# Generic Method for the Extraction of Quaternary Amines and Polybasic Drugs from Biological Fluids using ISOLUTE® HCX-Q Mixed-Mode SPE Columns and 96-well Plates

## INTRODUCTION

The use of ISOLUTE® HCX mixed-mode sorbents is widely accepted for providing high purity extracts of basic drugs from biological fluids. The dual retention mechanism, provided by non-polar and **strong** cation exchange functional groups, allows for a rigorous interference elution step resulting in low levels of co-extracted materials, thus minimizing the risk of ion suppression effects in LC-MS applications. This approach is suitable for drugs that can be eluted under basic conditions. However, quaternary amines and polybasic drugs cannot be eluted under these conditions. **ISOLUTE HCX-Q**, containing non-polar and **weak** cation exchange functional groups, was designed to allow elution of these compounds under acidic elution conditions.

The ISOLUTE HCX-Q sorbent utilizes a combination of weak cation exchange and C8 non-polar retention mechanisms. The analyte is initially retained by a non-polar (hydrophobic) retention mechanism, which is unaffected by high or variable ionic strength of the sample matrix. The pH control and salt removal at the sample application stage ensures that the polybasic drug or quaternary amine is retained by a robust cation exchange retention mechanism. The two retention mechanisms are shown in **Figure 1**. An interference elution step containing both aqueous buffer and a water miscible organic solvent (e.g. methanol) is used to remove many non-polar interferences. The quaternary amines are eluted by breaking both non-polar and ionic interactions, providing recovery of the analyte.

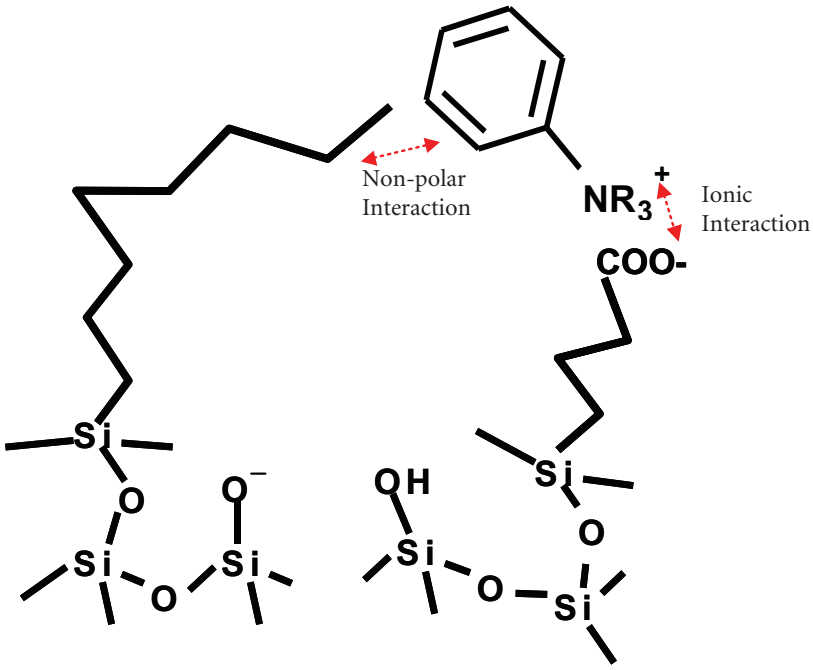


**Figure 1. Multiple interactions on ISOLUTE HCX-Q**

### *Extraction Procedure*

This procedure utilises ISOLUTE HCX-Q 25 mg/1 mL column or 96-well SPE plate configurations, optimized for the elution of basic drugs in very low volumes. Solvent volumes should be scaled up or down as necessary for other column configurations.

### *Sample Pre-treatment*

Dilute the 100 µL biological fluid sample with 0.05 M ammonium acetate buffer, pH 7 (1:4, v/v). Mix thoroughly.

### *Column Conditioning and Equilibration*

Condition the column with methanol (1 mL) followed by 0.05 M ammonium acetate buffer, pH 7 (500 µL).

### *Sample Application*

Apply the sample (500 µL) at a flow rate of 1-2 mL/minute.

### *Interference Elution*

Rinse the column with methanol/0.05 M ammonium acetate buffer, pH 7.0 (20:80, v/v, 500 µL).

The buffer ensures that the sorbent surface remains negatively charged during the interference elution step.

Increase and optimize the proportion of methanol to improve extract cleanliness, checking for analyte breakthrough.

### *Analyte Elution*

Elute analytes with 0.2 M monochloroacetic acid in methanol (2 x 125 µL).

Analyte elution is achieved by simultaneously suppressing ionization of the sorbent carboxylic acid functional groups and eliminating non-polar interactions through the use of the acidified methanol elution solvent. The use of higher concentration acid in the elution solvent may be necessary for some analytes.

If the final analysis technique is GC, evaporate the elution solvent to dryness and derivatize the analyte(s) using a suitable derivatization agent.

This method is also available in Application Note **IST1072A** Extraction of Quaternary Amines from Biological Fluids. This Application Note is based on work done by Lindegardh et al[1] using a suite of antimalarial quaternary amine drugs.

### *Reference:*

[1]Automated mixed-mode solid-phase extraction for simultaneous determination of atovaquone, proguanil and metabolites in plasma by HPLC.

Lindegardh, N; Bergqvist, Y. Department of Analytical Chemistry, Uppsala University, Sweden

Presented at Analytical Days 2002, Sweden

## ORDERING INFORMATION

| Description | Pack Size | Part Number |
|---|---|---|
| ***ISOLUTE HCX-Q SPE Columns*** | | |
| ISOLUTE HCX-Q 25 mg/1 mL columns | 100 | 986-0002-A |
| ISOLUTE HCX-Q 100 mg/1 mL columns | 100 | 986-0010-A |

ISOLUTE HCX-Q is also available in the 1 mL tab-less column format. 1 mL columns can also be supplied fitted with caps for use with Gilson ASPEC™ automation systems. Contact Biotage for further information.

| Description | Pack Size | Part Number |
|---|---|---|
| ***ISOLUTE-96 fixed well 96-well plates for high throughput*** | | |
| ISOLUTE-96 HCX-Q 25 mg fixed well plate | 1 | 986-0025-P01* |
| ISOLUTE-96 HCX-Q 100 mg plate fixed well plate | 1 | 986-0100-P01* |
| ***ISOLUTE Array flexible wells for high throughput*** | | |
| ISOLUTE Array HCX-Q 25 mg wells | 100 | 986-0025-R |
| ISOLUTE Array HCX-Q 100 mg wells | 100 | 986-0100-R |
| ***ISOLUTE Array flexible well 96-well plates for high throughput*** | | |
| ISOLUTE Array HCX-Q 25 mg plate flexible well plate | 1 | 986-0025-RP |
| ISOLUTE Array HCX-Q 100 mg plate flexible well plate | 1 | 986-0100-RP |

* Part numbers for single plate. To order a pack of 5 plates, replace the suffix P01 with P05.

The 96-well formats are fully compatible with the VacMaster-96 Sample Processing Manifold. Contact Biotage for further information on the 96-well formats.

For alternative column configurations, contact Biotage.



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