



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Toxicology Quality Assurance and Procedures Manual

8.5 Basic Drug Procedure

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).

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 (H₂O)
n-Butyl chloride (1-chlorobutane) (C₄H₉Cl)
Ammonium hydroxide (NH₄OH)
1 N Sulfuric acid (H₂SO₄)
Chloroform (CHCl₃)

8.5.5 Standard and Reagent Preparation

The following are examples of how to prepare the standards and reagent used in this procedure.

8.5.5.1 Standards

Basic Drug Reference Standard Solutions

See Basic Drug Appendix.



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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 H₂O.

8.5.5.2 Prepared Reagents

1 N Sulfuric Acid (H₂SO₄)

Add 13.9 mL concentrated sulfuric acid to H₂O 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).

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8.5.7 Reporting

Results can be reported if the following criteria are met:

8.5.7.1 Qualitative

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

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

8.5.7.2 Quantitative

8.5.7.2.1 All the qualitative result criteria above must be met.

8.5.7.2.2 Sample drug concentrations below the lowest calibration point may be reported as “less than....” the lowest calibration point.

8.5.7.2.3 Sample drug concentrations greater than the highest calibration point where the results are necessary for interpretation in the case shall be handled using the following methods:

- Reanalyze using smaller sample amounts (including dilutions).
- Reanalyze using higher standard concentrations.

8.5.7.2.4 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.

8.5.7.3 Results

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

8.5.7.3.2 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.

8.5.7.3.3 Qualitative results shall be expressed as “positive” and include any clarifying remarks, if applicable.

8.5.7.3.4 Quantitative results shall be reported in $\mu\text{g/mL}$. Results less than 1.0 $\mu\text{g/mL}$ shall be reported to the hundredths decimal place. Results 1.0 $\mu\text{g/mL}$ or greater shall be reported to the tenths decimal place.

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8.5.7.3.5 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.