Sample Preparation by Mixed-Mode SPE Using ISOLUTE® HCX

This technical note describes the extraction of basic drugs from biological fluids using ISOLUTE® HCX mixed-mode SPE products.

Sample preparation techniques such as protein precipitation, supported liquid extraction or non-polar SPE may not be selective enough to give extracts of sufficient purity for low level analysis. In these cases, the selective mixed-mode approach to the extraction of basic drugs is a suitable alternative, giving very high purity extracts with minimal levels of co-extracted material.

The ISOLUTE® HCX series of mixed-mode SPE sorbents (ISOLUTE HCX, HCX-3 and HCX-5) are based on a combination of strong cation exchange and non-polar (C8, C18 and C4 respectively) chemistries. Basic drugs are retained by two primary retention mechanisms (see Figure 1). This allows a rigorous interference elution regime to be used to elute interferences retained by either non-polar or cation exchange interactions alone. Only analytes with both non-polar and basic characteristics are extracted using the ISOLUTE HCX series of sorbents, providing an extremely pure final extract.

The mixed-mode approach for extraction of ionizable drugs from biological fluids is extremely robust. The initial retention mechanism for the analytes is non-polar (hydrophobic), and is unaffected by the high or variable ionic strength of the matrix. The initial hydrophobic interaction is a function of the sorbent chain length, with shorter chains (e.g. C4) being less retentive than longer chains (e.g. C18). If retention of non-ionizable compounds is minimized, a cleaner extract will result.

For further information on the use of ISOLUTE HCX-3 and HCX-5, please see Technical Note TN113.V.1.

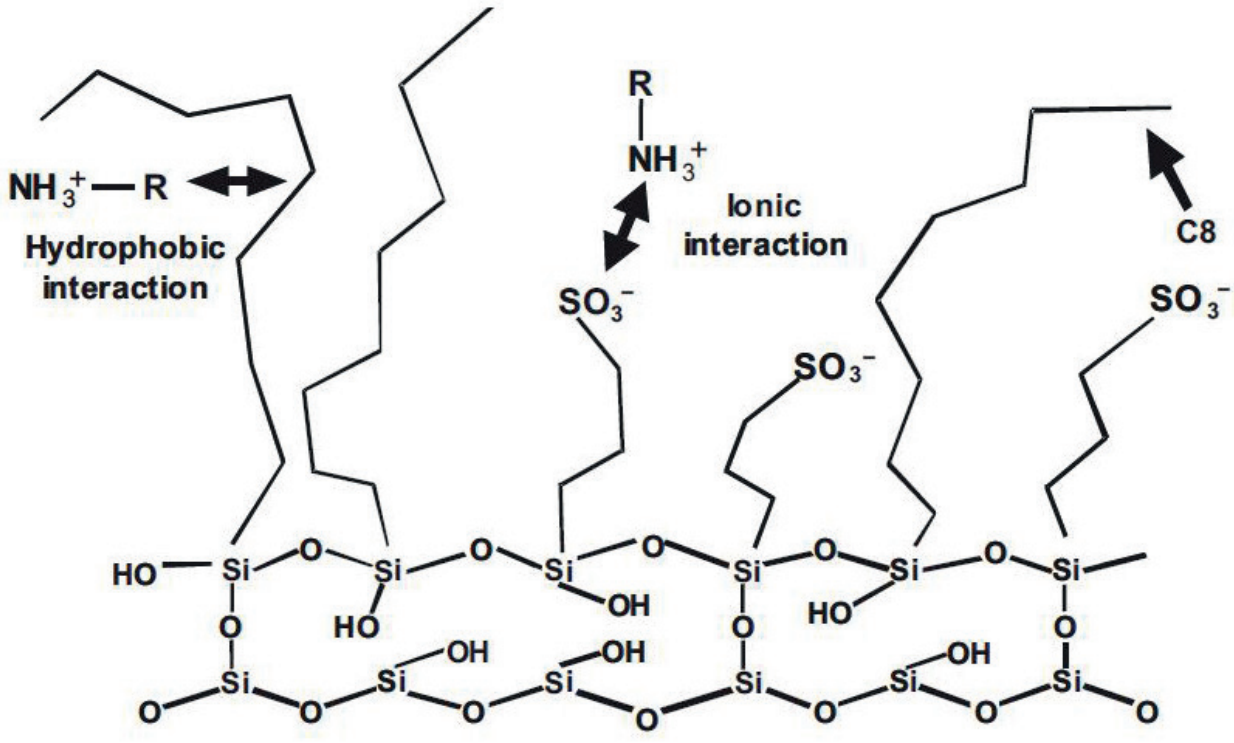

Figure 1. Multiple interactions on ISOLUTE® HCX mixed-mode columns.

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Extraction Protocol

Evaluate ISOLUTE® HCX, 25 mg/1 mL columns (p/n 902-0002-A), tabless columns (p/n 902-0002-AG) or 25 mg plates (p/n 902-0025-P01) using the procedure detailed below. Process using a VacMaster®-10 or -20 vacuum manifold, VacMaster-96 vacuum manifold, Biotage® PRESSURE+96 Positive pressure manifold, or automated liquid handling system (such as Biotage® Extrahera™).

Vacuum Settings

At all stages, use a short pulse (approx. 1 second) of low vacuum (‹ -5" Hg) or pressure (1–3 psi), unless otherwise stated.

Sample Volume

This procedure is optimized for a biological fluid sample volume of 100 µL. Sample should be diluted 1:1 (v/v) with appropriate buffer before applying to the column (total volume of buffered sample applied is 200 µL).

Note: Work in our R&D laboratory has shown that 25 mg ISOLUTE SPE columns and plates have sufficient capacity for extraction of up to 1 mL plasma sample without analyte breakthrough. Test conditions: 1 mL plasma spiked at 0.1 mg/mL analyte concentration and diluted 1:1 with buffer before applying to the column (total volume of buffered sample applied is 2 mL).

Sample Pre-treatment

Dilute the sample (100 µL of plasma or urine) with ammonium acetate buffer (0.05 M, pH 6.0, 100 µL) to give a 200 µL total sample volume at 1:1 dilution. Mix thoroughly.

Column Conditioning

Condition each well with methanol (1 mL). Use gravity or a short pulse of vacuum or pressure to initiate flow. This will ensure efficient wetting of the hydrophobic frits, promoting even flow of sample through the wells.

Column Equilibration

Rinse wells with ammonium acetate buffer (0.05 M, pH 6.0, 250 µL). Load all wells prior to applying a short pulse of vacuum or pressure to initiate flow.

Sample Loading

Apply 200 µL buffered sample. Load all wells prior to applying a short pulse of vacuum or pressure to initiate flow.

Interference Elution

Elute acidic and neutral interferences with:

- Ammonium acetate buffer (0.05 M, pH 6.0, 250 µL)
- Acetic acid (1 M, 250 µL)
- Apply vacuum or pressure for 30 seconds to dry sorbent bed
- Methanol (250 µL)

For each solvent, load all wells and allow to soak for 1 minute prior to applying a short pulse of vacuum or pressure.

Analyte Elution

For 96-well plates: Place collection plate in base of manifold. Ensure correct alignment (position A1 of collection plate directly underneath position A1 of extraction plate), and that extraction plate outlet Luer tips extend into the top of the collection plate. This will prevent sample cross contamination. Spacers are available to ensure optimum penetration. For columns: Ensure collection vessels are in place.

Elute basic analytes with methanol/NH_4OH (95:5, v/v, 2 x 100 µL). This will suppress ionization of the drug, overcoming both cationic and non-polar retention mechanisms, allowing elution of the analytes.

Apply the first 100 µL aliquot and allow to soak for 2–4 mins. If the aliquot has not reached the top frit at the end of the soak time, apply a short vacuum or pressure pulse.

Apply the second 100 µL aliquot and allow to soak for a further 2–4 mins. Apply low vacuum or pressure for 1 minute to complete elution.

Evaporate this elution solvent and re-constitute the sample in a solvent compatible with the analytical technique. For LC-MS the mobile phase is suggested.

Care should be taken to avoid losses of thermally labile or volatile analytes at this stage.

Reagents

1. Methanol
2. 0.05 M ammonium acetate buffer pH 6 Ammonium acetate 97+% reagent, FW 77.08. Dissolve 3.854 g in 1 L of water and adjust pH using 1 M acetic acid (0.9635 g in 250 mL of water).
3. 1 M acetic acid Acetic acid glacial 99.99+%, FW 60.05. Add 6 mL of acid to 50 mL of HPLC grade water in a 100 mL volumetric flask, make up to volume with water.
4. Methanol/ammonium hydroxide (95:5, v/v) Add 5 mL of ammonium hydroxide, FW 35.05, to 50 mL of HPLC grade methanol in 100 mL volumetric flask, make up to volume with methanol.

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Ordering Information

Part Number	Description	Quantity
ISOLUTE®-96 Format		
902-0025-P01	ISOLUTE®-96 HCX 25 mg plate	1
ISOLUTE® Column Format		
902-0002-A	ISOLUTE® HCX 25 mg/1 mL	100
Tabless ISOLUTE® Column Format		
902-0002-AG	ISOLUTE® HCX 25 mg/1 mL (tabless)	100

Other configurations are available, please contact Biotage for details.

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