Effect of Temperature on the Analysis of Catecholamines using Sub-2µm ZirChrom®-PBD

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

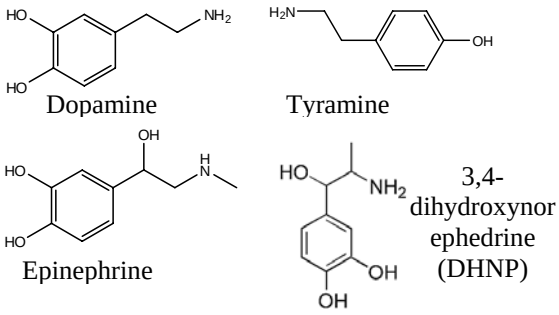

Figure 1. Structures of Catecholamines

Introduction

Catecholamines are historically difficult molecules to elute on zirconia-based HPLC columns due to the strong interaction between the catechol group and the Lewis acid site dominated surface. Previous work has been done to optimize separation conditions (1) & (2) allowing for a fast, multi-mode separation. In this application note we take the work a step further exploring the effect of temperature coupled with a smaller, sub-2µm, particle size. The unique stability of ZirChrom®-PBD enables a much wider temperature (up to 150 °C) and pH (pH 1 – 14) range for method development. Elevated temperature speeds separation through the following three main effects (3). Firstly, the viscosity of the mobile phase is decreased, enabling higher flow rates with existing equipment without increasing backpressure. Secondly, higher temperature increases the diffusion rate of analytes minimizing the any losses in efficiency at higher flow rates (4). Finally, at elevated temperature, the kinetics of the faster interactions between the analytes and stationary phase will lower the overall analysis time; often reducing or eliminating peak tailing. A decrease in mobile phase viscosity 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 employ these particles without the use of specialized UHPLC instrumentation.

Experimental

Four catecholamines 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:	85/15 Acetonitrile/10mM Ammonium Dihydrogen Phosphate, 30mM Ammonium Acetate, adjusted to pH 3.4 with HCl
Injection Vol.:	5 µl
Detection:	UV at 254 nm

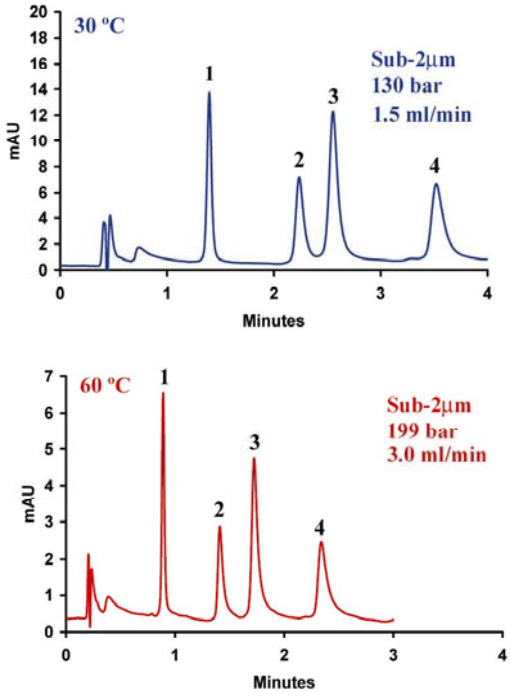

Figure 2. Separation of Catecholamines. **1**=Tyramine, **2**=Epinephrine, **3**=Dopamine, **4**=3,4-dihydroxynorephedrine

Figure 2 shows the separation of catecholamines at 30 and 60 °C on a sub-2µm ZirChrom®-PBD column. This very mild increase in temperature has allowed a two-fold increase in flow rate and has reduced the analysis time by one minute while keeping the back pressure within normal operating parameters 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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