In this application note we examine the effect of temperature on a sub-2μm zirconia-based phase for the analysis of nine common pharmaceuticals. Reducing the particle size and increasing the temperature increases both the efficiency and speed of separation without sacrificing resolution or column stability.

Finally, the viscosity of the mobile phase is decreased, enabling higher flow rates with existing equipment without increasing backpressure.

The decrease in mobile phase viscosity provided by high temperature is especially important for method development with sub-2μm particles as it helps to overcome the higher back pressures inherent in small particle HPLC and allows the average user to take advantage of the increased efficiency provided by the smaller particles without the use of specialized UHPLC instrumentation.

**Experimental**

Nine pharmaceuticals were separated using a ZirChrom®-PBD column. The separation conditions were as follows:

- **Columns**: Sub-2 μm ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number: ZR03-0546-1.9)
- **Mobile Phase**: 22/78 Acetonitrile/20mM Potassium Phosphate, pH 12.0
- **Injection Vol.**: 2 μl
- **Temperature**: 75°C
- **Back Pressure**: 246 bar
- **Flow Rate**: 2.5 mL/minute
- **Detection**: UV at 254 nm

Figure 2 shows the separation of nine pharmaceutical compounds at 75 °C on a sub-2μm ZirChrom®-PBD column. The instrument used in the analysis was a very basic commercially available HPLC system with minimal modification to eliminate as much void volume in the system as possible. As shown in Figure 2, the
increase in temperature allowed an increase in flow rate, reducing the analysis time while keeping back pressure well below the 400 bar operating limit for standard HPLC equipment.

This method can be tailored to your specific application needs. ZirChrom technical support can help to optimize and transfer this method to your site. Please contact ZirChrom technical support at 1-866-STABLE-1 or support@zirchrom.com for details.

**References**

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Visit [www.zirchrom.com](http://www.zirchrom.com) for more information about using ultra-stable, high efficiency ZirChrom columns at high temperature.