



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Toxicology Quality Assurance and Procedures Manual

8.4 Acid/Neutral Drug Procedure

8.4 ACID/NEUTRAL DRUG PROCEDURE

8.4.1 Purpose

To qualitatively and/or quantitatively identify acidic and neutral 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).

8.4.2 Specimen Requirements

Samples for analysis will be presumptively positive for barbiturates or suspected to contain a relevant drug per case circumstances. Acceptable samples for this analysis include blood and urine. For additional samples see Alternative Matrices (section 6.6).

8.4.3 Apparatus and Equipment

Disposable 15 mL culture tubes (2) 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
Evaporation station
11 mm autosampler vials, inserts, and caps
11 mm crimper
GC/FID, GC/MS, ChemStation software, compatible computer, and printer

8.4.4 Reagents and Standards

Negative blood, urine, or other matrix as needed
Reference standards
Aprobarbital stock reference standard solution [0.5 mg/mL] (internal standard)
Water (H₂O)
Potassium phosphate, monobasic (KH₂PO₄)
Ethyl ether ((C₂H₅)₂ O)
Toluene (C₇H₈)
Hexane (C₆H₁₄) (acetonitrile saturated)
Acetonitrile (C₃H₂N) (hexane saturated)

TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Toxicology Quality Assurance and Procedures Manual

8.4 Acid/Neutral Drug Procedure



8.4.5 Standard and Reagent Preparation

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

8.4.5.1 Standards

Acid/Neutral Drug Reference Standard Solutions

See Acid/Neutral Drug Appendix.

Aprobarbital Reference Standard Solution [0.5 mg/mL] (Internal Standard)

Dissolve 10 mg of aprobarbital and dilute to 20 mL with an appropriate solvent (e.g., reagent alcohol, methanol, etc.).

8.4.5.2 Prepared Reagents

Saturated Aqueous Potassium Phosphate Solution, Monobasic

Dilute potassium phosphate crystals with H₂O making sure there are still crystals present in the bottom of the solution.

Hexane/Acetonitrile Saturate

Combine hexane and acetonitrile in same container to saturate.

8.4.6 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. Prepare working reference calibrator and/or control standards from stock reference standards. See Acid/Neutral Drug Appendix for examples.
4. Pipette 1 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.
5. Pipette 20 µL of internal standard into each sample to make a final concentration of 10.0 µg/mL.
6. Add 1 mL of saturated potassium phosphate solution and mix.
7. Add 6 mL of ethyl ether.
8. Add 6 mL of toluene, mix/rotate sample for approximately 3-5 minutes, and centrifuge until separated (approximately 15 minutes).
9. Transfer the upper layer (toluene/ether) to another clean, labeled 15 mL culture tube. Discard the bottom aqueous layer.
10. Evaporate to dryness with heat (optional) and a dry gas (e.g. nitrogen) in an evaporation station.
11. Reconstitute the residue with 2 mL of hexane (acetonitrile saturate), mix/rotate, and transfer to a clean, labeled 5 mL conical tube.
12. Add 300 µL of acetonitrile (hexane saturate), mix/rotate sample for approximately 3 minutes, and centrifuge until separated (approximately 1-2 minutes).



TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division

Toxicology Quality Assurance and Procedures Manual

8.4 Acid/Neutral Drug Procedure

13. Transfer bottom layer (acetonitrile) to an 11 mm autosampler vial with insert and seal with cap. Discard the upper layer.
14. Analyze and quantitate the samples by GC/FID and confirm by GC/MS (full scan mode).
Note: Additional hexane washes can be used if bottom layer still appears discolored (after step 12). In this case, aspirate the upper (hexane) layer, add 1 mL of hexane (acetonitrile saturate), mix/rotate sample for approximately 3 minutes, and centrifuge until separated (approximately 1-2 minutes). Continue to step 13.

8.4.7 Reporting

Results can be reported if the following criteria are met:

8.4.7.1 Qualitative

8.4.7.1.1 Retention times of drugs identified are within $\pm 2\%$ of those of a calibrator or control standard of similar concentration on GC/FID unless otherwise noted in the case file.

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

8.4.7.2 Quantitative

8.4.7.2.1 All the qualitative result criteria above must be met.

8.4.7.2.2 Sample drug concentrations below the lowest calibration point may be reported as "less than...." the lowest calibration point.

8.4.7.2.3 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.

8.4.7.2.4 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.

8.4.7.3 Results

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

8.4.7.3.2 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.

TENNESSEE BUREAU OF INVESTIGATION

Forensic Services Division



Toxicology Quality Assurance and Procedures Manual

8.4 Acid/Neutral Drug Procedure

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

8.4.7.3.4 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.

8.4.7.3.5 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.).

8.4.8 References

Foerster, E.H., Demsey, J., & Garriott, J.C. “A Gas Chromatographic Screening Procedure for Acid and Neutral Drugs in Blood.” Journal of Analytical Toxicology, 3 (1979): 87-91.