Optimal Flow Rates for RediSep® Normal Phase Columns in 4, 12, 40, and 120 gram sizes

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Chromatography Application Note AN03

Application Overview

Liquid chromatography columns have an ideal flow rate or range of flow rates where the column is most efficient. This measurement of column performance is commonly referred to as a van Deemter curve where the height equivalent to a theoretical plate (HETP) is plotted against the flow rate. In this case, the plate count is plotted vs. flow rate for a given column. The narrower a peak is, the greater the number of theoretical plates will be. Flow rate through the column is optimized for greatest theoretical plate count.

Low flow rates can reduce column efficiency due to diffusion. Diffusion is the result of the amount of time the sample spends in the tubing and column before it is passed through the detector.

At higher than ideal flow rates, efficiency is lost due to turbulence. The effect of turbulence is the same as diffusion. The resulting peak is broadened at the base and has a rounded peak. In some cases, it is possible for a column to overpressure before the efficiency trends downward.

General Method

Normal Phase columns are installed on an Isco CombiFlash® Sq 16x which is programmed to run various flow rates. Normal phase 2-peak standard is 4-methoxyacetophenone and acetophenone. Mobile phase A is hexane and mobile phase B is ethyl acetate. The standard is analyzed for theoretical plate count and the plate counts are plotted vs. flow rate. Each column size has a method optimized for that column size. The flow rate is the only parameter that has been altered during any single run to establish efficiency curves.

Effective column volume for the normal phase columns can be determined by using chloroform as a mobile phase and injecting heptane as an unretained standard. The time interval from injection to detection is the instrument and column void volume. Instrument void volume is subtracted and remainder is the effective interstitial void volume of the column.

Analytical Results

RediSep 4 gram Normal Phase Columns

Using the stated method the determined column volume is 4.8 mL. The optimum flow rate for these columns is approximately 18 mL/min. The 4 gram column has a very flexible flow range where performance is very similar from 16–22 mL/min and a broader range of 12–25 mL/min that could be used. This has been determined by testing columns with a standard. By altering flow rates the column efficiency and thus the theoretical plate count is altered, this data is then plotted vs. flow rate (Figure 1).

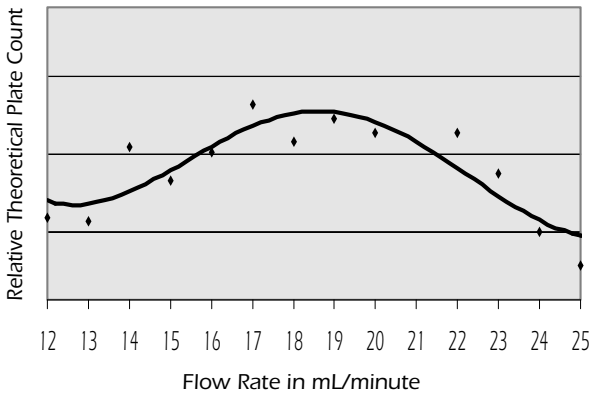

Figure 1: 4g Normal Phase Efficiency Curve

RediSep 12 gram Normal Phase columns

Using the stated method the determined column volume is 16.8 mL. The optimum flow rate is approximately 30 mL/min. The 12 gram column has a very flexible flow range where performance is very similar from 25–40 mL/min and a broader range of 15–50 mL/min that could be used. This has been determined by testing columns with a standard. By altering flow rates the column efficiency and thus the theoretical plate count is altered, this data is then plotted vs. flow rate (Figure 2).

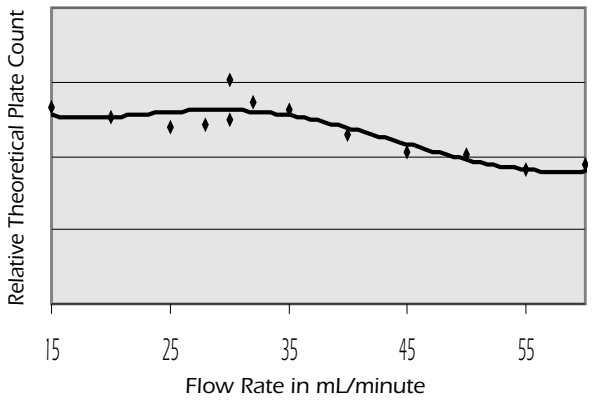

Figure 2: 12 g Normal Phase Efficiency Curve

RediSep 40 gram Normal Phase Columns

Using the stated method the determined column volume was established at 48 mL.

For efficiency curves, data is acquired at many flow rates to minimize variability. Standard used was normal phase two peak standard for 40 g.

The 40 gram column has a very flexible flow range where performance is best at 35–40 mL/min and the broader usable range is 25–50 mL/min. This has been determined by testing columns with a standard. By altering flow rates the theoretical plate count is altered, this data is then plotted vs. flow rate (Figure 3).

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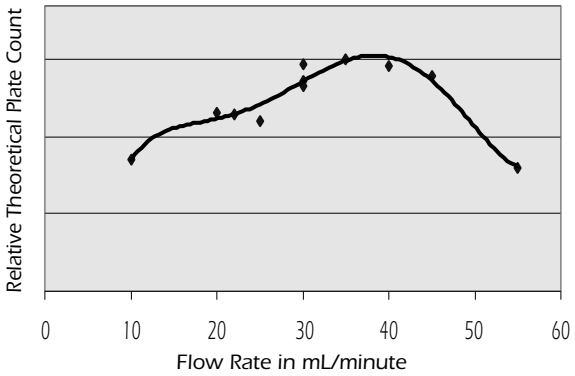

Figure 3: 40g Normal Phase Efficiency Curve

Redi*Sep* 120 gram Normal Phase Columns

Using the stated method the determined column volume was established at 192 mL.

For efficiency curves data is acquired at many flow rates to minimize variability. Standard used was normal phase two peak standard for 120 g.

The 120 gram column has a very flexible flow range where performance is best from 75–95 mL/min and a usable range of 60–120 mL/min. The limiting factor for the upper flow rate is apparently back pressure rather than column performance. This has been determined by testing columns with a consistent standard. By altering flow rates the theoretical plate count is altered, this data is then plotted vs. flow rate (Figure 4).

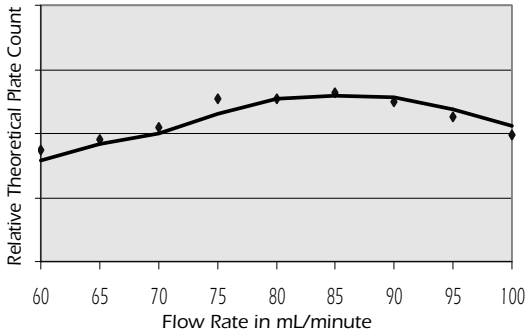

Figure 4: 120g Normal Phase Efficiency Curve

Summary

The column volume for Redi*Sep* 4 g Normal Phase columns is 4.8 mL, optimum flow rate is approximately 18 mL/min with a range of 16–22 mL/min.

The column volume for Redi*Sep* 12 g Normal Phase columns is 16.8 mL, optimum flow rate is approximately 30 mL/min with a range of 25–40 mL/min.

The column volume for Redi*Sep* 40 g Normal Phase columns is 48 mL, optimum flow rate is approximately 40 mL/min with a usable range of 25–50 mL/min.

The column volume for Redi*Sep* 120 g Normal Phase columns is 192 mL, optimum flow rate is approximately 85 mL/min with a range of 60–120 mL/min.

Table 1 contains additional parameters concerning required air purge time to clear the column of solvent at the conclusion of a run. When used, solid sample load cartridges must also be purged. Add one minute to the air purge time for the 5 gram cartridge size; add 2.5 minutes for the 25 gram size.

Table 1: Redi*Sep* Column Data

Column Size	Low Flow Rate	High Flow Rate	Ideal flow rate	Column Volume	Air Purge
4 gram	16 mL/min	22 mL/min	18 mL/min	4.8 mL	2 min
12 gram	25 mL/min	40 mL/min	30 mL/min	16.8 mL	3 min
40 gram	25 mL/min	50 mL/min	40 mL/min	48 mL	4 min
120 gram	60 mL/min	120 mL/min	85 mL/min	192 mL	5 min

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