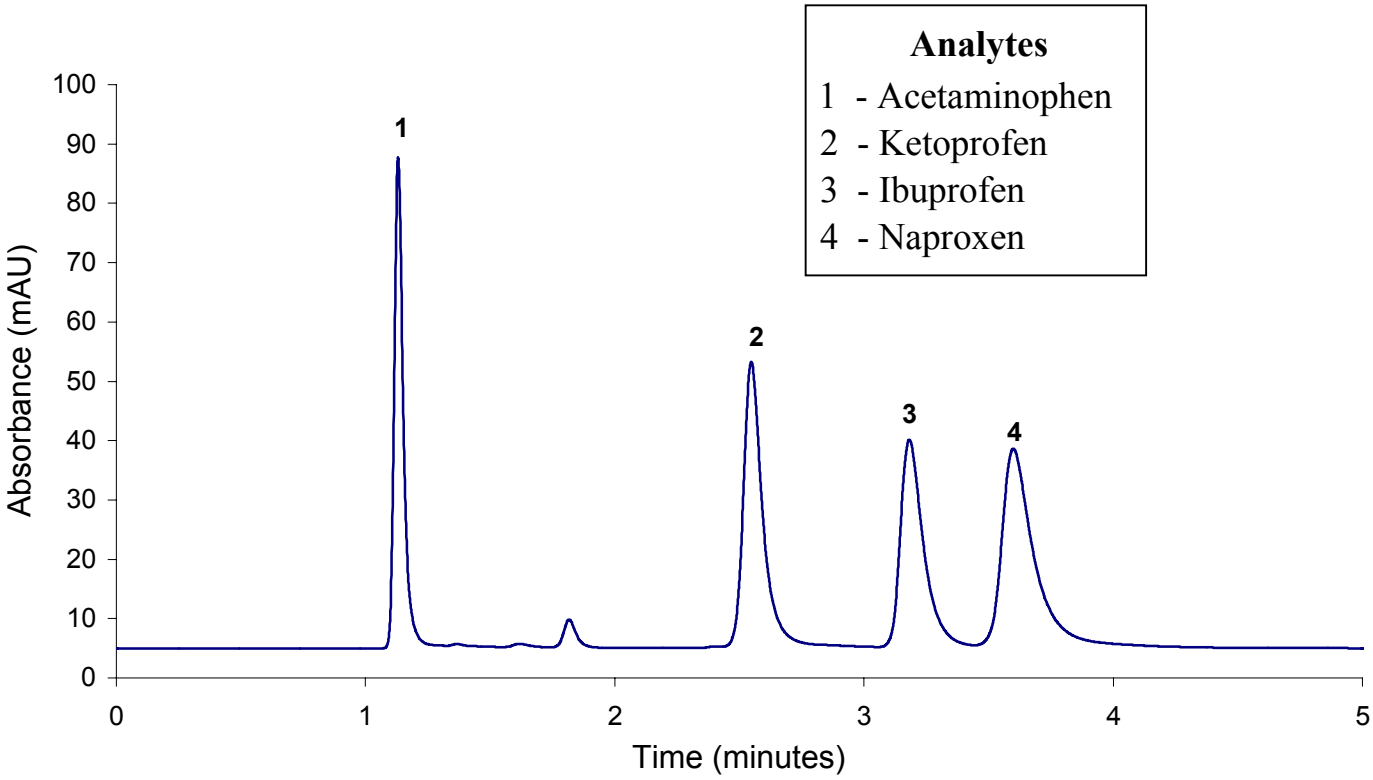
ZirChrom®

Technical Bulletin #224

... For Peak Performance

Separation of NSAIDs



LC Conditions

Column: DIAMOND BOND™ C18, 100 mm × 4.6 mm i.d.

Mobile Phase: 50/50 A/B

A: Acetonitrile

B: Water with 50 mM Phosphoric acid at pH 1.75

Flow rate: 1.0 mL/min.

Temperature: 65 ºC

Injection volume: 1 µL

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.

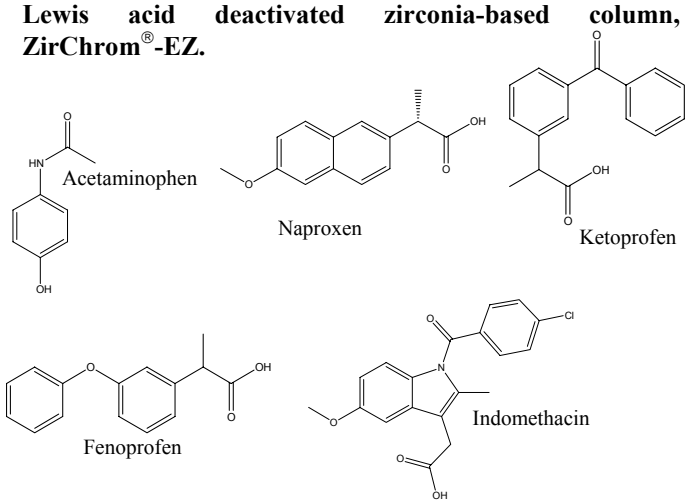


Figure 1: Structures of Non-steroidal anti-inflammatory drugs.

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.

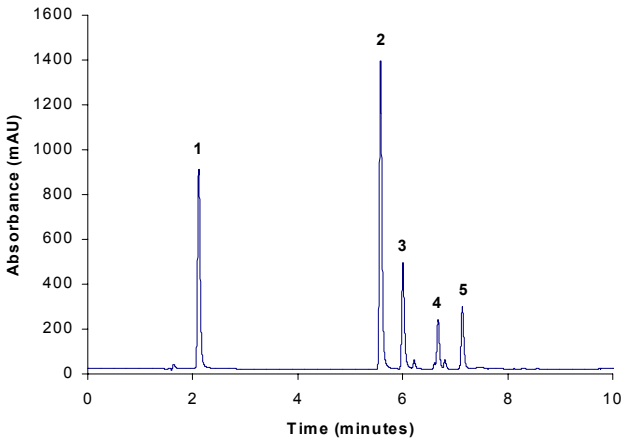


Figure 2: Separation of 1=Acetaminophen, 2=Naproxen, 3=Ketoprofen, 4=Fenoprofen, and 5=Indomethacin.

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.

ZirChrom Separations, Inc.
617 Pierce Street, Anoka, MN 55303
1-866-STABLE-1
support@zirchrom.com

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

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

Technical Bulletin #297

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®-MS.

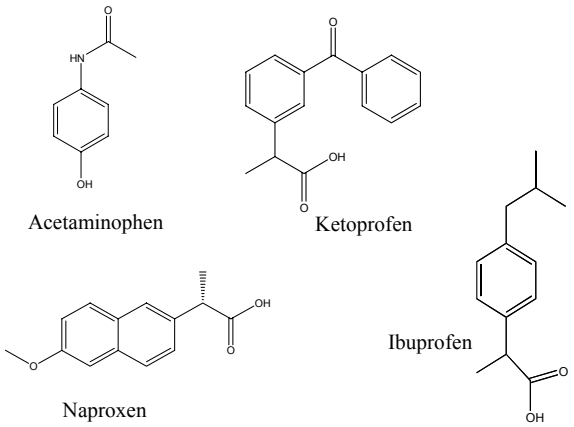

Figure 1: Structures of Non-steroidal anti-inflammatory drugs.

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®-MS 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

Four non-steroidal anti-inflammatory drugs were separated at 35ºC using a ZirChrom®-MS column. The separation conditions were as follows:

Column:	ZirChrom®-MS, 50 mm x 4.6 mm i.d. (Part Number: MS01-0546)
Mobile Phase:	Isocratic elution: 40/60 A/B A: acetonitrile B: 10mM ammonium acetate, pH 5.0
Temperature:	35 ºC
Flow Rate:	1.0 ml/min.
Injection Vol.:	5 µl
Pressure Drop:	68 bar
Detection:	UV at 254 nm

Four non-steroidal anti-inflammatory drugs were separated using simple acetonitrile/water isocratic elution and a LC/MS friendly acetate buffer. The selectivity of all four compounds is excellent which allows for a very good separation using only a short 5 cm column.

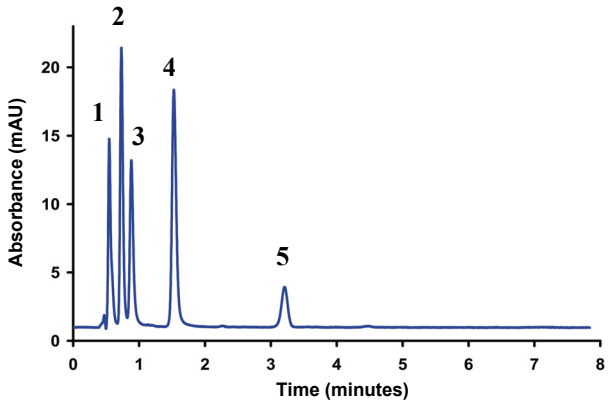

Figure 2: Separation of 1=Acetaminophen, 2=Ketoprofen, 3=Naproxen, 4=Ibuprofen, and 5=Impurity.

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*, *14*, 2875-2889, **1991**.

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LC/MS Compatible Separation of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) on ZirChrom®-SAX

Dr. Bingwen Yan and Dr. Clayton McNeff
ZirChrom Separations, Inc.

Technical Bulletin #307

In this application note we demonstrate the unique selectivity and versatility of the ZirChrom®-SAX column. The ZirChrom®-SAX column is a strong anion-exchanger that has a hydrophobic character to the stationary phase. The mixed-mode retention characteristic of the cross-linked polyethyleneimine-coated strong anion exchanger, ZirChrom®SAX, enables an LC/MS-compatible isocratic separation of four non-steroidal anti-inflammatory drugs (NSAIDs) in less than 4 min.

Figure 1: Compound structures

Introduction

Traditional silica methods for the separation of acetaminophen, ibuprofen, naproxen (common pain relievers/anti-inflammatories) and ketoprofen (used to control joint pain and swelling associated with rheumatoid arthritis) often require complex gradients and mobile phases to achieve satisfactory selectivity. NSAIDs have a carboxylate moiety, which makes them a effective Lewis base (Figure 1). It is well know that Lewis bases can interact strongly with zirconia-based columns, sometimes resulting in poor peak shapes. The strength of different common Lewis bases on zirconia has been previously reported (1). Although NSAIDs carboxylic acid moiety and formate are very near each other in elutropic strength, keeping the pH of the separation near the pKa of NSAIDs' carboxylic acid moiety increases the likelihood that it is protonated and thus will be efficiently displaced from the Lewis acid sites on zirconia by the smaller and more acidic formate anion. The resulting innovative approach uses the mixed-mode retention characteristics of the zirconia-based strong anion exchange phase, ZirChrom®-SAX, and a LC/MS-compatible ammonium formate buffer to resolve non-steroidal anti-inflammatory drugs quickly using isocratic conditions (Figure 2).

Experimental

Four non-steroidal anti-inflammatory drugs were prepared in an aqueous solution and injected on a ZirChrom®-SAX column. The separation conditions are as follows.

Column:	ZirChrom®-SAX, 50 x 4.6 mm i.d. (part number: ZR06-0546)
Mobile Phase:	80/20 ACN/15 mM ammonium formate, pH=4.0 (adjusted with formic acid)
Flow rate:	1.0 ml/min.
Temperature:	35 °C
Injection Vol.:	1.0 µl
Detection:	UV at 254 nm

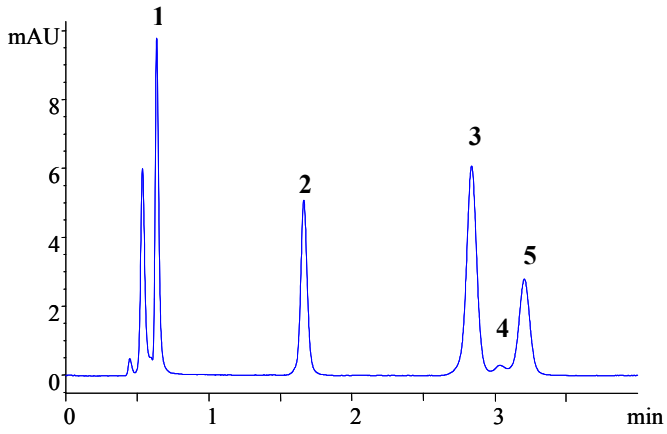

Figure 2. Separation of NSAIDs: 1=Acetaminophen, 2=Ibuprofen, 3=Naproxen, 4=Impurity, 5=Ketoprofen.

ZirChrom columns combine the high efficiency usually associated with silica columns with complete chemical and thermal stability.

References

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

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