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General Approach to the Extraction of Basic Drugs from Biological Fluids Using ISOLUTE® HCX Mixed-Mode SPE Columns

The extraction of drugs from biological fluids using a purely non-polar retention mechanism can lead to extracts that contain a large amount of non-polar co-extracted material, which can interfere with the subsequent analysis. Many drugs with a generally non-polar structure also contain a basic group such as a primary or secondary amine, and this is utilized in this approach for the extraction of basic drugs using ISOLUTE® HCX SPE columns.

ISOLUTE HCX columns are based on cation exchange and C8 mixed mode chemistries. Basic drugs are therefore retained by two primary retention mechanisms – ionic and non-polar (see Figure 1). This allows a more rigorous interference elution regime to be used, leading to a very clean final extract, as many non-polar interferences which are retained by a non-polar interaction alone, can be eluted selectively, prior to elution of the drug (see Figure 2).

This procedure utilizes the ISOLUTE HCX 130 mg/10 mL configuration, optimized for the extraction of basic drugs from 5 mL of urine, and ideal for applications with GC-MS end points.

Extraction Procedure

Format

ISOLUTE® HCX 130 mg/10 mL (XL),
part number 902-0013-H

Sample Pre-Treatment

Dilute the urine sample (5 mL) 1:1 (v/v) with 0.05–0.1 M phosphate buffer, pH 6.0. Mix thoroughly.

Column Conditioning and Equilibration

Condition the column with methanol (1 mL) followed by 0.05–0.1 M phosphate buffer, pH 6.0 (1 mL).

Sample Application

Apply the sample at a flow rate of 1–2 mL/min.

Interference Elution

Rinse the column with 0.05–0.1 M phosphate buffer, pH 6.0 (2 mL), followed by 1 M acetic acid (1 mL). This will ensure ionization of the basic drug during the following rinse step.

Dry the column for 10 minutes by vacuum aspiration or positive pressure. Rinse the column again with methanol (1 mL). Interferences that are not retained by ionic interactions will be eluted with the methanol, leading to a cleaner final extract.

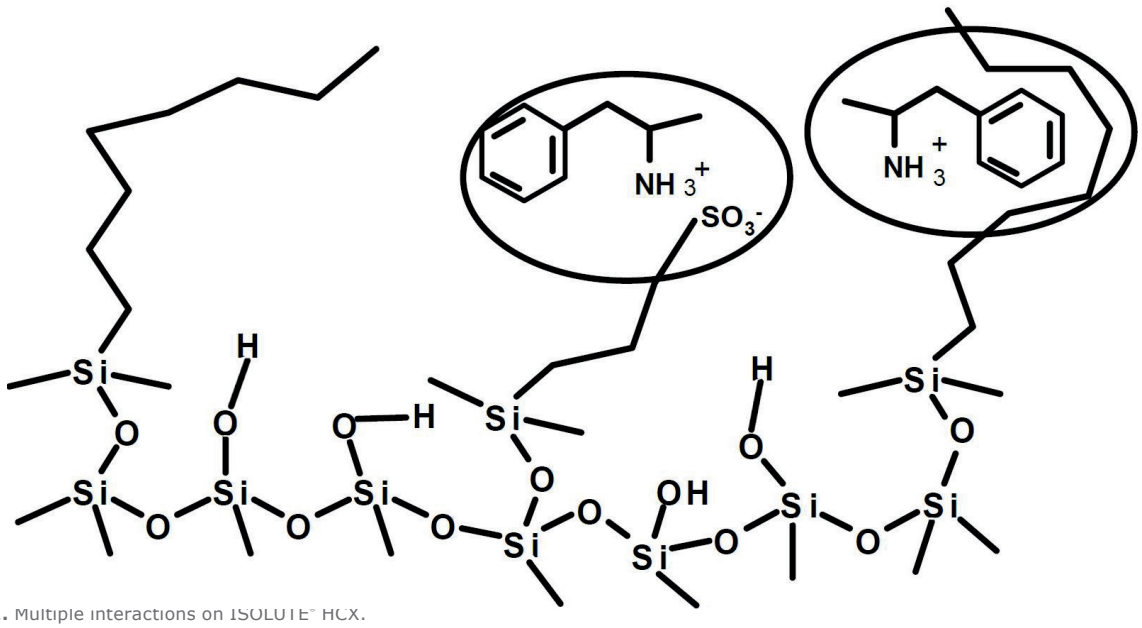

Figure 1. Multiple interactions on ISOLUTE® HCX.

Biotage®

Alalyte Elution

Elute analytes with ethyl acetate containing 2–5% ammonia (sg 0.88, v/v, 1 mL). This will suppress ionization of the basic drug,breaking both the ionic and non-polar retention mechanisms, and allowing elution of the analytes.

If the final analysis technique is GC, evaporate the elution solvent to dryness and derivatize the analyte(s) using a suitable derivatization agent.

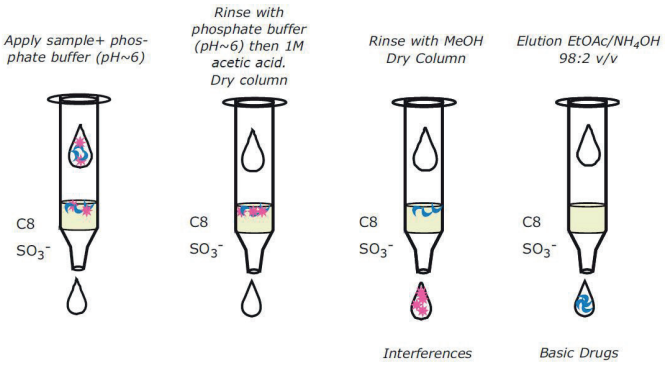

Figure 2. The rigorous washing procedure possible with ISOLUTE® HCX.

Ordering Information

Part Number	Description	Quantity
902-0013-H	ISOLUTE® HCX 130 mg/10 mL columns	50

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