Sample Preparation by Mixed-Mode SPE Using ISOLUTE® HAX

This technical note describes the extraction of acidic drugs from biological fluids using ISOLUTE® HAX 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 acidic drugs is a suitable alternative, giving very high purity extracts with minimal levels of co-extracted material.

ISOLUTE HAX mixed-mode SPE sorbent is based on a combination of strong anion exchange and non-polar chemistries. Acidic 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 anion exchange interactions alone. Only analytes with both non-polar and acidic characteristics are extracted using the ISOLUTE HAX sorbent, 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. Loading sample at acidic pH will therefore minimize retention of small organic acid endogenous compounds, as they will not retain by hydrophobic interaction alone. This ensures additional extract cleanliness, particularly when extracting urine samples.

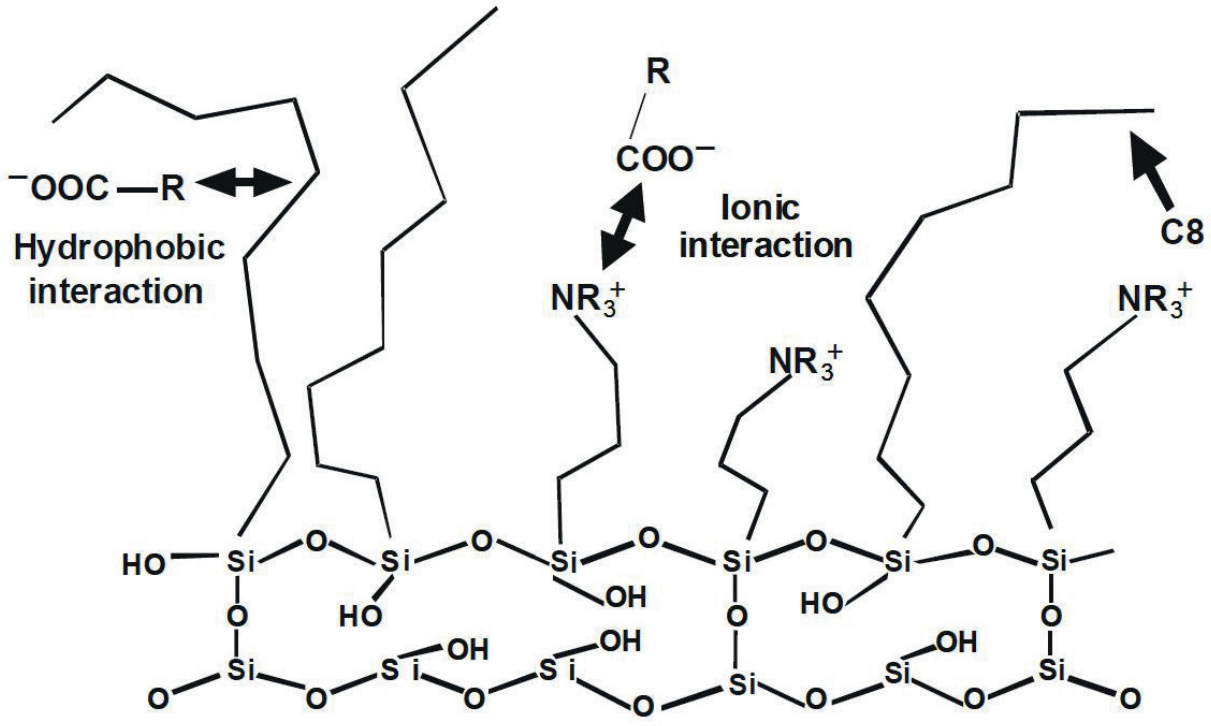

Figure 1. Multiple interactions on ISOLUTE® HAX mixed-mode columns.

Biotage®

Extraction Protocol

Evaluate ISOLUTE HAX, 25 mg/1 mL columns (p/n 903-0002-A), tabless columns (p/n 903-0002-AG) or 25 mg plates (p/n 903-0025-P01) using the procedure detailed below. Process using a Biotage® VacMaster™-10 or 20, vacuum manifold, Bioage® VacMaster™96 vacuum manifold (96-well plates); Biotage® PRESSURE+96 positive pressure manifold or automated liquid handling system such as Biotage® Extrahera™.

Vacuum Settings

At all stages, use a short pulse (approximately 1 second) of low vacuum (‹ -5" Hg), or positive 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 ISOLUTE 25 mg SPE columns have sufficient capacity for extraction of up to 1 mL plasma sample without analyte breakthrough. Test conditions: 1 mL plasma spiked at 0.1 µg/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 formic acid (2%, pH 2, 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 formic acid (2%, pH 2, 250 µL). Load all wells prior to applying a short pulse of vacuum or pressure to initiate flow. Sample loading Apply 200 µL acidified sample. Load all wells prior to applying a short pulse of vacuum or pressure to initiate flow.

Sample Loading

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

Interference Elution

Elute basic and neutral interferences with:

- Ammonium acetate buffer (0.1M, pH 7, 250 µL)
- Apply vacuum or pressure for 30 seconds to dry sorbent bed
- Methanol / water (50/50, v/v, 250 µL)*
- Apply vacuum or pressure for 30 seconds to dry sorbent bed

*Evaluate increasing the % methanol at this stage to improve extract cleanliness. Check for analyte breakthrough.

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: Ensure correct alignment (position A1 of collection plate directly underneath position A1 of extraction plate), and that extraction plate outlet Luer tips extend below the rim of the collection plate. This will prevent sample cross contamination. Spacers are available to ensure optimum penetration. For columns: Ensure collection vessels are in position.

Elute acidic analytes with methanol/acetic acid (98:2, v/v, 2 x 100 µL). This will suppress ionization of the drug, breaking both anionic 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. 2% Formic acid, pH 2. Add 2.083 mL formic acid (96%) to 100 mL volumetric flask, and make up to volume with HPLC grade water.
3. 0.1 M Ammonium acetate pH 7. Ammonium acetate 97+% reagent, FW 77.08. Dissolve 7.708 g in 1 L of HPLC grade water.
4. Methanol / water (50:50, v/v) Add 50 mL methanol to 100 mL measuring cylinder, make up to volume with HPLC grade water.
5. Methanol / acetic acid (98:2, v/v) Add 2 mL acetic acid, glacial 99.99+% to 50 mL of methanol in 100 mL volumetric flask, make up to volume with methanol.

Biotage®

Ordering Information

Part Number	Description	Quantity
ISOLUTE®-96 Format		
903-0025-P01	ISOLUTE®-96 HAX 25 mg plate	1
ISOLUTE® Column Format		
903-0002-A	ISOLUTE® HAX 25 mg/1 mL	100
Tabless ISOLUTE® Column Format		
903-0002-AG	ISOLUTE® HAX 25 mg/1 mL (tabless)	100

Other configurations are available, please contact Biotage for details.

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 68162810
Fax: +86 21 68162829
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA
Tel: +82 31 706 8500
Fax: +82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

INDIA
Tel: +91 11 45653772
india@biotage.com

Distributors in other regions are listed on www.biotage.com

Literature Number: TN127.V.1

Biotage®