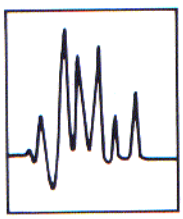
TROUBLESHOOTING

Recordkeeping: Plan Ahead to Minimize Downtime

JOHN W. DOLAN and VERN V. BERRY



With the beginning of a monthly column on LC troubleshooting, we would like to share our plans for the months to come. Our purpose is to help you make more productive use of your liquid chromatograph. We anticipate that beginning chromatographers will benefit especially from these discussions. We need input from readers — both beginning and expert — on problems most often encountered in LC operations. It should be noted that troubleshooting is a constantly changing skill: new instruments, columns, techniques, and test procedures often cause us to substitute new methods for old. As individuals, we are exposed only to a narrow view of problems and troubleshooting, but with this column as a forum, we can share our knowledge. We solicit your comments, questions, problems, solutions, and articles on LC troubleshooting that will make us all more productive scientists. Initially our discussion of troubleshooting will concentrate on problem isolation and correction in a general sense. In the following months the emphasis will shift to specific portions of the liquid chromatograph: the pump, column, injector, detector, and to column maintenance. We would like periodically to offer a solutions-oriented article in a question-and-answer format. Please direct your input to: Dr. J. W. Dolan, IBM Instruments, Inc., 40 W. Brokaw Rd., San Jose, CA 95110; or Dr. V. V. Berry, Salem State College, Chemistry Department, Salem, MA 01970.

OPERATOR'S MANUAL: A KEY RESOURCE

Before we begin to look at specific areas, it is worth noting that the operation and service manuals provided by LC manufacturers are invaluable aids to troubleshooting. Specifications for system performance will give you an idea of the expectations you should have for normal operation. These manuals also provide a reference to determine if a system has been fixed following failure. Most manuals also include a troubleshooting section, which generally highlights the most common problems encountered with a specific instrument. A list of commonly required parts and procedures for replacement may also be found in the manuals. Although this may seem like restating the obvious, the manual is all too often ignored — it is relegated to a shelf or drawer and is rarely consulted for help after the initial installation is complete.

THE LOGBOOK: INEXPENSIVE INSURANCE

A logbook for recording the history of use of each liquid chromatograph is another tool that should be used for troubleshooting. This is especially handy for troubleshooting problems in instruments that are used by several operators. Logbooks reveal when pump seals and detector lamps have been changed, whether recent eluents (such as ion-pair agents) are compatible with present detectors, and whether previous samples can explain ghosting or column blockage. Although most chromatographers acknowledge the value of logbooks, in practice few use them unless laboratory management so dictates.

The logbook may be as simple or as complete as the operator desires; even the simplest record kept on a paper taped to the liquid chromatograph is better than nothing. The simplest record should include dates when pump seals, detector lamps, guard columns, silica saturator columns, and solvent filters are changed, or when other services are performed on the instrument. One easy way to keep track of these records is to place self-adhesive labels (2 in. x 3 in., for example) on particular parts of the system. Write your notes on the appropriate label. Be sure to use pencil for your records when using this method, because solvent will inevitably smear ink.

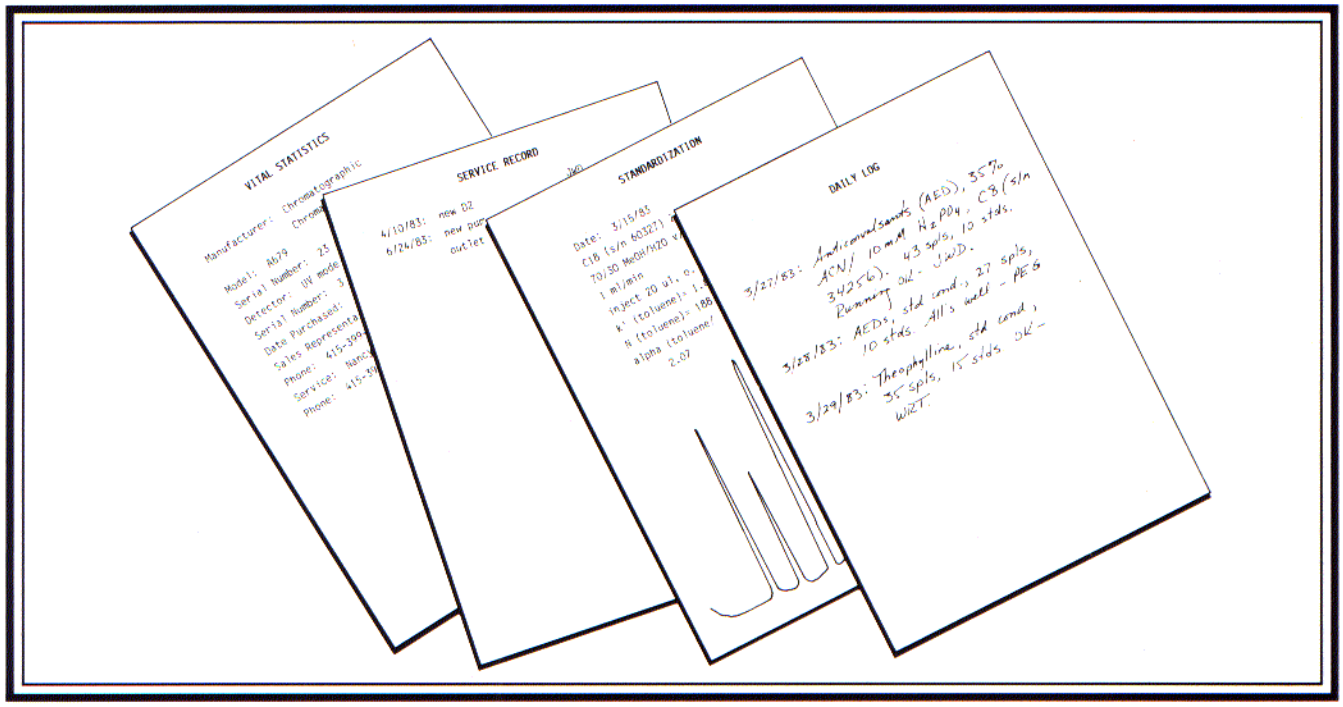
At the other end of the spectrum is keeping a complete logbook. This should contain the service record of the instrument as well as the use record. You might want to record who used the system and the specific details of the analysis: mobile phases, flow rates, column serial numbers, types and number of samples analyzed, and any other pertinent information.

In most cases, as a matter of convenience, a logbook that is somewhere between the extremes described above will be used, such as a spiral-bound notebook kept in the vicinity of the instrument and a regular reminder for all users to record in it regularly. Tape or staple the business card of the manufacturer's service engineer to the cover of the logbook for ready reference. The logbook information is like insurance — it's not very useful until you need it, and by then it is too late to obtain it if you have been negligent.

STANDARDIZATION: KNOWING WHAT IS NORMAL

Standard conditions: Standardization of the liquid chromatograph from time to time is as important as the regular use of a logbook. Take a standard chromatograph that represents the optimum conditions in the chromatographic mode you use most. For columns, the evaluation conditions recommended by the manufacturer of your column are adequate. In the reversed-phase mode, an octadecyl stationary phase with about 70% methanol/water at 1 ml/min and a UV detector at 254 nm are typically used. Test probes are usually uracil (for dead volume measurement) and a variety of substituted aromatics such as phenol, nitrobenzene, acetophenone, methyl benzoate, and toluene. Normal-phase, ion-exchange, steric-exclusion, and specialty columns vary widely in the choice of test conditions. Check the literature sent with your column for specific recommendations.

Key parameters: The capacity factor, selectivity, and chromatographic efficiency should be recorded and the chromato-



gram saved for future reference (labeled with the date, conditions, and operator's name). If you are not sure how to measure the important chromatographic parameters, check in L.R. Snyder and J.J. Kirkland's *Introduction to Modern Liquid Chromatography* (1979). We will discuss these parameters in detail in a later column. Initially, these parameters should be compared to the performance data supplied by the column manufacturer. If the values deviate by more than 10–20%, the entire system should be examined to determine if it is operating properly. For example, a slipped ferrule on the column outlet was found to cause broad peaks in one system.

Extracolumn effects in many liquid chromatographs will prevent you from obtaining column efficiencies that match those of the manufacturer when using microbore or "fast" LC columns (3-μm particles, 3–7.5 cm long). A standard chromatogram, however, will still allow you to determine if column deterioration has occurred since installation.

Standardize your analysis: If you are using the liquid chromatograph for routine, repeated analyses, such as for quality assurance or in a clinical laboratory, you may also want to standardize the instrument under routine operating conditions. A reference chromatogram of a typical standard or sample will provide you with a reference point for spotting early failures when no major changes in operating conditions have been made.

With a standard chromatogram in your logbook, it is a simple exercise to repeat the experiment whenever an abnormality in the LC system is suspected. You now have before and after data, which allow you to quantify changes in performance.

STANDARD STORAGE CONDITIONS: THINK OF TOMORROW

A daily starting point may be as important as the measurement of the performance of the liquid chromatograph. This is especially important if more than one user is involved in instrument operation. Choose a set of conditions for storage of an idle liquid chromatograph. You can usually combine this step with your routine washout procedure. Most commonly, this involves converting to a stronger mobile phase, which is also selected to remove strongly retained components from the column and to remove any buffer salts from the LC system.

In a reversed-phase analysis of anticonvulsant drugs, for example, one might be using 30% acetonitrile and 70% phosphate buffer with an octyl bonded-phase column. By changing to 80% acetonitrile and 20% water, strongly retained materials will be eluted and residual phosphate buffer removed. This will extend the system lifetime by removing corrosive chemicals and will prepare the instrument for the next user. With a standard set of conditions for system storage, you can quickly start up the system for a new assay without worrying about buffer precipitation or interferences from the previous analysis. For the lowest risk of column deterioration, these storage conditions should be the same as those used for the column when you received it from the manufacturer.

We have just discussed some of the tools you can use to maintain the upper hand when problems occur with your instrument. The wise use of the operator's manual, logbooks, standardization techniques, and standard storage conditions will go a long way in reducing instrument downtime.

BIBLIOGRAPHY: WHERE TO FIND HELP

The following list of references may be useful for finding solutions to problems that are not addressed in the operator's manual for your liquid chromatograph. If you have other references that you find especially useful, please send us the information for inclusion in a future column. Several instrument manufacturers also have specific troubleshooting publications and most provide free telephone troubleshooting consultation.

Johnson, E.L., and R. Stevenson, *Basic Liquid Chromatography*. Palo Alto: Varian Associates, 1978.

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Yost, R.W., L.S. Ettre, and R.D. Conlon, *Practical Liquid Chromatography: An Introduction*. Norwalk, Connecticut: Perkin-Elmer Corp., 1980. ■

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