
QSCREEN Extraction and Analysis by Liquid Chromatograph Quadrupole Time-of-Flight Mass Spectrometer (LC-QTOF-MS)

1.0 Purpose - This procedure specifies the required elements for the extraction for LC-QTOF-MS analysis and for the subsequent data analysis and interpretation.

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

3.0 Definitions

- Refer to Toxicology Definitions list.

4.0 Equipment, Materials and Reagents

4.1 Equipment

- Centrifuge
- Mechanical Pipettes
- Class A volumetric flasks
- TurboVap or equivalent evaporator

4.2 Materials

- Glass conical test tubes
- Vortexer
- Pipette tips
- LC vials with pre-slit septa caps

4.3 Reagents

- Deionized water
- Negative blood

4.4 Commercial Reagents (HPLC Grade or higher)

- Water
- Methanol
- Acetonitrile

4.5 Primary Reference Standards

- See QTOF Screen list

4.6 Prepared Reagents – Refer to [Toxicology Solution Prep Guidelines](#) for instructions on how to prepare the reagents required by this procedure.

4.7 Prepared Standards –Standards may be prepared by the Forensic Scientist in any amount provided that the component ratios are kept constant.

4.7.1 Stock Internal Standard Solution (SIS)

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- 4.7.1.1** Prepare a solution containing 20 µg/mL of mepivacaine reference standard.
- 4.7.1.1.1** Example – In a 50 mL volumetric flask, dilute 1.0 mL of a 1.0 mg/mL solution of mepivacaine and fill to the mark (QS) with methanol.
- 4.7.1.2** Lot number: Eight digit format year/month/day
- 4.7.1.2.1** Example: 20191231
- 4.7.1.3** Expiration: One year.
- 4.7.1.4** Store in freezer.
- 4.7.1.5** **QC check:** Prepare an ISW. Pipette 10 µL of the ISW into a test tube. Then follow steps outlined in **5.4.6** through **5.4.10**. For a QC check to be successful, the internal standard must be identified and meet all acceptance criteria.
- 4.7.2 Internal Standard Working Solution (ISW)**
- 4.7.2.1** Pipette 125 µL of the SIS solution into a 5 mL volumetric flask and (QS) to volume with methanol.
- 4.7.2.2** Expiration: Prepare daily.
- 4.7.2.3** QC check: N/A
- 4.7.3 QSCREEN Cutoff Stock Solution (QCS)**
- 4.7.3.1** Prepare solutions containing the compounds shown on QTOF Screen list. Place no more than 25 compounds in any one solution.
- 4.7.3.2** In a 25 mL volumetric flask, pipette the required volume of the compound indicated on the QTOF Screen list and fill to mark (QS) with methanol.
- 4.7.3.3** Lot number: QSCREEN followed by eight digit format year/month/day followed by an alpha character to differ between multiple solutions prepared on the same day.
- 4.7.3.3.1** Example: QSCREEN20191231-A
- 4.7.3.4** Expiration: Six months.
- 4.7.3.5** Store in freezer.
- 4.7.3.6** **QC check:** Prepare a QCW. Pipette 125 µL of QCW into a test tube. Then follow steps outlined in **5.4.5** through **5.4.10**. For a QC check to be successful, all analytes in the QCS must be identified and meet all acceptance criteria.
- 4.7.4 QSCREEN Cutoff Working Solution (QCW)**
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- 4.7.4.1 A QCW will be prepared for each QCS.
 - 4.7.4.2 Pipette 0.1mL of the QCS into a 5 mL volumetric flask and (QS) to volume with methanol.
 - 4.7.4.3 Expiration: Prepare Daily.
 - 4.7.4.4 QC check: N/A

5.0 Procedure

5.1 Allow all solutions and samples to be analyzed to equilibrate to room temperature.

5.2 Add water to the TurboVap if needed.

5.3 Sample Preparation

5.3.1 Ensure that all body fluids are homogenous.

5.3.2 If a homogenous sample cannot be obtained, a notation shall be made in the worksheet detailing the condition of the sample and its handling.

5.3.3 Add 900 uL of 50:50 methanol: acetonitrile solution to an appropriately labeled glass conical tube.

5.3.4 **Cutoff Control Preparation** – A cutoff control will be prepared for each QCW.

5.3.4.1 Pipette 250 µL of the QCW into a glass test tube and dry down using the TurboVap at 50°C.

5.3.4.2 Pipette 200 µL of negative blood to each QCW to obtain the appropriate cutoff concentrations.

5.3.4.3 Vortex for 1 min.

5.3.4.4 Proceed to 5.3.7.

5.3.5 **Negative Control Preparation** – 100 µL of negative blood pipetted according to 5.3.7.

5.3.5.1 One negative control will be prepared per 20 case specimens.

5.3.6 **Case Specimen Preparation** - 100 µL of case specimen pipetted according to 5.3.7.

5.3.7 While vortexing the methanol/acetonitrile mixture, pipette 100 µL of specimen to be extracted.

5.4 Extraction Procedure

5.4.1 Add 10 µL of the ISW to each specimen.

5.4.2 Vortex for 1 min.

5.4.3 Centrifuge tubes for 20 minutes @ 4000rpm.

- 5.4.4 Transfer the supernatant to a clean labeled glass conical tube.
- 5.4.5 Evaporate to dryness using a TurboVap at 50 °C.
- 5.4.6 Add 500 µL of 80:20 water: methanol solution.
- 5.4.7 Vortex 0.5 min.
- 5.4.8 Centrifuge for 10 min @ 4000rpm.
- 5.4.9 Transfer reconstituted specimens to labeled LC vials and cap.
- 5.4.10 Analyze samples on a LC-QTOF-MS as specified in the [Toxicology Liquid Chromatograph Quadrupole Time-of-Flight Mass Spectrometer \(LC-QTOF-MS\) procedure](#).

5.5 Data Processing and Acceptance Criteria

- 5.5.1 Process the QCWs using the QSCREEN processing method.
 - 5.5.1.1 For compounds contained within the QCW, update the peak heights in the QSCREEN processing method to be 90% of the peak height in the QCW.
- 5.5.2 Process the run using the updated QSCREEN processing method.
- 5.5.3 Save the data with the name of the procedure and the extraction date added to the end.
 - 5.5.3.1 Example: QSCREEN20191217
- 5.5.4 **Analyte Identification Criteria**
 - 5.5.4.1 The retention time shall be within 2% of the target value.
 - 5.5.4.2 The isotope ratio shall be within 20% of the target value.
 - 5.5.4.3 The mass error shall be no higher than 5 ppm
 - 5.5.4.4 The library match shall be at least 70%.
 - 5.5.4.5 The mass spectra of a substance may not contain any ions at a relative abundance equal to or greater than 50% that are not present in the reference standard.
- 5.5.5 **Quality Control Acceptance Criteria**
 - 5.5.5.1 The negative control fails for an analyte if there is a peak that meets the criteria set in 5.5.4.
 - 5.5.5.2 The QCW will fail for an analyte if it doesn't meet the criteria set in 5.5.4.

5.5.5.3 The QCW and Negative Control will fail for all analytes if the internal standard doesn't meet the criteria set in **5.5.4**.

5.5.5 QC Data Packet – assembled including the following items:

- Summary page with FA workstation reference
- Completed extraction worksheet
- LC-QTOF-MS sequence list
- Calibration Reports
- Instrument Tuning Report
- Testmix Analysis Reports
- Experiment and Method Report
- Reports of all controls

5.5.6 The quality control data packet will be named beginning with “QSCREEN” (capitalization optional), followed by eight digit format year/month/day ending with the instrument name. A suffix may be added to differentiate multiple runs.

5.5.6.1 Example: QSCREEN20191217-QTOF-T-X

5.5.7 All quality control data packets shall be administratively and technically reviewed prior to use of the associated case data for reporting.

5.5.8 The reviewed data packet shall be uploaded to the workstation managed files and approved. The review and approval will be indicated by signing the summary page prior to uploading to FA.

5.6 Calculations – n/a

5.7 Uncertainty of Measurement – There is no measurement uncertainty associated with compound identification. However, there is an associated measurement uncertainty with the recovery of compounds around threshold values. To account for this, the peak height cutoff is set at 90% of the peak height of the compounds in the QCWs.

5.8 Reporting

5.8.1 For an analyte to be reported as identified in a case specimen it must meet all criteria in **5.5.4**.

5.8.2 Refer to the [Drug Toxicology Reporting](#) procedure for reporting.

5.9 Record the following in the case record:

- Case specimen report.

6.0 Limitations – n/a

7.0 Safety

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

8.0 References

- **SCIEX VMethod Application** – Forensic Toxicology Screening on the SCIEX X500R QTOF System
- **QTOF Screen List**

9.0 Records

- Quality control data packet
- Case specimen report

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	<u>Eye Contact:</u> Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician. <u>Inhalation Exposure:</u> If inhaled, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. <u>Ingestion:</u> Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician. <u>Skin Contact:</u> Take off contaminated clothing and shoes immediately. Wash off with soap and plenty of water. Consult a physician. <u>Spills:</u> 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	
	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	<u>Eye Contact:</u> Flush eyes with water as a precaution. <u>Inhalation Exposure:</u> If inhaled, move person into fresh air. If not breathing, give artificial respiration. Consult a physician. <u>Ingestion:</u> 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). <u>Skin Contact:</u> Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. <u>Spills:</u> 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).