Section Five
Quality Assurance

5.12 Solution Preparation

5.12.1 BACKGROUND
Refer to references.

5.12.2 SCOPE
This section describes the proper preparation of solutions and buffers used in ISP Forensic Services toxicology methods for the extraction of drug compounds from blood and urine specimens.

5.12.3 EQUIPMENT AND SUPPLIES
5.12.3.1 Glassware
Adequately sized beakers, volumetric flasks, graduated cylinders and volumetric pipettes
5.12.3.2 Laboratory balance
5.12.3.3 pH Meter and/or Indicator Strips
5.12.3.4 Appropriate buffer solutions for pH meter
5.12.3.5 Stirring hotplate
5.12.3.6 Magnetic stirrers
5.12.3.7 Safety Equipment
- Chemical Fume Hood
- Acid Resistant Apron
- Laboratory Coat
- Safety Goggles and/or face Shield
- Laboratory Gloves

5.12.3 REAGENTS
All chemicals must be ACS Grade or better.
5.12.4.1 Acids
- Acetic, Glacial
- Hydrochloric
- Phosphoric
- Sulfuric
- Formic (this may be ACS or LCMS grade)

5.12.4.2 Salts
- Ammonium Chloride
- Potassium Hydroxide
- Potassium Phosphate Monobasic
- Potassium Phosphate Dibasic
- Sodium Acetate Trihydrate

- Sodium Bicarbonate
- Sodium Hydroxide
- Sodium Phosphate Monobasic
- Sodium Phosphate Dibasic
- Sodium Tetraborate Decahydrate

5.12.4.4 Solvents

- Methanol

5.12.5 PROCEDURES

Preparation of the following solutions must be recorded on corresponding preparation logs. Solutions may be made in different volumes by adjusting reagent ratios.

Note: Appropriate safety equipment must be worn during the preparation of solutions to minimize exposure to caustic/corrosive solutions. The order of the addition of chemicals may be crucial to minimize exothermic reactions. Refer to appropriate MSDS sheets for more information on handling chemicals.

5.12.5.1 Acetic Acid

5.12.5.1.1 **1.0M Acetic Acid (500mL)**

Place approximately 400mL DI water into a 500mL volumetric flask. Add 29mL **glacial acetic acid**, mix. QS to 500mL.

A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.1.2 **20% Acetic Acid (500mL)**

Place approximately 300mL DI water into a 500mL volumetric flask. Add 100mL glacial acetic acid, mix. QS to 500mL.

A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.2 Ammonium Chloride

5.12.5.2.1 **Saturated Ammonium Chloride (500mL)**

Place approximately 300mL DI water in a beaker and heat/stir over low heat. Add **ammonium chloride** until the solution is saturated. QS to 500mL.

A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.3 Borate Buffers

5.12.5.3.1 **Borate Buffer, pH 9.2**

Place approximately 500mL DI water into a 1000mL beaker. Heat and stir while adding 50g sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$). Once dissolved, allow to cool. Bring volume up to approximately 950mL with DI water. Verify pH and adjust as necessary to pH 9.2 ±0.2 with 1N KOH or 100mm HCl. Place solution in 1000mL volumetric flask and QS with DI water.

Solution is stable for at least six months. After six months, analyst is to verify pH prior to each use. If pH is outside preparation tolerance, the buffer should be remade. Further, a positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.3.2 **Borate Buffer, pH 12**

Place approximately 500mL DI water into a 1000mL beaker. Heat and stir while adding 50g sodium tetraborate ($Na_2B_4O_7 \cdot 10\ H_2O$). Once dissolved, allow to cool. Bring volume up to approximately 900mL with DI water. Add 25mL 10N NaOH and stir. Verify pH and adjust as necessary to pH 12 ±0.2 with 10N NaOH or 6N HCl. Place solution in 1000mL volumetric flask and QS with DI water.

Solution is stable for at least six months. After six months, analyst is to verify pH prior to each use. If pH is outside preparation tolerance, the buffer should be remade. Further, a positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.4 Formic Acid for LC/MS Mobile Phases

5.12.5.4.1 **0.1% Formic Acid in LCMS Grade Water (500mL)**

Place approximately 300mL LCMS Grade water into instrument solvent bottle. Add 500µL of Formic Acid, mix . QS to approximately 500mL.

Prepare fresh when needed, make appropriate volume adjustments if needed.

5.12.5.4.2 **0.1% Formic Acid in LCMS Acetonitrile (500mL)**

Place approximately 300mL LCMS Grade Acetonitrile into instrument solvent bottle. Add 500µL of Formic Acid, mix . QS to approximately 500mL.
Prepare fresh when needed, make appropriate volume adjustments if needed.

5.12.5.5 Hydrochloric Acid

5.12.5.5.1 **0.1M/100mM Hydrochloric Acid (500mL)**
Place approximately 300mL DI water into a 500mL volumetric flask. Add 4.2mL **concentrated hydrochloric acid**, mix. QS to 500mL.
A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.5.2 **1% HCl in Methanol (10mL)**
Add approximately 5mL of methanol to a 10mL volumetric flask. Pipet 100µL of **concentrated HCl**, QS and mix. Store in a brown glass bottle.
Solution is stable for six-months. A positive and negative control will be run with each use. Remake as indicated by control data, or every 6 months.

5.12.5.6 Potassium Hydroxide (KOH)
Note: As the addition of KOH to water will generate significant heat, exercise due caution.

5.12.5.6.1 **1M/1N Potassium Hydroxide (100mL)**
Dissolve 5.6g **potassium hydroxide** in approximately 80mL DI water in a 100mL volumetric flask. QS to 100mL.
A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.6.2 **11.8N Potassium Hydroxide (1000mL)**
Gradually(!) add 662g **potassium hydroxide** to approximately 600mL DI water, stir on stir plate to dissolve. Allow to cool (this takes awhile) and QS in a 1000mL volumetric flask.
A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.7 Potassium Phosphate Buffers

5.12.5.7.1 **Saturated Potassium Phosphate Buffer (1000mL)**
Place approximately 1000mL DI water in a beaker and heat/stir over low heat. Add **potassium phosphate monobasic** until the solution is saturated. Allow solution to cool. Adjust pH to approximately 1.8 with **concentrated phosphoric acid**.
A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.7.2 **0.1M/100mM Potassium Phosphate Buffer (100mL) - Adjusted to pH 6**
Dissolve 1.36g **potassium phosphate monobasic** in approximately 90mL DI water in a 150mL beaker. Adjust to pH 6.0 with 1–M **potassium hydroxide.** QS in a 100mL volumetric flask.
Store in colored-glass container (red or brown). Solution is stable for at least six months. After six months, analyst is to verify pH prior to each use. If pH is outside preparation tolerance, the buffer should be remade. Further, a positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.8 Sodium Acetate Buffers

5.12.5.8.1 **0.1M/100mM Acetate Buffer, pH 4.5 (500mL)**
Dissolve 2.93g **sodium acetate trihydrate** in 400mL DI water in a 600mL beaker. Add 1.62mL **glacial acetic acid**, and mix well. Adjust to pH 4.5±0.1 with **glacial acetic acid** or **100mM acetic acid.** QS to 500mL in a 500mL volumetric flask.
A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.8.2 **0.1M/100mM Acetate Buffer, pH 5.0 (500mL)**
Prepare as with pH 4.5 buffer (5.12.5.9.1). Adjust pH to 5.0 ±0.1.
A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.8.3 **2.0M Acetate Buffer, pH 4.8 (1000mL)**

Dissolve 141.4g **sodium acetate trihydrate** in approximately 800mL DI water. Add 55.2mL **glacial acetic acid**. Adjust to pH 4.8 and QS to 1000mL.

A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.9 Sodium Bicarbonate

5.12.5.9.1 **50mM Sodium Bicarbonate, pH 11 (500mL)**

Dissolve 2.1g **sodium bicarbonate** in 500mL DI water. Adjust to pH 11 as needed.

A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.10 Sodium Hydroxide (NaOH)

Note: As the addition of NaOH to water will generate heat, exercise due caution.

5.12.5.10.1 **2N NaOH (1000mL)**

Place approximately 250mL DI water into a 1000mL beaker. Gradually add 80g **NaOH.** Transfer to 500mL volumetric flask and QS to 500mL. (Caution: Exothermic)

A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.10.2 **10N NaOH (500mL)**

Place approximately 400mL DI water into a 1000mL beaker. Gradually add 200g **NaOH.** Transfer to 500mL volumetric flask and QS to 500mL. (Caution: Exothermic)

This reagent is used in the preparation of other reagents; those reagents are checked with each use.

5.12.5.11 Sodium Phosphate

5.12.5.11.1 **100mM Sodium Phosphate Dibasic (200mL)**

Dissolve 2.84g **sodium phosphate dibasic** in approximately 160mL DI water. QS to 200mL and mix.

Store in glass container. A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.11.2 **100mM Sodium Phosphate Monobasic (200mL)**
Dissolve 2.76g **sodium phosphate monobasic** in approximately 160mL DI water. QS to 200mL and mix.
Store in glass container. A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.12 Sodium Phosphate Buffers

5.12.5.12.1 **0.1M/100mM Sodium Phosphate Buffer (1000mL) Adjusted to pH 6**
Dissolve 1.70g **sodium phosphate dibasic** (Na_2HPO_4) and **12.14 sodium phosphate monobasic** ($NaH_2PO_4 \cdot H_20$) in approximately 800mL DI water in a 1000mL volumetric flask. QS to 1000mL. Adjust to pH 6.0 ±0.1 with 100mM **monobasic sodium phosphate** (to lower pH) or 100mM **dibasic sodium phosphate** (to raise the pH).
Check pH prior to use for blood toxicology casework; if pH outside preparation tolerance, remake buffer. Store in colored-glass container (red or brown). A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.5.13 Sulfuric Acid

5.12.5.13.1 **0.05M/0.1N Sulfuric Acid**
Place approximately 800mL distilled/deionized (DI) water into a 1L volumetric flask. Add 2.7mL **concentrated sulfuric acid**, mix. QS to 1L.
A positive and negative control will be run with each use. Remake as indicated by control data.

5.12.6 QUALITY ASSURANCE

5.12.6.1 Refer to toxicology Analytical Method 5.2 for balance intermediate check and calibration requirements.

Note: Balances properly monitored by drug discipline analysts fulfill quality assurance requirements. Additional check need not be performed.

5.12.7 REFERENCES

5.12.7.1 Shugar, G.J., Shugar, R.A. and Bauman, L. *Grades of Purity of Chemicals* pp. 145-154, *pH Measurement*. pp. 232-234. *in:* Chemical Technicians' Ready Reference Handbook, McGraw Hill: New York, 1973.

5.12.7.2 Ansys, Inc. SPEC Extraction Methods

5.12.7.3 United Chemical Technologies, Inc. Applications Manual.

Revision History

Section Five
Quality Assurance

5.12 Solution Preparation

Revision # Issue Date History

0	05-07-2007	Combined urine solution preparation (2.6) and blood solution preparation (3.8).
1	08-20-2008	Removed obsolete solutions, added reference for balance check requirements, clarifications.
2	7/8/2011	For solutions known to have a long shelf life, language was added to allow use longer that previously indicated. The following statement is in place of a definite expiration date. *A positive and negative control will be run with each use. Remake as indicated by control data.* Solutions that were no longer being used in toxicology analytical methods were removed. Solutions that were duplicated but listed in different volumes were removed. A statement allowing different volumes of solutions to be made was added. A statement was added to the safety note referencing MSDS sheets. Numbering updated.
3	4/9/2013	Added preparation instructions for LC/MS Mobile phase solutions using Formic Acid. For solutions known to have a long shelf life, language was added to allow use longer that previously indicated. The following statement is in place of a definite expiration date. *A positive and negative control will be run with each use. Remake as indicated by control data.* Numbering updated.
4	04/22/2015	Formatting and grammar corrections. Minor modification to scope statement. Changed grade requirement for formic acid.