

Work-up of Organic Synthesis Reaction Mixtures Using ISOLUTE® HM-N



One of the most common work-up techniques in medicinal chemistry is liquid-liquid extraction (LLE). 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

Typical solvents used for SLE are those used in traditional LLE, such as DCM, chloroform, ethyl acetate, hexane, butyl acetate and toluene. If the organic solvent contains a water miscible polar modifier 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 are processed using gravity flow, and therefore can be left unattended. Compared to traditional LLE procedures, this flow-through approach improves productivity and allows simultaneous processing of twenty samples at any one time using the Biotage® Gravity Rack.

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

Processing Options

ISOLUTE HM-N columns can be processed on the Biotage® Gravity Rack. For high throughput work-up, the ISOLUTE HM-N tabless 0.3 and 1 mL aqueous volume columns can be processed on the Mettler Toledo MiniBlock™ and other liquid handlers that process tabless SPE and work-up columns. The 1 mL aqueous volume tabless 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.

Table 1.

Formats	Reaction Mixture Volume	Maximum Aqueous Load
Standard columns	5–40 mL	0.3 to 20 mL as specified in description
Tabless 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 µL/g

Removal of Water Soluble Impurities from Reaction Mixtures

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. See Figure 1 (page 2) for a schematic representation. 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.

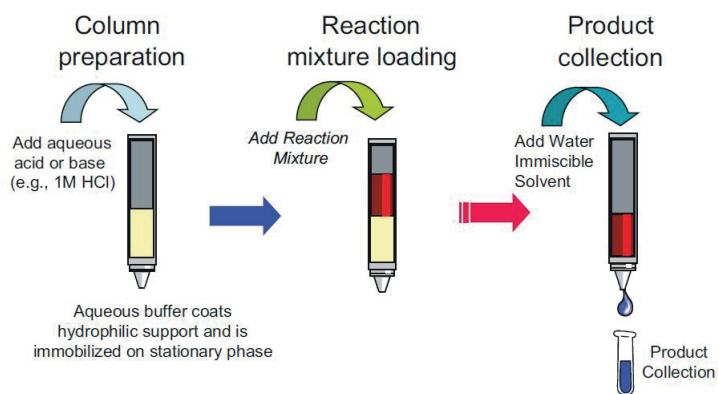


Figure 1. Schematic representation of salt scrubbing procedure.

Procedure

1. Load aqueous buffer using gravity flow. 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 mixture as it passes through the column. 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 µL of water per gram of material. This capacity may vary depending on the solvent used.

Procedure

1. Load the reaction mixture onto the column using gravity flow. Residual moisture will be absorbed onto the support material.
2. Collect the dried reaction mixture as it passes through the column.

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

3. 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.
4. Allow to stand for 5–15 minutes for complete absorption.
5. Load a suitable water immiscible organic solvent under gravity and collect the filtrate (contains the target compound(s)).

Ordering Information

Part Number	Description	Quantity
ISOLUTE® HM-N Columns		
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-FM	ISOLUTE HM-N (10 mL) aqueous volume	50
800-1300-FM	ISOLUTE HM-N (20 mL) aqueous volume	50
ISOLUTE® HM-N Tabless Columns for High Throughput Applications		
800-0040-BMG¹	ISOLUTE HM-N (0.3 mL) aqueous volume, Tabless	100
800-0100-CMG²	ISOLUTE HM-N (1 mL) aqueous volume, Tabless	100

¹ ISOLUTE tabless 3 mL columns are compatible with the Mettler-Toledo Miniblock™ System.

² ISOLUTE tabless 6 mL columns are compatible with the ISOLUTE® Array-24 System. Contact Biotage for further information.

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