







REVIEWED

By Anne Nord at 9:32 am, May 08, 2020

5/7/2020

BW

Worklist: 4218

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2020-0743	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-0753	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-0757	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0771	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0787	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0793	1	BCK	AM 27 Blood THC Quant by LC-QQQ	

BW

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

Extraction Date: 5/6/20

Analyst: Britany Wylie

Plate lot#: 200303

Plate Expiration: 9-3-2020

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: 20A52255 **Urine Blank:** 41520

Column: UCT Selectra DA 100 x 2.1mm 3um

LCMS-QQQ ID: 69679

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 250ul 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.
- 4. Pipette **500µL 0.1% formic acid in water blood sample** 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 r² 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: Urine cases only evaluated for Carboxy-THC

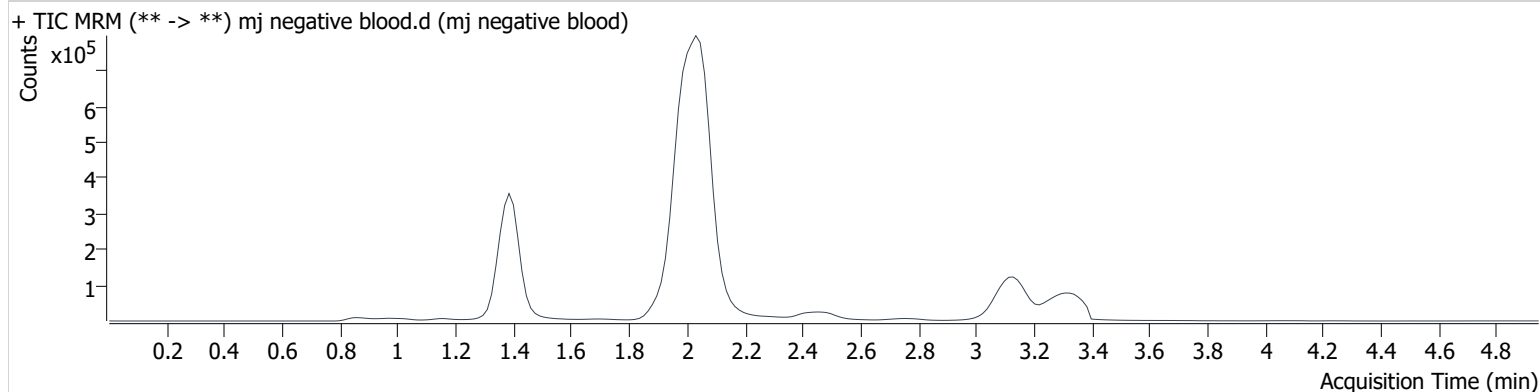
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 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	5/6/2020 2:14:09 PM		

Sample Info.

Sample Chromatogram

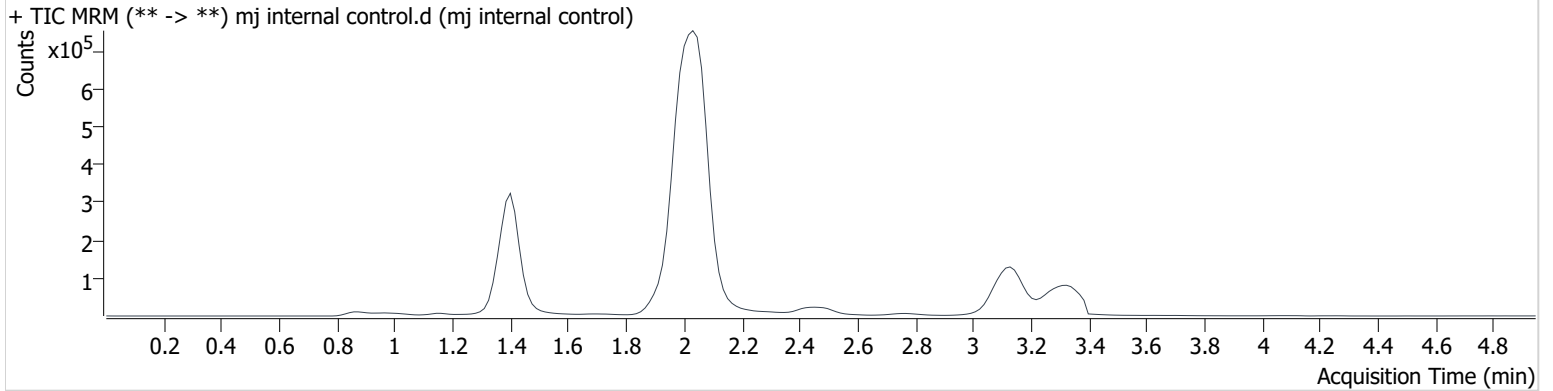


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 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 5/6/2020 2:06:27 PM
Sample Info.

Sample Chromatogram



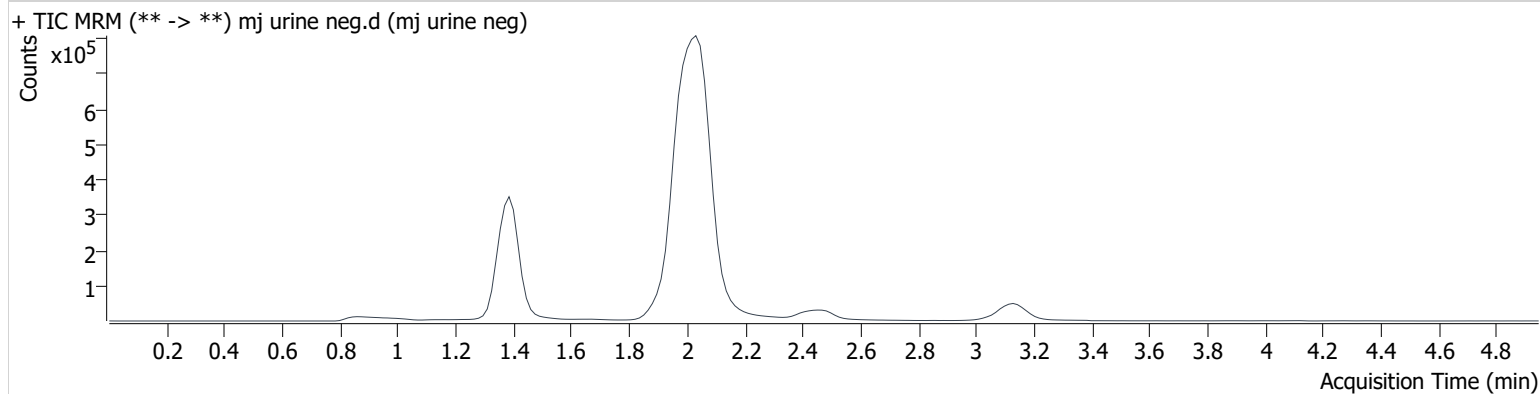
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	81584	∞	9.4	∞	805035	5.297 ng/ml
THC-COOH	1.415	95803	∞	191.2	∞	502374	16.426 ng/ml
THC	3.153	25188	∞	25.1	∞	669504	4.260 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 PM

Instrument	69679	Data File	mj urine neg.d
Type	Sample	Sample	mj urine neg
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-F2	Comment	
Injection Volume	10		
Acq. Date-Time	5/6/2020 3:31:01 PM		
Sample Info.			

Sample Chromatogram



AM #27 Cannabinoids

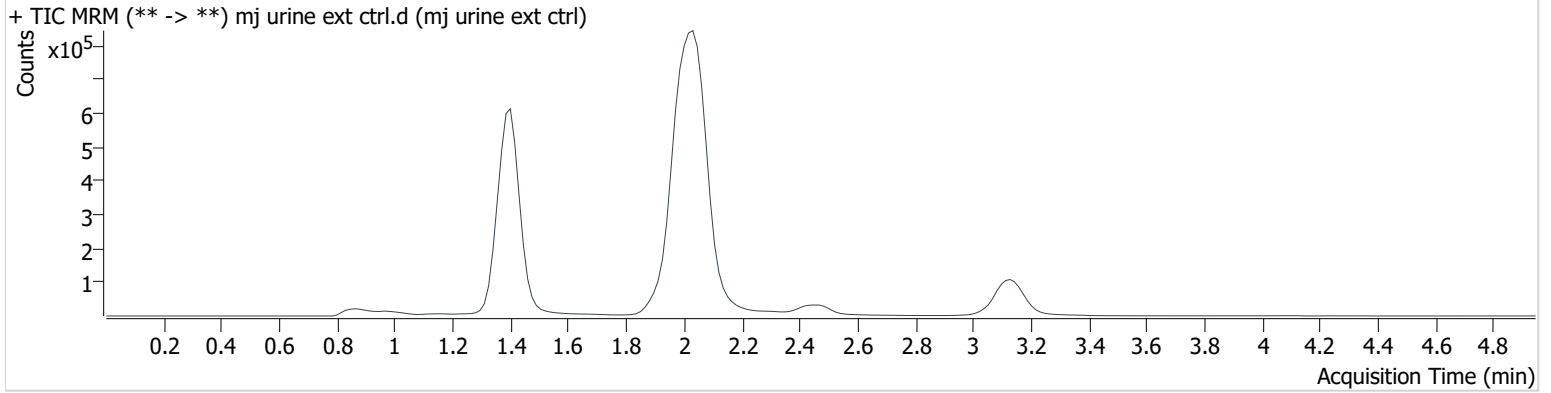
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Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 PM

Instrument	69679	Data File	mj urine ext ctrl.d
Type	Sample	Sample	mj urine ext ctrl
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-G2	Comment	
Injection Volume	10		
Acq. Date-Time	5/6/2020 3:38:45 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	384179	∞	10.8	∞	1341598	14.736 ng/ml
THC-COOH	1.415	303791	∞	181.7	∞	612135	40.531 ng/ml
THC	3.153	106770	∞	23.7	∞	653035	17.359 ng/ml

Toxicology AM method 27/26 external prep information

working solution 1 ug/ml in meoh C-THC, THC-OH, THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 75 ul THC-OH in 9767.5 ul meOH

Ppd 2/13/20 Exp: 8/13/20 lot 21320 by AMN

Drug	lot	expiration
C-THC	FE07171501	9/1/2020
THC-OH	FE07721601	7/1/2021
THC	FE001041701	3/1/2022

AM 27/26 blood control 100 ul working solution lot (91319) in 9900 ul blood lot (20A52255)

ppd 02/13/20 Exp 08/13/20 lot b81320 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-THC by AMN

AM 27/26 urine control 400 ul working solution lot (21320) in 9600 ul urine lot (32420)

ppd 4/17/20 Exp 9120 lot u101720 Concentration 30 ng/ml THC, THC-OH and 60 ng/ml C-THC by BAW

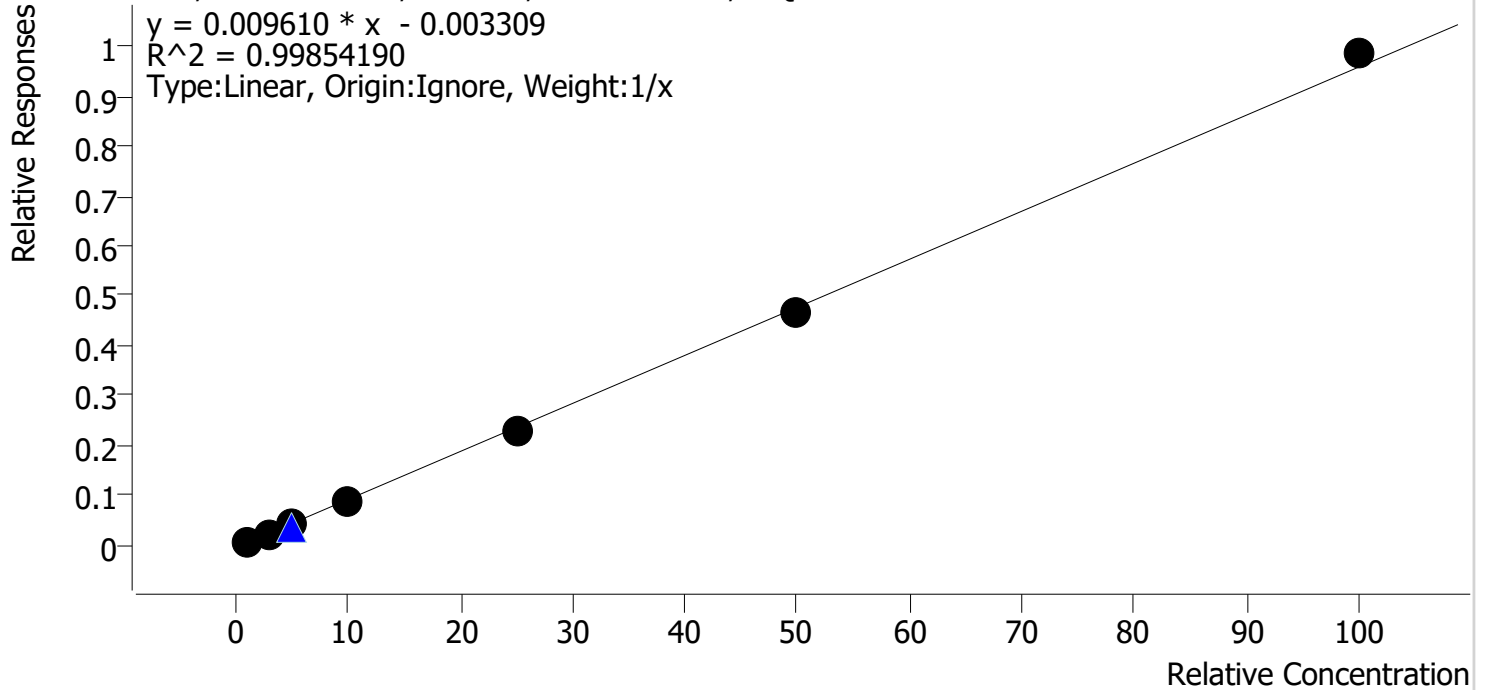
Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Last Cal. Update 5/6/2020 5:38 PM
Analyst Name ISP\datastor
Analyte THC

BW

Internal Standard THC-d3

THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	1.2	120.7
mj cal2	2	✓	3.0	2.9	95.5
mj cal 3	3	✓	5.0	4.6	92.4
mj cal 4	4	✓	10.0	9.5	94.5
mj cal 5	5	✓	25.0	24.0	96.0
mj cal 6	6	✓	50.0	49.1	98.2
mj cal 7	7	✓	100.0	102.8	102.8

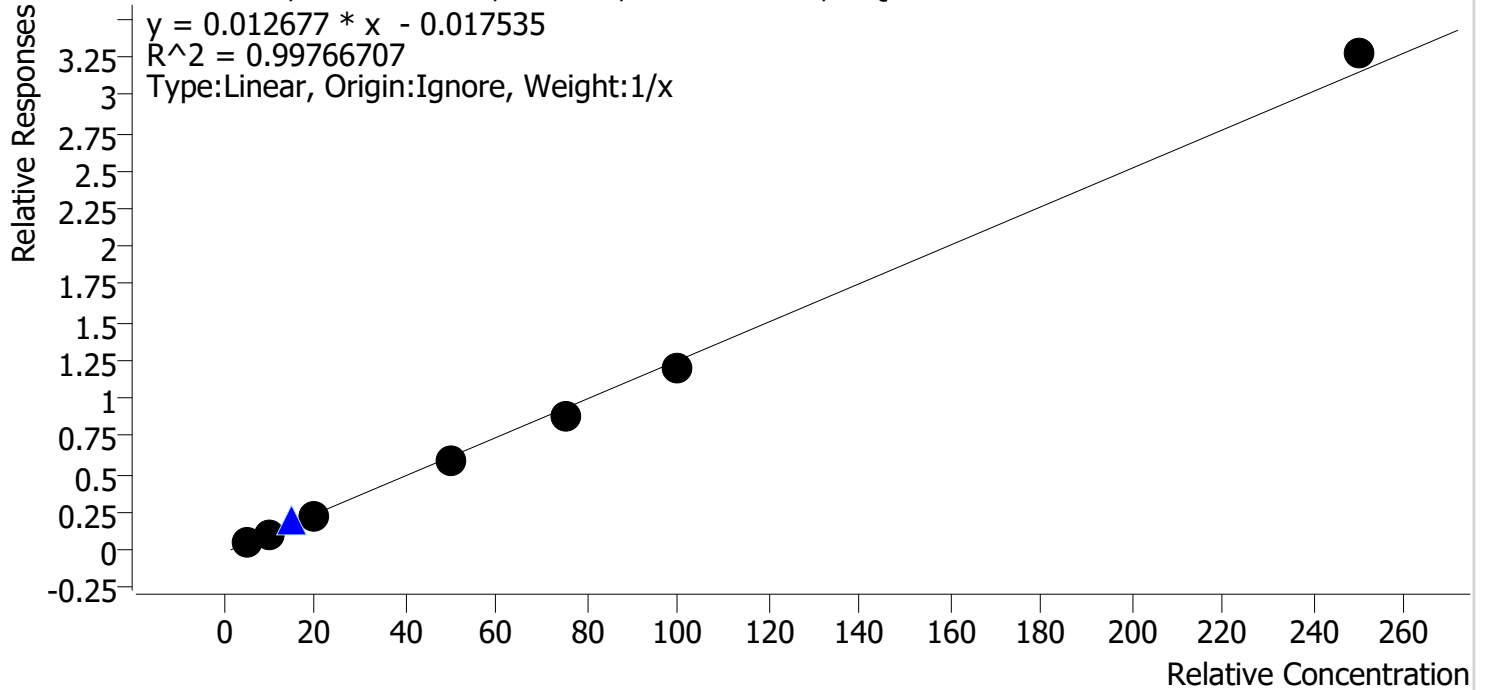
Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Last Cal. Update 5/6/2020 5:38 PM
Analyst Name ISP\datastor
Analyte THC-COOH

BW

Internal Standard **THC-COOH-d9**

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.6	112.9
mj cal2	2	✓	10.0	10.0	99.7
mj cal 3	3	✓	20.0	19.2	96.1
mj cal 4	4	✓	50.0	48.4	96.7
mj cal 5	5	✓	75.0	70.7	94.3
mj cal 6	6	✓	100.0	96.5	96.5
mj cal 7	7	✓	250.0	259.6	103.8

Compound Calibration Report

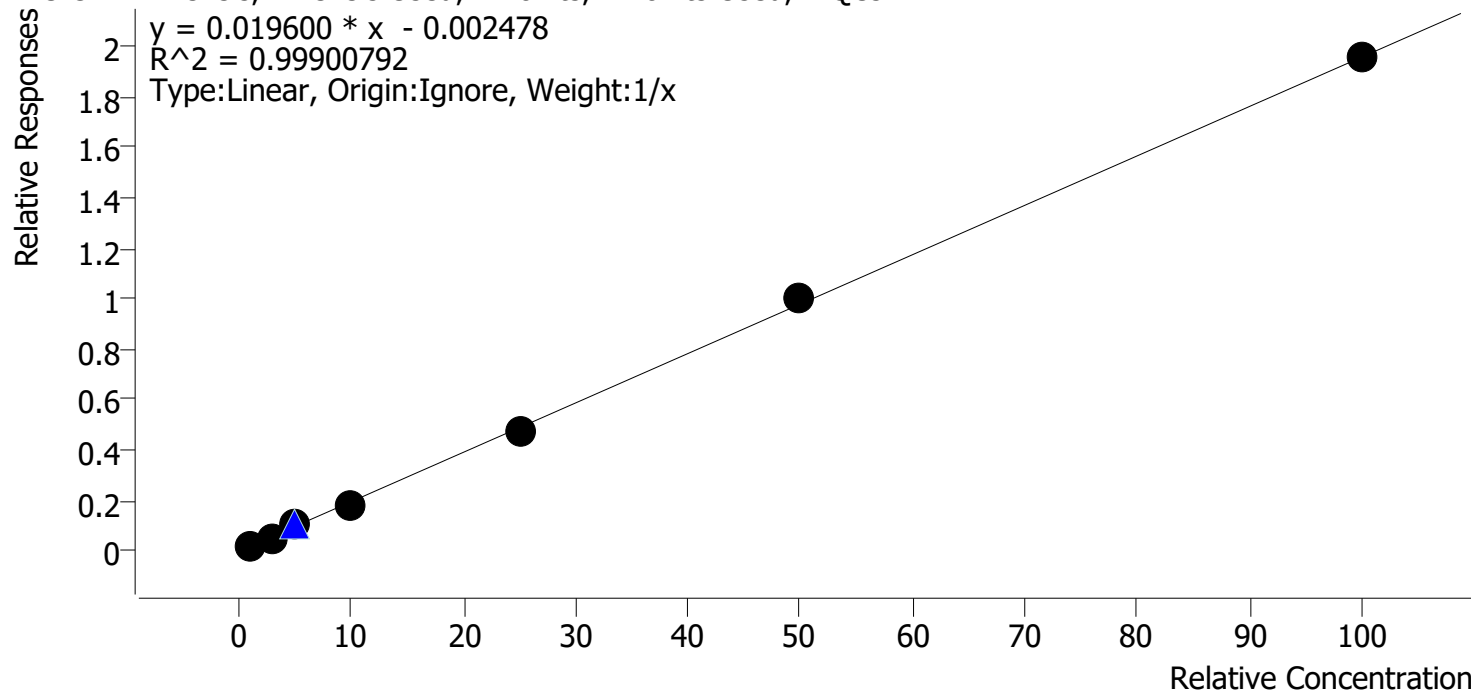
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Last Cal. Update 5/6/2020 5:38 PM
Analyst Name ISP\datastor
Analyte THC-OH

BW

Internal Standard

THC-OH-d3

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	1.1	109.2
mj cal2	2	✓	3.0	2.6	86.4
mj cal 3	3	✓	5.0	5.5	109.7
mj cal 4	4	✓	10.0	9.5	94.7
mj cal 5	5	✓	25.0	24.6	98.3
mj cal 6	6	✓	50.0	50.9	101.9
mj cal 7	7	✓	100.0	99.9	99.9

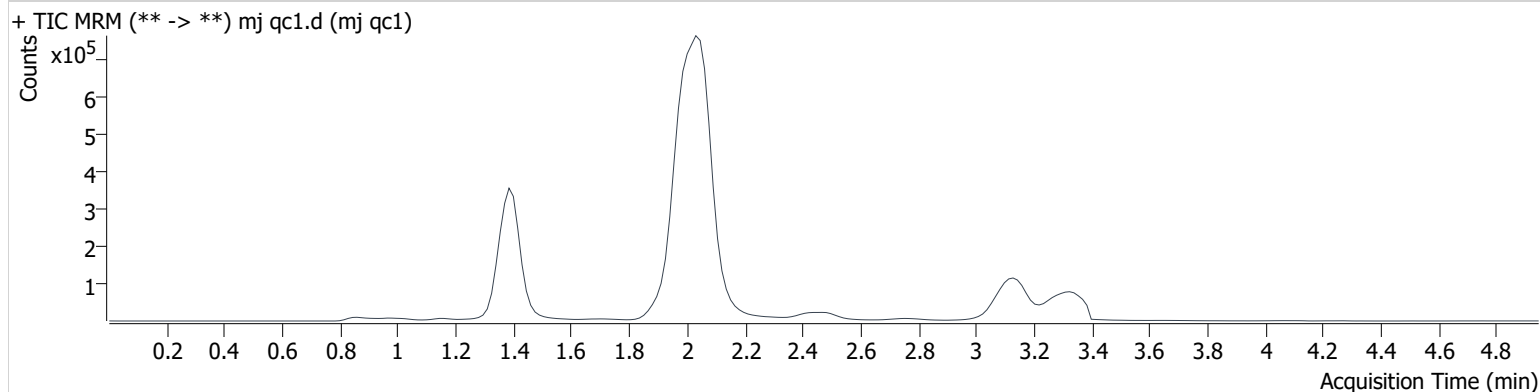
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 PM

Instrument	69679	Data File	mj qc1.d
Type	Cal	Sample	mj qc1
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	5/6/2020 1:12:23 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	20831	∞	10.9	∞	1100362	1.092 ng/ml Low
THC-COOH	1.415	33993	∞	156.8	∞	629464	5.643 ng/ml Low
THC	3.153	5304	∞	28.4	∞	639992	1.207 ng/ml Low

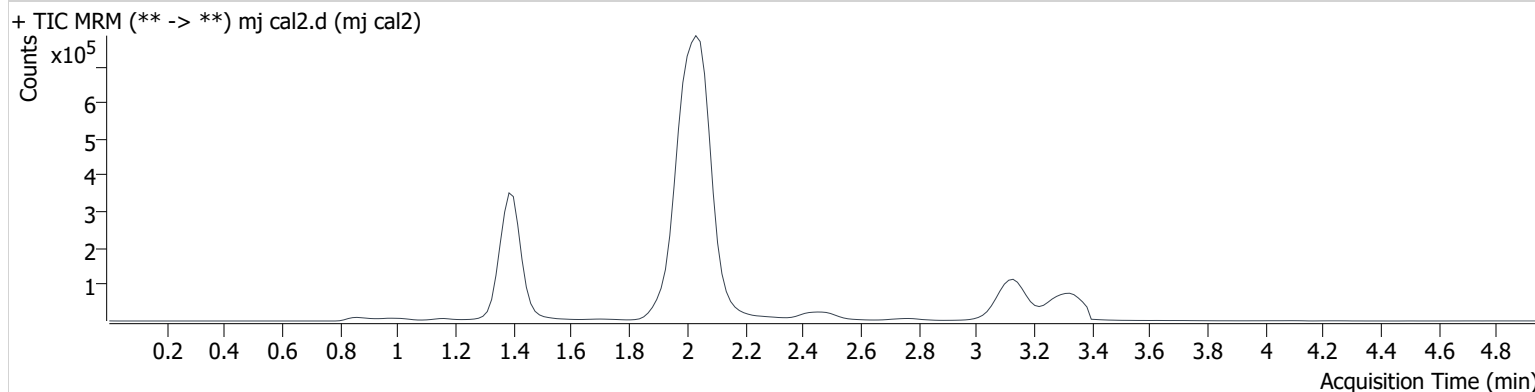
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 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	5/6/2020 1:20:07 PM		

Sample Info.

Sample Chromatogram



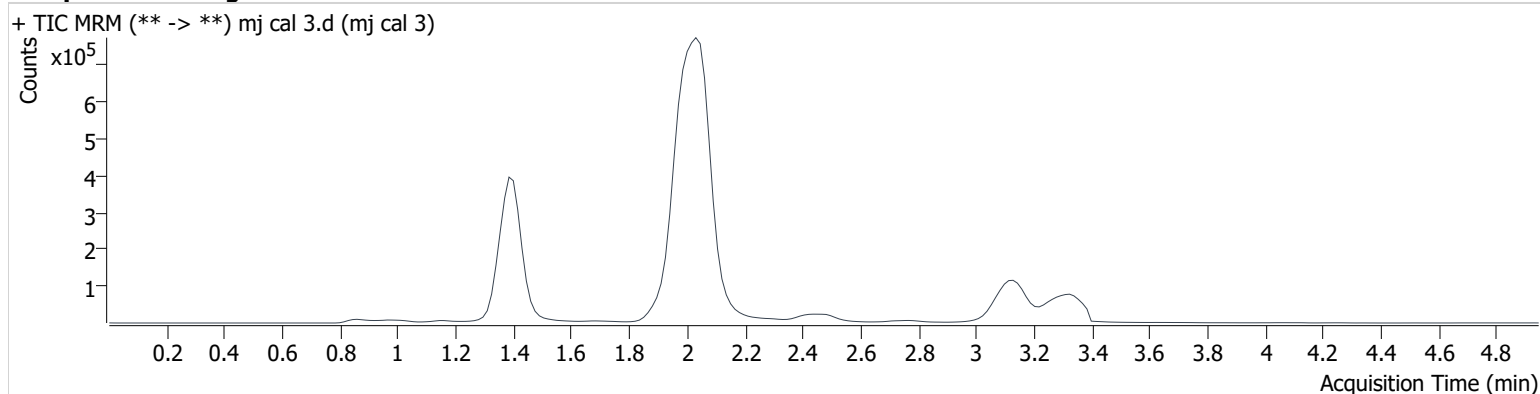
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	47905	∞	12.0	∞	991536	2.591 ng/ml Low
THC-COOH	1.415	62757	∞	180.1	∞	576504	9.970 ng/ml Low
THC	3.153	14336	∞	27.3	∞	592072	2.864 ng/ml Low

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 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	5/6/2020 1:27:51 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	107532	∞	10.3	∞	1024091	5.484 ng/ml
THC-COOH	1.415	136119	∞	199.1	∞	601992	19.220 ng/ml
THC	3.138	25329	∞	25.7	∞	616437	4.620 ng/ml

AM #27 Cannabinoids

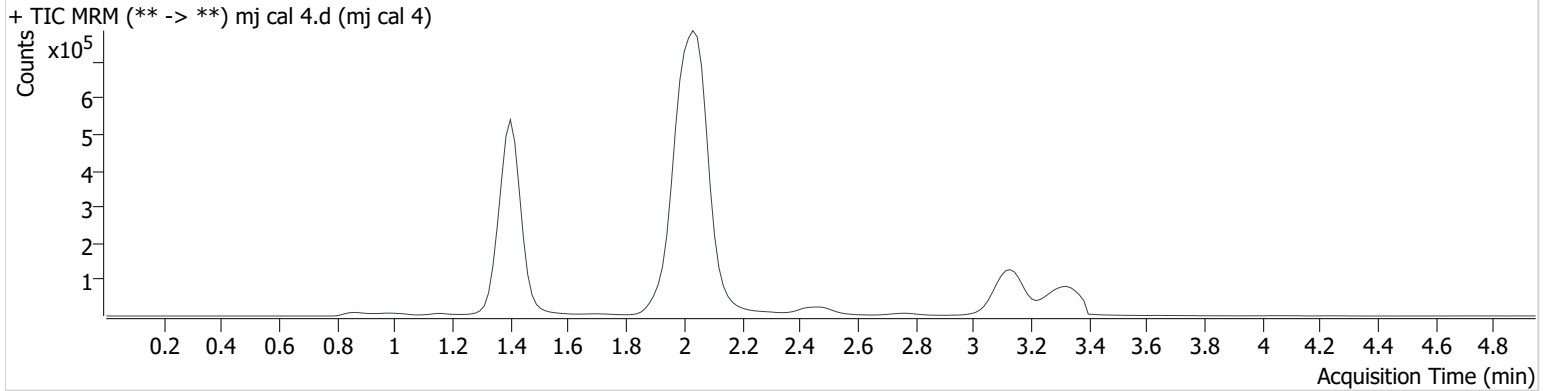
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Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 PM

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	5/6/2020 1:35:33 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	180821	∞	11.6	∞	987131	9.472 ng/ml
THC-COOH	1.415	343122	∞	187.5	∞	576172	48.360 ng/ml
THC	3.153	54872	∞	25.0	∞	627076	9.450 ng/ml

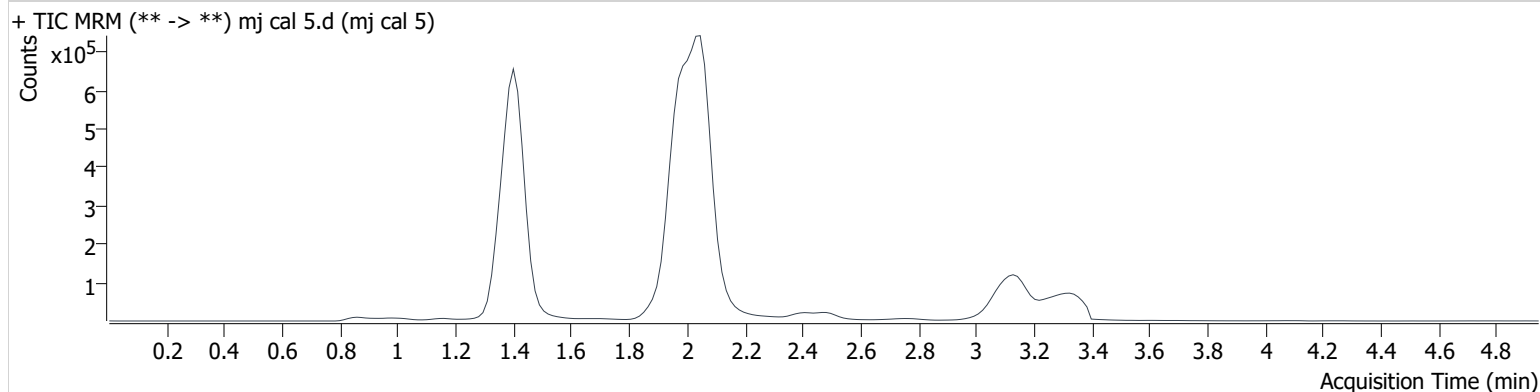
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 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	5/6/2020 1:43:16 PM		

Sample Info.

Sample Chromatogram



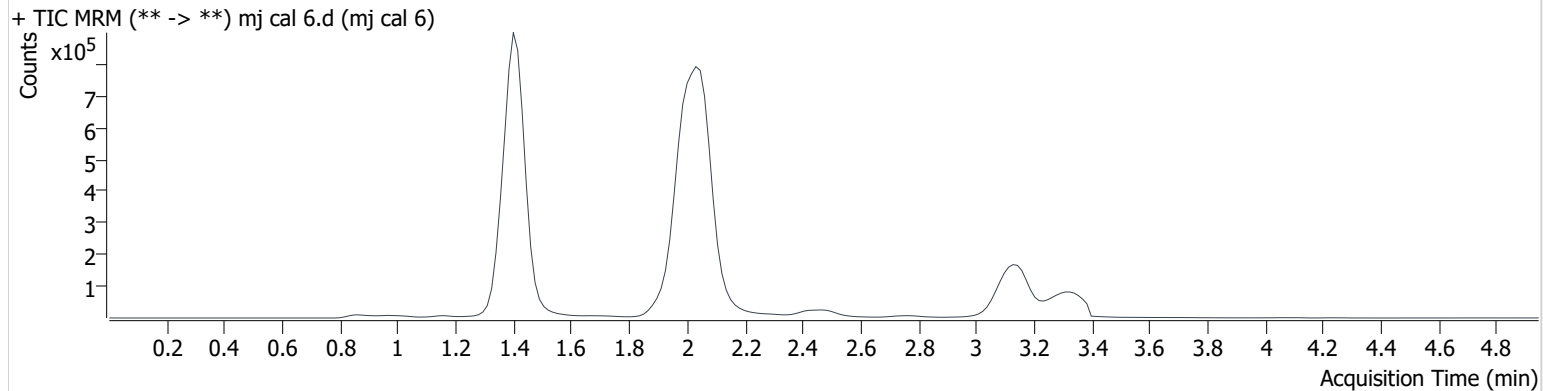
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	492619	∞	11.2	∞	1028421	24.565 ng/ml
THC-COOH	1.415	516164	∞	183.0	∞	587404	70.700 ng/ml
THC	3.153	134717	∞	24.2	∞	592689	23.998 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 PM

Instrument 69679 **Data File** mj cal 6.d
Type Cal **Sample** mj cal 6
Acq. Method AM 27 THC quant.m **Operator** Brittany Wylie
Sample Position P3-F1 **Comment**
Injection Volume 10
Acq. Date-Time 5/6/2020 1:51:00 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	970654	∞	11.5	∞	974671	50.936 ng/ml
THC-COOH	1.415	699281	∞	182.4	∞	579886	96.508 ng/ml
THC	3.153	307997	∞	25.1	∞	657493	49.092 ng/ml

AM #27 Cannabinoids

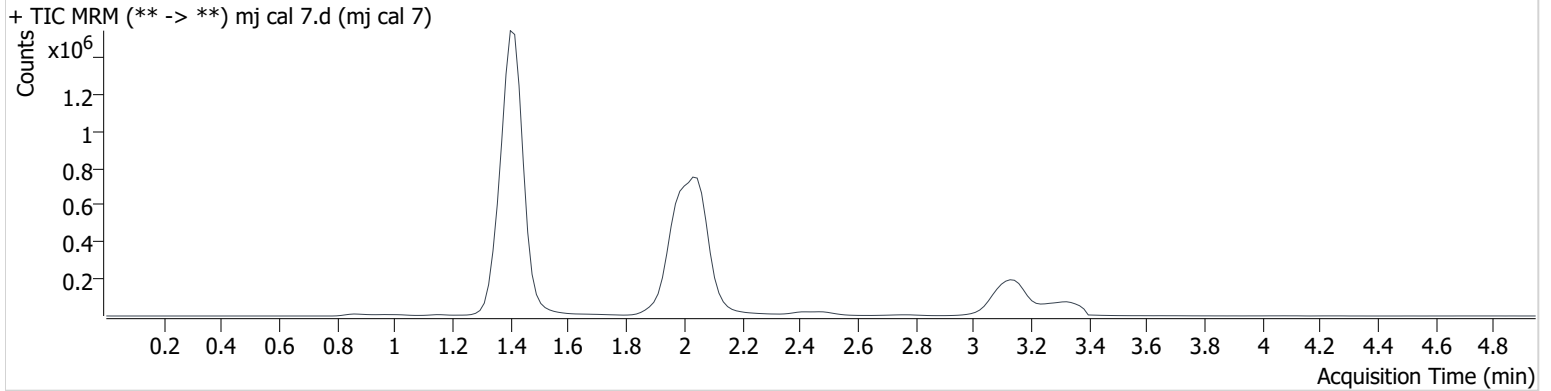
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Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-6-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/6/2020 5:38:32 PM

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	5/6/2020 1:58:44 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	1839135	∞	11.7	∞	940838	99.859 ng/ml
THC-COOH	1.415	1645891	∞	176.7	∞	502808	259.599 ng/ml
THC	3.153	605310	∞	25.0	∞	614997	102.769 ng/ml