Toxicology Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

1.0 Purpose - This procedure specifies the required elements for the calibration and use of the Waters Acquity Ultra Pressure Liquid Chromatograph (UPLC) in conjunction with a Waters Xevo TQD.

2.0 Scope – This procedure applies to the Toxicology sections in the Raleigh, Triad, and Western locations of the State Crime Laboratory.

3.0 Definitions

- **Calibration –** The introduction of a substance with a known mass to the mass spectrometer for the purpose of calibrating the mass scale.
- **Molecular ion –** Ion produced when a molecule gains or loses an electron. Also referred to as a parent or precursor ion.
- **Multiple Reaction Monitoring (MRM) –** A specific experiment of a triple quadrupole instrument in which a parent ion is passed through the first quadrupole into a collision cell (second quadrupole) filled with Argon. The parent ion is fragmented and a specific ion is passed through to the third quadrupole for detection.
- **Performance verification –** The initial confirmation of the reliability of a previously or externally validated method or instrument.
- **Product ion –** Ion produced from the intentional collision of a molecular ion with argon in the collision cell. Also referred to as a daughter ion.
- **Quality control (QC) check –** Periodic confirmation of the reliability of equipment, instrumentation, and/or reagents.
- **Tuning –** A method of achieving optimal peak intensities by optimizing the voltages, interface lenses and gas flows for a specific analyte under a set of specific operating conditions.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Waters Xevo TQD with Waters Acquity UPLC consisting of a Column Manager, Autosampler, and Binary Solvent Manager
- Nitrogen generator
- Computer running MassLynx software

4.2 Materials

- Sample vials and caps
- Acquity UPLC BEH C18, 1.7 µM columns or other columns as needed
- Frits

4.3 Commercial Reagents

- Argon Gas, Grade 5.0

4.4 Reference Material Standards

- Mass Scale and Resolution set-up solution
- Multi-component drug solutions

4.5 LC Solutions - Refer to Toxicology Solution Prep Guidelines for instructions on how to prepare the mobile phases and wash solutions required by this procedure.

5.0 Procedure

5.1 Instrument Performance Verification for New Instrumentation

5.1.1 New Toxicology LC-MS/MS instruments shall be installed by a manufacturer representative and shown to meet manufacturer requirements.

5.1.2 The Toxicology LC-MS/MS Key Operator or designee shall conduct performance verification on new LC-MS/MS instruments prior to use for casework.

5.1.2.1 Performance verification shall include a successful instrument resolution and calibration.

5.1.2.2 The performance verification shall include the infusion of certified reference materials using Intellistart.

5.1.2.3 A new entry for the instrument shall be made in the Resource Manager section of FA prior to use in casework. The new entry shall include the following:

5.1.2.3.1 Manufacturer's serial number.

5.1.2.3.2 Unique section identifier for the new instrument.

5.1.2.3.3 Notation under "Verification Date" to reflect the date the performance verification was completed.

5.1.2.4 The data shall be filed and maintained in the FA instrument resource by the Toxicology LC-MS/MS Key Operator.

5.2 Maintenance

5.2.1 Record all maintenance in the instrument logbook at the time it is performed.

5.2.2 The Toxicology LC-MS/MS Key Operator or designee shall update the instrument log and file any generated data in the instrument notebook when the instrument is returned to service.

5.2.3 Routine Maintenance - The routine maintenance schedule is a suggested minimum guideline. The maintenance schedule will be determined by the Toxicology LC-MS/MS Key Operator or designee based upon instrument use and performance.

5.2.3.1 Frit

- Replace weekly when in use.
- Post-maintenance check: Successful LC-MS/MS system check.

5.2.3.2 Column

- Replace as needed and record the serial number of the new column in the instrument logbook
- Post-maintenance check: Successful LC-MS/MS system check.

5.2.3.3 Needle Assembly

- Inspect weekly when in use, for ease of movement, or if plugged. Replace as needed.
- If the needle is replaced - Characterize the needle Z-axis, the needle seal, and characterize the needle and sample loop volumes.
- Post-maintenance check: Successful LC-MS/MS system check.

5.2.3.4 Pump Oil

- Inspect monthly-top off as needed.
- Change annually.
- Post-maintenance check: Successful Resolution/Calibration.

5.2.3.5 Clean Sample Cone

- Clean quarterly or as needed.
- Post-maintenance check: Successful LC-MS/MS System Check.

5.2.3.6 Exhaust Trap Bottle

- Inspect weekly.
- Empty when it is more than 10 % full.
- Post-maintenance check: n/a

5.2.4 Non-routine Maintenance

5.2.4.1 When non-routine maintenance is performed, the instrument shall be out of service until the non-routine maintenance is evaluated by the Toxicology LC-MS/MS Key Operator or designee to determine the need for additional instrument checks or recalibration prior to analyzing samples.

5.2.4.1.1 Maintenance that may affect chromatography requires a post-maintenance LC-MS/MS system check. The retention times of the analytes may need to be updated in the data analysis method as a result of the maintenance. The chromatography shall be examined for Gaussian peak shape.

5.2.4.2 The Toxicology LC-MS/MS Key Operator or designee shall update the instrument log when the instrument is ready to be used for casework and file any generated data in the instrument notebook located near the instrument.

5.2.5 LC-MS/MS system check

5.2.5.1 An LC-MS/MS system check shall be performed daily when the instrument is in use.

5.2.5.2 Prepare a multi-component reference material standard solution containing the appropriate internal standard.

5.2.5.3 Analyze the sample using the appropriate instrumental methods (LC, MS, and Tune).

5.2.5.4 Process the data using the appropriate instrumental method.

5.2.5.5 Ensure all MRM transitions are present for each compound and internal standard in the solution.

5.2.5.6 Examine the chromatography for Gaussian Peak shape.

5.2.5.7 Ensure the signal to noise is greater than 10:1.

5.2.5.8 Ensure that the analyte/internal standard area and retention times are consistent with previous system checks.

5.2.5.9 If acceptable, initial the report and store according to **5.6.4**.

5.2.5.10 If unacceptable, document the reason for the failure on the report and store according to **5.6.4.** Prepare a new sample to be analyzed and evaluated according to the above procedure. Notify the LC-MS/MS Key Operator of the failure.

5.2.6 Shutdown

5.2.6.1 A successful LC-MS/MS system check shall be performed following any LC or MS shutdown.

5.2.6.2 The shutdown shall be noted in the maintenance log.

5.3 Calibration – Mass Scale and Resolution

5.3.1 Calibration of the mass scale and resolution shall be done every 6 months or additionally as needed using a Waters Mass Scale and Resolution Set-Up Solution. Can use either Sodium Rubidium Iodide or Sodium Cesium Iodide.

5.3.2 Perform the calibration along with the resolution in positive ion mode using the Intellistart program.

5.3.3 The peak widths for the MRM resolution shall be determined in the method validation. Perform another resolution if it fails to meet acceptance criteria. If the problem persists, notify the Toxicology LC-MS/MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.

5.3.4 The calibration report shall show at minimum six matches out of seven tested mass references.

5.3.5 Record each resolution and calibration in the instrument log along with initials and date.

5.3.6 Initial the Resolution and Calibration report and mark any parameters that are out of specification. File the report in the FA instrument resource.

5.4 Standards and Controls

5.4.1 Internal standards, positive and/or negative controls are detailed in the Toxicology Section technical procedure used for sample preparation.

5.4.2 System flush

5.4.2.1 The needle shall be flushed after each injection with both Weak Wash and Strong Wash.

5.4.2.2 The plungers and tubing paths shall be rinsed with Seal Wash after every injection.

5.5 Sampling

5.5.1 Refer to the Toxicology Unit technical procedure used for sample preparation.

5.6 Instrument Procedure

5.6.1 If an instrument problem or error message occurs, the Forensic Scientist who discovers the issue shall document the issue in the activity log. If the issue cannot be corrected immediately, the Forensic Scientist shall mark the activity log to show that the instrument is out of service, notify the Toxicology LC-MS/MS Key Operator or designee and notify all other Forensic Scientists affected.

5.6.2 A logbook shall be maintained near each instrument.

5.6.3 The logbook shall contain:

5.6.3.1 The date, sequence name, initials of operator, and comments.

5.6.3.2 The date of maintenance, description of maintenance performed, parts replaced, and the initials of the person performing or documenting the maintenance.

5.6.4 All LC-MS/MS Activity logs, post maintenance data, and LC-MS/MS system checks generated shall be archived yearly in the FA instrument resource.

5.6.5 Projects

5.6.5.1 MassLynx uses projects to contain methods for the instrument components, sequences, data, and report formats that will be used in both the acquisition of data as well as its processing.

5.6.5.2 A new project shall be created each day the instrument is used. The current date shall be used in the name of the project.

5.6.5.3 The project subfolder "**ACQUDB**" shall contain the MS Method file, MS Tune file, and the Inlet method files necessary to perform the required analysis. These method files were created during the method validation for the analysis being performed.

5.6.5.4 For quantitative analyses, the project subfolder "**MethDB**" shall contain the appropriate data processing method. This method was created during method validation for the analysis being performed.

5.6.6 Sequences

5.6.6.1 The sequence shall be entered and printed prior to starting the instrument.

5.6.6.2 The sequence and the loading of the instrument shall be reviewed by another section employee prior to starting the run. The sequence shall be initialed and dated by reviewers.

5.6.6.3 The current date shall be used in the name of a sequence.

5.6.7 Data Files

5.6.7.1 Data files names shall include a reference to the procedure and a number series to ensure that files are distinguishable. Example (BCLLE01, BCLLE02, etc.).

5.6.7.2 Data files associated with casework shall not be deleted or overwritten.

5.6.7.3 Notify the Toxicology LC-MS/MS Key Operator or designee if the disk drive(s) become full.

5.6.8 For quantitative methods refer to the Toxicology Unit technical procedures used for sample preparation for data analysis, identification and reporting.

5.7 Calculations –N/A

5.8 Uncertainty of Measurement – N/A

6.0 Limitations

6.1 Introduction of improperly prepared samples may lead to poor sensitivity and carryover.

7.0 Safety

7.1 Refer to the State Crime Laboratory Safety Manual.

7.2 The Mass Spectrometer may be hot. Avoid touching hot areas and wear protective gloves while performing maintenance.

8.0 References

Skoog, Douglas A., James Hollar and Timothy A. Nieman. *Principles of Instrumental Analysis*, *5th Ed*., Garcourt Brace & Company, 1998.

Waters Xevo TQD Operation Training Manual, March 2013.

Waters Acquity UPLC Instrument Manuals.

Waters Xevo TQD Instrument Manuals.

9.0 Records

- LC-MS/MS logbook

10.0 Attachments- N/A

Revision History		
Effective Date	Version Number	Reason
03/14/2014	1	Original Document
08/29/2014	2	4.5 – Added preparation clarification
04/06/2016	3	Updated Issuing Authority 2.0– modified scope 4.3 – Removed reagents related to change in 4.5 4.5 – Solution prep instructions moved to Toxicology Solution Preparation Guidelines 5.2.3.3 Changed “daily” in first bullet point to “weekly when in use,” Inserted 5.2.5.1, 5.2.5.9 Modified 5.2.5.8 5.2.6 – Replaced “Calibration verification check” with “LC-MS/MS system check” 5.3.1 Changed “quarterly” to “every 6 months” 5.3.3 – Replaced 1.0 +/- 0.15 Da with “determined in the method validation” 5.6.4 and 5.6.5 – consolidated and modified to reflect electronic record storage. 5.6.8.3 – removed archival.
02/22/2019	4	4.2 – updated materials 4.5 – Removed “wash” in title, inserted “mobile phase” in reference statement 5.1.2.3 – Now 5.1.2.4, restructured for storage in FA instrument resource 5.2.3.2 – replaced lot with serial 5.2.3.4 - Replaced Successful Calibration Verification check with Resolution/Calibration 5.2.3.5 - Replaced Successful Calibration Verification check with LC-MS/MS System Check 5.2.5.8 – New 5.2.5.9 – Corrected line reference 5.2.5.10 corrected line reference 5.3.1 – Inserted solution choices for Calibration and Resolution 5.3.2 – Removed requirement for negative mode Resolution 5.3.6 – Changed storage location to FA instrument resource 5.4.1 – Removed reference to Drug Chemistry section 5.6.4, 5.6.5 – Consolidated, removed res/cal reports due to duplication with 5.3.6

		5.6.7.4 – Removed 5.6.8 - Reword 7.2 - Removed