Generic Method for the Extraction of Basic Drugs from Biological Fluids using ISOLUTE® Mixed-mode SPE Columns and 96-well SPE Plates

Chemistry Data Sheet

The extraction of basic drugs from biological fluids using a purely non-polar retention mechanism (e.g. C4, C8 or C18) can lead to extracts that contain a large amount of non-polar co-extracted material that can interfere with subsequent analysis. Conversely, extraction mechanisms based on ion exchange interactions can be non-robust due to the variable ionic strength of the sample matrix.

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 generic approach to the extraction of basic drugs using ISOLUTE® mixed-mode SPE columns.

The ISOLUTE family of mixed-mode SPE columns (HCX, HCX-3 and HCX-5) are based on a combination of strong cation exchange and non-polar (C8, C4 and C18 respectively) chemistries. Basic drugs are therefore 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 cationic characteristics are retained on the column to be eluted in an extremely pure final extract.

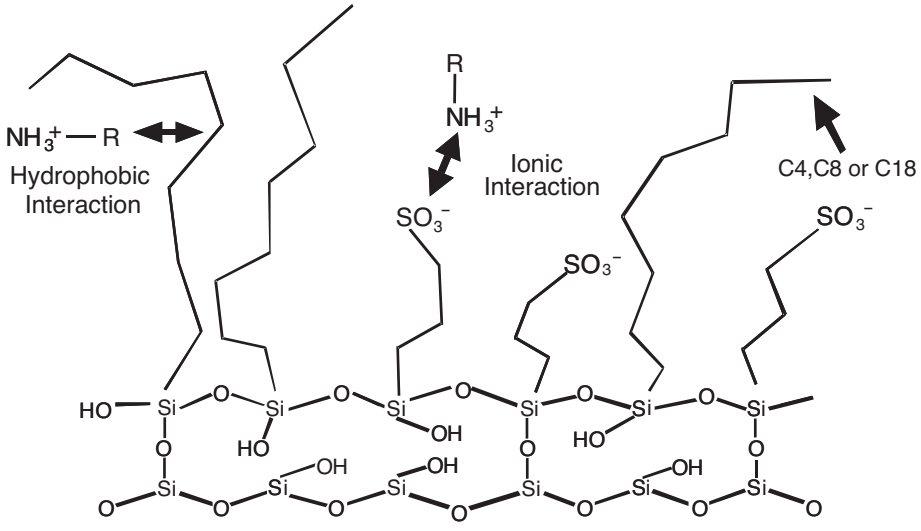

Figure 1. *Multiple interactions on ISOLUTE HCX, HCX-3 and HCX-5 mixed-mode SPE columns*

The mixed-mode approach for extraction of ionizable drugs from biological fluids is very robust. The initial retention mechanism for the analytes is non-polar (hydrophobic), and this interaction is unaffected by the high or variable ionic strength of the matrix. The initial hydrophobic interaction is a function of the 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 (**see Figure 2**).

Biotage

Generic Method for the Extraction of Basic Drugs from Biological Fluids

Column: Screen ISOLUTE HCX, HCX-3 and HCX-5, 100 mg/1 mL using the procedure detailed below. Choose the mixed-mode SPE column that combines high recoveries with the cleanest extract.

For high throughput applications, the ISOLUTE family of mixed-mode sorbents is available in both the ISOLUTE-96 Fixed Well Plate and modular ISOLUTE Array 96-well Plate. When using the 96-well plate format, it is recommended that 25 mg bed masses are used, scaling the sample and solvent volumes in the procedure from 1 mL to 250 µL.

1. Sample pre-treatment: Dilute the sample (1 mL of plasma or urine) with ammonium acetate buffer (0.05 M, pH 6.0, 1 mL). Mix thoroughly
2. Column conditioning: Condition column with methanol (1 mL) at a flow rate of 1 mL/min
3. Column equilibration: Rinse column with ammonium acetate buffer (0.05 M, pH 6.0, 1 mL) at a flow rate of 1 mL/min
4. Sample application: Apply 1 mL buffered sample at a flow rate of 1 mL/min
5. Interference elution: Elute acidic and neutral interferences with
   a) Ammonium acetate buffer (0.05 M, pH 6.0, 1 mL)
   b) Acetic acid (1 M, 1 mL)
   c)) Dry column for 30 seconds
   d) Methanol (1 mL)
6. Analyte elution: Elute basic analytes with methanol/NH_4OH (95:5, v/v 1 mL) This will suppress ionization of the drug, overcoming both cationic and non-polar retention mechanisms, allowing elution of the analytes.

This generic SPE method has been successfully applied to the extraction of a wide range of basic drug classes, including beta-blockers, tricyclic antidepressants, anti-psychotics and anti-ulcer drugs.

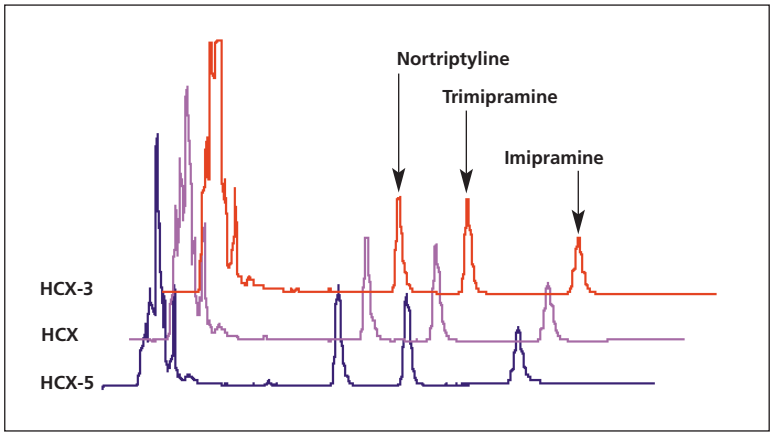

Figure 2. Extraction of tricyclic antidepressants from plasma using ISOLUTE mixed-mode sorbents. In general, the least hydrophobic sorbent (HCX-5) gives the cleanest extract.

ISOLUTE Mixed-mode SPE Columns Product Ordering Information

Part Number			Description	Quantity
HCX	HCX-3	HCX-5		
902-0002-A	905-0002-A	906-0002-A	25 mg/1 mL	100
902-0005-A	905-0005-A	906-0005-A	50 mg/1 mL	100
902-0010-A	905-0010-A	906-0010-A	100 mg/1mL	100
902-0010-B	-	-	100 mg/1mL	50
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

ISOLUTE Mixed-mode High Throughput Product Ordering Information

ISOLUTE Array wells				
	10 mg/1 mL	25 mg/1 mL	50 mg/1 mL	100 mg/1 ml
HCX	-	902-0025-R	902-0050-R	902-0100-T
HCX-3	-	905-0025-R	-	905-0100-T
HCX-5	906-0010-R	906-0025-R	-	906-0100-T
ISOLUTE Array plates				
HCX	-	902-0025-RP	902-0050-RP	902-0100-TP
HCX-3	-	905-0025-RP	-	905-0100-TP
HCX-5	-	906-0025-RP	-	906-0100-TP

ISOLUTE-96 plates			
	25 mg	50 mg	100 mg
HCX	902-0025-P01	902-0050-P01	902-0100-P01
HCX-3	905-0025-P01	-	905-0100-P01
HCX-5	906-0025-P01	-	906-0100-P01

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