Training Procedure for Liquid Chromatography-Tandem Mass Spectrometry

1.0 Purpose – This procedure provides an outline for training in the analysis of drug toxicology cases using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/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 procedure: Toxicology Liquid Chromatography-Tandem Mass Spectrometry LCMSMS.

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

3.1.3 Understand LC theory and concepts.

3.1.4 Understand Tandem Mass Spectrometry theory and concepts.

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

3.1.6 Successfully perform Resolution and Calibration.

3.1.7 Use the MS/MS to infuse a compound and create an acquisition method.

3.1.8 Successfully complete a written exam with a minimum score of 85 %.

3.2 Terms to define

- Atmospheric Pressure Ionization (API)
- Atmospheric Pressure Chemical Ionization (APCI)
- Calibration
- Collision-Induced Dissociation (CID)
- Efficiency
- Electrospray Ionization (ESI)
- Eluotropic Series
- Flow Rate
- Gradient
- Ion Trap
- Isocratic
- Matrix Effects
- Multiple Reaction Monitoring (MRM)
- Normal Phase Chromatography
- Plate Number
- Quadrupole
- Resolution
- Retention Factor
- Reverse Phase Chromatography
- Selected Ion Monitoring (SIM)

- Selectivity (Separation Factor)
- Tuning

3.3 Reading Assignments

3.3.1 McDonald, P., *The Quest for Ultra Performance in Liquid Chromatography*, USA, Waters Corporation, 2009.

3.3.2 Balogh, M., *The Mass Spectrometry Primer*, USA, Waters Corporation, 2009.

3.3.3 Arsenault, J. and McDonald, P., *Beginners Guide to Liquid Chromatography*, USA, Waters Corporation, 2009.

3.3.4 Grumbach, E., Arsenault, J, and McCabe, D., *Beginners Guide to UPLC*, USA, Waters Corporation, 2009.

3.3.5 Honour, J., "Development and Validation of a quantitative assay based on tandem mass spectrometry." *Annals of Clinical Biochemistry*, Volume 48 (March 2011): 97-111.

3.3.6 Page, J. et al, "Ionization and Transmission Efficiency in an Electrospray Ionization-Mass Spectrometry Interface." *Journal of the American Society for Mass Spectrometry*, Volume 18 (2007): 1582-1590.

3.3.7 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.

3.3.8 Chambers, E. et al., "Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses." *Journal of Chromatography B*, Volume 852 (June 2007): 22-34.

3.3.9 Moffat, A., Osselton, M.D., and Widdop, B. (ed.), *Clarke's Analysis of Drugs and Poisons 3rd edition*, London, Pharmaceutical Press, 2004, 379-391 and 500-534.

3.3.10 Toxicology Section Toxicology Liquid Chromatography Tandem Mass Spectrometry LCMSMS procedure

3.4 Practical/Laboratory Exercises

3.4.1 Read the assigned literature.

3.4.2 Prior to working with any chemicals, solutions, or standards, read and comprehend the SDS for each chemical used in the procedure. This includes the determination of which PPE and engineering / administrative controls are required when handling each.

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

3.4.4 Observe the LC-MS/MS Key Operator or designee perform the routine maintenance required in the procedure Toxicology Liquid Chromatography Tandem Mass Spectrometry LCMSMS procedure.

3.4.5 Successfully perform the routine maintenance required in the procedure: Toxicology Liquid Chromatography Tandem Mass Spectrometry LCMSMS .

3.4.6 Successfully perform a resolution and calibration that meets the requirements stated in the procedure: Toxicology Liquid Chromatography Tandem Mass Spectrometry LCMSMS. What is the significance of each requirement?

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

3.4.8 Using the LC-MS/MS software review the data files provided by the Toxicology Training Coordinator.

3.4.9 What change would occur in the TIC and the MRM if the quadrupole voltage were increased or decreased?

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

3.4.11 Review with a senior analyst and demonstrate the use of LC-MS/MS software to tune a compound provided by the Toxicology Training Coordinator or designee.

3.4.12 Propose structures for the daughter ions determined in **3.4.10**.

3.4.13 Review with a senior analyst and demonstrate the use of the LC-MS/MS software to determine the signal to noise ratio (S/N) of a chromatographic peak.

3.4.14 Review with a senior analyst and demonstrate the use of the LC-MS/MS software to develop a quantitative method.

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

3.5 Study Questions

3.5.1 Name three advantages that liquid chromatography has over gas chromatography?

3.5.2 What advantages does Ultra Pressure Liquid Chromatography (UPLC) have over High Pressure Liquid Chromatography?

3.5.3 State the Van Deemter equation and define each term in the equation.

3.5.4 Give an example of each diffusion process referred to in the van Deemter equation.

3.5.5 What are two effects of band spreading?

3.5.6 What are three possible sources of band spreading?

3.5.7 What are three advantages to using a gradient instead of an isocratic run?

3.5.8 What types of compounds are best analyzed by normal phase chromatography?

3.5.9 Define adsorption chromatography.

3.5.10 Define partition chromatography.

3.5.11 What is a protic solvent? Give three examples of a protic solvent.

3.5.12 What is an aprotic solvent? Give three examples of an aprotic solvent.

3.5.13 What are the three parts of a liquid chromatograph?

3.5.14 What purpose do additives in a mobile phase serve?

3.5.15 What is a Taylor cone?

3.5.16 Explain electrospray ionization.

3.5.17 What effect would salts and phosphate buffers have on ESI?

3.5.18 What are the two modes of a tandem mass spectrometer?

3.5.19 What are the molecular ions produced in each of the two modes?

3.5.20 What is a product ion scan? What is the function of each quadrupole?

3.5.21 What is a precursor ion scan? What is the function of each quadrupole?

3.5.22 What is a constant neutral loss scan? What is the function of each quadrupole?

3.5.23 What makes a collision cell different from a traditional quadrupole?

3.5.24 Explain collision induced dissociation (CID)?

3.5.25 Design an experiment to determine ion suppression/enhancement.

4.0 Safety

This document provides an outline for training on procedures that are written in additional detail in specific Toxicology Section documents. To see safety hazards for particular procedures, refer to that specific procedure.

5.0 Records

Toxicology Drug Training Checklist

Training Section Completion Summary

Revision History		
Effective Date	Version Number	Reason
9/23/2020	2	References to Drug Chemistry Section removed References to Toxicology Unit replaced with toxicology section 3.1.1- added LCMSMS 3.3.10 – added LCMSMS 3.4.2 New 3.4.4 – removed Toxicology; added LCMSMS 3.4.5- removed procedure; added LCMSMS 3.4.6- removed procedure; added LCMSMS 4 New