Training Procedure for Liquid Chromatography Quadrupole Time-Of-Flight Mass Spectrometry

1.0 Purpose – This procedure provides an outline for training in the analysis of drug toxicology cases using Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS).

2.0 Scope - This procedure applies to trainees in the Toxicology Section of the State Crime Laboratory.

3.0 Procedure

3.1 Objectives

3.1.1 Review and understand the Toxicology Section Technical Procedure for QSCREEN Extraction and Analysis and the Toxicology Liquid Chromatograph Quadrupole Time-of-Flight Mass Spectrometer procedure.

3.1.2 Become familiar with the components of the LC-QTOF-MS.

3.1.3 Understand LC theory and concepts.

3.1.4 Understand Time of Flight theory and concepts.

3.1.5 Gain practical knowledge of the operation and maintenance of the LC-QTOF-MS.

3.1.6 Successfully perform a tune, calibration, and routine maintenance, analyze a Testmix, and process data.

3.1.7 To understand the hazards associated with all the chemicals used in the procedure including 1) methods and observations that may be used to detect the presence or release of a hazardous chemical, 2) the physical, health, and other associated hazards of a hazardous chemical, and 3) measures employees can take to protect themselves and others from these hazards, including environmental and administrative controls, emergency procedures, and personal protective equipment to be used.

3.2 Terms to define

- Turbospray
- Turboionspray
- Calibration
- Tuning
- Efficiency
- Flow rate
- Gradient
- Matrix effects
- Normal phase chromatography
- Quadrupole
- Time-of-Flight
- Resolution
- Retention factor

- Selectivity
- Isotope ratio
- Mass error
- Information dependent acquisition (IDA)
- Data dependent acquisition (DDA)
- SWATH

3.3 Reading Assignments

- Allen, Darren R. and McWhinney, Brett C., "Quadrupole Time of Flight Mass Spectrometry: A Paradigm Shift in Toxicology Screening Applications." Clinical Biochemistry Review, Volume 40 (2019): 135-146.

- Arsenault, J. And McDonald, P., Beginners Guide to Liquid Chromatography, USA, Waters Corporation, 2009.

- Banerjee, Shibdas and Mazumdar, Shyamalava, "Electrospray Ionization Mass Spectrometry: A Technique to Access the Information beyond the Molecular Weight of the Analyte", International Journal of Analytical Chemistry, Volume 2012: 1-40.

- Dickson, Kennedy and Mata, Dani, "Comparative Analysis of ELISA Immunoassay and LC-QTOF for Opiate Screening", Journal of Analytical Toxicology, Volume 44, (2020): 410-413.

- Fu, Lijuan, et al., "Single-Injection Screening of 664 Forensic Toxicology Compounds on a SCIEX X500R QTOF System", SCIEX 2017.

- He, Xiang and Taylor, Adrian, "Forensic Identification and Quantification Workflows delivered on a Revolutionary Designed QTOF and SCIEX OS Software", SCIEX 2016

- Marin, Stephanie J. et al., "Comparison of Drug Detection by Three Quadrupole Time-of-Flight
  Mass Spectrometry Platforms", Journal of Analytical Toxicology, Volume 39, (2015): 89-95.

- Matuszewski, B.K., Constanzer, M.L., and Chavez-Eng, C.M., "Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS." Analytical Chemistry, Volume 75 (July 2003): 3019-3030

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

- SCIEX X500 QTOF System User Guide, August 2017SCIEX X500 QTOF System User Guide, August 2017

- Taylor, Adrian, et al., "Using MS/MS ^{ALL} with SWATH® Acquisition for Forensic Designer Drug Analysis with SCIEX X500R QTOF System and SCIEX OS Software", SCIEX 2016.

3.4 Practical/Laboratory Exercises

3.4.1 Read the assigned literature.

3.4.2 Attend a lecture on LC-QTOF-MS theory, operation and maintenance given by the Toxicology Training Coordinator or designee.

3.4.3 Read and comprehend the SDS for each chemical used in the Toxicology Section Technical procedure for Toxicology LC-QTOF-MS.

3.4.4 Observe the Toxicology LC-QTOF-MS Key Operator or designee perform the routine maintenance required in the Toxicology Section Technical procedure for Toxicology LC-QTOF-MS.

3.4.5 Successfully perform the required routine maintenance in the Toxicology Section Technical procedure for Toxicology LC-QTOF-MS.

3.4.6 Successfully perform a positive and negative MS check (tune) and calibration.

3.4.7 Prepare and analyze a Testmix solution.

3.4.8 Observe the Toxicology Training Coordinator or designee prepare to use a LC-QTOF-MS, setup a sequence, run a sequence and analyze data files.

3.4.9 Using the LC-QTOF-MS software review the data files provided by the Toxicology Training Coordinator.

3.4.10 Review an IDA acquisition method with the Toxicology Training Coordinator or designee.

3.4.11 Process positive and negative control samples provided by the trainer and evaluate their LC-QTOF-MS data as required by the current extraction and LC-QTOF-MS technical procedures.

3.4.12 Prepare an oral presentation, approximately 10 minutes in length, demonstrating an understanding of Liquid Chromatography Quadrupole Time-of-flight Mass Spectrometry and present it to the Toxicology Training Coordinator.

3.4.13 Complete a written exam.

3.5 Study Questions

3.5.1 Describe the components of an LC-QTOF-MS system.

3.5.2 What is flight path and why is it important to TOF/QTOF analysis?

3.5.3 What are the different types of flight paths?

3.5.4 What does performing a positive and negative MS check do and why is it important?

3.5.5 Explain mass resolution. How is it important to QTOF analysis?

3.5.6 What is mass error? How is it calculated?

3.5.7 What is isotope ratio?

3.5.8 What is collision energy (CE)? What CE is used in the QSCREEN?

3.5.9 Why is a gradient used in LC analysis?

3.5.10 What could happen if the additives in the mobile phases are not present or in the wrong proportion?

3.5.11 Explain Turbospray ionization. Explain Turboionspray. What are the main differences and why is it important to know which is being used.

3.5.12 Explain how high resolution mass spectrometry (HRMS) works.

3.5.13 How can HRMS be used to accurately predict the structure of an unknown compound.

3.5.14 Define Candidate, Confirmation, and Smart Confirmation library searches. Explain the advantages for each of them.

3.5.15 Explain intensity factor and intensity threshold. What can happen if you aren't careful when adjusting these settings?

3.5.16 What is the difference between an IDA and DDA method?

3.5.17 What are the advantages of using a QTOF over a TOF system?

4.0 Records

- Training Section Completion Summary
- Drug Toxicology Training Checklist

Revision History		
Effective Date	Version Number	Reason
	1	Original Document