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Systematic approach to the isolation, correction, and prevention of liquid chromatographic column problems

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ABSTRACT

A practical approach to correcting liquid chromatographic (LC) column problems is presented. Emphasis is placed on keeping contaminants that originate in the mobile phase or sample from reaching the column. Column evaluation prior to routine use and whenever chromatographic problems are observed is strongly suggested. A flowchart is presented as a guide for the isolation and correction of chromatographic problems that arise during the routine use of an LC method.

INTRODUCTION

Columns are available for all modes of liquid chromatography (LC) including normal-phase, reversed-phase, size-exclusion, ion-exchange (ion and cation), ion-pairing, hydrophobic interaction, and affinity chromatography. The broad range of LC applications makes it impossible to list all of the potential problems associated with column use and maintenance. Despite this complexity, the underlying similarity of modern LC columns (typically packed with 3–20 μm diameter, silica or polymeric, porous particles of 60–4000 Å porosity, coated with a bonded phase) makes it possible to provide some general guidelines for extending column life.

Columns generally arrive from the manufacturer in good condition and remain that way until they are installed in an LC system. Once in operation, the LC column and resulting separations can experience a wide range of problems. Although columns generally are considered to be consumable items with a finite lifetime, proper care and maintenance can greatly extend this life and improve separation performance.

In this paper we describe a systematic approach to understanding, minimizing, and correcting LC column problems. Taking advantage of this approach requires a good understanding of the entire LC system, as well as the current column and method being used. This will be aided by a thorough knowledge of the nature of the samples being analyzed. This knowledge, coupled with an appropriate level of pre-

ventive maintenance, careful observation, and good documentation minimizes problems related to column performance. When problems do arise, a systematic approach to troubleshooting helps to minimize downtime, and may extend the useful life of the LC column.

VALIDATION

The most important skill in column maintenance is the ability to recognize that a problem exists. Typical signs of column degradation are changes in system pressure, peak shape, band retention, selectivity, and/or efficiency [1,2]. Examples of these symptoms are shown in Fig. 1. Although each of these can be indicative of a deteriorating column, they can also be caused by non-column problems, including mobile-phase contaminants, poor gradient reproducibility, extra-column volumes, temperature changes, secondary retention effects, and/or sample degradation.

The best way to ensure the quality of an LC column is to validate its performance when it is received and continue to use the same validation protocol throughout its lifetime (whenever a columns problem is suspected). Most commercial LC columns are tested by the manufacturer; a copy of the performance results and test conditions usually is supplied with the column. The manufacturer's test protocol should be used to validate the column upon receipt. This not only ensures that the column has survived storage and shipment, but also helps identify system-related problems, such as excess extra-column volume, which can reduce column performance.

It is also useful to validate a new column using the method and calibration standards for which the column will be used. This helps to identify method- and sample-related problems, which often can be corrected by a slight modification to the method (e.g., use of mobile-phase additives). If such calibration is not possible, separation of a well-characterized standard representative of the sample type to be used is a viable alternative. An example of this approach was described by Mant and Hodges [3], who characerized and monitored a silica-based reversed-phase column with a set of peptides which were sensitive to changes in stationary phase load and increases in silanol interactions.

PREVENTIVE MAINTENANCE

The best way to maximize column performance and lifetime is to practice regular preventive maintenance. The most important factors to consider are the sample preparation technique, the mobile phase selection and preparation, and the proper operation and maintenance of the LC instrument. Proper attention to detail in these areas, plus the appropriate use of in-line devices such as filters, guard columns, and precolumns, can greatly extend the useful life of an LC column.

Sample preparation
Many LC column problems can be prevented by properly preparing samples before injection. The major sample preparation techniques for modern LC are extensions of those used in classical LC. Often, however, they require more rigor to protect expensive, small particle columns. These techniques, which have been reviewed recently by Wehr and Majors [4], should meet several objectives to help prevent column
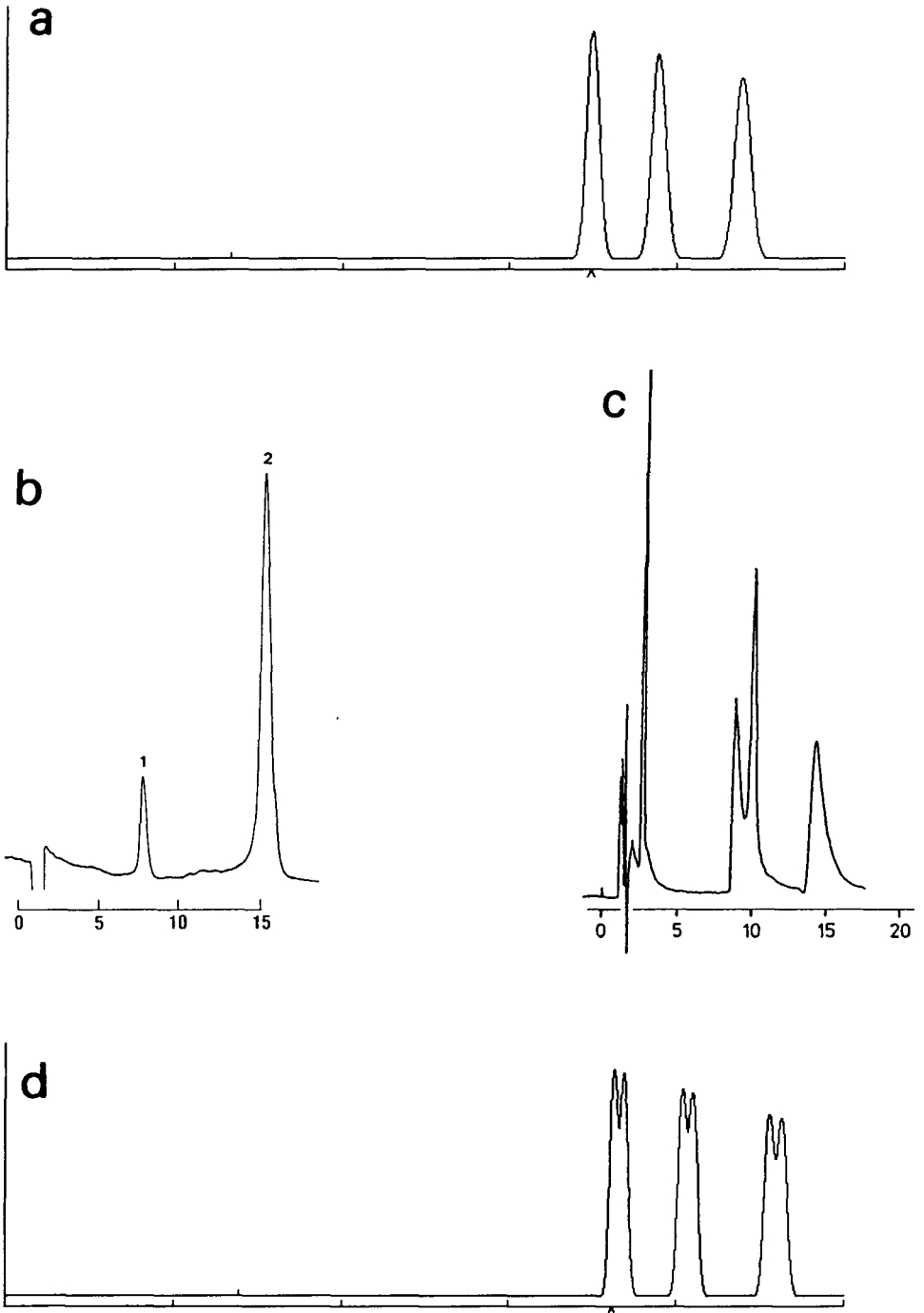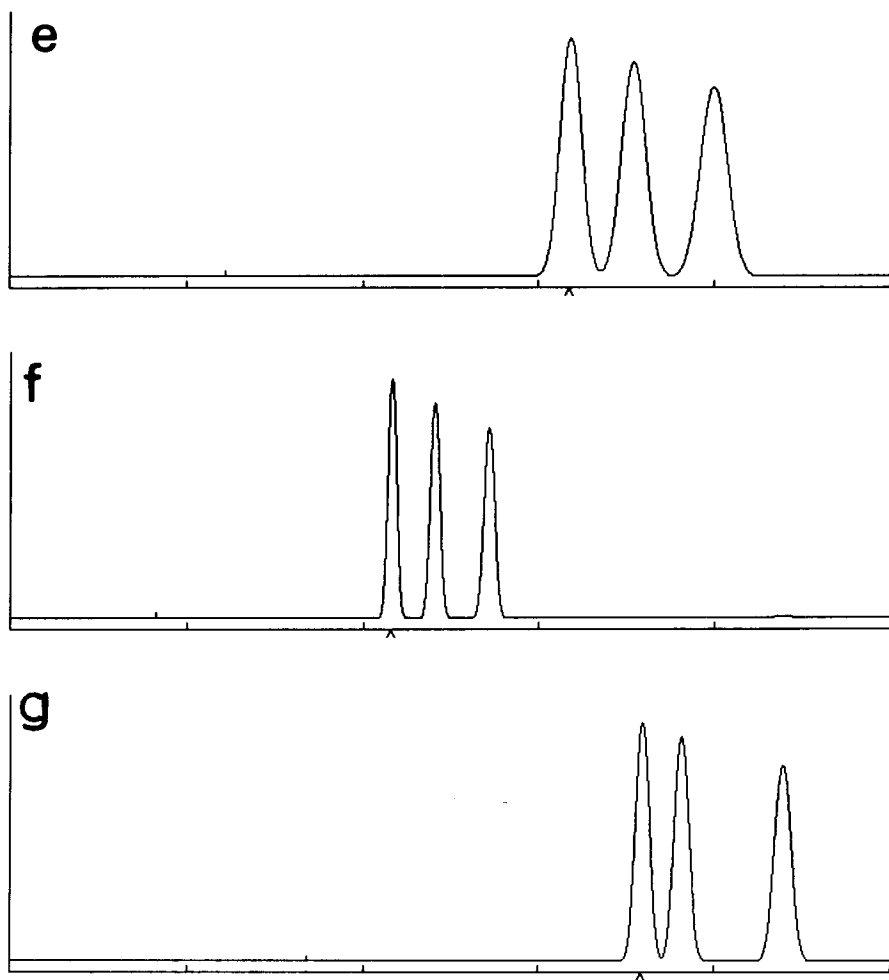
Fig. 1.

Fig. 1. Examples of non-ideal peak behavior due to column problems. (a) Normal column performance; (b) fronting peaks (from ref. 10, with permission); (c) tailing peaks (from ref. 11, with permission); (d) split peaks; (e) broadened peaks; (f) change in retention; (g) change in selectivity. Time scales in min.

problems. These include: (a) dissolving the sample in a solvent compatible with the starting mobile phase; (b) optimizing the sample concentration for proper column loading; (c) removing particles (usually to 0.2–0.5 μm) to prevent blockage of the column; and (d) simplifying the sample matrix by removing unwanted materials that might alter the performance of the column.

Sample preparation requirements vary with the type of sample and the potential for column and assay problems. The cost of sample preparation and the potential increase in variability of results must be balanced against the cost of column replacement and the impact of dirty samples on precision. As a minimum, samples should be dissolved in the mobile phase (or in a solvent weaker than the starting mobile) and any particles removed by filtration before injection.

Mobile phase selection and preparation

Commercial LC columns generally come with instructions for proper use and care. If these guidelines and a few other general rules are followed, many column degradation problems can be prevented.

Commercial high-performance liquid chromatographic (HPLC)-grade organic solvents, which have been purified and filtered especially for LC use, often can be used directly as received. Many mobile-phase-related column problems come from "bad" water or modified aqueous solutions. High purity water can be obtained from commercial sources, or freshly prepared using laboratory water purification systems; all water should be filtered through an $0.2\text{-}\mu m$ filter prior to LC use to remove microbial contaminants. Whenever salts, buffers, or other modifiers are added to LC mobile phases, these solutions should be filtered to 0.5 μm prior to use. To prevent microbial growth during use, thoroughly degas all aqueous solutions prior to use, and change the solutions daily (unless they contain at least 10% organic solvent or 0.05% sodium azide).

Mobile phase pH is an important parameter in the operation of silica-based LC columns. Most manufacturers recommend a mobile phase of $2 < pH < 7.5$, but even within these limits, problems can occur. At $pH > 7$, silica slowly dissolves, greatly shortening column life. Although one can use a precolumn (a silica-based column upstream from the injector which will presaturate the mobile phase with dissolved silica), this can also create problems [2]. At $pH < 2$, the silicon-carbon bond is susceptible to cleavage;; the result can be a gradual loss of stationary phase from silica-based LC columns. Glajch *et al.* [5] have reported losses of up to 80% of the original stationary phase from alkyl-bonded silica columns run at pH 2 in only 96 h. The extent of loss varies with column type; it affects both column life and column performance (especially retention time repeatability). Selecting the proper silica- or polymer-based column helps minimize pH-related problems.

Solvent compatibility is an important consideration when polymeric packings (organic packings such as cross-linked polystyrene) are used. An incompatible solvent or too sudden a change in solvent may cause the packing particles to shrink or swell, disturbing the integrity of the column bed. Many different types of polymers are used as base supports for LC columns; carefully read and follow the manufacturer's recommendations for use.

Regardless of column type, ensure that the mobile phases are miscible with each other, with the sample, and with the injection solvent. This especially true for gradient LC using organic solvents and buffers. Mobile-phase-strength effects can cause precipitation of sample or buffer inside the column, resulting in column blockage or a packing void.

When not in use, LC columns should be flushed with 5–10 column volumes of either an organic–water mixture or pure water with 0.05% sodium azide and the ends tightly sealed. These conditions minimize column degradation form precipitation, chemical attack, or microbial growth during storage.

LC SYSTEM MAINTENANCE

Although there are dozens of commercial LC systems in use today, most affect LC column performance and life in a similar manner. LC systems typically come with

documentation describing the capabilities and limitations of the system, as well as guidelines for routine maintenance. A thorough understanding of the system, a regular preventive maintenance and validation schedule, and a well-documented system log book work together to minimize system-related column problems, reduce downtime, and maximize system performance.

Major system-related problems result from contaminants which can block or foul the column. Certain parts, such as pump seals and injector rotors, always wear with use, creating particulate matter. This wear is accelerated when the system is operated at high pressures, under adverse conditions (e.g., high buffer concentrations), or when use is extended beyond the normal replacement interval. Most LC systems work well in the range of 3 < pH < 8, but special precautions must be taken for separations requiring caustic (pH > 9) or corrosive (low pH or halide salts) mobile phases, to prevent column contamination and other system related problems. Special LC systems can be acquired wih increased resistance to aggressive chemicals.

Improperly functioning hardware can also cause column problems by subjecting the column to pressure shocks. Slow rotation of injectors or use of very large sample loops can induce pressure shocks which may disrupt a column packing bed. Pump problems such as severe leaks, leaking or contaminated check valves, or cavitation from a blocked inlet-line filter can also result in pressure shocks. Most pulse dampeners will not control these extremes in pressure. In fact, pulse dampeners based on a diaphragm or membrane backed by a viscous fluid may rupture under these conditions, further contaminating the LC column. Every LC system should have a reliable pressure gauge which should be routinely monitored to ensure proper system operation.

A properly functioning system minimizes retention time variations during consecutive LC runs. When the LC system is used to mechanically proportion solvents, proper pump operation is critical to good performance. Minor leaks within the system, selective volatilization of a mobile-phase component, or changes in the column temperature also can give rise to changing retention times. The use of helium to degas and blanket the solvent, plus a column oven to stabilize temperature will help to minimize retention variations.

Column performance also can be degraded by extra-column effects resulting from excessive system volumes and sample mixing outside the column [6]. Problem areas can include all tubing, fittings, and other system components from the point the sample is injected until it leaves the detector. Excess extra-column volume increases bandwidths, with earlier bands in the chromatogram being affected more than later band (especially for isocratic separations). When significantly broader peaks (and sometimes band tailing) for earlier peaks relative to later peaks is observed, extra-column effects are suspected. Extra-column effects become more significant as peak volumes decrease.

Initial validation tests of the type described above should identify any incompatibilities between column and system. Properly integrated LC system components with a minimum of extra-column volume will help to minimize these problems.

Changes in column efficiency due to extra-column effects can occur. These usually arise after the LC system has been serviced or modified. Good documentation in a system log book can be very useful in identifying such problems. When a drop in column efficiency is noted following a system modification, carefully inspect all tub-

ing, fittings, and connections to identify problems such as wrong tubing I.D., poorly cut tubing, or improperly assembled fittings. A good (but not perfect) way to discriminate column *vs.* extra-column band broadening is to substitute a new column into the system to see if the problems go away.

IN-LINE FILTERS AND GUARD COLUMNS

Even after all of the sample-pretreatment, mobile-phase, and system-related precautions mentioned above have been taken, we strongly recommend that an in-line filter and/or guard column be placed between the injector and the main LC column. For assays using fairly pure samples, an in-line filter (0.5 μm) may be sufficient. This will help prevent blockage of the column inlet frit by particles from the sample, mobile phase, or LC system.

For more complex samples, we recommend use of a guard column. Guard columns remove materials which irreversibly bind to the column packing or are so large that they block either the column frit or the packing bed.

Several manufacturers sell bulk pellicular packings (30–40 μm) which can be dry packed into a guard column at a very low cost. Prepacked cartridge-type guard columns also are cost-effective. For very "dirty" samples, the best guard column is one prepacked with the exact same material (support, particle size, pore size, and bonded phase) as the separation column. A matched guard column removes materials which harm the main column, minimizes extra-column effects, and prevents differences in separation chemistry from degrading the separation.

COLUMN TROUBLESHOOTING

Despite rigorous attention to preventive maintenance, all LC columns age with use. Column aging is characterized by changes in: (a) system backpressure, (b) peak shape, (c) band retention, and/or (d) selectivity. It is important to properly identify the nature and extent of the problem (*e.g.*, consider Fig. 1).

An LC column troubleshooting flowchart is shown in Fig. 2. (Note that Fig. 2 is useful only for correcting a problem in a previously satisfactory method; problems due to poor method design are not included.)

Changes in system backpressure

When a significant change (*e.g.*, 50%) in backpressure occurs (that cannot be attributed to mobile phase viscosity changes), the problem should be isolated and corrected as soon as possible. A decrease in system pressure is generally due to a leak or loss of flow from the pump, and not specifically related to the column (unless the leak occurs at the column fittings). An increase in system pressure usually is caused by a blockage in the column. To identify the point of blockage, start by disconnecting the column at the detector inlet and work upstream. When a large drop in system pressure is observed, the component directly downstream is blocked (remember to take the normal column pressure into account). If the point of blockage is at the head of the column (or guard column), and no changes in peak shape, retention or selectivity have occurred, backflushing with the mobile phase may help. If the pressure increase is sample related (*e.g.*, sample components have accumulated on the frit at
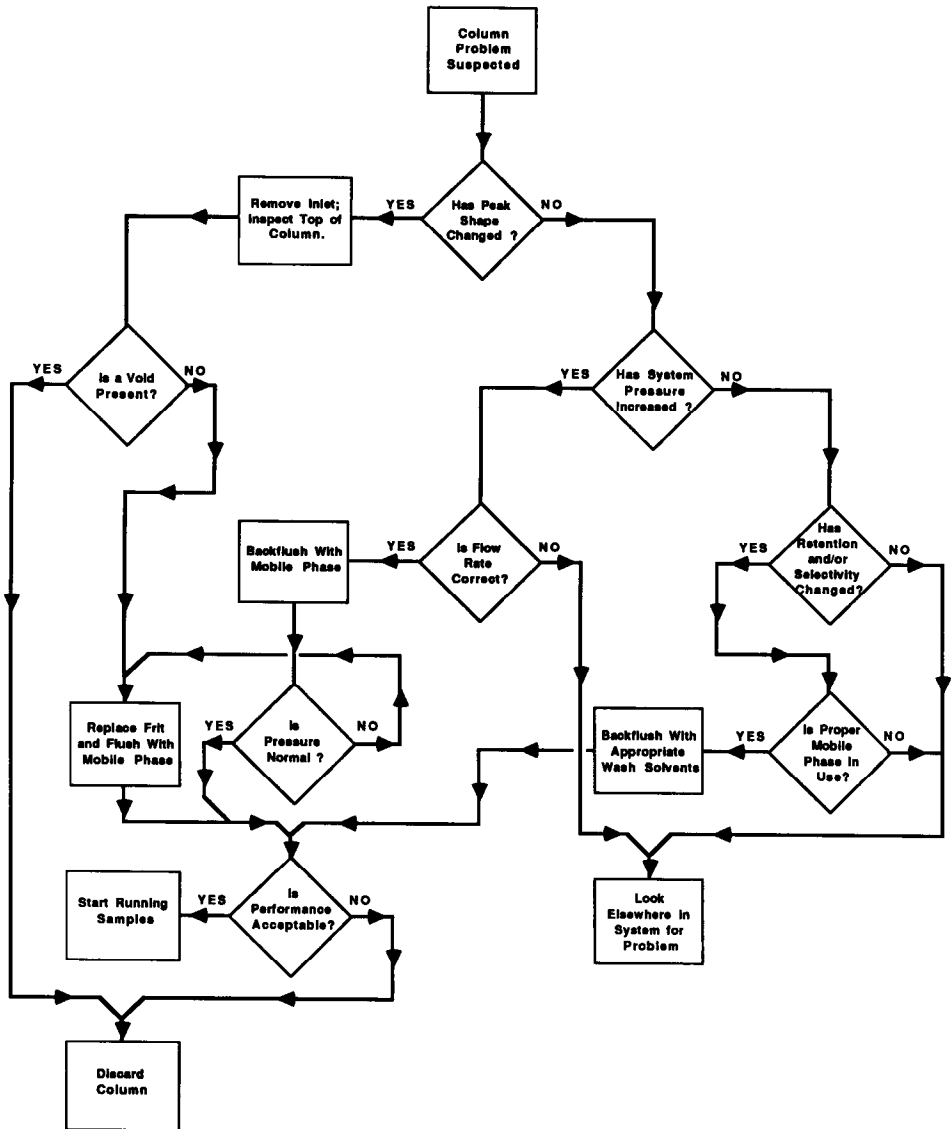
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                              ┌──────────┐
                              │  Column  │
                              │ Problem  │
                              │Suspected │
                              └──────────┘
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Fig. 2. Column troubleshooting flowchart.

the head of the column), then washing with a strong solvent or blackflushing may restore the original pressure. If this washing or backflushing does not restore an acceptable operating pressure, replace the inlet frit. If any problems are noted with the top of the column bed, follow the procedures described below for restoring changes in peak shape. If the column still fails to give acceptable performance, change to a new column.

Changes in peak shape

Column degradation should be suspected when changes in peak shape (such as those shown in Fig. 1) occur, which are not related to sample, method, or system problems discussed earlier. It is important to validate these using the appropriate test protocol. Because a change in peak shape often is due to a void in the LC column packing bed, the first action should be removal of the inlet fitting and inspection of the packing bed. Reversing the flow and backflushing the column may cause a void to shift and destroy a well-packed column bed, so it is best to inspect the top of the column first. If a void is found, replace the column. Filling a column void is seldom cost-effective. (If it is necessary to fill a void, use the technique of ref. 7 in which the void is filled and then the column is reversed before use.) If no void exists, replace the inlet frit, reassemble the column, and test using the appropirate validation protocol. If the column fails to return to an acceptable level of performance, replace it.

Changes in retention or selectivity

Column contamination and/or loss of bonded phase should be suspected when changes in peak retention or selectivity (such as those shown in Fig. 1f or g) occur, which are not related to sample, method, or system problems discussed earlier. Validate any suspected changes before proceeding.

When changes in retention or selectivity are due to column contamination, reverse the column in the system and backflush to waste (*not* through the detector). There are many protocols for removing contaminants from LC columns; the best procedure depends on the nature of the sample. If non-polar materials are bound to a reversed-phase column, successive flushes with increasingly non-polar solvents (taking care to ensure solvent miscibility) may help to remove the contaminants. If metal ion contamination is suspected, flushing with a chelating agent, such as EDTA, may be helpful. Contamination from biological materials may require flushing with acids or bases (much more compatible with polymeric columns), non-ionic detergents, and/ or chaotropes (guanidine, urea, etc.). If complete flushing (10–20 column volumes) with strong solvents fails to restore an acceptable level of column performance, the procedure described above for remedying changes in peak shape can be tried as a last resort prior to replacing the column. (For specific column flushing procedures, see ref. 1.)

A gradual loss of bonded phase usually is characterized by a decrease in all retention times. It also can cause increased tailing of basic compounds due to increased silanol interactions on silica-based columns. As reported by Glajch *et al.* [5], this loss may be slow and not affect column performance even after 50% of the stationary phase is gone. If a significant loss of bonded phase has occurred (*e.g.*, if retention is not restored by column cleanup as described above), replace the column.

CONCLUSIONS

Modern LC columns are expensive, so simple precautions that extend column life can significantly improve the analysis cost per sample. Maintaining any LC column requires an understanding of the column, method, samples, and LC system. A column validation protocol helps to quickly distinguish column problems from other method-, system-, and sample-related problems. Preventive maintenance, especially

during mobile-phase and sample preparation, is critical for ensuring good perform-ance and long column life. Finally, as problems do arise, a systematic approach to troubleshooting and repair minimizes downtime and maximizes the potential benefits of each procedure. (For further information concerning LC column maintenance and troubleshooting, including specific recommendations, see refs. 1, 8, 9.)

REFERENCES

1 J. W. Dolan and L. R. Snyder, *Troubleshooting LC Systems*, Humana Press, Clifton, NJ, 1989.
2 C. T. Wehr and R. E. Majors, *LC · GC*, 5 (1987) 942.
3 C. T. Mant and R. S. Hodges, *LC · GC*, 4 (1986) 250.
4 C. T. Wehr and R. E. Majors, *LC · GC*, 5 (1987) 548.
5 J. L. Glajch, J. J. Kirkland, and J. Koehler, *J. Chromatogr.*, 384 (1987) 81.
6 S. R. Bakalyar, K. Olsen, B. Spruce and B. G. Bragg, *LC · GC*, 6 (1988) 876.
7 J. Vendrell and F. X. Aviles, *J. Chromatogr.*, 356 (1986) 420.
8 R. E. Majors, *Column Watch*, a monthly feature in *LC · GC*.
9 J. W. Dolan, *LC Troubleshooting*, a monthly feature in *LC · GC*.
10 P. A. Asmus, J. B. Landis, and C. L. Vila, *J. Chromatogr.*, 264 (1983) 241.
11 J. H. Knox and R. A. Hartwick, *J. Chromatogr.*, 204 (1981) 3.