8.5 BASIC DRUG PROCEDURE

8.5.1 Purpose

To qualitatively and/or quantitatively identify basic drugs in submitted evidence using liquid-liquid extraction followed by instrumental analysis with gas chromatography/flame ionization (GC/FID) and gas chromatography/mass spectrometry (GC/MS). The list of drugs that are analyzed as part of this method, and their corresponding calibrator and control levels can be found in the appendix.

8.5.2 Specimen Requirements

Acceptable samples for this analysis include blood and urine. For additional samples see Alternative Matrices (section 6.6).

8.5.3 Apparatus and Equipment

Disposable 15 mL culture tubes and screw caps
Disposable 5 mL conical centrifuge tubes and screw caps
Volumetric pipettes and disposable tips
Assorted volumetric glassware
Disposable transfer pipettes
Sample mixer
Centrifuge
11 mm autosampler vials, inserts, and caps
11 mm crimper
GC/FID, GC/MS, ChemStation software, compatible computer, and printer

8.5.4 Reagents and Standards

Reference standards
Mepivacaine working reference standard [2.5 μg/mL] (internal standard)
Water (H_2O)
n-Butyl chloride (1-chlorobutane) (C_4H_9Cl)
Ammonium hydroxide (NH_4OH)
1 N Sulfuric acid (H_2SO_4)
Chloroform ($CHCl_3$)

8.5.5 Standard and Reagent Preparation

Standards and reagents may be made at different volumes than as listed below based on the needs of the laboratory. If starting materials of differing concentrations are used for standard preparation, the calculations used to determine the final concentration will be documented. (e.g. initial standard concentration used for preparation is a 100 ug/mL ampule instead of a 1 mg/mL ampule)

8.5.5.1 Standards

Basic Drug Reference Standard Solutions
See Basic Drug Appendix.
Mepivacaine Stock Reference Standard Solution [1 mg/mL] (Internal Standard)
Dissolve 10 mg of mepivacaine or 11.48 mg of mepivacaine HCl and dilute to 10 mL with an appropriate solvent (e.g., reagent alcohol, methanol, etc.).

Mepivacaine Working Reference Standard Solution [2.5 μg/mL] (Internal Standard)
Add 250 μL of mepivacaine stock reference standard solution [1 mg/mL] and dilute to 100 mL with H_2O.

8.5.5.2 Prepared Reagents

1 N Sulfuric Acid (H_2SO_4)
Add 13.9 mL concentrated sulfuric acid to H_2O and dilute to 500 mL.

8.5.6 Procedure

1. Allow all reference standards and case samples to equilibrate to room temperature before beginning procedure.
2. Label, check, and load/unload all samples in accordance with the “Sample Pipetting Check List’ (see Appendix section).
3. Pipette 2.5 mL of corresponding case sample, calibrator, positive control, or negative control into the appropriately labeled 15 mL culture tube.
   Note: Smaller sample volumes may be analyzed on a case-by-case basis.
4. Pipette 250 μL of internal standard into each sample to make a final concentration of 0.25 μg/mL.
5. Add 4 mL of n-butyl chloride.
6. Add 200-250 μL of ammonium hydroxide, mix/rotate sample for approximately 3 minutes, and centrifuge until separated (approximately 15 minutes).
   Note: If an emulsion is present, add a small amount of sodium sulfate, mix, and centrifuge until separated (approximately 2 minutes).
7. Transfer the upper layer (n-butyl chloride) to a clean, labeled 5 mL conical tube. Discard the bottom layer.
8. Add 1.5 mL of 1.0 N sulfuric acid, mix/rotate sample for approximately 3 minutes, and centrifuge until separated (approximately 15 minutes).
9. Aspirate top layer (n-butyl chloride).
   Note: If an emulsion is present, bubble with nitrogen.
10. Add 75 μL of chloroform and 400-500 μL ammonium hydroxide to the remaining aqueous layer, mix/rotate sample for approximately 3 minutes, and centrifuge until separated (approximately 15 minutes).
11. Aspirate top layer (aqueous layer).

12. Transfer the bottom (chloroform) layer to an 11 mm autosampler vial with insert and seal with cap.
13. Analyze and quantitate the samples by GC/FID and confirm by GC/MS (full scan mode).

8.5.7 Reporting

Results can be reported if the following criteria are met:

8.5.7.1 Presumptive

8.5.7.1.1 The Basic Drug Procedure may serve as a presumptive test for gabapentin if the peak in the case on the mass spectrometer sample matches the retention time of the control and there is a mass spectral match. This presumptive result must be confirmed by LCMSMS in order to be reported qualitatively.

8.5.7.2 Qualitative

8.5.7.2.1 Retention times of drugs identified are within ±1% of those of a calibrator or control standard of similar concentration on GC/FID unless otherwise noted in the case file.

8.5.7.2.2 Mass spectrums of drugs identified are consistent with those of analyzed reference standards.

8.5.7.2.3 Drugs that quantitate at higher than the highest calibrator are at risk of carrying over into the following sample. Scientists will carefully examine cases where this may have occurred and repeat the analysis of any cases where the contribution of carryover cannot be eliminated.

8.5.7.3 Quantitative

8.5.7.3.1 All the qualitative result criteria above must be met.

8.5.7.3.2 The concentration of the lowest calibrator is administratively set as the method LOQ for that drug. Sample drug concentrations will only be reported at levels greater than or equal to the concentration of the lowest calibrator for that drug.

8.5.7.3.3 Sample drug concentrations greater than the highest calibration point where the results are necessary for interpretation in the case shall be handled by reanalyzing the sample using smaller sample amounts including the dilution factor in the reported result.

8.5.7.3.4 Sample drug concentrations greater than the highest calibration point where the results are not necessary for interpretation in the case may be reported as "greater than…." the highest calibration point.

8.5.7.4 Results

8.5.7.4.1 Peaks on at least one of the chromatograms in the case file shall be labeled with the names of the identified drugs.

8.5.7.4.2 Any quantitative or retention time report result not used in a case shall either be lined through and initialed or all the data used shall be highlighted.

8.5.7.4.3 Qualitative results shall be expressed as "positive" and include any clarifying remarks, if applicable.

8.5.7.4.4 Quantitative results shall be reported in μg/mL. Results less than 1.0 μg/mL shall be reported to the hundredths decimal place. Results 1.0 μg/mL or greater shall be reported to the tenths decimal place.

8.5.7.4.5 When a definitive conclusion cannot be made, the reason shall be documented on the report (e.g., "insufficient sample for analysis", "sample unsuitable for analysis", "results are inconclusive due to sample condition", etc.).

8.5.8 References

Foerster, E.H., Hatchett, D., & Garriott, J.C. "A Rapid, Comprehensive Screening Procedure for Basic Drugs in Blood or Tissues by Gas Chromatography." Journal of Analytical Toxicology 2 (1978): 50-55.