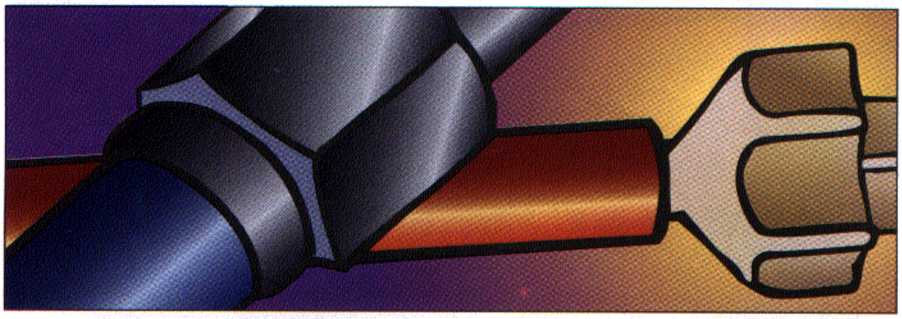
LC Troubleshooting



Maintaining Autosampler Performance

John W. Dolan

A properly operating autosampler is an essential tool for precision in a liquid chromatography system.

Most of today's liquid chromatography (LC) systems use an autosampler for sample introduction. In many cases, the autosampler's precision can be the limiting factor in the overall precision of the system. An autosampler injecting reasonable volumes of standards should be able to provide standard deviations for peak heights and peak areas of less than 1%. This month's "LC Troubleshooting" reviews the operation principles of autosamplers and highlights some of the problems that users may encounter.

INJECTION STYLES

Most autosamplers rely on six-port injection valves to introduce sample into the high-pressure mobile-phase stream. These valves generally operate in one of two modes — filled-loop or partially filled–loop injection. (These same injection options apply for manual injectors.)

Figure 1 illustrates filled-loop injection. The valve in the figure has a stationary ring and a rotating center or rotor. The LC pump and column are connected to adjacent ports. The sample loop is connected across the center of the valve, and the sample inlet and waste lines are connected to the remaining two ports. With filled-loop injection, the volume capacity of the loop determines the volume of sample that is injected. That is, a 20-µL sample loop provides a 20-µL sample volume.

In operation, the loop is filled with sample by introducing sample into the sample port until it flows out of the waste port. In practice, 40 µL of sample may be introduced, but only 20 µL will occupy the loop — the rest of the sample will flow to waste.

When the valve is in the load position, the loop can be filled. In this position, the valve shunts the mobile phase from the pump directly to the column. When the valve receives an inject signal, the valve rotor turns 60° to connect the pump and column at opposite ends of the loop. Then the contents of the loop are pumped onto the column so the chromatographic separation can take place. At the same time, the sample port can be flushed to waste if users want a flushing cycle.

In the intermediate position between the load and inject positions, the pump is snubbed off and cannot deliver mobile phase to the column. As a result, the pressure rises at the pump and drops at the column. When the pump and column are reconnected at the end of the switching cycle, a pressure surge is delivered to the column. In years past, columns were very sensitive to this pressure shock, and the voiding caused by valve switching was common. Today's columns are more robust and less susceptible to this sort of damage. However, users must rotate the valve quickly to minimize pressure buildup.

Some autosamplers are designed with a bypass circuit that shunts some of the mobile phase around the valve so constant flow is maintained to the column. Figure 2 shows one of these designs. The bypass passage acts as a splitter — less than 5% of the flow goes through the bypass in either the load or inject positions. However, 100% of the flow goes through the bypass in the intermediate position. This design solves the problem of stopped flow during part of the valve cycle, but it introduces another problem. If the tubing on the valve side of the bypass becomes blocked or partially blocked, the split ratio can change and cause much more of the flow to go through the bypass. This flow dilutes the sample as it is injected and can have the same effect as injecting the sample in a much larger volume of mobile phase.

Peak broadening, especially with early eluted peaks, occurs when the bypass ratio is too large. One solution for this problem is the Make Before Break valve (Rheodyne, Cotati, California), which incorporates a bypass passage that is connected only when the valve is rotated. In the load and inject positions, the passage is disconnected to eliminate the potential of variable split ratios.

An alternate way to use the injection valve is the partially filled–loop mode shown in Figure 3. In this model, the same valve is used but with a loop that is larger than the desired injection volume. For example, a 100-µL loop fitted to the valve would inject only 20 µL of sample. Under these circumstances, a carefully measured volume of sample is introduced into the loop, and the remainder of the loop is filled with solvent or — in most cases

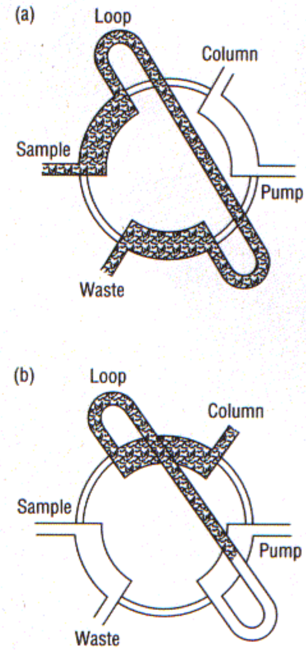


FIGURE 1: Six-port sample injector in the filled-loop mode. Shown are the (a) load and (b) inject valve positions. (Courtesy of LC Resources Inc., Walnut Creek, California.)

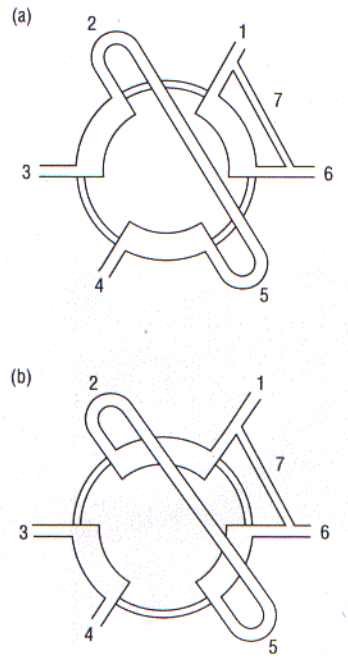


FIGURE 2: Pressure bypass circuit for sample injection valve in the (a) load and (b) inject positions. Ports: 1 = to column; 2,5 = sample loop; 3 = injection port; 4 = waste; 6 = from pump; 7 = bypass tubing. (Reprinted with permission from reference 1.)

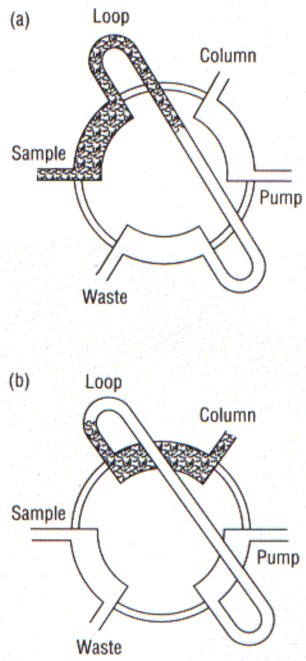


FIGURE 3: Six-port sample injector in the partially filled–loop mode. Shown are the (a) load and (b) inject valve positions. (Courtesy of LC Resources Inc.)

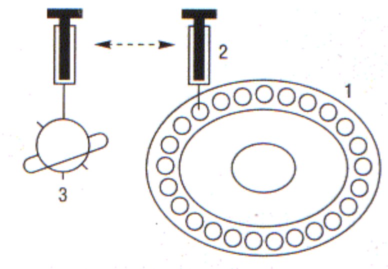


FIGURE 4: Autosampler schematic. 1 = sample tray filled with vials, 2 = syringe, 3 = injector. (Courtesy of LC Resources Inc.)

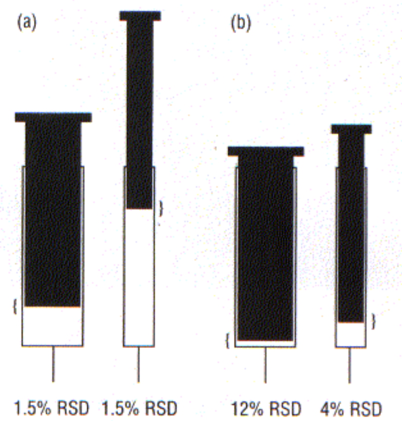


FIGURE 5: The effect of syringe size on precision for (a) 50- and (b) 10-μL injections. The larger and smaller syringes have 1-mL and 100-μL volumes, respectively. (Courtesy of LC Resources Inc.)

— mobile phase. When the loop is rotated to the inject position, the sample is pumped onto the column.

It is especially important that the valve is plumbed to backflush the loop onto the column, as illustrated in Figure 3, when the valve is operated in the partially filled mode. If the pump and column connections were reversed, the sample would have to pass through the entire loop before it reaches the column. This configuration probably would cause no problems with a sample that occupied a significant part of the loop. However, autosamplers often are fitted with 1-mL or larger loops. It is easy to imagine the band broadening that would occur if a 10-μL sample had to travel through 1 mL of tubing before it reached the column.

All autosamplers come plumbed in the correct backflush configuration. When disconnecting the valve for cleaning or other maintenance, users always should note which tubing is attached to which port on the valve so connections won't be reversed inadvertently. Mark the tubing with a self-adhesive label; the valve body generally is stamped with numbers for each port. With the filled-loop mode (Figure 1), the flow direction doesn't matter, but it is good practice to plumb the valve the same way every time.

When injection valves are filled manually in the partially filled mode, chromatographers must take care to avoid precision problems caused by the valve's filling characteristics (1,2). With autosamplers, however, the syringe is controlled by a precise stepper motor that reduces these variations.

AUTOSAMPLER DESIGN

Autosamplers have many designs, but most are based on the schematic shown in Figure 4. In this basic design, a syringe needle is inserted into the sample vial on the autosampler tray. A mechanized syringe withdraws the desired sample volume from the vial. The autosampler moves the needle to the injection port on the valve and forces sample into the sample loop, similarly to manual injections. Then the valve rotates, and the autosampler injects the sample. In one popular modification of this design, the needle becomes part of the high-pressure stream and is flushed onto the column during the injection process.

In practice, the syringe is mounted on a stepper-driven motor that moves the plunger very precisely to pick up the desired sample volume. The syringe usually is stationary because of the mechanism's bulk, and a long piece of tubing connects the syringe to the sample needle. The syringe, connecting tubing, and needle are all potential sources of autosampler problems.

SYRINGE PROBLEMS

Many autosamplers are designed to accept syringes of various sizes for different injection volumes. For example, an analyst could use a 100-μL syringe for 1–100 μL injections and a 1-mL syringe for 100 μL–1 mL injections.

A couple of years ago my laboratory had problems related to syringe selection, which is explained with the help of Figure 5. The laboratory had two autosamplers of the same brand. When we made 50-μL injections, we observed a peak area relative standard deviation (RSD) of approximately 1.5% for six injections with each system. This level of performance was expected from the systems. However, when we switched to 10-μL injections, the error rose to approximately 12% for one system and 4% for the other. With the smaller injection, we expected the error to be approximately 4–5% RSD, not 12%.

When my co-workers and I investigated the problem, we found that one autosampler was fitted with a 10-mL syringe and the second system had a 100-μL syringe. The explanation of the problem was simple. Whenever the syringe is moved under the influence of the stepper motor, some error in the position of the syringe is inherent (this occurrence is illustrated by the brackets [{,}] in Figure 5). This positioning error translates into volumetric error, and thus the observed RSD for repetitive injections. After injecting a 50-µL volume, the positioning error was small compared with the total movement of the syringe plunger in both cases, so the RSD was roughly the same. However, the large syringe's plunger movement was quite small compared with the positioning error when the small injection volume was selected, so the error contribution was larger, generating a larger volumetric error. The fix was simple — we used the 100-µL syringe on both autosamplers. After this switch, the performance of the two LC systems was equivalent.

The practical lesson is to make sure the syringe selection matches the desired injection volume. Not all autosamplers offer a selection of syringe sizes, but you should know if your autosampler has this option.

TUBING PROBLEMS

As discussed above, it is common for the syringe to be stationary with the needle connected to the syringe via a piece of connecting tubing. This tubing not only forms a flexible hydraulic connection between the two parts but also isolates the sample from the syringe itself. Because the syringe connection involves connecting fittings and different size passages, it can be a source of sample carryover. For this reason many autosamplers are designed so that the sample never reaches the syringe; rather it is fully contained in the connecting tubing, which can be efficiently flushed. This means that the connecting tubing must have sufficient volume to contain the entire sample for injection — as much as 1 mL in many cases.

Filling the connecting tubing and syringe with liquid forms a noncompressible hydraulic connection that enables the syringe movement to be translated accurately into sample aspiration. On the other hand, if the tubing or syringe contains a significant amount of air, the compressibility of the air will result in injection-volume errors. For this reason, you may obtain better precision if the autosampler flush solvent is degassed and the syringe mechanism is purged daily to remove unwanted air. We have found that flushing daily with degassed wash solvent greatly improves the reliability of our autosamplers.

NEEDLE PROBLEMS

The needle can be the source of most autosampler problems. The most common needle problem is blockage from a piece of vial septum. You may need to experiment with vial septa to find the best one for your autosampler. In my laboratory, we have the best luck with septa that have polytetrafluoroethylene (PTFE) film on one or both surfaces. PTFE layers lower the incidence of septum coring, in which a piece of septum breaks off, becomes lodged in the needle, and creates a partial or complete blockage.

Another factor influencing the frequency of septum coring problems is the type of needle used. Most LC autosamplers use a flat-tipped needle that tears its way through the septum rather than a pointed needle such as those used in gas chromatography autosamplers. If the edges of the tip of the needle become roughened, it will cut through the septum like a core borer, and the likelihood of septum fragments entering the needle increases. Some autosamplers use a needle with a rounded tip and a hole in the side (called a *side-port needle*) to eliminate coring problems. It is important to use replacement needles that match autosamplers' original needles.

The height of the needle relative to the bottom of the sample vials must be adjusted for optimum performance. If the needle height is too large, the autosampler will be unable to inject from vials that contain small sample volumes. This failure can show up as decreased precision because the sample above the needle tip is withdrawn without a problem; however, air can be drawn into the needle if the desired injection volume is too large. The result is the same as putting insufficient sample in a vial when everything is adjusted properly. If the needle depth is too great, the needle will hit the bottom of the vial. In some autosamplers, this touch will cause an error and shut down the system; in other systems, the needles will bend; and in still others, spring-loaded needles will accommodate small errors in adjustment. The autosamplers in my laboratory require readjustment of the needle height when we change from standard sample vials to micro vials. It is a simple adjustment, but it must be done for optimum performance.

Autosamplers also may require occasional adjustment of the needle seal on the injection valve. Two seal designs are popular. In one design, the flat-tipped needle is pressed against a seal with a hole in the center. Only the tip of the needle contacts the seal, so the seal must be intact and the needle tip must be smooth. The second type of seal is the same as the one used in manual injectors — the needle slides into a sleeve that fits tightly around the needle.

WASH SOLVENT

The autosampler wash solvent flushes the syringe, connecting tubing, and needle to minimize sample carryover. With most autosamplers, the wash solution is not injected, so its composition does not have to match the mobile phase. If the wash is injected, workers must take care to ensure that the wash solvent is not stronger than the mobile phase. The primary function of the wash solvent is to remove any sample residue before to the next injection, so its composition should be selected for this purpose.

Generally, I avoid using buffers in the wash solvent because if the wash solvent evaporates, it leaves abrasive crystals behind that can cause seal damage and other problems. To reduce problems with microbial growth and solvent deterioration, it is a good idea to change the autosampler wash solvent as frequently as you change the mobile phase. As mentioned above, I find better autosampler performance if I degas the wash solvent each day.

Most or all of the wash solvent flows out the waste line from the injection valve and ends up in the waste container. Be sure that the transfer line from the valve to the waste container is clean. If the line becomes blocked with buffer crystals or other sample-related debris, the needle may be unable to fill the loop properly because of back pressure. The result can be irreproducible injections.

CONCLUSIONS

To get the best performance from an autosampler, chromatographers must pay attention to the details. Because autosamplers play such an important part in overall LC system precision, it is wise to use a check list, standard operating procedure, or other method to make sure that vital maintenance and adjustment procedures are performed in a timely manner. The list doesn't have to be very long.

In my laboratory, the basic procedure requires changing the wash solvent daily, degassing it, and purging the system with the fresh wash solvent. Because the wash solvent purges the waste line, we don't need to worry about sample buildup in the waste line. We ensure that the appropriate syringes and loops are installed and check for proper needle height adjustment when switching between normal sample vials and microvials. You should remember to check your autosampler's manual for specific recommendations.

REFERENCES

(1) S.R. Bakalyar and B. Spruce, "Technical Note 5," Rheodyne (Cotati, California, 1983).

(2) J.W. Dolan and L.R. Snyder, *Troubleshooting LC Systems* (Humana Press, Totowa, New Jersey, 1989), pp. 243–246.

"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Walnut Creek, California, and a member of the Editorial Advisory Board of LC•GC. *Direct correspondence about this column to "LC Troubleshooting,"* LC•GC, *859 Willamette Street, Eugene, OR 97401, e-mail John.Dolan@LCResources.com.*

ERRATUM

The last installment of "LC Troubleshooting" (*LC•GC* **15**[5], 424 [1997]) listed an incorrect pK_a value for phosphate. The correct values are 2.1, 7.2, and 12.3.