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Guard and scavenger columns

Extending the lifetime of LC columns

The column is the heart of any liquid chromatograph, yet despite the sophistication of modern LC technology, the column remains the most problematic component of the LC system. With typical C18 columns priced in the $150–$300 range and some specialty columns, such as amino acid columns, costing more than $1000, column replacement can be quite expensive (1). The cost is even more prohibitive if one considers the loss in productivity that is caused by premature column failures and the time spent in verifying column performance. Therefore, it behooves the practicing chromatographer to use every available practical means for extending column lifetime.

Column lifetime is affected by many factors, both physical and chromatographic. Physical considerations include characteristics of the packing material (for example, particle size, shape, and porosity), column hardware design, and the stability of the packed bed. Chromatographic factors are problems caused by the mobile phase and the sample. Given today's improved column design and packing technology, physical problems are less likely. Mobile phase problems, however, such as dissolution of silica supports under high pH mobile phases (2), can indeed cause premature failure unless the column is protected by a silica presaturator (3). In practice, most premature column failures can be prevented through proper column care and maintenance and the judicious use of protector columns (1,4).

Protector columns include both guard columns and scavenger columns. The term *scavenger column* is used here because *precolumn* can be interpreted as any column that is located before the analytical column, including a guard column. The extension of column lifetime by such devices is well documented in the literature (1,5,6). Although some chromatographers continue to disregard these recommendations, a recent survey indicated that guard columns are used by over three-quarters of practicing chromatographers (7). The use of scavenger columns to protect analytical columns from mobile phase contaminants is more sporadic. With the increasing popularity of smaller-particle columns (for example, 3-μm columns, which are less forgiving if misused) (8,9), the need for column protection becomes even more critical.

The objectives of this article are to review the characteristics of protector columns and to demonstrate their effectiveness in extending column lifetime. The characteristics of guard and scavenger columns — such as dimensions, packings, dispersion, convenience, and sample capacities — will be discussed. The need for using these devices is demonstrated by controlled experiments using contaminated mobile phases and samples. Their benefits are further illustrated using model laboratory analysis situations that compare lifetimes with and without column protection. Finally, practical guidelines for prolonging column lifetime will be presented. Because reversed-phase columns are currently used in 60% to 80% of LC applications, C18 bonded-phase columns were used in this study. Conclusions and recommendations, however, are applicable to other LC modes.

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EXPERIMENTAL

The liquid chromatographic system used in this study consisted of either a series 4 or a series 10 pump, a model 7125S injection valve, an LC-95 variable-wavelength UV detector, and a model 056 recorder or an LCI-100 integrator. Automated injection for studying column lifetime was performed by an ISS-100 or an LC-600 autosampler. All equipment used was from Perkin-Elmer Corp. (Norwalk, Connecticut).

The analytical columns used in this study were Pecosphere 3×3C, 3.3 cm × 4.6 mm, C18 cartridge columns packed with 3-μm particles. For the anion chromatography and the clinical studies, 8.3 cm and 12.5 cm lengths were used, respectively, for 3- and 5-μm columns. The scavenger columns used were 3.3 cm × 4.6 mm, 8-μm C18 cartridge scavenger columns. Guard columns of various dimensions were evaluated and dry-packed with 40-μm, pellicular C18 material. All columns were from Perkin-Elmer.

The number of theoretical plates for a column was determined by the width-at-half-height method using *t*-butylbenzene as the test solute. This method was used as an indicator of column efficacy. The mobile phase was 60:40 (v/v) acetonitrile/water; flow rate was 2.0 mL/min.

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SCAVENGER COLUMNS

Figure 1 is a schematic diagram of the location of a scavenger column and a guard column in an LC system. The scavenger column, placed between the pump and injector, "scavenges" unwanted material from the mobile phase and protects the analytical column from particulate matter, pump-seal-wear particles, and irreversibly adsorbed chemical contaminants that may be present in the mobile phase. In isocratic analyses, the size of the scavenger column is relatively unimportant. In gradient analyses, however, a small scavenger column is necessary to minimize the gradient delay volume of the system.

A scavenger column functions differently from a silica presaturator (3). A scavenger is an effective filter and adsorber, whereas a silica presaturator prevents silica dissolution in the analytical column. A silica presaturator is only required under aggressive mobile phase conditions such as high pH. For maximum protection, the scavenger column is packed with the same type of packing material as the analytical column; but, because the sample does not pass through the scavenger column, its packing need not be a high-efficiency microparticulate. Larger (37–53 μm) particles can be used instead (1). Nevertheless, microparticulate packing is strongly recommended for the following reasons:

- Microparticulates are uniformly sized and highly purified materials, whereas the use of lower-grade silicas or bonded phases can lead to contamination problems. Plugging problems resulting from fines are also less likely with high-quality microparticulates.
- Smaller-particle packed beds are more effective filters than their larger-particle counterparts.

We recommend 8–13 μm particles in small (for example, 3.3 cm × 4.6 mm) scavenger columns because they are readily available, do not impart excessive back pressure, and permit rapid solvent changeover. They must, however, be slurry-packed to yield columns with good breakthrough characteristics. The limitation of small columns is their low capacities. In our experience, however, such a scavenger column can be used under typical laboratory conditions in the LC system for months before replacement is needed.

Bacterial growth in aqueous mobile phase: The growth of bacteria in an aqueous mobile phase can be a disaster for columns (10). Because such growth is not always visible, its presence in the mobile phase might not be apparent. Moreover, growth rate is accelerated in buffer solutions and at temperatures above ambient. This problem is aggravated for LC systems in which the solvent reservoirs are located above the electronic module — a heat source that effectively serves as an incubator. Filtering the aqueous mobile phase through a 0.5-μm filter membrane before use generally is not sufficient if the supply of mobile phase is to be used for more than 2–3 days. In this regard, several treatment procedures have been advocated:

- prepare and filter fresh mobile phase daily
- use sterile reservoirs and mobile phases
- saturate the aqueous mobile phase with chloroform
- add 0.004% sodium azide as a bactericide
- add 1% acetonitrile or methanol to retard bacterial growth

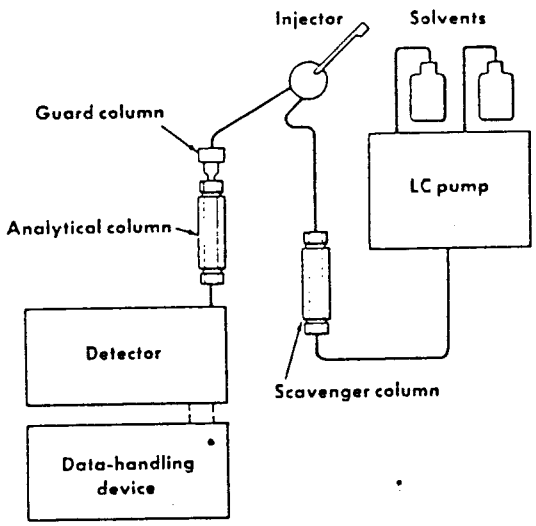

FIGURE 1: Schematic diagram of an LC system.

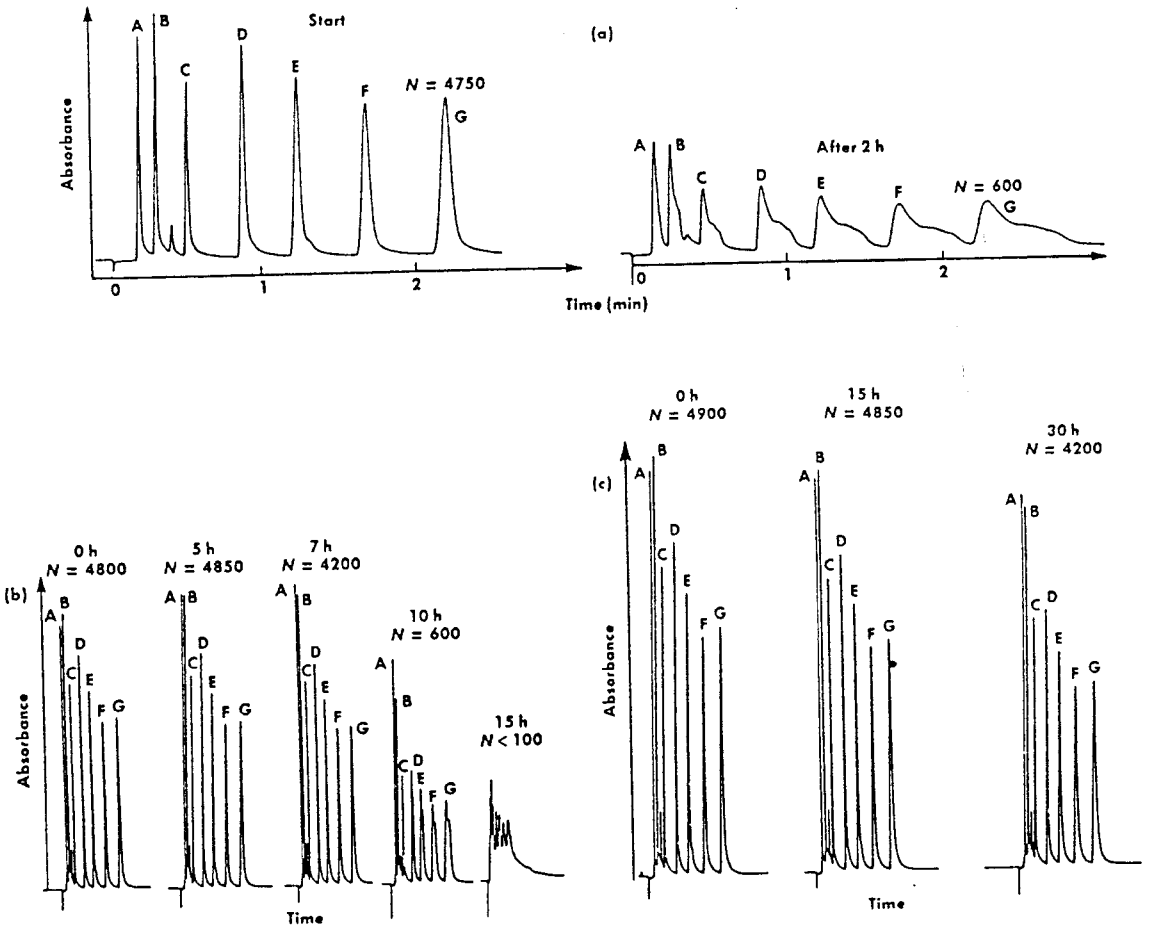

FIGURE 2: Degradation of column performance resulting from bacterial growth in an aqueous mobile phase for (a) an unprotected column, (b) a column protected by a 0.5-μm in-line filter, and (c) a column protected by a 0.5-μm in-line filter and a 3.3 cm × 4.6 mm, 8-μm C18 scavenger column. Analytical column: 3.3 cm × 4.6 mm, 3-μm C18; mobile phase: 60:40 acetonitrile/water; flow rate: 2.0 mL/min; detection: UV 254 nm; injection volume: 6 μL. Peaks: A = uracil, B = phenol, C = nitrobenzene, D = toluene, E = ethylbenzene, F = isopropylbenzene, G = t-butylbenzene.

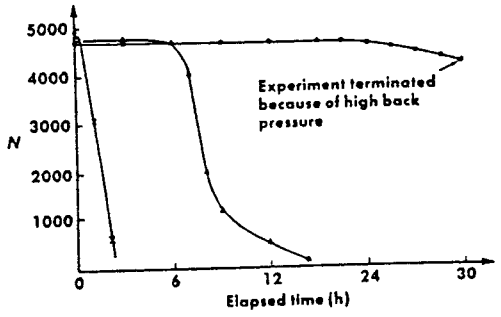

FIGURE 3: Effect of column protection on column-performance degradation resulting from bacterial growth in an aqueous mobile phase: (×) unprotected column, (▲) column protected by a 0.5-μm in-line filter, (●) column protected by a 0.5-μm in-line filter and a 3.3 cm × 4.6 mm, 8-μm C18 scavenger column.

None of these procedures appears to be completely satisfactory because of inconvenience or potential alteration of mobile phase properties. Some treatment is clearly needed, however, if the same bottle of aqueous mobile phase is to be used for several days.

To demonstrate the harmful effects of bacterial growth on column lifetime, we allowed bacteria to grow in water for several days until the water was visibly cloudy. The water contained both particulate (fibrils) and chemical (metabolites and breakdown products) contaminants. Using the contaminated water, we next prepared several liters of mobile phase (60:40 acetonitrile/water). Although this example is not typical of ordinary laboratory conditions, it does represent a limiting "worst case" mobile phase, which allowed us to observe column degradation rapidly and under controlled conditions.

Three column-lifetime experiments were performed using artificially contaminated mobile phase: without any column protection, with a 0.5-μm in-line filter, and with a 0.5-μm in-line filter before an 8-μm C18 scavenger column. In each experiment, plate count was measured at the outset and monitored periodically using a test mixture. The

3×3C C18 columns were used because their performance can be verified rapidly and because they are known to be more susceptible to particulate contamination.

The experimental results are illustrated in Figures 2a–2c. The performance of the unprotected column was drastically degraded — from 4750 plates to 600 plates — within 2 h of passing 120 mL of contaminated mobile phase through the column (Figure 2a). Figure 2b demonstrates the loss in column performance under similar conditions, but with the protection of a 0.5-μm in-line filter (Scientific Systems, Inc., State College, Pennsylvania), which is standard on many liquid chromatographs. Column performance was maintained for at least 5 h. By the 7-h mark, efficiency loss was observable. By 10 h, peak splitting had occurred, and by 15 h, the chromatogram was unrecognizable and showed a dramatic reduction in retention times. The efficiency loss could not be reversed by solvent flushing or back flushing. The in-line filter, although offering some protection against particulate contamination, did not prevent premature column failure.

Figure 2c illustrates the effectiveness of the scavenger column. The scavenger column is placed after the 0.5-μm in-line filter to prevent clogging of its inlet frit. After 15 h, no performance loss is visible. By 30 h, some efficiency loss is evident, although the performance is still quite acceptable. After 30 h, the experiment was terminated because of the high back pressure generated by the scavenger column. Reversing the direction of flow through the scavenger column did not reduce the back pressure. The comparative data of Figure 3 illustrate the effectiveness of the scavenger column in extending column lifetime.

Anion analysis using reversed-phase ion-pair chromatography: Ion-pair chromatography using indirect UV detection provides excellent sensitivity and selectivity in the analysis of common inorganic anions (11). An 8.3 cm × 4.6 mm, 3-μm C18 column was used with a mobile phase consisting of 1 m*M* tetrabutylammonium hydroxide (TBAOH), adjusted to pH 8.0, and a saturated solution of potassium hydrogen phthalate (KHP). Mobile phase was prepared fresh daily from purified chemicals and was filtered through a 0.5-μm filter membrane before use.

During development of the reversed-phase approach to anion analysis, it was found that the column would fail within 100 injections or with the passage of 5–6 L of mobile phase through the column (Figure 4a). The cause of column failure is not known, although the corrosiveness of TBAOH, the presence of contaminants in the KHP, and silica dissolution have been implicated (1,12). Under the above experimental conditions, column failures were accompanied by a red coloration at the top of the column. Also, column voiding was not observed. These two observations imply that column failure is a result of irreversible adsorption of a contaminant rather than silica dissolution.

Column lifetime was found to be extended significantly by using a 3.3 cm × 4.6 mm, 8-μm C18 scavenger column. Column performance remained essentially intact after more than one thousand injections, that is, after the passage of more than 20 L of mobile phase through the column (Figure 4b).

Column lifetime testing: To further verify the importance of scavenger columns, a 3×3C C18 column was subjected to extended column-lifetime testing under the chromatographic conditions given in Figure 5. Filtered mobile phase and clean test samples were used with a scavenger column. After 12 days of continuous testing and 8750 injections, column performance and pressure drop (ΔP) remained unchanged. Without the scavenger column, premature column failures resulting from efficiency loss with accompanying pressure increases were observed within 1–2 days.

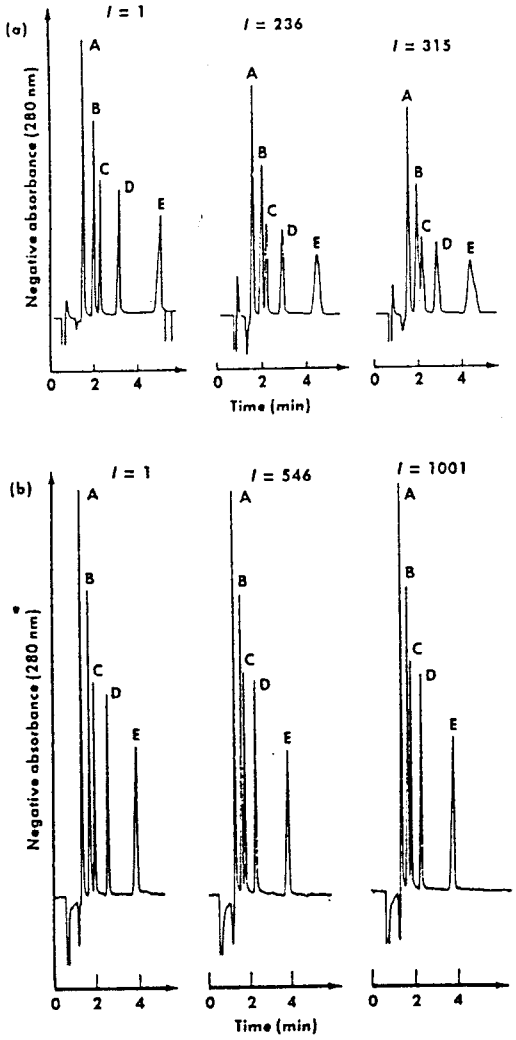

FIGURE 4: Column performance for varying number of injections (*I*) during reversed-phase ion-pair chromatography for (a) an unprotected column and (b) a column protected by a 3.3 cm × 4.6 mm, 8-μm C18 scavenger column. Analytical column: 8.3 cm × 4.6 mm, 3-μm C18; mobile phase: 1 m*M* TBAOH adjusted to pH 8.0 with a saturated solution of KHP; flow rate: 1.5 mL/min; detection: indirect photometric; injection volume: 20 μL, 50 ppm of standard. Peaks: A = Cl^-, B = NO_2^-, C = Br^-, D = NO_3^-, E = SO_4^{2-}.

The results of the above experiments, in addition to the experiences of chromatographers, suggest that a scavenger column is a useful device for extending column lifetime and should be considered a necessary component in

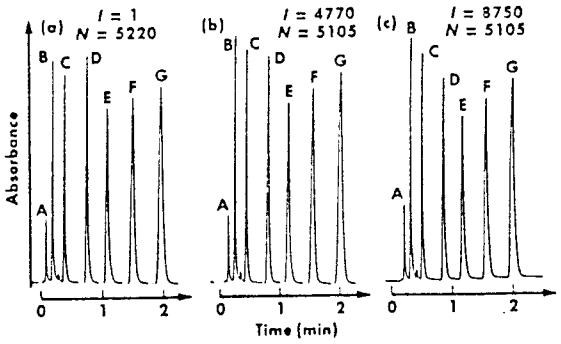

FIGURE 5: Column-lifetime testing over a 12-day period with a scavenger column in place. Chromatographic conditions same as in Figure 2 except flow rate was 1.5 mL/min; ΔP: (a) 7.6 MPa and (b–c) 8.0 MPa. Peak identities: same as in Figure 2.

TABLE I: COMPARISON OF PELLICULAR AND MICROPARTICULATE GUARD COLUMNS

Factor	Type of Packing: Pellicular (37–50 μm)	Type of Packing: Microparticulate (3–10 μm)
Extracolumn dispersion	some	none
Capacity	low	high
Method of repacking	dry-packing	slurry-packing
Pressure increase	slight	yes
Need for packing identical to that of analytical column	less important	important

TABLE II: EFFECT OF GUARD COLUMNS ON EFFICIENCY

Dimensions and Void Volume of Pellicular* Guard Column	Theoretical Plate Count** $k' = 1.5$	Theoretical Plate Count** $k' = 9$
No guard column in place	3700	5140
3.3 cm × 2.1 mm, 110 μL	3200	5030
3.3 cm × 2.6 mm, 170 μL	2100	4600
5.0 cm × 4.6 mm, 830 μL	1500	2900

*guard columns packed with 40-μm pellicular C18 material

**flow rate: 2.0 mL/min; see text for other chromatographic conditions

any LC system. A scavenger column traps substantial amounts of harmful materials, including fine particulates and chemical contaminants, that can pass through an in-line filter or pellicular guard column. Most importantly, a scavenger unit offers column protection without affecting the chromatography and/or adversely affecting the pressure drop, convenience, and reliability of the system.

GUARD COLUMNS

A guard column is placed between the injector and the analytical column to protect the analytical column from sample contaminants, and highly retained solutes, as well as from wear particles from the rotor seal of the injection valve. Because the sample passes through it, the guard column must be of low volume and contain a packing material similar to the analytical column. Both the advantages and the limitations of using microparticulate or pellicular materials in guard columns have been cited (1). Some pros and cons are summarized in Table I. In practice, pellicular guard columns are more popular because they are less expensive and they can be repacked more easily. They have low sample capacity, however, and offer less protection than microparticulate-packed guard columns.

Effect of pellicular guard columns on efficiency: Guard columns packed with pellicular materials in the 37–50 μm range can cause a significant loss in efficiency when used with the most recently developed small-particle columns because of their contribution to extracolumn dispersion. Extracolumn effects are dependent on column dimensions, particle diameter, and the capacity factor (k') of the peak in question and can be predicted by considering band variances (13,14). To demonstrate the contribution of guard columns to band broadening, several pellicular guard columns of varying dimensions were evaluated. The 3×3C analytical column was selected because of its sensitivity to extracolumn effects. Larger guard columns cause greater efficiency degradation (Table II). This effect is even more severe for low-k' compounds. The graph of Figure 6 illustrates the loss of efficiency. Clearly, the small, 3.3 cm × 2.1 mm guard column, having a void volume of 110 μL, is preferred for short, small-particle columns.

Contaminated samples: To illustrate the effect on performance resulting from the injection of "dirty" samples, a series of controlled experiments was carried out. The results are summarized in Table III. A sample of unfiltered espresso coffee was used, representing a "worst case" sample. The sample contained particulates, pigments, lipids, and other highly retained solutes. Three identical 3×3C C18 columns were tested under different conditions: without guard column protection, with a pellicular guard column, and with a microparticulate guard column. The plate count of each column was measured at the start of the run and, subsequently, after the injection of increasing volumes of the contaminated sample. All plate counts were measured with the guard column removed to permit direct measurement of the effectiveness of the guard column in removing particulates and contaminants. Without any protection, performance loss was severe. As is shown in Figure 7, plate counts degraded from 5170 to 2400 and 670, respectively, after injecting 40 μL and 200 μL of the coffee sample. Table III suggests that the pellicular guard column can offer significant protection, although the microparticulate guard column is preferred for maximum protection.

Clinical samples: In other well-documented studies, chromatographic profiles of serum ultrafiltrate from a large number of patients were examined to correlate the levels of nucleosides and bases with various disease states (15,16). The use of pellicular guard columns extended the lifetime of the 12.5 cm × 4.6 mm, 5-μm C18 column

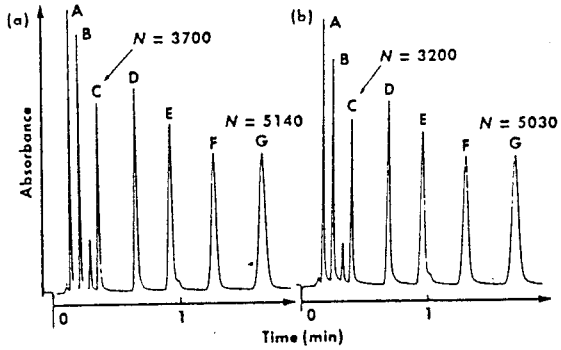

FIGURE 6: Column performance (a) without guard column and (b) with 3.3 cm × 2.1 mm, 30–40 μm pellicular guard column. Chromatographic conditions and peak identities same as in Figure 2.

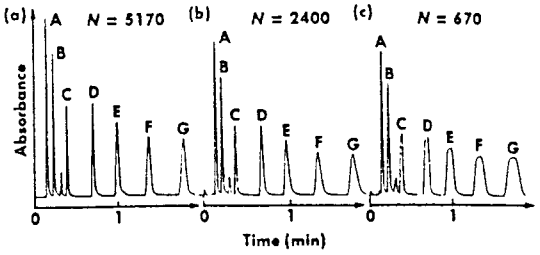

FIGURE 7: Column performance (a) before injection of espresso contaminant, (b) after injection of 40 μL of contaminant, and (c) after injection of 200 μL of contaminant. Chromatographic conditions and peak identities same as in Figure 2.

TABLE III: LOSS OF COLUMN EFFICIENCY RESULTING FROM INJECTIONS OF CONTAMINATED SAMPLES

Guard Column	Theoretical Plate Count for Total Injected Volume* of:				
	0 μL	40 μL	200 μL	400 μL	800 μL
No guard column in place	5170	2400	670	—	—
Pellicular C18 3.3 cm × 2.1 mm, 40 μm	5030	4350	3250	3000	—
Microparticulate C18 3.3 cm × 4.6 mm, 3 μm	4850	—	—	—	4850

*flow rate: 2.0 mL/min; see text for other chromatographic conditions

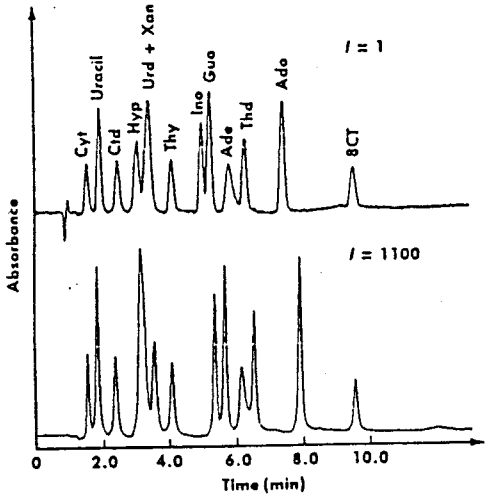

FIGURE 8: Effect of guard column on column lifetime during reversed-phase chromatography of serum ultrafiltrate. Column: Pecosil 12.5 cm × 4.6 mm, 5-μm C18 (Perkin-Elmer) with a 3.3 cm × 4.6 mm, 8-μm pellicular guard column attached; mobile phase: A = methanol and B = 0.02 *M* KH_2PO_4 (pH 5.6), linear gradient from 0.3% A to 24% A in 7 min; flow rate: 2.0 mL/min; detection: UV 254 nm at 0.08 AUFS; injection volume: 100 μL of nucleoside base standard. (Courtesy of reference 15.)

from 300–400 injections to more than one thousand injections (50-μL ultrafiltrate per analysis) (Figure 8). It was concluded in this case that guard columns need to be repacked every 50 analyses.

PRACTICAL GUIDELINES

In addition to some form of column protection, chromatographic cleanliness is required for extending column lifetime. Four practical guidelines are offered in Table IV. Clean and filtered mobile phases prepared from high purity chemicals should be used. Aqueous mobile phases should be treated with an appropriate antimicrobial agent or with 1%–2% of an organic solvent to retard bacterial growth. A small, microparticulate-packed scavenger column (for example, 3.3 cm × 4.6 mm, 8–13 μm C18) is recommended for additional mobile phase cleanup of irreversibly adsorbed contaminants. For silica-based columns, a silica presaturator packed with high-porosity silica is a must when using high pH mobile phases (pH > 8). Contaminated samples must be cleaned before injection onto the column. A small pellicular guard column (for example, 3.3 cm × 2.1 mm, 40-μm C18) is recommended for general use. For extra protection of expensive columns, a microparticulate guard column can be used. For routine analyses, a schedule for repacking the guard column or replacing the scavenger column should be established and followed.

CONCLUSIONS

Evidence for the effectiveness of protector columns is overwhelming, and the resulting economic benefits are irrefutable. Scavenger and guard columns are relatively

inexpensive and, when properly designed, can improve column longevity without incurring efficiency loss (for conventional and high-speed columns), pressure or gradient-delay-volume increase, or inconvenience. Modern LC systems should include scavenger and guard columns as necessary and integral components.

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TABLE IV: PRACTICAL GUIDELINES FOR EXTENDING COLUMN LIFETIME

Use clean mobile phase
Use scavenger columns
Clean up contaminated samples
Use guard columns: • small pellicular guard columns for general use • microparticulate guard columns for critical analyses or expensive columns

REFERENCES

(1) R. Majors, *LC, Liq. Chromatogr. HPLC Mag.* **2**, 16-20 (1984).

(2) J.R. Benson and D.J. Woo, *J. Chromatogr. Sci.* **22**, 386-399 (1984).

(3) J.G. Atwood, G.L. Schmidt, and W. Slavin, *J. Chromatogr.* **171**, 109-115 (1979).

(4) F.M. Rabel, *J. Chromatogr. Sci.* **18**, 394-408 (1980).

(5) G.D. Reed and L.R. Loscomb, *Chromatographia* **15**, 15-17 (1982).

(6) P.M. Ueland and E.S. Olheim, *J. Chromatogr.* **276**, 157-162 (1982).

(7) R. Majors, *LC, Liq. Chromatogr. HPLC Mag.* **3**, 774-780 (1985).

(8) M.W. Dong and J.R. Gant, *LC, Liq. Chromatogr. HPLC Mag.* **2**, 294-303 (1984).

(9) R. Majors, *LC, Liq. Chromatogr. HPLC Mag.* **2**, 202-208 (1983).

(10) J.W. Dolan and V.V. Berry, *LC, Liq. Chromatogr. HPLC Mag.* **1**, 542-543 (1983).

(11) P.A. Perrone and J.R. Gant, *Res. Devel.* **26**, 96-100 (1984).

(12) P.A. Perrone and J.R. Gant, "Advances in Anion Analysis: Reversed-Phase Ion-Pair Chromatography with Indirect Photometric Detection," Liquid Chromatography Technical Bulletin 1 (Perkin-Elmer Corp., Norwalk, Connecticut, 1984).

(13) L.R. Snyder and J.J. Kirkland, *An Introduction to Modern Liquid Chromatography*, 2nd Ed. (Wiley Interscience, John Wiley & Sons, New York, 1979), p. 228.

(14) J.J. Kirkland, W.W. Yau, H.J. Stoklosa, and C.H. Dilks, *J. Chromatogr. Sci.* **15**, 303-316 (1977).

(15) R.C. Simpson and P.R. Brown, *J. Chromatogr. Sci.* **23**, 89-93 (1985).

(16) M. Zakaria, in *HPLC in Nucleic Acid Research: Methods and Applications*, P.R. Brown, ed. (Marcel Dekker, New York, 1984), pp. 365-388. ■