

Automated Column Selection and Switching Systems for HPLC

W.S. Letter

The great popularity of high performance liquid chromatography (HPLC) systems today empowers chemists to determine the chemical purity of most types of samples. Advances in HPLC instrument design, computerized data analysis, and plotting software have added to the increased reliability and potential accuracy of modern instruments. However, the process of column selection and method development still consumes much time.

Often users must try different columns and mobile-phase mixtures sequentially to find a satisfactory separation in a reasonable amount of time. Additionally, some samples require analysis using more than one column connected in series; they may even need a change in column type and mobile phase. When these activities are performed manually, chromatographers spend a great deal of time and effort. This installment of "Sample Prep Perspectives" will describe several HPLC valving systems used for automated column selection and column switching that can improve method development and sample analysis. Table I lists some of the obvious advantages of these systems.

AUTOMATED COLUMN SELECTION

During method development, certain samples may require users to screen several column types to find the column that provides the best separation. This column-selection technique often involves user intervention (for example, stopping the pump, manually removing and re-installing a column or turning a valve, flushing, and reequilibrating the system) before the next analysis can begin. Often these systems use valves to divert the solvent flow from one column to another column, as described elsewhere (1-8).

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Chromatographers can use column-selection and -switching valves to improve the development and performance of high performance liquid chromatography methods. This installment of "Sample Prep Perspectives" describes the use of multiport, high-pressure valves that help in many applications. These column-selection systems can screen, test, flush, equilibrate, and develop methods automatically.

Modern electrically actuated column selection valves can be automated to perform unattended column selection, flushing, and reequilibration. Actuator switching times of less than 100 ms allow column changeover to

occur quickly and reliably. An automated system can reduce the time it takes to develop new methods by screening one or more samples against a group of columns that contain different stationary phases. Mobile-phase composition still can be varied as an important method development variable, but the stationary phase also can be varied with an automated system. When using automated systems, users are free to perform other work and monitor the instrument's progress instead of returning to the system at the ideal time for installing and reequilibrating new columns. The net result is a faster, more reliable screening process that easily allows tasks such as automated column testing, method development, performance verification, and chiral method development to occur in an after-hours environment, if desired.

Figure 1 depicts a column selection system that uses nine HPLC columns and a bypass line (4). The system can select columns in a systematic manner and develop methods with little user intervention.

Automated column selection systems maintain each column hydraulically sealed in liquid, which greatly reduces the potential of chemical contamination and exposure to air. In Figure 1, a bypass line is included to facilitate solvent changeover and to permit flow injection of samples for liquid chromatography-mass spectrometry (LC-MS) analysis. Columns can be selected or skipped in any

TABLE I: Advantages of HPLC Column Selection and Switching Systems

Automated Column Selection	Automated Column Switching
No need to disconnect columns	No need to disconnect columns
More reproducible	More reproducible
Closed in-line system	Closed in-line system
Use of bypass line allows fast solvent changeovers and flow injection for LC-MS	Backflushing and column bypass
Use of second pump allows simultaneous analysis and column flushing with two columns	Heart-cutting and fractionation possible
	High throughput and shorter total analysis times

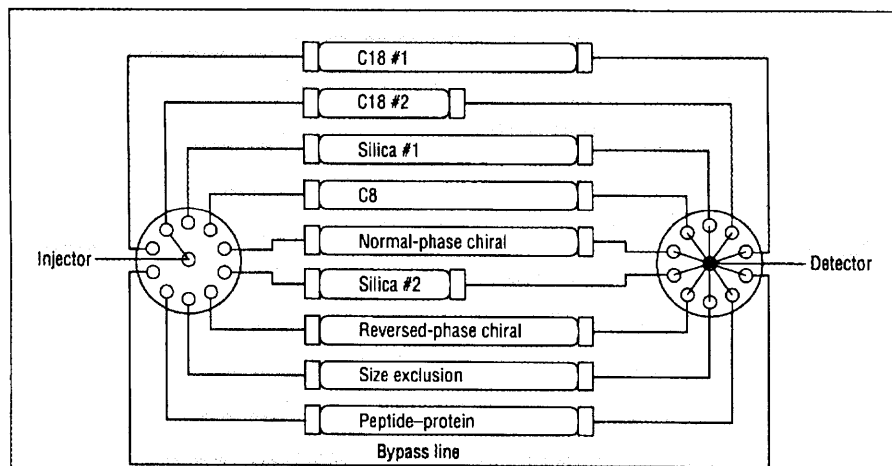


FIGURE 1: Example of an automated column selection system (4).

order and can be automatically flushed and reequilibrated, if desired, at the end of each run. Automatically flushing columns after each use minimizes potential sample contamination and leaves the column in an appropriate mobile phase for use during the next analysis. I recommend using short lengths of narrow-bore connecting tubing (0.007-in. i.d.) and narrow valve ports (0.010-in. i.d.) to minimize band broadening in these systems.

Most column selection systems still require users to wait for columns to be flushed and reequilibrated before they can begin a second analysis. However, if users want to repeat the same type of analysis repetitively and have a second gradient pump, a solution allows immediate analysis after the first analysis is finished (2,8). The system for repetitive analysis has a 10-port valve controlled through an external timetable that uses external contact closures to control the column swap.

Figure 2 shows a modified version of Figure 1 in which analysis occurs first on one of two identical columns (8). This example uses two separate pumps. One pump is dedicated to flushing and regenerating the first column, and the other pump runs the analysis method on the second column. After the first analysis is completed, the columns are switched and the second column undergoes the same flushing and regeneration process, the first column is used for the next analysis. This type of system can reduce the analysis time for repetitive analyses by 50%.

AUTOMATED COLUMN SWITCHING

If a sample requires purification, concentration, and analysis using two or more stationary phase types, chromatographers frequently use multidimensional chromatography such as LC-LC. Multidimensional column chromatography is a powerful technique for the separation and cleanup of multicomponent mixtures. In this technique, fractions from one chromatographic column are transferred selectively to one or more secondary columns for additional separation. Multidimensional chromatography can be used for

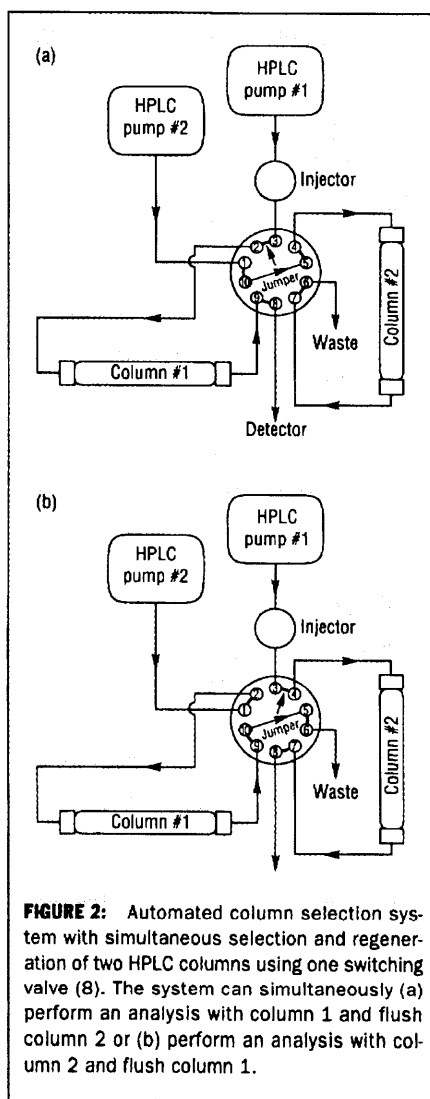


FIGURE 2: Automated column selection system with simultaneous selection and regeneration of two HPLC columns using one switching valve (8). The system can simultaneously (a) perform an analysis with column 1 and flush column 2 or (b) perform an analysis with column 2 and flush column 1.

- trace enriching of selected analytes.
- improving the resolution of complex sample portions (maximum resolution can be

achieved by using different modes, stationary phases, and mobile phases), and

- increasing sample throughput by the use of heart-cutting, backflushing, front- or end-cutting, and recycle chromatography methods.

In HPLC, multidimensional chromatography can be performed off-line or on-line. For the off-line technique, users collect fractions of solute at the detector exit from the first column and reinject the collected fraction onto the secondary column. This approach usually works, unless the job is a repetitive task. If the task must be repeated, an on-line system is much more effective. For the on-line approach, better known as *column switching*, chromatographers must couple two columns using high-pressure switching valves that either trap defined volumes or the collected samples, usually in a loop, and direct them to the second column (a process called *heart-cutting*). Alternatively, switching valves can be plumbed to divert the mobile phase containing the desired solutes from the first columns to the second columns for defined periods of time (a process called *on-column concentration*).

Others have described numerous innovations in the development of column-switching systems (9–18). Chromatographers can use column-switching to select specific columns for analysis or connect more than one column in series. The systems also can backflush specific sample components from one column to waste, which leaves only the peak of interest on the second column.

Figure 3 depicts a valving configuration for column switching (18). This system can be used for conventional HPLC analysis, and it can perform more advanced functions such as backflushing, heart-cutting, trace enrichment (on-column concentration), and front- and end-cutting when needed. The in-line design of this type of system also can reduce sample contamination and loss. Automating column-switching systems usually can be accomplished using the timed-event tables included with HPLC instrument-control software.

If you have an HPLC system that incorporates a system of valves that allows selective column backflushing and each column to be placed in-line selectively, such as those depicted in Figure 3, then you can develop an on-line column switching method without difficulty. You must carefully program the times at which the various valve-switching events must occur and any delay volumes. Next, you should test the entire process with the actual volume of sample that you intend to use. The entire switching method can be developed on-line without collecting each peak manually. The in-line design permits chromatographers to change the method easily using timed-event tables. The tables list the specific event times when each switching, backflush, and concentrating event occurs.

LC-MS DIVERTER VALVE

With the increasing popularity of LC-MS and LC-MS-MS systems, laboratory workers

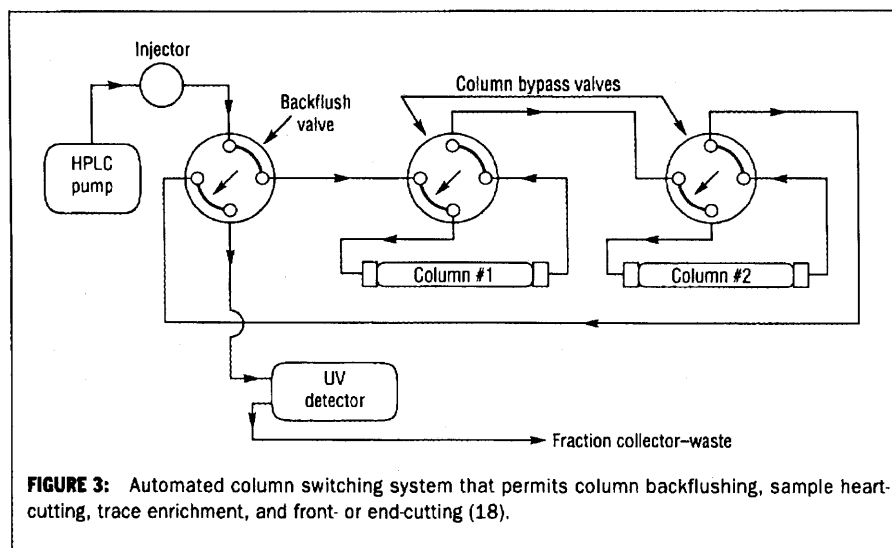


FIGURE 3: Automated column switching system that permits column backflushing, sample heart-cutting, trace enrichment, and front- or end-cutting (18).

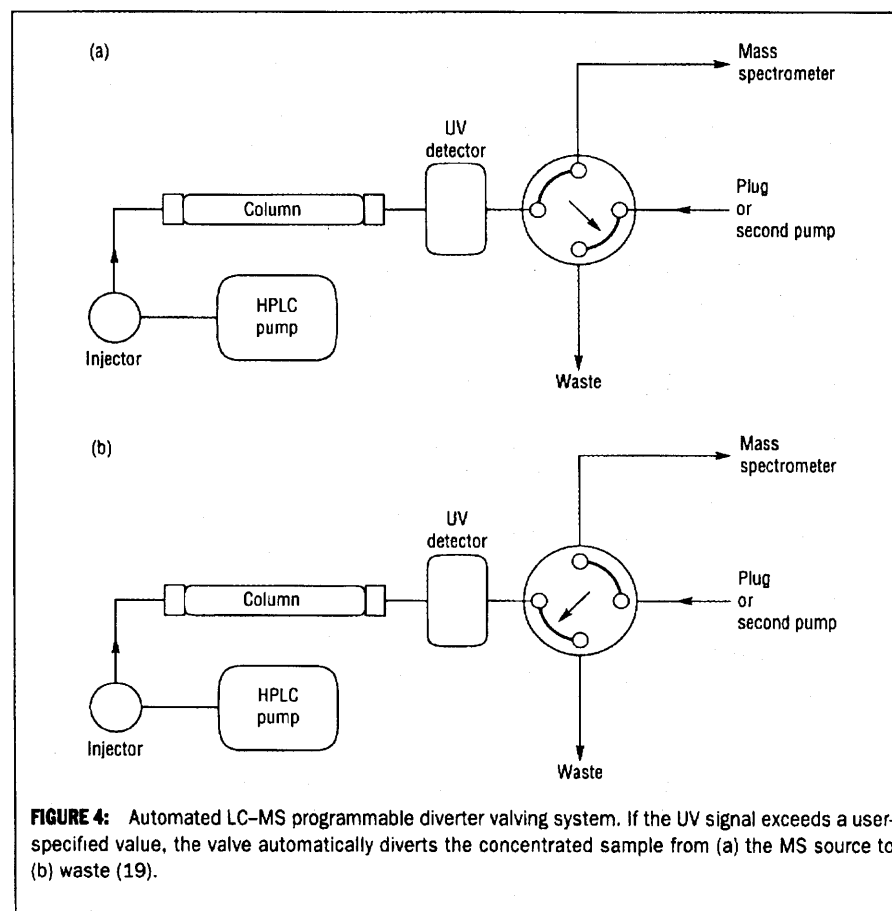


FIGURE 4: Automated LC-MS programmable diverter valving system. If the UV signal exceeds a user-specified value, the valve automatically diverts the concentrated sample from (a) the MS source to (b) waste (19).

are paying much more attention to sample throughput and cleanliness. Figure 4 shows an improvement to an existing two-position column-switching valve that is equipped with a microprocessor. This system provides potential UV detection applications for use with LC-MS systems (19). The device uses the output from a UV detector to monitor the absorbance signal and divert the solvent flow away from the MS source when the UV signal

exceeds a specific, user-defined value (between 1 and 2000 mAU). Diverting the flow to waste, instead of sending the highly concentrated sample to the MS source, reduces the potential contamination of the mass spectrometer. As an option, the device allows a second pump to supply the MS source with solvent, and the main flow is diverted to waste. For documentation purposes, the unit automatically generates a chart mark output each

time the microprocessor switches the valve diverter.

CONCLUSION

Chromatographers can use column-selection and -switching valves in various ways to improve the development and performance of HPLC methods. Multiport, high-pressure valves coupled to electric valve actuators allow many different tools to be constructed for solving diverse applications. These devices can save valuable time by automating tasks that are performed manually.

The use of column selection systems to screen, test, flush, equilibrate, and develop methods automatically can save time and provide more data for decision making. When necessary, using in-line column switching systems also can reduce the amount of wasted sample and improve the reliability of the methods used. As a bonus, these column-selection and -switching tasks often can be performed after hours, which saves even more time in the laboratory and increases overall productivity.

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