Section Five
Toxicology Quality Assurance

5.13 LCMS-QQQ Instrument Maintenance and Operation

5.13.1 BACKGROUND

Recent instrument improvements have led to the accepted use of Liquid Chromatography Mass Spectrometry Mass Spectrometry use in the toxicology field.

5.13.2 SCOPE

This method is used for maintenance and operational instructions for the Liquid Chromatograph Mass Spectrometer Mass Spectrometer (LC-QQQ or LC Triple Quad) used by the Idaho State Police Forensic Services Toxicology Section.

5.13.3 EQUIPMENT AND SUPPLIES

5.13.3 Agilent 6410B LC/MS/MS system and Mass Hunter software

5.13.4 REAGENTS

5.13.4.1 0.1% formic acid in water (mobile phase A)
5.13.4.2 0.1% formic acid in acetonitrile (mobile phase B)
5.13.4.3 LCMS Tuning Solution
5.13.4.4 De-ionized water
5.13.4.5 LC/MS grade water
5.13.4.6 LC/MS grade acetonitrile
5.13.4.7 LC/MS grade methanol
5.13.4.8 LC/MS grade formic acid

5.13.5 INSTRUMENT MAINTENANCE

5.13.5.1 Refer to Manufacturer's Recommendation for Scheduled Preventative Maintenance. (Note: Preventative Maintenance is not required as long as response and successful tuning is completed).

5.13.6 INSTRUMENT OPERATION

5.13.6.1 **Instrument and run set up**

5.13.6.1.1 Clean the electrospray ion source (if necessary)

5.13.6.1.2 Turn LC/MS/MS on and run Check Tune (May be run in only Positive mode as AM acquisition is for this mode), review tune report. If successful, continue with pre-run instrument start-up. If unsuccessful, perform Autotune then re-run Check Tune.

5.13.6.1.2.1 NOTE: Successful Check Tune must have been run within one week of case sample analysis.

5.13.6.1.3 Add fresh solvent to the solvent bottles (be sure to reset the solvent levels in the acquisition software).

5.13.6.1.4 Run the system using the background check method to evaluate the system. The maximum intensity for any background ion should be < 100,000 area counts, and ideally < 10,000 area counts.

5.13.6.1.5 In MassHunter Acquisition, load the appropriate acquisition method based on the analysis to be run on the instrument (eg. Benzos_Z-Drugs_ACN_FA or Cannabinoids method). Allow column temperature and LC pressure to stabilize. Verify that the binary pump ripple is <1%.

5.13.6.1.6 Open or start a new worklist. Enter the calibrators, blanks, controls and samples as needed.

5.13.6.1.7 Select Worklist then Worklist Run Parameters, and create a Data path for this Batch (e.g. 110808BZ).

5.13.6.1.8 Also in Worklist Run Parameters, select Acquisition Cleanup/Standby, to put the instrument in Standby after the Worklist, or if a Not Ready Timeout occurs.

5.13.6.1.9 Save the Worklist (using Save As to create a new worklist file)

5.13.6.1.10 Allow the instrument to stabilize for at least 15 minutes from the time it is turned ON.

5.13.6.1.11 Begin the Worklist by clicking on the Multiple Vial icon on the top center of the MassHunter Acquisition screen.

5.13.6.2 **Data Analysis**

5.13.6.2.1 Open MassHunter Quantitative Analysis.

5.13.6.2.2 Select File/New Batch.

5.13.6.2.3 Navigate to the MassHunter/Data directory, and open the folder containing the data files for the current Batch. Assign a name to the Batch (e.g. 110808BZ), and select Open.

5.13.6.2.4 Select File/Add Samples, Select All, and OK to add all the samples to the Batch. Any column rinse injections will not contain meaningful results, and can be removed from the Add Samples list.

5.13.6.2.5 Select Method/Open/Open and Apply from Existing File.

5.13.6.2.6 Navigate to the location of the Quantitative Analysis Data Analysis Method (Ex – benzos.quantmethod or cannabinoids.quantmethod), select it, and select Open. In this example, the benzo.quantmethod is stored in the MassHunter/data analysis methods directory.

5.13.6.2.7 When the method has been opened and applied, the Batch Table appearance will change, but the results will not yet be populated.

5.13.6.2.8 Select Analyze Batch, or F5, to complete the Batch analysis, and Save the Batch.

5.13.6.2.9 The Batch Table view will show the Batch Table with results, Compound Information, and the Calibration Curve. Navigation by Compound can be accomplished by using either the arrows or the drop-down menu in the Compound section of the Batch Table.

5.13.6.2.10 To update the retention times and qualifier ion ratios for the current Batch, go to Method/Edit, or use F10, to enter the Method Editor view of MassHunter Quantitative Analysis. Review the retention times and qualifier ion ratios from the calibrators, and make updates as appropriate.

5.13.6.2.10.1 Qualifier Ratios are updated by selecting Update, then Average Qualifier ratios, select the compounds desired and click OK.

5.13.6.2.11 To return to the Batch Table and apply the updated retention times and qualifier ion ratios, save the updated method and select the Exit button, answer Yes, and in the Batch Table select Analyze Batch, or F5.

5.13.6.3 **Batch Review**

5.13.6.3.1 The lab criterion for acceptable calibration curve R^2 will be defined in the appropriate analytical method.

5.13.6.3.2 A **minimum of four calibration points** are required for a valid curve. If the confirmation decision point is removed from the curve, the new administrative cutoff will be the lowest calibrator that meets quality assurance requirements.

5.13.6.3.3 Outliers are highlighted in the Batch Table with the color codes blue and red, for below or above acceptable limits (respectively).

5.13.6.3.4 The default criterion for Accuracy is that each calibrator result should agree with the target value ± 20%.

5.13.6.3.5 The default criteria for a defining a positive result are:

5.13.6.3.5.1 Retention time within ± 5% of the average of the calibrators.

5.13.6.3.5.2 Qualifier ion ratios within ±20% of the average of the calibrators.

5.13.6.3.5.3 The sample must have a concentration greater than the decision point calibrator (see specific levels of reporting defined in the appropriate analytical method).

5.13.6.3.5.4 Inconclusive sample criteria for reporting is defined in the specified analytical method.

5.13.6.3.6 Manual integration should not be needed frequently. When it is needed, it is enabled with the Start/End Manual Integration Tool in the Compound Information section of the Batch Table.

5.13.6.3.7 Manual integration is accomplished by left-clicking and dragging on the black boxes at peak start and end. Spurious peaks can be deleted by selecting the Start/End Manual Integration tool, right clicking in Compound Information, and selecting Zero Peak.

5.13.6.3.8 Review the results for each analyte in the Batch. Check for outliers, R^2 values, and check QC values.

5.13.6.3.9 When Batch review is complete, Save the Batch a second time.

5.13.6.4 **Generating Reports**

5.13.6.4.1 Select Report/Generate and navigate to the report template (Ex – ISP_Summary_07_ LCMS_1Qual), select it, then select OK. Once the report has generated, print it, then select the ISTD template report (Ex – Quant_Report_ISTD_Calibration_B_05_00) and print it. Alternatively, the generated reports may be saved as electronic files (Ex – pdfs) and stored electronically per any requirements in the ISP-FS Quality Manual.

5.13.6.4.2 The Queue Viewer, which allows you to track the report generation process, will open automatically. Depending on the size of the Batch, report generation may take approximately 5-20 minutes.

5.13.7 QUALITY ASSURANCE REQUIREMENTS

5.13.7.1 Refer to toxicology analytical methods 5.8 and 5.10 for additional quality assurance and reference material authentication requirements.

5.13.8 ANALYSIS DOCUMENTATION

5.13.8.1 Case results are to be recorded in the LIMS system.

5.13.8.2 Reports for the batch and controls, if printed, will be stored centrally in the lab in which the analysis was performed. A copy of data for controls may be stored electronically in a central location and need not be included in individual case files. When necessary, a copy of control printouts can be prepared from the centrally stored document.

5.13.8.3 The data from the run will be stored electronically, and if it is on a computer, will be backed up at least every two months.

5.13.9 REFERENCES

5.13.9.1 This method was developed in conjunction with Patrick Friel from Agilent during application training (July 23-26, 2012).

Revision History

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Toxicology Quality Assurance

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Revision No.	Issue Date	Revision/Comments
0	07/26/2016	Original Issue in SOP format.