Training Procedure for Extraction Chemistry

1.0 Purpose - The two basic extraction techniques used in forensic toxicology are liquid-liquid extraction and solid phase extraction (SPE). This training will explore the fundamentals and extraction methods used to isolate drugs from blood, urine and serum.

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

3.0 Procedure

3.1 Objectives

3.1.1 Review the Toxicology technical procedures.

3.1.2 Understand the concepts of acid and base.

3.1.3 Be able to determine based on structure whether a drug has acid or base properties.

3.1.4 Be able to identify the solubility of different drug forms in different solvents.

3.1.5 Be knowledgeable of solid phase and liquid-liquid extraction principles.

3.1.6 Successfully complete the extractions and GC-MS/LC-MS/MS analyses performed in Toxicology.

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

3.2 Study Questions

3.2.1 What is a Lewis acid? What is a Bronsted-Lowery acid?

3.2.2 What is a Lewis base? What is a Bronsted-Lowery base?

3.2.3 What are a conjugate acid and a conjugate base?

3.2.4 Explain the difference between a strong acid and a weak acid/strong base and a weak base. Give some examples of each.

3.2.5 Explain pKa. Discuss the Henderson-Hasselbach equation.

3.2.6 Generally, what are the products when an acid is combined with a base?

3.2.7 What types of organic functional group impart basic characteristics? Give an example of each type.

3.2.8 What types of organic functional group impart acidic characteristics? Give an example of each type.

3.2.9 Generally, what occurs when an inorganic acid is added to the base form of a drug?

3.2.10 Discuss salting out and its use in extractions.

3.2.11 Describe a liquid – liquid extraction.

3.2.12 Describe a Solid Phase Extraction.

3.2.13 What is the importance of pH when performing an extraction?

3.2.14 Explain the use of polar/non-polar solvents when performing an extraction. Give examples of both types of solvents.

3.2.15 What is the difference between Normal and Reverse Phase extractions?

3.2.16 Describe some common SPE sorbents used in forensic toxicology.

3.2.17 Describe a protein precipitation extraction.

3.2.18 What is derivatization?

3.2.19 What are some common derivatizing reagents used in forensic toxicology?

3.2.20 What types of functional groups might require derivatization?

3.3 Practical/Laboratory Exercises

3.3.1 For the following laboratory exercises, all quality control criteria outlined in the associated procedure must be met. If quality control criteria is not met, the extraction must be repeated.

3.3.2 With the Training Coordinator or designee, extract a set of quality control samples associated with each of the SPE procedures and analyze all associated data.

3.3.2.1 Solid Phase Extraction involves several additions to an SPE column. Answer the following questions:

3.3.2.1.1 Why is buffer added to the columns and samples?

3.3.2.1.2 Why is methanol added to the columns?

3.3.2.1.3 Why is water added to the columns?

3.3.2.1.4 Why is hexane added to the columns?

3.3.2.1.5 Why does methanol not remove the drugs found in the basic fraction of the blood?

3.3.2.2 Demonstrate ability to extract the quality control samples associated with SPE and analyze all data associated with extraction.

3.3.3 With the Training Coordinator or designee, extract all quality control samples associated with the liquid-liquid extractions and analyze all associated data

3.3.3.1 Answer the following questions associated with the Liquid-Liquid Extraction of Phenethylamines:

3.3.3.1.1 Why is NaCl added to the blood?

3.3.3.1.2 Why is buffer added?

3.3.3.1.3 Why is ammonium hydroxide added?

3.3.3.1.4 When n-butyl chloride is added, in what layer can the drugs of interest be found? Why?

3.3.3.1.5 Why is a 2% solution of HCl in methanol added?

3.3.3.1.6 Why is 0.5 N H2SO4 added?

3.3.3.1.7 Why is hexane added?

3.3.3.1.8 What is in the hexane which is aspirated out?

3.3.3.1.9 What is the purpose of adding acetic anhydride?

3.3.3.2 Answer the following questions associated with the Blood Cannabinoid Liquid-Liquid Extraction:

3.3.3.2.1 Why is 10% acetic acid added?

3.3.3.2.2 Why is 9:1 hexane:ethyl acetate added?

3.3.3.2.3 Why do we reconstitute BCLLE samples in 50:50 acetonitrile:water?

3.3.3.3 Demonstrate ability to extract the quality control samples associated with the liquid-liquid extractions and analyze all data associated with extraction.

3.3.4 Extract and analyze a set of practice samples with the associated quality control samples provided by the Toxicology Training Coordinator, to include all extraction procedures currently used.

3.3.5 Successfully extract and analyze competency samples provided by the Toxicology Training Coordinator, to include all extraction procedures currently used.

3.4 Required Reading

Toxicology technical procedures

Bell, Suzanne. *Forensic Chemistry*. 1st Ed. Pearson Prentice Hall, 2006. Chapter 4.

Chen, Xiao-Hua, et al. "Isolation of Acidic, Neutral, and Basic Drugs from Whole Blood Using a Single Mixed-Mode Solid-Phase Extraction Column" *Journal of Analytical Toxicology*, Vol. 16, November/December 1992.

Foerster, E.H. et al. "A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood or Tissues by Gas Chromatography." *Journal of Analytical Toxicology*, Vol. 2, March/April 1978.

Juhascik, M. P. and Jenkins, A. J. "Comparison of Liquid/Liquid and Solid-Phase Extraction for Alkaline Drugs" *Journal of Chromatographic Science*, Vol. 47, August 2009

4.0 References

4.1 Baselt, Randall C. *Disposition of Toxic Drugs and Chemicals in Man*. 8th Ed. Foster City, California: Biomedical Publications, 2008.

4.2 Sears, Robert. "Liquid – Solid Extraction in Toxicology:" Forensic Toxicologist Certification Board.

4.3 Streitwieser, Andrew, Jr. and Clayton H Heathcock. *Introduction to Organic Chemistry*. MacMillan Publishing Co., 1976. p 494.

4.4 Van Horne, K.C. *Sorbent Extraction Technology*. Analytical International, Inc.: 1990

4.5 Williams, Phillip L., Robert C James, and Stephen M Roberts. *Principles of Toxicology*. John Wiley and Sons: New York, 2000.

5.0 Records

Toxicology Drug Analysis Training Checklist

Training Section Completion Summary

Revision History		
Effective Date	Version Number	Reason
02/12/2016	1	Original Document
02/22/2019	2	1.0 Purpose: correct "There" to "the"; "liquid" to "liquid-liquid" Added 3.3.1 through 3.3.6