

General Approach to the Extraction of Basic Drugs from Biological Fluids using ISOLUTE® Confirm 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 the ISOLUTE approach for the extraction of basic drugs using ISOLUTE Confirm HCX SPE columns.

ISOLUTE Confirm 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**).

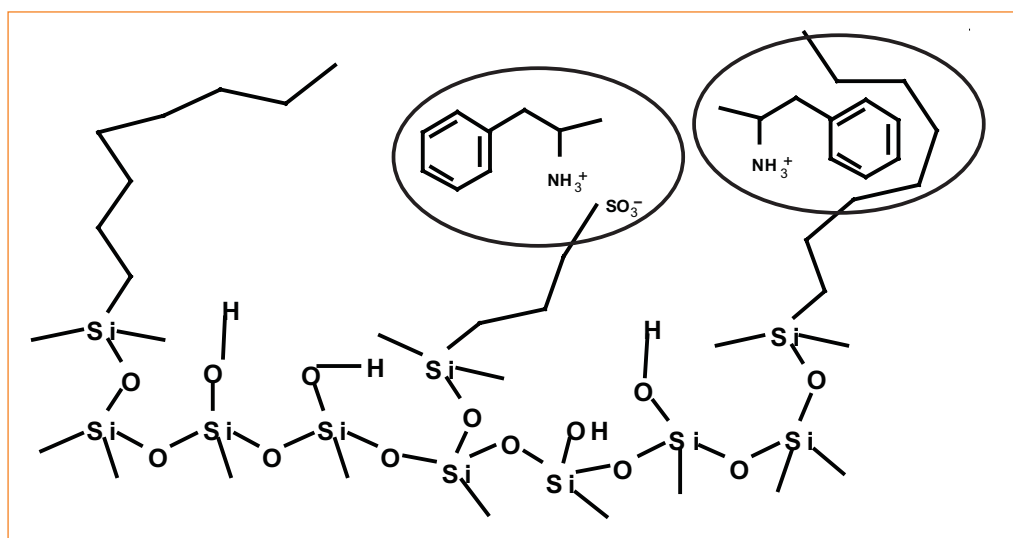


Figure 1. Multiple interactions on ISOLUTE Confirm HCX

Extraction Procedure

This procedure utilizes the standard ISOLUTE Confirm HCX 130 mg/10 mL configuration, optimized for the extraction of basic drugs from 5 mL of urine. Solvent volumes should be scaled up or down as necessary for other column configurations.

Sample Pre-treatment

Dilute the biological fluid sample 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. 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.

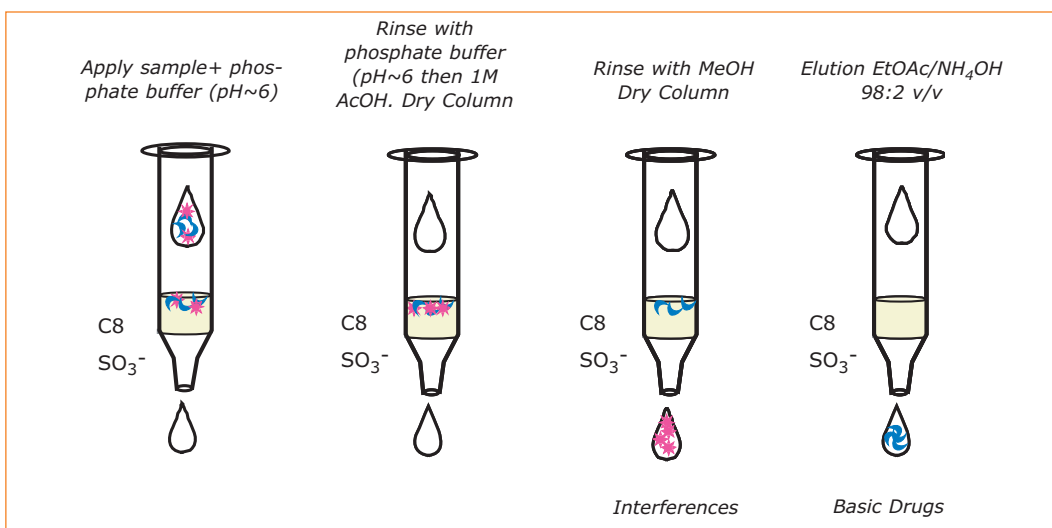


Figure 2. The rigorous washing procedure possible with ISOLUTE Confirm HCX

Analyte 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.

Specific protocols are available from Biotage for the extraction of the most common drugs of abuse using ISOLUTE Confirm HCX, including codeine and morphine, cocaine and benzoylecgonine, phencyclidine, amphetamine and methamphetamine and barbiturates.

ISOLUTE Confirm HCX Product Ordering Information

Part Number	Description	Quantity
902-0002-A	25 mg/1 mL	100
902-0005-A	50 mg/1 mL	100
902-0010-A	100 mg/1 mL	100
902-0013-A	130 mg/1 mL	100
902-0013-B	130 mg/3 mL	50
902-0013-C	130 mg/6 mL	30
902-0013-H	130 mg/10 mL	50
902-0020-B	200 mg/3 mL	50
902-0020-H	200 mg/10 mL	50
902-0030-B	300 mg/3 mL	50
902-0030-C	300 mg/6 mL	30
902-0030-H	300 mg/10 mL	50

Also available in 96-well plate format. See the latest Biotage Analytical Sample Preparation Catalog for configurations and ordering information.



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