## LC TROUBLESHOOTING

# Liquid Chromatography Pump Refinements

**John W. Dolan**

***Twenty years of design refinements have led to the improved performance of today's LC pumps.***

Last month's "LC Troubleshooting" (1) reviewed the basic operation of reciprocating-piston liquid chromatography (LC) pumps, which are based on modifications of the single-piston pump (Figure 1). Twenty years ago, commercial pumps consisted of little more than the parts shown in Figure 1. Since then, however, many innovations have improved the convenience and performance of LC pumps. This month's installment discusses three areas of improvement: on-line mixing, compressibility compensation, and pulse dampening.

## ON-LINE MIXING

Today, the manual preparation of mobile phase remains an operational inconvenience and a common source of errors. Because early LC pumps could pump only one solvent or mobile-phase mixture, hand-mixed isocratic mobile phases were the norm. Then someone realized that two LC pumps, each pumping from a different reservoir, could feed solvent into a mixing tee to create a mobile-phase mixture. The solvent ratios in the mixture could be adjusted by varying the flow rate of the two pumps relative to each other. Because the mobile-phase components were mixed on the high-pressure (outlet) side of the pump, this technique was called high-pressure mixing (Figure 2). Early innovators built their own mixers from compression fittings and custom-built mixing chambers. Waters Associates (Milford, Massachusetts) was probably the first to sell a commercial apparatus, and with it the company introduced a convenient way to generate gradients by varying the pump speed over time.

High-pressure mixing increased the convenience and precision of LC pump operation. The second pump was an added expense, but, with a few simple plumbing changes, operators could use it to drive a second LC system. The fact that solvents did not mix until they were under pressure minimized excess gas in the mobile phase and thus ensured the reliability of pump operation.

One problem, especially common in earlier pumps, was that delivery accuracy varied with flow rate. Often, the pumps would be less precise or less accurate when the flow rate was reduced to $\leq$0.1 mL/min. For example, to produce a 50:50 (v/v) methanol–water mixture pumped at 1 mL/min, one pump pumps methanol at a 0.5-mL/min rate and the other pumps water at a 0.5-mL/min rate; the pumps perform well in this delivery region. To produce a 99:1 (v/v) methanol–water mobile phase, however, the methanol is pumped at a 990-µL/min rate, and the water is pumped at a rate of only 10 µL/min. At such low flow rates, delivery precision and accuracy are generally poor, making the accuracy of the mobile-phase composition questionable. To produce gradient mobile phases, the pumps must change speeds during the gradient — an additional potential source of trouble. Newer LC pumps perform better at low flow rates, so this problem is less important today than it was in the past.

For many years, high-pressure mixing was the only practical way to mix solvents on-line, and it remains popular today. As chromatographers became interested in three- and four-component mobile phases, however, the attraction of high-pressure mixing diminished because each solvent required a separate pump, drastically increasing equipment costs. These considerations, along with technical innovations such as low-cost microprocessors, paved the way for low-pressure mixing.

In low-pressure mixing (Figure 3), a manifold is used to combine two or more solvents on the pump's low-pressure side. Because the solvents are premixed, only one pump is required. The precision and accuracy of mobile-phase preparation depend on the performance of the solenoid-controlled proportioning valves and the corresponding controller. In practice, to obtain a 35:15:50 (v/v/v) methanol–acetonitrile–water mixture, first the methanol proportioning valve is opened for 35% of the mixing cycle, then the acetonitrile valve is opened for 15% of the cycle, and finally, the water valve is opened for the remaining 50% of the cycle. Different manufacturers use different valve times and sequences to achieve the best proportioning possible for their pumps. With today's equipment, low-pressure mixing can provide excellent performance.

Two obvious advantages of this technique are reduced equipment costs (only one pump is required) and better flow precision (the flow rate is constant, and only the mobile-phase composition changes). On the other hand, low-pressure mixing systems are prone to bubble problems. Because the mobile-phase components are mixed at or below atmospheric pressure, any gas released during mixing can cause check-valve or flow problems in the pump. For this reason, you must thoroughly degas the mobile-phase components before use. Another, more insidious problem involves the partial blockage of the inlet-line sinker frits, which trap unwanted par-

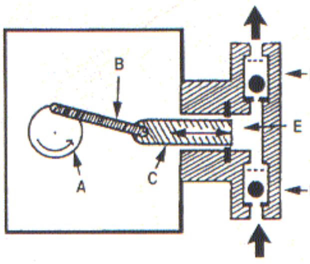

**FIGURE 1:** The reciprocating-piston pump. A = cam, B = connecting rod, C = piston, D = outlet check valve, E = cylinder, F = inlet check valve. (Reprinted with permission from reference 2.)

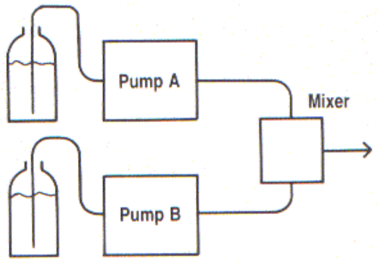

**FIGURE 2:** Schematic of high-pressure mixing apparatus. (Reprinted with permission from reference 2.)

ticles in the reservoir and prevent them from entering the rest of the system. If one of these frits becomes partially blocked, delivery from its reservoir may be reduced, thus creating a partial vacuum in the mixer when that reservoir is selected. The delivery shortage is compensated for when the next (free-flowing) reservoir is selected. Thus, when one or more inlet-line frits become blocked, the mobile-phase mixture may be inaccurate.

The choice between high- and low-pressure mixing is determined largely by the user's personal preference and the manufacturer's specifications. Manufacturers design their mixing systems to match their pumping systems. Many variations exist, such as the coupling of low-pressure proportioning with high-pressure mixing and the use of low-pressure pumps to supply solvents to the mixer. All of these techniques can be used successfully when the equipment is working properly. No matter which technique your system uses, mobile-phase degassing will improve pumping reliability.

## SOLVENT COMPRESSIBILITY

All solvents are compressed when subjected to increased pressure. The degree of compressibility depends on the solvent or mixture used and the amount of dissolved gas. Obviously, solvent compression can reduce the flow rates generated by LC pumps. Furthermore, the true volumetric flow may vary, depending on the solvent type or the mobile-phase composition. This variation can be especially worrisome during gradient elution runs.

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Pump manufacturers have devised ways to correct for solvent compressibility. Two compressibility compensation techniques are common. The simplest is to adjust the pump speed for an average solvent so that the adjusted flow setting actually delivers the desired flow rate. This method works fairly well because most LC solvents have a similar degree of compressibility, and the resulting small differences in flow are constant and seldom noticed. A more sophisticated technique involves measuring the compressibility and dynamically adjusting the pump; this method also is widely used.

## PRESSURE PULSATIONS

Reciprocating-piston pumps have inherent pressure cycles (illustrated in Figure 4a for a single-piston pump). In pumps with a round cam (Figure 1), half of the pump cycle is delivery and half is fill, so half of the time no solvent is flowing and the pressure is essentially zero. During the delivery cycle, the speed of the piston varies, producing the flow–pressure profile shown in Figure 4a. In the early days of LC, when columns were unstable and unable to accommodate pressure fluctuations, such pulses could ruin a column in short order. Consequently, most systems used some type of pulse dampener to minimize pump pulsations. Most dampeners relied on a Bourdon tube, gas ballast, or pressure gauge to provide a spring that made the final pressure more even. Several pump-design modifications have minimized pressure-pulsation problems.

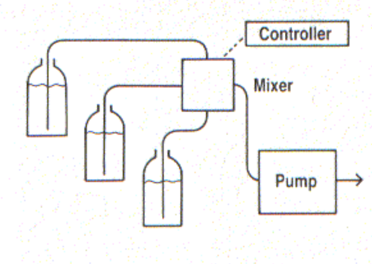

FIGURE 3: Schematic of low-pressure mixing apparatus. (Reprinted with permission from reference 2.)

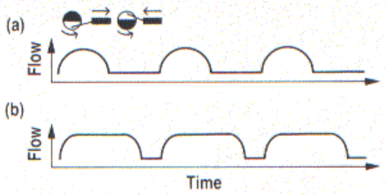

FIGURE 4: Output of (a) normal and (b) shaped-cam single-piston pumps. Inset shows cam rotation and direction of piston travel. (Reprinted with permission from reference 2.)

**Modified cam:** One way of compensating for pressure fluctuations is to use a cam that is not round. This compensation can be accomplished by using a shaped cam or by changing the pump control software to vary the piston speed during the pump cycle. The effect of modifying the pump cycle is illustrated in Figure 4b. First, the fill stroke (when the pressure is zero) is drastically shortened. Such pumps are referred to as rapid-refill pumps. In Figure 4b, the fill cycle has been reduced from 50% to ~20% of the total pump cycle.

The second way to compensate for pressure fluctuations is to adjust the piston speed during the delivery stroke so that the flow is as constant as possible. After the rapid-fill cycle, the piston rapidly moves forward to compress the solvent and begin delivery. The piston then slows to produce the desired flow rate. After the piston has emptied the pump chamber, the pump enters the next rapid-fill cycle. The resultant flow is much more pulse-free than before. When coupled with a pulse dampener, this type of pump produces satisfactory output. Commercial pumps of this design are in widespread use today.

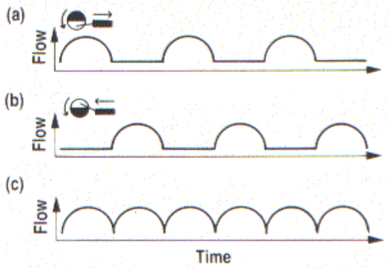

FIGURE 5: Dual-piston pump output, including (a) output from piston 1, (b) output from piston 2, and (c) the combined output of pistons 1 and 2. Insets show cam rotation and direction of piston travel. (Reprinted with permission from reference 2.)

**Dual-piston pumps:** Another way to reduce pressure pulses is to use a dual-piston pump. In this case, one motor drives both pistons, but, as Figures 5a and 5b show, the pistons are 180° out of phase: one pump head delivers solvent while the other fills. The combined output is shown schematically in Figure 5c. Manufacturers can further improve the performance of a dual-piston pump by using the shaped-cam techniques discussed above.

Dual-piston pumps are in widespread use today. From time to time, three-piston pumps have been produced commercially, but acceptance has been limited because, although the third piston improves the pump's pressure and flow stability, mechanical problems resulting from an additional set of check valves and other hardware tend to reduce operational reliability.

**Tandem-piston pumps:** A modification of the dual-piston pump has led to a third popular pump design — the tandem-piston pump, in which two pistons work in tandem rather than in parallel. This pump's operation is shown in Figure 6. During the first phase (Figure 6a), the top piston delivers mobile phase to the column in the normal manner while the bottom chamber fills. This portion of the cycle is identical to that of the dual-piston pump. During the second phase, the top chamber fills, and the bottom piston delivers solvent at twice the nominal flow rate. As a result, solvent is fed to the column while the pump fills the top chamber. This is accomplished in practice by using pistons with differing diameters or stroke lengths.

This pump design reduces pressure pulsations because solvent flows from the pump continuously. If you program the movement of the two pistons properly, the flow rate and pressure should remain constant. An additional advantage of this design is that it includes three check valves instead of the four used by dual-piston pumps; because check valves are the most troublesome pump component, using a pump that has fewer check valves should improve pump reliability.

Manufacturers have included many other design innovations in commercially available

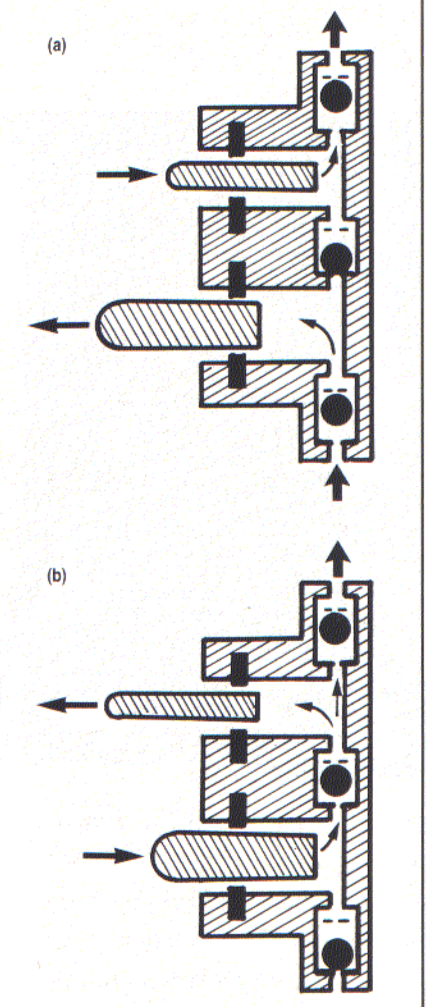

**FIGURE 6: Operation of a tandem-piston pump in which (a) the top piston delivers solvent to the column while the bottom chamber fills and (b) the bottom piston delivers solvent to the column and simultaneously fills the top chamber. (Reprinted with permission from reference 2.)**

pumps, such as syringe pumps, diaphragm pumps, and pumps with mechanically driven check valves. Although you face many choices when selecting a pump, you can take comfort in knowing that the years spent in refining pump design mean that nearly every pump on the market should provide years of reliable operation.

## REFERENCES

(1) J.W. Dolan, *LC•GC* **9**(4), 270–274 (1991).
(2) L.R. Snyder and J.W. Dolan, *Getting Started in HPLC, User's Manual* (LC Resources, Lafayette, California, 1985).

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*Continued from page 343.*

ing peak. If the splitting appears only on early peaks and is clean-looking, then the autosampler is the likely cause. Make sure the injection speeds are high enough to deliver sample in less time than about one-fourth of the peak width at the base. Slow injection speeds can split peaks when the plunger is not depressed immediately upon entering the inlet. A small amount of sample evaporates from the needle before the plunger action ejects the major portion; if the injection occurs too long after the evaporation, the events can appear as two distinct peaks. Jagged peak edges usually are caused by component overloading (injecting too much sample) or by solvent flooding the beginning of the column in on-column or splitless injection. You can remedy solvent flooding by installing a retention gap between the inlet and the column.

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**Tailing peaks:** Peak tailing is often present. Autosamplers are rarely a source of peak tailing, but under certain conditions they can cause this problem. If you observe the same tailing with manual injection, then the autosampler is not at fault. Instead, the column may be too active for the component, or a connection at the inlet or detector may be poor. The detector make-up gas, if needed, may have been turned off or set too low. The column entrance may be incorrectly positioned in the inlet. Other causes of peak tailing include injections that are too slow and, at high speeds, injections that are too large. In the second case, rapid sample vaporization forces sample vapors up into cooler inlet areas; carrier gas flow then slowly sweeps the vapors back into the column.

**Poor precision:** Users often report that autosampler injections are not repeatable. This is a subjective area because the observed-result standard deviations represent the sum of the deviations from injection, separation, detection, peak integration, and report generation. The best way to evaluate systematic repeatability is to use a synthetic test mixture that contains only hydrocarbons. For a system using a flame ionization detector, 50–100 ng/component should enter the column from injection. Using such a test mixture prevents the occurrence of too-large or too-small peaks, and the peaks will be free from column adsorption or activity. If you are concerned about repeatability, keep a mixture of pure hydrocarbons on hand for packed-column work, and dilute it as necessary for capillary split, splitless, or on-column injection. Choose components that are eluted under the same conditions as your sample analysis and make sure that you can obtain satisfactory results without peak tailing or splitting. Finally, fill the sample vials with enough liquid for all of the injections that you programmed.

Using the autosampler, make 10 injections and determine the standard deviations of the peak areas. Values < 3% relative standard deviation (RSD) are good; values > 5% RSD are not. If the results are good, then the problem is caused by the analytical sample in combination with the inlet, column, detector, or data-handling system — not the autosampler. If you obtain poor results, then the autosampler is the likely culprit. Before blaming the autosampler, however, repeat the experiment using your best manual injection technique. If the RSD values you obtain manually are considerably better than those produced using the autosampler, then the autosampler is definitely at fault. If not, then the problem may lie in the inlet or data-handling system. Check the split-vent flow rate and the split ratio or the splitless injection timing. Make sure that the data-handling system is correctly set up and that no obvious problems exist, such as integrating peaks on a rising baseline or incompletely resolved peaks. Are the flame ionization detector's combustion gases well regulated?

If you are convinced that the autosampler is at fault, look for a partially blocked syringe or air bubbles in the syringe as injection occurs. Is there enough solvent in the solvent reservoirs and enough sample in the sample vials? In a back-loading system, make sure that the solvent and sample can flow freely through the syringe and needle. Remove the syringe temporarily and make sure the plunger moves smoothly and easily. Make any necessary replacements, and try again.

## CONCLUSION

Autosamplers are complex mechanical devices. Although designed to last a long time and to operate under diverse conditions, they contain moving parts that are repeatedly cycled and narrow passages through which sample must flow. The best way to keep an autosampler running well is to keep it clean and maintain it regularly.

Autosampler parameters cover a wide range of conditions and must be set correctly for the best results. When evaluating the source of a problem, remember that the autosampler is used for the first of several steps in the separation and detection process — it may only appear to be the cause of injection, separation, detection, or data-handling problems. You can often identify the true problem source by isolating individual GC components for evaluation.

## REFERENCES

(1) J.V. Hinshaw, *LC•GC* **7**(11), 904–907 (1989).
(2) P.H. Silvis, *LC•GC* **7**(7), 562–569 (1989).

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