HPLC Analysis of Organic Acids Using ZirChrom®-EZ

Bingwen Yan, Ph.D. and Clayton V. McNeff, Ph.D.
ZirChrom Separations, Inc.

Technical Bulletin #282

The Lewis acidity of zirconia-based supports for HPLC has historically presented problems in the analysis of analytes containing Lewis base moieties, particularly in LC/MS applications where volatile mobile phase additives are required. In this application note we demonstrate the utility of a new Lewis acid deactivated zirconia-based support, ZirChrom®-EZ. This new deactivated phase provides a wide range of applicability without the need for non-volatile mobile phase additives.

Introduction

The Lewis acid-base chemistry of zirconia-based chromatographic supports has been studied in detail (1). The elutropic series of Lewis base mobile phase additives for the chromatography of Lewis base analytes using un-modified zirconia supports suggests that phosphate and fluoride salts are the additives of choice for analytes with wide ranging functionalities (2). While these types of additives work well in applications with UV/Vis detection, their use is almost entirely prohibited in LC/MS applications due to their relatively low volatility.

ZirChrom®-EZ presents an alternative to conventional zirconia-based reversed-phase supports for applications requiring volatile mobile phase additives. The deactivation of Lewis acid sites on the zirconia surface allows the chromatography of Lewis base analytes such as carboxylates, sulfates, and phosphates using mobile phase additives of the users choice including conventional LC/MS compatible buffers (such as acetate and formate) throughout the pH range of 1-10.

Experimental

A mixture of organic acids was separated at room temperature using a ZirChrom®-EZ column with a simple organic/water mobile phase with no additives. The separation conditions were as follows:

Column:	50 mm x 4.6 mm i.d. ZirChrom®-EZ (part # EZ01-0546)
Mobile Phase:	40/60 acetonitrile/water
Flow Rate:	1.0 ml/min.
Injection Vol.:	1 µl
Pressure Drop:	60 bar
Detection:	UV at 254 nm
Temperature:	30 °C

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The separation of organic acids is shown in Figure 1. Excellent peak shape is obtained for all four analytes with this simple organic/water mixture containing no mobile phase additives. It is important to note that these analytes would be irreversibly adsorbed to unmodified zirconia-based supports using the same mobile phase without any additives.

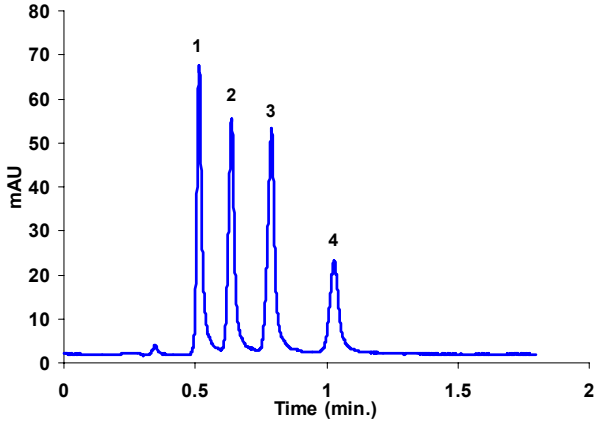

Figure 1. Separation of organic acids on ZirChrom®-EZ. Solutes: 1 = 4-hydroxybenzoic acid, 2 = 4-ethyoxybenzoic acid, 3 = 4-propoxybenzoic acid, 4 = 4-butoxybenzoic acid.

This new ZirChrom®-EZ phase represents a significant advancement of the zirconia-based technology for HPLC. The deactivation of the zirconia surface acidity greatly expands the possibilities for the use of ZirChrom®-EZ in LC/MS applications with any of the volatile mobile phase additives traditionally associated with LC/MS.

ZirChrom's technical support team has extensive experience with this and other reversed-phase supports and would be happy to help you with your particular application.

References

(1) Blackwell, J. A.; Carr, P. W. *Journal of Liquid Chromatography* **1991**, *14*, 2875-2889.
(2) Blackwell, J. A.; Carr, P. W. *Analytical Chemistry* **1992**, *64*, 863-873.

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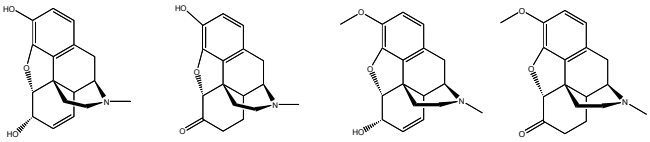
Fast, LC/MS Compatible Separation of Opioids on ZirChrom®-EZ

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 ºC 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 ºC 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.

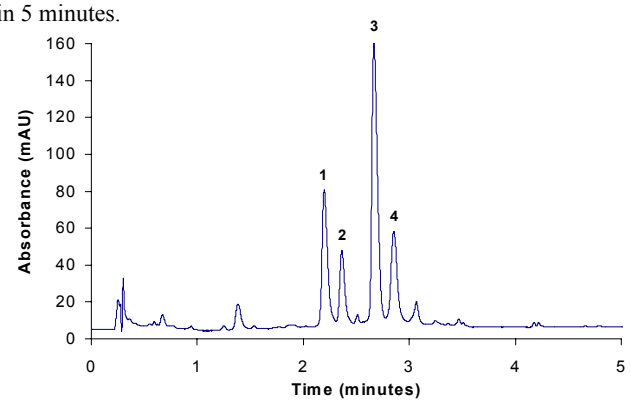


Figure 2: Separation of 1=Morphine, 2=Hydromorphone, 3=Codeine, 4=Hydrocodone on ZirChrom®-EZ at 35 ºC.

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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LC/MS Compatible Separation of Non-Steroidal Anti-Inflammatory Drugs

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

Technical Bulletin #285

The Lewis acidity of zirconia-based supports for HPLC has historically presented problems in the analysis of analytes containing Lewis base moieties, such as carboxylates, particularly in LC/MS applications where volatile mobile phase additives are required. In this application note we demonstrate the utility of a new Lewis acid deactivated zirconia-based column, ZirChrom®-EZ.

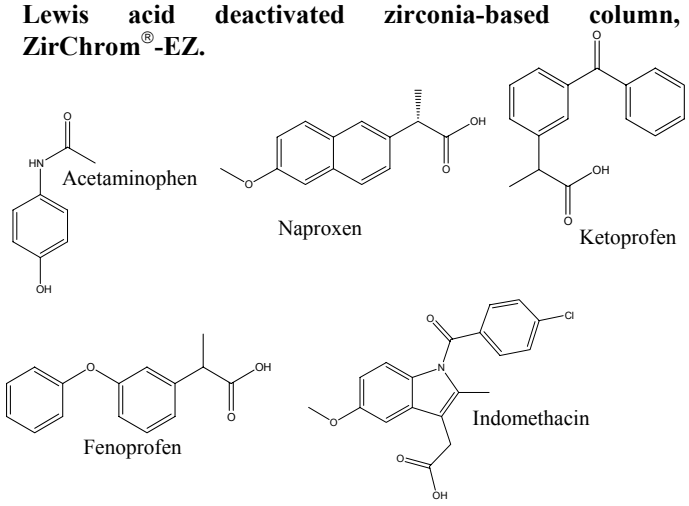

Figure 1: Structures of Non-steroidal anti-inflammatory drugs.

Introduction

Historically, the Lewis base carboxylic acid moiety on non-steroidal anti-inflammatory drugs required the use of a Lewis base mobile phase additive of a higher strength in the elutropic series (such as phosphate or fluoride) (1). While these types of additives work well in applications with UV/Vis detection, their use is almost entirely prohibited in LC/MS applications due to their relatively low volatility.

The deactivation of Lewis acid sites on the surface of the ZirChrom®-EZ particle allows the chromatography of Lewis base analytes using mobile phase additives of the users choice including conventional LC/MS compatible buffers (such as acetate and formate) throughout the pH range of 1-10.

Experimental

Five non-steroidal anti-inflammatory drugs were separated at 35ºC 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: A: acetonitrile
B: 20 mM ammonium acetate, pH 5.0

Time	%A	%B
0	10	90
10	90	10

Temperature: 35 ºC with Metalox™ 200-C Column Heater
Flow Rate: 1.0 ml/min.
Injection Vol.: 10 µl
Pressure Drop: 168 bar
Detection: UV at 254 nm

Five non-steroidal anti-inflammatory drugs were separated using simple acetoniltrile/water gradient elution and a LC/MS friendly acetate buffer.

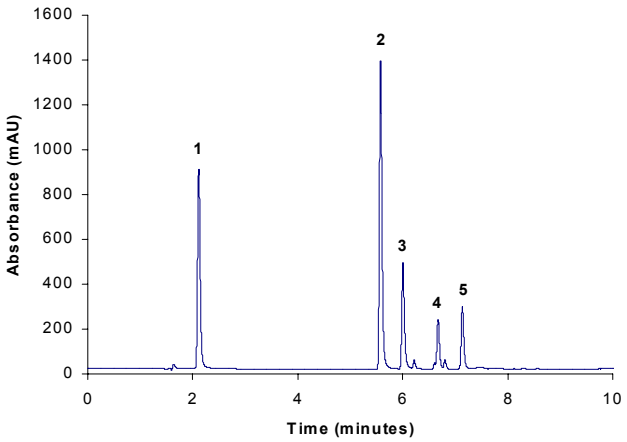

Figure 2: Separation of 1=Acetaminophen, 2=Naproxen, 3=Ketoprofen, 4=Fenoprofen, and 5=Indomethacin.

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.

References

(1) Blackwell, J. A.; Carr, P. W. *Journal of Liquid Chromatography* **1991**, *14*, 2875-2889.

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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.

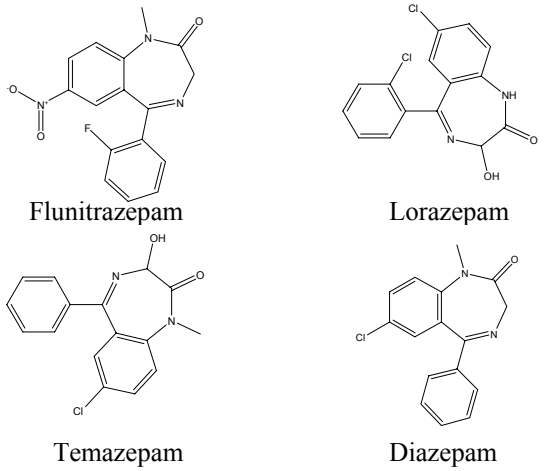

Figure 1: Chemical structures of four benzodiazepines.

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 35ºC 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 ºC 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.

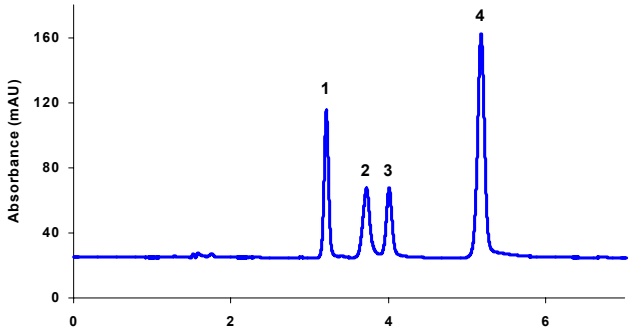

Figure 2: Separation of 1=Flunitrazepam, 2=Lorazepam, 3=Temazepam, and 4=Diazepam on ZirChrom®-EZ

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 for details.

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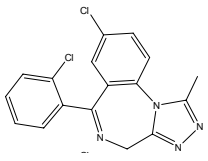
Separation of Triazolam from Hydroxytriazolam on ZirChrom®-EZ

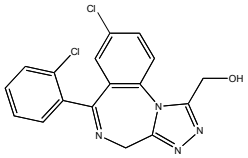
Clayton McNeff, Ph.D., Dwight Stoll, and Kelly Johnson
ZirChrom Separations, Inc.

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®-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 35ºC 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 ºC 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.

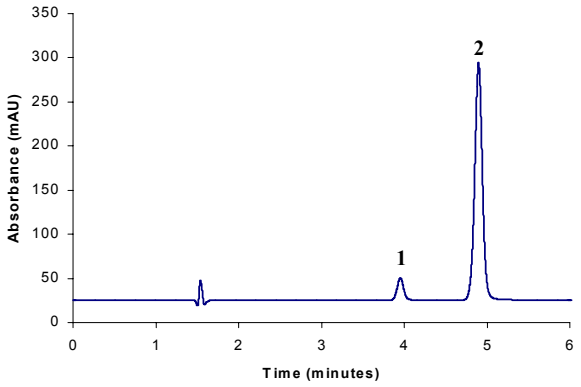


Figure 2: Separation of 1=Hydroxytriazolam and 2=Triazolam, on ZirChrom®-EZ

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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LC/MS Compatible Separation of Cocaine and Benzoylecognine

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

Technical Bulletin # 288

The Lewis acidity of zirconia-based supports for HPLC has historically been problematic, particularly in LC/MS applications where volatile mobile phase additives are required. In this application note we demonstrate the utility of a new zirconia-based column, ZirChrom®-EZ, for the separation of cocaine and benzoylecongnine using an LC/MS compatible mobile phase.

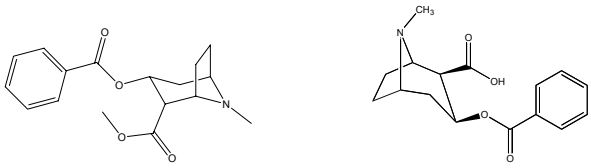

Figure 1: Structures of Cocaine and Benzoylecongnine

Introduction

Historically, the Lewis base carboxylic acid moiety on bezoylecognine required the use of a Lewis base mobile phase additive of a higher strength in the elutropic series (such as phosphate or fluoride) to obtain good peak shape (1). While these types of additives work well in applications with UV/Vis detection, their use is almost entirely prohibited in LC/MS applications due to their relatively low volatility.

The use of a ZirChrom®-EZ column allows the chromatography of Lewis base analytes using volatile LC/MS compatible buffers (such as acetate and formate) throughout the pH range of 1-10.

Experimental

A mixture of cocaine and bezoylecognine was separated at 35 ºC 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

Time	% A	% B
0	90	10
5	10	90

A: 20mM ammonium acetate, pH 6.0
B: acetonitrile

Temperature: 35 ºC with Metalox™ 200-C column heater
Flow Rate: 2.0 ml/min.
Injection Vol.: 10 µl
Pressure Drop: 168 bar
Detection: UV at 254 nm

Excellent peak shape and baseline resolution were obtained for cocaine and benzoylcongnine on the ZirChrom®-EZ column using simple gradient elution conditions and a LC/MS compatible acetate buffer.

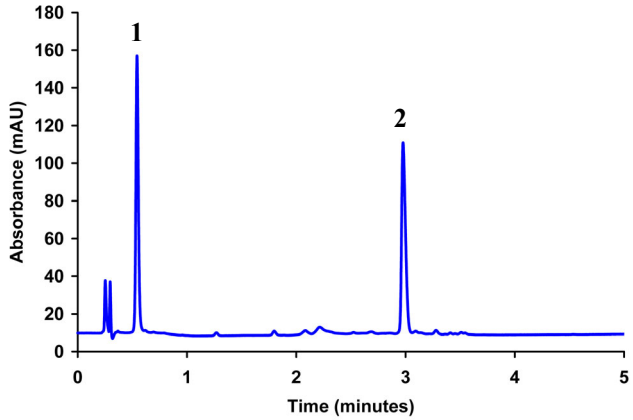

Figure 2: Separation of 1=Benzoylecongnine and 2=Cocaine

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.

References

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Fast LC/MS Compatible Separation of Tricyclic Antidepressants

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

Technical Bulletin # 289

The chromatography of the tricyclic family of antidepressants on C18-silica phases has traditionally resulted in broad and tailed peaks in the neutral pH range where most silica phases are stable. ZirChrom®-EZ is a new zirconia-based reversed-phase column that has mixed mode retention characteristics which allow chromatography of these highly basic amines with excellent peak shape and efficiency.

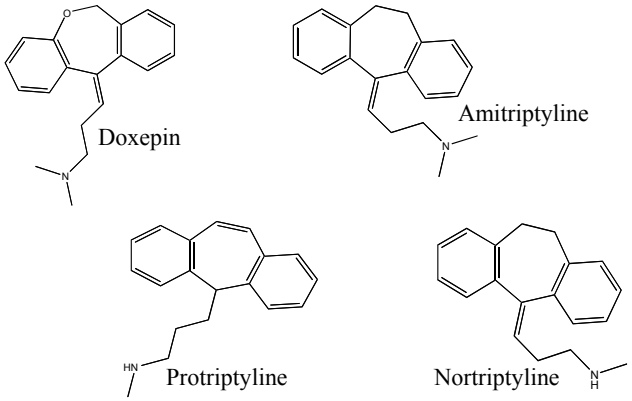

Figure 1: Structures of tricyclic antidepressants

Introduction

The chromatography of the tricyclic antidepressants on C18-silica phases has traditionally been so problematic that amitriptyline is commonly used as a probe solute for quantifying silanophilicity of silica phases. The surface chemistry of zirconia-based phases is dominated by Lewis acid sites, rather than the Bronsted acid sites which dominate the surface chemistry of silica phases. The mixed-mode retention character of ZirChrom-EZ® (cation-exchange and reversed-phase) allows separations that were previously difficult using conventional silica C18 phases. This application note shows the exceptional separation of four tricyclic antidepressants in less than three minutes.

Experimental

A mixture of four tricyclic antidpressants was separated at 35 ºC 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:	Isocratic elution: 35/65 A/B A: 20mM ammonium acetate, pH 6.0 B: acetonitrile
Temperature:	35 ºC with Metalox™ 200-C column heater
Flow Rate:	2.0 ml/min.
Injection Vol.:	5 µl
Pressure Drop:	110 bar
Detection:	UV at 254 nm

Four tricyclic antidepressant pharmaceuticals were separated using simple acetoniltrile/water isocratic elution and a LC/MS friendly acetate buffer. The selectivity of all four compounds is excellent which allows for a very fast separation using only a short 5 cm column.

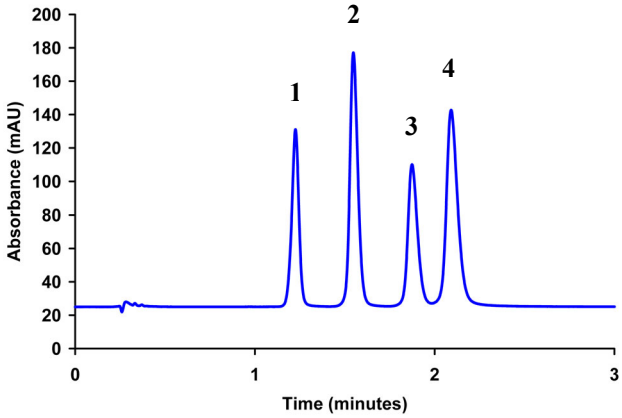

Figure 2: Separation of 1=Doxepin, 2=Protriptyline, 3=Amitriptyline, and 4=Nortriptyline

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.

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