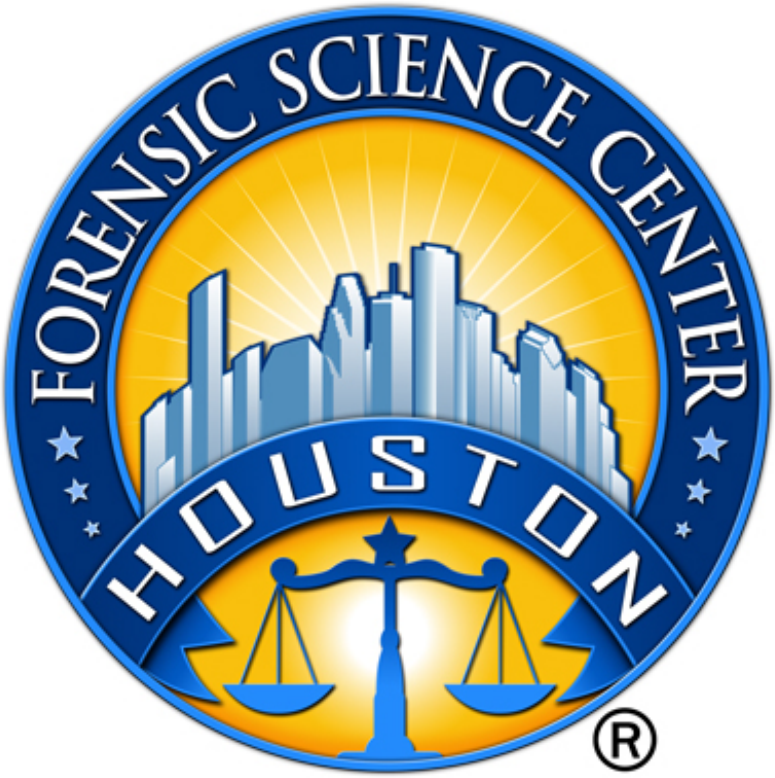

Toxicology Section
Training Manual
(Version 3.9)

Comparative and Analytical Division

Table of Contents

1. Introduction

1.1. Aim

1.1.1. Forensic Toxicology involves the detection and quantification of drugs or other toxic substances in the body and interpretation of the effects of those drugs on the body as they pertain to criminal or death investigations.

1.1.2. The purposes of the Toxicology Training Program are:

1.1.2.1. To educate the trainee in the theories, principles, and methods of Forensic Toxicology so the trainee can perform the work competently and effectively communicate that knowledge and understanding to a jury in a court of law.

1.1.2.2. To document training in each functional and relevant area of the Houston Forensic Science Center (HFSC). The Toxicology Training Program should familiarize the trainee with the relevant policies and procedures of HFSC and document competency and proficiency in each discipline of casework.

1.2. Training Program Layout

The HFSC Toxicology Training Program contains multiple training modules in the format described below.

1.2.1. **Purpose:** To provide training in all functional areas of casework according to established standard operating procedures (SOP).

1.2.2. **Scope:** The training covers the SOPs implemented within the HFSC Toxicology Section. Validation and uncertainty of measurement of analytical assays currently in service within the Toxicology Section are also included.

1.2.3. **Format:** Each practical training module addresses essential Toxicology policies and procedures. Upon completion of a particular training module, the trainee may need to repeat a procedure several times to allow for practice. Each training module is divided up into the following segments:

1.2.3.1. **Required Reading** – Trainees must familiarize themselves with the documentation listed under this section. HFSC-related documents are located in Qualtrax. Additional references are provided but may not be applicable, depending on the training and experience of the trainee and trainer. Additional resources are available on site. Non-HFSC references can be found in the Toxicology Section Library or in the Training Folder of the Forensic Toxicology Site online.

1.2.3.2. **Laboratory Exercise** – Hands-on training may include observation of a qualified analyst performing analysis, working under supervision, and eventually performing the test independently. Based on the trainee's previous experience and training, laboratory exercises can be completed using the primary biological matrix for the method (i.e., blood). The performance of analysis of the secondary matrix (i.e., urine) will be evaluated during the competency test. If laboratory exercises are completed using both biological matrices (blood and urine), the competency test may consist of

just the primary biological matrix for the method. If a competency consists of multiple GC-MS and/or LC-MS/MS modules, selected drugs but not all assays will be included as part of the competency test. Where applicable, laboratory exercises are intended to familiarize the trainee with practical aspects of each technical SOP. An experienced analyst or supervisor will monitor the training and be responsible for reviewing the trainee's progress.

1.2.3.3. **Study Questions** – These questions are designed to develop knowledge of the scientific principles behind each standard operating procedure. Answers to questions may be written or discussed with the appropriate trainer/supervisor. These concepts should serve as stimuli to aid the trainee's learning process, rather than required tasks. Additional references are listed at the beginning of the training module to aid the trainees in reviewing the fundamental information for each particular analysis. Study Questions may be used to assess the trainee's knowledge of the training module(s) as part of his/her written or oral competency exam.

1.2.3.4. **Competency** – A competency test is given once trainees successfully complete the relevant laboratory exercise and study questions. Before trainees can assume responsibility for casework, they must demonstrate qualifications in that area by performing a competency test.

1.2.3.4.1. Evaluations involve 1) the analysis of mock casework or re-analysis of adjudicated casework or old proficiency samples (if applicable), 2) writing of test reports (if applicable), and 3) a written or oral examination to assess the individual's knowledge of the discipline, category of testing, or task being performed (if applicable). The appropriate trainer will provide samples with analyte concentrations unknown to the trainees.

1.2.3.4.2. The evaluation criteria for the competency written/oral examination are whether the trainee accurately describes the key concepts, effectively communicates the technical knowledge, correctly conveys scientific principles and procedures associated with the assay, and satisfactorily addresses all, if any, follow-up questions.

1.2.3.4.3. The examination is graded as either pass or fail. "Pass" means the trainee meets all applicable evaluation criteria with a 100% passing score. This may occur after completion of one or more follow-up questions. "Fail" means one or more responses are not fully satisfactory and the deficiency is too significant to address via a follow-up. In this instance, the trainee will receive remedial training on the deficient topic(s) and try again to pass the exam with a 100% passing score.

1.2.3.4.4. Multiple GC-MS modules can be combined into one competency test and multiple trainees may work together on the competency samples, each working on different assays. This mimics Toxicology Section's workflow for

casework. Specific details for individual assays are given in each training module.

1.2.3.5. **Documentation of Completion** – Completion of each module is documented in Form LAB-076 Training Checklist, activity log, and/or equivalent electronic record. The records will be retained in the trainee's training and development file in the section's electronic storage for accreditation and certification purposes.

1.3. Qualifications of Trainers and Trainees

1.3.1. Trainers are qualified analysts and/or technicians who have been authorized to perform the tasks described in the training module.

1.3.2. Trainees are analysts and/or technicians employed at HFSC after meeting the requirements of education, experience, and skills and who have passed the required background check and drug screen.

1.4. Training Schedule

1.4.1. The Toxicology Training Program is intended to be self-motivating. Within a timeframe established by the section management, the trainee should proceed at his or her own pace. Typical timelines for training modules included in this manual are listed below. The date of the training initiation meeting with the section management and/or trainer is considered the start date of the training. The end date is the date of the authorization memo.

1.4.1.1. Typical timelines of analysis training modules:

1.4.1.1.1. Sections 2-6 (part of onboarding training): 60 days
1.4.1.1.2. Section 7: 4 months
1.4.1.1.3. Section 8: 3 months
1.4.1.1.4. Section 9: 6 months
1.4.1.1.5. Section 10-17: 1-2 months per section
1.4.1.1.6. Section 18: 1 month
1.4.1.1.7. Section 19: one day
1.4.1.1.8. Section 20 (to be included in applicable sections)
1.4.1.1.9. Section 21: 6-12 months

1.4.1.2. Typical timelines of review training modules:

1.4.1.2.1. Alcohol batch/case TR (section 7): 1 month
1.4.1.2.2. Alcohol case AR (section 7): 2 weeks
1.4.1.2.3. ELISA batch TR (section 8): 6 weeks
1.4.1.2.4. Qualitative case TR (section 9): 2 weeks
1.4.1.2.5. Qualitative case AR (section 9): 2 weeks
1.4.1.2.6. Drug batch TR (section 18): 3 months
1.4.1.2.7. Positive targeted case TR (section 18): 1 month

1.4.1.2.8. Positive targeted case AR (section 18; the trainee should have completed the section 20 training): 1 month

1.4.2. Segments of the Toxicology Training Manual may be waived, used independently, and/or pursued out of order, depending on the trainee's prior education, training, and experience, as well as the needs of HFSC and the Toxicology Section. Deviations will be noted in the authorization memo of the training module.

1.4.3. Not all training modules have a competency test (e.g., safety, review). Additionally, one competency test may be issued for multiple training modules (e.g., cocaine analysis, cannabinoids analysis etc.). It is not necessary to perform a competency test for each of the analytical methods if they use the same technique (e.g., liquid chromatography-tandem mass spectrometry).

1.4.4. The documentation of prior education, training, and experience as well as updated statements of qualifications, training certificates, and other training-related documents should be uploaded onto Qualtrax in a timely manner.

1.4.5. Each trainee is responsible for maintaining his/her Personal Training and Development file where all training-related records are stored.

1.4.6. Training tasks (e.g., reading, study questions, laboratory exercise) can be repeated as needed. Competency can be retaken after remedial training as documented in the training records.

1.4.7. Trainee completes the HFSC on-board training prior to or in conjunction with the section training.

1.4.8. Trainee is expected to read the materials provided by Texas Forensic Science Commission (TFSC) and obtains an appropriate license from TFSC prior to doing casework. This process can occur in conjunction with the section training.

1.4.9. Continuing education is achieved via reading scientific articles and other references, participating in the section discussions, conducting research projects and/or attending internal and external seminars, workshops, webinars, conferences, meetings, and other scientific programs. Competency is monitored as described in the HFSC Quality Manual.

1.5. Assessment of Training Program

1.5.1. Status and feedback of a training module are discussed throughout progression of a training module between the trainee and the trainer. This can also be relayed to the section management during the section meeting, one-on-one meeting, training group meeting, and other means.

1.6. Post Training

1.6.1. The first casework analysis for each analytical technique (i.e., HS-GC, ELISA, GC-MS, and LC-MS/MS) will be observed by a more experienced analyst. This is to support the less experienced analyst as s/he may have questions due to possible administrative differences between training vs. casework analyses. The observation is documented in batch records.

1.6.2. Proficiency testing procedure and requirements follow the current HFSC Quality Manual. It will be performed on two different rounds of four specimens per calendar year at a minimum.

1.6.2.1. Acceptability criteria of quantitative results in proficiency tests are ±10% of the consensus result or ±2 standard deviations of the consensus result (whichever is greater) for blood alcohol analysis and ±20% of the consensus result or ±2 standard deviations of the consensus result (whichever is greater) for drug analyses.

1.6.2.2. For qualitative results, positive samples shall be positive and negative samples shall be negative.

1.6.2.3. If a proficiency test result falls outside of the acceptability criteria, the section will investigate the cause of the failure, which may include re-analysis of the sample by another analyst, sending the sample to another laboratory for independent testing, analyzing another sample from the same lot from the proficiency vendor, analyzing the sample using a different technique (e.g., GC-MS instead of LC-MS/MS), and/or other experiments depending on the circumstances surrounding the sample.

1.7. Reauthorization

1.7.1. If a forensic scientist has been on an extended leave for a period of 6 months or longer, s/he must successfully complete a competency test prior to resuming casework. The competency test must include a practical examination and written report, if applicable, as described in the HFSC Quality Manual. This documentation is to be reviewed and approved by the Quality Division.

2. Quality Management System

The Quality Management System is the organizational structure, responsibilities, procedures, processes, and resources for implementing quality management. It includes all activities which contribute to quality, directly or indirectly. Every staff member is responsible for ensuring compliance with the Quality Management System.

2.1. Aim

2.1.1. During this training module, trainee will be introduced to the architecture of the Quality Management System including documentation, structure, roles, and responsibilities.

2.2. Required Reading

2.2.1. HFSC Quality Manual (current version)

2.3. Documentation of Completion

2.3.1. Completion of the training requirement is documented on Form LAB-076, activity log, and/or equivalent electronic record.

3. Security

3.1. Aim

3.1.1. This training module provides the necessary security-related information to ensure a secure workplace, including protecting staff members, physical assets, evidence, systems/data/passwords, vehicles, and employee information/privacy and to protect staff in the field or while traveling.

3.2. Required Reading

3.2.1. HFSC Security Manual (current version)

3.3. Laboratory Exercise

3.3.1. Understand the HFSC visitor policy; escort a visitor with another analyst.

3.4. Documentation of Completion

3.4.1. Completion of the training requirement is documented on Form LAB-076, activity log, and/or equivalent electronic record.

4. Safety

4.1. Aim

4.1.1. This training module provides the necessary safety information to prevent accidents, injuries, and illnesses, to recognize and eliminate hazards, and to maintain a safety-conscious environment.

4.2. Required Reading

4.2.1. HFSC Health and Safety Manual (current version)

4.3. Laboratory Exercise

4.3.1. The section safety representative will provide you with an overview of safety measures in HFSC.

4.3.2. New employees must complete blood borne pathogen and chemical safety training during the Employee Safety Orientation.

4.4. Study Questions

Before completing these questions, you should have received the current HFSC Health and Safety Manual and received an orientation from the Toxicology Safety Representative. Review the responses with the trainer.

4.4.1. Where are the safety showers, eye wash fountains, fire extinguishers, fire blankets, First Aid kit and spill kits located?

4.4.2. How are we alerted to chemical hazards in the laboratory?

4.4.3. Where are the safety data sheets (SDSs) stored and who is responsible for their upkeep?

4.4.4. Evidence and procedures used in the laboratory pose biological and chemical hazards. What steps are taken to ensure laboratory safety?

4.4.5. Who is the Toxicology section's safety representative?

4.4.6. In the event of a chemical spill, what is the first thing you should do?

4.4.7. In the event of an injury or cut, what is the first thing you should do?

4.4.8. What are "universal precautions"?

4.4.9. How do we dispose of biohazard waste?

4.4.10. How do we dispose of "sharps" waste?

4.4.11. Which biohazard waste container would you dispose blood that has organic solvents mixed with it?

4.4.12. True or false: Glass pipettes can be disposed into a biohazard box.

4.4.13. True or false: Biohazard sharps containers can be overfilled as long as they are kept upright and closed.

4.4.14. While opening a blood or urine sample it slips out of your hand and breaks. What should you do?

4.4.15. What measures are taken to secure compressed gas cylinders in the laboratory?

4.4.16. Which cabinet would you store a bottle of ammonium hydroxide, hydrochloric acid, and methanol?

4.4.17. True or false: You do not have to wear safety glasses when filling up wash vials.

4.4.18. What steps are taken to secure drug standards in the laboratory? What security steps are taken when you prepare a new drug standard?

4.4.19. Explain how to use a fire extinguisher. What does the acronym PASS stand for?

4.5. Documentation of Completion

4.5.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record.

5. Toxicology Section Operation

5.1. Aim

5.1.1. During this training module, trainees learn the operational activities, policies, and procedures of the Toxicology Section. The Toxicology Analytical Manual provides specific information on functional aspects of laboratory operations.

5.2. Required Reading

5.2.1. HFSC Toxicology Analytical Manual

5.2.2. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Part I. Introduction and Part II. Methodologies if the trainee has not previously received forensic toxicology-related education.

5.3. Study Questions

5.3.1. How often are the analytical and top-loading balances calibrated by an external vendor?

5.3.2. How often are weights used in the toxicology section calibrated by an external vendor?

5.3.3. What is the check-in process when the weights are received back from the external vendor?

5.3.4. Where are the Toxicology Laboratory Forms?

5.3.5. When are performance checks done on the balances?

5.3.6. How often is the pH meter calibrated?

5.3.7. What are the required maintenance steps for the Millipore water purification system? How do we check the purity of the Millipore water is acceptable?

5.3.8. Briefly describe the process for inventorying certified reference material purchased from an external vendor.

5.3.9. What are the requirements for all labels placed on reagents and drug standards made in-house?

5.3.10. How often is the performance check for the heat blocks performed?

5.3.11. How are the temperatures of the refrigerators and freezers monitored?

5.3.11.1. What are the acceptable ranges for each?

5.3.12. How often are pipettes calibrated by an external vendor? When is a performance check done on a pipette and how are performance checks and calibration documented?

5.3.13. What is the difference between “To Deliver” and “To Contain” on a volumetric pipette?

5.3.14. Describe the process for verifying the following:

5.3.14.1. Internal Standard

5.3.14.2. Calibrator

5.3.14.3. In-house control with theoretical target

5.3.14.4. External control

5.3.14.5. Reagent

5.3.14.6. Blank matrix

5.3.15. What is a system suitability? How often is a system suitability run on Headspace and GC-MS Instruments?

5.4. Laboratory Exercise

5.4.1. Perform a pipette precision and accuracy exercise that will be maintained in the trainee's training record.

5.4.2. Observe the following quality control measures being performed:

5.4.2.1. Balance Performance Check

5.4.2.2. pH meter Performance Check

5.4.2.3. Heat Block Thermometer Performance Check

5.4.2.4. Downloading of Temperature Monitoring Report

5.4.2.5. Uploading documents to e-Discovery website

5.4.2.6. Toxicology housekeeping tasks as applicable (e.g., glassware cleaning, waste disposal, supply management/inventory, outsourcing, etc.)

5.4.3. Observe the preparation of a drug standard.

5.4.4. Observe the preparation of a reagent.

5.5. Documentation of Completion

5.5.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record.

6. Evidence Handling and Documentation

6.1. Aim

6.1.1. This training module familiarizes the trainee with evidentiary processes such as chain of custody, documentation, secure packaging, repackaging, identification and preservation of evidence. Specific evidentiary and security-related policies and procedures of the Toxicology Section will be addressed.

6.2. Required Reading

6.2.1. HFSC Toxicology Analytical Manual

6.2.2. HFSC Client Services & Case Management Division Standard Operating Procedures: Toxicology Procedures

6.3. Additional Resources

6.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction)

6.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 13 (Sampling, Storage and Stability)

6.3.3. Garriott's Medicolegal Aspects of Alcohol, 6th Ed., Y.H. Caplan and B.A. Goldberger, 2015, Lawyers & Judges Publishing Company – Ch. 10 (Collection and Storage of Specimens for Alcohol Testing)

6.3.4. Specimen Selection, Collection, Preservation and Security. Bradford D. Helper and Daniel S. Isenschmid, Postmortem Toxicology of Abused Drugs, pp. 13-30, S. B. Karch, Ed., 2007, CRC Press, Washington DC.

6.4. Laboratory Exercise

6.4.1. Accessioning/LIMS

6.4.1.1. The trainee will learn about evidence handling and security policies and procedures prior to implementation of casework. The trainer will indicate the date(s) of this training. Trainer will also demonstrate each step described in the current Evidence Description and Review Form (LAB-032) or equivalent form in LIMS. The trainee needs to show his/her competence in following the proper procedure for evidence handling and preservation.

6.4.1.2. The trainee will observe at least three cases accessioned by qualified personnel from the Client Services/Case Management Division and read the Toxicology procedures in the CS/CM SOP.

6.5. Documentation of Completion

6.5.1. Completion of each training requirement is documented on Form LAB-076, activity log, and/or equivalent electronic record.

7. Analysis of Alcohol and Other Volatiles by Headspace Gas Chromatography-Flame Ionization Detector

7.1. Aim

7.1.1. This module provides training on procedures, practice, interpretation, and theory of the qualitative or quantitative analysis of ethanol, methanol, acetone, and isopropanol in biological specimens using headspace sampling and dual column gas chromatography with flame ionization detection (GC/FID).

7.2. Required Reading

7.2.1. HFSC Toxicology Analytical Manual
7.2.2. Validation package for appropriate Headspace GC-FID Instrument(s)
7.2.3. Uncertainty of Measurement package for Volatiles analysis

7.3. Additional Resources

7.3.1. Caplan, YH and Goldberger, BA., eds., Garriott's Medicolegal Aspects of Alcohol, 6th ed. Lawyers & Judges Publishing Company, Inc., 2015.
7.3.2. Levine, B and Kerrigan, S., eds., Principles of Forensic Toxicology, 5th Ed. Springer, 2020. – Ch. 19 (Alcohol).
7.3.3. Bernosky-Smith, MA, Shannon, EE, and Roth, AJ. Alcohol effects on simulated driving in frequent and infrequent binge drinkers. Hum Psychopharmacol. 2011;23:216-223.
7.3.4. National Institute on Alcohol Abuse and Alcoholism. No. 28 PH 356 April 1995. Alcohol and Tolerance. https://pubs.niaaa.nih.gov/publications/aa28.htm.
7.3.5. Jones, AW. Evidence-based survey of the elimination rates of ethanol from blood with applications in forensic casework. Forensic Sci Int. 2010;200:1-20.
7.3.6. Laurens, JB, Sewell, FJJ, and Kock, MM. Pre-analytical factors related to the stability of ethanol concentration during storage of ante-mortem blood alcohol specimens. J Forensic Leg Med. 2018;58:155-163.
7.3.7. Tiscione, NB, Vacha, RE, Alford, I, Yeatman, DT, and Shan, X. Long-term blood alcohol stability in forensic antemortem whole blood samples. J Anal Toxicol. 2015;39:419-425.
7.3.8. Shan X, Tiscione, NB, Alford I, and Yeatman, DT. A study of blood alcohol stability in forensic antemortem blood samples. Forensic Sci Int. 2011;211:47-50.
7.3.9. Winek, T, Winek CL, and Wahba, WW. The effect of storage at various temperatures on blood alcohol concentration. Forensic Sci Int. 1996;78:179-185.
7.3.10. Winek, CL and Louette, JP. Effect of short-term storage conditions on alcohol concentrations in blood for living human subjects. Clin Chm. 1983;29:1959-1960.
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7.3.12. Tiscione N. The impact of hemolysis on the accuracy of ethanol determinations. J Anal Toxicol. 2015;39:672-673.

7.3.13. Zittel, DB and Hardin, GG. Comparison of blood ethanol concentrations in samples simultaneously collected into expired and unexpired venipuncture tubes. J Anal Toxicol. 2006;30:317-318.
7.3.14. Miller, MA, Rosin, A, Levsky, ME, Patel, MM, Gregory, TJD, and Crystal, CS. Does the clinical use of ethanol-based hand sanitizer elevate blood alcohol levels? A prospective study. Am J Emerg Med. 2006;24:815-817.
7.3.15. Logan, BK and Jones AW. Endogenous ethanol 'auto-brewery syndrome' as a drunk-driving defence challenge. Med Sci Law. 2000;40:206-15.
7.3.16. Jones, AW. Salting-out effect of sodium fluoride and its influence on the analysis of ethanol by headspace gas chromatography. J Anal Toxicol. 1994;18:292-293.
7.3.17. Chang, J and Kollman, SE. The effect of temperature on the formation of ethanol by Candida albicans in blood. J Forensic Sci. 1989;34:105-109.
7.3.18. Penetar, DM, McNeil, JF, Ryan, ET, and Lukas, SE. Comparison among plasma, serum, and whole blood ethanol concentrations: impact of storage conditions and collection tubes. J Anal Toxicol. 2008;32:505-510.

7.4. Important Concepts

7.4.1. Pharmacology
- 7.4.1.1. Volatiles
- 7.4.1.2. Specimen type
- 7.4.1.3. Specimen Preservation
- 7.4.1.4. Fermentation
- 7.4.1.5. Pharmacokinetics – Absorption, Distribution, Metabolism, and Excretion
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- 7.4.1.7. Combination with Other Drugs
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7.4.4. Report Writing
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- 7.4.4.2. Reporting Guidelines
- 7.4.4.3. LIMS
- 7.4.4.4. Technical/Administrative Review

7.5. Study Questions

7.5.1. Pharmacology

7.5.1.1. What is a standard drink? How many grams of ethanol per standard drink, and give common examples.

7.5.1.2. Name the chemicals in gray top tubes contained in HFSC DWI kits. What is the purpose of each one? How much is in each tube?

7.5.1.3. What is the purpose of the expiration date on the gray top tubes? What are the implications of testing a tube with an expiration date that has passed?

7.5.1.4. Define absorption. How does ethanol absorb into the body?

7.5.1.5. What is the average time it takes to absorb a standard drink? Is there an average absorption rate?

7.5.1.6. What are some factors that can influence absorption? Include examples of what can cause absorption to occur more quickly or more slowly.

7.5.1.7. Define elimination. What are the main mechanisms of ethanol elimination?

7.5.1.8. What range of elimination rates has been observed in the literature? What elimination range does HFSC Toxicology utilize in interpretation, and why?

7.5.1.9. What are some factors that can affect elimination? Include examples of what can cause elimination to occur more quickly or more slowly.

7.5.1.10. Define retrograde extrapolation (RE). What assumptions are made during RE, and why are they important?

7.5.1.11. Practice Retrograde Extrapolation and Widmark Calculations (apply the current SOP, show your work and state any assumptions made).

7.5.1.11.1. Stop-3:49AM Test-5:00AM BAC-0.068 Last drink – 11:00PM prior

7.5.1.11.2. Stop-5:11AM Test-10:00AM BAC-0.068 Last drink – 1:00AM

7.5.1.11.3. Stop-1:36AM Test-4:40AM BAC-0.057 Last drink – 1:00AM

7.5.1.11.4. Stop-2:43AM Test-7:34AM BAC-0.071 Last drink-midnight

7.5.1.11.5. Male, 145lbs, BAC-0.080, How many drinks?

7.5.1.11.6. Female, 150lbs, BAC-0.243, How many drinks?

7.5.1.11.7. Male, 215lbs, 4 drinks, what is the BAC?

7.5.1.11.8. Female, 178lbs, 7 drinks, what is the BAC?

7.5.1.12. What is the quantitative relationship between whole blood and serum/plasma? From where is the range derived? What are the rules for reporting serum/plasma results, as described in the SOP?

7.5.1.13. Describe the stages of acute alcohol intoxication from low to high blood alcohol concentration.

7.5.2. Analytical Procedures

7.5.2.1. What are the purposes of an internal standard? Provide at least three. Name the internal standard used here. How much is added when preparing samples and at what concentration?

7.5.2.2. What are quality controls (QCs) and why are they included with every run?

7.5.2.3. What are negative controls and why are they included with every run?

7.5.2.4. Why are both aqueous and whole blood controls used?

7.5.2.5. What is the difference between a standard, calibrator, and a control?

7.5.2.6. Why are case samples run in duplicate in the ALC assay (when we only run singly in GC and LC methods)?
7.5.2.7. What is the purpose of the SS? What is considered a passing SS?
7.5.2.8. Explain how to prepare a liquor sample for analysis.
7.5.2.9. Define carryover. How do we monitor for carryover in each batch? What is the highest concentration of an unknown that can be obtained via the ALC method without concerns for carryover? How do we know this?
7.5.2.10. What is tolerance? Discuss how tolerance can affect a person's behavior while ethanol is on board.

7.5.3. Headspace GC
7.5.3.1. Define headspace.
7.5.3.2. Define Henry's Law. How does the HS-GC incorporate Henry's Law into the analytical process?
7.5.3.3. Define gas chromatography.
7.5.3.4. Why are two capillary GC columns used?
7.5.3.5. What is the difference between the two columns?
7.5.3.6. Define retention time.
7.5.3.7. What is a carrier gas? What carrier gas is used in the ALC method?
7.5.3.8. How is the detector flame ignited? What gases are used by the FID, and what are the flow rates of each?
7.5.3.9. What is the purpose of heating the vials? What are the agitation settings for our method?
7.5.3.10. Explain how the HS-GC instrument works.
7.5.3.11. Draw a schematic of an FID and describe how it works.

7.5.4. Report Writing
7.5.4.1. How do you evaluate the quality of a run? Describe what to look for to determine if a run is successful.
7.5.4.2. In what units are results reported? For blood? For beverages?
7.5.4.3. Define uncertainty of measurement. How is UM reported for alcohol results?
7.5.4.4. For calibrators and controls, what are the acceptance criteria?
7.5.4.5. Define limit of quantification (LOQ) and limit of detection (LOD). What are the LOQ and LOD for our procedure?
7.5.4.6. Locate a recently completed batch in the section shared storage. Manually calculate the BAC for the MQC in a batch (both columns) based on responses of ethanol, internal standard, and the calibration curve. Show all math and specify the batch name. (HINT: y = mx + b)

7.6. Laboratory Exercise

7.6.1. Depending on experience, the trainee may observe a qualified analyst performing an analysis.
7.6.2. Discuss instrument availability with the trainer. The trainee will use a headspace GC-FID system to analyze samples. For operation of the instrument, see the trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.

7.6.3. Depending on the trainee's level of experience, the trainee may now perform alcohol analysis for training purposes. Locate the calibrators, controls, and internal standards for training in the refrigerator. For analysis of blood samples, the trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee may evaluate the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.

7.6.4. Repeat the procedure as necessary. By the time the trainee takes the competency test, the trainee should be well practiced and familiar with the technique and should have performed at least three to five consecutive, successful alcohol analyses.

7.6.5. The trainee will learn how to draft alcohol case reports. Emphasis will be placed on creating reports in LIMS that are consistent with current formatting and content guidelines.

7.6.5.1. The trainee will observe a qualified analyst draft at least five alcohol case reports and then draft at least five case reports that will be reviewed by a qualified analyst. This must be completed to the satisfaction of the qualified analyst.

7.6.6. The trainee will complete the applicable study questions from section 20. The trainee will then complete at least 2 practice testimony question and answer sessions with the trainer or a qualified analyst.

7.6.7. Inform the trainer if further assistance is necessary.

7.7. Competency

7.7.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test.

7.7.2. A qualified analyst will assign the trainee mock case samples that have been previously analyzed. Unaided, the trainee will perform the volatile analysis according to the SOP. Treat the samples like regular casework including the reporting of results.

7.7.3. The analysis results must be in agreement with the original results obtained from qualified analysts or theoretical target concentrations as applicable (i.e., within 20% difference unless otherwise specified in the training records. Some analytes may have larger %difference due to instability; trainer will evaluate acceptability of the result and may consider a result with >20% difference as passing upon review of literature and/or re-analysis of the sample by another analyst) and the standards and controls must meet the acceptance criteria set forth in the SOP.

7.7.4. The trainee will have a mock trial on one of the case samples that was present in their competency test samples when possible. The trainee must successfully complete the mock trial before they can complete casework.

7.7.5. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to alcohol analysis. Once the results have been discussed and

approved, the trainee will receive an authorization memo and may begin independent casework upon issuance of TFSC Forensic Analyst license.

7.8. Review of Alcohol Case Reports

7.8.1. After at least six months of alcohol analysis experience from the current and/or previous employment, the trainee is eligible to learn how to perform technical and administrative review for alcohol case reports. Emphasis will be placed on the report writing review process in LIMS that are consistent with current formatting and content guidelines.

7.8.1.1. Technical Review

7.8.1.1.1. The trainee will observe technical review of a batch of alcohol case reports. The trainee will then perform technical review on at least five alcohol batches that a qualified analyst will subsequently review. The trainee must demonstrate his/her abilities to successfully technically review the batch and case records to the satisfaction of the qualified analyst.

7.8.1.2. Administrative Review

7.8.1.2.1. The trainee will observe administrative review of at least five alcohol case reports. The trainee will then perform administrative review on at least one batch of alcohol case reports that a qualified analyst will subsequently review. The trainee must demonstrate his/her abilities to successfully administratively review the case reports to the satisfaction of the qualified analyst.

7.9. Documentation of Completion

7.9.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

8. Drug Screen by Enzyme-Linked Immunosorbent Assay (ELISA)

8.1. Aim

8.1.1. This module provides training on procedures, practice, interpretation, and theory of immunoassay techniques used in the Toxicology Section. ELISA is used to screen for twelve common drugs or drug classes. The performance and reliability of the ELISA is paramount because in most cases these results will subsequently determine which confirmatory analyses are performed. An automated ELISA is used to screen a large number of samples for amphetamine, barbiturates, benzodiazepines, buprenorphine, cannabinoids, carisoprodol, cocaine metabolite, fentanyl, methamphetamine, opiates, oxycodone/oxymorphone, and phencyclidine (PCP).

8.2. Required Reading

8.2.1. HFSC Toxicology Analytical Manual
8.2.2. Validation package for ELISA

8.3. Additional Resources

8.3.1. TECAN Operating Manual Freedom EVO 75 BG/N: 30023958.02
8.3.2. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 13 (Immunoassay)
8.3.3. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 16 (Immunoassays)
8.3.4. Handbook of Workplace Drug Testing, 2nd Ed., J.D. Ropero-Miller and B.A. Goldberger, 2009, AACC Press – Ch. 2 (Screening: Immunoassays)
8.3.5. Origin of Morphine and Codeine in Biological Fluids, Mahmoud A. ElSohly, Handbook of Workplace Drug Testing, pp 225-238, 1995, Ray H. Lui and Bruce A. Goldberger Eds., AACC Press, Washington DC.
8.3.6. Adulteration of Urine Specimens, J. T. Cody, Handbook of Workplace Drug Testing, pp 181-208, 1995, Ray H. Lui and Bruce A. Goldberger Eds., AACC Press, Washington DC.

8.4. Study Questions

8.4.1. Explain the advantages and disadvantages of screening for the presence of drugs.
8.4.2. Briefly describe how the ELISA works. What is the relationship between absorbance and concentration?
8.4.3. Explain the following terms as they apply to ELISA; antigen, antibody, monoclonal/polyclonal antibody, microplate, substrate, horseradish peroxidase, cut-off, sensitivity, and specificity.
8.4.4. Is ELISA a homogeneous or heterogeneous immunoassay? Explain.
8.4.5. What is cross-reactivity and what causes it? Explain the advantages and disadvantages.
8.4.6. Define a "false positive" and "false negative".
8.4.7. State the formula for calculating sensitivity (%) and specificity (%) for qualitative screening methods.

8.4.8. Name the chemical compound that is the primary target of the antibody in each of the ELISA assays currently in use.
8.4.9. Describe how to determine whether a run meets the acceptance criteria.
8.4.10. How do we decide whether a sample is presumptive positive or negative?
8.4.11. Describe the purpose of the calibrator and controls used in each run. Where are they positioned?
8.4.12. Define "percent binding". How is it calculated?
8.4.13. At the end of a run the % CV for the opiate negative control is 30%. What are the possible reasons for this? What should be done?
8.4.14. Define analytical sensitivity. What is the difference between analytical sensitivity and limit of detection (LOD)?
8.4.15. What is the significance of the Negative Control?
8.4.16. The % CV and the average absorbance are calculated automatically using the Excel spreadsheet. How are these values calculated manually?
8.4.17. What is a "matrix effect", what causes it, and how can it be minimized?
8.4.18. What food product might affect your opiate ELISA results?
8.4.19. What are our current cut-offs and where did we get these values?
8.4.20. List substances, other than methamphetamine, that might produce a positive methamphetamine ELISA result.
8.4.21. Is the methamphetamine ELISA reactive towards the active ingredient of the Vicks inhaler? If so, why?
8.4.22. The target drug in the "cocaine" ELISA is benzoylecgonine (BE). What is BE? Give two conditions of how it can be formed in our samples. How do we deter its formation?
8.4.23. Is our ELISA procedure identical to the procedure recommended by the vendor in the package insert? Why is this and how do we know our results are reliable?
8.4.24. What is the required maintenance for the ELISA equipment? How often is it performed?
8.4.25. How do you verify a new lot of PBS buffer? What is the acceptance criterion?
8.4.26. If a within-run positive control fails, what case samples must be reanalyzed?

8.5. Laboratory Exercise

8.5.1. The trainee should familiarize himself/herself with the ELISA training literature and may observe a qualified analyst perform the screening test. The trainee will need at least four hours of instrument time to perform this practice run.
8.5.2. Depending on the trainee's level of experience, the trainee may now perform ELISA analysis for training purposes. The trainee should prepare a curve and a minimum of 10 samples in accordance with the SOP. The trainer will demonstrate how the calibrators and control samples are prepared and may provide additional samples for analysis. Follow the SOP to analyze the samples using the appropriate acquisition methods. Review the results with the trainer or a qualified analyst.
8.5.3. Repeat the procedure as necessary. By the time the trainee takes the competency test, the trainee should be well practiced and familiar with the technique and should have performed at least five consecutive, successful ELISA analyses (three blood runs and two

urine runs) if the trainee does not have prior ELISA experience. The trainee with prior ELISA experience should perform at least one blood and one urine runs.

8.5.4. The trainee will learn how to draft ELISA case reports (i.e., negative and presumptive positive reports where the only toxicology analysis performed was ELISA). Emphasis will be placed on drafting reports in LIMS that are consistent with the current formatting and content guidelines.

8.5.4.1. The trainee will observe a qualified analyst draft at least five ELISA case reports and then draft at least five case reports that will be reviewed by a qualified analyst. This must be completed to the satisfaction of the qualified analyst.

8.5.5. If the trainee has not completed the voir dire questions in section 20, they must be completed. The trainee should complete the applicable study questions in section 20. The trainee will then complete at least one practice testimony question and answer session with the trainer or a qualified analyst.

8.6. Competency

8.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.

8.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform the ELISA analysis according to the SOP.

8.6.3. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to ELISA analysis. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

8.6.4. If the trainee has not completed a mock trial at HFSC at this point a mock trial will need to be performed and documented on the appropriate form. See 20.4.3 for details.

8.7. ELISA Batch Review

8.7.1. After performing at least 10 ELISA casework batches, the trainee will learn how to perform batch review of ELISA batches.

8.7.2. The trainee will observe at least one ELISA batch review by a qualified analyst. The trainee will then perform batch review of at least five ELISA batches that a qualified analyst will then review. The trainee must demonstrate their ability to successfully batch review to the satisfaction of the qualified analyst.

8.8. Documentation of Completion

8.8.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

9. Drug Screen and Qualitative Confirmation by Gas Chromatography-Mass Spectrometry

9.1. Aim

9.1.1. The trainee will gain an understanding of the principles and practice of screening and qualitative confirmation for multiple drugs in biological samples using solid phase extraction (SPE) and GC-MS analysis. This technique is able to detect a number of drugs commonly encountered in forensic settings. With no derivatization occurring for this technique and the extraction technique not optimized for specific drug(s), some compounds may not be detected unless their concentrations are sufficiently high. The relative retention time to the internal standard and characteristic mass spectra are used to screen for a particular drug via the Deconvolution Reporting Software (DRS). The response above the cut-off calibrator, peak shape, MS spectra, and library matching confirms the presence of the drug via MS.

9.2. Required Reading

9.2.1. HFSC Toxicology Analytical Manual

9.2.2. Validation and Verification packages for GC-MS Drug Screen and Qualitative Confirmation (BSD) Analysis

9.3. Additional Resources

9.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), Ch. 16 (Method Validation), and Part III (Analytes)

9.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 19 (Gas Chromatography) and Ch. 21 (Mass Spectrometry)

9.3.3. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications

9.4. Study Questions

9.4.1. Describe liquid-liquid and solid phase extractions stating the advantages and disadvantages of both.

9.4.2. Explain the effects of pH on extractions.

9.4.3. What is the relationship between pH, pKa, and ionization?

9.4.4. List at least three different types of SPE sorbents and how they interact with the substances being extracted?

9.4.5. SPE is used to isolate basic, acidic, and neutral drugs from an aqueous biological sample. Drugs are manipulated based upon hydrophobic and ionic interactions. Explain in simple terms the mechanism of the extractions we use in the toxicology section.

9.4.6. Define the following terms:

9.4.6.1. Matrix

9.4.6.2. Functional group

9.4.6.3. Polarity
9.4.6.4. Henderson-Hasselbach equation
9.4.6.5. Basic molecules
9.4.6.6. Acidic molecules
9.4.6.7. Neutral molecules
9.4.6.8. Amphoteric molecules

9.4.7. The SPE columns are co-polymeric. They contain non-polar and charged moieties. Are the SPE columns negatively or positively charged?
9.4.8. What is the purpose of the phosphate buffer (pH 6) for sample dilution?
9.4.9. At pH 6, are the alkaline drugs positive, neutral, or negative?
9.4.10. What is the significance of washing the columns with dilute acetic acid?
9.4.11. What are co-extractive interferences?
9.4.12. Why are QCs and blanks included with every run?
9.4.13. What is the purpose of the internal standard? What factors should be taken into account when selecting an internal standard?
9.4.14. Explain why relative retention times are preferable for the identification of drugs.
9.4.15. Draw a schematic diagram of a gas chromatograph and describe the function of each component.
9.4.16. List three different modes of sample introduction and state the advantages and disadvantages of each.
9.4.17. Briefly describe the difference between pulsed split and split injections, as well as the difference between pulsed splitless and splitless injections.
9.4.18. What temperature should the injection port be under normal circumstances and why?
9.4.19. What is an injection port liner? What is it made out of? Why is it used?
9.4.20. What type of GC column is used in the toxicology section for GC-MS drug screen analysis? What types of compounds are separable on the column?
9.4.21. What is a split ratio? How is it calculated?
9.4.22. Describe the advantages and disadvantages of isothermal vs. temperature programming.
9.4.23. Why is it necessary to regulate the carrier gas flow? How is this done? What factors influence the optimum flow rate for a given carrier gas? If the carrier gas is too fast or too slow how will it affect peak shape? How will it affect the detector?
9.4.24. What are the possible causes and remedies for the following GC problems?
9.4.24.1. No peaks
9.4.24.2. Peak tailing
9.4.24.3. Leading peaks
9.4.24.4. Split peaks
9.4.24.5. Baseline drift
9.4.24.6. Column bleed
9.4.25. When and why are GC columns conditioned? Describe the process.
9.4.26. Define the following GC terms:
9.4.26.1. Height equivalent theoretical plate
9.4.26.2. Mobile phase

9.4.26.3. Stationary phase
9.4.26.4. Resolution
9.4.26.5. Partition coefficient
9.4.26.6. Theoretical plate
9.4.26.7. Make-up gas
9.4.26.8. Van Deemter plot
9.4.26.9. Flow rate
9.4.26.10. Relative retention time
9.4.26.11. Signal to noise

9.4.27. What is mass spectrometry? Draw a schematic of a mass spectrometer and explain the functions of each component.
9.4.28. How does a quadrupole mass filter operate?
9.4.29. Diagram and explain the components of the Agilent 5975 EI Source.
9.4.30. How does an electron multiplier work?
9.4.31. What vacuum conditions are necessary in the ionization source and the analyzing regions of an MS and why? What types of vacuums do we use in the Toxicology section?
9.4.32. Describe the importance of autotuning and explain the Autotune report.
9.4.33. Describe the following MS terms:
9.4.33.1. Mass-to-charge ratio
9.4.33.2. Molecular ion
9.4.33.3. Quantifier ion
9.4.33.4. Qualifier ion
9.4.33.5. Base peak
9.4.33.6. Mass resolution
9.4.33.7. Relative abundance
9.4.33.8. Scan rate
9.4.34. What is an extracted ion profile? How would you use it in drug identification?
9.4.35. What is DRS? How does it work?
9.4.36. How does probability-based library matching work?
9.4.37. What are the reference libraries we use in the Toxicology section?
9.4.38. What might be the cause of differences between reference library spectra and the spectra from an extracted sample? Explain.
9.4.39. What are the current drugs included in the GC-MS drug screen analysis? What are their cut-off concentrations?
9.4.40. Explain the difference between full scan and selected ion monitoring. What are the advantages and disadvantages of each mode?
9.4.41. Explain the difference between the GC-MS drug screen and the GC-MS qualitative drug confirmation in terms of the extraction procedure, acceptance criteria, and purpose of testing.

9.5. Laboratory Exercise

9.5.1. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Trainee may observe a qualified analyst performing the BSD analysis.

9.5.2. Discuss instrument availability with the trainer. The trainee will use the GC-MS system to analyze samples. For operation of the instrument, consult the trainer or a qualified analyst. Additional instructions are available in the Toxicology Manual and instrument user manual.

9.5.3. Depending on the trainee's level of experience, the trainee may now perform an analysis for training purposes; a minimum of three consecutively acceptable runs of positive and negative control samples in triplicate at each concentration level along with calibrators and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. A qualified analyst may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee may evaluate the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. The results will be reviewed by a qualified analyst.

9.5.3.1. The trainee must successfully complete at least one BSD batch regardless of his/her level of experience.

9.5.3.2. The trainee will learn how to draft qualitative (full scan) toxicology case reports based on BSD results. Emphasis will be placed on the use of LIMS to draft qualitative toxicology reports and enter appropriate batch information into LIMS.

9.5.3.3. The trainee will observe a qualified analyst drafting at least five case reports and then draft at least five case reports that a qualified analyst will subsequently review. This must be completed to the satisfaction of the qualified analyst.

9.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test, the trainee should be well practiced and familiar with the technique.

9.5.5. Inform the trainer if further assistance is necessary.

9.5.6. Observe routine maintenance on the GC-MS and then perform supervised routine maintenance on a GC-MS.

9.5.7. The trainee should complete the applicable study questions in section 20. The trainee will then complete at least one practice testimony question and answer session with the trainer or a qualified analyst.

9.6. Competency

9.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.

9.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform the analysis according to the SOP. Treat the samples as regular casework until the reporting of results.

9.6.2.1. After acceptable batches are complete and have been reviewed by a qualified analyst, the trainee will then draft and issue qualitative toxicology reports. These reports will be reviewed by a qualified analyst. The trainee must demonstrate their

ability to successfully draft qualitative toxicology reports and enter appropriate batch information in LIMS to the satisfaction of the qualified analyst.

9.6.3. In order to qualify, the analysis results must be in agreement with the target results (i.e., positive samples must be positive and negative samples must be negative) and the standards and controls must meet the QC criteria for qualitative analysis described in the SOP.

9.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to GC-MS drug screen and qualitative confirmation analyses. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

9.6.5. If the trainee has not completed a mock trial at HFSC, at this point a mock trial will need to be performed and documented on the appropriate form. See 20.4.3 for details.

9.7. Qualitative Case Review

9.7.1. After completion of the batch review training of the GC-MS drug screen and qualitative confirmation analysis, the trainee will learn how to perform technical and administrative reviews for qualitative toxicology case reports. Emphasis will be placed on LIMS to review reports that are consistent with the current formatting and content guidelines.

9.7.2. Technical Review

9.7.2.1. The trainee will observe technical review of at least five qualitative toxicology case reports. The trainee will then perform technical review on at least ten qualitative toxicology case reports that a qualified analyst will subsequently review. The trainee must demonstrate his/her abilities to successfully technically review the case reports to the satisfaction of the qualified analyst.

9.7.3. Administrative Review

9.7.3.1. The trainee will observe administrative review of at least five qualitative toxicology case reports. The trainee will then perform administrative review on at least ten qualitative toxicology case reports that a qualified analyst will subsequently review. The trainee must demonstrate his/her abilities to successfully administratively review the case reports to the satisfaction of the qualified analyst.

9.8. Documentation of Completion

9.8.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

10. Drug Confirmation by Liquid Chromatography-Tandem Mass Spectrometry

10.1. Aim

10.1.1. The trainee will gain an understanding of the principles and practice of qualitative and quantitative confirmation for drugs in biological samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS). This training is designed to familiarize the trainee with operation and maintenance of LC-MS/MS system and to provide an overview of important factors associated with LC-MS/MS analysis. This training is to learn theoretical concepts and hence does not include a competency test. The training of LC-MS/MS analyses and associated competency tests are described in subsequent LC-MS/MS training modules.

10.2. Required Reading

10.2.1. HFSC Toxicology Analytical Manual

10.3. Additional Resources

10.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), Ch. 15 (Quantitative Analytical Methods), Ch. 16 (Method Validation), Ch. 17 (Metrological Traceability and Measurement Uncertainty), and Part III (Analytes)

10.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 20 (High Performance Liquid Chromatography) and Ch. 21 (Mass Spectrometry)

10.3.3. CHROMacademy, Fundamentals of LC-MS Video Training Course and other resources

10.3.4. 2019 HFSC LCMS Training handouts

10.4. Study Questions

10.4.1. Name at least two advantages that liquid chromatography has over gas chromatography.

10.4.2. List main components of a HPLC system.

10.4.3. Explain how the run time, pressure, column length, and resolution change as the LC column particle size decreases.

10.4.4. Describe required characteristics of HPLC mobile phase.

10.4.5. Explain mobile phase additives. What are some common additives? Why are they used?

10.4.6. Describe the difference between isocratic elution and gradient elution. What are three advantages to using a gradient elution instead of an isocratic elution?

10.4.7. Describe the differences between reversed-phase HPLC and normal phase HPLC. Which one is commonly used in forensic applications? Why?

10.4.8. Describe three factors affecting resolution in LC.

10.4.9. What are the possible causes and remedies for the following LC problems?

10.4.9.1. No peaks

10.4.9.2. Broad peak

10.4.9.3. Peak tailing
10.4.9.4. Split peaks
10.4.9.5. Low pressure
10.4.9.6. High pressure
10.4.9.7. Retention time shifts
10.4.10. List main components of a mass spectrometer.
10.4.11. How does a triple quadrupole mass analyzer work? Describe the purpose of each quadrupole.
10.4.12. Describe the mechanism of collision induced dissociation.
10.4.13. Explain electrospray ionization. Why is it called a soft ionization technique?
10.4.14. List three things to consider when using APCI.
10.4.15. Explain the two ionization modes (polarity) of a mass spectrometer. What are the molecular ions produced in each of the two modes?
10.4.16. Describe the following MS terms.
10.4.16.1. Molecular ion
10.4.16.2. Fragment (product) ion
10.4.16.3. Mass resolution
10.4.16.4. Mass calibration
10.4.16.5. Instrument tuning
10.4.17. Describe the relationship among the number of MRM transitions, dwell time, and cycle time in terms of the number of data point (peak quality) and the signal-to noise.
10.4.18. Explain the difference among product ion scanning, precursor ion scanning, and MRM in LC-MS/MS analysis.
10.4.19. What are the possible causes and remedies for the following MS problems?
10.4.19.1. Reduced sensitivity
10.4.19.2. Poor peak shape
10.4.19.3. Failed mass calibration
10.4.19.4. High background spectrum
10.4.20. What is ionization suppression/enhancement and how can it affect LC-MS/MS analysis? How can it be minimized?
10.4.21. Define the term pKa.
10.4.22. What is the relationship between pH, pKa and ionization?
10.4.23. How do you decide what pH to use during an extraction to maximize recovery?
10.4.24. Why are “optimal” pHs not always used?
10.4.25. What are co-extractive interferences?
10.4.26. Why are QCs and blanks included with every run?
10.4.27. What is the purpose of the internal standard? What factors should be taken into account when selecting an internal standard?
10.4.28. Why are deuterated internal standards preferred?
10.4.29. How do the quantity and concentration of internal standard affect quantitative analysis?
10.4.30. What are “standards,” “calibrators” and “controls”?
10.4.31. Define the terms, limit of detection, limit of quantification, analytical sensitivity, precision, and accuracy.

10.4.32. What is the advantage of MRM acquisition over full scan? What is the significance of the ion ratios?

10.4.33. What is the benefit of using external controls?

10.5. Laboratory Exercise

10.5.1. Trainer will provide an overview of LC-MS/MS system in the laboratory, showing how to operate the instrument and check/maintain its performance.

10.5.2. If the trainee has not completed the voir dire questions in section 20, they must be completed. The trainee should complete the applicable study questions in section 20. The trainee will then complete at least one practice testimony question and answer session with the trainer or a qualified analyst.

10.5.3. If the trainee has not completed a mock trial at HFSC, at this point a mock trial will need to be performed and documented on the appropriate form. See 20.4.3 for details.

10.6. Documentation of Completion

10.6.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

11. Amphetamines Confirmation by Liquid Chromatography-Tandem Mass Spectrometry

11.1. Aim

11.1.1. The trainee will gain an understanding of the principles and practice of solid phase extraction (SPE) and analysis via LC-MS/MS for the detection and quantification for amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxy-N-ethylamphetamine (MDEA) in biological samples. Deuterated internal standards and multiple reaction monitoring (MRM) in positive electrospray ionization (ESI) mode are used.

11.2. Required Reading

11.2.1. HFSC Toxicology Analytical Manual
11.2.2. Validation package for Amphetamines Confirmation analysis
11.2.3. Uncertainty of Measurement package for Amphetamines Confirmation analysis

11.3. Additional Resources

11.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), and Ch. 25 (Amphetamines/Sympathomimetic Amines)
11.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 20 (High Performance Liquid Chromatography) and Ch. 21 (Mass Spectrometry)
11.3.3. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications
11.3.4. Drug Effects on Psychomotor Performance, R.C. Baselt, 2001, Biomedical Publications
11.3.5. National Highway Safety Traffic Administration, Drugs and Human Performance Fact Sheets, Methamphetamine (And Amphetamine) and Methylenedioxymethamphetamine (MDMA, Ecstasy)

11.4. Study Questions

11.4.1. SPE is used to isolate basic drugs from an aqueous biological sample. Methamphetamine is isolated based upon hydrophobic and ionic interaction. Explain in simple terms the mechanism of the extraction.
11.4.2. What is the purpose of adding acidic methanol to the extract after SPE?
11.4.3. A positive methamphetamine ELISA result may indicate either methamphetamine or another cross-reacting substance like ephedrine or pseudoephedrine. How is methamphetamine distinguished from ephedrine?
11.4.4. How are methamphetamine and amphetamine related biologically?
11.4.5. How can d- and l-methamphetamine be resolved using LC-MS/MS? How is l-methamphetamine different from d-methamphetamine pharmacologically?

11.4.6. What type of column is used in the LC? What type of compounds can be separated on this column?
11.4.7. What is the LOQ of each analyte in the method?
11.4.8. Describe the sequence of an example run for this method.
11.4.9. What are the acceptance criteria for reporting results?
11.4.10. Explain the mechanism of action of amphetamines/sympathomimetic amines.
11.4.11. Describe the general effects of amphetamines on driving performance.

11.5. Laboratory Exercise

11.5.1. Discuss instrument availability with the trainer. The trainee will use a LC-MS/MS system to analyze samples. For operation of the instrument, see trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.
11.5.2. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Depending on level of experience, the trainee may observe a qualified analyst performing amphetamines confirmation (AMP) analysis.
11.5.3. Depending on the trainee's level of experience, the trainee may now perform AMP analysis for training purposes; a minimum of three consecutively acceptable runs of positive control samples in triplicate at each concentration level along with calibrators, a negative control, and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee evaluates the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.
11.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test the trainee should be well practiced and familiar with the technique.
11.5.5. Inform the trainer if further assistance is necessary.

11.6. Competency

11.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.
11.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform AMP analysis according to the SOP. Treat the samples as regular casework until the reporting of results.
11.6.3. In order to qualify, the analysis results must be in agreement with the target results (i.e., within 20% difference unless otherwise specified in the training records. Some analytes may have larger %difference due to instability; trainer will evaluate acceptability of the result and may consider a result with >20% difference as passing upon review of literature

and/or re-analysis of the sample by another analyst) and the standards and controls must meet the amphetamines acceptance criteria set forth in the SOP.

11.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to amphetamines analysis. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

11.7. Documentation of Completion

11.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record**.** Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

12. Benzodiazepines and Zolpidem Confirmation by Liquid Chromatography-Tandem Mass Spectrometry

12.1. Aim

12.1.1. The trainee will gain an understanding of the principles and practice of solid-phase extraction (SPE) and analysis via LC-MS/MS for the detection and quantification for 7-aminoclonazepam, zolpidem, α-hydroxyalprazolam, oxazepam, nordiazepam, clonazepam, lorazepam, alprazolam, temazepam, and diazepam in biological samples. Deuterated internal standards and dynamic multiple reaction monitoring (dMRM) in positive electrospray ionization (ESI) mode are used.

12.2. Required Reading

12.2.1. HFSC Toxicology Analytical Manual
12.2.2. Validation package of Benzodiazepines Confirmation analysis
12.2.3. Uncertainty of Measurement package for Benzodiazepines Confirmation analysis

12.3. Additional Resources

12.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), and Ch. 20 (Benzodiazepines)
12.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 20 (High Performance Liquid Chromatography) and Ch. 21 (Mass Spectrometry)
12.3.3. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications
12.3.4. Drug Effects on Psychomotor Performance, R.C. Baselt, 2001, Biomedical Publications
12.3.5. National Highway Safety Traffic Administration, Drugs and Human Performance Fact Sheets, Diazepam

12.4. Study Questions

12.4.1. An enzyme immunoassay is used to identify presumptive positive samples for benzodiazepines and zolpidem. What is our ELISA target drug and why?
12.4.2. What are the limitations of benzodiazepine immunoassays?
12.4.3. What is the LOQ of each analyte in the BNZ method?
12.4.4. Describe the sequence of an example run for the BNZ method.
12.4.5. What are the acceptance criteria for reporting results?
12.4.6. Describe the general benzodiazepine structure.
12.4.6.1. Explain possible structural modifications that can affect potency and duration of action.
12.4.7. List common therapeutic uses of benzodiazepines and zolpidem.
12.4.8. Explain the mechanism of action of benzodiazepines and zolpidem.

12.4.9. Benzodiazepines are generally highly protein-bound. What is their major binding protein? What is the implication of >80% protein binding to the extraction procedure?
12.4.10. Why is use of MRM especially important for detection of benzodiazepines?
12.4.11. Describe factors to be considered when developing urine vs. blood specimen analysis for benzodiazepines.
12.4.12. Which metabolites of benzodiazepines are pharmacologically active?
12.4.13. Chlordiazepoxide can be broken down in blood to what compounds?
12.4.14. Describe the general effects of benzodiazepines and zolpidem on driving performance.

12.5. Laboratory Exercise

12.5.1. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Depending on level of experience, the trainee may observe a qualified analyst performing benzodiazepines/zolpidem confirmation (BNZ) analysis.
12.5.2. Discuss instrument availability with the trainer. The trainee will use a LC-MS/MS system to analyze samples. For operation of the instrument, see trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.
12.5.3. Depending on the trainee's level of experience, the trainee may now perform BNZ analysis for training purposes; a minimum of three consecutively acceptable runs of positive control samples in triplicate at each concentration level along with calibrators, a negative control, a hydrolysis control (urine) and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee evaluates the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.
12.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test, the trainee should be well practiced and familiar with the technique.
12.5.5. Inform the trainer if further assistance is necessary.

12.6. Competency

12.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.
12.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform BNZ analysis according to the SOP. Treat the samples as regular casework until the reporting of results.
12.6.3. In order to qualify, the analysis results must be in agreement with the target results (i.e., within 20% difference unless otherwise specified in the training records. Some analytes may have larger %difference due to instability; trainer will evaluate acceptability of the result and may consider a result with >20% difference as passing upon review of literature

and/or re-analysis of the sample by another analyst) and the standards and controls must meet the benzodiazepines acceptance criteria set forth in the SOP.

12.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to benzodiazepines analysis. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

12.7. Documentation of Completion

12.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

13. Cannabinoids Confirmation by Liquid Chromatography-Tandem Mass Spectrometry

13.1. Aim

13.1.1. The trainee will gain an understanding of the principles and practice of liquid-liquid extraction (LLE) and analysis via LC-MS/MS for the detection and quantification of Δ9-tetrahydrocannabinol (delta9-THC), 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THC-COOH), cannabidiol (CBD), and Δ8-tetrahydrocannabinol (delta8-THC) in biological samples. Deuterated internal standards and multiple reaction monitoring (MRM) in positive electrospray ionization (ESI) mode are used.

13.2. Required Reading

13.2.1. HFSC Toxicology Analytical Manual
13.2.2. Validation package of Cannabinoids Confirmation analysis
13.2.3. Uncertainty of Measurement package for Cannabinoids Confirmation analysis

13.3. Additional Resources

13.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), and Ch. 24 (Cannabis)
13.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 20 (High Performance Liquid Chromatography) and Ch. 21 (Mass Spectrometry)
13.3.3. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications
13.3.4. National Highway Safety Traffic Administration, Drugs and Human Performance Fact Sheets, Cannabis/Marijuana (Δ9-tetrahydrocannabinol, THC)

13.4. Study Questions

13.4.1. Cannabis contains more than four hundred different chemical compounds, of which over sixty are cannabinoids. Our procedure targets delta9-THC, 11-OH-THC, THC-COOH, CBD, and delta8-THC. Why is this?
13.4.2. An enzyme immunoassay is used to identify presumptive positive samples. What is our ELISA target molecule and why?
13.4.3. THC has a tricyclic 21 carbon structure with two chiral centers. What is the significance of these stereoisomers with respect to ELISA and LC-MS/MS analysis?
13.4.4. Cannabinoids are unstable under some conditions. What precautions are taken to minimize their instability?
13.4.5. Can prescription drug use cause a positive cannabis result by either ELISA or LC-MS/MS?
13.4.6. Most pharmacokinetic studies report THC concentrations in serum or plasma. What is the concentration relationship between whole blood samples and plasma?
13.4.7. Enzyme hydrolysis (beta-glucuronidase) or more commonly alkaline hydrolysis is used to free THC-COOH from glucuronide. The efficiency of the hydrolysis should be measured

using a glucuronidated standard. In general terms, what are the advantages and disadvantages of both these methods? Which hydrolysis method do we use currently and why?

13.4.8. What is the LOQ of each analyte in the method?

13.4.9. Describe the sequence of an example run for this method.

13.4.10. What are the acceptance criteria for reporting results?

13.4.11. Describe the general effects of THC on driving performance.

13.5. Laboratory Exercise

13.5.1. Discuss instrument availability with the trainer. The trainee will use a LC-MS/MS system to analyze samples. For operation of the instrument, see trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.

13.5.2. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Depending on level of experience, the trainee may observe a qualified analyst performing cannabinoids confirmation (THC) analysis.

13.5.3. Depending on the trainee's level of experience, the trainee may now perform THC analysis for training purposes; a minimum of three consecutively acceptable runs of positive control samples in triplicate at each concentration level along with calibrators, a negative control, a hydrolysis control (urine), and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee evaluates the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.

13.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test, the trainee should be well practiced and familiar with the technique.

13.5.5. Inform the trainer if further assistance is necessary.

13.6. Competency

13.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.

13.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform the THC analysis according to the SOP. Treat the samples as regular casework until the reporting of results.

13.6.3. In order to qualify, the analysis results must be in agreement with the original results obtained from qualified analysts (i.e., within 20% difference unless otherwise specified in the training records. Some analytes may have larger %difference due to instability; trainer will evaluate acceptability of the result and may consider a result with >20% difference as passing upon review of literature and/or re-analysis of the sample by another analyst) and

the standards and controls must meet the cannabinoids acceptance criteria set forth in the SOP.

13.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to cannabinoids analysis. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

13.7. Documentation of Completion

13.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

14. Carisoprodol/Meprobamate Confirmation by Gas Chromatography-Mass Spectrometry

14.1. Aim

14.1.1. The trainee will gain an understanding of the principles and practice of liquid-liquid extraction (LLE) and analysis via GC-MS for the detection and quantification of carisoprodol and meprobamate in biological samples. Deuterated internal standards and selective ion monitoring (SIM) in electron ionization (EI) mode are used.

14.2. Required Reading

14.2.1. HFSC Toxicology Analytical Manual
14.2.2. Validation package of Carisoprodol/Meprobamate Confirmation analysis
14.2.3. Uncertainty of Measurement package for Carisoprodol/Meprobamate Confirmation analysis

14.3. Additional Resources

14.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), and Ch. 21 (Miscellaneous Central Nervous System Depressants)
14.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 19 (Gas Chromatography) and Ch. 21 (Mass Spectrometry)
14.3.3. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications
14.3.4. National Highway Safety Traffic Administration, Drugs and Human Performance Fact Sheets, Carisoprodol (and Meprobamate)

14.4. Study Questions

14.4.1. Describe the relationship and structural difference between carisoprodol and meprobamate, including the metabolic pathway, potency and half-life.
14.4.2. Are carisoprodol and meprobamate considered acidic, basic, or neutral drugs?
14.4.3. An enzyme immunoassay is used to identify presumptive positive samples. What is our ELISA target molecule and why? What is the cross-reactivity of the other analyte?
14.4.4. How stable is carisoprodol in blood in vitro?
14.4.5. Can use of another drug cause a positive carisoprodol result by either ELISA or GC-MS? Do known method limitations/interferences put us at a risk to report false positives? Why or why not?
14.4.6. Explain the structural differences between commercially-available deuterated analogs of meprobamate and their implications in SIM analysis. Which is the form utilized in the HFSC CAR method? What would happen if the incorrect form was used?
14.4.7. What are the sources of carisoprodol/meprobamate calibrator and control solutions?
14.4.8. Why do we add ammonium hydroxide to each sample? How do we calculate 1M ammonium hydroxide?

14.4.9. Why is a hydrolysis step not included in analysis of urine samples for the CAR method while many other confirmation methods include a hydrolysis step?
14.4.10. What is the LOQ of each analyte in the method?
14.4.11. Describe the sequence of an example run for this method.
14.4.12. What are the acceptance criteria for reporting results?
14.4.13. What are the brand names of the prescription drugs containing carisoprodol or meprobamate? What are the indications for the drugs?
14.4.14. Describe the general effects of carisoprodol and meprobamate on driving performance.

14.5. Laboratory Exercise

14.5.1. Discuss instrument availability with the trainer. The trainee will use a GC-MS system to analyze samples. For operation of the instrument, see trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.
14.5.2. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Depending on level of experience, the trainee may observe a qualified analyst performing carisoprodol/meprobamate confirmation (CAR) analysis.
14.5.3. Depending on the trainee's level of experience, the trainee may now perform CAR analysis for training purposes; a minimum of three consecutively acceptable runs of positive control samples in triplicate at each concentration level along with calibrators, a negative control and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee evaluates the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.
14.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test, the trainee should be well practiced and familiar with the technique.
14.5.5. Inform the trainer if further assistance is necessary.

14.6. Competency

14.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.
14.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform the CAR analysis according to the SOP. Treat the samples as regular casework until the reporting of results.
14.6.3. In order to qualify, the analysis results must be in agreement with the original results obtained from qualified analysts (i.e., within 20% difference unless otherwise specified) and the standards and controls must meet the acceptance criteria set forth in the SOP.

14.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to cannabinoids analysis. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

14.7. Documentation of Completion

14.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

15. Cocaine Confirmation by Liquid Chromatography-Tandem Mass Spectrometry

15.1. Aim

15.1.1. The trainee will gain an understanding of the principles and practice of solid phase extraction (SPE) and analysis via LC-MS/MS for the detection and quantification for benzoylecgonine (BE), cocaine, and cocaethylene (CE). Deuterated internal standards and multiple reaction monitoring (MRM) in positive electrospray ionization (ESI) mode are used.

15.2. Required Reading

15.2.1. HFSC Toxicology Analytical Manual
15.2.2. Validation package for Cocaine Confirmation analysis
15.2.3. Uncertainty of Measurement package for Cocaine Confirmation analysis

15.3. Additional Resources

15.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), and Ch. 23 (Cocaine).
15.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 20 (High Performance Liquid Chromatography) and Ch. 21 (Mass Spectrometry)
15.3.3. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications
15.3.4. Drug Effects on Psychomotor Performance, R.C. Baselt, 2001, Biomedical Publications
15.3.5. National Highway Safety Traffic Administration, Drugs and Human Performance Fact Sheets, Morphine and Cocaine

15.4. Study Questions

15.4.1. Explain the extraction of cocaine, BE, and CE in terms of their acid/base characteristics and polarity.
15.4.2. Discuss the instability of cocaine and some of its metabolites.
15.4.3. Blood samples should be collected into grey top tubes which contain sodium fluoride preservative and potassium oxalate as anticoagulant. Occasionally, blood samples are submitted without preservative (e.g., lavender-, red-, green-, and yellow-top). What does this mean in terms of cocaine analysis in blood?
15.4.4. Stability of cocaine and its metabolites in urine is markedly different. Explain the reason.
15.4.5. Cocaine metabolism is quite complex, involving more than a dozen metabolites in all. Our analysis is limited to MRM for cocaine, BE, and CE. Why is this?
15.4.6. What is the significance of cocaethylene?
15.4.7. Both BE and ecgonine methyl ester (EME) are further metabolized to ecgonine. Although the pathways have not been fully studied, enzymatic hydrolysis of the benzoyl

ester (BE) and chemical hydrolysis of EME is suspected. Why don't we analyze for ecgonine?

15.4.8. Presumptively positive urine samples are identified by immunoassay. What is the target molecule and why?

15.4.9. Could artificial production of BE occur during the extraction? Would this introduce a systematic error during quantitative blood analysis?

15.4.10. What is the LOQ of each analyte in the method?

15.4.11. Describe the sequence of an example run for this method.

15.4.12. What are the acceptance criteria for reporting results?

15.4.13. Describe the general effects of cocaine on driving performance.

15.5. Laboratory Exercise

15.5.1. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Depending on level of experience the trainee may observe a qualified analyst performing cocaine confirmation (COC) analysis.

15.5.2. Discuss instrument availability with the trainer. The trainee will use a LC-MS/MS system to analyze samples. For operation of the instrument, see trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.

15.5.3. Depending on the trainee's level of experience, the trainee may now perform a COC analysis for training purposes; a minimum of three consecutively acceptable runs of positive control samples in triplicate at each concentration level along with calibrators, a negative control, and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. In addition to an in-house control, the trainee should also include an external control. The trainee may evaluate the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.

15.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test, the trainee should be well practiced and familiar with the technique.

15.5.5. Inform the trainer if further assistance is necessary.

15.6. Competency

15.6.1. Upon satisfactory completion of the training and laboratory exercises, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.

15.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform the COC analysis according to the SOP. Treat the samples as regular casework until the reporting of results.

15.6.3. In order to qualify, the analysis results must be in agreement with the target results (i.e., within 20% difference unless otherwise specified in the training records. Some analytes may have larger %difference due to instability; trainer will evaluate acceptability of the result and may consider a result with >20% difference as passing upon review of literature and/or re-analysis of the sample by another analyst) and the standards and controls must meet the cocaine acceptance criteria set forth in the SOP.

15.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to cocaine analysis. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

15.7. Documentation of Completion

15.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

16. Opioids Confirmation by Liquid Chromatography- Tandem Mass Spectrometry

16.1. Aim

16.1.1. The trainee will gain an understanding of the principles and practice of solid phase extraction (SPE) and analysis via LC-MS/MS for the detection and quantification for morphine, oxymorphone, hydromorphone, O-desmethyltramadol, codeine, 6-acetylmorphine, oxycodone, hydrocodone, tramadol, norbuprenorphine, buprenorphine, norfentanyl, fentanyl, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and methadone in biological samples. Deuterated internal standards and multiple reaction monitoring (MRM) in positive electrospray ionization (ESI) mode are used.

16.2. Required Reading

16.2.1. HFSC Toxicology Analytical Manual
16.2.2. Validation package for Opioids Confirmation analysis
16.2.3. Uncertainty of Measurement package for Opioids Confirmation analysis

16.3. Additional Resources

16.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), and Ch. 22 (Opioids)
16.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 20 (High Performance Liquid Chromatography) and Ch. 21 (Mass Spectrometry)
16.3.3. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications
16.3.4. Drug Effects on Psychomotor Performance, R.C. Baselt, 2001, Biomedical Publications

16.4. Study Questions

16.4.1. In MRM, only a few, significant ions are monitored. What is the advantage of this technique over full scan spectra?
16.4.2. Describe the chemical characteristics of hydrocodone, hydromorphone, oxycodone and oxymorphone as they relate to extraction conditions. Are they acidic or basic drugs?
16.4.3. How are hydrocodone, hydromorphone, oxycodone and oxymorphone related chemically? How are they related biologically?
16.4.4. What are the molecular weights of hydrocodone, hydromorphone, oxycodone and oxymorphone? What is the molecular weight difference between each opioid? What, then, is the molecular ion in the MRM acquisition method for each analyte?
16.4.5. Explain the difference between opiates and opioids. Classify each analyte in the method.
16.4.6. What is the metabolic pathway of hydrocodone, hydromorphone, oxycodone and oxymorphone? Are any of the opioids converted to an active metabolite?
16.4.7. Describe relative potency of the opioids in the method compared to morphine.
16.4.8. What is the antidote for opioid overdose?

16.4.9. What is the LOQ of each analyte in the method?
16.4.10. Describe the sequence of an example run for the method.
16.4.11. What are the acceptance criteria for reporting results?
16.4.12. Describe the general effects of opioids on driving performance.

16.5. Laboratory Exercise

16.5.1. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Depending on level of experience the trainee may observe a qualified analyst performing opioids confirmation (OPI) analysis.
16.5.2. Discuss instrument availability with the trainer. The trainee will use a LC-MS/MS system to analyze samples. For operation of the instrument, see trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.
16.5.3. Depending on the trainee's level of experience, the trainee may now perform an OPI analysis for training purposes; a minimum of three consecutively acceptable runs of positive control samples in triplicate at each concentration level along with calibrators, a negative control, and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee may evaluate the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.
16.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test, the trainee should be well practiced and familiar with the technique.
16.5.5. Inform the trainer if further assistance is necessary.

16.6. Competency

16.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.
16.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform the OPI analysis according to the SOP. Treat the samples as regular casework until the reporting of results.
16.6.3. In order to qualify, the analysis results must be in agreement with the target results (i.e., within 20% difference unless otherwise specified) and the standards and controls must meet the opioids acceptance criteria set forth in the SOP.
16.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to opioids analysis. Once the results have been discussed and

approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

16.7. Documentation of Completion

16.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or electronic equivalent record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

17. Phencyclidine Confirmation by Liquid Chromatography-Tandem Mass Spectrometry

17.1. Aim

17.1.1. The trainee will gain an understanding of the principles and practice of solid phase extraction (SPE) and analysis via LC-MS/MS for the detection and quantification for phencyclidine in biological samples. Deuterated internal standards and multiple reaction monitoring (MRM) in positive electrospray ionization (ESI) mode are used.

17.2. Required Reading

17.2.1. HFSC Toxicology Analytical Manual
17.2.2. Validation package for Phencyclidine Confirmation analysis
17.2.3. Uncertainty of Measurement package for Phencyclidine Confirmation analysis

17.3. Additional Resources

17.3.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 9 (Specimen Preparation/Extraction), Ch. 11 (Chromatography), Ch. 14 (Mass Spectrometry), and Ch. 26 (Hallucinogens and Psychedelics)
17.3.2. Clarke's Analytical Forensic Toxicology, 2nd Ed., A. Negrusz and G. Cooper, 2013, Pharmaceutical Press – Ch. 3 (Drugs of Abuse), Ch. 14 (Extraction), Ch. 20 (High Performance Liquid Chromatography) and Ch. 21 (Mass Spectrometry)
17.3.3. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications
17.3.4. Drug Effects on Psychomotor Performance, R.C. Baselt, 2001, Biomedical Publications
17.3.5. National Highway Safety Traffic Administration, Drugs and Human Performance Fact Sheets, Phencyclidine (PCP)

17.4. Study Questions

17.4.1. Explain the extraction of PCP in terms of its acid/base characteristics and polarity.
17.4.2. Explain chemically what is taking place with each of the "wash" steps in the PCP procedure.
17.4.3. What ion transitions are acquired by the LC-MS/MS in the MRM mode for PCP?
17.4.4. What is the LOQ of the method?
17.4.5. Describe the sequence of an example run for this method.
17.4.6. What are the acceptance criteria for reporting results?
17.4.7. Describe pharmacokinetic properties of PCP.
17.4.8. What are observable signs and symptoms of individuals intoxicated with PCP?
17.4.9. Describe the general effects of PCP on driving performance.

17.5. Laboratory Exercise

17.5.1. Discuss instrument availability with the trainer. The trainee will use a LC-MS/MS system to analyze samples. For operation of the instrument, see trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.

17.5.2. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Depending on level of experience, the trainee may observe a qualified analyst performing phencyclidine confirmation (PCP) analysis.

17.5.3. Depending on the trainee's level of experience, the trainee may now perform a PCP analysis for training purposes; a minimum of three consecutively acceptable runs of positive control samples in triplicate at each concentration level along with calibrators, a negative control, and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee evaluates the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.

17.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test, the trainee should be well practiced and familiar with the technique.

17.5.5. Inform the trainer if further assistance is necessary.

17.6. Competency

17.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.

17.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform the PCP confirmation analysis according to the SOP. Treat the samples as regular casework until the reporting of results.

17.6.3. In order to qualify, the analysis results must be in agreement with the target results (i.e., within 20% difference unless otherwise specified in the training records. Some analytes may have larger %difference due to instability; trainer will evaluate acceptability of the result and may consider a result with >20% difference as passing upon review of literature and/or re-analysis of the sample by another analyst) and the standards and controls must meet the PCP acceptance criteria set forth in the SOP.

17.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to PCP analysis. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

17.7. Documentation of Completion

17.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

18. Novel Benzodiazepines Confirmation by Liquid Chromatography-Tandem Mass Spectrometry*

*Note: a new SOP

18.1. Aim

18.1.1. The trainee will gain an understanding of the principles and practice of solid-phase extraction (SPE) and analysis via LC-MS/MS for the detection and quantification for 8-aminoclonazolam, clonazolam, flualprazolam, flubromazolam, bromazolam, etizolam, and flubromazepam in biological samples. Deuterated internal standards and dynamic multiple reaction monitoring (dMRM) in positive electrospray ionization (ESI) mode are used.

18.2. Required Reading

18.2.1. HFSC Toxicology Analytical Manual
18.2.2. Validation package of Novel Benzodiazepines Confirmation analysis
18.2.3. Uncertainty of Measurement package for Novel Benzodiazepines Confirmation analysis

18.3. Additional Resources

18.3.1. Disposition of Toxic Drugs and Chemicals in Man, 12th Ed., R.C. Baselt, 2020, Biomedical Publications
18.3.2. Manchester et al. The emergence of new psychoactive substance (NPS) benzodiazepines: A review. Drug Testing and Analysis. 2018;10:37-53.
18.3.3. Waters et al. The use of a quantitative structure-activity relationship (QSAR) model to predict GABA-A receptor binding of newly emerging benzodiazepines. Science & Justice. 2018;58:219-225.
18.3.4. Heide et al. Blood concentrations of designer benzodiazepines: Relation to impairment and findings in forensic cases. Journal of Analytical Toxicology. 2020;44:905-914.
18.3.5. Papsun et al. Flualprazolam blood concentrations in 197 forensic investigation cases. Journal of Analytical Toxicology. 2021;45:226-232.
18.3.6. Rohrig e al. Driving impairment cases involving etizolam and flubromazolam. Journal of Analytical Toxicology. 2021;45:93-98.

18.4. Study Questions

18.4.1. Which drugs are classified as novel benzodiazepines?
18.4.2. Which ELISA is used as a screening test for NBZ? What is the cross-reactivity value for each analyte?
18.4.3. How were the analytes in the NBZ method selected?
18.4.4. What is the LOQ of each analyte in the NBZ method?
18.4.5. Describe the sequence of an example run for the NBZ method.
18.4.6. What re the acceptance criteria for reporting results?
18.4.7. Describe the mechanism of action of novel benzodiazepines.
18.4.8. Describe the general effects of novel benzodiazepines.
18.4.9. How are novel benzodiazepines different from and/or comparable with prescription benzodiazepines?

18.5. Laboratory Exercise

18.5.1. Read the appropriate SOP. If any questions arise, ask a qualified analyst. Depending on level of experience, the trainee may observe a qualified analyst performing novel benzodiazepines confirmation (NBZ) analysis.

18.5.2. Discuss instrument availability with the trainer. The trainee will use a LC-MS/MS system to analyze samples. For operation of the instrument, see trainer. Additional instructions are available in the Toxicology Manual and instrument user manual.

18.5.3. Depending on the trainee's level of experience, the trainee may now perform NBZ analysis for training purposes; a minimum of three consecutively acceptable runs of positive control samples in triplicate at each concentration level along with calibrators, a negative control, a hydrolysis control (urine) and a matrix blank is required for inexperienced trainees and a minimum of one acceptable run for experienced trainees. The trainee should prepare calibrators and QC samples in accordance with the SOP. The trainer may demonstrate how the calibrators and QC samples are prepared and may provide additional samples for analysis. The trainee evaluates the run by including internal and external (if available) QCs. Follow the SOP to analyze the samples using the appropriate acquisition method. Review the results with the trainer or a qualified analyst.

18.5.4. Repeat the procedure as necessary. By the time the trainee takes the assigned competency test, the trainee should be well practiced and familiar with the technique.

18.5.5. Inform the trainer if further assistance is necessary.

18.6. Competency

18.6.1. Upon satisfactory completion of the laboratory exercise and the study questions, the trainee is deemed ready for a competency test. The trainee may need to practice the technical procedure several times.

18.6.2. A qualified analyst will assign the trainee mock case samples that have been fortified at different analyte concentrations unknown to the trainee and/or that have been previously analyzed. Unaided, the trainee will perform NBZ analysis according to the SOP. Treat the samples as regular casework until the reporting of results.

18.6.3. In order to qualify, the analysis results must be in agreement with the target results (i.e., within 20% difference unless otherwise specified in the training records. Some analytes may have larger %difference due to instability; trainer will evaluate acceptability of the result and may consider a result with >20% difference as passing upon review of literature and/or re-analysis of the sample by another analyst) and the standards and controls must meet the benzodiazepines acceptance criteria set forth in the SOP.

18.6.4. Review the competency test sample results with the trainer. Store the obtained results in a retrievable format with the original results and give the data to the supervisor for approval. The trainee will also be given a written or oral examination evaluating the trainee's knowledge related to benzodiazepines analysis. Once the results have been discussed and approved, the trainee will receive an authorization memo and may begin independent casework upon receipt of TFSC Forensic Analyst license.

18.6.5. If the trainee has already been authorized and performed casework using the benzodiazepines confirmation analysis, a competency test is not necessary. Completion of at least one laboratory exercise and study questions will be considered sufficient.

18.7. Documentation of Completion

18.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

19. Positive Targeted Toxicology Case Reports: Report Writing, Mass Spectral Batch Review, and Case Review

19.1. Aim

19.1.1. This training module familiarizes the trainee with positive toxicology report writing and the technical and administrative review processes for these reports. It also familiarizes the trainee with batch review of all drug screening and confirmation methods using mass spectral data.

19.2. Required Reading

19.2.1. HFSC Toxicology Analytical Manual

19.3. Laboratory Exercise

19.3.1. Report Writing

19.3.1.1. The trainee will learn how to draft positive toxicology case reports based on targeted assays. Emphasis will be placed on the use of LIMS to draft reports that are consistent with current formatting and content guidelines. NOTE: This does not include toxicology presumptive positive reports. Analysts who were authorized to author these reports during ELISA training will continue to perform this task if needed.

19.3.1.2. The trainee will observe a qualified analyst drafting at least five case reports and then draft at least five case reports that a qualified analyst will subsequently review. This must be completed to the satisfaction of the qualified analyst.

19.3.2. Batch Review

19.3.2.1. After performing at least 6-8 batches of drug screening and confirmation analyses, the trainee will learn how to perform batch review of drug screening and confirmation by mass spectrometry.

19.3.2.2. [Full scan] The trainee will observe batch review of at least one full scan batch by a qualified analyst. The trainee will then perform batch review of at least four full scan batches that a qualified analyst will subsequently review. The trainee must demonstrate his/her ability to successfully batch review to the satisfaction of the qualified analyst.

19.3.2.3. [Selective-ion monitoring (SIM)/selective reaction monitoring (SRM)] The trainee will observe batch review of at least two SIM/SRM batches by a qualified analyst. The trainee will then perform batch review of at least ten SIM/SRM batches that a qualified analyst will subsequently review. The trainee must demonstrate his/her ability to successfully batch review to the satisfaction of the qualified analyst.

19.3.3. Case Review

19.3.3.1. After completion of the batch review training, the trainee will learn how to perform technical and administrative review for positive toxicology case reports.

Emphasis will be placed on LIMS to review reports that are consistent with the current formatting and content guidelines.

19.3.3.2. Technical Review

19.3.3.2.1. The trainee will observe technical review of at least five positive toxicology case reports. The trainee will then perform technical review on at least twenty positive toxicology case reports that a qualified analyst will subsequently review. The trainee must demonstrate his/her abilities to successfully technically review the case records to the satisfaction of the qualified analyst.

19.3.3.3. Administrative Review

19.3.3.3.1. The trainee will observe administrative review of at least three positive toxicology case reports. The trainee will then perform administrative review on at least twenty positive toxicology case reports that a qualified analyst will subsequently review. The trainee must demonstrate his/her abilities to successfully administratively review the case records to the satisfaction of the qualified analyst.

19.4. Documentation of Completion

19.4.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record. Trainer should ensure that the trainee takes Qualtrax acknowledgement tests for necessary laboratory forms associated with the analysis.

20. Review Refresher

20.1. Aim

20.1.1. This training module is to provide reviewers with an understanding of what critical components must be checked and compared among documentation in the case record for each service. Other review criteria such as requirements versus preference, defect categorization, and request complexity scale may also be included as applicable.

20.1.2. The section will go over its top defects and locations based on the data in the review dashboard and the process weaknesses by reviewing defects identified in the post-mortem audit during the weekly section meetings.

20.2. Required Reading

20.2.1. HFSC Toxicology Analytical Manual

20.2.2. Relevant laboratory forms pertaining to administrative and technical reviews

20.3. Additional Resources

20.3.1. JusticeTrax Review DUI Guidelines

20.4. Laboratory Exercise

20.4.1. All qualified technical and administrative reviewers, both new and experienced, participate in the training annually (i.e., calendar year, excluding the year when the technique is introduced in the workflow) as required by the HFSC Quality Manual. Others (e.g., trainees) may also attend the training.

20.4.2. Each operation group by instrument type (i.e., alcohol, immunoassay, GC-MS, and LC-MS/MS) will provide this annual review refresher training by scheduling a meeting and going over the relevant review checklists during the meeting.

20.4.3. The supervisor of the operation or the designee will facilitate the meeting.

20.5. Documentation of Completion

20.5.1. Completion of this group training is documented in Qualtrax as a Quality Awareness event.

21. Testifying in Court

21.1. Aim

21.1.1. This training module prepares the trainee for serving as an expert witness. The trainee learns how to effectively testify in court regarding toxicology analysis and results of volatiles and other drugs, as well as the effects of those drugs on the human body.

21.1.2. Scientists authorized to perform an analysis are considered authorized to testify to the procedures and principles of the analysis as well as the general effects of the drugs and other topics related to HFSC operation and based on the current scientific literature. This is different from interpretative opinions regarding the toxicological results described in Section 22.

21.2. Required Reading

21.2.1. ANSI/ASB Best Practice Recommendation 037, First Edition, 2019. Guidelines for Opinions and Testimony in Forensic Toxicology.

21.3. Study Questions:

21.3.1. The study questions may be conducted during Analysis of Alcohol and Other Volatiles by Headspace (section 7), Drug Screen by ELISA (section 8), or Drug Screen and Qualitative Confirmation by GC-MS (section 9) depending on the analyst training and level of experience.

21.3.2. Voir Dire – Part I

21.3.2.1. Please state your name for the record.

21.3.2.2. What is your title and where are you employed?

21.3.2.3. How long have you been employed at HFSC?

21.3.2.4. What are your job duties?

21.3.2.5. Please describe your education background.

21.3.2.6. What specific training have you received that qualifies you to perform your duties?

21.3.2.7. Have you performed this type of work before you were employed at HFSC?

21.3.2.8. Please describe your previous experience.

21.3.2.9. Do you belong to any professional organizations or have any certifications?

21.3.2.10. Have you published any peer-reviewed articles in your field?

21.3.2.11. Approximately how many analyses have you performed on biological specimens?

21.3.2.12. Have you been qualified as an expert witness before?

21.3.2.13. If so, approximately how many times?

21.3.2.14. Is the lab you work for accredited?

21.3.2.15. If so, by whom is your lab accredited?

21.3.2.16. Can you please explain to the Ladies and Gentlemen of the jury why accreditation is important?

21.3.3. Alcohol Analysis – Part II

21.3.3.1. Can you explain to the Ladies and Gentlemen of the jury the science behind blood alcohol testing?

21.3.3.2. What instrumentation is used to perform this type of testing?

21.3.3.3. Is this technique generally accepted in the field of forensic toxicology?

21.3.3.4. Does your laboratory use this technique to perform alcohol analysis?

21.3.3.5. Let me show you (blood tube), previously marked as State's Exhibit #___. How can you identify this vial?

21.3.3.6. What was the condition of the evidence when you received it?

21.3.3.7. Did you follow the proper protocol for when analyzing this specimen?

21.3.3.8. I'm showing you what has been marked as State's Exhibit # ___ (report). Do you recognize this?

21.3.3.9. How do you recognize it? What is it?

21.3.3.10. Is this a true and correct copy of the lab results stemming from the analysis of the specimen you analyzed?

21.3.3.11. To your knowledge, has it been altered in any way?

21.3.3.12. Was it made at or near the time of the events we are discussing?

21.3.3.13. Was it made in the ordinary course of business?

21.3.3.14. What were the results?

21.3.3.15. Can you explain what uncertainty of measurement is?

21.3.3.16. During the course of your training, have you also learned about the physical effects of ethanol on the human body?

21.3.3.17. What are the effects on the body, specifically the body's central nervous system?

21.3.3.18. How could this affect a person's ability to operate a motor vehicle?

21.3.3.19. How long does it take an individual to fully absorb ethanol after their last drink?

21.3.3.20. What is retrograde extrapolation?

21.3.3.21. What are the 3 factors that are included in the retrograde extrapolation formula?

21.3.3.22. What is an alcohol elimination rate and is it a constant unit of time?

21.3.3.23. What is the minimum time frame your laboratory uses from time of last drink to the time of stop? Why?

21.3.3.24. Given the following facts, can you determine the defendant's BAC at the time of stop? (NEED: time of last drink, time of stop, and time of draw)

21.3.3.25. Explain the Widmark equation. What is the purpose of the calculation?

21.3.3.26. What additional information is necessary to perform the equation?

21.3.3.27. The defendant stated only 3 standard drinks were consumed. Given the following facts, is that consistent? (NEED: weight and # of drinks)

21.3.4. Drug Analysis – Part III (EIA, conducted during section 8)
- 21.3.4.1. I am showing you what has previously been marked as State's Exhibit __. Do you recognize this?
- 21.3.4.2. How do you recognize it? What is it?
- 21.3.4.3. What are the results?
- 21.3.4.4. What is ELISA drug screening?
- 21.3.4.5. What does "presumptive positive" mean? Why is the name of the positive drug group not reported?
- 21.3.4.6. What is the ELISA cutoff calibrator? How do you determine something to be positive?
- 21.3.4.7. What does the scope of analysis for ELISA indicate? What do the listed drugs mean?
- 21.3.4.8. How do you store blood samples? Why is refrigeration important?

21.3.5. Drug Analysis – Part IV (GC-MS, conducted during section 9)
- 21.3.5.1. What is a GC-MS Drug Screen?
- 21.3.5.2. What confirmation testing was performed?
- 21.3.5.3. What is GC-MS SIM analysis? How does it work?
- 21.3.5.4. What does "ng/mL" mean?
- 21.3.5.5. Can you describe the uncertainty of measurement for the jury?
- 21.3.5.6. How do you know the results your laboratory obtained are reliable and accurate?

21.3.6. Drug Analysis – Part V (LC-MS/MS, conducted during section 10)
- 21.3.6.1. Describe LC-MS/MS analysis.
- 21.3.6.2. Can you briefly describe how you obtained your results?
- 21.3.6.3. How do you know the instrument was working properly the day of your analysis?
- 21.3.6.4. How do you know the results your laboratory obtained are reliable and accurate?
- 21.3.6.5. What does "ng/mL" mean?
- 21.3.6.6. Can you describe the uncertainty of measurement for the jury?

21.4. Laboratory Exercise

21.4.1. Observe testimony of at least one analyst (if possible).

21.4.2. Go over the study questions with the experienced analyst.

21.4.3. Participate in a mock trial.
- 21.4.3.1. Qualified analysts will evaluate the trainee's performance and submit evaluation forms to the manager.
- 21.4.3.2. The mock trial may be conducted during Analysis of Alcohol and Other Volatiles by Headspace (section 7), Drug Screen by ELISA (section 8), Drug Screen and Qualitative Confirmation by GC-MS (section 9), or Drug Confirmation by Liquid

Chromatography-Tandem Mass Spectrometry (section 10), depending on the analyst training and level of experience.

21.5. Documentation of Completion

21.5.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record.

22. Interpretative Toxicology

22.1. Aim

22.1.1. This training module prepares the trainee to interpret toxicological test results in relation to pharmacodynamic and pharmacokinetic properties of the drugs. The trainee obtains a working knowledge of the general effects of various psychoactive drugs on human performance and behavior. S/he subsequently applies the knowledge to provide literature-supported court testimony and expert opinions for driving under the influence of drug (DUID) cases. Graduate level education in Toxicology, Pharmacology, or other relevant subjects and/or applicable previous experience may substitute all or a part of this training module.

22.2. Required Reading

22.2.1. ANSI/ASB Best Practice Recommendation 037, First Edition, 2019. Guidelines for Opinions and Testimony in Forensic Toxicology.

22.2.2. Pharmacology

22.2.2.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 2 (Human Performance Toxicology), Ch. 7 (Pharmacokinetics), and Ch. 8 (Pharmacodynamics).

22.2.2.2. The Pharmacological Basis of Therapeutics, 13th Ed., Brunton et al., 2017, McGraw-Hill Education – Section I and Section II Ch. 13-14.

22.2.3. Cannabis

22.2.3.1. Principles of Forensic Toxicology, 4th Ed., Ch. 19 (Cannabis).

22.2.4. CNS depressants (barbiturates, benzodiazepines, GHB, hypnotics, muscle relaxants)

22.2.4.1. Principles of Forensic Toxicology, 4th Ed., Ch. 14 (Benzodiazepines), Ch. 15 (GHB), and Ch. 16 (Miscellaneous CNS Depressants).

22.2.4.2. The Pharmacological Basis of Therapeutics, 13th Ed., Ch. 19 (Hypnotics and Sedatives).

22.2.4.3. Drummer. Benzodiazepines – Effects on Human Performance and Behavior. Forensic Science Review. 2002;14:1-14.

22.2.4.4. Robertson and Marinetti. Carisoprodol – Effects on Human Performance and Behavior. Forensic Science Review. 2003;15:1-7.

22.2.5. CNS stimulants (cocaine, sympathomimetic amines)

22.2.5.1. Principles of Forensic Toxicology, 4th Ed., Ch. 18 (Cocaine) and Ch. 20 (Amphetamines/Sympathomimetic Amines).

22.2.5.2. Isenschmid. Cocaine – Effects on Human Performance and Behavior. Forensic Science Review. 2002;14:62-100.

22.2.5.3. Logan. Methamphetamine – Effects on Human Performance and Behavior. Forensic Science Review. 2002;14:134-151.

22.2.5.4. Logan. 3,4-Methylenedioxymethamphetamine – Effects on Human Performance and Behavior. Forensic Science Review. 2003;15:12-28.

22.2.6. Narcotic analgesics (opioids)
22.2.6.1. Principles of Forensic Toxicology, 4th Ed., Ch. 17 (Opioids).
22.2.6.2. The Pharmacological Basis of Therapeutics, 13th Ed., Ch. 20 (Opioids, Analgesia, and Pain Management).
22.2.6.3. Stout and Farrell. Opioids – Effects on Human Performance and Behavior. Forensic Science Review. 2003;15:30-58.
22.2.7. Dissociative drugs (PCP, ketamine)
22.2.7.1. Principles of Forensic Toxicology, 4th Ed., Ch. 21 (Hallucinogens).
22.2.7.2. Mozayani. Phencyclidine – Effects on Human Performance and Behavior. Forensic Science Review. 2003;15:62-73.
22.2.8. Hallucinogens (LSD, psilocybin)
22.2.8.1. Principles of Forensic Toxicology, 4th Ed., Ch. 21 (Hallucinogens).
22.2.9. Inhalants
22.2.9.1. Principles of Forensic Toxicology, 4th Ed., Ch. 28 (Inhalants).
22.2.10. Therapeutic drugs
22.2.10.1. Principles of Forensic Toxicology, 4th Ed., Ch. 22-26 (anticonvulsants and antiarrhythmics, antidepressants, antipsychotics, antihistamines, and nonnarcotic analgesics).
22.2.10.2. The Pharmacological Basis of Therapeutics, 13th Ed., Ch. 15-17 (Drug Therapy of Depression and Anxiety Disorders, Pharmacotherapy of Psychosis and Mania, and Pharmacotherapy of the Epilepsies) and Ch. 30 (Antiarrhythmic Drugs).
22.2.11. Novel psychoactive substances
22.2.11.1. Mohr et al. Reports of Adverse Events Associated with Use of Novel Psychoactive Substances, 2017-2020: A Review. Journal of Analytical Toxicology. 2022;46:e116-e185.
22.2.12. Statistics
22.2.12.1. Principles of Forensic Toxicology, 5th Ed., B. Levine and S. Kerrigan, eds. 2020, Springer – Ch. 18 (Statistics for Forensic Toxicology)

22.3. Additional Resources

22.3.1. Disposition of Toxic Drugs and Chemicals in Man, 11th Ed., R.C. Baselt, 2017, Biomedical Publications
22.3.2. Drugs and Driving Literature List, Society of Forensic Toxicologists, http://www.soft-tox.org/duid_literature

22.4. Study Questions

22.4.1. Pharmacology
22.4.1.1. Describe the differences between pharmacokinetics and pharmacodynamics.
22.4.1.2. Explain absorption, distribution, metabolism, and elimination: the relationship among the parameters and the factors affecting each parameter.
22.4.1.3. Describe phase I and phase II metabolism and first-pass effect.

22.4.1.4. Illustrate zero order, first order, and non-linear (two-compartment) elimination kinetics.

22.4.1.5. Describe the dose-response curves in relation to efficacy and potency. Explain the receptor affinity.

22.4.1.6. Differentiate among full agonist, partial agonist, inverse agonist, and antagonist: give an example of each.

22.4.1.7. Describe P450 enzyme inhibition and induction and how inhibitors/inducers may change the effects of target drugs.

22.4.2. Individual classes of drugs (drugs included in the section's test panels)

22.4.2.1. General pharmacokinetic properties: half-life, V_d, and pKa.

22.4.2.2. General detection windows in blood and urine.

22.4.2.3. Metabolic pathway including active and/or inactive metabolites.

22.4.2.4. General storage stability of the drugs.

22.4.2.5. Therapeutic dose, concentration ranges, effects, and mechanism of action.

22.4.2.6. Adverse effects including the effects on driving and documented concentrations.

22.4.2.7. Withdrawal symptoms and time of onset.

22.4.2.8. The characteristic signs and symptoms noted by the Drug Recognition Experts.

22.4.3. Interpretation

22.4.3.1. What case information and observations at the scene can change your interpretation based solely on drug concentrations?

22.4.3.2. In each scenario, state 1) whether the detected drugs would have contributed to the incident/behavior, 2) in broad terms, how much and when the individual would have taken the drug (if possible), 3) how the drug would have affected the individual's behavior, and 4) whether the observed signs/symptoms are consistent with the general effects of the drug. Support your opinion with appropriate references.

22.4.3.2.1. A 57-year-old male was stopped for an improper turn. DRE observed elevated blood pressure, dilated pupil size, and increased pulse rate. The individual showed 3/8 WAT and 2/4 OLS clues. Blood samples were collected 1 hour after the stop, which had alcohol at 0.06 g/100 mL, THC at 10 ng/mL, 11-OH-THC at 3 ng/mL, and THC-COOH at 50 ng/mL.

22.4.3.2.2. A 42-year-old female had been observed weaving. Subsequently she crossed over the centerline and hit a car approaching from the opposite direction, causing multiple injuries to the driver. During SFST, she had difficulty following instructions and maintaining balance (4/8 WAT and 3/4 OLS clues); HGN was present. Blood samples were collected 2 hours after the crash. Blood alcohol test was negative, but the toxicology test indicated the following drugs: alprazolam at 120 ng/mL, nordiazepam at 500 ng/mL, and morphine at 90 ng/mL.

22.4.3.2.3. A 35-year-old male was stopped for a broken taillight. He did not exhibit any impaired signs during the SFST but showed agitation. Blood samples were collected 2 hours after the stop. Blood alcohol test was negative, but the toxicology test indicated the following drug: phencyclidine at 5 ng/mL.

22.4.3.2.4. A 29-year-old male was stopped for speeding and driving through a red traffic light. At the stop, he was combative and could not stand still. The SFST was not performed. Blood samples were collected 5 hours after the arrest. He expressed sleepiness and fatigue at the blood draw. Blood alcohol test was negative, but the toxicology test indicated the following drug: benzoylecgonine at 2000 ng/mL.

22.4.3.2.5. A 73-year-old female was stopped for going 20 mph below the speed limit and swerving. She had difficulty getting out of the car and could not complete the SFST because she fell down and could not stand up without support. Blood samples were collected 2 hours after the stop. Blood alcohol test was negative, but the toxicology test indicated the following drugs: carisoprodol at 1.5 µg/mL, meprobamate at 3 µg/mL, oxycodone at 200 ng/mL, hydrocodone at 100 ng/mL, and alprazolam at 70 ng/mL. She had prescriptions for all these drugs.

22.4.3.2.6. A 25-year-old female reported having been sexually assaulted a day before. She reportedly experienced confusion, dizziness, hyperthermia, blurred vision, and short-term memory loss. A urine sample was collected 23 hours after the incident. The toxicology test indicated the following drugs: nortriptyline at 100 ng/mL, fluoxetine at 150 ng/mL, and norfluoxetine at 100 ng/mL. She stated she did not take these drugs nor have prescriptions for them.

22.4.3.2.7. A 30-year-old male reported having been sexually assaulted a day before. He reportedly experienced drowsiness, nausea, and visual disturbance and eventually lost consciousness. A urine sample was collected 48 hours after the incident. The toxicology test indicated the following drug: gamma-hydroxybutyric acid at 200 ng/mL.

22.5. Laboratory Exercise

22.5.1. Explore various ways to find relevant references. Suggest referring to the literature search tips on the SOFT website (http://www.soft-tox.org/files/Literature_search.pdf).

22.5.2. Practice testimony questions associated with each scenario.

22.5.2.1. [For all scenarios in 21.4.3] In your expert opinion, was the individual impaired at the time of the incident?

22.5.2.2. [For all scenarios in 21.4.3] What can you say about the toxicology test results of this case?

22.5.2.3. [For 21.4.3.2.1] The defendant smoked marijuana for medical purposes to relieve his back pain and his blood alcohol level was below the legal limit. Wouldn't his performance have been improved with marijuana since he would no longer have suffered from excruciating back pain?

22.5.2.4. [For 21.4.3.2.2] She dropped her phone and was looking for it at the time of the crash. Also, she was shocked from the crash and extremely nervous when the officer conducted the SFST. Would the circumstance explain her observed behavior rather than those prescription drugs?

22.5.2.5. [For 21.4.3.2.4] Wouldn't cocaine improve his driving performance since it is a stimulant? Additionally, what the lab found is an inactive breakdown product of cocaine.

22.5.2.6. [For 21.4.3.2.5] Do you agree that the defendant's tolerance to her prescription drugs minimized the potential effects of the drugs? Her behavior can be explained by her age and the shock of being stopped by a police officer.

22.5.3. Develop an analytical method and perform appropriate validation experiments. Or perform validation and/or verification of an existing method. Trainee may have completed this requirement during the course of employment prior to this training module. When possible, publish the findings in a peer-reviewed journal and present them at a professional conference.

22.6. Competency

22.6.1. Upon satisfactory completion of the study questions and the laboratory exercise, the trainee is deemed ready for a competency test. Peer-review publication and conference presentation are not required for the trainee to take the competency test.

22.6.2. The competency test consists of 1) three toxicology case scenarios with multiple interpretive toxicology and pharmacology questions for each. The trainee must provide satisfactory written responses in 6 hours. This is an open-book test; and 2) an oral evaluation of responses to the questions from one of the case scenarios mimicking interpretative aspect of toxicology testimony.

22.7. Documentation of Completion

22.7.1. Completion of each training requirement is documented on Form LAB-076, activity log and/or equivalent electronic record.