The Use of ISOLUTE® HM-N for Rapid Sample Preparation

This technical note details the use of ISOLUTE® HM-N, a diatomaceous earth material, for use in rapid sample preparation by supported liquid extraction.

ISOLUTE® HM-N is a modified form of diatomaceous earth that can efficiently absorb aqueous samples. ISOLUTE HM-N is chemically inert and stable in the pH range 1–13. These characteristics make it a versatile material that plays an important role in many sample preparation applications.

When analyzing lipophilic compounds in complex aqueous matrices such as biological fluids, clean-up is usually required before analysis. Traditionally, liquid-liquid extraction in a separating funnel has often been used to provide this sample clean-up. ISOLUTE HM-N disposable supported liquid extraction columns can be used as a simple alternative to liquid-liquid extraction in a separating funnel, or as an effective way of removing water from a sample.

ISOLUTE® HM-N is Available in Several Formats

ISOLUTE® HM-N Columns

- Supported Liquid Extraction (SLE) for biological fluids
  Note: for high sensitivity, LC-MS/MS or GC-MS analyses, and high throughput applications, we recommend the use of the optimized ISOLUTE® SLE+ product range.
- Removal of water from aqueous samples
- Minimizing sample preparation procedures for viscous matrices and emulsions
- Dealing with unusual or difficult matrices, e.g. milk, equine urine

Bulk ISOLUTE® HM-N Material

- Pre-loading reaction mixtures onto silica flash columns
- Accelerated Solvent Extraction (ASE®)
- Supercritical Fluid Extraction (SFE)

ISOLUTE® HM-N Supported Liquid Extraction Columns

Supported Liquid Extraction using ISOLUTE HM-N is analogous to traditional liquid-liquid extraction using a separating funnel. ISOLUTE HM-N has a high capacity for retaining aqueous samples. When an aqueous sample is applied, the sample spreads over the hydrophilic surface in a very thin layer, and the aqueous phase is adsorbed. An efficient liquid-liquid extraction occurs when a suitable, water immiscible organic solvent is applied. The high surface area at the interface between the organic and aqueous phases increases efficiency, and eliminates the possibility of emulsion formation. The analytes are then eluted as the solvent passes through the column. (See Figure 1 for illustration of procedure).

For ionizable compounds, extraction efficiency may be enhanced by the use of pH control to suppress ionization (see Appendix A for two pH unit rule, and protocols for extraction of ionizable compounds).

There are several application fields in which Supported Liquid Extraction using ISOLUTE HM-N columns is be particularly appropriate, for example:

- Extraction of lipophilic compounds from biological fluids.
- Minimizing sample preparation procedures for viscous matrices and emulsions. These columns are suitable for applications where scientists are seeking a simple approach to sample preparation, minimizing method development.
- Dealing with unusual or difficult matrices. Analysts dealing with matrices that are challenging for standard SPE columns e.g. milk, turbid aqueous samples that may plug conventional SPE columns, equine urine and other viscous matrices, may find these columns useful.
- Removal of water from aqueous samples. Medicinal chemistry applications involve procedures that require isolation of compounds from aqueous solutions. Supported Liquid Extraction using ISOLUTE HM-N columns can be used for phase transfer – from aqueous solvent to organic, and for removal of water from samples.

Biotage®

How to Use ISOLUTE® HM-N: 3 Easy Steps

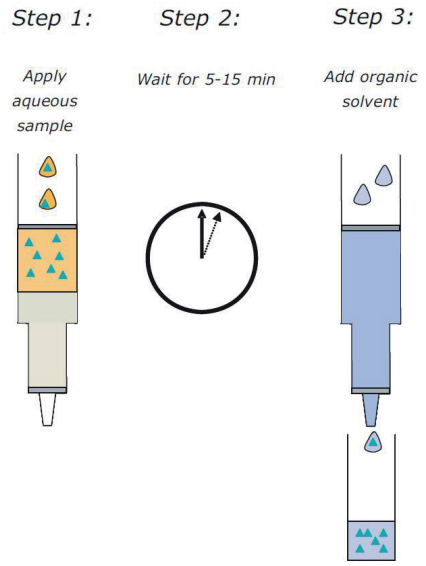

Figure 1. Schematic of Supported Liquid Extraction procedure.

Step 1

Apply the aqueous sample so that it permeates no more than three quarters (75%) down the bed height of the column. No more than the maximum sample volume for each column configuration should be applied to the column. Please refer to the maximum sample volume guidelines.

Step 2

Wait for 5–15 minutes (optimize as required).

Step 3

Apply a suitable water immiscible organic solvent (see overleaf for approximate elution volumes), and collect the analytes.

Important Notes for ISOLUTE® HM-N Column Use

- **Do not overload the column**. This could lead to breakthrough of the aqueous sample, and contamination of the final extract.
- Buffer or internal standards should be added to the sample and mixed thoroughly prior to applying it to the column.
- Do not use a solvent for elution that contains more than 10% water miscible component. This could lead to column overload and extract contamination.

How to Select the Correct Column Size

Selection of the correct column size is based on the volume of the aqueous sample. Always use a column of equal or greater capacity than the sample volume. The capacity of the column is included in the column description.

Table 1. Sample and Elution Volumes.

Description	Maximum Sample Volume (mL)	Suggested Elution Volume
ISOLUTE HM-N (0.3 mL sample)	0.3	3
ISOLUTE HM-N (1.0 mL sample)	1.0	8
ISOLUTE HM-N (3.0 mL sample)	3.0	12
ISOLUTE HM-N (5.0 mL sample)	5.0	16
ISOLUTE HM-N (10.0 mL sample)	10	24
ISOLUTE HM-N (20.0 mL sample)	20	40

Column capacity is included in the product description.

Biotage® Gravity Rack for Processing ISOLUTE® HM-N Columns

Biotage has developed a free standing rack system specifically for the gravity processing of ISOLUTE® HM-N columns. All of the rack components are made from solvent resistant polyethylene materials. The 20-port rack can process up to twenty 5 mL sample volume (E) columns (or ten 20 mL 'F' columns) simultaneously.

The Gravity Rack is supplied as standard with stainless steel needles. Optional PTFE needles, stopcocks and stopcock/needle units are also available, which offer high solvent resistance and can be used for processing requiring high sample purity. See the ordering information on page 3.

Ordering Information

Part Number	Description	Quantity
ISOLUTE® HM-N Columns		
800-0040-BM	ISOLUTE HM-N (0.3 mL sample)	100
800-0100-CM	ISOLUTE HM-N (1.0 mL sample)	100
800-0220-DM	ISOLUTE HM-N (3.0 mL sample)	100
800-0350-EM	ISOLUTE HM-N (5.0 mL sample)	100
800-0700-FM	ISOLUTE HM-N (10.0 mL sample)	50
800-1300-FM	ISOLUTE HM-N (20.0 mL sample)	50
Biotage® Gravity Rack and Needle Options		
123-2016	Gravity rack with 16 mm collection tube rack	1
123-2019	Gravity rack with 19 mm colection tube rack	1
121-0001	PTFE stopcock/needle unit	10
121-0002	PTFE needle unit	10
121-0003	Stainless steel needle	20
121-0004	Stainless steel needle retainer	10

Appendix

Optimized Extraction of Ionizable Compounds

The following simple protocols increase recoveries of ionizable compounds when extracted using ISOLUTE® HM-N columns. The protocols ensure that the compounds are neutralized (i.e. carry no charge) when applied to the column, enhancing the transfer from the aqueous to organic phase.

Extraction of Basic Compounds

1. Dilute sample with 0.5 M NaOH (1:1, v/v)
2. Load sample onto ISOLUTE HM-N column
3. Wait 3–5 minutes
4. Elute with hexane: 3-methyl-1-butanol (98:2, v/v)*

Column choice and solvent volumes used should be as described earlier in this Chemistry Data Sheet.

* Other water immiscible solvents (or solvent combinations) may also be appropriate. Elution solvents should be optimized for individual analytes.

Extraction of Acidic Compounds

1. Dilute the sample with 0.1 M phosphate buffer, pH 2 (1:1, v/v).
2. Load sample onto ISOLUTE HM-N column
3. Wait 3–5 minutes
4. Elute with hexane: 3-methyl-1-butanol (98:2, v/v)*

Column choice and solvent volumes used should be as described earlier in this Technical Note.

* Other water immiscible solvents (or solvent combinations) may also be appropriate. Elution solvents should be optimized for individual analytes.

The Two (2) pH Unit Rule

The pK_a of a molecular functional group is defined as the pH at which 50% of this group in solution is charged, and 50% is uncharged. Each pH unit change affects the percentage of charged or uncharged groups by a factor of 10, so it is sensible to perform extractions at pH at least 2 pH units from the pK_a value, to ensure that 99.5% of the functional groups are in the desired state.

Table 2.
e.g Effect of pH on the dissociation of a weak acid with a pK_a value of 4.0.

pH	% Free Acid (unchanged)	% Dissociated (charged)
4.0	50	50
3.0	95.0	5.0
2.0	99.5	0.5

Table 3.
e.g. Effect of pH on the dissociation of a weak base with a pKa value of 9.0.

pH	% Free Base (unchanged)	% Dissociated (charged)
9.0	50	50
8.0	95	5.0
7.0	99.5	0.5

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