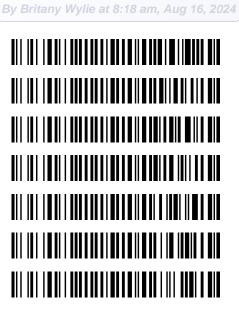
REVIEWED

8/15/2024

Worklist: 6898

LAB CASE	<u>ITEM</u>	ITEM TYPE	DESCRIPTION
C2024-1393	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-1394	1	BCK	AM 27 Blood THC Quant by LC-QQQ
C2024-1421	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2024-1422	1	UCK	AM 27 Urine Cannabinoids Confirmation by LC-QQQ
C2024-1447	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
C2024-1466	1	ВСК	AM 27 Blood THC Quant by LC-QQQ
C2024-1468	1	ВСК	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: <u>8/13/24</u> Plate lot#: 240513 Mobile phase A: 0.1% Formic Acid in LCMS Water Blank Blood Lot: 24C52043 Column: UCT Selectra DA 100 x 2.1mm 3um Analyst: <u>Anne Nord</u> Plate Retest Date: 11/13/2024 Mobile phase B: 0.1% Formic acid in Acetonitrile Blank Urine Lot: 6524 LCMS-QQQ ID: 69679

Pre-Analytic:

- \boxtimes 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- \boxtimes 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.

Analytic:

- \boxtimes 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- ☑ 2. Using a calibrated pipette, pipette 1000µL blood or 1000µL urine in wells of analytical (standards) plate. Pipette ID: K52558G
- ☑ 3. Urine hydrolysis add 100 ul BG turbo, and 200 ul BG turbo buffer to the urine samples in wells of the analytical plate.
- ☑ 4. Add 500µL of 0.1% formic acid in water in the wells of the analytical plate.
- ☑ 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- δ. Transfer 700-800μL of blood+acid or urine+acid mixture to corresponding wells of SLE+ plate. Amount transferred: 750 μL
- 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)
- \boxtimes 8. Wait 5 minutes.
- 9. Add 2.25mL MTBE. (Add in 3 increments of 750uL)
- \boxtimes 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)
- \boxtimes 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 75401 and evaporate to dryness at approx. 35°C.
- ☑ 16. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- \boxtimes 1. Create batch and process data.
- \boxtimes 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r² values ≥ 0.98 for each analyte
- 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
- \boxtimes 4. Did all QCs pass for each analyte? (if not, describe in comments section)
- \boxtimes 5. Enter QCs into control charting.
- Solution 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: The end of run blood control and the negative urine did not inject they were re-constituted and injected 8/15/24

Hydroxy-THC was not evaluated for the urine samples due to an interfering peak.

	1	2	3	4	5	6
а	cal 1	Internal control urine	1421-1			
b	cal 2	negative blood	1422-1			
с	cal 3	1393-1				
d	cal 4	1394-1				
e	cal 5	1466-1				
f	cal 6	1468-1				
g	cal 7	1447-1				
h	Internal control (blood)	negative urine				

Plate position 3

c2024-___-



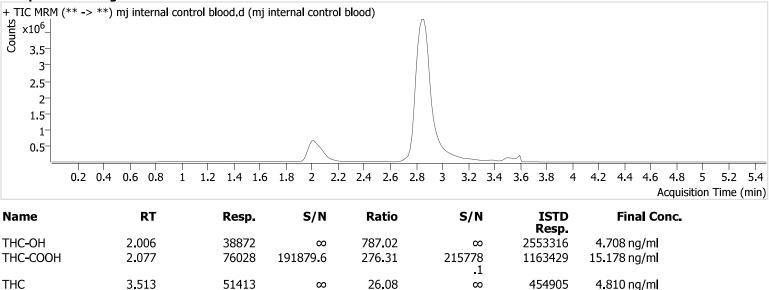
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TypeQCAcq. MethodtheSample PositionP3Injection Volume10	679Data File Samplec quant 50 50.mOperator Comment-H1Operator Comment13/2024 5:25:14 PM
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mj internal control blood.d mj internal control blood

Anne Nord

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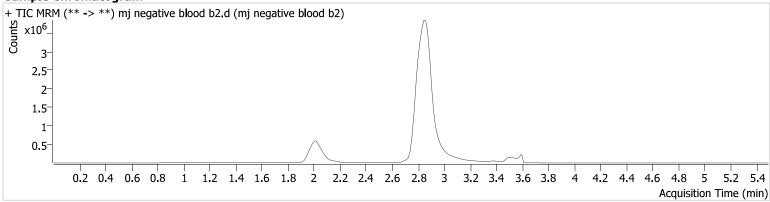


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mj negative blood b2.d mj negative blood b2 Anne Nord

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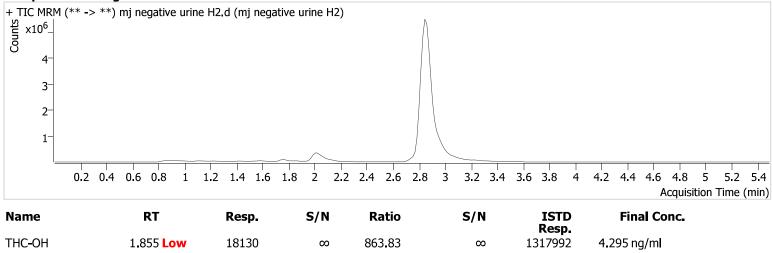
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Sample Info.		

mj negative urine H2.d mj negative urine H2 Anne Nord

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Sample Chromatogram



THC-OH was not evaluated in urine samples due to interfering peaks

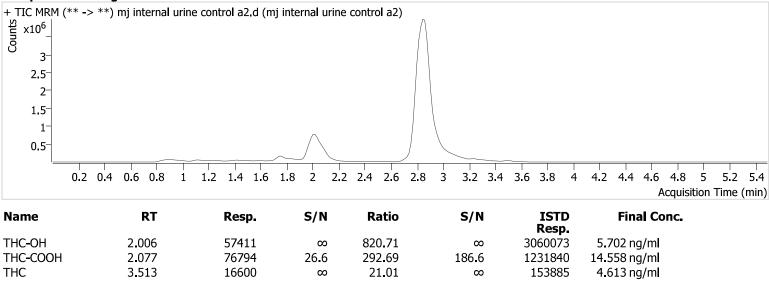
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Acq. Date-Time 8/13/2024 7:23:57 PM Sample Info.		69679 QC thc quant 50 50.m P3-A2 10 8/13/2024 7:23:57 PM	Data File Sample Operator Comment
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mj internal urine control a2.d mj internal urine control a2 Anne Nord

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Sample Chromatogram



THC-OH was not evaluated in urine samples due to interfering peaks

 Batch results
 D:\MassHunter\Data\2024\am 27-28\081324\QuantResults\am27.batch.bin

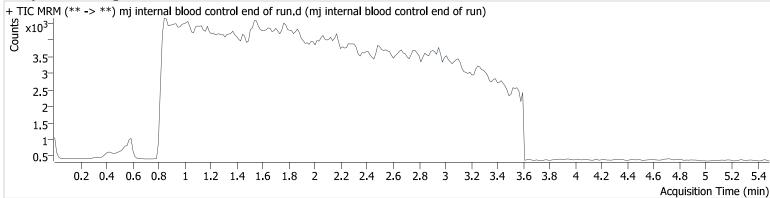
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 8/15/2024 11:06:03 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info.	69679 QC thc quant 50 50.m P3-H1 10 8/13/2024 7:30:33 PM	Data File Sample Operator Comment
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mj internal blood control end of run.d mj internal blood control end of run Anne Nord

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Sample Chromatogram



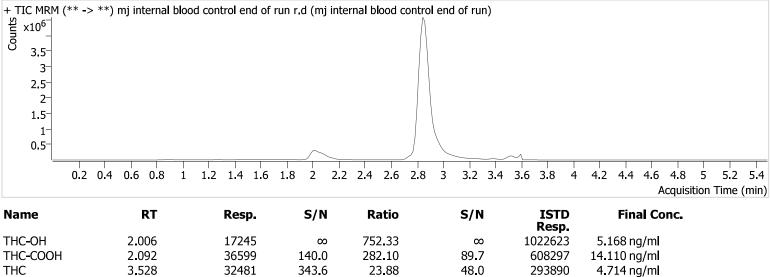
Sample did not inject it was reconstituted and injected 8/15/24

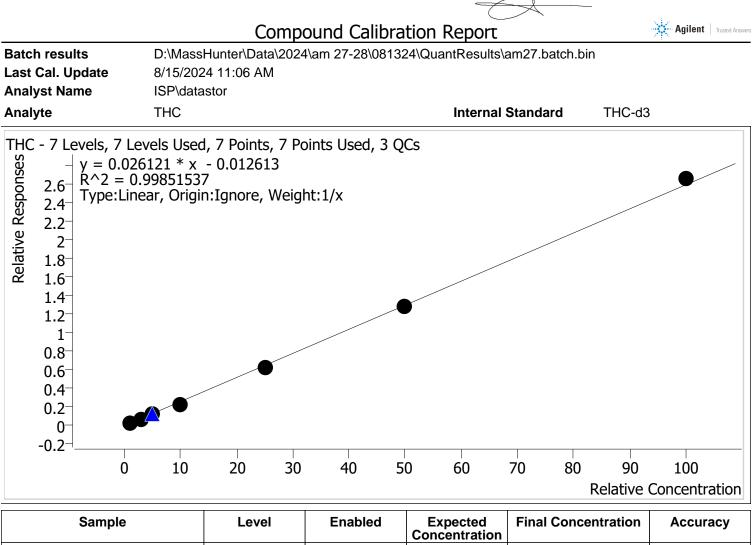


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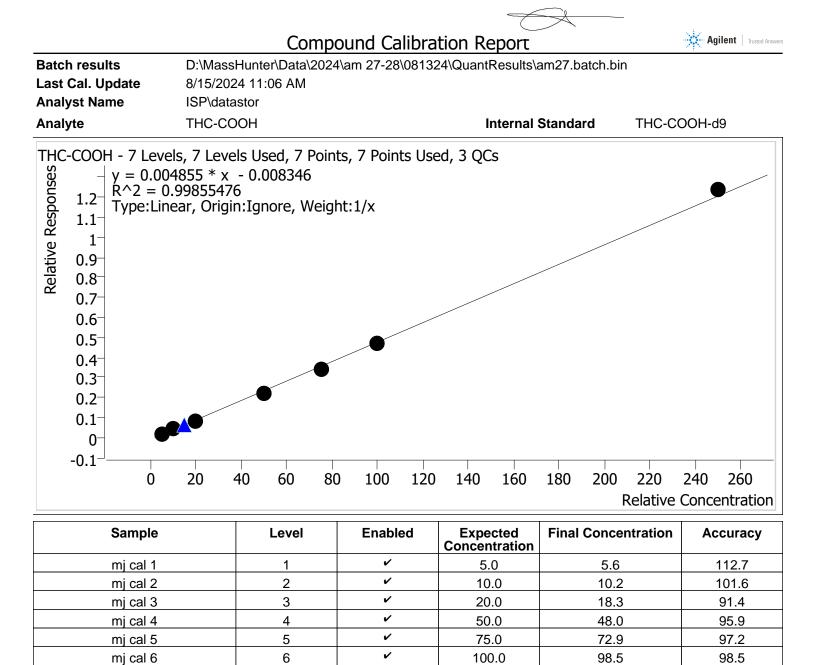
mj internal blood control end of run r.d mj internal blood control end of run Anne Nord

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			Concentration		,,
mj cal 1	1	~	1.0	1.2	115.3
mj cal 2	2	~	3.0	2.8	92.4
mj cal 3	3	~	5.0	5.2	104.5
mj cal 4	4	~	10.0	9.0	90.5
mj cal 5	5	 ✓ 	25.0	24.1	96.3
mj cal 6	6	~	50.0	49.3	98.5
mj cal 7	7	~	100.0	102.4	102.4



r

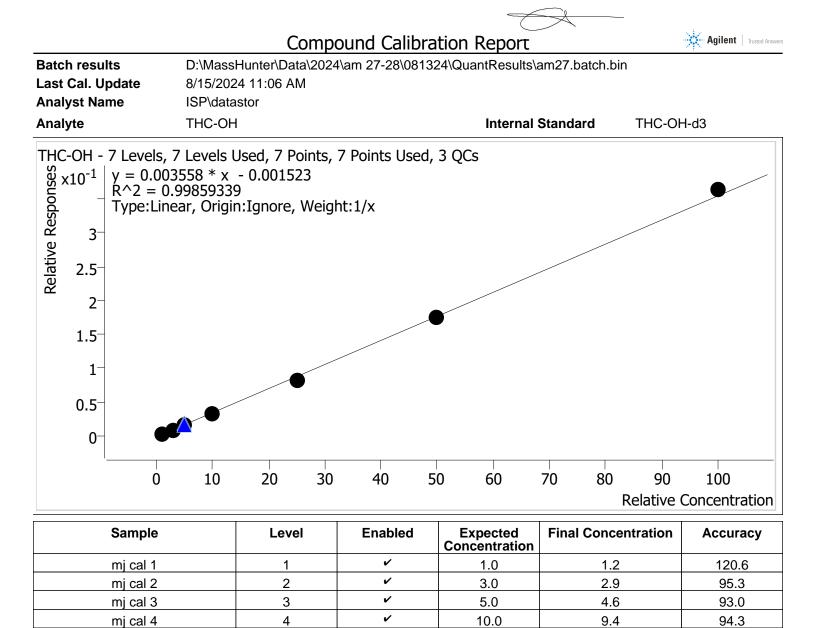
250.0

256.5

7

mj cal 7

102.6



r

r

r

25.0

50.0

100.0

5

6

7

mj cal 5

mj cal 6

mj cal 7

23.8

49.6

102.5

95.1

99.2

102.5

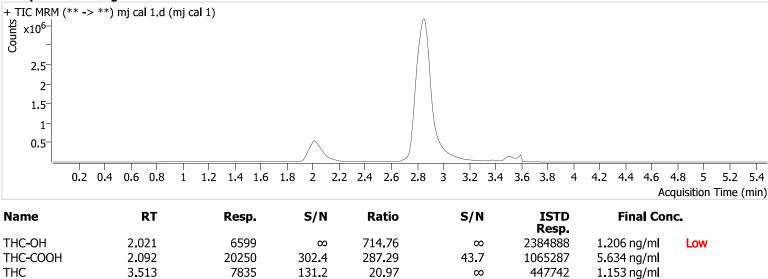


Batch resultsD:\MassHunter\Data\2024\am 27-28\081324\QuantResults\am27.batch.binCalibration Last Update8/15/2024 11:06:03 AM

mj cal 1.d mj cal 1

Anne Nord

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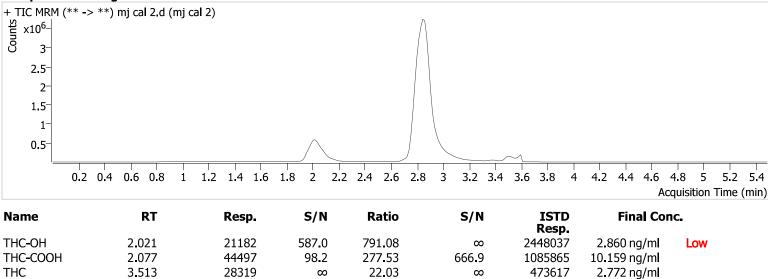


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mj cal 2.d mj cal 2 Appo Nord

Anne Nord

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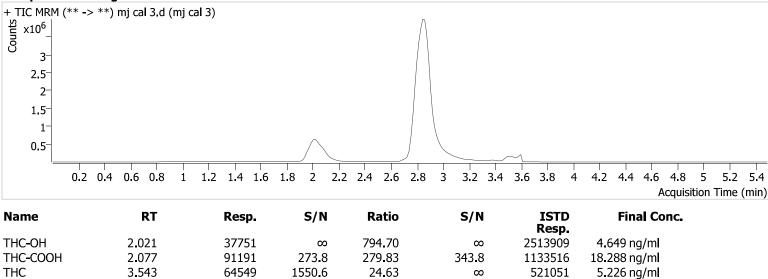


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mj cal 3.d mj cal 3

Anne Nord

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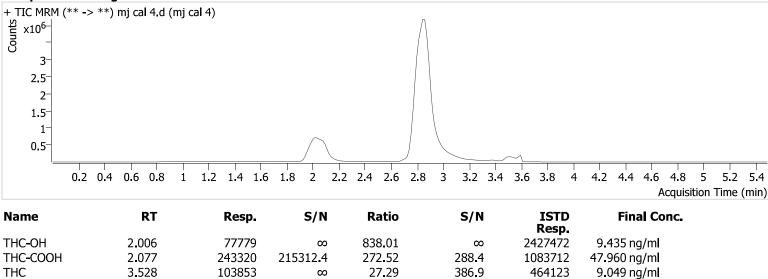
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Instrument69679TypeCalAcq. Methodthc quantSample PositionP3-D1Injection Volume10Acq. Date-Time8/13/2024Sample Info.X	50 50.m Data File Sample Operator Comment
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mj cal 4.d mj cal 4

Anne Nord

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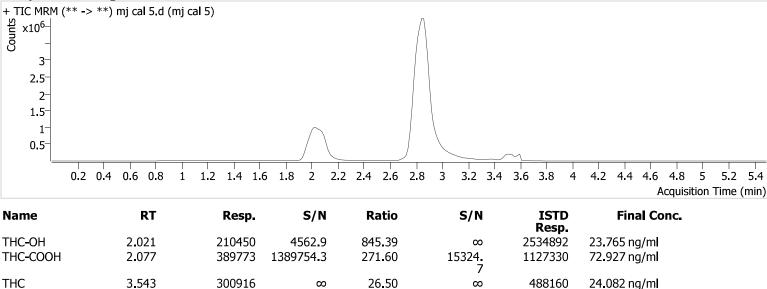


Batch resultsD:\MassHunter\Data\2024\am 27-28\081324\QuantResults\am27.batch.binCalibration Last Update8/15/2024 11:06:03 AM

Instrument69679TypeCalAcq. Methodthc quant 50 50.mSample PositionP3-E1Injection Volume10Acq. Date-Time8/13/2024 5:05:30 PMSample Info.Sample Info.	Data File Sample Operator Comment
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mj cal 5.d mj cal 5 Anne Nord

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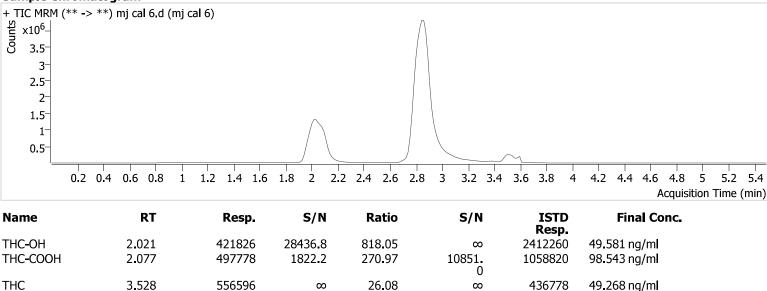


Batch results D:\MassHunter\Data\2024\am 27-28\081324\QuantResults\am27.batch.bin Calibration Last Update 8/15/2024 11:06:03 AM

mj cal 6.d mj cal 6

Anne Nord

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Batch results D:\MassHunter\Data\2024\am 27-28\081324\QuantResults\am27.batch.bin Calibration Last Update 8/15/2024 11:06:03 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info.	69679 Cal thc quant 50 50.m P3-G1 10 8/13/2024 5:18:40 PM	Data File Sample Operator Comment
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mj cal 7.d mj cal 7 Anne Nord

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