

# Improvements in Reverse Phase Medium Pressure Liquid Chromatography (MPLC) as an Alternative to Preparative HPLC

## Overview

Preparative HPLC has long been a mainstay in organic chemistry labs for purifying compounds on reverse phase. New Flash chromatography systems (MPLC) with higher pressures and flow rates, such as the *CombiFlash* Rf, allow the use C18 columns with reduced particle size to give results approaching those of preparative HPLC systems. Purifications of various compounds were compared using the *CombiFlash* Rf system, and high performance *RediSep* Rf Gold C18, standard *RediSep* Rf C18, and prep HPLC columns.

## Discussion—Comparison of Preparative HPLC to Flash

### Flexibility—able to switch solvent systems

Teledyne Isco's *CombiFlash* Rf system can be changed easily between normal and reverse phase solvent systems with little more than a flush of the system with a solvent miscible to both solvent systems used. HPLC systems are generally configured only to run reverse phase C18 chromatography.

### Sample Loading Options

*CombiFlash* MPLC systems allow a variety of loading techniques. Samples can be dissolved then adsorbed on a variety of materials prior to loading or injected as a liquid. The main advantage of MPLC injection mechanisms is the high sample recovery. The compounds are easily transferred to the column during the sample run.

**Solid Load Cartridges** — Solid loading has many advantages over liquid injection. It makes the best use of the automation capabilities of modern MPLC instruments such as the *CombiFlash* Rf. Solid loading allows “walk-away” automation where the sample is placed on the system, the user presses a button to start the run, then walks away to work elsewhere. The system equilibrates the column, loads the sample, collects the peaks

and re-equilibrates the column without further intervention. Solid load cartridges allow compounds that have limited solubility in the mobile phase to be easily loaded into a MPLC system without also injecting a slug of strong solvent that reduces column effectiveness. HPLC systems require the compounds to be completely soluble for injection, thus requiring strong solvents which reduce the ability of the mixture to initially bind to the column. There are many sorbent choices for solid load cartridges. Compounds loaded onto C18 columns can be adsorbed onto Celite; this material weakly interacts with most compounds so they elute onto the column easily. C18 cartridges are also available as a disposable pre-column to extend the life of the C18 column. For normal phase purification, compounds can be adsorbed onto silica, Celite, or other materials.

Compounds can be loaded onto solid load cartridges pre-packed with celite or C18. Typically, the material is dissolved in the strong solvent and air is blown through the cartridge (often using a vacuum) to dry the strong solvent. The Teledyne Isco Solid Load Cartridge Dryer (60-2200-010) allows fast drying of multiple samples on a vacuum system.

**Liquid Loading** — Liquid loading is also an option on flash systems. The sample is injected directly onto the column, or through the valve on *CombiFlash* systems with a syringe. By washing, and applying the wash to the column, nearly 100% of the compound is transferred for purification.

### Filtering of Solvents

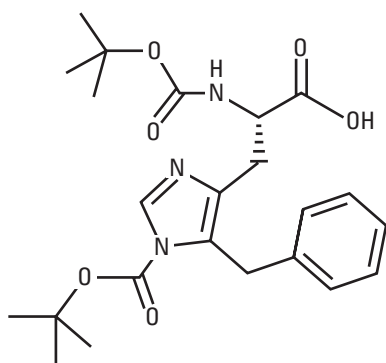
MPLC systems do not require that solvent be filtered or degassed. The pumps are robust and are designed to tolerate a small amount of particulates in the solvent. Air bubbles do not cause MPLC systems to lose their prime. Since solvents do not need filtering, there is less time required to start purification.

## Results and Discussion

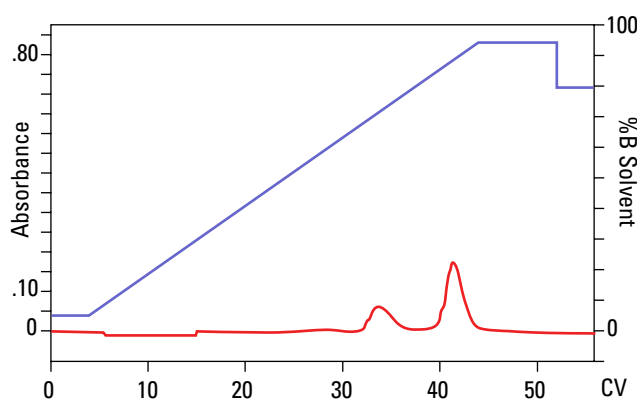
### Comparison of RediSep Rf C18, RediSep Rf Gold C18, and HPLC

A mixture containing 5-benzyl-N- $\alpha$ -N-im-di-*t*-Boc-L-His<sup>1</sup> (Compound A) was purified on a CombiFlash Rf system using high performance RediSep Rf Gold C18 and standard RediSep Rf C18 columns. The solvent system was 5–95% ACN:H<sub>2</sub>O) both containing 0.1% TFA. All experiments were performed with a detection wavelength of 214 nm. Figures 1 and 2 demonstrate that the RediSep Rf Gold C18 column allowed much higher loading and provided a sharper peak as compared to a standard RediSep Rf C18 column.

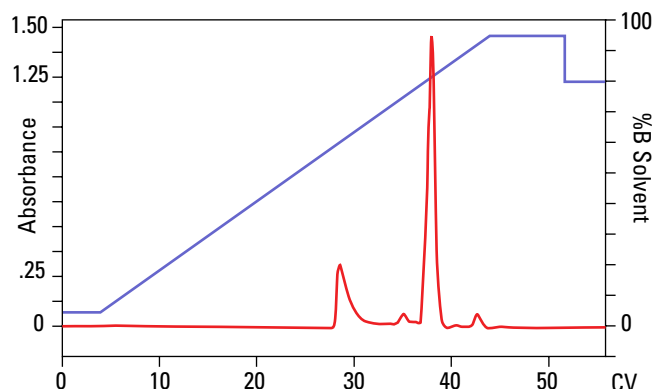
Compound A



(*S*)-3-[5-benzyl-1-(*tert*-butoxycarbonyl)-1*H*-imidazol-4-yl]-2-*tert*-butoxycarbonylamino)propanoic acid

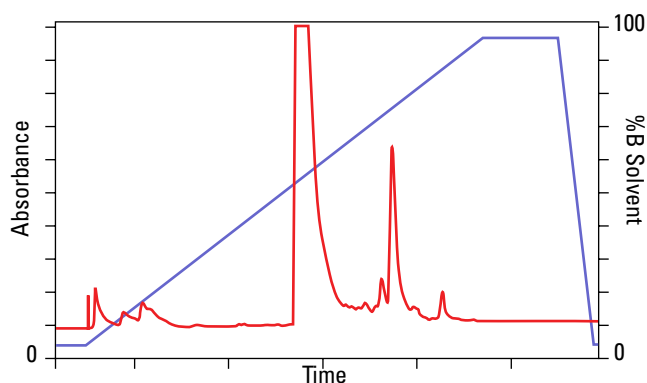


**Figure 1: CombiFlash Rf system chromatogram of 10 mg purification** of Compound A on a 13g RediSep Rf C18 column



**Figure 2: CombiFlash Rf system chromatogram of 46 mg purification** of Compound A on a 15.5g RediSep Rf Gold C18 column

The mixture was also purified on an HPLC system using the same solvent system (Figure 3).

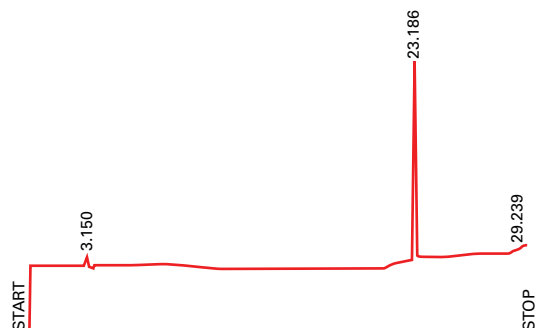


**Figure 3: Waters DeltaPrep 4000 system purification of 10 mg sample** of Compound A using a Vydac 10x250 mm column, 5 $\mu$  particle size

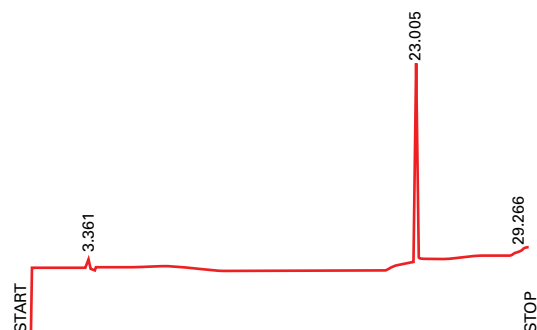
**Time Savings** – The CombiFlash Rf system was able to purify five samples in the time required to complete the first sample on the HPLC system. In the time required to filter the solvent and sample for prep HPLC, the CombiFlash Rf system completed the first two purifications. After the sample was injected on the prep HPLC system, three additional samples were purified on the CombiFlash Rf system by the time the prep HPLC run was complete — over a 13-fold increase in productivity.

1. The collaboration of Dr. David Smith at Creighton University, School of Pharmacy, is gratefully acknowledged.

**Compound Purity** – The *CombiFlash* Rf system yielded purified compounds with the same purity profile as the prep HPLC system. The main products from the high performance *RediSep* Rf Gold C18 column and the *Vydac* column were run on an analytical system (Waters 484 Detector with 600E Controller, 600 Pump, HP 3395 Integrator with a Waters Symmetry 300Å, 5µ 4.6×250 mm column. Gradient from 5–100% ACN in H<sub>2</sub>O, both containing 0.1% TFA over 30 minutes). The analytical HPLC of both purifications (Figures 4 and 5) are identical.



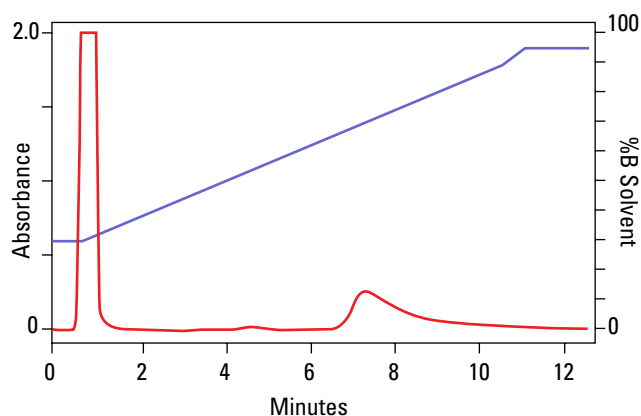
**Figure 4: Analytical HPLC of Compound A purified by a *CombiFlash* Rf system** using a *RediSep* Rf Gold C18 column



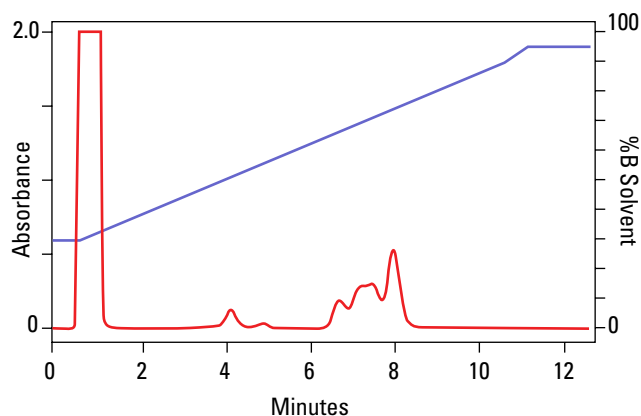
**Figure 5: Analytical HPLC of Compound A purified on a *DeltaPrep* 4000 system** using a *Vydac* 10x250 mm column, 5µ particle size

## Purification of Protected Aminoglycoside Antibiotics on a *CombiFlash* Companion<sup>®</sup>

The optimal flow rate for *RediSep* Rf Gold C18 columns is similar to that of silica columns of a similar dimension—35 mL/minute for the 15.5 gram column. However, some older flash systems are unable to run at optimal flow rates due to the back pressure generated. The *CombiFlash* Companion system is limited to 50 psi back pressure, or a 26 mL/minute flow rate for the high performance *RediSep* Rf Gold C18 column. Despite the lower flow rate, high performance C18 columns still show improved resolution compared to standard C18 Flash columns on a *CombiFlash* Companion system.



**Figure 6: Protected Aminoglycoside purified on a standard C18 Flash column** on a *CombiFlash* Companion system



**Figure 7: Protected Aminoglycoside purified on a *RediSep* Rf Gold C18 Flash column** on a *CombiFlash* Companion system

## Conclusion

The combination of high performance RediSep Rf Gold C18 columns and CombiFlash systems together provide purification on reverse phase that approaches results from prep HPLC. The CombiFlash Rf system is

easy to setup and use. The software is intuitive, allowing high productivity even from occasional users. The integrated design saves valuable bench space.

**Table 1: RediSep Rf C18 Columns**

| Sample Size    | Column Size | Teledyne Isco Part Number | Description                     |
|----------------|-------------|---------------------------|---------------------------------|
| 4.3–86 mg      | 4.3 g       | 69-2203-410               | 4.3 g C18 column, package of 2. |
| 13–260 mg      | 13 g        | 69-2203-411               | 13 g C18 column, package of 1.  |
| 26–520 mg      | 26 g        | 69-2203-412               | 26 g C18 column, package of 1.  |
| 43–860 mg      | 43 g        | 69-2203-413               | 43 g C18 column, package of 1.  |
| 86 mg – 1.72 g | 86 g        | 60-2203-416               | 86 g C18 column, package of 1.  |
| 130 mg – 2.6 g | 130 g       | 69-2203-414               | 130 g C18 column, package of 1. |
| 240 – 4.8 g    | 240 g       | 69-2203-418               | 240 g C18 column, package of 1. |
| 360 mg – 7.2 g | 360 g       | 69-2203-415               | 360 g C18 column, package of 1. |

**Table 2: RediSep Rf Gold High Performance C18 Columns**

| Sample Size    | Column Size | Teledyne Isco Part Number | Description  |
|----------------|-------------|---------------------------|--|
| 4.3 – 110 mg   | 5.5 g       | 69-2203-328               | 5.5 g High Performance C18 columns, package of 2 columns.                                    |
| 13 – 300 mg    | 15.5 g      | 69-2203-334               | 15.5 g High Performance C18 column, package of 1 column.                                     |
| 26 – 600 mg    | 30 g        | 69-2203-335               | 30 g High Performance C18 column, package of 1 column.                                       |
| 43 – 1000 mg   | 50 g        | 69-2203-336               | 50 g High Performance C18 column, package of 1 column.                                       |
| 86 mg – 2 g    | 100 g       | 69-2203-337               | 100 g High Performance C18 column, package of 1 column.                                      |
| 130 mg – 3 g   | 150 g       | 69-2203-338               | 150 g High Performance C18 column, package of 1 column.                                      |
| 240 – 5.5 g    | 275 g       | 69-2203-339               | 275 g High Performance C18 column, package of 1 column.                                      |
| 360 mg – 8.3 g | 415 g       | 69-2203-341               | 415 g High Performance C18 column, package of 1 column.<br>Contact factory for availability. |

**Table 3: RediSep Rf Empty and C18 Prepacked Solid Load Cartridges**

| Cartridge Size | Teledyne Isco Part Number | Description  |
|----------------|---------------------------|--|
| 5 g            | 69-3873-235               | Empty universal Rf sample load cartridges (holds up to 5 gram), package of 30. <sup>a</sup>  |
| 25 g           | 69-3873-240               | Empty universal Rf sample load cartridges (holds up to 25 gram), package of 30. <sup>a</sup> |
| 65 g           | 69-3873-225               | Empty Rf sample load cartridges (holds up to 65 gram), package of 12.                        |
| 5 g            | 69-3873-237               | Prepacked universal Rf sample load cartridge, 5 gram C18, package of 5. <sup>a</sup>         |
| 25 g           | 69-3873-242               | Prepacked universal Rf sample load cartridge, 25 gram C18, package of 4. <sup>a</sup>        |

a. These cartridges (200 psi, one-piece design with locks, no sleeve) require a new Solid Load Cartridge Cap (SLCC) on CombiFlash Rf systems.  
Part Numbers: 5 g SLCC, 60-5237-047; 25 g SLCC, 60-5237-048.

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