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A Practical User Guide for the Determination of Optimal Purification Gradients for the Gilson PLC 2020

Application Note PHA0211

Keywords

Purification, Pharmaceuticals, Optimal Solvent Gradients, Isolation Gradients, Organic Compounds, PLC 2020, Preparative Chromatography

Introduction

This study was performed by Janice Chin, Courtney Cullis, Kenneth Gigstad, Matthew Jones, and He Xu from Millennium Pharmaceuticals, Inc., Cambridge, MA, USA.

This application note provides a practical guide on the efficient use of the Gilson PLC 2020 Personal Purification System along with an experimentally derived correlation table for the selection of 'optimal' solvent gradients or 'isolation' gradients to be used for routine purifications of pharmaceutical organic compounds.

Simple touch screen control allows for quick modifications of preparative methods based on retention times obtained from standard HPLC analytical methods. The flexibility to set preparative solvent gradients for purification injections using method modifications or variables caters to an 'open access' laboratory environment where the main focus is to quickly elute products of interest at an 'optimal' time off the column, with minimal interference, and with reduced solvent waste. The following guidelines were compiled to ensure optimal preparative methods were obtained for each sample.

Figure 1. Gilson PLC 2020 Personal Purification System

Gilson, Inc. World Headquarters
Middleton, WI 53562 USA
Telephone: 800-445-7661

Materials & Methods

Materials

All solvents used were HPLC grade or higher. All reagents were ACS grade or better.

Analytical System:

System: Agilent 1100 HPLC System with Diode Array Detector
Mobile Phase: Acetonitrile:H_2O solvent gradients with 0.1% formic acid
Column: Waters Symmetry, C18, 3.5 micron, 4.6 x 100 mm

Preparative System:

System: Gilson PLC 2020
Mobile Phase: Acetonitrile:H_2O solvent gradients with 0.1% formic acid
Column: Waters SunFire™ C18 OBD Column, 5 micron, 19 x 150 mm

Methods – Sample Preparation

Effective sample preparation will aid in maintaining the system in good working order and will help to increase the longevity of the column. Ensure that a thorough work up has been carried out on the sample of interest prior to running on the HPLC system.

- To prepare the sample, dissolve compound (30 mg crude) in 2ml of Dimethyl Sulfoxide (DMSO), Methanol (MeOH), or water (or a combination thereof). Do not use any solvents that are incompatible with the mobile phase or could potentially damage the column.
- Ensure complete sample dissolution and filter before injecting the solution onto the system.
- Run an analytical analysis on your sample (see **Figure 2**) using a standard gradient to obtain a retention time and λ max for the peak of interest.

The following analytical system gradient was established as a general method with a flow rate of 1 mL/min.

Table 1. General Analytical System Gradient

Time (min)	**%A** 99% Water:1% Acetonitrile:0.1% Formic Acid	**%B** 95% Water:5% Acetonitrile:0.1% Formic Acid
0.00	95	5
10.00	0	100
12.50	0	100
13.00	95	5

Figure 2. Simulated Analytical System Chromatography Using Standard Gradient

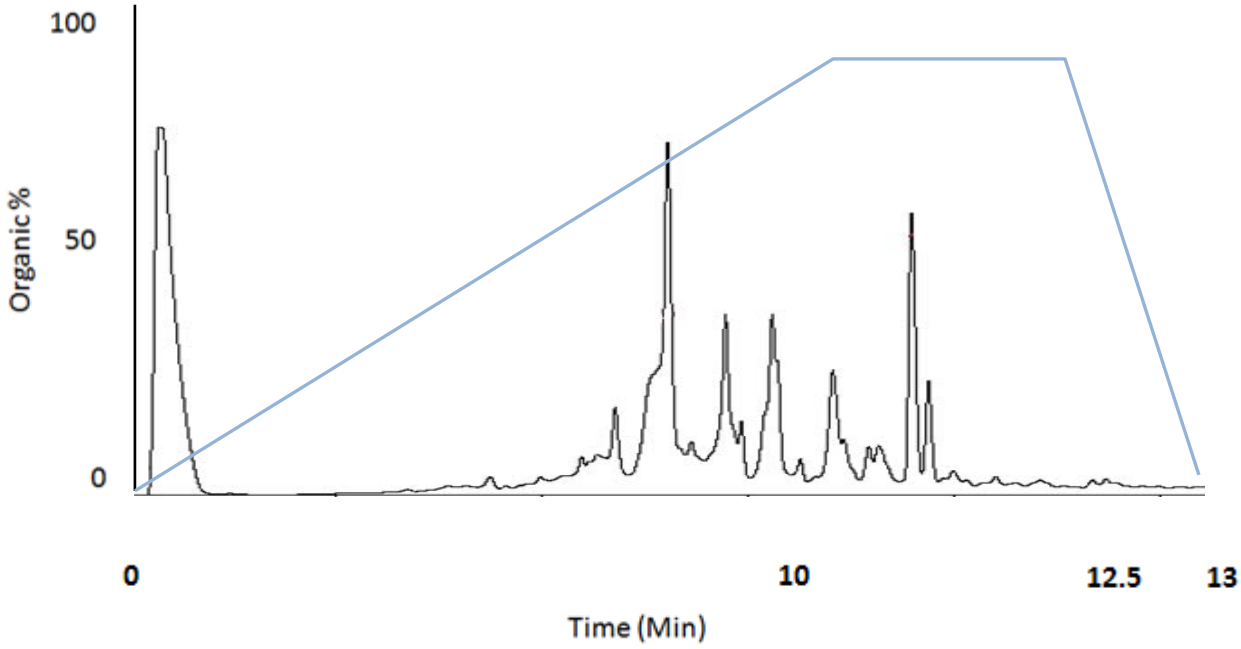
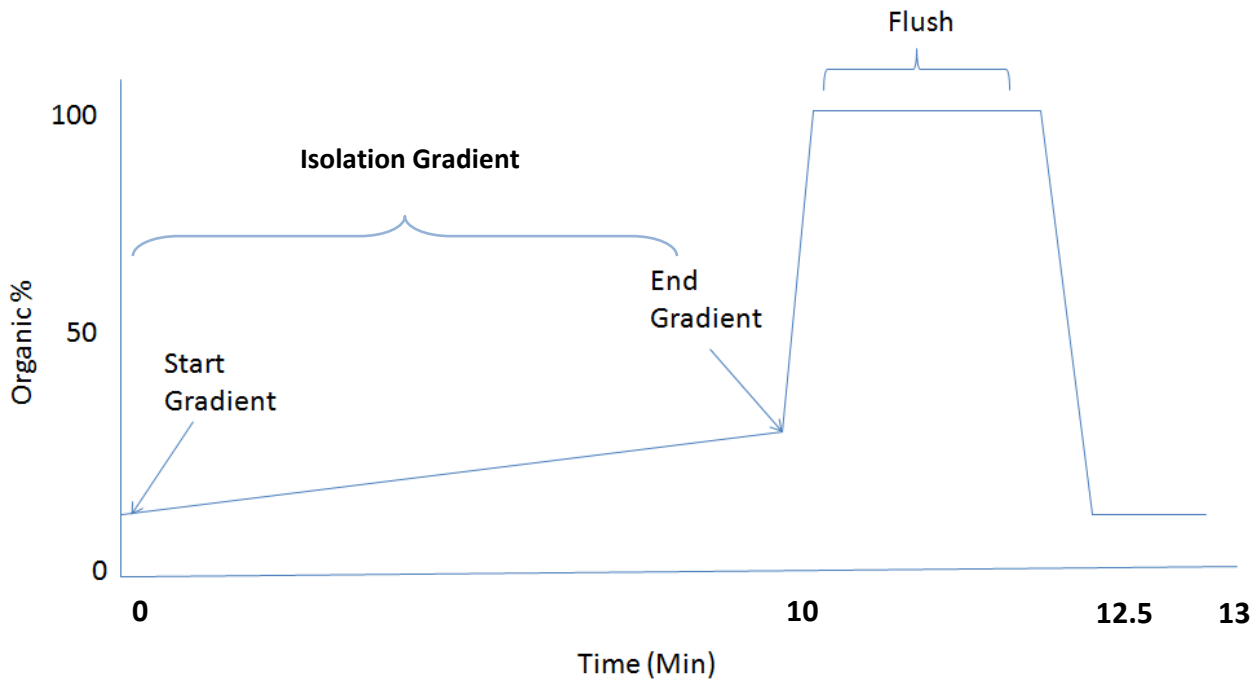


Figure 3. Simulated Gradient View of a 'General or Isolation Analytical Gradient Method'

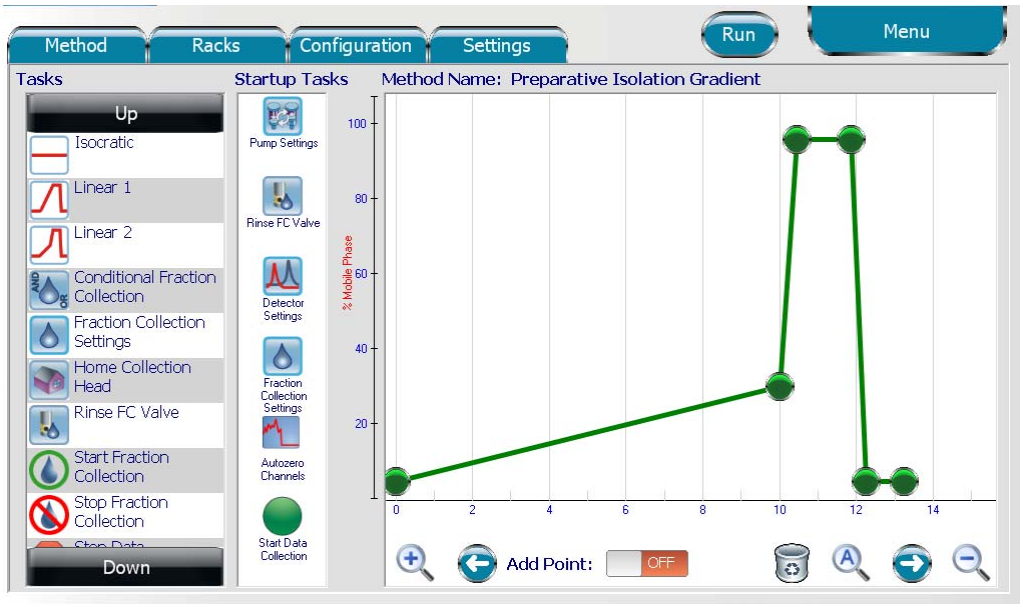


Based on the simulated standard analytical gradient, the slope or shallow gradient window remains a constant; only the start and end % organic change according to the retention time of the peak of interest. The new preparative isolation gradient, optimizes the separation of the peak from other interfering compounds through the use of this very shallow gradient window (consisting of the start gradient and end gradient). The flush time eliminates any late eluting compounds from interfering with subsequent injections.

Methods – Building the New Preparative Isolation Gradient

- To begin, go to the main screen in the PLC 2020 touch screen software.
- Choose *Method Builder*, then *Menu*, then *Open*. Select to open the *Preparative Isolation Gradient*.
- A screen similar to **Plot 1** will be displayed where the first mobile phase point (start gradient) shows a starting solvent mixture of CH_3CN/H_2O and a line ramping to the ending CH_3CN/ H_2O mixture (end gradient). The subsequent time points provide a wash and re-equilibration sequence.

Figure 4. PLC 2020 View of Method: Preparative Isolation Gradient



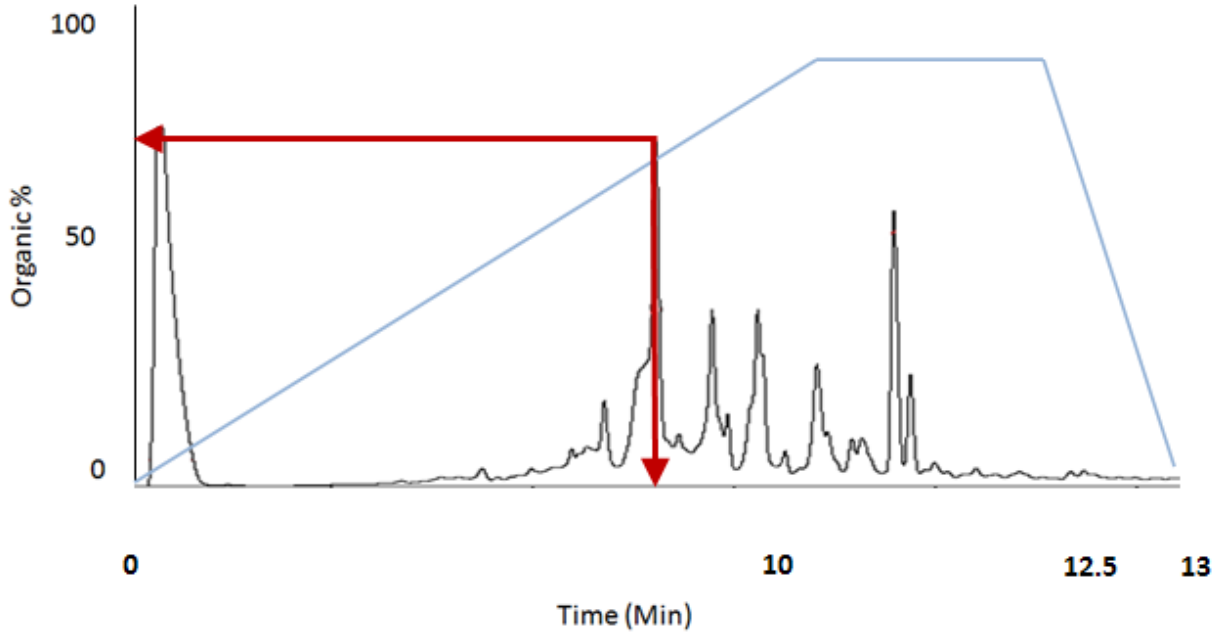
- To choose the appropriate gradient, use the observed retention time from the standard analytical method to locate the start and end values for the preparative column gradient from the Analytical to Preparative Conversion Table (**Table 2**).

Table 2. Analytical to Preparative Conversion Table

Analytical Retention time (min)	Prep column gradient	Approx retention time of product peak on Prep column	Analytical Retention time (min)	Prep column gradient	Approx retention time of product peak on Prep column
2.6	2-20	5.9	6.1	25-50	8.2
3.4	2-30	8.1	6.3	25-50	9.8
3.5	2-30	8.5	6.5	25-50	9.3
3.6	2-40	7.0	7.3	30-60	10.3
4.4	5-27	11.7	8.4	35-75	11.6
4.7	5-30	11.9	8.9	30-75	12.8
4.8	5-35	10.7	9.2	40-90	11.7
5.0	5-35	11.0	10.1	50-80	11.0
5.1	5-40	12.8	11.0	55-90	10.5
5.3	15-45	11.0	11.5	60-90	12.0
5.4	15-45	13.0	13.0	75-95	13.3
5.6	20-45	11.8			

- For example - the retention time of the peak of interest of a hypothetical sample is found to have a retention time of 8.5 minutes on the standard analytical method (see **Figure 3**). According to Table 2, a compound with an 8.4 minute retention time indicates a 35-75% CH_3CN/H_2O gradient could be used. This should result in the elution of the compound on the prep system at ~11.6 min.

Figure 5. Simulated Determination of Preparative Gradient from Analytical System Standard Gradient



- Alternatively, a compound with an 8.9 minute retention time (listed in **Table 2**) suggests using a 30-75% CH_3CN/H_2O gradient to elute material at 12.8 minutes. Either of these choices will probably work for the compound of interest. Factors (purity, amount, close elution of impurities, *etc*) may play a role in selection or modification of gradients selected.

- To set the preparative gradient of 35-75% CH_3CN/H_2O, double-click on the first mobile phase node to set the start gradient. Touch the keypad icon next to Acetonitrile. Enter a value of 35, touch the *Done* button, and then touch the *Apply* button (see figures 6 and 7). Deselect the start gradient node (touch in the white space).

- Set the End Gradient by following the same process. Double-click on the node, touch the keypad icon next to Acetonitrile, and enter a value of 75. Touch the *Done* button, and then touch the *Apply* button.

Figure 6. Using the PLC 2020 Touchscreen to Set the Acetonitrile Value for the New Preparative Gradient

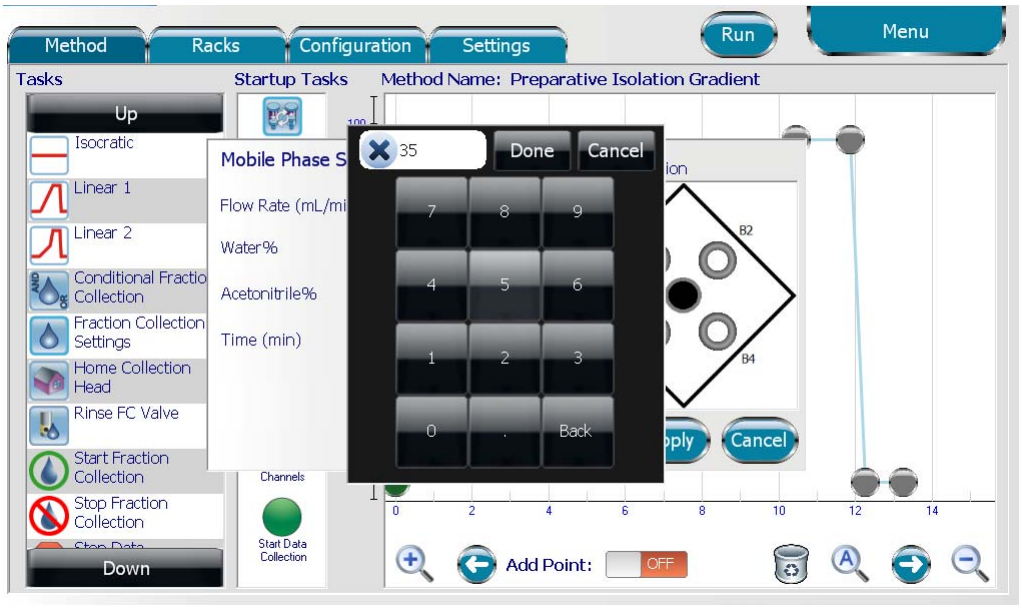


Figure 7. PLC 2020 Mobile Phase Settings for the New Preparative Gradient

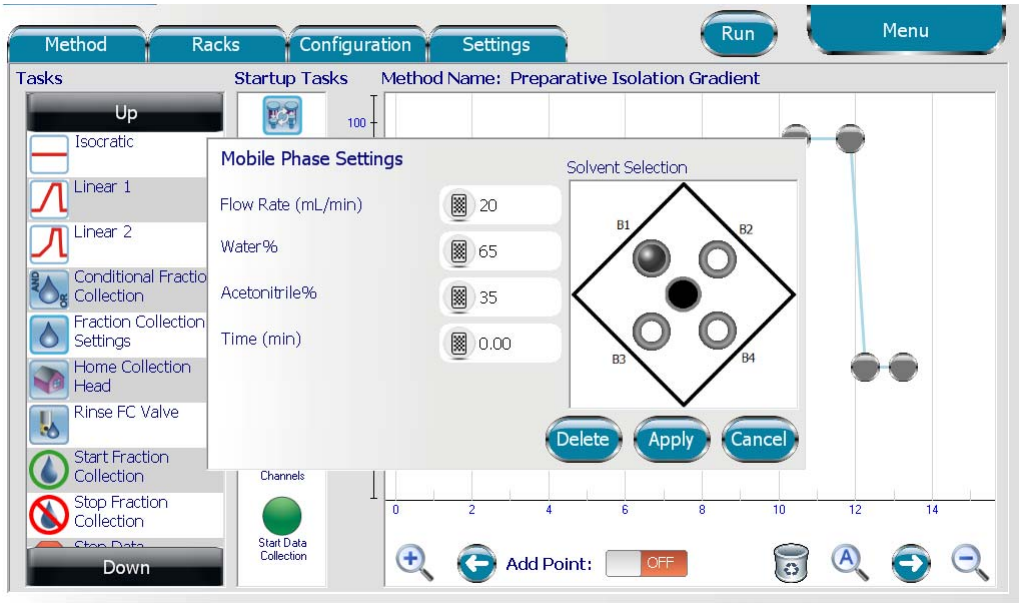
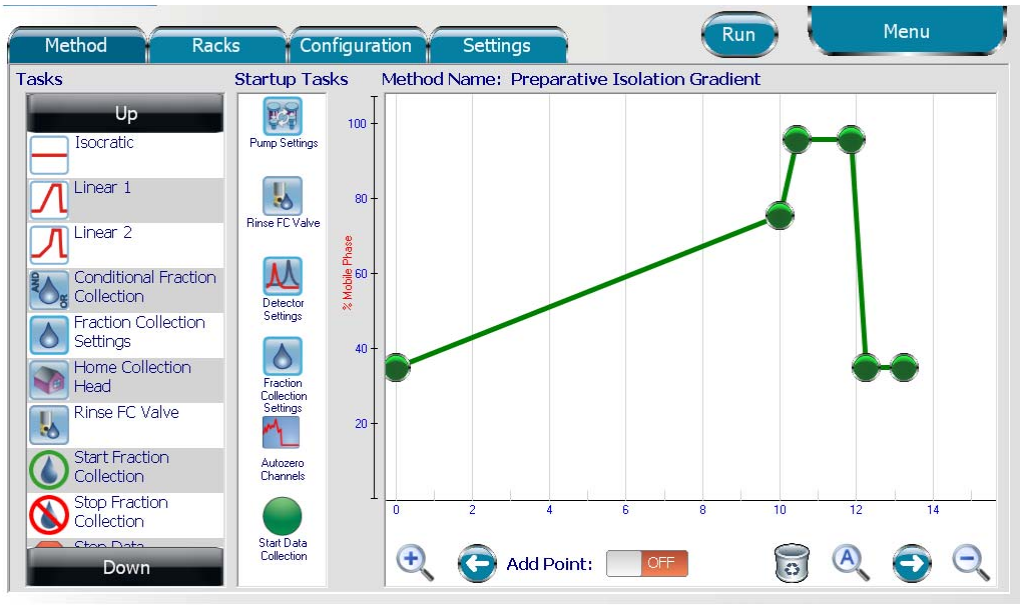


Figure 8. Modified New Preparative Gradient



- It is possible to add mobile phase nodes to a run, just remember to change the flow rate for each node that you add, since the default flow rate may not match the flow rate chosen for the run.
- Alternatively, mobile phase variables may be used so the changes can be made directly in the run screen.

- Once finished setting the method, select ‘menu’, then ‘save as’ to appropriately label the protocol for future recall. Select ‘run’ from the menu to proceed to the next section of operation.

Results & Summary

Purification of pharmaceutical organic compounds with the Gilson PLC 2020 using retention time criteria from analytical injections allows for quick elution of the compounds of interest at an 'optimal' time off the column, with minimal interference, and with reduced solvent waste. A practical guide to efficient use the PLC 2020 with an experimentally derived correlation table for the selection of 'optimal' solvent gradients or 'isolation' gradients offers simple, routine purification of organic compounds.

The simple PLC 2020 touch screen control allows for quick modifications of preparative methods based on retention times obtained from standard HPLC analytical methods. The flexibility to set preparative solvent gradients for purification injections using method modifications or variables caters to an 'open access' laboratory environment.

The enclosed guidelines represent the simplicity of establishing preparative compound purification conditions based on an analytical standard gradient injection.

References

A Novel Method for Semi-Preparative Purification of Natural Compounds Using Isolation Gradients, 2006; Gilson, Inc. (www.gilson.com)