

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

ISOLUTE 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).

This procedure utilizes the ISOLUTE HCX 130 mg/10 mL configuration, optimized for the extraction of basic drugs from 5 mL of urine, and ideal for applications with GC-MS end points.

## Extraction Procedure

### Format

ISOLUTE® HCX 130 mg/10 mL (XL),  
part number 902-0013-H

### Sample Pre-Treatment

Dilute the urine sample (5 mL) 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 or positive pressure. 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.

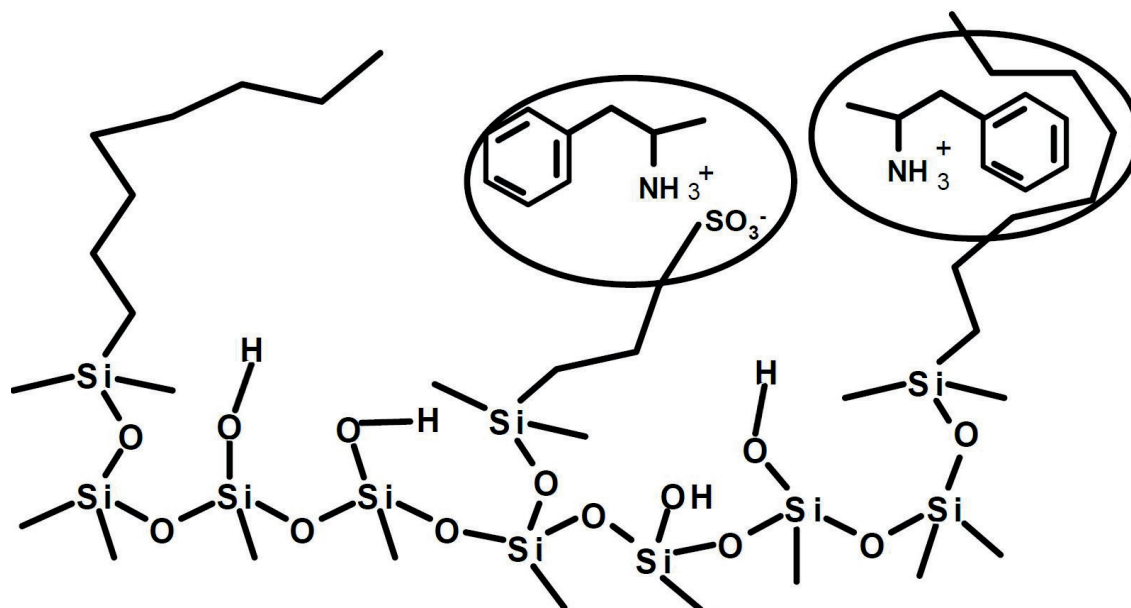
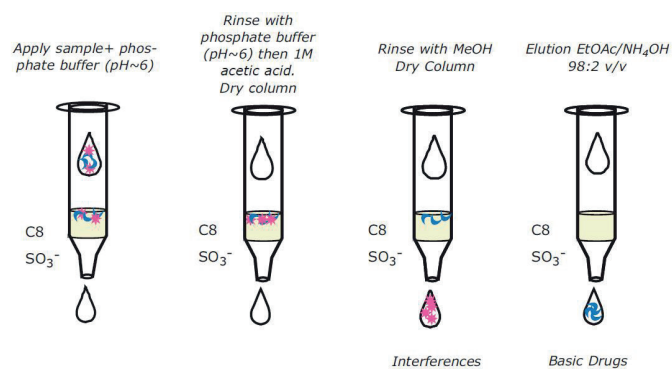


Figure 1. Multiple interactions on ISOLUTE® HCX.

## Alalyte 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.



**Figure 2.** The rigorous washing procedure possible with ISOLUTE® HCX.

## Ordering Information

Part Number	Description	Quantity
902-0013-H	ISOLUTE® HCX 130 mg/10 mL columns	50

### 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](http://www.biotage.com)

### Literature Number: TN113.V.1

© 2020 Biotage. All rights reserved. No material may be reproduced or published without the written permission of Biotage. Information in this document is subject to change without notice and does not represent any commitment from Biotage. E&OE. A list of all trademarks owned by Biotage AB is available at [www.biotage.com/legal](http://www.biotage.com/legal). Other product and company names mentioned herein may be trademarks or registered trademarks and/or service marks of their respective owners, and are used only for explanation and to the owners' benefit, without intent to infringe.