

SPESolid Phase
Extraction

Application Note

World Leaders in Sample Preparation

SPE180**ISOLATION OF ACIDIC BASIC AND NEUTRAL
DOPING AGENTS FROM EQUINE AND CANINE URINE****Spe-ed™ Cartridge**

Cat. No. 3721- Scan ABN 70u frit LRC, 500mg. Process with *Spe-ed Mate*.

Sample Preparation**Acid / Neutral Drugs:**

(phenylbutazone, lasix, naproxen, flunixin, theophylline, theobromine, metolozone).

Add 1mL of 0.1 N NaOH to 4mL of urine and incubate at 25°C for 10 minutes. Adjust pH to 6.0 with 0.1 M phosphate buffer.

Basic and Glucuronide Conjugated Drugs:

(clenbuterol, fentanyl, opiates, mazindol, amphetamines)

Add 3mL of 0.1M acetate buffer (pH 5.0) and 1mL of glucuronidase (7,000 units) to 5mL of urine. Incubate for 2 hours at 60°C. Adjust pH to 6.0 with 0.1 M phosphate buffer.

**Cartridge
Conditioning**

5mL methanol; 5mL water; 5mL 1 M acetic acid
DO NOT ALLOW CARTRIDGE TO RUN DRY!

Sample Addition

Add prepared urine sample (acidic/neutral and basic /glucuronide aliquots) to the cartridge. Aspirate sample slowly through the cartridge at 2mL/minute.

Cartridge Wash

5mL of water followed by 5mL of 0.1 M acetic acid.

Dry Cartridge

Air-dry cartridge by aspirating air through the sorbent bed for 10 minutes.

**Elute Acid/Neutral
Drugs**

10mLs of dichloromethane. Evaporate to dryness under N₂ at 50°C. Re-dissolve residue in 50uL of ethyl acetate*.

Elute Steroids

10mLs ethyl acetate. Evaporate to dryness under N₂ at 50°C. Re-dissolve residue in 50uL of ethyl acetate*.

Applied
Separations

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Wash Cartridge

10mLs methanol.

**Elute Basic /
Glucuronide
Conjugate Drugs**

10mLs of 2% ammonium hydroxide in dichloromethane:
isopropanol: (80:20) evaporate to dryness under N₂ at 50°C.
Reconstitute in 50uL of ethyl acetate*.

*After drying the eluate one can reconstitute the residue in any solvent appropriate to the analytical method.

Note: *Since sample matrix interferences and concentrations may vary from sample to sample, it may be necessary to adjust the wash and elution solvent/solution strength and/or volume to optimize isolation.*

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