Autosamplers — Symptoms and Solutions

Autosamplers perform the first steps in the chain of events that conducts solutes from vial to final chromatographic separation report. Any errors that arise during autosampling will propagate through separation, detection, and quantitation and will affect the final results. Therefore, proper autosampler setup, operation, and maintenance are critical to obtaining the best possible results. Commercial manufacturers produce devices suitable for dedicated autosampling of specific solid, liquid, or gaseous sample types as well as more universal devices that can incorporate a number of add-on modules for sample derivatization, extraction, and filtration (see Table I). I discussed thermal desorbers in the September 2000 issue of *LCGC* (1), and headspace sampling was discussed in terms of its use with high-speed gas chromatography (GC) separations in the same issue (2). I’ve limited the discussion in this column to GC liquid autosamplers, but many of the principles and ideas presented here are applicable to other autosampler types as well.

Liquid autosampling in GC comprises two fundamental steps: First, the syringe acquires liquid sample for injection. Second, the syringe injects sample into the inlet system. Each step requires specific mechanical motions at well-regulated speeds. Sample-acquisition choreography depends upon the autosampler mechanism and the sample type; sample-injection choreography depends upon the injection technique and the sample type. Various more-sophisticated sample, solvent, and even reagent sequences are possible; some autosamplers combine sub- or supernatant sample extraction, derivatization, or standard addition into the initial sample acquisition steps. A more recent addition is the capability to perform solid-phase micro-extraction. Interested readers should contact the manufacturers listed in Table I for more detailed information about these extended capabilities.

Types of Liquid Autosamplers

Liquid autosamplers can be divided into two basic categories when considered in terms of the path the sample takes through the syringe. **Front-loading autosamplers** mimic hand motions that move the syringe plunger up and down to pull sample up

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<tr>
<th>Product</th>
<th>Manufacturer†</th>
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* Some models are designed to fit various instruments, and others are specific to the manufacturers’ products. This table does not include all GC liquid autosampler manufacturers. The information presented here is based on information from the companies’ web sites and from individuals; neither the author nor *LCGC* are responsible for inaccuracies.

† Agilent Technologies (Wilmington, Delaware); Konik (Miami, Florida); Leap Technologies (Carrboro, North Carolina); OI Corp. (College Station, Texas); PerkinElmer Instruments (Wilton, Connecticut); Shimadzu Scientific Instruments, Inc. (Columbia, Maryland); SRI Instruments (Torrance, California); ThermoQuest/Finnigan (Austin, Texas); Varian Instruments (Walnut Creek, California).
into the barrel and then inject it by mechanical force. Back-loading autosamplers use gas pressure to push liquid from the sample vial or solvent reservoir through connecting tubing into the syringe barrel and then out the needle, always moving the liquid in the same direction. Hybrid autosamplers combine the sampling method of a front-loading autosampler with the solvent rinsing of a back-loading autosampler. In either case, a clean and relatively low-viscosity liquid sample will produce the best results. Dirty or highly viscous samples are problematic for reasons that I will discuss below.

Front-loading autosamplers: In front-loading autosamplers, the syringe moves into positions over the sample vial, solvent reservoirs, the inlet, and waste receptacles, as Figure 1 shows, and then deposits or removes liquid as required. The amount of liquid consumed is small, so these autosamplers can successfully handle samples of 100 µL or less. Only the syringe needle, barrel, and plunger contact the sample before injection, and this minimized contact reduces the amount of solvent needed to clean the syringes between samples and the levels of sample-to-sample carryover in normal operation.

Sample acquisition into the syringe barrel depends upon efficient plunger suction; a leaking or worn syringe will cause problems with reproducibility and sample-to-sample carryover. Sample cleanliness is paramount because small amounts of particulate matter will wear out the syringe plunger rapidly, and nonvolatile residues can cause the plunger actuation force to increase and eventually stick the plunger firmly in place. In front-loading devices, the syringe plunger goes through many more actuations per sample injection — approximately 16–20 — than in back-loading devices. That’s roughly 2000 actuations per full autosampler tray or 10,000 actuations per five-day workweek at one tray per day. For these reasons, most workers prefer polytetrafluoroethylene (PTFE)–tipped syringe plungers in front-loading autosamplers.

A too-volatile solvent, such as diethyl ether or methylene chloride (with atmospheric pressure boiling points of 34 °C and 40 °C, respectively) will boil easily under the reduced pressure conditions inside the syringe barrel and needle during plunger motion. The resulting bubbles seri-ously detract from accuracy and reproducibility. Even with autosampler tray cooling to minimize sample temperatures, hexane probably is the most volatile solvent that chromatographers can use successfully and consistently.

Back-loading autosamplers: In a back-loading autosampler, a second dedicated sampling needle removes liquid from the sample vials and solvent reservoirs. Instead of depending upon syringe plunger suction, gas pressure positively displaces sample from the solvent or sample containers through the second needle and connecting tubing, into the rear or top of the microsyringe itself. Liquid flows through the microsyringe and into waste receptacles during washing or sample acquisition. The plunger acts as a two-way valve in this case. When the plunger is in the up position, as Figure 2 shows, liquid can travel from the connecting tubing into the syringe and downward into a waste receptacle. This type of arrangement can flush the syringe very efficiently, but it also consumes more sample and solvent volume than a front-loading device. For injection, sample fills the syringe, the plunger moves into position at the programmed volume set point, and the syringe then injects the sample into the inlet.

A back-loading device also is susceptible to contaminated samples. Small particles can clog the interconnecting tubing, and nonvolatile residue deposits can cause the plunger actuation force to increase to the point of stalling as well. However, unlike a front-loading device, only a few syringe plunger actuations occur per injection, so the syringes tend to last longer. Finally, back-loading devices better tolerate more volatile solvents because the system remains under positive pressure during sample transport. However, the rapid changes in internal pressure that occur during sampling at temperatures close to the liquid’s boiling point sometimes cause bubble formation because of the release of entrained gas from the liquid at nucleation points along the tubing and connections.

Hybrid autosamplers: Hybrid autosamplers that mix features of both front- and back-loading systems also are possible. These devices load the sample through the syringe needle, as does a front-loading system, and rinses the syringe from a larger solvent reservoir, as does a back-loading system, as shown in Figure 2. This fusion of the two operational modes blends the low carryover and sample consumption
of a front-loading autosampler with the efficient cleaning obtained with a back-loading autosampler. As Table I illustrates, however, the overwhelming majority of liquid autosamplers operate in a full front-loading mode.

**Autosampler Troubleshooting**

So, what can go wrong with liquid autosampling systems? The more complex the system, the more possibilities for problems. The electromechanical components are driven by precision stepper motors, their positions are ascertained by accurate sensors, and the interplay between motion commands and position is monitored closely by the controlling software and hardware. Nonetheless, mechanical components may wear out or break from usage or because of external factors that include sample problems, installation problems, and maintenance problems. The remainder of this “GC Connections” column examines some of these problems in terms of their symptoms and presents some solutions to fix or prevent these difficulties.

The most common problem sources are dirty or inappropriate samples and poor maintenance procedures. As I mentioned above, a dirty sample will quickly plug or immobilize a syringe. Some autosampler parts such as septa and syringes require periodic replacement. The frequency of replacement depends upon sample characteristics and on the type of duty cycles that the sampling technique demands from the autosampler system.

**Syringe Problems**

**Small or missing peaks:** For the syringe, one of the most common problems manifests as very small or missing peaks. Assuming that the inlet system functions correctly, the column isn’t broken, and the detector works, this symptom almost always indicates a blocked syringe needle or the absence of enough sample volume in the vials to reach the tip of the sampling needle. Each autosampler and vial type requires a minimum liquid amount. Conservatively, standard 2-mL vials need at least 0.5 mL, and micro vials or inserts require 50 µL. Smaller sample volumes may lie under the bottom needle position, so the syringe draws no sample. On autosamplers with adjustable needle depths — for sample-extraction purposes — be sure that the needle depth is set appropriately.

If the needle dips below the liquid surface but little or no sample enters the column, check the syringe for leaks or blockage. Remove the syringe from the autosampler and attempt to manually introduce some pure solvent into it. Inspect the syringe barrel for the presence of a bubble-free solvent plug. If necessary, attempt to unblock the syringe or clean it.

If liquid is drawn, but several bubbles appear that cannot be expunged by repeated plunger motions, then the syringe plunger may be so worn that air can leak in around it. In this situation a new syringe is necessary — it is impossible to repair a syringe that leaks in this manner. Considering how many plunger cycles a syringe goes through in the course of a busy week, sample cleanliness is extremely important. Because real-world samples are never pristine, regular syringe replacement is the best course to avoid syringe problems. The frequency of replacement will depend upon the sample, but a weekly new syringe installation isn’t unusual. The cost of instrument downtime is very high in many laboratories, so a small extra supply cost to properly manage syringe life is worthwhile.

Finally, never let a syringe dry out with sample in it during idle periods. Make sure the autosampler program calls for multiple solvent rinses after each injection, and consider manually rinsing out syringes that won’t be used for a while, even if only for one day.

**Bent needle:** Some autosamplers seem to be prone to bending needles. It is not a design flaw; it’s a symptom of improper installation, maintenance, or operating conditions. The most common cause of bent needles is misalignment with the inlet septum nut. Some autosamplers are self-aligning and include sensors that adjust the syringe position slightly when the alignment shifts. Others rely on precision-molded parts to bring the parts together in the right way. However, if the autosampler tower isn’t installed securely on the instrument, and if an operator doesn’t check the needle–inlet alignment upon installation and upon starting up each day, needles will bend. Sometimes needles will bend without the problem being detected by the autosampler electronics, so it’s very important to check needles often.

Another source of bent needles is using the wrong septum. Needles encounter a design flaw; it’s a symptom of improper installation, maintenance, or operating conditions. The most common cause of bent needles is misalignment with the inlet septum nut. Some autosamplers are self-aligning and include sensors that adjust the syringe position slightly when the alignment shifts. Others rely on precision-molded parts to bring the parts together in the right way. However, if the autosampler tower isn’t installed securely on the instrument, and if an operator doesn’t check the needle–inlet alignment upon installation and upon starting up each day, needles will bend. Sometimes needles will bend without the problem being detected by the autosampler electronics, so it’s very important to check needles often.

**Jammed plunger:** Syringe plungers are the most active parts of a liquid autosampler. An incorrect installation will jam the plunger on the first motion. As noted above, as many as 15–20 plunger actuations may occur per sample. If the sample isn’t clean, then particles or nonvolatile residue may build up and eventually jam the plunger. In the case of non-PTFE-tipped plungers, operation without sufficient liquid for lubrication will remove microscopic glass particles that can interfere with the precision plunger–barrel fit and jam the plunger just as surely as a dirty sample. By keeping samples clean and using sufficient solvent rinses, chromatographers largely can avoid this particular problem.

**Electrical and Mechanical Problems**

Modern autosampling systems deliver remarkable long-term performance, considering the number of motions, speed, and accuracy that heavy-duty sampling demands of them. Fifteen years ago, the
previous generation of autosamplers suffered from balky mechanical designs and a lack of electrical–mechanical and software integration, which made them much less reliable and much less flexible than more recent designs. Today’s autosamplers, though, are dedicated robots with even more complex and precise mechanisms than earlier designers could access. They also present more possibilities for failure, but the chances of any one component failing are radically less than in earlier designs. The overall probability of failure is reduced considerably.

When a modern autosampler does experience an electrical or mechanical problem, the autosampler’s software and sensors very likely will sense the problem, gracefully pause or halt the analysis sequence, and alert an operator. A simple example is a missing solvent vial. In this case, most autosampling systems will present a message to that effect and then wait for an operator to correct the condition and signal back to the autosampling system that it should continue. A more extreme example would involve a broken drive belt, and in that case the autosampler sensors would detect the failure of parts to move into position on command. The controlling software would then stop attempting to move the failed component, signal that a serious failure has occurred, and halt the analysis sequence until an operator restarts it. At this point, it’s often a good idea to run the autosampler diagnostics to try to pinpoint the problem. Make a written record of the diagnostic results for future reference.

Serious failures of this type may require a trained service person for the repair. In particular, the installation and alignment of newly installed components is critical. Most chromatographers find it fairly easy to disassemble an autosampler and remove a broken part, but they usually don’t have the tools and information required to put it back together. Autosamplers’ high speeds and precise motions require tight tolerances and precision alignment. As I have often said, bringing in a trained professional who can repair an instrument quickly and get it running is well worth the extra outlay in the long run.

**Chromatographic Problems**

Another class of autosampler problems arises when the autosampling system appears to function normally, but the chromatographic results are not as good as expected. In these cases, chromatographers can use a process of elimination to help isolate the problem at the autosampler, the inlet, or the column and identify what remedial action might be appropriate.

The autosampler is the first link in the separation and detection chain, and poor injection is reflected all the way to the data-handling system. The simplest method for locating a problem that might be autosampler-related is to remove it as a potential problem source by making several manual injections. Those manual injections will help determine if the autosampler is at fault. Although manual injections will not reproduce the exact injection conditions that an autosampler would deliver, serious autosampler problems often will disappear or moderate with manual injection. In most cases, it is appropriate to establish a test mixture that includes the target analytes or their analogues at known concentrations that lie well within the capability of the chromatographic system. If trace analysis is the objective, then use a standard mix with concentrations that are 20–50 times greater than the method detection limit for the system in use. With splitless injection and a flame ionization detector, this amount might signify concentrations in the 10–1000 ppb range. For 50–100 µL large-volume injections, the concentrations might be somewhat lower. Record test chromatograms when the system is functioning well to establish a checkpoint for later verification. Verification may include more system-suitability tests for separation, resolution, and reproducibility (see below).

If manual injections produce similar problems to those of the autosampling injections, then look at the inlet system next and check for correct inlet installation, flow rates, and valve timing. If manual injection provides better results than autosampling, then examine the autosampler and its program settings. For normal autosampling, try varying autosampler parameters such as injection speed, injection volume, and the number of pre- and postinjection washes to see if these settings make any difference. Check to be sure that the selected program parameters are appropriate for the injection technique. Be alert for suspicious noises during sampling and visually check the autosampler, syringe, vials, robot arm, and any other active components as they operate.

**Determining Repeatability**

One reason that analysts use autosamplers is to obtain better results repeatability than manual injections will yield. Chromatographers have certain expectations for repro-
ducibility of results and retention times that may or may not be met by autosampling. In general, if an analysis itself — the injection technique, the separation, or the detection — is not prone to high reproducibility, then adding an autosampler will make little or no difference. For example, if peaks come out of a column at 1.5 times the detector noise level, then autosampling will do little to improve results. Likewise, if an inlet system is operating far outside its normal design parameters — such as performing splitless injections of 10 μL of liquid sample at a 5-mL/min vent flow rate — then adding an autosampler will improve nothing.

Chromatographers can easily measure retention time and area count or amount reproducibilities. The simplest way is by using a data system that includes these calculations in its reporting software. Without that function, some data systems support the transfer of results into a spreadsheet, where the reproducibilities can be computed. Most scientific calculators support these calculations as well.

The reproducibility of retention times or results most often is expressed in terms of the standard deviation of a series of at least 10 replicate injections. Fewer than 10 data points may provide values that are not representative of data in the long run, and many more than 10 points would be a waste of time if they were collected just for the sake of the one measurement. The standard deviation (s) of a set of n data points for a set of measured points that is a representative but relatively small sample of the entire data population is given by the following equation:

\[ s = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (y_i - \bar{y})^2} \]  

where, \( y_i \) are the individual data points and \( \bar{y} \) is their mean value.

Another useful figure is the relative standard deviation (RSD) given by this equation:

\[ \text{RSD} = \left( \frac{s}{\bar{y}} \right) \times 100 \]  

Most instrument manufacturers will specify the kind of reproducibility that their devices can deliver under a set of clearly defined circumstances, and those numbers usually represent the best performance that the instruments can deliver. It follows that those numbers are seldom attained in real-world analyses. However, a well-designed test mixture can come close to simulating the best possible circumstances if the methodology itself permits it. If a test mixture contains only inert analytes at easily handled concentrations, and if the methodology uses only standard injection methods at reasonable volumes, then it may be possible to get the same excellent results. Manufacturers often will specify the exact conditions, column, and sample they used to get their best results, so it is possible to reproduce those conditions in cases that demand this kind of fundamental crosscheck.

If, however, the real sample yields performance that is significantly worse than expected or required, some judicious checking and measuring can help to determine whether the autosampler is to blame, something else in the analytical system is at fault, or the expectations simply are too high for the chosen situation.
First, determine the RSD for a standard mixture that represents the actual sample. Make 10–15 replicate autosampler injections under the same conditions as intended for the actual analysis. If the area count RSDs are less than approximately 5%, then be glad the results are that good. For situations in which the peak area RSD is more than 2%, it may be advantageous to use an internal standard and normalize results to their area counts for each run, thereby largely eliminating small run-to-run area fluctuations that affect all peaks in the analysis.

For RSDs greater than 5%, check to see if the autosampler is causing a problem by making a second set of 10 manual injections of the same volume amount of sample. If the manual injection RSD is roughly the same or worse than the autosampler, then the problem, if there is one, lies elsewhere; for example, in the inlet or data-handling system. If, however, manual injections produce a substantial improvement over autosampler injections, then it’s time to examine the autosampler closely and determine what is the exact fault. Remove the autosampler syringe if possible and make some additional manual injections with it to see if the syringe is at fault. Check the syringe for leaks or blockage, as described above, and replace it if necessary. Ensure that each vial contains sufficient sample and that the solvent wash supplies are full. Check the waste receptacles for cleanliness and be sure that they haven’t filled up.

**Conclusion**

Autosamplers are precision electromechanical devices that are designed to repeatedly deliver the same amount of sample at the same speed and with the same mechanical motions for extended periods. Combined with a well-conceived analytical separation, autosamplers can significantly augment result repeatability and extend analytical capabilities with specialized injection techniques.

Modern autosamplers include an array of sensors and associated software that provide the required motion precision and detect when something goes wrong. In many cases, however, more subtle failures lead to worse-than-expected results. Chromatographers can diagnose those occurrences by isolating an autosampler and its syringe from the rest of the analytical separation chain to ascertain the problem source. GC systems seldom deliver their best possible performance for real-world samples, because of externally imposed effects from polar or labile components, injection system limitations, or unreasonable demands on detector sensitivity. In those cases, a well-constructed test mixture free of such effects will help determine if the hardware or the methodology is the culprit behind worse-than-expected results.

**References**


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**Metachem**

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