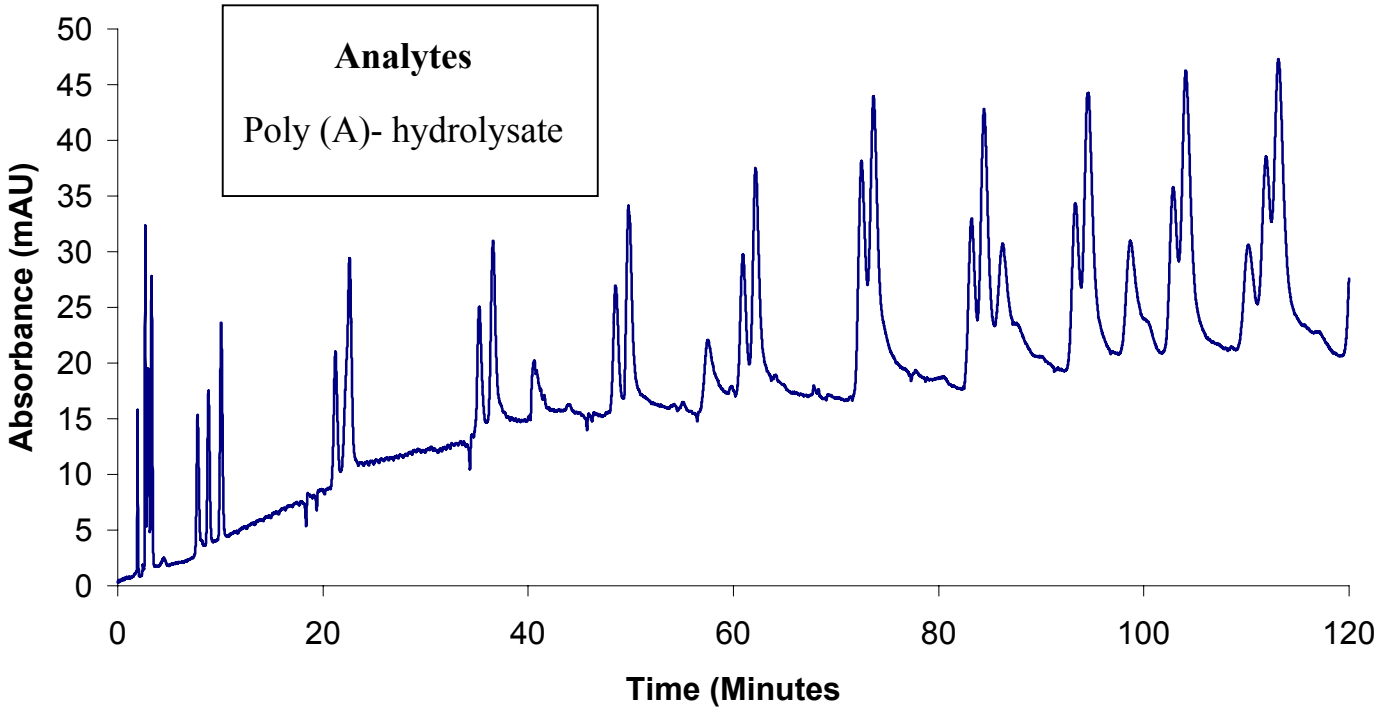
ZirChrom®

Technical Bulletin #166

... For Peak Performance

Oligonucleotide Separation on ZirChrom®-SAX



LC Conditions

Column: ZirChrom®-SAX, 50 mm x 4.6 mm i.d.
Mobile Phase:
A: 0.02 M potassium phosphate dibasic and 0.04 M NaCl at pH 8.5
B: 0.20 M potassium phosphate dibasic and 1.0 M NaCl at pH 8.5
Gradient: 5 to 95 % B over 90 minutes

Flow rate: 1.0 mL/min.
Temperature: 100 ºC
Detection: 254 nm
Injection: 25 µl

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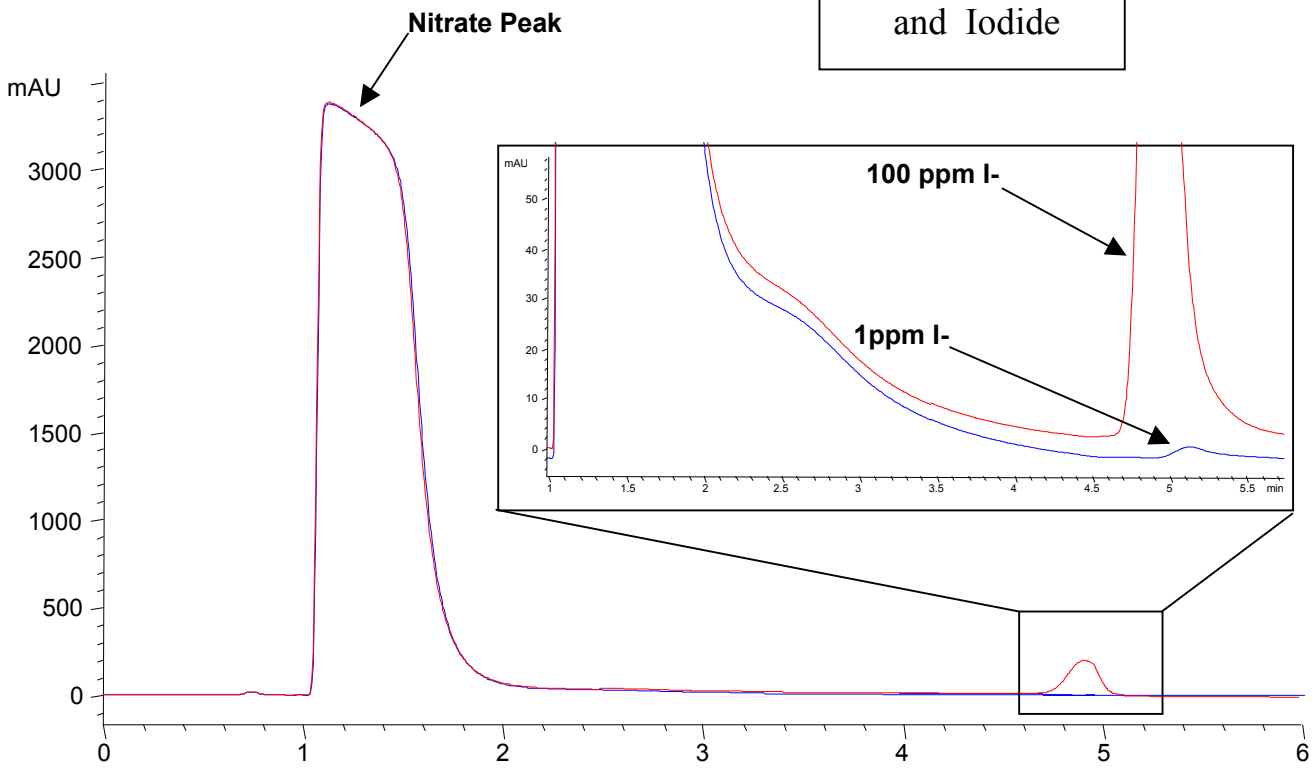
Technical Bulletin #214

... For Peak Performance

Trace Iodide Separation on ZirChrom®-SAX

Analytes
2M nitrate
sample matrix
and Iodide



LC Conditions

Column: ZirChrom®-SAX, 50 x 4.6 mm
Mobile Phase: 25mM ammonium phosphate,
275mM NaCl at pH 8.0

Flow rate: 1.0 mL/min.
Temperature: 30°C
Detection: 226 nm

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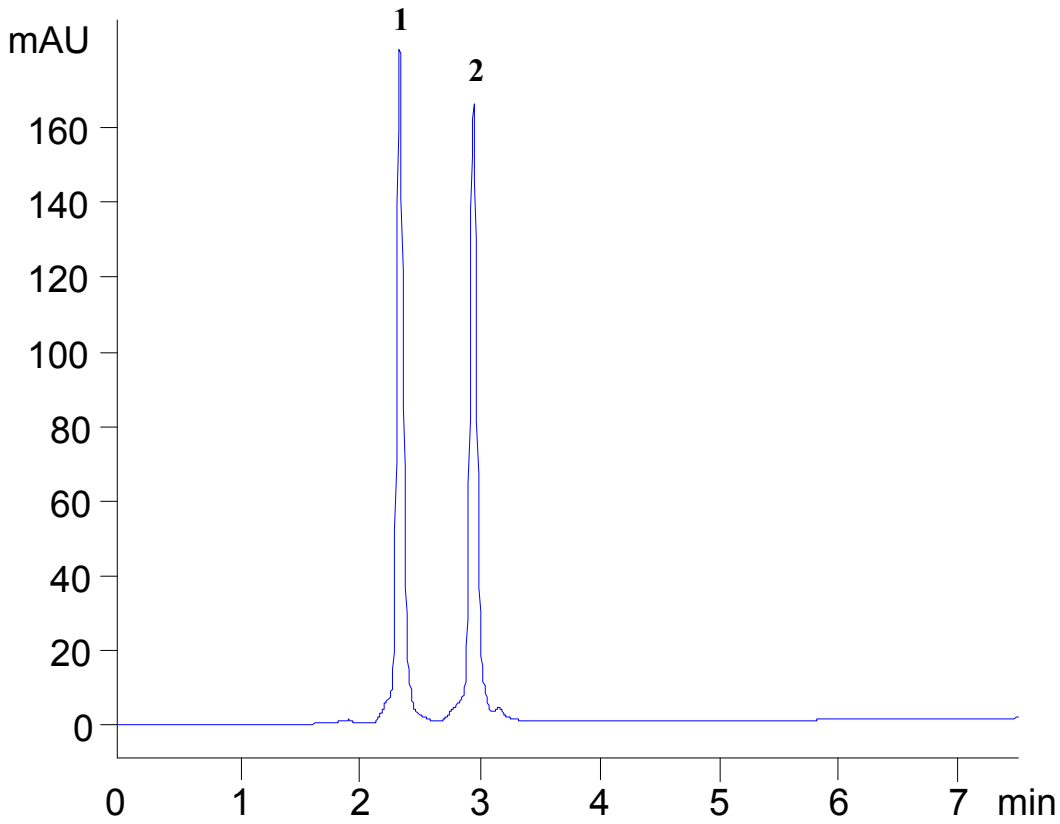
Technical
Bulletin #220

... For Peak Performance

Fertilizer Plant Effluent Ions on ZirChrom®-SAX

Analytes

1 - Cyanate
(1.0 mg/ml)
2 - Nitrate
(.02 mg/ml)



LC Conditions

Column: ZirChrom®-SAX, 150 x 4.6 mm
Mobile Phase: 25mM sodium fluoride, 175mM sodium chloride at pH 10.0
Flow rate: 1.0 mL/min.

Temperature: 30°C
Detection: 205 nm
Injection Vol.: 5 ul

High Performance Biomolecule Separations Using Zirconia-based Ion Exchange Phases

Technical Bulletin #267

Clayton V. McNeff, Peter W. Carr
ZirChrom Separations, Inc.

Silica and polymer-based phases used for the separation of biomolecules have traditionally suffered from such limitations as substrate instability in alkali media and shrinking/swelling of the particles upon changes in mobile phase composition. The extraordinary chemical and physical stability of zirconia-based phases allows much greater flexibility in method development for biomolecules. This note shows a separation of a polynucleotide hydrolysate using a ZirChrom®-SAX.

Introduction

The long-term reliability of an HPLC method depends greatly on the ruggedness of the stationary phase. In bonded silicas, an Si-O-Si bond is used to attach functional groups to the silica surface. It is well known that this bond is subject to chemical attack, especially at low pH. The silica itself dissolves readily in aqueous mobile phases at high pH. Even sophisticated silica bonding technologies have not solved this problem[1]. The basic instability of bonded silicas causes retention drift, short column life, and frequent replacement of the column and re-qualification of the HPLC system. This is expensive both in terms of actual expenditures and in terms of lost productivity.

Zirconia-based Phases for Ion Exchange Chromatography

Porous zirconia coated with polyethyleneimine (PEI) has been shown to be very useful for the separation of nucleosides, nucleotides, oligonucleotides, oligodeoxynucleotides and proteins[2]. The PEI coating can be crosslinked with a hydrophobic crosslinker and subsequently quaternarized to produce the stationary phase known as ZirChrom®-SAX which exhibits a mixed-mode characteristic of both reversed-phase and ion-exchange retention mechanisms. Because of the mixed-mode characteristic of the phase, temperature can be used as an effective tool in method development to selectively moderate the contributions of the ion-exchange and reversed-phase retention mechanisms to the overall retention.

References

(1) J. J. Kirkland et. al., Anal. Chem. 61, 2-11 (1989).

(2) C. McNeff et al., Anal Chem. 67, 2350-2353 (1995).

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support@zirchrom.com

Experimental

A hydrolyzed sample of Poly-G (oligonucleotide) was separated at elevated temperature using a ZirChrom®-SAX column. The separation conditions were as follows:

Column:	4.6 mm x 50 mm ZirChrom®-SAX
Mobile Phase:	Gradient elution from 5-95% B A: 0.02 M potassium phosphate dibasic and 0.04 M NaCl, pH 8.5 B: 0.20 M potassium phosphate dibasic and 1.0 M NaCl @ pH 8.5
Temperature:	100 °C
Injection Vol.:	25 µl
Flow rate:	1.0 ml/min.
Detection:	UV at 254 nm

Results

The chromatogram below illustrates the capability of a zirconia-based ion-exchange phase to resolve nucleotide oligomers in a hydrolysate of poly-G. The separation is performed in a phosphate buffer at 100 °C, further demonstrating the extraordinary chemical stability of the stationary phase.

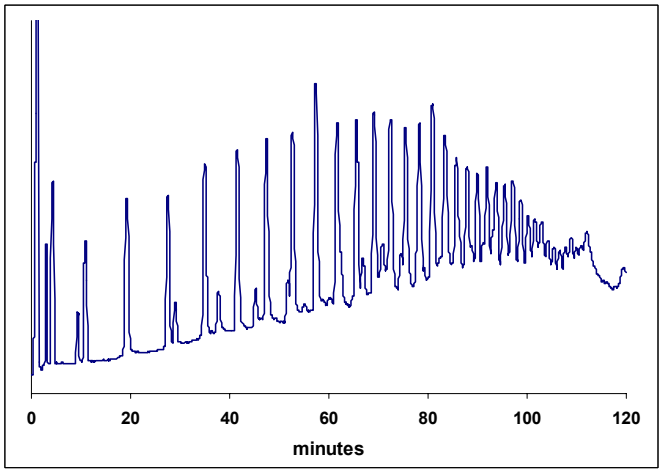

Figure 1: Separation Poly-G Hydrolysate

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Analysis of Inorganic Sulfate in Water with ZirChrom®-SAX

Dr. Clayton McNeff, ZirChrom Separations
Dwight Stoll, ZirChrom Separations

Technical Bulletin #274

Scientists at a leading cattle feed additive company, SarTec Corporation, needed a robust, linear method for the quantification of inorganic sulfate in water. ZirChrom method developers found the solution on ZirChrom®-SAX. The new method provides quantitation of inorganic sulfate (Na_2SO_4) in under 3 minutes by indirect UV detection (Figure 1).

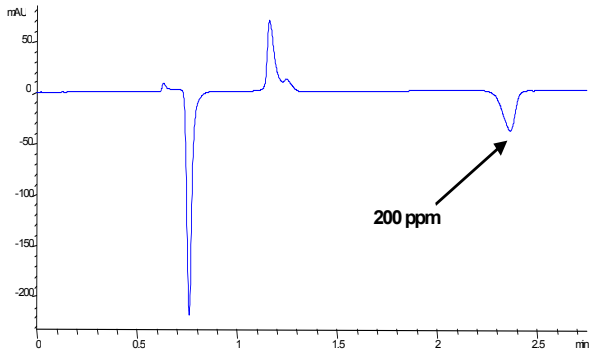

Figure 1: Analysis of Inorganic Sulfate in Drinking Water.

Introduction

Consumption of water with high levels of sulfate anion has been shown to lead to higher incidence of PEM (polioencephalomalacia) in beef cattle (1). The scientists at SarTec needed a way to reliably quantitate the amount of sulfate in water before they could test their hypothesis for its removal.

Experimental

A set of sulfate standards was made through serial dilution. Four replicates were performed for each of the concentrations.

Column:	4.6 mm x 150 mm ZirChrom-SAX
Mobile Phase:	2mM ethylenediaminetetra(methylphosphonic) acid (EDTPA), 20mM 2-(N-morpholino)ethane sulfonic acid (MES), 5mM Sodium Chloride
Injection Vol.:	10 ul
Pressure Drop:	195 bar
Detection:	UV at 220 nm
Flow Rate:	2.5 ml/min
Temperature:	50 ºC

This method of sulfate quantitation is reproducible, fast and produces a very linear standard curve (figure 2). The ZirChrom-SAX method allowed scientists at SarTec to quickly determine the sulfate concentration of water samples before and after experimental procedures to remove sulfate anions.

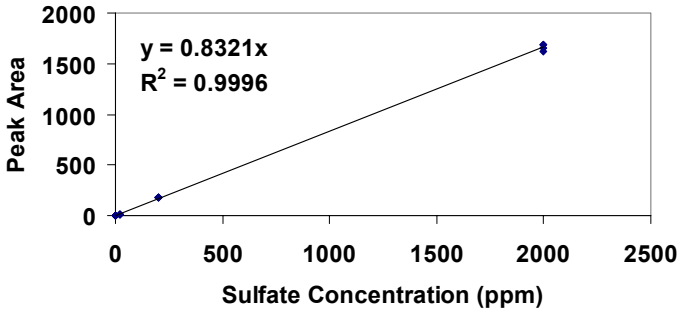

Figure 2: Standard Curve for Sulfate Analysis on ZirChrom®-SAX

Dr. Peter Greuel of SarTec corporation said "Not only is the analysis fast but so was the method development process. We are extremely happy with the results we've seen and look forward to working with ZirChrom in the future."

References

(1) J.J. Wagner, Continental Beef Research, Lamar, CO; G.H. Loneragen and D.H. Gould, Colorado State University, Ft. Collins, CO.

Acknowledgments

Dr. Peter Greuel, General Manager, SarTec Corporation

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"Green" Analysis of Diet Soft Drinks Containing Caffeine and Aspartame

Dwight Stoll and Dr. Clayton V. McNeff
ZirChrom Separations, Inc.

Technical Bulletin #278

The popularity of diet soft drinks containing aspartame requires the analysis of thousands of samples for quality control purposes each year. These analyses are typically carried out with some fraction of organic modifier in the eluent, leading to the generation of thousands of gallons of hazardous organic waste. This note shows the "green" analysis of several commercial diet soft drinks in combination with a 100% water mobile phase.

Introduction

The analysis of caffeine, aspartame, and benzoate from diet soft drinks is not especially challenging, as one can find this type of application in nearly every column manufacturers catalog. However, the typical conditions employed for reversed-phase separations of these compounds involves anywhere from 5-25% organic modifier. Whether this organic component of the eluent is methanol or acetonitrile, the added handling costs associated with either recycling or disposing of the used eluent can often be a major portion of the total analysis cost.

The extraordinary chemical and thermal stability of zirconia-based stationary phases allow the chromatographer to use a wide range of mobile phases and column temperatures to develop rapid separation methods while retaining method robustness. ZirChrom®-SAX is an anion-exchange material that is built on microporous zirconia and contains a substantial amount of hydrophobic cross-linker that imparts mixed-mode ion-exchange/reversed-phase characteristics. The separation of caffeine, aspartame, and benzoate shown here in a 100% aqueous mobile phase demonstrates the capability of the mixed-mode column to separate these compounds of wide-ranging chemical properties. The 100% aqueous mobile phase containing dilute amounts of phosphate and carbonate is completely environmentally friendly, eliminating the need for costly handling and disposal of the eluent. Finally, the thermal stability of the ZirChrom®-SAX material allows the separation to be carried out at 50 °C to decrease overall analysis time.

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Experimental

Samples of several commercial soft drinks were filtered with 0.45 µm filters and injected neat on a ZirChrom®-SAX column using the following conditions.

Column:	3.0 mm x 100 mm ZirChrom®-SAX
Mobile Phase:	10mM Ammonium phosphate, 5mM Ammonium carbonate, pH 6.6
Flow rate:	1.0 ml/min.
Temperature:	50 °C
Injection Vol.:	5.0 µl
Pressure Drop:	205 bar
Detection:	UV at 210 nm

The separation is shown in Figure 1. Under these conditions, the separation of caffeine, aspartame, and benzoate is achieved with good peak shape and baseline resolution, in under 4 minutes. Similar separations are obtained for samples of 5 different diet soft drinks indicating that this is a widely applicable method for diet soft drink analyses.

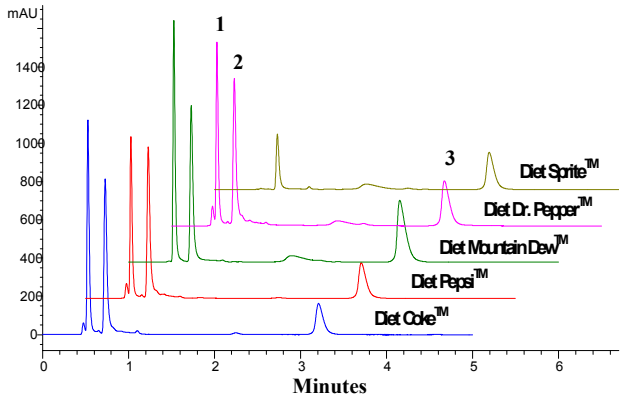

Figure 1. Analysis of several diet soft drinks.
1=Caffeine, 2=Aspartame, 3=Benzoate

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Water-Soluble Vitamin Analysis on ZirChrom®-SAX

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

Technical Bulletin #305

Traditionally the analysis of water-soluble vitamins by reversed-phase HPLC has been complicated by the lack of retention for these compounds on conventional silica C18 columns. Other analytical approaches, such as ion-pair chromatography, have also failed to yield successful and reproducible results. Here we demonstrate efficient baseline resolution of six water-soluble vitamins in six minutes using a ZirChrom®-SAX column. This method can be combined with ZirChrom's ProTain® In-Line Protein Removal System for the analysis of these compounds in biological samples.

Introduction

In this application note we focus on the HPLC analysis of Vitamin C and five B-complex vitamins; Vitamin B_1 (thiamine), Vitamin B_2 (riboflavin), Vitamin B_3 (nicotinic acid form), Vitamin B_3 (nicotinamide form), and Vitamin B_6 (pyridoxine). All of these vitamins are water-soluble.

Chromatographers oftentimes struggle in their attempts to analyze water-soluble vitamins by HPLC. Many water-soluble vitamins are very polar. Thiamin (Vitamin B_1), pyridoxine (Vitamin B_6) and ascorbic acid (Vitamin C), for example, show almost no retention on conventional C18 columns. Reversed-phase analytical methods employing ion-pair reagents have been offered as a potential solution to this problem, but these methods tend to suffer from column-to-column reproducibility problems due to the somewhat unpredictable way ion-pairing reagents interact with the silica surface and the bonded phase.

In this technical bulletin we present a unique method for the analysis of water-soluble vitamins using a ZirChrom®-SAX HPLC column. ZirChrom®-SAX, an anion exchange material, is polyethleneimine-coated zirconia containing a substantial amount of hydrophobic cross-linker which imparts both ion-exchange and reversed-phase characteristics. The mixed mode retention characteristics of the ZirChrom®-SAX column create the unique selectivity ideal for this application (Figure 1).

For the analysis of water-soluble vitamins in serum, or other samples containing biological matrices, we recommend the addition of the ProTain® In-Line Protein Removal System; consisting of one guard holder (part# 850-00-2) and a set of three ProTain® inserts (part# PT01-0246). Please see technical bulletins #275 and #291 for further information on the use of ZirChrom's ProTain® In-Line Protein Removal System.

Experimental

Six water-soluble vitamin standards were prepared in an aqueous solution and injected on a ZirChrom®-SAX column. The separation conditions are as follows.

Column:	ZirChrom®-SAX, 150 x 4.6 mm i.d. (part number: ZR06-1546)
Mobile Phase:	50 mM Ammonium dihydrogenphosphate, pH 4.5
Flow rate:	1.0 ml/min.
Temperature:	30 °C
Injection Vol.:	5.0 µl
Detection:	UV at 254 nm

The separation is shown in Figure 1. Under these conditions the separation of Vitamin C and the four B-complex vitamins is achieved, with good peak shape and baseline resolution, in 6 minutes.

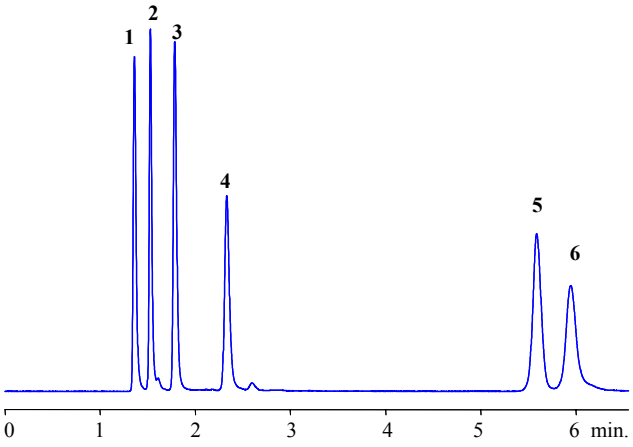

Figure 1. Analysis of Water-Soluble Vitamins.
1=Thiamine (Vit. B_1), 2=Pyridoxine (Vit. B_6),
3= Nicotinamide (form of Vit. B_3), 4=Riboflavin (Vit. B_2),
5=Nicotinic acid (form of Vit. B_3), 6=Ascorbic acid (Vit. C)

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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.

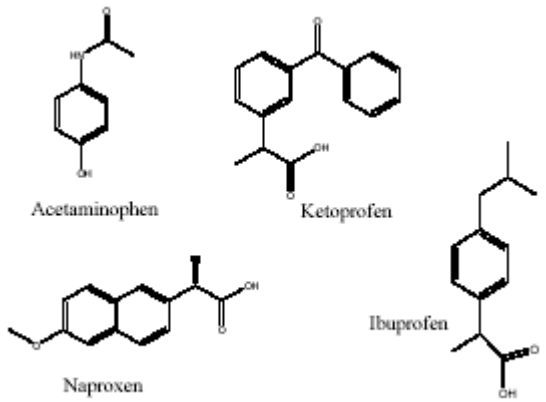

Figure 1: Compound structures

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

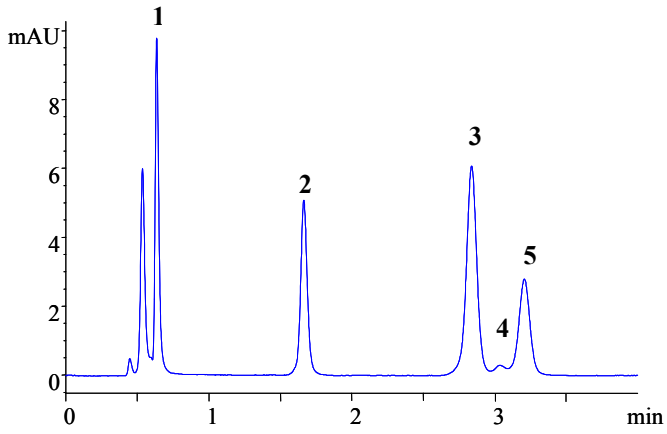

Figure 2. Separation of NSAIDs: 1=Acetaminophen, 2=Ibuprofen, 3=Naproxen, 4=Impurity, 5=Ketoprofen.

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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Isolation of Glyphosate in a Broad Leaf Herbicide using ZirChrom-SAX

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

Technical Bulletin # 320

Attempts at isolation using silica based columns of glyphosate, and an important process impurity, peak C, in a broad leaf herbicide are often hindered by lack of resolution, poor peak shape and short column lifetime. ZirChrom®-SAX provides baseline resolution of all process components employing an aqueous isocratic method with good peak shape and excellent column lifetime.

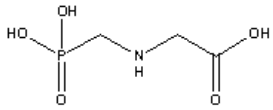

Figure 1: Structure of glyphosate

Introduction

Glyphosate [N-(phosphonomethyl) glycine] is a regulated, broad spectrum, postemergence herbicide, sold under the trade names Roundup (Monsanto Co., St. Louis, MO) and Rodeo (Dow AgroSciences, Indianapolis, IN)(1). Process testing requires the isolation of glyphosate and its impurities. Although selectivity for this analysis is improved using phosphate buffer and high pH employing these conditions may result in very short column lifetimes for silica based ion exchangers. The unique selectivity and superior stability of the ZirChrom®-SAX column allow method developers to choose the mobile phase that results in optimum selectivity without compromising column lifetime.

Experimental

A broad leaf herbicide was diluted 1:100 in mobile phase and separated at 30ºC using a ZirChrom®-SAX column. The separation conditions were as follows:

Column:	ZirChrom®-SAX, 150 mm x 4.6 mm i.d. (Part Number: ZR06-1546)
Mobile Phase:	20mM Ammonium Phosphate, pH 8.5
Temperature:	30 ºC with Metalox™ 200-C column heater
Flow Rate:	1.0 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 254 nm

All components are well separated with good peak shape including peak D isomers and the peak C impurity peak.

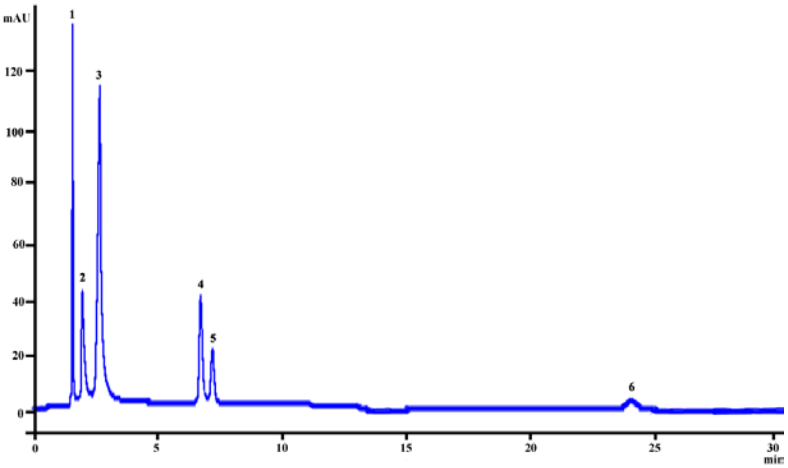

Figure 2: 1=Isopropylamine 2=Unknown 3=Glyphosate 4,5=peak D isomers 6=peak C (Impurity).

This method can be tailored to your specific application needs. ZirChrom technical support 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

(1) *Farm Chemicals Handbook*; Berg, Gordon L. Ed.; Meister: Willoughby, OH, 1989; p C147.

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Green Separation of Caffeine, Benzoate and Sorbate in Energy Drinks

Merlin Bicking, Ph.D. and Kelly S. Johnson
ACCTA and ZirChrom Separations, Inc.

Technical Bulletin # 339

In this application we examine the superior selectivity of the ZirChrom®-SAX phase for caffeine, benzoate and sorbate in energy drinks.

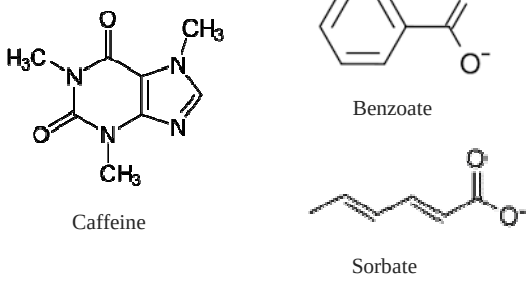

Figure 1: Structures of caffiene, sorbate and benzoate.

Introduction

Benzoate and sorbate are used as preservatives in many foods, including energy drinks. Traditionally the analysis of these compounds using silica C18 phases is hindered by low retention and poor selectivity.

The unique multimodal selectivity of Zirchrom®-SAX can take advantage of differences between the ionized forms, allowing for both ion exchange and reversed phase modes of separation.

Here we present an isocratic method that provides excellent selectivity and retention, resulting in 6 minute baseline resolution of caffeine, sorbate, and benzoate using UV detection at 230 nm. For comparison, we present the analysis of the same compounds using both neutral and acidic conditions on a leading silica C18 column.

Experimental

Three compounds in a common energy drink caffeine, sorbate and benzoate, were separated in 6 minutes using a ZirChrom®-SAX column. The separation conditions were as follows and as noted in Figure 2:

Column:	ZirChrom®-SAX, 100 mm x 4.6 mm i.d. (Part Number: ZR06-1046)
Mobile Phase:	10mM $NH_4H_2PO_4$ + 5 mM NH_4CO_3
Temperature:	50 ºC
Flow Rate:	1.5 ml/min.
Injection Vol.:	5 µl
Detection:	UV at 230 nm

Figure 2 clearly demonstrates that the neutral condition on a silica C18 column has too little retention of these compounds and that the acidic condition does not separate sorbate and benzoate. In comparison, the ZirChrom®-SAX column separates all three of the compounds in siz minutes using isocratic conditions and UV detection.

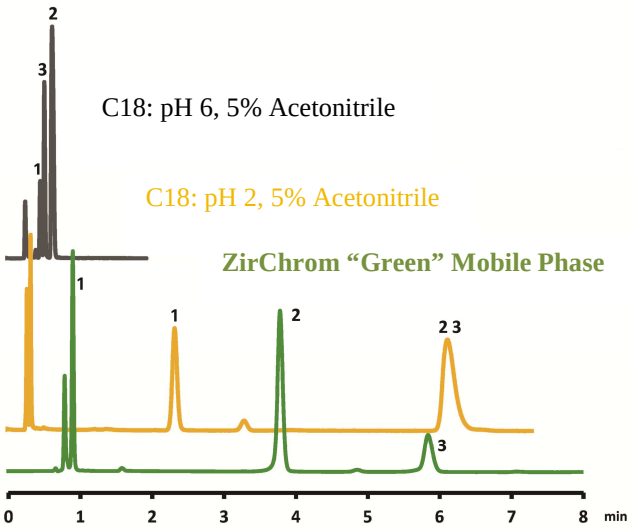

Figure 2: 1 =Caffeine, 2 = Sorbate, 3=Benzoate

This method can be tailored to your specific application needs. ZirChrom technical support 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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