



SPE Application Note for Acidic, Neutral and Basic Drugs from Urine

The following method addresses the extraction of all three classes of drugs of abuse from urine using non-polar and cation exchange retention mechanisms on the ISOLUTE Confirm HCX mixed mode column. This protocol was optimized to eliminate the use of dichloromethane in the elution of the acidic / neutral drug fraction.

EXTRACTION PROCEDURE

ISOLUTE® SPE Column: ISOLUTE Confirm HCX 130 mg/10 mL, Part #902-0013-H

Pre-treatment: To urine (5 mL) in a clean dry test tube, add 0.1M phosphate buffer, pH 6.0 (2 mL). Vortex the sample, and check that the pH is 5.0-7.0. Adjust if necessary.

Solvation: Solvate the column with methanol (2 mL).

Equilibration: Rinse the column with deionized water (2 mL) followed by 0.1M phosphate buffer, pH 6.0 (3 mL).

Sample application: Apply the sample to the column at a flow rate of 1-2 mL/min

Interference elution: Elute interferences with 0.1M phosphate buffer, pH 6.0 (1 mL) and dry the column under full vacuum for 5 mins. Add 1.0 M acetic acid (1 mL) and dry the column for a further 5 mins under full vacuum. Add hexane (1 mL).

Analyte elution: Acidic/neutral drug fraction. Elute the neutral and acidic drugs with ethyl acetate:hexane (25:75, v/v, 3 mL). Dry the column for 2 mins under full vacuum.

Interference elution. Elute interferences with methanol (6 mL) and dry the column under full vacuum for 2 mins.

Basic drug elution. Elute the basic drugs with ethyl acetate: ammonium hydroxide (98:2, v/v, 3 mL).

Structure Various.

Structural considerations N/A



Matrix considerations

The matrix is aqueous, with relatively high ionic strength. There are many possible interference compounds, so this method uses a mixed retention mechanism, to allow extensive clean-up steps.

Analytical method

Analyte dependant.

Reagents

General comments

1. Reagents
 - a) 1.0 M potassium hydroxide. Weigh potassium hydroxide (28 g) into a 500 mL volumetric flask. Add deionized water (400 mL), dissolve the potassium hydroxide, and make up to the mark with deionized water.
 - b) 0.1M phosphate buffer, pH 6.0. Weigh potassium hydrogen orthophosphate (13.61 g) into a one litre volumetric flask. Add deionized water (900 mL) and dissolve the potassium dihydrogen orthophosphate. Adjust the pH to 6.0 (+/- 0.1) with 1.0M potassium hydroxide, and make up to the mark with deionized water.
 - c) 1.0M acetic acid. Add glacial acetic acid (5.75 mL) to a 100 mL volumetric flask. Add deionized water (70 mL), mix thoroughly, and make up to the mark with deionized water.
 - d) 25:75 (v/v) ethyl acetate:hexane. Add ethyl acetate (25 mL) and hexane (75 mL) to a reagent bottle and mix thoroughly.
 - e) 98:2 (v/v) ethyl acetate:ammonium hydroxide. Add concentrated ammonium hydroxide (2 mL) to a 100 mL volumetric flask. Add ethyl acetate (80 mL). Mix thoroughly, and make up to the mark with ethyl acetate.
2. For reproducible and high recoveries, it is important to control the flow to 1-2 mL/min
3. The drying steps employed during the interference elution stage are important in order to maximize recoveries.
4. The ammonium hydroxide/ethyl acetate solution should be made up daily to obtain the required recoveries.
5. Previous # IST2006



ISOLUTE column part numbers represent the product configuration of choice for use with a vacuum sample processing station. For 96-well and alternative column configurations compatible with any SPE automation system, please contact Biotage.

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