Strategies for the Flash Purification of Highly Polar Compounds

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Abstract

Highly polar compounds, such as basic molecules and water soluble dyes, pose unique challenges for purification. They can bind to the media, or in the case of C18, elute without media interaction thereby preventing purification of the compound in both cases. Highly aqueous purifications on a new Flash C18 media is demonstrated using dyes. HILIC is also explored to purify polar compounds on silica, diol, and amine columns.

Background

Some of the most difficult compounds to purify are those containing highly polar groups. These compounds show poor solubility in organic solvents. They may exhibit poor binding on C18, and very tight binding on silica. The use of new Flash columns that work well with highly aqueous solvents allow purification of these difficult compounds.

Experimental and Results

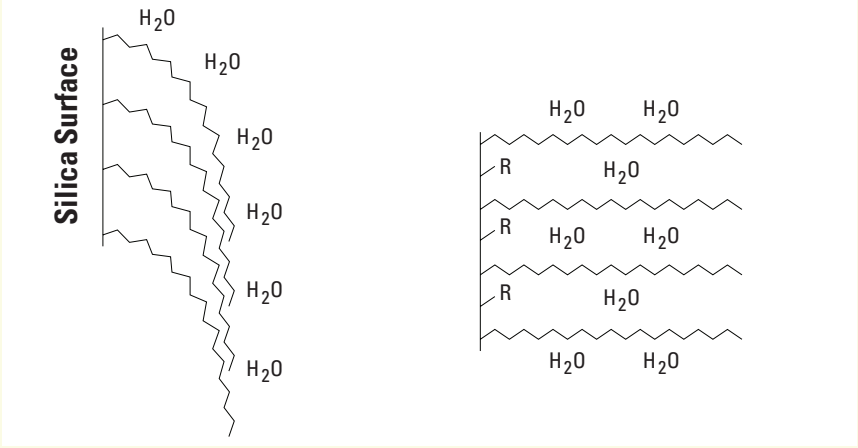
All experiments were run on a Combi*Flash*® Rf 200 (PN 68-5230-006, Teledyne Isco, Lincoln, NE). Pure chemicals were obtained from Sigma-Aldrich (St. Louis, MO); dyes were obtained from Hilton Davis (Cincinnati, OH). Other details are described in each section below.

Highly Aqueous C18

Standard C18-bonded phases are subject to phase collapse when used to purify highly polar compounds with solvent systems containing more than 80% water. Symptoms of phase collapse include poor retention and irreproducible runs where the same compounds show differing retentions when repeatedly run under the same conditions.

Redi*Sep* Rf Gold® C18 Aqueous columns are end-capped with a proprietary hydrophilic group that reduces phase collapse (**Figure 1**).

Figure 1 **C18 chains undergo phase collapse under highly aqueous conditions (left). Hydrophilic groups (right) reduce phase collapse and improve retention.**

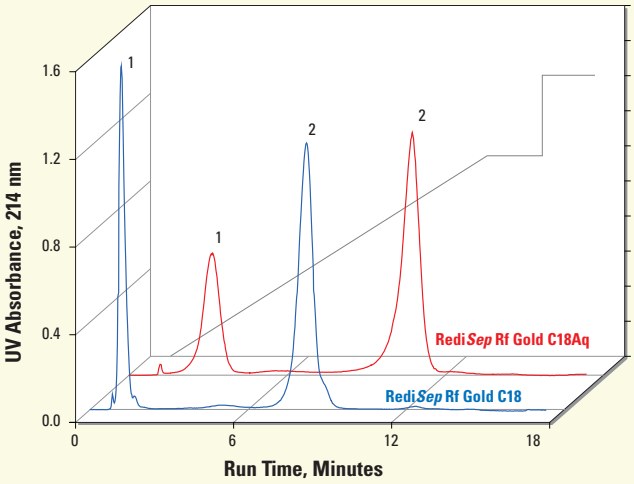


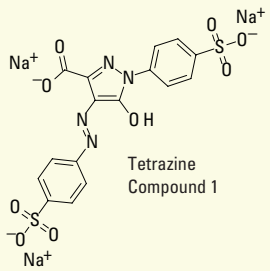
Water Soluble Dyes

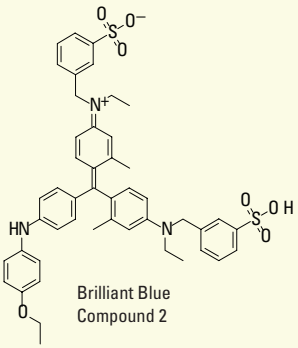
Tetrazine and brilliant blue (50 mg each) were dissolved in 2.0 mL water. Of this solution, a 1.0 mL sample was injected onto a 30 g Redi*Sep* Rf Gold C18 column (PN 69-2203-335) and a 30 g Redi*Sep* Rf Gold C18Aq column (PN 69-2203-560). The columns were run in a water/methanol gradient using the gradient profile shown in **Figure 2**.

The brilliant blue dye was retained on both types of C18 but was retained to a greater degree on the Redi*Sep* Rf Gold C18Aq column. Of greater interest is the improved retention of the tetrazine on the Redi*Sep* Rf Gold C18Aq.

Figure 2 **Comparison of Redi*Sep* Rf Gold C18Aq and Redi*Sep* Rf Gold C18 with a sample requiring a highly aqueous mobile phase for retention.**







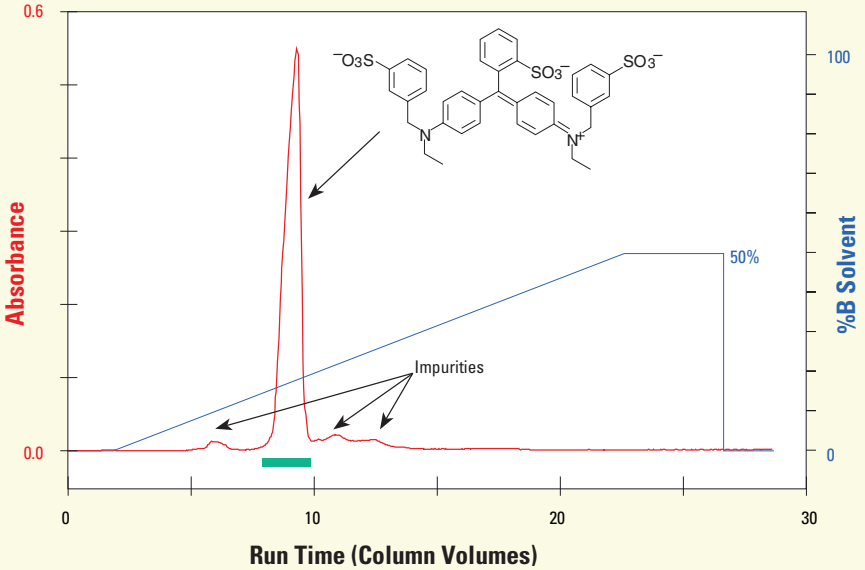
Bonded Phase HILIC
(Hydrophilic Interaction LIquid Chromatography)

Diol column

Diol columns take their name from diol groups bonded to silica. The diol acts, with a few exceptions, as a normal phase column when used with water. Being less polar than silica, compounds are less likely to irreversibly bind to diol.

Erioglaucine dye was adsorbed onto Celite® by dissolving in water and mixed with Celite to make a 10% sample load when dried. Methanol was added to make a slurry and the mixture was evaporated to dryness and then placed in a solid load cartridge. The sample (0.05 g dye) was run on a 15.5 g Redi*Sep* Rf Gold diol column (PN 69-2203-515). 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 the All-wavelength Collection feature in the Combi*Flash* Rf 200 system (**Figure 3**). The dye was successfully resolved from the impurities.

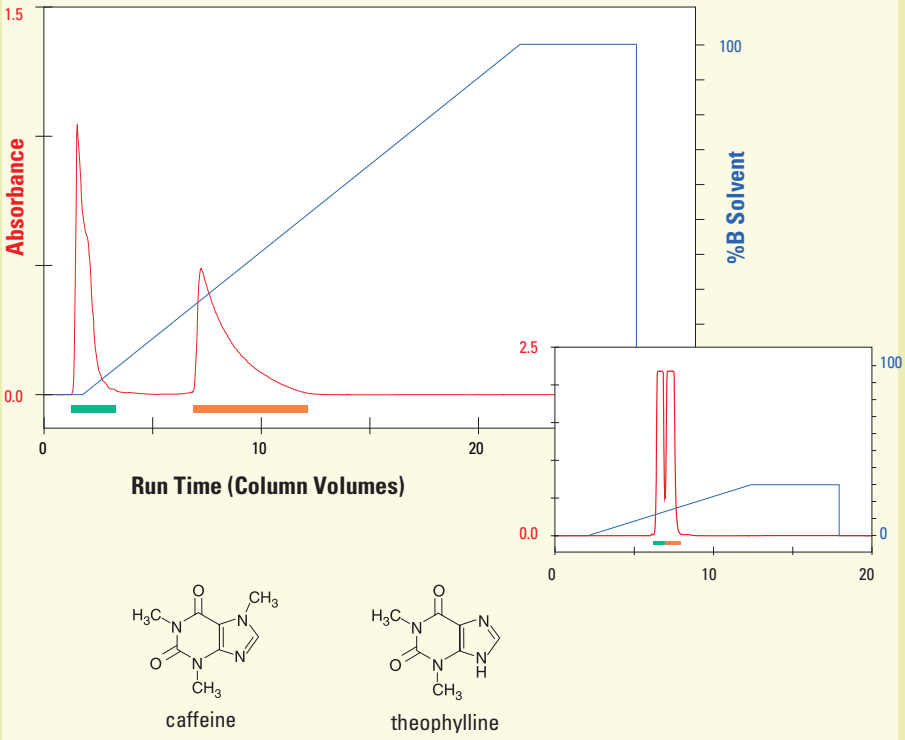
Figure 3 Erioglaucine dye purified on a Redi*Sep* Rf Gold diol column.



Amine Column

Silica and amine columns were loaded with 0.15 g sample each, consisting of 1:1 caffeine and theophylline and run with the solvent gradients depicted in **Figure 4**. The amine column was a 15.5 g Redi*Sep* Rf Gold Amine column (PN 69-2203-505) run an acetonitrile/water (B solvent) gradient. A 12 g Redi*Sep* Rf Gold Silica (PN 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; even greater resolution could be achieved by reducing the maximum gradient to 50% water.

Figure 4 Xanthine alkaloids are better resolved on an amine column in HILIC mode compared to a silica column (inset) running a dichloromethane/methanol gradient.

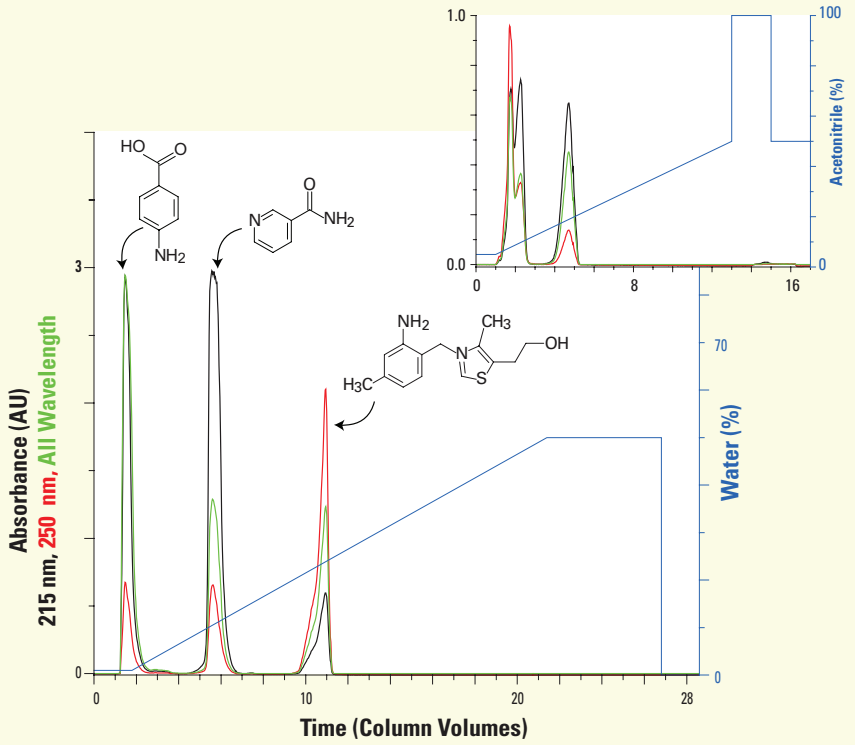


Silica HILIC

Redi*Sep* Rf Gold silica columns are made with silica sufficiently pure to prevent stationary phase leaching when highly polar solvents such as methanol and water are used.

Water soluble vitamins were run because they have a range of functional groups and show poor resolution using a C18 column. For this experiment, 4-aminobenzoic acid, niacinamide, and thiamine hydrochloride were separately adsorbed onto silica (20% loading) and 150 mg of each vitamin/silica mixture were mixed in a 5 g Solid Load Cartridge (PN 69-3873-235, Teledyne Isco). Solvent A was water containing 0.1% TFA; Solvent B was acetonitrile. The gradient in **Figure 5** was run on a 12 g Redi*Sep* Rf Gold silica column.

Figure 5 **Water soluble vitamins purified on a Redi*Sep* Rf Gold silica column using an acetonitrile/water gradient. The inset shows the mixture exhibits poor selectivity on a C18Aq column designed for purifying polar molecules.**



For the C18 experiment (**Figure 5, inset**), 100 mg of each vitamin was dissolved in 1.0 mL 1:1 acetonitrile:water; 0.3 mL of this mixture (30 mg each vitamin) was run on a 15.5 g Redi*Sep* Rf Gold C18Aq column. Solvent A was acetonitrile, solvent B was water; both solvents contained 0.1% TFA. The compounds were determined to elute in the reverse order compared to the silica column.

For both experiments, the detector simultaneously measured absorbance at 215, 250 nm, and All-Wavelength Collection (200 – 300 nm, 2 min peak width).

The vitamins showed excellent resolution and peak shape on silica, but failed to resolve on C18.

Figure 6 **Teledyne Isco Redi*Sep* Rf columns are available in a variety of sizes and media types.**

Conclusions

- Redi*Sep* Rf Gold C18Aq is useful for compounds that elute under highly aqueous conditions.
- Redi*Sep* Rf Gold amine and diol columns work well in aqueous normal phase conditions.
- Silica columns can be run in HILIC mode to purify difficult compounds or those that fail to be resolved using C18.
- No single technique works with all types of polar compounds, but the use of the Teledyne Combi*Flash* system and Redi*Sep* Rf Gold columns offers the flexibility of several purification routes.

Part Numbers (PN) are Teledyne Isco catalog numbers, unless otherwise stated.

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