

















Worklist: 4596

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
C2020-1991	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-1999	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2018	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-2054	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2074	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2092	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2094	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
C2020-2110	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2113	3	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2129	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
C2020-2149	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3146	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3147	1	BCK	AM 27 Blood THC Quant by LC-QQQ	
P2020-3147	2	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2020-3197	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ	
P2020-3211	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: 11/5/20Analyst: Britany WyliePlate lot#: 200723Plate Expiration: 1-23-2021**Mobile phase A:** 0.1% Formic Acid in LCMS Water**Mobile phase B:** 0.1% Formic acid in Acetonitrile

MTBE

LCMS Methanol

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 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

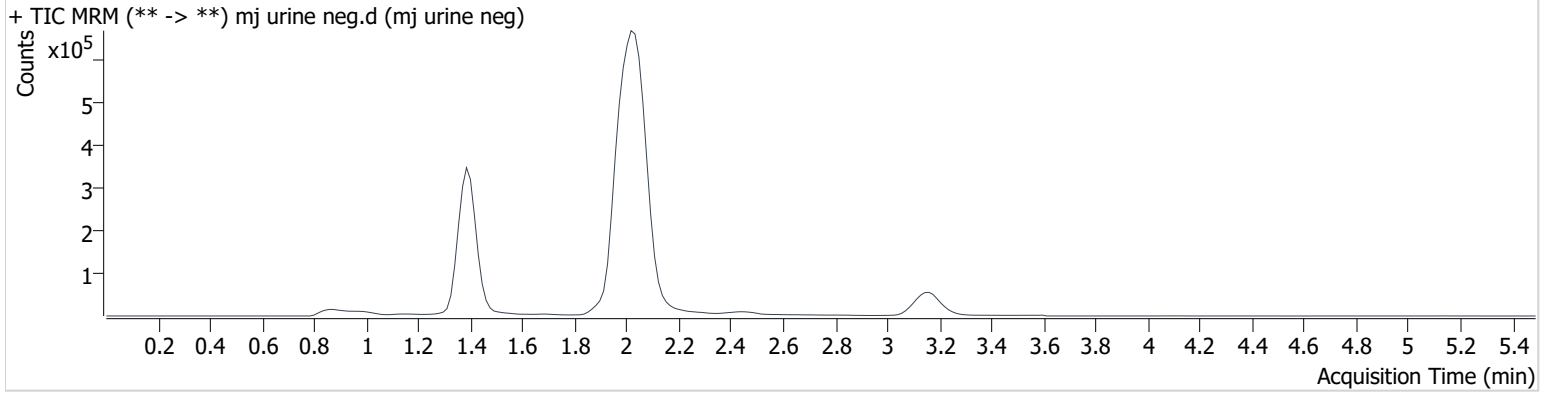
COMMENTS: *negative blood control wrong plate position on initial injection, corrected position and reinjected and evaluated.*

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 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-A2	Comment	
Injection Volume	10		
Acq. Date-Time	11/5/2020 6:20:08 PM		
Sample Info.			

Sample Chromatogram

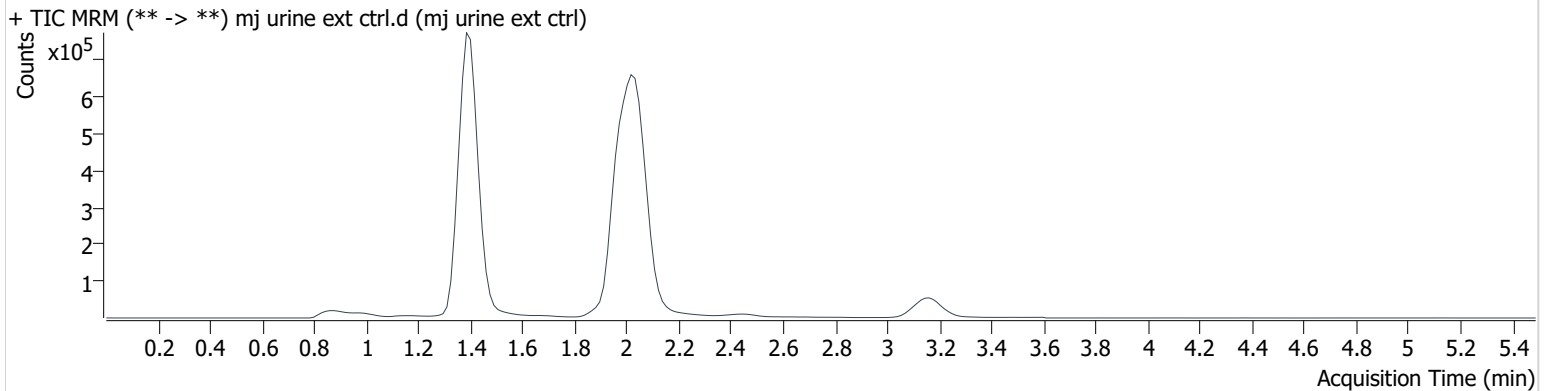


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 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-B2 **Comment**
Injection Volume 10
Acq. Date-Time 11/5/2020 6:26:52 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	1556574	∞	13.4	∞	1221466	39.300 ng/ml
THC-COOH	1.416	223589	401.2	33.9	592.2	432440	37.449 ng/ml
THC	3.167	89295	∞	25.3	4044394 5581323 5.0	296564	11.926 ng/ml

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

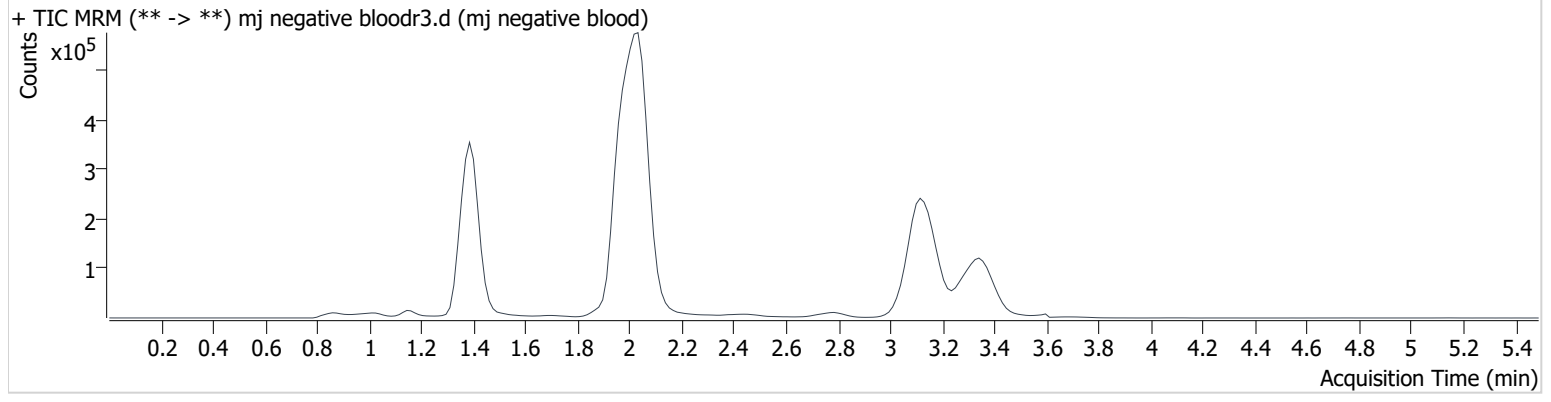
ppd 8/26/20 Exp 7/1/21 neg urine lot 73020	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	10/4/2020
ppd 10/5/20 Exp 7/1/21 neg urine lot 10120	lot 10520	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 PM

Instrument	69679	Data File	mj negative bloodr3.d
Type	Sample	Sample	mj negative blood
Acq. Method	AM 27 THC quant.m	Operator	Britany Wylie
Sample Position	P3-E3	Comment	
Injection Volume	10		
Acq. Date-Time	11/5/2020 9:07:34 PM		
Sample Info.			

Sample Chromatogram

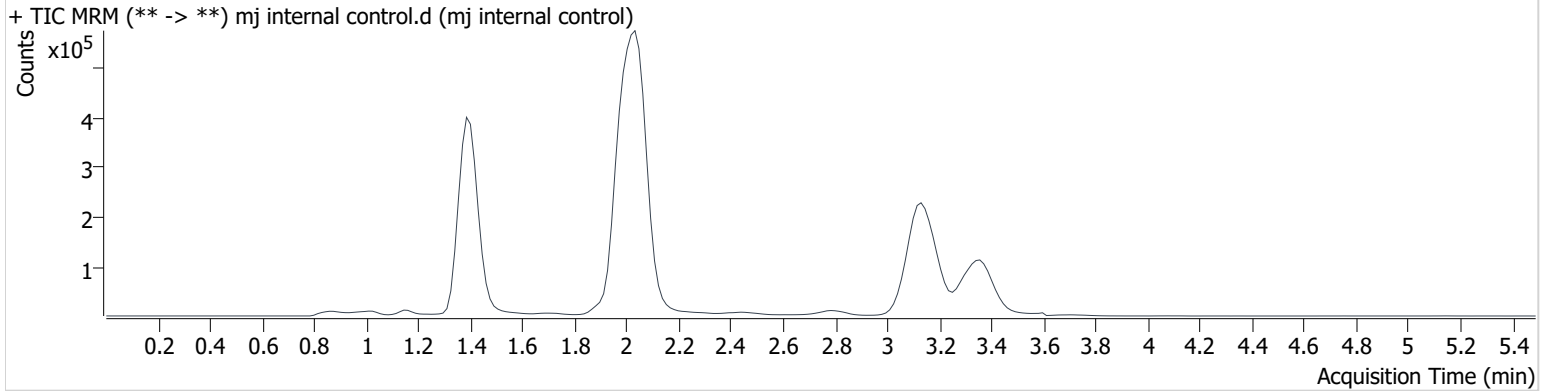


AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 PM

Instrument 69679 **Data File** mj internal control.d
Type QC **Sample** mj internal control
Acq. Method AM 27 THC quant.m **Operator** Brittany Wylie
Sample Position P3-H1 **Comment**
Injection Volume 10
Acq. Date-Time 11/5/2020 4:39:48 PM
Sample Info.

Sample Chromatogram



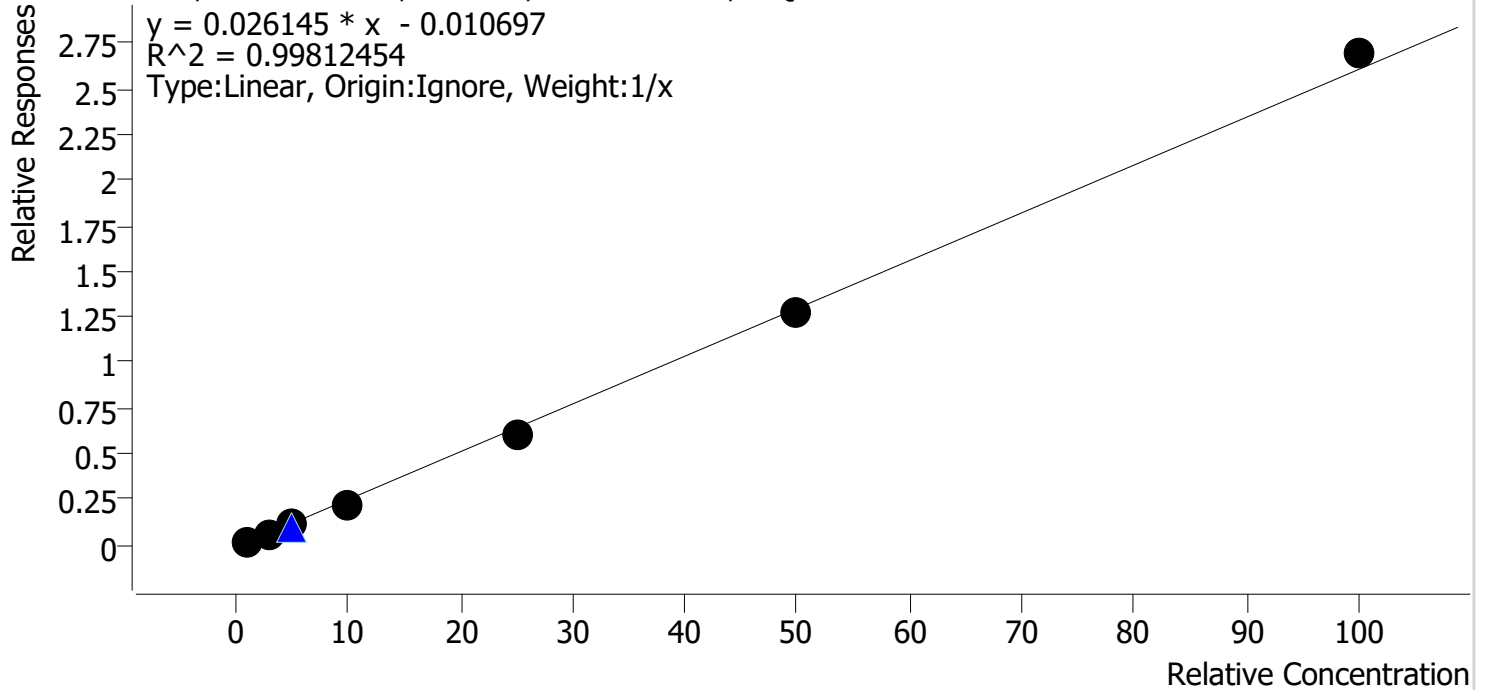
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	180737	∞	10.4	∞	1171393	4.813 ng/ml
THC-COOH	1.416	94886	183666.3	35.2	342.3	482202	14.934 ng/ml
THC	3.182	74529	∞	24.9	∞	703003	4.464 ng/ml

Compound Calibration Report



Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Last Cal. Update 11/5/2020 10:07 PM
Analyst Name ISP\datastor
Analyte THC **Internal Standard** THC-d3

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

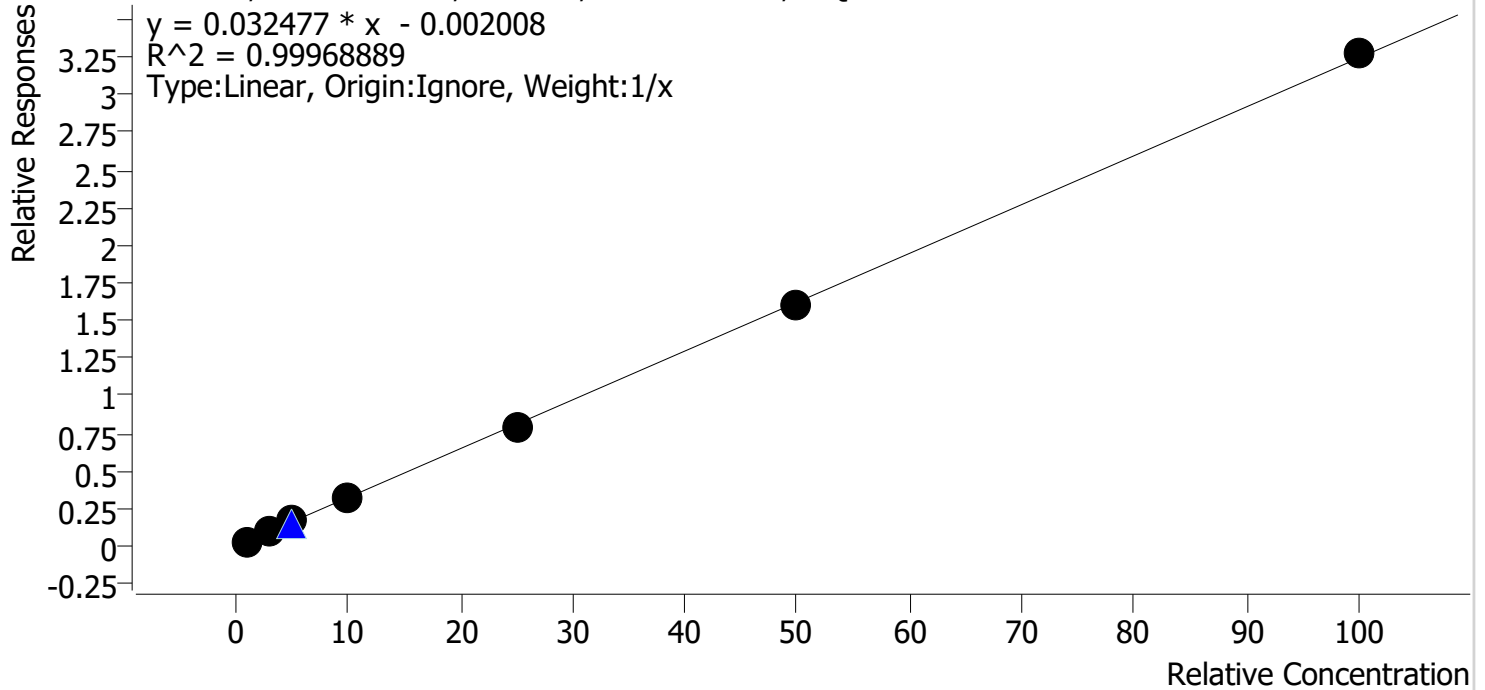


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
mj qc1	1	✓	1.0	1.2	117.1
mj cal2	2	✓	3.0	2.9	98.3
mj cal 3	3	✓	5.0	4.9	97.7
mj cal 4	4	✓	10.0	9.1	91.0
mj cal 5	5	✓	25.0	23.7	94.9
mj cal 6	6	✓	50.0	48.9	97.8
mj cal 7	7	✓	100.0	103.3	103.3

Compound Calibration Report

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Last Cal. Update 11/5/2020 10:07 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	0.9	94.0
mj cal2	2	✓	3.0	3.2	107.9
mj cal 3	3	✓	5.0	5.1	102.5
mj cal 4	4	✓	10.0	9.7	97.3
mj cal 5	5	✓	25.0	24.6	98.6
mj cal 6	6	✓	50.0	49.5	98.9
mj cal 7	7	✓	100.0	100.9	100.9

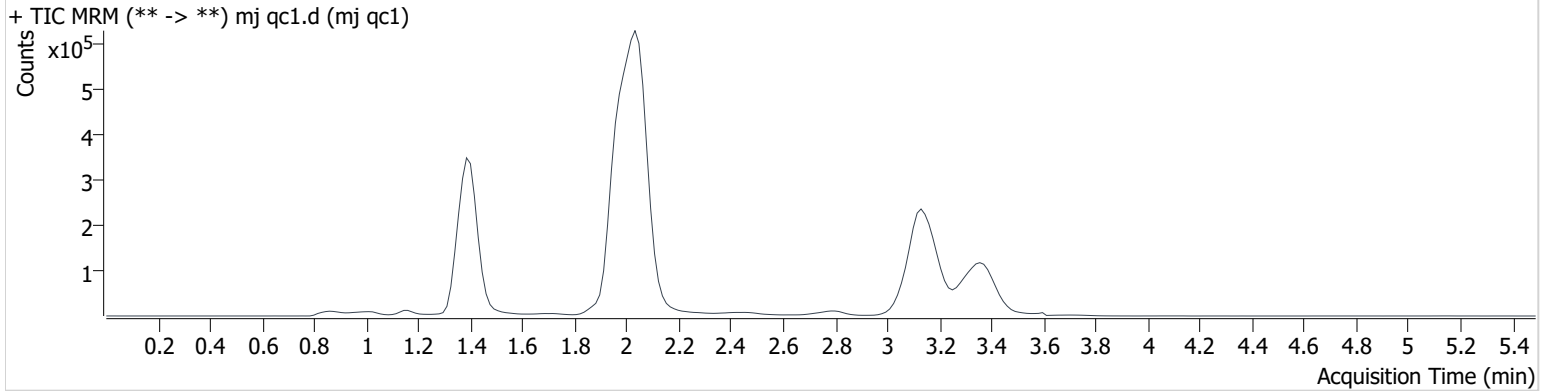
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 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	11/5/2020 3:52:38 PM		

Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	35341	∞	13.7	∞	1239800	0.940 ng/ml Low
THC-COOH	1.431	30164	58.7	35.1	37.1	483945	5.482 ng/ml Low
THC	3.182	15272	∞	31.3	∞	767002	1.171 ng/ml Low

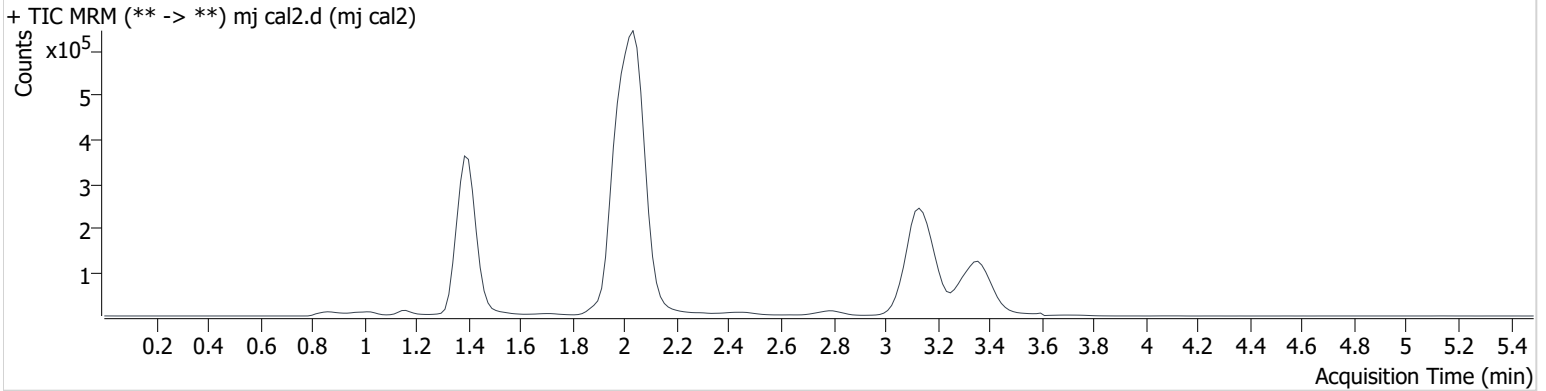
AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 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	11/5/2020 3:59:22 PM		

Sample Info.

Sample Chromatogram



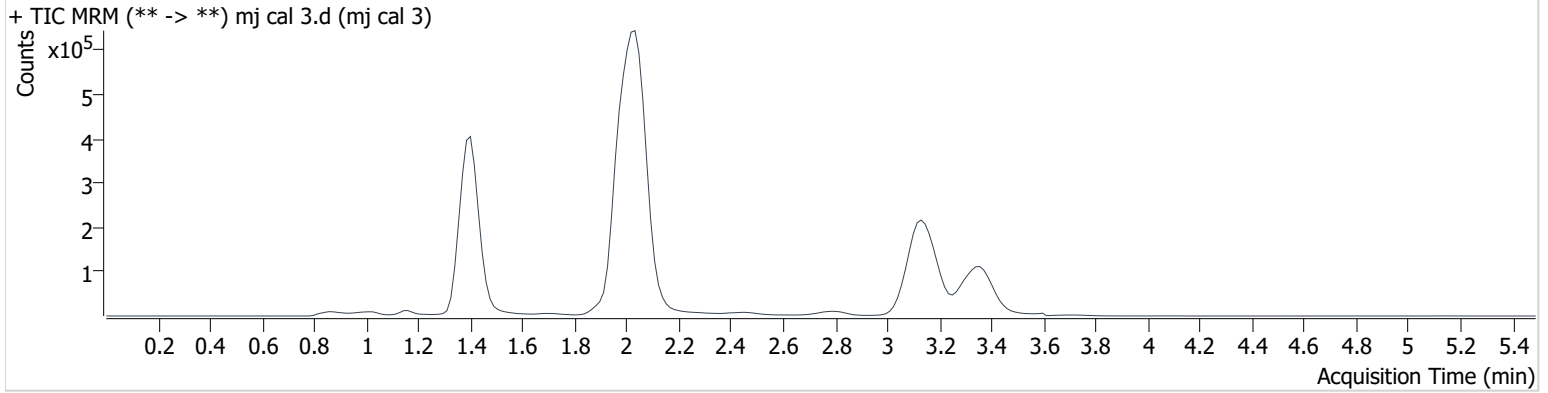
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	116596	∞	10.0	∞	1131097	3.236 ng/ml
THC-COOH	1.416	60438	99.4	34.5	141.8	479216	9.967 ng/ml Low
THC	3.182	50615	∞	28.4	3341649 1708880 .3	762160	2.949 ng/ml Low

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 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	11/5/2020 4:06:07 PM		

Sample Chromatogram



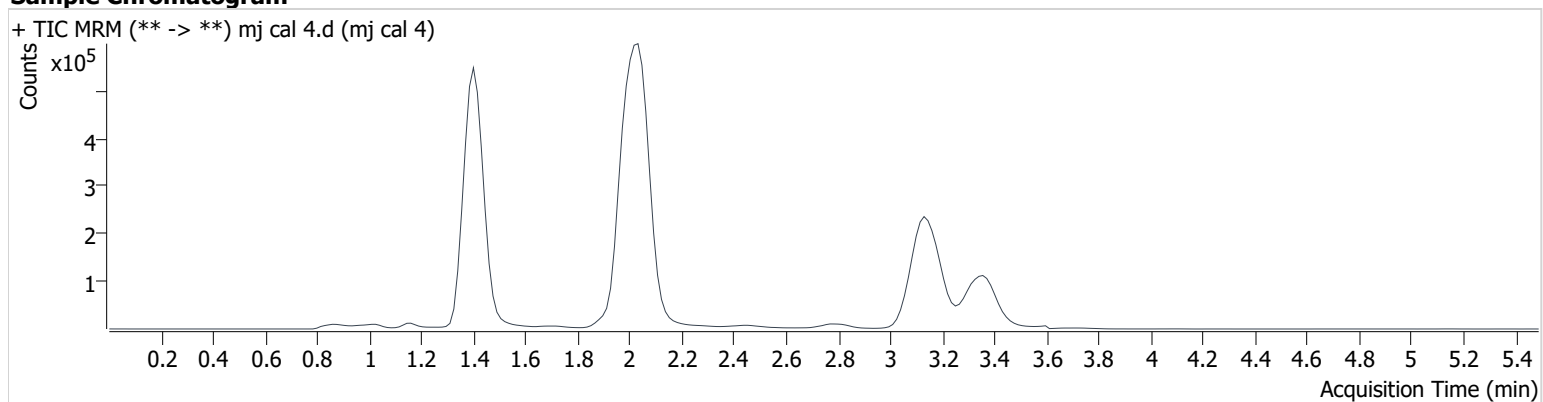
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	181672	∞	10.5	∞	1104444	5.127 ng/ml
THC-COOH	1.416	123355	225107.0	35.0	150.1	463916	19.793 ng/ml
THC	3.182	78631	∞	26.1	∞	672176	4.883 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 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 11/5/2020 4:12:51 PM
Sample Info.

Sample Chromatogram



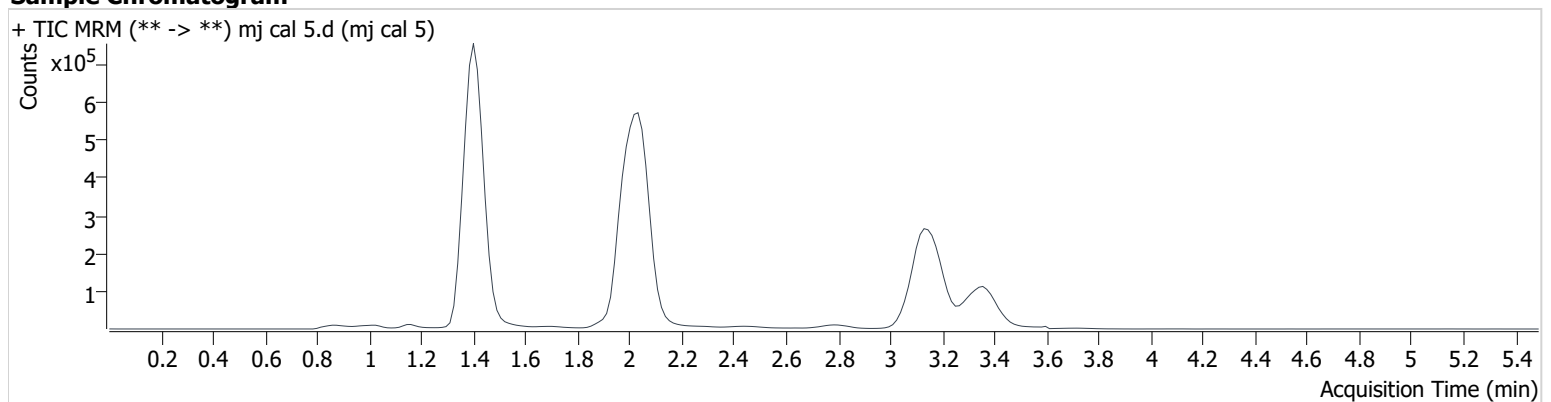
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	365724	∞	11.7	∞	1164521	9.732 ng/ml
THC-COOH	1.416	300801	151.0	36.7	210402.3	462989	46.775 ng/ml
THC	3.182	158719	∞	24.0	∞	698884	9.095 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 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 11/5/2020 4:19:35 PM
Sample Info.

Sample Chromatogram



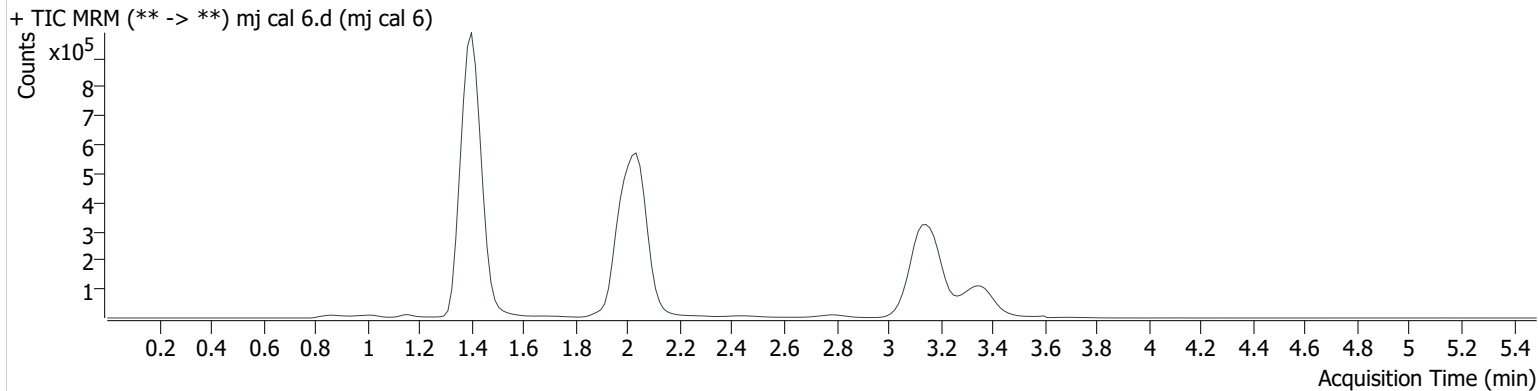
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	938453	∞	11.6	∞	1175760	24.638 ng/ml
THC-COOH	1.416	480639	3256.8	35.9	400113.9	468463	73.229 ng/ml
THC	3.182	420696	∞	23.1	∞	689806	23.736 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 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 11/5/2020 4:26:20 PM
Sample Info.

Sample Chromatogram



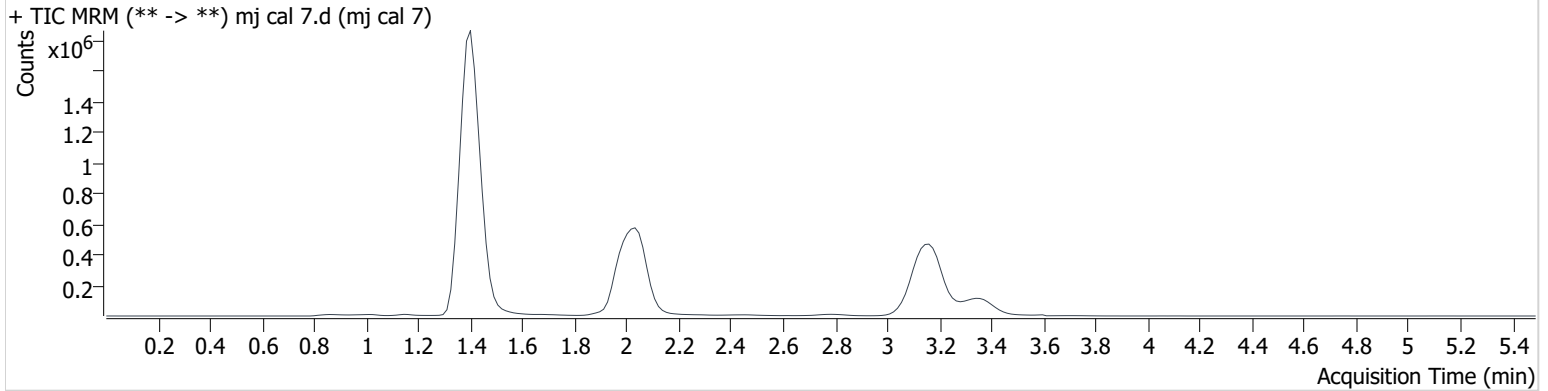
Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	1837422	∞	11.9	∞	1145430	49.454 ng/ml
THC-COOH	1.416	619467	8146.6	36.3	1602.1	450717	97.723 ng/ml
THC	3.182	863070	∞	24.2	∞	680916	48.889 ng/ml

AM #27 Cannabinoids

Batch results D:\MassHunter\Data\2020 Data\am 27-28 110520\QuantResults\mj.batch.bin
Calibration Last Update 11/5/2020 10:07:35 PM

Instrument 69679 **Data File** mj cal 7.d
Type Cal **Sample** mj cal 7
Acq. Method AM 27 THC quant.m **Operator** Brittany Wylie
Sample Position P3-G1 **Comment**
Injection Volume 10
Acq. Date-Time 11/5/2020 4:33:04 PM
Sample Info.

Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
THC-OH	1.391	3740950	∞	12.3	∞	1142592	100.874 ng/ml
THC-COOH	1.416	1512615	1694323.0	37.0	1585.5	415501	257.031 ng/ml
THC	3.182	1815606	∞	24.3	∞	675083	103.276 ng/ml

	1	2	3	4	5	6
A	IS + Cal. 1	neg urine	c2113-3	p3211-1		IS + QC_1
B	IS + Cal. 2	ext ctrl	c2129-2	c2018-1		IS + Cal. 7
C	IS + Cal. 3	c1991-2	p3147-2	c2094-1- did not go through SLE		IS + Cal. 6
D	IS + Cal. 4	c1999-1	p3197-1	c2094-1		IS + Cal. 5
E	IS + Cal. 5	c2054-1	neg blood			IS + Cal. 4
F	IS + Cal. 6	c2074-1	c2149-1			IS + Cal. 3
G	IS + Cal. 7	c2092-1	p3146-1			IS + Cal. 2
H	IS + QC_1	c2110-1	p3147-1			IS + Cal. 1

All wells to contain 100 µl of residual DMSO

Case #: 2020- -