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.

**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.

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