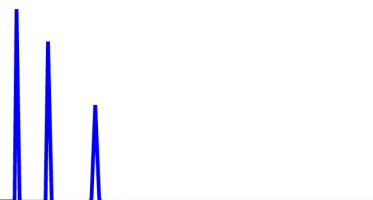




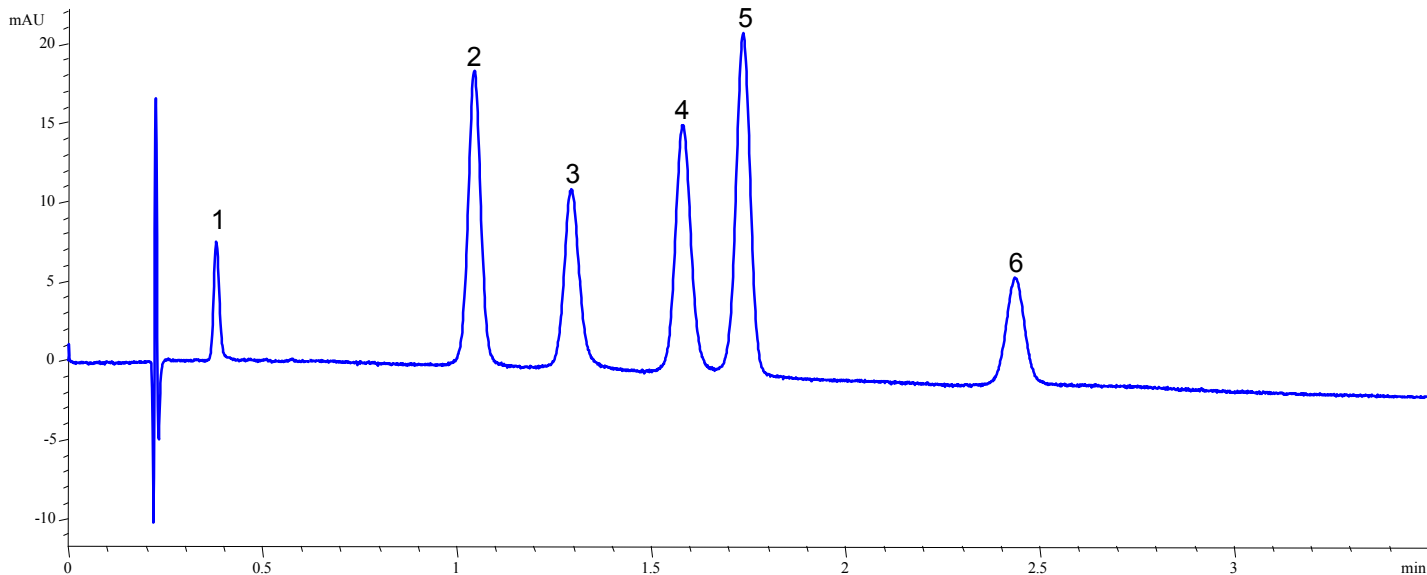
ZirChrom®

Technical  
Bulletin #261



... For Peak Performance

## Benzodiazepine Antidepressants on ZirChrom®-PBD



Analytes	
1 - 7-aminoflunitrazepam	4 - Nordiazepam
2 - Clonazepam	5 - Diazepam
3 - Alprazolam	6 - Medazepam

### LC Conditions

Column: ZirChrom®-PBD, 50 mm × 4.6 mm i.d.

Mobile Phase: Gradient Elution

Flow rate: 2.5 mL/min.

Temperature: 40 °C

Injection volume: 2 µL

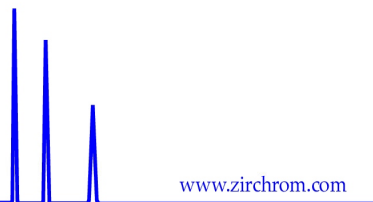
Detection: 254 nm

Back Pressure: 175 bar

Time (Minutes)	% A	%B
0	80	60
3	20	40

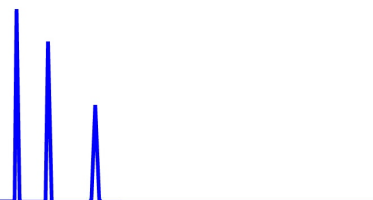
A: 20mM Acetic Acid pH to 5.0 using Ammonium Hydroxide

B: ACN



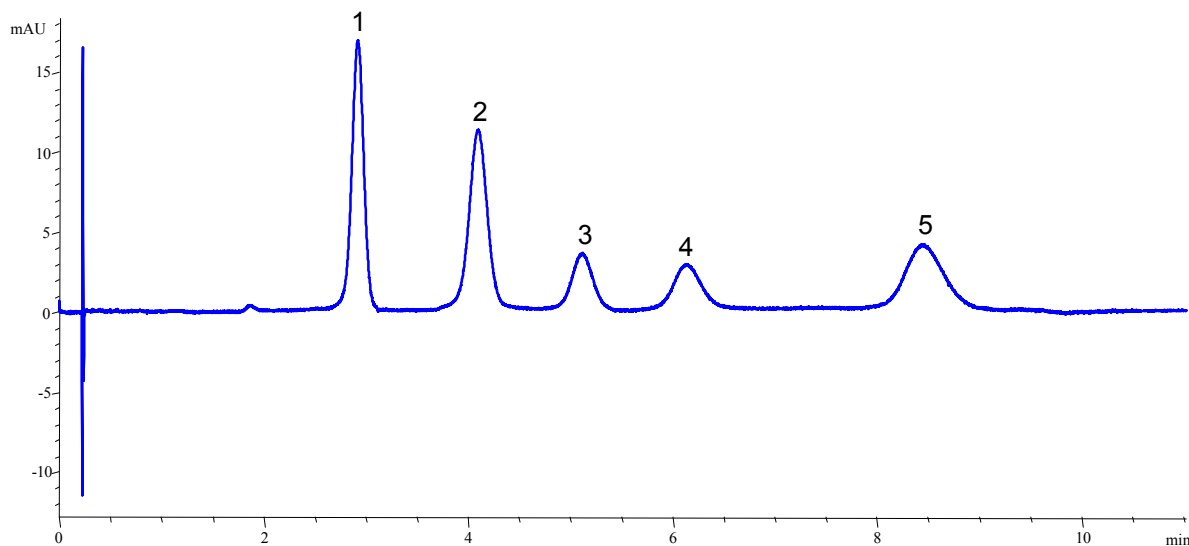


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## Benzodiazepine Antidepressants on ZirChrom®-PBD



### Analytes

1 - Flunitrazepam 2 - Chlordiazepoxide 3 - Triazolam 4 - Desalkylflurazepam 5 - Midazolam

### LC Conditions

Column: ZirChrom®-PBD, 50 mm × 4.6 mm i.d.

Mobile Phase: Isocratic Elution 12.5/87.5 A/B

 A: 20mM Acetic Acid pH to 5.0  
 using Ammonium Hydroxide

B: ACN

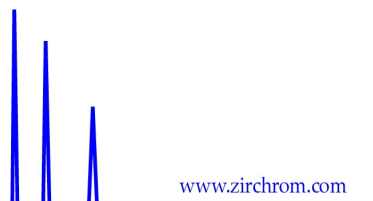
Flow rate: 2.5 mL/min.

Temperature: 40 °C

Injection volume: 2 µL

Detection: 254 nm

Back Pressure: 175 bar





# LC/MS Compatible Separation of Benzodiazepines on ZirChrom®-EZ

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

## Technical Bulletin # 286

At ZirChrom® we have compared the elution sequences of benzodiazepines antidepressants on reversed-phase zirconia and silica C18-based columns and found that poorly resolved compounds on silica are well separated on zirconia and vice versa. We report here the separation of four benzodiazepines under isocratic elution conditions and an LC/MS compatible acetate buffer.

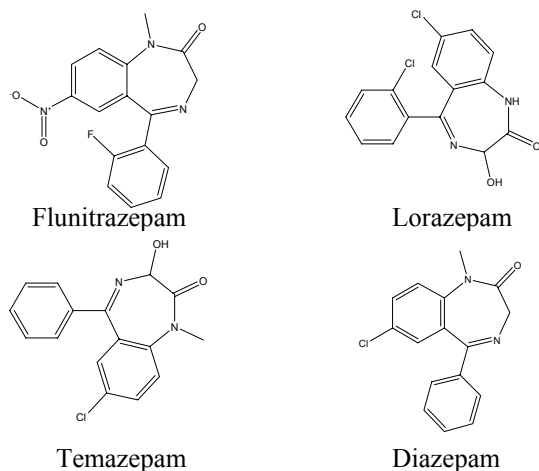


Figure 1: Chemical structures of four benzodiazepines.

### Introduction

Benzodiazepines are an important class of amine containing antidepressants. The use of a ZirChrom®-EZ column allows for the separation of these four benzodiazepine compounds in under six minutes.

### Experimental

A mixture of four benzodiazepines (flunitrazepam, lorazepam, temazepam, and diazepam) 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:	Isocratic elution: 35/65 A/B A: acetonitrile B: 20mM ammonium acetate pH 5.0
Temperature:	35 °C with Metalox™ 200-C column heater
Flow Rate:	1.0 ml/min.
Injection Vol.:	2 µl
Pressure Drop:	168 bar
Detection:	UV at 254 nm

Four benzodiazepines were separated using isocratic elution conditions and an LC/MS compatible acetate buffer. Peaks obtained were efficient and symmetrical.

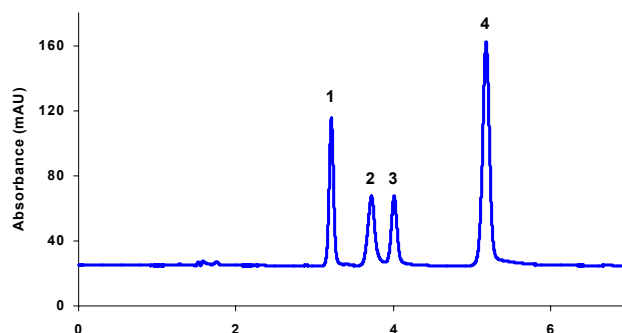


Figure 2: Separation of 1=Flunitrazepam, 2=Lorazepam, 3=Temazepam, and 4=Diazepam on ZirChrom®-EZ

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](mailto:support@zirchrom.com) for details.

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

**ZirChrom Separations, Inc.**  
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[support@zirchrom.com](mailto:support@zirchrom.com)

Visit [www.zirchrom.com](http://www.zirchrom.com) for more application notes using ultra-stable, high efficiency ZirChrom columns.

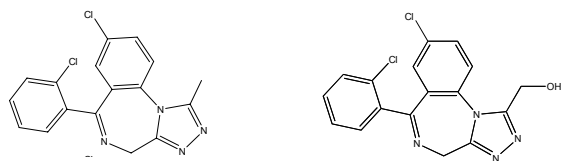


# Separation of Triazolam from Hydroxytriazolam on ZirChrom<sup>®</sup>-EZ

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

## 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<sup>®</sup>-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

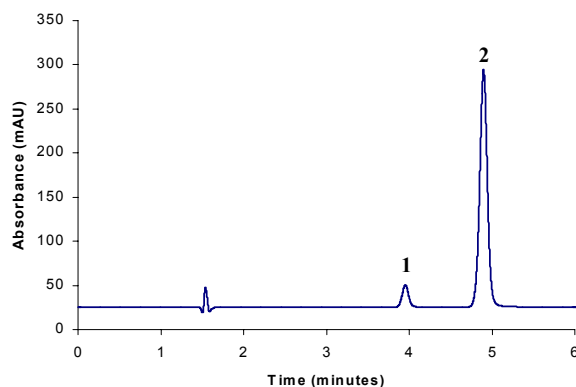
Previous to the development of the Lewis acid deactivated ZirChrom<sup>®</sup>-EZ phase, no suitable condition was found for the elution of hydroxytriazolam from any zirconia-based reversed-phase support. The deactivation of Lewis acid sites on the surface of the ZirChrom<sup>®</sup>-EZ support not only 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<sup>®</sup>-EZ column. The separation conditions were as follows:

Column: ZirChrom<sup>®</sup>-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<sup>™</sup> 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<sup>®</sup>-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](mailto:support@zirchrom.com) for details.

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

### ZirChrom Separations, Inc.

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Visit [www.zirchrom.com](http://www.zirchrom.com) for more application notes using ultra-stable, high efficiency ZirChrom columns.