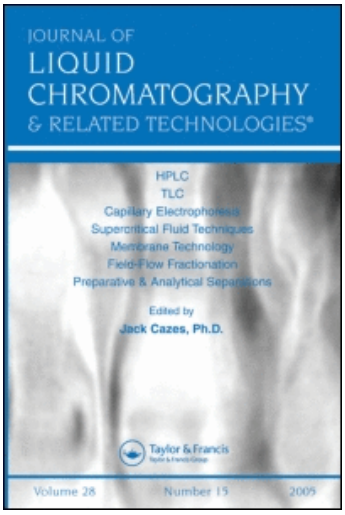


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# Practical Applications of Monolithic Columns to Pharmaceutical Process Development

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# Practical Applications of Monolithic Columns to Pharmaceutical Process Development

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**Abstract:** High performance liquid chromatography (HPLC) is among the most widely used analytical techniques in the process development of drug substances. With the goal of reducing analysis time, there has been considerable focus on high-speed HPLC separations. Recently, commercially available monolithic columns have proven to be one of the most promising developments in the area of fast chromatographic separations. In this work, pressure drop, column efficiency, and retention behavior of monolithic columns (Chromolith type) were evaluated and compared with those of conventional columns packed with porous stationary-phase particles. It was demonstrated that high-speed HPLC separations could be achieved with monolithic columns at acceptable pressure drops without significantly compromising column efficiency. The applications of monolithic columns in pharmaceutical process development, such as reaction monitoring, column fraction screening, and analysis of mother liquors and unstable analytes, are described with respect to the quality of separations, as well as, enhanced throughput and speed of analysis. It is concluded that the use of monolithic columns offers a significant advancement over currently available techniques in the high-speed and high-throughput analysis of pharmaceutical compounds. Some practical aspects in utilizing monolithic columns for fast separations are also discussed.

**Keywords:** Reversed-phase liquid chromatography, monolithic columns, fast separations, process development, pharmaceuticals

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## INTRODUCTION

High performance liquid chromatography (HPLC) has been widely used in the pharmaceutical industry for the analysis of raw materials and intermediates, monitoring of reaction mixtures, and characterization of active pharmaceutical ingredients. However, in recent years, the demand to improve the productivity of analyses, as well as to provide enabling information to make better real-time processing decisions has significantly raised the level of interest in high-speed liquid chromatography. The most straightforward approach to high-speed separations is the use of short columns packed with 3–5 μm particles in combination with high flow rates.[1] However, both column efficiency and resolution are usually compromised by the length of the column. This loss in efficiency and resolution is more pronounced at high flow rates beyond the optimum velocity. Such an approach to rapid analyses is of little practical value for complicated pharmaceutical process streams.

Using packing materials of smaller diameters in combination with a reduced column length is an ideal way to achieve high-speed HPLC separations without significantly sacrificing column efficiency.[2–5] Small particles yield enhanced column efficiency by virtue of relatively small intra-particulate mass transfer resistance as a result of short diffusion distance. However, high column back-pressures of a short column packed with small particles can still be a limitation, since pressure drop increases more rapidly with decreasing particle size than with decreasing column length or column efficiency. Although ultra-high pressure liquid chromatography (UHPLC) has been developed to overcome the conventional pressure limits, no such systems ($P > 15{,}000$ psi) are commercially available yet.[6–10]

Another approach to achieving fast HPLC separations is the use of high temperatures.[11–15] The mobile-phase viscosity is considerably reduced at elevated temperatures resulting in lower back pressures, which allows the use of higher flow rates. In addition, fast separation with high efficiency can be achieved at high temperatures due to the enhanced diffusivity and reduced mass transfer resistance of the mobile phase. However, not many efficient stationary phases are currently available that can withstand high temperatures. Another concern is the possible degradation of labile analytes at elevated temperatures, which may ultimately limit the wide use of high-temperature HPLC, unless the analysis time cycle can be reduced to the point at which it favorably competes with thermal degradation kinetics.

The recently introduced monolithic columns offer an alternative for high-speed HPLC separations.[16–21] Commercially available monolithic columns are silica based and prepared via a sol–gel process, which create unique bimodal structure properties.[22,23] These silica rod columns have 1.5–2 μm through-pores and narrow skeleton sizes, allowing high flow rates and

providing low mass transfer resistance. The total porosity of monolithic columns is approximately 15% higher than that of conventional packed columns. These properties provide the possibility of achieving high column efficiency with very high flow rates. The applications of monolithic columns have been focused mainly on high-throughput separations for biological samples in drug discovery.[24–26] Few applications have been reported in the area of drug substance process development.

Pharmaceutical process development often generates numerous sample types, including reaction mixtures for process monitoring, batch and waste layers from extractions or isolations for concentration measurement, or column fractions from large-scale preparative chromatography and isolated solids for impurity profile analysis. In today's competitive environment, high-throughput analytical techniques are essential to both the optimization of development time cycle as well as real-time decision-making. Various analytical techniques have been used to support process development, optimization of the synthetic route, and quality control. The majority of these analyses have been performed by HPLC because of the detailed information obtained.[27] Therefore, it is logical that high-speed HPLC separations can have a significant effect on productivity in drug development.

In this work, pressure drop, retention, selectivity, column efficiency, and resolution of monolithic columns have been investigated. An overview of some practical applications of monolithic columns to pharmaceutical analyses is presented, including extremely rapid monitoring of reaction mixtures, high sample volume analyses of preparative HPLC fractions, fast separations of very complex crystallization mother liquor streams, and fast analysis of labile analytes.



## EXPERIMENTAL

### Chemicals and Materials

HPLC grade acetonitrile and methanol used in this study were purchased from Fisher (Springfield, NJ, USA). HPLC grade water was generated by a Milli-Q water system (Millipore, Bedford, MA, USA). Uracil, phosphoric acid (85 wt%), toluene, ethylbenzene, propylbenzene, butylbenzene, amylbenzene, aniline, *N*-methylaniline, and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzonitrile were obtained from sigma–Aldrich (St. Louis, MO, USA). Potassium dihydrogen phosphate was purchased from Fisher (Fairlawn, NJ, USA). Process samples containing various drug substances and intermediates were prepared and provided by the Process Research Department, Merck Research Laboratories (Rahway, NJ, USA) and will be addressed with each application described later.

Chromolith SpeedROD RP-18e columns ($50 \times 4.6\,mm^2$ I.D.) and Performance RP-18e columns ($100 \times 4.6\,mm^2$ I.D.) were purchased from E. Merck (Darmstadt, Germany). The YMC-Pack Pro $C_{18}$ columns ($50 \times 4.6\,mm^2$ I.D., $3\,\mu m$) and the Waters Symmetry column ($50 \times 4.6\,mm^2$ I.D., $3.5\,\mu m$) were purchased from Waters (Milford, MA, USA). The Platinum EPS $C_{18}$ column ($50 \times 4.6\,mm^2$ I.D., $1.5\,\mu m$) was specially ordered from Alltech Associates, Inc. (Deerfield, IL, USA). The Ace5 C18 column ($250 \times 4.6\,mm^2$ I.D., $5\,\mu m$ particles) was purchased from MAC-MOD Analytical (Chadds Ford, PA, USA).

### Chromatography Conditions

HPLC experiments were carried out using an HP 1100 LC system (Wilmington, DE, USA) equipped with a vacuum degasser, a quaternary pump, an autosampler, a thermostated column compartment, and a variable wavelength detector. Chromatograms were acquired and processed using a PE Nelson data system equipped with a Turbochrom software package (version 6.1.2.0.1: D19) (PE Nelson, San Jose, CA, USA). The analytes were dissolved in acetonitrile at a concentration of $0.5\,mg\,mL^{-1}$ and $5\,\mu L$ was injected into the HPLC system unless otherwise noted.



## RESULTS AND DISCUSSION

### Pressure Drop

Pressure drop is one of the major limitations of high-speed separations when a conventional HPLC system and a column packed with small particles are utilized. Low pressure drops are desirable when high flow rates are used to achieve fast separations. Pressure drops for a Chromolith SpeedROD monolithic column ($50 \times 4.6\,mm^2$) were compared with those for $1.5\,\mu m$ and $3.5\,\mu m$ particle-packed columns. Figure 1 demonstrates that the pressure drop for the Chromolith monolithic column is approximately three times lower than that for the $3.5\,\mu m$ particle-packed column and nine times lower than that for the $1.5\,\mu m$ packed columns. In other words, if 300 bar is a practical operation limit for most HPLC systems, the flow rate for the $50 \times 4.6\,mm^2$ Chromolith column can be as high as $9\,mL\,min^{-1}$, while the maximum flow rates for the Waters Symmetry $3.5\,\mu m$ packed column and the Alltech $1.5\,\mu m$ packed column are only 2.8 and $1.3\,mL\,min^{-1}$, respectively. The low resistance to flow in monolithic columns is primarily due to its favorable configuration of large through-pores and high total porosity, which is approximately 15% higher than conventional packed columns.[19]

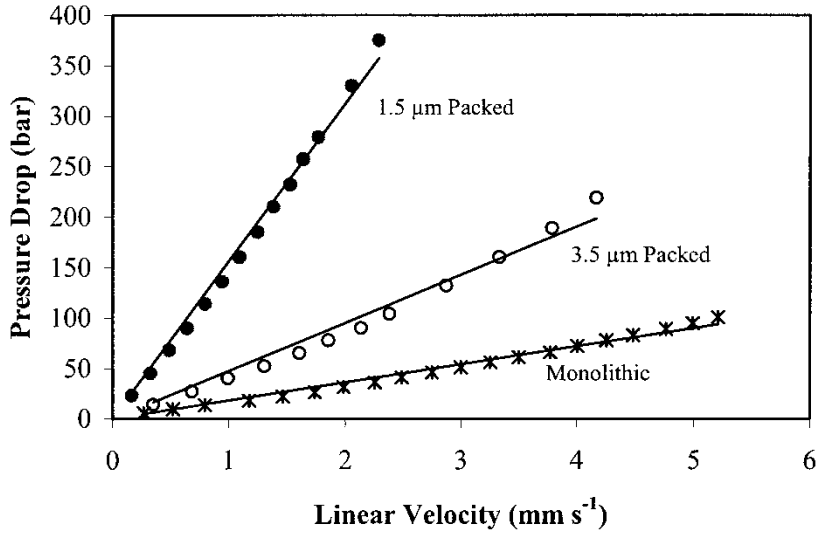


***Figure 1.*** Relationship between column pressure drop and linear velocity for monolithic and conventional packed columns in reversed-phase chromatography. Conditions—mobile phase: 70 : 30 acetonitrile–water (v/v); unretained marker: Uracil; UV detection: 254 nm; temperature: 25°C; injection volume: 2 μL; column dimension: $50 \times 4.6\,mm^2$; column type: E. Merck Chromolith SpeedROD $C_{18}$ monolithic column,1.5 μm packed Platinum EPS $C_{18}$ column, and 3.5 μm packed Waters Symmetry $C_{18}$ column.



Thus, HPLC analysis using monolithic columns can be carried out at much higher flow rates compared with packed columns.

## Column Efficiency

van Deemter plots for monolithic (Chromolith SpeedROD, $50 \times 4.6\,mm^2$) and packed (YMC-Pro $C_{18}$, $50 \times 4.6\,mm^2$, 3 μm) columns were compared in Fig. 2. It is seen that the monolithic column has a similar plate height to that of 3.0 μm packed column at the optimum linear velocity. However, at higher flow rates, the plate height of the monolithic column increases less significantly with increasing linear velocity. This suggests that the separation on the monolithic column can be performed at high flow rates without significantly sacrificing column efficiency. This chromatographic behavior is due to the unique structural feature of monolithic columns. The small-sized interconnected skeletons inside the silica rod reduce the diffusion path length or the mass transfer resistance from the stagnant mobile phase in the pores, while the large through-pores increase the column permeability.[19]

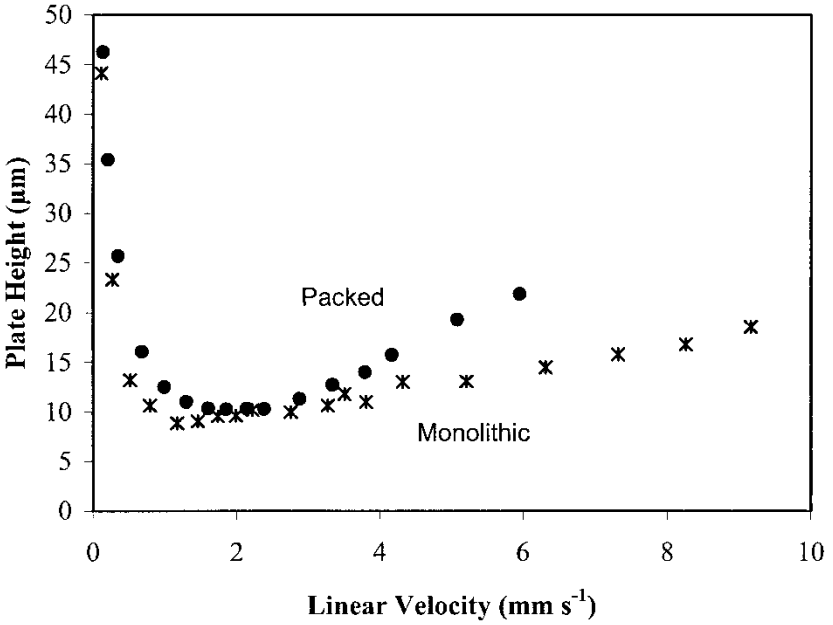


***Figure 2.*** van Deemter plots for packed and monolithic columns. Conditions—Test solute: amylbenzene; unretained marker: Uracil; UV detection: 254 nm; temperature: 25°C; injection volume: 2 μL; column dimension: 50 × 4.6 $mm^2$. column type: Packed YMC-Pro $C_{18}$ column using 70 : 30 acetonitrile–water (v/v) and E. Merck Chromolith SpeedROD $C_{18}$ monolithic column using 38 : 62 acetonitrile–water (v/v).



## Retention and Selectivity

The retention factors of amylbenzene were determined using acetonitrile/0.1% phosphoric acid mobile-phase system on both $C_{18}$ Chromolith and YMC-Pro $C_{18}$ packed columns. Figure 3 illustrates the retention factors obtained with the two columns at various acetonitrile compositions. It is seen that the average value of retention factors for the Chromolith column is approximately half that for the packed column. This suggests that the $C_{18}$ Chromolith column is less hydrophobic than the $C_{18}$ packed column. The lower retention on the Chromolith column may be explained by the column's high porosity. A Chromolith column has higher porosity and thus lower silica density, even though both the Chromolith and packed YMC-Pro $C_{18}$ columns have a similar silica surface area per gram ($300\,m^2\,g^{-1}$).[20] The lower density means a lower surface area per unit volume or a lower-phase ratio for the monolithic column, which can contribute to its lower retention factor. The difference in the retention factors must be considered when a method is transferred from a $C_{18}$ packed column to a $C_{18}$

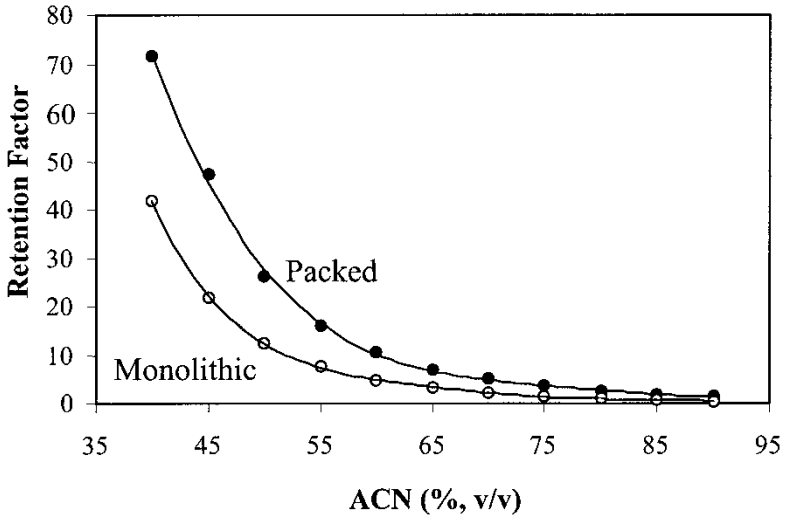


*Figure 3.* Retention factors of amylbenzene for packed and monolithic columns. Conditions—5 mL $min^{-1}$ at various acetonitrile concentrations. Other conditions are the same as in Fig. 2.



monolithic column. For example, approximately 15–25% lower percentages of acetonitrile in water should be used for the monolithic column (Chromolith type) to match the retention factor for that of the packed column.

In chromatography, selectivity is defined as the ratio of the retention factors of two different components.[19,29] A mixture containing benzene, toluene, aniline, and *N*-methyl aniline was used to compare the selectivity of the monolithic column (Chromolith SpeedROD, 50 × 4.6 $mm^2$) with that of the packed column (YMC-Pro $C_{18}$, 50 × 4.6 $mm^2$, 3 μm). The selectivity factors ($\alpha$) for three different pairs (toluene/benzene, *N*-methyl aniline/aniline, and *N*-methyl aniline/toluene) were plotted at different percentages of acetonitrile, as illustrated in Fig. 4. The $\alpha$ values of the three pairs of components are comparable on both $C_{18}$ Chromolith and YMC-Pro $C_{18}$ columns indicating that the separation mechanism for both columns is likely similar.

## Practical Considerations

As high flow rates and low retention factors are typically observed in fast separations with monolithic columns, the potential for extra column band broadening effects must be investigated.[30,31] Thompson and Carr have shown that the detector time constant can contribute significantly to the extra column

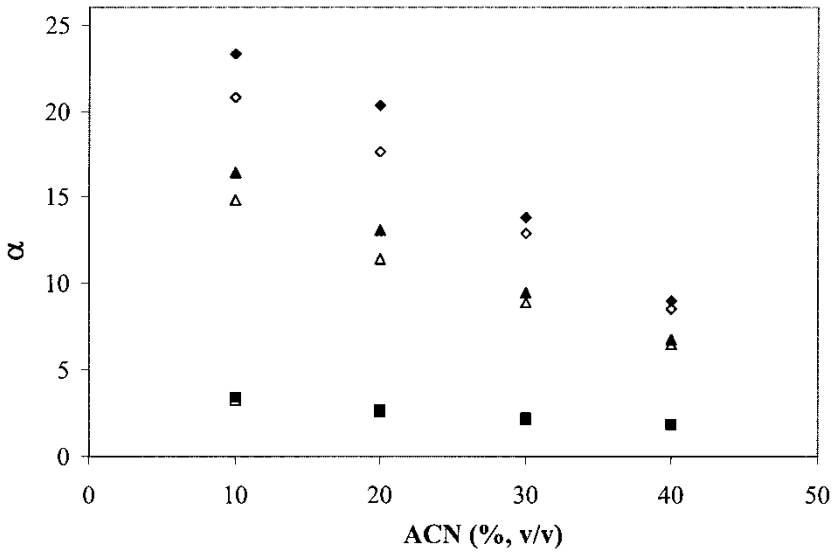


***Figure 4.*** Comparison of alkylbenzene selectivities in monolithic and packed columns. Conditions—mobile phase: 10 mM $K_2HPO_4$ in water/acetonitrile; temperature: 25°C; UV detection: 220 nm; column type: Chromolith SpeedROD RP-18e column ($50 \times 4.6\,mm^2$ I.D.) and YMC-Pack Pro $C_{18}$ column ($50 \times 4.6\,mm^2$ I.D., 3 μm); flow rates: 5 mL $min^{-1}$ for the monolithic column and 1 mL $min^{-1}$ for the packed column. (◆) selectivity of toluene vs. aniline on packed column; (◇) selectivity of toluene vs. aniline on monolithic column; (▲) selectivity of *N*-methylaniline vs. aniline on packed column; (Δ) selectivity of *N*-methylaniline vs. aniline on monolithic column; (■) selectivity of toluene vs. benzene on packed column; (□) selectivity of toluene vs. benzene on monolithic column.

band broadening, and low time constants are needed for high-speed separation.[31] In addition, the peaks produced in high-speed separations may be extremely narrow (less than 1 sec), thus the data acquisition speed should be high enough to maintain the resolution of these peaks. Separations at different detector time constants and at different data acquisition frequencies were illustrated in Fig. 5. At a data acquisition frequency of 20 Hz, a detector time constant of 0.06 sec was needed to fully resolve toluene and ethylbenzene (Fig. 5A). Similarly, at a detector time constant of 0.06 sec, a 20 Hz data acquisition frequency is needed to properly reconstruct peak shapes (Fig. 5B). Finally, short and narrow-bore extra-column tubing (100 μm I.D. used here) is recommended for the HPLC system, as this contribution to peak variance is proportional to the fourth power of the tubing diameter. In this study, all subsequent chromatographic data were acquired with a 0.06 sec detector time constant and 20 Hz frequency, unless otherwise noted.

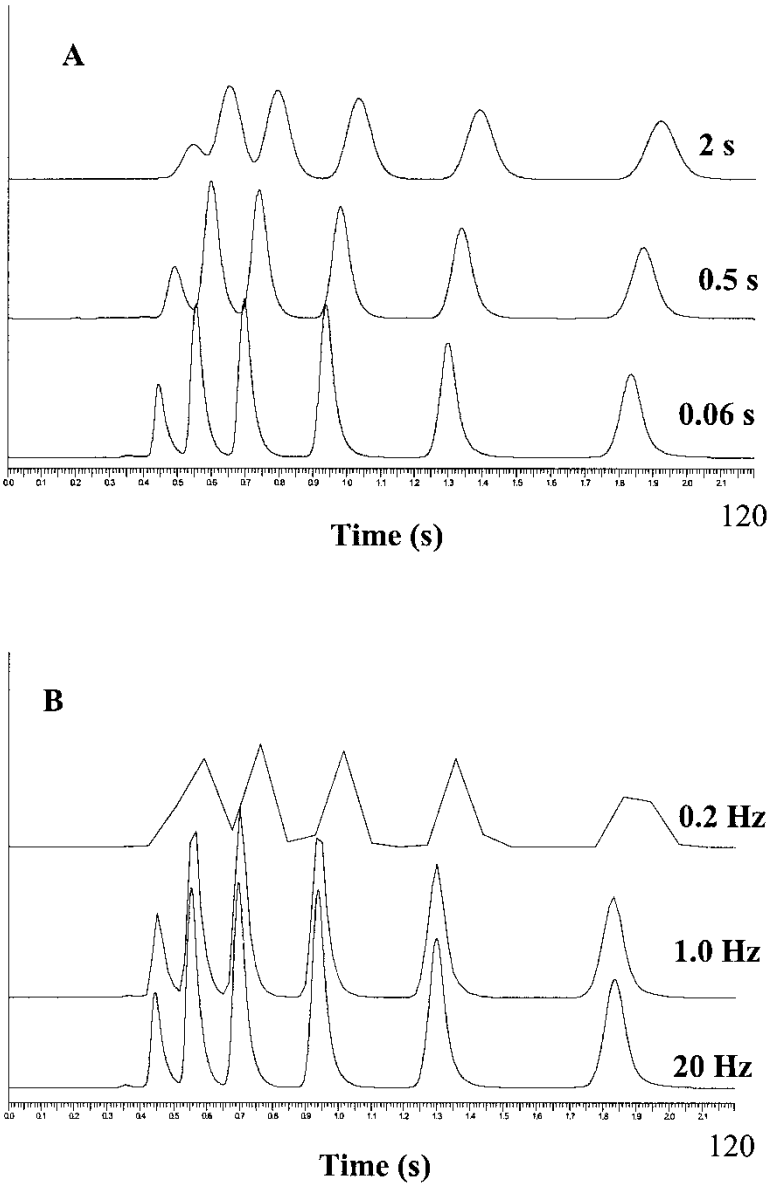


***Figure 5.*** Effects of time constant of the detector (A) and data acquisition frequency (B) on resolution. Conditions—columns: E. Merck Chromolith ($50 \times 4.6\,mm^2$); flow rate: $5\,mL\,min^{-1}$; injection volume: $2\,\mu L$; UV detection: 254 nm; temperature: 25°C; analyte concentration: $0.5\,mg\,mL^{-1}$ alkylbenzenes ($C_nH_{2n+1}C_6H_5$, from peaks 1 to 6, $n = 0–5$, respectively).

## Pharmaceutical Process Development Applications

In the development of synthetic processes for drug substances, HPLC has been routinely used for reaction monitoring, process optimization, and quality control among various other applications because of the favorable combination of high selectivity and sensitivity often attained. A large number of samples may be generated in the course of process development for a drug candidate. Particularly, real-time analysis results are critical for pilot plant samples in order to determine if further processing is needed. The analysis times for the typical HPLC methods using conventional packed columns ($250 \times 4.6\,mm^2$, 5 μm packing) range from 20 to 50 min in our research laboratory in order to achieve the needed chromatographic resolutions.

In reaction monitoring, reactant and major product peaks are usually of most interest, whereas minor impurities may be of less focus. Figure 6 shows a fast HPLC method using a monolithic column to monitor four sequential chemical reaction steps involving six major intermediates during the synthetic process for Aprepitant, a novel agent for the treatment of chemotherapy-induced emesis. By employing a flow rate of $5\,mL\,min^{-1}$ in

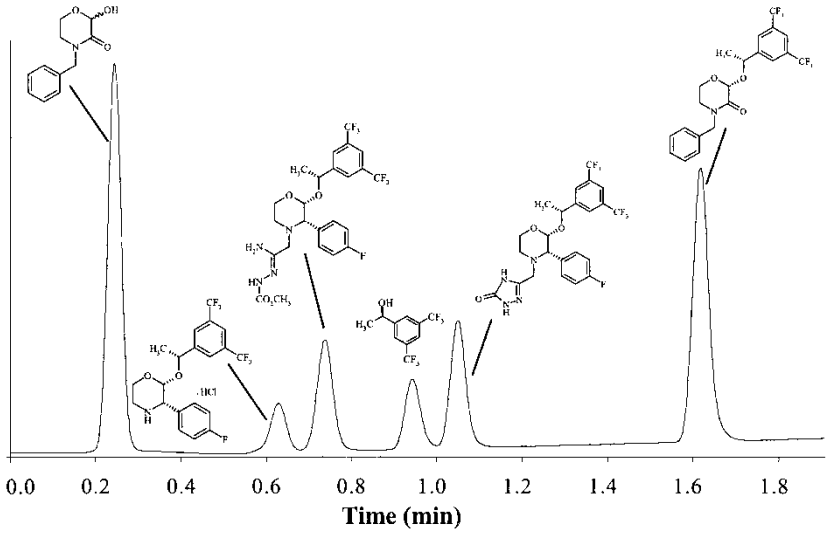


***Figure 6.*** Separation of Aprepitant in process intermediates. Conditions—flow rate: $5\,mL\,min^{-1}$; injection volume: 10 μL; UV detection: 220 nm; temperature: 35°C; mobile phase: 0.1% phosphoric acid in water and HPLC grade acetonitrile; gradient: linear ramp from 35 : 65 acetonitrile–water to 68 : 32 acetonitrile–water in 1.5 min, followed by linear gradient to 80 : 20 acetonitrile–water in 0.5 min. Peak identifications (from left to right): Lactam lactol, Sec Amine, ASA (Alkylated Sec amine), Chiral alcohol, Aprepitant drug substance, and Lactam acetal.

combination with a gradient, fast separations were achieved in a 2 min run time followed by a 1 min equilibration time. A previous HPLC method using a packed column (YMC ODS-AQ, $250 \times 4.6\,mm^2$, 5 μm) required 40 min, including a 10 min re-equilibration time (chromatogram not shown). The analysis time was reduced by approximately 14-fold for these process samples, which is not only important during the real-time monitoring of chemical processes, but also provides significant time savings during method development and validation studies.

Another ideal application in the pharmaceutical industry for monolithic columns is analysis of the numerous samples generated by large-scale preparative chromatography. With increasingly rapid development timelines for new compounds, the role of preparative chromatography to purify drug substances or their intermediates in lieu of more resource-intensive process, development research has increased, especially during early development when the goal is to obtain the safety/efficacy of a compound rapidly. In a typical preparative chromatography process for isolating a drug substance, as many as 20 fractions may be collected during elution of the parent compound. Each fraction needs to be assayed via analytical HPLC to assess purity prior to determining the rich-cuts and combining them for further processing. The total analysis time for these fractions would be 7.3 hr, if the previously described 50 min HPLC method using a conventional packed column is employed in the purification of an HIV protease inhibitor. However, using a fast HPLC method on a $50 \times 4.6\,mm^2$ $C_{18}$ monolithic column, analysis of each fraction may be achieved in 5 min and the total analysis time for all fractions is 1.3 hr, as illustrated in Fig. 7. Such time savings in a production environment is precious.

During development of an isolation process, complicated samples such as crystallization mother liquors frequently need to be screened for impurity identification and for tracking mass balance during a pilot plant process. Fast analysis of mother liquors is often extremely challenging due to the complexity of the sample matrix, which requires high efficiency as well as high speed. Figure 8 shows the separation of a typical mother liquor mixture for compound II using a Chromolith column. By coupling two 100 mm monolithic columns together, approximately 50 peaks, including the drug substance and process impurities, were resolved within 7 min, compared with 40 min when a conventional 5 μm packed column was used.

Another application of fast separations in the pharmaceutical industry is the analysis of analytes, which are unstable in protic environments, such as typical reversed-phase mobile phases. Several chromatographic approaches to address this analysis dilemma have been reported recently, including normal-phase HPLC in non-protic solvent systems and subambient reversed-phase HPLC on conventional bore HPLC columns.[32–35] However, the former approach often has limited applications for many

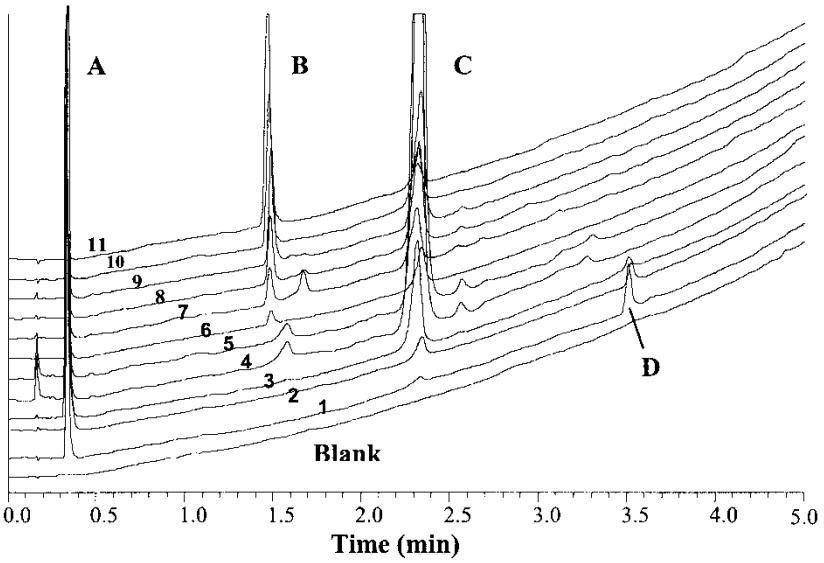


*Figure 7.* Chromatogram overlay of preparative chromatography fractions for an HIV protease inhibitor. Conditions—column: E. Merck Chromolith SpeedROD ($50 \times 4.6\,mm^2$); flow rate: $5\,mL\,min^{-1}$; injection volume: $2.5\,\mu L$; UV detection: 220 nm; temperature: 25°C; mobile phase: 10 mM $K_2HPO_4$ (pH 6.4), acetonitrile and methanol; gradient: 20 : 20 : 60 acetonitrile–methanol–10 mM $K_2HPO_4$ to 80 : 20 acetonitrile–methanol linearly in 5 min. Each sample contained two fractions. Peak identifications: (A) IPAC, (B) unknown impurity, (C) drug substance, and (D) unknown impurity 2.



compounds of pharmaceutical interest due to solubility considerations in normal-phase solvents. Although the latter approach often mitigates on-column decomposition, the required analyte residence times still frequently result in visible on-column degradation and concomitant poor peak shape, casting doubt on the accuracy of quantitative results. Figure 9 (chromatogram A) demonstrates the analysis of compound I in a typical acidic reversed-phase solvent system using a conventional column, where the residence time of the analyte is ca. 28 min. Significant on-column decomposition of the ester into the corresponding acid (II) at 9 min retention time is evident via the extreme peak asymmetry observed. In contrast, chromatogram B demonstrates analysis of the same compound on a reversed-phase monolith column. Several points should be noted in chromatogram B. First, the entire analysis is completed within 2 min, with a 1 min re-equilibration time, which provides quick turn around time to support processing applications. Second, components of interest are resolved within this 2 min analysis window with good peak shape, demonstrating that no chromatographic performance has

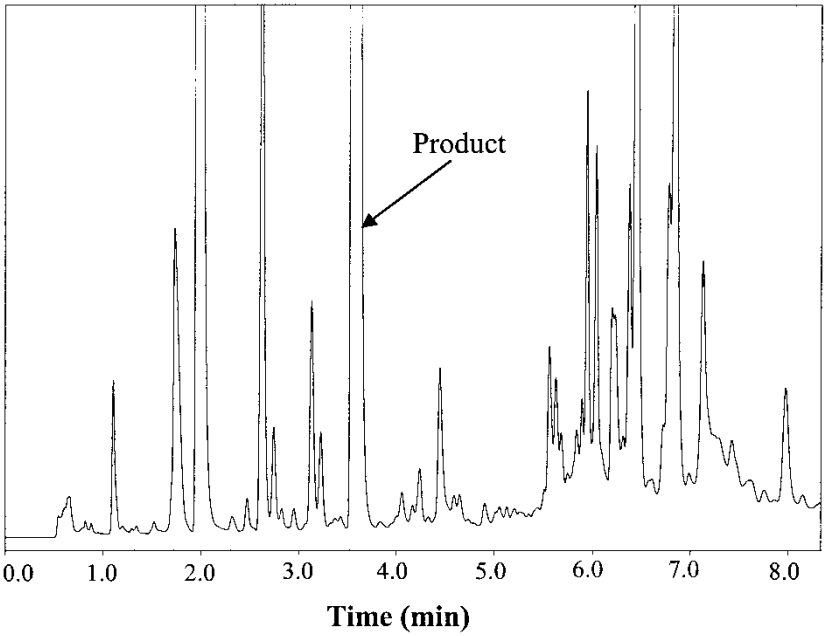


***Figure 8.*** Chromatogram of crystallization mother liquors from compound II. Conditions—flow rate: 4 mL min$^{-1}$; injection volume: 10 μL; UV detection: 220 nm; temperature: 40°C; mobile phase: 0.1% phosphoric acid in water and HPLC grade acetonitrile; gradient: 35 : 65 acetonitrile–water to 57 : 43 acetonitrile–water linearly in 4 min, then ramp up to 71 : 29 acetonitrile–water in 1.0 min, finally to 90 : 10 acetonitrile–water linearly.



been sacrificed in the name of speed, which is uncommon for such active esters in reversed-phase systems. Finally, the level of the corresponding acid degradate (II) is reduced by ca. 33 area% in chromatogram B vs. that observed with the conventional reversed-phase HPLC analysis (chromatogram A) as a result of reducing the column residence time by ca. 27 min. This highlights the extreme instability of the ester in this system and the criticality of fastest analysis possible to ensure that the highest quality data are achieved.

## CONCLUSIONS

This study demonstrates that the Chromolith monolithic column can be used for fast separations without significantly compromising required column efficiency. The selectivity of the $C_{18}$ monolithic column for neutral and basic compounds is comparable to that of conventional packed $C_{18}$ columns, although the

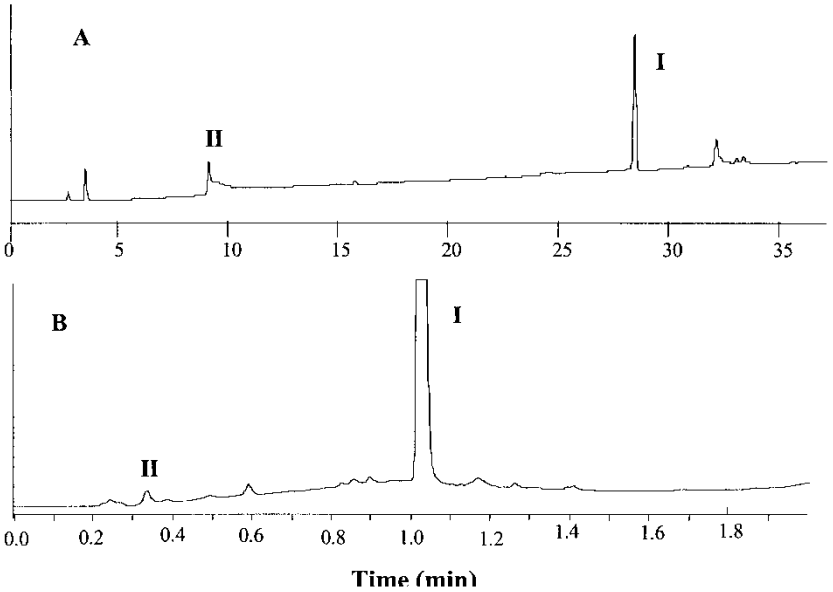


***Figure 9.*** Analysis of an unstable boronic acid ester. Conditions for Chromatogram A—flow rate: 1.0 mL $min^{-1}$; injection volume: 10 μL; UV detection: 210 nm; temperature: 25°C; mobile phase: 0.1% phosphoric acid in water and HPLC grade acetonitrile; Gradient A = 20 : 80 acetonitrile–water to 80 : 20 acetonitrile–water linearly in 30 min. Conditions for Chromatogram B—Flow rate: 7.0 mL/min; Injection volume: 10 μL; UV detection: 210 nm; Temperature: 25°C; Mobile phase: 0.1% phosphoric acid in water and HPLC grade acetonitrile; Gradient A = 10 : 90 acetonitrile–water to 90 : 10 acetonitrile–water linearly in 1 min, hold 1 min at final composition; B = 30 : 70 acetonitrile–water to 90 : 10 acetonitrile–water linearly in 1 min, hold 1 min at final composition.

Chromolith column provides less retention in reversed-phase HPLC. It is also shown that high-speed separations on monolithic columns can be used in pharmaceutical process development, in areas such as reaction monitoring, fraction analysis for preparative chromatography, mother liquor screening, and analysis of unstable analytes. Separation speeds for these process samples may be increased by as much as an order of magnitude without compromising chromatographic resolutions. Extra column effects, including detector time constant and data acquisition speed, must be considered when monolithic columns are used in order to obtain the optimum separation results. As monolithic technology progresses, the potential for development of additional achiral and chiral phases exists, which may further expand the range of samples amenable to analysis with these phases, and therefore expand the possibilities for rapid pharmaceutical development.

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