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

Troubleshooting Autosamplers, Part I

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This month's column discusses problems associated with HPLC autosamplers, specifically mechanical problems associated with sample-tray sensing and alignment and problems that cause excess or insufficient sample to be delivered to the sample-injection loop. Next month, the discussion will focus on autosampler injector problems as well as autosampler problems that result in poor precision and chromatographic problems. (Previous Troubleshooting columns [1,2] discussed other aspects of autosampler troubleshooting.)

Many autosamplers used today are available with modifications that allow them to be used for gas chromatography (GC). Therefore, while this discussion focuses on HPLC autosamplers, much of the information applies to GC autosamplers as well.

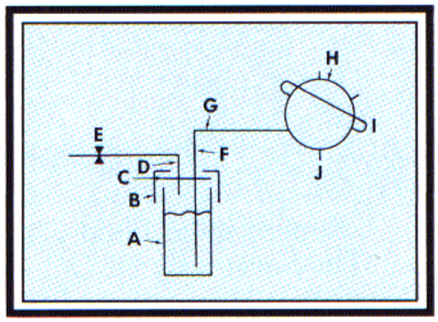

FIGURE 1: Schematic diagram of a displacement autosampler. A = sample vial, B = vial cap, C = septum, D = pressurizing needle, E = pressure control valve, F = sample needle, G = transfer tubing, H = injection valve, I = sample loop, J = waste line.

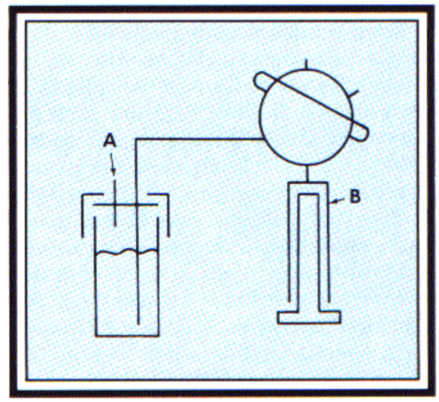

FIGURE 2: Schematic diagram of a syringe autosampler. A = vent needle (may be concentric to sample needle), B = sampling syringe; all other parts as in Figure 1.

TYPES OF AUTOSAMPLERS

Most HPLC autosamplers in use today fall into one of two categories: displacement or syringe (see Figures 1 and 2). In displacement autosamplers, the fluid in the sample vial is pressurized, causing an aliquot of the sample to be displaced through a sample needle into the sample loop of the injector. In syringe autosamplers, a motorized syringe draws an aliquot of sample from the sample vial into the sample loop. As can be seen in Figures 1 and 2, the principal difference between the two designs is that with displacement autosamplers sample is *forced* through the loop, but with syringe samplers it is *drawn* through the loop. Displacement autosamplers operate only in the filled-loop mode because it is not possible to control precisely the amount of fluid displaced from the sample vial into the injection valve sample loop. Syringe autosamplers, on the other hand, can operate in either the filled- or partially filled-loop mode because of the precise control of syringe movement. Although most autosamplers are either syringe or displacement models, a wide variety of modifications are incorporated into the various commercial designs, resulting in a range of features and functions.

In both types of autosamplers, the injector is commonly a six-port automated version of a widely used manual sample-injection valve. The injection valve is rotated either pneumatically or electrically.

This discussion will center on HPLC autosampler problems that are generally associated with mechanical components, sample delivery to the sample loop, injection valve operation, and chromatographic or precision problems. Other problems can occur with the electronic circuitry within the sampler, but troubleshooting these problems is beyond the scope of this discussion. Fortunately, many autosamplers have built-in electronic diagnostics, and service manuals may provide model-specific troubleshooting procedures. Most electronics problems with HPLC systems, however, require the expertise of a service engineer.

One general precaution is worth mentioning at the outset: Often during troubleshooting it is necessary to operate the unit with its covers removed — be careful to avoid such hazards as electrical shock and pinched fingers.

MECHANICAL PROBLEMS

The autosampler is the most mechanically complex component of the HPLC system; thus, the incidence of mechanical problems is relatively high for this module. The major problems are discussed below.

Sample needle: Problems with sample needles are common with autosamplers. Often, needles bend or become blocked, rendering the sampler useless. Needle design usually is unique for each autosampler model, and installation of the wrong needle will usually cause problems. It is wise to keep at least one spare needle on hand for each autosampler in operation.

Sample needles can bend for several reasons. First, if the vial septum is difficult to pierce, the needle can bend while penetrating the septum. If this occurs often, consider using another brand or style of septum. Second, needles can bend if the vial is not aligned properly; the needle hits the vial cap rather than the septum. This is usually a problem with tray alignment (see discussion below). Consult the service manual for tray-adjustment procedures. Third, the needle can bend if it hits the bottom of the vial. To minimize this problem, be sure that the needle is properly adjusted. Sometimes the needle-lowering (or vial-raising) mechanism may need adjustment. Some samplers (for example, the Wisp, Waters Chromatography Division, Millipore Corp., Milford, Massachusetts) use spring-loaded inserts for small sample volumes; these allow the needle to "bottom out" in the vial without causing damage.

Another major cause of sample needle problems is needle blockage. Most autosamplers use side-port needles instead of conventional syringe needles, which have a tapered end with the opening in the center. Side-port needles are less likely to be blocked by pieces of septa. If needle blockage is a recurring problem, consider two precautions. First, filter the samples before filling the vials. Hold the sample vial near a light; if the sample is opalescent or contains visible particulate matter, it should be filtered. Samples can be filtered directly into the vials through syringe-mounted 0.45-μm disposable filters.

Second, try using a different type of vial septum. Most users find good results with septa of silicon laminated to Teflon. The silicon rubber, however, may block the needle. If plain Teflon septa can be used, they may solve the problem. Otherwise, try a different brand of septa. If you are using the needles and septa recommended by the autosampler manufacturer and you still have problems, contact the manufacturer for advice.

Tray alignment: Sample vials are loaded into one or more sample trays that are then placed on the autosampler carrousel. The carrousel rotates under control of the method program until the vial of interest is under the sample needle. Sample selection varies from strictly sequential advancement to completely random access, depending on the unit's design and the control program.

Sequential sample selection requires a control mechanism that enables the sample carrousel to advance one position at a time. Carrousel advancement is usually sensed by a microswitch. In these models, switch malfunction is the most common fault. The switch can be damaged by spilled reagents or samples. Check the switch by depressing the sensing arm (or button) to determine if the rotation mechanism functions. If the tray does not rotate, unplug the unit, disconnect the switch, and check the switch with a volt-ohm meter (VOM). A defective switch should be replaced. (Note: If the switch failed because of a spill, take precautions to avoid spills; one simple practice is never to fill the sample vials while the sample tray is on the carrousel.)

Samplers with random-access features rely on a mechanical or optical sensor to detect the proper vial position. Sensing strips may be mounted on the sample tray or on each sample vial, depending on the particular brand and model. Optical sensors are particularly prone to malfunction caused by reagent or sample spills. The optical sensor or the sensing strip can become coated with chemical residues, resulting in sensing errors. The strips and sensors can generally be cleaned by gently wiping the optical surface with a cotton swab or a Kimwipe dampened with isopropanol. The Wisp autosampler verifies the presence of a vial by optically sensing a reflection off the top portion of the vial side. If the vial is nonreflective (because of dirt, labels, etc.), the vial sensing mechanism may not work.

Misalignment of the sample tray can cause three problems. First, if the wrong vial is in position for injection, sample mix-up can occur, invalidating the results. Second, a vial slightly out of alignment will often result in a bent sample needle, as discussed in the previous section. Third, with some models, a misaligned vial will cause the needle-plunger mechanism to crush the sample vial. Consult the service manual for specific alignment procedures for each autosampler model.

DELIVERY OF SAMPLE TO THE LOOP

This section focuses on problems that result when an improper amount of sample reaches the sample loop. Because displacement autosamplers and syringe autosamplers differ in the way the sample is delivered to the loop, each type will be discussed separately.

Displacement autosamplers: The plumbing diagram of a generalized displacement autosampler is shown in Figure 1. The vial is lifted (or the needle is lowered) so that the septum is pierced both by a pressurizing needle and by the sample needle. The pressurizing needle is shorter than the sample needle, so it stays above the surface of the sample. Gas pressure supplied through the pressurizing needle forces the sample out of the vial through the sample needle, into the sample loop, and then to waste. The pressure applied and the length of time the vial is pressurized determine how much sample is displaced. Of course, the sample viscosity and the resistance to flow in the connecting tubing also affect the sample delivery.

The Micromeritics (Norcross, Georgia) displacement autosampler incorporates a major design modification. With this sampler, the vial cap acts as a plunger, which is pressed downward during sampling, thus forcing the sample out through the sample needle. No auxiliary air pressure is needed for loop filling. The volume of sample delivered to the injector loop is determined by the distance the cap is pressed into the vial by the needle-plunger assembly.

The most common problem with a displacement delivery system is that the pressure is too high or the length of time is too long and the entire sample is displaced from the vial. This can result in air in the injection loop and, thus, an injection volume that is too small. When this occurs, there is not enough sample for multiple injections. The converse of this problem (too little pressure or not enough time) can result in an insufficient amount of sample reaching the loop. This also gives smaller injection volumes than expected. Any blockage in the connecting tubing can result in more flow resistance and a smaller sample injection than expected. For maximum precision, the pressure and time should be adjusted so that at least three sample-loop volumes pass to waste (3); any sample in excess of this amount is wasted. The operator's manual for the autosampler should contain guidelines for these settings. With some samplers, there is a "viscosity" switch that is used to make adjustments for changes in sample viscosity.

To limit the amount of sample delivered, reduce the pressure or the length of time the vial is pressurized. You can check to see that the pressure shut-off valve is working properly by immersing the pressurizing needle in a vial of water. Bubbles should flow in a steady stream when the valve is in the *on* position, but bubbles should not flow when the valve is turned *off*. (If bubbles appear, the valve should be replaced.) The delivery volume on the Micromeritics autosampler is adjusted by changing the displacement distance of the needle-plunger assembly. With all autosamplers, excess sample is required to fill the transfer tubing and flush the loop. This means that the sample loop may contain air bubbles if an insufficient amount of sample is placed in the vial.

If not enough sample is being delivered, check that the pressure and length of time are correct. Next, verify that the pressurizing needle is functioning properly by immersing it in a vial of water, as described above. Finally, check to be sure that the vial is sealed properly; a leaky vial cap or septum will thwart the sample-displacement system. If it has been determined that these components are functioning properly, the problem might be attributable to a blockage (or partial blockage) in the transfer tubing to the injection valve, in the valve itself, or in the waste line.

The blockage can be isolated by disconnecting the tubing in a stepwise manner. First, note the flow rate of the sample leaving the waste line. Generally, this can be done visually, although you may want to measure it volumetrically. Next, loosen the fitting where the waste line connects to the injector. If the waste line is blocked, the flow from the valve should be noticeably greater than from the waste-line exit. Continue working upstream in this fashion until you have isolated the blocked tubing. Sometimes you can clear the tubing by reverse-flushing, but it is best to replace the offending piece. In most cases, you will find that the blockage is located in the sample needle. For this reason, you may want to check the waste-line flow, then loosen the needle outlet, rather than to check the other tubing first.

Syringe autosamplers: The general problems of too much or not enough sample reaching the sample loop are the same for syringe autosamplers and displacement samplers, but the causes of the problems are slightly different. As shown in Figure 2, sample is drawn from the sample vial by the syringe. Because the injection valve is between the sample needle and the syringe, the sample is drawn into the sample loop for injection.

Over- or under-delivery of sample with syringe autosamplers is caused either by improper settings or by the use of the wrong syringe. Syringe movement generally is determined by a stepper motor, which is under software control. If the wrong volume is used in the control program, an incorrect volume will be delivered. Program entry errors are easily verified and corrected through a review of the program steps. The software converts volume commands into motor steps that withdraw the syringe the proper amount. Thus, the syringe must match the software, or the step size will be wrong. Although you should use only the syringe(s) recommended by the autosampler manufacturer, often you can switch syringe sizes by changing a "syringe-size" command in the control program.

Over-delivery of sample is most likely caused by a program error or by use of the wrong syringe. Under-delivery of sample is probably caused by a vacuum, blockage, or leak in the connecting tubing.

If a vacuum forms in the sample vial, the syringe may not be able to draw a sufficient volume of sample from the vial. To avoid this vacuum, be sure that the vent needle to the sample vial is open. The vent needle is often concentric, surrounding the sample needle; it is open to the atmosphere at the top. It is possible for the vent needle to become blocked if it is splashed by sample. The easiest way to determine if a vacuum has formed is to inject a (nonvolatile) sample with and without a septum on the vial. If there is a problem when the septum is used but no problem when the septum is not used, the vent needle is blocked. Clean or replace the blocked vent needle.

To isolate a blocked transfer tube or needle in a syringe autosampler, use a vial of water or methanol as a sample. First, determine how much sample is withdrawn from the vial through the entire system. Next, verify that the syringe is working properly, using the purge cycle available on most autosamplers. The purge cycle allows the syringe to fill with a wash fluid, after which the solution is expelled to waste. If this cycle works properly, the syringe is probably OK. Next, loosen the fittings in a stepwise manner from the sample needle to the syringe, dipping the free tube end in the sample vial to check for proper operation. Again, the most likely cause of problems is a blocked sample needle; you may want to replace the sample needle at the beginning. When the problem is isolated, replace the blocked tube.

Improperly connected tubing allows air to leak into the system while sample is withdrawn. This can result in bubbles being injected along with the sample, which causes smaller-than-expected injection volumes. In severe cases, air leaks can prevent the sample from being withdrawn from the vial. Be sure that the fittings are properly tightened; replace any that are questionable.

SUMMARY

Keeping the autosampler clean and well-adjusted is important for reliable autosampler operation. Proper tray alignment and tray sensor adjustment will help prevent sample mix-up and bent sampler needles. Filtered samples and use of the proper sample needle and vial septa will help prevent blockage of the sample needle and transfer tubing. Finally, adjustment of the sample displacement or syringe mechanism will ensure that the proper amount of sample reaches the sample loop.

REFERENCES

(1) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 834–836 (1984).

(2) J.W. Dolan, *LC•GC* **4**(5), 416 (1986).

(3) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3,** 1050–1052 (1985).

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