









REVIEWED

By Anne Nord at 11:43 am, May 22, 2020

5/21/2020 BW

Worklist: 4252

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2020-0824	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-0830	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-0831	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0862	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0863	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0906	2	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0915	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-0951	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

Extraction Date: 5/21/20Analyst: Britany WyliePlate lot#: 200303Plate 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 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: Urine cases only evaluated for Carboxy-THC

Curves Limited to:

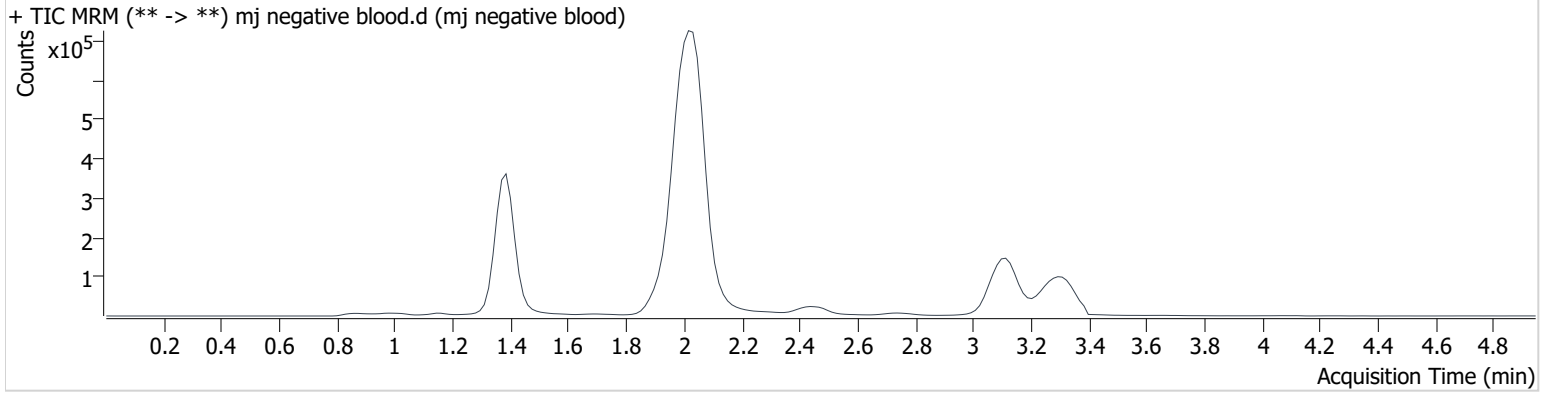
3-100 THC, Hydroxy-THC; 10-250 Carboxy-THC

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 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	5/21/2020 3:25:36 PM		
Sample Info.			

Sample Chromatogram



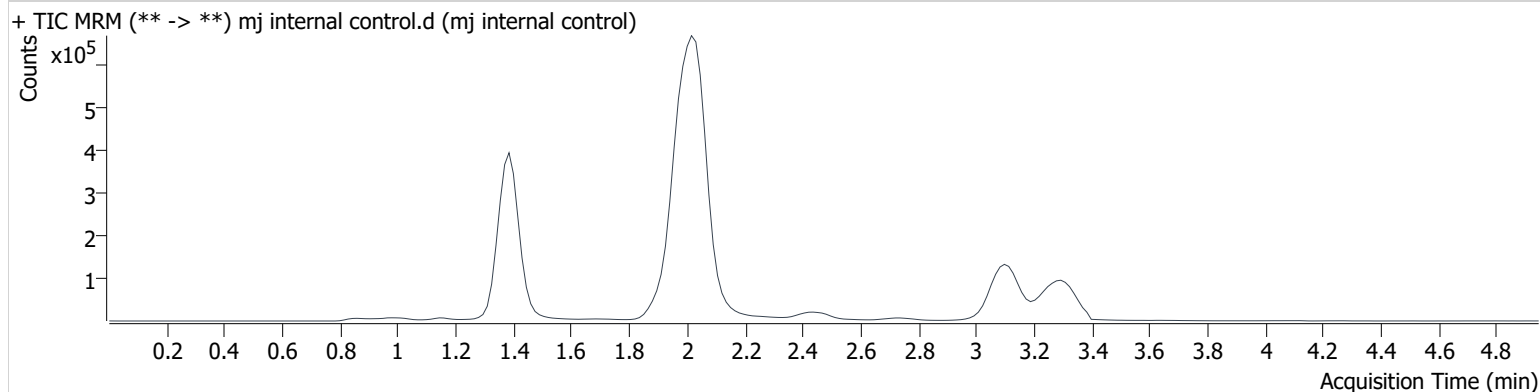
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 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	5/21/2020 3:17:52 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	104149	∞	10.2	∞	1019548	4.415 ng/ml
THC-COOH	1.415	97706	∞	221.1	∞	580288	15.496 ng/ml
THC	3.123	23619	∞	26.1	∞	641043	4.543 ng/ml

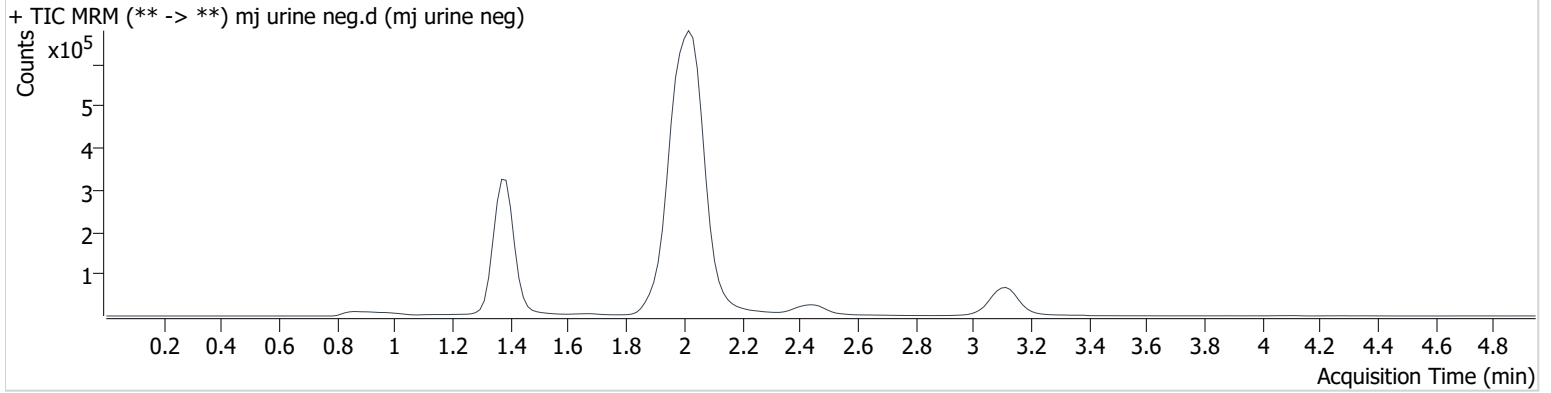
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 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-G2	Comment	
Injection Volume	10		
Acq. Date-Time	5/21/2020 4:57:50 PM		

Sample Info.

Sample Chromatogram

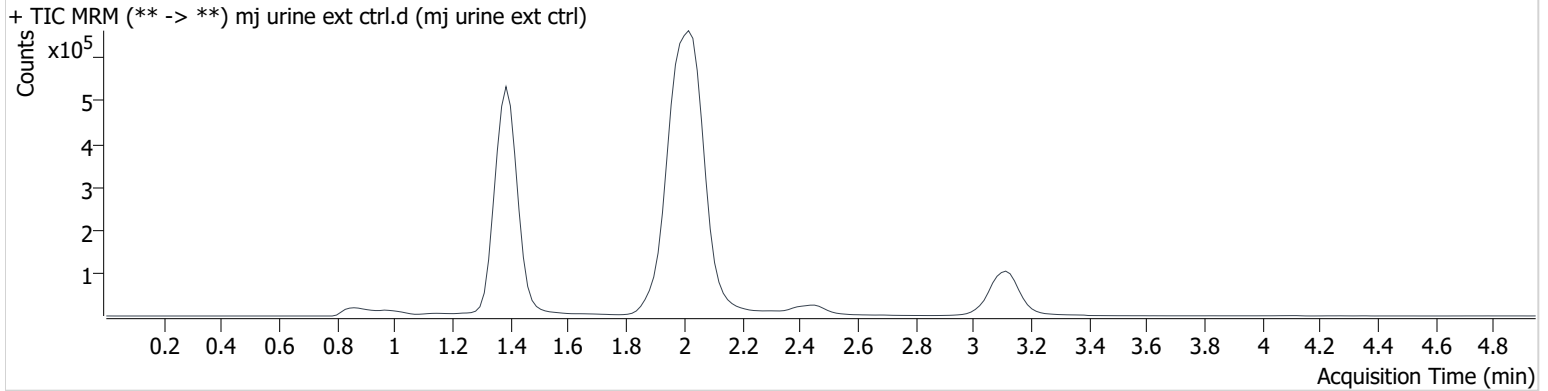


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 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-H2	Comment	
Injection Volume	10		
Acq. Date-Time	5/21/2020 5:05:35 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.375	306901	∞	11.6	∞	1185495	11.850 ng/ml
THC-COOH	1.400	253226	∞	194.2	∞	588930	37.569 ng/ml
THC	3.138	101262	∞	23.8	∞	647088	16.835 ng/ml

BW

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

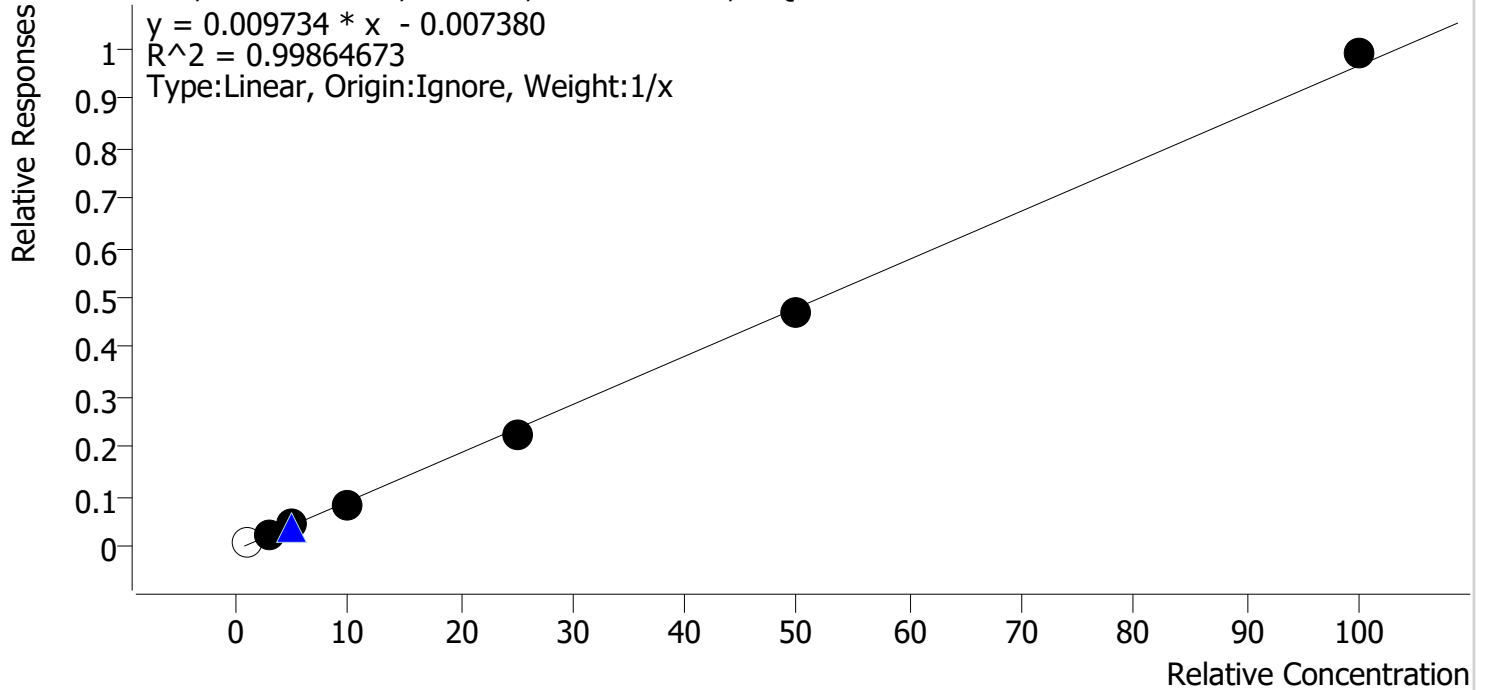
Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Last Cal. Update 5/21/2020 8:05 PM
Analyst Name ISP\datastor
Analyte THC

BW

Internal Standard THC-d3

THC - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	x	1.0	1.7	166.9
mj cal2	2	✓	3.0	3.2	106.5
mj cal 3	3	✓	5.0	5.2	103.9
mj cal 4	4	✓	10.0	9.4	93.7
mj cal 5	5	✓	25.0	24.0	95.8
mj cal 6	6	✓	50.0	48.8	97.7
mj cal 7	7	✓	100.0	102.5	102.5

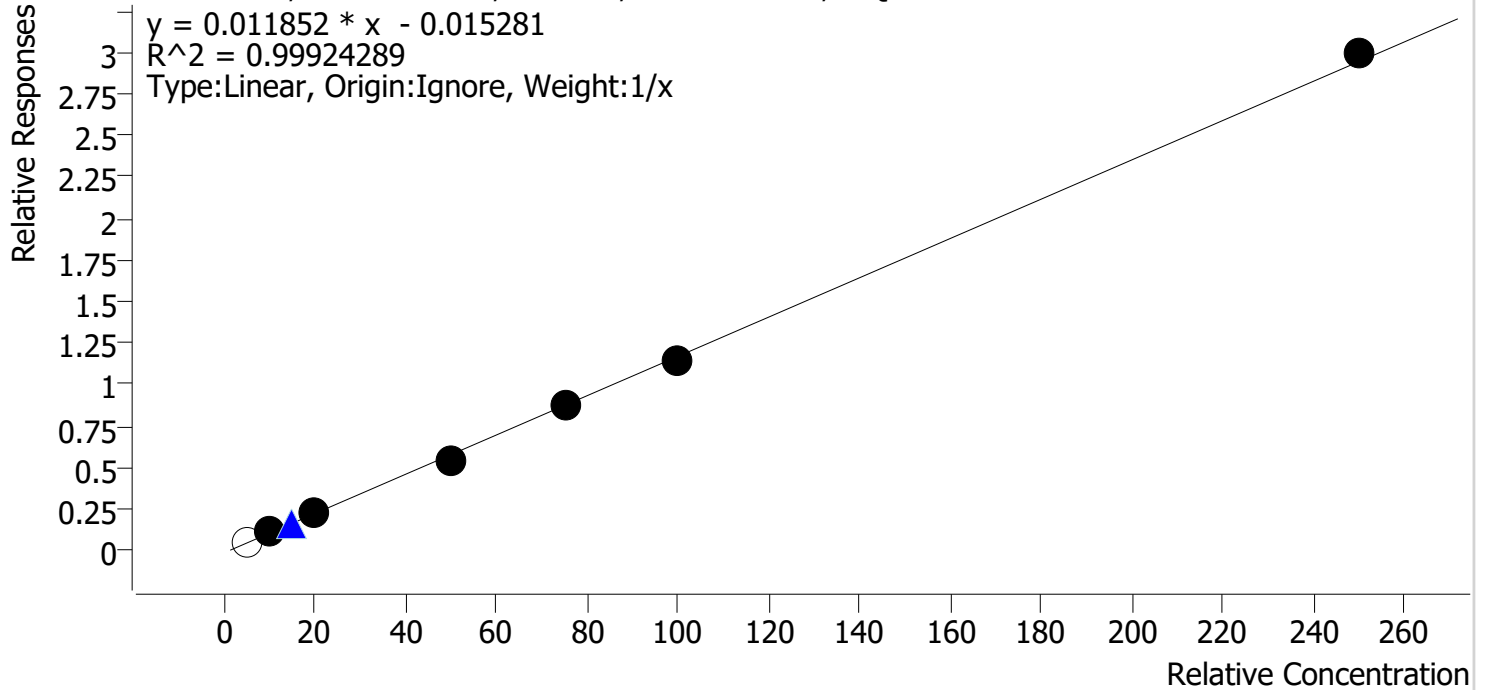
Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Last Cal. Update 5/21/2020 8:05 PM
Analyst Name ISP\datastor
Analyte THC-COOH

BW

Internal Standard THC-COOH-d9

THC-COOH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs



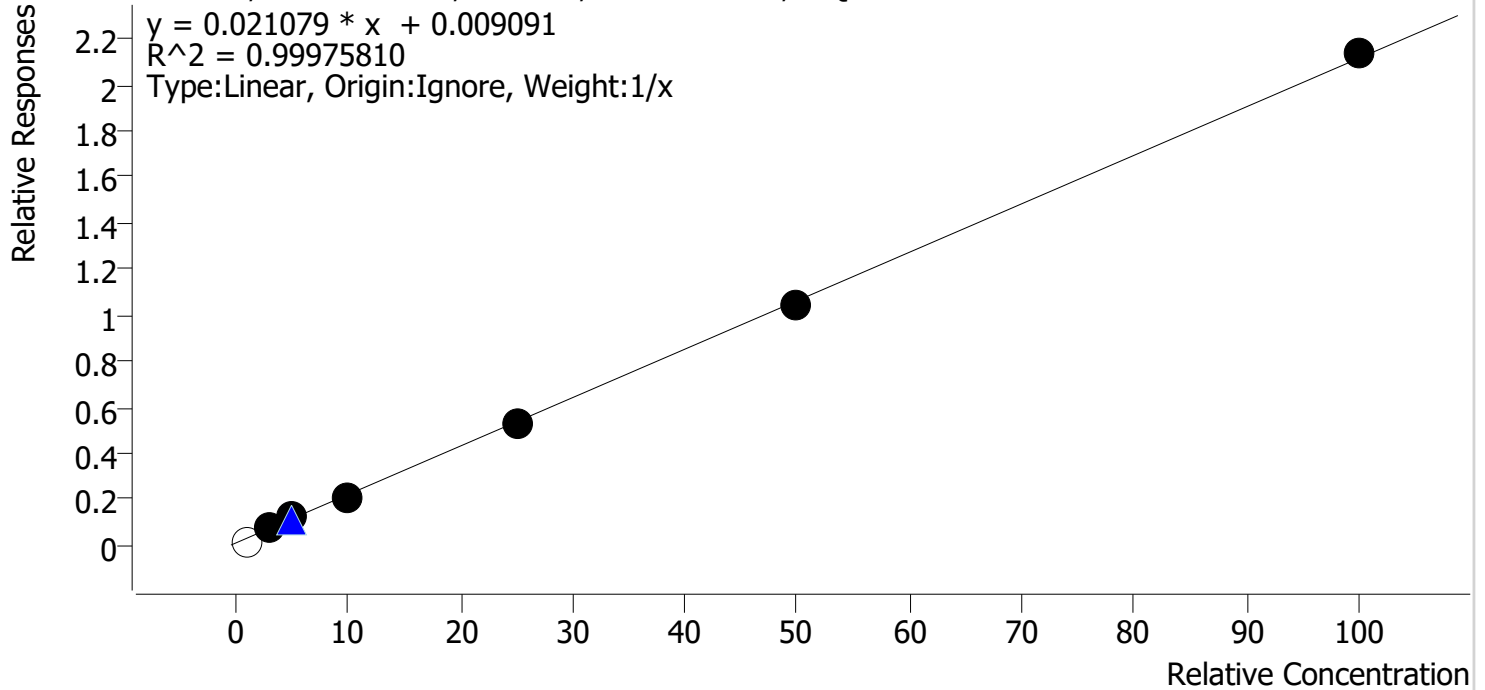
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	x	5.0	5.6	112.3
mj cal2	2	✓	10.0	10.5	104.9
mj cal 3	3	✓	20.0	20.1	100.6
mj cal 4	4	✓	50.0	47.6	95.2
mj cal 5	5	✓	75.0	74.7	99.7
mj cal 6	6	✓	100.0	98.0	98.0
mj cal 7	7	✓	250.0	254.0	101.6

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Last Cal. Update 5/21/2020 8:05 PM
Analyst Name ISP\datastor
Analyte THC-OH **Internal Standard** THC-OH-d3

BW

THC-OH - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 1 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	x	1.0	0.3	26.2
mj cal2	2	✓	3.0	3.1	101.7
mj cal 3	3	✓	5.0	5.1	102.8
mj cal 4	4	✓	10.0	9.7	96.5
mj cal 5	5	✓	25.0	24.8	99.2
mj cal 6	6	✓	50.0	49.4	98.9
mj cal 7	7	✓	100.0	100.9	100.9

AM #27 Cannabinoids

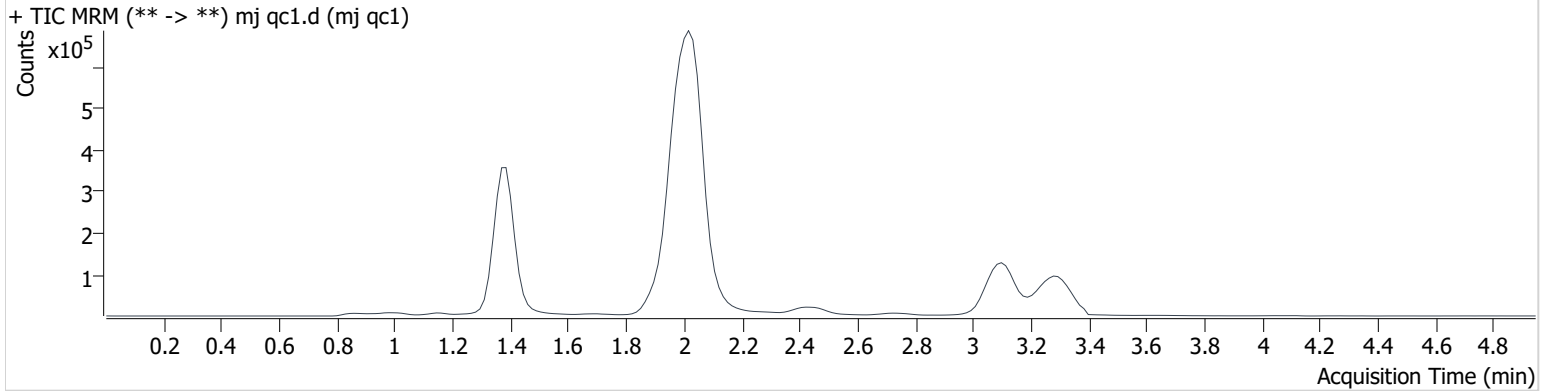
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Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 PM

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	5/21/2020 2:23:46 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.375	15892	∞	17.2 High	∞	1087085	0.262 ng/ml Low
THC-COOH	1.400	31435	∞	278.4 High	∞	612910	5.617 ng/ml Low
THC	3.123	5767	∞	33.2 High	∞	650537	1.669 ng/ml Low

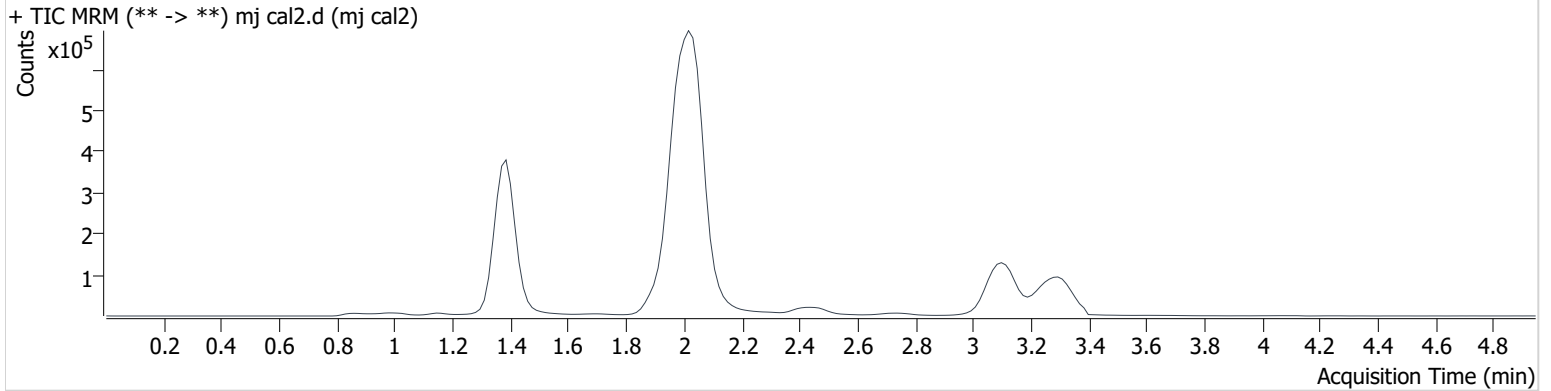
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 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	5/21/2020 2:31:30 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	78483	∞	9.5	∞	1069209	3.051 ng/ml
THC-COOH	1.415	66268	∞	232.5	∞	607439	10.494 ng/ml
THC	3.123	15443	∞	28.9	∞	651148	3.195 ng/ml

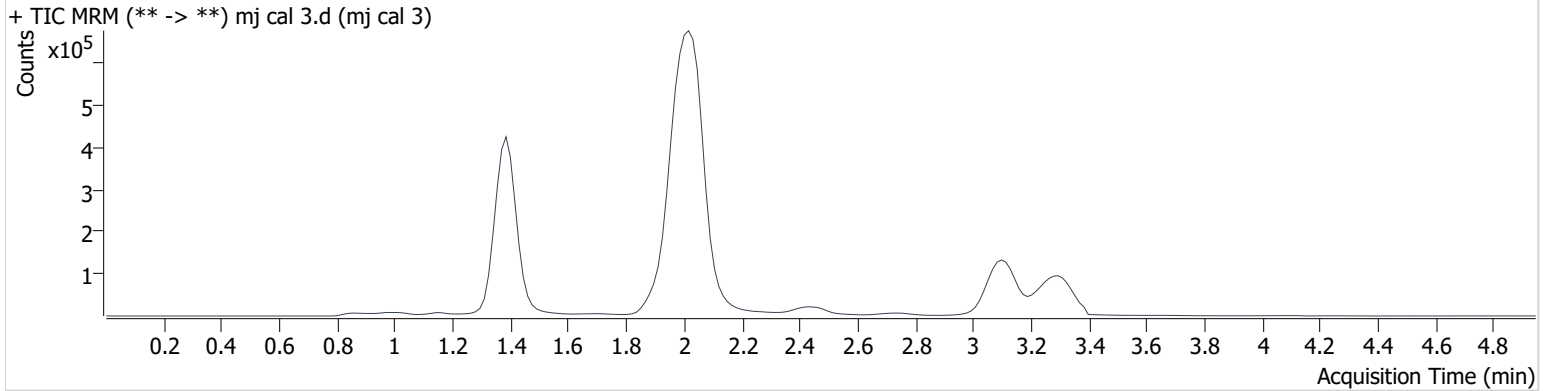
AM #27 Cannabinoids

BW

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 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	5/21/2020 2:39:14 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	124547	∞	10.6	∞	1060105	5.142 ng/ml
THC-COOH	1.400	132889	∞	212.4	∞	595730	20.111 ng/ml
THC	3.123	28075	∞	26.4	∞	650131	5.195 ng/ml

BW

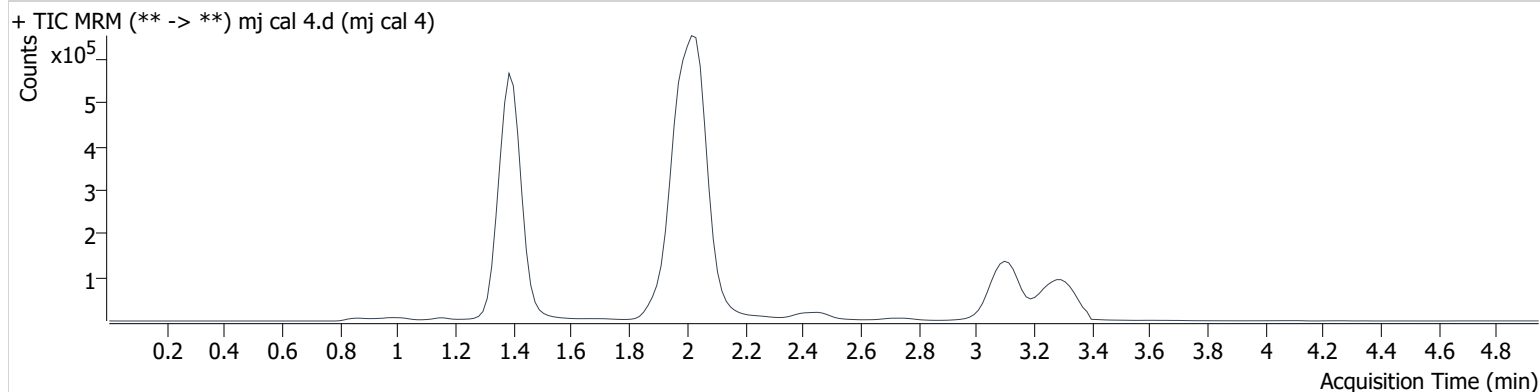
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 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	5/21/2020 2:46:58 PM		

Sample Info.

Sample Chromatogram



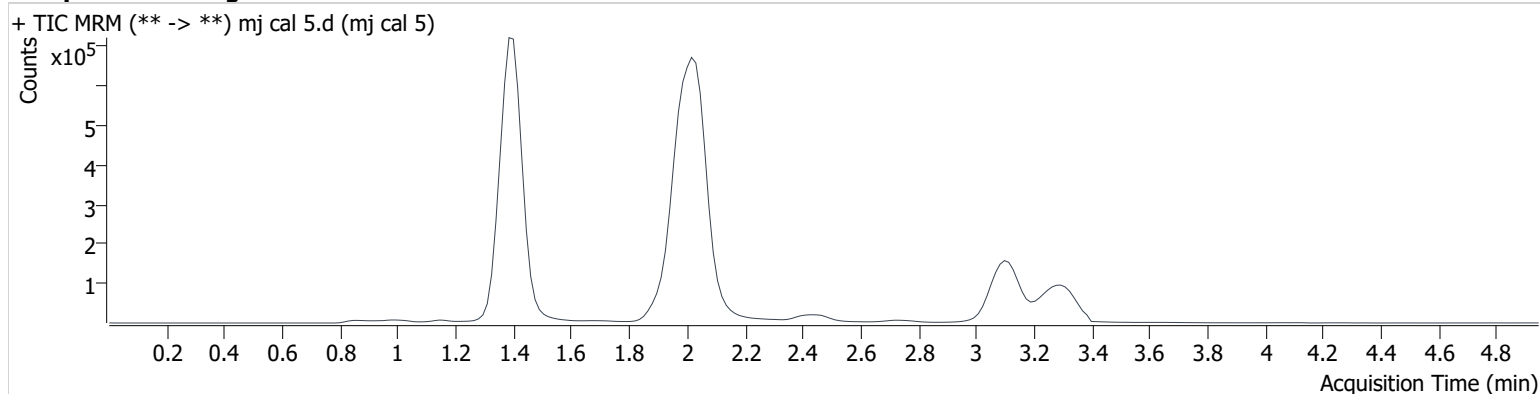
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.375	234960	∞	10.1	∞	1105579	9.651 ng/ml
THC-COOH	1.400	343160	∞	199.0	∞	625241	47.599 ng/ml
THC	3.138	56294	∞	26.5	∞	671522	9.370 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 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	5/21/2020 2:54:42 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.375	561923	∞	11.5	∞	1057035	24.788 ng/ml
THC-COOH	1.415	507344	∞	196.3	∞	582755	74.748 ng/ml
THC	3.123	150703	∞	25.0	∞	667566	23.950 ng/ml

AM #27 Cannabinoids

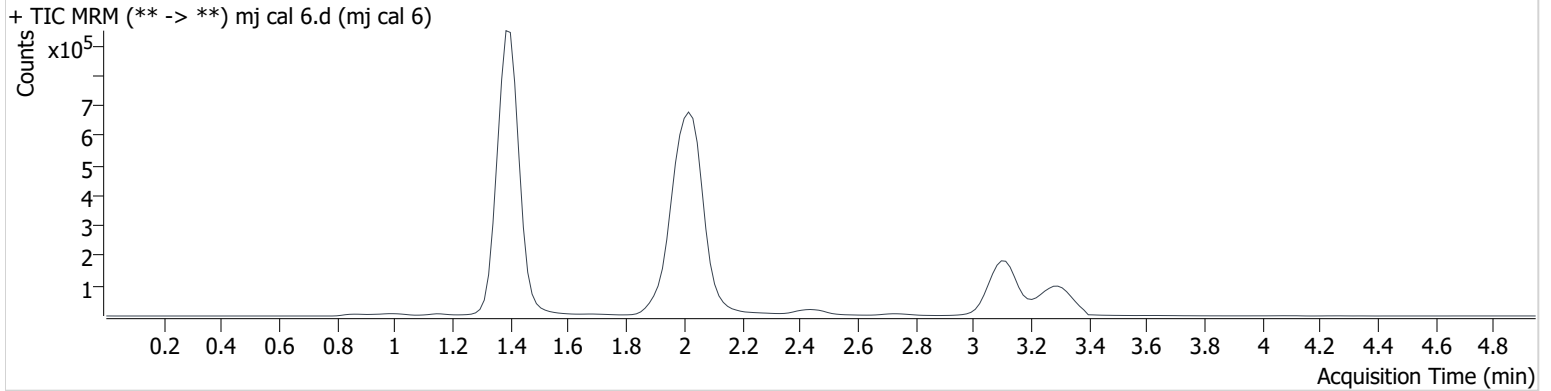
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Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 PM

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	5/21/2020 3:02:24 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	1108529	∞	12.2	∞	1054766	49.427 ng/ml
THC-COOH	1.400	661112	∞	195.5	∞	576606	98.032 ng/ml
THC	3.123	304129	∞	24.8	∞	650000	48.826 ng/ml

AM #27 Cannabinoids

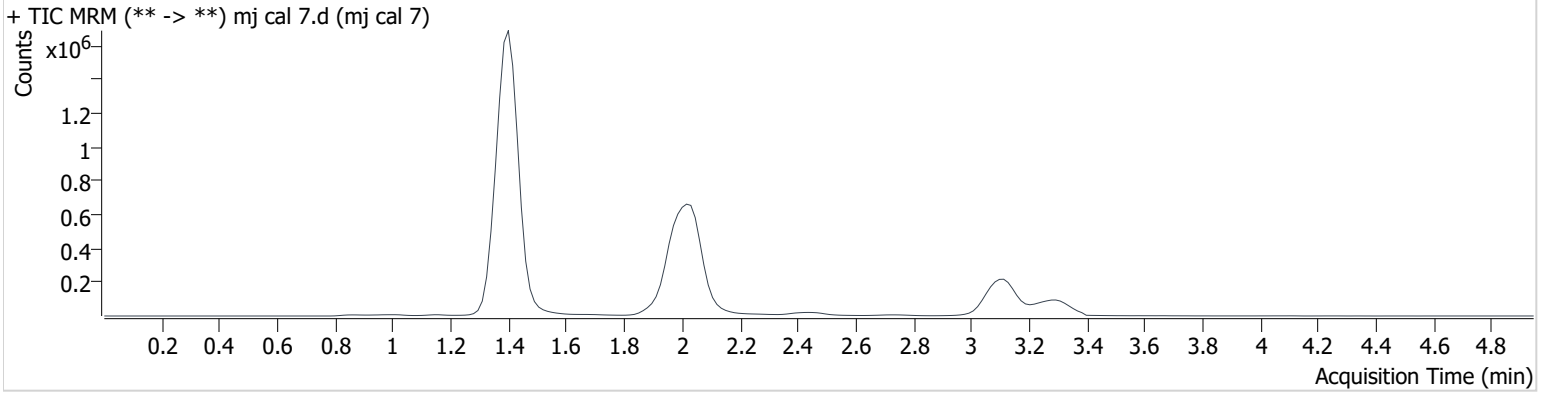
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Batch results D:\MassHunter\Data\2020 Data\am 27-28 5-21-20\QuantResults\thcq.batch.bin
Calibration Last Update 5/21/2020 8:05:41 PM

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	5/21/2020 3:10:08 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.390	2184177	∞	11.9	∞	1022156	100.941 ng/ml
THC-COOH	1.415	1569596	∞	191.3	∞	524036	254.015 ng/ml
THC	3.123	606147	∞	24.9	∞	612273	102.464 ng/ml