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HYDROLYSIS OF SPECIMENS FOR SOLID PHASE EXTRACTION

PRINCIPLE

Many drugs are metabolized by the process of glucuronidation. Glucuronides do not extract well in most common analytical procedures. This procedure is designed to hydrolyze the bond between a drug and glucuronic acid resulting in an increase in the amount of unconjugated (free) drug available for extraction from biological specimens. A single point calibrator at a concentration of 1000 ng/mL, a control at 200 ng/mL, a matrix blank and a hydrolysis control consisting of morphine glucuronide spiked into negative matrix to a concentration of 80 ng/mL, is run with each batch. Refer to the Opiate-BE procedure for preparation of additional solutions and standards as required.

SAFETY

The handling of all reagents, samples and equipment is performed within the guidelines which are detailed in the safety manual.

REAGENTS AND MATERIALS

1. **Deionized water**
2. **HCl, concentrated, 12.1 N** Fisher Scientific or equivalent
3. **100 mM phosphate buffer (pH 6.0)**

   Dissolve 3.4 g Na_2HPO_4 and 24.2 g NaH_2P0_4 H_2O in 1600 mL DI H_2O.

   Dilute to 2000 mL using DI H_2O. Mix. Adjust pH to 6.0 + 0.1 with 100 mM monobasic sodium phosphate (lowers pH) or 100 mM dibasic sodium phosphate (raises pH).

   Store at 5 ºC in glass.

   Stability: 1 month. Inspect each day before use for contamination.

4. **6N HCl**

   Add concentrated HCl to water (1:1). Mix thoroughly.

   CAUTION: Prepare this solution in a fume hood. Use appropriate safety equipment.

5. **Hydrolysis control solution**

Morphine glucuronide (10 mg/L)

Pipet 1 mL of 1.0 mg/mL morphine glucuronide into a 100 mL volumetric flask. Q.S. to 100 mL with methanol. Transfer to properly labeled container.

PROCEDURE

1. In properly labeled 16 x 150 screw-cap centrifuge tubes, place 1 mL samples, calibrator, and controls as described above, fortified with internal standard. Add a hydrolysis control by pipetting 1.0 ml of blank matrix to a properly labeled tube and fortifying with 40 µL of 10 mg/L morphine glucuronide control solution and the appropriate amount of internal standard solution.
2. Add 0.5 mL 6N HCl to all tubes. Vortex to mix Place the tube in a boiling water bath for 45 minutes. Remove the tube and allow it to cool to room temperature.
3. Add 2.0 mL deionized H_2O and 2.0 mL 100 mM phosphate buffer pH 6.0, cap, and mix by Vortex for 30 seconds. Centrifuge for 15 minutes at ≈ 3000 RPM
4. Filter sample through Whatman filter paper into a labeled 25 mL beaker.
5. Decant the supernatant into appropriately labeled Polychrom Clin II column and apply nitrogen at a pressure of 2-4 psi.
6. Proceed with analysis as in Opiate-BE procedure.