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**Gradient HPLC using capillary columns can provide extremely high-throughput methods with high peak capacity for a broad range of analytes without the need for high pressure.**

**M**aximizing the speed and resolution of high performance liquid chromatography (HPLC) continues to be an important goal in drug discovery and development. Many HPLC applications, including library screening and open-access analysis, also require a wide range of elution conditions to separate polar and hydrophobic species. To achieve these requirements, the HPLC system must be able to provide precise, rapid gradients with minimal delay volumes, high separation efficiencies, and rapid re-equilibration to the starting conditions.

One approach for improving speed and resolution has been to use smaller particles and shorter columns. Unfortunately, the use of particles smaller than 2  $\mu\text{m}$  results in a significant increase in the required operating pressure and requires specialized high-pressure instrumentation.

An alternative approach to high speed and resolution can be achieved using the unique physical properties of capillary columns (0.3 mm in this study). The advantages of capillary columns relative to conventional columns (> 1 mm) include rapid mixing in small volumes (reduced delay times), increased separation efficiencies, and increased column porosity (enabling higher linear velocities at conventional pressures). The advantages of capillary systems have not been widely recognized due to the lack of commercial instrumentation designed with minimal extracolumn variance and low delay volume. The Eksigent ExpressLC™ system provides high speed and resolution while maintaining quantitative precision.

## Experimental Conditions

HPLC: Eksigent ExpressLC-100 System

Column: Figure 1 — Zorbax SB300 C18, 3.5  $\mu\text{m}$ , 0.3  $\times$  50 mm

Figure 2 — Eksigent ChromXP C18, 3  $\mu\text{m}$ , 0.3  $\times$  50 mm

Mobile Phase: A: Water; Mobile Phase

B: Acetonitrile

Flow Rate: Figure 1 — 16  $\mu\text{L}/\text{min}$

Figure 2 — 16  $\rightarrow$  24  $\mu\text{L}/\text{min}$  over gradient

Sample: 8-bromoguanosine, diethyl phthalate, di-n-pentyl phthalate, dioctylphthalate

Injection Volume: 30 nL

## Results

Figure 1 demonstrates gradient (2 $\rightarrow$ 98% B) separations for a range of gradient times. Gradients ranging from 3 min to 15 s are shown. The sample mixture (prepared in 50:50 DMSO:Acetonitrile) includes a very polar (8-bromoguanosine) and a very hydrophobic compound (dioctyl phthalate). Quality peak shapes and reproducible retention times for 8-bromoguanosine require full re-equilibration to a nearly pure aqueous starting condition. Elution of the final component, dioctyl phthalate, provides an effective marker of the end of the chromatographic run since it is more retained than the majority of compounds of pharmaceutical interest.

# High Speed and Resolution in Gradient Capillary HPLC

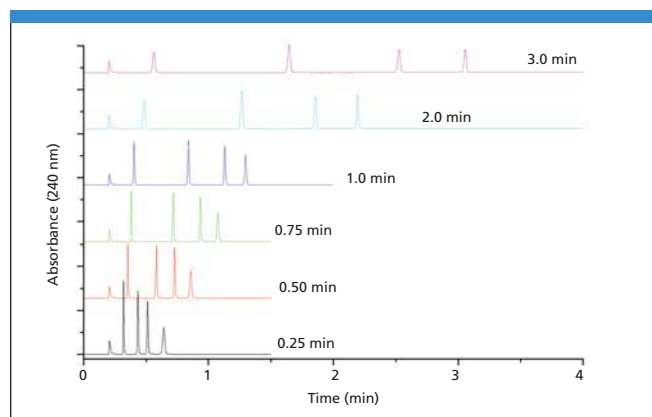
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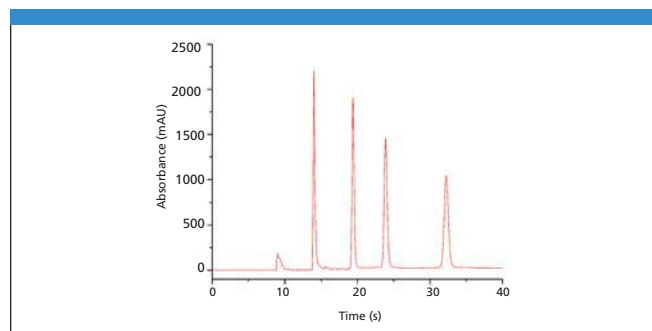
The chromatogram for a 15 s gradient is shown in Figure 2 where the separation is complete in less than 40 s and peak widths are in the range of 0.5 s FWHM. The full injection-to-injection cycle time for this method is  $\sim$ 1 min with retention time reproducibility of < 0.5% RSD.

## Conclusions

Gradient capillary HPLC provides the capability to conduct high-throughput analysis with high-separation efficiencies. Methods can be developed using a wide range of stationary phases for samples with extensive chemical diversity.



**Figure 1:** Gradient separations (2  $\rightarrow$  98% B) for a range of gradient times allowing optimization of peak capacity and analysis time.



**Figure 2:** Chromatographic separation using a 15 s gradient (2  $\rightarrow$  98% B) in a 1 min method provides injection-to-injection cycle times of 1 min.

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