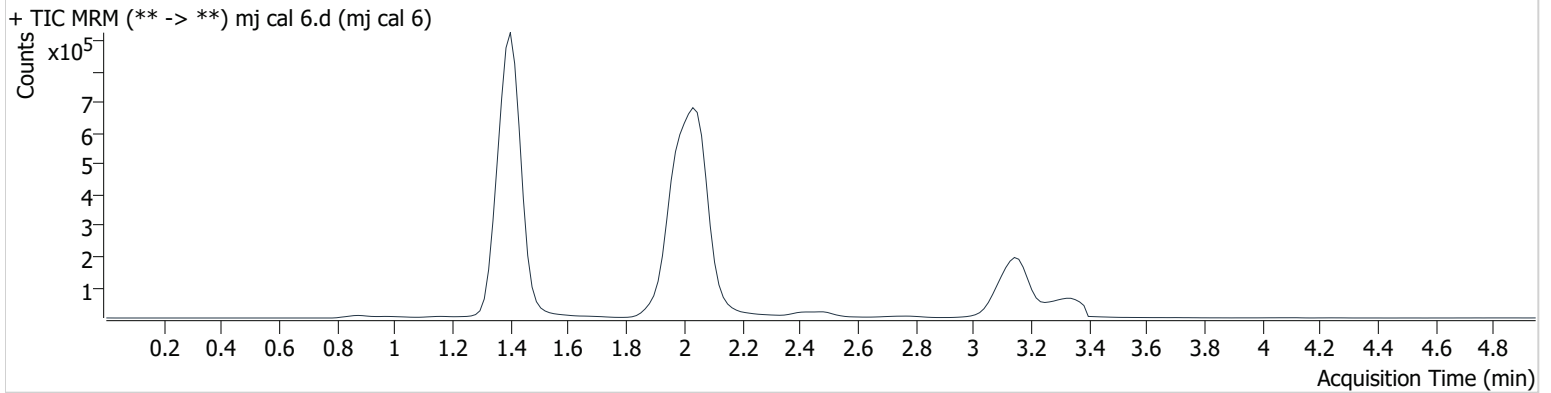
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj cal 6.d
Type	Cal	Sample	mj cal 6
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-F1	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 3:03:02 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	1035747	∞	11.7	13075.2	1268878	49.982 ng/ml
THC-COOH	1.415	688625	2368.0	47.0	4425.4	537566	99.765 ng/ml
THC	3.168	366313	∞	24.8	∞	851896	49.683 ng/ml

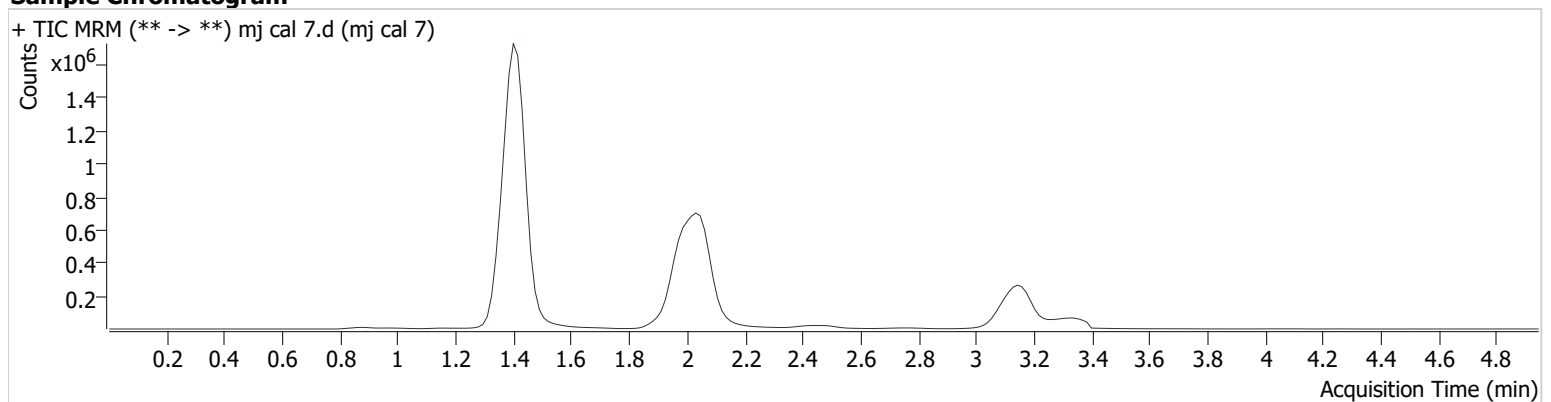
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AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 1-15-2020\QuantResults\thcq 1152020.batch.bin
Calibration Last Update 1/15/2020 6:14:17 PM

Instrument	69679	Data File	mj cal 7.d
Type	Cal	Sample	mj cal 7
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-G1	Comment	
Injection Volume	10		
Acq. Date-Time	1/15/2020 3:10:46 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	2045642	∞	12.1	∞	1246924	100.804 ng/ml
THC-COOH	1.415	1693911	1404.5	47.1	4779.4	518444	252.053 ng/ml
THC	3.168	766120	∞	25.4	∞	863685	101.723 ng/ml