Using the Solid Load Cartridge Bypass

on Combi*Flash*® Systems

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Abstract

On occasion, impurities precipitate in the solid load cartridge as compounds are purified. This can cause high back pressures that can be relieved by removal of the solid load cartridge. Peak*Trak*® versions 1.7 and newer have the ability to bypass the solid load cartridge during a run to reduce the back pressure. However, prematurely bypassing the solid load cartridge may leave the desired compound trapped on the solid load cartridge, resulting in a failed purification. This document describes how to determine when the desired compound has eluted from the solid load cartridge so that the Solid Load Cartridge Bypass feature may be used successfully.

Solid Load Cartridge Bypass Usage

1. Run a TLC plate

Before starting the run, one should run a TLC plate. Measure the R_f (Retention Factor) of the slowest eluting compound of interest. The R_f should be between 0.2 and 0.8. (This is the same range used by the Combi*Flash* Gradient Optimizer.) Note the percentage of solvent B used to run the TLC plate. If a compound displays tailing peaks as streaks on TLC, the R_f used corresponds to the bottom of the streak.

2. Load the Sample in the Solid Load Cartridge

When loading the solid load cartridge, determine the cartridge volume according to the selected cartridge size (Table 1). If the cartridge is only partially filled with silica, use the cartridge volume as if the cartridge was completely filled with silica.

Table 1:
Redi*Sep*® Solid Load Cartridge Volumes

Cartridge Size (g)	Cartridge Volume (mL)
5	10
25	45
65	110
260	500
750	1100

3. Run the Sample

Read the flow rate from the Status Bar in the upper right corner of the Peak*Trak* display (Figure 1). If the flow rate is operating at the programmed setting, the system is running fine. If the operating flow rate is slower than programmed, note the current flow rate from the display for use in the equation. If the flow rate is fluctuating, use the lowest displayed flow rate.

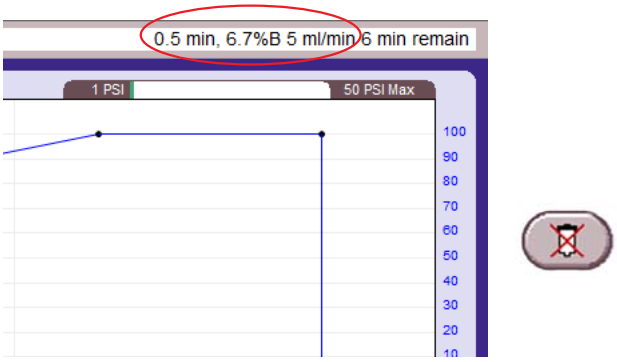

Figure 1: Combi*Flash* system status showing time and %B and Solid Load Cartridge Bypass button

Use the equation below to determine when to bypass the solid load cartridge:

$$T = \frac{\text{Cartridge Volume}}{(R_f \times \text{Flow Rate})}$$

T is the time, in minutes, to wait after the system reaches the %B solvent used to run the TLC plate. *Cartridge Volume* is read from Table 1. *Flow Rate* is the current flow rate displayed on the status bar, and R_f is the retention factor measured from the TLC plate.

Touching the Solid Load Bypass button causes the system to momentarily stop the pumps and move the injection valve so that the solid load cartridge is no longer in the flow path. the pumps then resume to move the compounds through the column.

Example

For example, a TLC run in 30% ethyl acetate in hexane indicates that the desired compound in a mixture has an R_f of 0.35. The sample mixture is then placed in a 5 g solid load cartridge and loaded on the Combi*Flash* system running at 60 mL/minute on an 80 gram Redi*Sep* Rf silica column. The time after reaching 30% ethyl acetate in a gradient is given by:

$$T = \frac{10\ \text{mL}}{(0.35 * 60\ \text{mL/minute})}$$

$$T = 0.48\ \text{minutes}$$

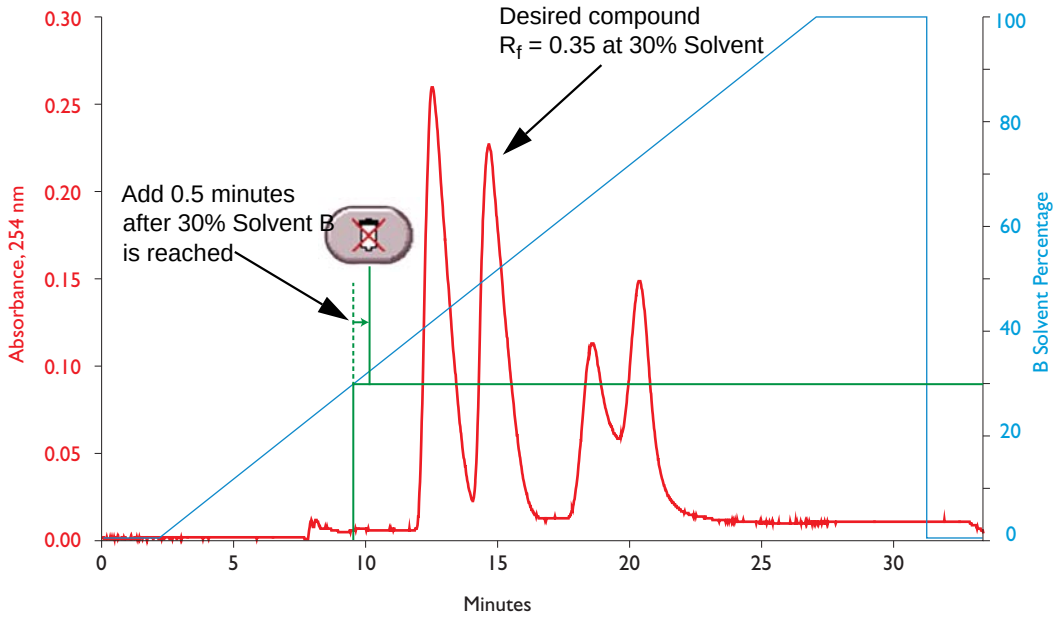

Figure 2: Purification of catechol and resorcinol
Solid load cartridge bypassed at 0.5 minutes past 30% B

During this run, the Solid Load Cartridge Bypass feature can be used 0.5 minutes or more after the gradient reaches 30% ethyl acetate without adversely affecting the purification of desired compounds (Figure 2).

The current %B and run time is displayed in the upper right corner of the Peak*Trak* display in the status bar. The actual percent B solvent used for the TLC plate does not matter because if the compound moves slower on the TLC plate from a low %B, it will take longer to elute and the equation will compensate for the longer elution time. In addition, the compound is moving during the gradient—the compound is likely to elute from the cartridge prior to the time predicted by the equation. Evidence for this is that two other peaks eluted after the desired compound in Figure 2.

Theory of Operation

Silica column methods are generally derived from Thin Layer Chromatography (TLC) plates. There is a well-known relationship between the elution time of a compound from a column and the retention factor on a TLC plate:

$$CV \frac{1}{R_f}$$

CV is the number of column volumes required to elute the compound and R_f is the retention factor measured from the TLC plate. If $R_f = 0.5$, the compound will elute in two column volumes. If the volume of the solid load cartridge, elution time in CV, and flow rate are known, it is possible to determine the time for the compound to elute from the solid load cartridge. Table 1 lists the approximate cartridge volume for various Redi*Sep* solid load cartridges. The listed volumes are approximate and represent a worst-case situation. For a partially filled cartridge, use the volume for the filled cartridge.

Conclusion

The Solid Load Bypass feature can be used to reduce backpressure. It is possible to determine when to use the Solid Load Cartridge bypass by using TLC data.The calculation used is conservative but allows the user to minimize the time that the solid load cartridge is in the flow path.

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