Toxicology Liquid Chromatograph Quadrupole Time-of-Flight Mass Spectrometer (LC-QTOF-MS)

1.0 Purpose - This procedure specifies the required elements for the calibration and use of the SCIEX Ultra Pressure Liquid Chromatograph (UPLC) in conjunction with a SCIEX X500R (QTOF-MS).

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

- SCIEX X500R QTOF Mass Spectrometer
- Nitrogen generator
- Computer running SCIEX OS software

4.2 Materials

- Sample vials and caps
- Kinetex 2.6 um Phenyl-Hexyl 100columns or other columns as needed

4.3 Commercial Reagents (HPLC Grade or higher)

- Acetonitrile
- Ammonium Formate
- Formic Acid
- Isopropanol
- Methanol
- Water

4.4 Reference Material Standards

- ESI Positive Calibration Solution for the SCIEX X500B System
- Acetaminophen
- Dihydrocodeine
- Lamotrigine
- Zolpidem
- Citalopram
- Verapamil
- Oxazepam
- Loratadine

4.5 QTOF 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-QTOF-MS instruments shall be installed by a manufacturer representative and shown to meet manufacturer requirements.

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

5.1.2.1 Performance verification shall include a successful MS Check and analysis of a system check solution.

5.1.2.2 The performance verification shall include the analysis of certified reference materials using SCIEX OS software.

5.1.2.3 A new entry for the instrument shall be made in the Resource Manager section of Forensic Advantage (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-QTOF-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-QTOF-MS Key Operator or designee shall update the instrument log and file any generated data in the FA resource when the instrument is returned to service.

5.2.3 After any maintenance, the instrument shall be labeled as being out of service until an MS check is performed successfully.

5.2.3.1 The Toxicology LC-QTOF-MS Key Operator or designee shall update the instrument log when the instrument is ready to be used for casework, by indicating the Calibration, Tune, and Testmix were successful.

5.2.4 **Routine Maintenance -** The routine maintenance schedule is a suggested minimum guideline. Instrument use may alter the need for maintenance. The maintenance schedule will be determined by the Toxicology LC-QTOF-MS Key Operator or designee based upon instrument use and performance.

5.2.4.1 Column

- Clean column every 300 injections or as needed.
- Replace after 1500 injections or as needed and record the serial number of the new column in the instrument logbook
- Post-maintenance check: Successful QTOF Testmix analysis.

5.2.4.2 Guard Column

- Replace after 150 injections or as needed
- Post-maintenance check: Successful QTOF Testmix analysis.

5.2.4.3 IPA Flush

- Place Mobile Phase lines in water and flush for 10 minutes. Then place in isopropanol for 30 minutes followed by water for another 10 minutes. Finally, run mobile phase through lines for 2 minutes
- Monthly
- Post-maintenance check: Successful QTOF Testmix analysis.

5.2.4.4 Pump Lubrication Bottles

- Fill as needed
- Post-maintenance check: n/a

5.2.4.5 Needle Assembly

- Inspect weekly when in use, for ease of movement, or if plugged. Replace as needed.
- Post-maintenance check: Successful QTOF Testmix analysis.

5.2.4.6 Pump Oil

- Inspect monthly-top off as needed.
- Change annually.
- Post-maintenance check: Successful Calibration, Tune, and QTOF Testmix analysis.

5.2.4.7 Clean Orifice Plate

- Clean daily or as needed with water followed by methanol.
- Post-maintenance check: Successful Tune and QTOF Testmix analysis.

5.2.4.8 Exhaust Trap Bottle

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

5.2.5 Non-routine Maintenance

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

5.2.5.1.1 Maintenance that may affect chromatography requires a post-maintenance MS check. The retention times of the analytes may need to be updated in the data analysis method as a result of the maintenance. Therefore, solution sets need to be analyzed and retention times updated prior to being placed back in service. The chromatography shall be examined for Gaussian peak shape.

5.2.5.2 The Toxicology LC-QTOF-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 FA resource.

5.2.6 Calibration – Mass Scale and Resolution – for Positive Ion mode

5.2.6.1 Using the ESI Positive Calibration Solution for the SCIEX X500B System, calibration of the mass scale and resolution shall be done prior to the start of a batch of samples and every 20 samples.

5.2.6.2 The calibration report shall show "**Calibration Succeeded**" for both the TOF MS and TOF MS/MS. Per **5.2.3.1**, the instrument log will be updated to indicate that the Calibration has been performed and passed.

5.2.6.3 The calibration report should be printed and stored in the appropriate FA instrument resource.

5.2.6.4 If the calibration fails to meet acceptance criteria, document the reason for failure on the instrument log. Repeat the calibration. If the problem persists, notify the Toxicology LC-QTOF-MS Key Operator or designee. The instrument shall remain out of service until the problem is corrected.

5.2.7 LC-QTOF-MS Tune

5.2.7.1 A Tune shall be performed daily when the instrument is in use.

5.2.7.2 Analyze the Positive ESI Calibration Solution for the SCIEX X500B System.

5.2.7.3 The Instrument Tuning Report should show "**PASS**" for the four checks performed. (Achieve Stable Spray/Modify, Channel Alignment, TOF MS Mass Check, and TOF MS/MS Mass Check. Per **5.2.3.1**, the instrument log will be updated to indicate that the Tune has been performed and passed.

5.2.7.4 The Instrument Tuning Report should be printed and stored in the appropriate FA instrument resource.

5.2.7.5 If unacceptable, document the reason for the failure on the instrument log**.** Analyze a new sample and evaluate according to the above procedure. Notify the LC-QTOF-MS Key Operator of the failure.

5.2.8 LC-QTOF-MS Testmix –Testmix may be prepared by the Forensic Scientist in any amount provided that the component ratios are kept constant.

5.2.8.1 A Testmix shall be analyzed after any maintenance or prior to the start of any batch of samples to ensure the performance of the instrument.

5.2.8.2 Prepare a solution from certified reference materials (CRMs) containing the following: 500 ng/mL of Acetaminophen, 40 ng/mL of Dihydrocodeine, 400 ng/mL of Lamotrigine, 40 ng/mL of Zolpidem, 40 ng/mL of Citalopram, 40 ng/mL of Verapamil, 80 ng/mL of Oxazepam, and 40 ng/ml of Loratadine.

5.2.8.2.1 Example – In a 250 mL volumetric flask, using 1 mg/ml CRMs, pipette 125 µL of Acetaminophen, 10 µL of Dihydrocodeine, 100 µL of Lamotrigine, 10 µL of Zolpidem, 10 µL of Citalopram, 10 µL of Verapamil, 20 µL of Oxazepam, and 10 µL of Loratadine, and fill to the mark (QS) with methanol.

5.2.8.3 Lot number: "QTM" followed by Eight digit format year/month/day

5.2.8.3.1 Example: QTM20191231

5.2.8.4 Expiration: Six months.

5.2.8.5 Store in freezer.

5.2.8.6 QC check: Successful analysis by QTOF with the identification of all components, no additional compounds should be identified.

5.2.9 Shutdown

5.2.9.1 Following any computer or instrument shutdown, a LC-QTOF-MS tune and Testmix analysis shall be performed and meet all acceptance criteria.

5.2.9.2 The shutdown shall be noted in the maintenance log.

5.3 Standards and Controls

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

5.3.2 System flush

5.3.2.1 The needle shall be flushed after each injection with Needle Wash.

5.4 Sampling

5.4.1 Refer to the Toxicology Section technical procedure used for sample preparation.

5.5 Instrument Procedure

5.5.1 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-QTOF-MS Key Operator or designee and notify all other Forensic Scientists affected.

5.5.2 A logbook shall be maintained near each instrument.

5.5.3 The logbook shall contain:

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

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

5.5.4 All LC-QTOF-MS Activity logs and post maintenance data, to include Calibration reports, LC-QTOF-MS Tunes, and Testmix analyses, generated shall be stored electronically in the FA instrument resource.

5.5.5 Projects

5.5.5.1 SCIEX OS 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.

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

5.5.5.3 The project subfolder shall contain the MS Method file and LC Method file necessary to perform the required analysis.

5.5.5.4 The project subfolder shall contain the appropriate data processing method.

5.5.6 Sequences

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

5.5.6.2 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.

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

5.5.7 Data Files

5.5.7.1 Data files names shall include a reference to the procedure and a number series to ensure that files are distinguishable. Example: QTOF01, QTOF02, etc.

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

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

5.6 Calculations – n/a

5.7 Uncertainty of Measurement –n/a

6.0 Limitations – n/a

7.0 Safety

7.1 Refer to Appendix 1 for chemical hygiene and safety precautions.

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.

SCIEX X500 QTOF System User Guide, August 2017

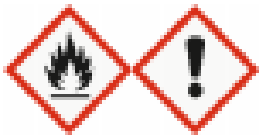
9.0 Records

- LC-QTOF-MS Instrument Log
- Installation paperwork
- Calibration Reports
- Instrument Tuning Reports
- Testmix Analysis Reports

10.0 Attachments- n/a

Revision History		
Effective Date	Version Number	Reason
12/31/2019	1	Original Document

Appendix 1 Chemical Hygiene and Safety Precautions

Acetonitrile DANGER		
	HEALTH	2
	FLAMMABILITY	3
	REACTIVITY	0
Detection of Release	A clear, colorless liquid with an ether-like odor.	
Signs/Symptoms of Exposure	Eye irritation, chest pain, tightness in the chest, nausea, emesis, tachycardia, hypotension, short and shallow respiration, headache, restlessness, semi consciousness, and seizures.	
PEL	OSHA TWA 20 ppm; NIOSH REL 20 ppm	
Associated Hazards	Highly flammable liquid and vapor. Harmful if swallowed, comes in contact with skin, or inhaled. Danger of skin absorption. Causes serious eye irritation.	
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: butyl-rubber (break through time = 480 minutes), nitrile (break through time = 1 minute)	
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep away from heat, sparks, open flames, and hot surfaces. Take measures to prevent the build-up of electrostatic charge. Use explosion-proof electrical, ventilation, lighting, and equipment. 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. **Inhalation Exposure:** If inhaled, 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. 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).	

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	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.	

Methanol DANGER		
	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).	

Isopropanol **DANGER**		
	**HEALTH**	**1**
	FLAMMABILITY	**3**
	REACTIVITY	**0**
Detection of Release	Colorless liquid with an alcohol-like odor	
Signs/Symptoms of Exposure	Eye irritant; Causes Central Nervous System impairment and Upper Respiratory Tract irritation.	
PEL	ACGIH Threshold Limit Values (TLV): 200 ppm (TWA)	
Associated Hazards	Highly flammable; causes serious eye irritation; and may cause drowsiness and dizziness.	
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 = 29 minutes)	
Safe handling, storage, disposal	Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep away from heat, sparks, open flames, and hot surfaces. Take measures to prevent the build-up of electrostatic charge. Use explosion-proof electrical, ventilation, lighting, and equipment. 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. **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:** Wash off with soap and plenty of water. 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).	