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Computer Assisted Design of Column Switching Instrument Methods for Reduced Chromatography Run Times

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ABSTRACT

Performing analyses in dual-column (tandem) mode, with off-line column regeneration, can increase chromatographic throughput by up to 100%. The technique requires a second pump, a second column, and a 2-position 10-port switching valve, together with a conventional gradient HPLC system, and has been known for many years. Considering the relatively low additional cost in relation to the increase in throughput, the technique is well suited for coping with increasing numbers of samples. The technique provides several additional benefits. First, throughput can be increased without changing existing (validated) methods. Second, with modern instrument technology, a system suitable for the technique does not occupy any additional bench space (compared to a standard HPLC system). And third, laboratories can increase throughput without needing additional staff to operate more instruments.

Many chromatography specialists have started to use tandem operation to achieve higher throughput with their HPLC equipment. However, setting up an instrument control program that automatically switches columns at the correct times and also controls two independent pumps is a time-consuming and error-prone process. It adds additional complexity to the method development process and can be difficult to accomplish even for well trained specialists. To date this has prevented the technique from gaining broader acceptance.

The Dionex Chromeleon® Tandem Wizard significantly reduces this complexity. It aids users in locating the correct switch point for their gradient methods, and then automatically creates a control program for handling both pumps and the column switching valve. This Wizard vastly speeds up the process of creating tandem operation methods, reduces user errors, and allows users, with no special training, to speed up their analytical runs immediately.

INSTRUMENT SETUP FOR COLUMN SWITCHING METHODS

In order to run column switching methods that decrease analytical run times, an HPLC requires a second pump, a column switching valve, and a second column (see Figure 1, right). With these modules the system provides two independent flow paths, one for analysis (blue), and one for column reconditioning (purple). The two columns are switched between the two flow paths. This setup maximizes the productivity of the analytical system by eliminating the time needed to recondition the analytical column.

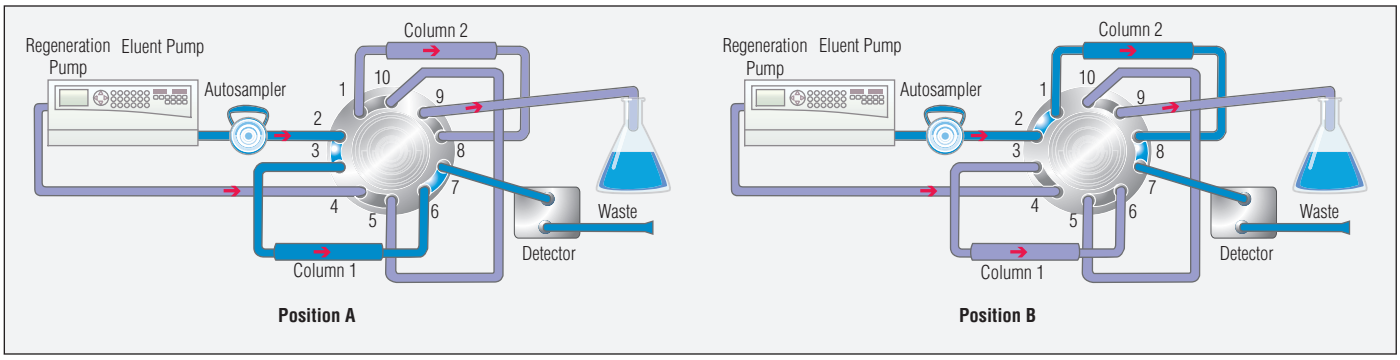

Figure 1. Instrument setup for column switching methods. Note that the pump in the figure is a dual pump (i.e., it contains two pumps in a single housing).

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INCREASING SAMPLE THROUGHPUT BY COLUMN SWITCHING

With column switching, it is possible to increase sample throughput by taking some of the chromatography steps off-line. We can see this by focusing on the different phases of a normal gradient run (Figure 2).

- Phase 1: Active part of the gradient where the compounds of interest elute
- Phase 2: Column wash phase (typically done at high percent of organic eluent)
- Phase 3: Column reequilibration phase at starting eluent conditions

Phases 2 and 3 are only required for column reconditioning. They do not provide chromatographic information about their sample. In other words, only phase 1 is a productive phase.

To reduce the cycle times, we can run phases 2 and 3 off-line using a separate flow path and a second pump. This operation mode is called dual-column tandem operation with off-line column reconditioning, or simply "tandem operation".

A typical pharmaceutical quality control gradient analysis run illustrates how sample throughput can be increased by using tandem operation. Figure 2 shows the chromatogram and the associated single column approach gradient profile.

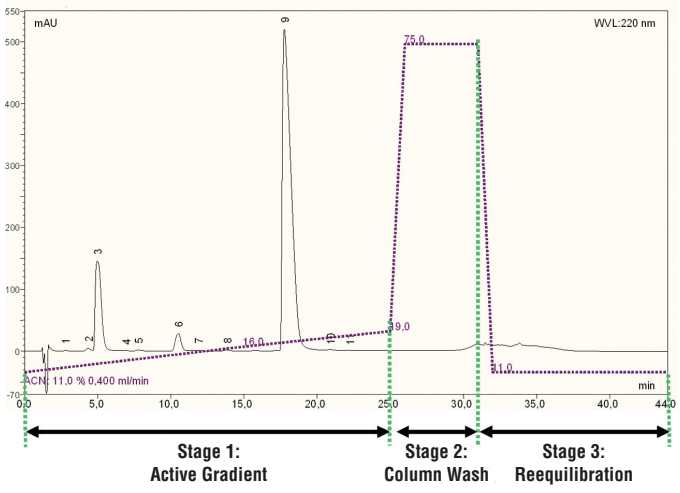

Figure 2. Gradient analysis for a pharmaceutical quality control laboratory.

In Figure 2, the chromatogram shows that the run time is 44 min, but that all peaks of interest elute during the active gradient, which ends after 25 min. By adopting a switching method that switches to the second column after 25 min (and adding a 1 min wash step to return the mobile phase to the starting conditions), the run time can be shortened to 26 min.

In this example, the run cycle was shortened by 19 min, or 41%, which increased the system's throughput by 69%.

DIFFICULTIES WITH CREATING TANDEM METHODS

Although the benefits of using column switching to improve sample throughput are well known, this technique has been under-utilized due to the complexity of setting up instrument control programs. There are several details that a chromatographer must take into account before they are able to create even a simple switching method. These are:

- Determining when to switch a column from active mode to equilibration mode
- Determining how long to purge the eluent lines for to ensure that the initial mobile phase proportions are used when the columns are switched
- Determining purge time requirements for the equilibration column
- Determining how to reduce gradient delay times

Also, the chromatographer needs to be easily able to create and visualize an instrument control method that sends different commands to the different pumps. Table 1 shows the steps required for a typical tandem method, and demonstrates just how difficult it can be to create and visualize the appropriate commands.

Table 1. Steps Required for a Typical Tandem Method

Starting Conditions		
0.000	PumpRight.Flow =	0.400 [ml/min]
	PumpRight.%B =	75.0 [%]
	PumpRight.%C =	0.0 [%]
	PumpLeft.Flow =	0.400 [ml/min]
	PumpLeft.%B =	11.0 [%]
	PumpLeft.%C =	0.0 [%]
Gradient		
18.250	PumpRight.Flow =	0.400 [ml/min]
	PumpRight.%B =	75.0 [%]
	PumpRight.%C =	0.0 [%]
19.250	PumpRight.Flow =	0.400 [ml/min]
	PumpRight.%B =	11.0 [%]
	PumpRight.%C =	0.0 [%]
25.000	PumpLeft.Flow =	0.400 [ml/min]
	PumpLeft.%B =	19.0 [%]
	PumpLeft.%C =	0.0 [%]
25.000	PumpLeft.Flow =	0.400 [ml/min]
	PumpLeft.%B =	11.0 [%]
	PumpLeft.%C =	0.0 [%]
31.250	PumpRight.Flow =	0.400 [ml/min]
	PumpRight.%B =	11.0 [%]
	PumpRight.%C =	0.0 [%]
	PumpLeft.Flow =	0.400 [ml/min]
	PumpLeft.%B =	11.0 [%]
	PumpLeft.%C =	0.0 [%]

COMPUTER ASSISTED DESIGN OF COLUMN SWITCHING METHODS

To simplify and accelerate both the creation and editing of column switching methods, a new instrument control Wizard and instrument control editor was introduced in Chromeleon version 6.7.

This Wizard guides users through the creation process in an easy to use and easy to follow step-by-step guide, while the editor allows users to visualize the method in an intuitive way.

The Wizard consists of two main windows that aid in the creation of tandem programs. In the first window (Figure 3), the gradient profile for the analytical method can be defined. After that, the "off-line recondition start time" can be defined. Essentially this time defines the end of the active gradient part of the method.

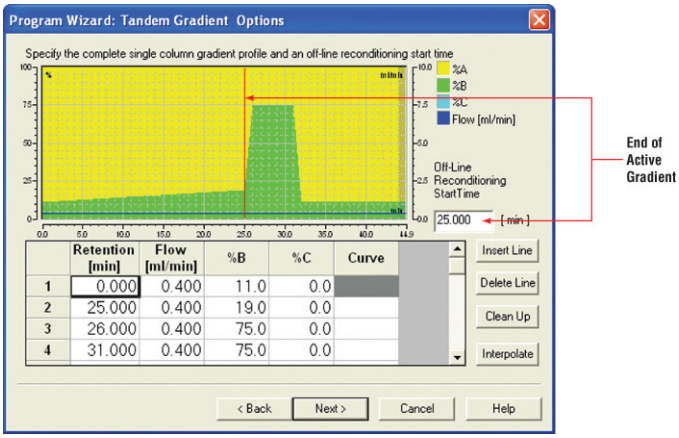

Figure 3. Defining the gradient profile and the "off-line recondition start time".

Once the gradient profile and the "off-line recondition start time" have been defined, Chromeleon automatically uses this information to create the commands for the analytical pump and for the reconditioning pump (Figure 4). In addition, it offers further options to optimize the control program. These options are:

- Void volume purge
- Bypass mode
- Reconditioning flow

Void Volume Purge

At the end of the active chromatographic run, the eluent lines between the point of gradient formation and the switching valve are not filled with the starting eluent composition. Therefore this void (delay) volume has to be purged with starting eluent composition before the off-line reconditioned second column can be switched into the analytical flow path. For this purpose purge volume, purge flow, and purge time can be specified. Chromeleon automatically enters default values based on the known system dead volume of the Dionex Summit® System.

Bypass Mode

The "Bypass Mode" is used to switch the autosampler out of the fluidic flow path. This reduces the gradient delay volume (as it significantly reduces the delay volume of the autosampler) and allows a faster response of a gradient change on the column thus providing faster elution of the analytes and hence shorter run times. The bypass time is calculated using the equation below.

$$\text{Bypass Time (min)} = \frac{(\text{Injection Volume } (\mu\text{L}) + \text{Volume Needle Seat to MSV } (\mu\text{L})) \times \text{Flush Factor}}{\text{Flow Rate } (\mu\text{L min}^{-1})}$$

Reconditioning Flow

This allows the user to select a higher flow rate for the recondition step, thus ensuring that the column is fully reconditioned before switching back to the active gradient.

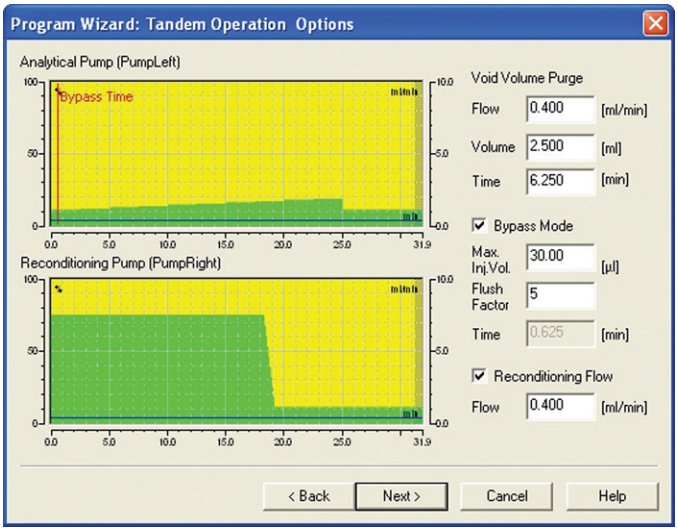

Figure 4. In the second step the gradient profiles for the active and reconditioning pumps are generated automatically. Additional options are available for optimizing the method.

By entering the appropriate values into these two Wizard dialogs, users are able to quickly create tandem instrument control programs, even for complex gradients. The built-in intelligence of the Wizard ensures that it is not possible for a user to enter invalid values, ensuring that a user can start working immediately with their instrument.

Once an instrument control program is created, it is also easy to modify because the new "editor" view provides a graphical overview of all pump settings (Figure 5). In addition, the editor provides an overview of the tandem settings and a consistency check to ensure that any changes made to the method do not affect the correct operation of the switching procedure (Figure 6).

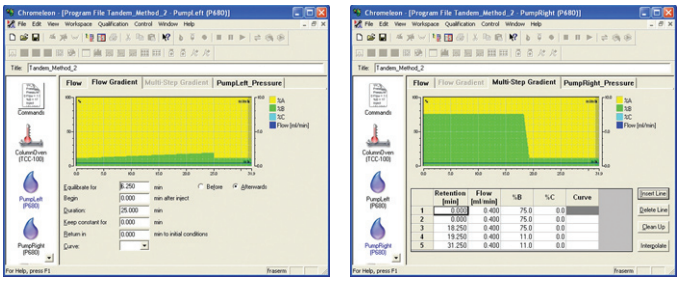

Figure 5. Graphical (and editable) views of the pump settings.

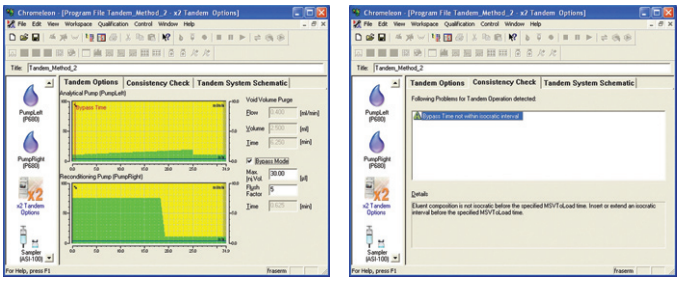

Figure 6. Overall graphical (and editable) view of the tandem settings. A consistency check is also provided to ensure that changes do not affect the switching procedure.

CONCLUSIONS

The computer aided design options in Chromeleon allow even inexperienced users to quickly create and run column switching methods. The main benefits are:

- In many cases, sample throughput can be increased significantly. In the example shown in Figure 2, throughput was increased by 69%.
- No special training is required to use this technique. The computer assisted design process offered by Chromeleon makes this technique easy to use. All a user has to do is let Chromeleon know the original method details, define the switching point, and the software does the rest.
- This feature allows laboratories to easily and immediately take advantage of the benefits offered by column-switching methods. That is, sample throughput can be significantly increased without having to change or revalidate existing analytical methods.

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