Technical Note 127

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 sorbents.

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.

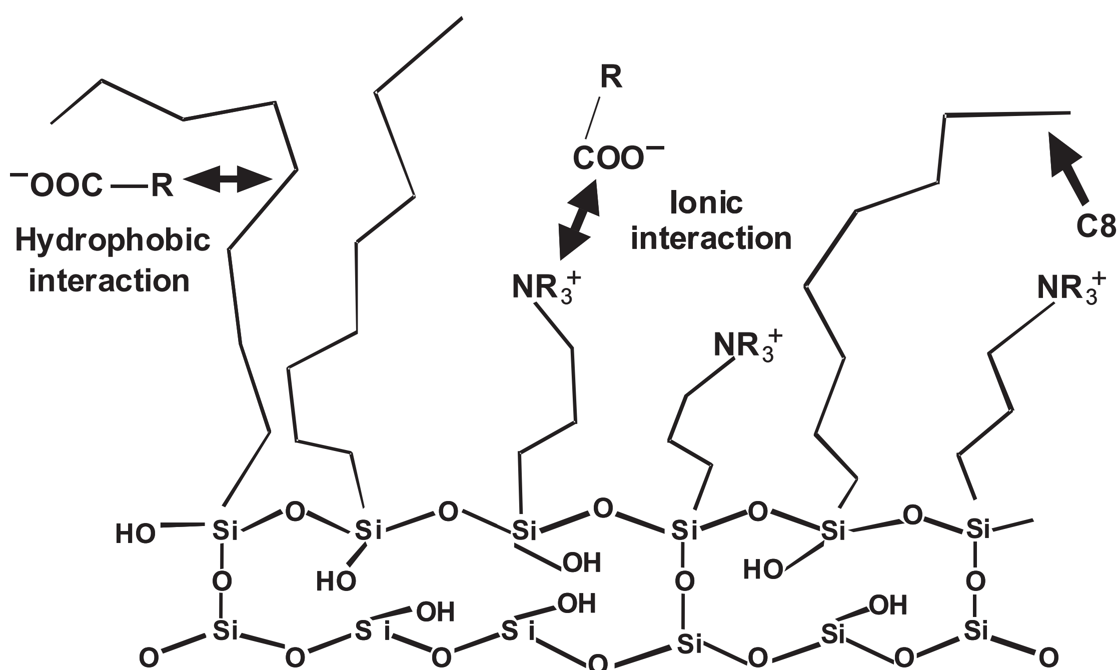

Figure 1. Multiple interactions on ISOLUTE HAX mixed-mode columns

Biotage

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.

EXTRACTION PROTOCOL

Evaluate ISOLUTE HAX, 25 mg/1 mL in the ISOLUTE Array® format using the procedure detailed below. Populate the plate with individual wells as required. Process using a VacMaster®-96 sample processing station or automated liquid handling system.

Vacuum settings

At all stages, use a short pulse (approximately 1 second) of low vacuum (< -5" Hg), 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

Place extraction plate on vacuum manifold. No collection plate should be used at this stage.

Condition each well with methanol (1 mL). Use gravity or a short pulse of vacuum 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 to initiate flow.

Sample loading

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

Interference elution

Elute basic and neutral interferences with:

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

Analyte elution

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 below the rim of the collection plate. This will prevent sample cross contamination. Spacers are available to ensure optimum penetration.

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 pulse.

Apply the second 100 µL aliquot and allow to soak for a further 2–4 mins. Apply low vacuum 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.

ORDERING INFORMATION

Description	Pack size	Part Number
ISOLUTE Array format		
ISOLUTE Array HAX 25 mg/1 mL wells*	100	903-0025-R
ISOLUTE Array HAX 25 mg/1 mL plate	1	903-0025-RP

* As with other Array products, loose wells can be processed on a standard VacMaster-10 or -20 Sample Processing Station equipped with Array Luer adaptors (p/n 120-1201). In order to process loose wells using a VacMaster-96 Sample Processing Station, a base plate (part number 120-1000-P01) and base plate sealing strips (part number 120-1200 for sealing unused positions) are required.

Description	Pack size	Part Number
ISOLUTE-96 format		
ISOLUTE-96 HAX 25 mg plate	1	903-0025-P01
ISOLUTE column format		
ISOLUTE HAX 25 mg/1 mL	100	903-0002-A
Tab-less ISOLUTE column format		
ISOLUTE HAX 25 mg/1 mL (tab-less)	100	903-0002-AG

Other configurations are available, please contact Biotage for details.

VacMaster-96 Sample Processing Station

Description	Pack size	Part Number
VacMaster-96 manifold only*	1	121-9600
VacMaster-96 Vacuum Control Unit	1	121-9601
VacMaster-96 Vacuum Control Unit with integral vacuum source	1	121-9602
VacMaster-96 with Vacuum Control Unit (121-9601)	1	121-9603
VacMaster-96 with Vacuum Control Unit (121-9602)	1	121-9604

* Option does not include a vacuum control unit.

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