REVIEWED By Sarah Collins at 11:56 am, Jul 26, 2024



| LAB CASE | <u>ITEM</u> | ITEM TYPE | DESCRIPTION |
|------------|-------------|-----------|---|
| M2024-2484 | 4 | ВСК | AM 27 Blood THC Quant by LC-QQQ |
| M2024-2682 | 2 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |
| M2024-2846 | 2 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |
| P2024-1446 | 1 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |
| P2024-1831 | 1 | BLOOD | AM 27 Blood THC Quant by LC-QQQ |
| P2024-1832 | 1 | BLOOD | AM 27 Blood THC Quant by LC-QQQ |
| P2024-1859 | 3 | ВСК | AM 27 Blood THC Quant by LC-QQQ |
| P2024-1873 | 1 | ВСК | AM 27 Blood THC Quant by LC-QQQ |
| P2024-1947 | 1 | ВСК | AM 27 Blood THC Quant by LC-QQQ |
| P2024-2000 | 3 | ВСК | AM 27 Blood THC Quant by LC-QQQ |
| P2024-2005 | 1 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |
| P2024-2006 | 1 | ВСК | AM 27 Blood THC Quant by LC-QQQ |
| P2024-2018 | 1 | ВСК | AM 27 Blood THC Quant by LC-QQQ |
| P2024-2081 | 2 | UCK | AM 27 Urine Cannabinoids Confirmation by LC-QQQ |
| P2024-2157 | 1 | BCK | AM 27 Blood THC Quant by LC-QQQ |



7/22/2024

AM# 27: Quantitation of THC and Metabolites in Blood and Urine by LC-MS/MS

Extraction Date: 07/22/2024 Plate lot#: 240513 Mobile phase A: 0.1% Formic Acid in LCMS Water Blank Blood Lot: Lampire 24052816 Column: UCT Selectra DA 100 x 2.1mm 3um Analyst: <u>Tamara Salazar</u> Plate Retest Date: 11/13/2024 Mobile phase B: 0.1% Formic acid in Acetonitrile Blank Urine Lot: POC021022 LCMS-QQQ ID: 069901

Pre-Analytic:

- \boxtimes 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. Using a calibrated pipette, pipette 1000µL blood or 1000µL urine in wells of analytical (standards) plate. Pipette ID: 42
- ☑ 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 6. 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) Manifold ID: 067104
- \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 and evaporate to dryness at approx. 35°C. SPE Dry ID: 067103
- ☑ 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.
- ☑ 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|-------------|-----------|--------------|--------------|--------------|-------------|
| A | IS + Cal. 1 | IS + QC_1 | | P2024-1446-1 | P2024-1947-1 | IS + QC_1 |
| В | IS + Cal. 2 | | | M2024-2846-2 | P2024-1873-1 | IS + Cal. 7 |
| С | IS + Cal. 3 | | | M2024-2682-2 | P2024-1859-3 | IS + Cal. 6 |
| D | IS + Cal. 4 | | | Neg Urine | P2024-1832-1 | IS + Cal. 5 |
| E | IS + Cal. 5 | | | P2024-2157-1 | P2024-1831-1 | IS + Cal. 4 |
| F | IS + Cal. 6 | | | P2024-2018-1 | M2024-2484-4 | IS + Cal. 3 |
| G | IS + Cal. 7 | | P2024-2005-1 | P2024-2006-1 | Neg Blood | IS + Cal. 2 |
| Н | IS + QC_1 | | P2024-2081-2 | P2024-2000-3 | IS + QC_1 | IS + Cal. 1 |

15

All wells to contain 100 μl of residual DMSO



Batch resultsD:\MassHunter\Data\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P5-A6 10 7/22/2024 2:55:44 PM Data File Sample Operator Comment MJ QC Control Blood.d MJ QC Control Blood Tamara Salazar 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 results
 D:\MassHunter\Data\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Sample AM 27 Agilent Method.m P5-G5 10 7/22/2024 3:21:58 PM Data File Sample Operator Comment MJ Negative Blood.d MJ Negative Blood Tamara Salazar 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 results
 D:\MassHunter\Data\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) QC AM 27 Agilent Method.m P5-H5 10 7/22/2024 10:47:50 PM Data File Sample Operator Comment Urine QC MJ.d Urine QC MJ Tamara Salazar 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 results
 D:\MassHunter\Data\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Sample AM 27 Agilent Method.m P5-D4 10 7/22/2024 8:10:30 PM Data File Sample Operator Comment Neg Urine MJ.d Neg Urine MJ Tamara Salazar 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

| Batch results Last Cal. Update Analyst Name | D:\MassHunter\Da 7/26/2024 10:50 A ISP\Datastor | a\2024∖AM 27 28∖ M | 072224 AN | /I 27 28 TS | QuantResul | ts\AM 27.batch | ı.bin | | |
|---|--|---------------------------------------|-----------|-------------|------------|--------------------|--------------|--|--|
| Analyte | THC | | | Interna | Standard | THC-D3 | THC-D3 | | |
| THC - 7 Levels, 7 Let $y = 0.009$ 1^{-1} $R^2 = 0.$ $R^2 = 0.$ $R^2 = 0.$ Type:Line 0.9^{-1} 0.8^{-1} 0.7^{-1} 0.6^{-1} 0.4^{-1} 0.3^{-1} 0.2^{-1} 0.1^{-1} 0^{-1} | vels Used, 7 Points 9659 * x - 0.00250 99950437 ear, Origin:Ignore, | s, 7 Points Used, 09 Weight:1/x | , 2 QCs | | 70 9 | | | | |
| 0 | 10 20 | 30 40 | 50 | 60 | 70 8 | 0 90 Relative C | oncentration | | |
| | | | | | | | | | |

| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|---------------------------|---------------------|----------|
| Cal 1 MJ | 1 | ~ | 1.0 | 1.1 | 109.3 |
| Cal 2 MJ | 2 | ~ | 3.0 | 2.9 | 97.8 |
| Cal 3 MJ | 3 | ~ | 5.0 | 4.9 | 98.3 |
| Cal 4 MJ | 4 | ~ | 10.0 | 9.6 | 96.0 |
| Cal 5 MJ | 5 | ~ | 25.0 | 24.7 | 98.8 |
| Cal 6 MJ | 6 | ~ | 50.0 | 49.0 | 98.0 |
| Cal 7 MJ | 7 | ~ | 100.0 | 101.8 | 101.8 |
| | | | | | |



AM #27 Cannabinoids Quant. Calibration Curve Report

| Batch results Last Cal. Update | D:\MassHunter\Data\2024\AM 27 28\ 7/26/2024 10:50 AM | 072224 AM 27 28 TS\QuantResult | s\AM 27.batch.bin |
|--|--|--------------------------------|-------------------|
| Analyte | THC-COOH | Internal Standard | THC-COOH-D9 |
| THC-COOH - 7 Levels 33 - y = 0.006 $R^2 = 0.9$ $R^2 = 0.9$ Type:Line | s, 7 Levels Used, 7 Points, 7 Point 067 * x - 0.003359 99977093 ar, Origin:Ignore, Weight:1/x | s Used, 2 QCs | • |



| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|---------------------------|---------------------|----------|
| Cal 1 MJ | 1 | ~ | 5.0 | 5.2 | 104.8 |
| Cal 2 MJ | 2 | ~ | 10.0 | 9.7 | 96.9 |
| Cal 3 MJ | 3 | ~ | 20.0 | 19.9 | 99.3 |
| Cal 4 MJ | 4 | ~ | 50.0 | 49.7 | 99.4 |
| Cal 5 MJ | 5 | ~ | 75.0 | 73.3 | 97.7 |
| Cal 6 MJ | 6 | ~ | 100.0 | 101.6 | 101.6 |
| Cal 7 MJ | 7 | ~ | 250.0 | 250.6 | 100.2 |



AM #27 Cannabinoids Quant. Calibration Curve Report

| | | | | | noius | s Quui | | andat | | | 1 CPOI | L | |
|---|--|--|---|---|--------------------------|----------|--------|-----------|-------------------|---------|------------------|-----------------------|---------|
| Batch resultsD:\MassHunter\Data\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.binLast Cal. Update7/26/2024 10:50 AMAnalyst NameISP\Datastor | | | | | | | | | | | | | |
| Analyte | | | THC-OF | ТНС-ОН | | | | Intern | Internal Standard | | | THC-OH-D3 | |
| Relative Responses | 2-OH - 1.6- 1.4- 1.2- 0.8- 0.6- 0.4- 0.2- 0- | 7 Levels, y = 0.01 R^2 = 0 Type:Lin | 7 Levels I 6323 * x 9.9998332 lear, Origin | Used, 7 P - 0.0053 0 n:Ignore, 20 | oints, 7 97 Weight | Points U | sed, 2 | QCs 60 | 70 | 80 R | 90 Selative C | • 100 Concentra | tion |
| | | Sampla | | Lov | | Enchlo | 4 | Expected | Final | Canaan | tration | A | |

| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|----------|-------|---------|---------------------------|---------------------|----------|
| Cal 1 MJ | 1 | ~ | 1.0 | 1.1 | 105.7 |
| Cal 2 MJ | 2 | V | 3.0 | 3.0 | 99.7 |
| Cal 3 MJ | 3 | V | 5.0 | 4.9 | 97.4 |
| Cal 4 MJ | 4 | ~ | 10.0 | 9.8 | 98.4 |
| Cal 5 MJ | 5 | ~ | 25.0 | 24.6 | 98.5 |
| Cal 6 MJ | 6 | V | 50.0 | 49.6 | 99.3 |
| Cal 7 MJ | 7 | v | 100.0 | 101.0 | 101.0 |



Batch resultsD:\MassHunter\Data\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P5-H6 10 7/22/2024 1:10:36 PM Data File Sample Operator Comment Cal 1 MJ.d Cal 1 MJ Tamara Salazar 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\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P5-G6 10 7/22/2024 1:23:55 PM Data File Sample Operator Comment Cal 2 MJ.d Cal 2 MJ Tamara Salazar 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 results
 D:\MassHunter\Data\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P5-F6 10 7/22/2024 1:37:02 PM Data File Sample Operator Comment Cal 3 MJ.d Cal 3 MJ Tamara Salazar 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 results
 D:\MassHunter\Data\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.bin

 Calibration Last Update
 7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P5-E6 10 7/22/2024 1:50:09 PM Data File Sample Operator Comment Cal 4 MJ.d Cal 4 MJ Tamara Salazar 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\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P5-D6 10 7/22/2024 2:03:16 PM Data File Sample Operator Comment Cal 5 MJ.d Cal 5 MJ Tamara Salazar 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\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P5-C6 10 7/22/2024 2:16:24 PM Data File Sample Operator Comment Cal 6 MJ.d Cal 6 MJ Tamara Salazar 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\2024\AM 27 28\072224 AM 27 28 TS\QuantResults\AM 27.batch.binCalibration Last Update7/26/2024 10:50:21 AM

Instrument Type Acq. Method Sample Position Injection Volume Acq. Date-Time Sample Info. Falco (069901) Cal AM 27 Agilent Method.m P5-B6 10 7/22/2024 2:29:31 PM Data File Sample Operator Comment Cal 7 MJ.d Cal 7 MJ Tamara Salazar 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.

