1.0 Purpose

This procedure provides guidelines for general use, routine maintenance, and performance qualification of the Agilent Ultivo LC-MS system.

2.0 Scope

This procedure applies to the Agilent Ultivo LC-MS system used in the QC Laboratory at Ion Labs.

3.0 Responsibility

3.1 It is the responsibility of QC Laboratory analysts to follow the guidelines for general use of the Agilent Ultivo LC-MS system.

3.2 QC Laboratory Management and/or AD personnel are responsible for ensuring analysts follow the guidelines set forth herein.

3.3 It is the responsibility of QC Laboratory Management, AD personnel, and/or outside contractors to perform maintenance and qualification of the Agilent Ultivo LC-MS.

3.4 It is the responsibility of QC Laboratory Management and/or AD personnel to keep this SOP current with the latest Ion Labs Practices.

4.0 Definitions

4.1 **QC** – Quality Control

4.2 **AD** – Analytical Development

4.3 **LC** – Liquid Chromatography

4.4 **MS** – Mass Spectrometry

4.5 **HPLC** – High Performance Liquid Chromatography

4.6 **ESI** – Electrospray Ionization

4.7 **QQQ** – Triple Quadrupole Mass Spectrometer

4.8 **MRM** – Multiple Reaction Monitoring

4.9 **ISTD** – Internal Standard

4.10 **PTFE** – Polytetrafluoroethylene

4.11 **DA** – Data Analysis

4.12 **IQ** – Installation Qualification

4.13 **OQ** – Operational Qualification

4.14 **PQ** – Performance Qualification

5.0 References

5.1 D-602, SOP, Labeling and Expiration Dating of Laboratory Chemicals

5.2 D-603, SOP, Chemical Waste Disposal

5.3 D-807, SOP, HPLC Operation, Maintenance, and Qualification.

5.4 Agilent Ultivo Triple Quadrupole LC-MS Concepts Guide

5.5 Agilent Ultivo Triple Quadrupole LC-MS System Quick Start Guide

5.6 Agilent MassHunter Workstation Software Quantitative Analysis Familiarization Guide

6.0 Safety Precautions

6.1 Solvents used as mobile phases are toxic and flammable. The minimum required personal protective equipment includes safety glasses, gloves, and enclosed shoes. Ensure that solvent reservoirs and waste containers are gas-tight.

6.2 HPLC systems operate at high pressure. Compressed liquids may cause eye injury if a sudden leak occurs.

6.3 The spray chamber drain tube and exhaust tube must be vented externally to the building (e.g. into a fume hood).

6.4 The vacuum pump exhaust tube must be vented externally to the building (e.g. into a fume hood).

6.5 Electrical faults could cause electrocution, explosion or fire. If an electrical fault is suspected, disconnect power from the instrument and have it serviced by a qualified individual.

7.0 Waste Handling and Disposal

7.1 Waste handling and disposal procedures are outlined in SOP D-603 Chemical Waste Disposal.

8.0 General Guidelines

8.1 For guidance on general use and maintenance of HPLC systems, consult SOP D-807 HPLC Operation, Maintenance, and Qualification Documentation.

8.2 The following parameters should be recorded in the laboratory notebook:

8.2.1 Instrument Ion Number

8.2.2 Instrument Cal Due Date

8.2.3 Column identifier

8.3 Use only LC-MS grade solvents and reagents. The use of solvents and reagents of lower purity will result in high background and decreased sensitivity.

8.4 Use only volatile mobile phase additives such as formic acid, acetic acid, ammonium formate, ammonium carbonate, triethylamine, and tridecafluoroheptanoic acid.

8.5 The bench that the Ultivo system sits on must be sturdy enough to hold the weight of the mass spectrometer and HPLC system (up to 275 lbs).

8.6 Do not attempt to move the mass spectrometer or HPLC system.

8.7 Ensure sufficient space for ventilation (min 6 inches on all sides).

8.8 The drain bottle should sit below the mass spectrometer and be connected to the ion source by a hose.

8.9 Make sure the leak drain connector on the lower right side of the mass spectrometer connects to a drain bottle.

8.10 Nitrogen (minimum 95.0% pure) is the only acceptable drying and nebulizing gas. Use of air, oxygen, or other gases, when combined with volatile solvents and high voltages in the spray chamber, can result in an explosion. Use of air, oxygen, or other gases also causes deterioration of parts in the Ultivo LC/TQ and negatively impacts instrument operation and sensitivity. Less than 0.1 ppm of hydrocarbons with the remaining gas must be oxygen and trace argon. At least 3 L/min is required at all times to prevent air from entering the mass spectrometer.

8.11 The stack on top of the mass spectrometer should not be taller than 30 inches. Make sure that solvent bottles at the top of the stack can be reached safely.

8.12 The spray chamber exhaust must not have positive pressure. Positive pressure in the spray chamber exhaust tubing and drain bottle can affect instrument performance and can contribute to excessive background contaminant levels.

8.13 The spray chamber exhaust and foreline pump exhaust must be vented with separate lengths of exhaust hose. These hoses can be connected into a common exhaust manifold. The separation of the exhaust minimizes the chances of foreline pump fluid vapor entering the spray chamber when drying gas is not flowing.

8.14 If a negative pressure vent is not available, the length of the tubing from the foreline pump and the drain bottle to the vent should each not exceed 460 cm (15 ft).

8.15 Exhaust gas venting must comply with all local environmental codes. Health hazards include chemical toxicity of solvents, samples, buffers, pump fluid vapor, and aerosolized biological samples.

8.16 Operating environment

8.16.1 Mass Spectrometer Electrical Supply: 200 to 240 Vac, 50/60 Hz

8.16.2 Mains supply voltage: Fluctuations not to exceed 10% of nominal supply voltage. Excessive fluctuations in the voltage of the power supply can create a shock hazard and can damage the instrument.

8.16.3 1260 HPLC system = 100-120 VAC with 15A supply circuit rating

8.16.4 Do not use extension cords with the LC-MS instrument. Extension cords cannot supply enough power and can present a safety hazard.

8.16.5 Operating Temperature: 15°C to 35°C (59°F to 95°F)

8.16.6 Humidity: < 85% RH at 35°C, non-condensing, non-corrosive atmosphere

9.0 Startup and Shutdown

9.1 System Startup

9.1.1 Perform this procedure to bring the system from the powered off state to standby mode.

9.1.2 The instrument should be properly installed with all power cables and gas lines connected.

9.1.3 Turn on power to the computer and monitor.

9.1.4 Check that the nitrogen gas supply is on and the supply pressure is 80 – 100 psi.

9.1.5 Turn on the front power switch of the LC-MS instrument. The vacuum system automatically starts to pump down the instrument and the electronics are turned on.

9.2 System On / System Standby

9.2.1 Perform this procedure when you need to switch between On and Standby modes. In Standby mode, source temperatures and gas flows are lowered but remain on to avoid contamination of the instrument. Standby mode is appropriate if the instrument will not be in use for up to one week.

9.2.2 Click the Data Acquisition icon on the desktop OR click Start > Agilent > Data Acquisition.

9.2.3 In the Instrument Status pane, hover the cursor over the QQQ module. An On switch and an Off switch will appear.

9.2.4 Select the appropriate switch to turn the system On or put it into Standby (Off).

9.3 System Shutdown

9.3.1 Perform this procedure when you need to turn the instrument off for an extended period of time or when maintenance will be performed that requires a vacuum shutdown.

9.3.2 Click the Data Acquisition icon on the desktop OR click Start > Agilent > Data Acquisition.

9.3.3 In the Instrument Status pane, right-click on the QQQ module and select Vent.

9.3.4 When the instrument has vented completely (High Vac reads 760 torr in the Actuals pane), toggle the power switch on the front of the instrument to turn it off.

10.0 Procedure for General Use

10.1 Click the Data Acquisition icon on the desktop OR click Start > Agilent > Data Acquisition.

10.2 The main window consists of multiple panes. You can show or hide a pane by using the commands in the View menu. You can drag a pane border to resize the pane. If you double-click the title bar of a pane, the pane "floats" outside of the main window. You can double-click the title bar again to "dock" the pane.

10.2.1 Instrument Status Pane

10.2.1.1 Shows the status of each device configured with the instrument (Error, Not Ready, Pre-run or Post-run, Running or Injecting, Idle, Offline, and Standby).

10.2.1.2 From the Status Pane, you can also set non-method control and configuration parameters for the LC devices and the MS instrument by using the right-click menu.

10.2.2 Actuals Pane

10.2.2.1 This pane shows the current value of selected instrument parameters. The parameters to be displayed can be configured using the right-click menu.

10.2.3 Chromatogram Plot Pane

10.2.3.1 This pane shows the chromatogram plot in real time.

10.2.3.2 Instrument parameters can also be plotted in this pane.

10.2.3.3 The plots that are displayed can be configured in the QQQ tab in the Method Editor window.

10.2.4 Spectrum Pane

10.2.4.1 This pane shows the spectral plot in real time when you are acquiring data or tuning the instrument.

10.2.4.2 A separate tab is added for each detector configured on the instrument.

10.2.5 Method Editor Pane

10.2.5.1 In this pane, the acquisition parameters for the method can be set up.

10.2.5.2 Checktune or Autotune can be run from the Autotune section of this pane.

10.2.6 Sample Run Pane

10.2.6.1 This pane allows the analysis of a single sample.

10.2.7 Worklist Pane

10.2.7.1 This pane allows the analysis of multiple samples.

10.3 Prepare the LC modules:

10.3.1 Switch the LC stream to Waste.

10.3.1.1 In the Instrument Status pane, right click the QQQ device.

10.3.1.2 Select LC > Waste

10.3.2 Purge the LC pump and begin equilibrating the LC system to initial conditions of the method as outlined in D-807.

10.4 In the Actuals pane, set up to view real-time parameter values:

10.4.1 Right-click the Actuals pane, and select the Setup command.

10.4.2 Add all parameters that you would like to monitor.

10.4.3 If desired, limits can be set for each parameter. If the parameter is outside of the set limits, the background of the parameter will turn red.

10.5 In the Chromatogram Plot pane, setup the signals to be monitored in real-time.

10.5.1 Right-click the chromatogram plot, and click Change.

10.5.2 In the Edit Signal Plot dialog box, select the desired display signal.

10.6 Perform Checktune or Autotune

10.6.1 Checktune should be performed at least once per week.

10.6.2 Autotune should be performed at least once per month.

10.6.3 Click the QQQ tab in the Method Editor window.

10.6.4 Click the Autotune section in the left pane of the QQQ tab.

10.6.5 Click the icon in the Autotune toolbar which locks the instrument for tuning.

10.6.6 Click the icon to perform checktune. Checktune takes approximately 3 minutes to run for each polarity.

10.6.7 Click the icon to perform autotune. Autotune takes approximately 30 minutes.

10.6.8 After checktune or autotune is complete, a tune report will be generated.

10.6.9 If both Checktune and Autotune fail, check the progress box in the Tune status list to see why the tune failed. Then, either correct the problem or contact Agilent for technical support or service.

10.6.10 Click the icon to unlock the instrument from Tune control.

10.7 Set up the Acquisition Method

10.7.1 At the top of the Method Editor pane, select DA.

10.7.1.1 Click the Qual tab.

10.7.1.1.1 Ensure that the Qual Automation checkbox is not selected.

10.7.1.2 Click the Quant tab.

10.7.1.2.1 Ensure that the Quant Automation checkbox is not selected.

10.7.2 At the top of the Method Editor pane, select the Pump tab.

10.7.2.1 Enter the flow rate.

10.7.2.2 Enter the initial mobile phase conditions.

10.7.2.3 Enter the pressure limits.

10.7.2.4 Enter the Stoptime.

10.7.2.5 Select Posttime > Off

10.7.2.6 Enter parameters for each step of the mobile phase gradient in the Timetable.

10.7.3 At the top of the Method Editor pane, Select QQQ.

10.7.3.1 On the left, select Method > Acquisition.

10.7.3.1.1 For each required time segment, select the Scan Type.

10.7.3.1.2 For each required time segment, enter all Acquisition Parameters specified in the analyte specific method.

10.7.3.2 On the left, select Method > Chromatograms.

10.7.3.2.1 Enter information for each chromatogram plot that should be displayed in the Chromatogram Plot pane.

10.7.3.3 On the left, select Method > Timetable.

10.7.3.3.1 It is often useful to divert flow from the LC to waste when no peaks are eluting. This is done by entering the desired time in the timetable, and selecting Type > Diverter and then Value > To MS or To Waste.

10.7.3.3.2 To switch flow from the LC to waste at the end of the run: select the "Post-run diverter position" check box, and "To waste".

10.7.4 Save the method.

10.7.4.1 All files related to a run (acquisition method, quantitation method, individual data files, report templates, etc.) should be stored under the D:\MassHunter\Data directory in analyte specific folders and organized by date.

10.7.4.2 Click Method > Save As or Method > Save.

10.8 Set up and run the samples.

10.8.1 In the Sample Run pane, click the Worklist tab at the bottom.

10.8.2 Right-click the upper left corner of the worklist.

10.8.3 Click Add Multiple Samples.

10.8.4 Click the Sample Information tab, and enter the required information.

10.8.5 Click the Sample Position tab, and specify the sample vial locations (ensure the correct sample tray type has been configured by right-clicking the autosampler device image).

10.8.6 Right-click the upper left corner of the worklist, and select Worklist Run Parameters.

10.8.7 On the Run Parameters tab, enter the correct path for the method.

10.8.8 Click the Data File Settings tab.

10.8.9 Select the folders where the data files should be saved.

10.8.10 Select the file naming options.

10.8.11 Click the Advanced Parameters tab, review the information, and click OK.

10.8.12 To start the run click the  or  icons, or select Worklist > Run.

10.9 After the run has completed, prepare the column for storage as outlined in D-807.

10.10 Process the data.

10.10.1 Click the Quantitative Analysis  icon on the Desktop or start the program by selecting Programs > Agilent > MassHunter Workstation > Quantitative Analysis (QQQ)

10.10.2 Click File > New Batch.

10.10.3 Navigate to the folder that contains the data to be processed, and enter a name for the new batch.

10.10.4 Click Create Batch.

10.10.5 The Add Samples dialog box will appear.

10.10.6 Select the samples to be processed, and then click OK.

10.10.7 If a Quantitation Method already exists for the type of analysis being performed, open the method and adjust settings as necessary.

10.10.7.1 Click Method > Open.

10.10.7.2 Select Open Method from Existing Batch.

10.10.7.3 Select the batch containing the existing quantitation method, and then select Open.

10.10.7.4 In the Method Tasks pane, select Retention Time Setup. In the Method Table, adjust the retention time for each analyte-of-interest.

10.10.7.5 In the Method Tasks pane, select Concentration Setup. Ensure that the standard concentrations listed in the Method Table are correct, or adjust as needed.

10.10.7.6 For existing methods, the integration parameters should generally not require adjustment. If necessary, adjust integration parameters from the Method Tasks pane under Advanced Tasks → Integration Parameters Setup.

10.10.7.7 After the quantitation method has been set up, select Exit from the Method Tasks pane.

10.10.7.8 Proceed to Section 10.10.9.

10.10.8 Generate a Quantitation Method:

10.10.8.1 Click Method > Edit.

10.10.8.2 At the top of the Quantitative Analysis window, click New, and select New Method from Acquired MRM Data.

10.10.8.3 In the Dialog box, select a data file corresponding to a standard with high concentration of all target analytes, and click Open.

10.10.8.4 Evaluate the automatically generated Quantitation Method for completeness/accuracy, and edit as required.

10.10.8.4.1 Under Method Setup Tasks in the left sidebar, click MRM Compound Setup.

- For each qualifier ion listed in the Method Table: select the row containing the qualifier ion, right click the row, and select delete. Select Yes to delete the qualifier ion.
- For each quantifier ion listed in the Method Table: select the row containing the quantifier ion, right click the row, and select New Qualifier.
- Select the correct product ion for the qualifier mass transition.
- Adjust the Rel.Resp. until the ratio displayed in the Compound Information pane is near 100%.
- Ensure that the Uncertainty listed for the Qualifier Ion is correct. A value of 20 is typical.

10.10.8.4.2 Under Method Setup Tasks in the left sidebar, click Method Setup Tasks > Retention Time Setup.

- For each analyte, click the corresponding row, and ensure that the analyte retention times and deltas are correct.

10.10.8.4.3 Under Method Setup Tasks in the left sidebar, click Method Setup Tasks > ISTD Setup.

- For each target analyte, ensure that the ISTD is correct. If not correct, click the down arrow in the ISTD Compound Name cell and select the correct ISTD.

- For each ISTD, enter the ISTD concentration. If the concentration is the same for all samples, a value of 1 is sufficient.

10.10.8.4.4 Under Method Setup Tasks in the left sidebar, click Method Setup Tasks > Concentration Setup.

- For the first target analyte, select the corresponding row, right click the row, and select New Calibration Level.
- Enter the level name (typically L1, L2, etc.) and enter the level concentration in units appropriate to generate the final result in the correct units (generally mcg/mL). If the product formula contains the target analyte in a form that is different from the reference standard (e.g. the formula contains thiamine mononitrate while the reference standard is thiamine hydrochloride), the number entered should reflect the working standard concentration in terms of the form that is present in the product profile. Calculation of the working standard concentration should be documented in the laboratory notebook.
- Repeat for each required calibration level.
- If other analytes share the same concentration levels, click Method > Copy Calibration Levels To …, then select the analytes to copy the levels to, then click OK.
- If other analytes have different concentration levels, repeat the process for each analyte.
- Select the correct units for each analyte in the Units column.

10.10.8.4.5 Under Method Setup Tasks in the left sidebar, click Method Setup Tasks > Calibration Curve Setup.

- Inspect and/or correct the CF, CF Origin, and CF Weight columns.

10.10.8.4.6 Under Advanced Tasks in the left sidebar, click Advanced Tasks > Integration Parameters Setup.

- In the method table, select the row for each analyte and ensure that the peak is properly integrated by viewing the chromatogram in the Compound Information pane. If necessary, correct the integration by adjusting the Int. and Int. Params. settings.

10.10.8.5 Click Validate to validate the method setup, and correct any errors or warnings that are found.

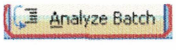
10.10.8.6 If no errors or warnings are found, click Exit.

10.10.8.7 Click Yes to apply the method to the batch.

10.10.9 Enter the sample amount for each sample in the Amt. column of the batch table. Units should be appropriately selected to generate the final result in the correct units.

10.10.10 Enter the sample dilution for each sample in the Dil. column of the batch table. Units should be appropriately selected to generate the final result in the correct units. If multiple dilutions were performed, this value should include all dilutions. For example, if the sample was dissolved in 50 mL and then diluted 1/10, the dilution would be 0.5 L.

10.10.11 Enter the weight of a single dosage unit from the product profile for each sample in the Tot. Amt. column of the Batch Table. Units should be appropriately selected to generate the final result in the correct units.

10.10.12 Click the 

icon to start data processing.

10.10.13 After processing is complete, click File > Save Batch.

10.11 Review the integration

10.11.1 Click the first row of the Batch Table.

10.11.2 Review the integration for the current analyte by viewing the chromatogram in the Compound Information pane.

10.11.3 Use the Next Sample icon ∨ at the top of the batch table to review integration for the current analyte in all samples.

10.11.4 Use the Next Compound icon > at the top of the batch table to review integration for all analytes.

10.11.5 If any chromatograms are not properly integrated, return to Integration Parameters Setup (Section 10.10.8.4.6) to make adjustments.

10.12 Review the Calibration

10.12.1 After processing, the batch table will be highlighted with flags and/or colors to indicate lines that contain any problem. Hover the cursor over the highlighted cell to show an explanation of the problem.

10.12.2 Make sure the Batch Table is set to single compound display mode by clicking the Single Compound/Sample View icon above the batch table.

10.12.3 For each calibration injection, change the sample type to Cal.

10.12.4 For each calibration injection, select the correct level name by clicking the drop down box in the Level column.

10.12.5 Click Home > Analyze Batch.

10.12.6 Review the calibration curve for each analyte.

10.12.7 Ensure that the coefficient of determination (R^2) for the calibration of each analyte meets the acceptance criteria listed in the test method.

10.12.8 Click Next Compound in the batch toolbar to cycle through each analyte.

10.13 Generate a report

10.13.1 Near the top of the screen on the Home tab, select Edit Report Method.

10.13.2 Click Add Template, and select the desired report template.

10.13.3 Click Save and Exit, then enter a filename to save the method as.

10.13.4 Click Generate Report.

10.13.5 Select the QuantReports folder.

10.13.6 Select the Report Method that was previously saved.

10.13.7 Press OK.

11.0 Preventative Maintenance

11.1 Refer to D-807 HPLC Operation, Maintenance, and Qualification for preventative maintenance of the HPLC portion of the LC-MS system.

11.2 Refer to the electronic help, which can be accessed from the Windows Start Menu by selecting Agilent > Ultivo LC-TQ Resources and then choosing Mass Spectrometer > Maintenance Guide, for instructions on how to perform regular maintenance on the LC-MS system.

12.0 Consumables

12.1 ESI-L Calibrant Solution, Agilent Part # G1969-85000

12.2 Abrasive mesh, Agilent Part # 8660-0827

12.3 Cotton swabs, Agilent Part # 5080-5400

12.4 SW60 foreline pump oil, Agilent Part # 6040-1361

12.5 Capillary 90, Agilent Part # G6303-80004

12.6 Canted Coil Spring, Agilent Part # G1460-2571

12.7 ¼-inch ID front capillary seal, Agilent Part # 0905-1475

12.8 Agilent Jet Stream heater replacement kit, Agilent Part # G1958-68000

12.9 Nebulizer (needle SS316 replacement) kit for Agilent Jet Stream, Agilent Part # G1958-60137.

13.0 Performance Qualification

13.1 The following tests are recommended for annual performance qualification (PQ) of the Ultivo mass spectrometer. For guidance on PQ of the HPLC system connected to the mass spectrometer, refer to D-807 HPLC Operation, Maintenance, and Qualification.

13.2 Supplies needed

13.2.1 OQPV Sulfa Standard for LCMS (Agilent Part No. 5190-0580)

13.2.2 ESI-L Low Concentration Tuning Mix (Agilent Part No. G1969-85000)

13.2.3 Methanol (LC-MS grade)

13.2.4 Water (≥18MΩ•cm)

13.2.5 C18 HPLC column (select dimensions to result in acceptable peak shape and separation of all components in the Sulfa Standard)

13.3 Vacuum Verification

13.3.1 A stable, high vacuum is required for high-sensitivity mass spectrometry.

13.3.2 Passing the vacuum verification is a pre-requisite for all other PQ tests listed in this procedure.

13.3.3 With the instrument in the Standby state, take five readings of the QQQ High Vac parameter over the course of 10 minutes. The parameter is located in the MassHunter Data Acquisition application in the Actuals pane.

13.3.4 Acceptance Criteria:

13.3.4.1 The mean High Vac reading is NMT 5.0×10^{-5} torr.

13.4 Mass Accuracy Verification

13.4.1 The built-in autotune function is performed to ensure proper mass accuracy of the mass spectrometer.

13.4.2 Perform autotune of the instrument:

13.4.2.1 Ensure that there is sufficient ESI-L Low Concentration Tuning Mix in the bottle located at the front right side of the mass spectrometer.

13.4.2.2 In the method editor pane, click on the QQQ tab.

13.4.2.3 Select Tune → Autotune.

13.4.2.4 Click the icon in the Autotune toolbar which locks the instrument for tuning.

13.4.2.5 Click the icon to perform autotune. Autotune takes approximately 30 minutes.

13.4.2.6 After autotune is complete, a tune report will be generated.

13.4.2.7 Click the icon to unlock the instrument from Tune control.

13.4.3 Acceptance Criteria:

13.4.3.1 Positive Mode:

13.4.3.1.1 Mass 1 = 117.89 – 118.29

13.4.3.1.2 Mass 2 = 321.85 – 322.25

13.4.3.1.3 Mass 3 = 621.83 – 622.23

13.4.3.1.4 Mass 4 = 921.81 – 922.21

13.4.3.1.5 Mass 5 = 1221.79 – 1222.19

13.4.3.2 Negative Mode:

13.4.3.2.1 Mass 1 = 112.79 – 113.19

13.4.3.2.2 Mass 2 = 301.80 – 302.20

13.4.3.2.3 Mass 3 = 601.78 – 602.18

13.4.3.2.4 Mass 4 = 1033.79 – 1034.19

13.4.3.2.5 Mass 5 = 1333.77 – 1334.17

13.5 Instrument Method Parameters

13.5.1 For the remaining tests, set up an acquisition method with the following parameters:

13.5.2 Sampler

13.5.2.1 Injection Volume: 1 µL

13.5.2.2 Enable Needle Wash: Selected

13.5.2.3 Mode: Flush Port

13.5.2.4 Time: 10 sec

13.5.3 Binary Pump

13.5.3.1 Flow Rate: adjust to result in pressure of 100 bar – 250 bar and retention time less than 1 minute depending on the column used.

13.5.3.2 Mobile Phase: H_2O/methanol (25/75)

13.5.3.3 Stoptime: 1.0 min

13.5.4 Column Oven

13.5.4.1 Temperature: 40 °C

13.5.5 QQQ

13.5.5.1 Acquisition

13.5.5.1.1 Ion Source: AJS ESI

13.5.5.1.2 Scan type: MRM

13.5.5.1.3 Polarity: Positive

13.5.5.1.4 Acquisition Parameters

Analyte	**ISTD**	**RT (min)**	**RT Window (min)**	**MS1 Res**	**Precursor (m/z)**	**MS2 Res**	**Product (m/z)**	**Dwell (ms)**	**Frag (V)**	**CE (V)**
sulfadimethoxine	no	0.5	1.0	Unit	311.0	Unit	156.0	200	150	24

13.5.5.2 Source Parameters

13.5.5.2.1 Ion Source: AJS ESI

13.5.5.2.2 Gas Temperature: 250 °C

13.5.5.2.3 Gas Flow: 12.0 L/min

13.5.5.2.4 Nebulizer Pressure: 35 psi

13.5.5.2.5 Sheath Gas Temperature: 400 °C

13.5.5.2.6 Sheath Gas Flow: 11.0 L/min

13.5.5.2.7 Capillary Voltage (Positive Setpoint): 3000 V

13.5.5.2.8 Nozzle Voltage (Positive Setpoint): 0 V

13.6 Linearity

13.6.1 The linearity test uses the Sulfa Standard to evaluate response linearity.

13.6.2 Connect a suitable column, and equilibrate the LC system with H_2O/methanol (25/75) and at a flow rate that results in pressure in the range 100 bar – 250 bar.

13.6.3 Perform at least three blank injections followed by a single 1 µL injection at each of five concentrations: 0.0100, 0.0250, 0.100, 0.500, and 1.00 mcg/mL.

13.6.4 Plot peak area of the sulfadimethoxine peak (m/z 156) versus concentration.

13.6.5 Perform linear regression of the data using the method of least-squares.

13.6.6 Acceptance Criteria:

13.6.6.1 The coefficient of determination (R^2) is NLT 0.980.

13.7 Precision

13.7.1 The precision test uses the Sulfa Standard to evaluate instrument precision.

13.7.2 Connect a suitable column, and equilibrate the LC system with H_2O/methanol (25/75) and at a flow rate that results in pressure in the range 100 bar – 250 bar.

13.7.3 Perform at least three blank injections followed by six replicate 1 µL injections of the 0.0100 mcg/mL Sulfa Standard.

13.7.4 Measure the peak area of the sulfadimethoxine (m/z 156) peak in the replicate injections.

13.7.5 Acceptance Criteria:

13.7.5.1 The %RSD of the sulfadimethoxine peak area in the six replicate injections is NMT 10.0%.

13.8 Carry-Over

13.8.1 The carry-over test uses the Sulfa Standard to evaluate instrument carry-over.

13.8.2 Connect a suitable column, and equilibrate the LC system with H_2O/methanol (25/75) and at a flow rate that results in pressure in the range 100 bar – 250 bar.

13.8.3 Perform at least three blank injections followed by a single 1 µL injection of the 1.00 mcg/mL Sulfa Standard and then a blank injection.

13.8.4 Calculate the amount of carry-over by dividing the peak area of the sulfadimethoxine peak (m/z 156) measured in the blank injection by the peak area of the sulfadimethoxine peak measured in the 1.00 mcg/mL Sulfa Standard. Multiply the carry-over result by 100 to obtain the value in percent format.

13.8.5 Acceptance Criteria:

13.8.5.1 Carry-over is NMT 1.00%.

13.9 Signal-to-Noise

13.9.1 The signal-to-noise test uses the Sulfa Standard to evaluate signal-to-noise.

13.9.2 Connect a suitable column, and equilibrate the LC system with H_2O/methanol (25/75) and at a flow rate that results in pressure in the range 100 bar – 250 bar.

13.9.3 Perform at least three blank injections followed by a single 1 µL injection of the 0.01 mcg/mL Sulfa Standard.

13.9.4 Calculate the signal-to-noise ratio for the sulfadimethoxine peak (m/z 156) using MassHunter software.

13.9.5 Acceptance Criteria:

13.10 Signal-to-Noise is NLT 1000.

14.0 Revision History

Revision	Date	Description of Changes	CCR #	By
0	04/05/21	New Document	N/A	S. Sassman
1	10/28/21	Add performance qualification section, remove things that don't apply to system, edit for clarification of meaning.	CC-21-0401	S. Sassman