

REVIEWED

By Anne Nord at 11:31 am, Dec 03, 2020

11/30/2020 BW

Worklist: 4635

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2020-2248	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-2278	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-2279	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-2281	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-2302	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2319	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2320	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2367	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-2368	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3350	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: 11/30/20

Analyst: Britany Wylie

Plate lot#: 200723

Plate Expiration: 1-23-2021

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

Mobile phase B: 0.1% Formic acid in Acetonitrile
Hexane

Blank Blood Lot: 20G20792 **Urine Blank:** 10120 **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

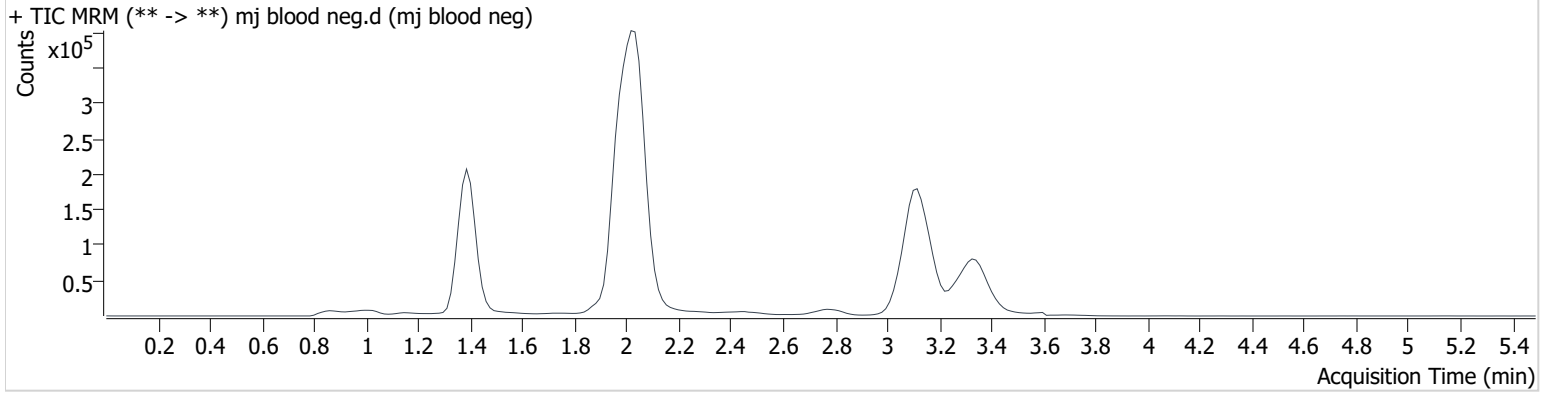
COMMENTS: Hydroxy-THC not evaluated in Urine samples, THC curve limitations: 3-100

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 PM

Instrument	69679	Data File	mj blood neg.d
Type	Sample	Sample	mj blood neg
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A2	Comment	
Injection Volume	10		
Acq. Date-Time	11/30/2020 5:36:33 PM		
Sample Info.			

Sample Chromatogram



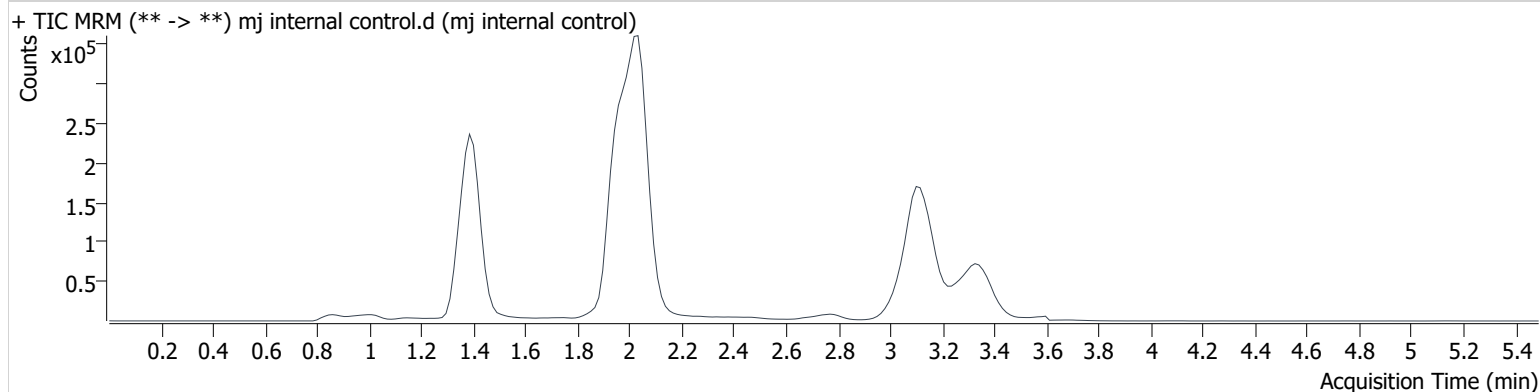
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 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	11/30/2020 5:29:50 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	93795	∞	13.4	∞	706363	4.132 ng/ml
THC-COOH	1.416	64255	43.1	36.1	20809.3	311510	15.152 ng/ml
THC	3.152	44814	∞	29.1	∞	411417	4.682 ng/ml

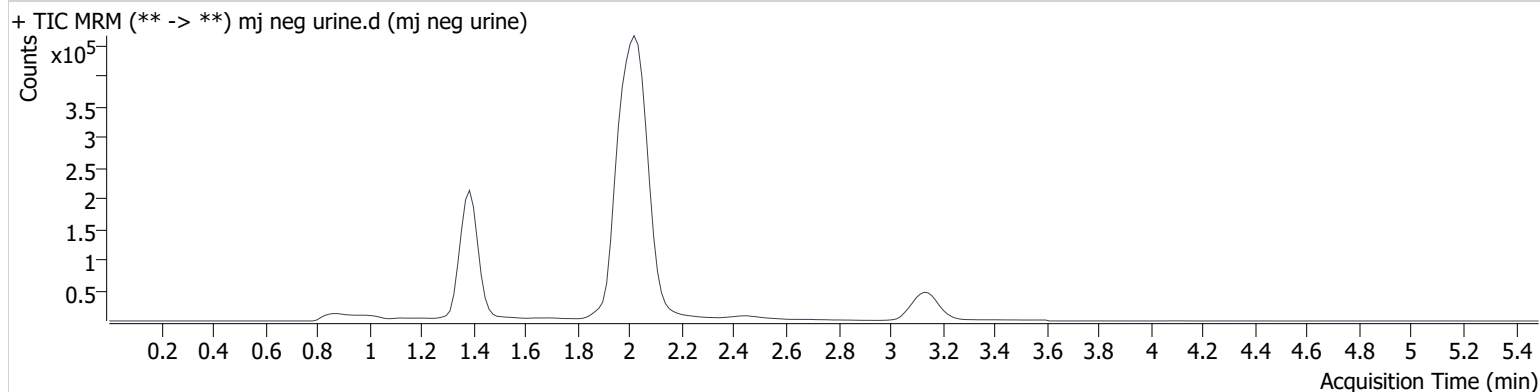
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 PM

Instrument	69679	Data File	mj neg urine.d
Type	Sample	Sample	mj neg urine
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-A3	Comment	
Injection Volume	10		
Acq. Date-Time	11/30/2020 7:23:28 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.466	15530	∞			756539	0.753 ng/ml Low

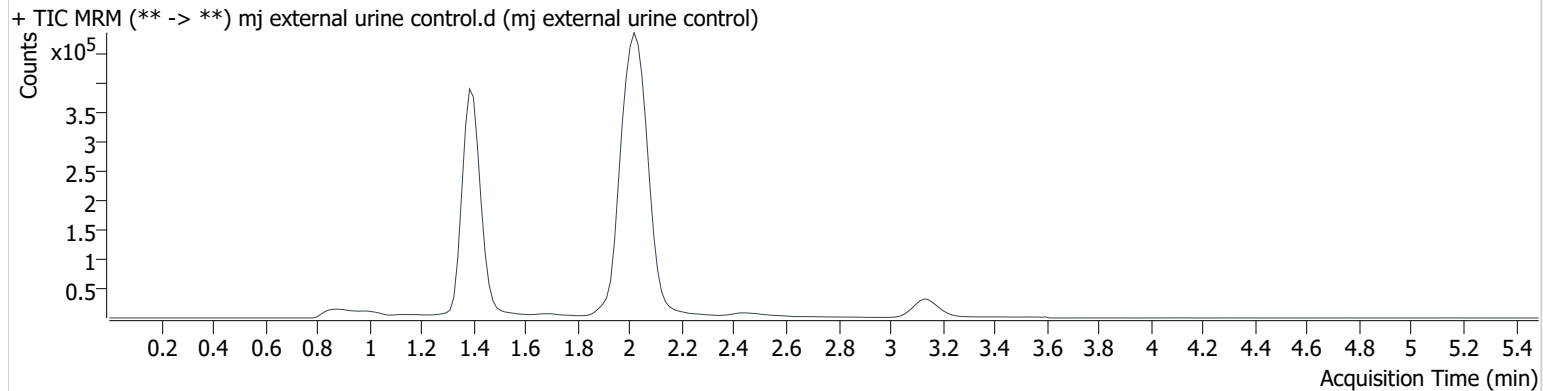
BW

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 PM

Instrument 69679 **Data File** mj external urine control.d
Type Sample **Sample** mj external urine control
Acq. Method AM 27 THC quant.m **Operator** Britany Wylie
Sample Position P3-B3 **Comment**
Injection Volume 10
Acq. Date-Time 11/30/2020 7:36:52 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.	
THC-OH	1.391	561365	∞	16.3 High	∞	689179	24.655 ng/ml	NE
THC-COOH	1.416	113148	219819.5	32.8	853.5	269064	29.376 ng/ml	
THC	3.167	22323	∞	25.3	2467532 7897717 .7	187161	5.073 ng/ml	

* NE: Not Evaluated - Hydroxy-thc not evaluated in urine
BW

Toxicology AM method 27/26 external prep information

BW

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 8/26/20 Exp: 7/1/21 lot 82620 by AMN

Drug	lot	expiration
C-THC	FE01061702	3/1/2022
THC-OH	FE07221601	7/1/2021
THC	FE01041701	3/1/2022

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

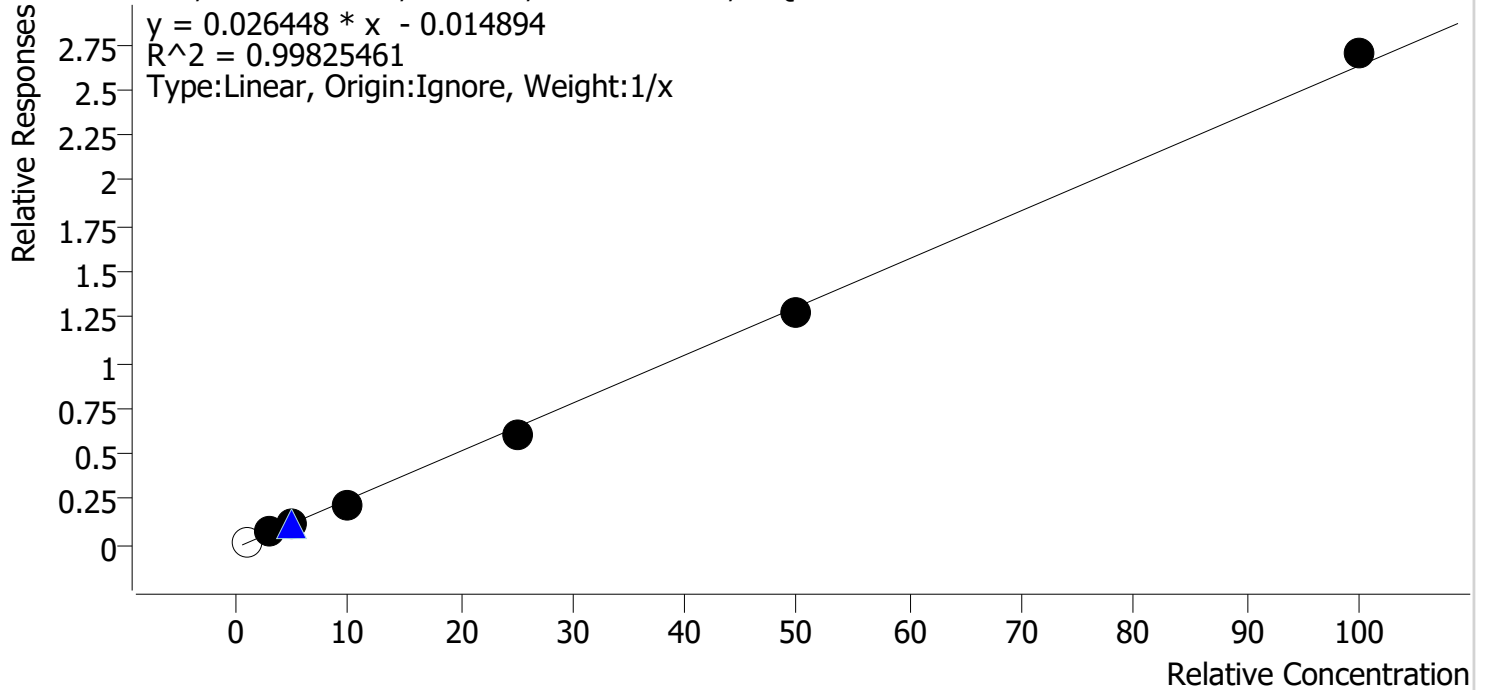
out of use

ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Last Cal. Update 12/2/2020 12:10 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

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

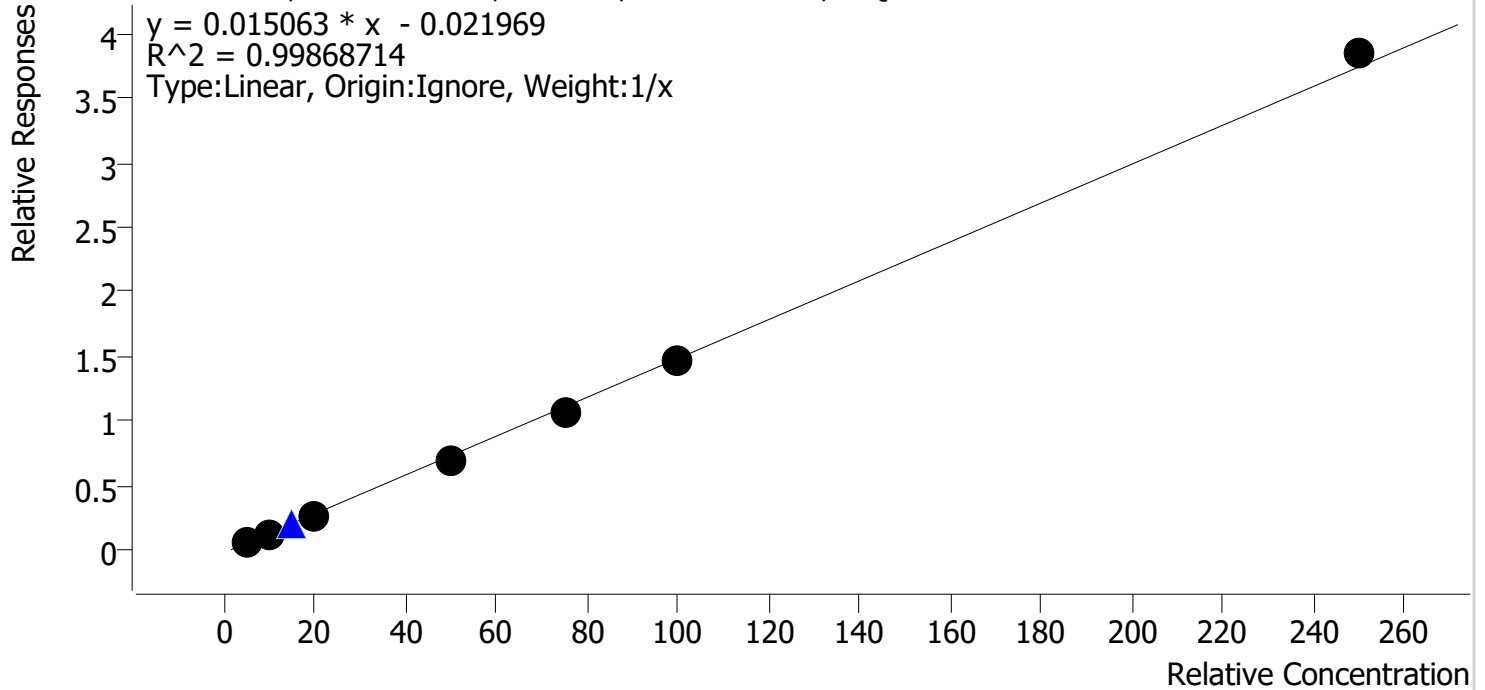


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	x	1.0	1.2	124.2
mj cal2	2	✓	3.0	3.3	108.9
mj cal 3	3	✓	5.0	5.2	103.9
mj cal 4	4	✓	10.0	9.2	91.5
mj cal 5	5	✓	25.0	23.7	94.9
mj cal 6	6	✓	50.0	49.1	98.2
mj cal 7	7	✓	100.0	102.5	102.5

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Last Cal. Update 12/2/2020 12:10 PM
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

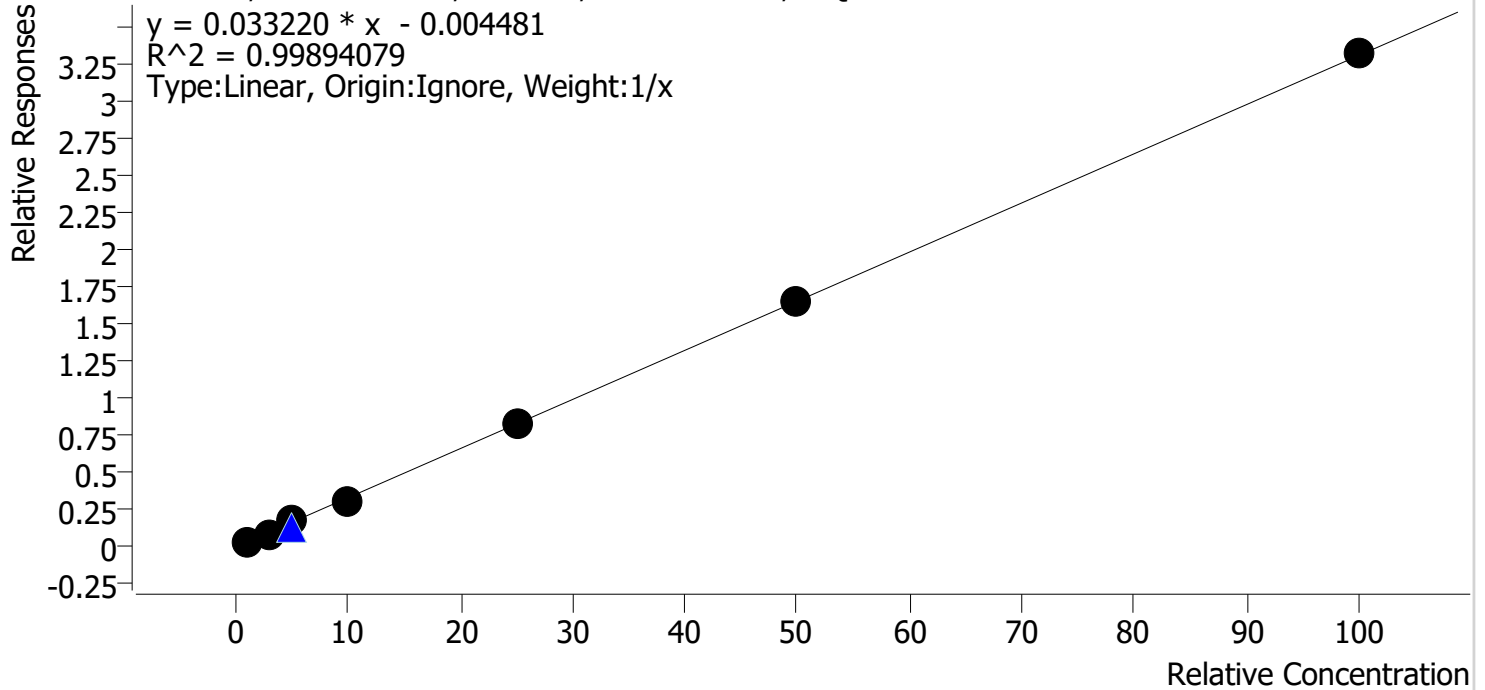


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	5.0	5.6	111.9
mj cal2	2	✓	10.0	9.9	98.7
mj cal 3	3	✓	20.0	19.3	96.5
mj cal 4	4	✓	50.0	47.7	95.3
mj cal 5	5	✓	75.0	72.5	96.7
mj cal 6	6	✓	100.0	98.3	98.3
mj cal 7	7	✓	250.0	256.8	102.7

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Last Cal. Update 12/2/2020 12:10 PM
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



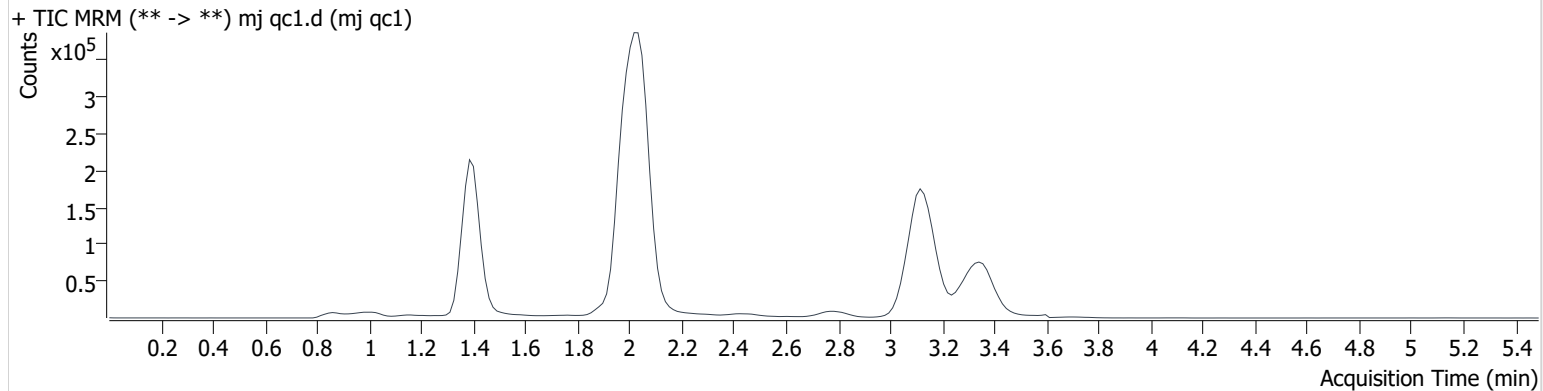
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	1.0	103.8
mj cal2	2	✓	3.0	2.7	91.2
mj cal 3	3	✓	5.0	5.6	112.9
mj cal 4	4	✓	10.0	9.2	91.9
mj cal 5	5	✓	25.0	24.9	99.7
mj cal 6	6	✓	50.0	50.1	100.3
mj cal 7	7	✓	100.0	100.3	100.3

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 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 11/30/2020 4:42:42 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	20217	∞	13.8	∞	673898	1.038 ng/ml Low
THC-COOH	1.416	18397	217.9	40.8	116.1	295251	5.595 ng/ml
THC	3.167	7264	27.2	34.9 High	12.8	404698	1.242 ng/ml Low

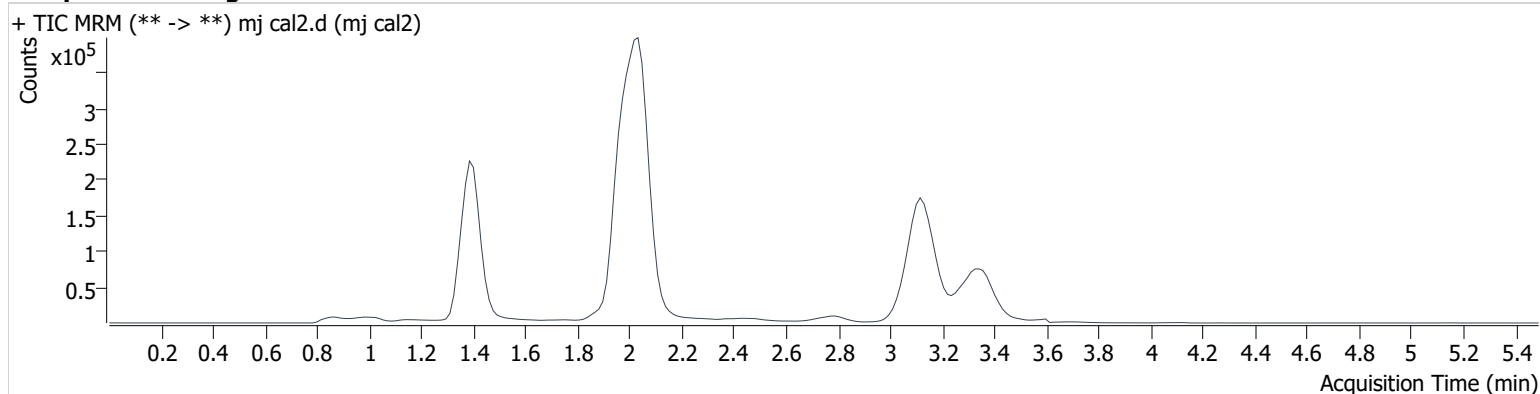
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 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	11/30/2020 4:49:27 PM		

Sample Info.

Sample Chromatogram



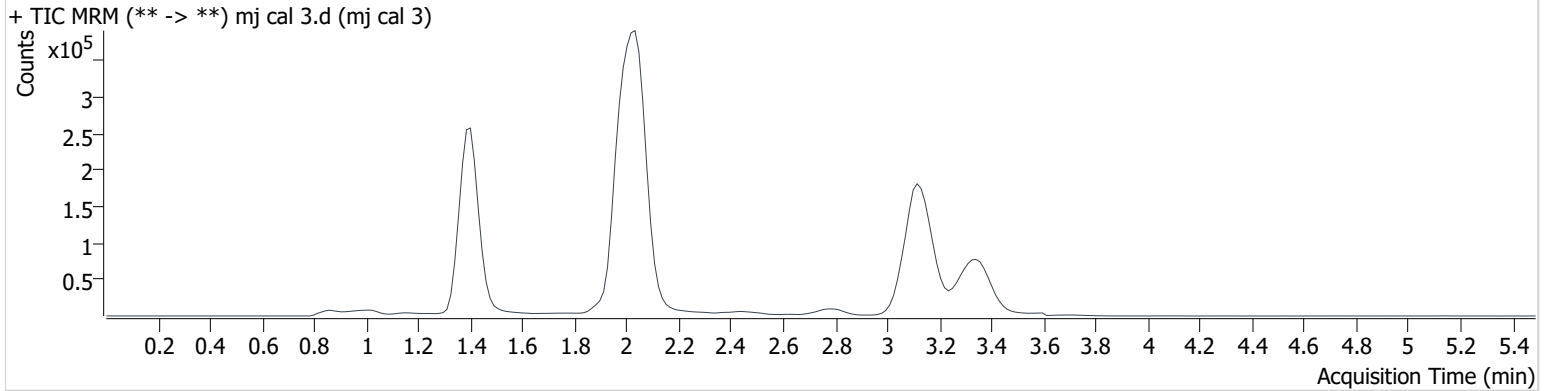
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	60026	∞	12.9	∞	694795	2.736 ng/ml Low
THC-COOH	1.416	37209	62.5	36.5	80.7	293819	9.865 ng/ml
THC	3.167	28607	∞	27.1	∞	400063	3.267 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 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 11/30/2020 4:56:11 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	125304	∞	10.3	∞	684815	5.643 ng/ml
THC-COOH	1.416	80741	286.9	36.5	130.1	300476	19.297 ng/ml
THC	3.167	50113	∞	25.9	1085279 0103145 .4	409131	5.194 ng/ml

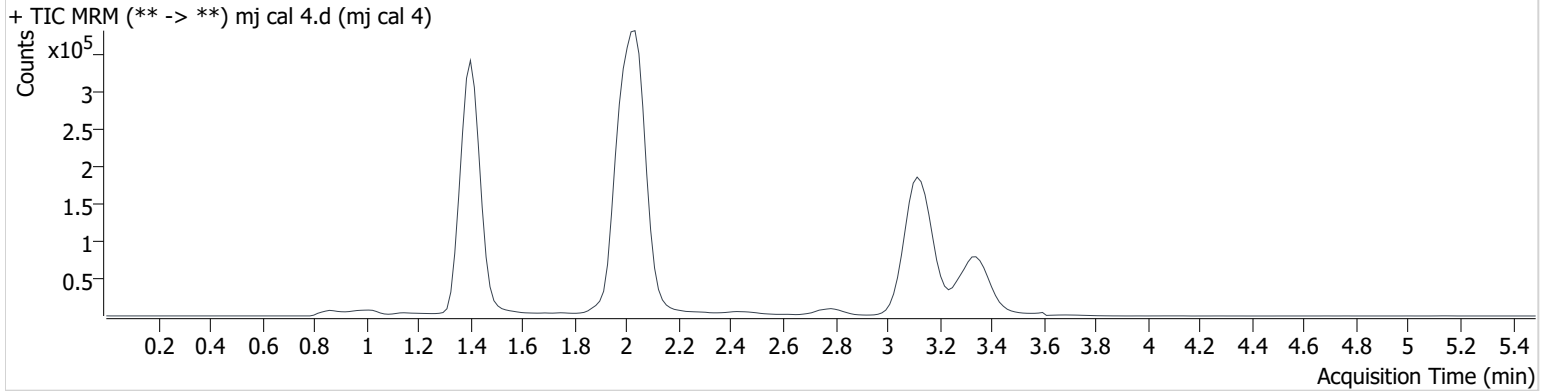
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 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	11/30/2020 5:02:55 PM		

Sample Info.

Sample Chromatogram



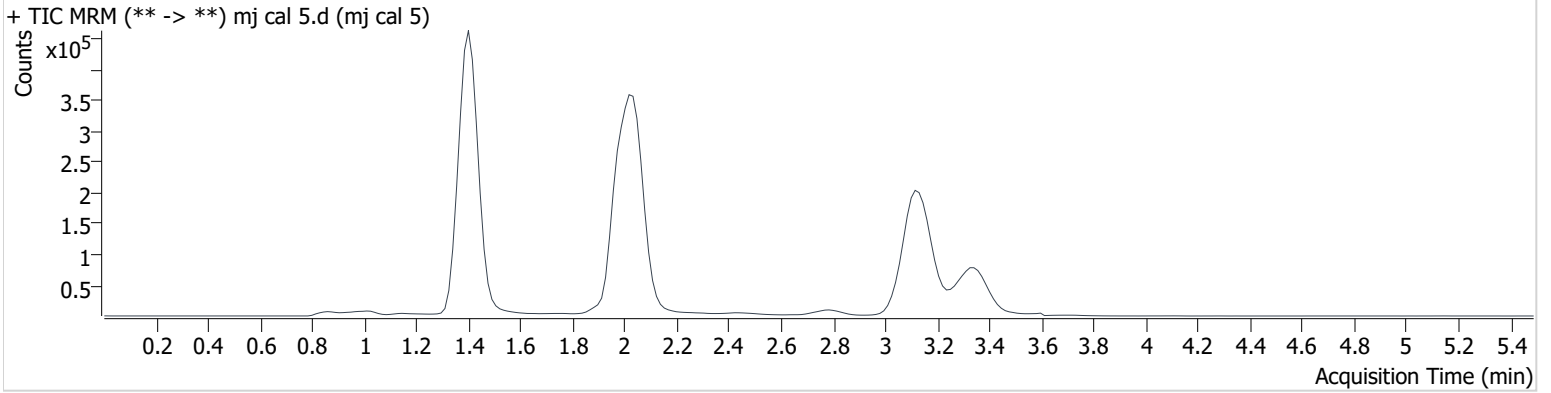
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	200611	∞	12.0	∞	667087	9.188 ng/ml
THC-COOH	1.416	208051	224.1	35.6	736.0	298986	47.654 ng/ml
THC	3.152	92136	∞	26.2	∞	405520	9.154 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 PM

Instrument 69679 **Data File** mj cal 5.d
Type Cal **Sample** mj cal 5
Acq. Method AM 27 THC quant.m **Operator** Brittany Wylie
Sample Position P3-E1 **Comment**
Injection Volume 10
Acq. Date-Time 11/30/2020 5:09:38 PM
Sample Info.

Sample Chromatogram



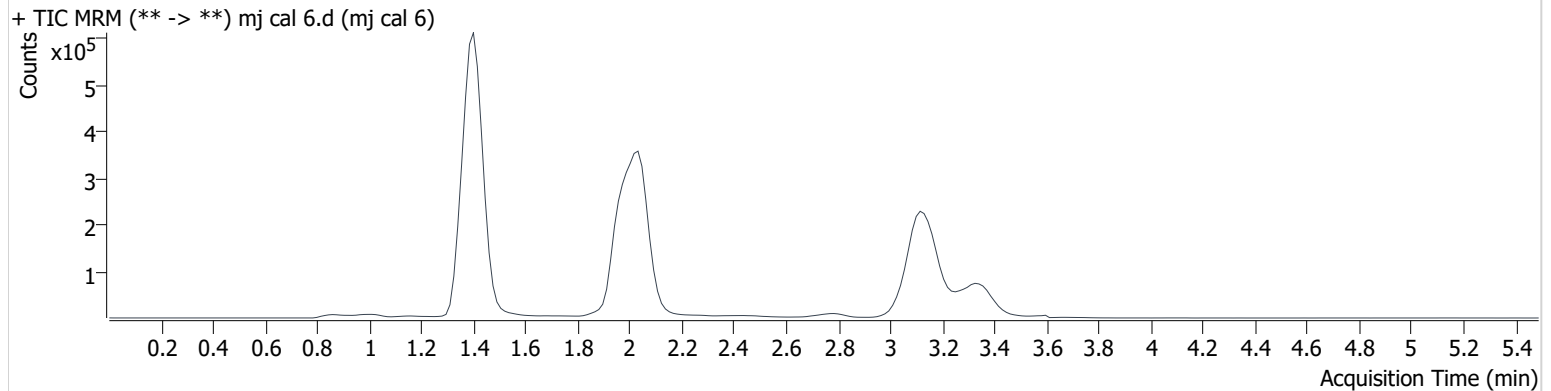
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	540987	∞	10.8	∞	657030	24.921 ng/ml
THC-COOH	1.416	313909	534.7	36.1	1708.2	293360	72.495 ng/ml
THC	3.167	236796	∞	25.9	∞	386539	23.726 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 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	11/30/2020 5:16:22 PM		

Sample Chromatogram



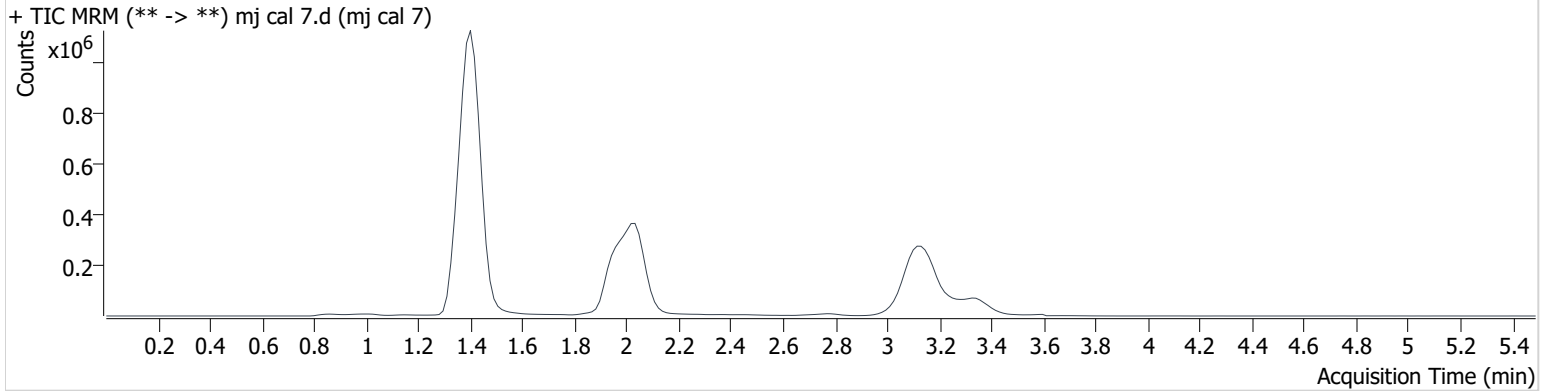
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	1110187	∞	11.3	∞	668518	50.126 ng/ml
THC-COOH	1.416	417781	799451.0	36.2	1950.1	286521	98.258 ng/ml
THC	3.152	494158	∞	24.6	∞	384798	49.119 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 113020\QuantResults\thcq.batch.bin
Calibration Last Update 12/2/2020 12:10:19 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	11/30/2020 5:23:06 PM		

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	2258302	∞	11.8	∞	678357	100.349 ng/ml
THC-COOH	1.416	1041955	2298084.1	36.6	385731.7	270861	256.836 ng/ml
THC	3.152	994857	∞	24.2	∞	368859	102.541 ng/ml

	1	2	3	4	5	6
A	IS + Cal. 1	neg blood	neg urine			IS + QC_1
B	IS + Cal. 2	c2248-1- did not flow through SLE	urine ext ctrl			IS + Cal. 7
C	IS + Cal. 3	c2278-1	c2302-1			IS + Cal. 6
D	IS + Cal. 4	c2279-1	c2319-1			IS + Cal. 5
E	IS + Cal. 5	c2281-1	c2320-1			IS + Cal. 4
F	IS + Cal. 6	c2367-1	c2248-1			IS + Cal. 3
G	IS + Cal. 7	c2368-1				IS + Cal. 2
H	IS + QC_1	P3350-1				IS + Cal. 1

All wells to contain 100 µl of residual DMSO

Case #: 2020- -