

HILIC Purification Strategies

for Flash Chromatography

Abstract

Water soluble compounds, such as anthocyanins, dyes, nucleotides, compounds containing carbohydrates, tannins or other polyphenols, and alkaloids can be difficult to purify because they are poorly retained on reverse phase columns while being strongly retained on silica. HILIC (**H**ydrophilic **I**nteraction **L**iquid **C**hromatography) is a technique that uses chromatography media that are compatible with water and are run as normal phase columns. The technique involves a gradient starting with an organic solvent and ending with a solvent mixture containing water. The use of water gives the technique the alternate name of *Aqueous Normal Phase*. The purification of alkaloids on an amine column and a synthetic dye on a diol column are demonstrated.

Background

Many compounds are difficult to purify due to their polarity and strong solubility in water. These compounds are insoluble in most normal phase solvents and irreversibly are retained on silica or alumina. Under reverse phase conditions, the compounds are poorly retained on C18.

The use of water-compatible normal phase media and solid load cartridges allow these compounds to be purified. Aqueous normal phase also exhibits different selectivity, allowing purification of compounds that may otherwise closely elute.

The gradient begins with a water miscible organic solvent (Table 1) and runs typically to 50% water. In general, less-polar solvents are preferred for the starting solvent in the gradient as they give greater control over the purification but other solvents may give differences in selectivity. Because the compounds elute using mostly organic solvents, there is less water to be removed allowing faster drying of the sample.

Table 1: List of solvents suitable for Aqueous Normal Phase Chromatography

Acetone	Ethanol
Acetonitrile	Methanol
Isopropanol	Tetrahydrofuran

Compounds should be adsorbed onto a material such as Celite¹ prior to the purification. Compounds dissolved in water and injected onto the column may not interact with the column media causing poor separation. Celite is useful because it adsorbs water while retaining the compound poorly.

Experimental and results

General procedure for sample loading

Samples were weighed and dissolved in a minimum volume of water. The sample weight was multiplied by nine to obtain the mass of Celite required for the experiment. The Celite and sample were mixed in a round bottom flask, and sufficient methanol was added to make a slurry. The mixture was evaporated to dryness.

Erioglaucine dye on diol

Erioglaucine dye was adsorbed onto Celite as described above and then placed in a solid load cartridge. The sample was run (0.05 g dye) on a 15.5 g RediSep Rf Gold[®] diol column (P/N 69-2203-495). Solvent A was acetonitrile; B was water containing 0.1% TFA. The gradient was run from 0 to 50% B. The compound was fractionated using All-Wavelength Collection.

The dye was resolved from the impurities.

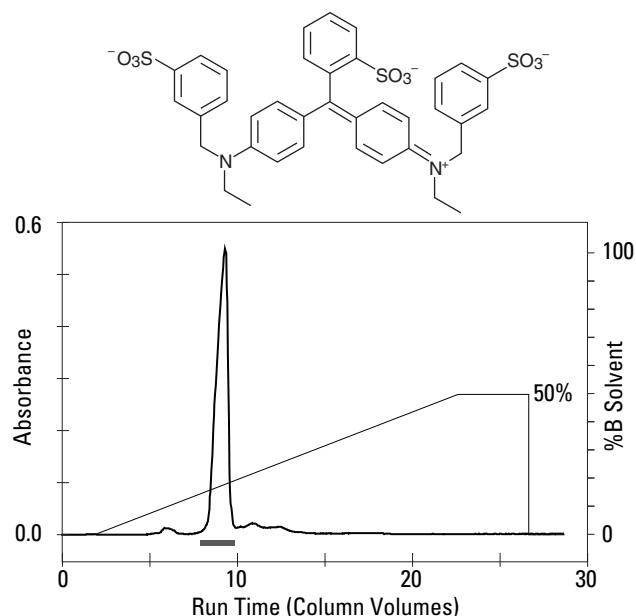


Figure 1: Purification of an erioglaucine dye on a diol column

1. Celite[®] is a registered trademark of Johns-Manville Corporation.

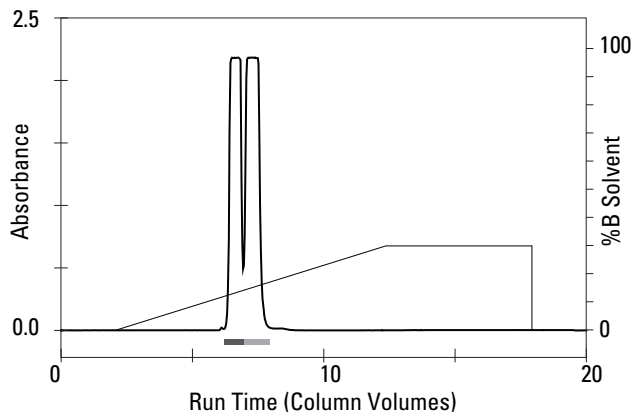
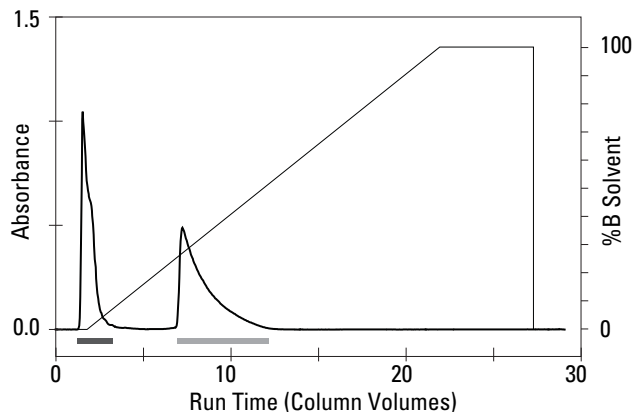
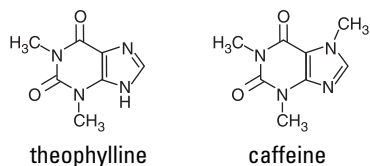


Figure 2: Purification of theophylline and caffeine on an amine column (left) using aqueous normal phase and silica (right) with a dichloromethane/methanol gradient.

Xanthine alkaloids on an Amine column

Both columns were loaded with 0.15 g total alkaloids and run with the solvent gradients depicted in Figure 2. The amine column was a 15.5 g RediSep Rf Gold Amine column (P/N 69-2203-505); a 12 g RediSep Rf Gold Silica (P/N 69-2203-345) column was eluted with a dichloromethane/methanol gradient.

The amine column under HILIC conditions exhibited greater resolution between the alkaloids compared to silica. In addition, the purification was achieved without the use of chlorinated solvents. Both compounds eluted with less than 50% water; greater resolution could be achieved by reducing the maximum gradient to 50% water.

Column Care

After using the columns, wash them with at least five column volumes of isopropanol. Store the column in isopropanol with the end caps in place.

Conclusion

Aqueous normal phase is a useful alternative to reverse phase purification for highly polar compounds. Strongly water soluble samples may be purified. The reduced amount of water required to elute the compounds makes solvent removal easier since organic solvents generally have lower boiling points compared to water. The use of HILIC conditions may eliminate the need for purification with chlorinated solvents.

RediSep Rf Gold is a registered trademark of Teledyne Isco, Inc.

Last modified October 21, 2010

Teledyne Isco, Inc.

P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 775-2965 • Phone: (402) 464-0231 • Fax: (402) 465-3001
E-mail: IscoService@teledyne.com

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