Analysis of Catecholamines on ZirChrom®-PBD

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Technical Bulletin # 328

Historically, catecholamines are 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. Catecholamines are zwitterionic and are also known to be good metal chelators. However, under the right mobile phase conditions the separations of catecholamines on zirconia-based stationary phases is facile and the multi-modal surface chemistry of zirconia allows for a unique selectivity. Here we report the separation of several catecholamines and the effect of ionic strength and percent organic on the resolution of these compounds: L-dopa, tyramine, epinephrine, dopamine, and 3,4-dihydroxynorphedrine.

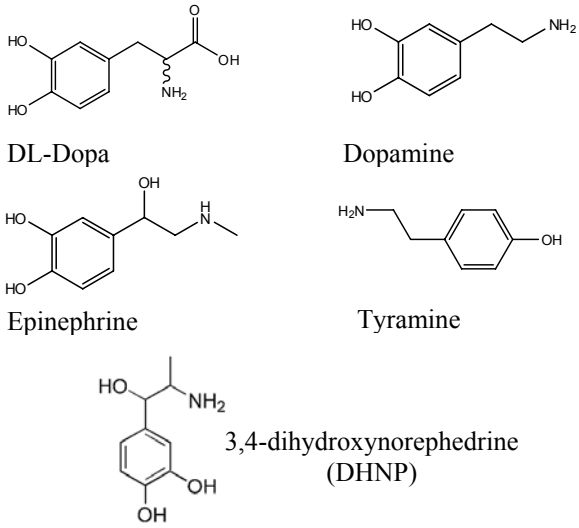

Figure 1. Structures of Catecholamines

Introduction

Catecholamines are hormones important in causing general physiological changes that prepare the body for physical activity such as the fight or flight response. The unparalleled stability of ZirChrom®-PBD allows for a much longer column lifetime and more robust separation when compared to traditional silica-based ion-pairing techniques. The multi-modal separation capabilities of ZirChrom®-PBD allow for the unique selectivity for these ionic molecules. To achieve optimum peak shape and selectivity for these ionic molecules a mobile phase containing both a sufficient amount of Lewis base additive (phosphate), ionic strength (acetate) and organic modifier was developed. The following details the separation of five catecholamines using a multi-modal separation on a ZirChrom®-PBD column.

Experimental

Five catecholamines were separated at 35 ºC using a ZirChrom®-PBD column. The separation conditions were as follows:

Column:	ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number: ZR03-0546)
Mobile Phase:	85/15 Acetonitrile/10mM Ammonium Dihydrogen Phosphate, 30mM Ammonium Acetate, pH 3.4
Temperature:	35 ºC
Flow Rate:	1.5 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 254 nm

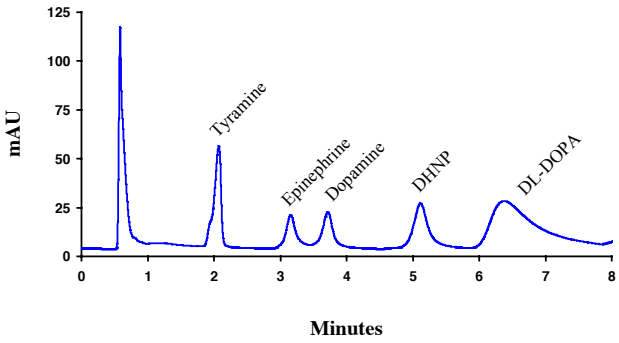

Figure 2. Separation of Catecholamines

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Comparison of Sub-2µm and 3µm ZirChrom®-PBD for the Separation of Catecholamines

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Technical Bulletin # 329

In this application note we examine the benefits of a smaller particle size for the analysis of catecholamines. As predicated theoretically, the decrease in particle size, from 3µm to sub-2µm, allows for a marked increase in efficiency (measured in plates/meter).

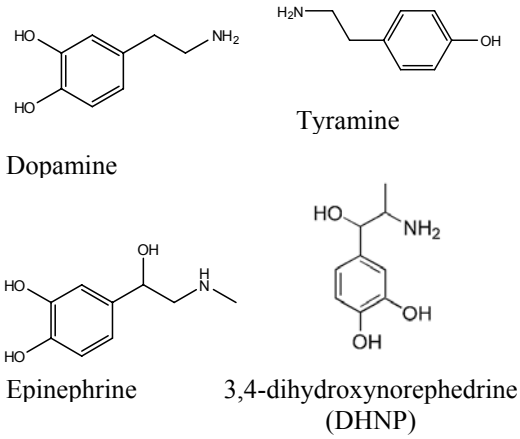

Figure 1. Structures of Catecholamines

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 (1) developed mobile phase conditions that enable facile, robust and multi-modal separation of these compounds. In this application note we take the work a step further exploring the effect of particle size on the efficiency of the peaks. Theoretical calculations predict, and recent work has demonstrated, that particle size is directly proportional to column efficiency (2) & (3). This increase in efficiency is useful when requiring a bit more resolution or to speed a satisfactory separation up by utilizing smaller particles in a shorter column size.

Experimental

Four catecholamines were separated at 30 ºC using a ZirChrom®-PBD column. The separation conditions were as follows:

Columns:	Sub-2 and 3 µm ZirChrom®-PBD, 50 mm x 4.6 mm i.d. (Part Number: ZR03-0546-1.9 & ZR03-0546)
Mobile Phase:	85/15 Acetonitrile/10mM Ammonium Dihydrogen Phosphate, 30mM Ammonium Acetate, adjusted to pH 3.4 with HCl
Temperature:	30 ºC
Flow Rate:	1.5 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 254 nm

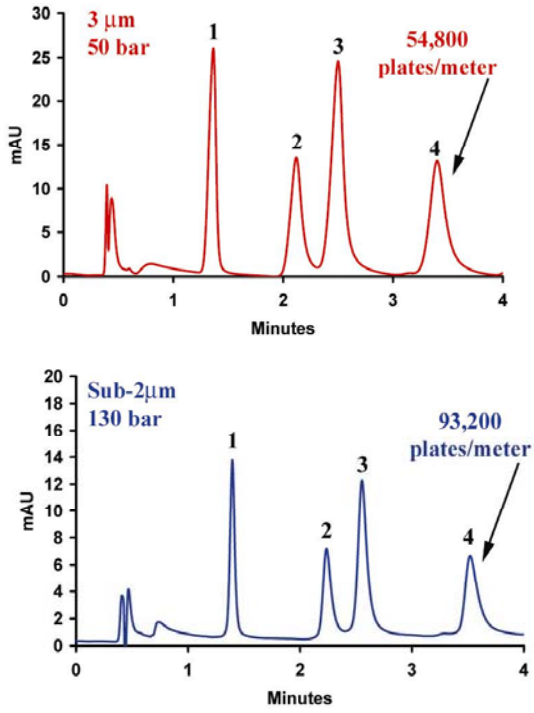

Figure 2. Separation of Catecholamines. **1=**Tyramine, **2=**Epinephrine, **3**=Dopamine, **4**=3,4-dihydroxynorephedrine

The data in Figure 2 support the hypothesis that smaller particles increase the efficiency of the column. Future work will explore the use of temperature and shorter column lengths to fully capitalize on the increased efficiency and thus resolution provided by sub-2µm particles.

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

(1) http://www.zirchrom.com/pdf/328.pdf
(2) Dolan, J.W., "The Perfect Method , Part 6" LCGC Europe, February (2008).
(3)) http://www.zirchrom.com/pdf/327.pdf

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

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Technical Bulletin # 330

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.

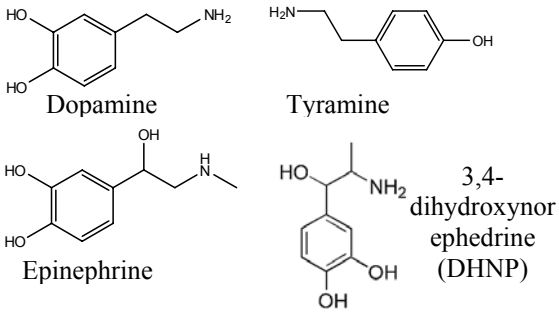

Figure 1. Structures of Catecholamines

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

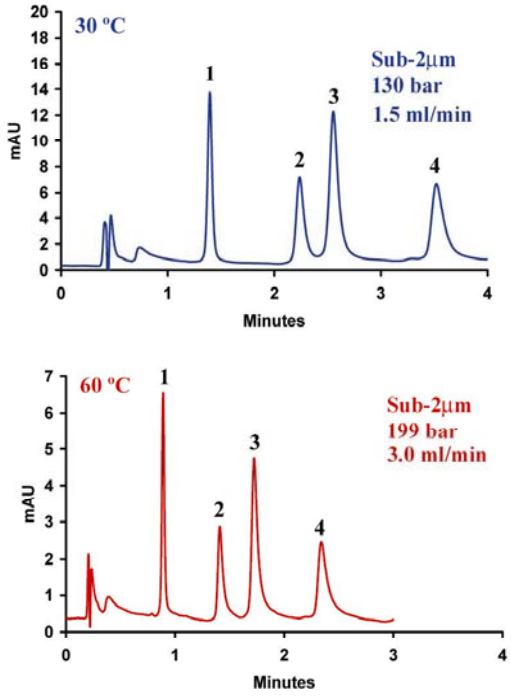

Figure 2. Separation of Catecholamines. **1=**Tyramine, **2=**Epinephrine, **3**=Dopamine, **4**=3,4-dihydroxynorephedrine

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

(1) http://www.zirchrom.com/pdf/327.pdf
(2) http://www.zirchrom.com/pdf/328.pdf
(3) Antia, F.; Horvath, C. J. Chrom. 435, 1-15 (1988)
(4) Li, J.W; Carr, P.W. Anal. Chem. 69(5), 837-843 (1997)

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