



## Work-up of Organic Reaction Mixtures using ISOLUTE® HM-N Supported Liquid Extraction (SLE) Columns

One of the most common work-up techniques in medicinal chemistry is liquid-liquid extraction (LLE) using a separating funnel. The technique is used for removal of water soluble impurities, solvent exchange, water removal or work-up of aqueous reaction mixtures.

### LLE in a Flow-through Column Format

Packed with a diatomaceous earth support, ISOLUTE® HM-N columns provide a simple and rapid alternative to LLE work-up. This hydrophilic inert support can be used instead of traditional LLE protocols, eliminating emulsion formation, increasing productivity and improving reproducibility. The technique is referred to as Supported Liquid Extraction (SLE).

### Easy Transfer of Traditional LLE Solvents

The same solvents used in LLE are also used in SLE (DCM, chloroform, ethyl acetate, hexane, butyl acetate and toluene). If the organic solvent contains a water miscible polar solvent such as isopropyl alcohol or acetone, dilute to less than 10% v/v polar modifier. As with traditional LLE, the use of acid or base to neutralize ionizable compounds is an important part of the procedure.

### Gravity Flow to Improve Workflow

ISOLUTE HM-N columns use gravity flow, and therefore may be left unattended. Compared to traditional LLE procedures, this flow-through approach improves productivity and can allow simultaneous processing of twenty samples at any one time using the IST Gravity Rack.

The disposable format eliminates the need for glassware washing, reducing the time taken for work-up and risk of contamination.

### Processing Options

ISOLUTE HM-N columns can be used with the IST Gravity Rack. For high throughput work-up, the ISOLUTE HM-N tab-less 0.3 and 1 mL aqueous volume columns are compatible with the Mettler Toledo MiniBlock™ and other liquid handlers that process tab-less SPE and work-up columns. The 1 mL aqueous volume tab-less columns are also compatible with the ISOLUTE Array-24 system. Contact Biotage for more details.

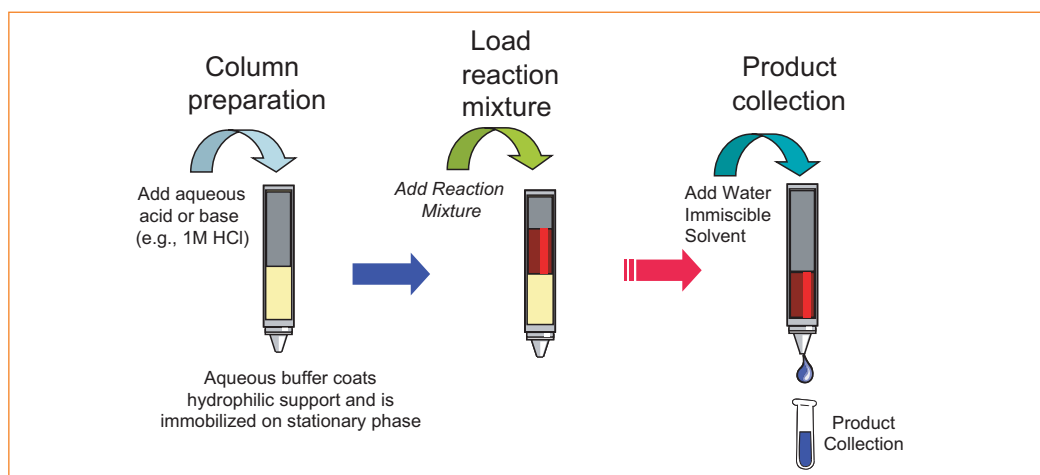
### Selecting the Appropriate Column Size

ISOLUTE HM-N columns are described by their capacity for aqueous systems. For example, an ISOLUTE HM-N 3 mL sample volume column can absorb up to 3 mL aqueous solvent.

Format	Reaction Mixture Volume	Maximum Aqueous Load
Standard columns	5-40 mL	0.3 to 20 mL as specified in description
Tab-less 3 and 6 mL columns for use with 48 and 24-well automation formats	3-6 mL	0.3 or 1 mL as specified in description
Bulk ISOLUTE HM-N	> 500 mL	750 $\mu\text{L/g}$

### Removal of Water Soluble Impurities from Reaction Mixtures (Salt Scrubbing)

Removal of water soluble impurities during reaction work-up is often referred to as 'salt scrubbing', as it is typically used to remove salts from a reaction mixture. (**Figure 1**). Initially, an aqueous buffer is immobilized onto the diatomaceous earth. The reaction mixture is loaded onto the column and water soluble components are transferred to the aqueous phase at the solvent interface.



**Figure 1.** Schematic representation of salt scrubbing procedure

#### Procedure

1. Load aqueous buffer using gravity. This will absorb onto the column. Allow 5-15 minutes for complete aqueous absorption to take place. The maximum aqueous load that can be applied to a particular column is stated in the column description, e.g. apply up to 1 mL of buffer to the ISOLUTE HM-N 1 mL column.

*To scrub bases, add 5% v/v 1 M HCl or similar acid*

*To scrub acids, add 5% v/v 1 M NaOH or similar base*

2. Load the water immiscible reaction mixture using gravity flow.
3. Collect the filtrate. The reaction mixture is now ready for further work-up or purification.

#### Water Removal

For reaction mixtures containing trace amounts of water, ISOLUTE HM-N can be used as a drying agent. ISOLUTE HM-N can absorb up to 750  $\mu\text{L}$  of water per gram of material. This capacity may vary depending on the solvent used.

#### Procedure

1. Load the wet reaction mixture onto the column. Residual moisture will be absorbed onto the support material.
2. Collect the dried filtrate.

ISOLUTE Sodium Sulfate Drying Cartridges can also be used for water removal. Contact Biotage for more information.

## Supported Liquid Extraction

For aqueous solvent mixtures, ISOLUTE HM-N can be used to transfer target compounds into water immiscible solvents. Request Chemistry Data Sheet **TN118 The use of ISOLUTE HM-N for Rapid Sample Preparation** for further details.

### Procedure

1. Load the aqueous reaction mixture onto the appropriate size column using gravity flow. (The maximum aqueous volume that can be applied to the column is referenced in the column description).
2. Allow to stand for 5-15 minutes for complete absorption.
3. Load a suitable water immiscible organic solvent under gravity and collect the filtrate (contains the target compound(s)).

## ISOLUTE HM-N Product Ordering Information

Part Number	Description	Quantity
800-0040-BM	ISOLUTE HM-N (0.3 mL) aqueous volume	100
800-0100-CM	ISOLUTE HM-N (1 mL) aqueous volume	100
800-0220-DM	ISOLUTE HM-N (3 mL) aqueous volume	100
800-0350-EM	ISOLUTE HM-N (5 mL) aqueous volume	100
800-0700-EM	ISOLUTE HM-N (10 mL) aqueous volume	50
800-1300-FM	ISOLUTE HM-N (20 mL) aqueous volume	50

## ISOLUTE HM-N Tab-less Columns for High Throughput Applications Product Ordering Information

Part Number	Description	Quantity
800-0040-BMG <sup>1</sup>	ISOLUTE HM-N (0.3 mL) aqueous volume, tab-less	100
800-0100-CMG <sup>2</sup>	ISOLUTE HM-N (1 mL) aqueous volume, tab-less	100

<sup>1</sup> ISOLUTE tab-less 3 mL columns are compatible with the Mettler-Toledo Miniblock™ System.

<sup>2</sup> ISOLUTE tab-less 6 mL columns are compatible with the ISOLUTE Array-24 System.

Contact Biotage for further information.



### www.biotage.com

UNITED STATES AND CANADA  
Main Office: +1 434 979 2319  
Toll Free: +1 800 446 4752  
Fax: +1 434 979 4743  
Order Tel: +1 434 220 2687  
Order Fax: +1 434 296 8217  
ordermailbox@biotage.com

UNITED KINGDOM  
Main Office: +44 1443 811811  
Fax: +44 1443 816552  
Order Tel: +44 1443 811822  
Order Fax: +44 1443 816816  
eurosales@eu.biotage.com

SWEDEN  
Main Office: +46 18 56 5900  
Fax: +46 18 59 1922  
Order Tel: +46 18 56 57 10  
Order Fax: +46 18 56 57 05  
order@eu.biotage.com

GERMANY  
Tel: +49 7624 90 80 0  
Fax: +49 7624 90 80 10  
separtis@eu.biotage.com

SWITZERLAND  
Tel: +41 61 743 90 15  
Fax: +41 61 743 90 18  
separtis@eu.biotage.com

AUSTRIA  
Tel: +43 2231 63167  
Fax: +43 2231 63520  
separtis@eu.biotage.com

JAPAN  
Tel: +81 422 28 1233  
Fax: +81 422 28 1236  
order@biotage.co.jp