T R O U B L E S H O O T I N G

Troubleshooting Autosamplers, Part II

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Last month's Troubleshooting column addressed autosampler problems related to sample-tray sensing and alignment, and problems related to delivery of sample to the sample loop (1). This month's discussion covers problems associated with sample injection valves, injection precision, and carryover.

INJECTOR PROBLEMS

Autosamplers generally feature automated versions of the manual injection valves commonly used in high performance liquid chromatography (HPLC). Typically, these are six-port valves connected to a pneumatic or electric valve actuator. When operating properly, the injection valve should rotate in <0.5 s; longer rotation times can result in a buildup of mobile-phase pressure before the valve while it is being rotated. This pressure buildup is released when the valve finishes rotating, and the pressure pulse can damage the column.

The rotational speed of pneumatically controlled valves is determined by the pressure and viscosity of the pressurizing gas. Normally, nitrogen or air is used to control the valves, with pressures no greater than ~60 psi. Higher pressures will cause the valve to rotate faster but may damage the actuator; consult the manufacturer's literature for specific recommendations. Valve-rotation speed also can be increased if a gas with lower viscosity is used. For example, helium will cause the valve to rotate faster than nitrogen at the same pressure. (If you do use helium, be sure to turn the tank off at night — a small leak can quickly drain the expensive helium supply.)

The rotational speed of electrically actuated valves is fixed, and they do not require a gas supply, so you don't need to be concerned about selecting the proper pressurizing gas or pressure. Autosamplers using electric actuators are also easier to move because they have fewer external connections.

If a pneumatically actuated injection valve does not rotate, it is probably because the gas pressure is too low. Be sure that the supply tank is not empty and that the regulator is adjusted properly. Both electric and pneumatic valves use an electrical signal to start and stop valve rotation; if a wire is loose, the valve will not rotate. Once you are sure that the proper signal and pressure are reaching the valve, you must determine if the problem is in the valve or in the actuator. Disconnect the valve body from the actuator and give the *load* or *inject* command to see if the actuator rotates. If rotation does not occur, the problem is with the actuator, the pressurizing system, or the input signal. If the actuator rotates properly without the valve in place, either the actuator is "weak" or the valve has too much resistance. If the valve resists rotation, try disassembling, cleaning, and readjusting it. At this point, problems are most conveniently isolated by substituting known good parts for questionable ones.

Other mechanical problems with autosampler injection valves include loop blockage, fittings problems, rotor wear, and poor adjustment (these problem areas were discussed in an earlier Troubleshooting article [2]).

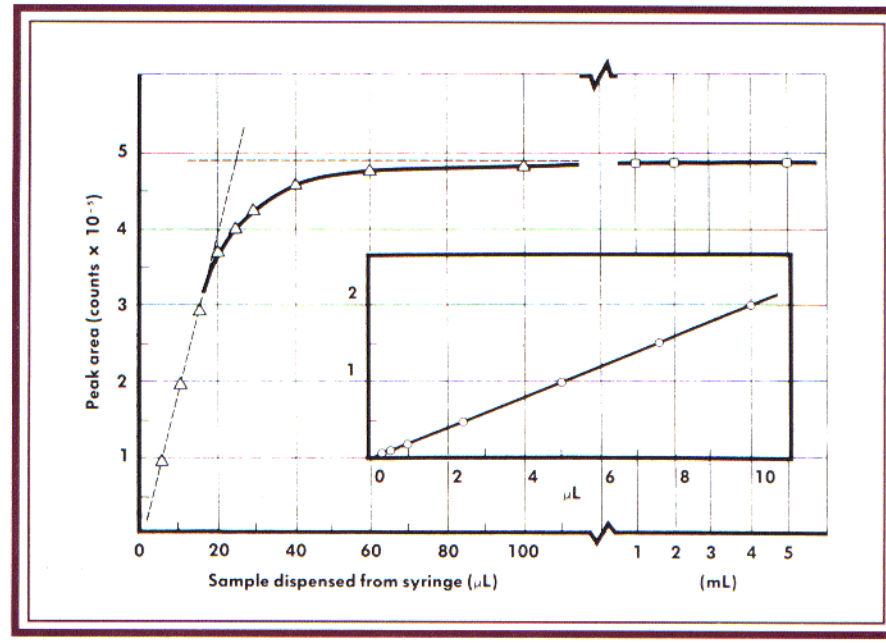

FIGURE 1: Linearity of filling of a sample-injection loop. (Courtesy of Rheodyne, Cotati, California.)

PRECISION PROBLEMS

Injection precision is a major concern when autosamplers are used (3,4). To check the precision of an autosampler, set the system to run 10 replicates of the same sample (from one or multiple vials). If the standard deviation of the sample response is outside the method specifications, the autosampler may be at fault. Unacceptable precision can arise from equipment problems or from poor technique. Partial or total blockages of connecting tubing, needles, or sample loops can result in insufficient sample reaching the sample loop. Loose or poorly assembled fittings allow air to leak into the system, causing poor precision because air is injected with the sample. As mentioned last month, a blocked vent needle can also cause poor precision (1). Isolation and correction of these equipment problems have been discussed (1).

Precision problems can arise from poor method design. Improper loop filling has been discussed (2) but bears repeating here. Figure 1 illustrates the filling characteristics of a typical sample-injection loop. In this case, a 20-μL loop was used; various aliquots of sample were placed in the loop (x-axis) and the corresponding signal measured (y-axis). The plot is linear below ~60% of the loop volume. A steady response for a full loop was not obtained until the loop had been flushed with ~300% of its nominal volume. Between 60% and 300% of the nominal loop volume, the response curve is nonlinear. This nonlinear region exists because of the laminar

flow characteristics of liquids in tubing. As was discussed in the earlier article, these filling characteristics mean that the loop should be used for injection of <60% of the nominal volume in the partial-fill mode or with >300% of the nominal volume in the filled-loop mode.

Displacement autosamplers, in which the sample is forced out of the sample vial and through the loop by air pressure, rely on filled-loop injection. For good precision with these samplers, be sure that at least 300% of the loop volume is flushed *through the loop*. This means that a 100-μL loop will require a minimum of 300 μL of sample, plus enough sample to fill all the connecting tubing between the vial and the loop. In some cases, 0.5 mL of sample or more is required for each injection.

Syringe autosamplers, which draw the sample into the sample loop using a syringe, can operate in either the filled-loop or partially filled loop mode. Again, be sure to use an adequate volume of sample for filled-loop injection. For partial-loop injections, the syringe must draw enough sample so that the loop contains no more than 60% of its nominal volume. To this must be added the tubing volume between the sample needle and the valve, typically 10–20 μL in modern autosamplers. When the autosampler is routinely used in the partially filled loop mode, it is convenient to mount a large-volume (0.5–1.0 mL) loop on the injector. Some samplers (for example, the Wisp, Waters Chromatography Division, Millipore Corp., Milford, Massachusetts) are designed to operate primarily with partial-loop injections and will accommodate ≤ 2.0-mL sample loops.

Other sources of autosampler imprecision are sample evaporation and sample degradation. Evaporative problems can be minimized by using tightly fitting vial caps and septa and by avoiding the use of volatile solvents. Sample degradation is a chemical problem that must be solved through the use of chemical stabilizers or refrigeration. Generally, these two problems will cause peak heights (areas) to change regularly over time. Solvent evaporation will concentrate the sample and thus give increasing heights over time; sample loss will give smaller heights. Sample degradation will cause smaller heights, and often additional peaks from breakdown products will appear in the chromatogram.

CARRYOVER

If the sample needle and transfer tubing are not sufficiently washed between injections, sample carryover from one injection to the next may occur. Check for carryover by making alternating injections of sample and solvent blank (water). If carryover occurs, you will see small sample peaks in the chromatograms for the blank injections. (You may need to select a more sensitive detector attenuation to see the carryover peaks.) If carryover is a problem, flush the autosampler thoroughly between injections. With some units, there is a separate wash cycle in which a wash solvent is drawn through the tubing from a wash vial or wash reservoir and disposed to waste. In that case, try increasing the wash volume or using two wash cycles instead of one. With all units, you can effectively wash the system by using excess sample in the filled-loop mode. The extra sample passing through the tubing and loop will remove residues from the previous sample. In extreme and rare cases, it may be necessary to inject a solvent blank between each actual sample. When running samples of greatly varying concentrations, inject the lowest-concentration samples first and the highest-concentration samples last. Any carryover will be less of a problem than if the samples were run in the reverse order. For example, a 1% carryover from a 10-μg sample won't affect the results of a 1-mg sample, but 1% carryover from a 1-mg sample would render results from a subsequent 10-μg sample unusable.

REFERENCES

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