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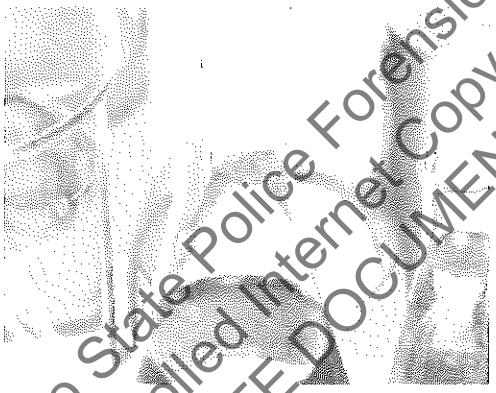

Discipline/Name of Document: Toxicology
5.12 Solution Preparation

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Quality Manager

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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 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

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5.12.4 REAGENTS
All chemicals must be ACS Grade or equivalent.
5.12.4.1 Acids
- Acetic, Glacial
- Hydrochloric
- Phosphoric
- Sulfuric

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

5.12.4.3 Enzyme
- β-Glucuronidase (Patella vulgata)

5.12.4.4 Solvents
- Methanol

5.12.5 PROCEDURES

Note: appropriate safety equipment should be worn during the preparation of solutions to prevent exposure to caustic/corrosive solutions. The order of the addition of chemicals may be crucial to prevent exothermic reactions.

5.12.5.1 Acetic Acid

5.12.5.1.1 **0.1M/100mM Acetic Acid (500mL)**
Place approximately 300mL distilled/deionized (DI) water into a 500mL volumetric flask. Add 2.9mL **glacial acetic acid**, mix. QS to 500mL.
Solution is stable for six months.

5.12.5.1.2 **1.0M Acetic Acid (500mL)**
Place approximately 400mL DI water into a 500mL volumetric flask. Add 29mL **glacial acetic acid**, mix. QS to 500mL.
Solution is stable for six months.

5.12.5.2 Acetate Buffers

5.12.5.2.1 **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 **100mM sodium acetate, glacial acetic acid** or **100mM acetic acid.** QS to 500mL in a 500mL volumetric flask.
Solution is stable for six months.

5.12.5.2.2 **100mM Acetate Buffer, pH 4.0 (100mL) [Varian]**
Add ≅80mL DI water to an 150mL beaker. Add 570µL glacial acetic acid. Stir to mix. Add 1.6mL 1.0M KOH. Resulting pH should be 4.0, adjust as

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necessary. Place solution into a 100mL volumetric flask and QS with DI water.
Solution is stable for six months.

5.12.5.2.3 **100mM Acetate Buffer, pH 4.0 (500mL) [Varian]**
Add ≅400mL DI water to an 600mL beaker. Add 2850µL glacial acetic acid. Stir to mix. Add 8.0mL 1.0M KOH. Resulting pH should be 4.0, adjust as necessary. Place solution into a 500mL volumetric flask and QS with DI water.
Solution is stable for six months.

5.12.5.2.4 **0.1M/100mM Acetate Buffer, pH 5.0 (500mL)**
Prior to pH adjustment, dilute 50mL of **1.0M Acetate buffer (5.12.4.2.3)** to 500mL with DI water. Adjust to pH 5 with **11.8N KOH**.
Solution is stable for six months.

5.12.5.2.5 **1.0M Acetate Buffer, pH 3.8 (100mL)**
Place ≈90mL DI water into a 250mL beaker. Add 5.7mL **glacial acetic acid** and stir well. Adjust to pH 3.8 with **11.8N KOH**. Transfer solution to a 100mL volumetric flask and QS to 100mL.
Solution is stable for six months.

5.12.5.2.6 **1.0M Acetate Buffer, pH 5.0 (500mL)**
Dissolve 42.9g **sodium acetate trihydrate** in 400mL DI water. Add 10.4mL **glacial acetic acid** and stir well. QS to 500mL. Adjust to pH 5.0±0.1 with **1.0M sodium acetate** or **1.0M acetic acid**.
Solution is stable for six months.

5.12.5.2.7 **2.0M Acetate Buffer, pH 4.8 (1000mL)**
Dissolve 141.4g **sodium acetate trihydrate** in ≈800mL DI water. Add 55.2mL **glacial acetic acid**. Adjust to pH 4.8 and QS to 1000mL.
Solution is stable for six months.

5.12.5.3 Ammonium Chloride

5.12.5.3.1 **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.
Solution is stable for 6-months.

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5.12.5.4 Ammonium Hydroxide

5.12.5.4.1 **7.4M Ammonium Hydroxide**

Place 50mL DI water into a 100mL volumetric flask. Add 50mL **concentrated ammonium hydroxide**, mix.

Make fresh.

5.12.5.5 Borate Buffer

5.12.5.5.1 **Saturated Borate Buffer , pH >9.5 (500mL)**

Place ≅250mL DI water into a 500mL volumetric flask. Stir while adding **sodium tetraborate (≅60g)** until solution is saturated. QS.

Solution is stable for six months.

5.12.5.5.2 **Borate Buffer, pH 9.2**

Place ≅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 ≅950mL with DI water. Verify pH and adjust as necessary to pH 9.2 ±0.2 with 1N NaOH/KOH or 1N HCl. Place solution in 1000mL volumetric flask and QS with DI water.

Solution is stable for six months.

5.12.5.5.3 **Borate Buffer, pH 12**

Place ≅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 ≅ 900mL with DI water. Add 25mL NaOH and stir. Verify pH and adjust as necessary to pH 12 ±0.2 with 10N NaOH/KOH or 6N HCl. Place solution in 1000mL volumetric flask and QS with DI water.

Solution is stable for six months.

5.12.5.6 β-Glucuronidase

β-Glucuronidase (Patella vulgata) lyophilized, 5,000 units/ml (10mL) {UCT}

Transfer appropriate units powder to a clean volumetric flask. QS with 1.0M Acetate buffer and mix well. Store at 2-8°C. Allow to come to room temperature prior to use. *Solution is stable for ~1-month.*

5.12.5.7 Hydrochloric Acid

5.12.5.7.1 **0.1M/100mM Hydrochloric Acid (500mL)**

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Place approximately 300mL DI water into a 500mL volumetric flask. Add 4.2mL **concentrated hydrochloric acid**, mix. QS to 500mL.

Solution may also be prepared by diluting 1N HCl.

Solution is stable for one year.

5.12.5.7.2 **1N HCl (500mL)**
Place approximately 400mL DI water into a 500mL volumetric flask. Add 42mL **concentrated hydrochloric acid**, mix. QS to 500mL.
Solution is stable for one year.

5.12.5.7.3 **1% HCl in Methanol (10mL)**
Add approximately 5mL of methanol to a 10mL volumetric flask. Pipet 100µL of **concentrated HCl**, QS and mix.
Solution is stable for one month.

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

5.12.5.8.1 **1N/1M Potassium Hydroxide (1000mL)**
Gradually add 56.0g **potassium hydroxide** to approximately 800mL DI water; stir on stir plate to dissolve. QS to 1000mL.
Solution is stable for one year.

1.0M Potassium Hydroxide (100mL)
Dissolve 5.6g **potassium hydroxide** in approximately 80mL DI water in a 100mL volumetric flask. QS to 100mL.
Solution is stable for one year.

5.12.5.8.2 **2N/2M Potassium Hydroxide (1000mL)**
Gradually add 112.0g **potassium hydroxide** to approximately 800mL DI water; stir on stir plate to dissolve. QS to 1000mL.
Solution is stable for one year.

5.12.5.8.3 **6N Potassium Hydroxide (1000mL)**
Gradually add 337g **potassium hydroxide** to approximately 600mL DI water, stir on stir plate to dissolve. QS to 1000mL.
Solution is stable for one year.

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5.12.5.8.4 **10N Potassium Hydroxide (500mL)**
Gradually (!) add 280g **potassium hydroxide** to approximately 300mL DI water in a minimum of a 800mL beaker, stir on stir plate to dissolve. Allow solution to cool (this takes awhile) and QS in a 500mL volumetric flask.
Solution is stable for one year.

5.12.5.8.5 **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.
Solution is stable for one year.

5.12.5.9 Potassium Phosphate Buffer

5.12.5.9.1 **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.**
Solution is stable indefinitely at room temperature.

5.12.5.9.2 **0.1M/100mM Potassium Phosphate Buffer (100mL) - Adjusted to pH 6**
Dissolve 1.36g **potassium phosphate monobasic** in ≈90mL DI water in a 150mL beaker. Adjust to pH 6.0 with 1.0M **potassium hydroxide.** QS in a 100mL volumetric flask.
Solution is stable for 6-months.

5.12.5.9.3 **100mM Potassium Phosphate Buffer (1000mL) Adjusted to pH 6 (Varian)**
Weigh 13.6g of potassium phosphate monobasic (KH_2PO_4) into a ≥1000mL beaker. Add ≅900mL DI water. Stir to dissolve. Adjust pH to 6.0 ±0.1 with 1M KOH while stirring. Bring up to volume with DI water in an 1000mL volumetric flask.
Solution is stable for 1 month. Store in glass container.

5.12.5.9.4 **100mM Potassium Phosphate Buffer (1000mL) Adjusted to pH 8 - 9 (Varian)**

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Weigh 13.6g of potassium phosphate monobasic (KH_2PO_4) into a ≥1000mL beaker. Add ≅900mL DI water. Stir to dissolve. Adjust pH to 8 - 9 with 10M KOH while stirring. Bring up to volume with DI water in an 1000mL volumetric flask.
Solution is stable for 1 month. Store in glass container.

5.12.5.9.5 **10mM Phosphate Buffer - pH 4**
Prior to pH adjustment, place 50.0mL of above 100mM Phosphate Buffer in a 500mL volumetric flask. Add approximately 400mL DI H_2O. Adjust pH to 4 with phosphoric acid while stirring. Bring up to volume with DI H_2O, mix.
Solution is stable for 1 month. Store in glass container.

5.12.5.9.6 **10mM Phosphate Buffer - pH 6**
Prior to pH adjustment, place 50.0mL of above (5.12.4.11.3) 100mM Phosphate Buffer in a 500mL volumetric flask. Add approximately 400mL DI H_2O. Adjust pH to 6 with 1N KOH or 1N HCL while stirring. Bring up to volume with DI H_2O, mix.
Solution is stable for 1 month. Store in glass container.

5.12.5.9.7 **10mM Phosphate Buffer - pH 8 - 9**
Prior to pH adjustment, place 50.0mL of above (5.12.4.11.3) 100mM Phosphate Buffer in a 500mL volumetric flask. Add approximately 400mL DI H_2O. Adjust pH to 8 - 9 with 10N KOH while stirring. Bring up to volume with DI H_2O, mix.
Solution is stable for 1 month. Store in glass container.

5.12.5.9.8 **1.5M Phosphate Buffer, pH 10.8 (100mL)**
Dissolve 26.1g **potassium phosphate dibasic** in approximately 90mL DI water in a 250mL beaker. Add 1.5mL of **11.8N potassium hydroxide** and stir. Transfer to a 100mL volumetric flask and QS to 100mL. Verify proper pH of 10.8.
Solution is stable for 6-months.

5.12.5.10 Sodium Acetate

5.12.5.10.1 **1.0M Sodium Acetate (100mL)**

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Dissolve 13.6g **sodium acetate** in 90mL DI water in a 100mL volumetric flask. QS to 100mL with DI water.
Solution is stable for 6-months.

5.12.5.8.2 **0.1M/100mM Sodium Acetate (100mL)**
Place 10mL **1.0 M sodium acetate** in a 100mL volumetric flask. QS to 100mL with DI water.
Solution is stable for 6-months.

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.
Solution is stable indefinitely at room temperature.

5.12.5.10 Sodium Hydroxide (NaOH)
Note: The addition of NaOH to water will generate heat, exercise due caution.

5.12.5.10.1 **0.45N NaOH (500mL)**
Gradually add 9g **NaOH** in 500mL DI water. (Caution: Exothermic)
Solution is stable for one year.

5.12.5.10.2 **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)
Solution is stable for one year.

5.12.5.10.3 **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)
Solution is stable for one year.

5.12.5.11 Sodium Phosphate

5.12.5.11.1 **100mM Sodium Phosphate Dibasic (200mL)**
Dissolve 2.84g **sodium phosphate dibasic** in ≈160mL DI water. QS to 200mL and mix.
Solution is stable for 1 month. Store in glass container.

5.12.5.11.2 **100mM Sodium Phosphate Dibasic (500mL)**

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Dissolve 7.1g **sodium phosphate dibasic** in ≈400mL DI water. QS to 500mL and mix.
Solution is stable for 1 month. Store in glass container.

5.12.5.11.3 **100mM Sodium Phosphate Monobasic (200mL)**
Dissolve 2.76g **sodium phosphate dibasic** in ≈160mL DI water. QS to 200mL and mix.
Solution is stable for 1 month. Store in glass container.

5.12.5.11.4 **100mM Sodium Phosphate Monobasic (500mL)**
Dissolve 6.9g **sodium phosphate dibasic** in ≈400mL DI water. QS to 500mL and mix.
Solution is stable for 1 month. Store in glass container.

5.12.5.12 Sodium Phosphate Buffer

5.12.5.12.1 **0.1M/100mM Sodium Phosphate Buffer (100mL) 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).
Solution is stable for 1 month. Store in glass container.

5.12.5.12.2 **0.1M/100mM Phosphate Buffer, pH 6 (250mL)**
Dissolve 0.42g **sodium phosphate dibasic** (Na_2HPO_4) and **3.03 sodium phosphate monobasic** ($NaH_2PO_4 \cdot H_20$) in approximately 200mL DI water in 250mL volumetric flask. QS to 250mL. Adjust to pH 6.0±0.1 with 100mM **monobasic sodium phosphate** (lowers pH) or 100mM **dibasic sodium phosphate** (raises pH).
Solution is stable for 1 month. Store in glass container.

5.12.5.13 Sulfuric Acid

5.12.5.13.1 **0.05M/0.1N Sulfuric Acid**

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Place approximately 800mL distilled/deionized (DI) water into a 1L volumetric flask. Add 2.7mL **concentrated sulfuric acid**, mix. QS to 1L.
Solution is stable for one year.

5.12.5.13.2 **1N Sulfuric Acid (H_2SO_4) (500mL)**
Place approximately 400mL DI water into a 500mL volumetric flask. Add 14mL **concentrated H_2SO_4.** QS to 500mL, mix.
Solution is stable for one year.

5.12.6 REFERENCES

5.12.6.1 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.

5.12.6.2 Ansys, Inc. SPEC Extraction Methods

5.12.6.3 United Chemical Technologies, Inc. Applications Manual.

5.12.6.4 Certify Methods Manual, pp. 8 - 12, Varian Sample Preparation Products, January 2001.

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Revision History

Section Five
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5.12 Solution Preparation

Revision #	Issue Date	Revisions
0	05-07-2007	Combined urine solution preparation (2.6) and blood solution preparation (3.8)

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