REVIEWED By Anne Nord at 2:11 pm, Dec 13, 2023

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Worklist:	6603
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LAB CASE	ITEM	ITEM TYPE	DESCRIPTION
M2023-4479	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4696	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4775	2	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4785	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4863	1	BCK	AM 27 Blood THC Quant by LC-QQQ
M2023-4931	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3511	2	BCK	AM 27 Blood THC Quant by LC-QQQ
P2023-3512	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: 12/8/2023 Plate lot#: 230627 Mobile phase A: 0.1% Formic Acid in LCMS Water Blank Blood Lot: Lampire 23E52981 LCMS-QQQ ID: 069901 Analyst: Celena Shrum Plate Retest Date: 12/27/2023 **Mobile phase B:** 0.1% Formic acid in Acetonitrile **Column**: Phenomenex Phenyl Hexyl (4.6x50mm, 2.6um)

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:

- ☑ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- □ 2. Urine hydrolysis (if applicable): add 1.5mL urine to blank plate, add 250µl 1N KOH. Shake and incubate at 40 degrees for 15 minutes.
- ☑ 3. Using a calibrated pipette, add 1000µl blood or 1000µl hydrolyzed urine into the appropriate wells of the analytical (standards) plate. Pipette ID: #42
- \boxtimes 4. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- S. Add 500µL of 0.1% formic acid in water to blood samples or 500µl of saturated phosphate buffer to urine samples to the appropriate wells of the analytical plate.
- \boxtimes 6. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- ☑ 7. Transfer **800µL of blood+acid mixture or urine+acid** to corresponding wells of SLE+ plate.
- ☑ 8. 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: 067104
- \boxtimes 9. Wait 5 minutes.
- ☑ 10. Add 2.25mL MTBE. (Add in 3 increments of 750uL)
- \boxtimes 11. Wait 5 minutes.
- ☑ 12. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- ☑ 13. Add 2.25mL Hexane. (Add in 3 increments of 750uL)
- \boxtimes 14. Wait 5 minutes.
- ☑ 15. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
- I6. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. SPE Dry ID: 067103
- Σ 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
- 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 lng/mL and OH-THC 3ng/mL (quantitative), Carboxy-THC: 5ng/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. THC concentrations of 1-3ng/mL will be reported qualitatively.
- \boxtimes 5. Did all QCs pass for each analyte? (if not, describe in comments section)
- \boxtimes 6. Enter QCs into control charting.
- ☑ 7. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Incorrect well positions were specified for M2023-4775-2 and M2023-4863-1. They were injected on 12/12/23. Due to QC accuracy for THC, samples between 1-5ng/mL will be reported qualitatively for THC.

	1	2	3	4	5	6
а					M2023-4931-2	QC 1
b					M2023-4863-1*	cal 100 ng
с					M2023-4785-1	cal 50 ng
d					M2023-4775-2*	cal 25 ng
e				M2023-4863-1*	M2023-4696-2	cal 10ng
f				M2023-4775-2*	M2023-4479-2	cal 5 ng
g				P2023-3512-1	NEG Blood	cal 3 ng
h				P2023-3511-2	QC 2	cal 1ng

*Additional aliquots were taken during sampling portion due to clots in the blood samples



Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Sample AM 27 Agilent Method.m P1-G5 10 12/8/2023 5:13:06 PM Data File Sample Operator Comment MJ Negative Blood.d MJ Negative Blood Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P1-A6 10 12/8/2023 4:46:52 PM Data File Sample Operator Comment MJ QC Control Blood.d MJ QC Control Blood Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.



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Acq. Method
Sample Position
Injection Volume
Acq. Date-Time
Sample Info.

Falco (069901) QC AM 27 Agilent Method.m P1-H5 10 12/8/2023 9:09:11 PM Data File Sample Operator Comment QC end MJ.d QC end MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.



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Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P1-H5 10 12/12/2023 10:45:08 AM Data File Sample Operator Comment QC end MJ_r.d QC end MJ_r Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





AM #27 Cannabinoids Quant. Calibration Curve Report

Bato Last Ana	h resu Cal. U lyst Na	lts pdate me	D:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.bin 12/12/2023 11:00 AM ISP\Datastor									
Ana	lyte		THC					Interna	al Standard	1	THC-D3	
Relative Responses	C - 7 Lo 0.9- 0.8- 0.7- 0.6- 0.4- 0.3- 0.2- 0.1- 0-	evels, 7 L y = 0.00 R^2 = 0 Type:Lin	evels Used 9672 * x 9.99917218 hear, Origin	, 7 Points + 2.4150 h:Ignore,	s, 7 Poir 077E-00 Weight	nts Used, 4 ::1/x 40	3 QCs	60	70	80	90	100
										F	Relative C	Concentration
												_

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	1.0	1.1	110.1
Cal 2 MJ	2	~	3.0	2.8	93.2
Cal 3 MJ	3	~	5.0	4.7	94.7
Cal 4 MJ	4	~	10.0	9.8	97.5
Cal 5 MJ	5	~	25.0	25.8	103.4
Cal 6 MJ	6	~	50.0	51.4	102.8
Cal 7 MJ	7	~	100.0	98.4	98.4



AM #27 Cannabinoids Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.bate Last Cal. Update 12/12/2023 11:00 AM Analyst Name ISP\Datastor						
Analyte	THC-COOH	Internal Standard	THC-COOH-D9			
THC-COOH - 7 Level Set 1.8 $y = 0.00$ $R^2 = 0$ $R^2 = 0$ Type:Lin y = 1.4	els, 7 Levels Used, 7 Points, 7 Points)7044 * x - 0.001373).99952487 near, Origin:Ignore, Weight:1/x	Used, 3 QCs				



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	5.0	5.4	107.6
Cal 2 MJ	2	~	10.0	9.3	92.6
Cal 3 MJ	3	~	20.0	19.9	99.6
Cal 4 MJ	4	~	50.0	49.9	99.8
Cal 5 MJ	5	~	75.0	73.5	98.1
Cal 6 MJ	6	~	100.0	102.6	102.6
Cal 7 MJ	7	~	250.0	249.4	99.7



AM #27 Cannabinoids Quant. Calibration Curve Report

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Batch	resul	ts D:\MassHunter\Data\2023\AM 27 28\1208:	23 AM 27 28 CS\QuantResults	AM 27.batch.bin	
	val. U∣ stNai	Date 12/12/2023 11:00 AM ne ISP/Datastor			
Analys	St Mai				
Analy	te	THC-OH	Internal Standard	THC-OH-D3	
Relative Responses H O	OH - 1.4- 1.2- 1- 0.8- 0.6- 0.4- 0.2-	7 Levels, 7 Levels Used, 7 Points, 7 Points Used, y = 0.014447 * x - 0.001885 R^2 = 0.99966647 Type:Linear, Origin:Ignore, Weight:1/x	3 QCs		

0-		<u> </u>										
·	0	10	20	30	40	50	60	70	80	90	100	
									R	elative C	Concentra	tion

Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1 MJ	1	~	1.0	1.1	111.9
Cal 2 MJ	2	~	3.0	2.9	96.4
Cal 3 MJ	3	~	5.0	4.7	94.5
Cal 4 MJ	4	~	10.0	9.7	97.0
Cal 5 MJ	5	~	25.0	24.7	98.7
Cal 6 MJ	6	~	50.0	50.6	101.3
Cal 7 MJ	7	v	100.0	100.2	100.2

Cal 1 MJ.d



Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-H6 10 12/8/2023 3:01:50 PM Data File Sample Operator Comment

Cal 1 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-G6 10 12/8/2023 3:15:04 PM Data File Sample Operator Comment Cal 2 MJ.d Cal 2 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.



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Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-F6 10 12/8/2023 3:28:11 PM Data File Sample Operator Comment Cal 3 MJ.d Cal 3 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-E6 10 12/8/2023 3:41:17 PM Data File Sample Operator Comment Cal 4 MJ.d Cal 4 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-D6 10 12/8/2023 3:54:24 PM Data File Sample Operator Comment Cal 5 MJ.d Cal 5 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-C6 10 12/8/2023 4:07:31 PM Data File Sample Operator Comment Cal 6 MJ.d Cal 6 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.





Batch resultsD:\MassHunter\Data\2023\AM 27 28\120823 AM 27 28 CS\QuantResults\AM 27.batch.binCalibration Last Update12/12/2023 11:00:15 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P1-B6 10 12/8/2023 4:20:37 PM Data File Sample Operator Comment Cal 7 MJ.d Cal 7 MJ Celena Shrum Only drugs and concentrations listed on the laboratory report itself are appropriate to be used for interpretation purposes. Any drugs or values included in the notes but not included on the report are used by laboratory personnel to make determinations/reach conclusions within the confines of the methods.

