

## 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 supported liquid-liquid extraction for rapid sample preparation.**

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 very versatile material that plays an important role in many sample preparation applications.

When analysing 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 liquid-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, particularly important in combinatorial chemistry applications. The high purity columns and frits ensure compatibility with high sensitivity LC and GC analyses.

### ISOLUTE HM-N is available in several formats

ISOLUTE HM-N material is available packed into columns and flexible format 96-well plates for high throughput applications.

#### ISOLUTE HM-N columns and ISOLUTE HM-N Array® (flexible plate) columns

- *Supported liquid-liquid extraction for biological fluids*
- *Removal of water from aqueous samples*
- *Minimising sample preparation procedures for viscous matrices and emulsions*
- *Dealing with unusual or difficult matrices, e.g. milk, equine urine*

ISOLUTE HM-N is also available as bulk material.

#### Bulk ISOLUTE HM-N material

- *Pre-loading polar samples onto silica flash columns*
- *Accelerated solvent extraction (ASE®)*
- *Supercritical fluid extraction (SFE)*



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## **ISOLUTE HM-N liquid-liquid extraction columns**

**Supported liquid-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.

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

There are several application fields in which liquid/liquid extraction using ISOLUTE HM-N columns is particularly appropriate, for example:

- **Extraction of lipophilic compounds from biological fluids**

- **Removal of water from aqueous samples**

Combinatorial chemistry applications involve procedures that require extraction of analytes from aqueous samples. Liquid/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.

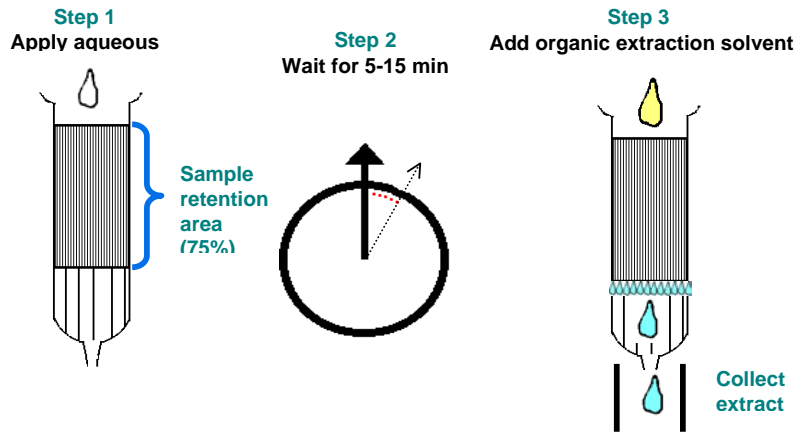
- **Minimising sample preparation procedures for viscous matrices and emulsions**

These columns are suitable for applications where scientists are seeking a simple approach to sample preparation, minimising 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.

## How to use ISOLUTE HM-N: 3 easy steps



**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 (optimise as required).

**Step 3** Apply a suitable water immiscible organic solvent (see Table I overleaf for approximate elution volumes), and collect the analytes.

### Important notes for HM-N column use

- **Do not overload the column.** This could lead to breakthrough of the aqueous sample, and contamination of the 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 I: Sample and elution volumes**

Description	Maximum sample volume (ml)	Suggested elution volume (ml)
ISOLUTE HM (0.3 ml sample)	0.3	3
ISOLUTE HMN (1.0 ml sample)	1.0	8
ISOLUTE HMN (3.0 ml sample)	3.0	12
ISOLUTE HMN (5.0 ml sample)	5.0	16
ISOLUTE HMN (10.0 ml sample)	10	24
ISOLUTE HMN (20.0 ml sample)	20	40

*Column capacity is included in the product description*

**Table II: ISOLUTE HM-N packed column ordering information**

Description	Pack size	Part number
ISOLUTE HM-N (0.3 ml sample)	100	800-0040-BM
ISOLUTE HM-N (1.0 ml sample)	100	800-0100-CM
ISOLUTE HM-N (3.0 ml sample)	100	800-0220-DM
ISOLUTE HM-N (5.0 ml sample)	100	800-0350-EM
ISOLUTE HM-N (10.0 ml sample)	50	800-0700-FM
ISOLUTE HM-N (20.0 ml sample)	50	800-1300-FM

## Gravity Rack for processing ISOLUTE HM-N columns

Argonaut has developed a free standing rack system specifically for the gravity elution of ISOLUTE HM-N columns, eliminating the need for a vacuum manifold to hold the columns and collection vessels during sample extraction. 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. See page 13 of the Argonaut Sample Preparation Products Catalogue for true-to-scale diagrams of these columns.

The gravity rack is supplied as standard with stainless steel needles. Optional PTFE needles and stopcocks are also available, which offer high solvent resistance and can be used for processing requiring high sample purity. See the ordering information below.

### Gravity Rack ordering information

Description	Part number
Complete with 10 mm diameter collection tube rack	123-2010
Complete with 12 mm diameter collection tube rack	123-2012
Complete with 16 mm diameter collection tube rack	123-2016
Complete with 19 mm diameter collection tube rack	123-2019

### Components for Gravity Rack collection tube size conversion

Description	Pack size	Part number
Top / mid plates for 10 mm diam. tubes	2	123-2110
Top / mid plates for 12 mm diam. tubes	2	123-2112
Top / mid plates for 16 mm diam. tubes	2	123-2116
Top / mid plates for 19 mm diam. tubes	2	123-2119
Blank top / mid plates for customised racks	2	123-2100

### Gravity Rack needle options

Description	Pack size	Part number
PTFE stopcock / needle unit	10	121-0001
PTFE needle unit	10	121-0002
Stainless steel needle	20	121-0003
Stainless steel needle retainer	10	121-0004

## 96-well format: ISOLUTE HM-N Array columns and plates

For high throughput screening (HTS) applications (e.g. purification of combinatorial libraries or screening pharmaceuticals in biological fluids), ISOLUTE HM-N is available in the versatile 96-well format, ISOLUTE Array. In this format, 200 mg of ISOLUTE HM-N is packed into each 2 ml Array column. These are suitable for extraction of 150 - 200 µl of aqueous sample. Packing techniques are optimised for efficient water removal or HTS drug extraction.

The columns can be used individually, or assembled into the plate format. Pre-assembled plates can also be purchased. The plate can be populated with as many or as few columns as required, making this a flexible, economical format.

### ISOLUTE HM-N Array ordering information

Description	Units / pack	Part number
200 mg / 2 ml Array columns	100	800-0200-T
Pre-populated plate, 96 x 200 mg / 2 ml Array columns	1	800-0200-TP

To process the ISOLUTE HM-N Array columns (P/N 800-0200-T) on a VacMaster<sup>®</sup>-96, an empty ISOLUTE Array base plate is required. When processing a partially populated plate, base plate sealing plugs are also required.

### ISOLUTE Array accessories ordering information

Description	Units / pack	Part number
ISOLUTE Array base plate	1	120-1000-P01
ISOLUTE Array base plate	5	120-1000-P05
Base plate plugs (strip of 8)	50	120-1200
Adaptors (to fit any standard vacuum manifold)	25	120-1201
Well removing tool	1	120-1202

## ISOLUTE HM-N Array plate processing

ISOLUTE HM-N Array columns require a short pulse of vacuum to load the aqueous sample onto the column. Following sample loading, analyte elution can be carried out under gravity. Processing of the populated plate can be carried out manually using the VacMaster-96 (see ordering information below), or by using automation systems designed for 96-well processing.

Up to twenty discrete Array columns can be processed using a VacMaster-10 or -20 sample processing station equipped with ISOLUTE Array Luer adaptors.

## VacMaster-96 for manual processing of 96-well plates

The VacMaster-96 manifold can be used to process up to 96 individual ISOLUTE Array wells simultaneously.

### VacMaster-96 manifold and vacuum control unit ordering information

Description	Part number
VacMaster-96 (no vacuum control included) <sup>1</sup>	121-9600
VacMaster-96 complete with VCU-1 (121-9601) <sup>2</sup>	121-9603
VacMaster-96 complete with VCU-2 (121-9602) <sup>3</sup>	121-9604
<b>Vacuum control options</b>	
VacMaster-96 vacuum control unit (VCU-1) <sup>2</sup>	121-9601
VacMaster-96 vacuum control unit with integral vacuum generator (VCU-2) <sup>3</sup>	121-9602

<sup>1</sup>This option is supplied for use with automated liquid handling systems with an in-built computer controlled vacuum source

<sup>2</sup>This option is for use in a laboratory with a vacuum source

<sup>3</sup>This option is for use in a laboratory with a compressed air source

IST's deep well collection plates are fully compatible with the VacMaster-96 for sample collection.

### Collection plate ordering information

Description	Pack size	Part number
Collection plate – 350µl	50	121-5201
Collection plate – 1ml	50	121-5202
Collection plate – 2ml	50	121-5203

Other commercially available collection plate formats (shallow or deep well) can be accommodated using a spacer system to raise the level of the collection plate, preventing well to well cross contamination.

## Appendix A

### Optimised extraction of ionisable compounds

The following simple protocols increase recoveries of ionisable compounds when extracted using ISOLUTE HM-N columns and plates. The protocols ensure that the compounds are neutralised (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 0.5M NaOH (1:1, v/v)
2. Load sample onto ISOLUTE HM-N column
3. Wait 3-5 mins
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 optimised for individual analytes.

#### Extraction of acidic compounds

1. Dilute the sample with 0.1M phosphate buffer, pH 2 (1:1, v/v)
2. Load the sample onto ISOLUTE HM-N column
3. Wait 3-5 mins
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 optimised 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% are 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.

E.g. Effect of pH on the dissociation of a weak acid with a  $pK_a$  value of 4.0.

pH	% free acid (uncharged)	% dissociated (charged)
4.0	50	50
3.0	95.0	5.0
2.0	99.5	0.5

E.g. Effect of pH on the dissociation of the conjugate acid of a weak base with a  $pK_a$  value of 9.0

pH	% free base (uncharged)	% dissociated (charged)
9.0	50	50
8.0	95	5.0
7.0	99.5	0.5

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