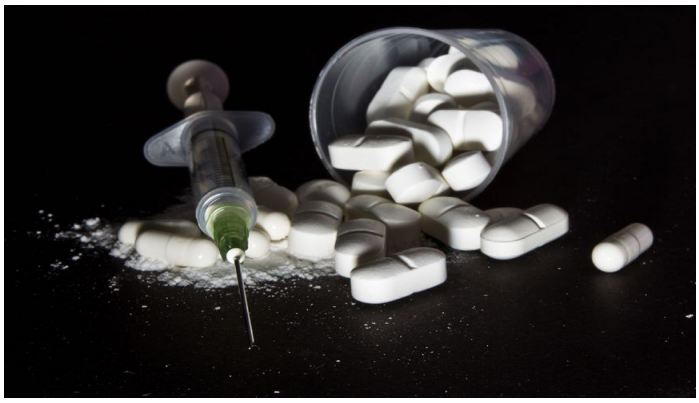
Abused Drugs In Canine Or Equine Urine Using: 500 mg XTRACKT® Extraction Column



UCT Part Numbers

XRDAH515
XtrackT DAU
500 mg 15 mL

Procedure:

1a. Prepare Sample-Enzymatic Hydrolysis of Glucuronides

a) To 5 mL of urine add internal standard(s) and 2 mL of ß-Glucuronidase 5,000 F units/mL Patella vulgata in 100 mM Acetate Buffer (pH 5.0).
b) Mix/vortex. Hydrolyze at 65 °C for 3 hours.
c) Centrifuge for 10 min. at 2000 rpm, discard pellet.

1b. Base Hydrolysis of Glucuronides

a) To 2 mL of urine add internal standard(s) and 100 µL of 10 N NaOH.
b) Mix/vortex. Hydrolyze at 60 °C for 20 minutes.
c) Centrifuge for 10 min. at 2000 rpm, discard pellet.

1c. Combine Hydrolysates

a) Combine both hydrolysis products with 5 mL of 100 mM phosphate buffer (pH 6.0).
b) Adjust sample pH = 6.0 ± 0.5 with 0.5 M Phosphoric acid.

2. Condition XtrackT® Extraction Column

a) 1 x 5 mL CH_3OH.
b) 1 x 5 mL D.I. H_2O.
c) 1 x 3 mL 100 mM phosphate buffer (pH 6.0).

Note: Aspirate at < 3 inches Hg to prevent sorbent drying.

3. Apply Sample

a) Load at 1 to 2 mL / minute.

4. Wash Column

a) 1 x 3 mL 100 mM phosphate buffer (pH 6.0).
b) 1 x 2 mL 1.0 M acetic acid.
c) Dry column (5 minutes at > 10 inches Hg).
d) 1 x 2 mL hexane.

5. Elute Acidic and Neutral Drugs

a) 1 x 4 mL methylene chloride; collect eluate at < 5 mL / minute.

6. Elute Steroids

a) 2 x 4 mL ethyl acetate; collect eluate at < 5 mL / minute.

7. Wash Column

a) 1 x 5 mL CH_3OH; aspirate.

8. Elute Basic Drugs

a) 1 x 5 mL methylene chloride / isopropanol / ammonium hydroxide (78:20:2).

9. Dry Eluate

a) Evaporate to dryness at < 40 °C.
b) Reconstitute with 100 µL ethyl acetate.

10. Quantitate

a) Spot onto TLC plate or inject 1 to 2 µL onto chromatograph

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