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 - see Toxicology Definitions list

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
- Methanol, HPLC grade
- Acetonitrile, HPLC grade
- Water, HPLC grade
- Ammonium Formate
- Formic Acid
- Nitric Acid

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

5.2.3.7 Filter

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

5.2.3.8 Glassware

- Clean quarterly or as needed by first washing it with organic solvent and then water. Next, rinse it with the solvent that will be put into it. If more aggressive cleaning is required, use the following procedure: Sonicate with 10% formic or nitric acid, then water, then methanol or acetonitrile, then water. Repeat two more times. Do not wash with detergent.

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 an appropriate neat positive control.

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.

5.4.3 Naming and Saving of Instrument Files

5.4.3.1 All instrument files created during performance checks, and all blanks and data files associated with case samples shall be saved on the instrument computer hard drive.

5.4.3.2 All instrument files listed above shall be placed into a compressed (.zip) file. The compressed file shall be named with the instrument identifier, year, and month in which it was collected.

5.4.3.3 The compressed (.zip) file shall be archived in the FA workstation object repository ("Manage Files") associated with the LCMSMS extraction on which it was collected.

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.

7.3 Use extreme caution when dismantling, installing, or transporting compressed gas cylinders. Cylinders shall not be moved without the cylinder cap securely in place.

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

- Refer to Appendix 1 for chemical hygiene and safety precautions for high risk and particularly hazardous substances.

Revision History		
Effective Date	Version Number	Reason
02/05/2021	5	Removed definitions 5.2.5.2 – Changed positive internal standard to neat positive control 5.2.3.7 and 5.2.3.8 – New 5.4.3 – New 7.3 New 10 – added statement Appendix 1 - New

Appendix 1:
Chemical Hygiene and Safety Precautions for High Risk and Particularly Hazardous Risk Substances

Formic Acid **DANGER: HIGH RISK SUBSTANCE** *	
	**HEALTH 3** **FLAMMABILITY 2** **REACTIVITY 0**
Detection of Release	Strong, penetrating odor.
Signs/Symptoms of Exposure	Irritant to nose, throat, and lungs; higher exposures can cause pulmonary edema (medical emergency). Headaches, dizziness, nausea and vomiting. Symptoms of acute ingestion (50 grams or more) can initially include salivation, bloody vomiting, a burning sensation in the mouth and pharynx, diarrhea, and/or severe pain.
PEL	OSHA TWA 5 ppm; NIOSH REL 5 ppm over 10 hours (Odor threshold = 49 ppm)
Associated Hazards	Flammable liquid and vapor. Harmful if swallowed. Corrosive- causes severe skin burns and serious eye damage. Toxic if inhaled. May damage kidneys.
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time = 6 minutes)
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep away from sources of ignition. Take measures to prevent the build-up of electrostatic charge. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage. Dispose in Hazardous Chemical Waste.
Emergency Procedures	**Eye Contact:** Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. Continue rinsing eyes during transport to hospital. **Inhalation Exposure:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. **Skin Contact:** Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. Small contained spill: wearing appropriate PPE, collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container. Dispose in Hazardous Chemical Waste. Large spills: Evacuate area and call 911 (Haz Mat).

Methanol

DANGER: HIGH RISK SUBSTANCE *

HEALTH	2
FLAMMABILITY	3
REACTIVITY	0

Detection of Release	Colorless liquid with a sweet, pungent odor.
Signs/Symptoms of Exposure	Headache, Nausea, Dizziness, Eye damage. May cause intoxication that includes central nervous system depression, headache, dizziness, nausea, lack of coordination, and confusion.
PEL	OSHA (TWA) 200 ppm
Associated Hazards	Flammable. Acute oral, dermal, and inhalation toxin. Toxic if swallowed, comes in contact with skin, or inhaled. Specific target organ toxicity of eyes.
Controls	Use under fume hood. Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product. Use eye protection. Handle with gloves. Wear lab coat. Gloves: nitrile (break through time less than 1minute), butyl-rubber (break through time greater than 8 hours)
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Use explosion-proof equipment. Keep away from sources of ignition. Take measures to prevent the build-up of electrostatic charge. Dispose in Hazardous Chemical Waste. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.
Emergency Procedures	**Eye Contact:** Flush eyes with water as a precaution. **Inhalation Exposure:** If inhaled, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. **Ingestion:** After swallowing: fresh air. Make victim drink ethanol (e.g. 1 drinking glass of a 40% alcoholic beverage). Call a doctor immediately (mention methanol ingestion). Only in exceptional cases, if no medical care is available within one hour, induce vomiting (only in fully conscious persons) and make victim drink ethanol again (approx. 0.3 ml of a 40% alcoholic beverage/kg body weight/hour). **Skin Contact:** Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. **Spills:** Avoid breathing vapors, mist, or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas. Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Small spills: Contain spillage, and then collect with non-combustible absorbent material, (e.g. sand, earth, diatomaceous earth, vermiculite) and place in container for disposal. Large spills: Turn off sources of heat if possible; evacuate area and call 911 (Haz Mat).

70% Nitric Acid DANGER: HIGH RISK SUBSTANCE *		
	HEALTH	3
	FLAMMABILITY	0
	REACTIVITY	2
Detection of Release	Irritating/pungent odor; Asphyxiating odor	
Signs/Symptoms of Exposure	Skin burns or irritation (may yellow skin); irritation to respiratory tract; dry/sore throat; nausea/vomiting	
PEL	OSHA TWA 2 ppm; NIOSH REL 2 ppm	
Associated Hazards	Oxidizing Liquid- may intensify fire; Skin corrosion/irritation- Causes severe skin burns and eye damage; Serious eye damage/eye irritation.	
Controls	Use under fume hood. Gloves. Eye protection. Lab coat.	
Safe handling, storage, disposal	Store in a cool area. Keep out of direct sunlight. Store in a dry area. Store in a dark area. Ventilation at floor level. Fireproof storeroom. Keep locked up. Provide for a tub to collect spills. Store only in a limited quantity. Keep away from heat sources, combustible materials, reducing agents, (strong) bases, cellulosic materials, organic materials, metal powders, and water/moisture. Dispose in Chemical Hazardous Waste.	
Emergency Procedures	**Skin Exposure**: Wash immediately with lots of water (15 minutes)/shower. Remove clothing while washing. Do not remove clothing if it sticks to the skin. Cover wounds with sterile bandage. Consult a doctor/medical service. If burned surface > 10%: take victim to hospital. **Inhalation Exposure**: Remove victim to fresh air. **Eye Contact**: Rinse immediately with plenty of water for 15 minutes. Do not apply neutralizing agents. Cover eyes aseptically. Take victim to an ophthalmologist. **Ingestion**: Rinse mouth with water. Immediately after ingestion: give lots of water to drink. Give milk to drink. Do not induce vomiting. Do not give activated charcoal. Do not give chemical antidote. Immediately consult a doctor/medical service. Call Poison Information Center. Take the container/vomit to the doctor/hospital. Ingestion of large quantities: immediately to hospital. **Spills:** Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Small contained spill: wearing appropriate PPE, collect with absorbent material, and place in container. Dispose in Hazardous Chemical Waste. Large spills: Evacuate area and call 911 (Haz Mat).	