Extraction of Acidic, Neutral and Basic Drugs from Urine using ISOLUTE® HCX Columns

Application Note

Introduction

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 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® HCX 130 mg/10 mL, Part number 902-0013-H

Matrix: 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.

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.

Condition: Condition 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 loading: 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).

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.

Aditional Information:

1. For reproducible and high recoveries, it is important to control the flow to 1–2 mL/min
2. The drying steps employed during the interference elution stage are important in order to maximize recoveries.
3. The ammonium hydroxide/ethyl acetate solution shoild be made up daily to obtain the required recoveries.
4. Previous # IST2006

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