GENERAL APPROACH TO THE EXTRACTION OF BASIC DRUGS FROM BIOLOGICAL FLUIDS USING NON-POLAR ISOLUTE SPE COLUMNS

This technical note describes the extraction of basic drugs from biological fluids using non-polar ISOLUTE SPE columns.

The extraction of drugs from biological fluids using a purely non-polar retention mechanism can lead to extracts containing 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. In order to obtain a clean extract, it is possible to utilise the ionic interactions between the exposed silanol groups of non-endcapped non-polar sorbents, and the basic moiety of the drug (**see Figure 1**). The drug is retained by both non-polar and ionic retention mechanisms, allowing a more rigorous interference elution regime to be used. This leads to a much cleaner 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.

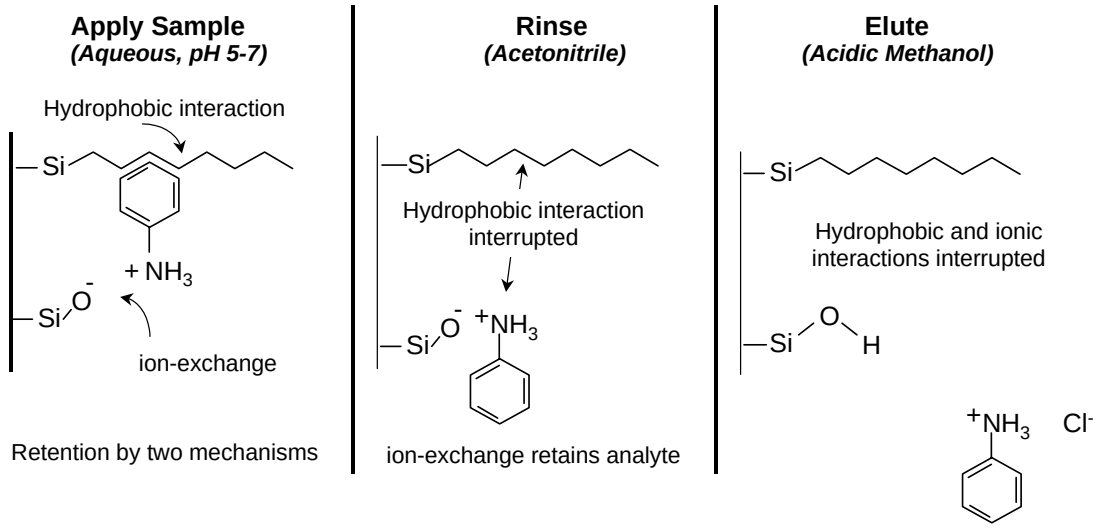

Figure 1: Representation of interactions present during retention and elution of basic analytes

As the different sorbents can have differing selectivities for analytes, we strongly recommend that the whole ISOLUTE range of non-polar, non-endcapped sorbents be screened for each application. A list of the sorbents available along with their sorbent reference number is included in this technical note. An application development kit containing the full range of non-polar, non-endcapped sorbents is also available.

Extraction Procedure

This procedure is based on the use of a 100 mg column. Solvent volumes should be scaled up or down as necessary for other column configurations.

Sample Pre-treatment

Dilute the biological fluid sample 1:1 (v/v) with 0.05-0.1M phosphate buffer, pH greater than 5.0-7.0. Mix thoroughly.

Column Conditioning and Equilibration
Condition the column with acetonitrile (1 mL) followed by 0.05-0.1M phosphate buffer of the same pH as that used for sample dilution (1 mL).

Sample Application
Apply the sample at a flow rate of 1-2 mL / minute.

Interference Elution
Rinse the column with acetonitrile/water (20:80, v/v, 2 mL). Dry the column by vacuum aspiration for 5mins. Rinse the column with acetonitrile (2 mL).

Evaluate the effect of rinse steps of water containing 1-15% methanol (1 mL) on the cleanliness of the final extract.

Analyte Elution
Elute analytes with methanol containing 1-5% triethylamine, or methanol containing 0.5% HCl.

Alternative elution solvents could include methanol containing 1% acetic or formic acid, or methanol containing 1% 1M ammonium acetate.

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

The Range of Non-polar ISOLUTE Non-endcapped Sorbents

Sorbent	Sorbent Reference #
C18	220
MF C18	240
C8	290
C6	380
C4	390
C2	320
CN	420
PH	360

Basic Drug Method Development Kit Part Number 203-0010-A

This kit contains ten 100mg/1mL columns of each of the above sorbents.
The kit is also available in 96-well plate format. Contact Argonaut Technologies for further information.

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