TROUBLESHOOTING

Common Column Problems

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In a recent survey conducted by *LC Magazine*, readers were asked to indicate their most frequently occurring column-related problem. A summary of the 284 responses is presented in Table I. This article will present an overview of the problems cited and their solutions, some of which will be discussed in greater detail in future "Troubleshooting" articles.

OVERVIEW

By far the largest percentage (63%) of problems cited were related to column degradation in one form or another. I suspect that many of these problems can be prevented by taking a little more care in sample preparation and column protection. Column-to-column reproducibility problems were encountered by 18% of the respondents. Some of these problems can be prevented, but others cannot and simply must be tolerated. Problems with column performance made up the last major category (12%) and, by the wording of the responses, I believe many instances of this type of problem could be avoided by modification of the LC method and/or the LC system. Let's look at these problem areas in more detail.

COLUMN DEGRADATION

Back pressure: Problems associated with increased column back pressure constituted the largest percentage (23%) of specific complaints, with many respondents indicating that blocked frits were the source of the problem. A procedure for frit replacement that should help you correct this problem when it occurs was described by C.T. Wehr in a recent "Column Watch" article (1). A much better way to address the problem, however, is to prevent column blockage from occurring altogether. The first step in preventing blockage is to use proper sample preparation techniques. It is very easy to check the sample's quality immediately prior to injection to see whether it needs to be filtered — just hold the sample vial up to a light and swirl it gently; if the sample liquid is cloudy or particulate matter is visible, filter the sample through a 0.5-μm filter. The relatively high cost of submicron sample filters generally discourages routine filtration of samples for good measure, but if you have frit blockage after fewer than 100 sample analyses, it may be worthwhile to filter every sample as a test. In that case, if the frit blockage problem disappears or is greatly delayed, it may be cost-effective to filter every sample.

If sample filtration is unnecessary or fails to solve the problem of column blockage, you should use a guard column. Almost one-half of the respondents to a recent survey indicated that they use guard columns on a routine basis (2). These columns act as both physical and chemical filters to protect the analytical column. A guard column will also help protect the analytical column from particulate contamination that arises from contaminated mobile phase or from degrading sample-injection valves. Another option is to use an in-line filter between the sample valve and the analytical column (or guard column if one is in use).

Some increase in back pressure can be expected in LC operation, but, if you are used to operating a chromatograph in a certain way and change to another method, an increase in back pressure may alarm you. Remember that an increase in mobile-phase viscosity will result in an increase in system pressure. Such increases in viscosity can arise from a reduction in column operating temperature — for example, changing from a method run at 35 °C to one run at room temperature. A change in mobile-phase components can change the viscosity: propanol, for example, is four times as viscous as methanol at room temperature. A change in mobile-phase composition can also increase back pressure. A 60:40 methanol/water solution produces about twice the column pressure as pure methanol. Other factors that will cause an increase in back pressure (assuming all other factors are held constant) are increased flow rate, increased column length, and reduced particle size. So be aware that some increases in system pressure are perfectly normal.

TABLE I: SURVEY RESPONSES*

Most Common Column-Related Problem	Percent of Respondents
Degradation	**63**
Pressure	**(23)**
Voids	**(9)**
Contamination	**(7)**
Unspecified	**(24)**
Reproducibility	**18**
Performance	**12**
Other	**8**

******LC Magazine***'s User Survey V question #22, "What is your most common column-related problem?" Based on 284 responses; total of percentages does not equal 100% because of rounding.**

Voids: Another specific problem in the area of column degradation was finding a depression, channel, or other form of void at the head of the column. Nine percent of the respondents indicated that their columns were subject to this problem. With the high level of technology used by column manufacturers today, it is safe to say that most column void problems originate with the user. It is true, however, that an occasional bad column slips through the manufacturer's quality-control department, but, for the most part, the days of being able to blame the manufacturer for column voids are now part of the past. The two major causes of column depressions are largely avoidable by proper operation of the chromatograph.

First, dissolving the column packing by using aggressive mobile phases should be avoided. Silica-based packings are not stable for very long when used with mobile phases of higher than pH 7. If it is necessary to use high-pH mobile phases, you can prolong column life by using saturator precolumns and guard columns and carefully flushing the mobile phase from the system when it is not in use (3). Because polymeric column packings are not stable in the presence of certain solvents, you should consult the manufacturer's data sheet for the specific column you are using.

Second, pressure pulses and excessively high pressures can cause the column packing to settle. Some form of pulse suppression should be used with all pumps; most pumps include this as a standard feature. If you suspect that a pressure shock is ruining your columns, gradually step up the flow rate to the desired level in 0.1 ml/min increments when you first turn on the pump. Ten years ago, this gradual start-up procedure was routine, but it is not usually necessary with today's more stable columns. Silica-based columns generally will withstand more pressure than the chromatograph is able to deliver, but polymeric columns often have an upper pressure limit of 1000 or 2000 psi. If you work with columns with upper pressure limits, set the automatic upper-pressure shutoff on your chromatograph to a value at least 10% below the limit.

Contamination: A small percentage (7%) of the respondents admitted to causing column deterioration. Most of them cited inadequate sample preparation, improper mobile-phase selection, and inappropriate sample/column combinations. The majority of these problems can be minimized by following the column-protection guidelines that were discussed previously.

Unspecified: The remaining column degradation problems were unspecified (24%). The lifetime of a column varies greatly from lab to lab and from assay to assay. You should keep a column log to determine the number of samples that can be run on a column before it is no longer usable (4). Column age given in months is not a valid measure of column life because one lab can run as many samples in a month as another lab does in a year. With a good column log as a reference, you will be able to determine whether columns are suddenly failing prematurely or whether a certain procedure drastically shortens column life. Carefully examine any new procedure for flaws if it appears to be especially hard on columns.

COLUMN REPRODUCIBILITY

Eighteen percent of the respondents indicated column-performance reproducibility problems. They reported reproducibility problems between columns from different manufacturers as well as between columns from the same manufacturer. You should not expect columns from different manufacturers to have the same separation characteristics, even for the "ideal" test compounds used to show column performance. There is nothing wrong with this disparity — the columns are just prepared from different silica and bonding reagents and are packed by different techniques. You should, however, expect column-to-column reproducibility between columns from the same manufacturer for most separations.

Assays involving the separation of both polar and nonpolar compounds may give differences in selectivity or peak spacing if retention is primarily caused by the bonded phase for one compound and by silica interactions for another (5). In these cases, certain mobile-phase additives, such as triethylamine, can mask column-to-column differences. In other cases, it may be wise to use another LC method, such as changing from reversed-phase to ion-pair chromatography. Some manufacturers do a better job than others of controlling performance variations from one batch of columns to the next, and for critical separations many manufacturers will allow you to buy (or reserve for future purchase) columns produced from the same bonding batch; these columns are about as close as you can get to identical columns.

Don't overlook some user-caused and normal degradation problems that may be disguised as problems of column-to-column reproducibility. Most assays, for example, result in irreversible adsorption of contaminants on the analytical column. As these contaminants build up, they can change column retention characteristics, often necessitating minor changes in mobile-phase composition to maintain adequate separation of sample components. If this is the case, don't forget to change back to the original mobile phase when the column is replaced. Once again, sample chromatograms from your logbook will help you trace these changes. Changes in column temperature, flow rate, mobile-phase preparation technique, and mobile-phase aging all can cause problems that may be misdiagnosed as column-reproducibility problems.

COLUMN PERFORMANCE

Performance-related problems were reported by 12% of the respondents. Some of these problems may be related to the areas discussed above, but many of the respondents indicated that the column did not perform as expected. Specific problem areas included resolution, peak capacity, column plate number, peak shape, usable temperature range, and retention time. In most of these areas, once the method is carefully developed, the characteristics of the column are fixed. If the method is optimized for a given column type and still found inadequate, it is best to change to another method. Although 60%–70% of today's LC separations are achieved using reversed-phase methods (2), normal-bonded-phase and ion-pair chromatography offer powerful alternatives for separations not possible with reversed-phase techniques.

OTHER PROBLEMS

The remaining problems varied, although the high cost of columns and the need for obtaining bulk packing material to repair columns were mentioned several times. There are not many alternatives to paying $300 or more for an analytical column. Some companies offer a lower grade of columns for a significantly lower cost; however, you should also expect lower performance. Some users are satisfied with repacking services offered by companies that take your old column, empty it, clean the packing, and repack the column. This service is also less expensive than a new column. The best way to lower the effective cost of the column while retaining high performance is to take extra care in sample cleanup and use a guard column to protect the analytical column. Doubling column lifetime by using these methods may reduce your column costs by 25% or more (after you account for the guard-column cost).

Bulk packing is not available from many of the major column manufacturers because of its proprietary nature, and many workers find that it is not worthwhile to try to repair a column once a void occurs; hence, the market for small amounts of packing is limited. There are, however, two other sources of small amounts of packing if you cannot obtain it from the column manufacturer.

An old column can be unpacked and the packing used to repair another column. First, clean the column carefully by backflushing with a strong mobile phase. Then, the packing can be extruded from the column by removing the inlet end fitting and frit and pumping mobile phase in the reverse direction through the column. Discard the first 2–3 cm of packing, which contains most of the irreversibly retained impurities (these often appear as a yellow band at the head of the column).

The other source of bulk packing is a company that provides column repacking services. These companies repack most brands of columns and often are willing to sell small amounts of bulk packing for column repair.

REFERENCES

(1) C.T. Wehr, *LC, Liq. Chromatogr. HPLC Mag.* **1**, 270–273 (1983).

(2) R.E. Majors, *LC, Liq. Chromatogr. HPLC Mag.* **2**, 660–666 (1984).

(3) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3**, 234–238 (1985).

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

(5) J.W. Dolan, *LC, Liq. Chromatogr. HPLC Mag.* **3**, 312–316 (1985).

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