TROUBLESHOOTING

LC User Survey: Detector Problems

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Last month, several autosampler problems reported by users in a recent *LC Magazine* user survey (1) were discussed (2). This month's Troubleshooting column will review some of the detector problems that were reported. The results of the data-system section of the survey will be covered next month.

TRENDS IN DETECTOR USAGE

Table I summarizes the detectors currently used by the survey respondents and compares usage to that reported in a similar survey done in 1984 (3). Although the statistical differences between the two sets of data have not been determined, it is interesting to speculate about possible trends in detector usage. Because the questions regarding variable-wavelength UV detectors were worded differently in the two surveys, there is a problem in comparing the data. Apparently, however, there has been a significant rise in the use of fixed-wavelength detectors. At least two factors might have contributed to the increase: First, more than 40% of the respondents reported doing most of their work at detector settings of 0.05 AUFS or less; respondents also reported that the amount of noise and drift and the degree of sensitivity were the most important criteria in purchasing new detectors. These factors support the choice of a fixed-wavelength detector over a variable-wavelength detector (assuming that the required detection wavelength is available in a single-wavelength model) because fixed-wavelength detectors generally are more sensitive. It is interesting to note that five times as many problems were reported for variable-wavelength UV detectors as for fixed-wavelength detectors, although the usage rate is only about 50% higher.

The increasing use of diode-array detectors is not surprising, as these relatively new systems have found general acceptance in the laboratory. The increased use of diode-array detectors probably accounts for some of the decrease in the use of stopped-flow scanning UV detectors. Although the diode-array and stopped-flow systems each provide similar information, diode-array detectors are faster and more convenient.

TABLE I: DETECTOR USAGE

Detector Type	Percentage of Total* 1984**	Percentage of Total* 1985†
UV–Vis		
variable wavelength	85	78
fixed wavelength	24	53
diode array	13	18
stopped-flow scanning	26	13
Refractive index	41	37
Fluorescence	33	31
Electrochemical	17	21
Conductivity	12	15
Postcolumn reaction	‡	9
Radioactivity	9	9
Mass spectrometric	5	5
Other	4	8

* totals exceed 100% because multiple answers were given
** from *LC* User Survey IV, December 1984 (3)
† from *LC* User Survey VII, December 1985 (1)
‡ data not available

Changes in the use of other detector types might not be significant. The drop in refractive index (RI) detector use could have been caused by the availability of more-sensitive alternatives such as low-wavelength UV monitoring. I suspect that the increase in electrochemical (EC) detector usage is a result of the fact that recent models are much easier to use and have fewer problems than earlier models. The increase in conductivity detection is probably a result of the widespread acceptance of ion chromatography. Postcolumn reactors were not listed on the 1984 survey.

UV DETECTOR PROBLEMS

Approximately 75% of the problems reported in the 1985 survey were related to UV detectors; with the exception of refractive index detector problems (see below), however, there were too few responses about other detectors to get any idea of specific problem areas. As mentioned above, problems reported for variable-wavelength UV detectors outnumbered the problems of fixed-wavelength detectors five to one. The major problems are listed in Table II. It is interesting to note that 11% of the respondents reported that they did

TABLE II: UV-DETECTOR PROBLEMS (1985)

Problem Type	Percentage of Problems Reported*
Noise, drift, sensitivity	47
noise	(26)
drift, stability	(12)
sensitivity	(9)
Lamp life	24
Bubbles	7
Cell contamination	6
Other	8
No problems	11

* totals do not equal 100% because of rounding

not have *any* detector problems — at least some of us are lucky!

Noise, drift, and sensitivity: Almost half of reported UV-detector problems were related to the noise, drift, or sensitivity of the detector. These problems are often interrelated; in fact, a noise problem for one chromatographer can be a sensitivity problem for another. These problems, along with possible solutions, will be discussed as a group.

First, several terms must be defined. By *noise,* we mean the random wanderings of the detector signal (as exhibited by the motion of the pen on a strip-chart recorder, for instance) over a period of less than ~30 s. Noise with a cycle of a few seconds or less results in a broad baseline that is several times the normal pen width. Over a longer time scale, baseline noise can even be confused with small peaks. For this reason many laboratories will not report a peak with a height that is less than 10 times that of the highest noise "peak." Noise can be related to pump stability, detector-cell cleanliness, lamp quality, detector time constant, mobile phase composition, and other factors.

Drift refers to longer-term variation in baseline position — generally over a period of tens of minutes or even hours. Drift is usually related to long-term changes in the environment, such as detector warm-up or fluctuations in temperature and mobile phase composition.

Sensitivity is detector response per unit concentration of a given compound. Thus, a more-sensitive detector will give a larger response for a given sample size than will a less-sensitive detector. The various factors often are combined in the *signal-to-noise ratio,* which expresses the detector response for a given sample mass in terms of the detector noise. A signal-to-noise ratio of 10 means that the detector response (generally peak height) is 10 times as large as the baseline noise (generally measured as peak-to-peak noise).

If you suspect that you have a detector problem related to noise, drift, or sensitivity, first verify that the problem really exists, then isolate its source. It is necessary to have a standard set of conditions to use as a reference in determining whether the system is operating properly. Reference conditions were discussed in detail in a previous column (4). The primary reference conditions — those defined by the detector manufacturer — are generally listed in the specifications section of the operator's manual. Typically, a manufacturer measures noise and drift at one or more wavelengths (for example, 200 nm, 254 nm, and 350 nm) with a dry (or nitrogen-filled) detector cell. Although such conditions are easy to check when the system is new, once it has been used, the detector's performance is more difficult to check against the specifications. It is much easier to use an "ideal" set of LC conditions for your system when it is operating at its best. The conditions used to test columns when they are new are a good choice. For example, use 60:40 methanol/water at a flow rate of 1 mL/min, a C18 column, and a temperature-stabilized environment. Set the detector at the most sensitive setting with a short time constant. After assembling your system, run it under those conditions for about an hour, recording the amount of noise and drift for future reference. It may be more convenient to use the "normal" operating conditions for an assay that is performed regularly. Simply save a 30-min trace of the chromatographic baseline about once a month as a reference for assessing future problems. Once you have this set of reference conditions to use as a yardstick of daily detector performance, you will be able to spot problems faster and with more certainty. When you suspect a problem, compare the detector performance with your reference chromatograms. Often you will find that the "problem" is really the expected outcome of altering an operating condition such as the detector time constant or wavelength setting.

Once you are certain that the problem is in the detector, determine whether the problem developed suddenly or gradually. Problems that appear suddenly are often caused by a system setting change or by the failure of a component. Noise spikes in the baseline may be traced to poorly degassed solvent, an improper changeover of mobile phase, or a failing lamp. A dirty detector cell will cause increased noise and is likely to trap microbubbles that otherwise would be swept through the cell. A gradual increase in baseline noise can be traced to lamp aging, a dirty detector cell, or column contamination. Drift is most often caused by changing lab temperature (if the column is not thermostated) or by a change in the mobile phase, such as that which occurs under gradient elution.

The best way to avoid noise and drift problems is to carefully control the system conditions. Use only HPLC-grade solvents and be sure to degas them thoroughly. Also, flush the column with strong solvent at the end of each day's run. If you plan to flush a lot of strongly absorbed material from the column, it is wise to disconnect the outlet of the column from the detector so that the detector cell does not become contaminated. Keep the column in a temperature-stabilized environment (wrap it with insulation or use a column oven), be sure to insulate the transfer line between the column and the detector, and keep the chromatograph away from drafty areas, such as heating or air-conditioning vents. Sometimes you can use sample preconcentration or on-column concentration to increase the sample size and thus improve the signal-to-noise ratio — large peaks mean that higher detector attenuations can be used, and fewer problems with noise and drift will be encountered.

Lamp life: The lifetime of deuterium lamps used in variable-wavelength UV detectors concerned 25% of the survey respondents. As was mentioned in a previous column (5), deuterium lamps have an expected lifetime of three to six months, versus a year or more for the mercury lamps used in fixed-wavelength UV detectors. It's important to remember that previously unused deuterium lamps have a shelf life, so if you install a "new" lamp that has been "aging" in a drawer, you might find that it has an unexpectedly short operational life. The performance of detector lamps generally deteriorates with age, resulting in increased noise. You should keep a careful record of lamp usage to determine the average lifetime of lamps in your laboratory. Then establish a preventive maintenance program so that lamps are automatically replaced at 75%–80% of their useful lifetime. With this practice, you might discard a lamp that is still functional, but you should recover your costs by spending less time troubleshooting problems caused by lamp failure.

Recently, some manufacturers have advertised extended-life deuterium lamps, but I have not seen data to substantiate claims of better overall performance. If you have experience in this area to share, please write to me c/o *LC•GC*, P.O. Box 50, Springfield, Oregon 97477.

Bubbles and dirty cells: Bubbles in the detector cell cause noise spikes in the chromatogram. Poorly degassed solvent is the most common source of bubble problems, especially if high-pressure mixing has been used. Often gas that is dissolved under normal system pressures (for example, 2000 psi) will form bubbles when the pressure is reduced in the detector cell. A combination of solvent degassing and a slight restriction on the detector-cell outlet will usually solve the problem (6).

A dirty detector cell also can lead to bubble problems. Dirty cell windows tend to trap microbubbles as they pass through the cell; the trapped microbubbles, which normally pass through the cell unnoticed, then form larger bubbles and cause noise spikes in the chromatogram. Cleaning the detector cell will usually solve the problem, but because cleaning is inconvenient and can cause additional problems, it is recommended that detector cells be cleaned only if contamination is suspected (6).

REFRACTIVE INDEX DETECTOR PROBLEMS

Problems related to drift and baseline stability accounted for 68% of the reported refractive index (RI) detector problems, which are almost always related to temperature instability. For this reason most RI detectors have some provision for thermostating the cell. For maximum stability, the column, transfer lines, and detector should be maintained at a constant temperature (for example, 35 °C). One manufacturer (Tecator, Herndon, Virginia) addresses this problem by enclosing a mobile phase preheating coil, the sample injector, the column, and the RI detector within the same temperature-controlled compartment; any RI user could take the same precautions.

CONCLUSIONS

Very few electronic problems or problems related to poor detector design or electronic problems were reported in the 1985 survey. Most of the problems involved either time-related degradation of components (for example, lamp life and detector-cell contamination) or changes instituted by the operator (poor degassing, improper temperature control, and incorrect system settings). The fact that a significant number of users reported trouble-free detector operation lends credence to the practice of preventive maintenance: parts that are prone to failure should be replaced *before* a problem occurs.

REFERENCES

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

(2) J.W. Dolan, *LC•GC Mag. Liq. and Gas Chromatogr.*, **4,** 416–420 (1986).

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(4) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **1,** 406–407 (1983).

(5) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **2,** 439–444 (1984).

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

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Readers are invited to contribute their troubleshooting tips to this column or to submit topics or questions for discussion in future articles. Write to The Editor, *LC•GC*, P.O. Box 50, Springfield, Oregon 97477.