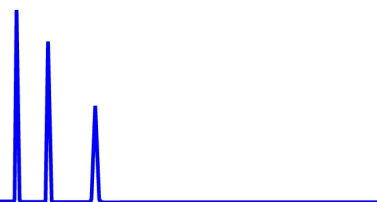




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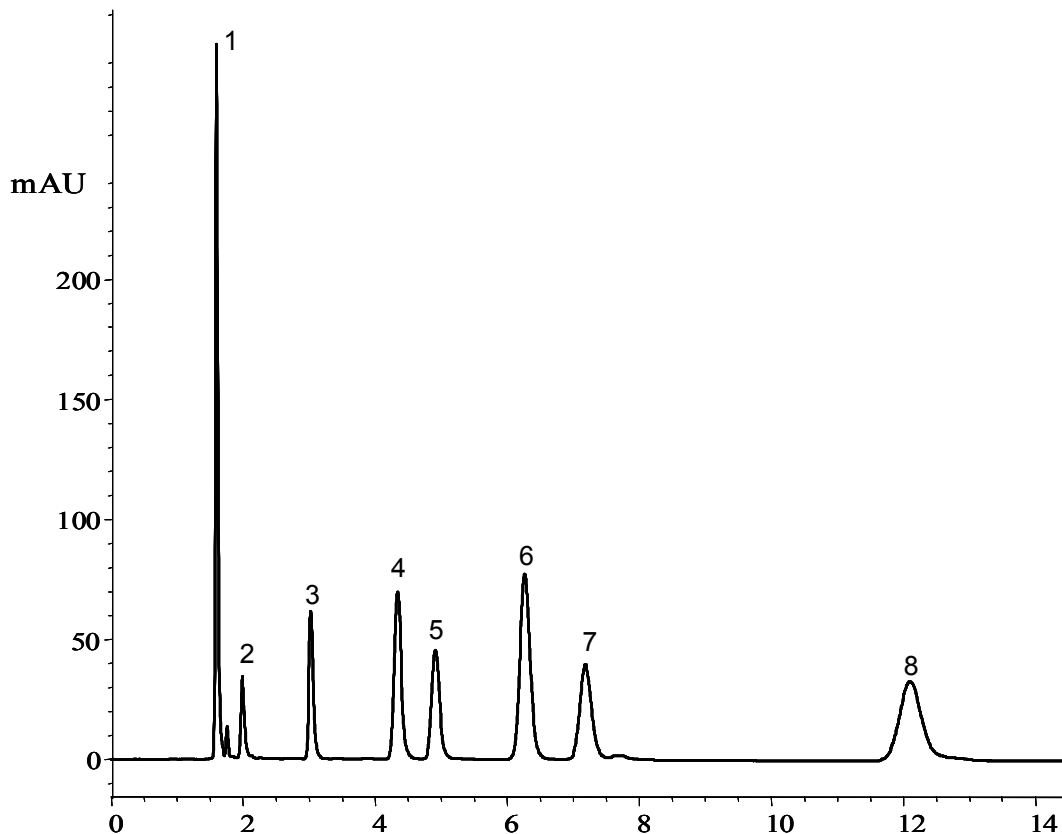


... For Peak Performance

Opioids on ZirChrom®-PBD

Analytes

- 1 - Aminophylline
- 2 - Morphine
- 3 - Phenacetin
- 4 - Codeine
- 5 - Hydrocodone
- 6 - Ethylmorphine
- 7 - Oxycodone
- 8 - Papaverine



LC Conditions

Column: ZirChrom®-PBD, 150 × 4.6 mm

Mobile Phase: 15/85 A/B

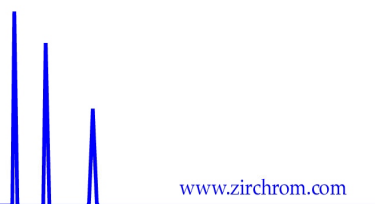
A: Acetonitrile

B: 50 mM potassium phosphate monobasic at pH 10.0

Flow rate: 1.0 mL/min.

Temperature: 30 °C

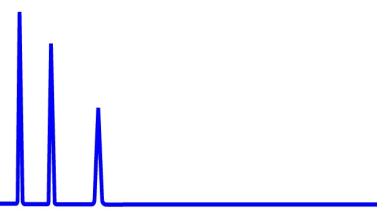
Detection: 220 nm





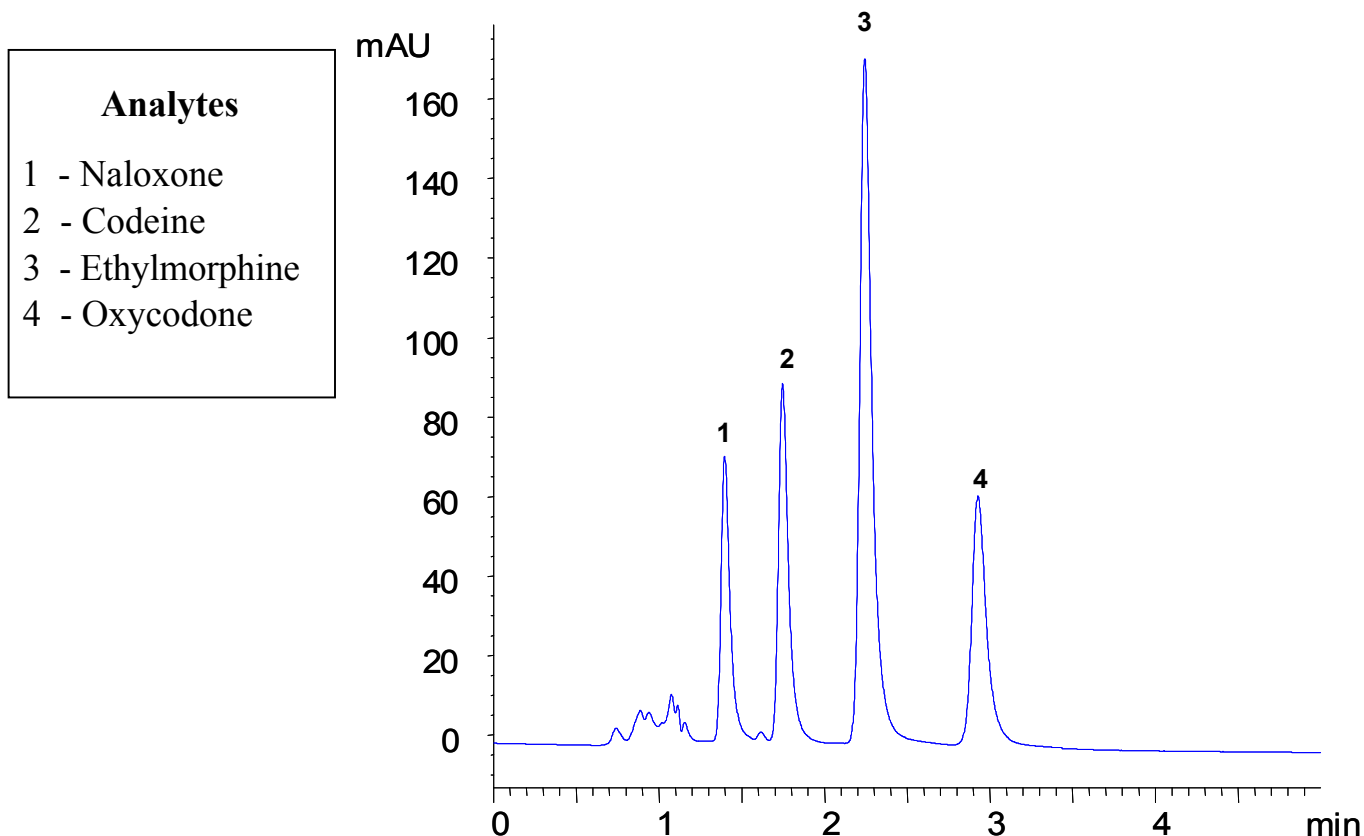
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... For Peak Performance

Separation of Opioids



LC Conditions

Column: **DIAMOND BOND™** 18, 100 × 4.6 mm

Mobile Phase: 26.5/73.5 A/B

A: THF

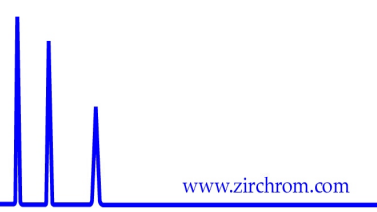
B: 20 mM Ammonium phosphate, pH 11.0

Flow rate: 1.0 mL/min.

Temperature: 40 °C

Injection volume: 1 µL

Detection: 220 nm



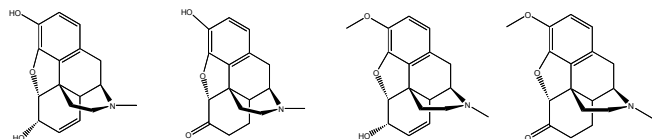


Fast, LC/MS Compatible Separation of Opioids on ZirChrom[®]-EZ

Clayton McNeff, Ph.D. and Dwight Stoll
ZirChrom Separations, Inc.

Technical Bulletin #283

The structural similarity of hydromorphone to morphine and hydrocodone to codeine requires a very selective stationary phase. Due to the fact that these compound pairs have identical molecular weights, a MS-detector is unable to distinguish between the parent compound and its metabolite. The unique characteristics of the ZirChrom[®]-EZ column allow for fast resolution of all four of these opioids using a simple acetonitrile/water gradient in combination with a MS-compatible ammonium acetate buffer at pH 5.0. The resulting method allows reliable quantitation by LC/MS.



Morphine M.W. 285.33 **Hydromorphone** M.W. 285.33 **Codeine** M.W. 299.36 **Hydrocodone** M.W. 299.36

Figure 1: Structures of parent opioid compounds and their metabolites.

Introduction

The opioids morphine and codeine are commonly analyzed using Liquid Chromatography/Mass Spectrometry (LC/MS) in the clinical laboratory because of the low limits of detection required. The structural similarity of these molecules presents a significant separation challenge. Generally, a MS-detector does not require as much resolution as a UV-detector. However, in the case of these four opioids the MS-detector cannot differentiate between the parent compound and its metabolite, which have identical molecular weights.

Experimental

A mixture of four opioids was separated at 35 °C using a ZirChrom[®]-EZ column. The separation conditions were as follows:

Column: ZirChrom[®]-EZ, 50 mm x 4.6 mm i.d.
(Part Number: EZ01-0546)
Mobile Phase: Gradient Elution
A: acetonitrile
B: 20mM ammonium acetate, pH 5.0

Time	%A	%B
0	10	90
5	90	10

Temperature: 35 °C with Metalox[™] 200-C Column Heater
Injection Vol.: 2 µl
Detection: UV at 254 nm

This method allows for baseline resolution of the metabolite and the parent compound using a MS-compatible ammonium acetate buffer in 5 minutes.

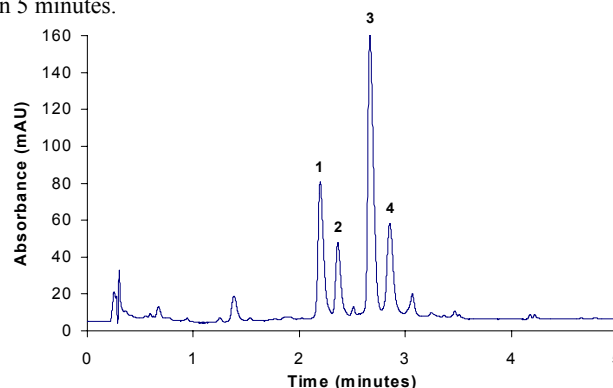


Figure 2: Separation of 1=Morphine, 2=Hydromorphone, 3=Codeine, 4=Hydrocodone on ZirChrom[®]-EZ at 35 °C.

ZirChrom's newest reversed-phase column, ZirChrom[®]-EZ, provides unique selectivity while simplifying the buffer selection process in the pH range of 1-10. This new ease-of-use capability, along with its orthogonal selectivity for pharmaceutical compounds, makes ZirChrom[®]-EZ well suited for LC/MS applications.

This method can be tailored to your specific application needs. ZirChrom method developers 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.

ZirChrom phases offer unique selectivity, high efficiency, and excellent chemical and thermal stability.

Acknowledgements

Randy Clouette, Clinical Reference Laboratory, (Lenexa, Kansas, USA)

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Visit www.zirchrom.com for more application notes using ultra-stable, high efficiency ZirChrom columns.