TROUBLESHOOTING

LC User Survey: Autosampler Problems

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A recent *LC Magazine* user survey polled readers on their use of autosamplers, detectors, data systems, and fraction collectors (1). This month's Troubleshooting column collates and discusses the most frequently reported autosampler problems (detector and data-system problems will be discussed in future columns). Some of the most common problems, such as needle blockage in autosamplers, probably have stimulated user innovation. If you have a clever way to minimize, solve, or prevent some of the problems mentioned here, please write to me c/o *LC•GC*, P.O. Box 50, Springfield, OR 97477. Perhaps others can benefit from your experience.

AUTOSAMPLER USAGE

Approximately 55% of the respondents reported using an autosampler with their LC systems. Of those, more than half run more than 75% of their sample loads with an autosampler. With labs becoming more dependent on automation, reliable autosampler operation is essential. The most frequently cited autosampler problems are listed in Table I.

TABLE I: FREQUENTLY CITED AUTOSAMPLER PROBLEMS*

Problem area	% of total
Sample-needle blockage	22
Poor precision	17
Mechanical problems	14
Tray position and sample identity	12
Carryover	9
Leaks	9
Seals	7
Features	6
Other	5

*some respondents stated more than one problem area (1)

SAMPLE-NEEDLE BLOCKAGE

Sample-needle blockage was the most frequently reported autosampler problem. From my experience, all users eventually experience needle blockage, which generally is caused by either particulate matter in the sample or pieces of septa from the vial.

Careful sample preparation should minimize the number of problems created by particulate matter. It is possible to test suspect samples by filtering them individually through a 0.5-μm filter. If the blockage problems disappear when samples are filtered, modify your sample-preparation scheme in order to remove particulate matter *economically*. Remember to account for your time as well as materials when calculating sample-preparation costs. Sometimes centrifugation can be an alternative to using more expensive disposable sample filters. In other cases, specialized cleanup devices, such as combined ultrafiltration–centrifugation techniques that simultaneously remove sample protein and filter the sample (for example, Centrifree from Amicon, Danvers, Massachusetts), combine several other cleanup steps while removing particulates.

When the sample needle becomes blocked with pieces of septum, determine the source of the problem. First, confirm that you are using the type of needle that is recommended by the autosampler manufacturer. A needle with a diagonally cut end (Figure 1a) is much more susceptible to blockage than a needle with a bent tip (Figure 1b). The first type tends to cut a plug from the septum, increasing the likelihood of blockage, whereas the bent tip partially protects the lumen of the needle, which minimizes blockage. A side-port needle (Figure 1c) is a better choice — assuming that it will work on your autosampler — because it is much less likely to cut a fragment from the septum. Do not use square-ended needles (Figure 1d), which are designed for manual use with injection valves. Also, be sure to use a needle with the proper internal diameter. Most autosamplers use 22- or 22s-gauge needles, which have the same 0.028-in. o.d. but different i.d.: 0.016 in. for the 22-gauge needle and 0.006 in. for the 22s-gauge needle. Clearly, the smaller needle is much more easily blocked and should be used only if the transfer volume must be kept to a minimum.

The choice of septum can also influence the frequency of needle blockage. Silicone or Teflon-faced silicone septa provide the best vial sealing but are more likely to cause needle blockage than Teflon septa. For aqueous samples, vial septa might not be necessary. On some autosamplers (for example, Micromeritics, Norcross, Georgia), the choice of septum is limited to a single type, so varying septum composition is not an option.

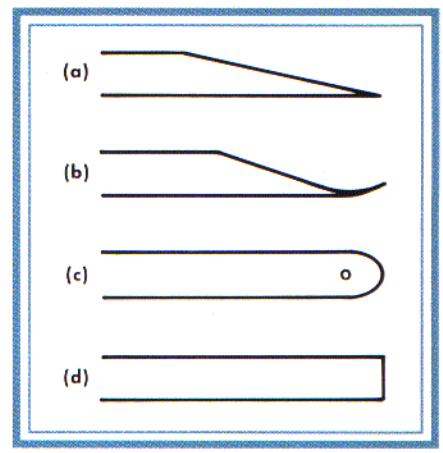

FIGURE 1: Syringe and autosampler needle styles: (a) 22-deg beveled tip (not recommended for chromatographic use), (b) 22-deg beveled with bent tip (recommended for septum penetration), (c) side-port tip (reduces blockage during septum penetration), (d) 90-deg bevel (for manual use with injection valves).

Transfer tubing in autosamplers is susceptible to blockage from the same sources that affect the sample needles. The choice of transfer tubing presents a dilemma because smaller-i.d. tubing is desirable in avoiding large transfer volumes, but larger-i.d. tubing is less likely to become blocked. Generally, the most successful compromise is to use 0.010-in. i.d. tubing of minimum length. Dilution of samples accompanied by injections of larger sample volumes (see discussion below) can minimize the importance of tubing volume as well.

INJECTION PRECISION

Seventeen percent of autosampler complaints were related to poor injection precision. Poor precision can usually be correlated with mechanical problems or with small sample sizes. For instance, blocked needles can cause precision problems because they restrict flow into the syringe or sample loop. A blockage in the needle or elsewhere in the tubing can cause cavitation (bubble formation), which can result in partially filled sam-

ple loops. Also, leaky fittings allow air bubbles to be drawn into the sample loop, and poorly adjusted syringe controllers will lead to erratic sample sizes. For autosamplers that rely on air pressure to force the sample into the loop, inadequate pressure, short filling times, and viscous samples can all contribute to improper sample-loop filling and, thus, to poor injection precision. If small sample volumes are used (10 μL or less), injection precision is expected to be lower than it is for larger samples because of variablity in the system's mechanical control. For example, an autosampler capable of picking up and injecting a sample with ±0.5 μL precision will be within ±0.5% for 100-μL injections but within only ±5% for 10-μL injections.

Careful and regular mechanical adjustment of the autosampler is required for maximum injection precision. The internal-standard calibration technique can also increase the precision of your assay. If the proper internal standard is used, its losses should parallel sample losses, and thus allow correction for imprecise sample preparation and injection.

Another, often overlooked, method for improving injection precision is dilution of the sample and injection of a larger volume. If the sample solvent is weaker than the mobile phase — for example, is half the strength of the mobile phase — a substantial increase in sample injection volume is possible with little or no loss in chromatographic performance. In the example mentioned earlier, in which 5% variability was found for a 10-μL injection volume, tenfold dilution would allow injection of 100 μL of sample and would reduce the injection imprecision to 0.5%. The larger sample is more convenient to prepare and often allows use of less-expensive autosampler vials. Sample dilution also reduces problems of band spreading that result from the sample passing through long pieces of connecting tubing.

MECHANICAL PROBLEMS

General mechanical troubles accounted for 14% of the reported problems, and when grouped with other mechanically related problems (for example, tray positioning), 30%–50% of autosampler problems could be classified as mechanical. Based on the present and earlier surveys (2,3), the mechanical reliability of autosamplers seems to be much lower than that of other parts of the LC system. Cleanliness and proper adjustment are keys to reliable autosampler operation. Buffer and other reagent spills can result in salt-deposit buildup, which can corrode or restrict autosampler movement. For this reason, sample vials should not be filled near the autosampler. If the sample tray is used to hold vials during filling, the tray should first be removed from the autosampler and washed whenever any spill residues are visible. Some autosamplers have segmented trays that can be removed for sample replenishment without stopping the autosampler. Reagent spills and sample residues that get on mechanical/optical sensors in the autosampler can cause malfunction. Check the operator's manual for cleaning procedures for these sensors or carefully wipe them with a methanol- or water-saturated cotton swab. Sample-tray alignment should be checked periodically (the operator's manual should have a procedure for this). An improperly aligned tray can result in bent sample needles or broken vials. The area around the waste container that is used during needle rinsing should be cleaned regularly on samplers that incorporate that feature. Rinse solutions that splash on other parts of the sampler mechanism can eventually cause malfunction. Finally, remember that the autosampler is a part of the LC system that should be flushed daily with nonbuffered mobile phase to remove potentially corrosive salt deposits in injector seals and other moving parts.

TRAY POSITION

Problems associated with sample-tray positioning were reported by 12% of the respondents. This is a more serious problem than some of those mentioned above because it suggests possible sample misidentification. Poor injection precision can be easily corrected by adding an internal standard, and blockage or mechanical failure is obvious because no injection is made, but improperly identified samples can have serious consequences, especially in health-related fields.

Autosamplers use either mechanical or optical sensors in positioning the tray so as to ensure selection of the proper sample. Be sure that the sensors are clean and are functioning properly. It is likely that operator error is responsible for some of the problems attributed to improper tray position. Operator error can be minimized if the vials are placed in the tray and then filled in sequence. Randomly filling vials (for example, vial 1, then 13, then 6) is a bad habit that is likely to cause mistaken sample identity. In critical cases, each vial should be labeled with a sample identifier. The regular use of standards can help solve sample-identity problems. If you use a standard whose chromatogram is easily distinguished from sample chromatograms, you will have a reference point for vial identification. For example, if you place a standard in positions 1, 11, 21, and so on, but observe that chromatograms 3, 13, and 23 correspond to the standards, you can readily identify the samples that follow. As a safety precaution, make a quick visual check of the chromatograms and the sample data before removing any vials from the tray to see if the standards correlate properly. That way you'll be able to rerun the samples if necessary.

SAMPLE CARRYOVER

Sample carryover from one injection to the next can be avoided in one of two ways. First, rinsing the sample needle between injections should eliminate carryover problems. In most cases, the action of drawing up sample and filling the loop will flush any residue of the previous sample from the system. Carryover problems can often be eliminated with longer loop-filling times if filled-loop injection is used. With autosamplers that use syringe injectors or in cases where loop flushing is not sufficient, a separate vial of wash

solution or a wash-solvent reservoir can be used. Many autosamplers provide for a sample needle and/or syringe rinse between samples; others require that the wash solution be placed in a separate vial between samples and injected as a "blank." In either case, carryover should be reduced to an acceptable level.

A second way of combating carryover problems is by minimizing the impact of carryover. Sample dilution, as discussed earlier, will reduce carryover problems, as well as increase precision, because autosamplers have a finite holdup volume (for example, 0.1 μL) representing residual sample. With a 10-μL sample, that much residue gives 1% carryover but would give only 0.1% carryover for a 100-μL sample. An alternative to sample dilution is to arrange your samples from low to high concentration. It is clear that a fixed-volume carryover will create fewer problems when a low-concentration sample is followed by a high-concentration sample rather than the other way around.

BUYING AN AUTOSAMPLER

A number of respondents indicated that their autosampler problems were caused by the presence or absence of certain features. That underscores the importance of purchasing the proper autosampler, which isn't always easy because there is more variability in the design and operation of autosamplers than in most other LC components. As with other system components, there are trade-offs between simple, mechanically controlled autosamplers and more complex samplers having more operational features. On one end of the spectrum are autosamplers that make a fixed number of injections per vial, each of the same volume and in sequence. Those samplers are simple, inexpensive, and usually very reliable. At the other end of the spectrum are samplers featuring air-bubble detection, optical (vial code) readers, random-access sampling, and random injection-volume selection. Because of their increased complexity, the more expensive samplers have more areas of potential failure, but they also improve reliability in other areas such as error checking and sample identification. Consider these items when buying an autosampler:

- Does it have the features that you *need* (as opposed to the ones you would like to have)?
- Does it have a good reputation in the marketplace? (Ask for references.)
- Is it easy to service, and are parts available? (Look through the service manual, or check on the manufacturer's service policy, and call the manufacturer to check on delivery time of parts or check with another user.)
- Is it designed to work well with *your* chromatograph? (Some work with only one brand.)
- Do you have other autosamplers of the same brand and model? (Spare-parts inventory costs, maintenance, and troubleshooting costs should be reduced when you stick with a single model.)

PREVENTIVE MAINTENANCE

Finally, good maintenance practices will help minimize autosampler problems. Keep thorough records of autosampler use and failure modes in order to establish a preventive-maintenance schedule. Follow the manufacturer's recommendations for cleaning, adjustment, and replacement of parts. Once a failure pattern for a certain part has been established, set up a schedule to repair or replace the part at about 75% of average failure time. For example, if you find that leaking seals are causing autosampler failure about once every six weeks, replace the seals after four weeks of operation. As with other LC components, early replacement of autosampler parts may at first seem like an unnecessary expense, but the cost will be quickly recovered in time saved from troubleshooting and repeating analyses *when* failure occurs.

REFERENCES

(1) *LC Magazine User Survey VII* (Aster Publishing Corp., Springfield, Oregon, December 1985).

(2) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3,** 420–423 (1985).

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