VERITY® HPLC System: Prep HPLC Scale Up

TECHNICAL NOTE TN231

TECHNICAL FEATURES

- Wide flow rate range from the VERITY® 3240 High Pressure Binary Gradient Pump
- Multiple injection modules on the GX-281 Liquid Handler
- Custom scale up task in TRILUTION LC to allow method adjustment by column choice
- Automated column switching

TECHNICAL BENEFITS

- Eases scaling-up purification work
- Automation of purification scale-up
- A single purification system for several different scales of work
- Walk-away column switching automation

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INTRODUCTION

Preparative high performance liquid chromatography (HPLC) is a well-established method for purifying target compounds. Method development is usually done at a smaller scale to define sufficient resolution between the peaks without wasting large amounts of sample. It is common to scale-up the purification from a smaller column to a larger preparative one. For example, moving from a 21.2 mm column to a 50 mm column to increase mass purified from milligrams to tens of grams. This enables more of the target compound to be purified per run and increases overall throughput.

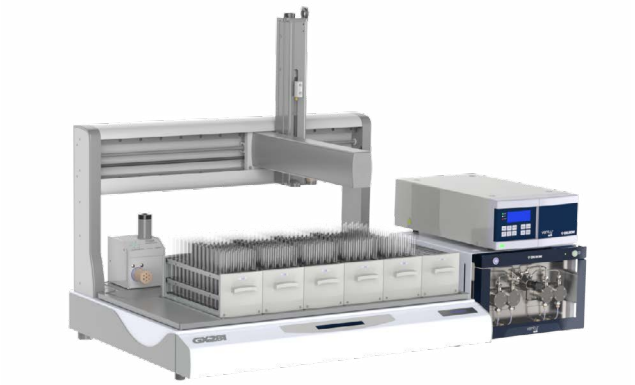

Figure 1
VERITY® HPLC Purification System

Scaling up a purification requires corresponding changes in flow rate, injection volume, and gradient conditions—depending on the columns. For a linear scale-up, calculations are used to translate parameters from the smaller to the larger column, for example, flow rate and injection volume.

$$\text{Flow Rate}_{\text{Column 2}} = \text{Flow Rate}_{\text{Column 1}} \times ((\text{Diameter}_{\text{Column 2}})^2/(\text{Diameter}_{\text{Column 1}})^2)$$

$$\text{Injection Volume}_{\text{Column 2}} = \text{Injection Volume}_{\text{Column 1}} \times ((\text{Diameter}_{\text{Column 2}})^2/(\text{Diameter}_{\text{Column 1}})^2)$$

GILSON®

TRILUTION® LC Software can automatically perform these calculations based on a column ID input, automatically calculating the scale-up flow rate and injection volume. This technical note demonstrates the use of a Gilson HPLC system to scale-up a purification. This system is controlled using TRILUTION® LC Software, which automates the HPLC method and provides full system control. The software automatically calculates the scaled-up flow rate and injection volume based on a column ID input.

The VERITY 3240 Pump in the purification system described here has a broad flow rate range, allowing for purifications on HPLC columns from 10 mm to 50 mm ID on the same system. The purification system is further enhanced by the GX-281 Liquid Handler, which includes the option for multiple injectors, accommodating both 1/16" and 1/8" sample loops, which allows for the wide range of sample injection volumes necessary for this kind of work.

Objective

The purification run aims to scale up a separation from a 21.2 mm ID column to a 50 mm ID column. Method and run parameters were set to automatically apply the appropriate scale-up parameters depending on the column selected at run time.

MATERIALS & METHODS

Materials

All chemicals were ACS grade or higher quality. Caffeine (Sigma-Aldrich C0750[1]), theophylline (Sigma-Aldrich T1633[2]), and phenol (Sigma-Aldrich P5566[3]) were dissolved in water. Two different test mixes were used: 1.25 mg/mL caffeine, 1.25 mg/mL theophylline, 7.5 mg/mL phenol and 2.5 mg/mL caffeine, 2.5 mg/mL theophylline, 15 mg/mL phenol. The higher concentration mix was only used in the secondary test with the 50 mm ID column.

Software and Hardware

- TRILUTION LC v4
- HPLC System
  - o GX-281 Liquid Handler
  - o GX Prep Solvent System
  - o GX Direct Injection Module (1/16", 5 mL loop)
  - o GX Direct Injection Module (1/8", 5 mL loop)
  - o VERITY 3240 Pump
  - o VERITY® 1741 UV-VIS Detector (0.1 mm, 1/8" flow cell)
  - o Two VALVEMATE® II Valve Actuators (2-position, 6-port valve)
- Phenomenex® Luna® Preparative HPLC column (50 x 21.2 mm, 5µm, product code 00B-4252-P0-AX) with guard column
- Phenomenex® Luna® Preparative HPLC column (50 x 50 mm, 5 µm, product code 00B-4252-V0-AX)

System Setup

In a preparative HPLC system, a 1/16" injector and sample loop are recommended for columns with a 30 mm ID or smaller, while for 50 mm ID or larger columns a 1/8" injector and sample loop is recommended. To accommodate these different injection options on a single HPLC system, dual injectors with different size valves and injection loops were installed. When this setup is used, VALVEMATEs route the mobile phase flow appropriately. The system used two GX Direct Injection Modules, one with a 1/16" injection valve connected to the 21.2 mm column and one with a 1/8" injection valve connected to the 50 mm column. The two VALVEMATEs were used to automatically select the appropriate injector and column as depicted in Figure 2.

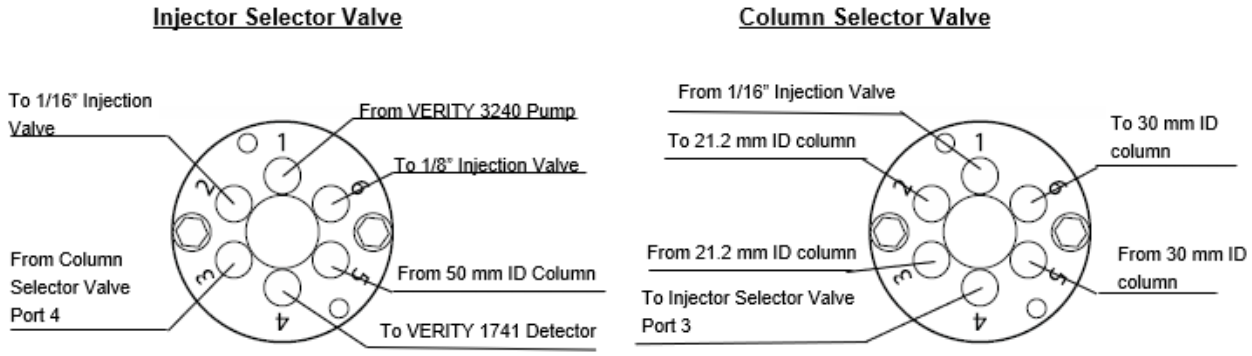

Figure 2
VALVEMATE II Plumbing Diagrams

RUN PARAMETERS

The run parameters are shown in Table 1. Two data channels of the VERITY 1741 Detector were utilized. A custom task was used to automatically set the mobile phase flow rate and injection parameters according to the user input column ID size. By incorporating this custom task, a single method can be used for both column IDs, applying the appropriate scale up parameters depending on the column selected at run time, translating the flow rate linearly. The custom task also automatically switches the valve modules to the appropriate positions to direct the mobile phase flow to the selected column. By including automated column switching with this setup, controlling the system is simple. Only the sample location and column ID need to be set (Figure 3).

	Method Name	Sample Name	#Sample Well	#Column ID
1	Scale Up Injection Method	Initial Sample	1	21.200
2	Scale Up Injection Method	Initial Sample	1	21.200
3	Scale Up Injection Method	Scale up Sample	1	50.000
4	Scale Up Injection Method	Scale up Sample	1	50.000

Figure 3
Run Time Sample List Entry

Table 1
Run Parameters

Parameters	Conditions
Mobile Phase	Solvent A: Water Solvent B: Methanol
Gradient Conditions	0 min: 25% B 0.75 min: 25% B 2.65 min: 75% B 2.85 min: 25% B 4.15 min: 25% B
Flow Rate	20 mL/min (21.2 mm ID column) 111.25 mL/min (50 mm ID column) [automatically calculated]
Injection Volume	500 µL (21.2 mm ID column) 2781.24 µL (50 mm ID column) [automatically calculated]
UV Detection	Channel 1: 254 nm Channel 2: 270 nm Data rate: 20 pt/sec
Fraction Collection Conditions	UV signal at 254 nm, slope 40 (front and back slope), peak width 0.2

RESULTS AND DISCUSSION

This technical note demonstrates the use of a Gilson HPLC system for purification scale-up with two different size preparative columns. The run with the 21.2 mm column was performed first (Figure 4), monitoring UV absorbance at two different wavelengths with the UV detector and collecting fractions as programmed in the method. TRILUTION LC automatically performed calculations at run time to calculate the injection volume and flow rate for the larger 50 mm column (Figure 5). The chromatograms from the 21.2 mm column and the 50 mm column were overlaid in Figure 6. This result overlay shows the retention time and peak similarity with the linear flow rate translation from the 21.2 mm column to the 50 mm column. Scaling up from the 21.2 mm column to the 50 mm column is greatly simplified using TRILUTION LC to automatically apply the appropriate scale-up parameters based on the user input column size. By fully automating the system, all necessary parameters are calculated, and the system automatically switches between columns at run time based on the user input column size.

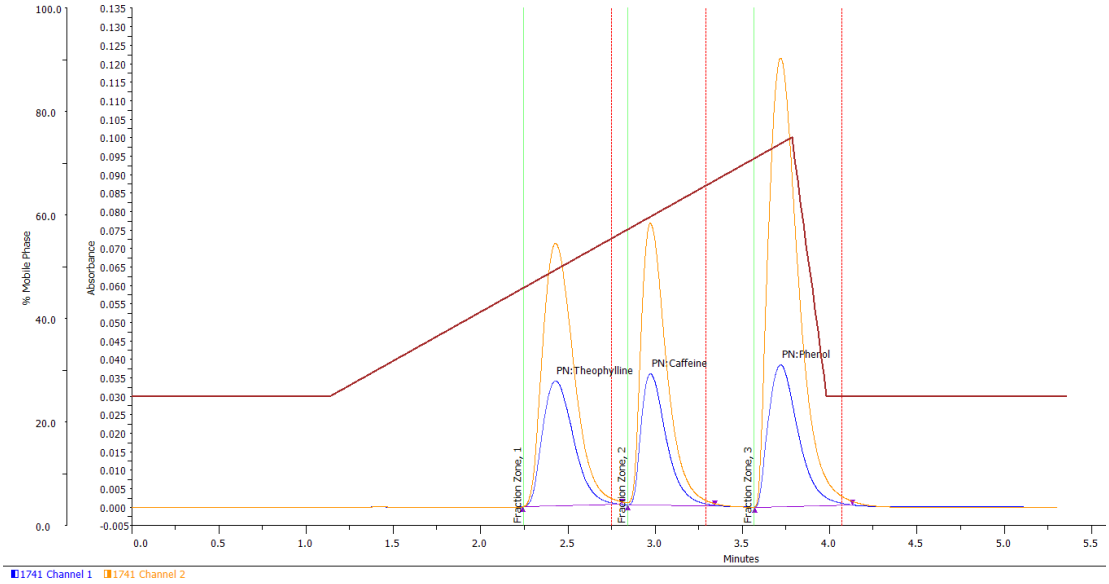

Figure 4

Separation with 21.2 mm ID column, 254 nm (blue) and 270 nm (orange)

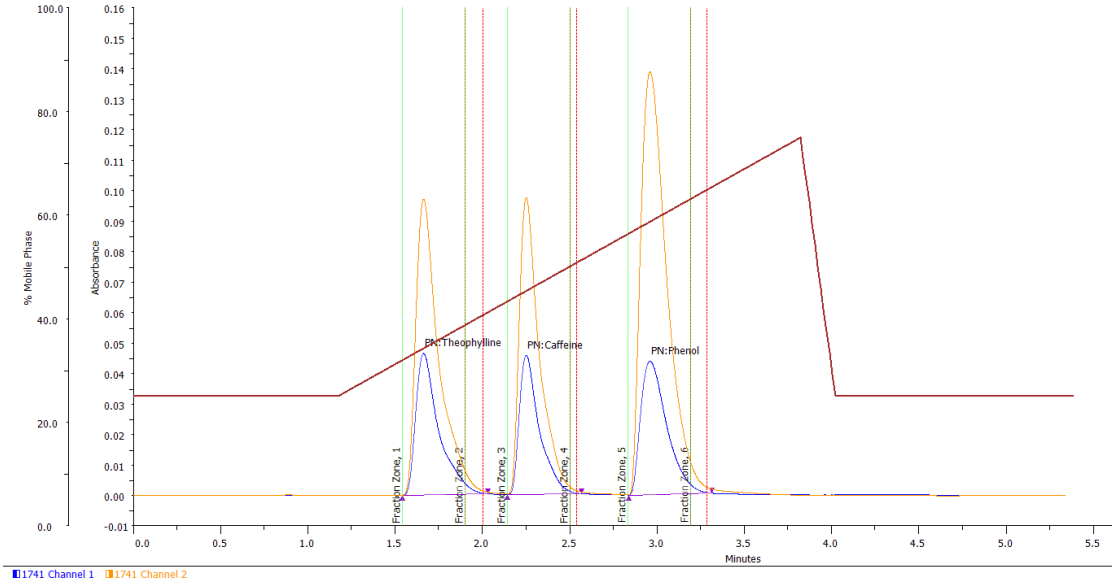

Figure 5

Separation with 50 mm ID column, 254 nm (blue) and 270 nm (orange)

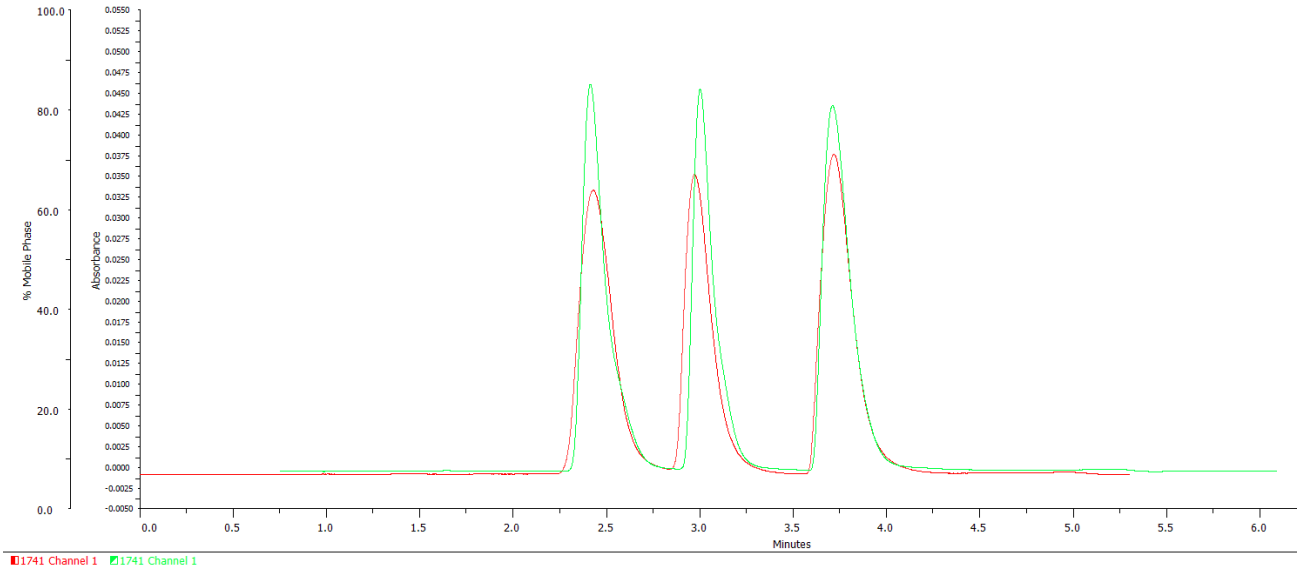

Figure 6

Separation with 21.2 mm ID column (red) and 50 mm ID column (green), 254 nm, 50 mm ID column data offset by time

Note: Data from the 50 mm column was offset by time to compensate for the time delay introduced to the 21.2 mm column by the system tubing.

A second test was performed to compare two injections of different sizes but the same amount on the 50 mm column; one with a larger injection volume of a lower concentration sample and the other with a smaller injection volume of a higher concentration sample. The chromatograms from the two injections on the 50 mm column (Figure 7) show how peak shape can be impacted by injection volume, as the peaks from the larger volume injection (green) are broader than the peaks from the smaller volume injection (red). When loading greater sample mass onto a column, it is preferable to increase the sample concentration rather than the sample volume, if possible.

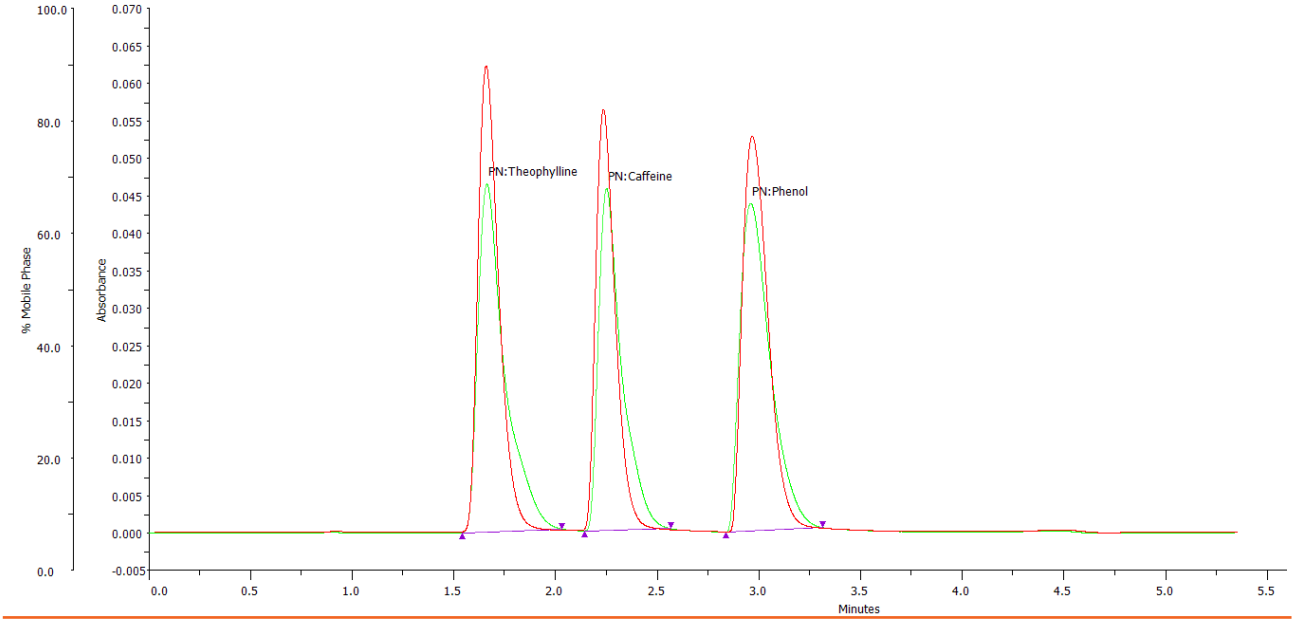

Figure 7

Separation of same sample concentration with 50 mm ID column, larger injection volume (green) and smaller injection volume (red)

CONCLUSIONS AND BENEFITS

- A Gilson HPLC system allows the connection of multiple columns to the same system, simplifying the scale-up process by requiring the user only to select the desired column at run time
- The VERITY 3240 Pump has a broad flow rate range allowing semi-preparative and preparative purification scales to be performed on one system with reproducible retention times
- Having the ability to add multiple injectors to the GX-281 allows easy and optimal running of multiple purification scales on a single system without compromising on injection volumes
- TRILUTION LC allows the use of a single method to achieve purification with different size columns, automatically adjusting necessary parameters according to the column at run time
- Different purifications from the same or different samples can be run at different scales from a single sample/run list, allowing for advanced sharing or open access to the system
- Adding automated column switching to the method allows the user to walk away from the system with the added flexibility of automatically changing between different size columns

REFERENCES

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2. Product information for theophylline. https://www.sigmaaldrich.com/US/en/product/sigma/t1633
3. Product information for phenol. https://www.sigmaaldrich.com/US/en/product/sial/p5566