Practical Examination of a Nonporous Silica Stationary Phase for Reversed-Phase Fast LC Applications

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Abstract

The performance of a nonporous silica reversed-phase high-performance liquid chromatographic stationary phase (1.5 µm) is examined and contrasted with that of a conventional stationary phase. The conventional column exhibits classic van Deemter behavior with respect to the impact of flow rate on efficiency, but the "fast" liquid chromatographic (LC) column's efficiency is not as strongly affected by flow rate because of its lessened contributions of longitudinal diffusion and stagnant mobile phase mass transfer effects and greater sensitivity to system void volume effects. Both columns exhibit roughly equivalent efficiencies on a per meter basis; however, the efficiency of the fast LC column was more strongly influenced by solute capacity factor. Retention by the fast LC column is more strongly influenced by mobile phase composition. In general, the fast LC column produces multispecies separations more rapidly with a weaker mobile phase than does the conventional column, which results in considerable reduction in solvent usage without a significant loss of efficiency. The performance of the fast LC column is strongly influenced by the system configuration, especially with respect to total system void volume.

Introduction

Reversed-phase high-performance liquid chromatography (HPLC) is typically performed with stainless steel columns that are 15–25 cm in length and contain stationary phases of structurally stable porous silica particles (5–10 µm in diameter) to which an appropriate functionality (e.g., C_{18}) has been chemically bonded (1). As manufacturing and column packing procedures have improved, shorter columns that contain smaller particles have become more widely used since they can produce separations with dramatically reduced analysis times and only slightly reduced performance properties. The practical benefits of this technology include increased laboratory productivity as the number of samples analyzed per unit time increases and decreased solvent-related costs.

The use of nonporous, small particle size stationary phases to produce high-speed, high-efficiency separations is well documented (e.g., 2–5). Recently, a "fast" liquid chromatographic (LC) column based on 1.5-µm nonporous silica microspheres has become commercially available. In view of the previous research results, the size and nonporous nature of these microspheres are anticipated to greatly impact stationary phase performance. In this study, the practical use of this fast LC column as a direct replacement for more conventional column configurations is assessed under isocratic elution conditions. Topics considered include flow rate and mobile phase composition effects, system configuration, and the ability of both column types to elute sets of model analytes.

Experimental

Columns

The "fast" LC (33 × 4.6 mm) contained 1.5-µm diameter nonporous silica (NPS) chemically bonded (C_{18}) microspheres. It was obtained from Micra Scientific (Northbrook, IL). The more "conventional" column (150 × 4.6 mm) was packed with 5-µm Alltech Adsorbosphere C_{18} stationary phase (Deerfield, IL). Both columns were used as received from the vendor.

General chromatographic conditions

Both columns were mounted on equivalent modular HPLC systems that consisted of an ABI Spectroflow 400 pump (Ramsey, NJ), an Alcott Model 235 electronically actuated injection valve (Norcross, GA), an Alcott Model 728 autosampler, an ABI Model 757 ultraviolet detector, an appropriate strip chart recorder, and a computer data acquisition system. The mobile phases consisted of acetonitrile–water mixtures that contained 0.015M ammonium acetate buffer. The injection size was 10 µL, the detector cell volume was 12 µL, and the detection wavelength was 215 nm. The experiments with the fast LC column had a detector time constant of 0.2 s and an integrator cycling rate of 2 Hz, and the experiments with the conventional column had a detector time constant of 0.5 s and an integrator cycling rate of 1 Hz. In general, the analyte concentration was 10 mg/L or less (10 ng injected).

In response to potential void volume issues associated with the fast LC system as defined above, an optimized system was configured. A Valco Model EQ-90 internal loop (60 nL) (Houston, TX) and a 2.5-µL detector flow cell were used. Tubing-related voids were minimized by directly connecting the column to the injector and bypassing the detector's internal heat exchanger by connecting the flow cell and the column with a small length of 0.007-in i.d. tubing. This optimized system was used to generate the van Deemter plot for the fast LC column.

Model analytes

Model analytes included a homologous series of alkyl phthalates (dimethyl, diethyl, dipropyl, dibutyl, and dioctyl phthalate) and pairs of compounds whose similar retention characteristics make them effective resolution monitors (acetophenone [AP] and 2-phenyl-2-propanol [PP]; aniline [AN] and acetanilide [AD]). The strong ultraviolet response of these analytes allowed column performance to be evaluated with small injection amounts, which minimized sample size related peak shape effects. The phthalates were chosen since they should exhibit a wide elution range due to their nearly 6 orders of magnitude range in octanol–water partition coefficients (6).

Generation of van Deemter plots

The effect of mobile phase flow rate on column efficiency was determined with dimethyl phthalate (DMP), diethyl phthalate (DEP), and dipropyl phthalate (DPP). Both columns were examined over a flow rate range of 0.15–1.5 mL/min, which is the lowest rate practically achievable with the HPLC systems used and the highest rate consistent with practical operating pressure constraints (3500 psi). Mobile phases that contained 20% or 45% acetonitrile were used to evaluate the fast LC and conventional columns, respectively, to produce similar capacity factors. The conventional column was also examined by using DMP as the marker and a 25% acetonitrile mobile phase. Triplicate injections of the test mixtures were made at each flow rate. Mean peak parameters were used to calculate column efficiency with the peak width at half height convention.

Mobile phase scouting

Elution characteristics of the phthalate markers were examined versus the acetonitrile content of the mobile phases at a flow rate of 1 mL/min. Each analyte was injected into equilibrated columns in triplicate for each mobile phase examined.

Impact of system configuration on the fast LC column

The efficiency of the fast LC column was examined with the use of three system configurations:

- 12-µL detector flow cell and "typical" column connecting tubing (10 cm of 0.010-in i.d. tubing between the injector and column and 10 cm of 0.020-in i.d. tubing between the column and detector)
- 12-µL flow cell and minimal connecting tubing (5 cm of 0.010-in tubing between the injector and column, direct connection between the column and detector)
- the optimized system described previously

DMP, DEP, and DPP were used as the analytes. The mobile phase contained 20% acetonitrile; the flow rate was 0.55 mL/min (which was necessary due to pressure limitations of the 2.5-µL flow cell). The test mixture was injected in triplicate into each system configuration.

Model separations

Roughly similar separations of the following mixtures were obtained with both columns: DMP, DEP, and DPP; DBP and DOP; PP and AP; and AN and AD. A mobile phase flow rate of 1 mL/min was used for both columns. The second system configuration was used for separations with the fast LC column. The separations for both columns are used to estimate solvent required to achieve a particular analysis.

Results and Discussion

An analyst who is considering the implementation of a new column technology is faced with several practical performance-related issues:

- What are the selectivity properties of the new stationary phase?
- How will the column respond to changing mobile phase composition?
- How will column performance be affected by mobile phase flow rate?
- How will the performance of this column compare with respect to columns in more routine use (especially with respect to the first three points)?
- How rugged or stable is the column in the face of the repetitive analysis of real world samples?

These issues were addressed in this study.

van Deemter plots

The effect of mobile phase flow rate on the efficiency of HPLC separations was attributed by van Deemter to three competing dispersion effects (7): (*a*) flow inequities in the column packing; (*b*) axial (longitudinal) diffusion in the mobile phase; and (*c*) resistance to mass transfer.

The flow dependence of each of these terms gives rise to the classic shape of an efficiency versus mobile phase velocity (van Deemter) curve. As anticipated and shown in Figure 1, the conventional column exhibited classic van Deemter behavior at several mobile phase compositions. An optimum column efficiency was observed for all three analytes. These van Deemter plots show a strong efficiency dependence on mobile phase velocity at both low and high values, which is consistent with the strong influence of effects of *b* and *c* on column performance. The magnitude of reduced plate height, the shape of the curves, and the relatively small effect of solute capacity factor on plate height all suggest that this chromatographic system is well controlled with respect to void volume contributions.

The van Deemter plot for the fast LC column, which was obtained with a system whose extra-column void volume was minimized, is shown in Figure 2. Only in the case of the most strongly retained species (DPP) was there evidence of any axial diffusion effects. The absence of a marked increase in plate

height at higher values of reduced mobile phase velocity also suggests that stagnant mobile phase mass transfer effects are minimized by the nonporous packing. However, the lack of the classic van Deemter shape of the plots for DMP and DEP, coupled with their relatively low height values, suggests that even the "optimized" system possesses a significant void volume, some of which may be related to column packing efficiency.

Thus, the fast LC column can be operated at high and low flow rates with no significant loss of efficiency for well-retained compounds. High flow rates would decrease the analysis time, and low flow rates might be necessitated when the system's pressure considerations are important.

A meaningful comparison of the efficiencies of two columns requires that the data be generated under conditions that are comparable and clearly defined. The maximum observed efficiency for both columns was obtained with the use of DPP as a marker. For the conventional column, the maximum efficiency of 76,000 plates per meter was obtained with a 45% acetonitrile mobile phase at a flow rate of of 0.75 mL/min (capacity factor = 17.6). For the fast LC column, the highest efficiency of 192,500 plates per meter was obtained with a mobile phase of 20% acetonitrile at a flow rate of 1.5 mL/min (capacity factor = 19.0, optimized system configuration). This result compares with the manufacturer's reported value of 274,000 plates per meter for a 0.5-mL injection of benzo[*a*]pyrene (capacity factor, approximately 30). In this separation, the mobile phase was water–acetonitrile–THF (60:32:8, v/v) at a flow rate of 0.85 mL/min.

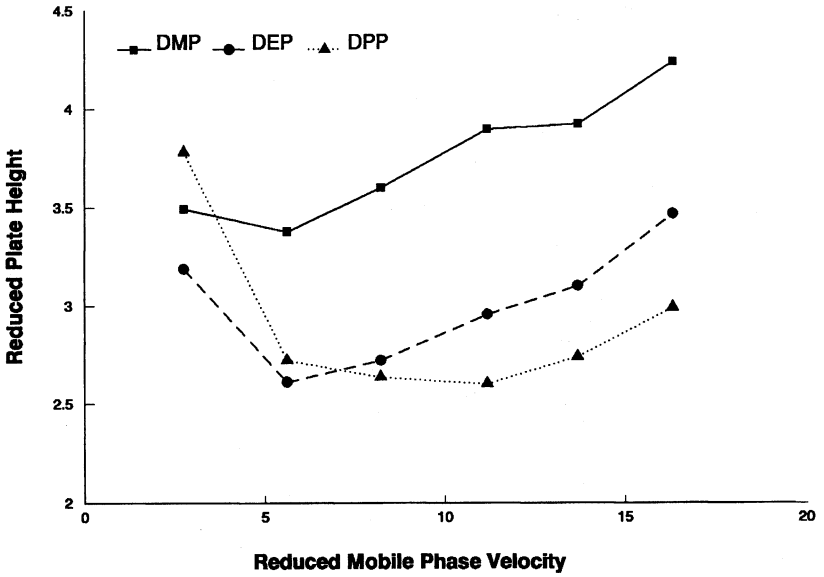

Figure 1. Reduced plate height versus reduced mobile phase velocity (van Deemter plot) for the conventional column. The mobile phase was 25% acetonitrile. The capacity and tailing factors (at 10% peak height) are as follows: DMP, 2.5 and 1.20; DEP, 5.6 and 1.08; DPP, 15.4 and 1.05.

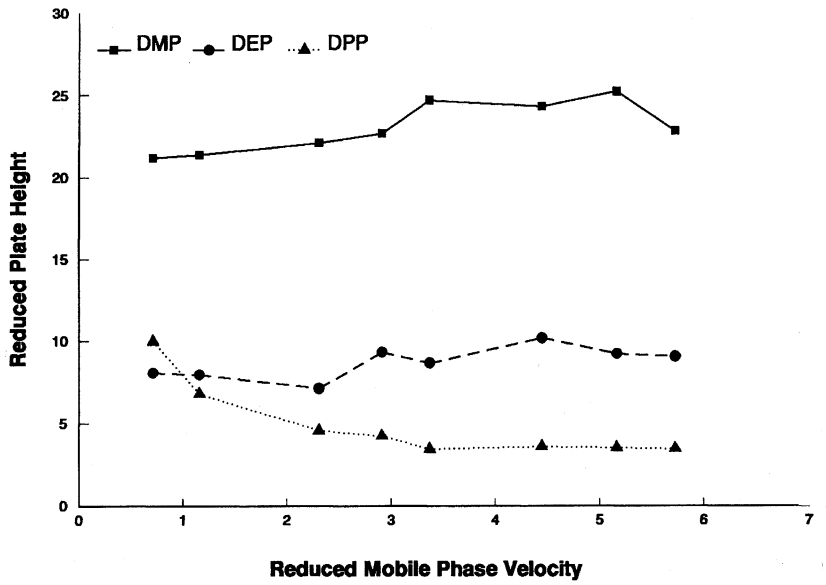

Figure 2. Reduced plate height versus reduced mobile phase velocity (van Deemter plot) for the fast LC column. The mobile phase was 20% acetonitrile. The capacity and tailing factors (at 10% peak height) are as follows: DMP, 0.9 and 1.26; DEP, 3.5 and 1.21; DPP, 19.0 and 1.08.

For DEP, the conventional column's maximum efficiency was also 76,000 plates per meter, which was obtained with a 45% acetonitrile mobile phase at a flow rate of 0.5 mL/min (capacity factor = 5.8). The maximum efficiency for DEP on the fast LC column (93,400 plates per meter) was obtained with the 20% acetonitrile mobile phase at a flow rate of 1.0 mL/min (capacity factor = 3.7). Because of the differences in capacity factor values, the DEP comparison is biased in favor of the conventional column. An appropriate comparison of the efficiencies for DMP could not be obtained because of the large difference in capacity factor values for DMP on the two columns.

Thus, under elution conditions that produce similar analyte retention, the two columns are roughly equivalent in performance.

Effect of mobile phase composition on retention

The impact of mobile phase composition on the elution of the phthalates for both column types is shown in Figures 3 and 4. In general, the capacity factor is linearly related to the organic modifier fraction of the mobile phase for both systems. The behavior of all the markers is roughly colinear, which is consistent with the fact that they are structurally similar members of the same homologous series. The two systems differ, however, with respect to their relative sensitivity to a change in the mobile phase composition. The mean slope of the elution profiles for the fast LC and conventional columns are –0.066 and –0.035, respectively. Thus, the capacity

factor in the fast LC column changes roughly twice as fast as that of the conventional column in response to a changing mobile phase composition. In addition, equal capacity factors are achieved for the fast LC column with less organic modifier. Although this behavior results in reduced solvent consumption in fast LC separations, the smaller amount of organic modifier, coupled with the increased sensitivity to changes in the mobile phase composition, means that fast LC separations are more strongly influenced by relatively small changes in the mobile phase proportions. Thus, in a practical sense, the fast LC separations are potentially less rugged.

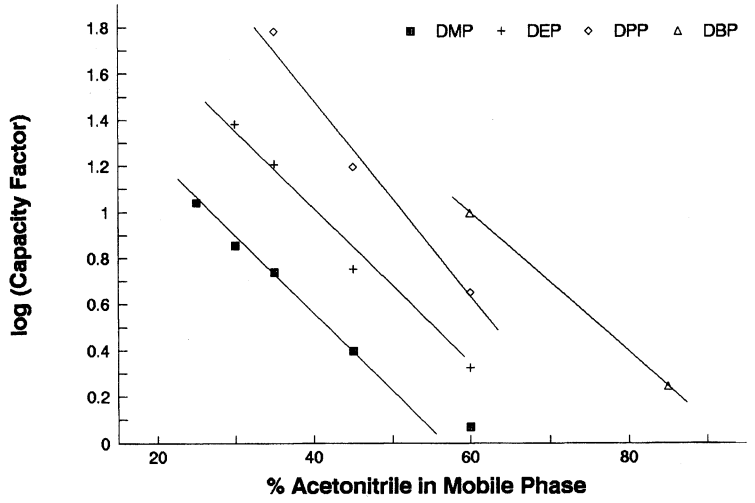

Figure 3. Effect of mobile phase composition on the elution of the phthalate markers for the conventional column. The log of the capacity factor is plotted versus the percentage of acetonitrile in the mobile phase. A flow rate of 1 mL/min was used.

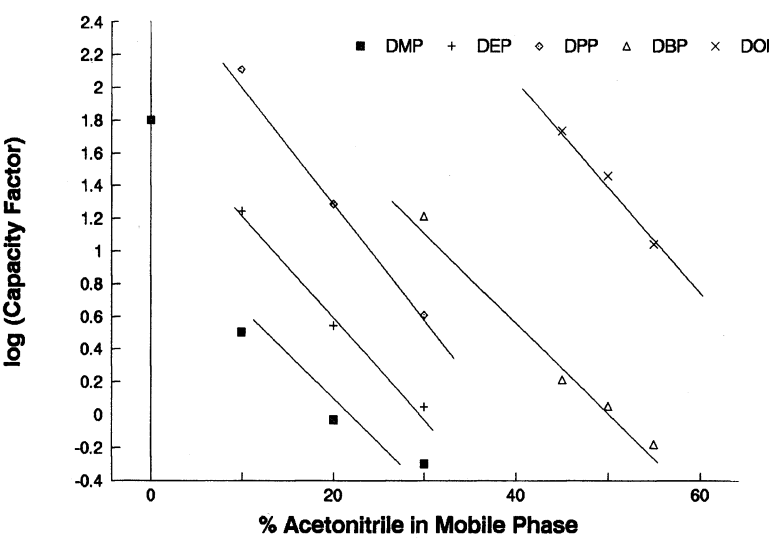

Figure 4. Effect of mobile phase composition on the elution of the phthalate markers for the fast LC column. The log of the capacity factor is plotted versus the percentage of acetonitrile in the mobile phase. A flow rate of 1 mL/min was used.

Examination of Figures 3 and 4 indicates that there exists no single mobile phase that can be used to produce workable separations with both columns. For example, consider the separation of DMP, DEP, and DPP. For the fast LC column, use of a mobile phase that contains more than 20% acetonitrile would be inappropriate since the capacity factor for DMP would be less than 1. For the conventional column, use of a mobile phase that contains less than roughly 45% acetonitrile would be inappropriate due to the large capacity factor for DPP (approximately 20) and corresponding excessive analysis time.

Impact of system configuration on the fast LC column

Since the bed volume of fast LC columns is small, the relative impact of extra-column voids in the chromatographic system on column performance increases. The need to minimize extra-column contributors to band broadening in applications that involve fast LC is well documented (8–12). In this study, cursory attention was paid to three typical contributors to extra-column void volume: injector configuration, connecting tubing size, and detector cell volume.

The results of this evaluation are shown in Table I. Minimizing the total volume of connecting tubing yields a significant increase in system efficiency. However, although the optimized system yielded enhanced operating efficiency, it is not practically useful given its difficulty in construction and its poor analyte sensitivity (due to the small sample injection volume).

Table I. Effect of System Configuration on the Efficiency of the Fast LC Column

Configuration	Mean value (n = 3)*		
	DMP	DEP	DPP
1	526 (15)	1444 (44)	2655 (3)
2	718 (25)	1973 (27)	3011 (124)
3	1200 (10)	2900 (53)	4165 (75)

*Standard deviation given in parentheses.

Model separations

Separations of the phthalate mixture obtained with both columns are shown in Figure 5. It is representative of the behavior exhibited by all analytes examined. While workable separations can be achieved with both columns in all cases, the use of the fast LC column results in much shorter analysis times and reduced solvent consumption (Table II). For the pairs AP/PP and AN/AD, the elution order of the markers is different in both columns, which indicates a selectivity difference.

Conclusion

The fast LC column examined herein is capable of producing high efficiency separations of the model compounds. With respect to the issues raised previously, the following observations are pertinent.

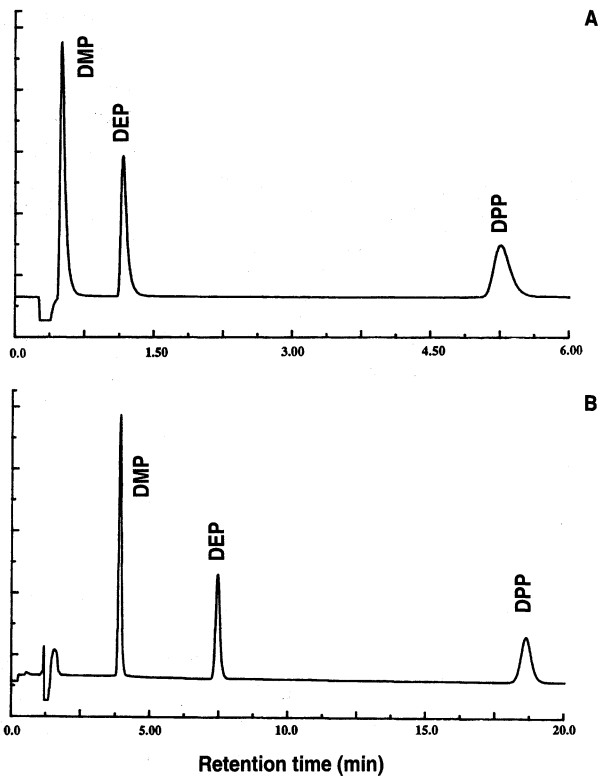

Figure 5. Typical chromatograms for the separation of DMP, DEP, and DPP. In chromatogram A, the fast LC column was used with the second column configuration. Twenty percent acetonitrile was used in the mobile phase. In chromatogram B, the conventional column was used with 45% acetonitrile in the mobile phase.

Table II. Elution Information for the Various Analyte Mixtures

Analytes	Column	Mobile phase (% organic modifier)	Total analysis time (min)*	Solvent use (mL)†
DMP/DEP/DPP	Fast LC	20	6.0	1.2
	Conventional	45	20.0	9.0
DBP/DOP	Fast LC	50	8.7	4.4
	Conventional	85	23.0	19.6
AP/PP	Fast LC	5	1.7	0.1
	Conventional	30	9.0	2.7
AD/AN	Fast LC	0	2.7	0.0
	Conventional	30	4.5	1.4

* Total analysis time is the injection to injection time that allows for baseline stabilization.
† Solvent use is the amount of organic modifier (acetonitrile) required to produce a single analysis.

The selectivity of the fast LC stationary phase was different from that of the more conventional packing in two respects. For several analyte pairs of closely eluting compounds, the elution order was reversed. For the analogous series of phthalates, the fast LC stationary phase greatly expanded the capacity factor window that encompassed the analytes examined. In other words, analytes whose capacity factors differed by a factor of 6 on the conventional column differed by more than a factor of 20 on the fast LC column.

The capacity factors of analytes on the fast LC column were roughly twice as sensitive to changes in mobile phase compositions as they were on conventional column. This behavior suggests that separations on the fast LC column may be less rugged than those on the conventional column. Additionally, the ability to fine tune multicomponent separations on the fast LC column by making small changes in mobile phase composition may be constrained.

The efficiency of the fast LC column is less sensitive to changes in mobile phase flow rate, especially for early eluting species whose performance is most strongly influenced by residual system void volumes.

The performance of the fast LC column is more strongly influenced by system void volume. The presence of significant system voids is readily confirmed by poor efficiencies for early eluting species and by large differences in analyte efficiency as a function of analyte capacity factor.

The ruggedness of the fast LC column is of potential concern with respect to two issues: the stability of the bonded phase (especially at low pH) and plugging of the small pore column frits by sample and system particulates. Neither of these issues was quantitatively addressed in this study. However, it is qualitatively observed that such columns used in the analysis of pharmaceutical products and plastic extracts have retained their original level of performance over extended periods of use and storage (several months and several hundred injections).

In general, the fast LC column produces workable separations in a shorter time period and requires mobile phases that contain proportionally less organic modifier. Thus, the fast LC column offers the benefits of increased laboratory efficiency because per-sample analysis time and solvent use decreases. Additionally, the faster analysis times may help maintain sample stability during lengthy automated analytical runs. However, effective use of the NPS column technology requires careful control of system dead volume and the use of small injection volumes. Use of these types of columns under less than optimal void and injection volume conditions results in reduced column efficiencies and nonsymmetric peak shape (e.g., tailing).

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