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 utilize 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.

As the different sorbents can have differing selectivities for analytes, we recommend that the whole range of ISOLUTE[®] 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.

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 5.0–7.0. Mix thoroughly.



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



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 or positive pressure for 5 mins. 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 derivatize the analyte(s) using a suitable derivatization agent.

The Range of Non-Polar ISOLUTE® Non-endcapped Sorbents

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

*ISOLUTE[®] CN can also be used as a polar SPE sorbent.

FUROPE

Main Office: +46 18 565900 Main Office: +1 704 654 4900 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

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

IAPAN

Tel: +81 3 5627 3123 Fax: +81 3 5627 3121 jp_order@biotage.com jp-1-pointsupport@biotage.com kr-1-pointsupport@biotage.com

CHINA

Tel: +86 21 68162810 Fax: +86 21 68162829 cn_order@biotage.com cn-1-pointsupport@biotage.com

KORFA Tel: +82 31 706 8500 Fax: +82 31 706 8510 korea info@biotage.com

INDIA Tel: +91 11 45653772 india@biotage.com

Distributors in other regions are listed on www.biotage.com

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