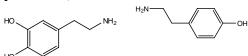


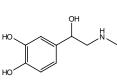
In this application note we examine the benefits of a smaller particle size for the analysis of catecholamines. As predicated theoretically, the decrease in particle size, from 3µm to sub-2µm, allows for a marked increase in efficiency (measured in plates/meter).

Tyramine

HC



Dopamine



ОН

NH<sub>2</sub>

Epinephrine

3,4-dihydroxynorephedrine (DHNP)

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

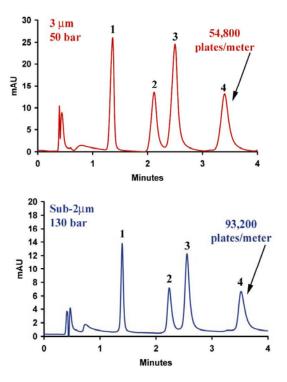
Four catecholamines were separated at 30 °C using a ZirChrom<sup>®</sup>-PBD column. The separation conditions were as follows:

Columns:	Sub-2 and 3 μm ZirChrom <sup>®</sup> -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 °C
Flow Rate:	1.5 ml/min.
Injection Vol.:	5 μl
Detection:	UV at 254 nm

# Comparison of Sub-2µm and 3µm ZirChrom<sup>®</sup>-PBD for the Separation of Catecholamines

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**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 <a href="mailto:support@zirchrom.com">support@zirchrom.com</a> 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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