Preventive Maintenance of Sample-Injection Valves

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For some analysts, sample injectors for liquid chromatography (LC) can provide years of problem-free service, but for others, these simple mechanical devices are very failure-prone. This month's "LC Troubleshooting" gives you a basic understanding of valve design and some guidelines for proper use and preventive maintenance.

INJECTOR DESIGN

All sample-injection valves in use today are based on the six-port valve such as that shown in Figure 1. The valve consists of a fixed *body* and a rotating *seal* (or *rotor*). In addition, several *ports* arranged around the valve connect the valve to the rest of the LC system. The *sample loop* couples two of these ports. The sample is introduced through the *injection port*, and excess sample is directed to waste through the *waste port;* the remaining ports connect the valve to the pump and column. Some valve models have a different number of ports (for example, Rheodyne [Cotati, California] often has seven ports) or internal bypass channels (for example, Waters U6K [Waters Chromatography Division of Millipore, Milford, Massachusetts]), and different brands look slightly different, but they all operate on the same principles.

Before a sample is injected, the valve rotor is moved to the *load* position (Figure 1a) so that the sample loop connects the injection and waste ports. Generally, a syringe is used to dispense sample into the loop. After the loop is filled, the valve is rotated to the *inject* position (Figure 1b) and the sample is washed onto the column. This cycle is repeated for each new injection.

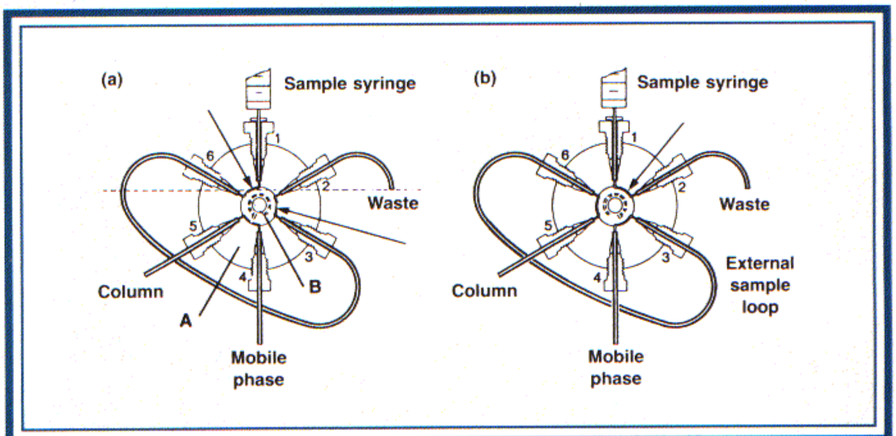

FIGURE 1: Six-port injection valve in (a) load position and (b) inject position. A = Valve body; B = rotor or seal. Port numbering: 1 = injection port (arrow indicates connecting passage); 2 = waste; 3 and 6 = connections for sample loop; 4 = mobile phase from pump; 5 = to column. Courtesy of Valco Instruments Inc.

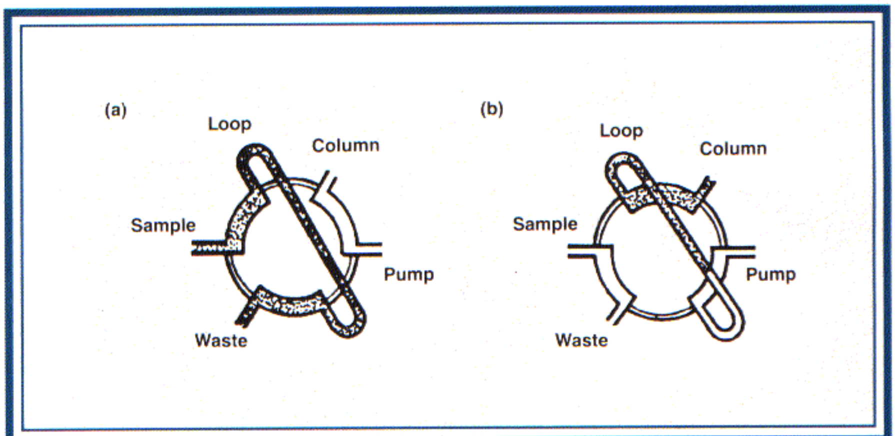

FIGURE 2: Filled-loop injection. Valve in (a) load position and (b) inject position. Reprinted from reference 6 with permission.

INJECTION TECHNIQUES

Two common techniques are used to dispense the proper amount of sample into the sample loop: *filled-loop* and *partial-loop* injection. These techniques have recently been discussed in some detail (1–3), so only a brief description will be given here.

With the filled-loop technique (illustrated in Figure 2), the entire loop is filled with sample so that the loop volume dictates the injection volume. To change the injection size, the loop is changed. Typical volumes for conventional injection loops range from 5 μL to 2 mL; special products are available to extend this range. When the filled-loop technique is used, the key to achieving reproducible injections is to overfill the loop by at least three loop volumes. That is, if you use a 10-μL loop, dispense at least 30 μL of sample into the loop. This procedure ensures that all of the previous loop contents are removed and that the loop contains only fresh sample. See references 1 and 4 for further discussion of this technique.

In the partial-loop technique, illustrated in Figure 3, only part of the sample loop is filled with sample. For example, 10 μL of sample might be placed in a 25-μL sample loop for injection; the remainder of the loop is filled with the previous loop contents (typically, mobile phase or wash solvent). This technique gives the user the flexibility to inject any sample volume desired, but the reproducibility of injections depends on the user's ability to reproducibly dispense a specific aliquot from a syringe. With the partial-loop technique, one is limited to loop volumes of <50% of the nominal loop volume (for example, <25 μL in a 50-μL loop). When >50% of the loop volume is used, laminar flow characteristics cause the center of the sample stream to flow out the waste end of the loop before all the previous contents have been washed out (1,4). Under these conditions it is hard to predict exactly how much sample is in the loop. One way around this problem is to inject a tiny bubble just ahead of the sample in order to isolate the sample from the previous loop contents (5), allowing >50% of the loop volume to be used.

When manual injections are made, generally it is preferable to use the filled-loop injection technique because it easily provides reproducible results. Partial-loop injections are more often used for exploratory runs in which the best sample size is not yet known, or for autoinjectors, which rely on very precise automated syringe-filling techniques.

INJECTORS FOR AUTOSAMPLERS

Most autosamplers use mechanically driven versions of manual-injection valves. Valve rotation is controlled by pneumatic or electric valve actuators. Although many autosamplers use a mechanized syringe to place the sample in the loop, they are designed so that the sample never contacts the syringe in order to simplify syringe washing. One common design uses the syringe to draw the sample into the loop. A needle is connected to the valve at the waste port (Figure 1). In order to fill the loop, the needle is lowered into a sample vial, and the syringe draws sample into the loop. In an alternate design, the syringe draws sample from the vial into a holding coil, then dispenses it into the loop in the same manner as for manual injections.

ENHANCING INJECTION

For a maximum column plate number and minimum loss of resolution, the injected sample volume should be <20–30% of the volume of the first peak of interest (1). For practical purposes, this means injecting the smallest convenient sample volume. For example, if a 10,000-plate, 15-cm column is used with a sample that elutes at $k' = 1$ (retention ≈ 2.5 min), the bandwidth is ≈0.1 mL. In this case, 20–30 μL of sample would be the upper limit for maximum system performance.

Another technique that effectively minimizes sample volume is using an injection solvent that is weaker than the mobile phase. When the injection solvent is less than ≈50% of the mobile phase strength (for example, 30% methanol/water with a 60% methanol/water mobile phase), the band gets compressed at the head of the column. This reduces band broadening — much as a small injection would — yet allows larger injections than do the guidelines given above.

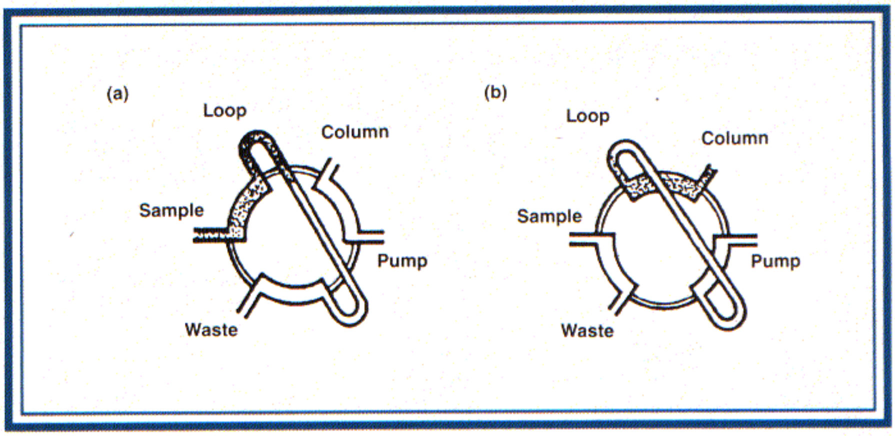

FIGURE 3: Partial-loop injection. Valve in (a) load position and (b) inject position. Reprinted from reference 6 with permission.

TABLE I: VALVE-BLOCKAGE ISOLATION

Condition	Position: Inject	Position: Load	Loosen Port*: 2	Loosen Port*: 3	Loosen Port*: 6	Blockage Location
Pressure high	+**	–	0	–†	–†	Port 6
	+	–	0	–	+	Port 3 or loop
	–	+	0	0	0	Misaligned rotor
	+	+	0	–†	–†	Port 5
	+	+	0	+†	+†	Port 4
Hard to fill loop§	+	+	+†	0	+‡	Port 1 or syringe
	+	+	+‡	0	–†	Port 2
	+	+	–	0	0	Waste line
	–	+	0	0	+	Port 6
	–	+	0	+	–	Loop

* based on Figure 1; other valve configurations may require some adjustment to table
** (+) condition true in this position; (–) condition absent in this position; (0) not applicable
† test in *inject* position
‡ test in *load* position
§ hard to push sample through loop in *load* position or to waste in *inject* position
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When partial-loop injections are made, it is important that the valve is plumbed as illustrated in Figures 1–3 so that the sample reaches the column before the previous loop contents. This prevents dilution of the sample, which would cause band broadening at the head of the column.

PREVENTING VALVE PROBLEMS

Most injection-valve problems are preventable, as is demonstrated by analysts who get years of trouble-free use from an injector. The primary cause of problems is dirt. Particulate matter entering the valve can lodge in the moving parts, causing scratches and leakage, or can block the connecting tubing or sample loop. The simplest preventive measure for valve contamination is to keep particulate matter from entering the valve in the first place. Some LC systems require that a 5-μm in-line filter be placed between the pump and the valve; the filter prevents contaminants originating in the pump or a saturator column from reaching the valve. Filtration of all samples will keep sample contaminants from getting to the valve, but this remedy is both inconvenient and expensive. Instead, filter any samples that have visible particulate matter or are cloudy or opalescent.

If you encounter valve blockage, you can use the troubleshooting chart in Table I to help determine where the blockage is located. For example, if you observe that the pressure is high in both the *load* and *inject* positions, try loosening the loop at ports 3 and 6 (Figure 1). If the pressure drops when either port is loosened, the problem is in port 5 or in the line leading from it. If the pressure stays high, however, port 4 or the line from the pump is blocked. Once the blockage is found, first try to backflush the blocked passage. If this is unsuccessful, replace blocked tubing. You can often clear a blocked valve port by disassembling the valve and sonicating the problem part in soapy water. Rinse the valve with clear water and try blowing the passage clear with compressed air. It is not wise to use a syringe-cleaning wire or other mechanical probe to try to clear a blocked valve because the probe can cause permanent damage. It is better to return a blocked valve to the manufacturer for reconditioning (this will cost much less than a new valve).

A second cause of valve problems is wear of the rotor seal. Although all seals wear out eventually, the wear can be greatly reduced by a few simple preventive maintenance techniques. First, prevent abrasive particulates from entering the system, as discussed above. Second, avoid letting buffered or corrosive mobile phases stand in the valve for extended periods. Instead, flush the valve (and the entire system) with unbuffered mobile phase at the end of each day's use. Third, you can adjust the pressure limit on some valves to reduce the wear rate. Most valves are designed to work at pressures as high as 6000 psi, but in practice most workers operate their systems at <2500–3000 psi. If the rotor pressure is reduced so that the pressure limit is 3500 instead of 6000 psi, the valve will work perfectly well, yet the rotor wear rate will be reduced.

Finally, some valve problems result from incompatibility of the sample or mobile phase with the valve materials. Most commonly, injection valves are constructed of stainless steel with a polymeric rotor. Valves are available made of ceramic, special alloys, and plastics. If you suspect that chemical interactions are causing problems with your LC system or assay, consult the valve manufacturer for specialty valves.

ISOLATING PRECISION PROBLEMS

Precision or reproducibility problems show up as irreproducible peak heights (or areas) in the chromatogram. Before you can correct the problem, you must isolate its source. Often the injection valve is suspect, although the chances are good that the problem is elsewhere. If you suspect that the valve is the problem, it is best to test the valve in the most reliable injection mode — filled loop — with a small sample that you know is stable (for example, a toluene sample with a methanol/water mobile phase for reversed-phase LC). Use a 10- or 20-μL loop and overfill it by loading at least 60 μL of sample. Leave the syringe in the loop while rotating the loop to the *inject* position, and leave the loop in the *inject* position until the sample elutes. If the reproducibility problem remains after several such injections of sample from the same vial, you have determined that the injection technique and sample are not at fault. (If the problem disappears, check for sample stability problems.) If the problem persists, and you still suspect the injector, it is helpful to substitute a known good injector in order to confirm your suspicions. More likely causes, however, are system problems such as mobile phase, column, or hardware malfunction. A detailed discussion of the isolation of problems can be found in reference 1.

SUMMARY

LC sample-injection valves are simple mechanical devices that should perform reliably for a long time if they are cared for properly. The best preventive maintenance techniques are to keep contaminants from reaching the valve (by using the proper mobile phase and by sample filtration) and to flush the entire LC system at the end of each day's work in order to remove buffers. The use of proper injection techniques will assure reproducible performance from sample to sample and day to day.

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"LC Troubleshooting" editor John W. Dolan is president of LC Resources Inc. of Lafayette, California, USA, and is a member of the Editorial Advisory Board of LC•GC.