

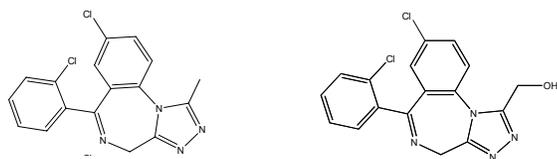


Separation of Triazolam from Hydroxytriazolam on ZirChrom[®]-EZ

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Technical Bulletin # 287

Historically chromatography of the hydroxy-metabolites of benzodiazepines such as triazolam has been hindered by the irreversible adsorption of these metabolites on the Lewis acid sites of zirconia-based HPLC columns. ZirChrom's new Lewis acid deactivated reversed phase column, ZirChrom[®]-EZ, allows the elution and separation of these analytes with excellent peak shape and high column efficiency.



Triazolam

Hydroxytriazolam

Figure 1: Structures of triazolam and its hydroxylated metabolite

Introduction

Previous to the development of the Lewis acid deactivated ZirChrom[®]-EZ phase, no suitable condition was found for the elution of hydroxytriazolam from any zirconia-based reversed-phase support. The deactivation of Lewis acid sites on the surface of the ZirChrom[®]-EZ support not only allows elution of both triazolam and its hydroxylated metabolite, but excellent peak shape can also be obtained using relatively simple buffers. The non-volatile buffers such as phosphate and fluoride traditionally used with zirconia-based reversed-phases are not required; rather, more conventional volatile buffers may be used including typical LC/MS compatible buffers (such as acetate and formate).

Experimental

A mixture of triazolam and its metabolite, hydroxytriazolam was separated at 35°C using a ZirChrom[®]-EZ column. The separation conditions were as follows:

Column: ZirChrom[®]-EZ, 150 mm x 4.6 mm i.d.
(Part Number: EZ01-1546)
Mobile Phase: 30/70 A/B
A: acetonitrile
B: 20mM ammonium acetate, pH 5.0
Temperature: 35 °C with Metalox[™] 200-C column heater
Flow Rate: 1.5 ml/min.
Injection Vol.: 5 µl
Pressure Drop: 168 bar
Detection: UV at 254 nm

The facile separation of triazolam from hydroxytriazolam using isocratic elution conditions in under six minutes is shown below in Figure 2.

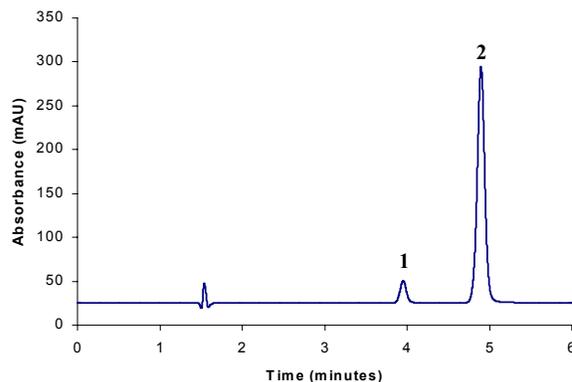


Figure 2: Separation of 1=Hydroxytriazolam and 2=Triazolam, on ZirChrom[®]-EZ

ZirChrom-EZ combines the superior stability of zirconia-based phases with the simplicity of operation of silica columns.

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