GLYCOPYRROLATE (ROBINUL) FROM EQUINE URINE BY LC-MSMS USING: 500 mg CLEAN UP® CCX2 EXTRACTION COLUMN

Part #: CUCCX25Z

1. **SAMPLE PREPARATION**

   Buffer 5 mL of urine to pH 7.0 by adding 3 mL of 100 mM phosphate buffer (pH 7.0).

   Add (12.5 ng) of mepenzolate (internal standard).

   Add 5 mL of water to the sample.

   Vortex or shake thoroughly.

   Centrifuge for 5 min at 800 rpm.

2. **CONDITION CLEAN UP® EXTRACTION COLUMN**

   1 x 3 mL CH_3OH.

   1 x 3 mL D.I. H_2O.

   1 x 1 mL 100 mM phosphate buffer (pH 7.0).

3. **APPLY SAMPLE**

   Decant supernatant onto SPE column.

   Load at 1 to 2 mL / min.

4. **WASH COLUMN**

   5 mL of CH_3OH.

   5 mL of D.I. H_2O.

   Dry column (5 min > 10 inches Hg).

5. **ELUTE GLYCOPYRROLATE**

   1 x 4 mL CH_3OH/0.5 M NH_4OAC buffer, pH 3.0 (95:5).

6. **DRY ELUTE**

   Evaporate to dryness at 60°C.

   Reconstitute with 100 µL CH_3OH.

7. **QUANTITATE**

   Inject 10 µL onto HPLC.

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