TROUBLESHOOTING

Autosamplers

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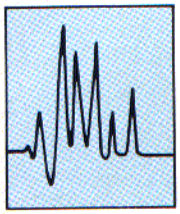

Autosamplers are widely used to increase sample throughput, to relieve the tedium of manual injections, and to reduce the labor costs associated with routine LC analysis. Typical autosamplers provide the dual benefits of improved assay precision and reduced assay cost. Although there are many commercially available autosamplers, all have four essential parts that mechanically automate the manual-injection process (Figure 1). Samples are held in uniform-size sample vials, which vary in capacity from a few microliters to several milliliters. Each vial is sealed with a septum that is part of the cap or is held on by the cap. Sample vials are held in a tray that permits serial — or sometimes random — access for injection. Usually the tray or the vial displays the vial number for transmission to a data system. A needle is used to penetrate the septum and draw the sample from the vial. Depending on its design, the needle may be movable or fixed, and it can be replaced in the event of bending or breakage. The final essential element of the system is a sample-injection valve that introduces the sample onto the column. This valve may be operated electrically or pneumatically and holds the sample in a sample loop prior to injection. This article will cover troubleshooting these common components. Valuable troubleshooting tips that are specific to particular autosampler models can be found in instruction manuals provided by manufacturers.

PROBLEM ISOLATION

The most common symptom of autosampler malfunction is a deterioration in the reproducibility of a particular assay. Most often this problem appears as increased variation in peak heights. Using the internal-standard method of calibration minimizes changes in precision, but, to spot problems, carefully observe the heights of standard peaks. Keeping a good logbook that allows day-to-day comparison of the performance of the entire LC system is therefore very important. If you observe a drop in precision, determine whether the problem lies in the autosampler (and not the rest of the system) by disconnecting the autosampler and performing manual injections. If precision levels return to normal, the problem is in the autosampler; if not, look elsewhere in the system.

To facilitate comparison of day-to-day performance, it is good practice to use a standardized sequence of calibration standards and samples in liquid-chromatographic runs. One group of readers has found the sample-analysis protocol shown in Table I to be useful for verifying proper system performance during routine assays (1).

For this protocol, two standards are prepared and assayed. The first injection is ignored because it is the one most likely to be affected by start-up problems. In a test for precision, responses should agree to within 1.5% after correction for concentration. When one standard is used to determine the contents of another standard (which is treated as an unknown) in a test for accuracy, the results should be similar to the designated content. It is important that the standards be prepared in a *blank* sample matrix and treated in the same manner as the samples. If the standard contains an extraneous component, this impurity can be used as a test for resolution. When quantified, this component can serve as another test for accuracy by comparison with previous results. Standards that are chromatographed throughout the analysis should produce a low relative standard deviation to confirm the reproducibility of a particular assay.

PROBLEMS WITH SAMPLES

There are two general causes of autosampler failure: the samples themselves or electrical problems with the instrument. Perhaps the most common sample-related problem is mislabeling. It is good practice to label sample vials individually, even if they are to be placed in numbered trays. If necessary, the samples can then be injected manually for positive identification. Because samples may sometimes remain unattended in the sample tray for 12 h to 24 h prior to analysis, sample stability is another key concern in using an autosampler. Evaporation can occur if the vials are improperly capped, resulting in sample loss or in a change of concentration, depending upon the nature of the sample. The sample can become contaminated by bits of septum that become dislodged by a dull sample needle or by material extracted from the septum if improper septa are used. Just as a sample can become contaminated by a bit of septum, the system can become blocked or damaged by particulate matter from a sample. Samples may require filtering prior to injection.

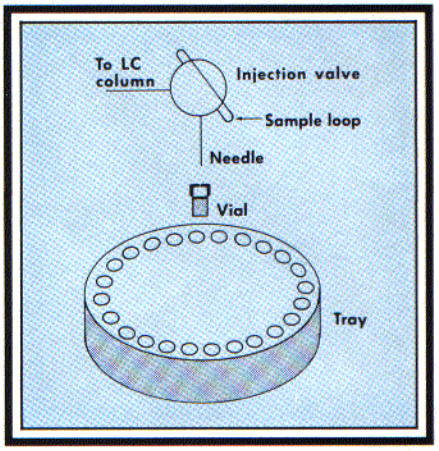

FIGURE 1: Basic components of an autosampler.

Be aware that sample-matrix effects and storage conditions can affect the precision of an assay. The following example illustrates the need to pay careful attention to sample handling. In one reader's lab, plasma drug samples were diluted in a saline solution and frozen in autosampler microvials. After thawing, injecting, and chromatographing, subsequent injections from a given vial resulted in reduced heights for all peaks in the chromatogram. Fresh standards behaved normally but frozen standards, under the same conditions, showed the same chromatographic pattern as the original samples. Manual injections, however, yielded normal peak heights. It was subsequently discovered that, as the samples froze, the top part of the vial froze first, forcing a concentration of salts and analytes at the bottom of the vial. Upon thawing, a density and concentration gradient persisted in the vial as a result of the narrow diameter and lack of agitation. Thus, when the first sample was drawn from the bottom of the vial, it contained a high concentration of drug. Each subsequent sample drawn had progressively lower concentrations of drug as the level of liquid in the vial dropped. Manual injection, on the other hand, caused agitation sufficient to mix the contents and to provide reproducible concentrations. The moral: Be sure samples are thoroughly mixed before proceeding with an injection (2).

AUTOSAMPLER PROBLEMS

Problems associated with the sample tray are usually easy to fix. The tray revolves, stopping when the sample is under the sampler needle. This operation is controlled by a mechanical or an optical sensor on the tray itself. If misalignment occurs, adjust the sensor and/or tray-stop so that the tray stops in the proper position. Some autosamplers allow for variation in vial alignment, whereas others will crush the vials if the alignment is not exactly right. If the autosampler "hunts" for but does not find a vial, does not stop, or does not send the vial number to the data system, the problem usually resides in the sensor mechanism. Most tray sensors use a mechanically activated microswitch to sense a bump or dent in the tray. This switch can be checked with an electrical continuity tester and replaced or adjusted as necessary. Problems with optically read sample trays arise because of sensor failure or, more commonly, because a sample has splashed on the sensor or on the encoding strip. Regular cleaning of these parts usually eliminates this type of problem.

Mechanical problems involving the sample vial or the sampler needle are often interrelated. The needle can become blocked from a bit of septum or particulate matter in a sample. The primary symptom of blockage is a sudden drop in peak heights. Bent needles can result from improperly aligned vials or from septa that are too tough; dull needles also can be bent. To avoid these problems it is important to use the proper needle/septum combination recommended by the autosampler manufacturer. Needle bending also can occur if the needle hits the bottom of the vial during sampling. This can happen if the needle is out of adjustment, if a needle of improper length has been installed, or if vials that are incompatible with the autosampler are used. Care also should be taken to keep the needle assembly clean. Buildup of buffer salts can block the vent needle in two-needle systems, resulting in poor assay precision. Because of the high potential for needle failure in autosamplers, several spares should be kept on hand to minimize downtime.

Sample-injection valves in autosamplers often are automated versions of manual-injection valves and are operated by pneumatic or electric actuators.

The supply of air to pneumatically operated valves needs to be maintained at a constant level; if the supply runs low during unattended operation the autosampler will stop working and valuable samples could be wasted. Also, autosamplers with pneumatically operated valves often use compressed air to force the sample into a sample loop. Air is fed into the sealed sample vial, forcing the sample out through the sample needle and into the sample loop. When the loop has been flushed and filled, the valve is rotated and the sample is injected. The pressure of the air in the sample-displacement circuit needs to be carefully regulated. If the pressure is too high, sample will be wasted by forcing too much of it through the loop; if the pressure is too low, a poorly filled loop will result and, thus, poor precision for the assay.

Electrically operated valves often use a syringe to fill a sample loop. Instead of forcing sample from the vial into the loop, the syringe draws on the outlet end of the loop, pulling sample from the vial into the loop. This adds flexibility of sampling and allows either full- or partial-loop injections, although full-loop assays are almost always more precise.

Wear of the sampler valve rotors, or seals, is much more common in autosamplers than in manually operated injection valves because the former perform far more injection cycles each day. Rotor wear can result in blocked lines or column frits, so it is worthwhile to use an in-line filter between the autosampler and the LC column to catch any rotor debris.

Valve timing is critical, particularly with autosamplers that displace the sample by means of air pressure and with LC systems that use gradient elution. What appears to be a sample or detector problem could merely be a case of turning the valve with no sample in the loop or of placing the sample on the column at the wrong point in the gradient. Timing adjustments vary for each type of system, so it is best to consult the operator's manual if injection problems are encountered. Another timing problem that can be encountered, particularly during installation, is reversal of the sample and load cycles of the valve. If the valve is improperly plumbed, the sample loop can be in the wrong position during the load cycle. In this case, the bypass channel in the valve acts as the sample loop. This tiny "loop," which often has a capacity of 0.5 μl or less, can cause the LC system to appear to have problems with detector sensitivity, so be sure to follow plumbing directions carefully.

OTHER CONSIDERATIONS

Requirements for using wash vials to minimize sample carryover are dictated by the needs of the particular assay. The degree of sample carryover should be determined by injecting a concentrated sample or standard and then following with several injections of a blank. (It will be necessary to increase detector sensitivity for the blank injections.) It is then easy to calculate the level of carryover. Generally, if similar samples are run, such as in a quality-control assay, the fractional percentage of carryover shown by most autosamplers is insignificant. In other assays, such as drug screening, carryover may influence the assay of the next sample's contents. This problem can be addressed by alternating sample vials with wash vials or by rerunning samples that show apparently high analyte levels.

Because of size considerations, autosamplers often require much longer connecting lines than ordinarily are used in LC systems. These lines may require changes in sample-preparation procedures to ensure that good injections are made. As a rule of thumb, it is wise to prepare samples in a solution that is half the strength of the mobile phase or weaker. This rule permits on-column concentration of the sample when it is injected and compensates for broadening that may occur as a result of using excessively long connecting tubing. Long connecting tubing can also increase the apparent retention time of components as compared with times produced with manual injection. This phenomenon occurs because data systems usually are started when the sample valve turns; with an autosampler, however, the sample may take several seconds to travel from the valve to the column.

TABLE I: INJECTION SEQUENCE

(1) Standard A
(2) Standard A
(3) Standard B
(4) Impurity standard(s)
(5) Samples
(6) Standard A
(7) Samples
(8) Standard B
(9) Impurity standard
(10) Samples
(11) Standard A
(12) Samples
(13) Standard B

CONCLUSIONS

As with problem diagnosis in the rest of the LC system, it is useful to have on hand a standard assay to ensure proper operation of autosamplers. Isolating problems to the autosampler can be accomplished by comparing autosampler and manual injections. When it has been determined that the problem is in the autosampler, make sure the sample has been handled properly and that the proper sample is being assayed. Check that the sample is being drawn from the vial, that the proper sample loop is being used on the valve, and that the valve is injecting in the proper time sequence. To minimize downtime, carefully follow the manufacturer's recommended preventive-maintenance procedures.

REFERENCES

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(2) S.J. Bannister, Key Pharmaceuticals, personal communication (July 1984).

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