Troubleshooting

Parallel Chromatography — Double Your Money

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How to get more out of that expensive detector.

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Column-switching techniques can have many applications for liquid chromatography (LC) separations. In our laboratory, we use column switching routinely to desalt samples or remove strongly retained materials from injected samples before they reach the analytical column. We also use a column-switching setup that can run the same sample through an array of six different columns in an overnight run to help speed column selection during method development. Use of column-switching valves in applications similar to these is common in many laboratories.

It has been interesting to observe the evolution of LC methods using tandem-quadrupole mass spectrometry detection (LC–MS-MS) during the past decade. LC–MS-MS has become the standard system for the analysis of pharmaceutical compounds in biological samples (bioanalytical applications) to support pharmacokinetics, toxicology, and other studies of drug behavior in the body. In the early days of bioanalytical LC–MS-MS, workers depended almost exclusively upon the selectivity of MS, using LC only to get rid of excess protein. Runs of less than 2 min were common. Throughout the years, however, workers became aware of the importance of ion suppression from background materials or other drugs. As a result, chromatography began to play a more important role in LC–MS-MS. Now runs of 4–8 min are common, often with much improved precision and accuracy compared with the faster runs of earlier times. The selectivity of MS still plays an important role, reducing resolution requirements when compared with the same separation obtained using LC with UV detection. Typically, 50 mm × 2.1 mm columns packed with 3- or 3.5-µm particles are operated at flow rates of 0.2–0.5 mL/min for fast runs in either isocratic or gradient modes.

It is not surprising that economics also enters the equation when considering the development and application of LC–MS-MS methods. Time often is a very important factor. In a service laboratory such as ours, clients always seem to want the data sooner. With an LC–UV method, LC systems are relatively inexpensive, and an extra system commonly is available, so splitting the sample set and running on two systems often is a viable solution to reduce the overall sample analysis time. However, taking the same approach for an LC–MS-MS sample set might not be an option — one doesn't keep a spare LC–MS-MS system "just in case." With triple-quadrupole MS systems starting at $200,000, one might have a difficult time finding a second system that is not occupied with other work.

Time Constraints

A typical example of the need for rapid turnaround is a rising dose-tolerance study, in which subjects are dosed with a drug, plasma concentrations of the drug are measured, and a decision for the next dosing cycle is made based upon the results. Samples might be shipped from the clinic on Monday and delivered to the analytical laboratory on Tuesday. A decision based upon the analytical results needs to be made on Friday for weekend dosing and a repeat of the cycle. In the example we've used here, a run might comprise two 96-well plates, including standard curve and quality control samples as well as study samples. An 8-min cycle time for the method (see Figure 1) means that the batch takes about 26 h to run. If the sample preparation is completed by the end of the day on Tuesday so it can be put on the LC–MS-MS system before the end of the day, the batch won't be completed until Wednesday evening, meaning that data aren't available for integration, report formatting, and review by quality assurance and quality control until Thursday (unless the lab has an evening shift of workers). The client would get the results sometime on Thursday afternoon. Having results on Wednesday would reduce the

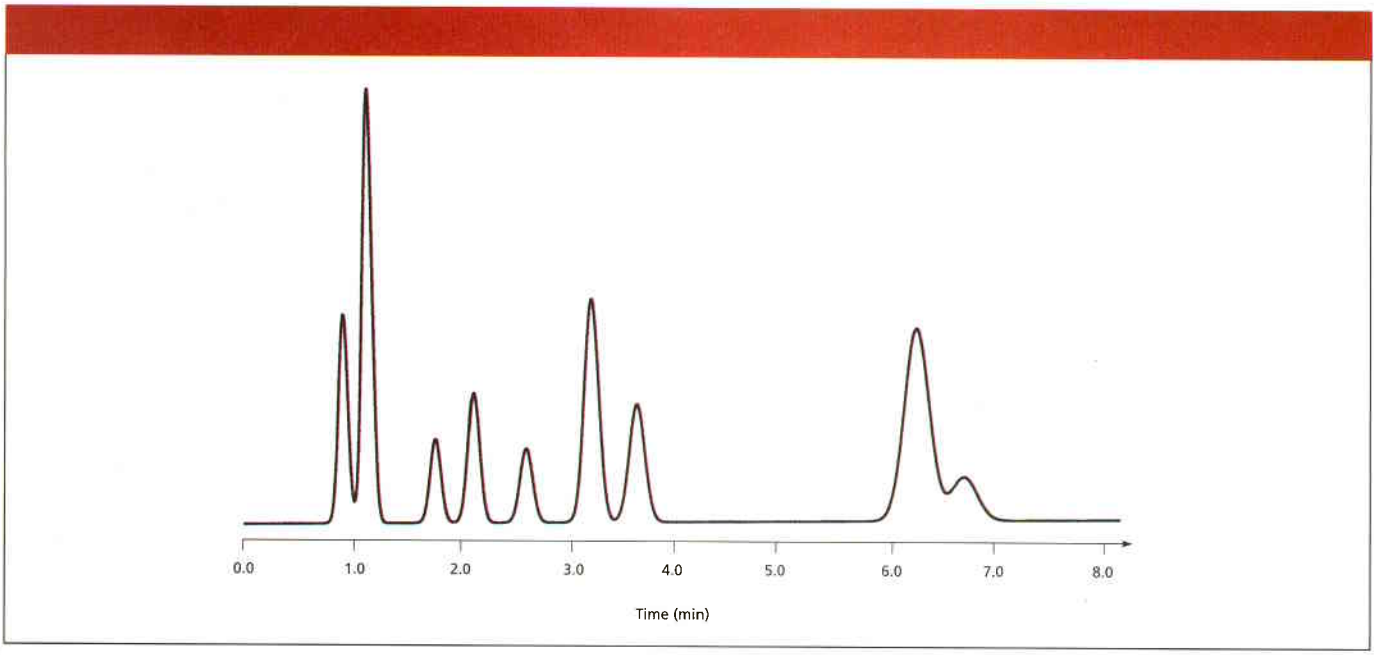

Figure 1: Simulated total ion chromatogram obtained for a hypothetical sample in an isocratic separation. Column: 50 mm × 2.1 mm, 3.5-µm; flow rate: 0.4 mL/min. Peaks of interest are the drug at 3.25 min and the internal standard at 3.65 min. See text for details.

client's stress level greatly on this project and facilitate information flow back to the clinic.

What can we do to improve things? In our laboratory, like most others, the LC–MS-MS units are kept very busy, so splitting the batch to run on two instruments means that another project will get bumped. Our first step should be to look at the chromatogram to see if there are some ways to take advantage of the separation from a chromatography standpoint. Figure 1 is a simulated total ion chromatogram of a hypothetical sample. In this case, the peak at 3.25 min is the analyte of interest, and the peak at 3.65 min is the internal standard. All the other peaks are of no interest. Note that after the last peak of interest is eluted, 4 min is spent washing off the unwanted peaks in the 6–7 min region. The net result is an 8-min cycle time.

This is a good example of a case in which parallel chromatography can be used effectively to shorten the run time. What would happen if we could send just the first half of the chromatogram to the mass spectrometer and then immediately feed in the first half of the chromatogram from another column while the first column is flushed? This would allow data for two runs to be collected in 8 min. We call this parallel chromatography, because two LC columns are running samples in parallel, with offset cycle times.

We have an additional complication in our laboratory. Each of our LC–MS-MS systems has a modern, fast-cycle autosampler. These have overhead of 20 s or less, meaning that when told to inject, they load and inject in less than 20 s. Our remaining autosamplers are an older model, quite suitable for longer LC–UV runs, but they take 1–2 min to complete the wash, load, and inject cycles. For a 15–25 min LC–UV run, this is of little consequence, but a 4-min LC–MS-MS run with a 2-min delay while the autosampler does its business is severely compromised. This means that if we are going to do parallel chromatography, we have to figure out how to do it with a single autosampler feeding both columns.

The Setup

The diagrams in Figure 2 show a plumbing configuration that supports parallel chromatography. The system requires two LC pumping systems (isocratic or gradient, depending upon the method), one autosampler, two six-port valves, two columns, and one mass spectrometer. The plumbing is configured so that LC system 1 is responsible for sample injection and elution, and LC system 2 is the column flushing system.

A typical timing sequence is summarized in Table I. At the beginning of the

Table I: Valve timing for parallel chromatography setup of Figure 2

Time	Valve Position	LC System	Activity
0	A	1	Injects sample on column 1, elutes sample to MS system
		2	Washes column 2 to waste with strong solvent
2.5	A	1	Continues to elute analytes from column 1
		2	Changes to mobile phase to equilibrate column 2
4	B	1	Injects sample on column 2, elutes sample to MS system
		2	Washes column 1 to waste with strong solvent
6.5	B	1	Continues to elute analytes from column 2
		2	Changes to mobile phase to equilibrate column 1
8 = 0	A	Both	Restart cycle

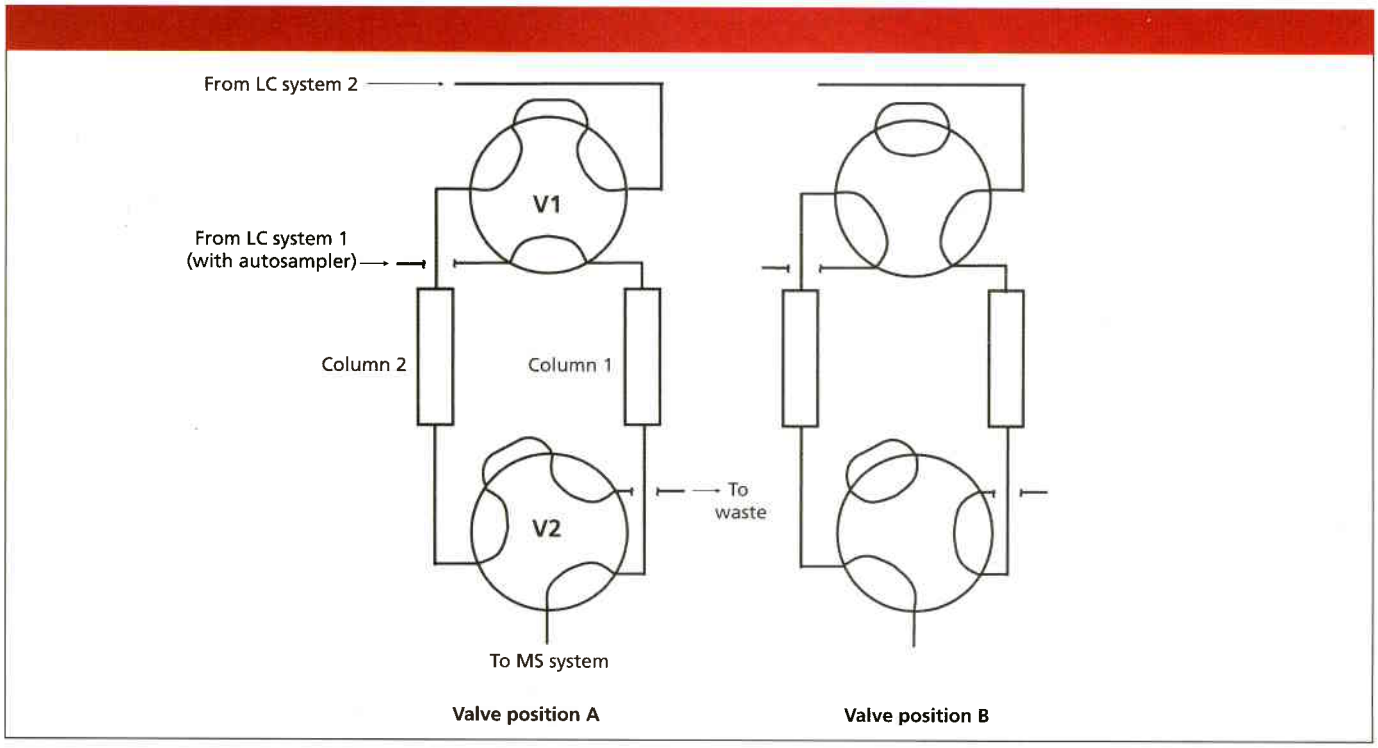

Figure 2: Valve configurations for a parallel chromatography system. See text for details.

sequence (position A in Figure 2), LC system 1 pumps mobile phase onto column 1, injects a sample onto column 1, and begins eluting the sample from the column into the mass spectrometer. At the same time, LC system 2 pumps a strong flush solvent through column 2. Just before the first half of the cycle is finished, LC system 2 changes back to mobile phase to reequilibrate column 2 in the mobile phase so that it is ready to receive sample when the valves switch. Halfway through the first cycle, the valves rotate to position B. This allows LC system 1 to inject sample onto the preequilibrated column 2. LC system 2 changes back to strong solvent and elutes the strongly retained materials from column 1. Just before the end of the cycle, column 1 is reequilibrated with mobile phase. In this manner, the effective run time is 4 min per sample, even though the actual run time per column is 8 min.

Deconvolution Required

Although the two columns used are nominally identical, you need to make sure that a standard curve and quality control samples are run on each column for use in quantification of the data collected from that column. It probably is simpler to make up two independent sample sets with calibrators and controls in separate 96-well plates (or vial racks if traditional vials are used) than to try to interleave the samples in a single plate. The autosampler is programmed to pick up the first sample from plate 1 and inject it on column 1, then the first sample from plate 2 gets injected on column 2, and so forth.

One complication of parallel chromatography is that the data must be sorted out when the run is finished. If the MS system allows you to collect data into two separate data files, you are in luck and can program the run so that the data from each column goes to a separate data file. More likely, you have to collect all the data in a single data file. This file then has to be split up after the run so that every other sample is put in an alternate file. Once the data are separated, the calibration plots and calculations can be done in the normal manner.

One more caution: what happens if the autosampler gets mixed up? You are definitely in trouble. A couple of tricks should help to keep the runs straight. First, you might place the quality control samples in different positions or in a different order in the two runs, so it would be obvious from the results which run you were observing. For example, plate 1 might have the 100-ng/mL quality control sample run first, whereas plate 2 might have a 10-ng/mL control sample in the same position. We have noticed that even though the columns are nominally the same, the retention times are a little different between the columns, so retention can be used to confirm which data set you are using. One could imagine adding an unretained marker to every sample in one of the plates, or using some other method to make one plate distinct from the other when the data were analyzed.

Variations on a Theme

In the setup of Figure 2, as described in Table I, both valves switch at the same time. If this were the only way you were going to use the system, the same process could be accomplished with a single 10-port valve, reducing equipment costs and simplifying the setup. Having two independent valves allows additional program steps to improve the throughput or method performance. For example, one could divert the first minute or two of the chromatogram to waste so that salts and other unretained materials are not directed into the mass spectrometer interface. Another use we have found with the two-valve system is to quickly flush out the dwell volume of LC system 1 before switching from one column to the other. Two six-port valves are more flexible for use in other applications, such as a precolumn cleanup step or other column switching setups.

Gradients can be handled just as easily as the isocratic example of Figure 1. LC system 1 would be programmed to run the elution portion of the gradient repeatedly.

LC system 2 would perform a strong-solvent flush and then re-equilibrate the off-line column. In both isocratic and gradient applications, system 2 could be run at a higher flow rate (remember that flushing and equilibration depend upon mobile-phase volume, not time) to shorten the cycle. In this manner, one could imagine our 8-min run split into 5 min of elution and 3 min of flushing and reequilibration.

The Payoff

So what is the net gain of all this extra plumbing? In the present example, with the 192-sample batch, we were looking at almost 26 h of elapsed time from the first to last injection. With the parallel chromatography method, the batch would be completed in about 13 h. The time saved in practical terms, however, is much greater than 13 h. In the first case, the batch is finished at the end of the day on Wednesday, but in the second case, all the samples will have been run by the time the day starts on Wednesday. This means the data work-up and quality review can take place on Wednesday, so the client gets the data 24 h earlier. Furthermore, the LC–MS-MS system is now available for other work and overtime is minimized — everybody wins.

Summary

The example presented here is just one of myriad possibilities for the use of column switching. In the present case, an expensive triple-quadrupole mass spectrometer with a high hourly burden rate was used more efficiently. This paid dividends to the lab and allowed the client to get data more quickly. Parallel chromatography is not limited to LC–MS-MS applications. It also can be used with conventional LC detectors, such as diode-array UV detectors, to increase throughput when instrumentation is limited. Consult the manufacturers of switching valves (see the Buyers Guide in each August's *LCGC* for contact information) for application notes related to column-switching techniques.

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