









10/12/2021

REVIEWED

By Britany Wylie at 2:06 pm, Oct 12, 2021

Worklist: 5281

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2021-2096		UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-2130		UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-2146		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-2152		UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-2157		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-2188		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-2193	3	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2021-2202		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-2211		BCK	AM 27 Blood THC Quant by LC-QQQ	
C2021-2219		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 10/7/21
Plate lot#: 210609

Analyst: Anne Nord
Plate Expiration: 12-9-21

Mobile phase A: 0.1% Formic Acid in LCMS Water MTBE
Mobile phase B: 0.1% Formic acid in Acetonitrile Hexane
LCMS Methanol

Blank Blood Lot: 21D52496 **Urine Blank:** 83121 **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:

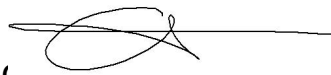
- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Urine hydrolysis: add 1.5 ml urine to blank plate, add 250 ul 1N KOH mix and incubate at 40 degrees for 15 minutes.
Pipette 1000µL blood (calibrated pipette) Pipette ID: k52558g in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 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 1ng/ml, OH-THC 3ng/mL (quantitative blood), Carboxy-THC: 5 ng/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:

Toxicology AM method 27/26 external prep informati



working solution 15 ug/ml in meoh C-THC, THC-OH, 7.5 ug/ml THC

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 150 ul THC-OH in 9692.5 ul meOH

Ppd 8/26/21 Exp: 8/26/22 lot 82621 by AMN

Drug	lot	expiration
C-THC	FE04151901	6/1/2024
THC-OH	FE06152002	6/1/2025
THC	FE04222001	5/1/2025

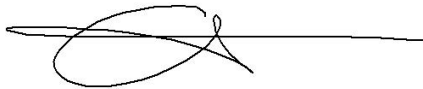
AM 27/26 blood control 100 ul working solution lot () in 9900 ul blood lot ()

		Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	
--	--	--	--

AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine

out of use

ppd 8/26/21 Exp 8/26/22 neg urine lot 5621	lot u82621	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	



	1	2	3	4	5	6
a	cal 1	neg blood	2130-1			
b	cal 2	2146-1	2152-1			
c	cal 3	2157-1	2193-3			
d	cal 4	2188-1	2219-1			
e	Cal 5	2202-1	urine external positive control			
f	cal 6	2211-1				
g	cal 7	urine negative control				
h	Internal control	2096-1				

C2021-____-__

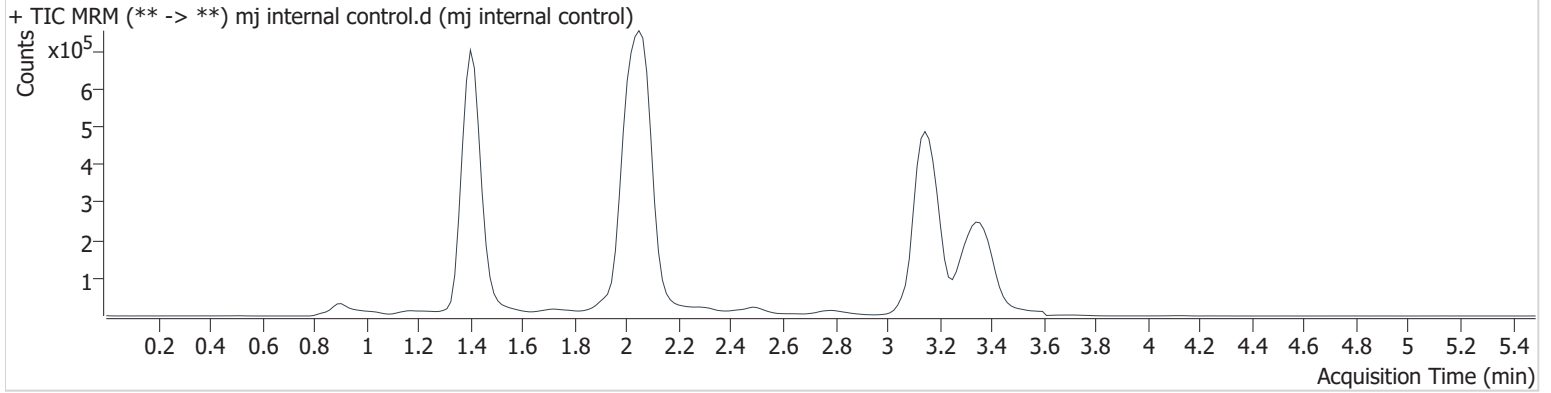
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj internal control.d
Type	QC	Sample	mj internal control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-H1	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 2:39:32 PM		

Sample Info.

Sample Chromatogram



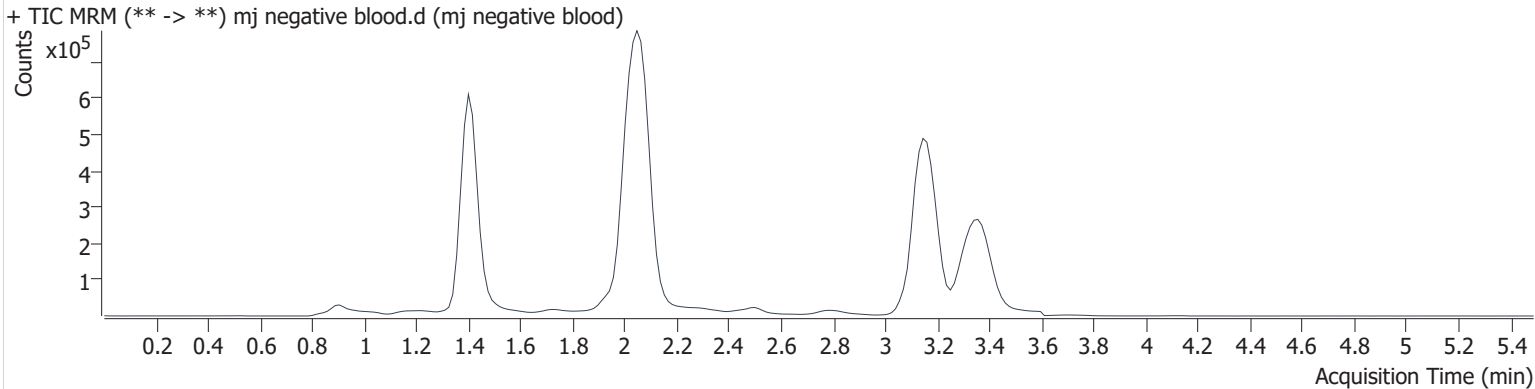
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	35973	∞	922.8	∞	2115903	5.022 ng/ml
THC-COOH	1.431	138992	641.7	33.7	189.5	711684	15.252 ng/ml
THC	3.167	256140	∞	24.8	451.2	2245708	4.577 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj negative blood.d
Type	Sample	Sample	mj negative blood
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 2:46:14 PM		
Sample Info.			

Sample Chromatogram

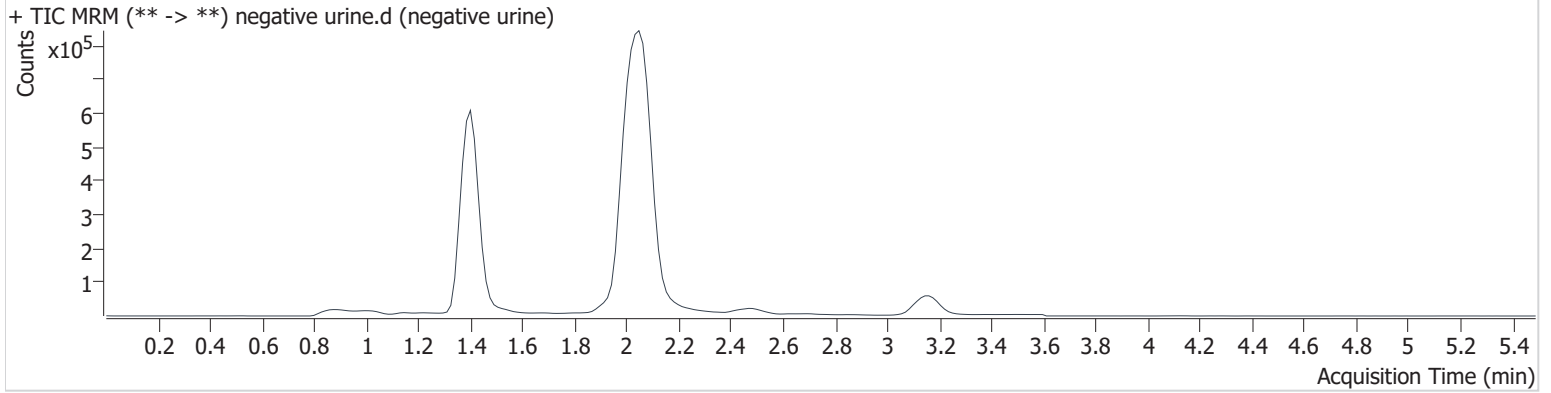


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	negative urine.d
Type	Sample	Sample	negative urine
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-G2	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 4:06:14 PM		
Sample Info.			

Sample Chromatogram

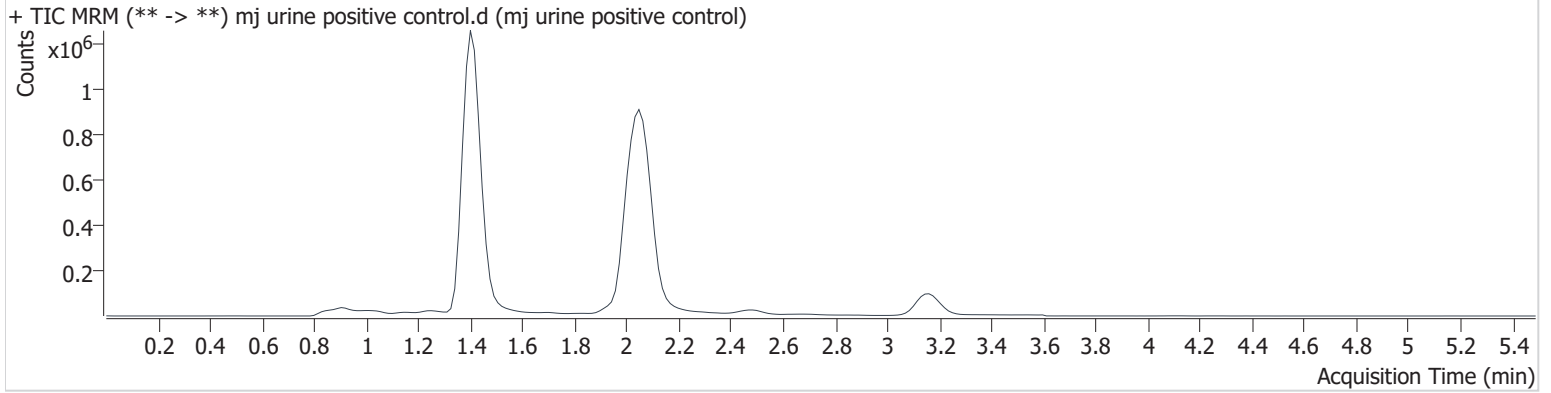


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj urine positive control.d
Type	Sample	Sample	mj urine positive control
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-E3	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 5:32:57 PM		
Sample Info.			

Sample Chromatogram

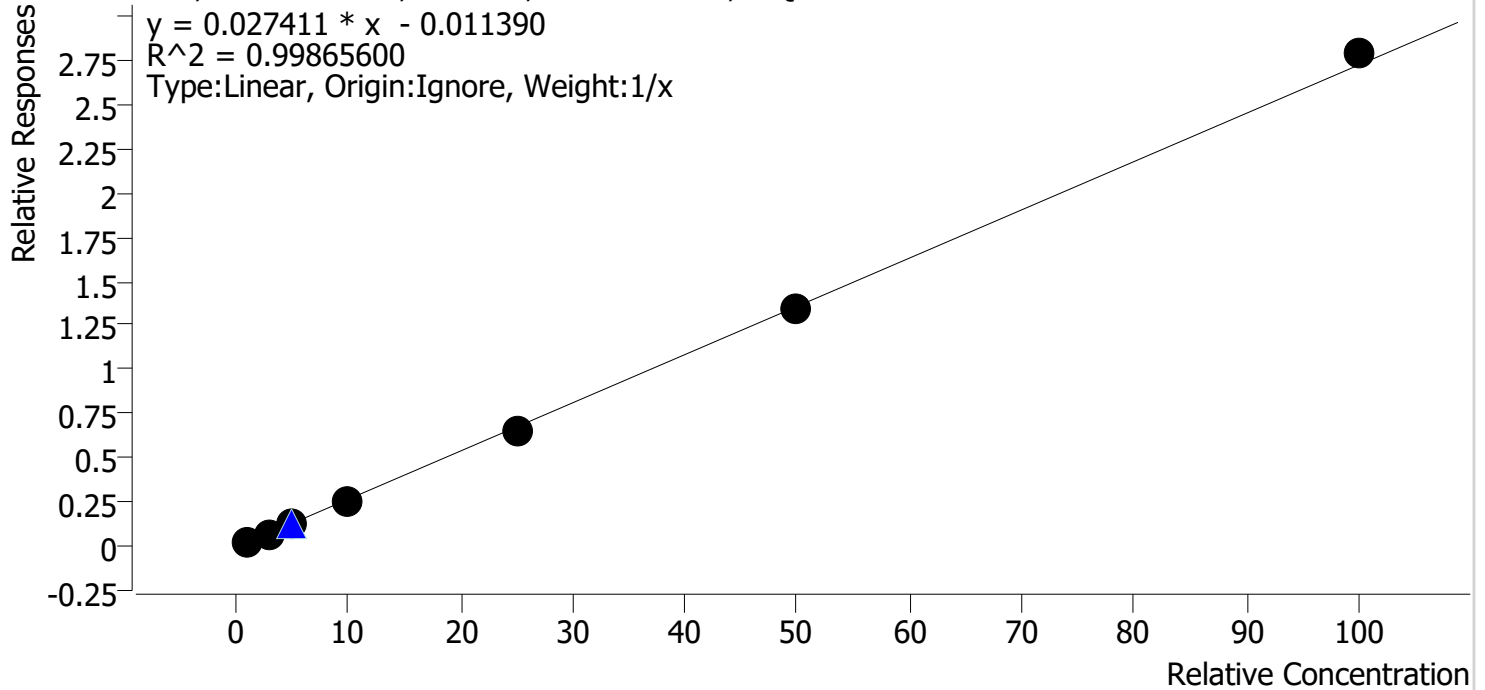


Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	258758	3585.7	864.7	∞	2347598	32.787 ng/ml
THC-COOH	1.431	317134	193.3	36.4	1519.8	519419	46.919 ng/ml
THC	3.167	58285	∞	25.0	134.2	555111	4.246 ng/ml

Compound Calibration Report

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Last Cal. Update 10/8/2021 7:59 AM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

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



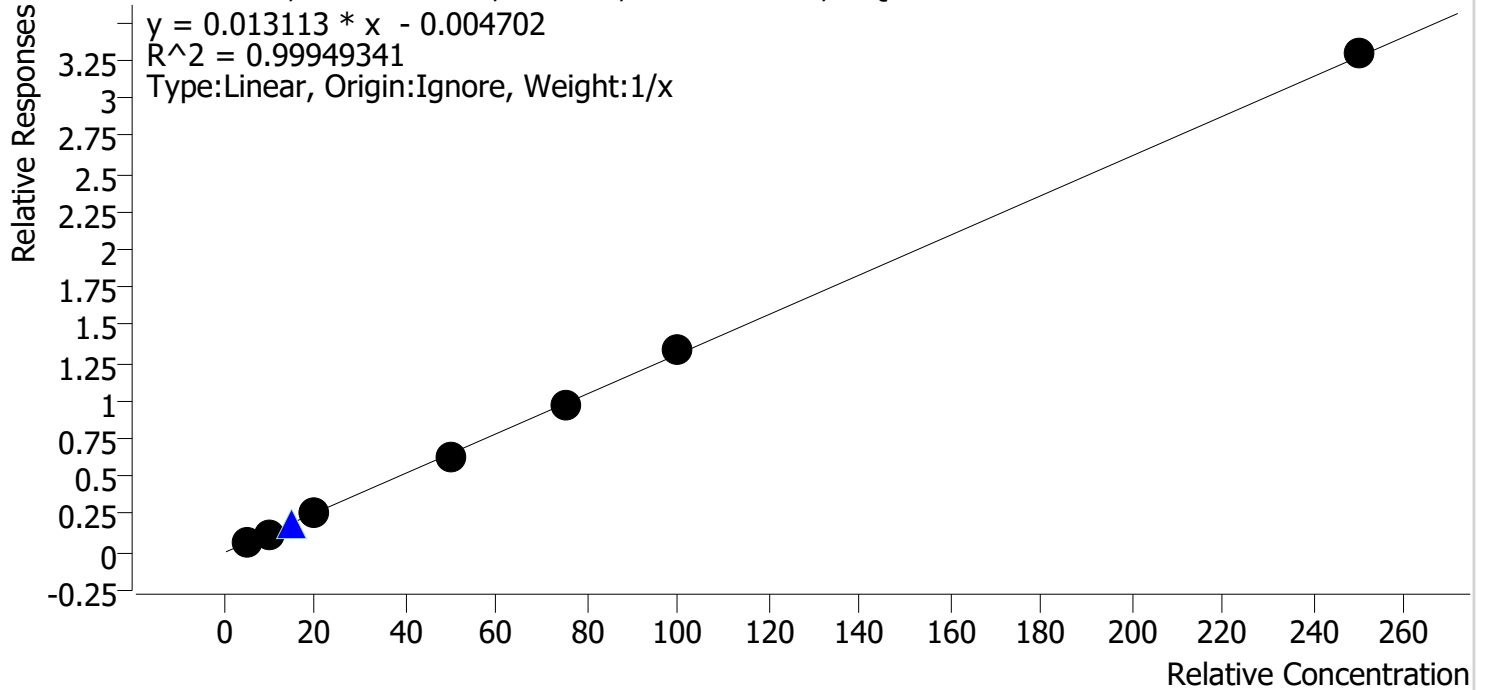
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	1.0	1.2	121.8
mj cal2	2	✓	3.0	2.9	95.2
mj cal 3	3	✓	5.0	4.6	91.8
mj cal 4	4	✓	10.0	9.3	93.3
mj cal 5	5	✓	25.0	24.1	96.5
mj cal 6	6	✓	50.0	49.6	99.1
mj cal 7	7	✓	100.0	102.3	102.3

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Last Cal. Update 10/8/2021 7:59 AM
Analyst Name ISP\datastor
Analyte THC-COOH **Internal Standard** THC-COOH-d9

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



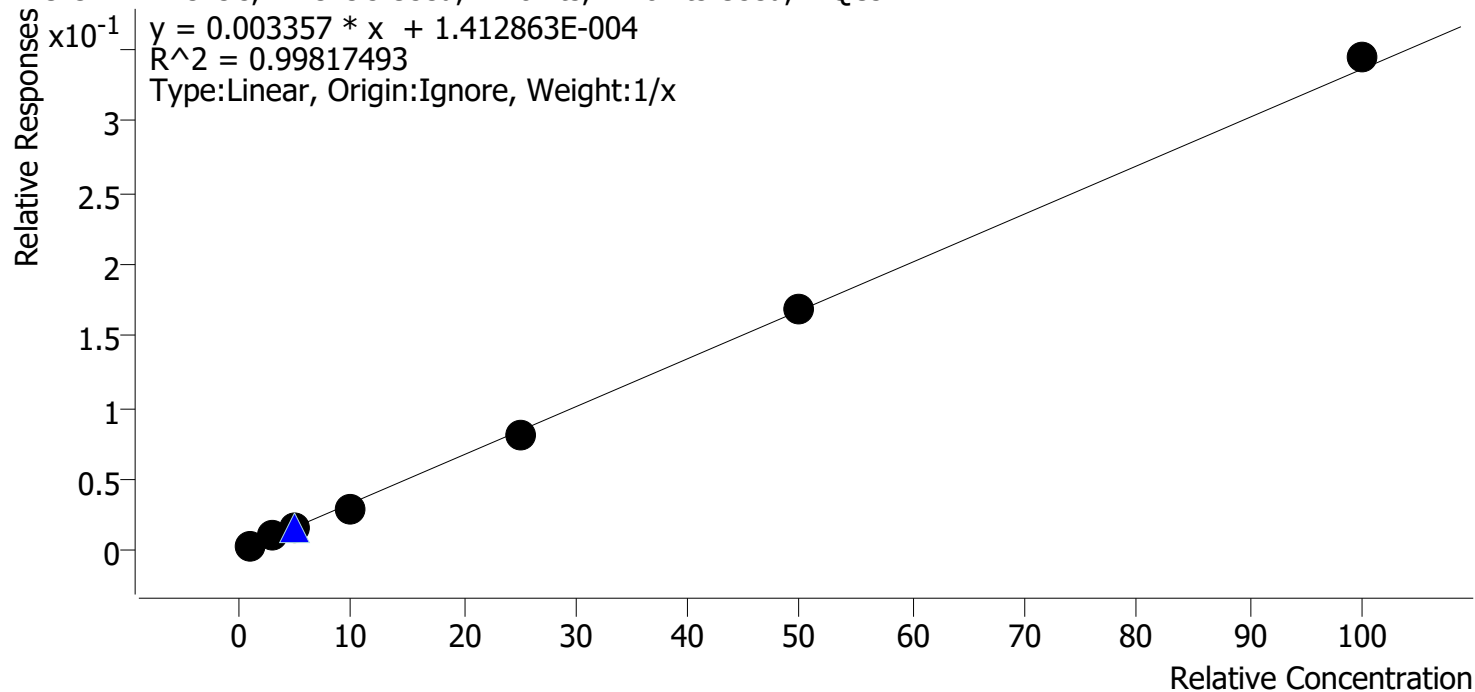
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj cal 1	1	✓	5.0	5.5	109.4
mj cal2	2	✓	10.0	9.6	96.1
mj cal 3	3	✓	20.0	19.4	97.2
mj cal 4	4	✓	50.0	47.9	95.8
mj cal 5	5	✓	75.0	74.6	99.5
mj cal 6	6	✓	100.0	101.4	101.4
mj cal 7	7	✓	250.0	251.5	100.6

Compound Calibration Report



Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Last Cal. Update 10/8/2021 7:59 AM
Analyst Name ISP\datastor
Analyte THC-OH **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 cal 1	1	✓	1.0	1.2	118.6
mj cal2	2	✓	3.0	3.0	100.9
mj cal 3	3	✓	5.0	4.7	94.5
mj cal 4	4	✓	10.0	8.8	88.2
mj cal 5	5	✓	25.0	23.8	95.2
mj cal 6	6	✓	50.0	50.0	100.1
mj cal 7	7	✓	100.0	102.4	102.4

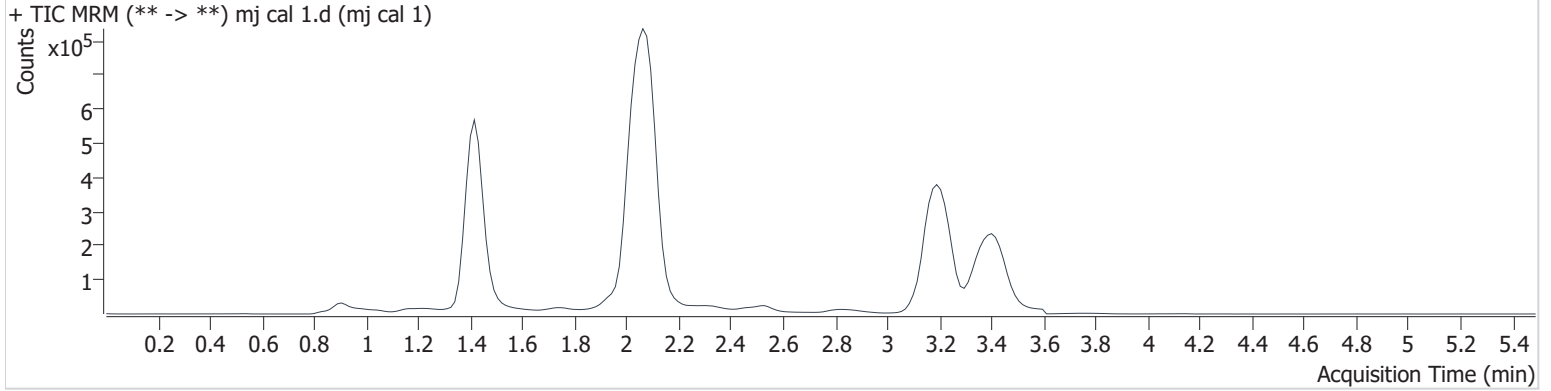
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj cal 1.d
Type	Cal	Sample	mj cal 1
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-A1	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 1:52:37 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.409	8061	∞	909.4	∞	1954555	1.186 ng/ml	Low
THC-COOH	1.446	39094	72.5	31.6	19.7	583203	5.470 ng/ml	
THC	3.212	41446	710.1	30.2	180.3	1883703	1.218 ng/ml	

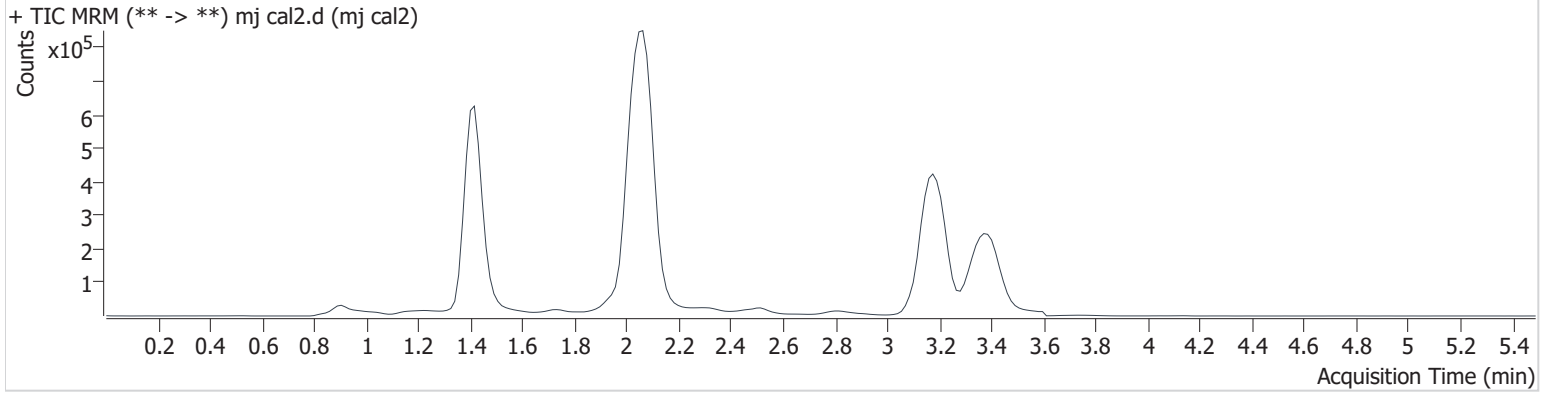
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj cal2.d
Type	Cal	Sample	mj cal2
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-B1	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 1:59:21 PM		

Sample Info.

Sample Chromatogram



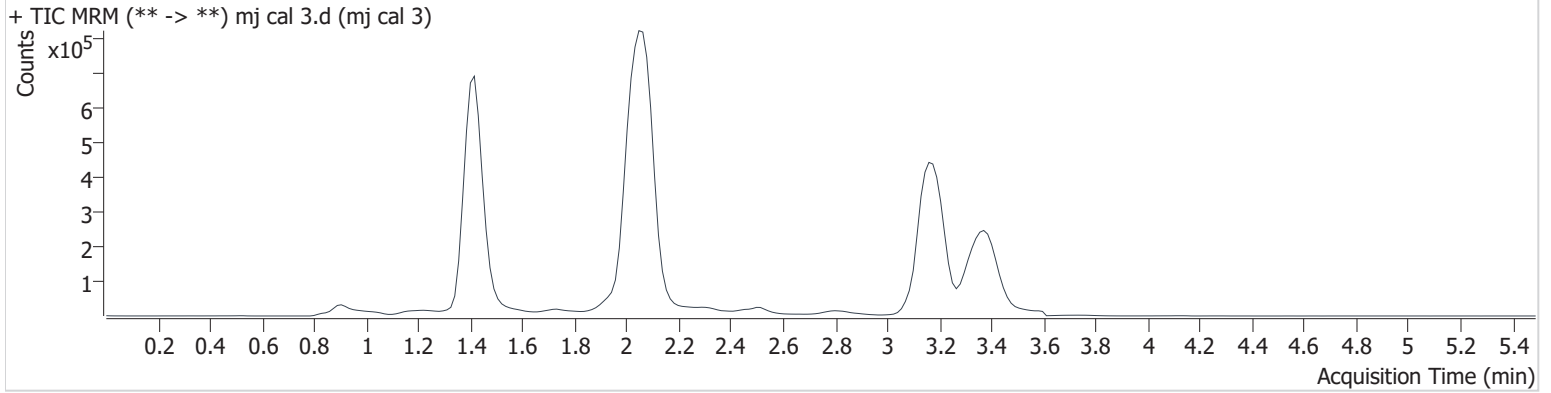
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	20315	∞	964.0	∞	1971655	3.027 ng/ml
THC-COOH	1.446	74677	94.7	36.0	3002.6	615800	9.606 ng/ml
THC	3.197	131990	∞	25.8	1953.1	1973315	2.856 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj cal 3.d
Type	Cal	Sample	mj cal 3
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-C1	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 2:06:03 PM		
Sample Info.			

Sample Chromatogram



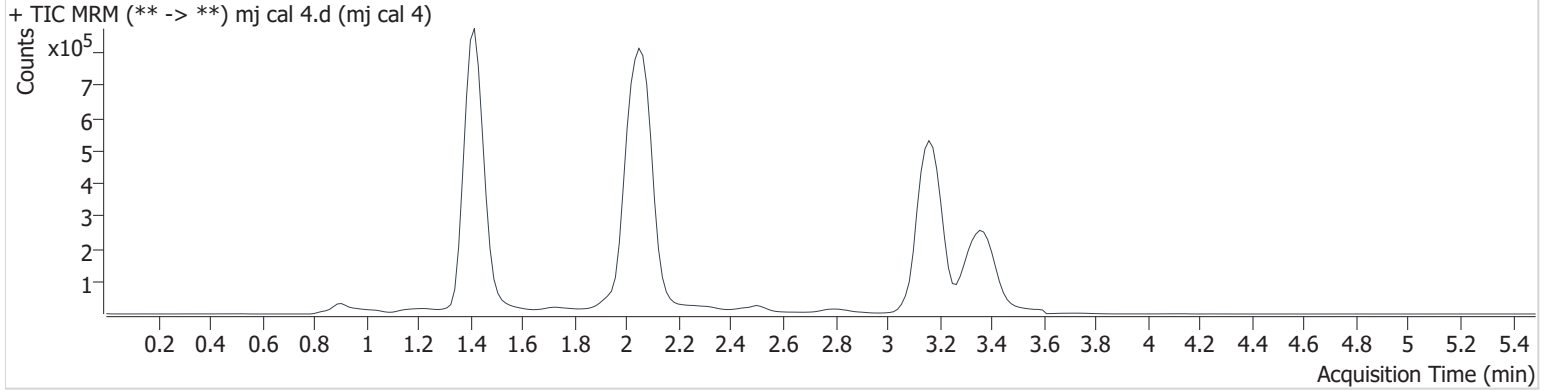
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	32522	1129193604	938.8	∞	2031112	4.727 ng/ml
THC-COOH	1.446	161906	269.8	35.7	129.1	647357	19.431 ng/ml
THC	3.197	232543	4267.3	24.7	541.0	2032919	4.589 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj cal 4.d
Type	Cal	Sample	mj cal 4
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-D1	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 2:12:45 PM		
Sample Info.			

Sample Chromatogram



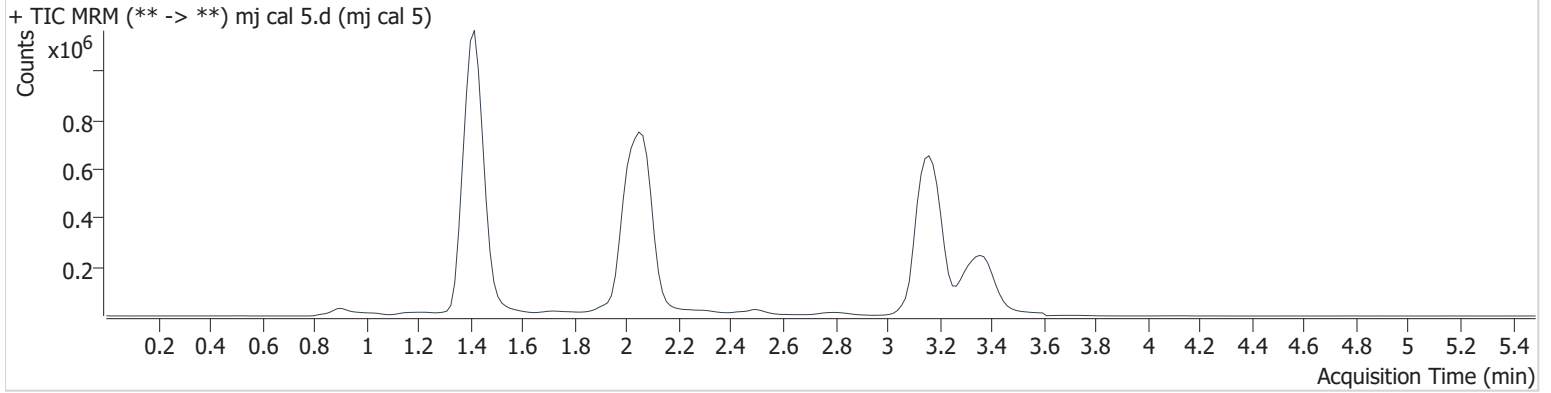
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	61037	1270611998	984.7	∞	2050656	8.823 ng/ml
THC-COOH	1.431	405663	1860.4	37.7	325.7	650762	47.896 ng/ml
THC	3.182	524837	27462.6	24.6	∞	2148387	9.328 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj cal 5.d
Type	Cal	Sample	mj cal 5
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-E1	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 2:19:27 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	165424	∞	962.6	∞	2065976	23.807 ng/ml
THC-COOH	1.431	640189	946.8	37.6	1319.3	657148	74.650 ng/ml
THC	3.182	1342331	∞	24.1	19858.4	2065703	24.122 ng/ml

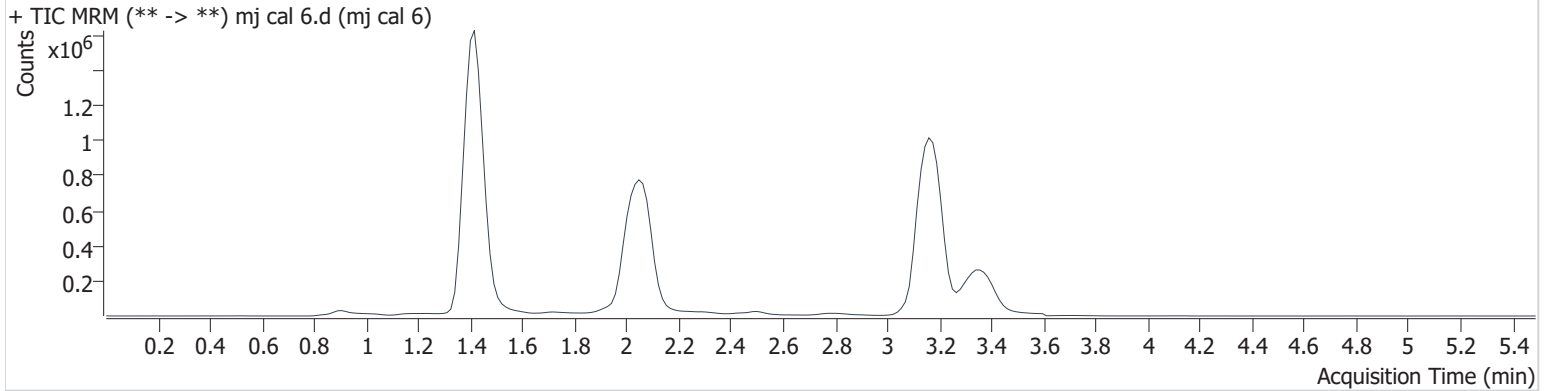
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj cal 6.d
Type	Cal	Sample	mj cal 6
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-F1	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 2:26:09 PM		

Sample Info.

Sample Chromatogram



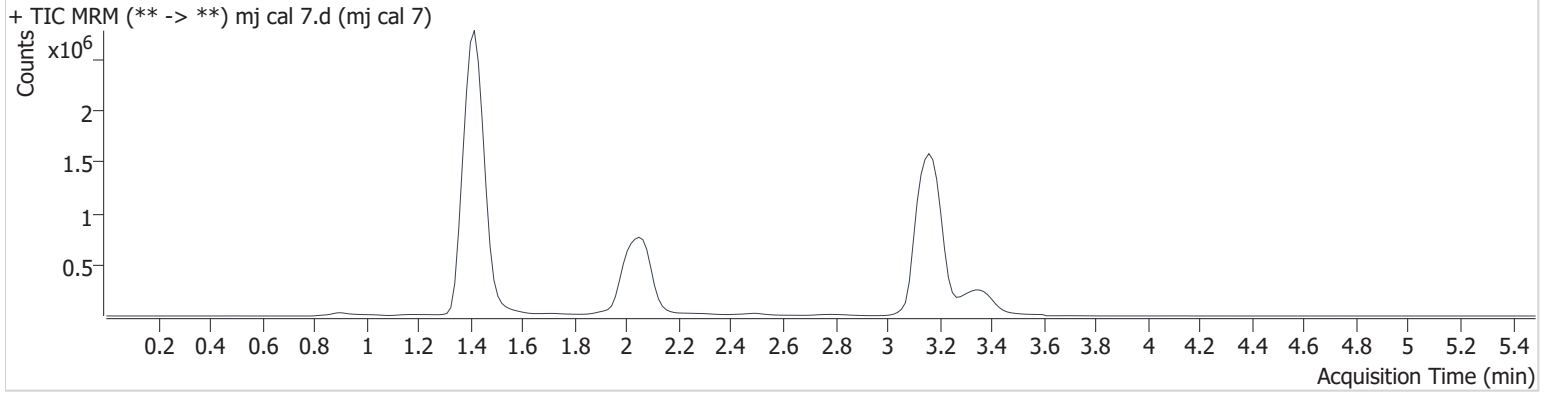
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	344592	∞	895.8	∞	2049059	50.047 ng/ml
THC-COOH	1.431	852347	2828.3	37.3	34289 3.2	643015	101.444 ng/ml
THC	3.182	2927038	∞	25.1	8790.9	2172756	49.562 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2021\am 27-28\100721\QuantResults\cann.batch.bin
Calibration Last Update 10/8/2021 7:59:21 AM

Instrument	69679	Data File	mj cal 7.d
Type	Cal	Sample	mj cal 7
Acq. Method	AM 27 THC quant.m	Operator	Anne Nord
Sample Position	P3-G1	Comment	
Injection Volume	10		
Acq. Date-Time	10/7/2021 2:32:51 PM		
Sample Info.			

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.409	712102	∞	860.7	∞	2070766	102.383 ng/ml
THC-COOH	1.431	2064985	4124.2	38.0	2282.2	627026	251.503 ng/ml
THC	3.167	6054621	∞	25.1	∞	2167419	102.326 ng/ml